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Citrus Research International, Nelspruit

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1 INTRODUCTION

CEO (CRI): Vaughan Hattingh

The CRI Group Alliance continued to provide the industry with wide ranging Research and Technical support services for this report period, being April 2008 to March 2009. CRI continued to manage its operations in the following Divisions: Research, Extension, Cultivar Development and the Citrus Improvement Scheme; with the overarching priority of Market Access.

The levy on export citrus fruit, as administered by the CGA, continued to provide the primary source of funds. A new source of additional funding was accessed from the Department of Science and Technology through the Post Harvest Innovation Programme, an initiative from the Fresh Produce Exporters Forum. Income derived through royalties from the commercialisation of new technologies continued to provide a valuable income stream for CRI. CRI retained the approach of identifying technologies with commercialisation potential, that arise through CRI activities, and engaging with the most suitable implementation partners, with a preference for utilising River Bioscience where appropriate. Thereby, wherever possible, CRI ensures that new technologies that arise from its activities, are made available to the industry in a way that ensures industry access at lowest commercially feasible prices and generates a royalty return, to support further research and reduce reliance on the levy.

Intense inflationary price increases were experienced in the first part of the financial cycle, presenting CRI with a major budget compliance challenge. This was compounded by the need to initiate large, unforeseen and unbudgeted projects, due to emerging biosecurity and market access concerns of critical importance to the industry's future. A combination of strict cost management, the fortuitous addition of funding from the Post Harvest Innovation fund (funds secured from the Department of Science and Technology through a FPEF initiative) and utilisation of reserve funds in the CRT, made it possible to conclude the season on a near break-even basis.

Concern about the threat that FCM poses to Market Access resulted in the industry implementation of a range of measures to reduce the risk. The disagreement between the European Union and South Africa about the European phytosanitary regulations pertaining to Citrus Black Spot (CBS) continued. An upsurge in interceptions of CBS infected fruit in Europe increased focus of attention on this issue. These developments have major potential consequence for the southern African citrus industry and CRI remained engaged in these matters, as a top priority.

The requisite data were generated to support the implementation of improved time temperature protocols for the export of all citrus types to Japan. The prospect of broadening access to the USA market progressed in terms of potentially providing access for the Northern Cape and for Pest Free Places of Production within Areas of Low Pest Prevalence, using the far northern Limpopo as a test case.

Within the Integrated Pest Management Programme there continued to be a strong focus on FCM and fruit flies. The second year of commercial implementation of the Sterile Insect Technique (SIT), through Xsit (Pty) Ltd., produced excellent results in the Citrusdal region. Research was undertaken in Letsitele to support roll out of the technology to other regions, but results were negative, necessitating further field trials that are planned for execution in both Limpopo and Eastern Cape. A new acaricide has been developed that will be of great value for the control of bud mite, once the registration process has been concluded.

In the Disease Management Programme, good progress was made in improving CBS control measures through elucidation of the critical infection period in the Eastern Cape and optimisation of copper spray programmes. Evaluations of several new potential chemical and biological control techniques were initiated. Deficiencies in the implementation of standard packhouse fungicide application recommendations were determined and communicated to the industry, so that corrective actions can be taken to reduce the extensive losses that ensue from the associated post harvest decay. Cutting edge research on spray technology progressed well, with the prospect of being able to reduce costs and increase efficacy through future application. Important advances were made in the diagnostics of Citrus Tristeza Virus and the development of alternative nematode control options.

Good progress was made in the Crop and Fruit Quality Management Programme through elucidating the role played by light and the mineral, nutrient and carbohydrate content of the flavedo, in rind quality and prevention of

rind breakdown. Development of chemical treatments to reduce the size of navel end openings progressed well. A multi-industry carbon footprint study was initiated to develop a standard industry carbon calculator, benchmarking the industry's carbon footprint and developing an industry carbon footprint strategy. The appointment of a DST-funded chair of post-harvest research at the University of Stellenbosch, holds the prospect of supporting more progress in this programme in the future.

Within the Cultivar Development division, the evaluation project progressed well and will result in a series of SA Fruit Journal publications. The natural mutation screening project produced numerous potential new selections that have entered shoot tip grafting procedures. A targeted CRI breeding programme has been designed for future implementation. Several new promising cultivars entered the country for local testing.

The Citrus Improvement Scheme continued to operate on a voluntary participation, non-statutory basis, with a high level of industry support. Fifteen cultivars or selections entered the shoot tip grafting process and 38 completed indexing for establishment at the CFB. Viroids were detected in two important navel cultivars. Supply of budwood was terminated, all nurseries and affected growers were notified, procedures were initiated to provide a viroid-free source and further research was initiated to better evaluate the risk. Ongoing difficulty in maintaining CTV cross protection in soft citrus varieties, resulted in a decision to replace the cross protection strain in soft citrus and the industry was accordingly advised of the situation.

Progress in conversion to a statutory scheme continued to be frustrated by delays in the appointment of the relevant responsible official in the DoA. The request to DoA to legislate an exclusion zone around the CFB remained outstanding. A survey was conducted to verify the absence of Citrus Greening Disease in the Eastern Cape Province and to monitor its distribution in the Western Cape. Isolated occurrence was detected near Port St Johns and a recommendation was made to DoA to eradicate, to maintain the Eastern Cape's greening Pest Free Area status. In the Western Cape, no additional infected Magisterial Districts were found.

The Citrus Foundation Block's sales recovered well during the report period, due to high seed sales, both locally and internationally. This enabled CRI to continue operating CFB on a financial self sufficiency basis, with sufficient income to provide for essential infrastructural developments in the forthcoming year. Infrastructure at the Citrus Foundation Block (CFB) was further expanded to improve phytosanitary risk management procedures. All open ground CFB plantings, with the exception of the seed source trees, were removed to minimise phytosanitary risk.

Twenty one citrus nurseries were inspected. Twenty were certified as being compliant with the CIS (as commercial citrus nurseries), including two new nurseries, and one received provisional certification. Six other nurseries are registered as farm nurseries.

The Extension highlight of the 2008/9 year was the very successful CRI Research Symposium, with a record 450 delegate attendance and a strong contingent of international presenters. The Regional Technology Transfer Group Network continued to provide a very effective technology implementation mechanism. The strategic partnership with the citrus consultants continued to provide good support to this network. The Citrus Cold Chain Forum continued to grow in strength with the conclusion of the first set of minimum industry standards for packaging material. The Cutting Edge was actively used as a quick and effective communication tool. The comprehensive review of research priorities was changed to a two-yearly cycle, with the next comprehensive review scheduled for mid-2009. The scope of extension services was expanded, by the appointment of a Transformation Extensionist in the South, to compliment the earlier successful deployment of a similar position in the northern region. These posts are fully funded by CGA and the employment platform is provided by CRI, within the Extension Division.

INLEIDING

Hoofuitvoerendebeampte (CRI): Vaughan Hattingh

Die CRI Groep Alliansie het weereens die bedryf van 'n wye reeks van Navorsing en Tegniese ondersteuningsdienste tydens die verslagperiode, April 2008 tot Maart 2009, voorsien. CRI het voortgegaan om sy bedrywighede binne die volgende Afdelings te bestuur: Navorsing, Voorligting, Kultivarontwikkeling en die Sitrusverbeteringskema; met Marktoegang as die oorkoepelende prioriteit.

Die heffing op sitrusvrugte wat uitgevoer word en deur die CGA geadmistrateer word, was steeds die primêre bron van befondsing. 'n Nuwe bron van addisionele befondsing is vanaf die Departement Wetenskap en Tegnologie, deur middel van die Na-Oes Innovasieprogram, 'n inisiatief vanaf die Vars Produsente Uitvoerdersforum, bekom. Inkomste wat vanaf tantieme vanaf die kommersialisering van nuwe tegnologie bekom is, was weereens 'n waardevolle bron van inkomste vir CRI. CRI het die benadering behou om tegnologie met die potensiaal vir kommersialisering, wat deur CRI aktiwiteite ontstaan, te identifiseer, in samewerking met die mees geskikte vennote vir implementering met die voorkeur om River Bioscience waar geskik, te gebruik. Hierdeur, waar moontlik, verseker CRI dat nuwe tegnologie wat deur sy aktiwiteite ontstaan aan die bedryf beskikbaar gestel word op so 'n wyse dat bedryfstoegang teen die laagste moontlike kommersiële pryse verseker word en tantieme as verdienstes gegeneer word om verdere navorsing te ondersteun en die afhanklikheid op hierdie heffing te verminder.

Hewige inflasionêre prystoename is in die eerste gedeelte van die finansiële siklus ondervind, wat daar toe gelei het dat CRI gelaat was met 'n groot uitdaging om binne die begroting te bly. Dit is vererger deur die behoefte om groot onvoorsiene en nie-begrote projekte te inisieer as gevolg van opkomende biosekuriteit en marktoegang bekommernisse wat van kritiese belang vir die bedryf se toekoms is. 'n Kombinasie van streng kostebestuur, die toevallige byvoeging van fondse vanaf die Na-Oes Innovasie Fonds (fondse verkry vanaf die Departement Wetenskap en Tegnologie deur 'n FPEF inisiatief) en benutting van reserwe fondse in die CRT, het dit moontlik gemaak om hierdie seisoen op 'n naby gelykbreek af te sluit.

Bekommernis oor die bedreiging wat VKM vir Marktoegang inhou het daartoe gelei dat die bedryf 'n reeks maatreëls geïmplementeer het om die risiko te verminder. Die geskil tussen die Europese Unie en Suid-Afrika oor die Europese fitosanitêre regulasies rakende Sitrus Swartvlek (SSV) het voortgegaan. 'n Styging in onderskepping van SSV-geïnfekteerde vrugte in Europa het meer aandag op hierdie aangeleentheid gefokus. Hierdie ontwikkelinge hou groot potensiële gevolge vir die suider-Afrikaanse sitrusbedryf in en CRI se betrokkenheid by hierdie sake het 'n hoë prioriteit gebly.

Die vereiste inligting om die implementering van verbeterde tyd/tempertuur protokolle vir die uitvoer van alle sitrustipes na Japan te ondersteun, is ontwikkel. Die vooruitsig om toegang tot die VSA mark uit te brei het gevorder in terme van die potensiële verskaffing van toegang aan die Noord-Kaap en aan Pesvrye Plekke van Produksie binne-in Areas van Lae Pes Voorkoms, met die verre Noordelike Limpopo as toetsgeval.

Binne die Geïntegreerde Plaagbestuurprogram het daar 'n sterk fokus op VKM en vrugtevlieë gebly. Die tweede jaar van kommersiële implementering van die Steriele Insek Tegniek (SIT), deur Xsit (Pty) Ltd., het uitstekende resultate in die Citrusdal streek opgelewer. Navorsing is ook in Letsitele onderneem om die uitbreiding van die tegnologie na ander streke te ondersteun, maar resultate was negatief, wat verdere veldtoetse genoodsaak het waarvan die uitvoering in beide Limpopo en Oos-Kaap beplan is. 'n Nuwe mytdoder is ontwikkel wat van groot waarde vir die beheer van knopmyt sal wees sodra die registrasieproses afgehandel is.

In die Siektebestuursprogram is goeie vordering in die verbetering van SSV -beheermaatreëls gemaak deur die vasstelling van die kritiese infeksie periode in die Oos-Kaap en die optimisering van koper spuitprogramme. Evaluasies van verskeie nuwe potensiële chemiese en biologiese beheertegniese is onderneem. Tekortkominge in die implementering van aanbevelings van standaard swamdoder toedienings in die pakhuis is bepaal en aan die bedryf gekommunikeer sodat korrektiewe aksies geneem kan word om omvattende verliese wat vanuit geassosieerde na-oes bederf voortvloei, te verminder. Gevorderde navorsing oor spuittegnologie het goed gevorder, met die vooruitsig dat dit moontlik sal wees om kostes te verminder en effektiwiteit te verhoog in toekomstige toepassings. Belangrike vordering is in die diagnostiek van Citrus Tristeza Virus en die ontwikkeling van alternatiewe beheeropsies vir nematodes gemaak.

Goeie vordering is in die Oes- en Vrugkwaliteitsbestuurprogram gemaak deur die vasstelling van die rol wat lig en mineraal-, voedingstof- en koolhidraatinhoud van die flavedo in die kwaliteit van die skil en die voorkoming van skilafbraak speel. Ontwikkeling van chemiese behandelings om die grootte van nawelend openinge te verminder, het goed gevorder. 'n Multi-bedryf koolstof-voetspoorstudie is onderneem om 'n standaard bedryfskoolstof- rekenaar te ontwikkel, die bedryf se koolstof-voetspoor se hoogtepunt vas te stel en ontwikkeling van 'n bedryfs-koolstof-voetspoor strategie. Die aanstelling van 'n DWT-befondste stoel vir Na-Oes navorsing by die Universiteit van Stellenbosch hou die vooruitsig van ondersteuning vir verdere vordering in hierdie program vir die toekoms in.

Binne die Kultivarontwikkelingsafdeling het die evaluasieprojek goed gevorder en sal tot 'n reeks van publikasies in die SA Vrugte Joernaal lei. Die natuurlike mutasie siftingsprojek het verskeie potensiele nuwe seleksies opgelewer wat nou in die proses van groeipuntenting is. 'n Geteikende CRI telingsprogram is vir toekomstige implementering ontwerp. Verskeie nuwe belowende kultivars is vir plaaslike toetsing ingebring.

Die Sitrusverbeteringskema opereer steeds op 'n vrywillige deelname, nie-statutêre basis met 'n hoë vlak van bedryfsondersteuning. Vyftien kultivars of seleksies is in die proses van groeipuntenting en 38 het indeksering voltooi vir vestiging in die Sitrus Grondvesblok (SGB). Viroiede is in twee belangrike nawel kultivars opgespoor. Voorsiening van enthout is gestop, alle kwekerye en geaffekteerde produsente is in kennis gestel, prosedures is onderneem om 'n viroied vrye bron te vind en verdere navorsing is begin om risiko beter te evalueer. Voortdurende probleme in die volhouding van die CTV kruisbeskerming in sagte sitrus kultivars het tot 'n besluit dat die kruisbeskermingsras in sagte sitrus vervang moet word, gelei en die bedryf is denuoreenkomstig van die situasie ingelig.

Vordering in die omskakeling na 'n verpligte verbeteringskema bly frustrerend weens die vertraging in die aanstelling van die relevante verantwoordelike beampte in die Department van Landbou, Bosbou en Visserye (DAFF). Die versoek aan DAFF om 'n buffer sone rondom die SGB te wettig, bly uitstaande. 'n Opname om die afwesigheid van Sitrus Vergroeningsiekte in die Oos-Kaap te verifieer en om die verspreiding in die Wes-Kaap te monitor, is uitgevoer. Geïsoleerde voorkoms is naby Port St Johns gevind en 'n aanbeveling vir vernietiging is aan DAFF gemaak om sodoende die Oos-Kaapse vergroenings pesvrye status te behou. In die Wes-Kaap is geen addisionele geïnfekteerde magistraatsdistrikte gevind nie.

Die Sitrus Grondvesblok se verkope het goed tydens die verslagperiode herstel as gevolg van hoë saad verkope, beide nasionaal en internasionaal. Dit het CRI in staat gestel om voort te gaan om die SGV op 'n finansiële self onderhoudende basis te bedryf met genoegsame inkomste om in belangrike infrastrukturele ontwikkelings in die komende jaar te voorsien. Infrastruktuur by die SGV is verder uitgebrei om die fitosanitêre risiko se bestuursprosedures te verbeter. Alle oopgrond aanplantings by die SGV, met die uitsondering van die bome wat as bronne vir saad dien, is verwyder om die fitosanitêre risiko te verminder.

Een en twintig sitruskwekerye is geïnspekteer. Twintig is gesertifiseer as in ooreenstemming met die Sitrusverbeteringskema (as kommersiële sitruskwekerye) insluitende twee nuwe kwekerye, en een het voorlopige sertifisering ontvang. Ses ander kwekerye is as plaaskwekerye geregistreer.

Voorligting se hoogtepunt van die 2008/9 jaar was die baie suksesvolle CRI Navorsingsimposium met 'n rekordgetal van 450 afgevaardigdes en 'n sterk afvaardiging van internasionale aanbieders. Die tegnologie-oordragnetwerk in die streke het voortgegaan as 'n baie effektiewe tegnologie implementeringsmeganisme. Die strategiese vennootskap met die sitrus konsultante het weereens goeie ondersteuning tot hierdie netwerk gelewer. Die Sitrus Koue Ketting Forum het in sterkte gegroei met die daarstelling van die eerste stel van minimum bedryfstandaarde vir verpakkingsmateriaal. Die Snykant is aktief as 'n vinnige en effektiewe kommunikasiemiddel gebruik. Die omvattende hersiening van navorsingsprioriteite is na 'n twee jaar siklus verander met die volgende omvattende hersiening wat vir mid-2009 geskeduleer is. Die doel van Voorligtingsdienste is uitgebrei met die aanstelling van 'n transformasie voorligter in die Suide om die vroeë suksesvolle aanstelling van dieselfde posisie in die noordelike streek aan te vul. Hierdie poste word volledig deur die CGA befonds en die werksplatform word deur CRI binne die Voorligtingsafdeling voorsien.

2 MARKET ACCESS TECHNICAL COORDINATION & BIOSECURITY

Coordinator: Vaughan Hattingh (CEO), assisted by Elma Carstens (CRI)

2.1 PROGRAMME SUMMARY

Expansion of distribution of the exotic fruit fly *Bactrocera invadens* in Africa is a major **biosecurity** concern. A steering committee was established, an action plan agreed upon, chemicals were stock-piled in preparation for eradication, surveillance networks were initiated, awareness campaigns undertaken and research initiated in preparation for potential future incursion. Biosecurity discussions were held with Angola. Seedlings from imported Floridian seed were destroyed to protect against the risk of Asiatic Greening. The **EU** continued to oppose SA's calls for relaxation of the EC CBS import regulations. Increased occurrence of CBS interception in the EU resulted in the EU notifying that it will send a delegation to South Africa to inspect SA's phytosanitary controls. Measures were implemented in South Africa to reduce occurrence of FCM in export fruit. Surveys

confirmed the absence of CBS in Western Cape Magisterial districts that were previously excluded from protection in relevant SA Regulations. The application to amend cold treatment conditions for all citrus types exported to **Japan** was submitted to SA-DoA by CRI. In pursuit of enhanced access to **USA**, the Northern Cape CBS-free areas were inspected by a USDA plant pathologist who submitted a favourable report. Potential Pest Free Places of Production were surveyed in the far Northern Limpopo and no evidence of CBS was found on any of the 19 farms inspected. Pursuit of access or enhanced access to the following additional markets received attention: **South-Korea, China, Thailand, Australia, Vietnam, Lebanon, Malaysia, Philippines** and **Syria**.

2.2 BIOSECURITY

The distribution of the exotic fruit fly, *Bactrocera invadens* expanded in 2008 and was reported in Namibia and Mozambique. DoA accordingly intensified restrictions on import of host material from these countries and others where the fly is known to occur. To prepare for an incursion, CRI convened a multi-industry workshop (including SA-DoA). An emergency action plan for *B. invadens* (*Bi*) was subsequently finalized and chemicals to initiate eradication efforts in response to an incursion were stockpiled. An official Bi Steering Committee, incorporating representation from DoA and various industries was constituted and came into operation. Information on the fly was compiled by CRI and distributed to create awareness in the industry.

Surveillance networks were expanded across southern Africa to improve the chances for early detection of an incursion. Unbudgeted research was initiated in collaboration with African countries where the fly already occurs, to (1) validate the efficacy of a post-harvest cold treatment, and (2) improve pre-harvest control techniques for this fly. River Bioscience was encouraged to obtain a SA Registration for Methyl Eugenol to use in eradication and control programmes and avoid the risks of Agro-Chemical companies profiteering from the industry's need for such materials when required to respond to incursions in the future.

Seedlings established with seed imported from Florida were destroyed (in accordance with an order issued by SA-DoA) in light of risks associated with potential seed transmissibility of Asiatic greening. Surveys were conducted to monitor the spread of African Greening in South Africa. An isolated occurrence was detected in an Eastern Cape site. CRI recommended to DoA that eradication be conducted to preserve the greening-free status of the Eastern Cape.

During this reporting period, meetings were held with the Angolan Minister of Agriculture, to discuss the threats posed (to African citrus industries) by citrus propagating material imported from South American countries where diseases of quarantine importance occur. The Angolan Ministry of Agriculture indicated that they would attempt to stop further imports from Brazil and would support a follow-up survey by SA plant pathologists to determine if any diseases had been introduced previously.

South Africa is an associate member of CLAM and therefore it was decided to present a proposal at the General Assembly of CLAM, during October 2008, for an inter-regional cooperation project on biosecurity threats posed by pest and disease incursions into African countries between these two regions. However, it became apparent that CLAM is not a suitable platform for such a project.

2.3 EUROPE

In August 2007, SA asked for a final ruling with regard to its call for revision of the EU import regulations pertaining to CBS on imported citrus fruit, since ample scientific evidence had been provided over the past years. In answer to this call, the European Commission forwarded an official request to the European Food Safety Authority (EFSA) in April 2008, to provide a scientific opinion on the data supplied by SA. The EFSA opinion was concluded in mid-December and was made available on their website on 20 January 2009.

EFSA's opinion did not support SA's view that fresh citrus fruit does not pose a threat for the spread of CBS to the EU. On the Citrus Industry's request, a letter was sent by SA-DoA to the European Commission's Standing Committee on Plant Health (SCPH), stating that South Africa is not in agreement with the outcome of EFSA's opinion. This letter pre-dated the EC's meeting on 10 March 2009, where EFSA's report on CBS was on the agenda.

In March 2009, side bar discussions were held between SA-DoA and EU Representatives at the IPPC meeting in Rome. The EU indicated that they had considered EFSA's report and that an official letter will be forwarded to South Africa. They also indicated that representatives from the EU will visit South Africa during the coming citrus export season, due to EU's concerns about the high interception rate of CBS infected consignments during the past three export seasons.

During the 2008 export season, the notices of CBS interceptions at European ports of entry rose sharply. This occurred despite warnings issued to the industry to remain vigilant in controlling CBS. The SA-DoA convened a workshop in August 2008 to address this issue, with a follow up workshop that was held in January 2009. It was decided that the CBS strike system would again be implemented as of 1 April 2009, with amendments as agreed at the workshop. The strike system was to be applied to exports to EU, Iran, Japan, India and Reunion. The decision was also taken that consignments of all citrus types destined for Spain, as well as all EU consignments that are out of the 21 days (soft citrus) or 28 days (hard citrus including grapefruit) protocols, will be subjected to a phytosanitary inspection in the port (by SA-DoA) prior to shipping. A zero tolerance for FCM would apply in these inspections.

Interceptions of FCM were reported by the EU during the previous export season and therefore the need for intensification of FCM control measures was communicated to the industry prior to commencement of the 2008/9 production cycle. The decision by the Industry Grading Committee, in November 2009, to implement a zero tolerance for decay during PPECB inspections at the packhouses in 2009 for all citrus types except grapefruit, together with SA-DoA phytosanitary inspections in the ports, was expected to assist in ensuring compliance with the need for intensified FCM control.

No official laboratory reports were received from SA-DoA on the official CBS surveys conducted (with CRI support) in 2007 and 2008 in the Western Cape magisterial districts of Knysna, George, Mossel Bay, Vredendal and Van Rhyndorp. It was however, reported at a Market Access meeting in February 2009, that no *Guignardia citricarpa* was isolated from any of the sites surveyed in these magisterial districts and therefore the whole of the Western Cape can be recognised as CBS free in South Africa's regulations pertaining to the movement of citrus propagating material.

During the previous reporting period, an official request was forwarded to the EU to remove the record of an interception of *Ceratitis cosyra* in citrus fruit exported from South Africa, since no technical justification was provided to support this finding. No response was received from the EU with regard to this matter.

2.4 JAPAN

The adoption of a revised cold treatment condition for the export of all citrus types to Japan remained one of the outstanding phytosanitary issues. The experimental work to support such a revision was concluded by CRI at the target temperature of 1⁰C for 16 consecutive days and the results of this experimental work were documented in 2008. The report was submitted to SA-DoA in January 2009, to proceed with communication with MAFF, in support of the proposed amendment to the current cold treatment conditions.

The other outstanding issues, namely the broadening of access for soft citrus cultivars and clarification of cultivars of sweet oranges that are allowed for export to Japan, were also not concluded during this reporting period. Several requests were made to Japan by SA-DoA for clarification of the permissible sweet orange cultivars. This included a side bar discussion at the IPPC meeting in Rome in April 2008.

For engagement with MAFF about the broadening of access for soft citrus cultivars, the definition of soft citrus in the Export Standards and Requirements needed to be amended, officially accepted and published. The amendment of this definition was requested by CRI in the previous reporting period. The amended definition was approved at the Variety Focus Group Meetings held in September 2008 and at the Citrus Grading Meeting in November 2008. However, when the Export Standards and Requirements for Soft Citrus for 2009 were published by SA-DoA, the amended definition for soft citrus was not included. By the end of this reporting period the Export Standards and Requirements for Soft Citrus had not yet been amended by DoA.

2.5 USA

The outstanding issues for this market were the reversion of the cold treatment for FCM from 24d to 22d, the recognition of the N. Cape-, western Free State- and southern North-West Provinces as a CBS-free area and the adoption of a system for recognising CBS free places of production in areas of low pest prevalence.

USDA-APHIS sent Dr José Hernández (Risk Manager - Plant Pathology: Commodity Imports Analysis & Operations) to inspect the new potential CBS-free areas in the Northern Cape, western Free State- and southern North-West Provinces. Dr Hernández visited the area together with a South African delegation (DoA and CRI-CGA) in August 2008.

In the official report prepared by Dr José Hernández and received by SA-DoA in November 2008 the addition of the new CBS-free areas in the Northern Cape, western Free State and southern North West Provinces was supported. As part of the feedback it was indicated that there is a need for USDA-APHIS and SA-DoA to agree on the logistics pertaining to the export of citrus from these areas and the promulgation of the relevant Regulation by USDA, before fruit can be exported. Although the USA is in the process of finalising the approval of the areas in the Northern Cape, Free State and North-West Province, it was indicated that a delay could be expected due to the take-over of the new administration in the USA and by the end of this reporting period no fruit had been exported to the USA from these areas.

In April 2008 a decision was taken to conduct a survey to determine the workability of the SOP in all the participating packhouses in the Western and Northern Cape. This SOP was drafted in 2007 by the Western Cape Producers for better management of FCM in support of the reversion of the cold treatment from 24d to 22d. Questionnaires were sent by CRI-CGA to all the participating packhouses as well as to the packhouses in the new potential areas in the Northern Cape. The outcome of the survey indicated that the SOP is workable and that it can be adopted. Based on these results the SA- DoA was requested by CRI-CGA to proceed with communication to USDA-APHIS to conclude this issue. A site visit to South African packhouses by USDA to verify implementation of the SOP is planned to take place during the 2009 packing season.

During December 2007 and January 2008 an official survey was conducted on the 19 farms in the far Northern region of the Limpopo Province that had applied for registration as CBS Pest Free Places of Production within the area of Low Pest Prevalence. The samples were sent to SA-DoA's Plant Health Laboratories in Stellenbosch for CBS analyses. CRI contracted QMS to assist with part of the sample analyses. Although DoA had not concluded a final report on this survey by the end of this reporting period, SA-DoA reported at a Market Access meeting in February 2009 that *Guignardia citricarpa* was not isolated from any of the farms surveyed. USDA-APHIS was requested to include a site visit of this region in support of SA's application to approve these areas for future export to the USA.

The Irradiation Framework Equivalency Work Plan was signed between South Africa and the USA. CRI trials to validate the efficacy of a FCM irradiation dosage were continued, but not concluded.

2.6 SOUTH KOREA

Reversion of the cold treatment for FCM from 24d to 22d, and inclusion of lemons, Grapefruit and soft citrus remain issues for this market.

It was not possible to demonstrate complete FCM non-host status of lemons. Due to the cold sensitivity of lemons and Grapefruit, a risk mitigation protocol with reduced cold treatment was drafted by CRI. However, consultation between DoA and South Korean authorities indicated that this protocol would not be accepted. CRI and DoA held a workshop to devise an alternative risk management protocol for lemons and Grapefruit to South Korea. CRI is to develop this draft protocol for further consideration.

No feedback was received from South Korean Authorities on SA's application to export soft citrus cultivars to this market.

2.7 CHINA

The revised citrus export protocol was signed in June 2006. The protocol was renewed in 2009 without changes to the terms. The outstanding issues pertaining to this market remain the reversion of the cold treatment protocol from 24d to 22d and the acceptance of bulk shipments.

2.8 THAILAND

All imports of fresh fruit and vegetables from South Africa to Thailand were suspended in January 2008. Despite several requests made by SA-DoA during this period, no feedback was received. Several official visits planned to Thailand during this reporting period, which would provide for opportunities to discuss all the outstanding issues, also did not take place.

2.9 AUSTRALIA

For this reporting period, no progress could be reported on the outstanding issues, despite several efforts made by SA-DoA. The issues outstanding were the acceptance by Australia of the cold treatment protocol for the fruit fly *Ceratitis rosa* and the non-host status of citrus for the fruit fly *Ceratitis quinaria*, as supported by documentation provided by CRI. Australia has acknowledged the receipt of South Africa's applications to gain access for several plant products (including citrus) but quoted lack of capacity for failure to make progress.

2.10 OTHER MARKETS

2.10.1 VIETNAM

Fresh citrus fruit was identified by the Vietnamese Authorities as one of the categories for which a PRA is needed to determine the import conditions. The PRA Data Requirement Form, as received from Vietnam, was completed by CRI and submitted to SA-DoA in August 2008. This information had not yet been submitted to Vietnam by SA-DoA, by the end of this reporting period.

2.10.2 LEBANON

The Pest Risk Analysis Questionnaire as requested by Lebanon, to determine the import conditions for all fresh citrus fruit from South Africa, was completed by CRI and submitted to SA-DoA in September 2007. By the end of this reporting period this information had not yet been submitted to Lebanon by SA-DoA, despite several requests made by Industry.

2.10.3 MALAYSIA

Upon SA request to clarify the import conditions for all fresh citrus fruit, specifications of the import conditions to Malaysia were obtained in September 2008. The conditions stipulated that citrus fruit would be allowed for import without a permit or a phytosanitary certificate. Fruit would however be inspected at the port of entry and consignments infected/infested with citrus greening disease and/or fruit flies, especially *Ceratitis capitata*, would be destroyed.

2.10.4 PHILIPPINES AND SYRIA

An official request was submitted to the **PHILIPPINES** to clarify their import conditions for all fresh citrus fruit. Feedback from the Philippines Authorities indicated that a PRA Data Requirement Form must be completed. In September 2008, CRI advised DoA to use the information as compiled for Vietnam to complete this PRA Data Requirement Form. By the end of this report period the PRA unit of the SA-DoA was still in the process of finalising the document and no information had been submitted to the Philippines Authorities.

No information was received from the **Syrian** Authorities about the official import requirements for all fresh citrus fruit from South Africa.

3 PROGRAMME: INTEGRATED PEST MANAGEMENT

3.1 PROGRAMME SUMMARY

By Sean D Moore (Manager: IPM Programme, CRI)

Challenges in the implementation of IPM-orientated research are mounting. Stricter residue tolerances have reduced chemical options for certain pests, the pest status of certain pests has elevated in certain export markets, and the threat of the appearance of new phytosanitary pests is very real. These challenges are being effectively met within the IPM research programme.

The awareness of the phytosanitary risk of false codling moth (FCM) by southern Africa's export markets is increasing. As pressure mounts to export fruit with negligible risk of FCM infestation, the FCM research project has once again attracted the lion's share of funding. Although Cryptogran has now been registered and widely used commercially for several years, a significant proportion of the research in this project was dedicated to improving field efficacy of Cryptogran. In addition, work is underway to determine whether there is any variation in host susceptibility or virus pathogenicity for both Cryptogran and Cryptex. The sterile insect technique (SIT) is now in its second year of commercial use in Citrusdal, covering 3500 ha of the valley. A lot of research effort has gone into improving the production of moths for SIT and the successful release of these moths. In addition, a pilot trial was conducted to expand the programme to Letsitele. With the rapidly waning chemical arsenal for FCM, it is encouraging that a new late-season chemical option was investigated. On the biocontrol front, EPNs are showing ever increasing promise, whereas the exploitation of larval parasitoids showed little potential. Finally, one experiment focussed on post-harvest disinfestation of FCM using potentiating CO₂ shock treatments with subsequent cold treatments.

A large portion of the total IPM funding also went towards fruit fly research. This project covered three main areas: improvement in the control of fruit flies, particularly through use of baits; understanding fruit fly distribution and potential distribution, particularly pertaining to Natal fruit fly; and the pre- and post-harvest control of the African invasive fruit fly, *Bactrocera invadens*. The impending appearance of the last mentioned in South Africa and the more stringent restrictions on the use of Malathion in fruit fly baits were the two major reasons for the considerable funding allocated to this project. It was determined that Natal fruit fly is a good candidate for climate related modelling of its distribution and potential distribution. This was well supported by valuable data on the temperature tolerance of the species. It was determined that M3 bait stations were highly effective in reducing fruit fly infestation of navel oranges, particularly in comparison with conventional bait applications. Good efficacy persisted, even where number (density) of bait stations was reduced. Further trials were conducted to not only better understand the performance of all available baits, but to identify new and promising baits. CRI's collaboration with ICIPE in Kenya and IITA in Benin to tackle the *B. invadens* threat progressed well. The methyl eugenol-based male annihilation technique shows good potential, as does post-harvest cold sterilisation for disinfestation of fruit. Finally, an innovative experiment to investigate the potential of entomopathogenic fungi (EPF) for controlling soil-borne life stages of both fruit flies and FCM progressed well, unearthing numerous naturally occurring EPF isolates.

As a result of the sudden and unexpected emergence of oleander mealybug as the dominant species in the mealybug complex in certain regions a few years ago, two experiments were initiated to study this species and its parasitoids. Although these have progressed well, progress on the parasitoid work has become more difficult due to the apparent recent demise in oleander mealybug numbers. This is evidently a cyclical trend, although explanations for this are lacking.

The biocontrol disruption, cosmetic pests and production pests projects focussed mainly on research driven by a dearth in chemical control options for pests covered by these projects. This is a problem likely to arise with increasing frequency, as market restrictions on chemical usage becomes more prevalent. Progress was made towards the registration of a new miticide for bud mite and grey mite control, with completion of residue and non-target tests. Unfortunately, insufficient progress was made with EPF trials against citrus thrips, attractant trials against citrus psylla, and chemical trials against leafhoppers and lemon borer moth – all because there were inadequate numbers of these pests. No effective repellents were identified for bees. Fortunately good progress was made with the development of a bait for the two major ant pest species occurring on citrus.

CRI's IPM research programme has been meaningfully supported by researchers at Stellenbosch University and Rhodes University, who have brought much needed skills to complement the programme. Rhodes University

launched a new dedicated Agricultural Entomology Unit (Waainek), specifically to collaborate with and service agricultural industries. Their relationship with the citrus industry has grown considerably and is now particularly strong. It is especially gratifying to see the contingent of young entomologists, of whom many are women, who have contributed to this research programme in the last year. Three of these students currently hold Citrus Academy bursaries. These are encouraging signs for our ability to meet future entomological challenges.

PROGRAMOPSOMMING

Uitdagings in die toepassing van navorsing op geïntegreerde plaagbeheer (GPB) is besig om toe te neem. Strenger residu-toleransie het chemiese opsies vir sekere plae verminder, die plaagstatus van sekere plae het vir sommige markte verhoog, en die gevaar van nuwe fitosanitêre plae is 'n werklikheid. Hierdie uitdagings word doeltreffend binne die GPB navorsingsprogram aangepak.

Suider-Afrika se uitvoermarkte word al hoe meer bewus van die fitosanitêre gevaar wat valskodlingmot (VKM) vir hulle inhou. Soos die druk verhoog om vrugte met minimale risiko vir VKM besmetting uit te voer, het die VKM navorsingsprojek weereens die grootste deel van befondsing binne in die program getrek. Alhoewel Cryptogran reeds 'n hele paar jaar geregistreer is en kommersieel wyd gebruik word, is 'n beduidende gedeelte van die navorsing in die projek op die verbetering van Cryptogran se doeltreffendheid in die praktyk gemik. Werk is ook aan die gang om te bepaal of daar enige variasie in gasheer gevoeligheid of virus patogenisiteit vir beide Cryptogran en Cryptex is. Die steriele insek tegniek (SIT) is tans in sy tweede jaar van kommersiële gebruik in die Citrusdal-area en dek 'n oppervlak van 3500 ha. Baie navorsing is gedoen om die produksie van motte vir SIT en die suksesvolle vrylating van die motte te verbeter. 'n Loodsproef is ook uitgevoer om die program na Letsitele uit te brei. Met die chemiese opsies vir VKM wat so vinnig afneem, is dit bemoedigend dat 'n nuwe chemiese opsie laat in die seisoen ondersoek is. Op die biologiese beheerfront toon entomopatogeniese nematodes al hoe meer belofte, maar die gebruik van larwale parasiete het nie veel potensiaal gewys nie. Laastens het een eksperiment op na-oes disinfestasië gefokus op die gebruik van kragtige CO₂ skokbehandeling wat opgevolg is deur kouebehandelings.

'n Groot gedeelte van die totale GPB befondsing is ook vir vrugtevlieg-navorsing gebruik. Hierdie projek het drie hoof areas gedek: verbetering in die beheer van vrugtevlieë, veral deur die gebruik van lokase; ondersoek na vrugtevlieg verspreiding en potensiele verspreiding, veral met verwysing na Natal vrugtevlieg; en die voor- en na-oes beheer van die Afrika indringer vrugtevlieg, *Bactrocera invadens*. Die waarskynlike verskyning van laasgenoemde in Suid-Afrika en die strenger beperkings op die gebruik van Malathion in vrugtevlieg lokase is die twee hoofredes vir die beduidende befondsing wat die projek getrek het. Dit is gevind dat Natal vrugtevlieg 'n goeie kandidaat is vir klimaats modellering om sy verspreiding en maandelikse verspreiding te bepaal, wat goed ondersteun is deur waardevolle data oor temperatuur toleransie van die spesies. Dit is bepaal dat M3 lokstasies hoogs doeltreffend is, gemeet aan die vermindering van vrugtevlieg besmetting van nawellemoene, veral in vergelyking met konvensionele lokaas toedienings. Goeie doeltreffendheid is behaal selfs waar die hoeveelheid (digtheid) lokstasies verminder is. Verdere proewe is uitgevoer, nie net om beskikbare lokase beter te verstaan nie, maar ook om nuwe en belowende lokmiddels te identifiseer. CRI se samewerking met ICIPE in Kenia en IITA in Benin om die *B. invadens* bedreiging na te vors het goed gevorder. Die methyl eugenol gebaseerde mannetjie uitwissingstegniek het goeie potensiaal getoon, so ook na-oes kouesterilisasie vir disinfestasië van vrugte. Laastens, 'n innoverende eksperiment om die potensiaal van entomopatogeniese swamme (EPS) vir beheer tydens die lewensstadiums van beide vrugtevlieë en VKM in die grond te ondersoek, het goed gevorder. Talle EPS isolate wat natuurlik voorkom is ontdek.

As gevolg van die skielike onverwagte verskyning van oleander witluis as die dominante spesie in die witluis spesie kompleks in sekere streke 'n paar jaar gelede, is twee eksperimente, om hierdie spesie en sy parasiete te bestudeer, aan die gang gesit. Alhoewel hierdie eksperimente goed gevorder het, het vordering met die parasiet-werk al hoe moeiliker geword as gevolg van die onlangese ooglopende afname in die voorkoms van oleander witluis. Dit wil voorkom dat hierdie 'n sikliese tendens is, wat moeilik is om te verduidelik.

Die projekte op bio-beheer ontwigting, kosmetiese plae en produksie plae het hoofsaaklik gefokus op navorsing wat deur 'n tekort aan chemiese beheer opsies, teen plae wat onder hierdie projekte gedek word,, gedryf is. Hierdie is 'n probleem wat heel waarskynlik al hoe meer gereeld gaan voorkom, as gevolg van al hoe strenger markbeperkings op die gebruik van chemiese middels. Vordering is gemaak met die registrasie van 'n nuwe mytdoder om knopmyt en grysmyt te beheer, met die voltooiing van residu en nie-teiken-effek toetse. Ongelukkig is onvoldoende vordering gemaak met EPS navorsing teen sitrus blaaspootjie, lokproewe teen

sitrusbladvlooi, en chemiese proewe teen bladspringers en suurlemoenboormot, omdat vlakke van al hierdie plae te laag was. Geen doeltreffende afweermiddels is vir bye geïdentifiseer nie. Gelukkig is goeie vordering gemaak met die ontwikkeling van 'n lokaas vir die twee hoof mierplaag-spesies wat op sitrus voorkom.

CRI se GPB navorsingsprogram is betekenisvol deur navorsers van Stellenbosch Universiteit en Rhodes Universiteit ondersteun, wat belangrike wetenskaplike kundigheid tot die program bygedrae het. Rhodes Universiteit het 'n nuwe, gefokusde Landbou Entomologie Eenheid (Waainek) gestig, spesifiek vir samewerking met en diensverskaffing aan landboubedrywe. Hulle verhouding met die sitrusbedryf het beduidend gegroei en is nou baie sterk. Dit is veral bemoedigend om te sien dat daar baie jong entomoloë is, insluitend baie vrouens, wat verlede jaar tot die program bygedra het. Drie van hierdie studente het tans Sitrus Akademie beurse. Hierdie is bemoedigende tekens van ons vermoë om die entomologiese uitdagings van die toekoms suksesvol aan te pak.

3.2 PROJECT: FALSE CODLING MOTH

Project Coordinator: Sean Moore

3.2.1 Project Summary

Due to the increasing severity of the perceived phytosanitary status of false codling moth (FCM) by South Africa's export markets, much time, manpower and resources have been invested in this project. Consequently, numerous experiments were conducted on FCM during the 2008/09 research cycle. An experiment on control of FCM with the sterile insect technique (SIT) (3.2.2) covered a wide range of subjects, including the rearing diet, egg sheet disinfection, the design of an insectary and moth release equipment, as well as an SIT pilot project in Limpopo Province. Rearing and release trials were geared towards improving understanding and execution of protocols. Although execution of the pilot trial in Limpopo worked well and moth recovery was initially good, there were no differences between fruit infestation in the SIT and control orchards. In the second experiment, *Agathis bishopi* parasitoids were released into each of 2 netted Lina navel orange trees on a farm in the Citrusdal area (3.2.3). This was after several pairs of FCM were released into both nets. Despite numerous live larvae being recovered over a period of several weeks, no parasitism was recorded. In the third experiment, two irradiation containers were built for trials with gamma-irradiation for post-harvest control of FCM in packed fruit (3.2.4). However, no real progress was made in the execution of trials. An experiment was conducted to identify, quantify and resolve persistence problems with Cryptogran and to improve field persistence through formulation and management practices (3.2.5). Where FCM pressure was high, an additional October application improved control. More frequent applications of Cryptogran at reduced concentrations were not adequately effective. Cryptogran performed significantly better than Cryptex in two field trials and marginally better in one. Cryptex performed better than Cryptogran in one field trial. Dithane was as effective an adjuvant with Cryptogran as was molasses and Agral 90. Cryptogran applied in an Isomate orchard significantly reduced FCM infestation. In the following experiment a field trial was conducted to test the entomopathogenic nematode (EPN) species, *Heterorhabditis bacteriophora*, for control of FCM larvae in the soil (3.2.6). After 2 days, high mortality of FCM larvae was recorded for 3 EPN concentrations. After 14 days the infectivity of the nematodes showed a sharp decline. However, after four months 45% mortality of FCM larvae was still recorded. A second experiment focussing on post-harvest disinfestation, revealed a significant increase in percentage mortality of 4th and 5th instar FCM larvae exposed to potentiating CO₂ shock treatments with subsequent cold treatments (3.2.7). In another experiment conducted with FCM viruses, laboratory assays showed a significant difference in pathogenicity of Cryptogran to 5th instar FCM larvae from Addo and from other field populations (and a laboratory colony) (3.2.8). In laboratory assays, Cryptogran was significantly more pathogenic than Cryptex against 1st instar FCM larvae, both from Addo and from an old colony. In another experiment no progress was made with the identification of new attractants and repellents for FCM (3.2.9). No product was therefore available that justified field testing. In another experiment conducted with SIT against FCM, sterile to wild FCM male ratios of 10:1 were frequently surpassed, unlike during the first trial season (3.2.10). Fruit infestation was also significantly lower during the second season. Flight, dispersal and recapture of released males were influenced by night temperatures. Rapid cold hardening could not be induced in FCM. Day temperatures above 40°C were detrimental to released moth survival. A new experimental insecticide, EXP5225, was moderately effective in controlling FCM (3.2.11 & 12). However, trials have not been completed. The final experiment in the project again looked at FCM virus. Despite genetic distinction between CrleGV-SA isolates in Cryptogran and Cryptex, surface dose-response bioassays showed no significant difference in pathogenicity (3.2.13). A new droplet assay technique was developed to examine pathogenicity.

Projekopsomming

As gevolg van die verhoogde bewustheid van die fitosanitêre plaagstatus van valskodlingmot (VKM) in Suid-Afrikaanse uitvoermarkte, is baie tyd, mannekrag en hulpbronne in hierdie projek belê. Gevolglik is 'n groot aantal eksperimente gedurende die 2008/09 navorsings siklus op VKM uitgevoer. 'n Eksperiment op die beheer van VKM met die steriele insek tegniek (SIT) (3.2.2) het 'n wye reeks onderwerpe gedek, soos die dieet, eiervelontsmetting, die ontwerp van insektarium- en motloslaattoerusting, asook 'n SIT loodsprojek in Limpopo Provinsie. Teel- en loslatingsproewe is uitgevoer om hierdie aspekte beter te verstaan en om tegnieke te verbeter. Alhoewel die loodsproef in Limpopo goed uitgevoer is en mot herwinnings oorspronklik goed was, was daar uiteindelik geen verskil in vrugbesmetting tussen SIT- en kontrole boorde. In die tweede proef is *Agathis bishopi* parasiete in elk van 2 netbedekte Lina nawelbome op 'n plaas buite Citrusdal losgelaat (3.2.3), nadat verskeie pare VKM in albei nette losgelaat is. Ondanks 'n hoë vlak van besmetting oor 'n tydperk van weke is geen parasitisme waargeneem nie. In die derde eksperiment is twee bestralingshouers vir proewe met gamma-bestraling vir na-oes beheer van VKM gebou. (3.2.4). Geen vordering met die proef is egter gemaak nie. 'n Eksperiment is uitgevoer om probleme met die nawerking van Cryptogran te identifiseer, te kwantifiseer en moontlik die formulering en bestuurspraktyke van die produk te verbeter (3.2.5). In gevalle waar VKM druk hoog was, het 'n adisionele Oktober bespuiting beheer verbeter. Meer gereelde toedienings van Cryptogran teen 'n laer konsentrasie was nie genoegsaam doeltreffend nie. In twee boordproewe het Cryptogran betekenisvol beter as Cryptex gevaar en effens beter in 'n ander proef. Cryptex het slegs in een proef beter as Cryptogran gevaar. Dithane is net so 'n doeltreffende byvoegmiddel by Cryptogran as wat molasse en Agral 90 is. Cryptogran wat in 'n Isomate-behandelde boord toegedien is, het VKM besmetting betekenisvol afgebring. In die volgende eksperiment is 'n boordproef uitgevoer om die entomopatogeniese nematode (EPN) spesie, *Heterorhabditis bacteriophora*, vir beheer van VKM larwes in die grond te toets (3.2.6). Na 2 dae was hoë mortaliteit van VKM larwes met 3 EPN konsentrasies waargeneem. Na 14 dae was daar 'n skerp afname in die infekteerbaarheid van die nematodes. Na vier maande is egter steeds 45% mortaliteit van VKM larwes verkry. 'n Tweede eksperiment wat op na-oes disinfestasië gefokus het, het 'n beduidende toename in persentasie mortaliteit van 4de en 5de stadium VKM larwes getoon met 'n kragtige CO₂ skokbehandeling wat opgevolg is deur koue behandelings (3.2.7). In nog 'n eksperiment wat met VKM virusse uitgevoer is, het laboratorium biotoetse 'n betekenisvolle verskil in patogenisiteit van Cryptogran tussen 5de stadium VKM larwes van Addo en die van ander veldpopulasies (en 'n laboratorium kultuur) gewys (3.2.8). In laboratorium biotoetse was Cryptogran beduidend meer patogenies as Cryptex teen 1ste stadium larwes van beide Addo en 'n ou laboratorium kolonie. In nog 'n eksperiment is geen vordering met die identifisering van nuwe lok- en afweermiddels vir VKM gemaak nie (3.2.9), gevolglik was geen produk vir veldtoetse beskikbaar nie. In nog 'n SIT eksperiment teen VKM-beheer is 'n steriele tot wilde mannetjie verhouding van 10:1 gereeld oorskry, anders as wat die geval in die eerste seisoen was (3.2.10). Gedurende die tweede seisoen was vrugbesmetting ook beduidend laer. Vlug, verspreiding en herwinnings van losgelate mannetjies is deur nagtemperatuur beïnvloed. Vinnige aanpassing by koue-toestande kon nie in VKM geïnduseer word nie. Dagtemperatuur bo 40°C is nadelig vir oorlewing van losgelate motte. 'n Nuwe eksperimentele insekdoder, EXP5225, het VKM tot 'n mate beheer (3.2.11-12), maar proewe is nog nie voltooi nie. Die finale eksperiment in die projek was weereens met die VKM virus. Ondanks genetiese verskille tussen die CrleGV-SA isolate in Cryptogran en Cryptex, het oppervlak dosis-gevoelige biotoetse geen betekenisvolle verskil in patogenisiteit getoon nie (3.2.13). 'n Nuwe druppel biotoets tegniek is ontwikkel om in die toekoms patogenisiteit te evalueer.

3.2.2 VORDERINGSVERSLAG: Bestryding van VKM met Steriele Insekloslatings

Proef 662 (2002-2010): J H en M Hofmeyr (CRI)

Opsomming

Valskodlingmot-(VKM)navorsing in dié proefreeks het 'n wye reeks onderwerpe, soos die dieet, eiervelontsmetting, die ontwerp van insektarium- en motloslaattoerusting, asook 'n Steriel-Insek Tegniek (SIT) loodsprojek te Limpopo Provinsie, ingesluit.

Twee alternatiewe dieetbestanddele, naamlik Gesifte meliemeel en Kremel-melkpoeier, is geëvalueer om onderskeidelik die standaard (duurder) Spesiale meliemeel en volroommelkpoeier te vervang. Produksiegewys het eersgenoemde swakker presteer, terwyl daar gevind is dat volroommelkpoeier deur Kremel vervang sal kan word.

'n Alternatiewe behandeling om die relatief-giftige standaard 20% formalien-eierbehandeling (1-2 sekonde dompeltyd) te vervang, naamlik 5% formalien met 'n dompeltyd van 20 sekondes, is ontwikkel. Toerusting om die grootskaalse ontsmetting van eiervelle met dié behandeling moontlik te maak, is ontwerp en met sukses getoets.

'n Proef is uitgevoer om die potensieel-nadelige uitwerking van die inwendige larwe-kleurstof, Calco Oil Red 2144 in die VKM-dieet, te ondersoek. Dit het geblyk dat Calco moontlik die produksie van VKM kan strem, terwyl die vrugbaarheid van motte wat daarmee geproduseer was, nie ernstig aangetas was nie. Daar is nie genoeg inligting ingesamel om die veiligheid van die kleurstof bo alle twyfel te bewys nie.

Probleme soos pypverstopings weens motskubbe en liggaamsvloeistof, asook kondensasie, in die sikloon-gebaseerde stelsel, is met die motversameltoerusting in die Xsit-insektarium ondervind. 'n Unieke prototipe apparaat is ontwerp wat bogenoemde probleme uitskakel. Die nuwe stelsel is met groot sukses getoets.

Motloslaattoerusting wat op 'n vierwielmotorfiets pas, is ontwerp en met sukses onder boordtoestande getoets. Die toerusting skakel veral kalibrasieprobleme uit wat met soortgelyke apparaat in die buiteland ondervind word. Loslaatkalibrasie met die nuwe toerusting is snelheidsafhanklik – dit laat meer motte vry wanneer daar vinniger gery word en andersom.

'n Loodsprojek is te Letaba Landgoed uitgevoer om die doeltreffendheid van Steriele-Insek Loslatings in 'n nuwe gebied te evalueer. Dit is uitvoerbaar bewys om bestraalde motte binne 19 uur van Citrusdal tot by Letaba, met behoud van motkwaliteit, te kry. Aanvanklike loslatings vanaf November 2008 het goed gewerk en 'n hoë oorvloedingsverhouding met wilde motte is gehandhaaf. Motvangste van losgelate motte het egter aan die einde van Januarie om 'n onbekende rede afgeneem. Vrugvalopnames het middel-Februarie begin en vrugbesmetting hoër as die ekonomiese drempelwaarde is in die SIT-gebied aangeteken. Daar was geen verskil tussen vrugval in die SIT- en die kontroleboorde nie.

Summary

False codling moth (FCM) research conducted in this report covered a wide range of subjects, viz. the rearing diet, egg sheet disinfection, the design of insectary and moth release equipment, as well as a Sterile Insect Technique (SIT) pilot project in Limpopo Province.

Two alternative diet ingredients, viz. Sifted maize flour and Kremel milk powder, were evaluated as replacements for (more expensive) Special maize flour and full cream milk powder. Production wise the Sifted flour did not perform as well as the Special flour, while Kremel was found to be suitable as a replacement for full cream milk powder.

An alternative treatment to replace the relatively toxic standard 20% formaldehyde egg treatment (1-2 second submersion) was developed, viz. 20 second submersion in 5% formaldehyde. A prototype egg sheet container to facilitate the mass disinfection of egg sheets with this treatment was designed and successfully tested.

An experiment was conducted to investigate the potentially detrimental effect of the internal larval dye, Calco Oil Red 2144 in the FCM rearing diet. It seemed that Calco has the potential to effect FCM production negatively, although fecundity and fertility were not seriously affected. Not enough evidence has been accumulated to prove Calco safe beyond all doubt.

Problems such as pipe blockages due to accumulation of moth scales and body fluids, as well as condensation, were encountered with the cyclone-based moth collection equipment in the Xsit insectary. A unique prototype apparatus was designed to eliminate the above problems. The new system was tested with excellent results.

Moth release equipment to fit on a quad all terrain vehicle, was designed and tested with good results under field conditions. The equipment especially eliminates calibration problems encountered with similar systems used internationally. Release calibration with the new equipment is speed dependent – more moths are released with increasing quad speed, and *vice versa*.

A pilot project was conducted at Letaba Estates to evaluate the efficacy of SIR in a new area. It was found feasible to transport irradiated FCM in 19 hours from Citrusdal to Letaba, with maintenance of moth quality.

Initial releases from November 2008 worked well and a high overflooding ratio with feral moths was maintained. However, trap catches of released moths decreased at the end of January for unknown reasons. Fruit drop evaluations started in mid-February and recorded higher fruit infestation than the economic threshold in the SIT area. There were no differences between fruit infestation in the SIT and control orchards.

Inleiding

Die oprigting en inbedryfstelling van 'n insektarium vir die massateel van valskodlingmot (VKM), wat deur Xsit (Edms) Bpk, bedryf word, is in die CRI-Jaarverslag vir 2007-2008 bespreek. Die eerste gamma-gesteriliseerde motte wat deur dié insektarium vir die onderdrukking van VKM geteel is, is gedurende die 2007-2008 seisoen in 1 500 ha in die Citrusdal-gebied losgelaat. Alhoewel goeie resultate met die Steriele-insek Loslatings (SIL) behaal is, was die insektarium in baie opsigte nog nie ten volle operasioneel nie. Alle toerusting wat in ander insektaria vir VKM-teling op veel kleiner skaal gebruik word, was totaal ontoereikend en moes nuut ontwerp word. Sekeres daarvan, soos groter teelflesse met spesiale papiermembraandeksels, die vlekvrystaalmandjies met polikarbonaatplastiek heuningkoekmateriaal vir pupering, 'n masjien vir die outomatiese insit van papiermembrane in die flesdeksels, 'n outomatiese teelflesvulmasjien, eierlé"panne" vir die versameling van eiers en motkabinette vir die versameling van pas-ontpopte motte, het van meet af met baie min probleme goed gewerk. Ander toerusting, soos die grotendeels gesentraliseerde lugversorgingstelsel, wat van kritiese belang in die teelproses was, het egter heelwat probleme gegee. Tekortkominge in sekere van die toerusting wat op klein skaal in die prototipe-stadium goed gewerk het, het gedurende massateling tevoorskyn gekom en moes aangepas word sonder om die teelproses beduidend te versteur. Voorbeelde hiervan is die ontsmettingstegniek met formalien vir eiervelle, die hitteversperring vir larwes in die larweteelkamers, die lugvervoerstelsel met geïntegreerde siklone vir motte van die motkabinette na 'n aangrensende koelkamer en die dieselgedrewe oond wat vir hitte-ontsmetting van die teelflesse gebruik word. Al die genoemde faktore het uiteraard 'n wisselend-nadelige uitwerking op motkwaliteit gehad. Motkwaliteit is ook ingeboet omdat die insekte na Stellenbosch vir gammabestraling vervoer moes word; Xsit se Kobalt₆₀-bron kon eers in Februarie 2008 in werking gestel word.

Baie tyd is aan die oplos van bogenoemde probleme afgestaan. Enkele van die stukke toerusting en sekere prosesse kon desnieteenstaande, aan die einde van die verslagjaar onder bespreking, nog steeds nie ten volle in bedryf gestel word nie. Met die huidige 2008-2009 seisoen nog nie agter die rug nie, is dit egter duidelik dat dit vir Xsit moontlik geword het om motte van goeie kwaliteit te teel.

3.2.2.1 Die giftigheid van formalien as 'n ontsmettingsbehandeling vir valskodlingmoteiers

Inleiding

'n Konsentrasie van 20% formalien (35% m/v) in water word as 'n standaard dompelbehandeling in VKM-insektaria gebruik om eiervelle te ontsmet voordat dit in teelflesse ingeënt word. 'n Proef is in Januarie 2005 uitgevoer om die invloed van formalien op VKM-eiers te ondersoek. Eiemortaliteit het van 39% tot 100% toegeneem soos die konsentrasie formalien van 10% tot 40% verhoog is (CRI-Jaarverslag vir 2004-2005, Afd. 3.4.5.11). In dié proef het 20% formalien byvoorbeeld 65% eiemortaliteit veroorsaak. In die Xsit-insektarium is probeer om die nadelige invloed te omseil deur groter getalle eiers per teelfles te gebruik. Dit het egter geblyk dat soveel meer eiers in teelflesse ingeënt moes word om 'n redelike motproduksie per fles te kry dat dit dikwels nadelig op eierproduksie ingewerk het én groter probleme met virusbesmetting veroorsaak het. 'n Ondersoek is geloods om die verskynsel verder te ondersoek.

Materiale en metodes

Twee proewe (Nov08 en Jan09) is uitgevoer om verlaagde formalienkonsentrasies se uitwerking op virusbesmetting en VKM-produksie te ondersoek. Die tegniek was in beide proewe dieselfde en word saam bespreek.

Onderskeidelik 20 en 9 heuningbottel-teelflesse is in Proewe Nov08 en Jan09 gebruik. Die teelflesse met dié is hitte-ontsmet en die volgende dag is elkeen met 'n gemiddeld van 700 eiers ingeënt. Die eiers is behandel deur elke eiervelletjie in die betrokke formalienmengsel te dompel en 1-2 sekondes lank met 'n tangetjie rond te skommel. Eiers van die kontrole-flesse is onbehandel ingeënt. Die flesse is vir larwe-ontwikkeling by 26°C geïnkubeer en ondersoek is daagliks uitgevoer om flesse met tekens van virusbesmetting te verwyder. Dié flesse is gehou totdat pupering in die flesse sonder virusbesmetting afgehandel was. Toe die eerste volwasse

larwes begin pupeer het, is die watterproppe verwyder en met riffelkartonproppe vir pupering vervang (CRI-Jaarverslag vir 2004-2005, Afd. 3.4.5.2). Proppe is met nuwes vervang sodra alle beskikbare openinge in die riffelkarton met kokonne gevul was. Die laaste proppe is 5 dae na die begin van pupering verwyder, ongeag die moontlike aanwesigheid van enkele oorblywende stadig-ontwikkelende larwes in die dieet. Die kartonproppe met kokonne is 'n week lank by 26°C gehou sodat pupering kon plaasvind. Die papies is daarna verwyder en getel. Produksie is gemeet aan die aantal papies wat in elke behandeling versamel is.

Resultates en bespreking

Die volgende behandelings is geëvalueer (Tabel 3.2.2.1):

Tabel 3.2.2.1. Formalienbehandelings wat in twee proewe geëvalueer is.

Proef	Konsentrasie formalien (%)	Dompeltyd (sekondes)
Nov08	0 (kontrole)	0
	5	1-2
	5	30
	10	1-2
	10	30
	20 (standaard)	1-2 (standaard)
Jan09	0 (kontrole)	0
	5	10
	5	20
	10	10
	10	20
	20 (standaard)	1-2 (standaard)

- **Virusbesmetting**

- *Proef Nov08:* Negentien van die 20 kontroleflesse was met virus besmet. Daar was lewendige larwes in slegs een van die 19 besmette flesse, wat meegebring het dat twee flesse se produksie geëvalueer kon word. Geen virusbesmetting is in enige flesse van die formalienbehandelings opgemerk nie.
- *Proef Jan09:* Alle kontroleflesse was virusbesmet (9 flesse van 9) en die produksie kon nie gemeet word nie. Een fles waarvan die eiers met die standaard 20% formalien behandel was, was heeltemal virusbesmet, terwyl daar enkele besmette larwes in 'n tweede fles van dieselfde behandeling was. Geen virusbesmetting is in enige flesse van die ander formalienbehandelings opgemerk nie.

Die hemisferiese eiers, wat deur die wyfemotte op die waspapier "vasgeplak" word, word dikwels in digte massas langs en opmekaar gelê, wat die vinnige indringing van formalien onder die eiermassas sal verhinder. Die manier waarop die eiers dus op die waspapier gelê word – meer of minder verspreid, kan derhalwe 'n uitwerking hê op die doeltreffendheid waarmee die formalien onder die eiers kan indring. Daar word dus aangeneem dat die 1-2 sekonde dompeltyd van die 20% formalienbehandeling te kort is om behoorlike virusontsmetting te verseker. Die skynbare goeie resultate met die 1-2 sekonde dompeltyd in proef Nov08 kan moontlik daaraan toegeskryf word dat die eiers in daardie proef meer verspreid op die waspapier gelê was as in Proef Jan09 (waar virusbesmetting in die 1-2 sekonde 20% formalienbehandeling opgemerk was).

Daar word internasionaal aanvaar dat virusse in die eiers van insekte oorgedra kan word. Dit is egter vreemd dat die flesse wat met eiers ingeënt is wat vir 10 to 30 sekondes in die formalien gedompel was, skoon gebly het. Dit is in skerp kontras met die hewige virusbesmettings in die twee proewe se onbehandelde kontrolebehandelings – wat 'n goeie maatstaf is van die besmettingsdruk waaraan die formalienbehandelings blootgestel was.

- **Produksie**

- *Proef Nov08:* Die onbehandelde kontrole-behandeling het die swakste produksie gelewer (Fig. 3.2.2.1). Dit kan moontlik aan die klein aantal herhalings (slegs 2) toegeskryf word. Dit kan egter ook wees dat daar 'n relatiewe ligte, onopsigtelike virusbesmetting in dié flesse was wat die produksie benadeel het.

Beide die konsentrasie formalien en die dompeltyd het gesamentlik of opsigself 'n invloed op eieroorlewing. In dié proef was die standaardbehandeling van 20% formalien die giftigste vir die eiers en het derhalwe die swakste produksie gelewer. Daar was baie min verskil tussen die 5% formalien- (dompeltye van onderskeidelik 1 en 30 sekondes) en 10% formalien-(1 sekonde dompeling)-behandelings. Die 10% formalienbehandeling wat 30 sekondes geduur het, het swakker presteer – waarskynlik weens 'n te lang dompeltyd.

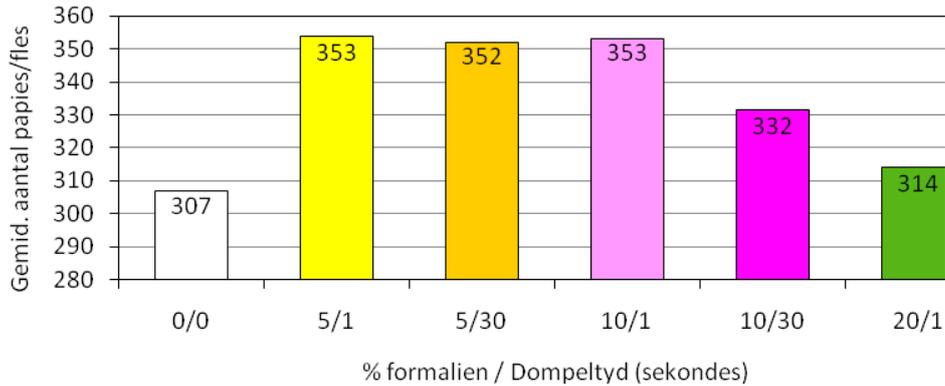


Fig. 3.2.2.1. Die uitwerking van formalienkonsentrasie en dompeltyd op VKM-produksie.

- *Proef Jan09:* Geen larwes in die kontrolebehandeling het weens virusbesmetting tot volwassenheid ontwikkel nie (Fig. 3.2.2.2). Produksie met die twee 5% formalienbehandelings was beter as met die 10% en 20% konsentrasies. In beide gevalle is die produksie nie deur die langer dompeltyd (20 teenoor 10 sekondes) benadeel nie. Dit is gewens om die dompeltyd so kort as moontlik te hou om die potensiële nadelige invloed op die eiers so klein as moontlik te hou, maar terselfdertyd te verseker dat virusonderdrukking nie benadeel word nie. Met die massabehandelingstegniek vir eiers wat in die Xsit-insektarium nodig is, is die dompeltyd om praktiese redes belangrik. Beide die 1-2 sekonde en 10 sekonde dompeltye is te kort om prakties-bruikbaar te wees. Daar is derhalwe besluit om die 20 sekonde dompeltyd in die toekoms in kombinasie met 'n 5% formalienkonsentrasie te gebruik.

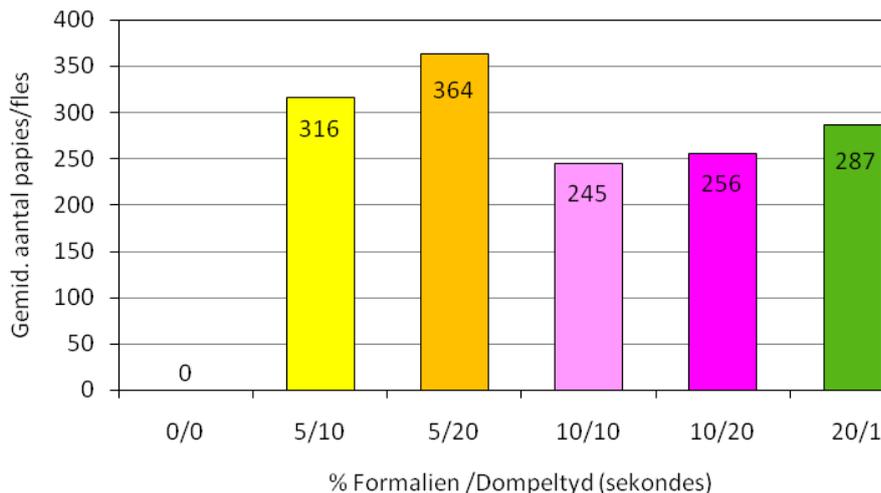


Fig. 3.2.2.2. Die uitwerking van formalienkonsentrasie en dompeltyd op VKM-produksie.

Gevolgtrekking en toekomstige doelwit

Die ondersoek na die formalienbehandeling van eiers word voortgesit. Die volgende stap is om die behandeling wat in die voltooide ondersoekwerk die toepaslikste geblyk het, naamlik 5% formalien met 'n 20 sekonde dompeltyd, onder semi-kommersiële toestande in die Xsit-insektarium te toets. Dit sal die behandeling van

volgrootte eiervelle (in teenstelling met die veel kleiner eiervelletjies wat algemeen gebruik word) in spesiale eiervelrame behels.

3.2.2.2 Kommersiële eierontsmetting met formalien

Inleiding

Die algemene werkswyse in kleiner VKM-insektaria (en tot dusver ook in die Xsit-insektarium) om VKM-eiers te steriliseer, is soos volg: Huishoudelike waspapier, 33 μm dik, word gebruik. Eiers word óf onder ronde huishoudelike meelsiwwe (deursnee ca. 160 mm) op waspapier gelê, óf in Xsit se geval, op A4-grootte waspapier. In beide gevalle word die eiervelle in kleiner velletjies, ca. 40 mm x 40 mm, geknip. Dié velletjies word een na die ander met 'n tangetjie opgetel, elkeen 1-2 sekondes lank in formalien gedompel en dan in 'n teelfles ingeënt. Die hele proses is uiteraard langdradig en is nie geskik om vir die massa-inenting van etlike duisende teelflesse per dag gebruik te word nie. Drie aspekte van dié proses is tydrawend, nl. (i) die eiervelle moet in kleiner stukkie geknip word, (ii) die eiervelletjies moet een-een ontsmet word en (iii) een eiervelletjie moet in elke teelfles geplaas word. Die geslote-houer teelproses wat in die VKM-teeltegniek gebruik word, laat nie toe dat daar aan laasgenoemde aspek verander word nie. Die eerste twee stappe kan egter aangepas word om beter produktiwiteit te bevorder.

Metodes en resultate

In die Kodlingmot SIT-insektarium te Osoyoos, Kanada, word eiers op gewakste bruinpapier gelê. Dié velle is heelwat groter as die A4-grootte wat in Xsit geproduseer word. Die eiervelle word in staalraam-“boeke” vasgeklem (Fig. 3.2.2.3) en daarna in ontsmettingsmiddel gedompel. Met dié tegniek in gedagte, is 'n toetshouer aanmekaar gesit wat uit 'n vel waspapier tussen twee yskasroosters bestaan het (Fig. 3.2.2.4). Die raam is vir 1-2, 5, 10, 20 en 30 sekondes lank in water gedompel om die uitwerking daarvan op die waspapier te ondersoek. Dit was dadelik duidelik dat die waspapier heeltemal pap raak na meer as net 1-2 sekondes se dompeling in die water. Alhoewel die papier in posisie gebly het, kon dit nie verwyder word sonder dat die papier in stukke geskeur het nie. Dikker waspapier, 40 μm dik, is vervolgens verkry en getoets. Dié papier is meer waterbestand, dus baie sterker en het goed in die toetshouer gewerk.



Fig. 3.2.2.3. “Boek”rame wat in die Kodlingmot SIT-insektarium te Osoyoos, Kanada gebruik om eiervelle te ontsmet.



Fig. 3.2.2.4. Toetshouer vir die hantering van eiervelle wat in formalien ontsmet moet word.

'n Prototipe eiervelhouer, in beginsel soortgelyk aan die Osoyoos-model, is vervolgens vir die Xsit-insektarium ontwerp (Fig. 3.2.2.5). In plaas van 'n "boek"-ontwerp, waar die "blaaie" vas aan die raam is, is die blaaie van die Xsit-houer los, sodat die eiervelle en blaaie alternatiewelik in die houer geplaas word (Fig. 3.2.2.6). Voorsiening is vir 10 eiervelle gemaak. Die ontwerp dra by tot beter ondersteuning van die eiervelle. Die blaaie sluit ook so styf opmekaar dat daar geen sydelingse beweging is wat die eiers kan beskadig nie.



Fig. 3.2.2.5. Prototipe vlekvrystaalraam vir die behandeling van eiervelle met formalien in die Xsit-insektarium.



Fig. 3.2.2.6. Eivervelhouer gedeeltelik uitmekaar gehaal om die positionering van die eivervelle te wys.

Gevolgtrekking en toekomstige doelwit

Die prototipe eivervelhouer word in die volgende verslagjaar aan semi-kommersiële toetse onderwerp om die doeltreffendheid van eierontsmetting te ondersoek. Indien suksesvol, sal volgrootte eivervelhouers vir kommersiële gebruik vervaardig word waarin meer as 10 eivervelle opmekaar en/of langsmekaar geplaas sal kan word.

Aandag word alreeds gegee aan die gemeganiseerde sny van die groot eivervelle in kleiner velletjies, waarna vroeër verwys is. Die ontwerp van dié snyer is egter afhanklik van eivervelle waarop die eierdigtheid grotendeels dieselfde is sodat die eivervelletjies, binne redelike perke, nagenoeg dieselfde aantal eiers per velletjie sal hê – daar word nog nie aan dié vereiste in die Xsit-insektarium voldoen nie. Standaardisering van die eierlêpatroon geniet egter heelwat aandag en die vooruitsig is goed dat die probleem opgelos sal kan word.

3.2.2.3 Die giftigheid van osoongas as 'n ontsmettingmiddel vir valskodlingmoteiers

Inleiding

Probleme wat in die algemeen met granulosevirusbesmetting én die hantering daarvan in 'n insektarium-omgewing ondervind word, is in die CRI-Jaarverslag vir 2007-2008 bespreek. Proewe is bespreek waarin die virusdodende uitwerking van osoon ondersoek is. Daar is gevind dat 'n sesuurlange behandeling met osoon (5 000 mg per uur) 'n kunsmatig-geïnduseerde virusbesmetting op eivervelle gedeeltelik onderdruk het. Daar is ook gevind dat 'n agtuurlange osoonbehandeling (5 000 mg per uur) nie die natuurlike eiermortaliteit op eivervelle verhoog het nie. Dié resultate was belowend genoeg om die studie voort te sit.

Materiale en metodes

'n Proef is uitgevoer om die giftigheid van osoongas vir eiers te ondersoek.

Twee standaardgrootte eivervelle (300 mm x 210 mm) is gebruik. Eiers op die een eivervelle was relatief yl versprei (laedigheid = LED), terwyl eiers op die ander eivervelle digter gelê was (hoëdigtheid = HED). 'n Halwe eivervelle van

elke kategorie is as onbehandelde kontroles eenkant gehou, terwyl die ander twee in 'n leë kamer aan 'n staander opgehang is. 'n Aeroqual-osoonlynproduseerder, wat 'n maksimum van 5 000 mg osoon per uur produseer, is gebruik. Die eiers is 14 uur lank aan die osoon blootgestel, waarna dit saam met die kontrole-eiervelle by 26°C geplaas is totdat alle lewensvatbare eiers in die kontrole uitgebroei het. Alle eiers in elk van 10 ewekansig-toegekende vierkante op elke eiervelle, 15 mm x 15 mm groot, is ondersoek en as uitgebroei of dood geklassifiseer.

Resultate en bespreking

Gewoonlik neem die "natuurlike" eiermortaliteit toe namate die eiers digter opmekaar op die eiervelle gelê word. In dié proef het die teenoorgestelde gebeur – die eiermortaliteit op die kontrole-eiervelle het afgeneem met toenemende eierdigtheid. Dit is moontlik dat die "hoë" eierdigtheid nie hoog genoeg was om eiermortaliteit te laat toeneem nie en dat die verskil slegs natuurlike variasie verteenwoordig. Die patroon van eierlegging is deurgaans uiters wisselvallig en dit maak die definiëring van digtheid baie arbitrêr en onakkuraat in terme van die onderskeid wat in die tipe studie onder bespreking getref moet word.

Die osoon het mortaliteit van die twee kategorieë eiers met gemiddeld 5,7% in vergelyking met die kontroles verhoog (HED = 3,9% en LED = 7,5%), wat nie baie is nie (Fig. 3.2.2.7). Die totale mortaliteit van 27% tot 38% in die twee osoonlynbehandelings is baie laer as byvoorbeeld die tot 65% eiermortaliteit wat deur ontsmetting met die standaard 20% formalienbehandeling veroorsaak word.

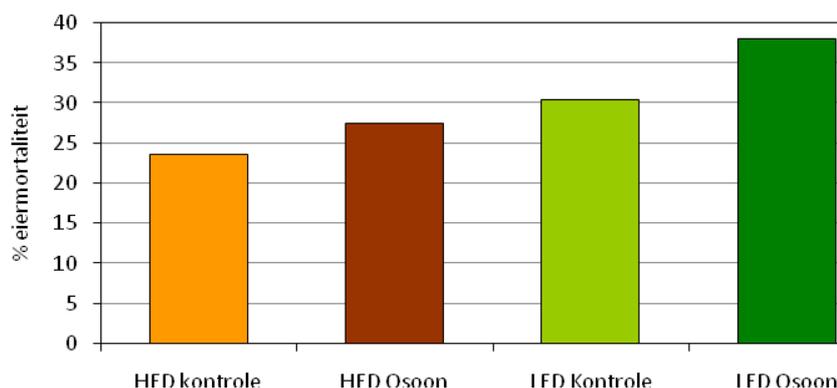


Fig. 3.2.2.7. Die giftigheid van osoon vir valskodlingmoteiers (HED = hoëdigtheid eiervelle; LED = laedigheid eiervelle).

Gevolgtrekking

Dit lyk op dié tydstip asof osoon 'n alternatief vir formalien-ontsmetting van eiervelle kan wees, mits die behandeling die granulosevirus bevredigend onderdruk.

Toekomstige doelwit

In die Inleiding is gemeld dat 'n sesuurlange osoonlynbehandeling granulosevirus op eiervelle gedeeltelik onderdruk. In 'n volgende studie moet bepaal word hoe lank 'n osoonlynbehandeling moet wees om bevredigende virusonderdrukking te verskaf en of dié behandeling, wat hopelik korter as 14 uur sal wees, minder giftig vir die eiers sal wees.

3.2.2.4 Produksiepotensiaal van Volroom- en Kremel-melkpoeier

Inleiding

Drie proewe is in die vorige CRI-jaarverslag (2007-2008) beskryf waarin die produksievermoë van twee alternatiewe, goedkoper dieëtbestanddele ondersoek is. Eerstens is die vervanging van die standaardtipe mieliemeel ("Special", SM) met 'n goedkoper, effens growwer mieliemeel ("Sifted", GM) ondersoek. Tweedens is

die produksievermoë van standaard Volroom- met Kremel-melkpoeier in een proef met mekaar vergelyk. Volroommelkpoeier is nie altyd beskikbaar nie en die toetsing van Kremel, 'n goedkoper, plantvet-gebaseerde melkpoeier, is derhalwe om strategiese redes nodig. 'n Finale proef is in die huidige verslagtydperk uitgevoer om die ondersoek af te sluit.

Material en metodes

Nege herhalings, wat elk uit 'n enkele heuningbottel-teelfles bestaan het, is per behandeling gebruik (Tabel 3.2.2.2):

Tabel 3.2.2.2. Kombinasies van mieliemeel en melkpoeier in proef geëvalueer

Dieet	Mieliemeel	Melkpoeier*
1	Spesiaal	1x Volroom
2	Spesiaal	1x Kremel
3	Spesiaal	1,4x Kremel
4	Gesif	1x Volroom
5	Gesif	1x Kremel
6	Gesif	1,4x Kremel

* 1x Volroom- en Kremel-melkpoeier = 18.3 g per 1 000 g dieet
 1,4x Kremel-melkpoeier = 25 g per 1 000 g dieet

Eenhonderd en veertig gram aangemaakte dieet (70 g dieët plus 70 g water) is in elke fles geplaas en hitte-ontsmet. Elke fles is daarna met formalien-ontsmette VKM-eiers op 'n velletjie waspapier, ingeënt. Die aantal eiers per fles is nie getel nie, maar die ewegroot eiervelletjies is voor inenting só uitgesoek dat almal op die oog af min of meer dieselfde eierdigtheid gehad het. Die flesse is na inenting met watterproppe toegemaak en by 26°C gehou totdat die larwes hul volwasse grootte bereik het. Die watterproppe is toe met riffelkartonproppe vervang. Laasgenoemde is 7 dae lank elke dag met nuwes vervang. Die kartonproppe met papies is 5 dae by 26°C gehou, waarna die papies verwyder en getel is. Tien wyfie- en 10 mannetjies papies per herhaling is elke dag ewekansig versamel en individueel op 'n analitiese weegskaal geweeg. Papies van die derde dag se produksie is toegelaat om te verpop en een paar motte van elke behandeling is in elk van 10 plastiekbakkies vir eierlegging geplaas. Die motte is 5 dae later verwyder. Tellings van eiermortaliteit is uitgevoer nadat alle lewensvatbare eiers in die standaardbehandeling (Spesiale mieliemeel plus Volroommelkpoeier) uitgebroei het.

Resultate en bespreking

- **Produksie**

- *Aantal papies:* In vorige proewe (CRI-Jaarverslag 2007-2008) is meer VKM deurgaans met die standaard Spesiale mieliemeel as met die Gesifte mieliemeel geproduseer (Proef 1: 407 papies per teelfles teenoor 378; Proef 2: 350 papies teenoor 285; Proef 3: Proef 3: 386 papies teenoor 379). In dié proef was die produksie ook groter met die Spesiale meel ongeag die tipe melkpoeier wat in die verskillende diëte gebruik was (Fig. 3.2.2.8).

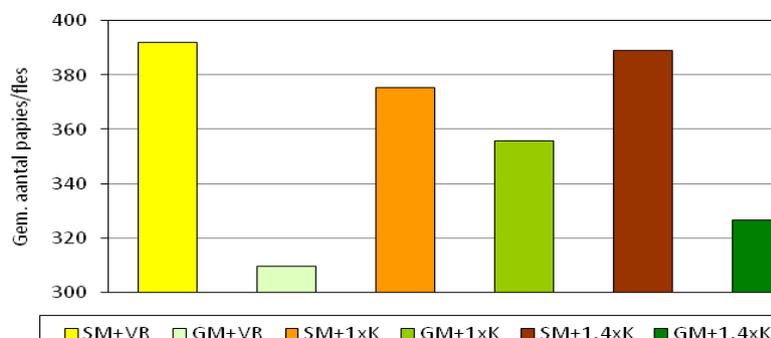


Fig. 3.2.2.8. Produksie van valskodlingmotpapies met alternatiewe mieliemeel geproduseer (SM = "Spesiale" mieliemeel; GM = "Gesifte" mieliemeel; VR = Volroommelkpoeier; K = Kremel-melkpoeier).

Meer papier is per fles met 1x Volroommelkpoeier as met 1x Kremel-melkpoeier in kombinasie met Spesiale meel geproduseer (392 papies per teelfles teenoor 375) (Fig. 3.2.2.9). Dié verskil in produksie is uitgewis toe die hoeveelheid Kremel met 36% verhoog is (389 papies). Dieselfde produksiepatroon is egter nie in die geval van die Gesifte meel gevind nie. Die resultate met Spesiale meel bevestig soortgelyke resultate (CRI-Jaarverslag 2007-2008) (386 papies per fles met 1x volroommelkpoeier teenoor 342 met 1x Kremel en 387 met 1,4x Kremel).

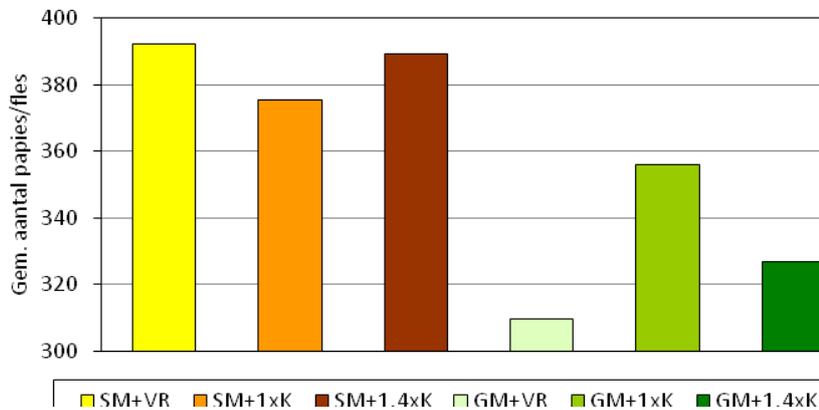


Fig. 3.2.2.9. Produksie van valskodlingmotpapier met alternatiewe melkpoeier geproduseer (SM = “Spesiale” mieliemeel; GM = “Gesifte” mieliemeel; VR = Volroommelkpoeier; K = Kremel-melkpoeier).

- *Massa van papies:* Ervaring met die heuningflesse dui daarop dat papiermassa benadeel word wanneer daar 'n produksie van 550 tot 600 papies per teelfles is. 'n Maksimum van minder as 400 papies is in dié proef geproduseer en die massa van nóg die wyfie- nóg die mannetjiespapier is in die verskillende behandelings aangetas (Fig. 3.2.2.10).

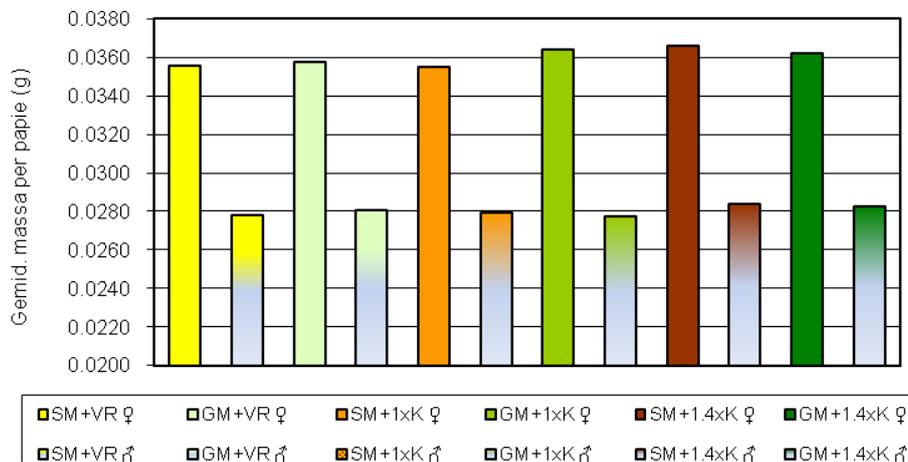


Fig. 3.2.2.10. Gemiddelde massa van valskodlingmotpapier met alternatiewe mieliemeel en melkpoeier geproduseer (SM = “Spesiale” mieliemeel; GM = “Gesifte” mieliemeel; VR = Volroommelkpoeier; K = Kremel-melkpoeier).

- **Ontwikkeling:** Indien die ontwikkeling van die larwes deur enige van die diëte aangetas was, sou vinnig-ontwikkelende larwes gouer begin verpop het en andersom. Daar is geen sulke duidelike neigings te sien nie (Fig. 3.2.2.11). Dit stem met twee van drie vorige proewe ooreen (CRI-Jaarverslag 2007-2008).

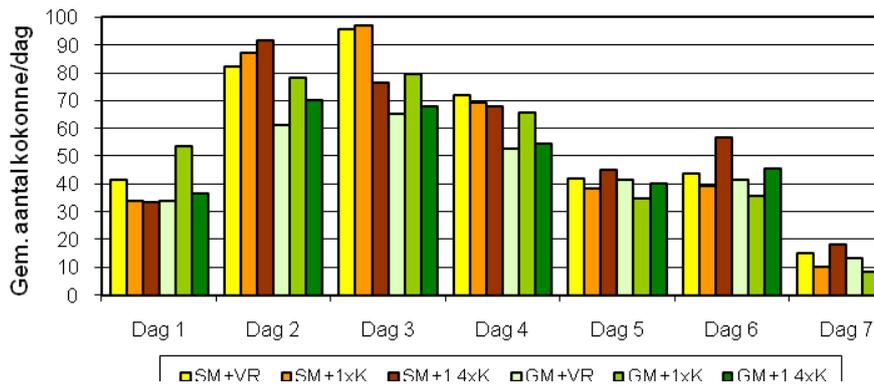


Fig. 3.2.2.11. Ontwikkelingstyd van VKM (uitbroeiing tot verpoping) met verskillende diëte.

• **Eierlegging:**

’n SIT-Insektarium wat vir die massateel van enige insek verantwoordelik is, moet aandag aan twee aspekte gee. Eerstens moet soveel lewenskragtige insekte as moontlik ekonomies vir loslating in boorde geproduseer word. Tweedens moet die insekte vrugbaar wees, aangesien die hele produksieproses daarvan afhang.

Die diëte met Spesiale én Gesifte meel wat Kremel bevat het, het beter eierlegging as die diëet met volroommelkpoeier tot gevolg gehad (Fig. 3.2.2.12). Die eiermortaliteit het egter toegeneem namate meer eiers in die verskillende behandelings gelê was.

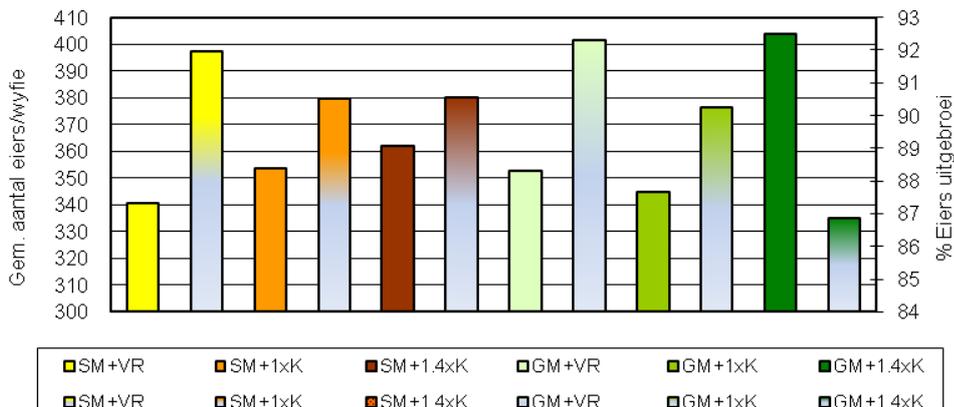


Fig. 3.2.2.12. Eierproduksie (egalig-gekleurde blokke: Aantal eiers per wyfie gelê) en –vrugbaarheid (tweekleur-blokke: % eiers uitgeborei) van VKM-wyfies wat op verskillende diëte geproduseer was.

Tellings het gewys dat eiermortaliteit eerder aan eierdigtheid as aan die diëte toegeskryf kan word. Wyfies verkies om hul eiers teen ander voorwerpe te lê, selfs teen of op ander eiers. Dié eienskap het tot gevolg gehad dat eiers in die hoeke van die paringshouers digter as op gelyk oppervlakte gelê is. Tellings van dooie eiers in hierdie twee gebiede het gewys dat meer eiers onuitgeborei bly wanneer hulle dig opmekaar gelê word, as wanneer die eierdigtheid laer is (Fig. 3.2.2.13). Dié verskynsel wys dat die aantal insekte wat vir eierlegging gebruik word, noukeurig vasgestel moet word, aangesien dit teenproduktief kan wees indien te veel eiers op ’n beperkte oppervlakte gelê word.

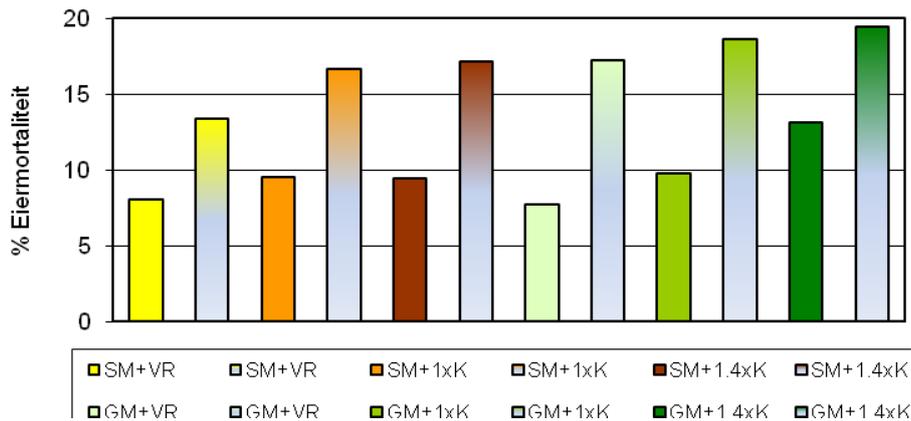


Fig. 3.2.2.13. Mortaliteit van eiers onder laer (egalig-gekleurde blokke) en hoër digtheid- (tweekleur-blokke) toestande.

Gevolgtrekking

Bogenoemde resultate wys dat Gesifte meliemeel nie so geskik as Spesiale meliemeel in die VKM-dieet is nie. Dit is moontlik dat die produksie sal styg indien VKM vir 'n aantal generasies lank met die growwemeeldieet geteel word. Volgens berig word is dié tipe meliemeel egter onlangs permanent van die mark onttrek en verdere spekulasie oor moontlike oplossings vir die probleem is tans onnodig. Verder het dit geblyk dat die Kremel-melkpoeier, indien nodig, wel as plaasvervanger vir volroommelkpoeier in die VKM-dieet gebruik sal kan word.

Toekomstige doelwit

Verdere ondersoeke na die VKM-dieet is nie uitgesluit nie, alhoewel daar nie verdere ondersoeke beplan word nie. Enige bykomende studies sal deur die behoeftes van die SIT-program bepaal word.

3.2.2.5 Invloed van Calco Oil Red-kleurstof op die teel van valskodlingmot

Inleiding

Calco Oil Red 2144 is 'n nie-giftige kleurstof wat by die dieet van Kodlingmot en Pienkbolwurm in insektaria in onderskeidelik Kanada en Amerika gevoeg word. Die kleurstof word saam met die voedsel deur die larwes gevreet, in die liggaam opgeneem en nie gedurende ver- en ontpopping afbreek nie. Sodanig gemerkte motte kan ná loslating in 'n SIL-program van wilde motte in lokvalle onderskei word deur hul pap te druk en die liggaamsinhoud vir tekens van rooi kleurstof te ondersoek. Die sukses van die loslatings kan sodoende bepaal word. Die uitwerking van Calco op ontwikkelende VKM is in die CRI-Jaarverslag vir 2007-2008 bespreek. Daar is in twee gevalle bevind dat meer insekte geproduseer word wanneer Calco by die dieet van VKM gevoeg word (709 papies met Calco teenoor 635 sonder Calco, asook 651 met Calco teenoor 621 sonder Calco). Dit was 'n onverwagte resultaat aangesien daar verwag is dat Calco óf geen óf 'n effens-nadelige invloed op larwe-ontwikkeling sal hê. Die studie is derhalwe met 'n verdere twee proewe voortgesit.

Materiale en metodes

Calco Oil Red word gewoonlik by Canola-olie gevoeg (4 ml per 1 000 g dieet) om dit makliker in die dieet te meng. Sonneblomolie is oor die algemeen goedkoper as Canola en is dit interessantheidsomhalwe by die proef ingesluit. Calco is teen konsentrasies van 0,01%, 0,0125% en 0,015% (0,1 g, 0,125 g en 0,15 g per 1 000 g dieet) by standaard VKM-dieet gevoeg en goed gemeng. Eenhonderd en veertig gram dieet is in elke fles geplaas en hitte-ontsmet. Elke fles is daarna met formalien-ontsmette VKM-eiers op 'n velletjie waspapier ingeënt. Die aantal eiers per fles is nie getel nie, maar die ewegroot eiervelletjies is voor inenting só uitgesoek dat almal op die oog af min of meer dieselfde eierdigtheid gehad het. Die flesse is na inenting met watterproppe toegemaak en by 26°C gehou totdat die larwes hul volwasse grootte bereik het. Die watterproppe is toe met riffelkartonproppe vervang. Laasgenoemde is vervang sodra die openinge vol kokonne gepak was. Die

kartonproppe met papies is 5 dae by 26°C gehou, waarna die papies verwyder en getel is. Tien wyfie- en 10 mannetjepapies is ewekansig per herhaling versamel en afsonderlik op 'n analitiese weegskaal geweeg. Papies van die eerste dag se produksie is toegelaat om te verpop en een paar motte van elke behandeling is in elk van 10 plastiekbakkies vir eierlegging geplaas. Die motte is 5 dae later verwyder. Tellings van eiermortaliteit is uitgevoer nadat alle lewensvatbare eiers uitgebroei het.

Resultate en bespreking

Proef 1: Geen kontrole-behandeling is by dié proef ingesluit nie. Die produksie, gemeet aan die aantal papies wat geproduseer was, het afgeneem soos die dosis Calco in die dieet verhoog is (Fig. 3.2.2.14). Dit lyk asof sonneblomolie saam met 0,015% Calco produksie effens verbeter het in vergelyking met die ooreenstemmende Canola-bevattende behandeling.

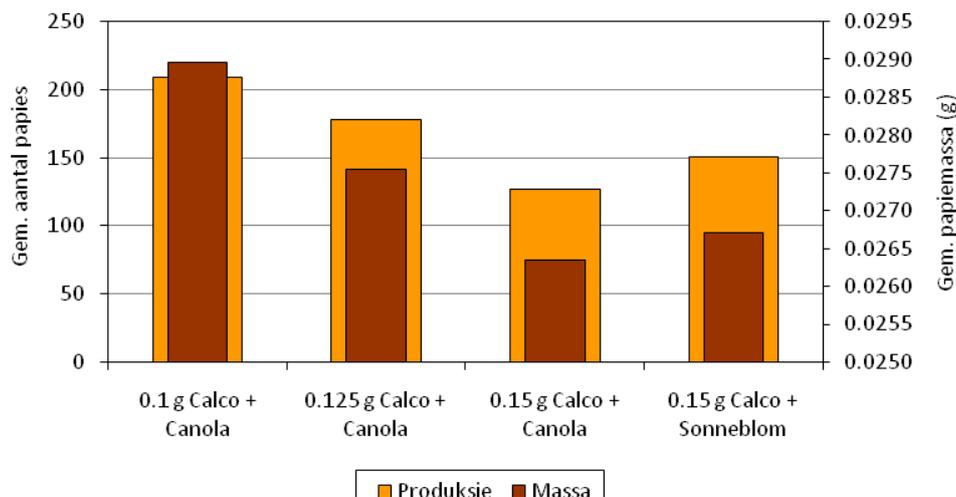


Fig. 3.2.2.14. Die invloed op produksiegrootte en papiemassa van VKM-dieet wat Calco Oil Red inwendige kleurstof bevat.

Die gemiddelde massa, dus grootte, van die papies, het, net soos die produksie, afgeneem met toenemende Calco-konsentrasie (Fig. 3.2.2.15). Die gemiddelde afname is egter klein – 'n speling van .004 g en 0.002 g onderskeidelik by die wyfie- en mannetjepapies, wat nie 'n opsigtelike verskil aan papiegrootte sal maak nie. Dié resultaat is teenoorgestelde van wat gewoonlik gebeur. Die papiemassa bly óf dieselfde met toenemende produksie (solank as wat daar nie oorproduksie in die flesse is nie) óf die insekte word kleiner wanneer die aantal insekte per fles die benutbare voedselvoorraad oorskry. In dié proef het die papies kleiner/licter geword met krimpende getalle. Dit het gebeur ten spyte daarvan dat die produksie per fles amper die helfte van die normale produksie (ongeveer 400 insekte per fles) was – waarskynlik as gevolg daarvan dat minder eiers as gewoonlik ingeënt was. Die afname kan daarop dui dat hoër konsentrasies Calco nie net ontwikkeling van larwes kan verhoed nie, maar selfs effens nadelig vir larwes is wat daarin slaag om tot volwassenheid te ontwikkel.

Die potensiele vrugbaarheid ("fecundity") van die VKM wat met die verskillende Calco-diëte geproduseer was, het min verskil – daar was slegs 'n verskil van 29 eiers tussen die beste en swakste behandeling (0,125 g Calco: 436 eiers en 0,15 g Calco: 407 eiers). Daar is nie 'n duidelike verband tussen eierlegging in die verskillende behandelings nie, wat waarskynlik daarop dui dat die onderlinge verskille onbelangrik is.

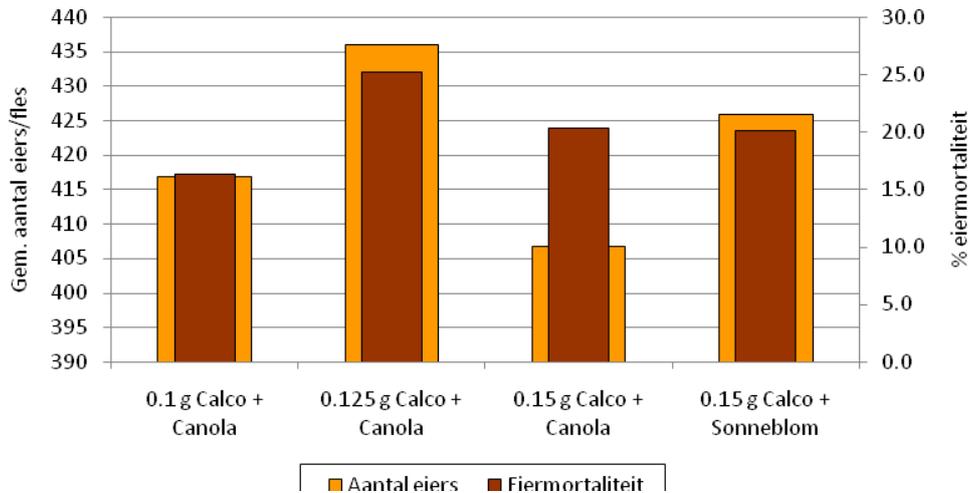


Fig. 3.2.2.15. Potensiële en werklike vrugbaarheid van VKM wat op verskillende Calco-bevattende diëte geteel is.

Die werklike vrugbaarheid (“fertility”) tussen die behandelings wissel van 16,4% eiermortaliteit (0,1 g Calco) tot 25% (0,125 g Calco). Eiermortaliteit in beide die 0,15 g Calco-behandelings was laer, wat wys dat daar waarskynlik nie behandelingsverskille bestaan nie. Die grootste oorsaak van eiermortaliteit in die proef was, soos vantevore, eiers wat in die hoeke van die paringshouers te dig opmekaar gelê was en groter eiermortaliteit veroorsaak het.

Proef 2: Daar is effens meer papies met die Calco-bevattende diëet as met die diëet sonder Calco geproduseer (Fig. 3.2.2.16). Die verskil is egter weglaatbaar klein (446 papies per fles teenoor 441). Die aantal papies per fles was naastebly soveel as wat per fles geproduseer behoort te word sonder dat die insekte weens ’n tekort aan benutbare voedsel opsigtelik kleiner word. Die gemiddelde massa-afname van die papies in die kontrole- en die Calco-behandeling was 0.004 g (0,005 g en 0.003 g by die wyfie- en mannetjiefapies onderskeidelik), wat net-net ’n merkbare verskil in papiegrootte verteenwoordig.

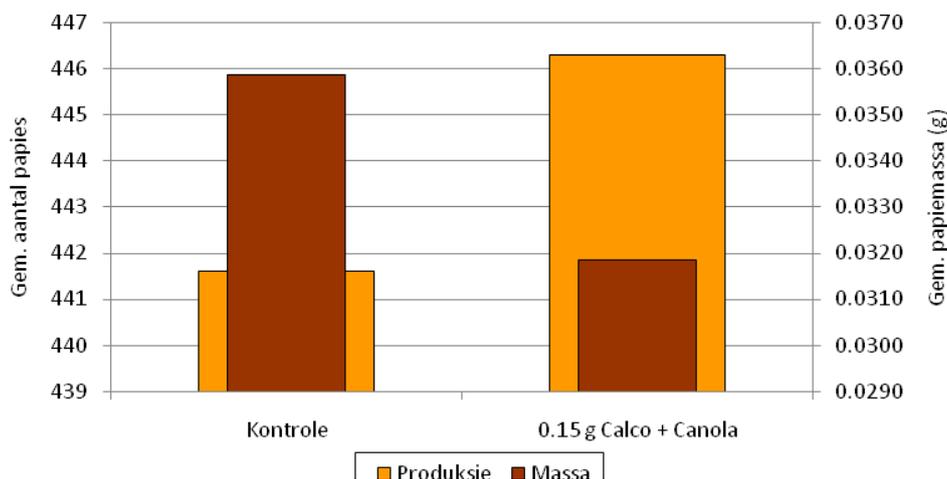


Fig. 3.2.2.16. Die invloed op produksiegrootte van VKM-diëet wat Calco Oil Red inwendige kleurstof bevat.

Die papies in die 0,15 g Calco/Canola-behandeling van proef 2 is gemiddeld 0,005 g swaarder as dié van dieselfde behandeling in proef 1. Dit is vreemd, aangesien daar normaalweg verwag sou kon word dat die papies van proef 2 ligter as dié van proef 1 sou weeg as gevolg van die heelwat beter produksie in eersgenoemde proef (gemiddeld 446 papies per fles teenoor 127).

Daar was 'n gemiddelde verskil van nagenoeg 60 eiers tussen die kontrole en die Calco-behandeling (kontrole: 472 eiers per wyfie en Calco: 411 eiers), wat 'n aanduiding kan wees dat die Calco 'n geringe invloed op die potensiele vrugbaarheid van VKM kan hê (Fig. 3.2.2.17). Die werklike vrugbaarheid in die twee behandelings was dieselfde (kontrole: 23,1% en Calco: 22,0%).

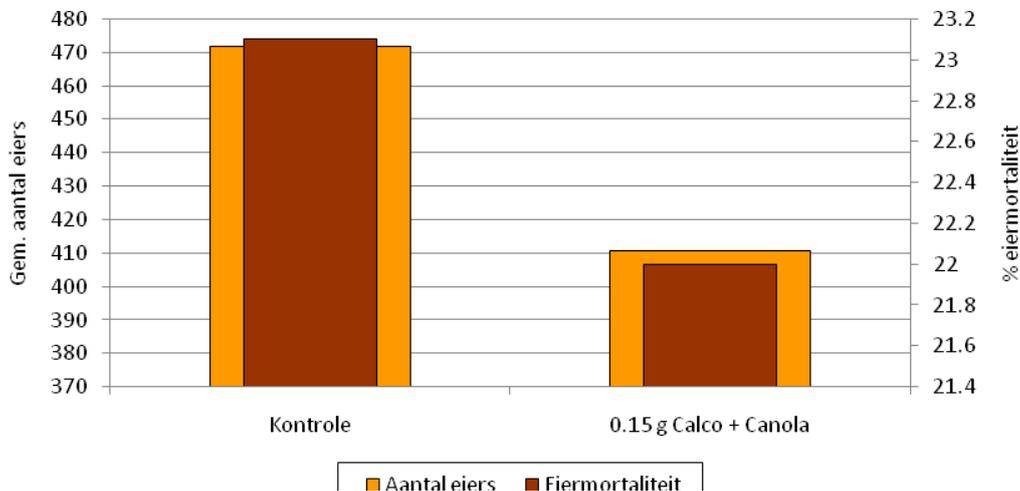


Fig. 3.2.2.17. Potensiele en werklike vrugbaarheid van VKM wat op 'n 0,15 g Calco-dieet geproduseer was.

Gevolgtrekking en toekomstige doelwit

Daar is nog te min proewe uitgevoer om 'n finale gevolgtrekking oor die ware uitwerking van Calco te maak. Alhoewel die Xsit-laboratorium alreeds geruime tyd Calco by hul larwedieet voeg en hulle met beide die produksie en insekgehalte tevrede is, is dit moontlik dat daarop verbeter kan word. Verdere proewe word in die vooruitsig gestel.

3.2.2.6 Hitteversperrings vir larwes

In die CRI-Jaarverslag vir 2007-2008 (Afd. 3.2.3.4) is verwys na 'n hitteversperring wat ontwikkel is om te voorkom dat volwasse larwes, nadat hulle uit die teelfesse geklim het, rondloop voordat hul pupeer. Dié versperring is met sukses in al 20 larwekamers van die Xsit-insektarium geïnstalleer en werk goed.

3.2.2.7 Die versameling van motte na ontpopping

In die CRI-Jaarverslag is verslag gelewer van motversamelingskabinette wat vir die Xsit-insektarium ontwerp is (Afd. 3.2.3.6). Dié kabinette (Fig. 3.2.2.18) was veronderstel om in kombinasie met sikloontoerusting (Fig. 3.2.2.19) gebruik te word om motte só te versamel dat hulle ná ontpopping sonder menslike ingryping versamel kon word.



Fig. 3.2.2.18. Motkabinette waarin die motte ontpop. Hulle val deur die tregtervormige deel onderaan die kabinette en word in die blou PVC-pype met 'n lugstroom teen 14 m/min na 'n aangrensende koelkamer vervoer.



Fig. 3.2.2.19. Sikloontoerusting in die koelkamer wat vir motversameling gebruik word. Die motte word vanuit die motkabinette bo-in die tregtervormige deel ingeblaas. Die windspoed word verminder as gevolg van die ontwerp van die sikloon en die motte val onbeskadig in die suil na onder waar hulle in 'n bak versamel word.

Alhoewel die toerusting redelik gewerk het toe dit nog skoon was, het beide die pype en siklone binne 24 uur binne-in begin aanpak met 'n neerslag van vetterige skubbe, wat gou tot 'n klipharde versperring, veral in die buie van die pype, maar ook aan die binnekant van die siklone, opgebou het. Dié neerslag het die windspoed beïnvloed en die motte is op groot skaal beseer – wat die vorming van die neerslag opsigself aangehelp het. Kondensasie het ook op die warm/kouelug interfasies tussen die mot- en koelkamer plaasgevind wat die probleem vererger het. 'n Soortgelyke stelsel word met sukses vir die versameling van Pienkbolwurm in die SIT-insektarium te Phoenix, Arizona, gebruik. Siklone, alhoewel met 'n totaal ander ontwerp, word ook in die Osoyoos-insektarium gebruik. Ooglopende tegniese tekortkominge aan die Xsit-stelsel kon nie na raadpleging met genoemde insektaria geïdentifiseer word nie. Die enigste ander oorsaak kan die morfologie van VKM wees. Ervaring het geleer dat die motte oorfloedige, los skubbe op hul liggame het wat hul baie maklik ontslae van raak. Dié skubbe veroorsaak groot besoedelingsprobleme in die insektarium wat met 'n verskeidenheid van maatreëls bestuur word en was waarskynlik die grootste oorsaak van die sikloonprobleem.

Motproduksie en -kwaliteit is dermate in die pype en siklone aangetas dat die hele stelsel verwyder moes word. Dit is op 'n tydelike basis met 2 l plastiekbottels vervang wat onderaan die tregtervormige deel van die motkabinette vasgeklamp is (Fig. 3.2.2.20). Die bottels word met leës omgeruil wanneer die motte tot op 'n diepte van nagenoeg 40 mm diep versamel het, waarna die bottels met motte na die koelkamer geneem word. Dié proses is uiters tydrowend en onproduktief en moes met iets beters vervang word.



Fig. 3.2.2.20. Motkabinette met plastiekbottels wat tydelik vir die versameling van motte gebruik word.

Die motloslaattoerusting wat vir installasie op vierwielmotorfiets ("Quads") ontwikkel is, is só ontwerp dat die motte met 'n waaier/pypstelsel nagenoeg 2-3 m ver van die rytuig af versprei word. Dié eienskap is as beginsel gebruik vir die ontwerp van 'n alternatiewe motversamelstelsel. Vereistes vir die nuwe apparaat is aan die ingenieursmaatskappy, Veritech Manufacturing te Somerset-Wes, voorgelê wat 'n prototipe gebou het. Dit bestaan slegs uit reguit pype – daar is geen buie waarin abnormale neerslae kan opbou nie. Daar word ook nie van 'n sikloon gebruik gemaak nie – dié is vervang met 'n eenvoudige, maar goed-beplande, lugbeheerhouer (plenum) wat verseker dat die lugsnelheid in die vervoerpyp so verminder word dat die motte sonder besering in 'n versameltrog in die koelkamer beland. Die stelsel skakel ook alle temperatuurverskille tussen verskillende dele van die stelsel uit wat kondensasie kan veroorsaak.

Die prototipe is verskeie kere in Veritech se werkswinkel getoets voordat dit in die Xsit-insektarium op een van die 5 rye motkabinette geïnstalleer is (Fig'e. 3.2.2.21, 3.2.2.22 en 3.2.2.23). Die prototipe is vervolgens aan 'n drie-weke lange semi-kommersiële toets onderwerp, waartydens nagenoeg 5,8 miljoen motte deur die stelsel

sonder enige instandhouding hanteer is. Die versamelde motte was deurgaans in 'n uitstekende toestand en geen beseerde individue is opgemerk nie. Die vervoerpype binne-in het aan die einde van die toetstydperk slegs geringe tekens van skubneerslae begin getoon. Die enigste byvoeging tot die stelsel is om deksels op die motversamelkis en motbakke in die koelkamer te sit om te verhoed dat motte van die motkabinetkamer (wat teen 26°C gehou word) uit die versameltrog en motbakke in die koelkamer vlieg of klim voordat hulle deur die koue (5°C) geïmmobiliseer word. Daar word beoog om die stelsel vroeg in die nuwe verslagjaar op alle motkabinette te installeer.



Fig. 3.2.2.21. Pyp onderaan die motkabinette waarmee die motte in 'n lugstroom teen 14 m/min na die koelkamer vervoer word.



Fig. 3.2.2.22. Prototipe lugbeheerhouer waarin die lugstroom se snelheid só verlaag word dat die motte onbeseer in 'n aangrensende koelkamer beland. Die lugbeheerhouer is vasgeheg aan die muur tussen die motkabinetkamer en die koelkamer.



Fig. 3.2.2.23. Prototipe versameltrog vir motte in die koelkamer. Die motte val deur 'n spleet onderaan die trog in verwyderbare bakke. Die punt van die trog grens aan die muur tussen die koelkamer en die motkabinetkamer. Motte word deur die blou pyp in die trog ingeblaas.

Gevolgtrekking

Die nuwe motversameltoerusting is 'n unieke ontwerp en is 'n goeie plaasvervanger vir die sikloontipe apparaat wat algemeen in massateelfasiliteite gebruik word.

Toekomstige doelwit

Op hierdie tydstip word verdere ondersoekwerk as onwaarskynlik beskou.

3.2.2.8 Motloslaattoerusting vir vierwielmotorfietse

Motte in 'n SIT-program kan breedweg op drie maniere in boorde losgelaat word, nl. (i) met die hand, (ii) met 'n girokopter of ligte vliegtuig of (iii) met gemeganiseerde apparaat op 'n vierwielmotorfiets ("quad"). Handloslatings, hetsy dit per voet of met twee arbeiders op 'n quad-motorfiets is, is onproduktief. Beide 'n girokopter en ligte vliegtuig het een groot nadeel, afgesien van hoë koste, nl. dat dit dikwels lewensgevaarlik is om laag te vlieg in bergagtige gebiede weens onverwagte lugstrominge en kan dus nie in alle sitrusboorde gebruik word nie. In die CRI-Jaarverslag vir 2006-2007 is melding van prototipe loslaattoerusting gemaak wat vir die loslaat van VKM in 'n SIL-program ontwikkel word. 'n Prototipe is vervaardig (Fig. 3.2.2.24) en boordtoetse het gewys dat die ontwerp tot 'n kommersiële model ontwikkel kan word. Die apparaat het egter twee nadele gehad, nl. (i) dat twee elektriese motors gebruik moes word wat deur 'n bykomende petrol-aangedrewe 240V-kragontwikkelaar aangedryf moes word en (ii) dat motloslatings nie quad-spoed afhanklik gemaak kon word nie. Daar is derhalwe besluit om 'n tweede prototipe te ontwikkel wat gesamentlik deur die quad-motorfiets se elektriese stelsel én aandrywing vanaf die agteras, aangedryf sou kon word.



Fig. 3.2.2.24. Eerste prototipe quad-gemonteerde apparaat vir steriele VKM-loslatings (240V-kragopwekker nie gewys nie).

Drie ingenieursmaatskappye is betrek wat verantwoordelik was vir (i) die hidroliese aandryfstelsel, (ii) die motloslaatstelsel en (iii) die elektroniese integrasie van beide voorgenoemde stelsels. Die voltooide prototipe het soos volg daar uitgesien (Fig. 3.2.2.25):



Fig. 3.2.2.25. Tweede prototipe quad-gemonteerde apparaat vir steriele VKM-loslatings.

Een van die twee skyfremme op die quad se agteras is verwyder en met 'n aandrywingstelsel vervang wat 'n hidroliese pomp aandryf. Laasgenoemde dryf 'n hidroliese motor aan wat aan 'n skroefvoerder gekoppel is (Fig. 3.2.2.26). Die skroefvoerder is onderaan 'n plastiekhouer gemonteer waarin etlike duisende motte geplaas kan word en is só gekalibreer dat dit 5 motte per lopende meter (1 000 motte per 200 m = 1 ha) kan loslaat. 'n Sentrifugale waaier is voorts aan 'n pypstelsel gekoppel sodat dit die motte in die bome kan inblaas. Die elektroniese beheerstelsel:

- (i) aktiveer die hele hidroliese stelsel,
- (ii) waarsku met behulp van alarms en 'n outomatiese enjinafsluitemeganisme wanneer 'n vasgestelde snelheid deur die quad oorskry word en voorkom sodoende dat die hidroliese pomp en –motor beskadig word; en
- (iii) waarsku audio-visueel deur middel van 'n optiese sensorstelsel dat daar óf 'n verstoping in die mothouer is óf wanneer alle motte losgelaat is (mothouer leeg).

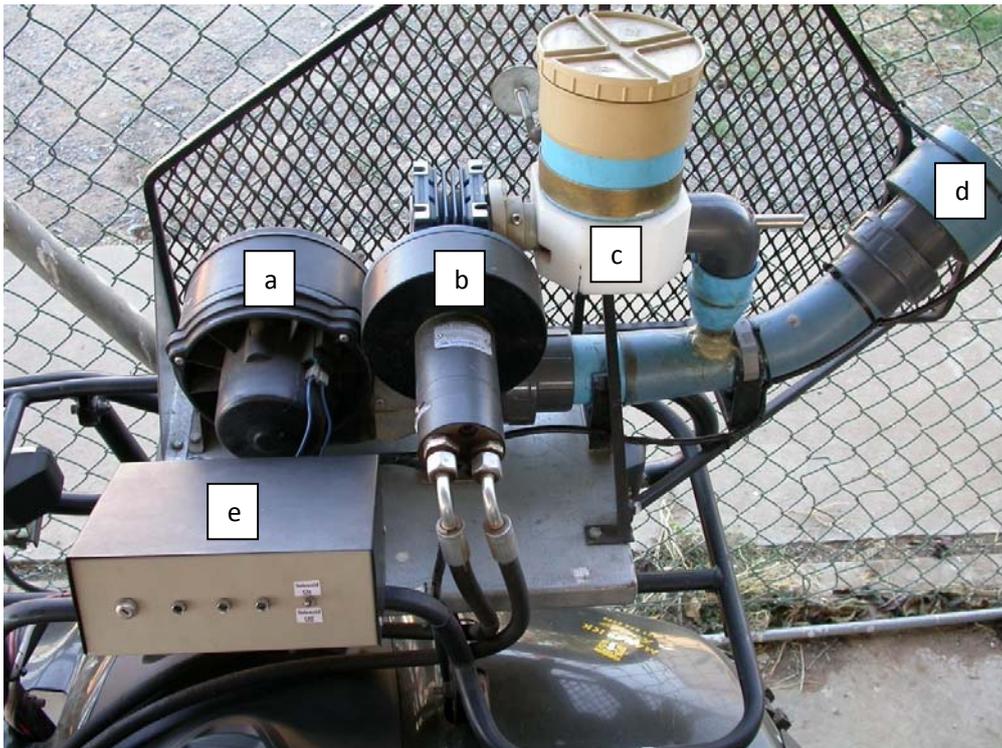


Fig. 3.2.2.26. Motloslaattoerusting op voorste drarak van quad gemonteer – gesien vanaf quad-drywer se kant (a = sentrifugale waaier; b = hidroliese motor; c = skroefvoerder met mothouer; d = loslaatpyp met optiese sensors; e = elektroniese beheer.

Die grootste voordeel van dié prototipe is dat die loslaat van die vasgestelde aantal motte deur die snelheid van die quad beheer word - hoe vinniger die bediener ry (binne toegelate perke), hoe vinniger word motte losgelaat en andersom. Met die Osoyoos-stelsel moet die loslaattoerusting voortdurend gekalibreer word om voorsiening te maak vir byvoorbeeld (i) boorde van verskillende groottes, (ii) stadige draaie aan die einde van boomrye en (iii) swak terrein wat wisselende snelhede noodsaak. Die snelheidsbeheerstelsel op die nuwe loslaattoerusting skakel herkalibrasie heeltemal uit.

Die prototipe is in verskeie boordproewe deur Xsit (Rob Stotter) gebruik om, in besonder, die akkuraatheid van die motkalibrasie en, in die algemeen, die gebruikersvriendelikheid van die apparaat, te toets. Daar is aanvanklik gevind dat verskillende getalle motte in opeenvolgende toetse met die apparaat losgelaat is – ten spyte daarvan dat die getalle motte wat in 'n besondere toets in opeenvolgende hektare losgelaat was, nagenoeg dieselfde was. Daar is vasgestel dat motte afkomstig van verskillende produksiegroepe in die insektarium, dikwels in grootte van mekaar verskil het. In die insektarium word die motte óf volumetries (bv. 'n gelykvol skep met 'n standaard maatlepel), óf per massa (met 'n weegskaal geweeg), in loslaateenhede van nagenoeg 1 000 motte elk afgemeet. Daar moet uiteraard gesorg word dat die motte wat aanvanklik vir kalibrasie-doeleindes gebruik word, gemiddeld van die grootste is wat normaalweg beskikbaar sal wees. 'n Standaard loslaateenheid sal derhalwe meer individue bevat wanneer die motte van daardie besondere produksiegroep gemiddeld kleiner as die standaard is – in van die toetse is loslaateenhede met 1 200-1 600 motte, wat kleiner as die standaard grootte was, waargeneem. By nadere ondersoek is dié probleem egter selfregulerend bevind, aangesien meer motte losgelaat word wanneer die loslaateenheid uit kleiner motte bestaan. Die toetse het egter bewys dat die apparaat in staat is om motte van standaard grootte herhaaldelik teen nagenoeg 1 000 individue per hektare los te laat.

Gevolgtrekking en toekomstige doelwit

Afgesien van die kalibrasie-kwessie, is sekere meganiese probleme ondervind, wat maklik reggestel is. Die enigste uitstaande probleem wat verdere ondersoekwerk regverdig, is die kwessie van vibrasie en skokke. Die apparaat is tans direk op die voorste drarak van die quad vasgebout, wat veroorsaak dat elke stamp en stoot wat die quad in ongelyke boorde ondergaan, na die apparaat oorgedra word. Motte wat in 'n afsonderlike koelkis

op die quad vervoer word, is aan dieselfde skokke onderworpe. Daar sal derhalwe 'n gepaste skokbrekerstelsel ontwerp moet word om abnormale slytasie en breekskade van die toerusting uit te skakel en agteruitgang van motkwaliteit te voorkom.

3.2.2.9 Uitbreiding van VKM-SIT na Limpopo-provinsie

Inleiding

Na afhandeling van die eerste seisoen van suksesvolle kommersiële SIL in Citrusdal het die sitrusbedryf besluit dat die tegniek in ander gebiede getoets moes word. 'n Proef, soortgelyk aan die loodprojek wat in CRI-jaarverslag vir 2005-2006 (Afd. 5) beskryf was, is derhalwe te Letaba-landgoed, Limpopo-provinsie, van stapel gestuur.

Metodes en resultate

• **Proefperseel**

- **Uitleg:** 'n Onreëlmatig-reghoekige blok van 38 ha sitrus, bestaande uit 13 ha Star Ruby-pomelo's en 25 ha Turkey-lemoene, is gebruik. Die boomryspasie was 6,5 m (noord-suid) en die boomspasiëring 3,5 m (oos-wes). Die proefperseel was deur onbewerkte grond (noorde- en noordoostekant) en 'n groot dam (noordwestekant), begrens. 'n Strook natuurlike bos het die proefperseel aan die suidoostekant van 'n klein, verwaarloosde nawelboord en boorde met Valencia-, Turkey- en middelseisoenlemoene geskei (Fig. 3.2.2.29).
- **Spuitprogram:** Slegs Agrimec/olie en Cryptogran is gedurende die proefverloop in die SIT-perseel toegedien. Agrimec is twee keer toegedien, onderskeidelik gedurende die middel van November en Desember. Cryptogran is eenkeer in elk van die Star Ruby- (8 April) en Turkey-boorde (19 April) toegedien.

- **Oorsprong, verpakking en versending van gammabestraalde motte:** Genoeg motte vir die loodprojek is kosteloos deur Xsit (Edms) Bpk te Citrusdal verskaf. Dié motte is in die Xsit-insektarium geteel en was inwendig met Calco Oil Red-kleurstof gekleur om hulle van wilde motte in die boorde te onderskei. Die motte is vooraf geïnaktiveer deur hulle by 4-5°C in 'n koelkamer te verkoel. Dit is gedoen om (i) hantering te vergemaklik, (ii) te verhoed dat hulle mekaar in die verpakkingshouers doodtrap en (iii) om die opbou van metaboliese hitte en die gevolglike vorming van kondensasie in die verpakkingshouers te verhoed. Eenduisend motte van beide geslagte is volumetries in elk van 40 vooraf-verkoelde Petri-bakkies (120 mm x 15 mm) afgemoot. Die Petribakkies met motte is in 'n polistireenkoelkis, 300 mm x 300 mm x 230 mm, met 30 mm dik wande, verpak, wat vervolgens in 'n groter polistireenkoelkis, 560 mm x 560 mm x 340 mm (75 mm dik wande) geplaas is. Laasgenoemde is in 'n aluminiumkis toegesluit. Die koelkiste is deurgaans voor gebruik oop in 'n koelkamer by 4-5°C gehou. Tien groot Seagull[®] vriesblokke (175 mm x 120 mm x 40 mm) is in die buitenste koelkis gepak om die binneste koelkis koud te hou. Geen vriesblokke is in die binneste koelkis gepak nie, omdat daar voorheen vasgestel is dat direkte blootstelling van die motte aan dié uiterste koue (tot -10°C) hulle lewensverwachting en werkverrigting ernstig benadeel. Die besendings motte is elke middag om 17:00 deur 'n koeriermaatskappy versamel en nagenoeg 19 uur later teen 14:00 die volgende middag op Letaba afgelewer. Die motte is kort na ontvangs in die proefperseel losgelaat. 'n Hobo-datalogger is by die meeste besendings in die binneste koelkis geplaas om die motversendingstemperatuur gedurende die reis te monitor.

- **Loslaattegniek en monitering van doeltreffendheid:** Agt en dertig bome is aan die begin van November 2008 in die proefperseel uitgesoek en gemerk. Die bome was elkeen nagenoeg 50 m in 'n noord-suid rigting en 70 m (oos-wes) van mekaar af geleë. Eenduisend motte (gemengde geslagte) is twee keer per week elke Dinsdag en Vrydag op elk van dié bome, asook 4-5 aangrensende bome, losgelaat.

• **Monitering van doeltreffendheid**

- **Vliegtoetsing:** Twee Petribakkies is by elke motbesending ingesluit vir die bepaling van motkwaliteit. Die motte moes met skemeraand losgelaat word om die vliegfiksheid van opeenvolgende besendings motte te toets.
- **Lokvalle:** Een Deltalokval, toegerus met Lorelei-geslagsferomoon, is in elk van 10 databome in die SIT-perseel opgehang. Die lokvalle was min of meer 200 m van mekaar af in die proefperseel versprei en nagenoeg 60 m vanaf die naaste loslaatpunte. Geen kontrole-lokvalle is gebruik nie. Weeklikse

lokvaltelling is van 21 November 2008 tot proefafsluiting (8 Mei 2009) uitgevoer. Die klewerige lokvalbodems is met elke telling verwyder, met kleefplastiek ("Gladwrap[®]") toegemaak en na die laboratorium vervoer waar die tellings uitgevoer is. Die mannetjies in elke lokval is as "SIT-motte" of "wilde motte" geklassifiseer. 'n Mannetjie is as 'n SIT-mot beskou indien hy die meeste van sy dorsale skubbe verloor het ('n teken dat die mot gehanteer en verpak was) of op sy rug in die lokval se kleefmiddel gelê het (die skubbedekking op die rûe van wilde motte voorkom dat hulle onderstebo in die kleefmiddel vasgevang word). Die aanwesigheid van Calco-kleurstof in die ingewande van die SIT-motte is nie as 'n onderskeidende kenmerk gebruik nie.

- **Vrugbesmetting:** Alle afvalvrugte onder die 10 lokvalbevattende databome is daaglik vanaf 16 Februarie 2009 bymekaar gemaak, oopgesny en vir simptome van VKM-besmetting ondersoek. Die aantal databome vir vrugvalmonitering is vanaf 24 Maart tot oestyd tot 5 per datapunt vermeerder (twee bykomende bome weerskante van elke lokvalboom). Soortgelyke datapunte in elk van 'n Star Ruby- en Turkeyboord, onderskeidelik 1 500 m en 2 500 m van die proefperseel af (4 000 m van mekaar af), is vir kontrole-doeleindes gebruik.

Resultate en bespreking

- **Sputprogram:** Nie een van die twee Agrimec-besputtings het 'n ooglopende invloed op lokvalvangste gehad nie. Die Star Ruby-boord is vier weke na die Cryptogran-toediening ge-oes en die proef is afgesluit. Enige invloed van die SIL op vrugval sou derhalwe nie deur die Cryptogran verdoesel gewees het nie, aangesien laasgenoemde se uitwerking eers ná vier weke sigbaar sou geraak het.
- **Versending van motte:** Versending van die motte het redelik volgens beplanning verloop. By twee geleenthede (middel-Desember en middel-April) is besendings egter etlike dae te laat deur die koerier afgelewer en die motte was gevolglik almal vrek by aankoms te Letaba. Dié probleem het geen ooglopende invloed op lokvalvangste daarna gehad nie.

VKM raak algaande onaktiewer namate die omgewingstemperatuur laer as ongeveer 16°C daal. Daar is derhalwe gepoog om die inhoud van die binneste koelkiste gedurende die reis na Letaba laer as dié temperatuur te hou. Dié aspek was bevredigend en die temperatuur was gewoonlik laer as 14°C wanneer die koelkiste by Letaba afgelewer is (Fig. 3.2.2.27).

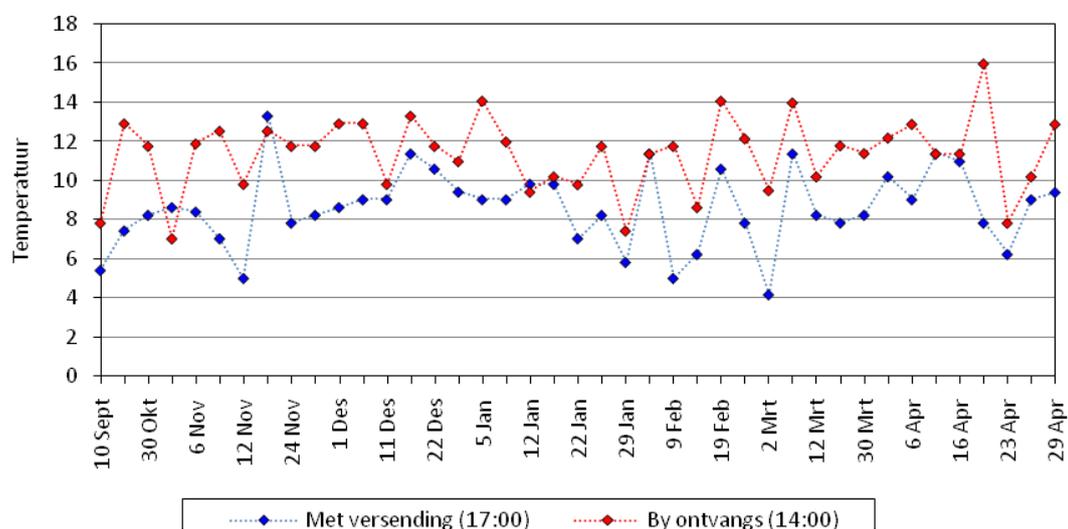


Fig. 3.2.2.27. Versendingstemperatuur in binneste, motbevattende koelkiste met vertrek uit Citrusdal (17:00) en 19 uur later met aankoms by Letaba Landgoed (14:00).

- **Vliegfiksheid:** Vliegtoetse is slegs by 6 geleenthede uitgevoer – 5 hiervan was in sterk sonlig kort nadat die motte by Letaba afgelewer was. Dié motte het nie aktief weggevlieg nie, maar na loslating op die grond geval – 'n voorspelbare verskynsel, aangesien VKM nie daardie tyd van die dag aktief sal vlieg nie, maar geneig sal

wees om op die warm grond te val en meesal net daar in die son dood te brand. Slegs een vliegtoets is laat in die seisoen volgens die voorgestelde protokol met skemeraand, wanneer vliegfikse motte aktief sal wegvlieg, uitgevoer. Dié motte was opsigtelik aktiewer as die voriges en die meeste het onmiddellik na loslating weggevlieg.

- **Motloslating:** Die SIT-motte is met die hand losgelaat, soos meesal in Citrusdal gedoen word. Die motverspreidingstegniek het egter heeltemal van die voorgestelde tegniek afgewyk. Daar word gewoonlik gepoog om SIT-motte so egalig oor die terrein te versprei as wat prakties moontlik is. In die eerste loodsprojek te Citrusdal in 2005-2006 is die motte op elke derde boom in elk van 75 vasgestelde werksrye, 42 m van mekaar af (500 motte per ry), losgelaat. In kommersiële SIT-boorde in Citrusdal word die motte op elke vyfde boom in elke agtste ry (ca. 48 m van mekaar af) losgelaat. Op Letaba is 1 000 motte (die volle inhoud van 'n Petri-bakkie) op elk van 38 eweredig-gespasieerde bome in die proefperseel losgelaat. Ondanks dié afwyking is goeie weeklikse vangste aangeteken (per geleentheid tot amper 200 mannetjies per lokval), wat daarop dui dat die mannetjies in staat was om relatief ver van die loslaatpunt af te versprei.

Die mannetjies is, soos in die geval van die Citrusdalse loodsprojek, gedurende die dag en gewoonlik in skerp sonlig, losgelaat. In beide proewe was motreaksie soos verwag – relatief min motte het weggevlieg. Die meeste motte het óf in die loslaatboom op blare tot rus gekom, óf in die skaduwee onder die boom op die grond geval en daar weggekruip.

- **Lokvalvangste:** Nagenoeg 70 SIT-mannetjies is gemiddeld per lokval na die eerste loslating te Letaba gevang (Fig. 3.2.2.28). Vir die daaropvolgende 3 weke het vangste tot slegs gemiddeld 23-30 mannetjies per lokval afgeneem. Daarna het die vangste toegeneem en 7 weke lank is meer as 40 mannetjies per lokval gemiddeld per week gevang. Die vangste het daarna in die week beginnende 31 Januarie, met een uitsondering, skielik tot minder as 40 mannetjies per lokval per week afgeneem. Slegs een vliegtoets is uitgevoer (raadpleeg “Vliegfiksheid” hierbo) en dit is derhalwe onmoontlik om vas te stel of dié skerp afname toegeskryf moet word aan motte wat van swakker kwaliteit as vroeër gedurende die proef was.

Heelwat minder wilde as losgelate mannetjies is gedurende die hele verloop van die proef gevang. Met slegs twee uitsonderings, naamlik in een lokval elk op 6 Maart (10 mannetjies) en 20 Maart (13 mannetjies), is deurentyd minder as 10 mannetjies per lokval gevang. Daar was min ooreenstemming in die vangspatrone van losgelate en wilde mannetjies tot die einde van Desember; daarna het die patrone redelik goed tot aan die einde van die proef ooreengestem.

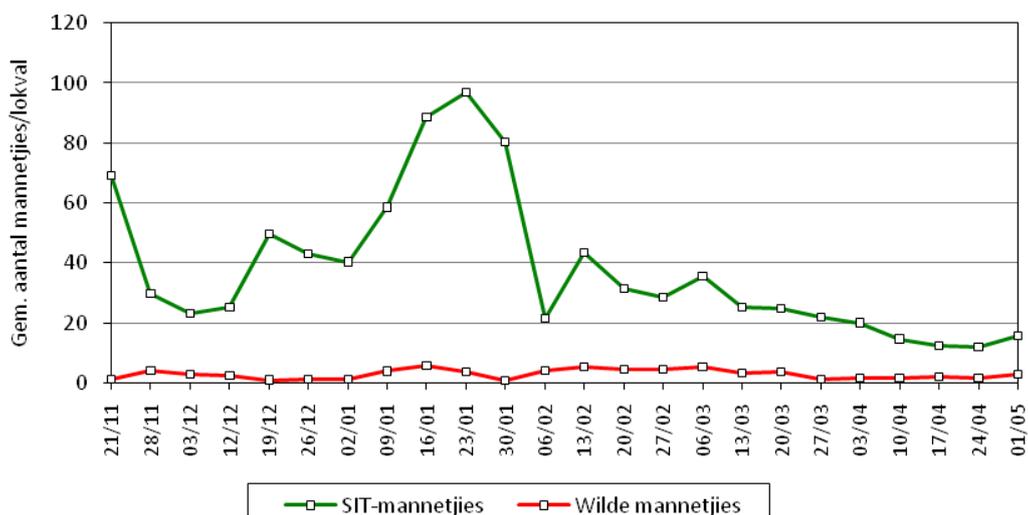


Fig. 3.2.2.28. Gemiddelde lokvalvangste van losgelate en wilde mannetjies in die SIT-proef te Letaba Landgoed.

Lokvalle in die SIT-perseel was soos volg versprei (Fig. 3.2.2.29):

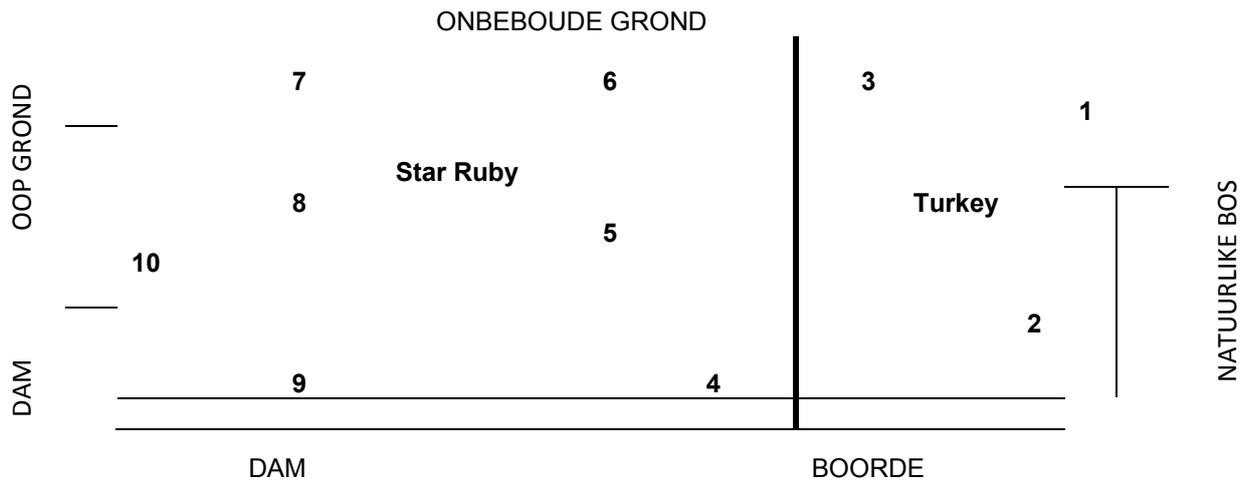


Fig. 3.2.2.29. Skematiese diagram van die SIT-perseel te Letaba Landgoed om die lokvalposisies (en meegaande databome vir vrugvalopnames) aan te dui.

- Lokvalnr. 1: Omring deur sitrusbome aan drie kante en natuurlike bos aan die vierde kant.
- Lokvalnr. 2: Dieselfde as lokvalnr. 1.
- Lokvalnr. 3: Omring deur sitrusbome aan drie kante en kaal, onbewerkte grond aan die vierde kant.
- Lokvalnr. 4: Omring deur sitrusbome aan drie kante en 'n dam aan die vierde kant.
- Lokvalnr. 5: Omring deur sitrusbome aan vier kante.
- Lokvalnr. 6: Omring deur sitrusbome aan drie kante en kaal, onbewerkte grond aan die vierde kant.
- Lokvalnr. 7: Omring deur sitrusbome aan drie kante en kaal, onbewerkte grond aan die vierde kant.
- Lokvalnr. 8: Omring deur sitrusbome aan vier kante.
- Lokvalnr. 9: Omring deur sitrusbome aan drie kante en 'n dam aan die vierde kant.
- Lokvalnr. 10: Omring deur sitrusbome aan drie kante en kaal, onbewerkte grond aan die vierde kant.

Te oordeel aan lokvalvangste was die wilde mannetjies relatief egalig deur die SIT-perseel versprei (Fig. 3.2.2.30). Die meeste mannetjies is in lokvalposisienr. 5 gevang wat in die middel van die SIT-perseel geleë was. Die minste mannetjies is by lokvalposisienr. 6 gevang, wat aan 'n stuk onbewerkte grond met min plantegroei gegrens het. Lokvalnrs. 3 en 7 wat aan dieselfde kant van die perseel was en ook aan die oop grond gegrens het, het in totaal meer mannetjies gevang, wat waarskynlik beteken dat die swakker vang by lokvalnr. 6 bloot toevallig was. Die natuurlike bos, wat naaste aan lokvalnrs. 1 en 2 was, het nie tot groter vangste bygedrae nie, ten spyte daarvan dat daar erkende VKM-gasheerplante, soos onder andere kasterolie (*Ricinus communis*) en *Acacia* spp., gegroei het.

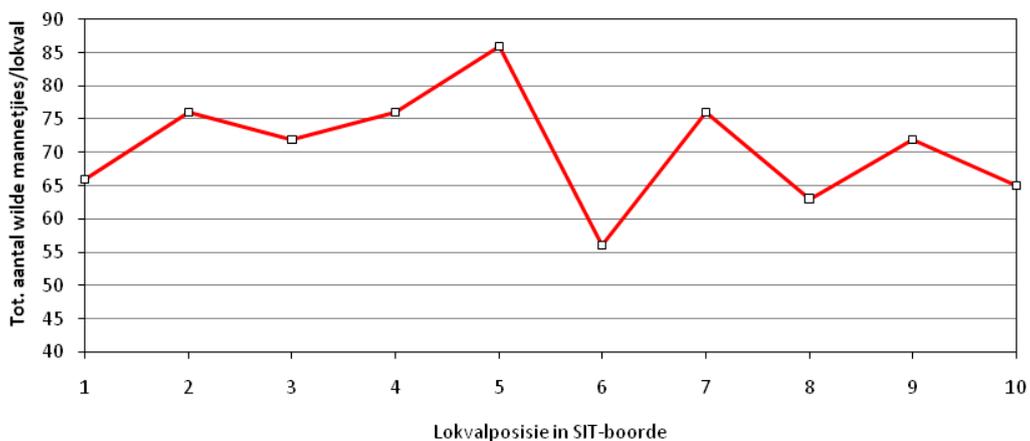


Fig. 3.2.2.30. Totale vangings van wilde mannetjies per lokvalposisie in die SIT-perseel te Letaba-landgoed.

Geen oortuigende verklaring kan vir die skielike afname in SIT-motvangste gegee word nie. Verskeie faktore kan egter oor die algemeen potensieel 'n oorsaak van kleiner vangste wees:

➤ **Omgewingstoestand:** VKM is aktief in boorde van sterk skemer tot nagenoeg 02:00 die volgende oggend. Enige gure omgewingsfaktor in dié tydperk sal motaktiwiteit derhalwe die meeste benadeel. Verskeie boordproewe het in die verlede gewys dat motaktiwiteit, gemeet in lokval- en paringstafelproewe, skerp afneem wanneer die nagtemperatuur laer as ongeveer 16°C daal. Daar is ook al waargeneem dat die motte onaktief gedurende reën is. Wind beïnvloed lokvalvangste, maar slegs tot dié mate dat meer mannetjies gedurende 'n windstorm, gemeet aan boomhoogte, in laaghangende lokvalle as in hoëhangende lokvalle gevang word. Daar is nog geen ander faktor opgespoor wat motaktiwiteit kan onderdruk nie. Daar word dus aanvaar dat slegs lae temperature oor 'n lang tyd (byvoorbeeld nie slegs gedurende een aand nie) motaktiwiteit kan demp. 'n Studie van Letaba se weerdata wys dat geen temperatuur, lugvog, reën of ander faktor aan die begin van Februarie só verander het dat dit lokvalvangste kon benadeel het nie. Die gemiddelde minimum nagtemperature het van 21 Maart af (Fig. 3.2.2.31) laer as 16°C gedaal, wat wel 'n voorspelbare afname in die lokvalvangste van beide losgelate en wilde mannetjies veroorsaak het.

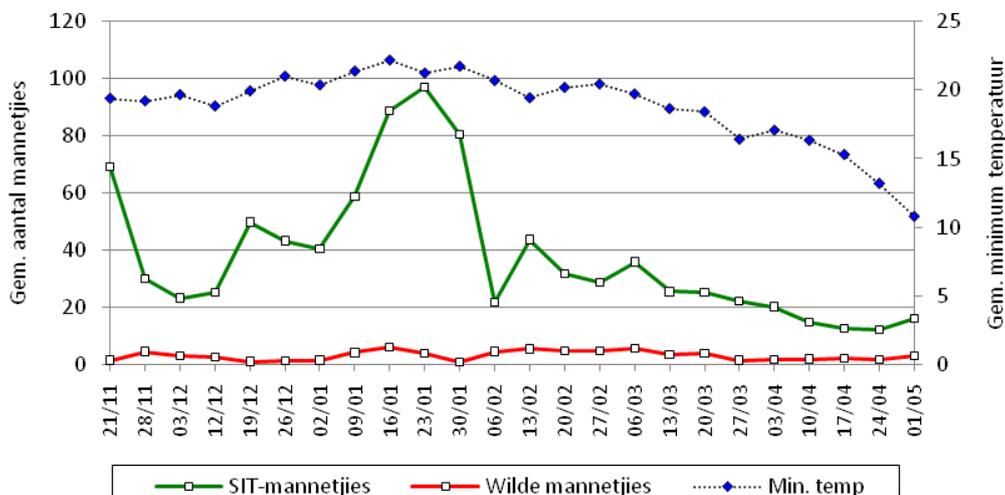


Fig. 3.2.2.31. Gemiddelde lokvalvangste van SIT- en wilde motte en gemiddelde minimum nagtemperature te Letaba Landgoed.

- **Motkwaliteit:** Motkwaliteit kan deur twee belangrike faktore bepaal word, naamlik:
- (i) *Aanvanklike kwaliteit van die motte ná produksie in die insektarium:* Die motte wat vir die proef gebruik was, was deel van die daaglikse produksie wat deur Xsit vir hul kommersiële SIT-loslatings gebruik was. Xsit se kwaliteitsevaluasies toon geen agteruitgang in motkwaliteit vir daardie tydperk nie - trouens, hul boorddoeltreffendheid het ietwat verbeter van die begin van Februarie af.
 - (ii) *Vervoer van die motte van Citrusdal na Letaba:* Daar is alreeds gemeld dat die koelkies temperature binne die verlangde bestek was. Dié aspek is egter nie 'n waarborg dat motkwaliteit nie deur die versending van die motte benadeel was nie. Alle besendings is op dieselfde wyse deur een koerier sonder afwyking van hul gewone prosedures hanteer. Die enigste alternatiewe wyse waarop motkwaliteit beoordeel sou kon word, is die vliegtoetse wat as deel van die evaluasieproses voorgestel, maar selde uitgevoer, was.
- **Lokvalplasing:** VKM-lokvalle is veronderstel om op uitgestrekte armhoogte bo die kop in bome opgehang te word. Lokvalle te Letaba is heelwat laer as die voorgeskrewe hoogte opgehang, wat normaalweg heelwat kleiner vangste tot gevolg sal hê. Soos die vrugte met verloop van die seisoen groter word, sou die takke al swaarder geword het en die lokvalle sou nog laer gesak het, wat beduidende kleiner vangste sou veroorsaak het. Alhoewel dié faktor moontlik nie binne die bestek van een week so 'n skielike afname in vangste sou veroorsaak het nie, kan dit egter nie as een van die oorsake vir die swakker vangste in die proef geïgnoreer word nie.

- Oorvloedingsverhouding:** Dit is nie altyd ooglopend nie, maar insekte word tot meerdere of mindere mate nadelig deur gammabestraling beïnvloed en kan as sodanig dikwels nie direk met hul wilde eweknieë in die natuur meeding nie. Daar word dus gepoog om dié agterstand uit te wis deur groot getalle bestraalde motte los te laat. Sodoende word die kans dat 'n SIT-mannetjie eerste 'n wilde wyfie sal opspoor, heelwat verbeter. Daar word gestrewe om 'n oorvloedingsverhouding (OV) van minstens 10 SIT-mannetjies tot 1 wilde mannetjie in die loslaatgebied te bewerkstellig. Na die kort tydperk van drie weke aan die begin van die proef, waarin relatief min mannetjies gevang was (OV van 7:1-11:1), het vangste vinnig toegeneem en die OV het sewe weke lank tussen 15:1 en 115:1 gewissel (Fig. 3.2.2.32). Aan die begin van Februarie het die vangste skielik afgeneem en die OV het vir die res van die proef weekliks op minder as 10:1 te staan gekom.

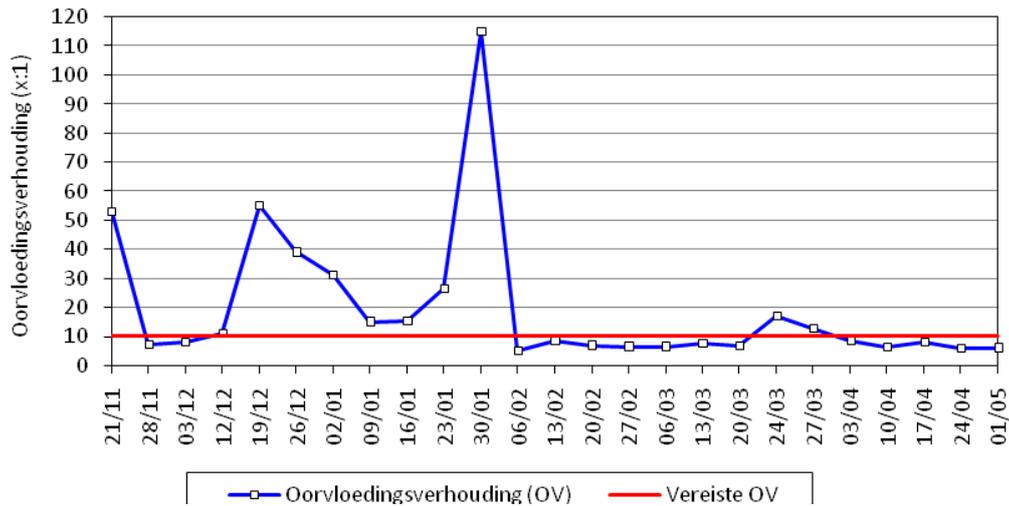


Fig. 3.2.2.32. Oorvloedingsverhouding van SIT- tot wilde mannetjies te Letaba Landgoed.

- Vrugbesmetting:** Evaluasie van vrugval het eers in die middel van Februarie begin. Vrugval in die SIT- en kontrolepersele is daaglik nagegaan, Saterdag en Sondag uitgesluit; data vir die week eindigende 3 April het verlore gegaan. Elke week se data van Maandag tot Vrydag is vir doeleindes van dié bespreking saamgevoeg. Met die tweede weeklikse kumulatiewe telling (27 Februarie) het die aantal besmette vrugte in die SIT-perseel die vrugvaldrempelwaarde (een besmette vrug per boom per week) oorskry (Fig. 3.2.2.33). Dié toename in besmette vrugte het vier weke na die skielike vermindering in oorvloedingsverhouding op 6 Februarie plaasgevind (Fig. 3.2.2.33). Die afname in SIT-motgetalle en toename in besmette vrugte kon 'n direkte verband met mekaar gehad het, aangesien ligter of hewiger vrugval gewoonlik 3-5 weke na, byvoorbeeld, onderskeidelik 'n doeltreffende insekdoderbespuiting of groter motaktiwiteit volg. Die vrugvalpatroon in die kontrolepersele was egter presies dieselfde, wat beteken dat daar geen gevolgtrekking oor die loslating van die steriele motte gemaak kan word nie. Geen vrugvaldata is vóór 20 Februarie versamel nie en dit sal onwys wees om te aanvaar dat vrugval vóór daardie datum dieselfde in beide die SIT- en kontrolepersele was.

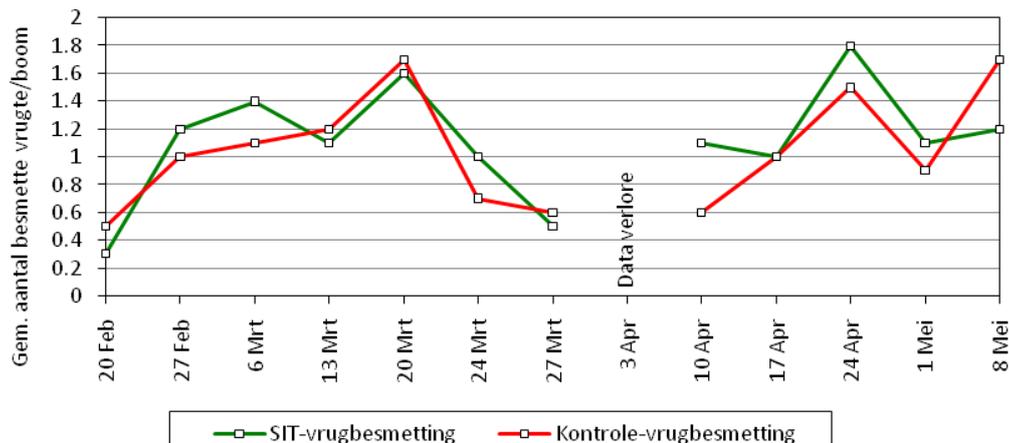


Fig. 3.2.2.33. Vrugbesmetting deur VKM in SIT- en kontroleboorde te Letaba Landgoed.

Die relatiewe hewige vrugbesmetting volg op klein lokvalvangste van wilde mannetjies; die drempelwaarde vir beduidende vrugbesmetting van 16 mannetjies/lokval/week is nooit gedurende die proef oorskry nie. Oorsake vir die vrugbesmetting kan een of meer van die volgende wees:

- (i) Die (klein) OV wat van die einde van Januarie bewerkstellig was, was moontlik nie groot genoeg om die paring van wilde motte met mekaar te verhoed nie.
- (ii) Wilde mannetjies in die lokvalle kon verkeerdelik as SIT-motte geïdentifiseer gewees het. Die maatstawwe wat vir die identifikasie van SIT-motte gebruik word, is nie foutloos nie. Dit is ook dikwels moeilik om met sekerheid tussen losgelate en wilde mannetjies op die lokvalbodems te onderskei – veral wanneer tellings nie in die boord uitgevoer nie, maar die klewerige bodems met kleefplastiek toegemaak en later in die laboratorium ondersoek word. Dit kon tot gevolg gehad het dat die relatiewe verhouding tussen losgelate en wilde motte in die lokvalle van die aangetekende getalle verskil het.
- (iii) Daar kon migrasie van bevrugte wyfies vanuit die omgewing aangrensend aan die SIT-perseel gewees het. Daar was skynbaar ietwat meer besette vrugte onder databome aan die boskant van die perseel opgetel – daar is egter geen aangetekende data om dit te bewys nie, aangesien alle afvalvrugte onder al die databome in die SIT-perseel elke dag in een monster saamgevoeg was en nie per datapunt ontleed was nie. Dieselfde geld ook vir die kontrole-perseel.
- (iv) Algemene boordsanitasie te Letaba Landgoed het eers in die middel van Februarie begin – teenstrydig met die praktyk in Citrusdal waar intensiewe en deeglike boordsanitasie alreeds in November begin. In laasgenoemde gebied het vrugvalopnames en boordondersoeke bewys dat laat en/of swak boordsanitasie sonder uitsondering groter vrugbesmetting tot gevolg het – selfs in 'n SIT-gebied.

Gevolgtrekking

Een aspek waaraan daar nog nie vantevore in VKM-navorsing aandag geskenk was nie, is die verenigbaarheid van verskillende VKM-bevolkings. Dit is nie onmoontlik nie dat bevolkings in verskillende gebiede onderling só van mekaar kan verskil dat die een gebied se motte nie met motte van dieselfde spesie in 'n ander gebied kan, of wil, meeding nie. Dit kan 'n aspek soos onaanloklikheid tussen wyfies en mannetjies van die twee bevolkings behels. Dit kan selfs wees dat, alhoewel sulke bevolkings wel geslagsverenigbaar is, daar afwykings in gedragpatrone is wat verenigbaarheid verhoed, byvoorbeeld 'n verskil in die tyd wat motte van die twee bevolkings snags geslagsaktief raak. Dit sal derhalwe nodig wees om minstens met behulp van lokvalproewe vas te stel of daar mededingendheidsverskille is tussen VKM van Citrusdal én die gebied waarin 'n volgende loodsprojek uitgevoer gaan word. Wanneer kommersiële VKM SIT na ander gebiede uitgebrei word, sal die probleem uiteraard opgelos word, aangesien 'n insektarium in elke gebied opgerig sal moet word. Die motkultuur van elke insektarium sal dan opgebou word met insekte wat in daardie besondere gebied versamel is.

Verdere doelwitte en werksplan

Die skynbare onvermoë van VKM SIT om vrugbesmetting in dié loodsprojek te keer, mag nie pogings keer om die benadering in ander gebiede as Citrusdal te ondersoek nie. Alhoewel vrugbesmetting nie na wense

onderdruk was nie, is heelwat inligting oor SIT-tegnologie ingewin wat in die toekoms van waarde sal wees. Soortgelyke navorsing moet uitgevoer word, maar met die klem op gehaltebestuur en projekuitvoering.

Dankbetuiging

Baie dankie aan Dr Martin Gilbert wat, afgesien van sy gewone verpligtinge by Letaba Landgoed, die uitvoer van die loodsprojek op sy skouers geneem het. Xsit (Edms) Bpk word bedank vir die nagenoeg 2,5 miljoen VKM wat gratis vir die projek verskaf is; groot waardering ook aan die Produksiebestuurder, Mnr Sarel Steyl, wat hulle eiehandig verpak het.

Tegnologie oordraging

Lesings is te Letsitele, Hoedspruit, Citrusdal en Stellenbosch aangebied.

3.2.3 FINAL REPORT: Understanding and improving biological control of false codling moth larvae Experiment 690 (April 2002 – March 2009): by Sean Moore, Wayne Kirkman (CRI) and Kierryn Keeton

Opsomming

Dit is bevestig dat *Agathis bishopi* die dominante parasietespesie van valskodlingmot (VKM) larwes in die Oos-Kaap is. Opnames van parasitisme van VKM larwes in Citrusdal het geen teenwoordigheid van *A. bishopi* opgelewer nie. Daarom is dit besluit om *A. bishopi* in die laboratorium vir klassieke biologiese beheer vrylatings op Citrusdal aan te teel. Tien pare VKM is in elk van 2 net-gedekte Lina nawel bome vrygelaat. Daarna is 60 en 53 parasiete in elk van die nette vrygelaat. Twee weke daarna, vir 'n tydperk van 6 weke, is larwes wat gevalde vrugte besmet het vir parasitisme ondersoek. Al is 155 lewendige larwes al te saam gekry, is geen parasitisme gekry nie. Geen verdere navorsing word op hierdie eksperiment beplan nie.

Summary

It was confirmed that *Agathis bishopi* is the dominant parasitoid species of false codling moth (FCM) larvae in the Eastern Cape. Surveys of FCM larval parasitism in Citrusdal revealed no presence of *A. bishopi*. It was therefore decided to laboratory rear *A. bishopi* for classical biocontrol release in Citrusdal. Ten pairs of FCM were released into each of 2 netted Lina navel orange trees. Subsequently, 60 and 53 parasitoids were released into each of the nets. Two weeks thereafter, for a period of 6 weeks, larvae infesting fruit which had dropped from the trees were inspected for parasitism. Despite a total of 155 live larvae being recovered, no parasitism was recorded. No further research is planned on this topic.

Introduction

From preliminary studies conducted in the Eastern Cape from December 2001 to May 2002 (Sishuba, 2003), two parasitoid species were found, namely *Agathis bishopi* (Nixon) and *Apophua leucotreta* (Wilkinson). From a study in the 2003/2004 season it was confirmed that *A. bishopi* was the dominant parasitoid in the Eastern Cape (Moore & Kirkman, 2004). Consequently, mass collections were made in order to initiate a laboratory culture, study the biology of *A. bishopi*, and conduct field releases in regions where *A. bishopi* was not found. Sishuba (2003) found no *A. bishopi* parasitizing FCM in surveys conducted in citrus orchards in Citrusdal, Western Cape Province. During the 2005, 2006 and 2007 seasons average levels of parasitism of FCM larvae in the Eastern Cape ranged from 10.0 - 14.5% over each full season (Moore & Kirkman, 2004 & 2005; Keeton, 2007). Parasitism during the 2007 season peaked at 39% in April. Rearing of the parasitoid proved difficult due to various factors. Despite this, adequate numbers were produced for a classical biological control trial in the Citrusdal area (Keeton *et al.*, 2007).

Materials and methods

Two 12 year old Lina navel orange trees on Boschklouf Farm in Citrusdal were covered individually with steel-frame supported nets. Each net had a zip-door to facilitate access to the tree. On 20 February 2008, 10 mating pairs of FCM adults were released into each net. On 25 and 26 February several male and female *A. bishopi* were released into each net (Table 3.2.3.1). Male and female parasitoids were only introduced to one another

the evening before being released into the nets. On 10 March, a further 10 pairs of parasitoids were released into each net.

Table 3.2.3.1. *A. bishopi* parasitoids released into netted navel orange trees on Boschloof Farm in Citrusdal.

Netted tree	Dates on which parasitoids emerged	Date on which parasitoids were released	Female parasitoids	Male parasitoids
1	11-19 Feb 2008	25-26 Feb 2008	7	4
	20-26 Feb 2008	25-26 Feb 2008	23	6
	1-2 March 2008	4 March 2008	10	10
Total			40	20
2	11-19 Feb 2008	25-26 Feb 2008	2	1
	20-26 Feb 2008	25-26 Feb 2008	24	6
	1-2 March 2008	4 March 2008	10	10
Total			36	17

Weekly from 25 March to 13 May 2008, fruit which had fallen from each tree was collected, packaged and couriered to CRI, PE for inspection for FCM infestation. FCM larvae were exhumed from the fruit and placed individually on artificial diet in glass vials, stoppered with cotton wool. Parasitism of these larvae was monitored.

Results and discussion

Over the full trial period of 7 weeks, 6 evaluations for parasitism were conducted in both of the nets. A total of 153 infested fruit were collected from tree 1 (Table 3.2.3.2) and a total of 99 infested fruit were collected from tree 2 (Table 3.2.3.3). Despite a combined total of 155 live larvae being recovered, no parasitism was recorded. This was surprising, as conditions seemed to be favourable. However, if parasitoids were unable to survive and establish in such a controlled environment, classical biological control releases into open orchards in this region (Citrusdal valley) would surely be superfluous.

Table 3.2.3.2. FCM larval infestation of dropped fruit from netted tree 1 on Boschloof Farm in Citrusdal, and parasitism of larvae.

Collection date	Infested fruit collected	Larvae recovered				
		Instar	Total	Dead	Live	Parasitised (with <i>A. bishopi</i>)
25-Mar	5	5	5	0	5	0
		Subtotal	5	0	5	0
01-Apr	3	3	1		1	
		5	2		2	
		Subtotal	3	0	3	0
08-Apr	1	0	0			
		Subtotal	0	0	0	0
16-Apr	2	2	1	1		
		Subtotal	1	1	0	0
23-Apr	6	2	8		8	
		3	1		1	
		4	1		1	
		Subtotal	10	0	10	0
13-May	136	2	17	1	6	
		3	30	10	20	
		4	45	2	43	

		Subtotal	92	13	69	0
Total	153		111	14	87	0

Table 3.2.3.3. FCM larval infestation of dropped fruit from netted tree 2 on Boschklouf Farm in Citrusdal, and parasitism of larvae.

Collection date	Infested fruit collected	Larvae recovered				
		Instar	Total	Dead	Live	Parasitised (with <i>A. bishopi</i>)
25-Mar	2	5	2		2	
		Subtotal	2	0	2	0
01-Apr	0		0			
		Subtotal	0	0	0	0
08-Apr	0		0			
		Subtotal	0	0	0	0
16-Apr	0		0			
		Subtotal	0	0	0	0
23-Apr	2	2	1		1	
		Subtotal	1	0	1	0
13-May	95	2	3	2	1	
		3	16	5	11	
		4	20	4	16	
		Subtotal	39	11	28	0
Total	99		44	11	33	0

Conclusion

Agathis bishopi showed no potential as a classical biological control agent for release in the Citrusdal area of the Western Cape. This was despite significant numbers of parasitoids being released in a controlled environment with fruit heavily infested with various FCM larval instars. It therefore appears that any expansion on this study in the Citrusdal region would be superfluous. Releases of *A. bishopi* in any other regions in South Africa would most likely not constitute classical biological control, as FCM occurs indigenously throughout the remainder of South Africa. It is therefore probable that *A. bishopi* also occurs naturally throughout most of South Africa.

Future research

No further research is planned on this topic. The only possible proposal for a future study is to develop a cage into which fruit removed during orchard sanitation can be placed in order to facilitate emergence of any possible larval parasitoids. The gauze of the cage will have to be of such porosity that parasitoids can pass through, but moths cannot.

Technology transfer

A paper entitled, "Rates of larval parasitism of false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), on citrus in South Africa" was presented at the International Conference of Entomology (ICE) 2008. Another paper on this work was presented at the 5th CRI Citrus Research Symposium.

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3.2.4 VORDERINGSVERSLAG: Ontwikkeling van 'n uitvoerprotokol vir die bestryding van valskodlingmotlarwes in verpakte vrugte met behulp van gammabestraling
 Proef 719 (2003-2010): J H en M Hofmeyr (CRI)

Summary

Two of several irradiation containers necessary for implementation of the study were manufactured. However, no real progress was made as gamma dose rate studies, which are a prerequisite to the main study, were delayed due to a heavy work load carried by the responsible nuclear scientist at iThemba LABS.

Opsomming

Twee van verskeie bestralingshouers wat vir die uitvoer van die studie nodig word, is vervaardig. Geen vordering met die proef is egter gemaak nie omdat dosistempostudies, wat 'n voorvereiste is, weens 'n druk werksprogram van die verantwoordelike kernwetenskaplike by iThemba LABS, vertraag is.

Inleiding

Sommige markte vereis dat sitrus 'n kouebehandeling van <-0.3°C vir 24 dae ondergaan om alle VKM-larwes in verpakte vrugte te dood. Dié behandeling is wisselend nadelig vir vrugkwaliteit en moet met 'n veiliger metode, wat ook fitosanitêr doeltreffend is, vervang word.

Weens 'n druk SIL-navorsingsprogram wat met die bou en inwerkingstel van die nuwe Xsit-insektarium te doen het, is geen navorsing gedurende 2007-2008 in Proef 719 uitgevoer nie. 'n Navorsingprotokol wat na aanleiding van vorige resultate opgestel is, is met die USDA bespreek. Verandering is aan die voorgestelde proeftegniek aangebring en gedurende die verslagjaar goedgekeur.

Navorsing deur Graham Barry dui daarop dat die kwaliteit van sitrusvrugte relatief maklik nadelig deur gammabestraling aangetas word. Dit veroorsaak dat 'n bestralingdosis wat laag genoeg is om fitosanitêre versekering teen VKM-larwes in sitrusvrugte te verskaf en terselfdertyd veilig vir vrugkwaliteit is, moeilik met beskikbare bestralingstegnieke ontwikkel sal kan word. Dit is egter noodsaaklik dat die navorsing voortgesit en afgehandel word, sodat die inligting beskikbaar sal wees wanneer bestralingstegnieke sodanig aangepas kan word dat dit prakties toegepas kan word.

Materiale en metodes

Baie voorwerpe wat bestraal moet word, kan nie net so direk behandel word nie. Voorwerpe soos motte, eiers en witluise bied as sodanig baie min weerstand teen gammastrale wanneer hulle direk bestraal word. Hulle sal derhalwe nie die verlangde dosis ontvang indien hulle nie in behoorlik-ontwerpte bestralingshouers behandel word nie.

iThemba LABS is verantwoordelik vir die vasstelling van dosistempo's vir die verskillende opstellings wat vir die bestralingsnavorsing (SIT-proewe, asook gamma-ontsmetting van VKM en witluis in verpakte vrugte) gebruik moet word. Daar is niemand anders beskikbaar wat dié tipe studie kan uitvoer nie en CRI is derhalwe van hulle afhanklik. Die organisasie is onderbeman en het selde tyd om die nodige werk wat CRI van hulle verlang, vinnig uit te voer. Daar is vordering, maar dit is baie stadig en wisselvallig.

’n Gespesialiseerde bestralingshouer vir die behandeling van VKM-eiers is deur iThemba se ingenieursdepartement vervaardig. ’n Dosistempostudie is vervolgens in Xsit se gammabestraller te Citrusdal deur iThemba uitgevoer. Die studie het egter misluk as gevolg van foutiewe chemiese dosimeters wat gebruik was en die studie moet herhaal word.

’n Tweede tipe bestralingshouer wat vir die bestraling van VKM-eiers en witluise op lemoene gebruik sal word, is deur JHH ontwerp en vervaardig.

Toekomstige doelwit

Twee verdere dosistempostudies, naamlik vir die bestraling van VKM-larwes in teelflesse en lemoene, moet afgehandel word voordat die gamma-ontsmettingstudie kan begin. Afhangende van vordering met die dosistempostudies word daar gehoop dat die navorsing in 2009-2010 afgehandel sal kan word.

3.2.5 PROGRESS REPORT: Investigating and improving field persistence of Cryptogran

Experiment 791 (April 2005 – March 2010): Wayne Kirkman, Sean Moore (CRI), Stephan Honiball (Ceder Biocontrol) and Johanna Matthewson (Saamfarm)

Opsomming

Die doel van hierdie eksperiment was om probleme met die nawerking van Cryptogran te identifiseer, te kwantifiseer en hopelik deur beter formulاسie en bestuurspraktyke die produk te verbeter. Die tegniek van druppeltoediening biotoetse is ontwikkel om kleiner verskille in dosisreaksie en UV-biotoetse tussen behandelings op te spoor. Brilliant Blue kleurstof is geskik bevind vir gebruik in die biotoetse. ’n Geskikte dosisreeks is bepaal en die tegniek is verder ontwikkel. Boordproewe het gewys dat ’n bykomende vroeë Cryptogran-bespuiting tydens Oktober nie die onderdrukking van lae VKM-besmettings verbeter het nie. Kleiner dosisse Cryptogran meer dikwels toegedien, was doeltreffender as die geregistreerde program. Die omgekeerde het vir beide behandelings gegeld wanneer die VKM-besmetting hewig was. Cryptogran is met Cryptex in 4 boordproewe vergelyk, uitgevoer in die Oos- en WesKaap. Cryptogran was beduidend doeltreffender as Cryptex in twee boordproewe en effens doeltreffender in een. Cryptex het in een proef beter as Cryptogran gevaar maar as gevolg van baie lae VKM aktiwiteit is resultate nie statisties betekenisvol nie. Halvering van die melassedosis het die doeltreffendheid van Cryptogran effens benadeel. Beter resultate is verkry met die gebruik van ’n dikker melasse. DiPel het opsigself, of in kombinasie met Cryptogran, geen invloed op VKM-onderdrukking gehad nie. Die byvoeging van Dithane by Cryptogran het VKM-onderdrukking verbeter. Die byvoeging van Cryptex aan Cryptogran het VKM-onderdrukking nie beduidend beter as Cryptogran alleen verbeter nie. Cryptogran wat in ’n Isomate boord toegedien is het VKM beduidend verminder tot ’n vlak waar geen VKM in die laaste 6 weke van ’n 17 weke proef gekry is nie.

Summary

This experiment aims to identify, quantify and resolve persistence problems with Cryptogran and to improve the field persistence through formulation and management practices. The technique of droplet-feeding bioassaying was developed in order to show smaller differences between treatments for dose-response and UV bioassays. Bioassays showed that Brilliant Blue dye was suitable for use in droplet-feeding bioassays. A suitable dose range for droplet-feeding bioassays was determined, and the technique was developed. Field trials indicated that, where FCM pressure was low, an additional early application in October did not improve FCM control. However, reduced concentrations applied more frequently were more effective than the registered programme. Where FCM pressure was high, an additional October application did improve control. However, more frequent applications of Cryptogran at reduced concentrations were not adequately effective. Cryptogran was compared with Cryptex in 4 field trials, conducted in the Eastern and Western Cape. Cryptogran performed significantly better than Cryptex in two field trials and marginally better in one. Cryptex performed better than Cryptogran in one field trial. However, results were not significant, as FCM levels were extremely low. Field trials showed that reducing the molasses concentration by half, resulted in lower efficacy of Cryptogran. Better results were obtained using a thicker molasses. DiPel had no effect on FCM, and did not improve efficacy when added to Cryptogran. Dithane was as effective an adjuvant with Cryptogran as was molasses and Agral 90. A combination of Cryptex and Cryptogran did not significantly improve FCM control over that of Cryptogran alone. Cryptogran applied in an Isomate orchard significantly reduced FCM infestation to a non-detectable level during the last 6 weeks of a 17 week trial.

Introduction

Field trials have been conducted with Cryptogran since the year 2000. Cryptogran is also in its fifth year of commercial use. Results from both field trials and commercial use have shown varying degrees of field persistence. A principal disadvantage of the use of baculoviruses in the field is their short residual activity due to their inactivation by UV irradiation (Huber, 1990; Shapiro, 1995). This has also been demonstrated for CrleGV (Moore, 2002), the virus in Cryptogran. A prerequisite for the success of Cryptogran as a means of controlling false codling moth (FCM) is to understand all of the factors affecting field persistence of the virus (not only UV irradiation) and to find ways to improve it. Environmental persistence can be improved by ensuring rain fastness and UV protection (Most & Quinlan, 1986). A different method of bioassaying (droplet-feeding bioassay) was developed, to show more accurate differences between products and UV protectants. Many of the trials were aimed at improving practical management practices and usage of Cryptogran. Timing of application was investigated in several trials. Reduced rates of molasses, as well as substitutes for it and Agral 90 were investigated. An integrated approach is necessary to control FCM. With this in mind, two trials were conducted to test the efficacy of Cryptogran within Isomate (mating disruption) treated orchards.

Materials and methods

Comparison of Cryptogran and Cryptex in a detached fruit bioassay

A detached-fruit bioassay was conducted, where the efficacy of Cryptogran and Cryptex against FCM were compared. Ninety Valencia oranges were harvested from the Citrus Foundation Block on 15 September 2008. The two virus suspensions were prepared at their respective registered rates, and applied to 30 oranges each using a knapsack sprayer. Distilled water was applied to another 30 fruit in a similar way, to be used as a control (Table 3.2.5.1). The fruit were allowed to dry on a mesh rack and were then inoculated with 3 neonate FCM larvae each. The fruit were then left for two weeks, after which they were inspected for penetration marks and the presence of FCM larvae. Results of treatments were compared using an ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 1996).

Table 3.2.5.1. Treatments applied to Valencia oranges in a detached-fruit bioassay on 27 September 2008, to compare the efficacy of Cryptogran and Cryptex

Treatments (Doses per 100 L water)	
1	Distilled water
2	Cryptex (3.3 ml) + molasses (250 ml) + Agral 90 (18 ml)
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)

UV bioassay: Cryptogran vs Cryptex

Claims were made by the suppliers of Cryptex that it is formulated with a UV protectant. A bioassay was conducted to compare the sensitivity to UV of Cryptogran and Cryptex. Cryptogran contains no specific UV protectant in the formulated product. It is an unpurified product, which contains insect body parts and dietary ingredients. Suspensions of Cryptex and Cryptogran were prepared at concentrations of 1.34×10^5 OB's / ml. Fifteen ml of each suspension was inoculated into each of 12 Petri-dishes. These Petri-dishes were then exposed to a germicidal UV lamp (UV-C, 254 nm wavelength) for 4 periods ranging from 120 minutes to 480 minutes. After exposure the suspensions were inoculated onto artificial diet which included agar, in 25-cell Sterilin bioassay trays and bioassayed against neonate FCM larvae. One larva was placed into each cell. Two distilled water treated controls were used and one control of each of Cryptogran and Cryptex, which were not exposed to UV. The bioassay trays were kept at 27°C for 7 days and evaluated for larval survival. Two replicates of this trial were conducted.

Droplet-feeding bioassays

Droplet-feeding bioassays are considered a more accurate way of conducting bioassays than surface-dose bioassays (Jones, 1998). It is possible that smaller differences in pathogenicity or UV protection could be detected using this technique. Various attempts were made to develop this technique for the bioassaying of viruses against neonate FCM larvae.

Brilliant Blue (BB) is a dye which has been commonly used for conducting droplet-feeding bioassays (Jones, 1998). The dye is clearly visible in the gut of neonate larvae once it has been ingested. The dye is generally added to virus suspensions at a concentration of 1% (Jones, 1998). As this method has not previously been used for bioassaying viruses against FCM, it was first necessary to determine whether BB had any effect on FCM. A bioassay was conducted whereby one group of 25 neonate larvae were fed BB (1%), and another 25 larvae were fed distilled water as a control. These larvae were then placed onto artificial diet containing agar and evaluated for larval mortality after one week. The treatments were replicated three times.

In order to determine the range of concentrations to be used in droplet-feeding bioassays, it was necessary to measure the volume of virus suspension ingested per neonate larva. To do this, a few hundred neonate larvae were exposed to a 2 µl droplet of a 1% BB solution. Once the droplet was entirely consumed, the larvae which showed signs of BB ingestion were counted, and so an average volume ingested per larva was calculated. This process was repeated several times. In another study, Lyndall Pereira da Conceicao (Rhodes University) weighed several neonate FCM larvae, before and after BB ingestion, and so also calculated the average volume ingested per larva.

Once a technique and a dose range had been established (Table 3.2.5.2), four bioassays were conducted to evaluate the new technique. The first two were conducted using old, previously used 25-cell bioassay trays. Few of these remain, and at the time were not commercially available, and so new 24-cell trays, with round cells, were used in the following two bioassays. Several droplets of virus suspensions in a 1% BB solution were placed inside a honey-jar lid, which contained approximately 100 – 200 neonate FCM larvae. The larvae were attracted to the droplets and drank from them. Once a larva had ingested the suspension, a clear blue line could be observed in the gut. After 5 minutes, these larvae were then selected and put onto agar-inclusive artificial diet in the cells of bioassay trays. Larvae which had crawled through the droplets, and were covered in the dye, were ignored and not used for the bioassays. Where possible, LD₅₀ values were calculated where probit analyses were conducted.

Table 3.2.5.2. Concentrations used in two-fold series dilution, droplet-feeding dose-response bioassays.

Treatment	Concentration of virus (OBs / ml)
1	Distilled water
2	2.27 x 10 ⁵
3	4.54 x 10 ⁵
4	9.08 x 10 ⁵
5	1.82 x 10 ⁶
6	3.63 x 10 ⁶
7	7.26 x 10 ⁶

Other bioassay improvements

Several droplet-feeding bioassays were conducted using the new 24-cell bioassay trays. In virtually all of the cases, control mortality was high and the results were variable. A droplet-feeding dose-response bioassay, as described in the previous section, was conducted to compare larval mortality in old 25 cell trays and the new 24-cell trays.

It was observed that the diet appeared to remain moister in the cells of the new trays. A trial was conducted to determine if this was the reason for the higher larval mortality when using these trays. A comparative bioassay was conducted, where nine 24-cell bioassay trays were prepared with artificial diet. In three trays, bulldog clips were used to keep the tray lids tightly closed (as always done in the past). In another three trays, elastic bands were used, which would possibly close the lids less tightly, and allow some desiccation of the diet. The final three trays were left open for 90 minutes longer than the other two treatments, so that the diet could dry out more before larvae were inoculated onto the diet and the lids were secured with bulldog clips. The bioassay was evaluated after 7 days for larval survival, and the condition and size of the surviving larvae were noted.

Field trial 1: Dunbrody Farm

This trial was partially reported on in the 2007/8 CRI annual report (Kirkman *et al*, 2008). However, evaluations continued into May 2009, beyond the previous reporting period. The trial was conducted to test the efficacy of Cryptogran when applied at different times of the year, both as single and multiple applications (Table 3.2.5.3). This included an application corresponding with a minor peak in FCM activity in October, indicated by pheromone trap catches. More frequent Cryptogran applications (monthly) at half the registered concentration (5 ml /100 L) were included, as well as Cryptex, an FCM virus product, produced by Andermatt in Switzerland. Two similar, adjacent orchards of Lane Late navel oranges were selected on Dunbrody Farm in the Sundays River Valley. The orchards, in which trees were spaced at 6 m x 2 m (rows x trees), were planted in 1997. The orchards were divided into 10 blocks of approximately 150 trees each. Each treatment was applied to 2 randomly selected blocks. Approximately 8500 L of spray mix per hectare was applied for all applications. Twelve data trees were selected per treatment (6 in each orchard). Dropped fruit from each tree were collected weekly, and analysed separately. Evaluations were done by inspecting and dissecting fruit in order to determine the cause of drop. FCM infestation was determined by the presence of a larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 1996).

Table 3.2.5.3. Timing of Cryptogran and Cryptex applications in an orchard of Lane Late navel oranges on Dunbrody Farm.

Treatment		Concentration (ml / 100 L water)	Application date				
1	Untreated control		-	-	-	-	-
2	Cryptogran	10		04 Dec 2007	-	14 Feb 2008	-
3	Cryptogran	10	24 Oct 2007	04 Dec 2007	-	14 Feb 2008	-
4	Cryptex	3.3		04 Dec 2007	09 Jan 2008	14 Feb 2008	-
5	Cryptogran	5	24 Oct 2007	04 Dec 2007	09 Jan 2008	14 Feb 2008	13 Mar 2008

Field trial 2: Far Away Farm

A similar trial to the previous one was conducted in the 2008/9 season. This included an application corresponding with the minor peak in FCM activity in October, indicated by pheromone trap catches. More frequent Cryptogran applications (monthly) at half the registered concentration (5 ml / 100 L) showed promise in the trial at Dunbrody Farm the previous season. This treatment was therefore again included – however, this time with and without molasses (Table 3.2.5.4). Two similar, adjacent orchards of Palmer navel oranges were selected on Far Away Farm in the Sundays River Valley. The orchards, in which trees were spaced at 6 m x 3 m (rows x trees), were planted in 1997. The orchards were divided into 12 blocks of approximately 135 trees each. Each treatment was applied to 2 randomly selected blocks, one in each orchard. Approximately 10 800 L of spray mix were applied per hectare for all applications. Twelve data trees were selected per treatment (6 in each orchard), and evaluation took place as described in the previous trial.

Table 3.2.5.4. Timing of Cryptogran applications in an orchard of Palmer navel oranges on Far Away Farm.

Treatment		Concentration (ml / 100 L water)	Application date					
1	Untreated control		-		-	-	-	-
2	Cryptogran	10			09 Dec 2008	-	12 Feb 2008	-
3	Cryptogran	10	30 Oct 2008		09 Dec 2008	-	12 Feb 2008	-
4	Cryptogran	5 +		17 Nov	09 Dec	15 Jan	12 Feb	19 Mar

	+ molasses	250		2008	2008	2009	2008	2008
5	Cryptogran	5		17 Nov 2008	09 Dec 2008	15 Jan 2008	12 Feb 2008	19 Mar 2008

Field trial 3: Dunbrody Estates – Boerboon Farm

A trial was conducted to compare the efficacy of Cryptogran when applied in March, April and May. The trial was conducted on Boerboon Farm in the Sundays River Valley, in an orchard of Powell navel orange trees. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 2002. Each treatment consisted of a single Cryptogran spray, applied to two randomly selected blocks of 20 trees each (5 trees in each of 4 rows), on 13 March 2008, 23 April 2008 and 26 May 2008. An average of 15.5 L of spray mixture per tree was applied. Twelve data trees were selected per treatment (6 per block), and evaluation took place as described in the previous trial.

Field trial 4: Molasses and other adjuvants

Due to a countrywide shortage of molasses, a field trial was conducted last season to test the effect of reduced rates of molasses on the efficacy of Cryptogran. In addition the efficacy of Cryptogran was tested with other additives, which could possibly be used if molasses was unavailable. This season some of the products which showed promise in the previous season's trial were included in a new trial. Some new additives were also tested, including a thicker Molatech molasses. There had been reports that Cryptogran applications in the Northern areas of South Africa had been particularly effective in controlling FCM when sprayed in combination with Dithane (for Citrus Black Spot control) (Deon Begemann, personal communication). This treatment was therefore included. Cryptex was tested as registered, but also in combination with Cryptogran, at full and half rates. It was speculated that certain populations could be more susceptible to certain strains of virus, so a combination of the two viruses was tested. The trial was conducted in the same orchard as the previous year i.e. an orchard of Lane Late navel oranges on Lone Tree Farm in the Sundays River Valley, (Table 3.2.5.5). The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 1999. The trial was laid out in a randomised block format, replicated 10 times. Treatments were applied with a Janisch hand-gun applicator on 10, 11 and 12 December, at an average rate of 21.9 L per tree for all treatments. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and Duncan's multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 1996).

Table 3.2.5.5. Various treatments applied to an orchard of Palmer navel orange trees on Lone Tree Farm in the Sundays River Valley on 10, 11 & 12 December 2008.

Treatment	All doses per 100 L water
1	Untreated control
2	Cryptogran (10 ml)
3	Cryptogran (10 ml) + Voermol molasses (250 ml) + Agral 90 (18 ml)
4	Cryptogran (10 ml) + Voermol molasses (125 ml) + Agral 90 (18 ml)
5	Cryptogran (10 ml) + Molatech molasses (125 ml) + Agral 90 (18 ml)
6	Cryptogran (10 ml) + Molatech molasses (250 ml) + Agral 90 (18 ml)
7	Cryptogran (10 ml) + Mannitol (1000) + Agral 90 (18 ml)
8	Cryptogran (10 ml) + White sugar (200 g) + Agral 90 (18 ml)
9	Cryptogran (10 ml) + Wetcit (100 ml)
10	Cryptogran (10 ml) + Voermol molasses (250 ml) + Wetcit (100 ml)
11	Cryptogran (10 ml) + Dithane (200 g) + Agral 90 (18 ml)
12	Cryptogran (10 ml) + Silicon (200 ml) + Agral 90 (18 ml)
13	Cryptogran (10 ml) + H & R medium spray oil (300 ml)
14	Cryptex (3.3 ml) + Voermol molasses (250 ml) + Agral 90 (18 ml)
15	Cryptogran (10 ml) + DiPel (12.5 g) + Agral 90 (18 ml)
16	DiPel (12.5 g)
17	Cryptogran (5 ml) + Cryptex (1.65 ml) Voermol molasses (250 ml) + Agral 90 (18 ml)
18	Cryptogran (10 ml) + Cryptex (3.3 ml) Voermol molasses (250 ml) + Agral 90 (18 ml)

Field trial 5: Patensie

This trial was also reported on in the previous annual report, but evaluations continued into April 2009. Cryptogran trials were last applied in the Gamtoos River Valley a few years ago. It was therefore decided to conduct a trial in an orchard of Palmer navel orange trees on Paksaam Farm in Patensie, Gamtoos River Valley. The orchard, in which trees were spaced at 6 m x 4 m (rows x trees), was planted in 1990. Cryptogran was applied to 2 blocks of 60 trees each using a tower mistblower, on 28 November 2007 and again on 7 February 2008. Two untreated blocks of similar size were left unsprayed. Dropped fruit from 6 data trees per block (12 trees per treatment) were collected weekly and evaluated for FCM infestation, as described for the previous trials.

Field trial 6: Cryptogran in Isomate-treated orchards

A trial was conducted to test the efficacy of Cryptogran when applied within an Isomate-treated orchard of Palmer Navel orange trees on Scheepersvlakte Farm in the Sundays River Valley. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 2000. The treatment was applied on 11 December 2008, using the grower's tower mistblower machine. Two separate blocks, consisting of 20 trees in 5 rows, were sprayed with Cryptogran (10 ml / 100 L), molasses (250 ml / 100 L) and Agral 90 (18 ml / 100 L). An average of 18.7 L of spray mix was applied to each tree. Six data trees were marked in the centre of each sprayed block, giving a total of 12 data trees. Similarly, 2 groups of 6 data trees each were marked in unsprayed areas, i.e. where only Isomate was applied. Dropped fruit from under these trees were collected weekly and analysed as described in previous field trials.

Field trial 7: Brakfontein, Citrusdal (Stephan Honiball)

The purpose of this trial was to compare various different virus (particularly Cryptogran) programmes in the Citrusdal area of the Western Cape. Simultaneously comparisons were made with a Cryptex programme. The trial was conducted in Block no. 53 on the farm Brakfontein, 10 km north of Citrusdal. The block consisting of 14 year old Robyn navel orange trees, which were grafted onto rough lemon rootstocks. The trees were spaced 6 m x 4 m, giving a total of 416 trees/ha. The trees did not touch each other, and were pruned a few months earlier to allow good spray penetration. The trial was laid out in a semi-commercial block format, each treatment replicated twice. Each block consisted of approximately 100 bearing citrus trees, therefore approximately 200 citrus trees were sprayed in total per treatment. Treatment one (the untreated control) consisted of only one block of a hundred trees, due to concerns expressed by the producer at leaving an untreated control. No guard trees were used between blocks. The trees were sprayed with the land owner's Cima mist blower at 2 bar pressure, and a full cover film spray was applied at a rate of approximately 10 500 L/ha or about 25 L spray mixture per tree. The different spray mixtures were mixed and applied according to the label instructions, and spraying commenced at 16h30 or soon thereafter on the specified dates. Spraying continued into the night until finished, to minimize the effect of harmful UV rays at certain times of the year. No rain fell during 48 h after spraying. A Hobo datalogger was used to measure the temperature at hourly intervals. A graph of the daily average minimum and maximum temperatures between 05h00 and 07h00 in the morning, and 14h00 and 16h00 in the afternoon, is presented in Fig. 3.2.5.1 and 3.2.5.2. Cryptogran and Cryptex were applied according to the schedule in Table 3.2.5.6.

Six data trees were used in the middle of each block for evaluation. Each data tree's data was kept separately to allow for statistical analysis. All dropped fruit were collected weekly beneath the trees from 3 weeks after spraying, and was carefully dissected in the field to determine the cause of drop. Fruit that were infected by the *Alternaria* fungus were discarded, since these fruit naturally attract FCM oviposition, and were already in a process of decay. Fruit containing live or dead FCM larvae or the characteristic granular frass, were counted as infested.

Table 3.2.5.6. The time of application and different dosages used in the Cryptogran comparative trial, October 2007 – April 2008.

Treatment no.	Product	Time of application	Concentration/100 L water	Batch no.
1	Untreated control			
2	Cryptogran	28-Nov-07	10 ml	7256
	Cryptogran	12-Feb-08	10 ml	7257
3	Cryptogran	24-Oct-07	10 ml	7256
	Cryptogran	28-Nov-07	10 ml	7256
	Cryptogran	12-Feb-08	10 ml	7257
4	Cryptex	28-Nov-07	3.3 ml	#3
	Cryptex	08-Jan-08	3.3 ml	#3
	Cryptex	12-Feb-08	3.3 ml	#3
5	Cryptogran	24-Oct-07	5 ml	7256
	Cryptogran	28-Nov-07	5 ml	7256
	Cryptogran	18-Dec-07	5 ml	7257
	Cryptogran	08-Jan-08	5 ml	7257
	Cryptogran	12-Feb-08	5 ml	7153
	Cryptogran	12-Mar-08	5 ml	7153

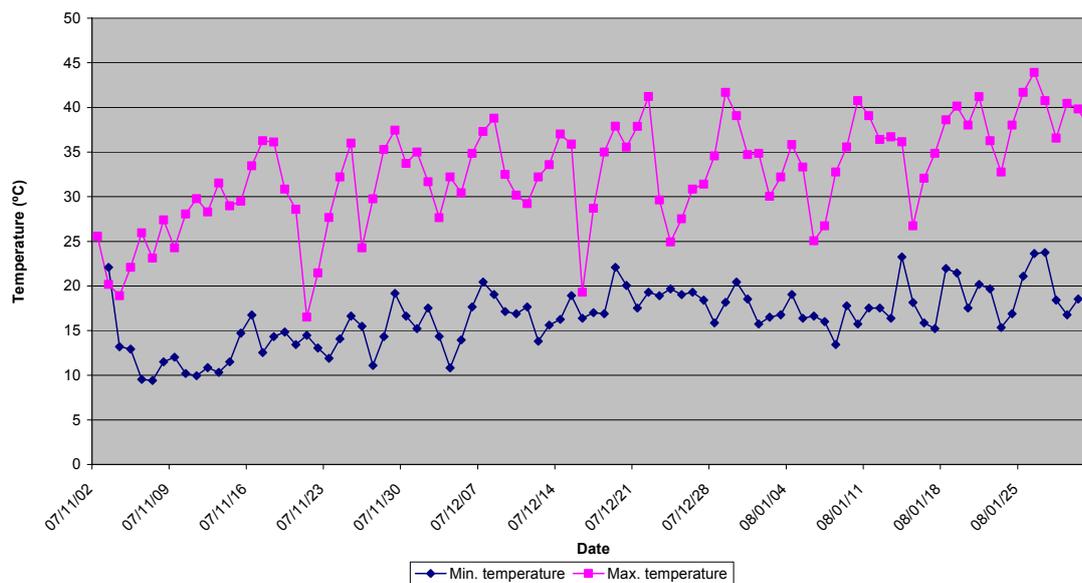


Fig. 3.2.5.1. Mean maximum temperatures during the trial at Brakfontein (November 2007 – January 2008).

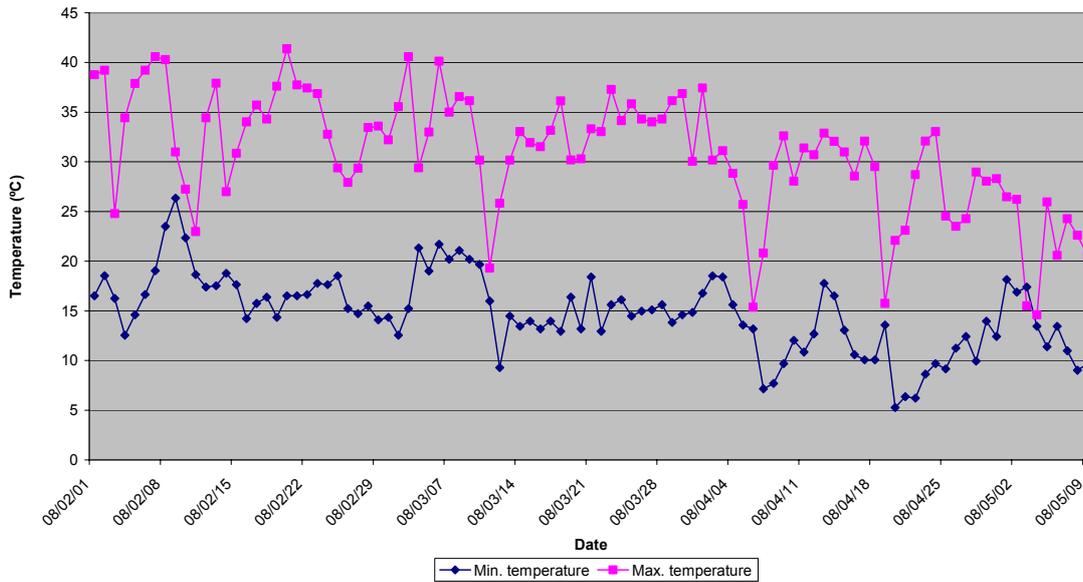


Fig. 3.2.5.2. Mean maximum and minimum temperatures during the trial at Brakfontein (February – May 2008).

Trial 8: Ouwerf, Citrusdal (Stephan Honiball)

This trial was conducted to compare the efficacy of a single late season application of Cryptogran with that of Cryptex. The trial was not initially planned, but was conducted as FCM levels in the previous trial conducted in Citrusdal were too low. The trial was conducted on the farm Ouwerf, 5km north of Citrusdal on the “Bo-River” Road. The trial (Block 4) consisted of a fairly uniform square block (+/- 5 ha in size) of Robyn navel orange trees. The planting date was 1985 and the trees were grafted on rough lemon rootstocks. The tree density was approximately 416 trees/ha (6 m x 4 m). A farm road divided the block in two near equal parts: a southern and a northern block. Each block was further subdivided in two, resulting in four equal quadrants (Fig. 3.2.5.3).

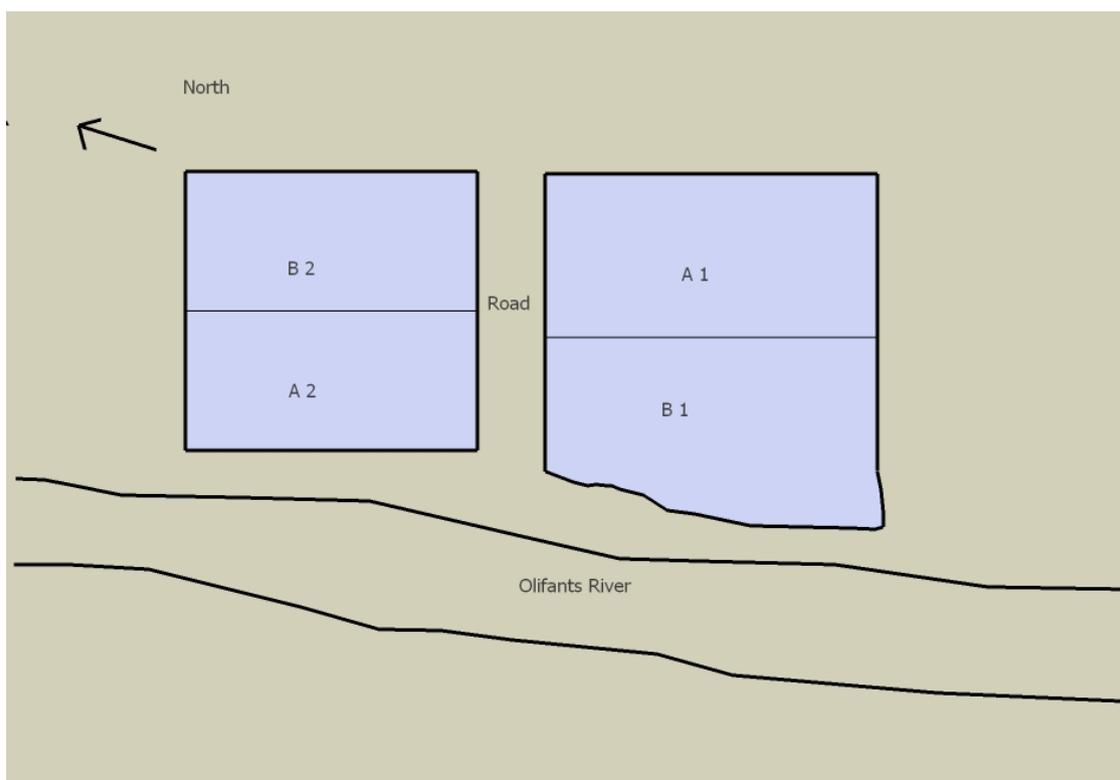


Fig. 3.2.5.3. Schematic layout of FCM virus trial at Ouwerf

Two of these quadrants (A1 and A2) were treated with a full cover Cryptogran spray (10 ml Cryptogran + 250 ml molasses + 18 ml wetter/100L water), while the other two quadrants were treated with a full cover Cryptex spray (3.3 ml Cryptex + 500 ml molasses/100 L water). The spray mixtures were applied using the farmer's hydraulic spray pump (Jacto Airbus – capacity – 2000 L), delivering 190 L/min at 20 Bar pressure). Citrus fruit and leaves were covered with spray mixture to the point of run-off, and sufficient spray mixture penetrated the trees to ensure good coverage of inside fruit also. Approximately 9 200L of spray mixture was applied per hectare, resulting in about 22 L spray mixture per tree. Due to phytosanitary constraints, an untreated control could not be retained. Cryptogran (Batch #: 8118) was sprayed during the morning on 26 May 2008, and Cryptex (Batch #: 4) the following day. No rain fell during the first week after spraying.

Eight data trees were used in the middle of each block for evaluation. Each data tree's dropped fruit were collected weekly and analyzed separately for the presence or absence of FCM larvae or characteristic granular frass. The fruit were collected from three weeks after spraying, to allow for previously infested fruit to drop off the trees. Many of the dropped fruit were in an advanced stage of decay, which hampered the evaluation process. This was especially true during the last three weeks of the trial, when intermittent rain showers expedited the decay process.

Trial 9: Magogong, Vaalharts (Johanna Mathewson)

This trial was conducted, as a general perception was reported that results with Cryptogran in the Vaalharts area were not very good. An orchard of Palmer navel orange trees on rough lemon rootstock on Magogong Farm in the Vaalharts region was selected for the trial. The orchard was planted in 1983 at a spacing of 7 m x 3 m (rows x trees). The orchard had been treated with Isomate (500 dispensers hung on 21 October 2008, 300 dispensers hung on 12 January 2009). However, some FCM infestation of fruit was still evident. The orchard was therefore considered suitable for the trial. The orchard (Plot 1 JX-2) consisted of 15 rows with 34 trees in each. The orchard was divided into quarters. Two diagonally opposite quarters were twice sprayed with Cryptogran (Table 3.2.5.7). The remaining two quarters were used as untreated control blocks.

Table 3.2.5.7. Application details for Cryptogran treatments in an orchard of Palmer navel oranges at Magogong Farm, Vaalharts.

Application date	24 Nov 2008	9 Feb 2009
Application time	16h00 – 20h45	16h00 – 18h20
Tractor	New Holland 72.86 Fiat Agri	New Holland 72.86 Fiat Agri
Gear	1/1	1/2
Spray machine	Oscillating tower mistblower	Oscillating tower mistblower
Whirlers	Alternating 45/56	56
Nozzles	Alternating D4/D5	D5
Pressure	20 bar	20 bar
Treatment (per 100 L water)	Cryptogran 10 ml Molasses 250 ml Agral 90 18 ml	Cryptogran 10 ml Molasses 250 ml Agral 90 18 ml
Volume applied/tree	20.7 L	16.6 L
Total number trees sprayed	255	255

Ten data trees were marked in the middle of each block. Two weeks after the first treatment, all fallen fruit was cleared from underneath the data trees. From 3 weeks after the first treatment, evaluations were initiated. Each week on the same day (Monday), fallen fruit lying underneath each data tree were picked up and individually assessed to determine the cause of drop. FCM infested fruit was recognised by the presence of an FCM larva or its characteristic frass. Evaluations were conducted from 15 December 2008 to 6 April 2009. FCM infestation between treated and untreated blocks was statistically compared using a Student t-test.

Results and discussion

Comparison of Cryptogran and Cryptex in a detached fruit bioassay

The detached-fruit bioassay showed that fewer larvae penetrated the Cryptogran-treated fruit than the Cryptex-treated fruit (Table 3.2.5.8). There was, however, no significant difference between the treatments, due to high variation ($P = 0.2540$). The bioassay needs to be repeated to make a reliable comparison between the two products using this method of bioassay.

Table 3.2.5.8. Infestation of Valencia oranges treated with distilled water, Cryptex or Cryptogran. Three neonate larvae were placed per fruit; 30 fruit per treatment, evaluated on 10 October 2008.

Treatments (doses per 100 L water)		Total no of larvae penetrated	Mean no of larvae/fruit
1	Untreated control	17	0.56a
2	Cryptex (3.3 ml) + molasses (250 ml) + Agral 90 (18 ml)	13	0.43a
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	11	0.37a

*Different letters in the same column denote significant differences between values ($P > 0.05$; Bonferroni multiple range test).

UV bioassays: Cryptogran vs Cryptex

Larval survival was low in both bioassays, and it would appear that there is no difference in the ability of the two products to withstand the effect of UV irradiation (Table 3.2.5.9) Cryptogran is a crude virus preparation, containing insect body parts and dietary ingredients. Some of the world experts in the field use of baculoviruses, such as Jones (1998), Johannes Jehle (personal communication) and Caroline Hauxwell (personal communication) agree that crude virus preparations persist longer than other formulations, and that formulation and additives do not play an important role in the field persistence of baculoviruses.

Table 3.2.5.9. Impact of UV-irradiation (sunlight) on Cryptogran and Cryptex (both at a concentration of 1.34×10^5 OBs / ml), measured by mortality of neonate FCM larvae in a dose-response bioassay.

Treatment no	Treatment (All virus (Cryptogran and Cryptex) treatments at a concentration of 1.34×10^5 OBs/ml)	Larval mortality (%) Replicate 1		Larval mortality (%) Replicate 2	
		Cryptogran	Cryptex	Cryptogran	Cryptex
1	Distilled water	12	20	24	24
2	Virus – no exposure to sunlight	96	96	96	100
3	Virus – exposed for 120 minutes	100	100	92	100
4	Virus – exposed for 240 minutes	92	96	100	100
5	Virus – exposed for 360 minutes	84	92	100	100
6	Virus – exposed for 480 minutes	80	88	92	100

Development of droplet-feeding bioassay techniques

Brilliant blue appeared to have no effect on FCM larvae in this bioassay. In fact, the mortality of larvae which ingested the 1% BB solution was marginally lower than for those which ingested distilled water (Table 3.2.5.10). BB was therefore shown to be a suitable dye to be used when conducting droplet-feeding bioassay with FCM.

Table 3.2.5.10. Impact of Brilliant Blue dye on neonate FCM larvae, measured by mortality of larvae in a dose-response bioassay.

Treatment			Larval mortality (%)	Average mortality per treatment (%)
1	Distilled water	Replicate 1	32	33.3
1	Distilled water	Replicate 2	36	
1	Distilled water	Replicate 3	32	
2	Brilliant Blue (1%)	Replicate 1	24	28.0
2	Brilliant Blue (1%)	Replicate 2	36	
2	Brilliant Blue (1%)	Replicate 3	24	

The trial was repeated 6 times. The number of larvae that consumed 2 µl of BB solution varied between 18 and 72. The variability was very high, and this was clearly not an accurate way to determine the average volume ingested. In some instances the larvae were hesitant to feed on the droplet, and so evaporation could have occurred, resulting in a lower average ingestion. It is possible that some larvae were a few hours older than others, and could have been more strongly attracted to the liquid. In several cases, larvae crawled through the droplet, broke the surface tension and spread the droplet over a larger area. Not all of the solution was ingested, as the larvae carried some of it away on themselves. Evaporation could also occur faster, as the solution was now spread over a larger surface area. These factors most likely have led to the high variation in the calculated volume ingested.

In the second method used by Lyndall Pereira da Conceicao (see 3.2.13 in this report), results were much more constant and conclusive.

The average mass of solution ingested was calculated by subtracting the average mass of larvae from the average mass of larvae after ingestion, i.e. $0.02240 - 0.01805 = 0.00435$ mg. If the average mass of a 50µl droplet of 1% BB solution is 49.45 mg, then the 0.00435 mg ingested per larva equates to 0.0044 µl of 1% BB solution ingested on average by each larva.

In the surface-dose bioassays used previously, the highest dose in the 5-fold series dilution was 76300 OBs / ml ($76.3 \text{ OBs} / \mu\text{l}$). If each larva ingests 0.044 µl, then they would ingest an average of 0.33572 OBs / larva. This concentration was therefore too low for even the lowest concentration in a range, as not all larvae would ingest any virus.

A literature review showed that the LD_{50} for *Spodoptera littoralis* was 5.91 OBs per larva (Jones, 1998). Hughes & Ware (1995) found the LD_{50} for *Trichoplusia ni* to be between 2 and 5 OB's / larva. Kadir *et al* (1999) reported a LD_{50} value of 1 to 8.9 OB's / larva for the PxGV-Taiwan against the diamond back moth, *Plutella xylostella*, and

9.5 - 30.2 OB's / larva for GmNPV and AcNPV against *Galleria mellonella* and *Autographa californica* respectively. It was therefore decided to conduct droplet-feeding bioassays with a two-fold series dilution. According to the calculations by Pereira da Conceicao, the weakest dose (2.27×10^5) would ensure that each larva would consume an average of 1 OB per larva (Table 3.2.5.11).

Table 3.2.5.11. Concentrations and related dosages used in two-fold dilution series, droplet-feeding dose response bioassays

Treatment	Concentration of virus (OBs / ml)	Average no of OBs ingested per larva
1	Distilled water	0
2	2.27×10^5	1
3	4.54×10^5	2
4	9.08×10^5	4
5	1.82×10^6	8
6	3.63×10^6	16
7	7.26×10^6	32

In the first bioassay old 25-cell bioassay trays were used. Unfortunately only enough neonate FCM larvae emerged to inoculate the first 4 treatments (Table 3.2.5.12). However, from the mortality recorded it appeared that a suitable dose range had been identified. The LD₅₀ value calculated was 4.54×10^5 , which would equate to 2 OB's per larva, and the LD₉₀ value was 7.05×10^5 (approximately 20 OB's per larva). More bioassays would need to be conducted to confirm this first calculation.

Table 3.2.5.12. Mortality of neonate FCM larvae when bioassayed against a dilution series of Cryptogran, in a droplet-feeding bioassay, using Sterilin 25-cell bioassay trays.

Treatment	Concentration of virus (OBs / ml)	Mortality of neonate FCM larvae (%)
1	Distilled water	20
2	2.27×10^5	52
3	4.54×10^5	56
4	9.08×10^5	72

In a second bioassay conducted in a similar way, a dose response was recorded but unfortunately the control mortality was too high (Table 3.2.5.13). A statistical analysis to calculate LD₅₀ and LD₉₀ was therefore not possible. However, the dose response did appear to indicate that the doses used were within the right range.

Table 3.2.5.13. Mortality of neonate FCM larvae when bioassayed against a dilution series of Cryptogran, in a droplet-feeding bioassay, using Sterilin 25-cell bioassay trays.

Treatment	Concentration of virus (OBs/ml)	Mortality of neonate FCM larvae (%)
1	Distilled water	40
2	2.27×10^5	36
3	4.54×10^5	48
4	9.08×10^5	60
5	1.82×10^6	72
6	3.63×10^6	68
7	7.26×10^6	76

Due to a shortage of the old 25-cell Sterilin bioassay trays, new 24 round-celled bioassay trays were used in the next two droplet-feeding bioassays. In both of these bioassays, control mortality was high, and the results were variable and unreliable (Table 3.2.5.14). Possible reasons for this, as well as attempts to improve the bioassay technique, are discussed under the next sub-heading. However, there was a general increase in mortality with increased concentration of Cryptogran.

Table 3.2.5.14. Mortality of neonate FCM larvae when bioassayed against a series dilution of Cryptogran, in a droplet-feeding bioassay, using 24-cell bioassay trays.

Treatment	Concentration of virus (OBs/ml)	Mortality of neonate FCM larvae (%)	
		Replicate 1	Replicate 2
1	Distilled water	50.0	37.5
2	2.27×10^5	45.9	66.7
3	4.54×10^5	66.7	45.9
4	9.08×10^5	66.7	41.7
5	1.82×10^6	58.4	70.9
6	3.63×10^6	83.4	66.7
7	7.26×10^6	87.6	75.0

Although this is not as clear as in the first bioassay where the 25-cell bioassay trays were used, it does appear that an acceptable dose range has been developed. Once the bioassay technique and equipment is improved, more bioassays will be conducted to ascertain whether the dose range is ideal, and then the droplet-feeding technique can be used for dose-response and UV bioassays.

Other bioassay improvements

In the first bioassay, larval survival was marginally higher in the 25-cell trays (Table 3.2.5.15). More importantly, a reasonable dose-response was observed when using these trays. Where the 24-cell trays were used, mortality was very high in all the treatments and no dose response was apparent.

Table 3.2.5.15. Mortality of neonate FCM larvae in a droplet-feeding dilution series bioassay with Cryptogran, using 2 different bioassay trays.

Treatment	Concentration of virus (OBs / ml)	Mortality of neonate FCM larvae (%)	
		25 cell trays	24 cell trays
1	Distilled water	28.0	29.2
2	2.27×10^5	56.0	70.9
3	4.54×10^5	60.0	100.0
4	9.08×10^5	60.0	95.9
5	1.82×10^6	72.0	100.0
6	3.63×10^6	80.0	95.9
7	7.26×10^6	88.0	100.0

In the second bioassay, survival was highest where bulldog clips were used to clamp the lids of the bioassays down (Table 3.2.5.16). However, larval development appeared slower in these trays, as noted in the size of larvae at the time of evaluation. Where elastic bands were used to keep the lids on, there was slightly lower survival, and several larvae had escaped from their individual cells and were wandering around in trays. Where the diet had been allowed to dry for 90 minutes longer before inoculation with larvae, survival was low, but the surviving larvae were larger and were further developed by at least one larval instar.

Table 3.2.5.16. Mortality of neonate FCM larvae in a droplet-feeding dilution series bioassay with Cryptogran, using 24-cell bioassay trays.

Treatment		% survival		Comments
1	Lids closed with bulldog clips	Replicate 1	79.2	Larvae small (2 nd instar), diet moist, 1 larva found ('wandering')
1		Replicate 2	70.9	
1		Replicate 3	75.0	
		Average	75.0	
2	Lids closed with elastic bands	Replicate 1	58.4	Larvae small (2 nd instar), diet moist, 6l larva found ('wandering')
2		Replicate 2	62.6	
2		Replicate 3	70.9	
		Average	63.9	
3	Diet allowed to dry out for 9 minutes longer; lids closed with bulldog clips	Replicate 1	54.2	Larvae better developed (3 rd instar), diet drier, no larvae 'wandering'.
3		Replicate 2	50	
3		Replicate 3	83.4	
		Average	62.6	

These results showed that the larvae developed faster where the diet was not as moist. Unfortunately, in two of the three trays where the diet was allowed to dry for longer, the survival was very low (54% and 50%). This was surprising, as higher mortality of larvae due to overly moist diet has been observed in the past, and the fact that the larvae were better developed in the drier diet would lead one to expect a better survival rate.

Unfortunately the trials did not give clear answers. Overall, the survival in untreated controls when using the 24-cell trays has been too low, and dose-responses have been variable. Also, the 24-cell trays are cumbersome to work with, as there are spaces between the cells, which make filling the cells with diet slow. These spaces also allow larvae to wander between the cells. They are therefore not ideal for our purposes. Subsequently a source has been found for the old 25-cell Sterilin bioassay trays, and orders have been placed.

Field trial 1: Dunbrody

In the second field trial, it appeared that an additional early October application of Cryptogran did not result in lower FCM infestation than where the normal December and February sprays were applied (Table 3.2.5.17) (Figure 3.2.5.4). This trial was sprayed against a relatively low level of FCM infestation (0.27 infested fruit per tree per week). The greatest reduction in infestation occurred where Cryptogran was applied more frequently (monthly) at half the registered rate (5 ml / 100L water). The Cryptex programme resulted in the smallest reduction in infestation, and was the only treatment that did not result in a significant reduction of FCM infestation (Table 3.2.5.17) (Figure 3.2.5.4).

Table 3.2.5.17. FCM infestation for different Cryptogran programmes in an orchard of Lane Late navel oranges on Dunbrody Farm, evaluated from 2 January to 5 May 2008.

Treatment		Conc. (ml / 100 L water)	Date of application				FCM infestation (mean fruit/tree/week)	Reduction in infestation (%)
1	Untreated control		-	-	-	-	0.27a*	
2	Cryptogran	10	-	04 Dec 2007	-	14 Feb 2008	0.12b	60.9
3	Cryptogran	10	24 Oct 2007	04 Dec 2007	-	14 Feb 2008	0.13b	56.3
4	Cryptex	3.3	-	04 Dec 2007	09 Jan 2008	14 Feb 2008	0.17ab	43.8

5	Cryptogran	5	24 Oct 2007	04 Dec 2007	09 Jan 2008	14 Feb 2008	13 Mar 2008	0.09b	68.8
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*Different letters in the same column denote significant differences between values ($P>0.05$, Bonferroni multiple range test).

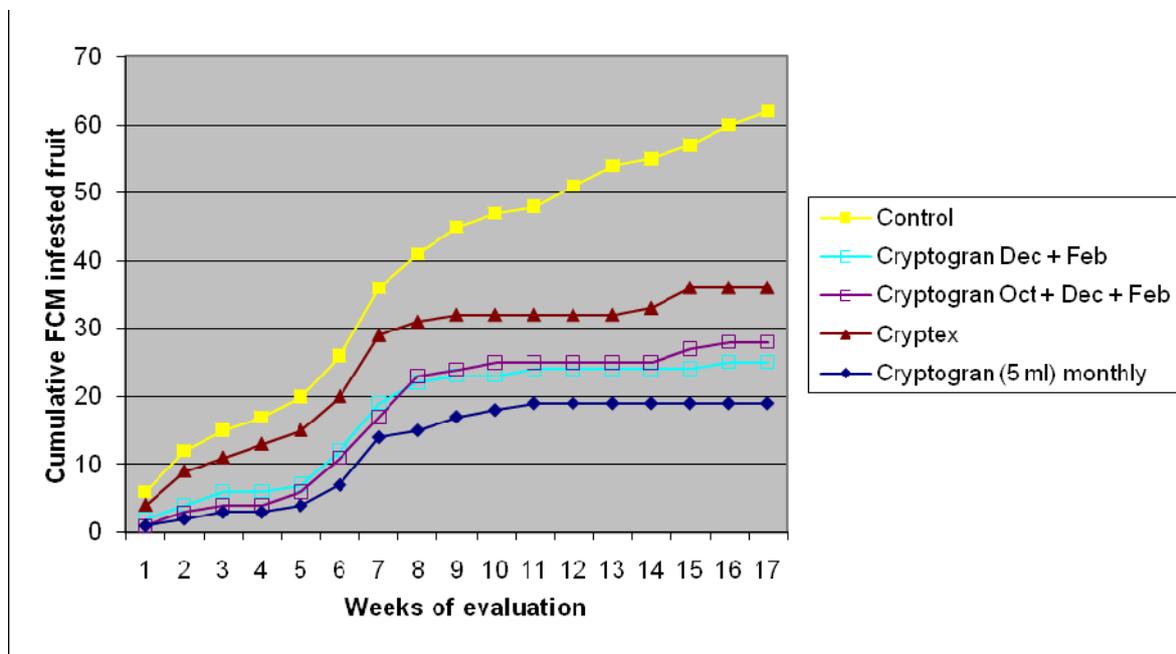


Fig 3.2.5.4. Cumulative number of infested fruit for various treatments and an untreated control, for the duration of evaluation, in an orchard of Lane Late navel oranges on Dunbrody Farm in the Sundays River Valley, evaluated from 2 January to 5 May 2008.

Field trial 2: Far Away Farm.

In the previous trial on Dunbrody Farm, where FCM infestation was low (0.27 infested fruit /tree/week), an additional early October application of Cryptogran did not result in lower FCM infestation than where the normal December and February sprays were applied. However, in this trial the infestation level was much higher (1.3 infested fruit/tree/week) (Table 3.2.5.18). The highest reduction in infestation was recorded where the early October application was included. Orchard sanitation had been particularly poor in these orchards. At the time of the October application, there were still many dropped fruit on the ground, as a result of out-of-season fruit not being removed. This was most likely the reason for high FCM levels. At the first evaluation, three weeks after the December applications, the infestation in the untreated control was an average of 3 infested fruit per tree per week. It is possible that the reason that the early application had an effect in this case, was high FCM pressure early in the season. However, the difference between this programme (treatment 3) and the standard December + February programme (treatment 2), was not significant. October sprays could be possibly be beneficial to growers, especially where FCM levels were high the previous season and the FCM inoculums at the start of the next season would therefore be high. It would appear that the lower concentrations of Cryptogran were unable to cope with the higher levels of FCM in this trial. Initially these applications had very little effect in reducing FCM populations (Fig 3.2.5.5.), and were significantly less effective than the treatments applied at the full rate of 10 ml per 100 L water. This indicates that concentration plays a very important role when controlling pests with a baculovirus. Surprisingly, the treatment without molasses (treatment 5) performed better than the treatment applied with molasses (treatment 4), although not significantly so. This could be explained by the fact that for some reason, FCM infestation appeared to be much higher in one corner of one of the two orchards used in this trial. One of the blocks to which treatment 4 was applied fell within this area, which probably resulted in this unexpected result. In all previous trials, Cryptogran has performed better when applied with molasses. Possibly more blocks per treatment should be used in future trials, to minimize the effect of variation in FCM pressure within a trial site. The trial will be monitored until the end of May, and the final results will be reported on in the next Annual Report.

Table 3.2.5.18. FCM infestation for different Cryptogran programmes in an orchard of Palmer navel oranges on Far Away Farm, evaluated from 6 January to 31 March 2009.

Treatment		Concentration (ml/100 L water)	Application date						Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control		-		-	-	-	1.3a		
2	Cryptogran	10			09 Dec 2008	-	12 Feb 2008	0.57bc	54.3	
3	Cryptogran	10	30 Oct 2008		09 Dec 2008	-	12 Feb 2008	0.50c	59.2	
4	Cryptogran + molasses	5 + 250		17 Nov 2008	09 Dec 2008	15 Jan 2009	12 Feb 2008	1.03ab	22.8	
5	Cryptogran	5		17 Nov 2008	09 Dec 2008	15 Jan 2008	12 Feb 2008	0.68bc	42.9	

*Different letters in the same column denote significant differences between values (P>0.05, Bonferroni multiple range test).

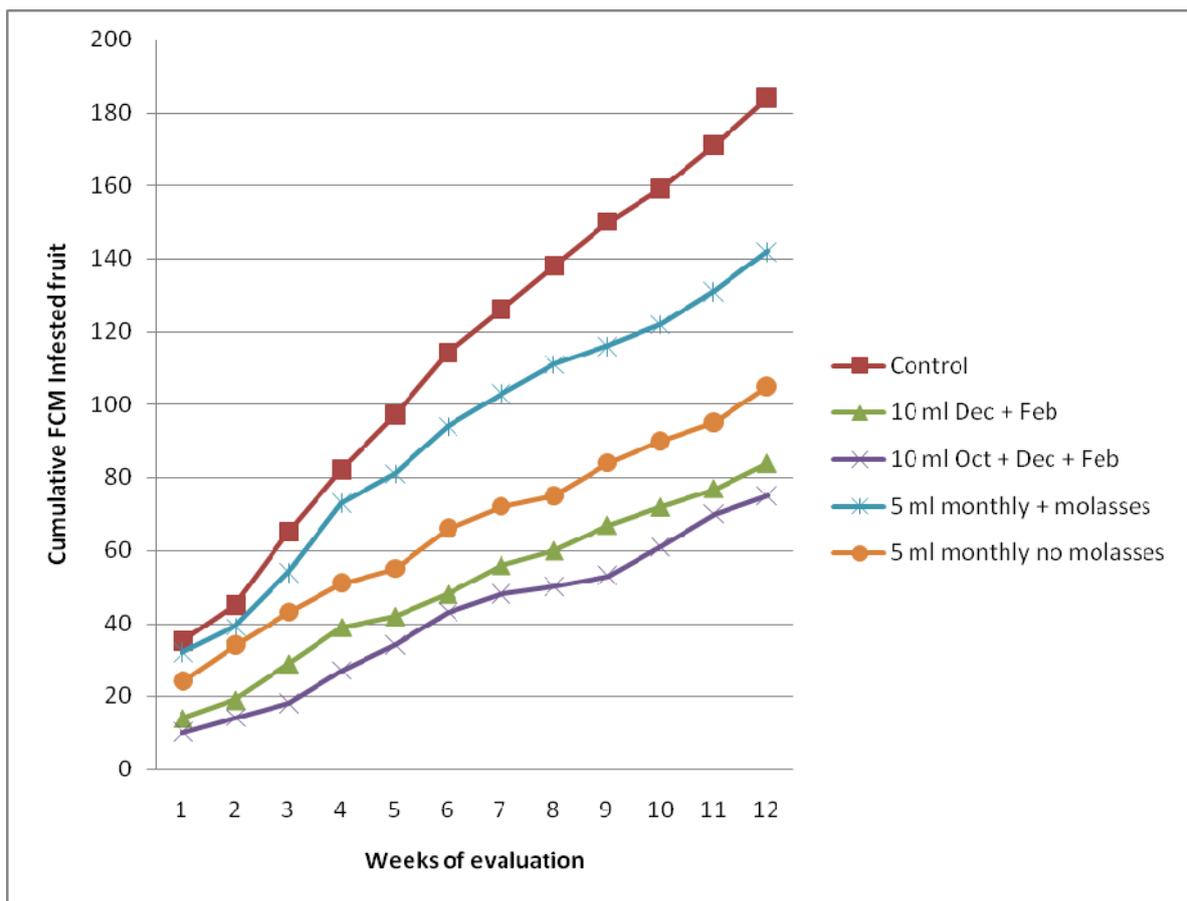


Fig 3.2.5.5. Cumulative number of infested fruit for various treatments and an untreated control in an orchard of Palmer navel orange trees on Far Away Farm in the Sundays River Valley; evaluated from 6 January to 31 March 2008.

Field trial 3: Boerboon

Unfortunately FCM infestation levels were very low throughout the period of evaluation. The treatment applied in February gave the highest percentage reduction in infestation (56.3%) over a period of 12 weeks (Table 3.2.5.19). The April and May applications both resulted in a 33% reduction in infestation, over 8 and 4 weeks of evaluation respectively. None of the treatments resulted in a significant reduction in infestation, due to high variability as well as very low infestation. The trial would need to be repeated to obtain meaningful results.

Table 3.2.5.19. Reduction in FCM infestation for various Cryptogran applications in an orchard of Powell navel orange trees on Boerboon Farm, evaluated from 16 April to 2 July 2008.

Date of Cryptogran application	Period of evaluation (weeks)	Mean infestation in untreated control	Mean infestation in treatment	Reduction in infestation (%)
13 March 2008	12	0.110a	0.49a	56.3
23 April 2008	8	0.125a	0.73a	33.3
26 May 2008	4	0.125a	0.83a	33.3

Field Trial 4: Molasses and other adjuvants

FCM control was not as effective when the rate of molasses was reduced, although these differences were not significant (Table 3.2.5.20). Where Voermol molasses was used at 250 ml/100 L, FCM infestation was reduced by 64.9%, and where the molasses concentration was lowered, FCM infestation was reduced by 59.5%. Where the thicker Molatech molasses was used at 250 ml/100 L, FCM infestation was reduced by 70.3%, and where this molasses concentration was lowered, FCM was reduced by 62.2%. The thicker Molatech molasses gave of the best results (70.3% reduction in infestation), and even when this molasses was used at 125 ml / 100 L, it resulted in a reduction of 62.2%. This was almost as effective as where Voermol molasses at 250 ml / 100 L was used (64.9% reduction in infestation). The Molatech molasses is supplied by River Bioscience.

Mannitol appears to be a good alternative to molasses (67.6% reduction in infestation), but is very expensive. Cheaper, sugar-alcohol alternatives could be investigated. White sugar also performed reasonably well (59.5% reduction in infestation), but was not quite as effective as molasses. Results with Wetcit (100 ml / 100 L) were poorer than where Agral 90 was used. This was surprising, as in previous trials Wetcit had improved the efficacy of Cryptogran + molasses. However, the product was applied at 200 ml / 100 L in previous trials, which may explain the poorer results. The greatest reduction in infestation was achieved where Cryptogran was applied with Dithane (without molasses). Further laboratory trials should be conducted to determine the reason for this, and the treatment should be replicated in further field trials. DiPel alone hardly gave hardly any FCM control (8.1% reduction in infestation), and did not increase the efficacy of Cryptogran when applied in combination with it. An application of a combination of Cryptogran and Cryptex at half their registered concentrations was less effective than Cryptogran alone (54.1% reduction in infestation). The combination of Cryptex and Cryptogran at their full registered rates, slightly improved FCM control. This was not significant. Medium spray oil and silicon were less effective adjuvants than molasses.

Table 3.2.5.20. FCM infestation for various treatments in an orchard of Palmer navel oranges on Lone Tree Farm, evaluated from 6 January to 10 February 2009.

Treatment	All doses per 100 L water	FCM infestation (mean fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	0.62a	
2	Cryptogran (10 ml)	0.37abc	40.5
3	Cryptogran (10 ml) + Voermol molasses (250 ml) + Agral 90 (18 ml)	0.22c	64.9
4	Cryptogran (10 ml) + Voermol molasses (125 ml) + Agral 90 (18 ml)	0.25c	59.5
5	Cryptogran (10 ml) + Molatech molasses (125 ml) + Agral 90 (18 ml)	0.23c	62.2

	ml)		
6	Cryptogran (10 ml) + Molatech molasses (250 ml) + Agral 90 (18 ml)	0.18c	70.3
7	Cryptogran (10 ml) + Mannitol (1000) + Agral 90 (18 ml)	0.20c	67.6
8	Cryptogran (10 ml) + white sugar (200 g) + Agral 90 (18 ml)	0.25c	59.5
9	Cryptogran (10 ml) + Wetcit (100 ml)	0.30bc	51.4
10	Cryptogran (10 ml) + Voermol molasses (250 ml) + Wetcit (100 ml)	0.32abc	48.6
11	Cryptogran (10 ml) + Dithane (200 g) + Agral 90 (18 ml)	0.17c	73.0
12	Cryptogran (10 ml) + Silicon (200 ml) + Agral 90 (18 ml)	0.32abc	48.6
13	Cryptogran (10 ml) + H & R medium spray oil (300 ml)	0.4abc	35.1
14	Cryptex (3.3 ml) + Voermol molasses (250 ml) + Agral 90 (18 ml)	0.3bc	51.4
15	Cryptogran (10 ml) + DiPel (12.5 g) + Agral 90 (18 ml)	0.43abc	48.6
16	DiPel (12.5 g)	0.58ab	8.1
17	Cryptogran (5 ml) + Cryptex (1.65 ml) Voermol molasses (250 ml) + Agral 90 (18 ml)	0.28bc	54.1
18	Cryptogran (10 ml) + Cryptex (3.3 ml) Voermol molasses (250 ml) + Agral 90 (18 ml)	0.20c	73.0

*Different letters in the same column denote significant differences between values ($P>0.05$, Duncan's multiple range test).

Field trial 5: Patensie

FCM infestation in the trial was low, but FCM control by Cryptogran was very good, with the two Cryptogran applications resulting in a 65.4% reduction in infestation over a 16 week period (Table 3.2.5.21; Fig 3.2.5.6).

Table 3.2.5.21. FCM infestation for a Cryptogran treatment and an untreated control in an orchard of Palmer navel orange trees on Paksaam Farm in the Gamtoos River Valley, evaluated from 20 December 2007 to 09 April 2008.

Treatment no	Treatment (dosages per 100 L water)	FCM infestation (mean fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	0.15a	
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	0.05b	65.4

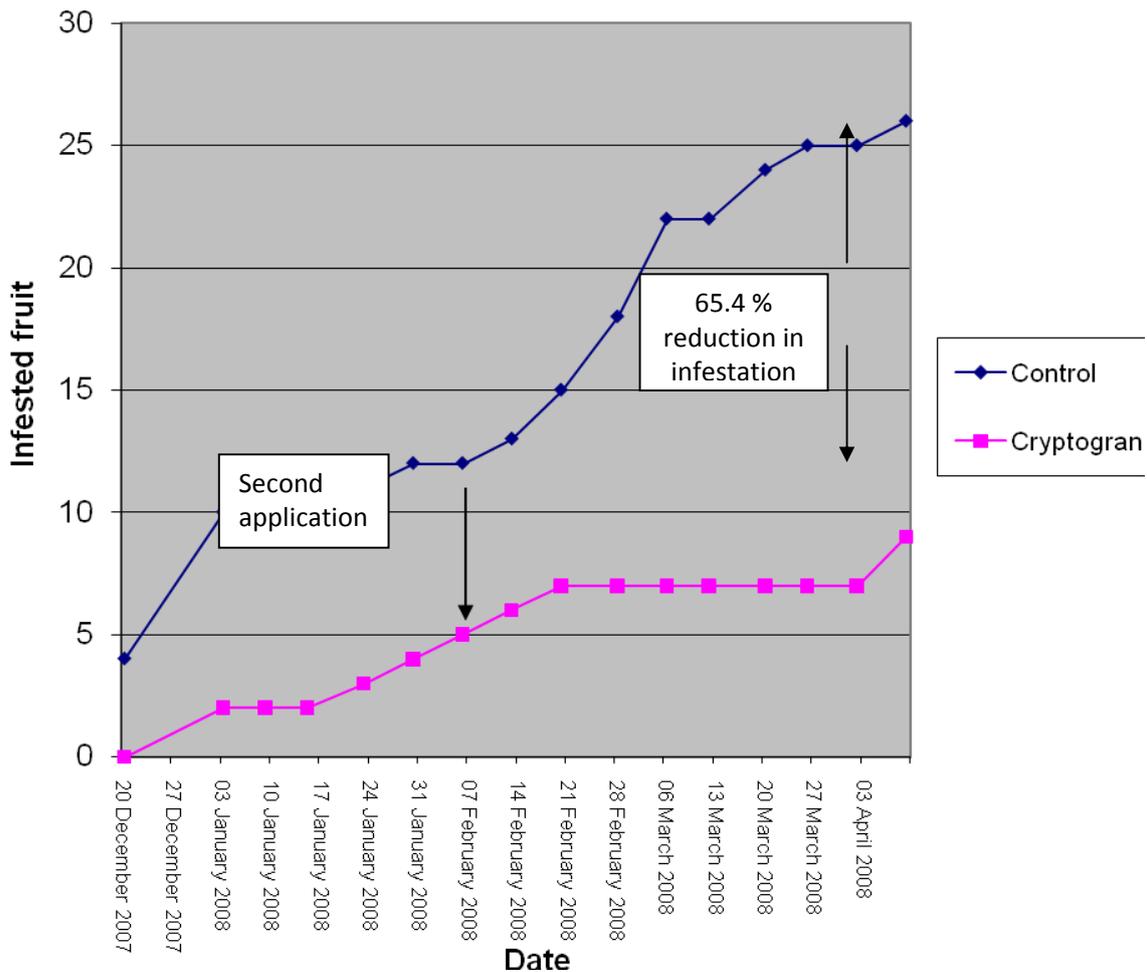


Fig 3.2.5.6. Cumulative number of infested fruit for a Cryptogran programme and an untreated control in an orchard of Palmer navel orange trees on Paksaam Farm in the Gamtoos River Valley, evaluated from 20 December 2007 to 9 April 2008.

Field trial 6: Cryptogran in Isomate-treated orchards

Unfortunately FCM levels remained very low throughout the period of evaluation, so the results were meaningless. The trial was evaluated for 6 weeks, from 6 January to 10 February 2009. During this period, there was an average FCM infestation of 0.15 fruit per tree per week for both the Isomate treatment and the Isomate + Cryptogran treatment. The trial will have to be repeated where higher FCM levels are present. If results are good, this integrated control option could be considered in areas where FCM pressure is high and where fruit is destined for sensitive markets.

Trial 7: Brakfontein, Citrusdal (Stephan Honiball)

The trial was evaluated for a 21-week period. FCM infestation over this full period was extremely low, impeding meaningfulness of results. Over the full evaluation period, fewer than 1.2 infested fruit were recorded per tree in the untreated control (Fig. 3.2.5.7). This is an average of less than 0.06 infested fruit per tree per week. Traditionally the threshold for intervention in the Citrusdal area was considered to be 1.0 infested fruit per tree per week. During 16 out of the 21 weeks of evaluation, no infested fruit were recorded in the untreated control. In addition, moth catches in pheromone traps were very low (Fig 3.2.5.8), with a slight increase in catches towards the tail-end of the trial, coinciding with a small spike in fruit infestation recorded during the last week of evaluation.

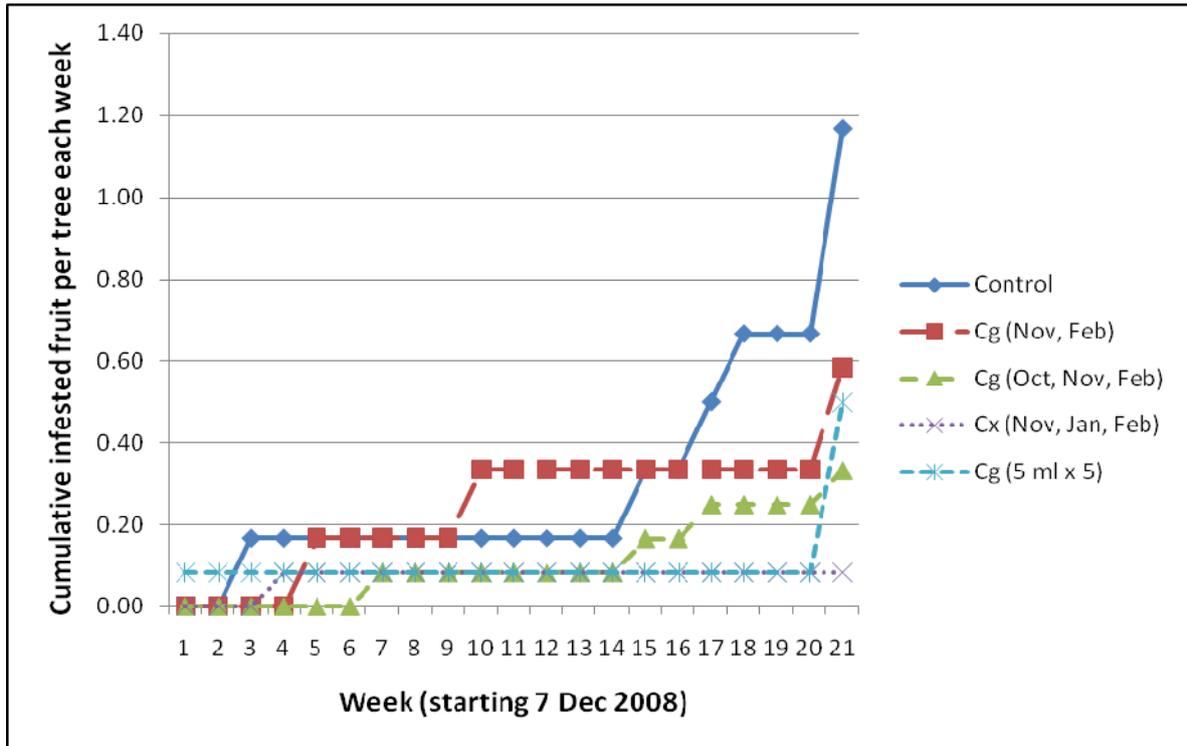


Fig. 3.2.5.7. Cumulative FCM infestation per tree per week for various virus programmes in an orchard of Robyn navel orange trees on Brakfontein Farm, Citrusdal, evaluated from 21 December 2007 to 9 May 2008 (Cg=Cryptogran; Cx=Cryptex).

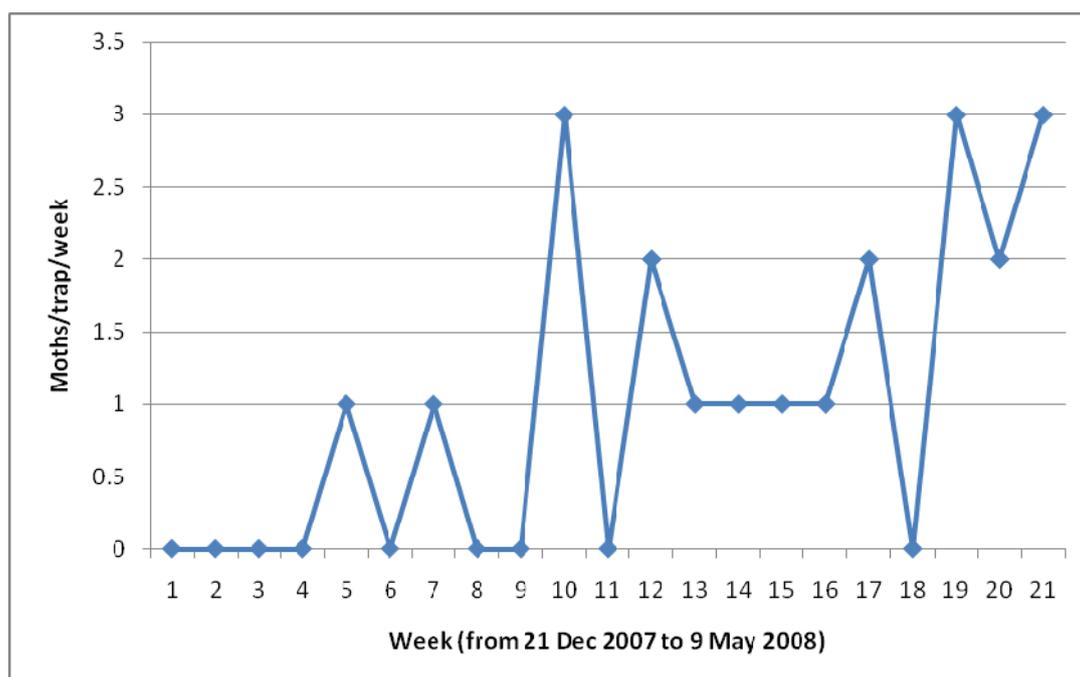


Fig 3.2.5.8. Number of adult male FCM caught in a delta trap using a Lorelei pheromone dispenser, each week from 21 December to 9 May.

Due to the very low level of FCM, no differences in infestation were statistically significant (Table 3.2.5.22). FCM infestation was reduced by between 50.0% and 92.9%, Cryptex being responsible for this highest level of reduction in infestation. This was in contrast to the majority of previous (and subsequent) comparisons in field efficacy on citrus between Cryptogran and Cryptex. Number of infested fruit recorded for treatment 5 (Cryptogran at 5 ml per 100 L water) was no different to that for treatment 4 (Cryptex), until the very last week of evaluation, when 5 infested fruit were recorded from the 12 data trees in treatment 5.

Table 3.2.5.22. Mean FCM infestation per tree per week for various virus programmes applied in an orchard of Robyn navel orange trees on Brakfontein Farm, evaluated from October 2007 to April 2008.

Treatment	All doses per 100 L water	Time of application	FCM infestation (mean fruit/tree/week)*	Reduction in infestation (%)
1	Untreated control		0.055a	-
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	Nov Feb	0.028a	50.0
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	Oct Nov Feb	0.016a	71.4
4	Cryptex (3.3 ml) + molasses (500 ml)	Nov Jan Feb	0.004a	92.9
5	Cryptogran (5 ml) + molasses (250 ml) + Agral 90 (18 ml)	Oct Dec Jan Feb Mar	0.022a	60.9

*Values in the same column followed by the same letter are not significantly different ($P > 0.05$; Bonferroni LSD multiple range test).

Trial 8: Ouwerf, Citrusdal (Stephan Honiball)

As it was not possible to retain an untreated control for this trial, it was not possible to measure the efficacy of the two products (applications). However, it was determined that there was no significant difference in infestation of fruit in the two treatments (Fig. 3.2.5.9). An average of 0.77 infested fruit per tree per week was recorded for Cryptogran, whereas an average of 0.82 infested fruit per tree per week was recorded for Cryptex – only a 5.4% difference. These can be considered as moderate, albeit unacceptable (from a phytosanitary perspective), levels of infestation.

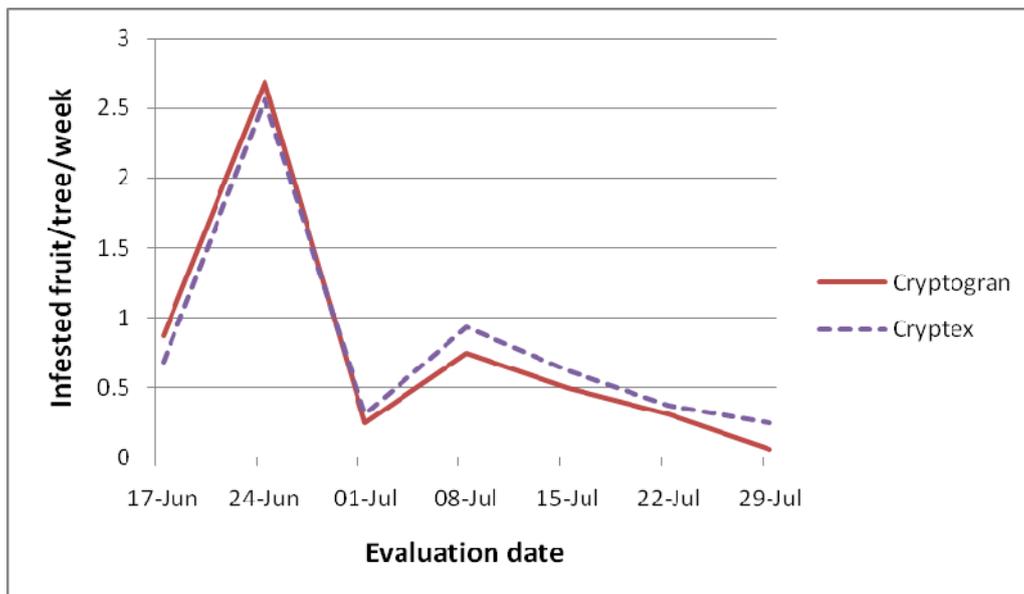


Fig. 3.2.5.9. FCM infestation per tree per week for various virus programmes in an orchard of Robyn navel orange trees on Ouwerf Farm, Citrusdal, evaluated from 17 June to 29 July 2008 (Cg=Cryptogran; Cx=Cryptex). FCM infestation in the A1 and B1 (southern) blocks was substantially higher than in the A2 and B2 (northern) blocks (Fig. 3.2.5.3). This may be explained by the poor sanitary practices in place on the adjacent farm. Comparing A1 (Cryptogran) and B1 (Cryptex) only, infestation was 1.4% lower for Cryptogran. Comparing A2 (Cryptogran) and B2 (Cryptex) only, infestation was 17.4% lower for Cryptogran.

FCM trap catches in this trial were very low. During only three of the weeks were any FCM caught: 1 moth on each of 27 May and 24 June and 2 moths on 15 July. This may be explained by the use of the mating disruption product, Isomate, in all other orchards on this farm.

Trial 9: Vaalharts (Johanna Mathewson)

FCM infestation at the trial site was generally low, peaking at an average of 0.8 infested fruit per tree in the untreated control during the first and third weeks of January 2009 (Fig. 3.2.5.9). Mean FCM infestation in the control over a 15 week evaluation period (excluding weeks 14 and 15 due to no FCM infestation) was 0.35 infested fruit per tree per week (Table 3.2.5.23). FCM infestation was lower in the Cryptogran blocks during each but one week. This was week 7, when infestation in sprayed and unsprayed blocks was equally low. During the last 6 weeks of evaluation, no infested fruit were recorded in the Cryptogran treated blocks, whereas FCM was recorded in the unsprayed blocks during 3 of these 6 weeks (Fig. 3.2.5.9).

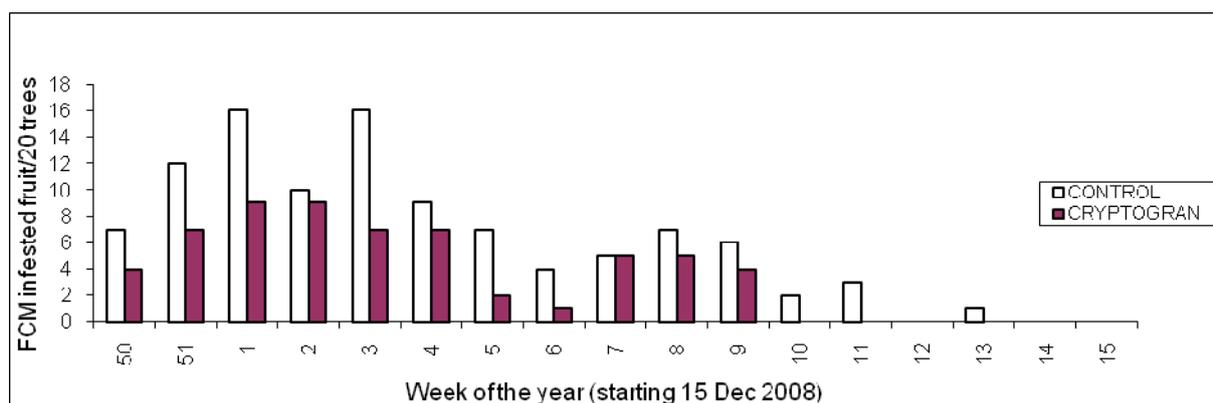


Fig. 3.2.5.9. Weekly FCM infestation per 20 data trees for a Cryptogran treatment and a control treatment in an orchard of navel orange trees on Magogong Farm, Vaalharts. Treatments applied on 24 November 2008 and 9 February 2009. Evaluations conducted from 15 December 2008 to 6 April 2009.

A t-test was conducted to determine whether there was any significant difference in FCM infestation of fruit between the control and Cryptogran treatments over a 15-week period. Data from weeks 14 and 15 was excluded, as no infested fruit were found during these last 2 weeks of evaluation (Fig. 3.2.5.9). At the 95% confidence level, there was no statistically significant difference. However, means were significantly different at the 90% confidence level.

Table 3.2.5.23. Mean FCM infestation per tree per week for Cryptogran and in the untreated control in an orchard of Robyn navel orange trees on Brakfontein Farm, evaluated from 15 December 2008 to 6 April 2009.

Treatment	All doses per 100 L water	Time of application	FCM infestation (mean fruit/tree/week)	Reduction in infestation (%)
1	Untreated control		0.35	
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	24 Nov 08 9 Feb 09	0.20	43.0%

Conclusion

A protocol and a dose-range has been developed for droplet-feeding bioassay, which should prove more accurate in showing smaller differences between treatments in dose-response and UV-bioassays. An additional, early application of Cryptogran coinciding with a minor FCM activity peak in October could be beneficial where FCM pressure is high, particularly as a result of poor FCM control during the previous season. Where FCM pressure was relatively low, reduced rates of Cryptogran applied more frequently were shown to be more effective than a standard Cryptogran programme at registered rates. Halving the molasses concentration reduced the efficacy of Cryptogran, but less so for the thicker Molatech molasses (available from River Bioscience) than Voermol molasses. The addition of Dithane to Cryptogran, instead of molasses and Agral 90, gave equivalent efficacy. This treatment will need to be replicated in further trials. Cryptogran was shown to be more effective than Cryptex in two trials. During the course of this study, in excess of twenty adjuvants and UV-protectants have been tested in combination with Cryptogran, and in virtually all cases molasses and Agral 90, as registered, has performed as well as any treatment and better than most. Management practices, such as timing of applications and effective spray coverage appear to be more important in controlling FCM, than the addition of UV-protectants and adjuvants.

Acknowledgments

River Bioscience is thanked, for the supply of Cryptogran for trials. Growers in the Sundays River Valley and the Gamtoos River Valley are thanked for making their orchards available and for assisting with the management of trial sites

Further objectives (milestones) and work plan

Further research will be continued to test potential UV-protectants and adjuvants, including sugar-alcohols, both in laboratory bioassays and in field trials, in an effort to improve the formulation of Cryptogran. The effect of lower concentrations applied more frequently, and the effect of October sprays will be tested again. Simulated rainfall trials will be repeated, as will trials to examine whether the navel end of navel oranges provides any protection of the virus against UV irradiation. The latter trials were initiated during the previous research cycle. Trials on other FCM susceptible varieties, such as Turkey Valencias and grapefruit, will be conducted. Cryptogran applications in Isomate-treated orchards will be re-evaluated.

Technology transfer

Wayne Kirkman and Sean Moore made various presentations at grower meetings. See Section 9 on Technology Transfer for details. Wayne Kirkman presented a poster at the International Congress of Entomology, entitled "Understanding and improving the residual efficacy of the *Cryptophlebia leucotreta* granulovirus" and made an oral presentation at the 5th Citrus Research Symposium, entitled "Factors influencing the field persistence of Cryptogran".

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3.2.6 PROGRESS REPORT: The use of entomopathogenic nematodes for the control of false codling moth

Experiment 793 (January 2007 – March 2009): A P Malan (US) and S D Moore (CRI)

Opsomming

'n Veldproef vir die beheer van valskodlingmot met *Heterorhabditis bacteriophora* is uitgevoer. Hokkies, gevul met boordgrond en 20 valskodlingmot larwes, is begrawe elk van 32 bome. Konsentrasies van 0, 20, 40 en 60 nematodes/cm² is met 'n gieter rondom elke boom toegedien. Na 48 uur is die hokkies uit die grond verwyder en evalueer vir mortaliteit 3 en 6 dae daarna. Insek mortaliteit is uitgedruk as die persentasie valskodlingmot larwes geïnfekteer met nematodes en die totale getal larwes wat terug gevind is. Op dae 7, 14 en 21 na die oorspronklike toediening is gevulde hokkies met valskodlingmot larwes weer onder dieselfde bome begrawe en op dieselfde manier evalueer vir mortaliteit. Daar is gevind na 2 dae in die grond hoë persentasie mortaliteit vir al drie nematode konsentrasies (tussen 90-99%) verkry word. Sewe dae na toediening het die effektiwiteit van die nematodes by die laagste konsentrasie, van 90% tot 16% gedaal; en vir die hoër konsentrasies na ongeveer die helfte van die oorspronklike effektiwiteit (57 en 59% onderskeidelik) gedaal. Na 14 en 21 dae het die effektiwiteit van die nematodes skerp gedaal. Na vier maande is gevulde hokkies weer by dieselfde bome begrawe en vir 6 dae in die grond gelos voor hulle opgegrawe en op dieselfde dag evalueer is. Daar is gevind dat die persentasie mortaliteit, insluitend die kontrole, van 37 - 45% gewissel het. Die onmiddellike effek van *H. bacteriophora* op die mortaliteit van valskodlingmot in die eksperimentele opset was baie beter as wat verwag is. Daar word vermoed dat die tydperk wat die hokkies in die grond gelaat is moontlik te kort was en dat dit verantwoordelik was vir die lae nawerking wat verkry is.

Summary

A semi-field trial for the control of false codling moth with *Heterorhabditis bacteriophora* was conducted. Cages filled with orchard soil and 20 final instar false codling moth larvae were buried underneath 32 trees. Concentrations of 0, 20, 40 and 60 nematodes/cm² were added to the soil with a watering can. Cages were left in the soil for 48 hours, removed and evaluated for mortality after 3 and 6 days. Insect mortality was expressed as the percentage of insects infected with nematodes and the total number of insects retrieved. On days 7, 14 and 21 after the original application, loaded cages were again buried underneath the same trees and evaluated in the same way for mortality. It was found that after the cages were in the soil for 2 days high mortality of false codling moth larvae, ranging from 90-99%, was recorded for the three nematode concentrations. On day 7 the efficacy of *H. bacteriophora* dropped for the lowest concentration of 20 IJ/cm² from 90% to 16% and for the two higher concentrations to approximately half of the initial efficacy (57 and 59% respectively). After 14 and 21 days the infectivity of the nematodes showed a sharp decline. After four months loaded cages were buried beneath the same trees and left in the soil for 6 day. It was found that the percentage mortality ranged from 37 - 45%, including the control. The immediate effect of *H. bacteriophora* on the mortality of false codling moth larvae was better than expected. The time period the cages were left in the soil was probably too short, which could be responsible for the low persistence found in this trial.

Introduction

Larvae of false codling moth feed inside the fruit, making chemical control very difficult. Current control includes chemical, mating disruption and biological control by using a *Cryptophlebia leucotreta* granulovirus, in an integrated pest management programme (Moore *et al.*, 2004). Problems with the development of resistance (Hofmeyr and Pringle, 1998) against commonly used insecticides and the success of the sterile insect technique make biological control a necessary option. Not one of the current control measures is targeted against the soil stages of false codling moth. Soil is the natural habitat for entomopathogenic nematodes. When the larvae leave the fruit, they fall onto the soil to burrow and pupate, offering entomopathogenic nematodes a window period to be used as a biological control agent against false codling moth pupae and emerging moths.

Entomopathogenic nematodes have not previously been considered for the control of false codling moth in field trials. In this study the viability of the use of entomopathogenic nematodes for the control of the soil stages of control of false codling are being investigated. The objective of this study was to test different concentrations of

a laboratory selected endemic nematode species, *Heterorhabditis bacteriophora*, in a semi-field trial. For false codling moth with up to five generations per year, persistence will especially be of value. The persistence of the nematodes was tested in the citrus orchards to determine if a long term effect could be achieved.

Materials and methods

Containment of insect larvae in the field trials: Wire mesh (40 mesh/425 µm aperture size) cages (Duncan *et al.*, 2003) were made by rolling 11 x 8 cm pieces of wire in a cylinder, fitted on both sides with plastic caps, glued together, except for one plastic cap, giving access to the cylinder. Unsterilized sieved soil from the trial orchard was used to fill the cylinder, together with 20 final instar false codling moth larvae. The cylinder was closed and secured with a rubber band to prevent larvae escaping. The cages were lightly sprayed with water and left in a closed container for 24 hours to give the larvae time to spin into cocoons.

Study orchard: Field trials were conducted in a 16 year old, high density Mihowase Satsuma citrus orchard (0.629 ha) on Carrizo citrange rootstock at the Welgevallen Stellenbosch University Experimental Farm. The initial application of the Trial 1 was during November 2008 and of Trial 2 February 2009. The orchard is planted 1.5 m between trees and 4 m between rows. Temperature and humidity for each row were monitored by using Hobo H8 Pro Series data loggers mounted on the lowest scaffold branch of the tree in the middle of each of four rows only for the period in which the cages with false codling moth larvae were buried in the orchard. The orchard was irrigated with micro jet sprinklers beneath each tree, every two days, with 2 mm water per hour for two hours. Sub-samples of soil were taken at each of 32 trees to make a representative sample of the orchard of 1 kg. Half of the soil was used for a five fraction soil analysis done by Bemlab, Stellenbosch. The other half of the soil was split into four plastic containers and five *G. mellonella* larvae added to determine the presence of endemic nematodes.

Nematode concentration: Cages loaded with sieved unsterilized soil from the trial orchard and 20 final instar false codling moth larvae were buried 10 cm from the base of each tree, just beneath the soil surface. The experimental design consisted of eight tree-plots for each treatment with two buffer trees between each tree in a complete randomised design. Three treatments of 20, 40 and 80 IJ/cm² and a control treatment of water only were applied. A watering can was used to add 500 ml of water, with the appropriate number of nematodes, in a 20 cm radius around the base of the tree on the same day after the cages were buried. The cages were retrieved from the soil after 2 days and sealed in plastic containers for another 24 hours in a growth chamber at 25 ± 2°C. Care was taken to use a different spade to dig up the cages for the control treatments. The soil was removed from the cage and then washed through a sieve to retrieve false codling moth larvae and cocooned insects. The cocoons were placed on a filter paper in a petri dish and opened with the aid of a stereo microscope to ensure that the larvae or pupae inside remained intact when removed. Mortality caused by infection was visually determined by the colour change in the larvae to brick red. Natural death was confirmed by larvae turning black or putrefying and touching the pupae to confirm death. The petri dishes were returned to the growth chamber for another 3 days and again evaluated for mortality and infection confirmed by dissection.

Nematode persistence: Cages loaded with unsterilized sieved orchard soil and 20 false codling moth larvae each were placed at the same trees 7, 14 and 21 days after the initial application of the nematodes. Mortality caused by infection was determined in an identical manner to that used for the first set of cages. After a period of 3 months, loaded cages were buried at each tree in the trial and after 6 days in the soil the mortality by infection was determined.

Results and discussion

Nematode concentration: The three nematode concentrations of 20, 40 and 60 IJ/cm² caused a high level of mortality of false codling moth larvae, ranging from 90-99% (Fig 3.2.6.1). No significant differences were found between the three *H. bacteriophora* concentrations applied. There were also no significant differences in mortality after 2, 3 and 6 days in the soil. An endemic nematode population was detected at three trees in trial 1 of the controls (4%). This endemic nematode was identified as *H. bacteriophora*. Usually results in field trial are somewhat disappointing but in this trial better than expected results were obtained. Concentrations could even be lowered to get an optimum field concentration.

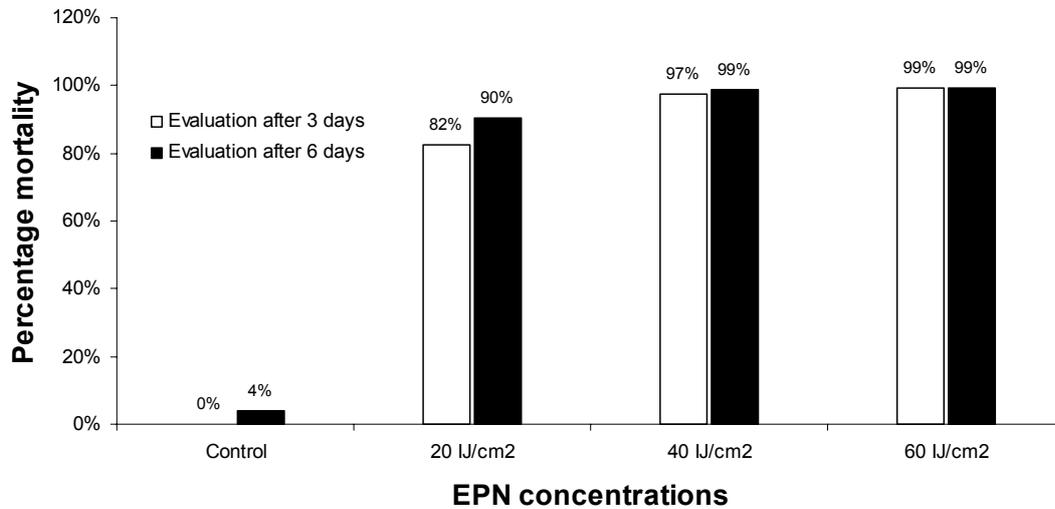


Fig. 3.2.6.1. Percentage mortality of caged false codling moth larvae in soil, inoculated with four concentrations of 0, 20, 40 and 60 nematodes per cm². Cages with insect larvae were removed from the soil after two days and mortality was evaluated on days 3 and 6.

Persistence: On day seven, the efficacy of *H. bacteriophora* dropped for the lower concentration of 20 IJ/cm², from 90% to 16% and for the two higher concentrations to approximately half of the initial efficacy (57 and 59% respectively). After 14 and 21 days, efficacy dropped to below 40 and 20% respectively, for all concentrations. On day 21 mortality of false codling moth larvae were very low (Fig. 3.2.6.2). Much better results regarding persistence were found in laboratory bioassays.

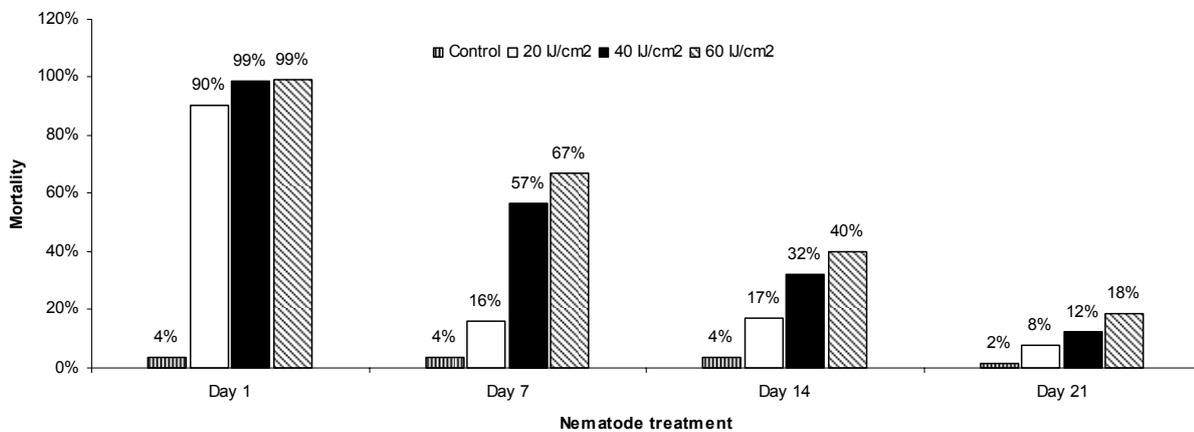


Fig. 3.2.6.2. Percentage mortality of caged false codling moth larvae 7, 14 and 21 days after the initial inoculum of four concentrations of 0, 20, 40 and 60 nematodes per cm² had been applied. Cages with larvae were left for two days in the soil and evaluated after 7 days for mortality.

After a period of four months, it was found that the percentage mortality varied between 37 and 45% with no significant difference (Fig. 3.2.6.3) between treatments, including the control. The cages were left in the soil for 6 instead of 2 days, which could have led to a higher mortality of false codling moth larvae. These results were very surprising and could be attributed to the evaluation method used. Duncan *et al.* (2003) left cages with one larva of the citrus root weevil for 7 days in citrus orchards and evaluated them for persistence 2 days after removal from the orchard.

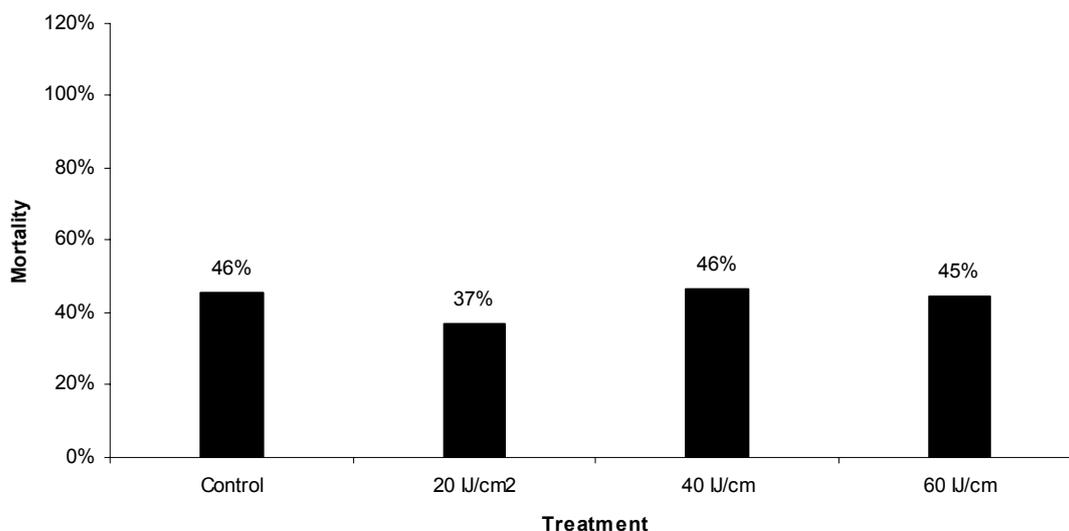


Fig. 3.2.6.3. Percentage mortality obtained for caged false codling moth larvae, three months after the original inoculum of 0, 20, 40 and 50 IJ/cm² nematodes had been applied. The cages were left in the soil underneath each of the 32 trees for 6 days and larvae were evaluated for infection on the day of removal.

Conclusion

Better than expected mortality of false codling moth larvae was obtained in a field trial, after application of *H. bacteriophora*. Lower concentrations of nematodes should therefore be tested. Field persistence was not as good expected, in comparison with laboratory tests. The evaluation method should however be adapted, as that could be responsible for low mortality evaluated.

Further objectives (milestones) and work plan

Semi field trials will be conducted with different nematode species and lower concentrations of nematodes. The application area under each tree will be enlarged and persistence will be evaluated using different evaluation methods.

Technology transfer

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3.2.7 **PROGRESS REPORT: Improvement of cold treatment conditions for the disinfestation of false codling moth in citrus fruit, using a potentiating CO₂ shock treatment**
Experiment 858 (June 2006-December 2009): Chirene Jelbert (SU)

Opsomming

Die valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick), is 'n inheemse plaag in Suid Afrika en endemies in Afrika, en is dus as kwarantyn plaag geag in uitvoer markte. 'n 24-dag koue periode teen -0.5 °C is tans die enigste na-oes disinfestasië behandeling vir VKM in sitrus. Die behandeling belemmer egter vrug kwaliteit, en nuwe markte vereis nou ook VKM disinfestasië prosedures. Daar is dus 'n groot behoefte vir die ontwikkeling van 'n alternatiewe na-oes disinfestasië behandeling wat minder skade rig aan vrugte self en meer meetbaar is vir markte met kort verskeepings tye.

'n Moontlike alternatiewe na-oes disinfestasië metode is ontdek toe Spaanse navorsers sensitiwiteit in *Ceratitis capitata* (Wiedemann) binne Fortune manderyne gevind het na potensieerende CO₂ skok behandelings. Die mees koud bestande VKM larwes is aan veskeie CO₂ skok, en koue behandelings blootgestel. 'n Aansienlike toename in persentasie mortaliteit was aanskou in die mees koue bestande VKM larwes wat aan die behandelings blootgestel is. Die mees doeltreffende behandeling word geïdentifiseer vir verdere werk op vrugte in verband met die effek van die behandeling op vrug kwaliteit en VKM sensitiwiteit binne die vrug.

Summary

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), is an indigenous pest to South Africa and endemic to Africa, and thus considered a quarantine pest by foreign markets. A 24-day cold treatment at -0.5 °C is currently the only post-harvest disinfestation treatment for FCM in citrus. This treatment is, however, detrimental to fruit quality, yet new markets also require FCM disinfestation procedures. There is thus an urgent need to develop alternative post-harvest disinfestation treatments which are less detrimental to fruit quality and more compatible to markets with short shipping times.

A possible alternative post-harvest disinfestation method was identified when Spanish Researches identified the sensitivity of *Ceratitis capitata* (Wiedemann) in Fortune mandarins to potentiating CO₂ shock treatments. The most cold tolerant FCM larvae were exposed to varying CO₂ shock treatments, followed by subsequent cold treatments conditions. A significant increase was found in the percentage mortality of 4th and 5th instar FCM larvae exposed to potentiating shock treatments with subsequent cold treatments. The most efficient treatments are being identified with which future bioassays will be conducted on fruit, to monitor the effect of such a combination treatment on fruit quality and the sensitivity of FCM larvae to these treatments inside the fruit.

Introduction

The False Codling Moth (FCM), *Thaumatotibia leucotreta* (Meyrick), is endemic to Africa and thus considered a quarantine pest by foreign markets. For any citrus shipment from Africa to the United States of America, a phytosanitary requirement exists where the cold sterile treatment, T107-k, of 24-days at -0.5°C must be applied. This treatment is currently the only accepted post-harvest disinfestation treatment for FCM and fruit-fly.

Permit regulations stipulate the treatment, T107-K, may not commence until all sensors are reading -0.5°C or below in the pulp of the fruit. When any given sensor logs a temperature that exceeds -0.27°C, the treatment shall be extended one third of a day for each day or part of the day the temperature is above -0.27°C. If the exposure period is extended, the temperature during extension must be 1.11°C or below. If the temperature exceeds 1.11°C at any time, the treatment is nullified and ships may be commanded to return the fruit to their country of origin. These treatment measures cause chilling injury and are detrimental to essential fruit quality aspects such as the rind colour which becomes pale.

Markets, such as China and Taiwan are now also demanding phytosanitary FCM disinfestation procedures. As no post harvest control strategies exist other than the damaging cold sterile treatment, T107-k, there is a dire need for an alternative post harvest disinfestation treatment.

Spanish researchers found that carbon dioxide gas (CO₂) diminishes the cold tolerance of third instar larvae of *Ceratitis capitata* (Wiedemann) in Fortune mandarins. CO₂ causes the insect's spiracle muscle to open, which in turn causes uncontrolled water loss and dehydration in larvae.

The research by Alonso *et al.* 2006, prompted this South African research project to look at using a combination of a potentiating CO₂ shock treatment in conjunction with a cold sterilisation treatment at -0.5°C. Hypothetically the treatment would cause an increase to the cold sensitivity of FCM larvae, leading to premature mortality.

If the treatment proved to be effective this could possibly alter the required length of treatment T107-k and reduce the detrimental effects on fruit quality. The treatments have to however meet the Probit 9 requirement. Initial experimental parameters were set very wide, as no previous data existed on a response dosage for FCM. As dose response data was collected, the need to change the experiment was recognized, and experiments were developed in phases.

Materials and methods

The initial research process involved rearing FCM in jars. The rearing process was investigated through a literature study and visit to the CRI offices in Port Elizabeth, where the rearing technique was demonstrated to the author. The technique was adopted, and FCM larvae were reared from eggs supplied by CRI PE. The experiments were conducted in phases to establish the most effective treatment.

Phase 1: Prepared jars of mature larvae were exposed to different CO₂ concentrations of 95%, 75%, 50% and 25%, for 8, 16, 20, and 24 hours at both 20°C and 25°C. Larvae were then subjected to either 1°C or -0.5°C for 10, 12, 14, 16, 18, 20 or 22 days and assessed for percent mortality over time. Lemon fruit were also subjected to the same treatments and assessed for fruit quality before and after treatments.

Phase 2: To make the experiment more manageable, a number of co-variables were removed from the initial experiment. Co-variables were removed on the basis of a number of critical observations that were made in the original experiment. The treatment co-variable of 95% CO₂ was removed from the experiment. From the remaining CO₂ treatments it was decided to keep the two most extreme points to try and show significant differences between treatments. Carbon dioxide is normally present in the atmosphere at a concentration of 0.03%. Thus for the control, ambient air (0.03% CO₂) was also included. Where the prepared jars of 4th - 5th instar larvae were exposed to the CO₂ gas treatments for varying time, the 8 and 24 hours co-variables were used. The CO₂ gas treatments were applied at a temperature of 20°C. The cold treatment temperature selected was -0.5°C, as this is the standard required temperature in export disinfestation treatments. Exposure times to cold treatments were also shortened to 6, 7.8, 10.14, 13.18 and 17.13 days at -0.5 °C to better fit a Probit regression model.

Phase 3: The CO₂ shock treatments were considered in different levels to motivate the use of a specific treatment for further experiments. A major consideration for the treatments that was used is the health effects associated with exposure to carbon dioxide. Research states that even a 'minimal' concentration of 2.8% CO₂ can cause dyspnoea in humans after a mere 30 minutes of exposure. In exposures ranging from 17% to 30% found that within 1 minute there could be a loss of controlled and purposeful activity, unconsciousness, coma, convulsions, and death.

Phase 4: It was decided to use the CO₂ treatment considered potentially least harmful for human safety and least damaging effect to fruit, yet still showing a dosage response of increased percentage mortality in reduced time within the FCM larvae. This combination of treatments is 25% CO₂ at 8 hours at -0.5° C for the altered varying amount of time, namely 6, 7.2, 8.64, 10.37, 12.44 and 14.93 days. Another CO₂ exposure treatment of 4.5% CO₂ was also incorporated to establish a possible lower dosage response level.

Results and discussion

Phase 1: Initial results with the above mentioned treatments indicated an average mortality of 97.2%. However, due to the largest percentage of jars presenting 100% mortality, which could not be used in the intended Probit analysis, the experiment needed to be changed. The lemon fruit showed an extreme breakdown in flesh firmness with the high concentrations of CO₂, especially 95% CO₂.

Phase 2: It was also noted that there were a number of larvae that had not achieved the most cold tolerant 4th and 5th instar stage, due to the normally variable developmental rates of eggs and larvae. These 2nd - 3rd instars were less cold tolerant and showed a higher percentage mortality within both the control and other treatments. To allow for a more accurate representation of the most cold tolerant instars only, a distinction was made between 2nd - 3rd instars and 4th - 5th instars through obvious visual aspects of size and colour. These aspects were compared and confirmed by a literature study before being implemented.

The adapted experiment was repeated three times after the first two experiments showed some problems in the form of variance in diet and number of larvae per treated jars. These initial variances were due to a large number of jars being affected by fungal infection and dried out diet. These problems were solved when a faulty laminar flow bench filter was replaced and the diet and liquid were increased to better suit the cold temperature exposure times that the jars with larvae were exposed to. It was found that the diet tended to harden and dry out more readily within these prolonged periods of time in the cold and was possibly the cause of death in larvae, instead of the combination of CO₂ and cold treatments.

Phase 3: The altered experiment showed the desired results for the prepared jars with 4th - 5th instar larvae. Data suggested that the increased exposure of carbon dioxide made the cold hardy 4th - 5th instar larvae more sensitive to cold temperatures, causing increased mortality in shorter periods of cold treatments. However, no statistically significant difference was found between the CO₂ treatments of 25% and 75% CO₂, as it seems that at 6 days the surviving larvae had already passed the dose response point. No treatment could thus be classified as being the most effective.

Phase 4: A dosage response to the CO₂ shock treatment of 25% concentration remained evident when compared to the control treatment (Fig 3.2.7.1). However, the results of the CO₂ shock treatment of 4.5% concentration were surprising as the dosage response became less evident over time and more in line with the results of the control treatment.

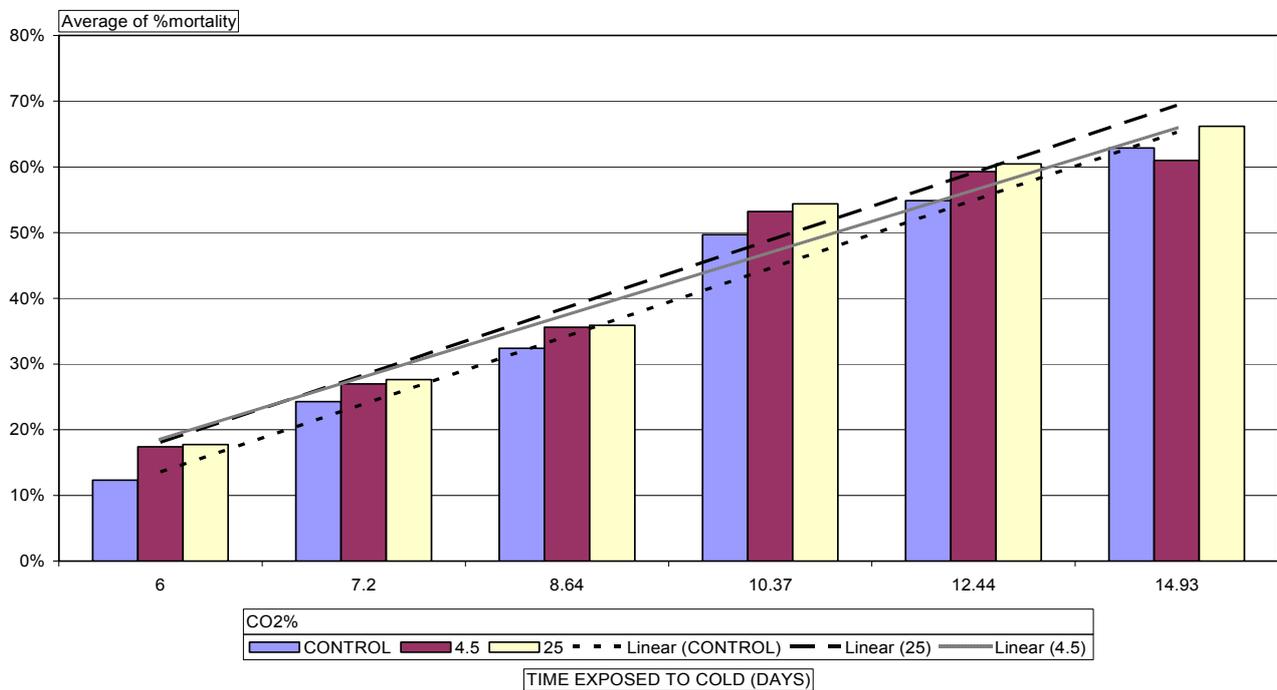


Fig. 3.2.7.1 Percent mortality in 4th - 5th instar larvae exposed to shock treatments of ambient (0.03% CO₂), 25% CO₂, and 75% CO₂, at a cold temperature of -0.5°C for varying amounts of time, namely 6, 7.2, 8.64, 10.37, 12.44 and 14.93 days.

A probit 9 analysis was attempted, using the Polo Plus Probit analysis programme. Although data in the Probit Regression appeared to be normal and fit a linear plot (Fig. 3.2.7.2), a large chi-square indicated a poor fit of

the data by the probit regression and large deviations occurred for expected probabilities near 0 and 1. If extrapolated, even the control indicated a possible Probit of 34 days.

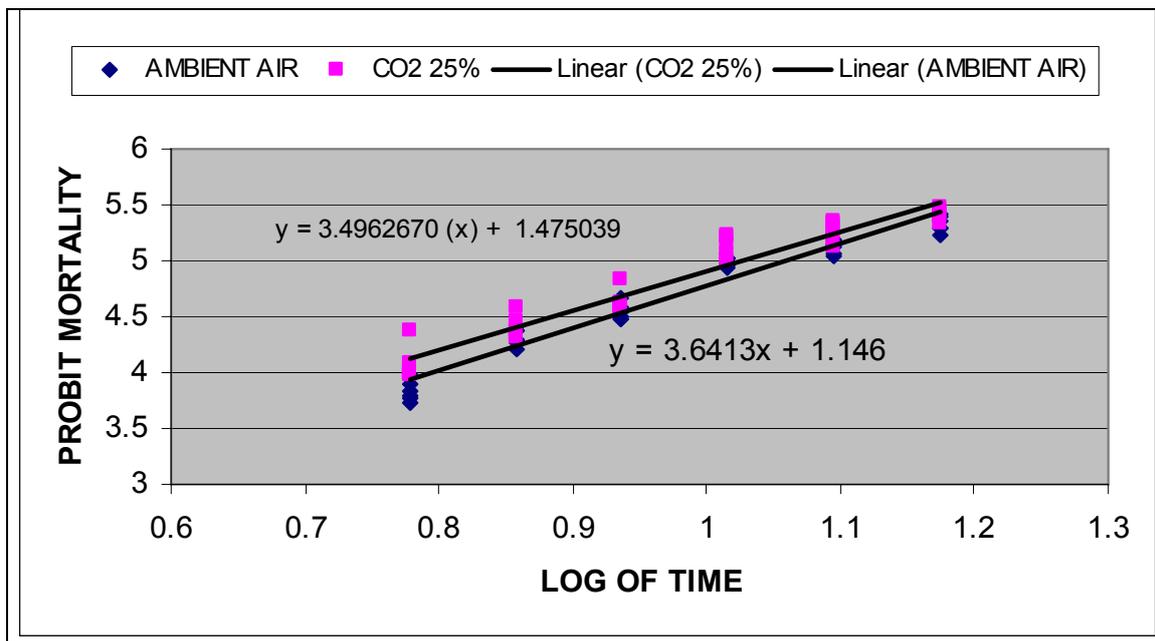


Fig. 3.2.7.2 Simple linear regression of probit mortality on the log of time of exposure for the CO₂ concentrations, 0.03 and 25%.

Results contradicted previous results from Phase 1 and 2. An investigation into these unexpected results was conducted. After discussions with assistants who had assessed percentage mortality, it was concluded that there was some confusion between 2nd to 3rd and 4th to 5th larval instars.

Conclusion

As the results of the experiments were contradictory, no conclusion could be made and the experiment will have to be repeated. It was also noted the control treatment uses air, containing oxygen (O₂), and the CO₂ shock treatments contain only CO₂ and N₂, with no O₂. No conclusion can thus be made on whether it is the CO₂ which is having an effect on the mortality of the FCM larvae or the anoxic effect due to the lack of O₂. The experiment thus has to be altered and repeated to accommodate this factor.

The new combination of treatments contains not only 25%, 4.5% or 0.03% CO₂, but also 21% O₂ with a balance of N₂. The larvae will be exposed to the gas treatments for 8 hours. The larvae will then be placed in a cold room at -0.5°C for 6, 7.2, 8.64, 10.37, 12.44 and 14.93 days. The experiment will also be conducted with 25%, 4.5% or 0.03% CO₂ with a balance of N₂ only.

Further objectives (milestones) and work plan

The altered experiment has been scheduled to be repeated in June and July 2009.

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3.2.8 FINAL REPORT: Geographic variation in the susceptibility of FCM populations to a granulovirus, CrleGV-SA

Experiment 878 (April 2007 – March 2009): JK Opoku-Debrah (NMMU) and Sean D. Moore (CRI)

Opsomming

Verskille in die gevoeligheid van valskodlingmot (VKM) bevolkings van verskeie sitrus produksiestreke in Suid-Afrika vir Cryptogran en Cryptex (albei kommersieel virus produkte vir beheer van VKM) is ondersoek. Eerstens is 'n maatstaf vir doeltreffendheid met laboratorium-geteelde VKM bepaal. Oppervlak dosis-respons biotoetse is met ongeformuleerde Cryptogran (CrleGV-SA) teen die 5 larwe stadiums van VKM uitgevoer. Die LC₅₀ en LC₉₀ waardes vir die biotoetse is uitgewerk as: 4.095×10^3 , 4.516×10^4 , 1.662×10^5 , 2.205×10^6 , 2.678×10^7 OBS/ml en 1.185×10^5 , 4.287×10^6 , 9.992×10^6 , 1.661×10^8 , 9.118×10^9 OBS/ml vir die 1^{ste}, 2^{de}, 3^{de}, 4^{de} en 5^{de} stadiums onderskeidelik. 'n Standaard protokol om die LC₅₀ en LC₉₀ waardes vir die 1^{ste} tot 5^{de} larwe stadiums met CrleGV-SA (Cryptogran) te bepaal is ontwikkel. Hierdie protokol is gebruik om biotoetse met boord-versamelde VKM larwes uit te voer. Gevoeligheid vir CrleGV-SA het met larwestadium afgeneem en het met tydsduur van blootstelling toegeneem. Die protokol is gebruik om 'n riglyn vir biotoetse met boord-versamelde VKM larwes te gee. Laboratorium biotoetse met Cryptogran (teen 1.661×10^8 OPs/ml) teen boord-versamelde VKM larwes van Addo, Kirkwood, Citrusdal, Clanwilliam en 'n ou laboratorium kultuur, het in een geval 'n betekenisvolle verskil in virulensie getoon. Hierdie verskil is tussen 5^{de} instars van die Addo populasie en die ander populasies (insluitend die ou laboratorium kolonie) opgelet. Vier geografiese uitkenbare VKM kolonies is van Addo, Citrusdal, Marble Hall en Nelspruit gestig. In laboratorium biotoetse met Cryptogran en Cryptex teen 1^{ste} stadium VKM larwes is Cryptogran teen albei die Addo kolonie en die ou kolonie, betekenissvol meer patogenies as Cryptex. Niesteenstaande is 'n groot variasie in respons met elke populasie gekry.

Summary

Differences in the susceptibility of false codling moth (FCM) populations in some key citrus growing areas in South Africa to Cryptogran and Cryptex - both commercially produced virus products used in the biological control of FCM, were investigated. An initial benchmark for pathogenicity was established with laboratory reared FCM. Surface dose-response bioassays were conducted with the five larval stages of FCM, using unformulated Cryptogran (CrleGV-SA). The LC₅₀ and LC₉₀ values for the assays were calculated to be: 4.095×10^3 , 4.516×10^4 , 1.662×10^5 , 2.205×10^6 , 2.678×10^7 OBS/ml and 1.185×10^5 , 4.287×10^6 , 9.992×10^6 , 1.661×10^8 , 9.118×10^9 OBS/ml for the 1st, 2nd, 3rd, 4th and 5th instars respectively. A standard protocol for determining the LC₅₀ and LC₉₀ values for the 1st to 5th FCM larval instars with CrleGV-SA (Cryptogran) has been established. This protocol was used in carrying out bioassays with field collected FCM larvae. Susceptibility to CrleGV-SA was found to decline with larval stage and increase with time of exposure. The protocol was used in guiding bioassays with field collected FCM larvae. Laboratory assays conducted with Cryptogran (at 1.661×10^8 OBS/ml) against field collected FCM larvae from Addo, Kirkwood, Citrusdal and Clanwilliam as well as an old laboratory colony, showed a significant difference in pathogenicity in only one case. This significant difference was observed between 5th instars from the Addo colony and the other field populations, as well as 5th instars from the old laboratory colony. Four geographically distinct FCM colonies from Addo, Citrusdal, Marble Hall and Nelspruit were also established. In laboratory assays with Cryptogran and Cryptex against 1st instar FCM larvae, Cryptogran was significantly more pathogenic than Cryptex against both the Addo and the old colony. However, a high level of heterogeneity was observed in responses within each population.

Introduction

The false codling moth (FCM), *Thaumatotibia* (= *Cryptophlebia*) *leucotreta* (Meyrick) (Lepidoptera: Tortricidae) continues to be a problem to the citrus industry, since the detection of a single larva in fruit marked for export can result in the entire consignment being rejected (Moore, 2002; Hattingh, 2006). The application of biological control agents such as Cryptogran (*Cryptophlebia leucotreta* granulovirus, CrleGV-SA) has been of much benefit to the citrus industry (Moore, 2002; Moore & Kirkman, 2004; Moore *et al.*, 2004).

Of late, there have been some global concerns regarding insect resistance to some of these very useful biological control agents (Fuxa, 1993). Fuxa reports of some observed variation in response between different *Spodoptera frugiperda* populations to three nucleopolyhedroviruses (NPVs) found in the same area. There are a number of reports where differences in susceptibility between geographically distinct insect host populations to baculoviruses were observed (Briese, 1986). According to Timm (2005), genetic studies with FCM in South Africa revealed some significant genetic variations. Genetic differences were observed over wider and local geographic regions in South Africa.

Recent reports indicate that some field populations of codling moths (CM), *Cydia pomonella* (L.) showed markedly reduced susceptibility to a commercially produced baculovirus, CpGV-M (Mexican isolate) (Fritsch *et al.*, 2005; Sauphanor *et al.*, 2006; Jehle *et al.*, 2006, 2008a & 2008b). In 2002 and 2003, the first accounts of CpGV-M showing reduced efficacy were observed in organic orchards (Jehle *et al.*, 2008b). In 2005, two CM populations with up to a 1000-fold reduced susceptibility to CpGV-M were reported in southern Germany (Fritsch *et al.*, 2005). By 2006, another resistant population was recorded in France (Sauphanor *et al.*, 2006). Most recently, 30 orchards with CpGV-M resistance have been recorded across Europe (Jehle, 2008b).

Cydia pomonella granulovirus (CpGV) just like *Cryptophlebia leucotreta* granulovirus (CrleGV-SA, Singh *et al.*, 2003) or Cryptogran and Cryptex (another CrleGV product which contains a different isolate to Cryptogran, produced by Andermatt Biocontrol Switzerland) are both viruses that belong to the same family Baculoviridae and genus granulovirus. These granuloviruses generally have a narrow host range with infection; being confined to one or more species within the same family as the original host. They are, also known only to infect insects in the order Lepidoptera (Federici, 1997).

Considering the previous reports of increased resistance to commercially produced baculoviruses in Europe, coupled with the marked genetic differences observed in some FCM populations in South Africa, it was imperative that we investigate whether a similar phenomenon occurs or could develop in South Africa, with FCM against Cryptogran and Cryptex.

In view of the above, the main objective outlined in this study was to investigate the susceptibility of various field populations of FCM from a range of geographic areas to Cryptogran and Cryptex. The protocol and results pertaining to this study are discussed.

Materials and methods

Establishment of geographically distinct FCM colonies

Four new and separate laboratory colonies of FCM were also established. These were established from FCM infested citrus fruit collected from individual orchards (each representing a geographically distinct population). Fruit were collected from Addo, Citrusdal, Marble Hall and Nelspruit for the establishment of new and separate laboratory colonies. All larvae used for the establishment of new and separate FCM colonies as well as those used for conducting assays were reared according to the protocol described by Moore (2002).

Small scale moth rearing using test-tubes

This procedure was employed in the establishment of an FCM laboratory colony from field collected individuals. The moth rearing process was carried out concurrently in separate rooms, with each room hosting one colony. The initial diet used for rearing the field collected larvae consisted of, 200 g dry ingredients of the standard diet plus 200 ml distilled water, uniformly mixed to form a paste (Moore, 2002). The contents were mixed in glass pie-dishes and heated in an oven at 180°C. After 25 minutes, the cooked diet was allowed to cool in a laminar flow cabinet. Once cool, individual diet plugs (approximately 5 to 7 mm thickness) were cut using the lip of a glass

test-tube (28 ml capacity) Thereafter, the diet plugs were inserted into the tubes (using sterilized glass rods) and pushed to the bottom of the tube. Fruit were individually cut open with the aid of sharp knives to locate larvae. A size 000 paint brush (sterilised in 2% sodium hypochlorite) was used in transferring individual larvae onto the diet plugs (Moore, 2002). Afterwards the test-tubes were corked with cotton wool. Thereafter, the larvae (held on the diets in the test-tubes) were sent to an incubation chamber with a temperature of about $27^{\circ} \pm 1^{\circ}\text{C}$ for development and growth to take place.

Upon emergence of adult moths the cotton wool stoppers were removed. To collect emerging moths a second test-tube was inverted over the first one to allow the moths to climb to the top of the inverted test-tube. Thereafter, all the moths emerging from the individual test-tubes were transferred into a sieve (approximately 15.5 cm in diameter) through a hole (approximately 25 mm diameter) cut in the middle of the sieve. The sieve was inverted onto a wax paper. The bottom of the test-tube was gently tapped with a finger to encourage the moths to fly or drop into the sieve. The hole in the sieve was then fitted with cotton wool soaked in water which served as a source of nourishment for the moths. The sieve was finally secured at both edges with sellotape in order to prevent moths from escaping through the space between the edge or mouth of the sieve and the wax paper.

Large scale moth rearing using jam jars

This procedure was employed as a continuation of the small scale moth rearing process, as well as, for the maintenance of stable laboratory colonies of FCM. For large scale moth rearing, 370 ml jam jars were used instead of the individual test-tubes. This was because each jam jar could hold enough larvae (approximately 300 to 400 FCM eggs), and was also found to be suitable for large scale moth rearing (Moore, 2002). FCM diet was prepared beforehand. Therefore, a mixture of 50 g standard dry diet and 50 ml distilled water (dH_2O) were weighed and dispensed into each jam bottle. Afterwards individual bottles were then stoppered with cotton wool and autoclaved at 121°C for 20 minutes and placed under a laminar flow hood to cool (Moore, 2002).

After 3 to 4 days most of the moths held in the sieve had started to lay eggs. Initially only a few eggs were laid (mostly singly) and were scattered on the wax sheet. In order to maximize the use of each available egg, the egg sheets had to be cut into several pieces before sterilizing in 10% formaldehyde solution (from 35 to 40% formaldehyde stock solution). The egg sheets were then transferred into the 370 ml jam jars (containing diet) using sterilized forceps (Moore, 2002). The jam bottles were finally sent to an incubation chamber with temperature of about $27^{\circ} \pm 1^{\circ}\text{C}$ for development and growth to take place.

After the first filial generation (F_1) the number of larvae pupating in the cotton wool stoppers fitted in the jam jars was inadequately small to maintain a stable laboratory colony. Therefore, individual pupae were gently removed (leaving a few cotton wool strands on the pupa) from the cotton wool by hand. The pupae were then held singly in the test-tubes until moth emergence.

At this stage, moths emerging from the pupa (attached to the cotton wool strands inside the test-tubes) were always held in the oviposition apparatus for egg laying. Eggs laid during the preceding 24 h on the wax paper sheets, in the oviposition apparatus were reared using the jam jars as previously outlined.

This process was repeated until the F_4 generation. After the F_4 generation the egg density and moth numbers had improved significantly, thus the use of the oviposition apparatus was discontinued. Instead an emergence box consisting of a ten compartment facility specially designed for the moths was used.

Several pieces of egg sheets (containing approximately 300 to 400 eggs) from the F_4 generation were cut into approximately 10 mm by 10 mm squares with a pair of scissors. The eggs were sterilized in 10% formaldehyde solution using sterilised forceps (Moore, 2002). One egg sheet was then placed into each jam bottle (containing diet) using sterile forceps. The jam bottles were then sent to the incubation chamber for development and growth to take place. When all the larvae in the jam jars had reached pupation, the jam bottles were finally sent to the moth emergence box. Thereafter, subsequent eggs laid during the preceding 24 h on the wax paper sheets fitted inside the wire mesh of the moth emergence box were always collected for the maintenance of the laboratory colonies or for use in conducting bioassays.

Bioassays

All laboratory trials in this study were conducted with the unformulated virus product. It was however not possible to obtain the unformulated Cryptex (unlike Cryptogran) as such the formulated product was acquired and purified accordingly. This was necessary since Cryptogran and Cryptex (commercially formulated products) are formulated with both CrleGV-SA (active ingredient) and UV protectants. The UV protectants enable the virus (CrleGV-SA) to persist in the field.

Twenty five larvae were tested at different concentrations of the virus, using a five-fold serial dilution technique, in order to establish a good dose-response curve. Individual larvae were then transferred onto standard FCM diets, which were altogether held in 30 ml capacity polypots (Evron, South Africa) (Moore, 2002). Hence, for the treatments the diets were surface inoculated with the virus whilst the controls were inoculated with distilled water. Bioassays conducted with the laboratory colonies of FCM, were replicated at least three times.

A lot of trial and error was involved in arriving at the appropriate series of concentrations that gave a good dose-response curve. This was therefore quite a lengthy process. In establishing a dose-response relationship for the laboratory reared FCM larvae, an agar-based diet was used. Assays were conducted with Cryptogran and Cryptex against 1st instar (neonate) larvae from both the old laboratory and the newly established Addo colony. Larvae were evaluated 7 days post-infection. Results from each bioassay were replicated at least three times. Bioassay results were only used if control mortality did not exceed 20%.

Purification, enumeration and serial dilution of virus inoculum

In order to get rid of all impurities and formulation additives and end up with a pure virus for bioassays, the virus purification protocol as described by Hunter-Fujita *et al.* (1998) and Moore (2002) using a glycerol gradient was adopted in this study. The two commercially produced CrleGV-SA products, Cryptex and Cryptogran were purified accordingly. A Thoma bacterial counting chamber (0.02 mm depth), at 400 times magnification under dark field light microscopy was used in counting virus particles.

Benchmark dose-response bioassays with FCM larvae

Firstly, a benchmark for pathogenicity was established. However, a benchmark for pathogenicity with CrleGV-SA (Cryptogran) against the 1st and 5th instar FCM larvae was previously established by Moore (2002). Therefore it was only necessary to establish another benchmark for pathogenicity against the other larval stages (2nd, 3rd and 4th). This benchmark was also used in guiding bioassays with CrleGV-SA (Cryptogran) against field collected FCM larval instars. Since Cryptogran and Cryptex are always targeted against 1st instar FCM larvae in the field, further comparative laboratory assays were conducted with two laboratory colonies - an old laboratory colony and a newly established colony from Addo.

Field collection of FCM larvae for laboratory assays

Mass collections of FCM infested citrus fruit were carried out in Addo and Kirkwood (both in the Eastern Cape) in order to isolate FCM larvae from them. Other collections were carried out in Citrusdal and Clanwilliam (both from the Western Cape). Although other mass collections of fruit were conducted in the Nelspruit and Marble Hall regions (both in the Mpumalanga Province), the number of larvae obtained from these fruit was inadequately small to warrant any reliable assays.

In assays conducted with the field collected FCM larvae, a non-agar based diet was used. This was because unacceptably high levels of mortality were recorded with the agar-based diet. Assays were conducted with Cryptogran (at 1.661×10^8 OBs/ml) against the field collected 2nd, 3rd, 4th and 5th instar FCM larvae from Addo, Kirkwood, Citrusdal and Clanwilliam as well as the old laboratory colony. Assays were replicated two or three times, where possible. This was due to the difficulty in obtaining enough larvae, as well as, forecasting the required larval populace - from a given batch of infested fruit for assays. Control mortality not exceeding 35% was used for data analysis (for the field collected larvae). The old laboratory colony was established in 1996 (Sean Moore pers. comm.). It is estimated that there are over 144 FCM generations to date. The parent material was obtained from Citrusdal, Zebediela (Limpopo Province) and the Eastern Cape (Sean Moore, pers. comm.). Therefore, the old population was of heterogeneous origin. All the field collected larvae were tested at a single

CrleGV-SA concentration of 1.661×10^8 OBs/ml. The field collected 2nd, 3rd, 4th and 5th instar FCM larvae were evaluated after 8, 10 and 14 days respectively.

Statistical analysis

A total of 150 larvae were used per trial, (with 25 larvae per treatment, including the control). Larvae or pupae were recorded as dead or alive post-inoculation. Data from the dose-response bioassays were analysed using PROBAN (Van Ark, 1995), a software programme used in the analysis of bioassay data. PROBAN corrected the control mortality according to Abbott's formula (Abbott, 1925). From this, the LC₅₀ (concentration required to elicit 50% mortality in the test insects) and LC₉₀ (concentration required to elicit 90% mortality in the test insects) were calculated. PROBAN transformed the doses to log₁₀ and the percentage response to empirical probits. Using this information the fit of the probit (regression) lines were calculated, as were the fiducial limits. Bartlett's test ($P < 0.01$) was employed in the comparison of probit lines (Van Ark, 1995). Differences between treatments for the assays conducted with the field collected larvae were analysed with SPSS 11.0 statistical package.

Results and discussion

Establishment of geographically distinct FCM colonies

A total of 1266, 663, 406 and 704 citrus fruit were sampled from individual orchards from Addo, Citrusdal, Marble Hall and Nelspruit respectively. The highest percentage of field collected larvae emerging into moths (first generation) was recorded with the 5th instars from all the field lines (Fig. 3.2.8.1).

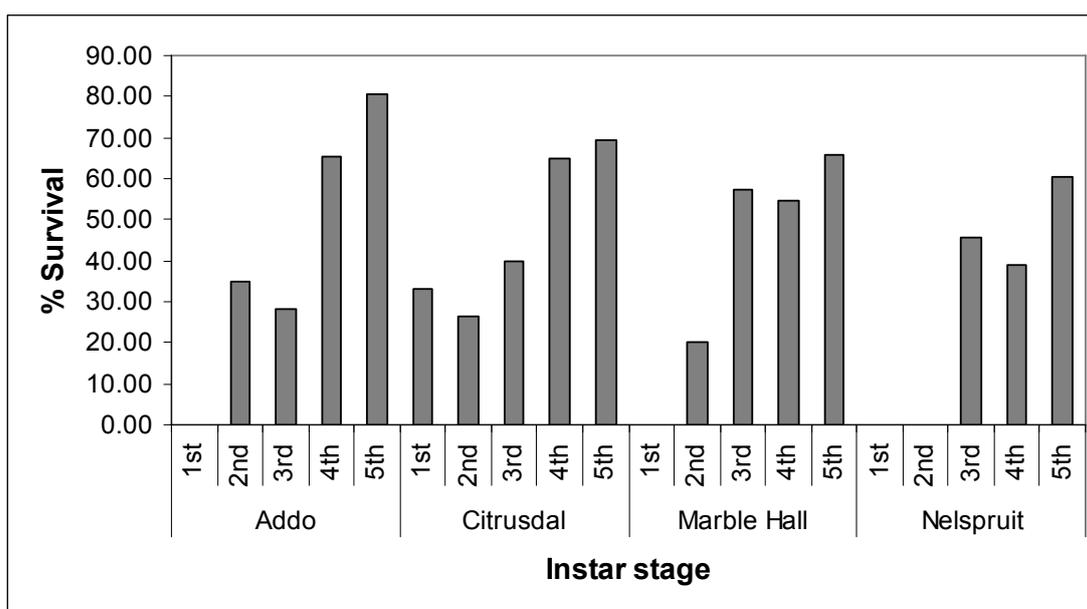


Figure 3.2.8.1 Percentage survival and development of field collected FCM larvae to adulthood.

Although it was possible to establish all instars of field collected FCM larvae on artificial diet, obtaining high numbers of fifth instar larvae would be highly advantageous, due to their higher survival rate than the other instars. An incubation room temperature of about $27^{\circ} \pm 1^{\circ}\text{C}$ was critical for the establishment and maintenance of the new colonies. Temperature is noted to play a vital role in speeding the growth rate of FCM larvae (Diaber, 1980; Van Der Geest, *et al.*, 1991; Moore, 2002).

On average the establishment of stable laboratory colonies from field lines was achieved by the fourth generation of FCM. Once the new laboratory colonies had become established, it was determined that their life-cycles (1st to 6th generation) were approximately 14 days longer than that of the old colony. But after the 7th generation, the duration of the life cycle had shortened to that of the old colony. It was also found that only by the F₅ generation was there sufficient oviposition to obtain adequate numbers of 1st instars to be able to conduct bioassays.

Benchmark dose-response bioassays with FCM larvae

In surface dose-response bioassays with CrleGV-SA against laboratory reared 2nd, 3rd and 4th instar larvae, the mean LC₅₀ values were calculated to be; 4.516 x 10⁴, 1.662 x 10⁵ and 2.205 x 10⁶ OBs/ml respectively. The LC₉₀ values for the 2nd, 3rd and 4th instars were calculated to be 4.287 x 10⁶, 9.922 x 10⁶ and 1.661 x 10⁸ OBs/ml respectively (Table. 3.2.8.1). The LC₅₀ and LC₉₀ values for the 1st and 5th instar FCM larvae established by Moore (2002) were calculated to be 4.095 x 10³, 2.678 x 10⁷ and 1.185 x 10⁵, 9.118 x 10⁹ respectively. In consequence, the full regime of the dose-response relationship for all instars was successfully established.

Table 3.2.8.1 Mean LC₅₀ and LC₉₀ for all FCM larval instars with CrleGV-SA.

FCM Instar	LC ₅₀	LC ₉₀	Exposure time (days)
1 st	4.095 x 10 ³	1.185 x 10 ⁵	7
2 nd	4.516 x 10 ⁴	4.287 x 10 ⁶	8
3 rd	1.662 x 10 ⁵	9.922 x 10 ⁶	8
4 th	2.205 x 10 ⁶	1.661 x 10 ⁸	10
5 th	2.678 x 10 ⁷	9.118 x 10 ⁹	14

*LC₅₀ and LC₉₀ values for 1st & 5th FCM instar were established by Moore (2002).

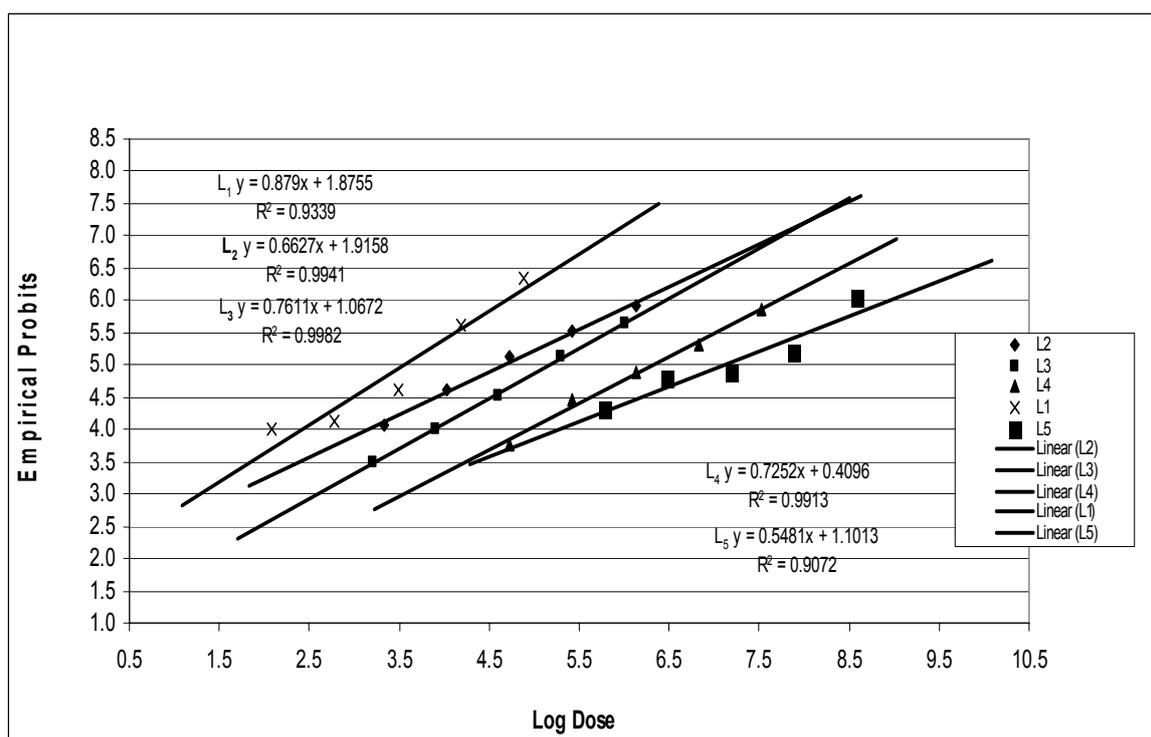


Figure 3.2.8.2 Comparison of dose-response probitlines for the 1st, 2nd, 3rd, 4th and 5th FCM instars * L₁ (first FCM instar), L₂ (second instar), *L₃ (third instar), *L₄ (fourth instar) and L₅ (fifth instar).

The regression equations calculated from the fitted lines were; $y = 0.879x + 1.8755$ ($R^2 = 0.9339$), $y = 0.6627x + 1.9158$ ($R^2 = 0.9941$), $y = 0.7611x + 1.0672$ ($R^2 = 0.9982$), $y = 0.7252x + 0.4096$ ($R^2 = 0.9913$) and $y = 0.5481x + 1.1013$ ($R^2 = 0.9072$) for the 1st – 5th FCM instars respectively. The R^2 values for all instars (1st to 5th) were all higher than 91%.

LC₅₀ and LC₉₀ values increased with larval stage (Table 3.2.8.1 & Fig. 3.2.8.2) reflecting a marked difference in susceptibility of larva with age. This phenomenon is described as maturation resistance (Jones, 2000). This

increasing resistance or lower susceptibility with larval stage has been reported by several authors (Hughes & Shapiro, 1997; Hunter-Fujita *et al.*, 1998; Escribano *et al.*, 1999; Jones, 2000; Moore, 2002). According to some authors, when larvae move from one instar to the next (molting), their midgut epithelial cells are normally sloughed off. As a result the new epithelial cells generated are rather thin and easily accessible by the virus, resulting in infection (Federici, 1997; Sun, 2005; Jehle *et al.*, 2006). However, unlike the early instars, the late instars (such as the 5th instar FCM larvae) on the other hand tend to have a much thicker peritrophic membrane which makes it difficult for the virus to penetrate and get access to the midgut epithelium in order to initiate infection (Federici, 1997).

In this study, the LC₅₀ values established for the larval instars, increased by 11, 3.6, 14 and 12 fold from one larval stage to the next (1st to 5th instars). There was a relatively small increase in the LC₅₀ value from the 2nd to 3rd instars, showing only 3.6 fold increase. Escribano *et al.* (1999) also observed a minor 3.9 fold increase in the LC₅₀ value from the 2nd to 3rd *Spodoptera frugiperda* larval instar. The LC₅₀ values increased with larval stage from 2.04 x 10⁵ OBs/ml for the 2nd instars to 8.05 x 10⁵ OBs/ml for the 3rd instars (Escribano *et al.*, 1999). This observation may indicate that susceptibility of lepidopteran larvae to baculovirus reduces to a lesser extent from the 2nd to the 3rd larval stage than between any of the other stages.

Field collection of FCM larvae for laboratory assays

A total of 8637 citrus fruit were collected from Crocodile Valley (Nelspruit), Schoeman Boerdery (Marble Hall), Rondegat (Clanwilliam), Jansekraal (Citrusdal), Lone Tree (Addo) and Tregaron (Addo) farms (from December 2007 to May 2008) for larval collection. The percentage of 1st, 2nd, 3rd, 4th and 5th instar FCM larvae collected from the infested fruits was recorded to be; 2.24%, 15.22%, 30.21%, 17.69% and 34.63% respectively (Table 3.2.8.2 & Figure 3.2.8.3).

Table 3.2.8.2 FCM larvae collected from navel oranges from a range of geographic regions from December 2007 to May 2008.

Date of fruit collection	Area	Province	Total number of fruits collected	Total number of FCM larvae collected				
				1 st	2 nd	3 rd	4 th	5 th
19/12/2007	Addo	Eastern Cape	852	6	63	188	113	80
08/01/2008	Addo	Eastern Cape	366	3	46	64	62	59
15/01/2008	Addo	Eastern Cape	286	0	11	58	49	32
19/01/2008	Nelspruit	Mpumalanga	318	0	2	7	3	5
23/01/2008	Addo	Eastern Cape	458	8	32	65	34	73
30/01/2008	Addo	Eastern Cape	543	12	49	73	54	125
15/02/2008	Citrusdal	Western Cape	902	0	2	4	5	26
15/02/2008	Clanwilliam	Western Cape	350	0	0	0	3	8
16/02/2008	Addo	Eastern Cape	661	0	19	62	25	108
20/02/2008	Kirkwood	Eastern Cape	892	6	44	125	94	246
28/02/2008	Clanwilliam	Western Cape	287	0	6	24	22	83
29/02/2008	Citrusdal	Western Cape	396	0	20	27	21	96
05/03/2008	Marble Hall	Mpumalanga	506	0	4	13	9	25
11/03/2008	Kirkwood	Eastern Cape	525	3	19	45	27	46
18/04/2008	Addo	Eastern Cape	399	5	60	101	22	46
24/04/2008	Citrusdal	Western Cape	178	23	55	10	1	2
25/04/2008	Clanwilliam	Western Cape	189	9	38	39	11	21
09/05/2008	Kirkwood	Eastern Cape	258	2	31	73	20	46
21/05/2008	Clanwilliam	Western Cape	155	0	7	22	23	52
21/05/2008	Citrusdal	Western Cape	116	0	16	40	11	13

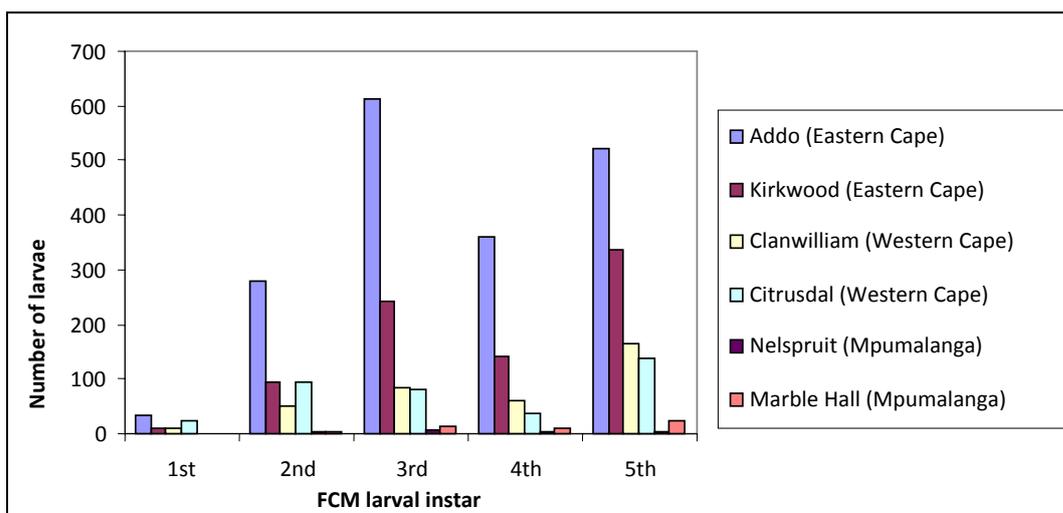


Figure 3.2.8.3 Total number of FCM larvae of each instar collected from a range of geographic regions in South Africa.

Surface dose-response bioassays with *Cryptogran* against field collected FCM larvae

Mean mortality of the field collected 2nd, 3rd and 4th instar FCM larvae ranged from 94.05 to 100%. There was no significant ($\chi^2 = 12.563$, $df = 11$, $p = 0.323$, Cramer's $V = 0.138$) difference in the response of these field collected 2nd to 4th instar larvae to CrleGV-SA (*Cryptogran*). It was felt that the virus concentration used was too high to pick up any small, or relatively small, differences in susceptibility between these instars.

However, mortality of 5th instar FCM larvae was significantly ($\chi^2 = 16.76$, $df = 3$, $p = 0.001$, Cramer's $V = 0.23$) lower for those collected from Addo, than those collected from the other three regions. The 5th instars from Addo exhibited a low percentage mortality of 35.0%. However, there was no significant ($\chi^2 = 0.622$, $df = 2$, $p = 0.733$, Cramer's $V = 0.053$) difference in the percentage mortalities between field collected 5th instars from Kirkwood (Eastern Cape), Clanwilliam (Western Cape) and Citrusdal (Western Cape) and the laboratory colony (Fig. 3.2.8.4).

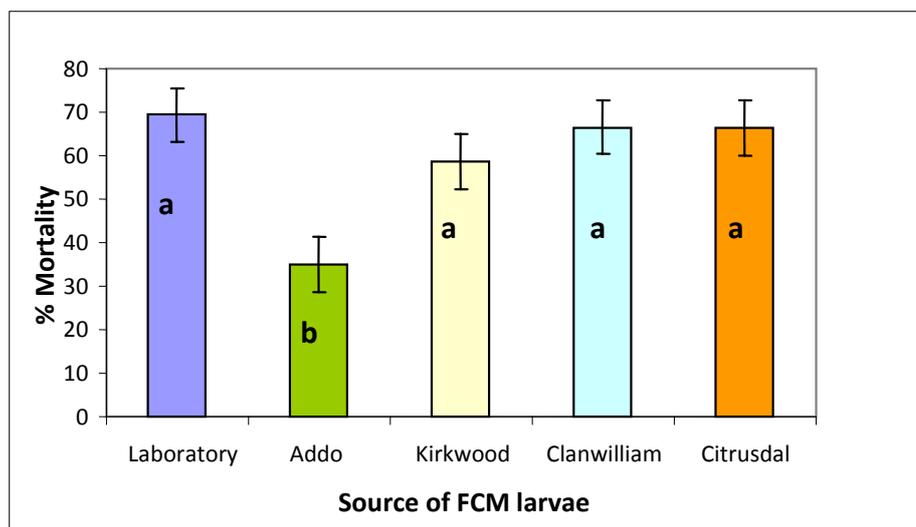


Figure 3.2.8.4 Mortality of both field collected and laboratory reared FCM larvae in bioassays with CrleGV-SA at 1.661×10^8 OBS/ml. (*Bars with the same letter are not significantly differently, $P > 0.05$).

Mortality of 5th instars collected from Addo was significantly lower than for those collected from Kirkwood, Clanwilliam and Citrusdal and for the laboratory colony. The 5th instars from Addo exhibited a low percentage mortality of 35.0% in contrast to the 58.60 to 69.33% mortality observed in the other populations. Although

mortality of 5th instar FCM larvae from Addo was significantly lower in laboratory assays, it is questionable whether this difference in mortality is large enough to be reflected in field applications targeted against neonate larva (1st instars).

A field trial conducted in the same orchard (Lone Tree Farm - Addo) from which the larvae were collected for the bioassays - showed an 86.7% reduction in fruit infestation with a Cryptogran application (Moore *et al.*, 2007). FCM infestation of fruit was evaluated weekly per tree for two months (January and February) (Fig. 3.2.8.5) (Moore *et al.*, 2007). Even if this difference in susceptibility of 5th instars in different populations is real and repeatable, it may be irrelevant, as field usage of the virus is exclusively against 1st instars.

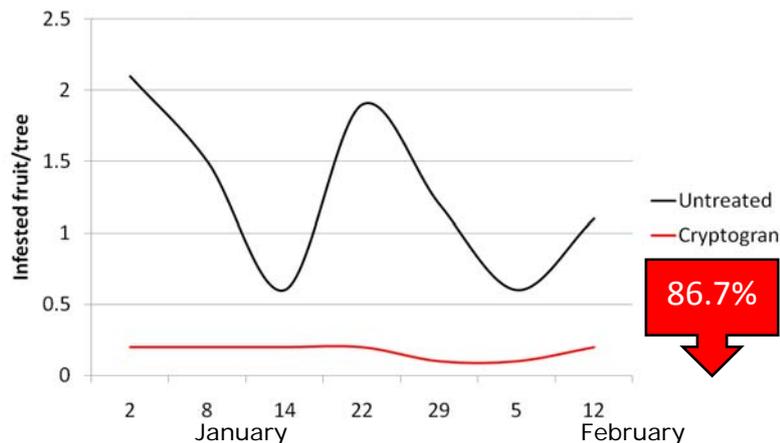


Figure 3.2.8.5 False codling moth infestation of navel oranges treated with Cryptogran and untreated at Lone Tree Farm (Addo, SRV, Eastern Cape), 2007 - 2008. *Source: (Moore *et al.*, 2007).

According to Jones (2000), intrinsic differences in individual larvae could contribute to variation in assay results. Yet again, the low number of individual larvae tested per treatment, per replicate probably contributed to this significantly low mortality recorded with the 5th instars from the Addo population. According to Jones (2000) an absolute minimum of 20 larvae should be tested at each dose, with each trial being replicated at least three times. However, this was difficult to achieve with the field collected larvae. This was probably due to the difficulty in obtaining enough larvae, as well as, forecasting the required larval populace - from a given batch of infested fruits for assays.

In a study on genetic variation among geographic populations of FCM by Timm (2005b), high levels of polymorphism were observed among FCM populations sampled from the Western Cape, Eastern Cape and Mpumalanga Provinces. However, genetic diversity was reported to be much higher within the Western Cape populations than both the Eastern Cape and Mpumalanga populations (Timm, 2005a & 2005b). Timm (2005a & 2005b) found that populations sampled from the same province appeared to be more closely related to one another than those collected from other provinces. The inference drawn was that, FCM populations were more or less locally adapted populations (Timm, 2005a). Timm (2005a) speculated that these locally adapted FCM populations could vary in their response to insecticide resistance or virus susceptibility (Timm, 2005a).

Interestingly, Timm (2005a) found that population genetic differentiation was similar in the Western Cape and Mpumalanga populations, but significantly lower in the Eastern Cape populations. She concluded that the Eastern Cape populations were more heterogeneous than the Western Cape and Mpumalanga populations (Timm, 2005a). However in this study, there was a notable difference in virus susceptibility even between 5th instars collected within the same province (Addo and Kirkwood). On the other hand, all field applications of CrleGV-SA commercial products (such as Cryptogran) are targeted against 1st instar larvae (other larval stages cannot be reached with the virus) (Moore, 2002; Moore & Kirkman, 2004).

Despite the significantly lower susceptibility observed with 5th instars (especially late 5th instars) from Addo, 5th instars are normally not accepted as good candidates for bioassays analyses. Hence it is speculated that small differences in larval age could also contribute to variation in assays. Especially when working with field larvae

which vary in age due to difficulty in synchronizing rearing conditions unlike in laboratory cultures. For instance it is noted that there is a higher probability of early 5th instars ingesting diet than is the case for late 5th instars, due to the increased tendency to enter into the pre-pupal stage observed with the latter (Catling & Aschenborn, 1978; Briese, 1981; Moore, 2002). In a similar experiment, Briese (1981) saw it justifiable to exclude the 4th (final) instars of the potato tuber moth, *Phthorimaea operculella* (Zeller), in laboratory assays.

Surface dose-response bioassays with Cryptogran and Cryptex against an old laboratory colony and the Addo colony

Data from each of the replicates from the bioassays conducted with each of the old and Addo colonies were pooled together, in order to compare their susceptibility to both Cryptogran and Cryptex (Table 4.6). There were four treatments-subject combinations in total.

The regression equations calculated from the slopes of the Old – Cryptex, Old – Cryptogran, Addo – Cryptex and Addo – Cryptogran treatments had the equations $y = 2.2047 + 0.6741x$ (SE of slope = 0.0793), $y = 2.2288 + 0.7790x$ (SE of slope = 0.0735), $y = 2.1381 + 0.7375x$ (SE of slope = 0.0845) and $y = 2.9957 + 0.5769x$ (SE of slope = 0.0671), respectively.

Table 3.2.8.3. Comparison of probit line slopes from dose-response bioassays with Cryptex and Cryptogran against 1st instar FCM larvae from two different laboratory colonies.

Line (Slope)	Addo - Cryptex	Addo - Cryptogran	Old - Cryptex	Old - Cryptogran
Addo - Cryptex		2.40	0.55	2.23
Addo - Cryptogran	0.42		0.23	0.93
Old - Cryptex	1.82	4.35*		4.05*
Old - Cryptogran	0.45	1.08	0.25	

NB* Old - Cryptex: Old colony treated with Cryptex; Old - Cryptogran: Old colony treated with Cryptogran Addo - Cryptex: Addo colony treated with Cryptex; Addo - Cryptogran: Addo colony treated with Cryptogran

The LC₅₀ value for the old colony treated with Cryptex increased by 1.15-fold in comparison to the Addo colony also treated with Cryptex. There was no significant difference (P<0.05) between these treatments. The LC₅₀ value of Cryptogran against the Addo colony was 1.45 times higher than that for the old colony. There was no significant difference (P<0.05) between these treatments. For the old colony, the LC₅₀ value of Cryptex was 3.8 times higher than the LC₅₀ value of Cryptogran. There was a significant difference (P<0.05) between these treatments. The LC₅₀ value of Cryptex against the Addo colony was 1.84 times higher than that of Cryptogran against the same colony. However there was no significant difference (P<0.05) between these treatments (Table 3.2.8.3).

Table 3.2.8.4. Mean LC₅₀ and LC₉₀ values for 1st instar FCM larvae from Addo and the Old colony treated with Cryptogran and Cryptex.

FCM Colony	CrleGV-SA Product	LC ₅₀	LC ₉₀
Old Colony	Cryptex	1.37 x 10 ⁴	1.25 x 10 ⁵
	Cryptogran	4.45 x 10 ³	2.62 x 10 ⁵
Addo Colony	Cryptex	1.19 x 10 ⁴	7.67 x 10 ⁵
	Cryptogran	6.45 x 10 ³	1.63 x 10 ⁵

In a laboratory study using surface-dose response bioassays with 1st instar FCM larvae of the old colony, Goble (2007) showed that Cryptogran was significantly more pathogenic than Cryptex. Goble (2007) determined the LC₅₀ and LC₉₀ for Cryptogran to be 4.054 x 10³ OBs/ml and 7.372 x 10⁴ OBs/ml whilst that for Cryptex they were 8.460 x 10³ OBs/ml and 1.950 x 10⁵ OBs/ml respectively. However, this significant difference in pathogenicity was established with the LC₉₀ values (Goble, 2007).

In this study, the LC₅₀ value for Cryptex against 1st instar larvae of the old colony was 5.38 times higher than that determined by Goble (2007) (Table 3.2.8.4). Also the LC₅₀ value of the Addo colony treated with Cryptogran showed a marginal decrease (1.59-fold) in pathogenicity in comparison to that reported by Goble (2007) with the old colony (Table 3.2.8.4). It is quiet common to observe variation between assays conducted by different researchers in different laboratories (Jones, 2000). However, Hunter-Fujita *et al.* (1998) implores that when assays are conducted frequently, variation between and within assays becomes less. According to Dulmage (1973) up to 3-fold differences can be expected for virus assays conducted under identical conditions. Similarly, in these studies, the LC₅₀ values of Cryptogran were determined to be significantly lower than those of Cryptex, indicating that Cryptogran was more pathogenic than Cryptex against both FCM colonies (the Addo and Old FCM colony) (Table 3.2.8.4). This observation further confirms that genotypically distinct virus isolates (Cryptogran and Cryptex) show high levels of variation in pathogenicity (Cory, 1997; Cory *et al.*, 2005).

The slopes of the probit lines from some of the bioassays against the Addo colony differed significantly for Cryptex ($F_{6, 27} = 3.761$) and Cryptogran ($F_{5, 23} = 19.517$). This suggests that there was a high level of variation in the response of the Addo colony to the two virus isolates. For instance, Cryptogran was significantly more pathogenic than Cryptex, based on their LC₅₀ values. However, based on their LC₉₀ values, Cryptex appeared to be slightly (but not significantly) more pathogenic than Cryptogran. The LC₉₀ value, for Cryptogran was 2.13 times higher than that for Cryptex, against the Addo colony. Results with the old colony showed the inverse (significant difference in pathogenicity was established based on both the LC₅₀ and LC₉₀ values). However, LC₅₀ values (not LC₉₀ values) have been accepted as the most accurate means of determining the potency of a virus (Hunter-Fujita *et al.*, 1998; Jones, 2000).

Timm (2005a) observed significantly high levels of genetic heterogeneity within FCM populations found in the Eastern Cape. Therefore these differences in pathogenicity in the Addo colony could explain why some members of the population were more susceptible to Cryptogran at lower concentrations (LC₅₀) than Cryptex, whilst other members appeared to be more susceptible to Cryptex than Cryptogran at higher concentrations (LC₉₀) of the virus.

In another study, Asser-Kaiser (2007) found that codling moth (CM) populations that developed resistance to *Cydia pomonella* granulovirus (CpGV-M (Mexican isolate)) products were actually a heterogeneous population (a mixture of both susceptible and resistant individuals). It was discovered that CpGV-M could still induce about 30-40% mortality, representing the susceptible individuals within the resistant populations (Asser-Kaiser *et al.*, 2007).

However, some authors contend that, the determination of the potency of a baculovirus using quantal response (mortality) alone does not fully reflect the efficacy of a virus (Sait *et al.*, 1994; Goulson & Cory, 1995). Other factors such as latent infection and sub-lethal effects have been noted to be of significant importance in this regard (Cory *et al.*, 1997). For instance, sub-lethal effects such as altered host development and growth are all essential parameters for determining the potency of a baculovirus. However, there is little knowledge on the mechanisms of sub-lethal infections (Cory *et al.*, 1997).

It might even be possible that the application of Cryptex and Cryptogran against 1st instar FCM larvae from both the Addo and old colonies could trigger other viruses (possibly new CrleGV-SA isolates yet to be discovered) out of their latent forms, thus influencing overall mortality results. To date, five FCM colonies have been established in the laboratory. It is not known whether these colonies carry any CrleGV-SA isolates in their latent forms, which could be present in their field populations. Although, a number of diseased FCM larvae were recovered from the field material used in this study, the causative agent (pathogens) of this infection is not yet known, and this should be investigated. Previous studies by Moore (2002) speculated on the possible existence of other South African isolates of CrleGV-SA that needed to be confirmed by future work.

It is becoming quite commonly believed that insects could be co-infected with different viruses with the 'parent' viruses only manifesting themselves after being triggered by other newly introduced viruses or through stress related factors encountered by the host (Longworth & Cunningham, 1968). For instance, in one study Cory *et al.* (2005) found that, an NPV isolated from a single pine beauty moth, *Panolis flammema* larva had twenty four (24) genetically different genotypes and that all these genotypes differed in their pathogenicity.

Conclusion

A benchmark for pathogenicity has been established with CrleGV-SA (Cryptogran) against FCM larvae. Susceptibility to CrleGV-SA has been found to decline with larval stage and increases with time of exposure. This protocol was used in guiding bioassays with field collected FCM larvae. Conducting of bioassays with field collected FCM larvae on a non-agar based diet was found to be more suitable than an agar-based diet. Changes in diet and methodology could contribute to variation in assays. Although mortality of 5th instar FCM larvae from Addo was significantly lower in laboratory assays, a field trial conducted in the same orchard as the larvae were collected, showed excellent control of FCM.

Four geographically distinct FCM colonies from Addo, Citrusdal, Marble Hall and Nelspruit have been successfully established. In surface dose-response bioassays Cryptogran was significantly more pathogenic than Cryptex on two FCM populations (Addo colony and an old colony). There were also differences in virus pathogenicity within the Addo population. This is probably indicative of a high level of heterogeneity within these populations. These findings may indicate that under laboratory conditions there is a high level of variation in susceptibility within, as well as between, certain geographically distinct FCM populations in South Africa. Even though it was not possible (due to time constraints) to carry out comparative assays using Cryptex and Cryptogran against the other colonies, this should be investigated in the near future.

Future research

1. Detailed studies using droplet assays (for LT in LC studies) as well as detached fruit bioassays on separate and mixed formulations of Cryptogran and Cryptex against the old and the four newly established FCM colonies should be investigated.

2. Moore (2002) obtained indications in his studies of the probable existence of other South African isolates of CrleGV-SA and stated that this needed to be confirmed by future work. It is therefore imperative that the existence of these suggested new CrleGV-SA isolates be investigated. The already established new laboratory colonies from the different field populations could serve as a strategic stock for the initial investigation of such new isolates. Consequently, it might even be possible to formulate multiple isolates of the CrleGV-SA thereby minimising the possibility of some FCM populations developing resistance to a particular isolate.

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3.2.9 VORDERINGSVERSLAG: Die ontwikkeling van middels wat as lok- of afdryfmiddels teen VKM-wyflies en mannetjies gebruik kan word
Proef 927 (2008): J H en M Hofmeyr (CRI)

Summary

No progress was made by Desense with the identification of new attractants and repellents for false codling moth. No product was therefore available that justified field testing.

Opsomming

Geen vordering is deur Desense met die identifisering van nuwe lok- en afdryfmiddels vir valskodlingmot gemaak nie. Daar was derhalwe geen verbindings wat toetsing in boordproewe geregtig het nie.

Inleiding

Tot op hede is slegs een produk vir die monitering van VKM beskikbaar, naamlik 'n geslagsferomoon wat jare gelede deur JHH ontwikkel is. Dié produk lok slegs mannetjies en VKM-gedragspatrone kon tot dusver slegs aan die hand van hul reaksie bestudeer word. Dit is ironies, aangesien baie min bekend is oor die gedrag van wyfietotte, wat uiteraard vir vrugbesmetting en oesskade verantwoordelik is. Verskeie proewe in die verlede om 'n lokmiddel vir wyfietotte te vind, was onsuksesvol.

Navorsing word alreeds geruime tyd deur C J Smit van Desense uitgevoer om chemiese verbindings te identifiseer wat moontlik as lokmiddels of afstootmiddels vir VKM gebruik kan word. Die doel van daardie navorsing is om verbindings te ontwikkel wat met of sonder geslagsferomoon tot produkte verwerk kan word waarmee beide geslagte VKM, opsigself of gesamentlik, deur middel van benaderings soos lok&vrek- of paringsontwrigting bestry kan word. Daar is besluit dat, aangesien Desense nie meer deur die sitrusbedryf befonds word nie, CRI slegs navorsingsgewys betrokke sal raak wanneer 'n besonder belowende verbinding opgespoor word. Alhoewel sekere kandidaatprodukte wel tot dusver geïdentifiseer is, word nie een van hulle skynbaar as 'n "finale" stadiumverbinding beskou nie. Daar is derhalwe geen navorsing in dié voorgestelde proef uitgevoer nie.

Toekomstige doelwit

Proefwerk hang af van die vordering wat deur Desense met die identifikasie van belowende kandidaatverbindings gemaak word.

3.2.10 PROGRESS REPORT: Monitoring the efficacy of Sterile Insect Technique to control false codling moth in the Citrusdal area (Western Cape Province, South Africa)
Experiment 928 (November 2007 – November 2010): by R. Stotter (SU)

Opsomming

Kommersiele vrylatings van gedeeltelik gesteriliseerde valskodlingmot (VKM) in die Citrusdal streek is vir amper 19 maande aan die gang. Monitering van verhoudings van steriel tot wilde VKM mannetjies in boorde het getoon dat die gewenste verhouding van 10:1 moeilik behaalbaar gedurende die eerste seisoen was. Dié verhouding is net gedurende een week bereik. Gedurende die tweede seisoen is dié gewenste verhouding elke week, behalwe een, behaal. Gedurende die tweede seisoen is baie minder wilde VKM as gedurende die vorige jaar gevang. Gemiddelde vrugbesmetting is ook betekenisvol laer as die vorige seisoen.

Weeklikse vlugtoetse wat met bestraalde geteelde VKM oor 'n jaar se tydperk uitgevoer is, het getoon dat vlug, verspreiding en hervangs van vrygelate motte deur nagtemperatuur beïnvloed is. Hierdie kan 'n invloed op die doeltreffendheid van SIT hê, veral gedurende die winter. Dit wil ook voorkom dat hervangs gedeeltelik afhangend is van tyd van vrylating, veral op warm dae. Dit kom voor dat temperature bo 40°C nadelig vir vrygelate mot oorlewing is.

Lae temperatuur toleransie van VKM is met laboratorium-geteelde motte ondersoek. 'n Positiewe verhouding is tussen temperatuur en oorlewing van VKM gekry, wat getoon het dat baie lae temperature al hoe meer noodlottig is. 'n Negatiewe verhouding is tussen oorlewing en tydduur van blootstelling gekry. Dit het gewys dat toenemende tyd teen 'n sekere temperatuur ook meer noodlottig was.

Eksperimente is gedoen om te toets of dit moontlik is om VKM se toleransie vir lae temperature met vinnige koue bestandmaking verbeter kon word. Die tekort aan positiewe reaksie met VKM het aangedui dat hierdie spesie 'n beperkte vermoë het om sy temperatuurtoleransie oor kort daaglikse tydskaal aan te pas.

Summary

Commercial releases of partially-sterile false codling moth (FCM) in the Citrusdal area have been underway for nearly 19 months. Monitoring of field ratios of sterile: wild FCM males has shown that the desired ratio of 10:1 was difficult to achieve during the first season, where this ratio was only achieved in one week of releases. During the second season, this desired ratio has been surpassed every week so far, except for one. The number of wild male FCM trapped during this season has been substantially lower than last year. Fruit infestation, on average, has also been significantly lower than last season.

Weekly flight tests conducted for a year with irradiated facility-reared FCM have shown that flight, dispersal and recapture of released males is influenced by night temperatures. This could have an influence on the effectiveness of SIT, particularly during winter. Recaptures also appear to be partly dependant on the time of release, especially on hot days. It seems that temperatures in excess of 40°C are detrimental to released moth survival.

The low temperature tolerance of FCM was investigated using lab-reared moths. A positive relationship was found between temperature and proportional survival in FCM, indicating that more severe low temperatures were increasingly lethal. A negative relationship between survival and duration of exposure was found, showing that increasing time at a given temperature was also more lethal.

Experiments were conducted to see whether rapid cold hardening could be induced in FCM to improve their tolerance to low temperatures. The lack of rapid cold hardening found in FCM suggests that this species has limited capacity to adjust its thermal tolerance over short, daily time-scales.

General introduction

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is a phytosanitary pest of citrus as well as various other species of cultivated and non-cultivated plants. This pest is not indigenous to the Citrusdal area, having arrived here in the early 1970s (Honiball, 2004).

Historically, control of this pest revolved around chemical control and stringent orchard sanitation. Strict regulations on residue levels of chemicals on citrus fruit have led to the investigation and implementation of alternative control measures. These include mating disruption, parasitoid releases and granulovirus applications. The associated registered control products have met with varying degrees of success in this geographical area. As such, an investigation into the potential use of Sterile Insect Technique against this pest was initiated in 2002. Myburgh (1963), Schwartz (1978) and Du Toit (1981) conducted preliminary studies on the effects of gamma radiation on FCM in South Africa. Bloem *et al.* (2003) recently published data on the radiation biology and inherited sterility of FCM.

Studies into the potential of the Sterile Insect Technique (SIT) to control FCM in the Citrusdal area were initiated in 2002 (Hofmeyr, 2004).

Preliminary investigations and a semi-commercial pilot project carried out in 2006/7 showed that this practice could be employed to control FCM in the Citrusdal area. A company was set up in 2007, and a rearing facility was built in Citrusdal.

Inundative releases of sterile FCM males commenced on 1 November 2007. The initial aim was to achieve a desired ratio of ten sterile FCM males to every one feral FCM male (Hofmeyr *et al.*, 2005). Releases take place twice a week over approximately 3500 ha of citrus orchards. In the first season (2007/8), irradiated FCM were

released in 1500 Ha of orchards. Approximately 3000 sterile FCM are released per hectare of citrus orchards per week.

Irradiated moths were initially dyed internally with Calco Red dye, added to the rearing diet, to enable one to distinguish between released and wild FCM. However, the use of this dye was halted last season due to fears that it was affecting moth quality and competitiveness. Research conducted last year showed that Calco dye was not adversely affecting moth quality or production, and so the use of this dye was resumed in September 2008 for the start of the second season of releases. Released FCM males are now more easily distinguishable from their feral counterparts.

This project aims to monitor the control achieved by these releases over a period of 3 years. In addition, the implementation of other control methods alongside SIT will be monitored, to gain an indication of the compatibility between the various other control practices and SIT.

Judd and Gardiner (2004) showed that complimentary action of SIT and mating disruption provided better control and more rapid eradication of codling moth (*Cydia pomonella*) in British Columbia, Canada. In addition, the use of egg parasitoids (*Trichogrammatoidea cryptophlebiae*) combined with SIT was suggested by Carpenter *et al.* (2004). Whilst *T. cryptophlebiae* is no longer commercially reared in South Africa, combinations of the two practices will be investigated during this project.

Furthermore, monitoring and evaluation of released FCM performance has been undertaken in order to gain insight into the influence of temperature and various other limiting factors on moth performance.

Very little is known about the effects of temperature on FCM. Temperature is a critical abiotic factor affecting insect population dynamics. At sub-lethal temperatures it determines the rate of acquisition and consumption of resources, thereby influencing growth, development and reproduction. Most importantly, temperature determines the likelihood of mortality and hence, population declines, especially at extremes (Hoffmann *et al.*, 2003; Chown and Terblanche, 2007). This project aims to investigate the lower and upper lethal temperature ranges for FCM, as well as the potential for rapid cold hardening to improve the performance of released irradiated FCM under low temperature conditions.

Materials and methods

Monitoring of the efficacy of Sterile Insect Technique (SIT) in the Citrusdal area.

Seven farms were selected in October 2007, based on FCM trapping history obtained from a local packhouse. The 7 farms selected had historically the highest trap catches of FCM males in the current SIT release area. On each of these farms, pheromone-baited delta traps were set in each orchard of a bearing age and size (≥ 10 years old), excepting lemon orchards. Yellow Delta traps are used, with sticky pads and the Lorelei FCM pheromone dispenser. A total of 77 traps were set out at the end of October 2007 to coincide with the first releases of sterile FCM on 2 November 2007. Traps were hung generally in the middle of the orchard, at least 10 trees in from the edge, on the southern side of the orchard, which is the windward side. Traps are generally hung at a height of 2 m above the ground, on the outside, and southern side of the tree. Traps are monitored every week, and the number of wild and sterile FCM is recorded. Initially, it was intended that facility-reared moths would have Calco red dye implemented into their diet, which would stain them pink internally, allowing them to be distinguished from wild FCM. However, fears arose that the Calco red dye was affecting the quality of released moths, so its use was suspended, pending further investigation. At the start of the second season in November 2008, the use of Calco red dye was continued after it was found that use of the dye had no adverse effects on FCM quality or production. Sterile FCM within traps are identified largely by sight, which is relatively easy, as the facility-reared moths lose most of their scales, and are easily distinguished from wild FCM (Fig 3.2.10.1). Identification is very accurate. If uncertainty arises, each moth can be squashed, and FCM reared on diet containing Calco red dye produce a pink smear, whilst wild FCM produce a yellow smear. Generally, each moth caught is squashed, to ensure for accurate identification.



Fig. 3.2.10.1. Sticky pad from a delta trap showing the difference in visual appearance of a facility-reared sterile FCM male (left) and a ferral FCM male (right).

In addition to trapping, 13 blocks of navel oranges are inspected weekly for fruit infestation by FCM. The blocks comprise mostly of Washington navels, and each block comprises 10 evenly-spaced marked trees. The same blocks were monitored in both seasons. Fallen fruit is collected from beneath these trees every week and inspected for FCM damage, starting on 1 December, until fruit is harvested 5-6 months later.

Monitoring flight ability and longevity of facility-reared sterile FCM.

This experiment is being conducted on the farm Sonderwater, just north of Citrusdal in a 4 ha block of 20 year-old Washington navel oranges. Twelve delta traps were arranged in 2 concentric squares containing (inside to outside) respectively 4, and 8 delta traps, around a central moth release point in the centre of the orchard (Fig 3.2.10.2). Trees in this block are spaced 4 m x 6 m (trees x rows). Traps are no further than 60 m from the release point. Lorelei FCM pheromone lures and sticky pads are used within the delta traps.

Approximately 3000 mixed FCM irradiated with 150 Gy are released every Tuesday and Friday when possible. Fresh moths are used that are at least 12-24 h old. Each batch of moths is lightly dusted with a fluorescent powder dye (Fig. 3.2.10.3) to distinguish between batches. Moths are sprinkled within the central release tree immediately after irradiation to ensure freshness. Traps are monitored every Tuesday and Friday with the aid of a UV lamp, and data is collected. An Escort® iLog RH data logger is kept permanently within a delta trap in the centre of the orchard to constantly monitor temperature and humidity.

Investigating the effect of age of released FCM on percentage recapture

Two experiments were carried out. The first one was carried out towards the end of Autumn on 22 April 2008, and the second was carried out in early Summer on 21 November 2008.

In each case, 1000 newly emerged male FCM were selected daily over a four day period from the XSIT rearing facility and stored in a cold room at 5°C for 4, 3, 2 and 1 days respectively. The FCM were then irradiated for 13.5 minutes with 150Gy and each treatment was dusted with a different colour of external fluorescent powder dye. These moths were then all released on a central tree in the orchard described in Fig. 3.2.10.2. Percentage recapture was recorded a week later with the aid of a UV lamp to identify moths from each treatment.

Investigating the effect of time of release on performance of released irradiated FCM males

As there is only currently 4 different coloured external fluorescent dyes available for research purposes at the XSIT facility, only a maximum of 4 treatments can be done per release experiment. It was unknown as to whether any of the available dyes (Fire orange, blue, saturn yellow and green) have adverse effects on released FCM.

A release trial was conducted using a single batch of moths of the same age to test for adverse effects from any particular external dye. Moths of approximately 6 h old since emergence were selected from a particular emergence cabinet at the XSIT Facility. Four hundred males were selected, and 100 of each were dyed orange, blue, yellow or green. The same negligible quantity of dye was used for each treatment. The moths were irradiated at 4 pm with 150 Gy for 13.5 minutes and released at 7pm on 20 January 2009, in a block of Washington Navel oranges on the organic farm Modderfontein, in a block layout identical to that used in Fig. 3.2.10.2.

Moths were recaptured over a period of a week in delta pheromone traps, and identified with the aid of a UV lamp.

Consecutive trials were then initiated to investigate any possible effects of time of release on moth survival and competitiveness, also based on recapture of male FCM.

These trials took place on 5 December 2008 and on 12 January 2009. Approximately 1000 male FCM were used for each treatment. Moths were collected from emergence boxes and irradiated 2 h before release. Moths were transported to the release site in a cooler box and releases were made at 10 am, 1 pm, 4 pm, and 7 pm respectively. Each treatment was dyed a different colour. Colours were selected at random, and differed in each trial, for each treatment. Recaptures were ascertained a week later with the aid of a UV lamp. Temperature and humidity data was collected with an ESCORT iLOG RH data logger placed inside a delta pheromone trap inside of a centrally positioned tree in the orchard.

Investigating the flight time of FCM males in citrus orchards in the Citrusdal area.

A trial was undertaken on 2 December 2008. Approximately 1000 facility-reared male FCM of approximately 12 h old (since eclosion) were irradiated with 150 Gy for 13.5 minutes. These FCM were lightly dusted with a fluorescent powder dye and released by quad bike into a Washington Navel orange orchard on the farm Middelpoos just outside of Citrusdal at 5 pm on 2 December 2008. The release was conducted along two rows spaced 5 rows apart. Three Delta traps with Lorelei FCM pheromone dispensers and sticky pads were hung at a height of 2 m in trees in the central row between the two release rows, approximately 40 m apart.

Traps were monitored hourly between 6pm and 12am, and again at 6am for 3 nights in a row, with the aid of a UV lamp. Trapped male FCM were counted and removed each hour, and data was collected.

Temperature data was collected with an ESCORT ILOG RH data logger placed inside a delta trap inside a tree in the middle of the orchard.

Low Temperature Tolerance of FCM

A facility-reared culture of FCM from XSIT in Citrusdal was used for these experiments. These moths are all reared at an almost constant temperature of approximately 26°C with a relative humidity of approximately 50%.

Adult FCM of approximately 24 h old of mixed sexes were selected from moth emergence cabinets and assayed for lethal temperatures under a range of conditions. Temperature tolerance was measured as proportion of survival after exposure to a constant temperature for a fixed period of time over a range of experimental test temperatures using circulating, programmable water baths (Grant GD200-R4, Grant Instruments, UK; accuracy: $\pm 0.1^\circ\text{C}$). To allow for sub-zero temperature operation the water baths were filled with a solution of propylene glycol and water (1:1 ratio). Lethal temperatures were determined for groups of 10 moths placed into 60ml non-airtight plastic vials, within water-tight ice cream tubs, and subjected to temperature treatments for a fixed time period (0 hrs = control group, 2, 4, 6, 8 and 10 hrs). A data logger (ESCORT ILOG RH) was also submerged within the water bath inside a water-tight plastic bag to record temperature throughout each experiment, and to ensure that the desired temperature was reached and maintained for the full duration.

After the set time, moths were removed from the plastic vials and placed in petri dishes at 25°C for 24 hours. Holes were cut into the lids of each petri dish and cotton wool soaked in sugar water was made available to the specimens. After 24 hours, survival was scored. Survival was considered as a co-ordinated response to gentle stimuli (e.g. normal walking behaviour or flight upon gentle prodding). This process was replicated at least four times per temperature and time combination to fully account for variation among individuals.

Once complete replicated survival curves were obtained for lower lethal temperatures of *T. leucotreta* across several durations, fully encompassing the range of 0 to 100% mortality, a range of sub-lethal temperature pre-treatments were used to determine if rapid cold hardening could be induced. The hardening experiments generally followed the methods outlined in Terblanche *et al.* (2008). Handling controls were included in the pre-treatments to confirm that if survival improves, it was a consequence of temperature treatment and not handling stress. A temperature of -3°C for 4 h, where 50% survival was estimated in preliminary trials, was used to discriminate the effects of pre-treatment.

Statistical analyses were undertaken using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) (*proc probit*) for non-linear modelling of lethal temperature curves with help from Dr. John Terblanche from the University of Stellenbosch.

Results and discussion

Monitoring of the efficacy of Sterile Insect Technique (SIT) in the Citrusdal area

Control achieved during the second season (2008/9) has been significantly better than during the first season (2007/8) (Fig. 3.2.10.4). A ratio of 10:1 has been easily achieved every week of releases except for one week. This is in comparison to the first season, where this ratio was only achieved in one week. This has been due to several reasons. Firstly, it is now the second year of releases on the farms concerned. Some level of population suppression has been achieved on these farms. In addition, orchard sanitation has vastly improved in the area since the initiation of the project, largely due to increased pressure placed on farmers, which has also reduced the size of the wild FCM population. The production and release methods of partially-sterile FCM have also vastly improved. Releases were also initiated 2 weeks earlier in the second season (mid-October) than in the first season (Early November). The achieved ratio decreased with the onset of winter, as was recorded during the pilot project in 2007 (Hofmeyr & Hofmeyr, Pers. Comm). This is due to low temperatures which seem to adversely affect the willingness or ability of the released FCM to fly and find mates.

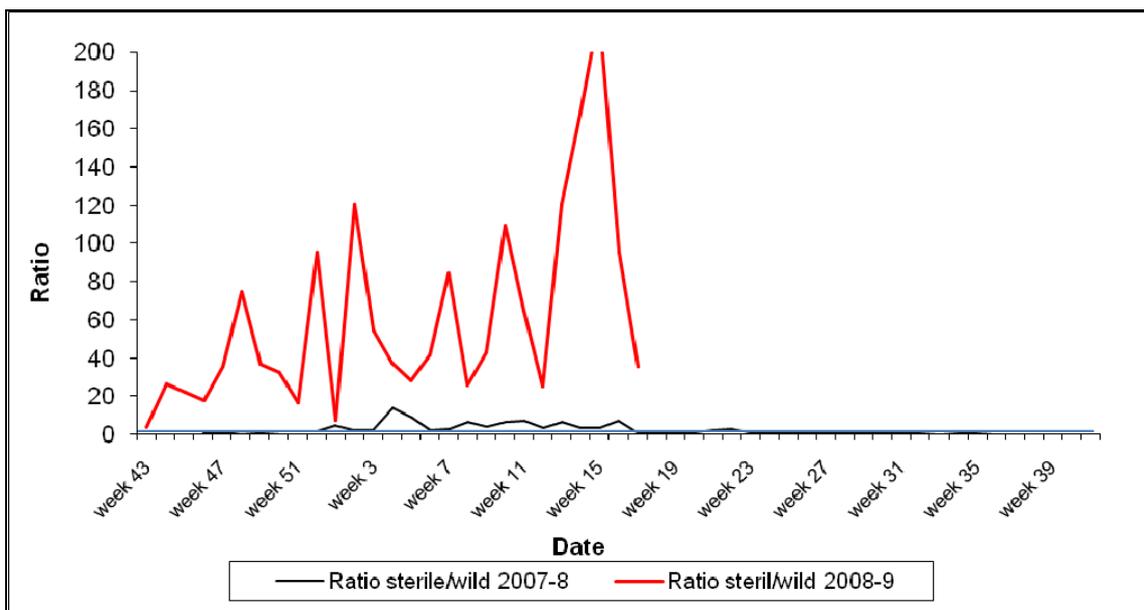


Fig. 3.2.10.4. Weekly ratios of sterile:wild FCM on 7 monitored farms in the Citrusdal area 2007-2009.

Trapping data from 77 delta traps shows the average weekly trap catches of wild FCM males to be consistently well below the threshold for Lorelei pipe traps of 10 males/trap/week. This threshold has often been surpassed in certain orchards, but the average is well below this value. Catches of wild FCM males have been significantly lower during the second season (Fig 3.2.10.5).

Catches of wild male FCM peaked between weeks 13 and 25 last season, with the highest numbers trapped in week 24.

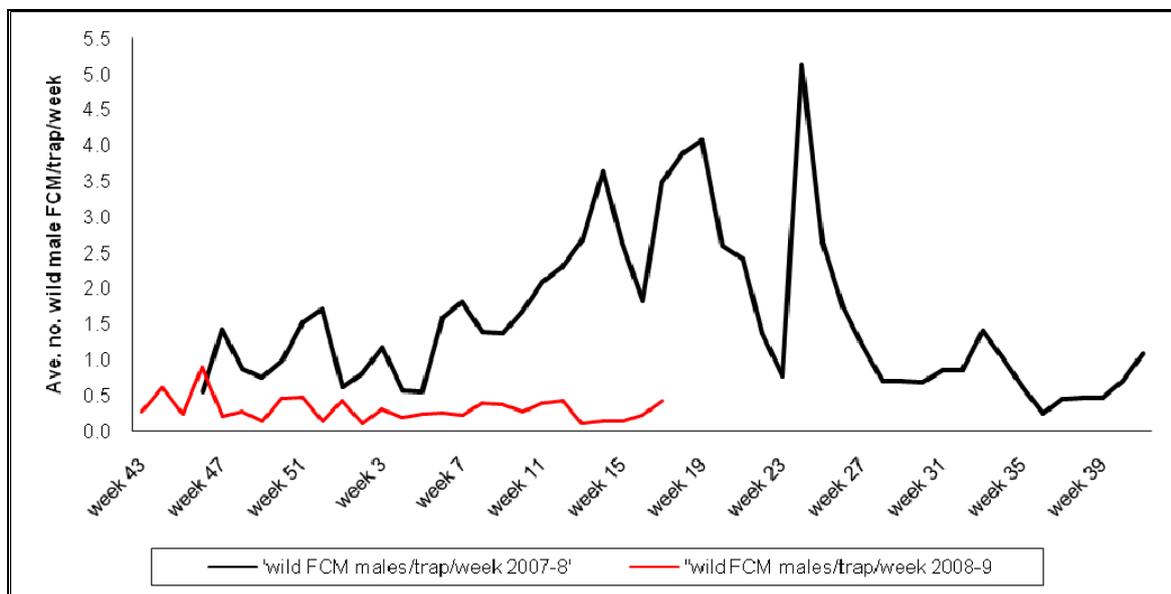


Fig. 3.2.10.5. Average catches of wild FCM per trap/week 2007-2009.

The average fruit infestation in the 13 monitored navel orange orchards has been consistently lower this season compared to last season (Fig. 3.2.10.6). During the first season, the suggested economic threshold of one infested fruit per tree per week was exceeded in weeks 17 and 18 only, and during the second season, infestation has not reached near this level as yet. Most of the monitored orchards have SIT as a stand-alone control practice along with strict orchard sanitation.

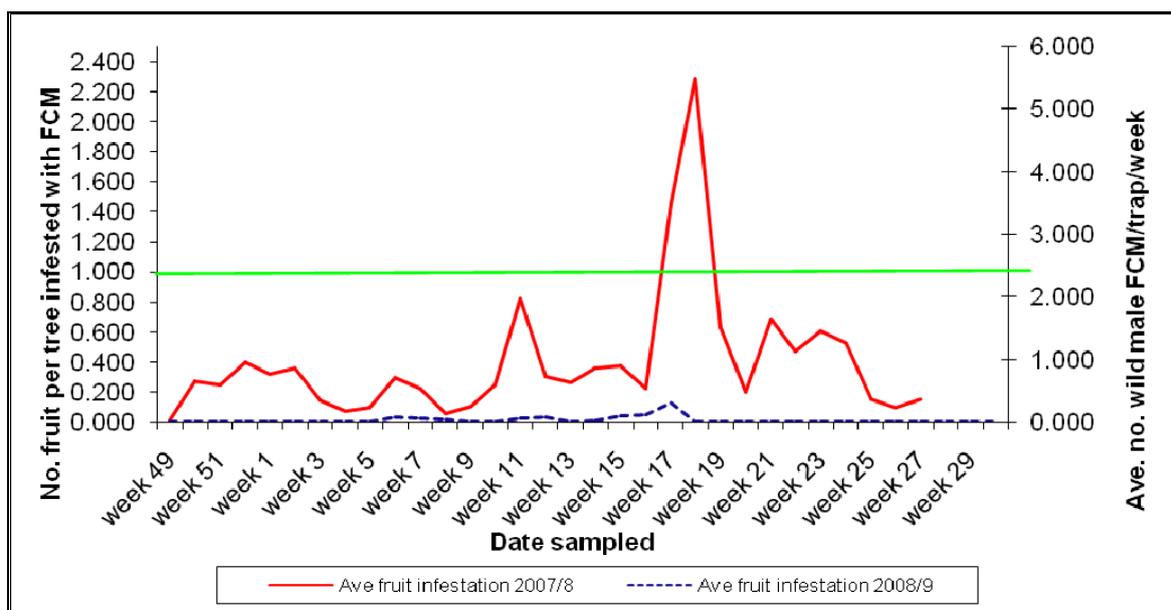


Fig. 3.2.10.6. Average infestation of navel oranges per tree per week in 13 monitored orchards 2007-2009.

Monitoring flight ability and longevity of facility-reared sterile FCM

Recapture of irradiated moths has been disappointingly poor (Fig. 3.2.10.7). Often, no released males are trapped at all. This may be attributed to low nightly temperatures at certain times of the year. It seems that when the average nightly temperature (between 7pm and 2am) falls below 15°C, no released moths are caught, and very few wild moths are caught either. When average nightly temperatures are above 20°C, recaptures are generally better (Fig. 3.2.10.8).

During winter, recaptures are consistently around 0%. This led to the halting of commercial releases of facility-reared irradiated FCM in mid-June last year. Unfortunately, late June is the peak time for wild FCM activity (personal records). This could mean that wild FCM in this area are more tolerant of lower temperatures than their facility-reared counterparts.

As so few released moths have been recaptured, one cannot draw any conclusions with regard to longevity. Generally, released moths are not recaptured for more than a week after release, except for occasional specimens recaptured during the colder periods. This could suggest that longevity is longer under lower temperatures and shorter during mid-Summer. The majority of recaptured moths are caught within 3 days after release. In terms of dispersal distance and dispersal direction, most released moths are recaptured within 30m of the release point (Fig. 3.2.10.9), with far fewer moths being captured at 60m or more from the release point. There is no particular pattern regarding dispersal direction, although slightly more male FCM were recaptured to the West of the release point. Hofmeyr and Hofmeyr (2004) found that male FCM irradiated with 200 Gy were recaptured up to 148 m from the release point, and were captured for up to 13 days after release. However, that particular experiment was carried out during October (Hofmeyr and Hofmeyr, 2004), when temperatures are over 20°C at night, and under 40°C during the day (Fig. 3.2.10.10).

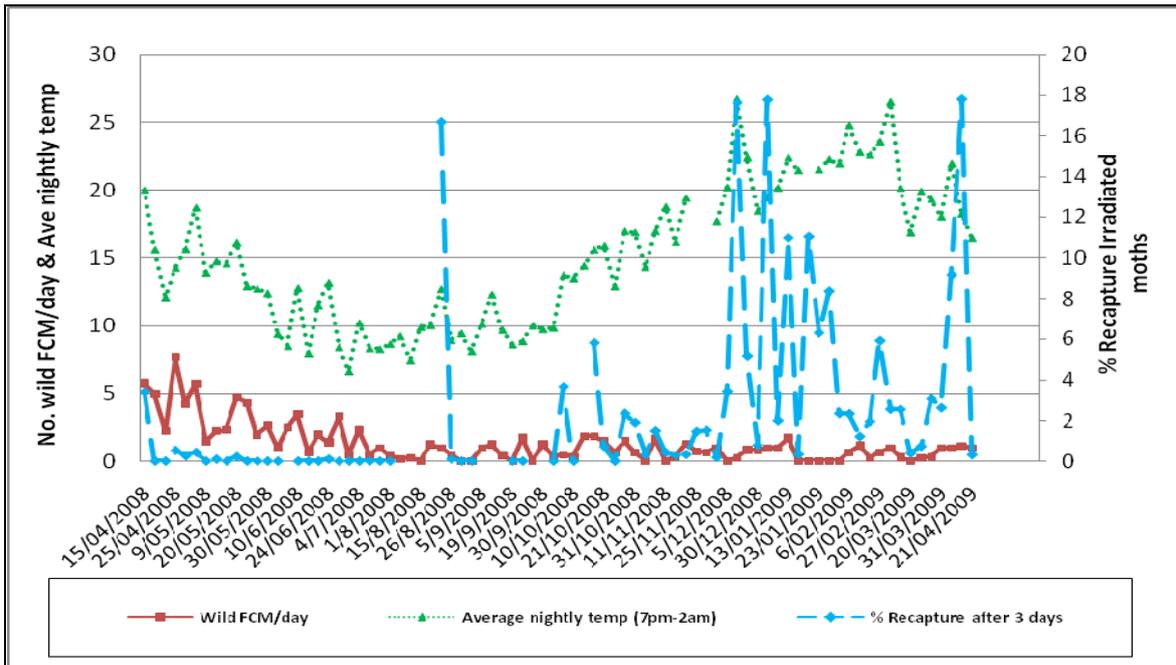


Fig. 3.2.10.7. Percentage recapture of dyed sterile FCM males released from a central point, compared with wild FCM catches and average nightly temperature between 7pm and 2am.

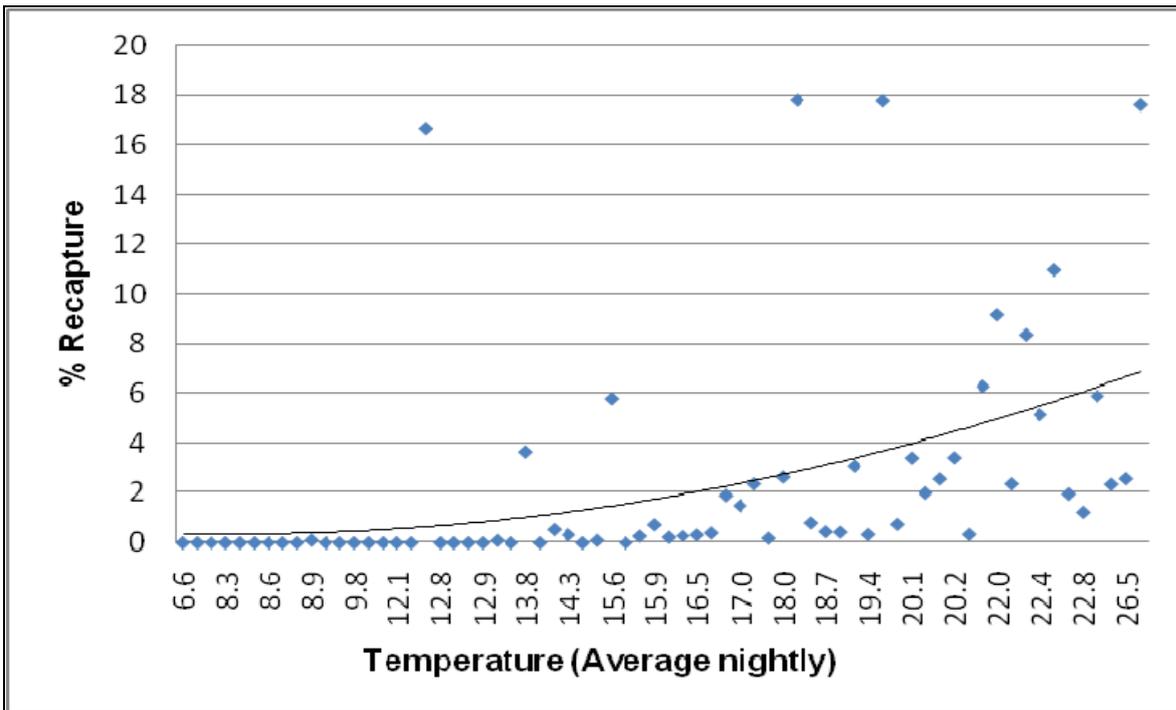


Fig. 3.2.10.8. Recapture of irradiated male FCM according to average nightly temperature between 7pm and 2am over three days. Also included is a polynomial trendline.

Investigating the effect of age of released FCM on percentage recapture

The first trial in April 2008 was conducted at a time when temperatures were rather low (average nightly temperature of 15.6°C between 7pm and 2am in the week concerned). No released FCM males were recovered during this experiment.

The second trial conducted in November 2008 produced better results. The average nightly temperature during this time was 19.8°C between 7 pm and 2 am (the assumed flight time of FCM males). In this case it was particularly evident that the younger (or fresher) the released FCM were, the higher the percentage of recapture was (Table 3.2.10.1; Fig. 3.2.10.11).

Table 3.2.10.1. Recapture of different-aged FCM released on 21st November 2008.

Treatment	Colour dyed	No. Male FCM Released	No. Male FCM recaptured	% recapture
4-day old cold room	yellow	887	5	0.3
3-day old cold room	orange	1068	2	0.1
2-day old cold room	green	1023	33	1.6
1-day old cold room	blue	989	37	1.9

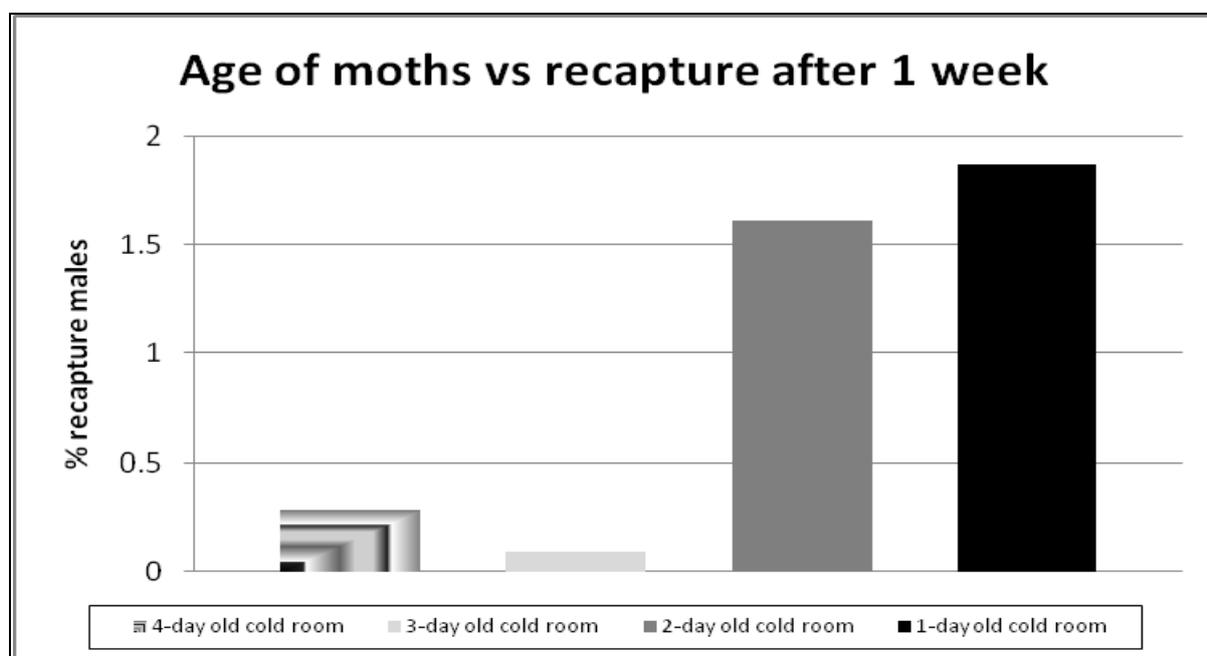


Fig. 3.2.10.11. Age of released irradiated FCM males vs percentage recapture in delta pheromone traps after a week.

These experiments bear some importance to the SIT practise for FCM suppression in that they prove that fresh moths should be used for releases and that moths should not be stored for more than 2 days prior to irradiation and release.

Investigating the effect of time of release on performance of released irradiated FCM males

It was ascertained that none of the available external powder dyes appeared to have more of an adverse effect on the released FCM than the others (Table 3.2.10.2 and Fig. 3.2.10.12). Between 13 and 15% recapture was observed for each treatment. It has not yet been ascertained as to whether external powder dyes have an adverse effect on recaptures of FCM.. However, research conducted by Stephens *et al.* (2008) on the effect of external powder dyes on painted apple moth showed that powder dyes reduced the ability of male PAM to detect

a component of the sex pheromone. However, field trials suggested that male PAM were able to overcome these negative effects and respond to female moths.

Table 3.2.10.2. Colour of external powder dye used to colour released FCM males versus percent recapture.

100 males dyed with each colour, ≤ 6 hrs old, irradiated 4 pm, released 7 pm, Modderfontein block 20.01.2009.		
	Recapture 23.01	% recapture
blue	13	13
green	14	14
orange	14	14
yellow	15	15

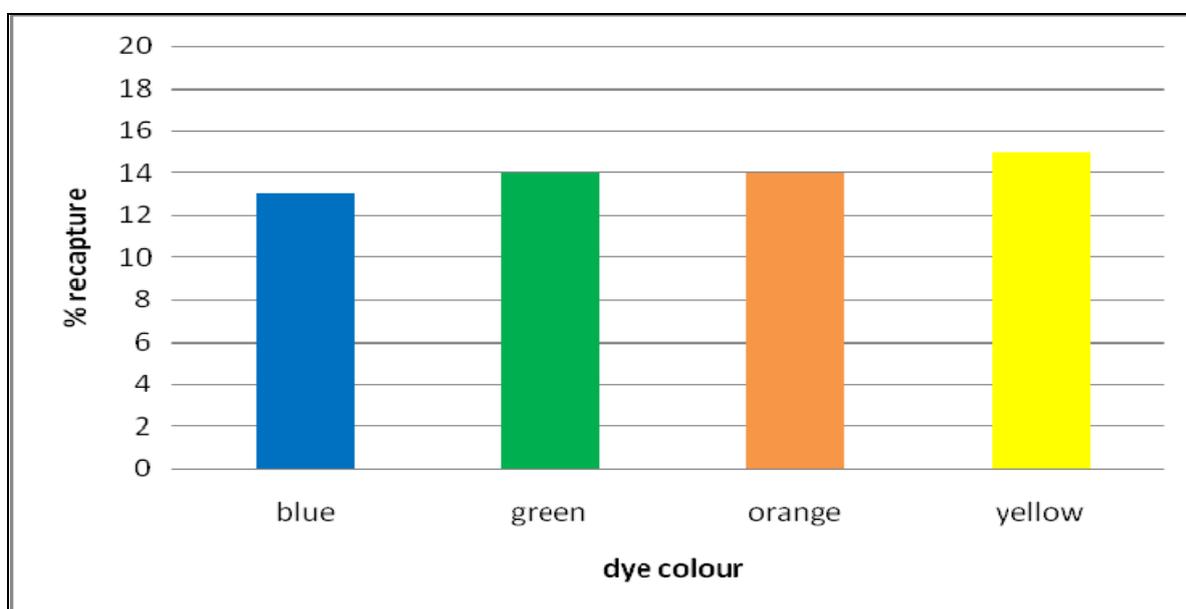


Fig. 3.2.10.12. External powder dye colour versus recapture of irradiated released male FCM.

The trials for effects of release times on moth performance produced varying results.

In the first trial conducted on 5/12/2008, recapture of moths was significantly higher for moths released at 7pm than at either 10 am, 1 pm or 4 pm. Releases at 4 pm produced the worst recaptures of just 2.3%, whereas recapture of males released at 7 pm was 14.8% (Table 3.2.10.3 and Fig. 3.2.10.13). Temperatures exceeded 40°C between 4 pm and 5 pm on the particular release day (Fig. 3.2.10.14). Many released moths were observed to fall directly onto bare soil during release. This can be detrimental to FCM, as the temperature of bare sand is probably slightly higher than the ambient temperature, and moths have been observed to die almost instantly when landing on bare sand on a hot day. The relatively poor recoveries from the 10 am and 1 pm releases could be explained by the increased time of exposure to the high daytime temperatures experienced on this particular day. Moths released at 7 pm were not exposed to these high temperatures during the day, and were released close to the peak flight time for FCM.

Table 3.2.10.3. Recapture of male FCM released at different times of day into a Washington Navel orange orchard on 5/12/2008.

Moths collected from moth cabinets and irradiated 2 h before release, and dyed different colours. Released at Modderfontein by hand on 5/12/2008				
Treatment	Colour	Number released	Number recaptured	Recapture (%)
Released 10 am	orange	1000	34	3.4
Released 1 pm	blue	1000	76	7.6
Released 4 pm	yellow	1000	23	2.3
Released 7 pm	green	1000	148	14.8

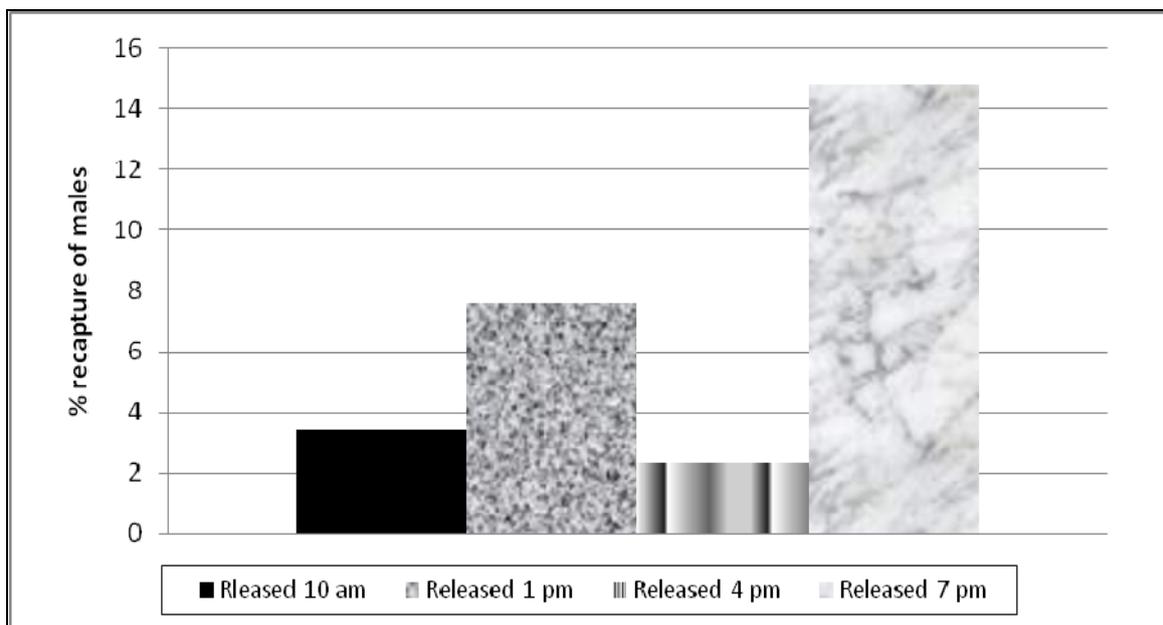


Fig. 3.2.10.13. Recapture of male FCM compared with time of release on 5/12/2008.

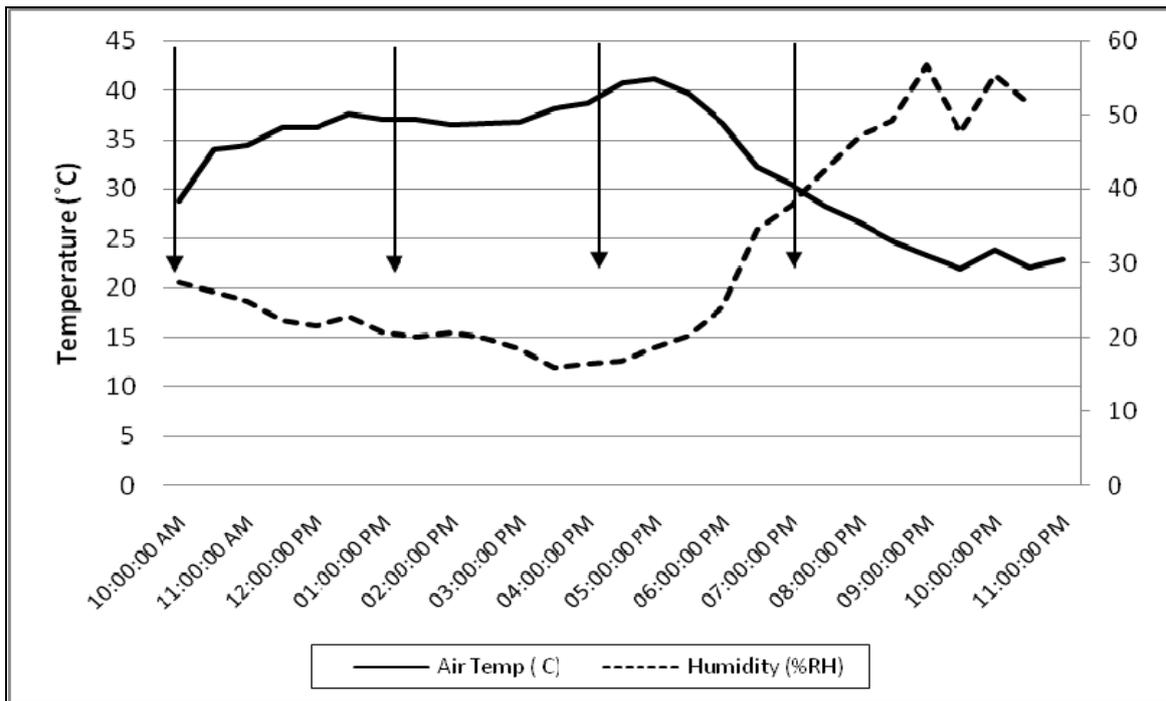


Fig. 3.2.10.14. Temperature and humidity between 10am and 11 pm on 5/12/2008. Arrows illustrate release times.

The second trial conducted on 12/01/2009 produced totally different results (Table 3.2.10.4 and Fig. 3.2.10.15.). In this case, the best recaptures of males were from releases conducted at 10am and 1pm, and the worst recaptures were from the release conducted at 7pm. It is unclear why this occurred.

Table 3.2.10.4. Recapture of male FCM released at different times of day into a Washington Navel orange orchard on 12/01/2009.

Moths collected from moth cabinets and irradiated 2 h before release, and dyed different colours. Released at Modderfontein by hand on 12/01/2009				
Treatment	Colour	Number released	Number recaptured	Recapture (%)
Released 10 am	blue	689	73	10.6
Released 1 pm	green	611	68	11.1
Released 4 pm	orange	800	69	8.6
Released 7 pm	yellow	622	20	3.2

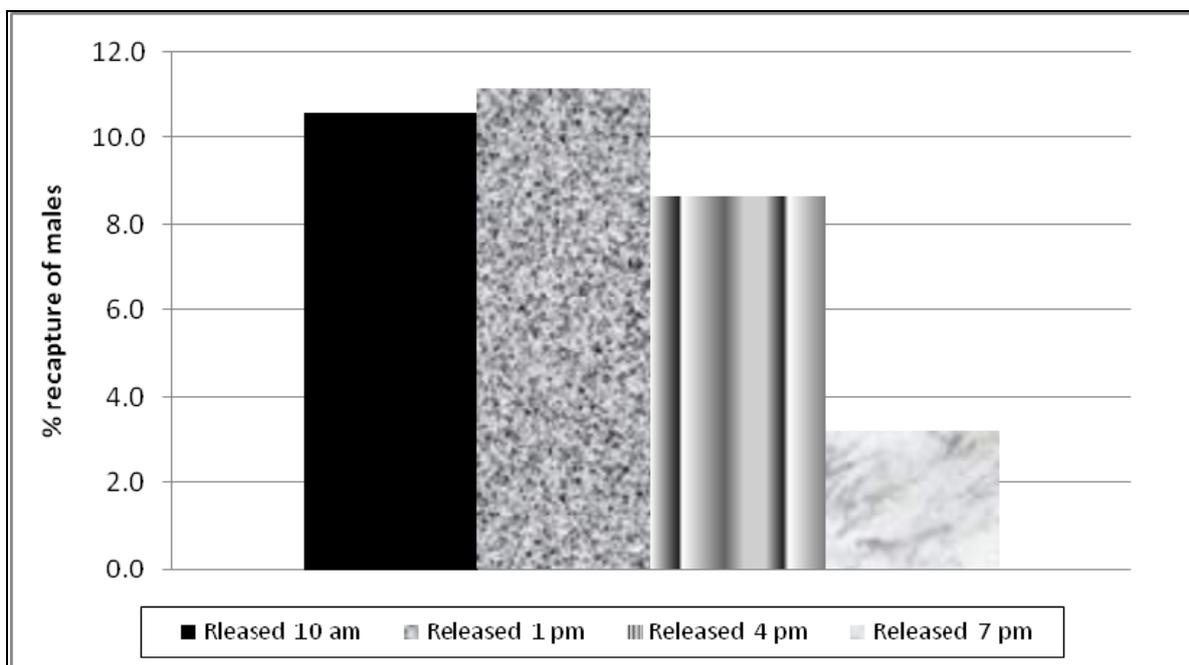


Fig. 3.2.10.15. Recapture of male FCM compared with time of release on 12/01/2009.

The temperatures on the release date (Fig. 3.2.10.16) were lower than in the previous trial, and this may be a reason for the better recaptures for releases conducted during the day, which was cooler than during the previous trial. This does not explain the low recaptures from the 7pm release though.

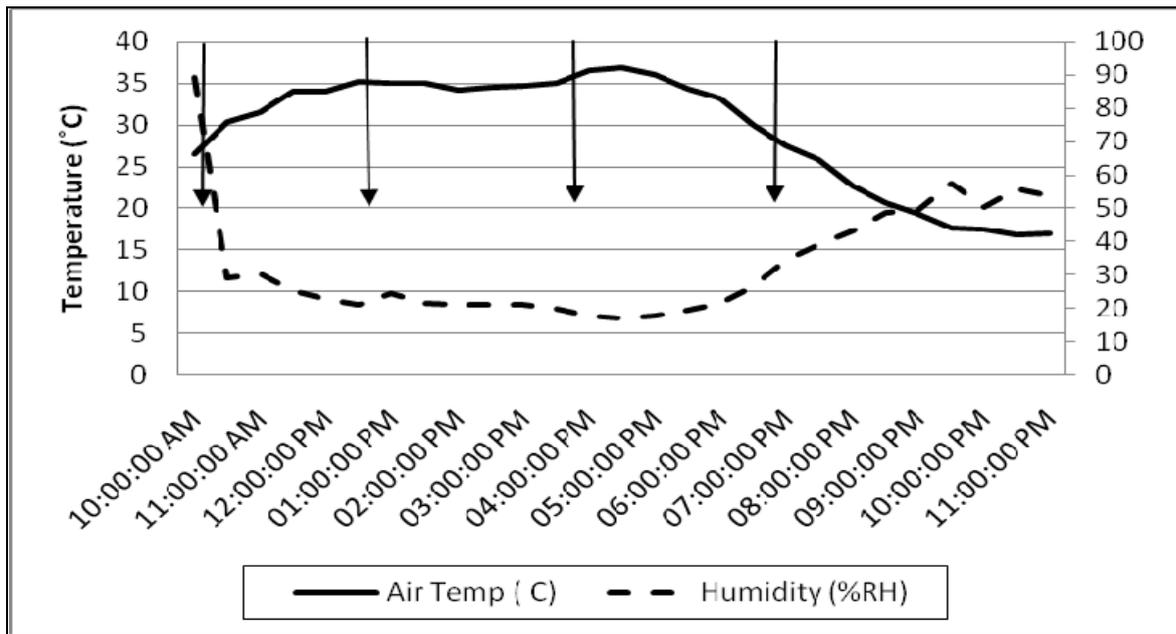


Fig. 3.2.10.16. Temperature and humidity between 10am and 11 pm on 12/01/2008. Arrows illustrate release times.

It therefore seems that release time alone does not determine moth performance, but that temperatures on any given day may also affect the performance of the released FCM. It must also be noted that moths released during the day tend to land on the ground or on a tree and appear to remain inactive until dusk, whereas moths released at dusk appear to fly almost immediately and disperse further from the release point. Therefore, one could expect some degree of predation on moths released during the day (particularly by birds).

Investigating the flight time of FCM males in citrus orchards in the Citrusdal area

Over the 3 nights concerned, released male FCM were trapped mostly on the 2nd night (3/12/2009). Very few male FCM were trapped between 6 pm and 8 pm. Most were trapped between 8 pm and 9 pm (Fig. 3.2.10.17), with very few caught after this time. During the second night, male FCM were trapped throughout the night, but also with a peak between 8 pm and 9 pm. Although there appears to be another peak in activity after 12 pm, it should be noted that this is over a 6 h period, and averages to about 4 moths per hour.

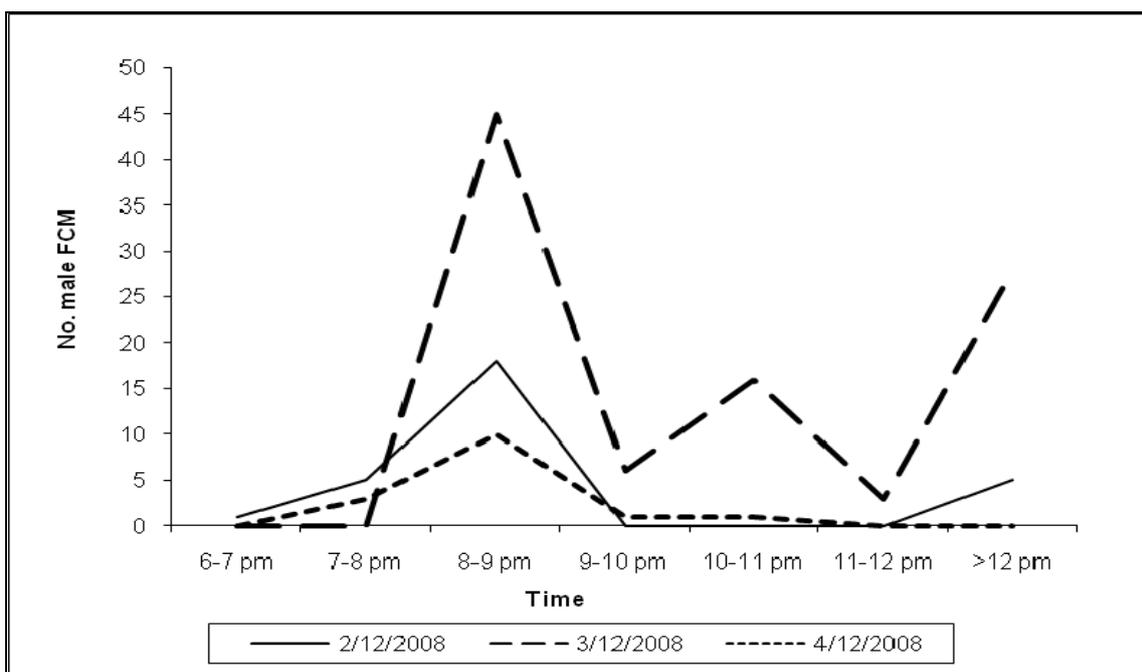


Fig. 3.2.10.17. Recapture over a period of 3 nights of male FCM released into a Washington Navel orange orchard on 2/12/2008, showing times of recapture on each night.

Temperatures during the 3 nights concerned are illustrated in Fig. 3.2.10.18, showing average hourly temperature. The temperatures were consistently above 15°C throughout the night, which could be deemed preferable for male FCM activity.

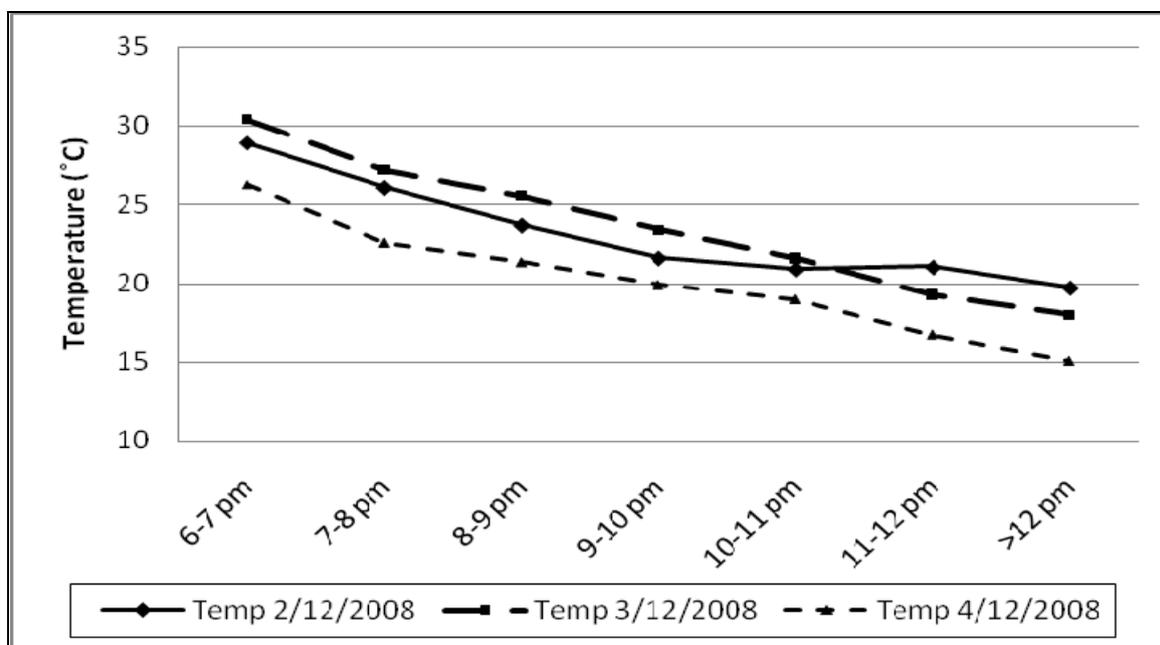


Fig. 3.2.10.18. Average hourly temperature recorded over 3 nights in a Washington Navel orange orchard in early December in the Citrusdal area.

From these results it could be said that the activity of released FCM is at a peak on the second night after release. This is consistent with other personal observations in orchards in the area. Whilst trap catches peaked between 8pm and 9pm, it cannot be assumed that this would be the case throughout the year, and would probably depend largely on season and on temperature on the particular day.

Further investigation is therefore required.

Low Temperature Tolerance of FCM

The effects of time and temperature on survival of FCM were highly significant. A positive relationship was found between temperature and proportional survival in FCM, indicating that mortality increased with decreasing temperatures (dropped to severely low levels) (Table 3.2.10.5; Fig. 3.2.10.19). A negative relationship between survival and duration of exposure was found, showing that increasing time at a given temperature was also more lethal (Table 3.2.10.5; Fig 3.2.10.19). In addition, a significant and positive effect was found on survival for the interaction between temperature x time. Plots of the relationship between proportional survival, temperature and time showed relatively simple patterns, but with an apparent local optimum in the range of -2°C for 4-8 hrs in *T. leucotreta* (Fig. 3.2.10.19).

Table 3.2.10.5. Summary estimates and results of probit non-linear analyses of the effects of time and temperature on survival of *T. leucotreta* (n=1400 moths, n=140 replicates).

Parameter	DF	Estimate ± SE	χ^2	P-value
Intercept	1	2.294 ± 0.188	149.62	<0.0001
Temperature	1	0.353 ± 0.055	42	<0.0001
Time	1	-0.187 ± 0.028	45.65	<0.0001
Temperature x Time	1	0.047 ± 0.011	19.26	<0.0001

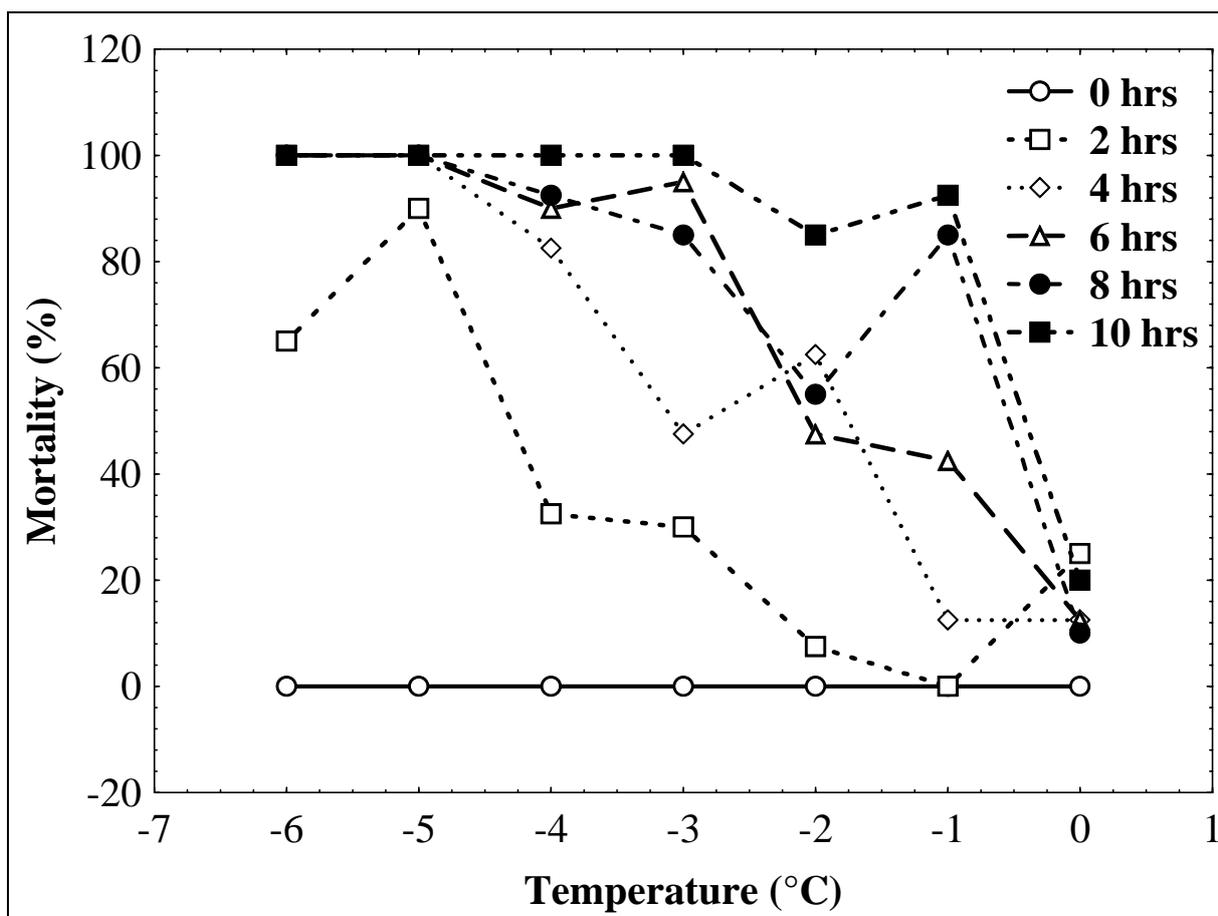


Fig. 3.2.10.19. Mean mortality of FCM at different temperatures for various durations. Each symbol is the mean of four replicates of 10 moths per replicate. 0 hrs treatment = handling control.

No significant effects of early adult age and gender on low temperature tolerance of FCM were detected at -3°C for 4 hours ($p > 0.53$ in all cases) (Table 3.2.10.6.; Fig. 3.2.10.20.), although it seemed that 2 day old male FCM had a slightly reduced tolerance of low temperature relative to 1 day old male FCM (Fig 3.2.10.20).

Table 3.2.10.6. Effects of gender and early adult age on low temperature tolerance of FCM.

Repetition	1 day old				2 days old			
	♂		♀		♂		♀	
	Survival	Mortality	Survival	Mortality	Survival	Mortality	Survival	Mortality
1	7	1	8	0	0	8	8	0
2	8	0	0	8	8	0	7	1
3	1	7	8	0	8	0	0	8
4	8	0	8	0	0	8	8	0
5	8	0	7	1	0	8	8	0
6	8	0	7	1	7	1	8	0
Total	40	8	38	10	23	25	39	9

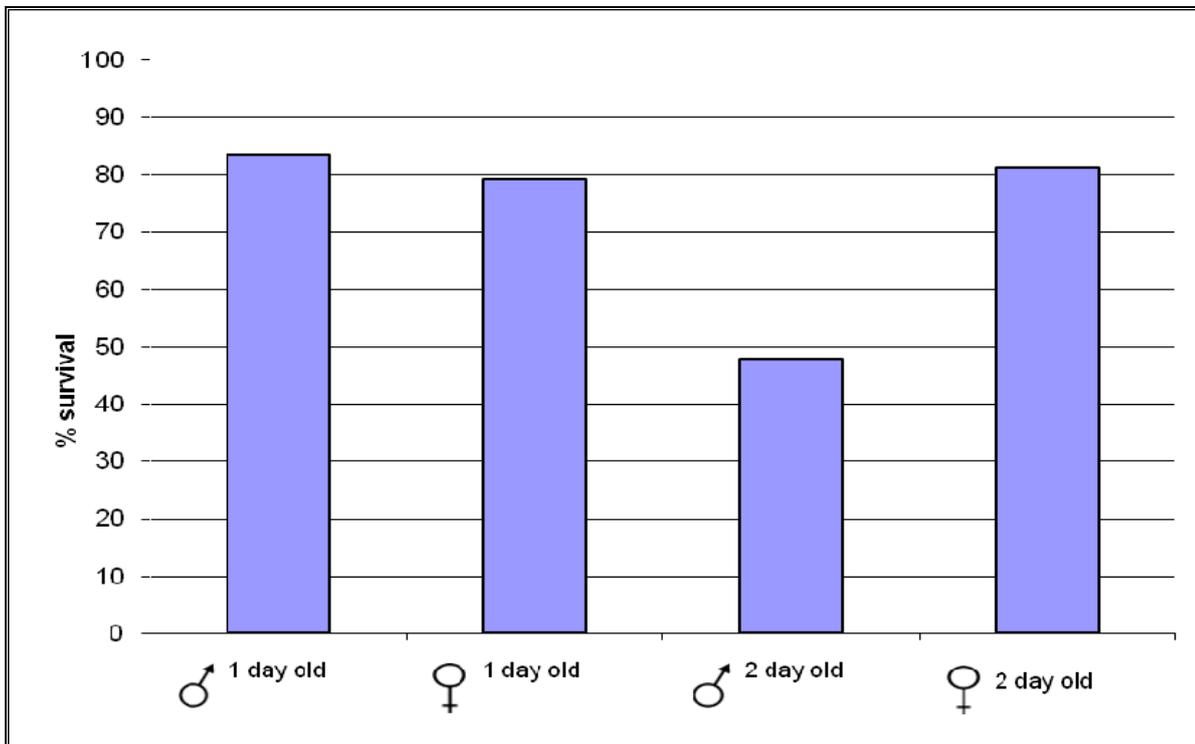


Fig. 3.2.10.20. Effects of gender and early adult age on low temperature tolerance of FCM.

Low temperature pre-treatments of 5°C for 2 or 4 h or 5°C with a 1 h return to 20°C did not improve survival of low temperature exposure (Fig. 3.2.10.21, Table 3.2.10.7). In addition, 8°C for 2 h or 8°C for 2 h with a gap at 20°C did not significantly improve low temperature survival (Fig. 3.2.10.21). A range of high temperature treatments (40°C) for 1 and 2 h and with a 1 h holding period at moderate temperatures (20°C) had little influence on low temperature survival (Fig. 3.2.10.21; Table 3.2.10.7). All effects were non-significant, whether analysed separately for low and high temperature pre-treatments, or all treatments combined (Table 3.2.10.7).

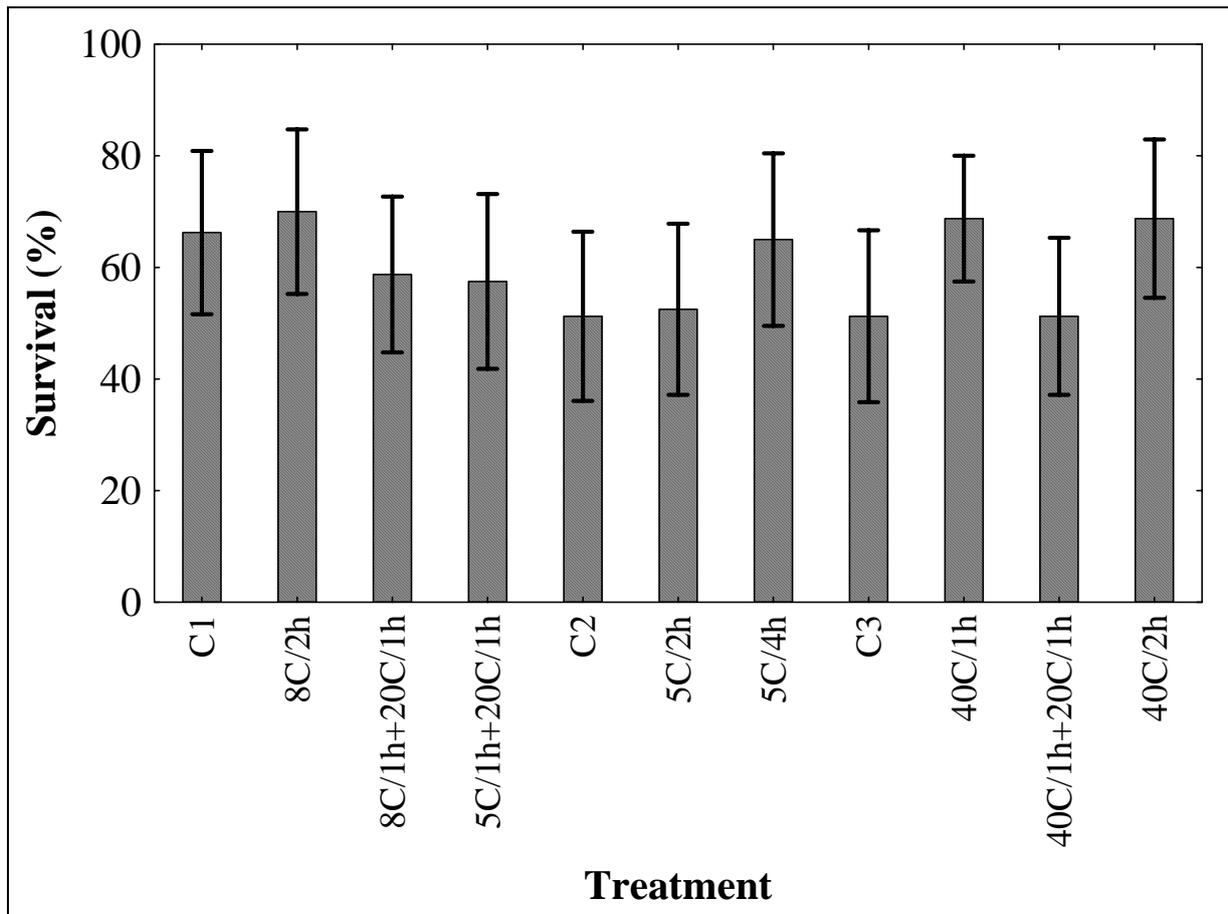


Fig. 3.2.10.21. Mean (\pm SE) survival of *T. leucotreta* at -3°C for 4 h across various low and high temperature pretreatments. C1=control day 1. C2=control day 2, C3=control day 3. 5C, 8C, 20C and 40C represent temperature treatments in degrees Celsius.

Table 3.2.10.7. Summary results of generalized linear models (GLZ) for the effects of various low and high temperature pretreatments on low temperature survival in *T. leucotreta*. Each treatment group consists of a minimum of 8 replicates of 8-10 moths per replicate. A binomial distribution and a logit link function were used with correction for overdispersion.

Treatment	DF	Estimate \pm SE	Wald χ^2	p
Low temperature pre-treatments				
1 Control 1	1	0.675 \pm 0.730	0.85	0.3555
2 8C/2h	1	0.847 \pm 0.753	1.27	0.2606
3 8C/1h+20C/1h	1	0.354 \pm 0.701	0.25	0.614
4 5C/1h+20C/1h	1	0.302 \pm 0.698	0.19	0.6651
5 Control 2	1	0.050 \pm 0.691	0.01	0.9423
6 5C/2h	1	0.100 \pm 0.691	0.02	0.8849
7 5C/4h	1	0.619 \pm 0.724	0.73	0.3923
	7		3.32	0.854
High temperature pre-treatments				
1 Control 3	1	0.050 \pm 0.635	0.01	0.9373
2 40C/1h	1	0.789 \pm 0.685	1.32	0.2498
3 40C/1h + 20C/1h	1	0.050 \pm 0.635	0.01	0.9373
4 40C/2h	1	0.789 \pm 0.685	1.32	0.2498
	4		2.66	0.6159
All treatments	11		5.9	0.8798

Irradiated released FCM do not appear to perform well (in terms of flight ability and active seeking of mates) during low temperatures occurring during late Autumn and Winter. In contrast, their feral counterparts are at a peak in population and activity at the end of June (mid-Winter). This could suggest that feral FCM are more tolerant of low temperatures than the facility-reared FCM. This could be due to the fact that the facility-reared FCM are reared at a constant temperature of 26C, whereas their feral counterparts develop at a fluctuating temperature. As it appears that facility-reared FCM cannot undergo rapid cold-hardening, this poses a problem

to the future of SIT in such an area, as releases of sterile FCM have to be halted at the onset of Winter. It is likely that feral FCM acquire a greater tolerance to low temperatures through fluctuating temperatures during their lifecycle.

Conclusions

SIT appears to be working effectively as a control practice against FCM in the Citrusdal area. Infestation of navel oranges has decreased drastically in the past two seasons, and the population of wild FCM has reduced in size considerably. It is largely apparent that the effectiveness of SIT is largely dependent on temperature. Activity of released male FCM increases with increasing night temperatures, and decreases considerably with the onset of winter. Effectiveness of released FCM may also depend on time of release and temperatures on the day of release. It also depends on the age of released moths, with younger moths (1-2 days old) being more active and more effective than older moths (3-4 days old). FCM are susceptible to low temperatures, and mortality increases with decreased temperature and with increased duration of exposure to low temperatures. A lack of rapid cold hardening observed in FCM could be the result of insufficient pre-treatments used and further investigation is required before conclusions can be made. However, it seems likely that FCM cannot undergo rapid cold hardening in the adult stage, and alternative methods need to be investigated to attempt to improve their tolerance of low temperatures.

Technology transfer

A talk was presented at the 2008 Citrus Research International research symposium in the Drakensberg, entitled "Spatial and Temporal Distribution of False Codling Moth across Landscapes in the Citrusdal area (Western Cape Province, South Africa)".

Publications

Spatial and Temporal Distribution of False Codling Moth across Landscapes in the Citrusdal area (Western Cape Province, South Africa). MSc thesis accepted February 2009. Graduated with *Cum laude*.

Current collaborations

Low temperature tolerance of false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) with Dr. John Terblanche from University of Stellenbosch.

Further objectives (milestones) and work plan

Monitoring of SIT in the Citrusdal area will continue for at least another year.

It will be necessary to look more in depth at the effects of combining this control technique with other control techniques. A trial is currently underway with a new compound, employing a new mode of chemical control for FCM. A trial was initiated in October 2008 to investigate the potential for augmentative control of FCM with monthly releases of egg parasitoids (*Trichogrammatoidea cryptophlebiae*) in combination with SIT. This trial was halted after two months due to a collapse of the rearing colony of parasitoids. This trial will have to be repeated, as well as a more comprehensive look at the effects of combining SIT with granulovirus applications or mating disruption.

Various trials need to be conducted to investigate the low temperature tolerance of the various life stages of FCM, as well as a more in-depth look at the possibilities of inducing rapid cold-hardening to improve low temperature tolerance.

Additionally, similar trials need to be conducted to investigate the high temperature tolerance of FCM. Once this has been completed, a comprehensive study can be conducted to optimise FCM rearing techniques to improve thermo-tolerance of released FCM according to season, if this is deemed possible.

Optimisation of release mechanisms and protocol also needs to be investigated further, to ensure optimal survival and performance of the released irradiated FCM.

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3.2.11 VORDERINGSVERSLAG: Doeltreffendheid van die insekdoder EXP5225 (200 g/l SC) in kombinasie met 'n Steriele-Insek Loslaatprogram
Proef 973 (2009): J H en M Hofmeyr (CRI)

Opsomming

Daar is 'n tekort aan doeltreffende insekdoders vir valskodlingmot(VKM)-onderdrukking. Dit is dus nodig om voortdurend aandag aan die toetsing en ontwikkeling van belowende produkte te gee. 'n Boordproef is volgens 'n vasgestelde protokol uitgevoer met die eksperimentele insekdoder, EXP5225 (200 g/l SC), in 'n Citrusdalse nawelboord wat deel van 'n Steriele-Insek Loslaatprogram was. Die produk is as enkeltoedienings, onderskeidelik 8 weke en 4 weke voor oestyd, asook as 'n dubbelbespuiting op dieselfde tye, getoets. Die besmettingsdruk was laag en die verskillende behandelings het nie beter as die kontrole-behandeling gevaar nie.

Summary

The number of effective insecticides for false codling moth (FCM) suppression is inadequate. It is therefore necessary to continually test and develop new products. An orchard experiment was conducted with the insecticide, EXP101/2009 (200 g/l SC) according to a prescribed protocol, in an orchard which participated in a Sterile Insect Release programme. The product was evaluated as single applications, respectively 8 and 4 weeks before harvest, as well as a double application at the same intervals. The natural infestation was light and the treatments did not perform better than the untreated control.

Inleiding

Ontwikkelingswerk is alreeds voorheen met die insekdoder EXP5225 deur die betrokke maatskappy uitgevoer. Geen verdere inligting oor die produk is bekend nie, behalwe dat die resultate goed genoeg was om verdere proefwerk te regverdig. Die doel van die proef onder bespreking was om vas te stel of die produk in kombinasie met Steriele-Insek Loslatings (SIL) die doeltreffendheid van VKM-bestuur kan verbeter.

Materiale en metodes

'n Plaas is in Citrusdal uitgesoek wat alreeds vir die tweede agtereenvolgende seisoen aan die SIL-program van Xsit (Edms) Bpk deelneem. Die proef is in 'n Washington-nawelboord met volwasse bome uitgevoer. Plantafstande in die boord was 6,5 m x 4,5 m en die bome het nie aanmekaar geraak nie. Die databome is gedurende die proefverloop slegs met die eksperimentele insekdoders behandel – daar is geen ander insekdoders in die boord toegedien nie.

Die proef is in 'n eenvoudige blokontwerp met enkelboomherhalings uitgelê. Tien blokke bome is gebruik, elk bestaande uit een herhaling van elke behandeling wat ewekansig in die blok toegeken was.

Die verskillende spuitbehandelings is teen hoë druk en hoë volume met handspuite toegedien wat aan 'n konvensionele trekker-aangedrewe hidroliese spuitenk gekoppel was. Die verskillende behandelings, wat hoofsaaklik uit enkelbespuitings, asook 'n enkele dubbelbespuiting bestaan het, is op 26 Februarie of 26 Maart (enkelbespuitings), asook beide datums (dubbelbespuiting), toegedien. Die behandelings en spuittye was soos volg (Tabel 3.2.11.1):

Tabel 3.2.11.1. Besonderhede van behandelings

Behandeling	Spuitye beoog vir kommersiële gebruik	Proeftoedienings*
Onbehandelde kontrole	Slegs Steriele-Insek Loslatings	
SIL + Meothrin: 30 ml/hl water	4 weke voor oestyd	8 weke voor oestyd
SIL + EXP5225: 17,5 ml/hl water**	4 weke voor oestyd	8 weke voor oestyd
SIL + EXP5225: 17,5 ml/hl water	8 weke voor oestyd	12 weke voor oestyd
SIL + EXP5225: 17,5 ml/hl water	8 weke plus 4 weke voor oestyd	12 weke plus 8 weke voor oestyd

*n Vierweke-lange tydsverloop is nodig om alle vrugte wat alreeds voor bespuiting besmet was, kans te gee om af te val. Die proeftoedienings is derhalwe nóg vier weke vroeër toegedien om te verseker dat daar genoeg tyd vir vrugvalopnames was.

**17,5 ml produk/hl water = 3,5 ml aktiewe bestanddele/hl water

n VKM-lokval van Xsit se SIL-program was in die proefblok geleë en weeklikse motvangste is aangeteken.

Vrugvalopnames is uitgevoer deur alle afvalvrugte een keer per week onder elke databoom te versamel, oop te sny en vir simptome van VKM-besmetting te ondersoek. n Vrug is as besmet beskou indien n lewendige of dooie VKM-larwe daarin gevind is en/of tipiese tekens van VKM-besmetting in die vorm van korrelrige uitwerpsels in die vrugvleis opgemerk is.

n Hobo-datalogger, wat die temperatuur uurliks gemeet het, is in een van die databome opgehang.

Resultate en bespreking

Datalogger-lesings wys dat gemiddelde nagtemperatuur in die proefboord (in die tydperk 20:00-02:00, wanneer die motte aktief is) tot die middel van Maart warm genoeg vir normale motaktiwiteit was (warmer as 20°C) (Fig. 3.2.11.1). Daarna het dit kouer geword en tot die eerste week in April het temperatuur hoofsaaklik tussen 15°C en 20°C gewissel. Dié temperatuur was waarskynlik, alhoewel laag, nog nie aanhoudend koud genoeg om n invloed op vrugbesmetting deur die motte te verhoed nie. Ná die eerste week in April was nagtemperatuur hoofsaaklik kouer as 15°C, wat ligter vrugval nagenoeg 4 weke later in die begin van Mei, tot gevolg kon gehad het.

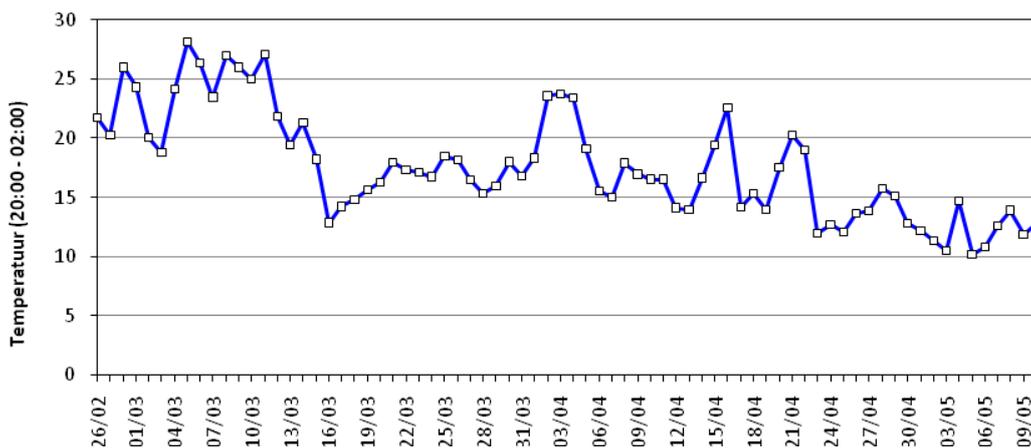


Fig. 3.2.11.1. Daaglikse temperature gedurende 20:00 tot 02:00 – die tyd wat valskoddingmot gewoonlik snags aktief is indien die temperatuur hoër as 16°C is.

Geen reën het binne 14 dae na enige van die twee bespuitingsreekse geval nie.

Die proef is uitgevoer in n gebied waar steriele motte gereeld twee keer per week in n SIL-program losgelaat word. Dit het beteken dat die natuurlike besmetting lig sal wees en n insekdoder-spuitprogram waarskynlik nie skouspelagtige verskille tussen die kontrole- en die spuitbehandelings sal lewer nie. Slegs 6 wilde mannetjies is

in die Xsit-lokval in die proefboord gevang in die tydperk 26 Februarie (eerste bespuitings) tot oestyd. Hiervan is 3 mannetjies op die laaste dag van die proef op 11 Mei gevang. Die ander drie mannetjies is een-een vroeër in die proefverloop gevang. Ander lokvalle op die plaas het net so min mannetjies gevang. Dié lae vangste wys dat daar geen algemene hewige vrugbesmetting in die boord of omgewing verwag sou kon word nie.

'n Doeltreffende VKM-doder se werking sal 3 tot 5 weke na toediening sigbaar raak. Op daardie tydstip sal alle vrugte wat alreeds voor bespuiting besmet was, van die bome afgeval het en daar sal 'n afname in die aantal besmette afvalvrugte wees. Vrugvalopnames in hierdie proef het vier weke na die eerste toedienings begin en enige bespuitingsinvloed sou waarskynlik alreeds met die eerste vrugondersoek, of kort daarna, waarneembaar gewees het. Min besmette vrugte is onder die meeste databome opgetel – insluitend die kontrole én die EXP-behandeling wat nog nie behandel was nie (EXP4w) en dus ook op daardie tydstip as 'n kontrole-behandeling beskou kon word (Fig. 3.2.11.2).

Resultate van twee van die spuitbehandelings was heeltemal teenstrydig. Vrugbesmetting op die bome van EXP8w en EXP8w+4w, wat beide 8 weke voor oestyd bespuit was, was uiteenlopend – vrugbesmetting op die eerste behandeling het toegeneem, terwyl dit in die tweede behandeling afgeneem het! Die tweede bespuiting van dié behandeling is eers op 26 Maart toegedien. Laasgenoemde bespuiting sou geen waarneembare uitwerking op vrugval tot nagenoeg 20 April gehad het nie en kan nie vir die afname in vrugval verantwoordelik gewees het nie. Die algemene afname in vrugbesmetting vanaf die einde van April is waarskynlik aan die kouer nagtemperature toe te skryf.

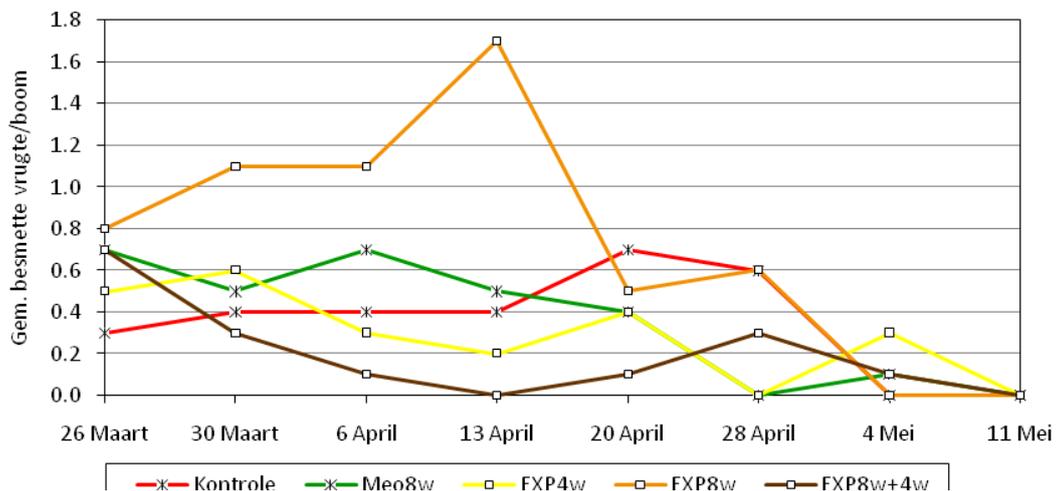


Fig. 3.2.11.2. Vrugbesmetting weens valskoddingmot in 'n insekdoderproef te Citrusdal.

Vrugbesmetting in die orige twee spuitbehandelings, Meo8w en EXP4w, was nagenoeg dieselfde as in die onbehandelde kontrole, wat verklaar kan word: EXP4w is op 26 Maart bespuit en enige uitwerking sou eers teen 28 April verwag word – dit was dus tot op 26 Maart in effek 'n kontrole-behandeling. VKM in Citrusdal het alreeds baie jare gelede weerstand teen Meothrin ontwikkel en die doeltreffendheid van dié produk kon dus so swak gewees het dat vrugbesmetting nagenoeg dieselfde as in die kontrole was.

Die besmetting op die kontrolebome was deurentyd lig en het nooit die drempelwaarde vir beduidende vrugval (een besmette vrug per boom per week) oorskry nie. Dit bring mee dat die lae vrugval in 3 van die 4 spuitbehandelings (EXP8w uitgesluit) nie aan behandelingsdoeltreffendheid toegeskryf kan word nie. Geen verklaring kan vir die relatiewe hewige besmetting in die EXP8w-behandeling gegee word nie. Dit is veral verontrustend dat EXP5225 wat op die regte tydstip toegedien was om so 'n besmetting te voorkom (26 Februarie), dit nie reggekry het nie.

Gevolgtrekking en toekomstige doelwit

Danksy die SIL-program was die natuurlike VKM-besmetting in die proefboord lig, wat beteken dat 'n bykomende insekdoderbespuiting vrugbesmetting tot die absolute minimum behoort te beperk. Dit het nie gebeur nie - die

skynbare swak resultaat met een van die EXP5225-behandelings plaas 'n vraagteken oor die produk se doeltreffendheid wat verdere ondersoek noodsaak.

3.2.12 PROGRESS REPORT: The efficacy of the insecticide EXP5225 (200 g/L SC) for control of false codling moth in the Eastern Cape

Experiment 973 (February – June 2009): Sean Moore & Wayne Kirkman (CRI)

Opsomming

CRI is gekontrakteer om doeltreffendheids proewe met 'n nuwe insekdoder, EXP5225, vir die beheer van valskodlingmot (VKM) binne 'n beheerprogram op nawellemoene in die Oos-Kaap uit te voer. EXP5225 is in 2 Palmer nawellemoenboorde in Februarie en Maart omtrent 4 weke uitmekaar uit gespuit. 'n Derde toediening word vir April beplan, omtrent 4 weke later, wat ook omtrent 4 weke voor oes is. Enkel en dubbel EXP5225 behandelings is toegedien. Van hulle is op 'n fondasie van vroer Cryptogran bespuitings (enkel en dubbel). Van 6 Januarie tot 31 Maart het 'n Cryptogran program (Desember en Februarie bespuitings) VKM besmetting met 54.3% verminder. 'n Februarie bespuiting van EXP5225 het van 10-31 Maart 'n 39.1% vermindering in VKM besmetting veroorsaak. Dieselfde behandeling op 'n Cryptogran fondasie (in Desember toegedien) was geensins meer doeltreffend nie. Weeklikse evaluasies sal tot oestyd, waarskynlik vroeg in Junie, voortgesit word. Tot dan kan geen betroubare gevolgtrekkings rondom die doeltreffendheid van EXP5225 gemaak word nie.

Summary

CRI was contracted to conduct efficacy trials with a new insecticide, EXP5225, for the control of false codling moth (FCM) within a control programme on navel oranges in the Eastern Cape. EXP5225 was applied in two orchards of Palmer navel orange trees in February and March, approximately 4 weeks apart. A third application is planned for April, approximately 4 weeks later, which will be around 4 weeks before harvest. Single and double EXP5225 treatments were applied; some were on a foundation of earlier Cryptogran sprays (single and double). From 6 January to 31 March, a Cryptogran programme (December and February sprays) reduced FCM infestation by 54.3%. From 10-31 March, a February spray of EXP5225 reduced FCM infestation by 39.1%. The same treatment on a foundation of Cryptogran (applied in December) was not any more effective. Weekly evaluations will be continued until harvest, which is anticipated to be early in June. Until then it would be premature to draw any conclusions about the efficacy of EXP5225.

Introduction

CRI was contracted to conduct efficacy trials with a new insecticide, EXP5225, for the control of false codling moth (FCM) in citrus orchards. The objective of this particular trial was to examine the efficacy of EXP5225 within an FCM control programme on navel oranges in the Eastern Cape.

Materials and methods

Orchards 40 and 41 on Far Away Farm in Sundays River Valley was selected for the trial. The orchards consisted of Palmer navel orange trees, planted in 1997 and spaced at 6 m x 3 m (rows x trees). Cryptogran had already been applied by CRI in one block of around 135 trees in each of the two orchards on 9 December 2008. Similarly sized untreated blocks had also been retained in each of the two orchards. The EXP5225 treatments were duplicated in the Cryptogran and untreated blocks (Table 3.2.12.1). In addition, Cryptogran was reapplied in February on certain of the trees in the Cryptogran blocks. Treatments were laid out as single-tree replicates in a randomised block design. There were 6 replicates per treatment per block; therefore, a total of 12 replicates per treatment. Cryptogran was applied at the registered rate of 10 ml per 100 L water plus 250 ml molasses and 18 ml Agral 90; at a volume of 19.05 L per tree (10573 L per ha). EXP5225 was applied (February and March) at 17 ml per 100 L water at a volume of 16.9 L per tree (9379 L per ha).

Table 3.2.12.1. Treatments and timing of application of Cryptogran and EXP5225 with an FCM control programme in orchards 40 and 41 (navel oranges) on Far Away Farm, Sundays River Valley.

Treatment		Application date			
		9 Dec	10/12 Feb*	9 Mar	? Apr**
1	Untreated control				
2	Cryptogran	X	X		
3	EXP5225		X	X	
4	EXP5225		X		X
5	EXP5225			X	X
6	Cryptogran	X	X		
	EXP5225				X
7	Cryptogran	X			
	EXP5225		X		X
8	Cryptogran	X			
	EXP5225			X	X

*EXP5225 was applied on 10 February; Cryptogran was applied on 12 February.

**A final application of EXP5225 will be made during April 2009.

EXP5225 sprays were applied approximately 4 weeks apart, at approximately 12 weeks (February spray) and 8 weeks (March spray) before the anticipated harvesting date. The April spray of EXP5225 will be applied approximately 4 weeks before harvest. In addition, the contractors had expressed their intention for sprays to be timed with (or shortly after) peaks in trap catches.

After application, the trial was evaluated in the following manner. Fruit drop from data trees was evaluated from three weeks after application, until shortly before harvesting began. Dropped fruit from each tree was collected weekly, and analysed separately. Evaluations were done by inspecting and dissecting fruit in order to determine the cause of drop. FCM infestation was determined by the presence of the larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation, 2001).

Results and discussion

The EXP5225 application on 10 February was not timed with a peak in FCM activity (Fig. 3.2.12.1). However, the March application was very closely timed with a flight peak. The idea was that results were likely to be better if sprays were timed with (or shortly before) a peak in emergence of neonate larvae from eggs, which would naturally follow within 1-2 weeks after a trap peak. This would only hold true if EXP5225 has a very short period of residual efficacy or a very rapidly waning residual effect. There is no indication that this is so. It is therefore questionable whether timing with FCM activity peaks is necessary. Calendar applications (e.g. 8 and 4 weeks before harvest) might be more sensible.

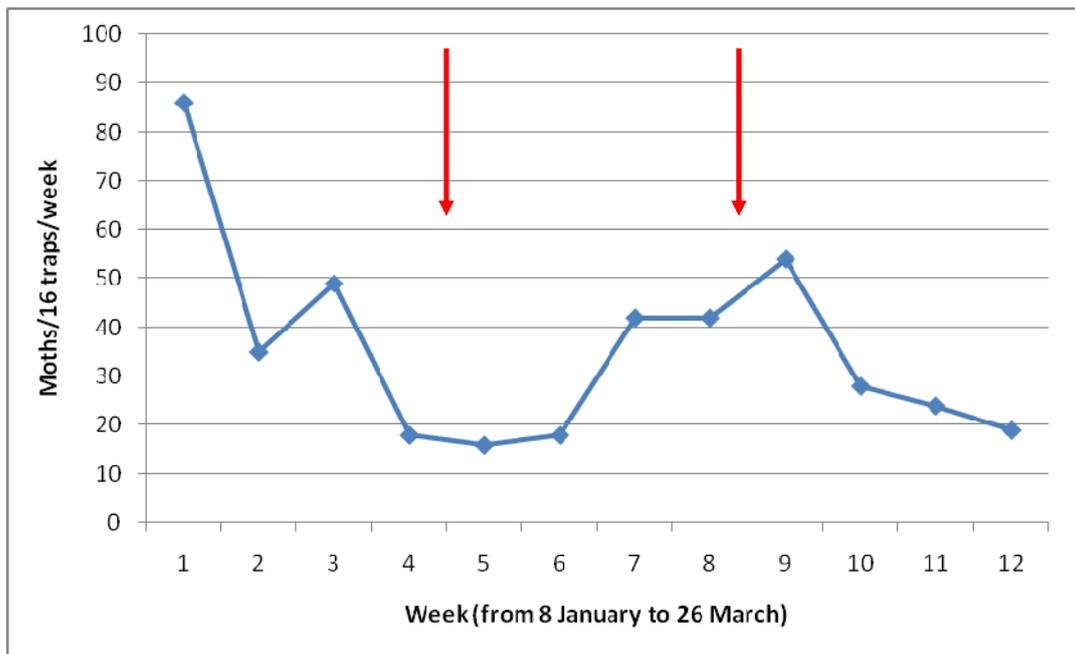


Fig. 3.2.12.1. Mean catches of male FCM adults per pheromone trap per week in 16 navel orange orchards in the general Addo region of the Sundays River Valley – the region in which Far Away Farm is situated. (Arrows indicate timing of EXP5225 treatments).

FCM infestation in the untreated control was moderate to high, averaging 1.28 infested fruit per tree per week in the untreated control, over the full evaluation period to 31 March 2009. However, evaluations will continue until harvest – projected to be early in June. The double Cryptogran programme was responsible for a 54.3% reduction in FCM infestation from 6 January to 31 March 2009 (Table 3.2.12.2). The results with Cryptogran were not as good as has become the norm in the Eastern Cape (Moore *et al.*, 2006). This was probably due to the poor (almost non-existent) orchard sanitation and the extremely dense large trees.

Table 3.2.12.2. FCM infestation of fruit for all programmes in a trial on Palmer navel oranges on Far Away Farm in Sundays River Valley. (Results from treatment 6 have not been included, as to end March 2009 this treatments was identical to treatment 2).

Treatment			Evaluation (2009)					
			6 Jan-31 Mar		10-31 Mar		31 Mar	
			Fruit infested /tree /week	Reduction in infestation	Fruit infested /tree /week**	Reduction in infestation	Fruit infested /tree /week	Reduction in infestation
1	Untreated control		1.28	-	0.96a	-	1.08	-
2	Cryptogran	Dec, Feb	0.58	54.3%	0.50b	47.8%	0.58	46.3%
3	EXP5225	Feb, Mar			0.60b	37.0%	0.67	38.0%
4	EXP5225	Feb*			0.58b	39.1 %	0.67	38.0%
5	EXP5225	Mar*					1.00	7.4%
7	Cryptogran	Dec			0.67ab	30.4%	1.25	-13.6%
	EXP5225	Feb						
8	Cryptogran	Dec					0.67	38.0%
	EXP5225	Mar						

*To be followed by another EXP5225 application in April.

**Values in the same column followed by the same letter are not significantly different ($P>0.05$; Bonferroni LSD multiple range test).

From 10-31 March, the February application of EXP5225 caused a 39.1% reduction in FCM infestation (Table 3.2.12.2). Surprisingly, the same treatment on a foundation of Cryptogran (applied in December) was not any more effective. In fact, this treatment was not even significantly different from the control. Little value can be placed on the results recorded on 31 March, as this is only one evaluation. One needs to look at efficacy over a protracted period of time. In addition, 31 March may have been a bit soon to see the results of treatments applied on 9 March, 3 weeks earlier.

Another EXP5225 treatment is scheduled to be applied in April, approximately 4 weeks after the March application. Weekly evaluations will be continued until harvest, which is anticipated to be early in June. This will enable a good and fair comparison between all treatment programmes.

Conclusion

Initial results with EXP5225 show moderate results. However, it is too soon to draw conclusions. A further application of the treatment must be applied and evaluations of efficacy must be continued. In June 2009, a full assessment of the efficacy of all treatment programmes will be possible. In addition, further issues need to be studied, such as timing of applications and spray volumes. This will need to be done in future trials. Comparisons with trials conducted in other regions and under different conditions will be made.

Further objectives (milestones) and work plan

A final application of EXP5225 is planned in April. Evaluations will continue until shortly before harvest. Further efficacy trials with EXP5225 are warranted. However, CRI's involvement will depend on research agreements.

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3.2.13 PROGRESS REPORT: Development of bioassay techniques, genetic characterisation and biological activity of South African *Cryptophlebia leucotreta* Granulovirus (CrleGV-SA) in biopesticides, Cryptex® and Cryptogran®

Experiment RU001-09 (January – July 2009): by L. L. Pereira da Conceicao, M. P. Hill (Rhodes University), S. Moore (CRI)

Opsomming

Cryptex® (Andermatt, Switzerland) en Cryptogran® (River Bioscience, Suid-Afrika) is geregistreerde biologiese produkte wat met Suid-Afrikaanse isolate van die granulovirus, CrleGV-SA, geformuleer is. Hierdie produkte word in die biologiese beheer van die valskodlingmot, *Cryptophlebia leucotreta* Meyrick, op sitrus in Suid-Afrika gebruik. Die twee biologiese insekdoders verskil geneties van mekaar en daarom is proewe ook gedoen om hulle biologiese aktiwiteit te vergelyk. Die metodes en tegnieke om biologiese aktiwiteit te bepaal is ontwikkel om meer vir veldtoestande op toepassing te wees. Hierdie sal ook kan lei tot verbetering in produkformulasie en toediening. Dit is gedoen deur die uitvoer van oppervlak en druppel dosis-respons bioetse met pasuitgebroeide larwes. In oppervlak bioetse is noodlotige konsentrasies (LC₅₀ en LC₉₀) vir Cryptex® geskat as 1.138×10^5 OPs/ml en 3.434×10^6 OPs/ml onderskeidelik, en vir Cryptogran® is hulle geskat teen 7.722×10^5 OPs/ml en 9.627×10^8 OPs/ml. Die druppel dosis-respons tegniek is vir die eerste keer ontwikkel vir *C. leucotreta*, deur larwes se gewigte te gebruik om volumes virus wat ingeneem is uit te werk. Volume per larwe is uitgewerk teen 0.0044 ± 0.0027 µl vloeistof. Noodlotige dosise (LD₅₀ and LD₉₀) van druppel bioetse moet nog uitgewerk word. 'n Volledige RFLP analiese met die gebruik van die korrekte molukulêre leër moet nog uitgevoer word om genetiese karakterisering van die virus isolate te voltooi. Ondanks die genetiese verskil tussen die twee produkte, volgens bioetse is daar geen betekenisvolle verskil in die patogeniese werking van Cryptex® en Cryptogran® nie.

Summary

Cryptex® (Andermatt, Switzerland) and Cryptogran® (River Bioscience, South Africa) are registered biological pesticides formulated using South African isolates of the granulovirus, CrleGV-SA. These products are used in the commercial control of the false codling moth, *Cryptophlebia leucotreta* Meyrick, on citrus in South Africa. The two biopesticides are genetically heterogenous and hence the biological activity between the two was tested and compared. The methods and techniques to determine biological activity were developed to better suit field conditions and aid in improving product formulation and application. This was determined by surface and droplet dose-response bioassays using neonate larvae. Surface dose lethal concentrations (LC₅₀ and LC₉₀) for and Cryptex® were estimated to be 1.138×10^5 OBs/ml and 3.434×10^6 OBs/ml respectively and for Cryptogran® were estimated as 7.722×10^5 OBs/ml and 9.627×10^8 OBs/ml. The droplet dose-response technique was developed for the first time on *C. leucotreta*. Larval weights were used to calculate volumes ingested by each larva. This was calculated as 0.0044 ± 0.0027 µl of liquid per larva. Lethal dosages (LD₅₀ and LD₉₀) for the two virus products are still to be calculated. A complete RFLP analysis using the correct molecular ladder needs to be carried out in order to complete the genetic characterisation. Despite the genetic distinction between the two products, according to the surface dose-response bioassays, the pathogenicity of Cryptex® and Cryptogran® was not significantly different.

Introduction

The false codling moth, *Cryptophlebia* (= *Thaumatotibia*) *leucotreta* Meyrick (1912) (Lepidoptera: Tortricidae) is regarded as a serious pest on citrus, cotton, maize and other crops in Africa south of the Sahara (Singh, 2001). It is particularly damaging on citrus as the bulk of South Africa's citrus is exported, generating over ZAR 2 billion in foreign currency (Moore, 2002). South Africa experiences losses of over ZAR 100 million per annum due to larval feeding resulting in premature fruit drop and foreign market rejection due to excess insecticide residues or loss of aesthetic value due to pest damage (Ludewig, 2003).

Recent advances in biological control have led to the genetic manipulation of pathogens as pest control agents to reduce pest populations (Ludewig, 2003). Biopesticides are a sustainable alternative to chemical pesticides as they are cost effective, not harmful to the consumer, they are specific to the target pest, have little or no harmful environmental effect (Erlandson, 2008), they cause no secondary pest outbreaks and mass production is possible (Singh, 2001).

Biological pesticides have been formulated for citrus using entomopathogenic viruses (Hunter-Fujita *et al.*, 1998), such as granuloviruses that belong to the family Baculoviridae. They have a very narrow host range (Wagner & Hewlett, 1999), are not harmful to the environment, are easily genetically engineered and occur as virions embedded in proteinaceous occlusion bodies (OBs) which protect them from UV light degradation and desiccation in the natural environment, and therefore make ideal pest control agents (Hunter-Fujita *et al.*, 1998; Erlandson, 2008). The *Cryptophlebia leucotreta* Granulovirus (CrleGV) is an ingested virus that is specific to the false codling moth and was first described from infected larvae from the Ivory Coast (CrleGV-IC) (Ludewig, 2003) and also occur from the Cape Verde Islands (CrleGV-CV3) and South Africa (CrleGV-SA). The wild type isolates from these 3 localities consist of a mixture of genotypes which can be separated and purified by *in vivo* cloning and have been genetically characterised by restriction analysis (Jehle *et al.*, 2003). Previous studies have shown that recombination occurs between genotypes of the isolates resulting in genetic diversity within this virus group (Jehle *et al.*, 2003). It is this genetic variation which enables the pathogen to adapt to changes that may occur in *C. leucotreta* which may otherwise result in resistance development ((Hunter-Fujita *et al.*, 1998).

Two registered biological control products using the naturally occurring South African *Cryptophlebia leucotreta* Granulovirus (CrleGV-SA) virus have been formulated for the South African pesticide market for augmentative use on citrus and other economically important crops in Integrated Pest Management (IPM) systems (Goble, 2007). These biopesticides are Cryptogran® (River Bioscience, South Africa) which is available in a suspension concentrate of 5×10^{10} virus OBs/ml (Internet Source 1) and Cryptex® (Andermatt, Switzerland) which is available in a virus concentration of 2×10^{13} OB/litre (Internet Source 2), which need to be further diluted for field application. It is important to note that the increased concentrations and dosages for viruses do not have an additive effect on the host (as one virus particle should theoretically be able to induce an infection) and rather an increased probability that one or more virus particles successfully passing through the host's natural barriers (Ridout *et al.*, 1993). In citrus, the best results are achieved when the biopesticide is applied after the main flowering period and again before harvest (Internet Source 1). This is necessary as FCM eggs are laid on the outside of the fruit and the biopesticides target the newly hatched larvae before they burrow into the fruit where they remain until pupation (Ludewig, 2003), therefore there is only one susceptible life stage (Lacey, 1997) and one chance for the larvae to ingest the virus as they burrow into the fruit so the correct product concentration used in the field is imperative. Both formulations are exceptionally sensitive to UV light, therefore late afternoon and evening application is recommended (Moore, 2002).

The accurate characterisation (both genetically and biologically) of biological pesticides is especially economically important. Not only will this type of knowledge help with improving the development of biopesticides, it will also aid in determining potential resistance build-up of the pest to the formulations. Ensuring that the characterisation of genetics and biological activities on these biopesticides is mandatory will make certain that formulations will always be of a high standard, and that generic products will be reliable in the field. Furthermore, that the end user can make informed decisions on the appropriate formulations (Hunter-Fujita *et al.*, 1998).

These two products, Cryptex® and Cryptogran® have been genetically but not biologically characterised (Goble, 2007). They have been shown to be two genetically distinct genotypes of the CrleGV-SA isolate that is presently the main component of each biopesticide (Goble, 2007). The aim of this study was to further develop the bioassay techniques used to determine biological activity to better suit field conditions as well as further characterise the biological activity of CrleGV on *C. leucotreta* using mortality as an index which is precise, biologically important and unequivocal (Rand, 1995). This was done by testing the pathogenicity of each product using surface dose-response bioassays with neonate FCM larvae to determine the lethal concentrations with the least variability in the curve (median lethal concentration, LC_{50} , required to kill 50 % of test insects) (Moore, 2002). Developing an appropriate single droplet dose-response technique to test the pathogenicity of each product and to determine the lethal dosages (median lethal dose, LD_{50} , required to kill 50% of test larvae) (Navon & Ascher, 2000) was also included in this report. The lethal concentrations and doses obtained from the experiments will be used to either confirm or reject the hypothesis that there is no significant difference in the pathogenicity between the registered biopesticide products, Cryptex® and Cryptogran®.

Materials and methods

The South African isolates of *Cryptophlebia leucotreta* Granulovirus (CrleGV-SA) used in this study were obtained from two companies which are currently manufacturing registered biological control products: Cryptogran® from the South African company, River Bioscience, and Cryptex® from Swiss company,

Andermatt. Both products were supplied as suspension concentrates in 25 ml Scholtt bottles by Dr S. Moore (Citrus Research International).

CrleGV-SA occlusion body (OB) purification and enumeration

Viral occlusion bodies (OBs) were purified according to Ludewig (2003); where 12 ml of product was added to 18 ml of 0.1% SDS. The total volume was transferred to two sterile Beckman JA-20 centrifuge tubes and centrifuged at 10 000 rpm for 30 minutes in a Beckman JA-20 rotor (Beckman, Johannesburg). The OB pellets were re-suspended in 3 ml of 0.1 % SDS. Two 25-90 % (v/v) glycerol gradients in 0.1 % SDS (one for each product) were prepared in Beckman SW-28 swing out rotor centrifuge tubes and rotating them on a BIOCOMP™ master gradient maker (Model 107, Johannesburg). The 3 ml re-suspended OB pellet was layered onto the gradient and the tube was centrifuged at 15 000 rpm for 15 minutes using the Beckman L-70 ultracentrifuge (Beckman, Johannesburg). The viral OB band was carefully auto-pipetted into two Beckman JA-20 centrifuge tubes. The tubes were filled with distilled water and centrifuges at 10 000 rpm for 14 minutes. The pellets were then again re-suspended and the spin repeated. The final pellet re-suspension as in 1.5 ml of distilled water and stored at -20 °C. The purified virus was sent to MSc student, John Opoku-Debrah at the CRI (Citrus Research International) in Port Elizabeth for virus enumeration by dark-field microscopy using a 0.02 mm depth Thoma bacterial counting chamber – a method suitable for baculoviruses (Moore, 2002).

Biological activity of viral isolates using dose-response bioassays

All larvae used in the experiments were 24-hour old neonates. Within bioassays, larvae were fed on an artificial diet consisting of (weight): 12.7% maize meal, 1.8% agar, 1.2% wheat germ, 0.6% brewer's yeast, 0.2% milk powder, 0.1% Nipagin and 0.05% sorbic acid. The bioassays were modified from Lacey (1997) and were not carried out in bioassay trays as described in Moore (2002). Instead, bioassays were conducted in independent, plastic tablet vials (size 10) obtained from a local pharmacy, each vial contained a layer of approximately 25 mm of artificial diet as opposed to the bioassay trays that had approximately 10 mm depth of diet. All bioassay experiments were conducted under a laminar flow cabinet to prevent contamination and incubated in constant environment (CE) rooms at 27 °C for designated time periods. The symptomatology of the infected larvae was observed for Cryptex ® and Cryptogran® for both surface and droplet dose-response bioassays (Fig 3.2.13.1).

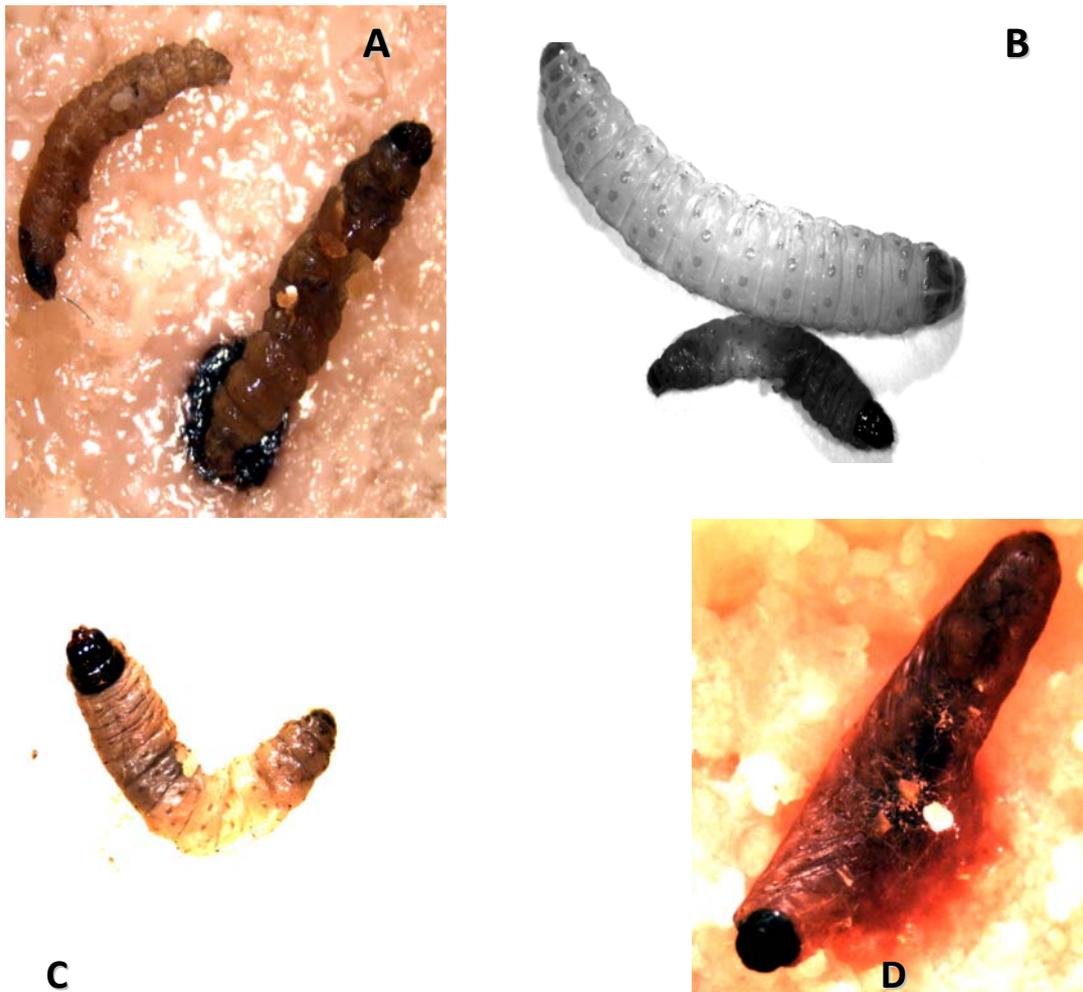


Figure 3.2.13.1. Symptomatically CrleGV infected third instar *C. leucotreta* larvae, [A] disseminating viral occlusion bodies, [B] comparison between uninfected fourth instar larva and infected third instar larva, [C] flaccid, darkening body of third instar larva and, [D] characteristic of infected, disseminating third instar cadaver. (Photos obtained from Goble, 2007).

Surface dose-response bioassays (LC)

Surface dose bioassays included five 5-fold serial dilutions of purified CrleGV products in distilled water. The highest concentration was 7.63×10^4 OBs/ml and distilled water was used for the control. Twenty five vials were used per treatment (Fig 3.2.13.2); 50 μ l of each viral dilution and of the control water was pipetted onto the centre of the diet and spread evenly over the surface by tilting the vials. One neonate larva was placed into each vial with a 000 paint brush, a cotton-wool plug was placed in the vial lid to absorb condensation and the vials were kept at $27^\circ\text{C} \pm 1^\circ\text{C}$ for 10 days. After this time period, each vial was opened and the larvae were recorded as either dead or alive. Non-responsive or missing larvae were recorded as dead. Some vials were found to be contaminated with fungus, *Aspergillus* spp. however the presence of this fungus did not appear to affect the larvae. Four replicates were conducted for each of the 6 treatments (doses) for each viral product with 25 larvae per dose. The replicates were combined and dose-response curves were calculated using PROBAN (Van Ark, 1995) probit analyses (Finney, 1971) computer software. This programme takes into account the mortality from the control insects and corrects the response from treated larvae according to Abbott's formula (Abbott, 1925). The lethal concentrations required to kill 50% of larvae (LC_{50}) and 90% of larvae (LC_{90}) were calculated using PROBAN. The data was log-transformed and percentage responses were transformed into empirical probits which linearizes the data (probit line). A χ^2 -test was used to determine if the fit of the probit lines and elevations were homogenous, parallel and therefore comparable (Rand, 1995). The value G, was used for the calculation

of the fiducial limits. The probits were then compared using Bartlett's F-test for homogeneity of residual variances.

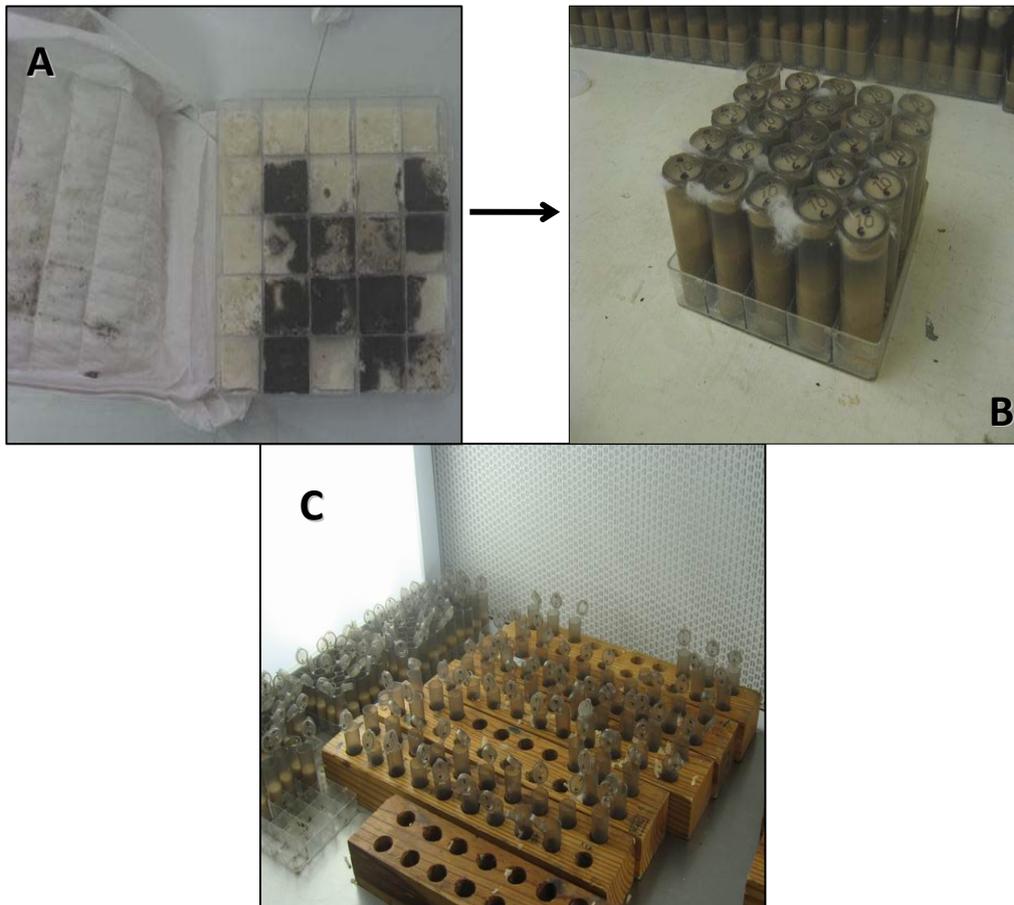


Figure 3.2.13.2. The change in bioassay technique from [A] the old bioassay trays containing cells that larvae and fungal contaminants were able to freely move between, to [B] independent cells with individual lids thus solving the problem of missing larvae, cross contamination and fungal contamination, [C] bioassay vials were placed in test tube racks to facilitate the pouring of artificial diet.

Development of droplet dose-response bioassays (LD)

The single droplet dose-response bioassay had not previously been conducted with false codling moth. As a neonate larva is only approximately 1 - 2 mm in length, it was not possible to successfully feed each larva a specific amount of virus. Instead, parafilm was placed on the inside of a jar containing newly hatched FCM larvae, once the parafilm contained over 100 larvae it was removed and placed in to petri dish (Fig 3.2.13.3). Ten μl droplets of distilled water containing 1% (w/v) brilliant blue dye (Navon & Ascher, 2000) were placed randomly amongst the larvae on the parafilm (Fig 3.2.13.3). The larvae readily drank from the droplets and 10 larvae (distinguished by a blue gut) were weighed on a Sartorius electronic micro-balance (Sartorius AG, Germany) before and after ingesting the fluid, as well as the weight of the dye-solution in 50 μl droplets. These were replicated 10 times. The average weights were calculated and used in the actual droplet dose-response technique. This experiment relies on the assumption that lepidopteran larvae will not stop ingesting liquid until completely saturated (Navon & Ascher, 2000). Therefore each larva should consume roughly the same amount of solution. Droplet dose-response bioassays to determine the effect of the 1% brilliant blue dye on the larvae was then conducted, with distilled water as the control. This experiment was replicated 3 times and the data analysed using a Student's 2 sample t-test.

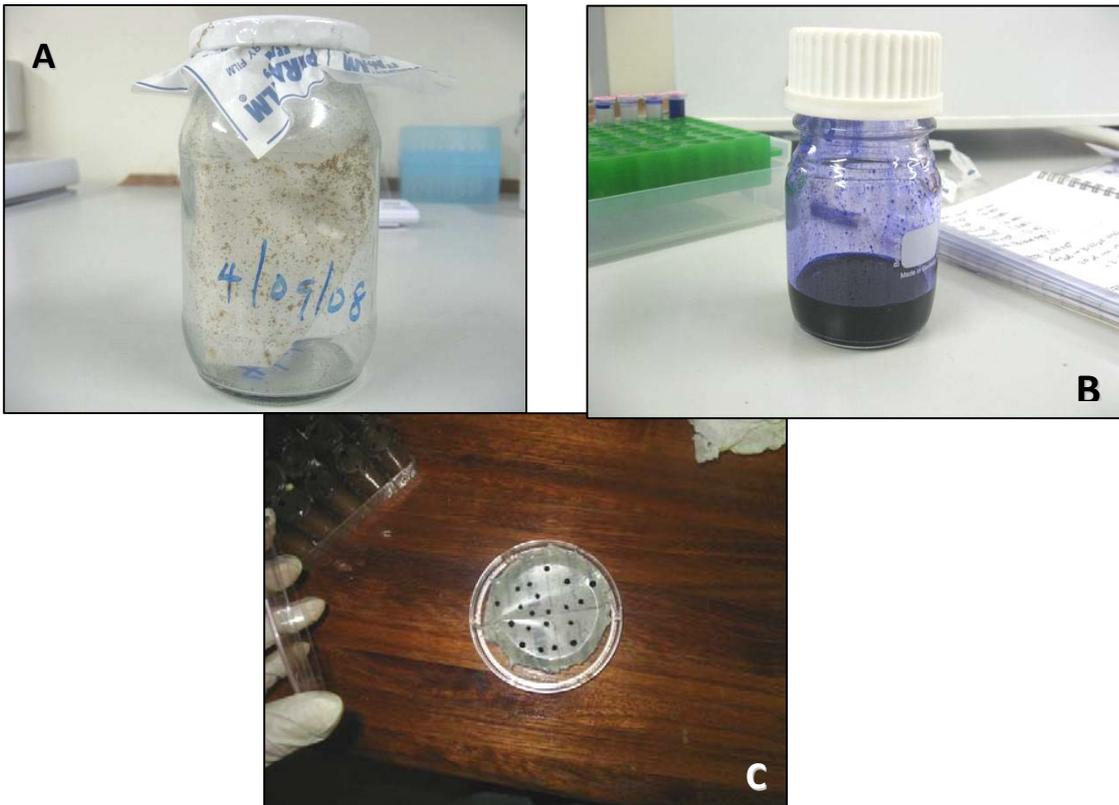


Figure 3.2.13.3. Droplet dose-response technique where [A] larvae were collected by placing parafilm onto the lid of the hatching jar, [B] a 1 % brilliant blue dye (w/v) and distilled water solution used in the droplet bioassays and, [C] the parafilm containing over 100 larvae from the hatching jar was placed into a Petri dish and 10 – 15 μ l droplets were randomly placed amongst the larvae and left to drink for 30 minutes.

The actual droplet dose-response bioassays were conducted in 25 tablet vials per treatment (Fig 3.2.13.2). Single droplet dose-response bioassays were generally conducted in the same way as the surface-dose bioassays with a few modifications. Five 2-fold serial dilutions were prepared with the highest concentration as 3.637×10^6 OBs/ml, with distilled water as a control. The larvae were placed onto parafilm containing 10 – 15 μ l droplets of a purified CrleGV viral concentration containing 1% Brilliant Blue dye and allowed 30 minutes to ingest the liquid. Larvae that had noticeably ingested the blue dye were selected and one larva was placed per vial in order to negate any transmission of virus from one larva to another (Moore, 2002). Three replicates were carried out for each viral isolate and the droplet dose-response curve was calculated using PROBAN (Van Ark, 1995), as was the surface dose-response curve. From these probit analyses, the lethal doses and lethal concentrations (LD_{50} and LD_{90} and LC_{50} and LC_{90} , respectively) were calculated.

Genetic characterisation

Cryptex® and Cryptogran® were genetically analysed and characterised by Goble (2007). However, the RFLP analysis was not completed, as the molecular marker used at the time did not include the largest and smallest DNA bands cut by the restriction enzymes for both products. Therefore viral DNA was extracted using the protocol from Ludewig (2003) and an RFLP including the correct molecular marker will be run on the DNA samples.

Results and discussion

CrleGV-SA OB purification and enumeration

In the dark-field microscopy of the glycerol purified viral isolates, Cryptex® yielded 3.58×10^{10} OB/ml while Cryptogran® yielded 5.00×10^{10} OB/ml. These purified viral stocks were diluted in the appropriate series and used in the surface and droplet dose-response bioassays.

Surface dose-response bioassays

The probit (regression) lines were fitted to the corrected responses (Table 3.2.13.1) with the linear equations of $y = 0.621 + 0.866X$ (SE of regression coefficient = 0.216) and $y = 2.563 + 0.414X$ (SE of regression coefficient = 0.125) for Cryptex® and Cryptogran® respectively (Fig 3.2.13.4) using PROBAN (Van Ark, 1995). The χ^2 test was used on each data set to determine the fit of each probit line at the test level of $\alpha = 0.05$, probit lines for both viral pesticides were considered homogenous with $\chi^2 = 1.041$ at 3 degrees of freedom with $p = 0.794$ for Cryptex® and $\chi^2 = 0.940$, (df = 3) with $p = 0.818$ for Cryptogran®. This homogeneity considers the deviations between observed and expected larval mortalities to be no larger or smaller than expected from normal sampling variation. Four identical experiments were conducted for each biopesticide. The data was combined for each of the five calculated viral doses (OBs/ml) and percentage larval mortality with standard errors (SE) per dose is shown in Table 3.2.13.1. The PROBAN analysis was programmed to account for 20% mortality of control insects as a conservative method of incorporating human error. The empirical probits per viral treatment and corrected percentage mortality were obtained from the PROBAN analysis and therefore did not show the standard errors for these values (Table 3.2.13.1). When calculating the fiducial limits for the probit lines a value G is determined, this indicates the response variations of the larvae between experimental replicates. If G is found to be greater than 1 (indicating extreme response variation) then no fiducial limits are calculated. G was calculated to be 0.2383 and 0.3500 for Cryptex® and Cryptogran® respectively. However, Moore (2002) states that G values above 0.25 are rather large and accuracy of the experimental procedures or the value of the probit lines may be questionable. The residual variances of the two probit lines were found to be homogenous with F-value = 1.107 (df. = 3, 3), $p = 0.9360$ at a test level of $P = 0.01$ (two-tailed test). Therefore the slopes and elevations of the two probit lines may be compared. The χ^2 test for parallelism was carried out on the probits at a $P = 0.05$ test level, χ^2 -value = 3.289 (df. = 1) with $p = 0.0660$, therefore concluding that the two lines are parallel and the elevations are comparable. A comparison of elevations (with adjusted means) was then performed on the data at a $P = 0.05$ test level and the elevations were found to be insignificantly different from one another (F-value = 0.036; d.f = 1, 7; $p = 0.8560$). This suggests that there is no significant difference in the pathogenicity between the two viral biopesticides Cryptex® and Cryptogran®.

Table 3.2.13.1. Mean percentage mortality for *C. leucotreta* neonate larvae from 4 replicates of a five-fold surface-dose response bioassay with CrleGV-SA, isolated from two viral biopesticides, Cryptex® and Cryptogran®. Calculated empirical probits and larval mortality recorded from each treatment were calculated using PROBAN (Van Ark, 1995), after correcting for control mortality.

Combined replicates	Cryptex			Cryptogran			
	Dose of CrleGV (OBs/ml)	Larval mortality (%)	Corrected response (%)	Empirical probit	Larval mortality (%)	Corrected response (%)	Empirical probit
Control		20.00 ± 0.00	NA	NA	20.00 ± 0.00	NA	NA
	1.221×10^2	21.00 ± 11.0	1.25	2.758	22.00 ± 7.7	2.50	3.040
	6.104×10^2	26.00 ± 10.1	7.50	3.560	28.00 ± 8.6	10.00	3.718
	3.050×10^3	25.00 ± 11.0	6.25	3.466	36.00 ± 11.3	20.00	4.158
	1.520×10^4	37.00 ± 3.8	21.25	4.202	39.00 ± 10.0	23.75	4.286
	7.630×10^4	56.00 ± 4.6	45.00	4.874	46.00 ± 5.2	32.5	4.546

The lethal concentrations for each biopesticide were calculated by PROBAN. Cryptex® LC₅₀ and LC₉₀ were estimated to be 1.138×10^5 OBs/ml and 3.434×10^6 OBs/ml while LC₅₀ and LC₉₀ for Cryptogran® were estimated to be 7.722×10^5 OBs/ml and 9.627×10^8 OBs/ml (Fig 3.2.13.4). Larval mortality above 50% was not obtained in any of the experiments and the control mortality was never zero, this implies that the LC₅₀ to LC_{99.9} values from both Cryptex® and Cryptogran® were an estimation of the actual values and provides a possible

explanation of the increasingly large variations in the fiducial limits around these LC values. Although the Cryptex® (red) probit gradient has a steeper elevation (regression coefficient = 0.866) than the Cryptogran® (green) probit gradient (regression coefficient = 0.414), this difference is not significant and is due to chance alone and is not the result of one viral product being more pathogenic than the other product (Fig 3.2.13.4).

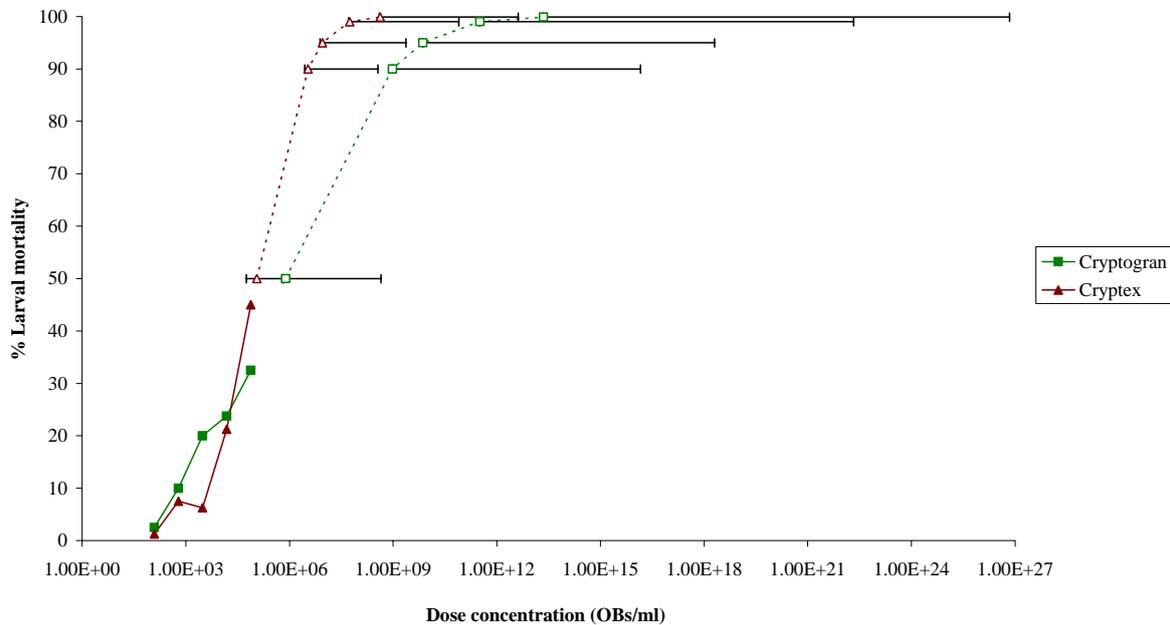


Figure 3.2.13.4. Dose-response plot of percentage mortality of 100 neonate *C. leucotreta* larvae to a five-fold dilution series surface dose-response bioassay with purified CrIeGV-SA from two biological control products, Cryptex® and Cryptogran®. The broken-line represents the PROBAN calculated lethal concentrations with LC₅₀ to LC_{99.9} values plotted together with the upper and lower fiducial limits shown as horizontal bars on the graph.

Development of droplet dose-response bioassays

It has been shown that the volumes ingested by lepidopteran neonate larvae are generally constant (Navon & Ascher, 2000) and therefore the amount imbibed by *C. leucotreta* was made on an estimation of weight. The larvae were weighed before and after ingesting the dye and virus dose, the average larval weight before liquid ingestion was found to be 0.0181 mg and 0.0224 mg after ingestion, therefore each larva ingests an average of 0.00435 mg. A 50 µl droplet of dH₂O weighed approximately 49.45 mg and therefore each larva would have ingested 0.0044 ± 0.0027 µl of liquid. From this it was possible to calculate the approximate number of occlusion bodies (OBs) ingested per larva per virus concentration and therefore ending the five two-fold dilution series with the lowest concentration equivalent to each larvae ingesting one OB (2.273 x 10⁵ OBs/ml) and the highest concentration amounting to 16 OBs/larva (3.637 x 10⁶ OBs/ml).

The bioassays that compared the effect of the 1% brilliant blue dye against the distilled water control on the neonate larvae were tested using a two sample t-test with equal variances (F = 0.1429; df = 2 & 2; p = 0.2500) and a normal distribution (D = 0.3333; p = 0.9963), where t = -0.3536 with df = 4 and p = 0.7415, therefore failing to reject the null hypothesis that the dye has no significant effect on the larvae compared to distilled water. Therefore 1% brilliant blue dye can be safely used in droplet dose-response bioassays.

Synthesis of surface and droplet dose-response bioassay techniques

The LC₅₀ and LD₅₀ values are considered the closest and most accurate determination of response frequency with the smallest range in fiducial limits and least variability within the curve (Navon & Ascher, 2000; Sparks, 2000). These values determined in this study are much higher than those proposed by Moore (2002) and Goble (2007). This may be as a result of the change in method from bioassay trays to separate, independent vials. The bioassay trays were problematic as they were susceptible to severe fungal contamination (*Aspergillus* spp.) and subject to cross-contamination of the virus and escaping larvae, as larvae were able to move freely between

cells (Fig 3.2.13.2). The depth of diet in the bioassay cells were only 5 – 10 mm which resulted in the larvae burrowing in and out of the diet plugs, this does not reflect field conditions as the neonate larvae only burrow into the fruit once and remain within the fruit until pupation (Singh, 2001). This therefore also increased the chance of a larva ingesting virus. The tablet vials had individual lids and therefore provided independent cells that solved the issue of missing or escaping larvae and reduced fungal contamination considerably (Fig 3.2.13.2). As each vial was filled with a layer of approximately 25 mm of artificial diet, which did not contain agar and was therefore preferred by the larvae, they were found to only burrow through the diet once. Larvae therefore had only one chance to come into contact with virus, therefore creating a more accurate reflection of field conditions. With this new method, more bioassays need to be performed in order to acquire a full series of results which are representative of the new methodology used. In addition, dose ranges which lead to mortality between 10% and 90% must be established, otherwise results are considered inaccurate (Jones, 2000).

Conclusion

The registered biopesticide formulations, Cryptex® and Cryptogran® were found to be genetically distinct viral isolates of the SA variant of CrleGV (Goble, 2007). However, this genetic distinction does not infer that the biological activity of the two isolates should differ. In this study, using surface dose-response techniques, it was found that there is no significant difference in the pathogenicity, in the laboratory, between the two viral biopesticides, Cryptex® and Cryptogran®. To obtain more accurate lethal concentrations and dosage values, more bioassays with wider concentration ranges that include the PROBAN estimations as a basis to begin with, should be conducted. The bioassays in this study should be considered as preliminary trials due to the method change as they did not comprise the correct range of viral concentrations. The methods developed in this paper are a step closer to emulating field conditions. This constructive technology can potentially aid in the improvement of biopesticide formulation (by aiding in the selection of more virulent isolates) and of biopesticide application (by reaching a more accurate determination of product quantities and thresholds needed for optimum control) (Hunter-Fujita *et al.*, 1998), thus preventing wastage. Selected geographically virulent isolates should have significant implications on the market, as the possibility of a genetic difference or mutation giving rise to a more pathogenic viral isolate are indeed real, even though this has not been the case in this study. Therefore further studies should genetically and biologically identify and characterise geographic isolates of CrleGV-SA in orchards around South Africa, so that empirical tests of these may improve biopesticide formulation and ultimately application in the field.

Technology transfer

A poster will be presented at the Entomological Society of southern Africa (ESSA) conference in July 2009, entitled: "Development of bioassay techniques and biological activity of South African *Cryptophlebia leucotreta* Granulovirus (CrleGV-SA) in biopesticides, Cryptex® and Cryptogran®." A paper will be submitted in an appropriate journal, entitled: "Development of bioassay techniques, genetic characterisation and biological activity of South African *Cryptophlebia leucotreta* Granulovirus (CrleGV-SA) in biopesticides, Cryptex® and Cryptogran®".

Further objectives (milestones) and work plan

The droplet dose bioassays need to be replicated before performing a probit analysis and estimating the LD values. A full RFLP electrophoresis gel of the DNA of Cryptex® and Cryptogran® that includes the correct size molecular marker needs to be run before the genetic characterisation of the 2 biopesticides can be completed.

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3.3 PROJECT: FRUIT FLIES

Project Coordinator: A. Manrakhan (CRI)

3.3.1 Project summary

Fruit flies are problematic to the citrus industry because they cause direct damage to fruits and most importantly they are quarantine pests that can restrict market access. Laboratory colonies of three fruit fly species: (1) Mediterranean fruit fly (Medfly), *Ceratitidis capitata*; (2) Natal fly, *C. rosa* and (3) Marula fly, *C. cosyra* continue to be maintained at the CRI facility to provide insect material for applied and basic research. Success of these experiments depends on quality of insect material provided. A study was carried out to determine the life history traits of Medfly reared on the standard bran diet and other carrot based diets (3.3.2).

Phytosanitary regulations associated with fruit fly pest species are often associated with potential distribution of the species. While Medfly is widely distributed across the world and now reported on all continents, Natal fly distribution is mainly restricted to the east and south of the African continent and also in some Indian Ocean islands. The distribution of Natal fly in different climatic regions in South Africa was determined by trapping (3.3.3). Data from the distribution will then be used to model its potential global distribution.

Control of fruit fly pests is mainly carried out through the use of poisoned protein baits applied either as sprays or stations. Reduced tolerances of insecticide residues in fruits have called for an optimization of the bait application technique. There were three experiments to address research on the bait application technique. Performances of currently used fruit fly baits (HymLure, GF 120 and M3 bait) were determined (3.3.4). Medfly and Natal fly were found to respond well to all three baits, in particular when fresh. Marula fly responded well to GF 120 and M3 bait but were not attracted as much to HymLure. An increase in HymLure concentration led to a corresponding increase in response of Medfly to this bait. A new bait, Prolure, was found to be attractive to all

three fruit fly species. The M3 bait station represents a viable alternative to liquid bait sprays for fruit fly control. A new strategy for deployment of M3 bait stations was explored with stations placed in decreasing numbers from perimeter to centre of an orchard (3.3.5). This new strategy was found to compare well with uniform placement of M3 bait stations and aerial bait spray application. Efficacy of three treatments for controlling fruit flies was determined in a citrus orchard (3.3.6). The three treatments were: (1) spray application of a mixture of HymLure and Malathion, (2) application of M3 bait stations, (3) application of M3 bait stations in combination with male M3s (stations loaded with Capilure). Use of M3 bait stations, with or without male M3s, was found to be more effective than a spray application of HymLure and Malathion in reducing fly numbers and fruit damage, although differences between the treatments were not statistically significant.

The new invasive Asian fruit fly pest, *Bactrocera invadens*, which is rapidly spreading across Africa and is already in southern Africa (Zambia, Mozambique and Namibia) constitutes a threat to all fruit industries, including the citrus industry, of South Africa. Research on post harvest treatment and control was carried out with collaborators in Kenya (ICIPE) and Benin (IITA), respectively. Results from the post harvest research studies have shown that the third larval instar of *B. invadens* was the least susceptible stage to cold treatment (3.3.7). As such, the third larval instar will be used in developing a cold treatment for oranges at 1.1°C. The efficacy of male annihilation technique for control of *B. invadens* was determined in mango orchards in Benin (3.3.8). This technique, which utilizes a combination of methyl eugenol, a powerful male attractant, and Malathion placed in stations was found to be effective in reducing populations of this invasive pest as well as fruit damage.

Research on ethyl formate as a fumigant for fruit fly and other phytosanitary pests (3.3.9) could not be conducted since no permission was granted for importation of this chemical from Australia. Two basic research projects were conducted at Stellenbosch University (3.3.10 & 3.3.11). Interspecific competition between Medfly and Natal fly on different host fruits (deciduous and citrus) was studied (3.3.10) and the cold tolerance of Natal fly was investigated (3.3.11). The aim of both projects was to provide a basis for understanding the geographical distribution and abundance of Natal fly. The use of entomopathogenic fungi for control of fruit flies was investigated in research conducted at Rhodes University (3.3.12). A number of potential fungal isolates for control of Medfly were isolated from soils in citrus orchards. A higher recovery of fungal isolates was obtained from soil samples taken from areas of indigenous vegetation neighbouring citrus orchards.

Projekopsomming

Vrugtevlieë is problematies vir die sitrusbedryf omdat hul direkte skade aan vrugte kan veroorsaak en meer belangrik, hulle is kwarantynplae wat marktoegang kan beperk. Daar is voortgegaan om laboratorium kolonies van drie vrugtevlieg spesies: (1) Mediterreense vrugtevlieg (Medvlieg), *Ceratitis capitata*; (2) Natalvlieg, *C. rosa* en (3) Maroelavlieg, *C. cosyra* by die CRI fasiliteit te onderhou om insekmateriaal vir toegepaste en basiese navorsing te voorsien. Sukses van hierdie eksperimente is afhanklik van die kwaliteit van die insekmateriaal wat voorsien word en 'n studie is uitgevoer om die kenmerke van die lewensiklus van Medvlieg wat op 'n standaard vesel dieet en ander wortel gebaseerde diete geteel is, te bepaal (3.3.2).

Fitosanitiere regulasies wat aan spesies van vrugtevlieë wat plae is, gekoppel is, is dikwels geassosieer met potensiële verspreiding van die spesie. Terwyl Medvlieg wyd verspreid is oor die wêreld en nou in alle lande aangeteken is, is Natalvlieg hoofsaaklik beperk tot die ooste en die suide van die Afrika kontinent en ook tot sommige eilande in die Indiese Oseaan. Die verspreiding van Natalvlieg in verskillende klimaatstreke in Suid-Afrika is met lokvalle bepaal (3.3.3). Inligting van die verspreiding sal gebruik word om sy potensiële globale verspreiding te modelleer.

Die beheer van vrugtevlieg plae word hoofsaaklik deur die gebruik van giftige proteïen lokmiddels, wat as bespuitings of as lokvalstasies toegedien word, gedoen. Verminderde toleransies van residue van insekdoders op vrugte het die optimisering van die lokmiddel- toedieningstegniek genoodsaak. Daar was drie eksperimente om die navorsing van die lokmiddel-toedieningstegniek aan te spreek. Prestasies van die vrugtevlieg lokmiddels wat tans gebruik word (HymLure, GF 120 en M3 lokmiddel) is bepaal (3.3.4). Daar is gevind dat Medvlieg en Natalvlieg goed op al drie lokmiddels reageer, veral wanneer dit vars is. Maroelavlieg het goed op GF 120 en M3 lokmiddels gereageer, maar is nie so sterk deur HymLure aangelok nie. 'n Toename in HymLure konsentrasie het gelei tot 'n ooreenstemmende toename in die reaksie van Medvlieg tot hierdie lokmiddel. 'n Nuwe lokmiddel, Prolure, is gevind om aantreklik vir al drie vrugtevlieg spesies te wees. Die M3 lokval stasie verteenwoordig 'n sigbare alternatief vir die vloeistof lokmiddel bespuitings vir die beheer van vrugtevlieg. 'n

Nuwe strategie vir die uitplasing van M3 lokval stasies is ondersoek, die stasies is so geplaas dat die getalle vanaf die buitekant tot die middel van die boord afneem (3.3.5). Hierdie nuwe strategie is gevind om goed te vergelyk met die uniforme plasing van M3 lokvalstasies en lugbespuitings van lokmiddels. Effektiwiteit van drie behandelings vir die beheer van vrugtevlug is in 'n sitrusboord bepaal (3.3.6). Die drie behandelings was: (1) bespuitings van 'n mengsel van HymLure en malathion, (2) aanwending van M3 lokval stasies, (3) aanwending van M3 lokval stasies in kombinasie met manlike M3s (lokvalstasies wat met Capilure gevul is). Gebruik van M3 lokvalstasies, met of sonder manlike M3s, is gevind om meer effektief te wees as 'n bespuiting van HymLure en malathion in die vermindering van vlieggetalle en vrugskade, alhoewel die verskille tussen die behandelings nie statisties belangrik was nie.

Die nuwe indringer Oosterse vrugtevlug plaag, *Bactrocera invadens*, wat besig is om vinnig oor Afrika te versprei en reeds in suider-Afrika is (Zambië, Mosambiek en Namibië) hou 'n bedreiging vir alle vrugtebedrywe, insluitende die sitrusbedryf, van Suid-Afrika in. Navorsing op na-oes behandelings en beheer is met medewerkers in Kenia (ICIPE) en Benin (IITA) onderskeidelik uitgevoer. Resultate van die na-oes navorsing het getoon dat die derde larwale instar van *B. invadens* die minste vatbaar is vir 'n koue behandeling (3.3.7). Die derde larwale instar sal dus in die ontwikkeling van 'n koue behandeling vir lemoene by 1.1°C gebruik word. Die effektiwiteit van die uitwissingstechniek wat op mannetjies gerig is vir die beheer van *B. Invadens*, is in mangoboarde in Benin bepaal (3.3.8). Die tegniek wat van 'n kombinasie van methyl eugenol, 'n kragtige lokmiddel vir mannetjies, en malathion gebruik maak is in lokvalstasies geplaas en is gevind om effektief te wees in die vermindering van die populasie van hierdie indringerplaag asook vrugskade.

Navorsing op ethyl formate as 'n berokingsmiddel vir vrugtevlug en ander fitosanitêre plaeg (3.3.9) kon nie uitgevoer word nie omdat toestemming vir die invoer van hierdie chemikalie vanaf Australië, nie toegestaan is nie.

Twee basiese navorsingsprojekte is by Stellenbosch Universiteit (3.3.10 & 3.3.11) uitgevoer. Interspesifieke kompetisie tussen Medvlug en Natalvlug op verskillende gasheer vrugte (sagte vrugte en sitrus) is bestudeer (3.3.10). Die koue toleransie van Natalse vrugtevlug is ook ondersoek (3.3.11). Die doel van beide projekte was om in 'n basis te voorsien om die geografiese verspreiding en volopheid van die Natalvlug te verstaan. Die gebruik van entomopatogeniese fungi vir die beheer van vrugtevlug is ondersoek in 'n projek wat by Rhodes Universiteit uitgevoer is (3.3.12). 'n Aantal potensiële swam isolate vir die beheer van vrugtevlug is van gronde in sitrusboorde geïsoleer. 'n Hoër verkryging van swam isolate is gevind met grondmonsters wat algemeen is van areas van inheemse plantegroei wat aangrensend aan sitrusboorde is.

3.3.2 PROGRESS REPORT: Fruit fly rearing

Experiment 407 (1999 onwards): by Aruna Manrakhan, Charl Kotze and Rooikie Beck (CRI)

Opsomming

Huidiglik word daar drie verskillende vrugtevlug spesies n.l.: *Ceratitis capitata*, *C. rosa* en *C. cosyra* te CRI Nelspruit geteel om insek materiaal te lewer vir verskeie navorsings projekte. Hierdie projekte sluit in die evaluasie van verskillende lokase en gifstowwe, die ondersoek na entomopatogeniese swamme met die oog op biologiese beheer en vrugtevlug gedrag en fisiologie. Die sukses van hierdie projekte berus alleenlik op die kwaliteit van die insek materiaal wat gelewer word, wat weer op sy beurt direk beïnvloed word deur die sintetiese voedingsmedium waarop teling plaasvind. Daarom is daar 'n studie geïnisieer om die effek van drie verskillende voedingsmedia op verskillende lewens eienskappe van *C. capitata* te bepaal. Agt verskillende lewens eienskappe is waargeneem: Larvale ontwikkelings tydperk, papier gewig, papier herwinning, volwasse ontpopping, volwasse oorlewing, vlieg vermoë sowel as bevrugting en vrugbaarheid. Die twee media met wortel as basis het vlieg met 'n korter papier ontwikkelings periode gelewer, sowel as meer papier. Terwyl die medium met semels as basis tot 'n hoër vlugvermoë onder die vlieg gelei het. Dit moet alhoewel ook gesê word dat al drie voedingsmedia baie lae vlugvermoëns getoon het en dat hierdie verskynsel verder ondersoek sal word. Verdere studies sal op die ander twee spesies herhaal word te CRI.

Summary

Three fruit fly species: *Ceratitis capitata*, *C. rosa* and *C. cosyra* are currently being reared at the CRI facilities in Nelspruit to provide insect material for various research projects: Evaluation of baits and toxicants for fruit fly control, investigation of entomopathogenic fungi as biocontrol agents, fruit fly behaviour and physiology. The

success of these experiments depends on the supply of quality insect material which in turn will be affected by the artificial diet used in rearing. A study was initiated to compare the nutritional effects of three types of diet on life history traits of *C. capitata*. Eight life history traits were studied: larval development time, pupal weight, pupal recovery, adult emergence, adult survival, flight ability, fecundity and fertility. The carrot-based diets produced flies with shorter larval development time and higher pupal recovery. The bran-based diet produced flies with a better flight ability. Flight ability was, however, generally low for all diets and should be further investigated and corrected. This study will be repeated with the two other fruit fly species being reared at CRI.

Introduction

Laboratory colonies of three species of fruit flies: *Ceratitis capitata*, *C. rosa* and *C. cosyra* are currently being maintained in Nelspruit to provide insect materials for different research projects. The three species are maintained on two types of diet. *C. capitata* is maintained on a diet consisting of bran while the other two species are reared on a carrot-based diet (without bran). An investigation was initiated to determine the nutritional effects of different diets on life history traits of *C. capitata* over five generations in the laboratory. The same studies will be carried out thereafter on the two other species with the aim of comparing the nutritional effects of different larval diets for the rearing of the three congeneric species.

Supply of materials for fruit fly research

Adult fruit flies were used for the evaluation of different fruit fly baits and toxicants in cages (Experiment 915). *C. capitata* and *C. rosa* eggs and larvae were provided to Ms Tarryn Anne Goble at Rhodes University for research on entomopathogenic fungi for fruit fly control (Experiment 930). *C. capitata* and *C. rosa* pupae were also shipped to Dr John Terblanche and Dr Juanita Heunis for research on cold tolerance of Natal fruit fly and fruit fly behaviour respectively.

Laboratory evaluation of diets on life history traits of Mediterranean fruit fly, *C. capitata*

Materials and methods

Eggs were collected from colonies of adult *C. capitata* maintained for about 200 generations on a bran-based diet and placed on moist blue blotting paper inside a 9 cm diameter petri dish. After 24-36 hours, 100 neonate larvae were introduced with a fine brush onto the surface of 150 g of each of three diets evaluated (Table 3.3.2.1) in plastic cups.

Each artificial diet cup was maintained in a larger plastic container that contains a thin layer of sand at the bottom to serve as pupariation medium. There were four cups of each diet type. Observations were made for each diet for 5 consecutive generations. At every generation, the following were recorded:

1. Larval stage duration defined as time from neonate larval introduction into diet to pupal formation. This was done by checking containers daily for pupae/pupariating larvae.
2. Percentage of pupae recovered from diet. This was calculated by counting the total number of pupae per cup obtained over the number of larvae introduced into each cup.
3. Weight of pupae. The weight of four lots of 20 pupae for each diet type was recorded 4 days after pupal formation. 20 pupae were selected from each container for weighing.
4. Adult emergence and adult survival. After recording weight of the four lots of 20 pupae for each diet, each lot was placed in a plastic container and provided with water, sugar and yeast. The percentage of adult emergence and adult survival was recorded 21 days after the first adult emergence.
5. Flight ability. Four lots of 20 pupae from each diet type were placed on a brown paper inside a 9 cm diameter petri dish. A black plastic tube 8.9 cm diameter and 10 cm long was placed over the petri dish containing the pupae. The tube was coated with talc powder to prevent flies walking up the tube. A transparent petri dish coated with a sticky insert was placed on top of the tube to trap fliers. The remaining pupae from each diet type were put together and placed inside separate rearing cages. Eggs were then collected from each diet type for inoculation into the respective diet type (as above). This was repeated for five consecutive generations.
6. Fecundity and Fertility. 10 pairs of 7-10 day old flies from each diet type were placed in individual plastic containers fitted with side nettings and containing sugar, yeast and water offered separately. The number of eggs laid per female was determined daily for a period of 10 days. Females laid eggs through the side nettings and a cup lined with a small volume of water was placed under each container to collect eggs. Hatch rate of the eggs were determined after 72 hours.

All experiments were carried out at room temperature and humidity. The average temperature during the study period was $24.50 \pm 0.02^{\circ}\text{C}$ and the relative humidity was about $63.64\% \pm 0.11\%$.

Table 3.3.2.1. Percentage composition of larval diets used in the study.

Ingredient	Bran (Standard)	Carrot: Yeast (2:1)	Carrot:Yeast (4:1)
Torula yeast, g	6.80	6.12	3.67
Carrot, g	6.12	12.24	14.69
Sugar, g	10.20		
Nipagin, g		0.13	0.13
Bran, g	19.04		
Sodium benzoate, g	0.26		
Ascorbic acid, g		0.06	0.06
Hydrochloric acid, ml	0.91		
Water, ml	56.67	81.58	81.58

Results and discussion

Three out of 8 life history traits of *C. capitata* varied significantly among the 3 diets evaluated (Table 3.3.2.2). All generations were pooled as there were no significant interactions between diet and generation for all life history traits examined except for larval development time ($F= 8.62$, $df=10,51$, $P=0.00$). The carrot-based diet, in particular with a 2:1 ratio of carrot powder to Torula yeast had the shortest larval development time and the highest pupal recovery rate. Dehydrated plant materials like carrot powder and dry yeasts in larval diets for fruit flies have been reported to achieve high yields of pupae (Tsitsipis, 1989). Kaspi *et al.* (2002) demonstrated that high levels of protein in larval diet reduced larval developmental time. It is likely that the carrot-based diet provided more protein in the diet than the bran-based diet. The protein content of the carrot-based diet was not known.

Interestingly, the percentage of adult fliers was significantly higher when flies were reared on a bran-based diet compared to carrot-based diet. In general, however, flight ability was low for all diets. The % fliers for a bisexual strain of *C. capitata* should be a minimum of 75 according to FAO/IAEA/USDA (2003) guidelines. This therefore should be further investigated and corrected. Flies reared are currently being used for bait evaluation tests in field cages and results are dependent on supply of flies with good flight ability. Some handling procedures such as sifting pupae from the media at critical times during adult development and high temperatures during larval development have been shown to contribute to poor flight ability (Calkins, 1989).

Conclusion

Larval diets for rearing of *C. capitata* were found in this study to influence some of its life history traits such as larval pupal development, pupal recovery and flight ability. This study also provided information on the characteristics of *C. capitata* being reared in the colonies at CRI on the bran-based diet. The poor flight ability observed in all diets will be further investigated.

Further objectives (milestones) and work plan

The study will be repeated for the two other species within the next report period. Experiments on Natal fruit fly have already commenced in April 2009 and will be ongoing. After the completion of the Natal fly experiments, the experiments on Marula fly will begin.

The Medfly and Natal fly colonies will be refreshed by setting up separate batches of laboratory reared females and wild males reared from infested fruits.

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Table 3.3.2.2. Effects of larval diet on larval developmental time, pupal recovery, pupal weight, adult emergence and survival, flight ability, fecundity and fertility of *C. capitata* over five generations (Average \pm SE).

Diet	Larval development time, days	% Pupal recovery	Pupal weight, g (20 pupae)	% Adult emergence	% Adult survival (21 days)	Flight ability (% fliers)	Fecundity, eggs/female/day	% Fertility
Bran	8.17 \pm 0.28 a	92.46 \pm 2.30 b	0.20 \pm 0.00 a	86.66 \pm 2.36 a	90.90 \pm 2.26 a	50.17 \pm 5.13 a	12.33 \pm 1.60 a	63.94 \pm 4.83 a
Carrot 2:1	7.71 \pm 0.34 b	97.08 \pm 1.39 a	0.21 \pm 0.00 a	92.15 \pm 1.86 a	94.19 \pm 1.96 a	25.61 \pm 2.88 b	12.27 \pm 1.59 a	69.67 \pm 4.08 a
Carrot 4:1	7.83 \pm 0.33 c	96.00 \pm 1.50 a	0.21 \pm 0.00 a	92.11 \pm 2.00 a	93.01 \pm 1.61 a	34.61 \pm 2.92 b	9.44 \pm 1.38 a	69.20 \pm 4.80 a
F	29.86	3.99	0.30	2.51	0.94	11.74	1.24	0.81
Df_{diet, total}	2,71	2,71	2,71	2,71	2,58	2,59	2,119	2,119
P value	0.00*	0.02	0.74	0.09	0.40	0.00	0.29	0.45

* Values in bold highlight the factors that have significant effects.

3.3.3. PROGRESS REPORT: Determine the potential global distributions for Natal fruit fly and false codling moth

Experiment 805 (March 2006 – March 2010): by M de Villiers (post-doctoral student at SU)

Opsomming

Natalvlieg, *Ceratitis rosa* Karsch, en valskodlingmot, *Thaumatotibia leucotreta* (Meyrick), is plaes van 'n verskeidenheid vrugte wat in Suid-Afrika geproduseer word. Weens fitosanitêre belang, verhoed hierdie plaes internasionale vrugtehandel. Indien hul huidige verspreiding bekend is, kan hul potensiele globale verspreiding gemodelleer word om sodoende die toepaslikheid van geassosieerde fitosanitêre regulasies te evalueer. "Bucket" valle met BioLure® as lokmiddel, en deltavalle met feromoon as lokmiddel is in verskillende klimaatstreke van Suid-Afrika geplaas om Natalvlieg en valskodlingmot onderskeidelik te monitor. Drie valle, wat maandeliks nagegaan is, is per monitorarea gebruik. Die volopheidsverspreiding van Natalvlieg het gebyk om ooreen te stem met klimaatsverskille, met laer getalle of afwesigheid in die droeër klimaatstreke van die land. Dit stel voor dat Natalvlieg 'n goeie kandidaat is om sy potensiele globale verspreiding, gebaseer op klimaat, te modelleer. Hierdie inligting is gebruik om 'n CLIMEX model vir hierdie plaag saam te stel. Hierdie model sal verder verfynd word ten einde die potensiele globale verspreiding van Natalvlieg betroubaar te voorspel. Die volopheidsverspreiding van valskodlingmot het nie ooreengestem met klimaatsverskille nie, wat voorstel dat relatiewe volopheid waarskynlik meer sensitief is teenoor faktore soos gasheerbeskikbaarheid as beperkinge wat deur die klimaatsreeks van die studiearea gestel word.

Summary

Natal fruit fly, *Ceratitis rosa* Karsch, and false codling moth, *Thaumatotibia leucotreta* (Meyrick), are pests of various fruit produced in South Africa. Due to phytosanitary concerns, these pests hinder international fruit trade. If their current distribution is known, their potential global distribution can be modelled and the relevance of associated phytosanitary regulations evaluated. Bucket traps baited with BioLure®, and delta traps baited with pheromone, were placed in different South African climatic regions to trap Natal fly and false codling moth respectively. Three traps, serviced monthly, were used per monitoring area. The abundance distribution of Natal fly seemed to correspond with climatic differences, with lower numbers or absence observed in the drier regions of the country, suggesting it is a good candidate to model its potential global distribution based on climate. This information was used to construct a CLIMEX model for this pest. This model will be further refined in order to reliably predict the potential global distribution of Natal fly. The abundance distribution of false codling moth did not correspond with climatic differences, suggesting that relative abundance may be more sensitive to factors like host availability than to restrictions imposed by the climatic range of the study area.

Introduction

Due to the importance of Natal fruit fly, *Ceratitis rosa* Karsch (Diptera: Tephritidae), and false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) as phytosanitary pests (EPPO 2007), the current distribution of these pests needed to be determined in South Africa in order to model their potential to invade other parts of the world.

Materials and methods

To determine the relative abundance of Natal fly and false codling moth (FCM) across South Africa, the following study areas were used: Stellenbosch, Citrusdal, Swellendam, Knysna, Hondeklipbaai, Onseepkans, Keimoes, Britstown, Jan Kempdorp, Addo, King William's Town, Bloemfontein, Pietermaritzburg, Nkwalini, Groblersdal/Marble Hall, Nelspruit, Komatipoort, Tshipise, Baltimore, Tzaneen and Rustenburg. These areas are representative of the different climatic regions of South Africa (Earle *et al.* 1996). In each of these areas, three Chempac bucket traps, baited with three-component BioLure®, were used to monitor Natal fly, and three yellow delta traps, baited with FCM pheromone, to monitor FCM. These traps were placed mostly in home gardens and in host plants. Traps were also placed in Harare, Zimbabwe. To determine the geographical limits of distribution of Natal fly within southern Africa, the following sites were used in addition to the above mentioned sites: Onrus/Vermont, Somerset West, Paarl, Riebeeck Kasteel, Piketberg, Porterville, Clanwilliam, Vanrhynsdorp, Beaufort West, Garies, Springbok, Olifantshoek, Gariëpdam and Vryburg. Again, three bucket traps, baited with BioLure®, were used to monitor Natal fly in each of these areas. All traps were rebaited and trap catches collected at a monthly basis, throughout the year.

The CLIMEX simulation model (Maywald & Sutherst 1991; Sutherst *et al.* 1999) will be used to analyse the information gathered and simulate the potential global distributions. This software was developed and distributed exclusively by Hearne Scientific Software (<http://www.hearne.com.au/>) and it enables the user to estimate the potential geographical distribution and seasonal abundance of a species in relation to climate.

Results and discussion

Trap catch data of Natal fly is given in Table 3.3.3.1. During the period April 2008 to March 2009, Natal fly was absent from Porterville, Citrusdal, Clanwilliam, Vanrhynsdorp, Swellendam, Beaufort West, Hondeklipbaai, Garies, Springbok, Onseepkans, Keimoes and Tshipise. The maximum monthly average over this period was very low (<10 flies/trap/month) in Britstown, Olifantshoek, Gariepdam, Groblersdal/Marble Hall and Baltimore, low (10-29.9 flies/trap/month) in Onrus/Vermont and Jan Kempdorp, moderate (30-99.9 flies/trap/month) in Somerset West, Paarl, Piketberg, Knysna, King William's Town, Nelspruit and Komatipoort, and high (100-499.9 flies/trap/month) in Stellenbosch, Riebeeck Kasteel, Addo, Bloemfontein, Pietermaritzburg, Nkwalini and Tzaneen. For Vryburg, Rustenburg and Harare, no monthly averages are available for this period, since samples were taken at irregular intervals. It seems as if the drier regions of the country are less suitable for Natal fly. A sensitivity of Natal fly to desiccation was also observed by Duyck *et al.* (2006) and Myburgh (1962) who studied the effect of relative humidity on pupae survival and mating respectively.

Trap catch data of FCM is given in Table 3.3.3.2. During the period April 2008 to March 2009, FCM was absent from Swellendam, Hondeklipbaai and Britstown. The maximum monthly average over this period was low (<10 moths/trap/month) in Onseepkans, Knysna, Addo, Groblersdal/Marble Hall, Komatipoort, Tshipise and Tzaneen, moderate (10-19.9 moths/trap/month) in Nkwalini and Nelspruit, high (20-49.9 moths/trap/month) in Jan Kempdorp, Pietermaritzburg and Baltimore, and very high (≥ 50 moths/trap/month) in Stellenbosch, Keimoes, King William's Town and Bloemfontein. For Citrusdal, Rustenburg and Harare, no monthly averages are available for this period, since samples were taken at irregular intervals. Keimoes, Onseepkans and Hondeklipbaai fall into the same climatic region, being dry (less than 250 mm rain a year), with hot summers and warm winter days (Earle *et al.* 1996). In this region, FCM was abundant at Keimoes, but was present in low numbers in Onseepkans (observed only once) and seemingly absent from Hondeklipbaai. Jan Kempdorp, Bloemfontein and Britstown also fall into one climatic region (Earle *et al.* 1996). In this region, FCM was absent from Britstown, but was abundant in Jan Kempdorp and Bloemfontein.

During the period April 2008 to March 2009, Mediterranean fruit fly, *C. capitata* (Wiedemann), was present in all these sampling areas. Marula fly, *C. cosyra* (Walker), was present in Baltimore, Groblersdal/Marble Hall, Harare, Nelspruit, Nkwalini, Pietermaritzburg, Rustenburg, Tshipise and Tzaneen. CLIMEX training was attended in Madrid, Spain during October 2008. A preliminary CLIMEX model was constructed for Natal fly.

Conclusion

Results from this year's data were similar than from the previous year. The abundance distribution of Natal fly again seemed to correspond with climatic differences, being mostly absent or present in lower numbers in the drier regions. This suggests that Natal fly is a good candidate to model its potential global distribution based on climate. FCM occurred across all climatic regions, again showing localised occurrence of both high numbers and apparent absence or low numbers within the same climatic regions. Therefore, FCM did not show an abundance distribution pattern corresponding with climatic regions. This suggested that relative abundance of FCM is probably more sensitive to factors like host availability than to restrictions imposed by the climatic range covered by the study area. Therefore, a CLIMEX model was constructed only for Natal fly. This model will be refined in order to give a reliable prediction of the potential global distribution of Natal fly.

Table 3.3.3.1. Abundance data of Natal fruit fly, *Ceratitis rosa*, across southern Africa.

Area	2008									2009		
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Addo	12.7	293.0	253.3	371.7	26.0*							
Baltimore	-	-	-	-	0	0	0	0.3	0.7	0.7	1.0	-
Beaufort West	0	0	0	0	0*							
Bloemfontein	130.0	77.3	3.0	3.3	2.3*							
Britstown	4.0	1.0	0.3	0	0*							
Citrusdal	0 ⁺		0 ⁺		0 ⁺		0 ⁺ *					
Clanwilliam	0 ⁺		0 ⁺		0 ⁺		0 ⁺ *					
Gariepdam				3.5 ⁺ *								
Garies	0	0	0	0*								
Groblersdal/Marble Hall	0.7	1.3	0.7	0.7*								
Harare				501.7 ⁺	-	-	-*					
Hondeklipbaai	0 ⁺	0	0		0 ⁺	0		0 ⁺			0 ⁺	-
Jan Kempdorp	7.3	11.0	9.0	0.7	-*							
Keimoes	0		0 ⁺	0	0*							
King William's Town	33.0	52.3	-	-	-*							
Knysna	72.7		53.0 ⁺	8.7		6.0 ⁺ *						
Komatipoort	4.7		13.7 ⁺	14.0	90.7*							
Nelspruit	83.3	33.3	22.0	12.0*								
Nkwalini		126.7 ⁺	119.0	50.7	65.7	98.7	8.0	5.7	7.3	28.0	16.7	17.0
Olifantshoek	4.0	4.0	0.7	0.3	0*							
Onrus/Vermont	24.0	6.0	4.3	4.0*								
Onseepkans	0	0	0	0	0*							
Paarl	87.0	11.0	7.3	5.0	1.0	0.7	5.7*					
Pietermaritzburg	236.3	417.0	276.3	158.0	72.0	7.3	19.3	19.0	39.0		144.7 ⁺	159.7*
Piketberg	5.3	40.0	26.3	-	-*							
Porterville	0	0	0	0	0	-*						
Riebeeck Kasteel	80.3	50.3	136.3	67.0	29.7*							
Rustenburg					26.3 ⁺ *							
Somerset West	96.7	82.7	36.0	19.7*								
Springbok	0	0		0 ⁺	0*							
Stellenbosch	225.7	123.0	76.7	35.0*								
Swellendam	0	0	0	0	0*							
Tshipise	0	0	0	0	0*							
Tzaneen		97.7 ⁺		50.7 ⁺	137.0*							
Vanrhynsdorp	0	0	0	0		0 ⁺ *						
Vryburg			62.0 ⁺	-	-	-*						

+ Collective samples, taken over more than one month – monthly averages not available; * End of two-year sampling period; - Data not obtained

Table 3.3.3.2. Abundance data of false codling moth, *Thaumatotibia leucotreta*, across southern Africa.

Area	2008									2009		
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Addo	0	3.3	2.3	2.7	0.3*							
Baltimore	-	-	-	-	5.3	8.3	8.3	20.3	29.7	20.3	10.7	-
Bloemfontein	51.7	68.3	5.7	0.7	1.3*							
Britstown	0	0	0	0	0*							
Citrusdal	36.0 ⁺		67.3 ⁺		27.0 ⁺		5.3 ⁺ *					
Groblersdal/Marble Hall	3.3	2.3	3.3	1.3*								
Harare				44.3 ⁺	-	-	-*					
Hondeklipbaai	0 ⁺	0	0		0 ⁺	0		0 ⁺			0 ⁺	-
Jan Kempdorp	12.7	20.3	4.7	0	-*							
Keimoes	184.0		252.0 ⁺	9.0	38.0*							
King William's Town	55.0	26.7	-	-	-*							
Knysna	0.7	0	0	0		0 ⁺ *						
Komatipoort	2.7		0.3 ⁺	1.0	0.7*							
Nelspruit	15.7	12.3	18.3	10.3*								
Nkwalini		5.0 ⁺	1.5	0	2.7	7.0	19.3*					
Onseepkans	0	1.3	0	0	0*							
Pietermaritzburg	14.3	16.3	34.3	23.3	27.7	9.0	9.3	4.0	11.3		46.7 ⁺	16.7*
Rustenburg					113.5 ⁺ *							
Stellenbosch	90.7	160.3	221.7	41.7*								
Swellendam	0	0	0	0	0*							
Tshipise	1.0	0	1.0	0.3	0.7*							
Tzaneen		3.0 ⁺		9.7 ⁺	1.0*							

+ Collective samples, taken over more than one month – monthly averages not available; * End of two-year sampling period; - Data not obtained

Technology transfer

A poster was presented at the International Congress of Entomology in Durban during July 2008 and a presentation at the Citrus Research Symposium in the Drakensberg during August 2008.

Further objectives (milestones) and work plan

Trapping will continue in Hondeklipbaai (FCM and Natal fly) and Nkwalini (Natal fly only) until two years' data is obtained. Trapping in Baltimore will continue until one year's data is obtained. This site was used to replace the Tom Burke site, used during the previous year, where cooperation was poor.

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3.3.4 PROGRESS REPORT: A new bait for more effective control of all *Ceratitidis* fruit flies

Experiment 915 (April 2008 - March 2009): by A Manrakhan, C Kotze, J H Daneel & R Beck (CRI)

Opsomming

Die behandeling met 'n proteïen-lokaas gemeng met 'n insekdoder, is die belangrikste metode om vrugtevlieëbeheer in sitrus produserende areas te verkry. 'n Verminderende toleransie ten opsigte van insekdoder residue op vrugte veroorsaak dat lokaas toediening geoptimaliseer moet word deur te soek na meer effektiewe lokase. Hierdie proef fokus eerstens daarop om die huidige vrugtevlieë lokase se doeltreffendheid te bepaal en ook om nuwe kandidaat lokase te evalueer.

Die evaluasie van die huidige gebruikte lokase: 1% Hym lure, 5% GF120 en M3 lokaas was gedoen in 'n nuwe boordhok te CRI. Kandidaat proteïen-lokase was vergelyk met Hym lure in binnenshuishokke. 'n Nuwe lokaas, Prolure, was geëvalueer in mango- en sitrusboorde gedurende die vrugdraende seisoen.

Die reaksie van Medvlieg was dieselfde teenoor Hym lure, GF120 en vars M3 lokaas. Medvlieg was nietemin duidelik meer aangelok deur vars Hym lure en vars GF120 as met 2 tot 4 weke oue M3 lokaas. In teenstelling hiermee was Natalvlieë ewe veel aangelok deur Hym lure, GF120 en M3 lokaas, afgesien van die ouderdom van die M3 lokaas.

Die konsentrasie van Hym lure en GF120 het 'n duidelike effek op hulle aanloklikheid vir Medvlieg. 'n Verhoogde Hym lure konsentrasie lei tot 'n ooreenstemmende verhoogte Medvlieg reaksie. In teenstelling hiermee, wanneer die konsentrasie van GF120 verhoog was bo die aanbevole 5% vlak, het dit nie gelei tot meer Medvlieg vangstes nie.

Twee nuwe kandidaat lokmiddels: (1) Mazoferm, 'n mielie proteïen-hidrolisaat en (2) Brouersgis was getoets en vergelyk met Hym lure. Die resultate van hierdie proewe was ongelukkig onoortuigend as gevolg van hoë getalle insekte in die kontrole lokvalle, moontlik as gevolg van versadigde dampe binne in die hokke. Nietemin, hierdie twee lokase sal weer geëvalueer word in oop buitelughokke.

Die nuwe lokmiddel, Prolure, was duidelik meer aanloklik vir Natalvlieë as Marulavlieë wanneer dit vergelyk was met 0.4% Hym lure. Hierdie belowende lokaas moet verder getoets word met verskillende gifstowwe.

Summary

The application of protein baits mixed with insecticide is a principal method of fruit fly control in citrus production areas. Reduced tolerance to insecticide residues on fruit requires that the bait application be optimized through a search for more effective baits. This experiment focused first of all on determining the performances of the currently used fruit fly baits. New candidate baits were also evaluated.

Evaluation of currently used baits: 1% HymLure, 5% GF 120 and M3 bait, was carried out in a new field cage at CRI. Candidate protein baits were screened against HymLure in cages placed indoors. A new attractant, Prolure, was evaluated in mango and citrus orchards during the fruiting season.

Responses of Medfly to HymLure, GF120 and fresh M3 bait were found to be similar. Medfly was, however, significantly more attracted to fresh HymLure and fresh GF 120 compared to 2 week and 4 week old M3 bait. In contrast, Natal fly was equally attracted to HymLure, GF 120 and M3 bait, irrespective of the age of M3 bait. Marula fly had a consistently low response to HymLure but responded well to GF 120 and M3 bait.

The concentration of HymLure and GF 120 had a significant effect on their attractiveness to Medfly. An increase in HymLure concentration led to a corresponding increase in Medfly response. In contrast for GF 120, an increase in its concentration beyond the 5% recommended dosage did not result in more catches of Medfly.

Two new candidate baits: (1) Mazoferm, a corn protein hydrolysate, and (2) Brewer's yeast were screened and compared to HymLure. The results of these tests were unfortunately not conclusive given the high counts on a control (blank) trap which possibly was as a result of saturation of odours within the cages. Nonetheless these two attractants should be re-evaluated in open cages.

The new attractant, Prolure, was significantly more attractive to Natal fly and Marula fly compared to 0.4% HymLure. This promising attractant would be tested with different toxicants.

Introduction

The use of protein baits mixed with insecticide termed as the bait application technique is one of the main methods of fruit fly control worldwide (Roessler, 1989). The technique works on an attract and kill principle whereby adult flies (in particular females) in search of food (protein) are attracted to the bait and in the process are killed by an insecticide mixed with the bait either upon contact or following ingestion of the mixture. Such poisoned bait mixture limits the use of insecticide and at the same time increases the efficacy of control. Poisoned baits can be applied either as foliar sprays (aerial or ground) or in discrete containers known as bait stations.

Various protein bait formulations are being used worldwide- acid-hydrolysed corn products such as NuLure, enzymatic yeast hydrolysate such as Torula Yeast, enzymatically hydrolyzed protein from corn processing such as Mazoferm E802 and enzymatically hydrolysed local brewery wastes such as Mauri's Pinnacle Protein Lure. In citrus production areas in South Africa, the commonly used baits in fruit fly control are HymLure, GF 120 and M3 bait (Ware & Grout, 2005). HymLure and GF 120 are applied either as ground or aerial sprays. HymLure is usually mixed with organophosphates such as Mercaptothion (Malathion) or Trichlorfon whilst GF 120 is a formulated bait mixture which contains the toxicant Spinosad and requires only dilution with water before application. The M3 bait is mixed with a toxicant and contained in a plastic material to form the M3 bait station. M3 bait stations are set at densities varying from 300 to 400 units per hectare. Although, effectiveness of the commonly used baits for suppressing fruit fly populations have been determined before, there are few publications on the performance (attractiveness) of the baits to local fruit flies. Buitendag & Naude (1994) evaluated HymLure in Sensus traps in the field and found it to be attractive to Natal fly but gave poor results for Mediterranean fruit fly (Medfly), *Ceratitidis capitata*. Steyn et al. (1997) compared the attractiveness of HymLure with Buminal, Nasiman, Marmite and Bovril in traps placed in a guava orchard. The authors found that Buminal was the most attractive bait to Natal fruit fly, *Ceratitidis rosa*. Studies conducted by Barry et al. (2003) have demonstrated that GF 120 is attractive to Medfly, in particular when the bait is fresh and placed at high densities on leaves. Interestingly, the latter authors found that fly attraction to GF-120 was limited to only several cm from the bait.

Attractiveness of baits is known to be species-specific. For instance, Barry et al. (2006) reported a stronger response of the melon fly, *Bactrocera cucurbitae* to protein baits compared to *B. dorsalis*. Protein deprived Medfly was found to respond higher to food odours compared to protein deprived *Ceratitidis fasciventris* and Marula fly, *C. cosyra* (Manrakhan & Lux, 2008). In a citrus production area with multiple pest species of

quarantine importance – Medfly, Natal fly and Marula fly, it would be important not only to have an effective bait but also a bait that would target all fruit fly species.

There is a growing interest to improve efficacy of the current bait application technique for fruit fly control. This is in the light of decreasing tolerances of insecticide residues on fruits and also high costs of control. A bait that is more attractive and palatable than HymLure, for instance, would allow for lower doses of toxicants as well as permit the use of reduced risk insecticides. Moreover, if a more effective bait is found for a bait station, fewer bait stations would then be required per unit hectare which would then lower the cost of this control technique.

The general aim of this study was therefore to develop more efficient bait application techniques for fruit fly control and the specific objectives were to: (1) evaluate new and currently used fruit fly baits, (2) investigate alternative toxicants (other than Malathion or Trichlorfon) that can be incorporated into baits and (3) investigate on new dispensing technology for bait stations through the use of cheaper and biodegradable substrates.

To date, attraction of Medfly, Natal fly and Marula fly to currently used baits was investigated in a new field cage. Different concentrations of HymLure and GF 120 were also evaluated in the field cage on Medfly only. Three candidate fruit fly baits were screened and compared to HymLure in indoor cages. A new attractant that was found promising in previous studies namely Prolure was further evaluated at different concentrations in traps in the field.

Materials and methods

Field cage bioassays- Attractiveness of currently used baits

Bioassays were initiated in October 2008 following the set-up of a new field cage at CRI Nelspruit. The cage is

4 m long, 3.2 m wide and 2.5 m high and is made up entirely of nylon screen with a 1 mm x 2mm mesh size. A plastic cover is fitted on top of the cage to protect against rain and direct sunlight. Eight potted Ruby Grapefruit trees were placed inside the cage. A 1.96 m high rotating pole fitted with 4 arms and nylon rope fitted in between the arms was fixed in the middle of the field cage. The distance between each arm was 1.26 m.

Experiment 1: Attractiveness of currently used baits at standard field rates

Insect material. Protein deprived, 10– 14 day old adult *C. capitata*, *C. rosa* and *C. cosyra* flies were used. Flies were fed only with sugar and water since emergence. For each test, 200 males and 200 females of each species were used. Species were evaluated separately.

Baits. Three currently used fruit fly baits were evaluated: (1) HymLure (Savoury Food Industries [Pty] Limited, South Africa) (2) GF 120 (Dow AgroSciences, South Africa), and (3) M3 bait (Green Trading, South Africa) which is used in M3 bait station. HymLure and GF 120 were evaluated at concentrations of 1% and 5% respectively representing the rates at which they are used for fruit fly control. Each bait was absorbed onto a 2.5 cm x 2.5 cm filter paper. The filter paper containing 2 ml of bait was then placed inside a 4 cm diameter white plastic cup covered with netting on top to allow dispersion of odour from the bait whilst preventing direct access of flies to the bait. For each test, HymLure and GF 120 were freshly prepared. For the M3 bait, three different ages were evaluated separately against the two other baits: (1) Fresh, (2) 2 weeks old and (3) 4 weeks old. M3 baits were aged by placing the cup containing the bait outdoors under a shed. A control was included in each test and consisted of a blank filter paper placed inside a similar plastic cup as above.

Experimental set-up. Flies were released into the field cage 1 hour before start of the test. A water dispenser was placed inside the cage to provide a water source for flies. Each bait container was suspended in the middle of an open ended and transparent cylinder (8 cm diameter x 8 cm length) lined with a transparent sticky insert. Cylinders used during tests were hung at equal distances on each arm of the rotating pole. Positions of baits on the wheel were allocated at random. The baits were exposed for 23 hours inside the cages after which they were removed. The time of fly response was during daytime. Baits were rotated every 2 hours during daylight hours (between 8:00 – 17:00). At the end of the test, baits were removed from the cage. Male and female flies captured on the sticky insert of each cylinder were counted. There were 4 replicates for each test. The positions of baits were moved one place at each replicate.

Experiment 2: Influence of dilution on attractiveness of liquid protein baits

Insect material. Protein-deprived, 10– 14 day old adult *C. capitata* flies were used. Flies were fed only with sugar and water since emergence. For each test, 200 males and 200 females were released.

Baits. Hym-Lure and GF 120 were tested separately at different concentrations. HymLure was tested at 0.4% (standard), 1% and 2% and GF 120 was tested at 1%, 5% (standard) and 10%.

The experimental set-up used was similar to experiment 1.

Screening of new baits in indoor field cages

Insect material. Protein-deprived, 8 – 10 day old adult *C. capitata*, *C. rosa* and *C. cosyra* flies were used. Flies were fed only with sugar and water since emergence. For each species, 25 males and 25 females were released separately in a cage.

Liquid baits. Two new baits: Mazoferm (Corn Products, Kenya) which is a commercially available corn protein hydrolysate and Brewer's yeast (Jahela Brewer's Yeast CC, South Africa) were compared to HymLure. All liquid baits were evaluated at a concentration of 1% (dilution with water).

Experimental set-up. Tests were conducted in nylon screen cages (60 cm x 110 cm x 85 cm) which were placed indoor. One potted Ruby Grapefruit tree was placed inside each cage to simulate a host environment. On a test day, flies were released in the cage one hour before placement of baits to allow flies to disperse. 2ml of each bait was absorbed onto a 2.5 cm x 2.5 cm filter paper and placed inside a 4 cm diameter white plastic cup covered with netting on top. A control was included in each test and this consisted of a blank filter paper placed in the plastic cup. Each cup was placed inside an open ended transparent cylindrical tube (8 cm diameter x 8 cm length) lined with a transparent sticky insert. At 15 00, the four cylinders: three fitted with baited cups and one with a cup containing a blank filter paper (control), were placed inside each cage. Cylinders were hung on 2 parallel lines at equal distances and were set just above the plant canopy. Positions of the cylinders were allocated at random and rotated for each replicate. Baits were exposed for a total period of 21 hours. During bait exposure, the cages were rotated every two hours during day time. At 12:00 the next day, all cylinders were removed from the cages. Male and female flies were counted and removed from the cylinder. Remaining flies inside the field cage were removed using an aspirator at the end of a test. For each species, there were 8 replicates.

Field evaluation of a new attractant, Prolure

Different concentrations of Prolure were evaluated in traps and compared to 0.4% HymLure in a mango orchard and a citrus orchard in Mpumalanga Province.

Study sites. Studies were conducted in a mango orchard in February 2009 during mango fruiting season at Oeversig Farm and in a Satsuma citrus orchard in April and May 2009 during the citrus fruiting season at Brackenhill Farm.

Baits. Four dilutions of Prolure were tested and compared with the recommended HymLure concentration for use in sprays. The treatments were: (1) 0.4% Prolure, (2) 1% Prolure, (3) 5% Prolure, (4) 10% Prolure, (5) 0.4% HymLure (Standard Bait), (6) water (negative control).

Traps. Baits were evaluated in Chempac Bucket traps (Chempac [Pty] Limited) which are McPhail type traps with yellow base and a transparent lid.

Experimental set-up. In both sites, the same set-up was applied. 200 ml of each bait were placed inside each trap (without insecticide). Traps were placed across 6 selected rows. Each row contained the 6 treatments placed at random. The rows represented replicates for each treatment. The layout of the treatments followed a Latin Square Design. There were therefore 36 traps in total. All traps were placed at a height of 1.5 m in the shaded side of the tree. Traps within a row were at a distance of about 10-30 m. Traps were emptied, checked and rebaited every 2-4 days. The traps were rotated within a row at each check. All specimens caught in traps were collected in vials and kept in 70% alcohol. Species were identified and sexed.

Statistical analysis

The relative attractiveness of the different baits for each species was calculated by dividing the number of flies responding to a particular bait on a test day over the total response to all baits (control inclusive) on that day. Data were transformed using arcsine square root function to stabilize variances before doing an Analysis of Variance (ANOVA) to compare responses between baits with interactions calculated between baits and species, baits and replicates and baits and sex (Statsgraphics Plus 5.1).

For data on evaluation of new baits in cages placed indoor, counts of flies of each species on each bait were averaged. Data were log transformed ($\log(x+1)$) and subjected to an ANOVA.

For the field evaluation of Prolure, catches per trap per day for each treatment and replicate were calculated by dividing the catches in a trap containing a particular treatment over the number of days that the trap was exposed. Data were then log transformed ($\log(x+1)$) and subjected to an ANOVA with interactions calculated between treatments and each three factors: species, replicate and sex.

Results and discussion

Attractiveness of currently used baits

The three fruit fly species varied in their responses towards the currently used baits (Table 3.3.4.1 & Figure 3.3.4.1). There were no significant differences between responses of male and female fruit flies towards the baits.

For *C. capitata* (Medfly), there were no significant differences in attractiveness to fresh M3 bait and freshly prepared GF 120 and HymLure. Responses of Medfly to M3 bait after 2 weeks and 4 weeks of aging were however significantly lower than freshly prepared HymLure and GF 120. Medfly was equally attracted to 1% HymLure and 5% GF 120 in all tests. For *C. rosa*, Natal fruit fly, there were no significant differences among the three baits tested, irrespective of the ages of the M3 bait. *C. cosyra*, Marula fly, had a consistently lower response to HymLure compared to the other baits which confirm previous field observations. Marula fly responded well to M3 bait and GF 120, with a preference to a 4 weeks old M3 bait. The interaction between bait and replicate was significant when tests were carried out with fresh M3 bait since there were quite some variations between the replicates in responses to the three fresh baits for Medfly.

Fly attraction to protein baits is usually associated with ammonia release (Bateman & Morton, 1981). The decrease in response of Medfly to M3 bait as it ages might be as a result of a reduction of ammonia release. Responses of Natal fly and Marula fly however were not affected by aging of M3 bait which might indicate different thresholds of ammonia releases between the three fly species with Medfly having the highest threshold. Species-specific responses to baits have previously been found and discussed to be as a result of differences in life history traits between species, namely fecundity which would thereby influence protein requirements and attraction to protein (Diaz-Fleischer et al, 2009). Medfly has a wider host range than Natal fly and Marula fly (De Meyer et al., 2002) and possibly higher net fecundity than the other two species.

Table 3.3.4.1. ANOVA for the comparisons of attractiveness of currently used baits to three local fruit fly pests.

Tests	Bait (df=3,95)		Replicate (df=3,95)		Species (df=2, 95)		Sex (df=1, 95)		Bait x Replicate (df=9, 95)		Bait x Species (df=6, 95)		Bait x Sex (df=3, 95)	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
With Fresh M3	13.96	<0.01*	0.01	1.00	0.20	0.82	0.08	0.78	3.04	<0.01	3.30	<0.01	0.75	0.51
With 2 weeks M3	22.05	<0.01	0.13	0.94	0.59	0.56	0.00	0.95	0.90	0.53	4.13	<0.01	0.90	0.44
With 4 weeks M3	24.50	<0.01	0.14	0.94	0.10	0.90	0.05	0.82	1.20	0.31	4.92	<0.01	0.23	0.87

* Values in bold highlight the factors that have significant effects ($P < 0.05$)

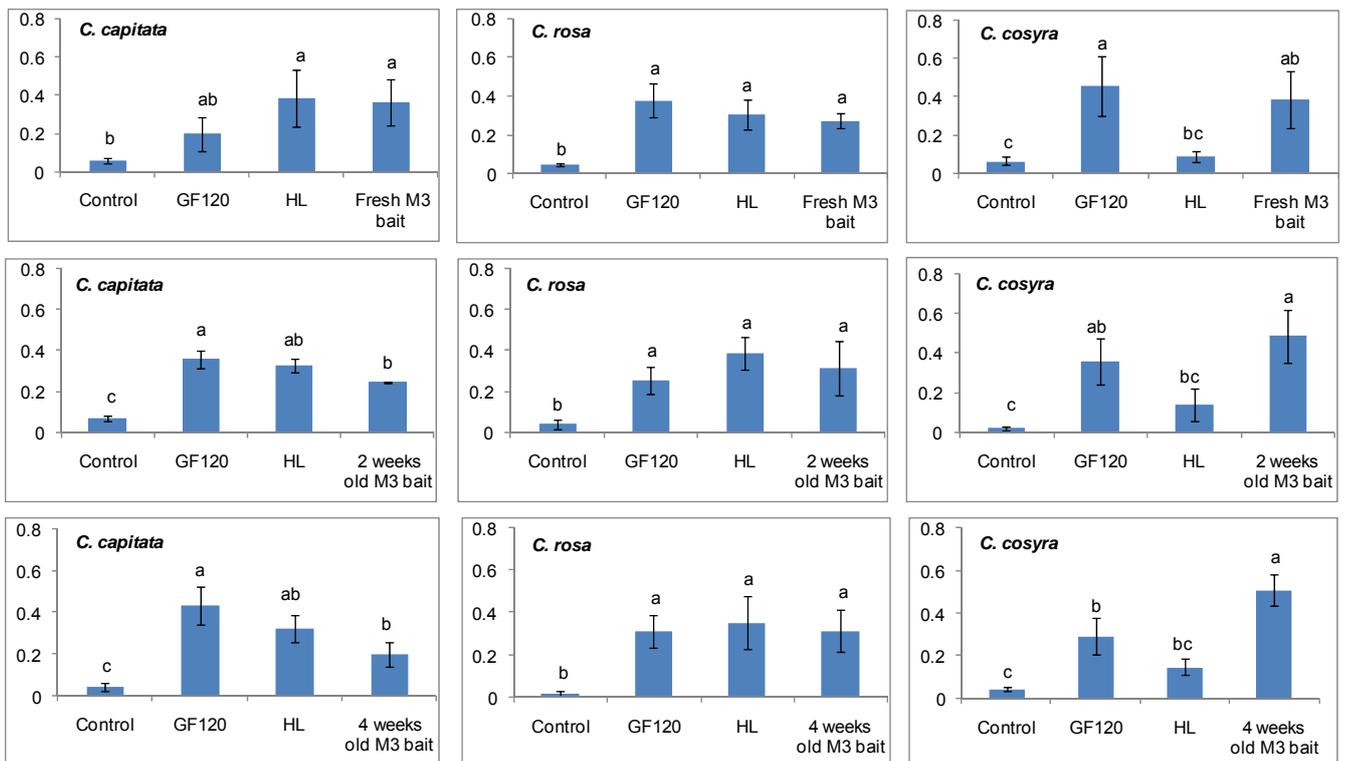


Figure 3.3.4.1. Percentage of response of three fruit fly species: *C. capitata*, *C. rosa* and *C. cosyra* to HymLure (HL), GF 120 and M3 baits at different ages (Average \pm SE). M3 baits of different ages were evaluated separately with freshly prepared GF 120 and HymLure.

Influence of dilution on attractiveness of liquid protein baits

Concentration of bait significantly influenced attractiveness of both liquid protein baits tested: HymLure and GF120 to Medfly (Table 3.3.4.2). Similar to the findings in experiment 1, there were no significant differences in responses to baits between males and females. Response of flies increased with increasing concentration of HymLure whilst with GF 120, the response was highest for the standard concentration of the bait used in the field (5%). Interestingly, response of flies was lower when the concentration of GF 120 increased beyond the standard 5% concentration.

In studies on melon fly, Fabre et al. (2003) also found that an increase in HymLure concentration led to an increase in fly response, in both low (0.5% - 2%) and high ranges (1% - 10%) of bait concentration tested. In a review on fruit fly control and monitoring in South Africa, Barnes (2000) discussed the use of bait formulation with a very low percentage of active ingredient of protein in South Africa (0.1% - 0.2%) compared to the rest of the world (1% - 40%). An increase in HymLure concentration could possibly increase efficacy of control when using this bait.

Table 3.3.4.2. Percentage (Average \pm SE) of *C. capitata* flies caught in cylinders containing HymLure and GF 120 at different concentrations.

Bait	Concentration				F _{df}	P
HymLure	Control	0.4%	1%	2%		
<i>C. capitata</i>	4.48 \pm 1.03d	19.52 \pm 5.40c	26.76 \pm 3.30b	49.24 \pm 9.32a	16.16 _{df=3,31}	<0.01
	Concentration					
GF 120	Control	1%	5%	10%		
<i>C. capitata</i>	3.26 \pm 0.56c	10.55 \pm 4.34c	58.09 \pm 3.28a	28.10 \pm 3.17b	17.36 _{df=3,31}	<0.01

Screening of new fruit fly baits

There were no significant differences (F = 0.66, df=3, 191, P=0.58) between the liquid baits and even control for all the species (Figure 3.3.4.2). It is likely that a saturation of bait odours occurred inside the cages with

freshly prepared liquid baits causing the flies to land at random on the cylinders. These liquid baits would have to be e-evaluated inside the new semi-field cage.

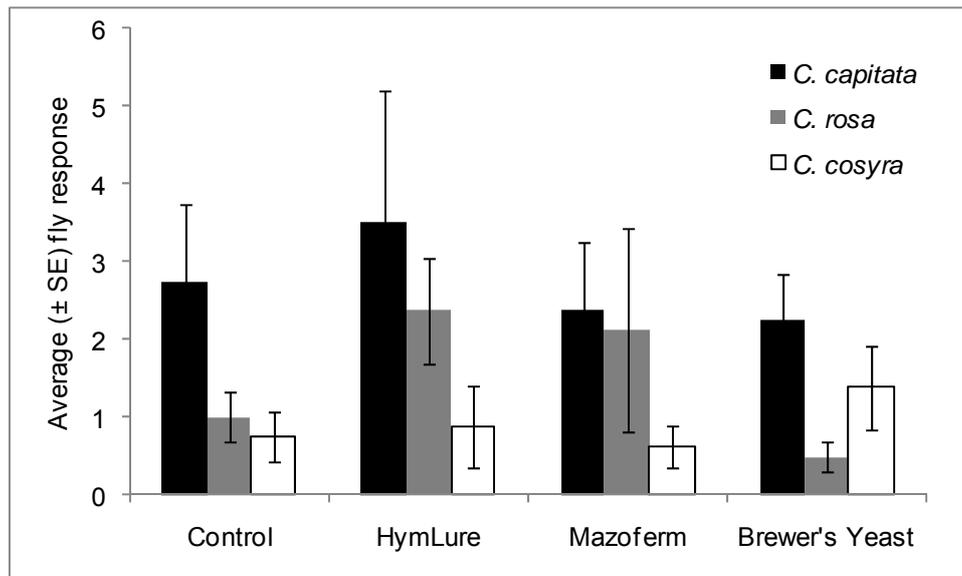


Figure 3.3.4.2. Response of *C. capitata*, *C. rosa* and *C. cosyra* to 1% Mazoferm, Brewers' Yeast and HymLure in indoor cages.

Field evaluation of Prolure

There was a significant Natal fly and Marula fly populations in both study sites whilst Medfly population was higher in the citrus orchard in Brackenhill and practically non-existent in the mango orchard in Oeversig. There were significant differences among the different concentrations of Prolure tested, 0,4% HymLure and the control (water) in both study sites (Figure 3.3.4.3). There were significant effects of species and sex on responses to baits in both study sites (Table 3.3.4.3). The interactions between treatment and species were also significant indicating that the species reacted differently to the treatments offered. There was no significant interaction between treatment and sex, indicating that both males and females reacted the same way to the treatments. As such, data was presented separately for each species and male and female catches were pooled together.

In both sites, the highest number of Natal flies was caught in the 1% Prolure concentration and for Marula fly catches were highest for the 10% concentration of Prolure. Medfly catches were highest at the 10% Prolure concentration in the citrus orchard at Brackenhill. A 0.4% Prolure concentration was significantly more attractive to Natal fly and Marula fly compared to a 0.4% HymLure.

ProLure seems to be a promising candidate that could be an alternative to HymLure and could be more effective against Marula fly.

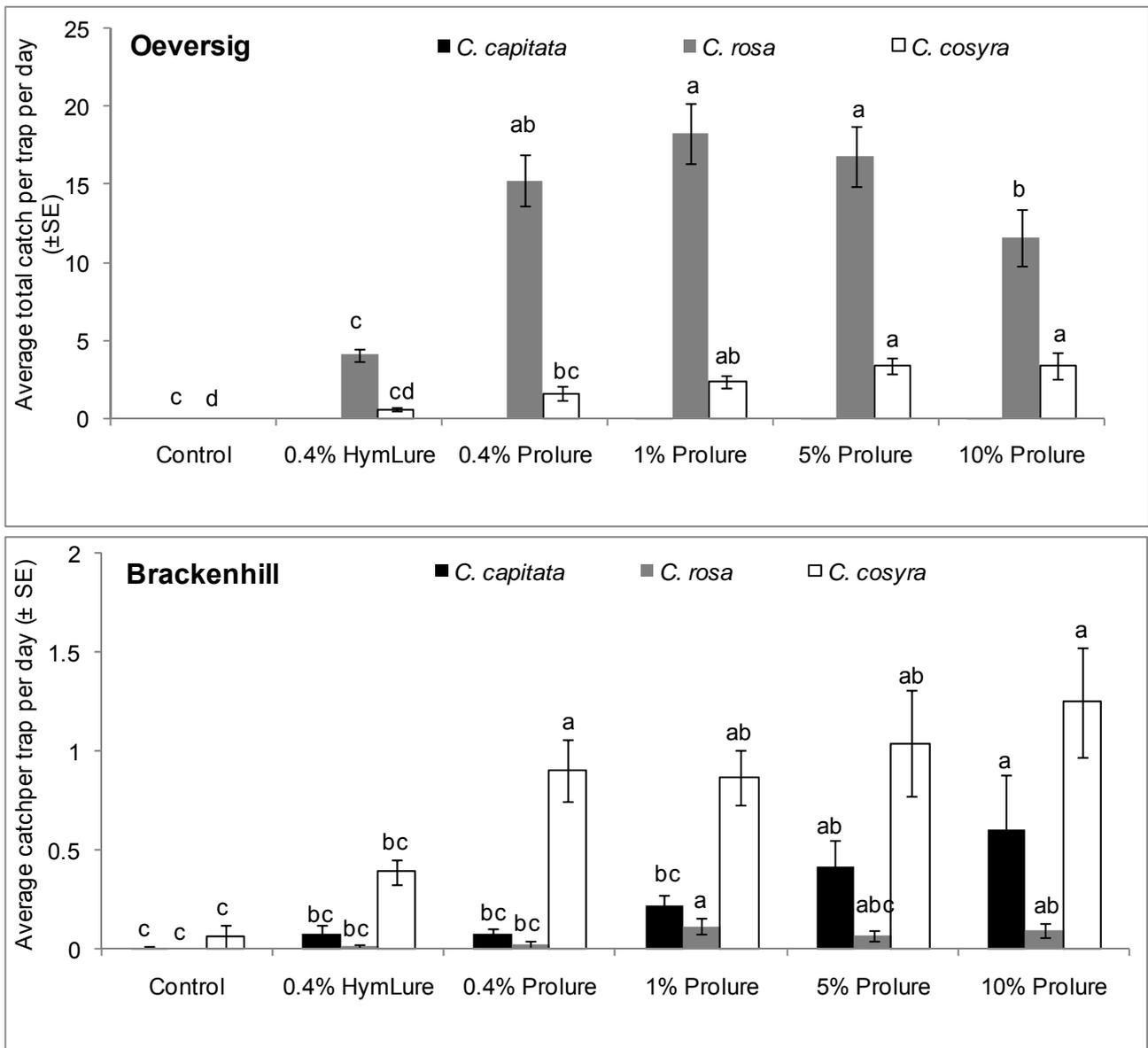


Figure 3.3.4.3. Average catch of *C. capitata*, *C. rosa* and *C. cosyra* per trap per day for four different Prolure concentrations, 0,4% HymLure (Standard) and water (negative control) in the mango orchard in Oeversig and citrus orchard in Brackenhill. For each species, treatments with different letters were significantly different ($P>0.05$).

Table 3.3.4.3. ANOVA for comparisons of catches in different concentrations of Prolure and 0.4% HymLure in Oeversig and Brackenhill.

Factors	Oeversig			Brackenhill		
	F	df _(corrected=1535)	P	F	Df _(corrected=1295)	P
Treatment	187.50	5	<0.01	17.72	5	<0.01
Species	1431.57	2	<0.01	101.30	2	<0.01
Sex	22.89	1	<0.01	23.33	1	<0.01
Replicate	2.96	5	0.01	2.93	5	0.01
Treatment x Species	83.15	10	<0.01	5	10	<0.01
Treatment x Sex	1.42	5	0.22	1.10	5	0.36
Treatment x Replicate	1.06	25	0.39	1.36	25	0.11

Conclusion

These studies clearly showed variability in responses of three important fruit fly species to baits used for control. HymLure was confirmed not to be an attractive bait for Marula fly and therefore in areas where this fly occurs, bait application technique using HymLure may not be as effective for control of the latter fly as it is for Medfly and Natal fly. A decrease in response to aging M3 bait was observed for Medfly. Options to prolong bait durability such as incorporation of materials into the bait to provide a more controlled release of volatiles or addition of ammonium acetate to boost attractiveness could be explored.

Results from studies on bait dilution indicated that an increase in efficacy of control of Medfly can be expected with an increase in HymLure concentration (up to 2%).

Finally, Prolure was found to be a promising bait for all three fruit fly species. Combination of this bait with different toxicants will be evaluated.

Technology transfer

Results on performance of currently used fruit fly baits were presented as part of fruit fly monitoring and control talks delivered at study groups of the Western Cape, Eastern Cape and Northern Cape in April and May 2008.

Further objectives (milestones) and work plan

1. Testing of Prolure with various toxicants. Different dosages of toxicants will be tested
2. Evaluation of candidate baits for sprays and stations.
3. Investigate on the use of biodegradable substrates for use in bait stations. Tests will be conducted in field cages.
4. Field evaluation of formulation of Prolure and selected toxicant and comparisons with HymLure/Malathion & GF 120 treatments.

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3.3.5 FINAL REPORT: Fewer fruit fly bait stations for larger areas

Experiment 925 (April 2008 - March 2009): by Aruna Manrakhan, Tim Grout and John-Henry Daneel (CRI)

Opsomming

Die M3-lokaasstasie bied 'n lewensvatbare alternatief vir giftige lokaastoedienings waarmee vrugtevlieë beheer word. Nietemin, die koste verbonde aan die lokaasstasie tegniek beperk die gebruik daarvan. Hierdie studie poog om 'n ondersoek te loods na die effektiwiteit van 'n perimeter lokaas strategie as 'n metode om die digtheid van lokaasstasies per hektaar te verminder. Die studie was gedoen in Valencia lemoenboorde in Nelspruit en Groblersdal in April en Junie 2008 waar geselekteerde boordblokke behandel was met M3-lokaasstasies, uitgeplaas in verminderende getalle vanaf die perimeter van die boordblok na die middel met die kern van die boord sonder enige M3's. In Groblersdal was die perimeter lokaas toediening vergelyk met lugbespuitings en 'n standaard M3 behandeling (lokaasstasies uitgeplaas elke 4 m reg deur die boord). Die vrugtevlieg populasie in al die behandelde blokke was gemoniteer met Capilure en Questlure gelaaide Sensus lokvalle. 'n Vrugskade opname was gedoen tydens oestyd in al die blokke op beide persele. Die aanwending van die M3-lokaasstasies met verminderende getalle vanaf die perimeter na die kern van die boord het wel die digtheid van die stasies wat per hektaar benodig word verminder en daar is gevind dat die metode instaat is om die vrugtevlieg populasie laag te hou tot oestyd om sodoende vrugtevliegskade te voorkom.

Summary

The M3 bait station represents a viable alternative to application of poisoned bait sprays for fruit fly control. However, the cost of the bait station technique remains a constraint to its use. This study aimed at determining the efficacy of a perimeter baiting strategy as a means of lowering the density of stations required per hectare. Studies were conducted in Valencia orange orchards in Nelspruit and Groblersdal in April and June 2008 where selected blocks were treated with M3 bait stations placed in declining numbers from the perimeter of the block to the centre, leaving a core central area untreated. In Groblersdal, additionally, this perimeter baiting treatment was compared to aerial bait sprays and uniform M3 baiting (bait stations applied every 4 m throughout the block). The fruit fly population in all treated blocks was monitored using Capilure and Questlure baited Sensus traps. A fruit damage assessment was also conducted at harvest in all treated blocks in both sites. The application of M3 bait stations with declining numbers from perimeter to centre reduced the overall density of stations required per hectare and was found to be able to sustain a low fruit fly population until harvest and prevented fruit fly damage.

Introduction

Fruit fly control in citrus production areas has to date relied mainly on spray application of a mixture of protein bait and insecticide (Ware, 2003). Organophosphates such as Malathion have been mostly used in these protein bait mixtures. Given that the maximum residue level of Malathion on citrus was lowered to 0.01 mg/kg in January 2009 (Hardman & Hattingh, 2008), fruit fly control will no doubt have to be shifted more and more to the use of either safer insecticides such as GF 120 or to the use of localized baiting in the form of bait stations. Mangan and Moreno (2007) define bait stations as discrete containers of attractants and toxins. In South Africa, the M3 bait station has been registered for use in fruit fly control since 2001. The bait station has been shown to provide effective control of fruit flies and the level of control achieved with the use of M3 bait stations has been shown to compare well with that of sprays of a mixture of protein hydrolysate (HymLure) and Malathion and sprays of GF 120 (containing spinosad) (Daneel et al., 2004). The advantages that come with the use of bait stations are the following (1) no danger of pesticide residues on fruits, (2) only a single application is required in the season and (3) it is an environmentally friendly technique. The recommended application rates of M3 bait stations vary with different citrus types from 300 to 400 per ha, depending on the susceptibility of fruits to fruit fly damage. The cost of the circular M3 bait station was prohibitive but the station was redesigned with a square shape to make it cheaper. Nonetheless, bait stations have not been widely adopted with costs (product and labour) being still the major constraint. With the use of a lower number of bait stations per hectare, this technique might become more cost competitive.

The use of M3 bait stations with declining numbers from perimeter of the orchard to the centre was previously evaluated by Ware et al. (2004) as a deployment strategy to lower the number of stations required

per hectare, but the numbers of flies were very low in this trial so definite conclusions could not be drawn. Perimeter baiting is based on the premise that in most cases flies invade commercial orchards from neighbouring vegetation (Aluja, 1996). This approach was further evaluated in the following research.

Materials and methods

Study sites

Studies were conducted in commercial Valencia orange orchards near Nelspruit and Groblersdal in April and June 2008, respectively, for a period of 8-12 weeks from colour break until harvest and a few weeks after harvest.

Treatments

Near Nelspruit, the study was carried out in a 6.5 ha block. The block was divided into an inner core area (0.4 ha) which was untreated and a perimeter area (6.1 ha) consisting of four sub-areas (outer, first middle, second middle and inner) with declining number of M3 bait stations towards the core (Figure 3.3.5.1). In the outer perimeter area (2.5 ha), M3 bait stations were hung on every tree (4 m apart). In the first middle area (1.6 ha), M3 bait stations were hung on every 2nd tree (8 m apart). In the second middle perimeter area (1.2 ha), M3 bait stations were hung on every 3rd tree (12 m apart). In the inner perimeter area just next to the core (0.8 ha), M3 bait stations were hung on every 4th tree (16 m apart).

Near Groblersdal, the perimeter M3 baiting treatment was compared to a uniform M3 bait station application (bait stations every 4 m), an aerial bait spray application and an untreated area (Figure 3.3.6.2). The perimeter baiting treatment was carried out in a 2.7 ha block which comprised an inner core area (0.2 ha) left untreated and a perimeter area (2.5 ha) consisting of only 2 sub-areas (outer and inner) that were treated with M3 bait stations. The perimeter baiting layout in Groblersdal was different to that of Nelspruit given the smaller size of the treated block. In the outer perimeter area (1.6 ha) stations were hung on every 2nd tree (every 4 m). In the inner perimeter area (0.9 ha) just outside the core, stations were hung on every 4th tree (every 8 m). The uniform M3 bait treatment was carried out in a 2.96 ha block adjacent to the perimeter M3 bait treatment block. For the uniform M3 bait treatment, M3 bait stations were hung every 2nd tree (every 4 m) in the entire block reaching a bait station density of 416 per hectare. A block under aerial bait spray application, approximately 100 m from the uniform M3 bait station block was selected and included as a treatment. Aerial sprays consisted of an application of a mixture of 750 ml HymLure and 250 ml Malathion UL per hectare. An untreated area (0.7 ha) adjacent to the perimeter M3 bait treatment block was also included in the study.

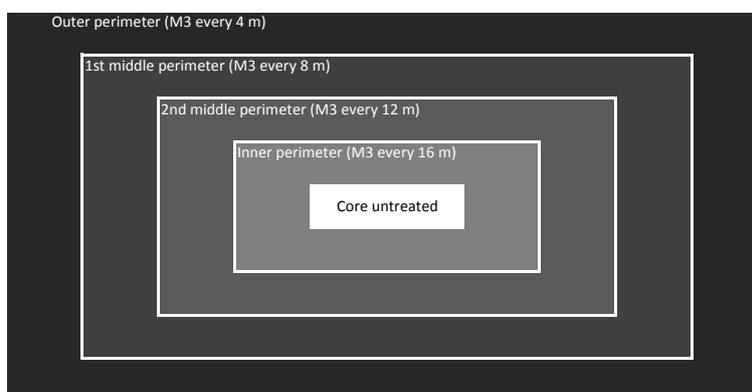


Figure 3.3.5.1. Layout of bait stations in the perimeter M3 baiting block near Nelspruit.

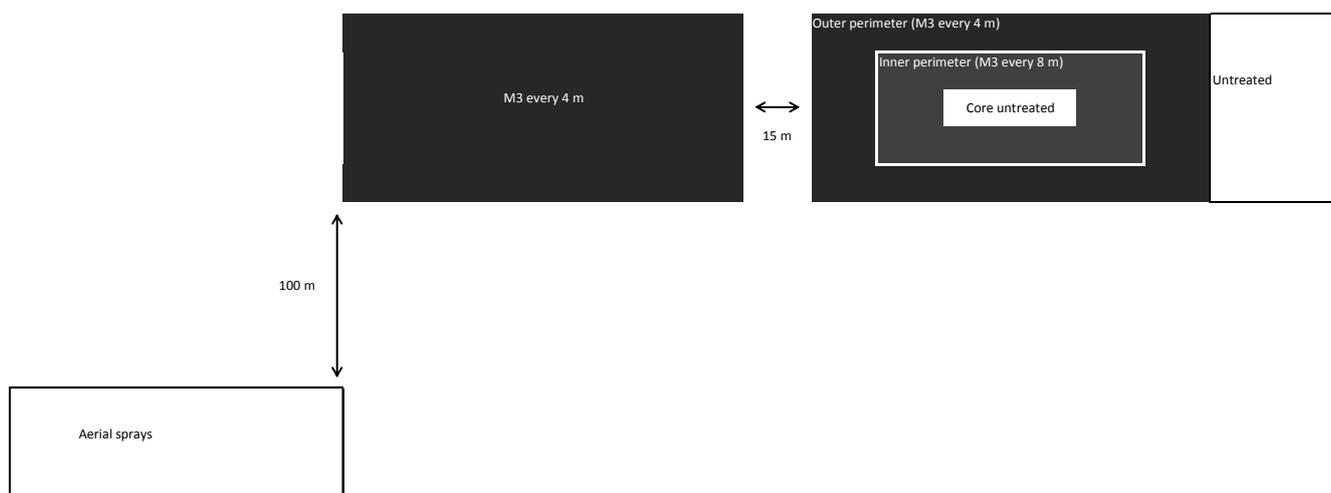


Figure 3.3.5.2. Layout of treatments near Groblersdal.

Fruit fly population monitoring

In all treatment blocks, the adult fruit fly population was monitored using Capilure and Questlure baited Sensus traps. A 1 cm x 1 cm Dichlorvos block was added to the trap to kill flies. Flies were collected from traps on a weekly to fortnightly basis and were taken to the laboratory for counting. Attractants and toxicants were replaced after 8 weeks.

Different numbers of traps were used in the two sites given that the sizes of blocks treated were different. In the M3 perimeter baited block in Nelspruit, 4 Capilure and 4 Questlure baited traps were placed in the core untreated area 16-28 m apart and 10 Capilure baited traps were placed in the outer perimeter area between 70-90 m apart. In Groblersdal, 10 Capilure and 6 Questlure baited traps were placed in total in each of the two M3 treatment blocks. Two of each Capilure and Questlure traps for each M3 treatment were placed in the central areas of the blocks which constituted the core area in the perimeter M3 treatment block. The remaining traps (both Questlure and Capilure) were placed in the border areas of each of the blocks. Distance between traps in the central area was about 18 m whilst distances between traps in the border area were about 40 m. In Groblersdal, 2 Capilure and 2 Questlure baited traps were placed in each of the aerial bait sprayed block and untreated block.

In both study sites, at harvest 500-1000 fruits were assessed for fruit fly damage on the tree in each treated block. In the M3 perimeter treatment, 500 fruits were assessed in the core area and 500 fruits in the perimeter area. 500 fruits were assessed in blocks treated with each of the other treatments in Groblersdal. Ten fruits from randomly selected 50 trees were checked for visible fruit fly damage symptoms such as punctures and exit holes.

Data analysis

Trap captures were presented as the mean number of flies caught per trap per week (\pm SE). Data were transformed to log ($x+1$) to stabilize variance before analysis. The General Linear Models procedure (Statgraphics plus 5.1) was then used to determine the effect of factors such as trap position, time and treatment on fly catches. Only two fruit fly species of interest were considered in the analysis: *Ceratitis capitata*, Mediterranean fruit fly (Medfly) and *C. rosa*, Natal fly, although other fly species like *C. cosyra* were also captured in Questlure baited traps near Nelspruit.

Results and discussion

Effectiveness of fewer M3s near Nelspruit

The position of Capilure baited traps (perimeter versus core) did not influence captures of Medfly and Natal fly males (Medfly: $F=0.10$, $df=1$, 165 , $P=0.75$; Natalfly: $F=0.61$, $df=1$, 165 , $P=0.43$) in the block treated with M3 bait stations. As such, catches from Capilure baited traps in all positions were pooled together for analysis. An increase in male Medfly population was recorded in the perimeter M3 treated block towards harvest ($F=6.5$, $df=11$, 165 , $P<0.001$) but catches did not exceed threshold level until after harvest (Figure 3.3.5.1). In contrast, Natal fly male population did not change significantly with time in the treated block ($F=0.83$, $df=11$, 165 , $P=0.61$) and stayed below the threshold level.

Catches of Medfly and Natal fly in Questlure baited traps did not change significantly over time (Figure 3.3.5.3) during the study period (Medfly: $F=1.18$, $df=11$, 95 , $P=0.32$; Natal fly: $F=1.14$, $df=11$, 95 , $P=0.34$) and did not exceed the threshold level until after harvest. Interestingly, peaks in catches of Medfly males in Capilure baited traps corresponded well with peaks in catches of Medfly females in Questlure baited traps. This indicates that both attractants Capilure and Questlure determined equally well fluctuations in fruit fly population in an M3 treated block, despite the fact that the M3 bait used for control and Questlure used for monitoring have protein as a common component and might compete with each other. No fruit fly infestation was recorded in fruits when a fruit damage assessment was conducted in the core and perimeter areas.

The density of M3 bait stations recommended for Valencia orange orchards is 300 units per hectare. In this study, the use of M3 stations with declining numbers from the perimeter of the orchard to the centre amounted to a density of only 187 stations per hectare. The perimeter M3 deployment strategy was able to sustain fruit fly population below the threshold level and prevent fruit fly damage. In Israel, Cohen & Yuval (2000) also found that perimeter mass trapping by placement of food baited traps only in the peripheral rows of the orchards provided effective control of Medfly.

Host availability has been shown to influence abundance of fruit flies (Israely et al., 1997; Puche et al., 2005) and volatile components of ripening fruit are major long-range stimuli that guide mature fruit flies towards host plants (Prokopy & Roitberg, 1989). In a long term study on population fluctuations of pest fruit flies in commercial mango orchards in Southern Mexico, Aluja et al. (1996) found that most flies were captured in the periphery of orchards and discussed the possibility of a seasonal fly population flux between neighbouring native vegetation, home gardens, isolated wild hosts and commercial orchards. The placement of bait stations in the perimeter areas is therefore likely to kill flies arriving at orchards.

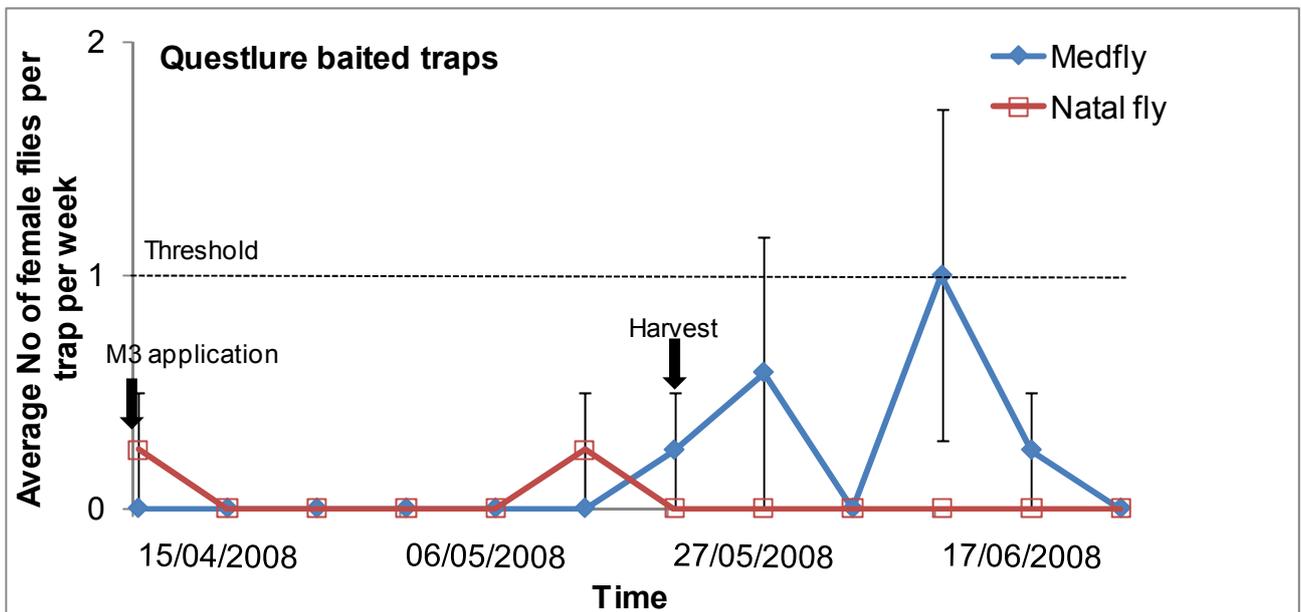
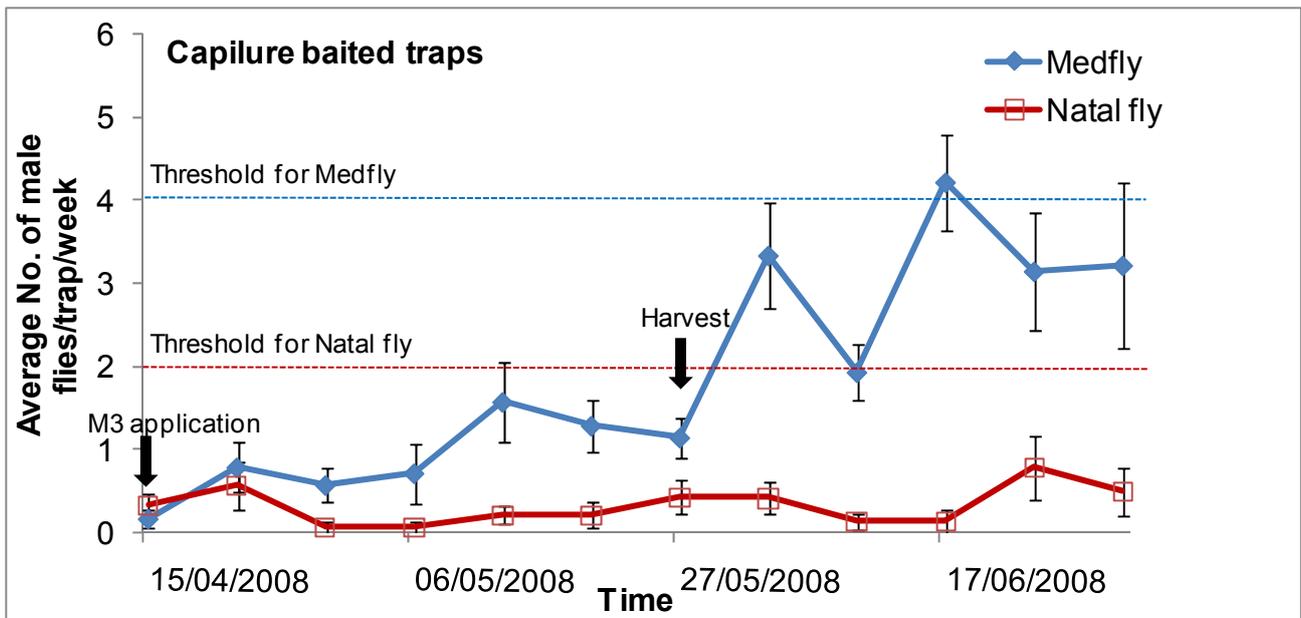


Figure 3.3.5.3. Fruit fly population in perimeter M3 baiting in Nelspruit. Dotted lines on graphs indicate threshold levels for the different fruit fly species in Capilure and Questlure baited traps. The threshold in a Questlure baited trap is 1 female (Medfly/Natal fly) per trap per week.

Comparison of two deployment strategies of M3 bait stations versus aerial bait sprays in Groblersdal

Similar to the results in Nelspruit, position of Capilure baited traps in the perimeter M3 treatment block did not influence fruit fly catches ($F=1.10$, $df=13, 69$, $P=0.38$) and therefore catches from traps in both perimeter and core sub-areas of that block were pooled together.

Medfly was the most abundant pest species captured in Groblersdal, constituting 94.7% of the catches in Capilure baited traps (all treatments pooled). Only one Natal fly was captured in a Capilure baited trap placed in the block with uniform M3 bait stations. No fly was captured in Questlure baited traps throughout the study period. Therefore only the data on Medfly catches in Capilure baited traps were analysed further.

Figure 3.3.5.4 shows the fruit fly population fluctuations in the different treatments. The fruit fly population in the block treated by aerial bait sprays was significantly higher compared to M3 treated blocks and even the untreated block adjacent to M3 treated blocks ($F= 36.59$, $df = 3, 167$, $P<0.001$). There was no significant difference in fruit fly population between the two types of M3 deployment. Although fruit fly catches were numerically higher in the untreated block compared to the two M3 treated blocks, differences were not

statistically significant. In all treatments except the aerial bait spray application, the threshold of 4 Medfly males per trap per week in Capilure baited traps was never exceeded, even after harvest.

Despite the presence of fruit flies in all blocks and higher catches in the aerial sprayed blocks, fruit fly infestation was not recorded at harvest in any of the different treatments.

T

he use of M3 bait stations with declining numbers from perimeter to centre leaving a core area untreated brought the total density of M3 stations down from 416 stations per ha, as used in the adjacent uniform M3 treatment, to 324 stations per ha (a decrease of 22.1%). Such a deployment strategy was found to be effective, similar to the results in Nelspruit, in sustaining low fruit fly population below threshold level up to harvest and also prevented fruit fly damage on fruits. It is likely that the M3 bait stations effected some degree of fruit fly control in the adjacent untreated area which could explain why fruit fly catches were also low in that untreated block and no damage was recorded.

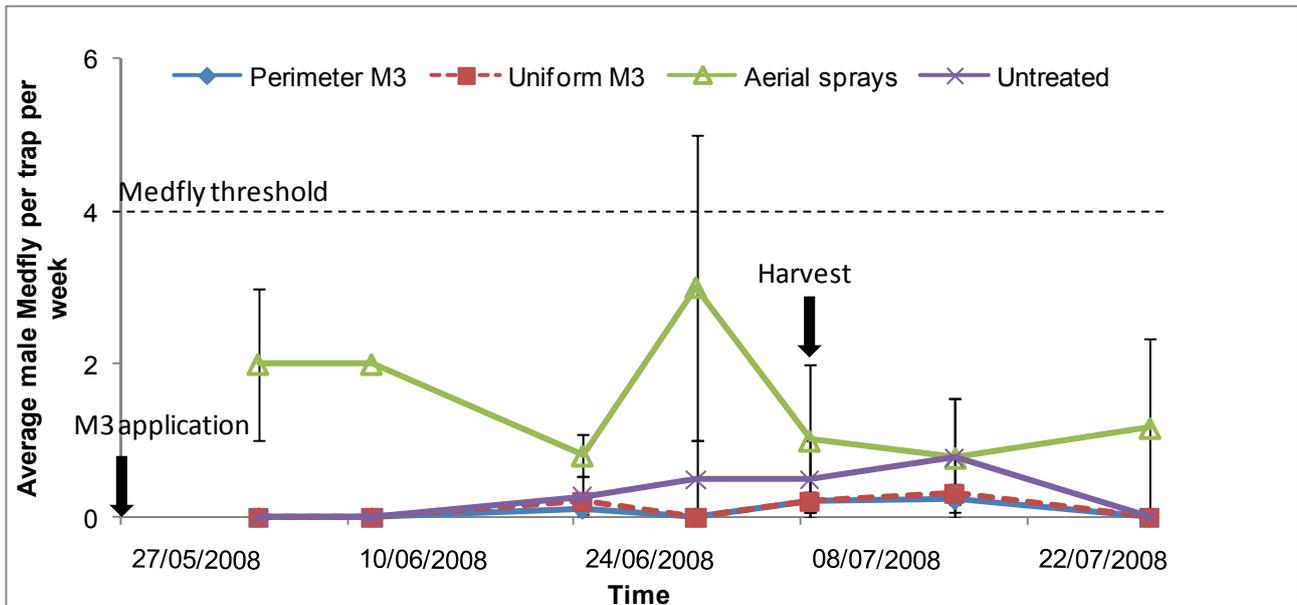


Figure 3.3.5.4. Fruit fly population fluctuations in citrus blocks under different treatments near Groblersdal: (1) perimeter M3 baiting, (2) M3 distributed uniformly, (3) aerial bait spray and (4) untreated. Dotted lines on graphs indicate threshold levels for the different fruit fly species in Capilure and Questlure baited traps.

Conclusion and future research

In this study, we showed that there is a potential to lower the number of M3 bait stations by deploying them in the perimeter of a block with declining numbers towards the centre and still maintain effective fruit fly control. Despite some weaknesses in the design of the experiments in not having another treatment to compare with the perimeter M3 baiting method in Nelspruit and having two different ways of deployment of M3 bait stations in the two study sites, the results in Nelspruit concurred with those in Groblersdal. In both study sites, fruit fly populations were kept below threshold levels until harvest in the perimeter-M3-baited blocks and no fruit fly damage was recorded.

The deployment of stations with varying intervals from perimeter to core might present some practical difficulties as we have ourselves experienced when laying out the trials. Nonetheless, the perimeter M3 baiting strategy should be further evaluated given its proven efficacy in sustaining low fruit fly population and preventing damage. However, in future tests, it would be preferable to have a fixed M3 baiting interval in the perimeter and vary only the sizes of core areas that can be left untreated.

Technology transfer

No technology transfer occurred between the period April 2008 and March 2009 but a paper is under preparation for the SA fruit journal on bait stations used in fruit fly control where the results of this study will also be presented.

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3.3.6 **PROGRESS REPORT: Fruit fly damage reduction and management of male fruit fly numbers** Experiment 929 (April 2008 – March 2009) by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

’n Proef is uitgevoer op nawel lemoene om die doeltreffendheid van 3 verskillende maniere van vrugtevliegbeheer te bepaal en te vergelyk. Hierdie is gedoen deur die vergelyking van vrugtevlieg vangstes met die gebruik van Capilure, Questlure en Ceratitislure lokmiddels sowel as vergelyking van vrugskade, vrugbesmetting en totale vrugval. Die 3 behandelings is Hymmlure en merkaptotien lokaas, M3 vrugtevlieg lokstasies en M3s plus mannetjie M3s. Die sogenaamde mannetjie M3s is met Capilure gelaai plaas van die standaard proteïen-hidrolisaat gebaseerde wyfie lokmiddel waarmee M3s gewoonlik gelaai word. Lokvalle is vir ’n 15 weke tydperk tot oestyd weekliks ondersoek. Vrugval, skade en besmetting is weekliks vir die laaste 11 weke voor oes ontleed. Al 3 behandelings het vrugtevlieg getalle verminder, gemeet deur Sensus lokval vangstes. In baie gevalle was hierdie verskillende statistiese betekenisvol. Al 3 behandelings het ook vrugtevlieg besmetting, vrugskade en selfs vrugval verminder. Hierdie verskil is veral vir die 2 M3 behandelings betekenisvol. Resultate het gewys dat M3s effens, maar nie statistiese betekenisvol nie, meer doeltreffend as die lokaas bespating was (beide wat vlieg getalle en vrugskade betref). Teen alle verwagtings het M3s ’n vinniger afname in mannetjie vrugtevlieg getalle as die bespating veroorsaak. Mannetjie M3s saam met standaard M3s het ’n vinniger afname in vrugtevlieg getalle veroorsaak as M3s op hulle eie. Hierdie verskil is gewoonlik nie volgehou nie. Heel waarskynlik as gevolg van die verval van die Capilure in die “mannetjie M3s” na omtrent 6 weke. Amper 12% van M3s wat gebruik is het binne 3 maande na hang uitgeloog. Dit het die werking van die M3s verminder. In die toekoms sal werk uitgevoer word om hierdie loog probleem te probeer verminder en om enige impak of doeltreffendheid van enige veranderinge in formulering te toets. Verdere proewe sal ook uitgevoer word om die werking van GF120 lokaasbespating met M3s te vergelyk. Hierdie proewe sal op ’n soortgelyke manier as hierbo beskryf is, uitgevoer word.

Summary

A trial was conducted on navel oranges to measure and compare the efficacy of 3 different treatments for controlling fruit flies. This was done by comparing fruit fly catches using Capilure, Questlure and Ceratitislure and by comparing fruit damage, fruit infestation and total fruit drop. The 3 treatments were baiting with Hymmlure and mercaptotien, M3 fruit fly bait stations and M3s plus male M3s. The so-called male M3s were loaded with Capilure, instead of the standard protein hydrolysate based female attractant

with which the M3s are usually loaded. Traps were checked weekly for a 15 week period until harvest. Fruit drop, damage and infestation were evaluated weekly for the last 11 weeks before harvest. All 3 treatments reduced fruit fly numbers, measured by Sensus trap catches. In many cases these differences were statistically significant. All 3 treatments used, also reduced fruit fly infestation, fruit damage and even fruit drop. These differences were particularly significant for the two M3 treatments, showing M3s to be marginally, but not significantly, more effective than baiting (both in reducing fly numbers and fruit damage). Against expectations, M3s caused a more rapid reduction in male fruit fly numbers than did baiting. Male M3s, together with standard M3s, caused a more rapid reduction in fruit fly numbers than did M3s alone, but this difference was generally not maintained, probably due to the expiry of the Capilure in the “male M3s” after about 6 weeks. Almost 12% of M3 used “leached” out within 3 months of hanging, which reduced their efficacy. In future, work will be conducted to try and reduce this leaching problem and to test the impact on efficacy of any changes to the formulation. Further trials will also be conducted to compare the efficacy of GF120 baiting and M3s, conducted in a similar manner as the trial described here.

Introduction

Fruit fly is regarded as one of the two most important phytosanitary entomological pests on citrus in South Africa. Three general ground-based modes of controlling fruit fly are available to citrus farmers. These are baiting with a protein hydrolysate and toxicant, baiting with GF120 and the use of M3 bait stations. All of these methods are considered to work adequately well. However, due to the seriousness of the fruit fly threat, it has never been possible to leave an untreated control, in order that fruit damage and reduction in fruit damage can be measured. This trial proposes to attempt to measure exactly this. Secondly, a problem which can exist with all modes of fruit fly control, is the possibility that male fly numbers can remain high, even when female flies are under good control. This trial proposes to measure male numbers where control measures are applied correctly, and to investigate a means of reducing male numbers for the sake of complying with good agricultural practice (GAP) protocols. A first trial was conducted from September to November 2007, comparing M3 bait stations with untreated controls (Moore & Kirkman, 2007). M3 bait stations proved to be totally effective in eliminating fruit fly damage in navel oranges, even where control measures were initiated when fruit fly numbers were already high and fruit was fully ripe, therefore highly susceptible. This was despite the bait stations being unable to noticeably reduce numbers of fruit flies caught in traps. The trial recounted in this report was conducted in a more conventional manner.

Materials and methods

Three adjacent orchards, of mixed cultivars and varieties, at the Citrus Foundation Block, Uitenhage, were selected for the trial. The orchards were divided into 8 approximately equal blocks. A common denominator between the 8 blocks was that each block had navel trees in the centre, which were used as data trees. Each block was between 6 and 9 rows wide and between 16 and 23 trees long. Each block therefore consisted of between 78 and 147 trees and was between 0.14 and 0.26 ha in size.

Four treatments were employed – each in 2 blocks. Treatments were randomly laid out. The 4 treatments were baiting with Hym lure (protein hydrolysate) and mercaptotion, M3 bait stations, M3 bait stations interspersed with male attracting stations (M3 + male M3), and an untreated control. M3 bait stations were hung at a density of 350 per hectare. The attractant used in the male M3s was Capilure, instead of the normal protein hydrolysate-based mixture. Alpha-cypermethrin was the toxicant, as per the norm for the standard female-attracting M3s. The male M3s were hung at a density of 10 per hectare.

On 11 March 2008 2 Questlure-loaded and 2 Ceratitislure-loaded Sensus traps were placed into each block. There was a delay in the acquisition of Capilure. Therefore, only a week later were 2 Capilure-loaded Sensus traps also placed into each block. Treatments were initiated on 18 March. Baiting and monitoring of traps was continued weekly for a 15-week period until 15 June. Fruit damage (determined as being fruit fly induced) and fruit fly infestation were evaluated from colour-break until harvest i.e. for an 11 week period from 15 April to 25 June. This was done by weekly evaluating fruit which had dropped under 10 data trees (all navel orange trees) in each block. Therefore, 20 data trees were evaluated for each treatment.

Data was statistically analysed by subjecting the means of all measurements (be they trap catches or fruit damage) for each treatment over the full evaluation period, to an ANOVA. Transformed means were then compared using a Bonferroni LSD multiple range test.

Results and discussion

Capilure loaded Sensus traps caught more flies than did traps with the other two lures (Table 3.3.6.1). However, this was only 29% more flies than caught by Ceratitislure. Capilure was almost useless at attracting female flies (as is well known). Questlure attracted the most female flies, with catches being around double those of Ceratitislure. Although it is known that male flies can be attracted, to a limited extent, by protein baits, only a negligible number of male flies were caught with Questlure, despite there obviously being large numbers of male flies in the trial orchards.

Table 3.3.6.1. Species and gender of total flies caught in all traps (with all lure types) over a 15 week period.

Lure type	Medfly				Natal fly			
	Male		Female		Male		Female	
	Total	%	Total	%	Total	%	Total	%
Capilure	1440	97.96	1	0.06	29	1.97	0	0
Questlure	1	2.32	29	67.44	0	0	13	30.23
Ceratitislure	1116	81.28	17	1.24	233	16.97	7	0.51

As there were far more Mediterranean fruit flies than Natal fruit flies caught (Table 3.3.6.1), and in order to present data more easily, differentiation between species has not been made in the ensuing 3 tables. These 3 tables compare fruit fly trap catches in the 4 different treatments (Tables 3.3.6.2 – 3.3.6.4).

Fruit fly numbers were consistently above the threshold for additional intervention i.e. 4 fruit flies per trap per week where Capilure is used in a Sensus trap (Ware, 2003; Table 3.3.6.2). In the table this would be indicated by 16 flies per set of 4 traps per week. Only once in the trial did numbers drop below this level. This was in the M3 treatment in the last week of monitoring (Table 3.3.6.2). The M3 treatment was the only treatment to reduce fly catches to a level significantly lower than that in the control blocks. The use of male M3s with normal M3s did not reduce average fruit fly catches over 15 weeks, relative to M3s on their own. However, during the first 6 weeks of trapping, catches were consistently lowest where male M3s had been included. It may be that it was necessary to replenish these male M3s with fresh Capilure attractants after 6 weeks, as is the recommendation for monitoring purposes in the Sensus traps.

Table 3.3.6.2. Male fruit flies caught per 4 Capilure-loaded Sensus traps per period (approximately a week) per treatment.

Date	Male fruit flies per trap per week			
	Control	Protein hydrolysate & malathion	M3	M3+♂M3
18/03	-	-	-	-
25/03	158	94	79	48
01/04	67	48	31	20
08/04	119	96	72	29
15/04	76	88	38	64
22/04	186	181	123	77
29/04	161	141	99	79
06/05	88	75	31	49
14/05	120	158	102	130
21/05	36	48	25	63
28/05	214	168	78	141
05/06	103	83	44	83
12/06	72	81	32	87
18/06	51	64	33	62
29/06	18	39	7	26
Average*	104.93b	97.43b	56.71a	68.43ab

*Mean values in the same row followed by the same letter are not significantly different ($P > 0.05$; Bonferroni LSD multiple range test).

The baiting with protein hydrolysate and mercaptothion was the only treatment which significantly reduced female fruit fly numbers caught in Questlure-loaded traps (Table 3.3.6.3). However, there was little difference between catches in bait sprayed blocks and catches in M3 + male M3 blocks. Catches of female fruit flies were higher in M3 treated blocks than in the untreated control. This was due mainly to extremely high catches in one particular trap which was near the edge of the orchard, adjacent to natural vegetation. This skewed the results against the M3 treatment. The M3 + male M3 probably gave a more accurate indication of the ability of the M3 bait station to reduce female fruit fly numbers. However, although there was little difference between female fruit fly catches with Questlure in the baited blocks and the M3 + male M3 blocks, the results deserve a more detailed analysis. The 9 females caught in the baited blocks in the first week may be an outlier which inflates the total numbers for this treatment. During the last 9 weeks before harvest only 1 female fly was caught in the baited blocks, whereas 11 female flies were caught in the M3 + male M3 blocks (Table 3.3.6.3). One would expect this to translate to lower levels of fruit damage and infestation where baiting had been conducted, but this was not so (Table 3.3.6.5).

Two further difficulties with the M3 bait station were noted. This was the leaching out (washing or drying out) of 11.8% of bait stations within 3 months after hanging. The other problem was vandalism by baboons, which physically removed a further 13.6% of bait stations. The bait stations, which had been removed by baboons were replaced when their absence was noted. The 11.8% of bait stations which had been wholly or almost wholly depleted of liquid, were tested for residual efficacy in a separate trial. By placing these M3s in Sensus traps, which were also loaded with dichlorvos tablets, it was ascertained that within the first week after hanging, these M3s still attracted 28% of the numbers of flies caught in Sensus traps loaded with fresh M3 bait stations. This was a positive result, indicating that bait stations which appeared to be leached out, may still have some efficacy in an orchard.

Table 3.3.6.3. Female fruit flies caught per 4 Questlure-loaded Sensus trap per period (approximately a week) per treatment.

Date	Female fruit flies per trap per week			
	Control	Protein hydrolysate & malathion	M3	M3+♂M3
18/03	2	9	5	3
25/03	4	1	2	0
01/04	3	1	0	1
08/04	0	1	1	0
15/04	0	1	0	1
22/04	0	1	3	0
29/04	0	0	2	2
06/05	0	0	3	0
14/05	2	0	10	1
21/05	1	0	5	2
28/05	11	0	4	5
05/06	5	0	2	0
12/06	6	1	3	1
18/06	5	0	3	0
29/06	3	0	8	0
Average*	2.80 bc	1.00a	3.40c	1.07ab

*Mean values in the same row followed by the same letter are not significantly different ($P>0.05$; Bonferroni LSD multiple range test).

Ceratitslure was not quite as effective as Capilure at attracting male flies and was not as effective as Questlure at attracting female flies (Table 3.3.6.1). However, it was more effective than both the other lures at simultaneously attracting both sexes of flies. The threshold for Ceratitslure for additional intervention against fruit fly is 4 male flies per trap per week or 2 female fruit flies (Ware, 2003). Rarely, in any of the treatments, were numbers of male flies below the threshold. This occurred most often for the M3 + male M3 treatment, where catches were below the threshold for 7 out of the 15 weeks. Despite the high numbers of male fruit flies caught, catches of female fruit flies were below the threshold during most weeks for all

treatments. All 3 treatments significantly reduced male fruit fly numbers, relative to the untreated control (Table 3.3.6.4). However, there was no difference between female fruit fly numbers.

Table 3.3.6.4. Fruit flies caught per 4 Ceratitislure-loaded Sensus trap per period (approximately a week) per treatment.

Date	Fruit flies per trap per week							
	Control		Protein hydrolysate & malathion		M3		M3+♂M3	
	M	F	M	F	M	F	M	F
18/03	165	9	146	9	106	12	72	2
25/03	202	8	97	0	88	1	26	3
01/04	111	2	40	0	28	0	9	0
08/04	89	3	45	0	29	0	6	0
15/04	88	0	32	0	26	1	16	0
22/04	106	0	26	0	22	1	11	0
29/04	73	1	43	0	20	0	10	0
06/05	25	0	49	0	12	0	8	0
14/05	52	0	24	0	17	0	17	0
21/05	7	1	5	0	3	0	3	1
28/05	131	0	55	0	36	0	46	0
05/06	145	0	42	0	38	0	32	0
12/06	68	0	40	0	31	0	23	0
18/06	60	0	36	1	18	0	19	0
29/06	27	0	14	0	5	0	11	0
Average males*	89.93c		46.27b		31.93ab		20.60a	
Average females*		1.60a		0.67a		1.00a		0.40a

*Mean values in the same row followed by the same letter are not significantly different ($P>0.05$; Bonferroni LSD multiple range test).

An approach which is often recommended and employed where M3 bait stations are used, is to first apply a protein hydrolysate and toxicant bait, before hanging M3s, as this is presumed to provide a better knock-down of flies than the M3s. However, the results obtained in this trial indicate the opposite. Results with all but one of the trap lures indicate that fruit fly numbers were lower during the first week after application, where M3 bait stations were used than where baiting was conducted (Tables 3.3.6.2 – 3.3.6.4). The only exception was female fruit fly counts in Ceratitislure-loaded traps (Table 3.3.6.4). It was not possible to measure the statistical significance of this observation.

Even before the cause of fruit drop was analysed, significantly fewer fruit were lost from trees in blocks treated with M3s (both M3 treatments) than untreated trees (Table 3.3.6.5). All 3 treatments significantly reduced the occurrence of blemishes (“stings”) which could be associated with fruit fly. Although fruit fly infestation was markedly lower for all 3 treatments than for the untreated control, this was only significantly so for the two M3 treatments. It is interesting that the treatments controlled fruit fly as well as they did, despite trap catches generally remaining high – often not significantly down from the untreated control and often not below the threshold for additional treatment. This may indicate that either the trap thresholds for intervention are too conservative or it may be a reflection on the small treatment block sizes. If the latter, then despite the treatments effectively controlling fruit flies, continual immigration of flies into treated blocks caused traps to give an exaggerated picture of the fruit fly threat to fruit.

Table 3.3.6.5. Fruit fly damage and infestation from fallen fruit per data tree per treatment (20 data trees per treatment) per week.

Date	Fruit drop				Fruit damaged				Fruit infested			
	Con	Ph+m ¹	M3	M3+ ♂M3	Con	Ph+m ¹	M3	M3+ ♂M3	Con	Ph+m ¹	M3	M3+ ♂M3
15/04	29	17	37	18	0	1	0	0	0	1	0	0
22/04	38	35	36	19	2	0	0	0	1	0	0	0
29/04	43	29	25	34	1	0	0	0	1	0	0	0
06/05	49	50	52	34	1	0	4	2	0	0	0	0
14/05	73	62	46	40	3	3	1	1	0	0	0	0
21/05	72	59	46	44	5	4	1	1	0	0	0	0
28/05	66	87	38	70	4	2	1	1	0	0	0	0
05/06	93	93	51	82	2	3	3	1	1	1	1	0
12/06	87	74	40	43	3	0	1	1	1	0	0	0
18/06	45	38	34	16	2	1	0	1	0	0	0	1
25/06	46	61	32	39	3	0	0	1	2	0	0	0
Mean/ tree/ week*	2.914b	2.750ab	1.986a	1.915a	0.118b	0.064a	0.050a	0.041a	0.027b	0.009ab	0.005a	0.005a

¹Protein hydrolysate and malathion bait spray.

*Mean values in the same row and section (i.e. fruit drop, penetration marks or infested) followed by the same letter are not significantly different (P>0.05; Bonferroni LSD multiple range test).

Conclusion

Baiting with protein hydrolysate, M3 bait stations and M3s + male (Capilure-loaded) M3s all reduced fruit fly numbers. All 3 treatments used also reduced fruit fly infestation, fruit damage and even fruit drop. These differences were particularly significant for the two M3 treatments, showing M3s to be marginally, but not significantly, more effective than baiting (both in reducing fly numbers and fruit damage). Interestingly, even though the treatments usually did not markedly or acceptably reduce trap catches, damage and infestation were still dramatically reduced. Against expectations, M3s caused a more rapid reduction in male fruit fly numbers than did baiting. Therefore, baiting as a “knockdown” before hanging M3s might not make sense. However, an initial combination of M3s and baiting might result in an even greater knockdown of flies. M3s + male M3s caused a more rapid reduction in fruit fly numbers than did M3s alone, but this difference was generally not maintained.

Further objectives (milestones) and work plan

Further work will be conducted to try and reduce the problem with leaching from the M3 bait stations and to test the impact on efficacy of any changes to the formulation. Further trials will also be conducted to compare the efficacy of GF120 baiting and M3s, conducted in a similar manner as previously done. Although funding has not been acquired for this work for the 2009-10 cycle, the work was initiated late in the 2008-09 cycle and will be completed.

Technology Transfer

A presentation on this work was made at the biennial CRI Citrus Research Symposium in 2008. This work will also be presented at the biennial congress of the Entomological Society of Southern Africa in 2009.

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3.3.7 PROGRESS REPORT: Cold disinfestation of *Bactrocera invadens* in citrus

Experiment 914 (Apr 2008 – Mar 2010) by T G Grout, J H Daneel, V Hattingh (CRI), S Ekesi, S A Mohamed and P W Nderitu (ICIFE, Kenya)

Opsomming

Dit is onvermydelik dat *Bactrocera invadens* uiteindelik in Suid-Afrika gaan aankom en wanneer dit gebeur, sal sommige van ons markte versekering vereis dat die koue sterilisasie behandeling wat tans gebruik word vir *Ceratitidis capitata*, ook *B. invadens* larwes suksesvol sal elimineer. Omdat ICIFE in Nairobi, Kenia 'n kultuur van *B. invadens* het en geïnteresseerd was om in oorleg met ons 'n na-oes tegniek te ontwikkel, het ons ingestem vir 'n gesamentlike navorsingsprojek. Die eerste twee fases van hierdie ondersoek het die ontwikkelings tempo van *B. invadens* in Valencia lemoene teen 28°C suksesvol aangetoon en ook dat die derde larwe instar die minste gevoelig is van die eier of larwe lewensstadiums, teen 'n koue behandeling van 1.1°C in lemoene. Verdere navorsing sal nou fokus op die derde instar om 'n koue behandeling vir lemoene teen 1.1°C te ontwikkel.

Summary

It is inevitable that *Bactrocera invadens* will eventually arrive in South Africa and when it does, some of our markets will require assurances that the cold disinfestation treatment used for *Ceratitidis capitata* will successfully eliminate any *B. invadens* larvae. As ICIFE in Nairobi, Kenya has a culture of *B. invadens* and was interested in collaborating on the development of post-harvest techniques, we agreed to conduct this joint research. The first two phases of this research have successfully shown the developmental rate of *B. invadens* in Valencia oranges at 28°C and that the third larval instar is the least susceptible of the egg and larval life stages to a cold treatment at 1.1°C in oranges. Further research will now focus on the use of the third instar in developing a cold treatment for oranges at 1.1°C.

Introduction

Bactrocera invadens was first found on the African continent in February 2003 by A. Manrakhan in Kenya using a protein baited trap. Since then it has spread west as far as Senegal, north to Sudan, south to Zambia, Namibia and Mozambique (approx. 18°S) and east to the Comoro Islands. It has not yet been recorded in Botswana, Malawi or Zimbabwe. This fly's primary host plant appears to be mango and it is out-competing Marula fly in this host in West Africa. In East Africa, Marula fly seems better able to compete at higher altitudes. *B. invadens* has been recorded as a pest of various *Citrus* species but grapefruit is the most common. This may be due to the fact that the fly most often occurs at low altitude. In Tanzania it is permanently present at altitudes between 380 and 520 m but occasionally occurs at altitudes as high as 1650 m when mango is not available at the low altitudes.

There is no doubt that *B. invadens* will be devastating to the fruit industry in the subtropical regions of South Africa, particularly the lowveld. The SA government is funding the monitoring of this fruit fly at a few locations near northern border posts and major cities in the country but is not providing any funds for research on control or disinfestation of the fruit fly. Once the fly arrives in the country, funds may be released for research on control but by then it will be too late to prevent an enormous economic impact on the citrus industry. Apart from the increased fruit loss expected from this fruit fly, export markets in subtropical or temperate regions such as China and Japan will require risk mitigation treatments or assurances that cold treatments used for *Ceratitidis* species are effective against *B. invadens*. Some research is being conducted on field control of this fruit fly in Kenya but as citrus is not an important crop in Kenya, nobody is planning work on cold disinfestation of citrus. By collaborating with ICIFE in Nairobi, who have a culture of *B. invadens*, it was possible for us to conduct this research in their facilities in order to determine whether the T107-a cold treatment of 14 d at 1.1°C recognised for *Ceratitidis capitata* by USDA APHIS (Anonymous 2009), is effective against *B. invadens*.

Materials and methods

Phase 1: Investigation of the larval development of *B. invadens* in citrus

In order to determine how long the different life stages took to develop at 28°C, three replicates of the following procedure were conducted using count 88 Valencia oranges. It was determined that 1 ml *B. invadens* eggs was equivalent to 16 000 eggs and approximately 35 eggs were required per fruit in 0.025 ml liquid, so the eggs were diluted with the appropriate amount of water to provide this ratio. Counts of the number of eggs in 10 aliquot samples per replicate later showed that a mean number of 32 eggs per fruit

were used in the first replicate and 33 in the second and third replicates. The eggs (not older than 24 hours) were inoculated into each orange on 14 August 2008 after first removing the calyx and punching a hole using a 6 mm diameter cork borer into the stem end of the fruit. Some *Torula* yeast (between 0.2 and 0.5 ml of a mixture with water in a 1:2 ratio) was also injected into the inoculation hole to provide additional nourishment for the larvae. The inoculation procedure was conducted as three batches of 130 fruit. Each batch was treated as a replicate. After inoculation the hole was plugged with sterilised cotton wool and this sealed with hot wax. Each orange was placed in a brown paper bag and stored at 28°C according to the replicate. From the first day through to the 15th day after inoculation, seven fruit were dissected daily from each crate and the number of fruit fly larvae in each instar determined. Dissected larvae were carefully measured under a stereomicroscope with the assistance of Leica Application Suite software and at least six larvae per day were mounted on slides and examined closely in order to determine what instars were represented. Although the primary objective for conducting this study was to determine how long it took for each larval instar to develop, a further 25 infested fruit per replicate were kept on sand and the number of pupae determined daily by sieving the sand from 19 August onwards. The pupae were allowed to develop and the number of adults emerging determined in order to gain more information about the life cycle of *B. invadens* at 28°C.

Phase 2: Determination of the most cold-tolerant life stage

ICIPE did not have a cold room that could work reliably at around 1°C so CRI paid for a new Zanotti split unit to be installed. Thirty-three cartons of export quality, count 88 Valencia oranges were acquired from a supplier in Nairobi for both replicates. The first replicate started with inoculation on 13 October 2008 and the second replicate on 8 December 2008. In each replicate, eggs, not more than twenty-four-hours old, were inoculated into 1 400 fruit as described above (35 per fruit). One group of 300 fruit was moved to the cold room (1.1°C±0.5°C) on the day of inoculation (egg stage) while the rest of the fruit were held at 28°C. Two days later (actually 36 h after inoculation to allow 12 h to reach 1.1°C) a second batch of 300 fruit (containing first instars) were moved into the cold room and four days (84 h) after inoculation (second instars) another batch of 300 fruit. After six days the last batch of 300 fruit (third instars) were moved into the coldroom. The remaining 200 fruit (four batches of 50 fruit per life stage group) were used as controls and held at 28°C then dissected six days after inoculation. Fifty fruit from the egg stage group were removed from the cold room on each of days 3, 5, 7, 9, 11 and 13 after commencement of the cold sterilization treatment and held at 28°C for 6 days. The fruit was then dissected and the number of living and dead larvae noted. Batches of 50 fruit from the first, second and third instar stage were removed at the same time intervals as the egg stage group and held at 28°C for 4, 2 and 1 days respectively, before being dissected. Two replicates were conducted and the susceptibilities of the different life stages to the cold were analysed with Probit analysis using 95% fiducial limits (Finney 1971).

Results and discussion

Phase 1: Investigation of the larval development of *B. invadens* in citrus

The results of the larval development studies at 28°C (Table 3.3.7.1) showed that first instar larvae appeared two days after inoculation and most second instar larvae four days after inoculation. Most larvae had reached the third instar by six days after inoculation. Compared to the short larval life stages, the formation of pupae took place over a long time (21 d), perhaps as a survival strategy or due to difficulty in getting out of the fruit (Fig. 3.3.7.1). The first larva left the fruit to pupate, seven days after inoculation. The first adults eclosed 17 d after inoculation and most adults eclosed between 20 and 26 d after inoculation (Fig. 3.3.7.1).

Table 3.3.7.1. Larval development at 28°C with time after inoculation for three replicates of 105 fruit each inoculated with 32 (Replicate 1) or 33 (Replicates 2 and 3) eggs.

Days after inoculation	Replicate 1		Replicate 2		Replicate 3		Mean size	Total no.	Instar ratio (%); First: Second: Third
	Size	No.	Size	No.	Size	No.			
1	-	0	-	0	-	0	-	0	No larvae
2	2.49	12	3.09	24	3.11	23	2.98	59	100:0:0
3	4.90	23	4.54	26	5.00	22	4.80	71	93:7:0
4	8.44	29	6.08	47	8.23	37	7.39	113	0:87:13
5	9.55	46	8.95	38	8.53	45	9.02	129	0:30:70
6	9.61	54	9.30	43	9.44	37	9.46	134	0:9:91
7	9.78	48	9.85	54	9.52	47	9.72	149	0:0:100
8	10.08	43	10.01	56	9.78	47	9.96	146	Third instar and pupae
9	9.94	57	9.83	55	10.33	33	9.98	145	
10	10.17	51	10.53	11	9.88	31	10.12	93	
11	10.07	36	9.86	27	9.63	32	9.86	95	
12	10.05	50	10.29	46	10.24	28	10.18	124	
13	10.07	34	9.99	44	9.96	38	10.00	116	
14	9.68	38	9.88	44	9.85	21	9.80	103	
15	10.36	32	10.36	44	10.12	27	10.30	103	
Total	-	553	-	559	-	468	-	1580	

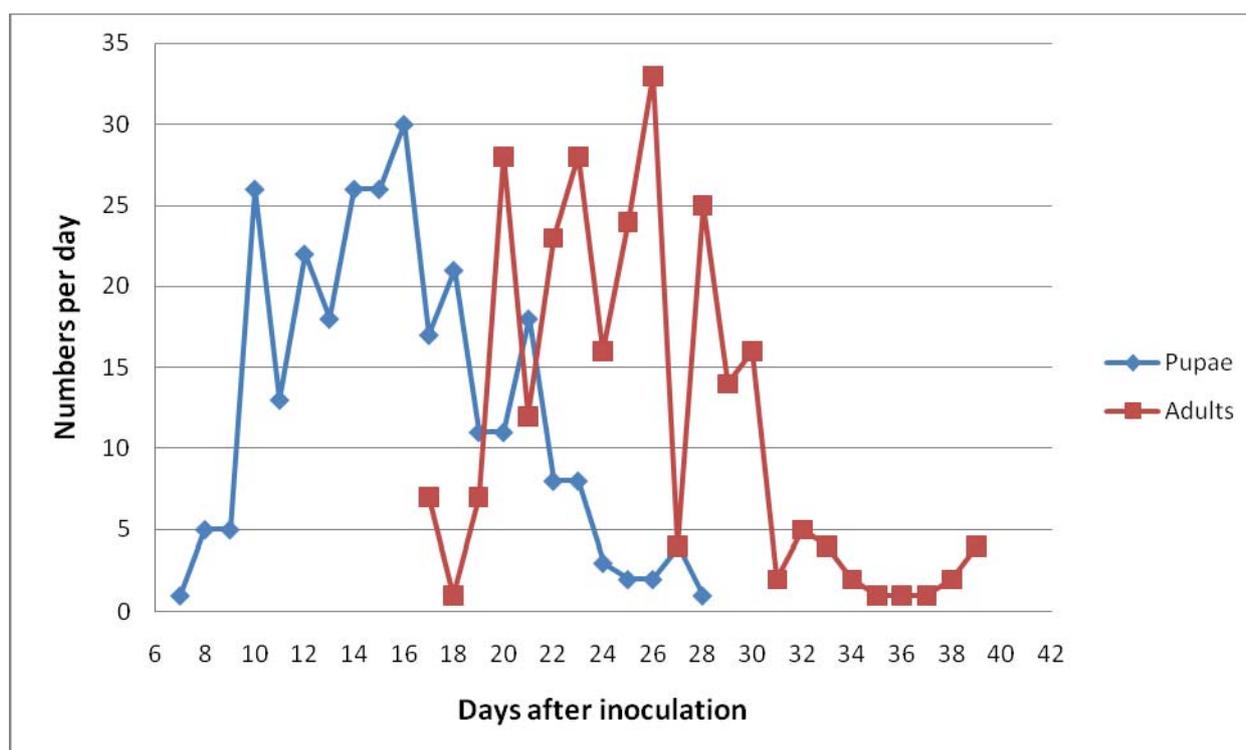


Figure 3.3.7.1. Total numbers of *B. invadens* pupae forming and adults eclosing per day from oranges on sand at 28°C.

Phase 2: Determination of the most cold-tolerant life stage

The cold room appeared to work well and when the few crates of fruit were moved into the room they were rapidly cooled to 1.1°C. Seven days after inoculation, fruit that had started the cold treatment at the egg stage had 100% larval mortality in both replicates (Tables 3.3.7.2 and 4) and the same applied to fruit that started treatment when larvae were in the first instar. With only two results from each of these life stages having mortalities less than 100%, Probit analysis was not possible. The corrected mortalities of second instars after having been exposed for seven days were 99.73% for the first replicate and 99.29% for the second replicate, whereas for third instars the corrected mortalities were 97.83% and 98.68%, respectively.

With Probit analysis, these differences between second and third instars were significant ($P < 0.05$) in the first replicate (Table 3.3.7.3) but not in the second replicate. However, the prediction for the LT 99.9% was higher for third instars than for second instars in both replicates. These results therefore suggested that the third instar was more tolerant to the cold treatment so this instar will be used in further evaluations of this treatment.

Table 3.3.7.2. Summary of results for Replicate 1 with controls evaluated on 19 October 2008.

STAGE	EXPOSURE PERIOD (DAYS)	NUMBER OF FRUIT	NUMBER OF EGGS TREATED	NUMBER OF LIVE LARVAE RECOVERED	MORTALITY (%)	CORRECTED MORTALITY (%)
EGGS	CONTROL	51	1785	333	81.34	
	3	50	1750	126	92.80	61.41
	5	50	1750	16	99.09	95.10
	7	50	1750	0	100.00	100.00
	9	50	1750	0	100.00	100.00
	11	50	1750	0	100.00	100.00
	13	52	1820	0	100.00	100.00
FIRST INSTAR	CONTROL	50	1750	347	80.17	
	3	50	1750	96	94.51	72.33
	5	51	1785	14	99.22	96.04
	7	50	1750	0	100.00	100.00
	9	51	1785	0	100.00	100.00
	11	51	1785	0	100.00	100.00
	13	50	1750	0	100.00	100.00
SECOND INSTAR	CONTROL	50	1750	364	79.20	
	3	50	1750	143	91.83	60.71
	5	50	1750	35	98.00	90.38
	7	51	1785	1	99.94	99.73
	9	50	1750	0	100.00	100.00
	11	50	1750	0	100.00	100.00
	13	51	1785	0	100.00	100.00
THIRD INSTAR	CONTROL	51	1785	361	79.78	
	3	51	1785	235	86.83	34.90
	5	50	1750	78	95.54	77.96
	7	52	1820	8	99.56	97.83
	9	50	1750	2	99.89	99.43
	11	51	1785	0	100.00	100.00
	13	50	1750	0	100.00	100.00

Table 3.3.7.3. Probit analysis comparing second and third instars in the first replicate.

Instar and replicate	Expected mortality (%)	Days	Standard Error	Upper fiducial limit	Lower fiducial limit
Second instar first replicate	50	2.72	0.256	Could not be calculated	Could not be calculated
	90	4.63	0.313		
	95	5.39	0.458		
	99	7.16	0.928		
	99.9	9.83	1.834		
Third instar first replicate	50	3.61	0.104	3.81	3.40
	90	5.77	0.138	6.07	5.52
	95	6.59	0.189	7.01	6.26
	99	8.46	0.348	9.26	7.86
	99.9	11.19	0.641	12.71	10.11
Comparison of lines: Elevations significantly different with F= 15.951, D.F. = 1 and 4, P=0.0160					

Table 3.3.7.4. Summary of results for Replicate 2 with controls evaluated on 14 December 2008

STAGE	EXPOSURE PERIOD (DAYS)	NUMBER OF FRUIT	NUMBER OF EGGS TREATED	NUMBER OF LIVE LARVAE RECOVERED	MORTALITY (%)	CORRECTED MORTALITY (%)
EGGS	CONTROL	53	1961	271	86.18	
	3	50	1850	80	95.68	68.71
	5	52	1924	11	99.43	95.86
	7	52	1924	0	100.00	100.00
	9	52	1924	0	100.00	100.00
	11	50	1850	0	100.00	100.00
	13	52	1924	0	100.00	100.00
FIRST INSTAR	CONTROL	53	1961	284	85.52	
	3	50	1850	71	96.16	73.50
	5	52	1924	15	99.22	94.62
	7	52	1924	0	100.00	100.00
	9	52	1924	0	100.00	100.00
	11	52	1924	0	100.00	100.00
	13	52	1924	0	100.00	100.00
SECOND INSTAR	CONTROL	53	1961	291	85.16	
	3	51	1887	114	93.96	59.29
	5	52	1924	15	99.22	94.75
	7	51	1887	2	99.89	99.29
	9	52	1924	2	99.90	99.30
	11	52	1924	0	100.00	100.00
	13	52	1924	0	100.00	100.00
THIRD INSTAR	CONTROL	51	1887	380	79.86	
	3	52	1924	131	93.19	66.19
	5	52	1924	29	98.49	92.52
	7	51	1887	5	99.74	98.68
	9	52	1924	0	100.00	100.00
	11	51	1887	0	100.00	100.00
	13	54	1998	0	100.00	100.00

Table 3.3.7.5. Probit analysis comparing second and third instars in the second replicate.

Instar and replicate	Expected mortality (%)	Days	Standard Error	Upper fiducial limit	Lower fiducial limit
Second instar first replicate	50	2.73	0.112	2.93	2.49
	90	4.42	0.136	4.73	4.18
	95	5.07	0.194	5.53	4.74
	99	6.56	0.378	7.51	5.95
	99.9	8.74	0.718	10.62	7.62
Third instar first replicate	50	2.47	0.123	2.69	2.20
	90	4.58	0.140	4.89	4.32
	95	5.45	0.216	5.96	5.08
	99	7.56	0.481	8.77	6.79
	99.9	10.92	1.023	13.61	9.32
Comparison of lines: Elevations not significantly different with F= 0.008, D.F. = 1 and 3, P=0.9340					

Conclusion

The first phase of research successfully provided the development times for different life stages in Valencia oranges at 28°C. The second phase of research indicated that the third larval instar was the least susceptible of the egg and larval life stages to a cold treatment at 1.1°C in Valencia oranges. The third larval instar will therefore be used in further development of a cold treatment for *B. invadens* in oranges at 1.1°C.

Further objectives and work plan

The third and fourth phases of the development of a cold treatment must be completed within the 2009-10 financial year. This should be possible with the use of Egyptian and South African Valencias and without any unforeseen delays.

Technology transfer

Other than informing various interested parties that the work is being conducted in collaboration with ICIPE, technology transfer has not been appropriate at this early stage.

References cited

- Anonymous. 2009. Treatment manual. USDA APHIS version 05/2009 – 34.
 Finney, D.J. 1971. Probit analysis. Cambridge University Press, Cambridge. 333 pp.

3.3.8 PROGRESS REPORT: Feasibility of using male annihilation techniques for suppression of *Bactrocera invadens* and *Ceratitidis* spp in mango orchards in northern Benin

Experiment 926 (Apr 2008 – Mar 2010) by R Hanna, D Gnanvossou, (IITA, Benin) T G Grout and J H Daneel (CRI)

Opsomming

Hierdie verslag weergee bevindinge van eksperimente gerig op die uitwissing van mannetjies in Noord-Benin met die gebruik van methyl eugenol – vir die onderdrukking van *Bactrocera invadens* – en terpinyl asetaat vir die onderdrukking van *Ceratitidis* spp. Methyl eugenol MATs het die populasie mannetjies van *B. invadens* teen minder as 10% van die populasies in kontrole boorde, gehou. Terpinyl asetaat was ook effektief in die onderdrukking van populasie mannetjies van *Ceratitidis* spp., maar was baie minder effektief as methyl eugenol in die onderdrukking van *B. invadens*. Besmettings van vrugtevlug (persentasie vrugte besmet) in die mid-seisoen mango kultivars, Eldon en Kent, is met 39.8% en 46.8% respektiewelik verminder, met 'n gesamentlike vermindering van 56.1% en 68.8% in die besmettingsindeks (papier/kg vrugte), respektiewelik. Gesamentlik dui die vermindering in die populasie mannetjies, vrug besmettingsvlakke en vrugtevlug populasie regenerasie aan dat die uitwissing van mannetjies van *Ceratitidis* spp en *B. invadens* 'n belowende aanslag is in geïntegreerde programme vir die onderdrukking van vrugtevlug in mangoboorde in Afrika. Hierdie eksperimente sal wel herhaal moet word, maar onder verskillende toestande om die veralgemening

van die gebruik van MAT in die bestuur van vrugtevlieg, spesifiek onder verskillende landbou-ekologiese toestande, te bewerkstellig. Weens die veranderinge in die fokus van navorsing in Benin sal dit waarskynlik in sitrusboorde in Tanzanië uitgevoer word.

Summary

This report summarizes the findings from male annihilation experiments in northern Benin using methyl eugenol - for suppression of *Bactrocera invadens* - and terpinyl acetate for suppression of *Ceratitis* spp. Methyl eugenol MATs maintained *B. invadens* male populations at less than 10% of its populations in the control orchards. Terpinyl acetate was also effective in suppressing *Ceratitis* spp. male populations, but was much less effective than methyl eugenol in the suppression of *B. invadens*. Fruit fly infestation (percent fruits infested) of mid-season mango cultivars Eldon and Kent were reduced by 39.8% and 46.8% respectively, with a concomitant reduction of 56.1% and 68.8% in infestation index (pupae/kg fruit), respectively. Taken together, the reduction in male populations, fruit infestation levels and fruit fly population regeneration indicate that male annihilation of *Ceratitis* spp and *B. invadens* is a promising approach in integrated programmes for the suppression of fruit flies in mango orchards in Africa. These experiments must be repeated, however, under different conditions to allow for generalization of MAT utility in fruit fly management, particularly under different agro-ecological conditions. Due to changes in research focus in Benin, this will probably be conducted in citrus orchards in Tanzania.

Introduction

Fruit flies have been known to be major pests of mango in West and Central Africa. Prior to 2004, only fruit flies belonging to several *Ceratitis* species were known to infest mango. In 2004, a new species, later described as *Bactrocera invadens* (Drew, White & Tsuruta) (Drew et al. 2005), was discovered in Benin and nearly simultaneously in six countries in West and Central Africa. This new species is presently found in 27 countries in sub-Saharan Africa. It is highly polyphagous, widely distributed, and can cause up to 70% losses in mango production. Guava and citrus are among the other cultivated crops most affected by *B. invadens*.

Subsequent to the invasions of Africa by *B. invadens*, a programme was initiated by IITA in West and Central Africa to develop integrated management programmes of fruit flies in tree fruit and vegetable crops. A similar programme was already underway by *icipe* in Eastern Africa.

To date, several fruit fly management options have been explored, including classical biological control of *B. invadens* with exotic parasitoids, new association biological control of *Ceratitis* spp. (i.e., using exotic parasitoids to control native species), use of GF-120 (spinosad) alone or in combination with entomopathogenic fungi, and promotion of weaver ants which have been recently shown to cause substantial reductions in fruit fly populations. More recently, IITA in collaboration with Citrus Research International (Nelspruit, South Africa) initiated studies on the feasibility of using male annihilation technique (MAT) for the suppression of *B. invadens* and *Ceratitis* spp. populations in mango orchards. This report provides a summary of the results from the studies conducted during the 2008 mango season in northern Benin.

Materials and methods

Six mango orchards, two in each of three villages, were selected for the experiment in March 2008. Five orchards were 2 ha in size while the sixth orchard had an area of 3 ha. The orchards were located in Tchaourou (08.98.177N 002.62.003E and 08.92.147N, 002.56.569E); Tchatchou (09.05.707N, 002.33.309E and 09.21.451N, 002.37.297E); and Alafiarou (09.01.443N, 002.41.364E and 09.01.449N, 002.38.861E). Orchard elevations ranged from 301 to 352 masl. All orchards contained mixed plantings of early, mid and late season mango varieties, with Gouverneur, Eldon and Kent being the dominant varieties. Vegetation surrounding the orchards was composed mainly of *Vitellaria paradoxa* C.F. Gaertn., *Annona senegalensis* Pers., *Anacardium occidentale* L. and *Sarcocephalus latifolius* (Smith) Bruce.

In each village, the two orchards were randomly designated to MAT and control treatments. Each MAT orchard had 40 M3 devices (supplied by Citrus Research International) per lure type at the rate of 20 devices per ha, except for the 3 ha orchard in which 60 devices of each lure were installed. The lures used in the MAT devices (MATDs) were methyl eugenol (ME) and terpinyl acetate (TA). Only one type of MATD was hung per tree at about 1.5 m above ground level near the middle of the tree canopy. Devices of the two lures were placed in alternative mango tree rows – 10 m between tree rows and 20 m between MATDs within a tree row.

MATDs were charged with 4 ml solution containing 3 ml of the lure (ME or TA) and 1 ml of undiluted malathion (500 g/L EC) toxicant. ME MATDs were recharged every month while TA MATDs were recharged at 15-day intervals due to higher volatility of TA. In each of the MAT orchards, five of each lure MATDs (four at the periphery and one in the centre) were each placed in a bucket trap to monitor male populations. Three MATDs of each lure were similarly used in each of the control orchards to monitor population trends of male *B. invadens* and *Ceratitis* spp. In addition, Torula yeast was used in McPhail traps in both treated and untreated areas because this lure was not in competition with the MAT lures. MATDs and traps were placed on 15 March 2008 and fruit flies were collected at 15 day-intervals through 19 July.

In addition to adult fruit fly population monitoring, 100 fruits per variety (10 from each of 10 trees) were collected on each sampling date during two consecutive weeks of the peak maturity period for that variety. The trees used for sampling were distributed as follows: four trees in the periphery and intermediate distance to the centre, and two trees in the centre of the orchard. All fruits were inspected for signs of infestation by fruit flies, weighed and incubated in plastic bins (with a 2 cm layer of moist sand in the bottom) in groups of 10 fruits to allow fruit fly larvae to leave the fruit and pupate. Pupae were removed at 3-4 day intervals and placed in Petri dishes with moist cotton and a small quantity of sugar and protein hydrolysate for emerging adults, which were later sexed and identified to species.

In addition to mango sampling, we collected (at two-week intervals) at least 20 fruits from each of several potential *B. invadens* and *Ceratitis* spp. hosts within 500 m from the borders of each of the experimental orchards. These included *S. latifolius*, *A. senegalensis*, *V. paradoxa* and *A. occidentale*. The identity and frequency of host species were recorded to assess the relative effect of fruit fly sources surrounding orchards on the efficacy of MAT to suppress fruit flies in the mango orchards.

Results and discussion

Ceratitis spp. populations in TA traps were 5-fold higher in MAT orchards compared with control orchards on the first sampling date (Figure 3.3.8.1). During subsequent dates, *Ceratitis* spp declined to substantially lower levels (with about 65% reduction at peak population density) in MAT compared with control orchards. To our knowledge, this is the first demonstration in sub-Saharan Africa of the utility of terpinyl acetate in the suppression of *Ceratitis* spp. populations.

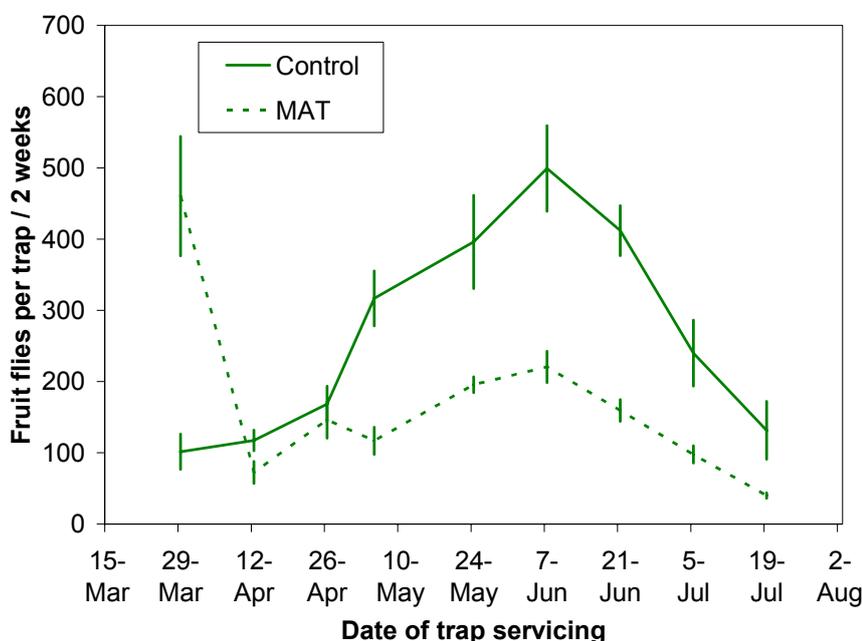


Figure 3.3.8.1. Population trends of male *Ceratitis* spp. in terpinyl acetate traps in control (solid line) and MAT orchards (dashed line).

Bactrocera invadens displayed considerably different dynamics than *Ceratitis* spp. Unlike *Ceratitis* spp., *B. invadens* populations were nearly absent until early May, which is typical for northern Benin, where *B. invadens* disappears during the dry season from January to April, and reinvades after the beginning of the

rainy season. As in the case of *Ceratitis* spp. in TA traps, *B. invadens* populations were relatively higher in the MAT orchards compared with control orchards on the first sampling date, but remained very low in all orchards during the month of April (Figure 3.3.8.2). The trend reversed by early May when *B. invadens* populations increased steeply in the control orchards reaching an average of 5024.8 ± 644.4 (mean \pm SE) individuals per trap per two week period by the last sampling date on 19 July, while *B. invadens* populations in the MAT orchards remained relatively flat and at levels of less than 10% of those in the MAT orchards and reaching a peak of 431.5 ± 153.1 individuals per trap per two weeks on 19 July. To our knowledge, this is also the first study to demonstrate the utility of MAT to suppress populations of *B. invadens* anywhere where this species has been found. Results from Torula yeast traps have not been included as the sorting of flies from these traps is still incomplete.

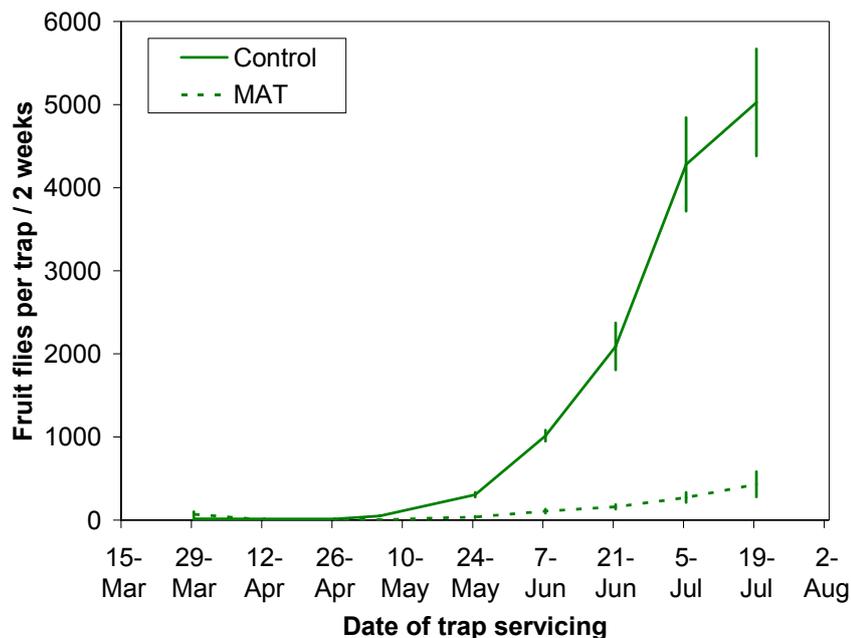


Figure 3.3.8.2. Population trends of male *Bactrocera invadens* in methyl eugenol traps in control (solid line) and MAT orchards (dashed line).

Fruit fly infestations of two mid-season varieties (Eldon and Kent) are presented in Table 3.3.8.1. These infestations mirrored the reductions in fruit fly adult populations, particularly those of *Ceratitis* spp. Percent of fruits infested by fruit flies (all species) were similar for the two varieties (49.8 ± 2.67 for Eldon and 46.2 ± 2.98 for Kent) in the control orchards, but in the MAT orchards infestations of both varieties were significantly less than in the control orchards, with infestation of Kent being less than those of Eldon. MAT reduced fruit fly infestations by 39.8% for Eldon and 46.8% for Kent. Unfortunately, this infestation refers to both *B. invadens* and *Ceratitis* spp. (mostly *C. cosyra*) because at the time of writing this report the identification of the emerging flies was not complete. As the TA MAT device was less efficient at controlling *Ceratitis* due to its volatility it is likely that many of the flies were *Ceratitis* spp.

Table 3.3.8.1. Fruit fly infestation levels in MAT and control orchards, 2008 mango season in northern Benin.

Mango variety	Fruit infestations (mean \pm SE)		Percent infestation reduction with MAT
	Control	MAT	
	Percent infested fruits		
Eldon	49.8 ± 2.67	30 ± 2.1	39.8
Kent	46.2 ± 2.98	24.6 ± 2.42	46.8
	Pupae per kg/fruit		
Eldon	26.3 ± 3.10	12.9 ± 2.38	56.1
Kent	19.7 ± 2.57	5.61 ± 1.11	68.8

The results of this one year study are very encouraging. Not only did MAT reduce adult male populations of both *Ceratitis spp.* and *B. invadens* adult male populations, but more importantly it led to substantial reductions in fruit fly infestations and in turn greater harvest of marketable mango. This is a first demonstration of the utility of MAT in fruit fly suppression in mango orchards in sub-Saharan Africa. However, it is necessary to repeat these studies for another mango season before generalization can be made on the utility of MAT in fruit fly suppression across years, agroecologies, and farming practices. Moreover, it is necessary to proceed to the next phase of integrating MAT with other approaches such as biological control, bait sprays/stations, sanitation, etc, to achieve sufficient control of fruit flies in mango orchards. MAT should also be tested in other systems such as citrus and guava orchards where *B. invadens* is the dominant species in Benin and where MAT is likely to be even more effective than in mango. There is also an urgent need to develop a means of reducing the volatility of TA (with significant reduction in its attractiveness) to reduce the frequency of recharging MAT devices and achieve greater reduction in total investment in this useful approach. Alternatively, Ceratitislure could be used in MAT devices for *C. cosyra*. Finally, it is necessary to test this MAT approach with alternative MAT devices that can be locally fabricated with little cost to the producer. Coconut husks have been tested in the Pacific Islands and have been shown to be quite effective as MAT devices. Fibre-board blocks have also been used elsewhere and are more readily available in most inland countries.

Conclusion

MAT devices made from M3s successfully lowered populations of *B. invadens* and *Ceratitis spp.* and their resultant infestation of mangoes when used as the sole method of control in northern Benin. For further work, Ceratitislure should be evaluated in MAT devices for *C. cosyra* and inexpensive fibre-board blocks should be used rather than plastic M3s. Combinations of MAT and baiting should be evaluated.

Acknowledgements

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Further objectives and work plan

IITA is now focusing on biological control with the release of exotic parasitoids. Government researchers in Tanzania would like to try MAT and baiting for area-wide control of *B. invadens* in citrus near Muheza so research in 2009 will involve collaboration with them and start with the protection of oranges in June.

Reference cited

Drew, R.A.I., K. Tsuruta and I.M. White. 2005. A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *Afr. Entomol.* 13: 149-154.

3.3.9 PROGRESS REPORT: Ethyl formate as a fumigant for fruit fly and other phytosanitary pests Experiment 913 (Apr 2008 - Mar 2011) by T G Grout and P R Stephen (CRI)

Opsomming

Afrox het nog nie toestemming van die Departement van Omgewingsake en Toerisme ontvang om hierdie GRAS chemikalie van Australië in te voer nie. Geen werk is dus met ethyl formate (Vapormate) gedoen nie en die tyd wat aan die navorsing geallokeer was, is vir ander navorsing aangewend. Indien Vapormate in die toekoms wel beskikbaar kom, sal dit ondersoek word omdat dit effektief teen sommige fitosanitêre plae kan wees. Navorsing word egter nie nou beplan nie.

Summary

Afrox have not received permission from the Department of Environmental Affairs and Tourism to import this GRAS chemical from Australia. No work was therefore conducted on ethyl formate (Vapormate) and the time allocated to this research was spent on other research. If Vapormate does become available in the

future it will be investigated, as it may be effective against some phytosanitary pests. However, research is not currently planned.

3.3.10 **PROGRESS REPORT: The role of interspecific competition in regulating populations of *C. capitata* and *C. rosa***

Experiment US/ENT-07-A (April 2008-March 2009): by N. Mfeka, P. Addison (SU), A. Manrakhan (CRI)

Opsomming

Die interspesifieke kompetisie tussen Mediterreense en Natalse vrugtevlieë is ondersoek deur na gasheervrug benuttings patrone van albei spesies te kyk. Tot dusver is eksperimente op appels, lemoene, druwe, pomelos, pruime en perskes uitgevoer. Alle eksperimente was uitgevoer teen 25°C, ± 60% RH en 12:2 L:D siklus. Volwasse vlieë is voorsien met water en 'n dieet van proteïene en suiker (1:4 verhouding). Die aanvaarding van vrugte vir eierlegging deur albei spesies het minimaal verskil. Persentasie vlieë wat papies gevorm het was oor die algemeen hoër vir Mediterreense vrugtevlieg as Natalse vrugtevlieg, behalwe in die geval van appels. Die persentasie van uitgebroeide eiers het gewissel van 54% vir appels vir Natal vlieg, tot 96% vir perskes vir Mediterreense vlieg. Die sigbaarheid van wonde in vrugte het gewissel van hoogs sigbaar op appels tot hoogs onopvallend op sitrus vrugte vir albei spesies. Papievorming is aangeteken vir alle vrugsoorte behalwe pomelos, waar geen papies ontwikkel het. In vrugte waar papies gevorm het, het persentasie papievorming gewissel van 4% in druwe tot 74% in perskes. Die ontwikkeling van vlieë tot volwassendheid was die hoogste op perskes en die laagste op druwe vir albei vrugtevlieg spesies.

Summary

Patterns of host-fruit utilisation by Medfly and Natal fly were investigated in order to determine the role of interspecific competition between these two species. So far, experiments on apple, orange, grape, grapefruit, plum, and peach have been conducted. All experiments were conducted at 25°C, ± 60 RH and 12:12 L:D cycle. Adult flies were fed on protein and sugar (1:4 ratio) and supplied with water. Acceptance of fruit for oviposition by both fly species differed slightly. Percentage pupation was generally higher for Medfly than Natal fly, except when reared on apples. Percentage egg hatch ranged from 54% for apples in Natal fly to 96% for peach in Medfly. The visibility of punctures on fruits ranged from highly visible on apples, to highly inconspicuous on citrus fruits for both species. Pupation was recorded for all fruits except grapefruit, which yielded no pupae. In fruits where pupae were recovered, pupation percentage ranged from 4% in grapes to 74% in peach. Peach yielded more adult flies emerging for both fly species whereas grapes yielded the lowest number of adult flies emerging.

Introduction

Both Medfly and Natal fly are classified as polyphagous species attacking a wide variety of fruits. Medfly has a wider host range than Natal fly, but they share many common hosts. In South Africa, both Medfly and Natal fly are serious pests of citrus, deciduous and subtropical fruits. The two species co-exist in many areas and attack a number of similar commercial and wild hosts. Currently there are two main techniques for fruit fly control in commercial orchards: 1) Bait Application Technique targeting both fly species and 2) Sterile Insect Technique (SIT) targeting Medfly only (In the Western Cape only).

In areas where SIT is implemented, it is feared that Natal fly populations could surge and increase in abundance in commercial hosts shared by the two species. It is therefore important to quantify competition between these two species with respect to commercial hosts they attack. Comparative host-demographic parameter studies must therefore be performed in order to determine the risk of displacement of Medfly by the Natal fly. The objectives for this study were therefore to: 1). Determine the suitability of commercial fruits for the development of the two species and 2). Investigate interspecific interactions of adults on host fruits and larval competition between the two species.

Materials and methods

A colony of Medfly and Natal fly was established from infested fruits collected in home gardens and orchards in various areas of the Western Cape. Emerging flies were reared on bananas to obtain an amount that was needed for experiments on host-specific demographic parameters. Various citrus and deciduous fruit types were used to quantify host-specific pre-adult survival and development of the two fly species. For each fruit

fly species, 5 test fruits were exposed to 100 two-week old flies (1:1 female: male ratio). This was replicated four times. For each fruit type tested, puncture response, duration of the egg stage and percentage egg hatch were determined for Medfly and Natal fly. Ten newly emerged larvae of each species were introduced to each fruit type with positive puncture response and egg development. Ten fruits of each fruit type were used. After larval introduction, fruits were placed on a layer of moist sterilized sand in plastic containers and incubated for 15 weeks or until all fruits were too dry for larval survival. Twenty-five newly emerging male-female pairs were then held individually in cages in order to determine adult history patterns. One fruit type was placed in each cage and changed daily to record puncture response. Mortality was recorded daily until the last female died. Eggs collected from the punctures were counted and placed on a moist filter paper inside a petri dish and incubated at 25°C to assess egg hatch every 12 hours, for 72 hours.

Results and discussion

Puncture response and rearing success of fruit species tested are shown in Table 3.3.10.1. Both Medfly and Natal fly have been reported to infest these fruits under field conditions. It is clear from the above table that different fruit types respond differently to attack by fruit flies. Punctures were highly visible on apple but very difficult to detect on citrus fruits. Grapefruit was attacked by both fly species but there was no pupae recovered for either of the fly species. Apples and grapes did yield to pupae but the counts were fairly low for Medfly. These findings are not consistent with those of Krainacker and Carey (1987) and Carey (1984), where no Medfly pupae were recorded when reared on apple and grape. For Natal fly, on the other hand, the number of pupae recovered from apples were three times higher than that of Medfly. Egg hatch, pupation and adult fly emergence differed between species and between host fruits, as shown in table 3.3.10.2. Generally, percentage egg hatch was higher for Medfly compared to Natal fly. Rate of pupation was higher in peach for both species, but as low as 4% for grape. Adult fly emergence ranged from 1% in grape to 68% in peach. Failure of larvae to develop in some fruits can be attributed to (1) pulp texture, for example in grape, the fruit is too watery for larval development whilst in apples, host tissue is too firm for newly hatched larvae to successfully penetrate and feed on the fruit and (2) presence of compounds within the fruit which might restrict larval growth.

Table 3.3.10.1. Puncture response and rearing success for Medfly and Natal fly reared on different fruit types.

	Scientific name	Common name	Puncture response(-/+)	Puncture rating*	Rearing success**
Medfly	<i>Malus communis</i>	Apple	+	1	P
	<i>Citrus sinensis</i>	Orange	+	3	P
	<i>Prunus persica</i>	Peach	+	3	P
	<i>Vitis vinifera</i>	Grape	+	2	P
	<i>Citrus paradisi</i>	Grapefruit	+	4	N
Natal fly	<i>Malus communis</i>	Apple	+	1	P
	<i>Citrus sinensis</i>	Orange	+	3	P
	<i>Prunus persica</i>	Peach	+	2	P
	<i>Vitis vinifera</i>	Grape	+	2	P
	<i>Citrus paradisi</i>	Grapefruit	+	4	N

*Visual rating after 48 hr exposure; 1= highly conspicuous(Clear or highly noticeable sting marks); 4= inconspicuous(Barely noticeable sting marks on the surface of the fruit).

** Host yielded at least one pupa (P), no pupae recovered (N).

+ At least one puncture present, - no puncture on fruit surface.

Table 3.3.10.2. Percentage egg hatch, pupation and adult emergence for Medfly and Natal fly reared on different fruit types.

	Host	Average egg hatch (%)	Pupation (%)	Adult emergence (%)
Medfly	Apple	96	11	8
	Orange	57	33	27
	Peach	97	74	68
	Grape	85	14	9
	Grapefruit	52	0	0
Natal fly	Apple	54	32	15
	Orange	67	32	22
	Peach	94	64	59
	Grape	61	4	1
	Grapefruit	68	0	0

Conclusion

The fruit fly species do share common hosts but their acceptance differs slightly, with Medfly utilising most fruits better than the Natal fly. Deciduous fruits are preferred by both fly species compared to citrus fruits, but more fruits are still to be tested.

Further objectives (milestones) and work plan

More fruit species will be investigated for host-specific demographic parameters for both fly species. Life history tables will be constructed for both fly species on different host fruits.

References cited

- Krainacker D. A, Carey J. R., and Vargas, R. I. 1987. Effect of larval host on life history traits of the Mediterranean fruit fly, *Ceratitis capitata*. *Oecologia*, 73: 583-590.
- Carey, J. R. 1984. Host-specific demographic studies of the Mediterranean fruit fly *Ceratitis capitata*. *Ecological Entomology*, 9: 261-270

3.3.11. PROGRESS REPORT: Cold tolerance of Natal fruit fly (*Ceratitis rosa*): geographic distribution and overwintering physiology (2009-2011) by Dr J.S. Terblanche (SU)

Opsomming

Die omgewingsfaktore wat die verspreiding en oorvloed van vrugtevlieë beïnvloed, word steeds swak verstaan. Dit het dramatiese implikasies vir area-wye plaagbestuur en ook vir potensiële indringer voorspellings. Hierdie projek beoog om temperatuur, een van die hoof abiotiese faktore, ten opsigte van die verspreiding van *Ceratitis rosa* en *C. capitata* te ondersoek. Inligting van twee hoof proefnemings, wat die termiese faktore van verdraagsaamheid op populasie vlak behels, is ingesluit. Die effek van ouderdom, geslag, voedingstoestand en potensiële interaksies op die kritieke termiese minimum en maksimum in volwasse *C. rosa* word spesifiek ondersoek. As verdere bylaag ondersoek ons die invloed van die tempo van temperatuur verandering op die kritieke termiese grense. Resultate toon 'n betekenisvolle invloed van ouderdom op die kritieke termiese grense, met maksimum termiese verdraagsaamheid by beide hoë en lae temperature in 9-14 dae oue vlieë. Die kritieke termiese maksimum het van 41.6 tot 42.8 (± 0.3)°C gewissel, terwyl die kritieke termiese minimum van 6.4 to 5.6 (± 0.4)°C gewissel het tussen 2 tot 28 dag oud vlieë. Alhoewel mannetjie en wyfie vlieë van sekere ouderdomme (bv. 9 dae oue kritieke termiese minimum) verskille getoon het in termiese verdraagsaamheid, toon geslag 'n klein mate van invloed op termiese verandering verdraagsaamheid. Voedingstoestand blyk 'n betekenisvolle invloed van gevoerde vlieë teenoor ongevoerde vlieë op beide boonste en onderste temperatuur-verdraagsaamheid te hê. Verder het verhitings en afkoeling tempo 'n betekenisvolle effek op termiese toleransie gehad. Die kritieke termiese maksimum was die hoogste teen 0.06°C/min (42.1 \pm 0.2°C) en die laagste teen 0.25°C/min (41.7 \pm 0.2°C). Die kritieke termiese minimum was die laagste teen 0.06°C/min (5.7 \pm 0.4°C) en hoogste teen 0.25°C/min (6.4 \pm 0.4°C). 'n Stadiger temperatuur verandering tempo het gelei tot verbeterde termiese toleransie wat aandui dat akute

fenotipiese plastisiteit by hoë en lae temperature oor die kort tydgrense mag voorkom. Hierdie resultate is noodsaaklik om die populasie dinamika van *C. rosa* onder agri-ekosistiem omstandighede te verstaan. Dit dien ook as belangrike grondige inligting in die verdere ondersoek in *C. rosa* en *C. capitata* vergelykings.

Summary

Environmental factors affecting the geographic distribution and abundance of fruit flies are poorly understood and have dramatic implications for area wide pest management and predictions of invasion potential. This project aims to explore one of the major abiotic factors potentially influencing *Ceratitits rosa* and *C. capitata* geographic distribution, namely temperature. Here we report data from two major experiments investigating the factors influencing thermal tolerance at the population level in *Ceratitits rosa* under semi-natural orchard conditions. Specifically, we investigated the effects of age, gender, feeding status and potential interactions among these factors on adult *C. rosa* critical thermal minimum and critical thermal maximum. In addition, we explored the effects of rate of temperature change on critical thermal limits in *C. rosa*. Results showed significant effects of age on critical thermal limits, with maximum thermotolerance at both high and low temperatures in 9-14 day old flies. Critical thermal maxima varied from 41.6 to 42.8 (± 0.3)°C across 2 to 28 day old flies. Critical thermal minima varied from 6.4 to 5.6 (± 0.4)°C among 2 to 28 day old flies. Gender had little effects on thermotolerance although at certain ages (e.g. day 9 critical thermal minimum) male and female flies had different thermal tolerance. Feeding status had a significant effect with fed flies significantly more tolerant at both upper and lower temperatures. Heating and cooling rates had significant effects on thermal tolerance. Critical thermal maxima were highest at 0.06°C/min (42.1 \pm 0.2°C) and lowest at 0.25°C/min (41.7 \pm 0.2°C). Critical thermal minima were lowest at 0.06°C/min (5.7 \pm 0.4°C) and highest at 0.25°C/min (6.4 \pm 0.4°C). Slower rates of temperature change resulted in improved thermal tolerance suggesting acute phenotypic plasticity at both high and low temperatures over these short timescales. These results are significant to understanding population dynamics under agro-ecosystem conditions in *C. rosa* and provide important baseline data for further comparisons among *C. rosa* and *C. capitata*.

Introduction

The Mediterranean fruit fly *Ceratitits capitata* (Diptera: Tephritidae) (Medfly), which originated from sub-Saharan East Africa (Baliraine et al. 2004), is considered one of the most invasive insect species, having spread and successfully established throughout much of the tropical-temperate parts of the world (Carey 1991; Vera et al. 2002; Malacrida et al. 2007). Medfly invasion and establishment success is probably facilitated by its highly polyphagous life-history (Malacrida et al. 2007), short generation times (Duyck et al. 2002; Grout & Stoltz 2007), and possibly rapid evolutionary adaptation (Huey et al. 2005; Malacrida et al. 2007; Leibhold & Tobin 2008). However, a major factor which contributes to the successful establishment after introduction of an insect species to a novel environment is its basal and inducible physiological tolerance to environmental stress (e.g. to temperature and water stress) (Richardson & Pysek, 2006; Huey et al. 2005; Chown et al. 2007).

Medfly is not the only tephritid with major invasion potential, and indeed, several other fruit flies (e.g. *Bactrocera*, *Dacus* spp.) have been introduced and successfully established in various locations around the world despite strict quarantine measures. In particular, the Natal fruit fly *Ceratitits rosa*, which is also a highly polyphagous congeneric species with a broad African distribution (De Meyer et al. 2008), has shown alarming invasion potential. On Reunion Island, *C. rosa* was able to rapidly outcompete and competitively exclude Medfly (White et al. 2001; Duyck et al. 2004) possibly owing to niche segregation (Duyck et al. 2006). At present the factors limiting geographic distribution and abundance of *Ceratitits* spp. in the Western Cape and indeed, South Africa as a whole, are poorly understood and represents a potentially major problem to fruitfly control and management strategies (Manrakhan & Addison 2007). However, the potential for establishing permanent populations (i.e. invasion potential) is also not well understood in these flies (De Meyer et al. 2008), hence several existing research projects to understand competition and behaviour already funded by the DFPT & CRI.

Variation in temperature tolerance might be a key mechanism explaining the differences in *Ceratitits* species geographic distribution and their potential invasiveness at global (Duyck et al. 2006; Gutierrez et al. 2008) and regional scales (De Meyer et al. 2008). Similarly, in the fruit fly *Bactrocera dorsalis*, predictions of geographic range under current and future climate scenarios suggests low temperature is a principal limiting factor in the U.S.A. (Stephens et al. 2007). By contrast, drought stress seems to be an important limiting factor in CLIMEX models, based on the Mediterranean distribution data, of global potential geographic distribution of *C. capitata* (Vera et al. 2002). Thus, different approaches to predicting distribution suggest different underlying mechanisms of the species in question. Understanding *Ceratitits* geographic distribution

and the potential limiting factors in the Western Cape is, however, currently limited by a lack of basic temperature tolerance information, particularly for the two key species *C. rosa* and *C. capitata*.

Recent studies suggest physiological differences between *C. rosa* and *C. capitata* from Reunion Island (Duyck et al. 2006) which might explain the species' distinct geographic distributions in southern Africa (De Meyer et al. 2008). On the basis of experiments undertaken for Reunion Island strains, Duyck et al. (2006) argued that *C. capitata* and *C. rosa* can be separated both ecologically and geographically. *Ceratitis rosa* succeeds in cooler (22-23°C), wetter (3000-3500mm rainfall) environments while *C. capitata* tend to inhabit warmer (24-26°C) and drier (0-1000 mm rainfall) regions (Duyck et al. 2006). Similar observations have been made for these species in southern Africa, in which *C. rosa*'s natural distribution is mainly limited to eastern, coastal regions with high annual rainfall, while *C. capitata* is generally more widespread (Manrakhan and Addison, 2007; and see De Meyer et al. 2008).

The studies by Duyck et al. (2006) and De Meyer et al. (2008) have provided some important insights into aspects of *Ceratitis* biology which might potentially limit the geographic distribution of *C. rosa* and *C. capitata* in the Western Cape. Modelling work predicting potential *Ceratitis* distributions in the Western Cape is presently being undertaken (CRI, M. de Villiers), and has been undertaken previously for *C. capitata* at a coarse global scale (Vera et al. 2002). However, several factors limit the application of the available data to predict the fine-scale distribution of these flies in South Africa of which four factors are perhaps most significant. First, comparable data for local populations is not available and, therefore, the degree of region-specific differences among populations, which could have been altered through local climatic adaptation in distinct ways (Hoffmann & Parsons 1997; Hoffmann & Willi 2008) is presently unknown. Such a process frequently occurs in traits of temperature tolerance in other Dipteran species (e.g. *Drosophila*, *Glossina* e.g. Hoffmann et al. 2005; Terblanche et al. 2006; reviewed in Hoffmann et al. 2003; Chown and Nicolson 2004) but its extent, and the rate at which such changes might accumulate, is unknown for Medfly and Natal fly from any region.

Second, overwintering strategy, a major potential source of re-introduction of *Ceratitis* species is not known for local South African populations. Regardless, similar data are available from other parts of the world. For example, in the Judean Hills of Israel *C. capitata* is unable to overwinter (Israely et al. 2004), and instead, there is a process of re-invasion each spring from adjacent agricultural regions (Israely et al. 2005). This type of data has direct pest management implications since it assists by knowing when and where to focus control efforts.

Third, Duyck et al. (2006) consider temperature tolerance from early life-stages as the temperature-dependent growth rate. However, it may be important to consider survival and limits to behavioural activity (e.g. low temperature flight activity thresholds, limits to mating), which play a role at different, often less severe, temperatures and may have subtle but distinct differences for biogeography predictions (Terblanche et al. 2006; Chown and Terblanche 2007). Moreover, temperature tolerance from the adult life-stage, which has not been given much attention to date, should also be considered since dramatic differences can occur between life-stages of various insect species (Bowler & Terblanche 2008). Knowledge of which life-stage is the most temperature-limited under natural conditions, an important component of any population dynamics or biogeography modelling, is presently unknown for *C. rosa* and *C. capitata* owing to variation in methodological approaches confounding comparisons among studies (Terblanche et al. 2008).

Finally, no studies of *Ceratitis* spp. to date have considered physiological responses to temperature treatment, a major mechanism used by insects to cope with temperature variation at daily (Meats 1976; Kelty and Lee 1999/2001; Overgaard & Sorensen 2008) and seasonal timescales (Terblanche et al. 2006; Chown and Terblanche 2007), but significantly, also upon introduction into new environments (Chown et al. 2007; Kristensen et al. 2008). For flies, much work has been undertaken on the family Drosophilidae, and most studies have focused extensively on *Drosophila melanogaster* and *D. simulans* (e.g. Hoffmann & Watson 1993; Jensen et al. 2007). By contrast, little or no work has been published on rapid heat- and cold-responses in Tephritidae for *Ceratitis capitata* or *C. rosa* (though see early work on *Dacus* by Meats (1973)). Furthermore, temperature responses can affect post-harvest control techniques, especially if fruit is temperature treated (cold sterilization) during distribution, as is presently the case for *Ceratitis*-infested fruits. Another aspect of significant concern is how global climate change might alter the rate and impact of invasive pest insects (Chown et al. 2007; see also Helmuth et al. 2005). Without some insight into the mechanisms underlying these species' thermal biology and their ability to compensate under changing weather conditions we are simply unable to make informed decisions for strategy and intervention plans. In consequence, several commercially important aspects of *Ceratitis* thermal biology require urgent investigation.

The results of this study will therefore provide an empirical framework for understanding the factors limiting geographic distribution of *C. rosa* and *C. capitata*, laboratory handling for SIR programmes, post-harvest

control and sterilization techniques, and will directly aid in the prediction of the potential invasiveness of *C. rosa* and *C. capitata* at various timescales. This information is regarded as critical for the effective integrated management of fruit fly species in the Western Cape.

The general aims of this project are to understand the thermal physiology potentially limiting the geographic distribution of *Ceratitis rosa* relative to *C. capitata* in the Western Cape, South Africa.

The key objectives outlined in the application for funding of this project were to determine:

- 1) baseline cold tolerance of Natal fruit fly (*C. rosa*) relative to Medfly (*Ceratitis capitata*);
- 2) if *C. rosa* or *C. capitata* show inducible cold tolerance at daily and seasonal time-scales;
- 3) overwintering strategy of *C. rosa*;
- 4) if thermal history influences cold tolerance and geographic distribution of *C. rosa*;
- 5) if *C. capitata* and *C. rosa* populations have permanently or reversibly adapted to local climatic conditions.

To date we have undertaken a systematic exploration of the population level factors influencing cold and heat tolerance in adult *Ceratitis rosa* (addressing key objectives 1,2,4 and 5 in *C. rosa*). Due to difficulties in obtaining sufficient numbers of *C. capitata* that do not have the heat-shock genetic modification for gender sorting, progress exploring these factors has been slower. However, we are now obtaining individuals from CRI and beginning to make progress but without sufficient data yet to analyse statistically.

Materials and methods

Experiment 1: Effects of age, gender and feeding status on adult *Ceratitis rosa* thermotolerance

In order to assess the role of age, gender and feeding status as potential factors influencing thermal tolerance within species, the first phase of this research project was to undertake experiments to assess these factors at the population level. Fruit flies (*C. rosa*) were reared in plastic cages in the laboratory on a 12:12 L:D photoperiod. Flies were provided with water, sugar, protein and were supplied with bananas for oviposition. The experiment employed a completely randomized design replicated on a minimum of 10 individual insects per age, gender and feeding status category. Individual *C. rosa* were placed in a double jacketed chamber or organ pipes connected to a programmable water bath (Grant GP200-R4, Grant Investments, UK) (as in e.g. Terblanche et al. 2006) filled with 1:1 water: propylene glycol to allow for subzero temperatures. A type K thermocouple connected to a Fluke 54 series 2 (Fluke Cooperation, China) digital thermometer (accuracy: 0.05°C) was inserted into the control chamber to record chamber temperature. For small insects (<1 g), body temperature is always in equilibrium with environmental temperature (Terblanche et al. 2007), in this case chamber temperature. Both critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) experiments started at a setpoint temperature of 25°C from which temperature increased (for CT_{max}) or decreased (for CT_{min}) at a rate of 0.25°C/minute until all the insects reached their CT_{max}/CT_{min}. Critical thermal maximum and CT_{min} was defined as the temperature at which each individual insect loses muscle function consequently losing the ability to respond to stimuli. This was done on different ages of *C. rosa* (2, 5, 9, 14, and 28 days old) and on both genders of the adult flies. The temperature at which each individual lost muscle function was recorded as CT_{min}/CT_{max} for that individual. Therefore, both upper and lower critical thermal limits for both genders and different ages were analysed using factorial ANOVA in Statistica 8 (Statsoft, USA) and Tukey-Kramer's post-hoc tests were used to identify statistically homogenous groups.

To determine the effect of feeding status on thermotolerance of *C. rosa* CT_{max} and CT_{min} experiments were performed again using programmable water baths, starting from 25°C increasing/decreasing temperature at a rate of 0.25°C/min (as in Terblanche et al. 2006). This experiment was conducted using 2 and 9 day old *C. rosa* and on both genders on starved and fed flies. Starved flies were deprived of any food for 48 hours before measuring thermotolerance. Fed flies were supplied with food (sugar water) *ad libitum* during their lifetime. However, before measuring thermotolerance, these flies were temporarily deprived of food for 6 hours before reintroducing the food. Flies were then closely monitored and recently fed flies were withdrawn from cages for the thermotolerance experiments. The experimental treatments were undertaken in random order. Individual flies were loaded into the organ pipes described above and CT_{max} and CT_{min} of each individual was recorded. Critical thermal limits for both genders and different ages were analysed using factorial ANOVA in Statistica Software.

Experiment 2: Effect of heating/cooling rate on adult *Ceratitis rosa* thermotolerance

To assess if rate of temperature change significantly affected thermotolerance in *C. rosa*, CT_{max} and CT_{min} were assessed using the methods described above but varying the rate of temperature change. Since

gender showed little influence on *C. rosa* thermotolerance (see Results), this experiment was performed using 2 day old fruit flies of mixed genders. Flies (n = 10) were loaded into the organ pipes, and after 20 minutes to reach equilibration, temperature was increased (for CT_{max}) or decreased (for CT_{min}) at three different rates (0.06, 0.12, and 0.25°C/minute) starting from 25°C. The CT_{min} and CT_{max} for each individual insect were recorded separately as described previously and this was repeated twice to yield sample sizes of n = 20 per treatment. Effects of rate of temperature change on critical thermal limits were analysed using factorial ANOVA in Statistica.

Results and discussion

Experiment 1: Effects of age, gender and feeding status on adult *Ceratitis rosa* thermotolerance

Critical thermal maxima significantly increased with age up until 14 days for both males and females of *C. rosa* after which it declines at 28 days of age (Figure 3.3.11.1; Table 3.3.11.1). There were no gender or age x gender interaction effects on CT_{max} of *C. rosa*. For CT_{min}, age was marginally non-significant, while gender was not significant. However, the interaction between gender and age was highly significant (Table 3.3.11.1). This interaction between gender and age was largely a consequence of the 9 day old flies which showed distinct differences in thermal tolerance among males and females. There were no significant gender differences on any other days of age in *C. rosa* (Figure 3.3.11.2). Why this is the case is not clear. However, one possibility is that age-related changes in thermal tolerance are a result of changes in body condition or body size. Thus, we undertook an Analysis of Covariance correcting for fresh body mass which still showed a significant age x gender interaction for CT_{min} (Table 3.3.11.2). This suggests that the variation in thermotolerance is not simply a consequence of changes in body size during adult development.

Both CT_{max} and CT_{min} were significantly enhanced with feeding but there was no significant age x feeding interactions on either CT_{min} or CT_{max} (Figure 3.3.11.2; Table 3.3.11.3). In these experiments age only had an effect on CT_{max} but not CT_{min}. These results suggest an important role for dietary nutrition and food availability in determining thermal tolerance. Clearly flies which are recently fed can withstand greater temperature extremes (by approximately 1-2°C).

Experiment 2: Effect of heating/cooling rate on adult *Ceratitis rosa* thermotolerance

Ramping rates significantly affected CT_{max} in *C. rosa* ($F_{(2, 57)} = 8.0$, $p < 0.0001$). Slow heating rates resulted in significantly higher CT_{max} for the fruit flies and conversely, faster heating rates resulted in a lower CT_{max} (Figure 3.3.11.3). These results suggest that more time during heating (i.e. a slower heating rate) gives the flies an opportunity to develop some heat protection, and therefore suggests that *C. rosa* has short-term phenotypic plasticity of high temperature tolerance. This is similar to rapid heat hardening or heat shock responses observed in *Drosophila*.

The rate of cooling also significantly affected CT_{min} ($F_{(2, 57)} = 13.22$, $p < 0.0001$). Slow cooling rates significantly lowered CT_{min} for *C. rosa* and the reverse was also true; faster cooling rates resulted in higher CT_{min} (Figure 3.3.11.3). This suggests that longer duration of exposure (i.e. lower rate of temperature change) allows the flies to reach lower chill coma temperatures. Consequently, this suggests that *C. rosa* has the potential to rapidly cold harden, a form of phenotypic plasticity previously demonstrated in a range of insect species.

These results are significant to understanding population dynamics under agro-ecosystem conditions in *C. rosa* and provide important baseline data for further comparisons among *C. rosa* and *C. capitata*.

Table 3.3.11.1. Summary ANOVA showing effect of age and gender on *Ceratitis rosa* thermal tolerance.

Effect	SS	F-value	d.f.	p-value
CTMax				
Age	7.984	4.7	4	<0.001
Gender	0.008	0.0	1	0.8901
Age x Gender	2.404	0.6	4	0.2321
CTMin				
Age	1.419	2.30	4	0.0646
Gender	0.449	2.91	1	0.0913
Age x Gender	3.853	6.25	4	<0.001

Table 3.3.11.2. Summary ANCOVA showing effect of age and gender on *Ceratitis rosa* CT_{min}.

Effect	SS	F-value	d.f.	p-value
Age	1.4706	2.425	4	0.053846
Gender	0.5646	3.724	1	0.056823
Age*Gender	3.7547	6.191	4	0.000193

Table 3.3.11.3. Summary ANOVA showing effect of age and feeding status on *Ceratitis rosa* thermal tolerance.

Effect	SS	F-value	d.f.	p-value
CTMax				
Age	8.128	50.26	1	<0.0001
Feeding status	5.565	34.41	1	<0.0001
Age x feeding status	0.010	0.06	1	0.8030
CTMin				
Age	0.242	1.42	1	0.2371
Feeding status	4.232	24.84	1	<0.0001
Age x feeding status	0.145	0.85	1	0.3600

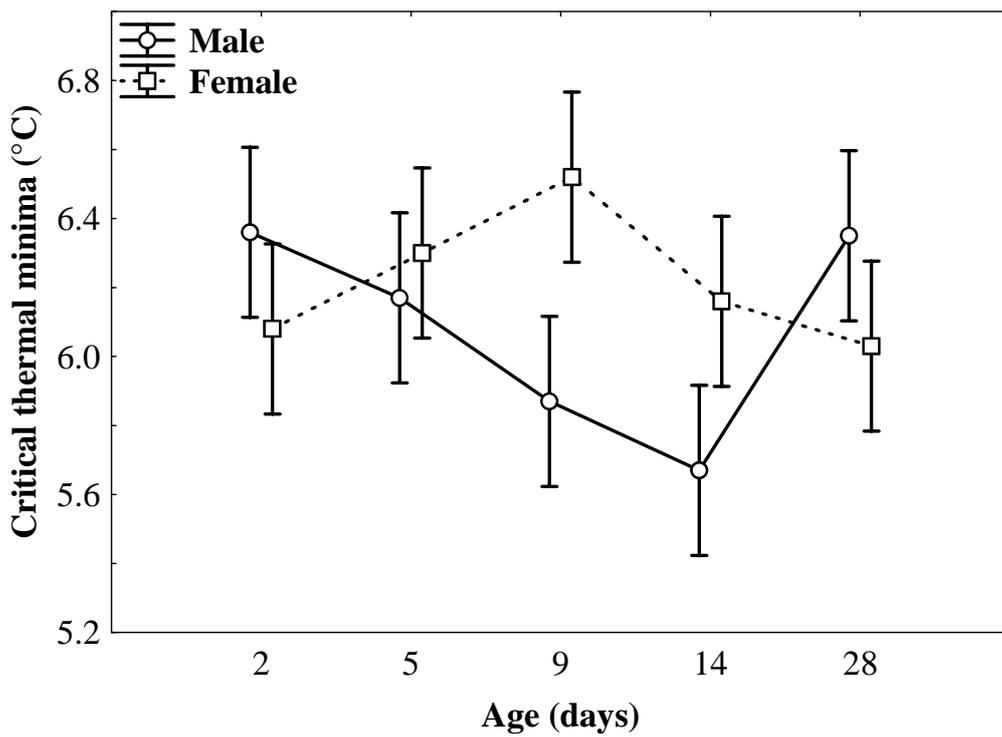
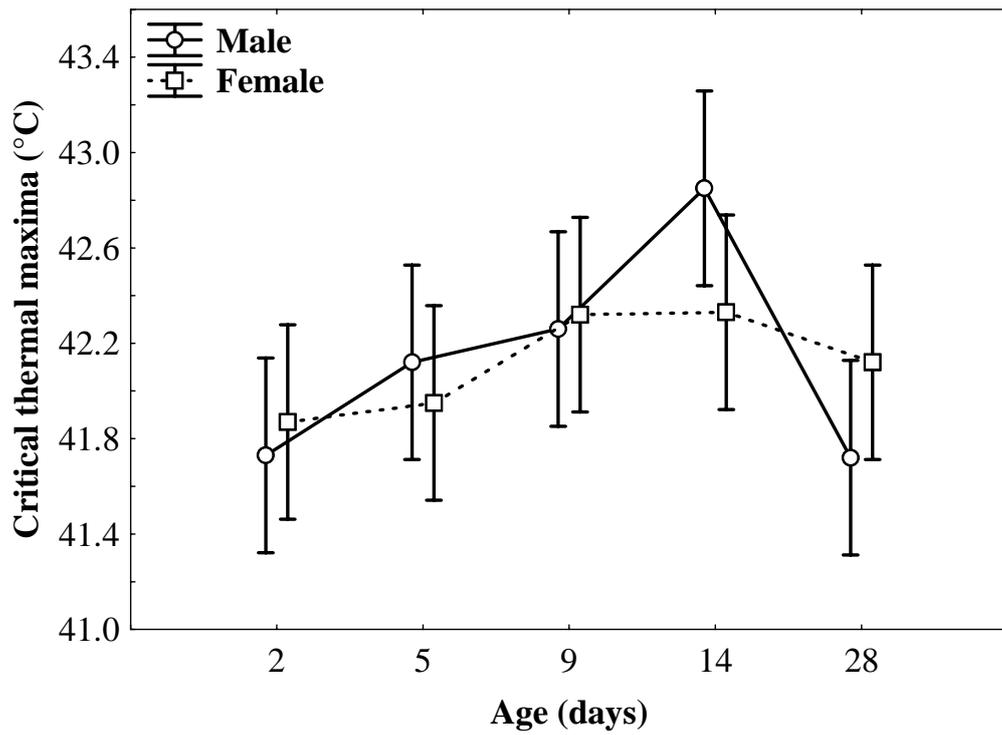


Figure 3.3.11.1. Effect of age and gender on *Ceratitis rosa* (A) CT_{max} and (B) CT_{min} (Error bars represent \pm 95% CLs).

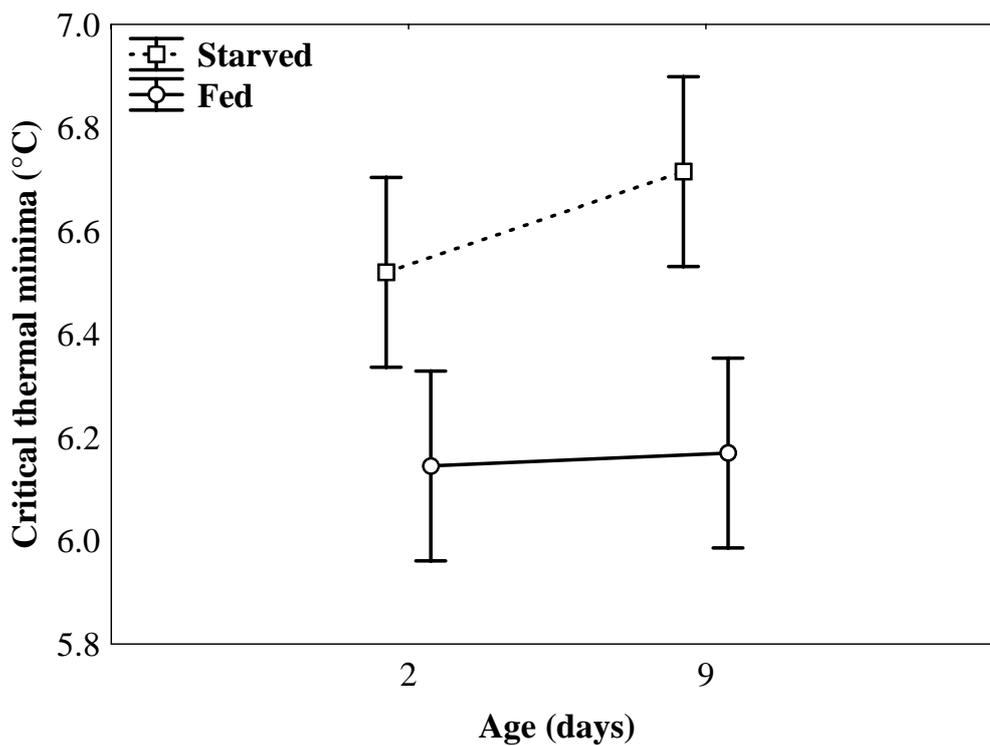
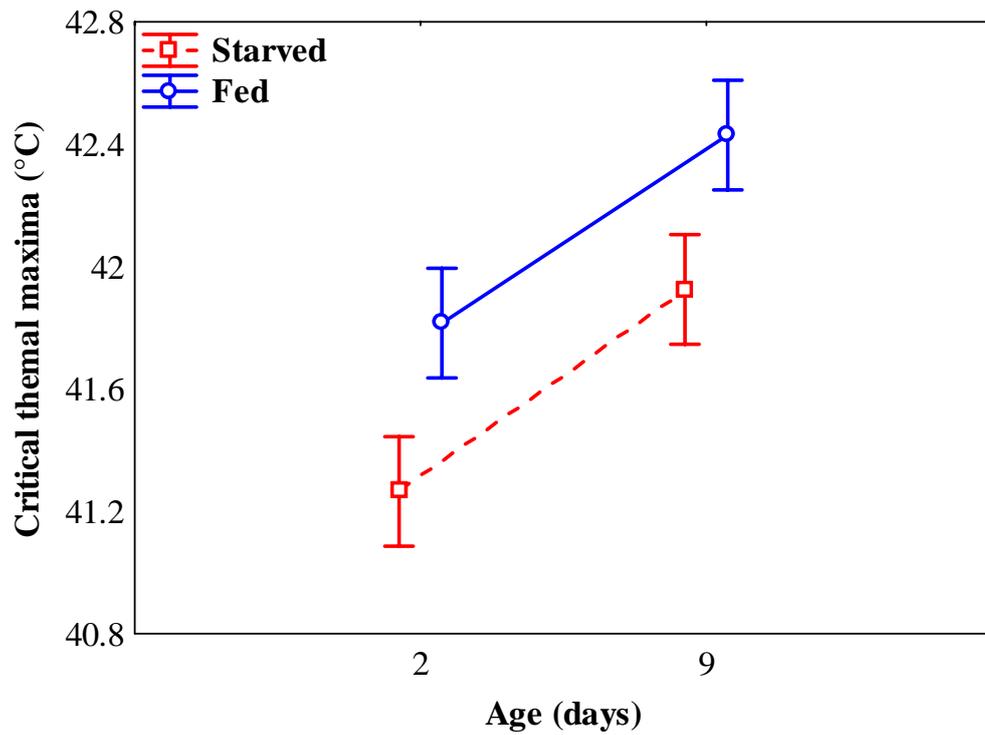


Figure 3.3.11.2. Effect of age and feeding status on *Ceratitis rosa* (A) CT_{max} and (B) CT_{min}. (Error bars represent ± 95% CLs).

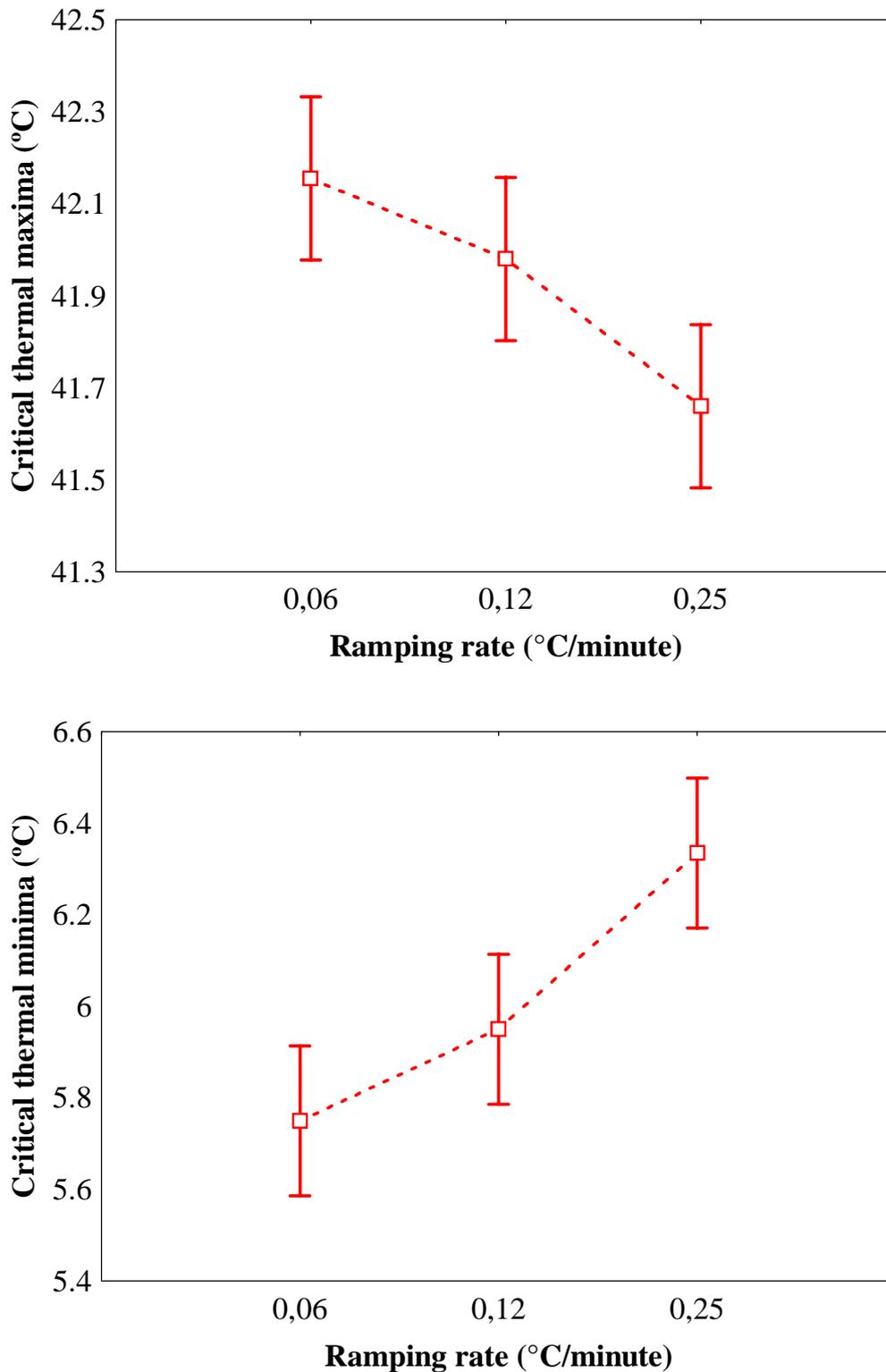


Figure 3.3.11.3. Effect of ramping rate on *C. rosa* (A) CT_{max} and (B) CT_{min}. (Error bars represent ± 95% CLs).

Conclusion

Clearly there is considerable variation in thermotolerance within adult *C. rosa*. This suggests further comparisons among species or populations from different geographic regions need to account for factors such as age and feeding status, while gender itself is not a major factor especially if early age flies are used. Further experiments on seasonal and daily adjustments in thermal tolerance in response to various temperature treatments will aid understanding phenotypic plasticity of thermal tolerance. These results are

significant for understanding population dynamics under agro-ecosystem conditions in *C. rosa* and provide important baseline data for further comparisons among *C. rosa* and *C. capitata*.

Further objectives & milestones

- 1) Aug-Oct 2009. Assay cold hardening and acclimation responses of *C. rosa*.
- 2) Nov-Apr 2010. Assay cold tolerance using lab-reared colony of *C. capitata*. Assay time-temperature interactions. Write up and submit manuscript 1.
- 3) May-Jul 2010. Assay field populations of *C. capitata* and *C. rosa*.
- 4) Aug-Oct 2010. Assay cold hardening and acclimation responses of *C. capitata*.
- 5) Nov-Jul 2011. Assay the influence of diet and developmental rearing conditions on thermal tolerance and hardening ability of *C. rosa* and *C. capitata*. Write up and submit manuscript 2.
- 6) May-Jul 2011. Write-up and prepare oral seminar for international conference.
- 7) Aug-Oct 2011. Write-up and submit Ph.D thesis.

Technology transfer

The PhD student on this project Mr C. Nyamukondiwa will be presenting results of this work at the ESSA meeting in July 2009 (Stellenbosch, South Africa).

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3.3.12 **PROGRESS REPORT: Investigation of Entomopathogenic fungi for control of false codling moth and fruit flies**

Experiment 930 (Feb 2008-April 2009): T. A. Goble; J. F. Dames and M. P Hill (Rhodes University)

Opsomming

’n Opname om die voorkoms van entomopatogeniese swamme te bepaal is onderneem. Grondmonsters van binne en buite sitrusboorde op beide konvensioneel en organies bestuurde plase in die Oos-Kaap van Suid Afrika is ondersoek. ’n Aangepaste metode is gebruik om grondmonsters met larwes van die sleutel sitrusplae, valskodlingmot ((*Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)) en Mediterreense vrugtevlieg

((*Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)) asook met die standard lokaas-insek, die wasmot ((*Galleria mellonella* (Linnaeus) Lepidoptera: Pyralidae)), te toets. Twee en sestig potensieel waardevolle entomopatogeniese swam isolate wat aan 4 genusse behoort, is van 288 grondmonsters ingesamel (voorkoms frekwensie van 21.53%). Die entomopatogeniese swam spesie wat die mees algemeen voorgekom het was *Beauveria bassiana* (Balsamo) Vuillemin (15.63%), gevolg deur *Metarhizium anisopliae* var. *anisopliae* (Metschnikoff) Sorokin (3.82%). *Lucanicillium psalliotae* (1.39%) en die spesies, *Metarhizium flavoviride* (0.35%) en *Conidiobolus coronatus* (0.35%) was slegs eenmalig vanuit die grondmonsters geïsoleer. Die insek wat die mees suksesvol entomopatogeniese swamme geïsoleer het was die standard lokaasinsek, *G. mellonella*, met 45 isolate, gevolg deur *C. capitata* met 11 en *T. leucotreta*, met 6 isolate. *C. capitata* was die enigste lokassinsek waarvan die swam spesies, *C. coronatus* en *L. psalliotae*, geïsoleer is. Die genus *Metarhizium* is glad nie deur *C. capitata* geïsoleer nie. Dit is bevind dat die grondmonsters wat van buite die sitrus boorde versamel is, 'n betekenisvol hoër voorkoms van entomopatogeniese swamme as die van binne die boorde, bevat het. Dit is die geval met beide die konvensionele en organiese plase. Daar is geen betekenisvolle verskil in die voorkoms van swamisolate tussen die tweekoppes boerdery nie.

Summary

A survey of the occurrence of entomopathogenic (EP) fungi was undertaken on soils from citrus orchards and refugia on conventionally and organically managed farms in the Eastern Cape Province in South Africa. An adapted method for baiting soil samples with the key citrus pests, false codling moth ((*Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)) and Mediterranean fruit fly ((*Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)) larvae, as well as with the standard bait insect, the wax moth ((*Galleria mellonella* (Linnaeus) Lepidoptera: Pyralidae)), was implemented. Sixty-two potentially useful EP fungal isolates belonging to 4 genera were collected from 288 soil samples, an occurrence frequency of 21.53%. The most frequently isolated EP fungal species was *Beauveria bassiana* (Balsamo) Vuillemin (15.63%), followed by *Metarhizium anisopliae* var. *anisopliae* (Metschnikoff) Sorokin (3.82%). *Lucanicillium psalliotae* (1.39%) and the species, *Metarhizium flavoviride* (0.35%) and *Conidiobolus coronatus* (0.35%) were only isolated once from soil samples. The standard bait insect, *G. mellonella*, was the most effective insect used to isolate EPF species, with a total of 45 isolates obtained, followed by *C. capitata* with 11 isolates recovered and *T. leucotreta*, with 6 isolates recovered. Interestingly, *C. capitata* was the only bait insect to isolate the fungal species, *C. coronatus* and *L. psalliotae*. The genus *Metarhizium* was never isolated from *C. capitata*. There was a significantly higher occurrence of EP fungi in soil samples taken from refugia compared to cultivated orchards of both organically and conventionally managed farms. No significant differences were observed in the recovery of fungal isolates when both farming systems were compared.

Introduction

The South African citrus industry is the second largest exporter of citrus worldwide after Spain (FAO: Citrus Fruit Annual Statistics 2006). Annually, up to 89 million cartons of citrus fruit are exported, generating an annual income of approximately 5.1 billion in foreign currency for South Africa (Edmonds, pers comm). As a result of the European Union's strict phytosanitary regulations, and drive to reduce harmful chemical pesticides (Mather & Greenberg, 2003) and to a lesser extent the recent consumer interest in organically produced foods (Zehnder *et al*, 2007), South African citrus growers exporting fruit to the overseas market have been forced to reconsider their pest control practices and in particular the use of chemical pesticides. Consequently, sustainable agriculture may rely increasingly on alternatives to conventional chemical insecticides for pest management that are environmentally friendly and reduce the amount of human contact with pesticides (Butt *et al*, 2001; Lacey & Shapiro-Ilan, 2003).

In South Africa, the false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), are regarded as important pests on citrus, mainly due to their broad host ranges and phytosanitary significance (Moore, 2002; Peña *et al*, 2002; Kirkman & Moore, 2007). Large consignments of exportable fruit may be rejected by phytosanitary inspectors due to the presence of just one larva of either of these pest species (Moore, 2002). False codling moth causes losses in excess of ZAR100 million per annum mainly due to pre-harvest fruit drop and post-harvest decay of fruit caused by the internal feeding of the larvae (Kirkman & Moore, 2007). Similarly, 'fruit flies' have been considered the most destructive fruit pests on citrus in South Africa and the global fruit industry (Ware, 2003). The biology of these insect pest species includes them dropping from their host plants to pupate in the soil or leaf litter below citrus trees. The most common method used to control fruit flies in South Africa is the attract and kill technique, where by a protein attractant is mixed with a toxicant (Ware, 2003). In some cropping systems in other areas of Africa however, the control of fruit flies has relied on the use of broad-spectrum chemical insecticides such as diazinon mixed into soils beneath host trees to kill fruit fly larvae and puparia (Dimbi *et al*, 2003). As

alternatives to chemical control, genetic methods (sterile insect techniques), natural enemies, microbial pesticides (viruses and fungi), botanical insecticides, insect growth regulators and semiochemicals (mating disruption and attract & kill), have been evaluated as facets of an Integrated Pest Management (IPM) strategy for both pests (Konstantopoulou & Mazomenos, 2005; Stibick, 2006).

The use of microbial control agents specifically EP fungi, have been investigated for the control of a wide range of orchard pests (Cross *et al*, 1999; Puterka, 1999; Lacey & Shapiro-Ilan, 2003; Alves *et al*, 2005; Castrillo *et al*, 2005; Dolinski & Lacey, 2007; Lacey & Shapiro-Ilan, 2008). Soil-inhabiting EP fungi are an important and widespread component of most terrestrial ecosystems and play a key role in regulating some soil-dwelling insect populations (Meyling & Eilenberg, 2007; Quesada-Moraga *et al*, 2007). In agroecosystems, they deliver important ecological services to cropping systems, such as biological control of insect pests (Meyling & Eilenberg, 2006). An improved understanding of the ecology of indigenous populations of these beneficial organisms is an important prerequisite for the evaluation of their contributions to pest control and in predicting the impact of agricultural practices on their populations (Meyling & Eilenberg, 2007). EP fungi which belong to the class Hyphomycete; species such as *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) are known to attack FCM and fruit fly species (Dimbi *et al*, 2003; Ekesi *et al*, 2003; Lacey & Shapiro-Ilan, 2003; Begemann, 2008). Previous researchers have shown that both fecundity and fertility in fruit flies can be reduced by EP fungi (Castillo *et al*, 2000; Ekesi *et al*, 2002; Dimbi *et al*, 2003; Dimbi *et al*, 2004). Ekesi *et al* (2002) reported that EP fungi were able to induce high mortality in puparia of *C. capitata* and significantly reduce adult emergence. Dimbi *et al* (2003), reported a mortality range from 7- 100% in *C. capitata* to twelve isolates of *M. anisopliae* at 4 days post-inoculation. In contrast very little work has been undertaken looking at the effects of EP fungi on FCM. In a study by Begemann (2008), various concentrations of *B. bassiana* in suspensions and as dry preparations were tested against the late instar larvae of FCM. This work revealed that *B. bassiana* spores were able to infect and kill FCM larvae and showed great potential for its control. Currently three preparations of *B. bassiana* are registered and available in South Africa (Begemann, 2008) for the control of plant pests such as weevils, aphids and nematodes.

Information on the natural history and ecology of EP fungi outside of their hosts will assist in isolate selection and development of control strategies for their utilization. Castrillo *et al* (2005) mentions that it is important that investigative collection studies continue and that microbial control efforts not be limited to currently available mycoinsecticides and their inundative releases. Meyling & Eilenberg (2007) discuss the idea of conservation biological control (CBC): 'the manipulation of farming management practices to enhance the living conditions of specific natural enemies of a pest, with the primary focus being suppression of that pest species.' Thus the idea serves to conserve what natural enemies/microbial populations are already there, rather than inundating the area with maladapted, non-indigenous strains or species.

The aims of the present study were to recover and identify indigenous isolates of EP fungi in the Eastern Cape Province in South Africa which could be potentially used for control of false codling moth and fruit flies in citrus production areas. This study also served to compare both the distribution and abundance of EP fungi in conventionally versus organically farmed citrus soils and in cultivated orchards versus orchard margin (refugia) areas.

Materials and methods

Insect Cultures

Thaumatotibia leucotreta (Lepidoptera: Tortricidae) larvae were obtained as late instar larvae from a continuous laboratory culture held at River Bioscience, Addo. *Ceratitis capitata* larvae were obtained as eggs from a continuous laboratory culture held at Citrus Research International, Nelspruit, and maintained on an appropriate diet until late instar larvae were needed. *Galleria mellonella*, known as the greater wax moth, is a standard insect whose larvae are used for the isolation of insect-pathogenic fungi, predominantly because these insects are highly susceptible to infection by EP fungi. *Galleria mellonella* was also included in the study and were obtained from absconded bee hives held at Grahamstown (33° 23' 54"S; 26° 25' 41"E) and were cultured on an appropriate diet (Meyling, 2007) and maintained in constant darkness at 24°C.

Soil sampling

A total of 288 soil samples were collected from three conventional citrus farms and three organic citrus farms (as defined by the EU council regulation 2092/91) in the Eastern Cape Province, South Africa (Figure 3.3.11.1). The locations of the sampled soils were recorded using global positioning system (GPS) equipment (Garmin: E-Trex). Conventional citrus sites included, Arundel farm near Addo (33° 30' 57"S; 25° 39' 11"E), Mosslands farm near Grahamstown (33° 23' 54"S; 26° 25' 41"E) and J & B Citrus near Cookhouse (32° 45' 56"S; 25° 45' 46"E). The organic sites included: Rosedale farm near Addo (33° 32' 21"S; 25° 41' 39"E); Hippo

Pools farm near Kirkwood (33° 24' 42"S; 25° 24' 34"E) and Olifantskop near Addo (33° 37' 14"S; 25° 40' 49"E) (Figure 3.3.12.1).

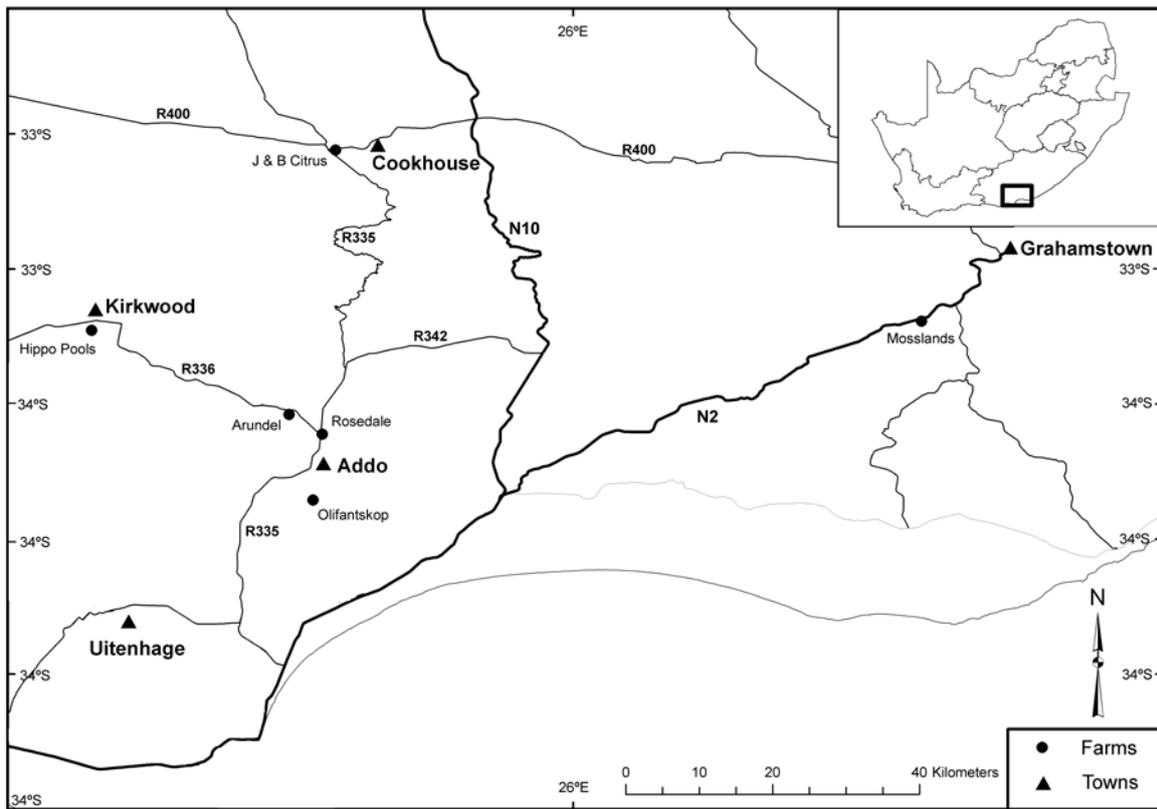


Figure 3.3.12.1. Map of a portion of the Eastern Cape Province with the locations of the six farms, marked with a bullet (•), sampled in this study.

At each of the 6 farms, three orchards were randomly selected. A total of 12 soil samples were collected per orchard, along two intersecting transects, until the end of the orchard was reached (Figure 3.3.12.2). Additionally, indigenous vegetation within 1-2 km of orchard sites was randomly sampled at 12 points, which were at least 18 m apart; hereafter referred to as refugia. Soil samples were collected with the use of a cylindrical soil auger (7 cm x 14 cm) volume 538 cm³ to a depth of 15 cm. Surface litter was removed and samples were placed in labeled, clear plastic bags (31x20 cm). Orchard soil samples were collected under tree canopies at the start of autumn (March) 2008, stored at 4°C and baited individually 2-6 months after sampling. All orchards had trees of similar sizes and the heights of the tree canopies were consistent.

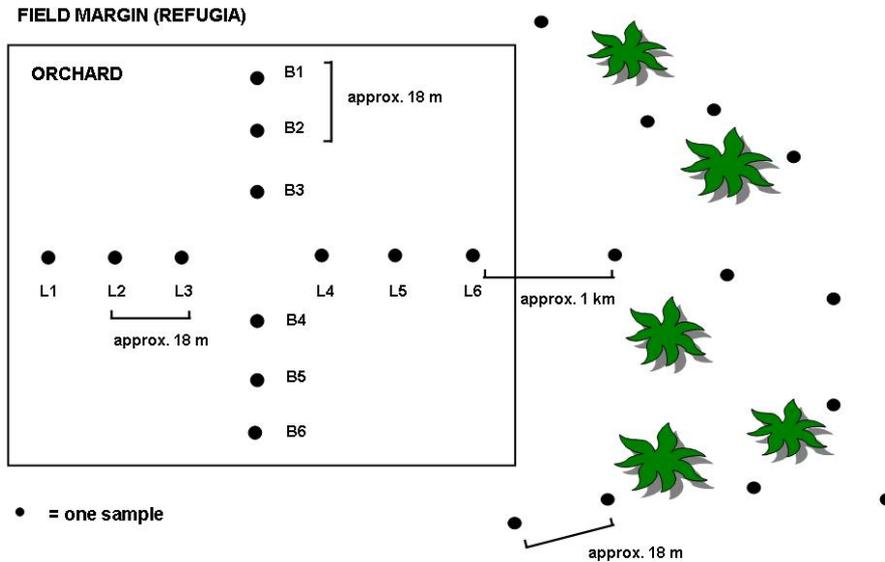


Figure 3.3.12.2. Sampling points in each orchard and the adjacent field margin (refugia).

Baiting procedures

In the laboratory, each soil sample was thoroughly mixed and then sieved through a metal sieve with a mesh size of 4 mm. Soil samples were divided into three 200 ml portions and transferred to 400 ml transparent plastic pots which were then sealed with perforated lids. If soil samples were too dry they were moistened with distilled water to maintain humidity during baiting. EP fungi were isolated from soil samples using variations of the 'Galleria bait method' (Zimmermann, 1986, Chandler *et al*, 1997; Ali-Shtayeh *et al*, 2002; Klingen *et al*, 2002; Keller *et al*, 2003; Meyling & Eilenberg, 2006). Three separate experiments were run to test *T. leucotreta*, *C. capitata* and *G. mellonella* larvae individually as bait insects. The *Galleria* bait method was conducted using 10 late instar larvae which were placed on the surface of each soil sample container. *Galleria mellonella* larvae were heat-treated according to Meyling (2007) to prevent excessive webbing in the soil. The same protocol was undertaken using 10 *T. leucotreta* and 10 *C. capitata* larvae per soil sample; however there was no heat-treatment applied to these larval species because excessive webbing in the soil was not experienced with these insects. All soil samples were incubated at 22°C in the dark. Containers were inverted daily for the first week to make bait insects penetrate the soil as much as possible. After initial baiting, samples were checked for the presence of dead larvae every 3-4 days for 3 weeks. All dead larvae were surface sterilised with 70% ethanol prior to incubation in a moisture chamber (Petri dish with moistened filter paper) to prevent opportunistic external saprophytic fungi from growing on the dead larval cadavers. Sporulating larvae and/or pupae were placed on appropriate media to isolate fungal cultures.

Isolation and identification of fungi

Fungal isolations were made using selective media adapted from Meyling (2007) with the following composition and preparation: 32.5 g SDA (Sabouraud Dextrose Agar, Merck) supplemented with 1 ml Dodine, 50 mg/L Chloramphenicol, 50 mg/L Ampicillin or 50 mg/L Rifampicin. Plates were incubated at 22°C in the dark. All potential EP fungi were identified microscopically using tape mounts, according to morphological characteristics described in taxonomic keys and other relevant literature (Barnett, 1960; Domsch *et al*, 2007). Selected strains were sent to the Mycology Unit at the Plant Protection Research Institute (PPRI) in Pretoria, for morphological identification. On completion of the identifications each isolate was allocated an accession number and stored in the South African National Collection of Fungi held at PPRI. On return of positive PPRI identifications and the allocation of accession numbers, those cultures were in turn used as a reference catalogue.

Quick screening method to detect entomopathogenic fungi

To separate EP fungi from opportunistic saprophytic fungal species, a rapid screening method was adapted from Ali-Shtayeh *et al* (2002). The isolated fungal cultures were first grown on SDA media for 10-12 days until sporulation was reached. Fungal suspension solutions were prepared by incising small pieces of hyphae and conidia from sporulating cultures and placing them into sterilized 1.5 µl microcentrifuge tubes supplemented with 1 ml of triple distilled water and 0.05% Triton X then vortex mixed. Five final instar *G. mellonella* larvae

were dipped into each fungal suspension for 2 seconds. The larvae were then placed into moisture chambers and incubated in the dark at 22°C. Petri-dishes were checked daily for the presence of dead larvae. Dead larvae were placed onto SDA agar until sporulation was again detected. Sporulating EP fungal cultures were labelled and sent to the Mycology Unit at PPRI in Pretoria, for morphological identification. Cultures that were not sent to PPRI were morphologically identified using the methods described above. All saprotrophic fungi found using the baiting procedures were discarded and were not considered in the present study.

Data analysis

Only EP fungal species were considered for all statistical analyses. Chi-squared (χ^2) tests were used to compare the recovery of EP isolates from the three bait insects used and specifically the recovery of *Beauveria bassiana* from these insects. Chi-squared tests were also used for the comparison of occurrence of EP fungi in soil sampled from organically versus conventionally farmed soil and for comparison between soils sampled in cultivated orchards versus refugia. Infections were registered qualitatively per sample pot. This meant that whether one or several larvae infected with the same EP fungal species were observed in the same sample pot, this was registered as one infection. All analyses are based on 288 soil samples from 6 locations (farms); 108 from organically farmed soil; 108 from conventionally farmed soil and 72 from refugia. In all analyses these ratios were adjusted accordingly.

Results and discussion

Bait insects and fungal species

The fungal species and the number of fungal isolates obtained during this study differed significantly according to the three insects used as bait (Figure 3.3.12.3). When the standard bait insect, *G. mellonella*, was used, a total of 45 EP fungal isolates were obtained and this was significantly ($\chi^2 = 40.13$, $df = 2$, $P \leq 0.005$) more than what was recovered from either *C. capitata* (11 isolates) or *T. leucotreta* (6 isolates). *Beauveria bassiana* was isolated significantly ($\chi^2 = 43.72$, $df = 2$, $P \leq 0.005$) more often than any other EP fungal species from all bait insects (Figure 3.3.12.3). More *Metarhizium anisopliae* var. *anisopliae* isolates were obtained using *G. mellonella* than any other bait insect and the only isolate of *Metarhizium flavoviride* was also obtained from this bait insect species. *Ceratitis capitata* was the only bait insect to isolate the fungal species, *Conidiobolus coronatus* and *Lucanicillium psalliotae*. *Lucanicillium psalliotae* is known as a nematophagous fungal species (Gan *et al*, 2006), but was included as an EP fungal species because of its capability of infecting arthropods (Pirali-Kheirabadi *et al*, 2007) and because of its close relatedness to *Lucanicillium lucanii* (a well-known entomopathogenic fungus) (Zare & Gams, 2008). The genus *Metarhizium* was not isolated from *C. capitata* (Figure 3.3.12.3).

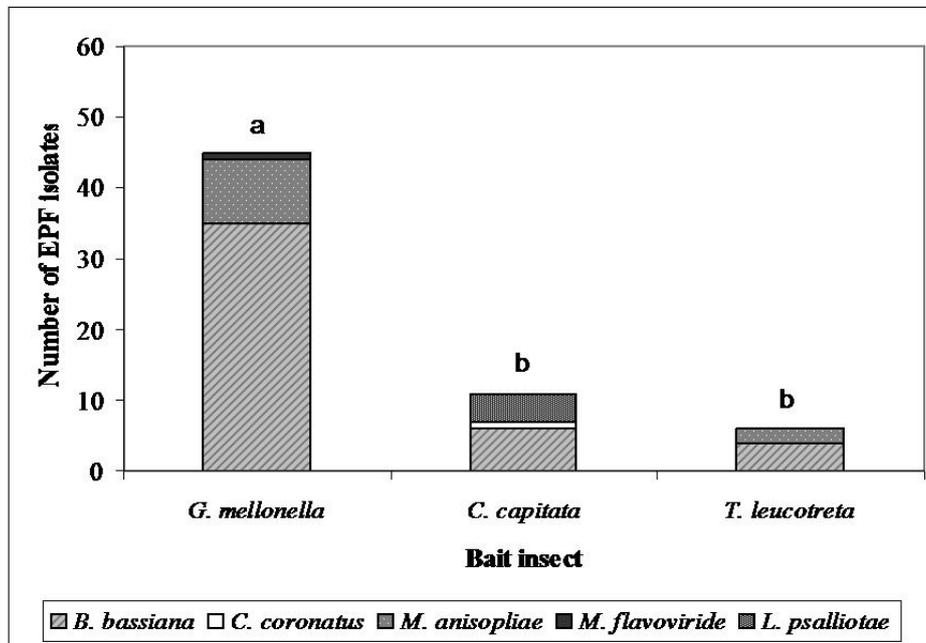


Figure 3.3.12.3. The effect of bait insect on the isolation of entomopathogenic fungal species.

The larvae of *G. mellonella* are known to be highly susceptible to insect pathogens (Chandler *et al*, 1997; Vilcinskis *et al*, 1997; Meyling 2007) and this may further explain the large number of EP fungal isolates

which were recovered from this insect bait species when compared to the other bait insects. Further the soil environment is an integral habitat for the completion of the lifecycles of both *T. leucotreta* and *C. capitata* therefore these bait species must be better adapted to the adversities of this environment compared to *G. mellonella*, to which the soil is not a natural habitat. More EP fungal isolates were recovered from *C. capitata* (11 isolates) than *T. leucotreta* (6 isolates); a possible explanation for this observation may be the biology of *T. leucotreta*. The late instar larvae of this insect species spin a cocoon around themselves prior to pupation (Moore, 2002; Stibick, 2006; Kirkman, 2007). This cocoon was often spun within 24 hours of the inoculation of the late instar larvae onto the test soil during experimentation. This feature of the biology of *T. leucotreta*, which does not occur in *C. capitata* (Thomas *et al*, 2001), may in fact minimize the possibility of the spores of EP fungi coming into direct contact with the cuticle of this insect species. The spores of EP fungi may adhere to the cocoon but because of the lack of direct contact with the insect cuticle may fail to germinate. Thus the cocoon may act as a barrier of protection against EP fungi and may reduce the general sensitivity of the assay/isolation of EP fungi using this bait insect. Furthermore, prior to pupation and cocoon spinning, Begemann (2008) explained that the migratory larvae of *T. leucotreta* in the pharate state were less susceptible to *B. bassiana* infection than non-migrating larvae, possibly due to the moulting process already taking place in pharate larvae. By shedding the larval skin when pupating, the migratory larva possibly rids itself of adhering conidia before the fungus can germinate. The late instar larvae of *C. capitata* remain relatively active for longer during the baiting process than the larvae of *T. leucotreta*, this may increase the chances of *C. capitata* larvae coming into contact with conidia and thus may explain the increased incidence of EP fungal isolates from this insect species. Secondly, it is possible that the larvae of *C. capitata* are more easily infected orally because of digging with their mouthparts, a possibility stipulated by Klingen *et al* (2002) when discussing another Dipteran larvae, *Delia floralis*. Finally, it may also indicate that this bait insect is more susceptible to fungal infection than *T. leucotreta*.

Abundance and geographic distribution of entomopathogenic fungi

In total, 62 EP fungal isolates belonging to 4 genera were recovered from 288 soil samples baited with all insect species at 22°C, an occurrence frequency of 21.53% (Table 3.3.12.1). Tables 3.3.12.3 & 3.3.12.4 (Appendix) refer to the location and ecological data of the 62 isolates of EP fungi collected from cultivated orchards and refugia, at the six farms in the Eastern Cape. Soil types and citrus rootstocks of a particular soil sample were obtained from farm owners. The soil types in South Africa: Oakleaf Caledon, Oakleaf Letaba, Oakleaf Limpopo, Oakleaf Richie and sterkspruit are classified by using unique combinations of topsoil and subsoil horizons (layers) to place a soil into a specific soil form. Certain other characteristics within the soil form are then applied to define the soil series. (Agricultural Research Council, 2009) The most frequently isolated EP fungal species was *B. bassiana*, which was recovered from 15.63% of all soil samples baited. This was followed by *M. anisopliae* var. *anisopliae*, which was found to occur in fewer soil samples at a lower frequency of 3.82 %. *L. psalliotae* was isolated with a frequency of 1.39%, while other species like *M. flavoviride* (0.35%) and *C. coronatus* (0.35%) were only isolated once from soil samples (Table 3.3.12.1). Only 1.38% (4 out of 288) of the soil samples collected yielded two EP fungal species in the same sample.

Table 3.3.12.1. Distribution and frequency (% positive samples) of entomopathogenic fungi in soil sampled from cultivated (orchards) and marginal lands (refugia) on six citrus farms in the Eastern Cape Province.

EPF species*	Conventional farms						Organic farms						% F **
	Mosslands		Arundel		J&B Citrus		Rosedale		Hippo Pools		Olifantskop		
	N= 36 orchards	N=12 refugia	N= 36 orchards	N=12 refugia	N= 36 orchards	N=12 refugia	N= 36 orchards	N=12 refugia	N= 36 orchards	N=12 refugia	N= 36 orchards	N=12 refugia	
All species	27.7	66.5	8.2	8.3	5.5	24.9	22.2	33.3	5.5	16.6	33.1	58.2	21.53
<i>B. bassiana</i>	11.1	41.6	5.5	0.0	5.5	16.6	22.2	33.3	5.5	8.3	27.7	41.6	15.63
<i>C. coronatus</i>	0.0	0.0	0.0	0.0	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.35
<i>M. anisopliae</i>	16.6	8.3	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	16.6	3.82
<i>M. flavoviridae</i>	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.35
<i>L. psalliotae</i>	0.0	8.3	0.0	8.3	0.0	0.0	0.0	0.0	0.0	8.3	2.7	0.0	1.39

*Percentage frequency of isolates found per farm
** Percentage frequency % F based on the total number of isolates found on all farms/ 288 soil samples

The distribution and frequency (% positive samples) of EP fungi in soil sampled from cultivated orchards and refugia on the six citrus farms in the Eastern Cape Province are shown in Table 3.3.12.1. The greatest diversity of EP fungal species was recorded from the conventional citrus farm, Mosslands. Eighteen isolates

belonging to four fungal species (three genera) were obtained. The only isolate of *M. flavoviride* was obtained from one soil sample taken in refugia of this farm. At Arundel, four isolates belonging to three species of EP fungi were recovered. Five isolates belonging to two EP fungal species were retrieved from J & B Citrus and the only isolate of *C. coronatus* found in this study was retrieved from a soil sample taken from refugia at this location. The farm, Rosedale, yielded the lowest diversity of EP fungal species. Only *B. bassiana* was retrieved from soil samples from this farm however a large number of isolates were obtained (12 isolates). At Hippo Pools, four fungal isolates belonging to two species were recovered. At Olifantskop, the largest number of EP fungal isolates was recovered (19 isolates) but the diversity of fungal species (three species) was lower than what was observed at Mosslands. On five of the six farms, Arundel being the only exception, *B. bassiana* was more often recovered from soil samples taken from refugia than from cultivated orchards. Conversely, *M. anisopliae* var. *anisopliae* was obtained predominately from cultivated lands on two of the three farms it occurred at.

Farming systems and field margins

More EP fungal isolates were recovered from organically (35 isolates) than conventionally (25 isolates) farmed soil samples but the result was not shown to be significant (Table 3.3.12.2 & Figure 3.3.12.4). When comparing cultivated lands and refugia, significantly more ($\chi^2 = 11.65$, $df = 1$, $P = 0.005$) entomopathogenic fungi were found in refugial habitats than cultivated land habitats (Figure 3.3.12.4). When treating cultivated lands and refugia separately, there were no significant differences observed in the occurrence of EP fungi from soil samples of both farming systems (Figure 3.3.12.4). *Beauveria bassiana* was isolated more frequently in samples from organically farmed soils (30 isolates) than from samples of conventionally farmed soils (15 isolates) and this was shown to be significantly different ($\chi^2 = 5.00$, $df = 1$, $P \leq 0.05$) (Table 3.3.12.2). In contrast, *M. anisopliae* var. *anisopliae* was isolated more frequently in samples from conventionally cultivated soils (eight isolates) than organically farmed soil samples, both cultivated and refugia (three isolates), although this result was not significantly different. *Lucanicillium psalliotae* occurred equally in both organically and conventionally farmed soil samples (Table 3.3.12.2), the single occurrence of *M. flavoviride* and *C. coronatus* were not considered in this table.

Table 3.3.12.2. Occurrence of entomopathogenic fungi in samples from organically and conventionally farmed soils.

Farm system	Pooled data	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>L. psalliotae</i>
Organic (%)	35 a	30 a	3 a	2
Conventional (%)	25 a	15 b	8 a	2

Different letters within a column denote significant differences using χ^2 tests, $P = 0.05$.

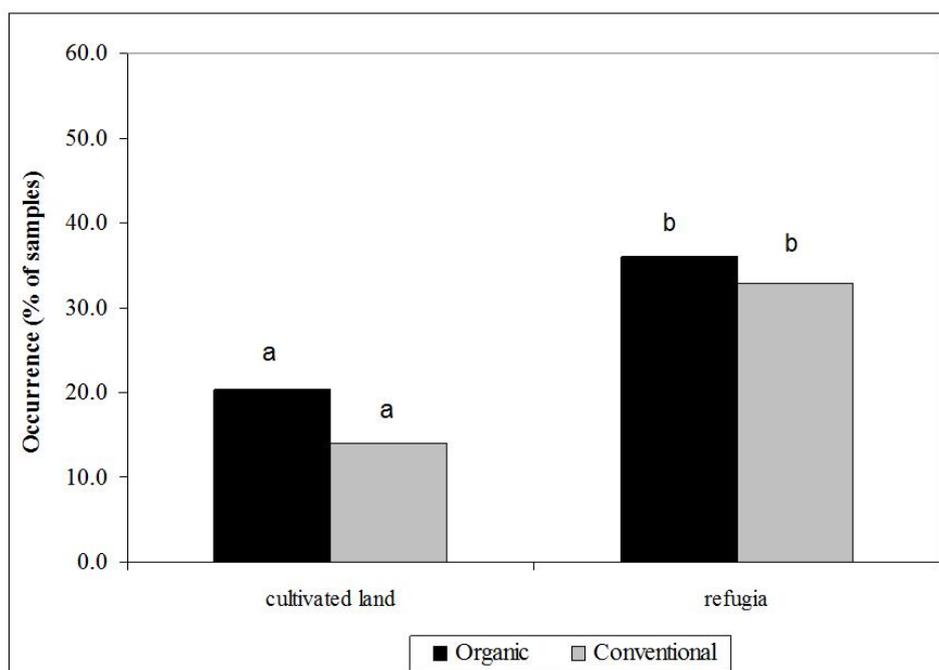


Figure 3.3.12.4. The occurrence of entomopathogenic fungi in soils sampled from cultivated and marginal (refugia) lands of organically and conventionally farmed soil. Letters above a column denote significant differences using χ^2 test, $P \leq 0.05$.

Fungal ecology

The 21.53% occurrence of five species of EP fungi in soils from the Eastern Cape, South Africa, is lower than the expected range in accordance with findings of other authors. Ali-Shtayeh *et al* (2002) found insect-pathogenic fungi to occur in 33.6% of the soil samples studied in irrigated vegetable fields and citrus orchard soils from Palestine. Other typical recovery rates include: 38.6 % in Mauritius (Sookar *et al*, 2008), 55.5% in China (Bing-Da & Xing-Zhong, 2008), 71.7% in Spain (Quesada-Moraga *et al*, 2007) and 96% in Switzerland (Keller *et al*, 2003), Chandler *et al* (1997) reported that EP fungi were common inhabitants of the soil biota but the diversity of species was low, usually with one or two species occurring frequently; this observation has been noted by several other authors (Keller *et al*, 2003; Quesada-Moraga *et al*, 2007). In the present study, *B. bassiana* and *M. anisopliae* var. *anisopliae* were the most commonly isolated EP fungal species obtained from all bait insects. The occurrence of *L. psalliotae* was low and was usually recovered from the larvae of *C. capitata*. Little literature exists on the distribution and occurrence of this nematophagous fungal species (Gan *et al*, 2007) which can also be entomopathogenic and capable of infecting other arthropod hosts (Evans & Whitehead, 2005; Pirali-Kheirabadi *et al*, 2007). Further, the single isolation of *M. flavoviride* was surprising. *M. flavoviride* has been documented in other studies and is considered to have a wide geographic distribution (Braga *et al*, 2001). Meyling & Eilenberg (2006) for example found *M. flavoviride* to occur commonly (21.9%) in soils of a Danish research farm in Copenhagen. The single occurrence of *C. coronatus* did not seem unlikely, as Hatting *et al* (1999), after a survey of the fungal pathogens of aphids from South Africa, revealed that *C. coronatus* was not isolated from field-collected wheat samples in South Africa but that the only record of this fungal species was obtained from a mycosed aphid cadaver in the aphid-rearing regimen held in Bethlehem, South Africa. As most EP fungal species are soil-borne pathogens, this study did not consider soil samples and thus the limited occurrence of *C. coronatus* must be carefully compared. Ali-Shtayeh *et al* (2002) however, found *C. coronatus* to be the most frequently isolated (31.4%) EP fungus in citrus orchard soils in Palestine. The limited number of fungal isolates obtained from the species *M. flavoviride*; *C. coronatus* and *L. psalliotae* may mean that these fungal species are locally rare or simply that they may not have been detected by the bait method protocol for various reasons which are discussed above. However, based on the studies mentioned, these species of fungi do occur with more prevalence world wide.

Many authors have noted the phenomenon of relevant isolations of *B. bassiana* from natural, undisturbed and shaded habitats and *M. anisopliae* from cultivated, sun-exposed and disturbed habitats (Bidochka *et al* 1998; Meyling & Eilenberg, 2007; Quesada-Moraga *et al*, 2007; Bing-Da & Xing-Zhong, 2008). Our study revealed a

similar pattern of distribution among these fungal species. On five of the six farms, Arundel being the only exception, *B. bassiana* was more often recovered from soil samples taken from refugia than from cultivated orchards. Sookar *et al* (2008) found that significantly more *B. bassiana* isolates were obtained in habitats of natural vegetation when compared to either vegetables or sugarcane plantations in Mauritius. Further, there was a significant difference in the recovery of this species from organically managed soils than from conventionally managed soils. Bidochka *et al* (1998) found *B. bassiana* to be affiliated with shaded, uncultivated habitats such as forests and Meyling & Eilenberg (2006) found *B. bassiana* to occur more frequently in hedgerow soils in Denmark than in field site soil samples. Mietkiewski *et al* (1997) observed that the isolation of *B. bassiana* from arable soils in the UK was usually associated with natural undisturbed habitats which were higher in organic matter and Quesada-Moraga *et al* (2007) suggested that soils with greater organic matter content have higher cation exchange capacities with greater organic matter enhancing conidia absorption. An increase in organic matter content in the soils is said to increase the presence of *B. bassiana* (Mietkiewski *et al*, 1997). However, Meyling & Eilenberg (2006) found that organic matter content and subsequent biological activity in the soil adversely affected the persistence of this fungal species due to antagonistic effects. Mainly because both *Beauveria* and *Metarhizium* are poor competitors for organic resources compared to opportunistic saprophytic fungi that are ubiquitous in the soil (Meyling & Eilenberg, 2007). Quesada-Moraga *et al* (2007) found that the occurrence of EP fungi was frequently associated with higher organic matter but the species, *B. bassiana*, was associated with soils of lower organic matter, which may relate to fungistatic compounds found in organic matter that have previously been shown to affect *B. bassiana* more than *M. anisopliae*. However in the present study *B. bassiana* was most often recovered from the soils in refugia on organically managed farms where organic matter content is expected to be higher due to organic farming practices such as composting.

In the present study, *M. anisopliae* var. *anisopliae* was obtained predominately from cultivated lands on two of the three farms it occurred at, Olifantskop being the only exception. *Metarhizium anisopliae* is thought to be strongly associated with soils from cultivated habitats, particularly field crops and is often referred to as an 'agricultural species' (Bidochka *et al*, 1998; Meyling & Eilenberg, 2007; Quesada-Moraga *et al*, 2007; Bing-Da & Xing-Zhong, 2008, Sookar *et al*, 2008). It has been suggested that *M. anisopliae* is more frequently isolated from cultivated habitats than *B. bassiana* because the conidia of *M. anisopliae* can persist longer without repeated infection of hosts (Quesada-Moraga *et al*, 2007). Filho *et al* (2001), suggest that some fungal species are more tolerant to pesticides than others and *M. anisopliae* is considered to be more tolerant to pesticides than *B. bassiana* (Quesada-Moraga *et al*, 2007); this together with a lower competitive ability suggested for *B. bassiana*, may add value to the explanation of *M. anisopliae* being found more frequently in cultivated lands in the present study. Furthermore, Quesada-Moraga *et al* (2007) found that *M. anisopliae* predominated in soils with a pH lower than 7 and were better adapted to slightly more acidic soils than *B. bassiana*; citrus soils tend to be more acidic (Obreza & Collins, 2002).

This work revealed a greater likelihood of finding EP fungi in refugia of both organically and conventionally farmed soils, but no difference was found between the two farming systems. Mietkiewski *et al* (1997) in Poland and Chandler *et al* (1997) in the UK both showed that the frequency of EP fungi in intensively cultivated soils was lower than in marginal habitats. This was not the case in a study by Klingen *et al* (2002) who found more EP fungi in arable fields of organically farmed soils but no differences were found between the two farming systems when comparing field margins (refugia). However there was a common result in that a positive relationship existed between occurrence of EP fungi and organically farmed soils. The occurrence of EP fungi is influenced by a complex set of abiotic and biotic factors such as pesticides (Filho *et al*, 2001), organic matter of the soil (Quesada-Moraga *et al*, 2007), desiccation, ultra-violet light (UV-B) (Braga *et al*, 2001), temperature and insect hosts. In light of some of the above mentioned factors, it appears that organically farmed soil might be a more suitable habitat for insect-pathogenic fungi, which Klingen *et al* (2002) mentions. The absence of some inorganic pesticides and fertilizers from organic farms might be one reason for the prevalence of EP fungal species; whereas the presence of insecticides and especially fungicides in some conventional citrus farming practices may have a direct killing effect on EP fungi (Khalil *et al*, 1985; Klingen *et al*, 2002, Saenz-de-Cabez Irigaray *et al*, 2003; Meyling & Eilenberg, 2007). Conversely, the use of compost (especially manure) or organic fertilizers may have a positive effect on the occurrence of EP fungal species because an increased carbon load in the soil is favourable for soil inhabiting insects, which themselves, are potential hosts for EP fungi (Klingen *et al*, 2002). Furthermore, the direct application of EP fungi in organic farming practices may also have a significant impact on the occurrence of EP fungi in these systems. The organic citrus farm, Rosedale for example, made extensive use of a *Beauveria bassiana* formulated product called BB plus® (Madumbi-BCP) prior to the sampling period. The high incidence of occurrence of *B. bassiana* isolates from Rosedale may almost certainly be some of the residual spores from the application of BB Plus®.

Conclusion

Knowledge of the ecology of indigenous populations of EP fungi, specifically *B. bassiana* and *M. anisopliae* var. *anisopliae* in agroecosystems in South Africa, as well as the environmental conditions and agricultural practices on these fungi, are crucial criteria for evaluating and manipulating these microbes in conservation biological control (CBC) (Meyling & Eilenberg, 2007). Based on this study, each farm had its own range and assemblage of EP fungi in the soils. These differences in occurrences challenge the CBC strategy. At Mosslands for example, indigenous populations of *M. anisopliae* var. *anisopliae* appear to be the more suitable candidate for environmental manipulation because this species was more associated with agricultural field soils (16.6%) than *B. bassiana* (11.1%). Furthermore, *M. anisopliae* is thought to be more pesticide resistant as the conidia of *M. anisopliae* can persist longer without repeated infection of hosts and *M. anisopliae* has a better competitive ability than *B. bassiana* (Quesada-Moraga *et al*, 2007). However, in cultivated fields at Rosedale, *M. anisopliae* var. *anisopliae* was rare, perhaps due to orchards which are planted in an east-west orientation which increases the amount of shade covering soils, particularly on the southern side of the canopy. Therefore *B. bassiana* (22.2%) would be a more suitable candidate for CBC at Rosedale.

Since most EP fungi are soil-borne microorganisms, the development and formulation of alternative control strategies using these fungi in compost-based products, targeted at larvae and pupae of the insect pests, *T. leucotreta* and *C. capitata* in the soil, may benefit existing IPM management of citrus in South Africa. The biology of these insect pest species includes them dropping from their host plants to pupate in the soil or leaf litter on the ground below citrus trees (Thomas *et al*, 2001; Peña *et al*, 2002; Stibick, 2006). By incorporating fungal spores and diatomaceous earth in a compost-based product which can be applied to the base of citrus trees, one may potentially reduce pupating pest numbers at the soil level. Richards *et al* (2005), incorporated diatomaceous earth with the fungal spores of *Aspergillus flavus*; severe cuticular lacerations on the lateral surfaces of the mid and hind legs of the small hive beetle, *Aethina tumida*, caused by the diatomaceous earth, were observed. These focal areas of severe cuticular laceration may permit increased fungal spore access and promote fungal infection in insect pests. This novel approach combined with the fact that the soil is not affected as much by environmental extremes but acts like a buffer, can increase fungal spore survival and persistence (Braga 2001; Ekesi *et al*, 2003). Since, most citrus orchards are perennial habitats, they may support larger more stable microbial communities than annual vegetable cropping systems, and thus they are more amenable to conservation biological control (CBC) and microbial products. Ali-Shtayeh *et al* (2002) found that EP fungi were more frequently isolated from soils under fruit trees than from soils in vegetable cropping systems. Since fungi rely on insect hosts to build-up significant population sizes by producing vast amounts of conidia, the availability of hosts in orchards must be considered. However, recent studies on *M. anisopliae* revealed that this EP fungal species may have rhizosphere competence; it was documented that different genes were expressed when the fungus was growing on exudates from bean roots compared with when it was grown on an insect cuticle (Wang *et al*, 2005). This study indicated that *M. anisopliae* has developed various adaptations to function as a pathogen or to grow saprophytically in the presence of plants in the rhizosphere (Bidochka, 2001; Meyling & Eilenberg, 2007). This ability of *M. anisopliae* has great potential in biological control because it means that if insect hosts become scarce in the habitat, which is likely in agriculture, the fungus can adapt and persist until hosts become available again. However despite this evidence, insect hosts should still be considered the primary source of organic matter for fungus population build up (Meyling & Eilenberg, 2007).

Further objectives (milestones) and work plan

1. Currently 12 EPF isolates are being tested against the three pest species *T. leucotreta*, *C. capitata* and *C. rosa* using conidia suspension bioassays (conc: 1×10^7 conidia/ml) to determine the 4 most virulent strains. These bioassays are also being used to determine and compare adult pest emergence, the number of adults mycosed and the number of pupae mycosed.
2. Once the 4 most virulent strains are determined, serial dilution bioassays using four doses of inoculum (1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 conidia ml⁻¹) will commence.
3. Isolates with the lowest LC₅₀ values will then be tested against 12, 48 and 96 hour old pupae to evaluate the effect of age on the susceptibility to the selected using a concentration of 1×10^8 conidia ml⁻¹.

Technology transfer

Entomology Society (Stellenbosch) 5-8 July 2009:

Oral presentation: The occurrence of EP fungi in citrus soils in the Eastern Cape Province and the virulence of different EP fungal isolates towards false codling moth, *Thaumatotibia leucotreta*, Mediterranean and Natal fruit fly, *Ceratitidis capitata* and *C. rosa*. **Goble T**, Dames J, Hill M & Moore S.

42nd Annual meeting of the Society of Invertebrate Pathology (Park City, Utah, USA) 16-20 August 2009:

Oral presentation: The occurrence of EP fungi in citrus soils in the Eastern Cape Province and the virulence of different EP fungal isolates towards false codling moth, *Thaumatotibia leucotreta*, Mediterranean and Natal fruit fly, *Ceratitidis capitata* and *C. rosa*. **Goble T**, Dames J, Hill M & Moore S.

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APPENDIX

Table 3.3.12.3. The location and ecological data of *Conidiobolus coronatus*, *Lucanicillium psalliotae*, *Metarhizium anisopliae* and *M. flavoviride* collected from cultivated and refugial soils in the Eastern Cape Province, South Africa.

Farm name	Farming type	Habitat	Sample area(Ha)	Soil type	Citrus rootstock	Bait type	Fungal genus or species	Accession no.
J&B Citrus	conventional	refugia	2.00	unknown	NA	FF	<i>Conidiobolus coronatus</i>	PPRI 9695
Arundel	conventional	refugia	1.00	unknown	NA	FF	<i>Lucanicillium psalliotae</i>	PPRI 9768
Mosslands	conventional	refugia	2.00	Red sandy	NA	FF	<i>Lucanicillium psalliotae</i>	PPRI 9767
Olifantskop	organic	orchard	0.80	loamy	rough lemon	FF	<i>Lucanicillium psalliotae</i>	PPRI 9766
Hippo Pools	organic	refugia	1.00	unknown	NA	FF	<i>Lucanicillium psalliotae</i>	RHO 14 B4
Arundel	conventional	orchard	0.50	loamy	single citrange	FCM	<i>M. anisopliae</i> group	PPRI 9561
Mosslands	conventional	orchard	0.98	Oakleaf Richley	rough lemon	FCM	<i>M. anisopliae</i> var. <i>anisopliae</i>	RHO F 14 2 B5
Mosslands	conventional	orchard	0.98	Oakleaf Richley	rough lemon	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9558
Mosslands	conventional	orchard	0.98	Oakleaf Richley	rough lemon	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	RHO 14 2 L6
Mosslands	conventional	orchard	0.98	Oakleaf Richley	rough lemon	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9562
Mosslands	conventional	orchard	0.98	Oakleaf Richley	rough lemon	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	RHO G 14 2 B5
Mosslands	conventional	orchard	0.25	Oakleaf Caledon	rough lemon	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9803
Mosslands	conventional	refugia	2.00	unknown	NA	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9559
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9802
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9800
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	<i>M. anisopliae</i> var. <i>anisopliae</i>	PPRI 9801
Mosslands	conventional	refugia	2.00	unknown	NA	<i>Galleria</i>	<i>Metarhizium flavoviridae</i>	PPRI 9560
<p>NA = not applicable, RHO = Rhodes University accession numbers</p> <p>FCM = <i>Thaumotobia leucotreta</i>, FF = <i>Ceratitis capitata</i>, PPRI = Plant Protection Research Institute accession numbers</p>								

Table 3.3.12.4. The location and ecological data of *Beauveria bassiana* collected from cultivated and refugial soils in the Eastern Cape Province, South Africa.

Farm name	Farming type	Habitat	Sample area (Ha)	Soil type	Citrus rootstock	Bait type	Accession no.
Arundel	conventional	orchard	0.50	loamy	carizzo citrange	<i>Galleria</i>	RHO Ar 17 L6
Arundel	conventional	orchard	0.50	loamy	carizzo citrange	<i>Galleria</i>	PPRI 9679
Mosslands	conventional	orchard	0.98	Oakleaf Richley	rough lemon	<i>Galleria</i>	PPRI 9555
Mosslands	conventional	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9774
Mosslands	conventional	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9773
Mosslands	conventional	refugia	2.00	Red sandy	NA	<i>Galleria</i>	PPRI 9554
Mosslands	conventional	refugia	2.00	Red sandy	NA	<i>Galleria</i>	PPRI 9557
Mosslands	conventional	refugia	2.00	Red sandy	NA	<i>Galleria</i>	PPRI 9593
Mosslands	conventional	orchard	0.82	Oakleaf Caledon	rough lemon	FCM	PPRI 9680
Mosslands	conventional	orchard	0.25	Oakleaf Caledon	rough lemon	FCM	PPRI 9592
Mosslands	conventional	orchard	0.25	Oakleaf Caledon	rough lemon	FCM	PPRI 9594
J&B Citrus	conventional	orchard	0.74	Oakleaf Caledon	rough lemon	<i>Galleria</i>	PPRI 9678
J&B Citrus	conventional	orchard	1.11	clay/sterkspruit	rough lemon	FF	PPRI 9693
J&B Citrus	conventional	refugia	2.00	unknown	NA	FF	PPRI 9556
J&B Citrus	conventional	refugia	2.00	unknown	NA	FF	PPRI 9553
Olifantskop	organic	orchard	0.80	loamy	rough lemon	<i>Galleria</i>	RHO OI 13 L1
Olifantskop	organic	orchard	0.80	loamy	rough lemon	<i>Galleria</i>	PPRI 9688
Olifantskop	organic	orchard	0.80	loamy	rough lemon	<i>Galleria</i>	RHO OI 15 L5
Olifantskop	organic	orchard	0.80	loamy	rough lemon	<i>Galleria</i>	RHO OI 15 B3
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	PPRI 9689
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	RHO OI 19 B1
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	RHO OI 19 B2
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	RHO OI 19 B3
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	RHO OI 19 B5
Olifantskop	organic	orchard	0.50	loamy	rough lemon	<i>Galleria</i>	RHO OI 19 B6
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9772
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9771
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9692
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9691
Olifantskop	organic	refugia	2.00	unknown	NA	<i>Galleria</i>	PPRI 9690
Rosedale	organic	orchard	0.90	Oakleaf Letaba	troyer citrange	<i>Galleria</i>	RHO Rose. 3 L2
Rosedale	organic	orchard	0.90	Oakleaf Letaba	troyer citrange	<i>Galleria</i>	PPRI 9681
Rosedale	organic	orchard	0.90	Oakleaf Letaba	troyer citrange	<i>Galleria</i>	RHO Rose. 3 B2
Rosedale	organic	orchard	0.90	Oakleaf Letaba	carizzo citrange	<i>Galleria</i>	PPRI 9682
Rosedale	organic	orchard	1.10	Oakleaf Limpopo	carizzo citrange	<i>Galleria</i>	RHO Rose.23 L1
Rosedale	organic	orchard	1.10	Oakleaf Limpopo	carizzo citrange	<i>Galleria</i>	PPRI 9687
Rosedale	organic	orchard	1.10	Oakleaf Limpopo	carizzo citrange	<i>Galleria</i>	RHO Rose 23 L4
Rosedale	organic	orchard	1.10	Oakleaf Limpopo	carizzo citrange	<i>Galleria</i>	RHO Rose 23 L5
Rosedale	organic	refugia	1.20	unknown	NA	<i>Galleria</i>	PPRI 9683
Rosedale	organic	refugia	1.20	unknown	NA	<i>Galleria</i>	PPRI 9686
Rosedale	organic	refugia	1.20	unknown	NA	<i>Galleria</i>	PPRI 9684
Rosedale	organic	refugia	1.20	unknown	NA	FCM	PPRI 9685
Hippo Pools	organic	orchard	0.40	loamy	rough lemon	FF	RHO HP 14 L5
Hippo Pools	organic	orchard	0.40	loamy	rough lemon	FF	RHO HP 14 B5
Hippo Pools	organic	refugia	1.00	unknown	NA	FF	PPRI RHO R1
NA = not applicable, RHO = Rhodes University accession numbers FCM = <i>Thaumotobia leucotreta</i> , FF = <i>Ceratitis capitata</i> , PPRI = Plant Protection Research Institute accession numbers							

3.4 PROJECT: COSMETIC PESTS Project coordinator: Tim G Grout (CRI)

3.4.1 Project summary

Citrus thrips was the primary cosmetic pest of citrus for the past 30 years or more but its pest status has now declined, perhaps due to improved survival of a suite of natural enemies in most citrus orchards. The remaining pests that cause cosmetic damage are mostly sporadic in their occurrence and therefore difficult to work with. Citrus grey mite is no exception, although in Highveld regions it is more common. Research showed that Envidor and RJU37PY were effective against this pest and will be suitable alternatives to Acarol. A simple washing technique proved to be an effective method of sampling for this mite, but this would require the use of a microscope (3.4.2). No progress was made in testing microbial isolates against citrus thrips due to the inability to find suitable numbers of citrus thrips to run bioassays (3.4.3). However, further progress was made with a less sporadic pest, citrus bud mite, with the submission of residue samples for analysis after spraying with the new acaricide RJU37PY which had previously been shown to have equivalent efficacy to Acarol (3.4.4). Research on the lemon borer moth showed no correlation between pheromone trap catches and infestation levels, but did show that DiPel provided effective control that was superior to Ultracide and mevinphos (3.4.5). Leafhoppers proved to be sporadic in 2008-9 and only one site could be found with barely sufficient numbers for a chemical trial. Results confirmed that methomyl SP (40-60 g/hl) and mevinphos SL (30-45 ml/hl) were effective for the control of citrus leafhopper when sprayed at approximately 5 000 L/ha (3.4.6). Further trials must be conducted to provide enough data for registration.

Projekopsomming

Sitrus blaaspootjie was vir die afgelope 30 jaar of meer die vernaamste kosmetiese plaag van sitrus, maar sy plaagstatus het nou afgeneem, moontlik weens die verbetering in oorlewing van 'n reeks van natuurlike vyande in die meeste sitrusboorde. Die oorblywende plaag wat kosmetiese skade veroorsaak is meestal sporadies in hul voorkoms en daarom moeilik om mee te werk. Sitrus grysmyt is geen uitsondering nie, alhoewel dit in die Hoëveld streke meer algemeen is. Navorsing het getoon dat Envidor en RJU37PY effektief was teen hierdie plaag en geskikte alternatiewe vir Acarol sal wees. 'n Eenvoudige wastegniek is bevestig as 'n doeltreffende metode vir monsterneming vir hierdie myt, maar dit benodig die gebruik van 'n mikroskoop (3.4.2). Geen vordering is gemaak met die toetsing van mikrobiële isolate teen sitrus blaaspootjie nie omdat geskikte getalle van sitrus blaaspootjies nie gevind kon word vir biotoetse nie (3.4.3). Verdere vordering is egter gemaak met 'n minder sporadiese plaag, sitrus knopmyt, met die inhandiging van residu monsters vir ontleding na bespuiting met die nuwe mytdoder, RJU37PY wat in vorige proewe ekwivalente doeltreffendheid aan Acarol getoon het (3.4.4). Navorsing op die suurlemoenmot het geen korrelasie tussen feromoon lokvalvangste en besmetting getoon nie, maar het wel gewys dat DiPel effektiewe beheer verskaf het wat beter as Ultracide en mevinphos was (3.4.5). Bladspringers het geblyk om sporadies in 2008-9 te wees en slegs een perseel, met skaars genoegsame getalle vir 'n chemiese proef, kon gevind word. Resultate het bevestig dat methomyl SP (40-60 g/hl) en mevinphos SL (30-45 ml/hl) effektief is vir die beheer van sitrus bladspringer wanneer dit teen ongeveer 5 000 L/ha gespuit word (3.4.6). Verdere proewe om genoeg data vir registrasie te voorsien, sal gedoen moet word.

3.4.2 PROGRESS REPORT: Improving management of citrus grey mite, *Calacarus citrifolii* Experiment 856 (2006-2008/9) by Tim G Grout, Peter R Stephen and Charl Kotze (CRI)

Opsomming

Sitrus grysmyt (CGM) word deur sekere sitrus produksieareas in die Hoëveld as 'n belangrike plaag gereken, maar die sporadiese aard van die plaag en die voorkomende benadering wat deur die produsente gevolg word om dit te beheer, beteken dat moeilik was om besmettings te vind. Twee proewe wat chemiese behandelings vergelyk het, is uitgevoer en het getoon dat Envidor teen 15 ml/hl net so effektief soos Acarol teen 30 ml/hl was en dat Torque teen 15 ml/hl en RJU37PY teen 150 ml/hl die tweede beste opsies was. Geen ander sitrusboorde met geskikte besmettings vir eksperimentele doeleindes is gevind nie. Monsters van gasheer anders as sitrus het net twee plante met groot getalle van eriophyoid myte wat soos CGM lyk, opgelewer. Groot genoeg monsters, om die identifikasie te bevestig, moet nog versamel word. 'n Wastegniek het geblyk om 'n bruikbare metode vir die monitering van die myt te wees en is ook baie meer sensitief as om blare vir die myte te deursoek. Hierdie tegniek moet in die toekoms gebruik word vir verkenning en evaluering van chemiese proewe. Navorsing op alternatiewe behandelings en die rol van gasheerplante anders as sitrus sal voortgaan wanneer besmettings vlakke dit toelaat.

Summary

Citrus grey mite (CGM) is considered an important pest by certain Highveld citrus production regions but the sporadic nature of the pest and the preventive approach used by growers to control it meant that infestations were difficult to find. Two trials comparing chemical treatments were conducted and these showed that Envidor at 15 ml/hl was as effective as Acarol at 30 ml/hl and that Torque at 15 ml/hl and RJU37PY at 150 ml/hl were the next best options. No other citrus orchards were found with suitable infestation levels for trial purposes. Samples of non-citrus host plants only resulted in two plants that hosted large numbers of eriophyid mites resembling CGM. Large enough samples must still be collected to confirm identification. A washing technique was found to be a useful tool for monitoring for this mite and much more sensitive than searching leaves for the mites. Further use should be made of this technique in scouting and evaluating chemical trials. Research on alternative treatments and the role of non-citrus host plants will continue when infestation levels permit.

Introduction

Citrus grey mite (CGM) has been listed as an important research priority at Rustenburg, Zimbabwe, Waterberg, Vaalharts and Ohrigstad/Burgersfort study groups. Tydeiid mites such as *Pronematus ubiquitous* may play a role in suppressing CGM as they are often associated together (Ueckermann and Grout 2007). Phytoseiid mites may also contribute to CGM's control and this may be why it is not as problematic on mature trees with a better microclimate for predatory mites as on young trees. Several acaricides are effective against this pest but scouting is difficult due to its small size. Use of the concentric ring blotch symptoms is also not practical because the symptoms appear several weeks after feeding by the mites, and possibly after the application of an effective treatment. A washing and filtering method, derived from a procedure described by de Lillo (2001), may be a useful alternative for evaluating acaricidal sprays and even for scouting. Some citrus growers believe that the mite is blowing into citrus orchards from indigenous bush. Attempts were made last year to verify this with the use of aerial traps in an orchard adjacent to bush that had had a severe CGM infestation, but although other mites were caught on the traps, no CGM could be found in the orchards or on the traps. Hopefully this work can be repeated if a suitable trial site is found.

As with other chemicals, options for the control of this pest are becoming limited because they are generally required after petal fall. However, as these mites belong to the same family as citrus bud mite there is a good chance that new products registered for the control of bud mite should be effective against citrus grey mite. Recent trials by CRI (Grout and Stephen 2008) have shown that RJU37PY, not previously registered on citrus in South Africa, is effective against bud mite and should also be evaluated against CGM, in addition to some other unregistered products.

A third aspect requiring investigation is the possibility that more than one species of "grey mite" exists. Within the eriophyid family, *Calacarus citrifolii* is exceptional in that it has more than one host plant. Apart from the possibility of being on indigenous host plants, it is known from granadilla, papaya, banana, *Poinsettia* and *Brunfelsia*. Now that molecular identification techniques are available, the identification of species on hosts other than citrus must be confirmed to determine whether natural vegetation can play a role in increasing populations in adjacent orchards.

Materials and methods

To evaluate the effect of 11 different acaricidal treatments (Table 3.4.2.1) on CGM, two trials were conducted at Mooinooi farm near Brits, one on mature Eureka lemon trees and one on young Valencia trees. Both sites had a history of CGM with most leaves on the lemons having concentric ring blotch symptoms and the Valencias showing a few symptoms at the time of spraying. On 10 April 2008, treatments were sprayed by hand using a high-pressure spray machine on two separate blocks of 10 lemon trees and two blocks of 20 Valencia trees. These treatment blocks were arranged randomly within the two replicate zones of the orchard. For data collection, eight double-tree replicates of each treatment were used for the Valencias and ten double-tree replicates for the lemons. Half of these data trees were from one block and half from the other block for each treatment. Untreated blocks of control trees were used that were close to the other treated blocks. The treatments were evaluated after 4 weeks (lemons) and 7 weeks (Valencias) using a washing and filtering method. This method comprised the washing of 10 terminals per double-tree replicate as follows: A terminal with 10-15 leaves was shaken in water containing 1.5% bleach and 0.2% dishwashing soap (Teepol) for 10-15 s. The washing mixture containing the mites was filtered through two stainless steel sieves: an 850 µm mesh to remove debris and a 25 µm mesh to retain the mites. For each replicate the mites retained in the 25 µm sieve were washed into a 50 ml bottle using the same solution. This final mixture

included a variable amount of dust particles and other fine debris. CGMs in the water mixture were counted under a dissecting microscope at 30X magnification using a counting grid under an 8.8 cm diameter petri dish. The mite mixture was swirled to mix thoroughly then poured into the petri dish. Only mites on black squares, comprising 10% of the surface area of the petri dish, were counted and the number then multiplied by 10. This was necessary due to the time required to count very high numbers of mites. The lemons were evaluated first on 7 May 2008 and the Valencias were evaluated on 26 May 2008. Numbers were transformed to $\text{Log}_{10}+1$ before analysis of variance and means compared using Student-Newman-Keuls test at $\alpha=0.05$.

The washing method was also used to survey for possible future trial sites as well as to sample alternate host plants. At each potential site, 20 terminals distributed through the orchard/plants were washed and samples taken as described above. On 5 February 2009, four farms were sampled at Ohrigstad and Burgersfort. On 12 February 2009, three farms in the Makopane/Mookgophong area were sampled for potential trial sites. At each farm, up to three orchards where CGM had previously been a problem were sampled. During February 2009, various host plants in Nelspruit were sampled using the washing method.

Results and discussion

An attempt to link developing leaf damage in the Valencias to the treatments failed as damage appeared in the best treatment as well as the untreated control. This confirmed that CGM damage takes several weeks to appear and that the use of symptoms to evaluate treatments would be unreliable. Using the washing method, high numbers of mites were recovered from the controls and weaker treatments, while acaricides known to be effective yielded relatively low mite numbers, showing that this method is sensitive to differing CGM population densities. Envidor at 15 ml/hl was as effective as Acarol at 30 ml/hl in both trials (Table 3.4.2.1). The former product has not been registered on CGM due to a lack of data and the difficulty involved in conducting trials. RJU37PY at 150 ml/hl was the next most effective treatment on lemons but on Valencias it was significantly worse than Torque at 15 ml/hl. The addition of Break-Thru to Smite did not significantly improve control but Smite alone was significantly worse than Acarol and not significantly different from Dithane at 200 g/hl on both citrus types. Abamectin treatments without oil were all significantly worse than Dithane. Acramite at 50 ml/hl had variable results but was not very promising.

Based on an estimate of 15 leaves per terminal washed, counts showed an average of 20 to 26 mites per leaf for the controls or ineffective treatments, much higher than physical observations of leaves in the field or laboratory. The better treatments had mean numbers of approximately 0.4 to 1 mite per leaf. Perhaps an infestation of 4 or more mites per leaf, determined by the above washing technique, should be treated to prevent possible leaf drop.

Table 3.4.2.1. Results of two trials conducted at Mooinooi in the Brits area during April and May 2008.

Treatments (Sprayed 10/04/2008)	Concentration (per 100 L water)	Mean no. CGM per double-tree sample (10 terminals)	
		Lemons: 07/05/2008	Valencias: 26/05/2008
Acarol (bromopropylate 50% EC)	30 ml	157.4 a	46.3 a
Torque (fenbutatin oxide 55% SC)	15 ml	227.7 ab	132.5 b
RJU37PY	150 ml	186.9 ab	251.3 c
Acramite (bifenazate 48% SC)	50 ml	333.9 b	2061.3 d
Envidor (spiroadiclofen 24% SC)	15 ml	124.5 a	55.0 ab
Abamectin (1.8% EC)	20 ml	1060.5 c	2183.8 d
Abamectin (1.8% EC)	40 ml	985.2 c	3967.5 d
Abamectin (1.8% EC)	60 ml	795.0 c	395.0 c
Dithane (mancozeb 80% WP)	200 g	453.0 b	288.8 c
Smite (etoxazole 10% SC)	30 ml	285.0 b	328.8 c
Smite + Break-Thru	30+3 ml	269.0 ab	328.8 c
Control		2929.5 d	3348.8 d

Means in the same column followed by the same letter are not significantly different ($P>0.05$ SNK test)

Out of 16 previously-infested sites, CGM was only found at four in very low numbers that were inadequate for further spray trials or aerial trapping (Table 3.4.2.2). Sampling of various garden plants using the washing technique only confirmed large numbers of eriophyoids resembling CGM on *Duranta* sp. and *Rhus*

leptodictya (Table 3.4.2.3). The latter is an indigenous tree that Van der Merwe and Coates (1965) also described as a host but sufficient numbers of mites have not yet been collected to confirm the identification of this mite. We found no mites on *Rhus lancea* but Van der Merwe and Coates (1965) listed this as a host plant and *Rhus pyroides* as a preferred host plant.

Table 3.4.2.2. Numbers of CGM recovered in washing samples of citrus foliage at sites where CGM was previously present.

Area	Farm	Orchard	CGM per sample of 20 terminals
Ohrigstad	Namoneng	Block 27	0
		Block 29	0
		Block 14	0
	Lushgrow	Block 2002A	0
	LeRoux Farming	Block G	0
Block B1		1	
Burgersfort	Dirishana	C1(Clementines)	0
		Cara Cara	0
Sterkrivier (Makopane)	Grootrivier	Block 4	0
		Block 5	0
		Block 7	2
Buffelsfontein (Mookgopong)	Bufland	Block 13*	3
		Block 15	10
Haakdoring (Mookgopong)	Prinsloo Boerdery	Block 4	0
		Block 1	0
		Lemons	0

*Site where aerial sticky traps were previously used near indigenous vegetation.

Table 3.4.2.3. Results of citrus grey mite survey on alternative host plants in Nelspruit.

Host plant (location)	Total apparent CGM per sample*
<i>Rhus lancea</i> (Kelkiewyn St)	0
<i>Rhus</i> spp. (Hunter St)	5
<i>Rhus leptodictya (amerina)</i> (Golden Drv)	161
<i>Brunsfelsia</i> (Golden Drv)	9
<i>Brunsfelsia</i> (DuPreez St)	0
<i>Brunsfelsia</i> (Kelkiewyn St)	0
Lime tree (Golden Drv)	0
Papaya (Golden Drv)	7
<i>Duranta</i> sp. (Wilger St)	186
<i>Cedrela</i> sp. (Nelson Mandela Drv)	0

*These mites resembled citrus grey mite but their identification must still be confirmed when large numbers can be collected with fresh plant material

Conclusion

Two trials comparing chemical treatments were conducted and these showed that Envidor at 15 ml/hl was as effective as Acarol at 30 ml/hl and that Torque at 15 ml/hl and RJU37PY at 150 ml/hl were the next best options. A washing technique was found to be much more effective for sampling for CGM than inspecting foliage.

Further objectives and work plan

If suitable infestations can be found in citrus orchards, research on alternative treatments will continue. Similarly, infestations on non-citrus host plants will be monitored with the washing technique until large enough numbers of mites can be found for verification of identification by PCR.

Technology transfer

The results of the chemical trials were presented as a poster at the Citrus Research Symposium in 2008. Further experience needs to be obtained with infestation levels before confirming an intervention threshold based on the washing technique.

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3.4.3 PROGRESS REPORT: Investigation of entomopathogenic fungi for control of citrus thrips Experiment 879 (2007/8-2009/10) by Tim G Grout and Kim C Stoltz (CRI)

Opsomming

'n Verdere twee NIPB isolate van *Metarhizium anisopliae* is bekom bykomend tot 'n isolaat van Tarryn Goble by Rhodes Universiteit. Spore is van die NIPB isolate versamel en gestoor, gereed vir die behandeling van sitrus blaaspootjie. Geskikte getalle van sitrus blaaspootjies kon egter nie in die veld versamel word om die evaluering te doen nie. Hierdie toetse sal uitgevoer word sodra daar 'n geskikte bron van sitrus blaaspootjies gevind word, maar verdere navorsing oor hierdie onderwerp word nie beplan alvorens 'n mikrobiese formulering nie in die veld geëvalueer kan word nie.

Summary

Another two PPRI isolates of *Metarhizium anisopliae* were obtained in addition to an isolate from Tarryn Goble at Rhodes University. Spores were harvested from the PPRI isolates and stored ready to treat citrus thrips. However, suitable numbers of citrus thrips could not be collected from the field to conduct evaluations. These tests will be conducted once a suitable supply of citrus thrips is found but further research on this topic is not planned until a microbial formulation can be evaluated in the field.

3.4.4 PROGRESS REPORT: Alternative chemicals for citrus bud mite control Experiment 916 (2007-2008/9) by Tim G Grout and Peter R Stephen (CRI)

Opsomming

'n Nuwe mytdoder wat effektief is teen sitrus knopmyt, RJU37PY is by vier plekke op twee verskillende sitrus tipes gespuit vir residu doeleindes. Vrugmonsters is 'n paar ure na bespuitings geneem en ook met interalle van 3, 7, 14 en 28 dae. Dit is gevries totdat dit vir ontleding gestuur kon word. Resultate van die ontledings is egter nog nie beskikbaar nie. Verdere proewe om die effektiwiteit van hierdie produk, in kombinasie met minerale olie, kan moontlik indien geskikte proefpersele gevind kan word, uitgevoer word. Geen ander proewe met nuwe mytdoders word beplan nie.

Summary

A new acaricide that is effective against citrus bud mite, RJU37PY was sprayed at four sites on two different citrus types for residue purposes. Fruit samples were taken a few hours after spraying then at intervals of 3, 7, 14 and 28 days and kept frozen until being sent for analysis. Results of the analysis are not yet available. Further efficacy trials involving combinations of this product with mineral oil may be conducted if suitable trial sites are found. No other trials with new acaricides are planned.

Introduction

Although tydeid mites are clearly associated with citrus bud mite (CBM) (*Eriophyes sheldoni*) and other eriophyoid mites and probably prey on eggs and early instars, control of CBM is dependent on the use of chemicals. The most effective time to spray CBM is between February and May to prevent damage to the main growth flush in July-August. Unfortunately, most effective acaricides can no longer be used after petal fall due to residue problems and propargite which is acceptable is no longer being produced. There is therefore a desperate need for alternative chemicals to be registered for the control of CBM that have a preharvest interval of less than three months. Last year, four orchard trials were conducted with a new acaricide RJU37PY that gave similar control of CBM to Acarol (Grout and Stephen 2008). Further research is now required to provide fruit samples for residue analysis and conduct non-target effects on natural enemies. Notes on the residue trials are provided below but the report on the non-target effects is under the Biocontrol Disruption project (3.6.3).

Materials and methods

Residue trials were conducted at four sites in Mpumalanga as follows: Lisbon lemons at Bakgat Boerdery Trust, Lemoneira lemons (Block D4) at Tekwane Estates and Valencias at two widely separated orchards (Town 7 and Xhosa 3B) at Crocodile Valley Citrus Company. Sprays of RJU37PY were applied to four or five trees (depending on the size and expected number of fruit) at each site on 1 July 2009 using a high-pressure (2 500 kPa) spray machine with hand guns (nozzle orifice 2.5 mm). Trees were sprayed with medium coverage to wet all foliage and fruit to the point of run-off. Dosages used were the single dosage of 150 ml/hl water and the double dosage of 300 ml/hl water. Fruit were picked for residue samples immediately after spraying, once the trees were dry, then again after 3, 7, 14 and 28 days. Control samples were picked on the day of spraying from the same orchard and after 28 days. Two samples (A and B) were picked from the trees on each occasion and each sample weighed approximately 2 kg.

The fruit were immediately frozen in chest deep freezers at CRI, Nelspruit after being picked. Considerable delays were experienced in getting a standard for the residue tests and the required protocol so the fruit remained frozen for several months before being transported to SABS for residue analysis. At the time of this report, results have not yet been finalised.

Conclusion

Residue samples were taken from two different citrus types from two sites each and sent for analysis. The company concerned will also conduct their own residue tests.

Future research

Further residue tests are not planned but spray combinations with mineral oil may be attempted if suitable sites are found. No other new acaricides are expected.

Reference cited

Grout, T.G. and P.R. Stephen. 2008. Progress Report: Alternative chemicals for citrus bud mite control. (2007-2008/9). pp. 194-197. In: CRI Group Annual Research Report 2007-8. Citrus Research International. Nelspruit, South Africa.

3.4.5 **FINAL REPORT: Control options for the lemon borer moth**
Experiment 933 (April 2008 – March 2009) by Sean D Moore and Wayne Kirkman (CRI)

Opsomming

Suurlemoenmot, *Prays citri*, is in 3 Eureka suurlemoenboorde in die Sondagsriviervallei, met die gebruik van feromoon gelaaië delta lokvalle, gemonitor. Gelyktydig is blomtrosse vir besmetting van *P. citri* larwes en papies gemonitor. Geen korrelasie tussen lokvalvangste en besmetting kon bepaal word nie. Na blomval is slegs weglaatbaar min eierlegging op vrugte gekry, met geen vrugskade nie. Baie larwes en papies is versamel, tot volwassendheid geteel en identifikasie van almal is as *P. citri* bevestig. Slegs een parasitoïed spesie is van *P. citri* larwes aangeteken. Identifikasie van die spesie is nog nie afgehandel nie. 'n Suijterproef teen larwale besmetting is uitgevoer. Die produkte Mevinphos, Ultracide en DiPel is gebruik. DiPel was van die 3 produkte, die mees doeltreffend. Geen verdere navorsing word vir hierdie eksperiment beplan nie.

Summary

Lemon borer moth, *Prays citri*, was monitored in 3 Eureka lemon orchards in Sundays River Valley, with the use of pheromone loaded delta traps. Simultaneously, blossom clusters were monitored for *P. citri* larval and pupal infestation. No correlation between trap catches and infestation could be identified. After petal drop, only negligible egg laying on fruit was recorded, with no damage to fruit. Numerous larvae and pupae were collected and reared to adulthood, confirming the identification of all of them as *P. citri*. Only one parasitoid species was recorded from *P. citri* larvae. Identification of the species is still being awaited. A spray trial was conducted against larval infestation. The products used were Mevinphos, Ultracide and DiPel. DiPel was the most effective of the 3 products. No further research is planned within this experiment.

Introduction

A few years ago, the first incidence of lemon borer moth damage was recorded on lemon fruit in the Eastern and Western Cape Provinces. Since then, its appearance has been an annual occurrence, particularly in imidacloprid treated orchards. Up to 5% crop reduction, attributed to the lemon borer moth, has been estimated in the Eastern Cape and up to 50% damage to fruit has been estimated in the Western Cape (Moore, 2003b). Originally, damage was attributed to *Cryptoblabes gnidiella*, which was recorded on lemons bearing the damage in question. However, subsequently it has been established that *Prays citri* is more likely primarily responsible for the damage, even though such damage has not previously been associated with *P. citri*. Some fairly elementary work has been conducted on chemical control options for the lemon borer moth. However, this knowledge is incomplete and as yet, no products are registered for its control. In addition, although it is known that *P. citri* has a number of larval parasitoids, little is known about their influence (and that of any other members of the natural enemy complex) on citrus in South Africa (Moore, 2003a).

Materials and methods

Monitoring

Three Eureka lemon orchards in Sundays River Valley were selected for the trial (Table 3.4.5.1). All 3 orchards had a history of lemon borer moth infestation and damage. All 3 orchards were treated with imidacloprid. Therefore, none of them were sprayed with anything more disruptive than abamectin (for citrus thrips control), a narrow spectrum and short residual "soft" product.

Table 3.4.5.1. Details of orchards in which the lemon borer moth, *P. citri*, was monitored during the 2008/09 citrus growing season.

Region	Trial sites		
	Barkley Bridge, Sundays River Valley	Addo, Sundays River Valley	Hermitage, Sundays River Valley
Farm	Blaartjiebrug	Lone Tree	Siyatemba
Farmer	Francois Joubert	Jacques Senekal	Johan Troskie
Orchard no.	2	32	1
Cultivar	Eureka lemon	Eureka lemon	Eureka lemon
Planting date	1998	1996	1996
Tree spacing (rows x trees)	6 x 4	6 x 4	6 x 3

P. citri female pheromone dispensers were obtained from Insect Science. Dispensers were placed into yellow delta traps (1 in each), also purchased from Insect Science. One loaded trap was placed into each of the orchards, in a similar position in all orchards i.e. 5th tree in 5th row on SW side of orchard. Traps were hung on 8 October and were checked weekly for 14 weeks i.e. until 19 January 2009. Pheromone dispensers were changed twice – on the first occasion after 4 weeks and then again after a further 5 weeks.

After checking traps and removing moths from the traps, blossoms were inspected for larval and pupal infestation. This was done on 10 blossom clusters on 10 data trees in each orchard. This was continued for 3-4 weeks until all blossoms had dropped off.

Once all blossoms had dropped off, inspections were conducted on fruit instead. These inspections were for eggs and gumming – penetration marks of neonate larvae. These eggs would have been laid by the second generation i.e. the moths eclosing from the pupae occurring on the earlier blossom.

Moth identification and parasitism

During the first 3 weeks of inspection, when larvae and pupae were found infesting blossom clusters, samples of each were collected from non-data trees and placed into vials. Fresh petals were included in the vials on which larvae could feed until pupation. Vials were kept in the laboratory at room temperature until either moths eclosed or parasitoids emerged. Any emerging parasitoids were placed into 70% alcohol and sent to the Biosystematics Unit of the PPRI-ARC for identification. Identification of eclosing moths was also confirmed.

Chemical control

Orchard 2 (a Eureka lemon orchard) on Blaartjiebrug Farm (Table 3.4.5.1), where blossoms were conspicuously infested with *P. citri* larvae and pupae, was used for the trial. A large section of the orchard was split into 4 quarters, each consisting of 8 rows with 16 trees in each row. A different treatment was applied to 3 of the quarters using a mistblower, and the last quarter was used as an untreated control. Sprays were applied as diffuse medium cover sprays at 8 L per tree on 27 October 2008. The treatments were Mevinphos EC (100 ml/100 L water); Ultracide EC (methidathion; 150 ml/100 L water) plus Agral 90 (18 ml/100 L water); and DiPel (Bt; 12.5 g/100 L water) plus Comodobuff (50 ml/100 L water). Although Ultracide is known to be one of the least effective organophosphates against Lepidoptera, it was included in the trial, due to its common usage in the region against other pests (e.g. citrus thrips and mealybug) at that time of year.

Efficacy of treatments was evaluated on 30 October and again on 6 November 2008. This was done by inspecting 10 blossom clusters on each of 10 trees positioned in the middle of each treatment block. Results were compared using ANOVAs and multiple range tests.

Results and discussion

Monitoring

Generally, moth catches at Lone Tree Farm were higher than at the other two sites (Tables 3.4.5.2 – 3.4.5.4). However, infestation of blossoms was highest at Blaartjiebrug Farm. There did not appear to be any correlation between trap catches and larval and pupal infestation. Even if one considered a lag-phase between the two or a possible relationship between one generation and the next, there was still no apparent relationship.

Table 3.4.5.2. *P. citri* pheromone trap catches and larval and pupal infestation in a lemon orchard on Blaartjiebrug Farm, Barkley Bridge.

Date	Moths/trap/week	Infestation (% blossom clusters infested)			Comments
		Larvae	Pupae	Total	
13 Oct 08	45	6	8	14	
23 Oct 08	21	16	2	18	
30 Oct 08	16	12	15	27	
6 Nov 08*	8	10	8	18	
		Infestation (per individual fruit)			
		Eggs	Gumming		
13 Nov 08	43	-	-		No signs of eggs or gumming
18 Nov 08	2	-	-		No signs of eggs or gumming
25 Nov 08	11	-	-		No signs of eggs or gumming
3 Dec 08	11	-	-		No signs of eggs or gumming
10 Dec 08*	12	-	-		Few eggs and gumming on out of season fruit near windbreak

18 Dec 08	52	-	-	As above
23 Dec 08	66	-	-	As above
6 Jan 09	78	-	-	No signs of eggs or gumming
13 Jan 09	63	-	-	No signs of eggs or gumming
19 Jan 09	63	-	-	Few eggs and gumming on out of season fruit near windbreak

*Date on which pheromone dispenser was changed.

Table 3.4.5.3. *P. citri* pheromone trap catches and larval and pupal infestation in a lemon orchard on Lone Tree Farm, Addo, Sundays River Valley.

Date	Moths/trap/week	Infestation (% blossom clusters infested)			Comments
		Larvae	Pupae	Total	
13 Oct 08	111	0	2	2	
23 Oct 08	39	1	1	2	
30 Oct 08	32	0	0	0	
		Infestation (per individual fruit)			
		Eggs	Gumming		
6 Nov 08*	17	-	-	-	No signs of eggs or gumming
13 Nov 08	35	-	-	-	No signs of eggs or gumming
18 Nov 08	110	-	-	-	No signs of eggs or gumming
25 Nov 08	5	-	-	-	No signs of eggs or gumming
3 Dec 08	5	-	-	-	No signs of eggs or gumming
10 Dec 08*	13	-	-	-	No signs of eggs or gumming
18 Dec 08	0	-	-	-	No signs of eggs or gumming
23 Dec 08	13	-	-	-	No signs of eggs or gumming
6 Jan 09	103	-	-	-	No signs of eggs or gumming
13 Jan 09	9	-	-	-	No signs of eggs or gumming
19 Jan 09	16	-	-	-	No signs of eggs or gumming

*Date on which pheromone dispenser was changed.

Table 3.4.5.4. *P. citri* pheromone trap catches and larval and pupal infestation in a lemon orchard on the Siyatemba Farm, Hermitage, Sundays River Valley.

Date	Moths/trap/week	Infestation (% blossom clusters infested)			Comments
		Larvae	Pupae	Total	
13 Oct 08	22	1	2	3	
23 Oct 08	15	0	1	1	
30 Oct 08	0	0	0	0	
		Infestation (per individual fruit)			
		Eggs	Gumming		
6 Nov 08*	8	0	0	0	No signs of eggs or gumming

13 Nov 08	53	0	0	No signs of eggs or gumming
18 Nov 08	34	0	0	No signs of eggs or gumming
25 Nov 08	31	0	0	No signs of eggs or gumming
3 Dec 08	31	0	0	No signs of eggs or gumming
10 Dec 08*	35	0	0	No signs of eggs or gumming
18 Dec 08	0	0	0	No signs of eggs or gumming
23 Dec 08	-	-	-	No access (gate locked)
6 Jan 09	-	-	-	No access (gate locked)
13 Jan 09	-	-	-	No access (gate locked)
19 Jan 09	80	0	0	No signs of eggs or gumming

*Date on which pheromone dispenser was changed.

In trials conducted a few years ago, larval and pupal infestation of blossom clusters was followed by egg laying (average of 1.9 eggs per fruit in the untreated control) and concomitant gumming on fruit (Moore & Kirkman, 2004). In this case, trap catches peaked at over 300 moths per trap per week, unlike the 2008 trial in which the highest trap catch was 111 moths per trap per week (Table 3.4.5.3). Admittedly, one difference was the use of bucket traps in 2004 as opposed to delta traps in 2008. It is not clear what difference this would make to catches. However, it is possible that the delta trap would catch higher numbers of moths than the bucket trap, due to its design. In this case, *P. citri* pressure in 2008 would be even lower than indicated by trap comparisons with the 2004 study. These 2008 catches may be too low to lead to a second generation ovipositing on and damaging fruit. Substantially more work would be required to try and establish a correlation between trap catches and infestation (of blossoms (1st generation) or fruit (2nd generation)), or to establish a trap threshold for intervention.

Moth identification and parasitism

All moths developing from larvae and pupae collected from blossoms, were confirmed to be *P. citri*. This confirmed the identification of the lemon borer moth and the appropriate usage of the *P. citri* pheromone dispenser in traps.

Only one parasitoid was found. Identification is still being awaited. There are a number of larval parasitoids known to attack *P. citri*. Of these, *Chelonus* sp. is the most important (Moore, 2003a).

Chemical control

Within 3 days after spraying, all 3 treatments had significantly reduced infestation (Table 3.4.5.5). There was no significant difference in efficacy between the treatments. A week later, infestation in the Ultracide treated block was no longer significantly different from the untreated control. At this stage, infestation in the DiPel treatment was the lowest. Being the most IPM-compatible of the products tested, this was encouraging. From the first to the second evaluation (7 days) there had been no decline in infestation for the two chemical treatments. However, infestation in the untreated control and where DiPel had been applied, had declined markedly.

Table 3.4.5.5. *P. citri* infestation of lemon blossom clusters after various treatments, applied on 27 October 2008 on Blaartjiebrug Farm, Barkley's Bridge.

Treatment		Infestation of blossom clusters (%)*	
		30 October 2009	6 November 2009
Untreated control	Larvae	12	10
	Pupae	15	8
	Total	27a	18a
Mevinphos	Larvae	3	6
	Pupae	4	0

	Total	7b	6b
Ultracide	Larvae	4	9
	Pupae	5	1
	Total	9b	10ab
DiPel	Larvae	5	3
	Pupae	7	1
	Total	12b	4b

*Total infestation values in the same column followed by the same letter are not significantly different (P<0.05; Bonferroni LSD multiple range test).

The ultimate test of the efficacy of these treatments would have been the measurement of impact on the ensuing generation. Although heavy infestations of the blossom might arguably result in crop reduction, far more severe damage has been observed on the fruit, caused by penetration of larvae hatching from eggs laid by the generation infesting the preceding blossom. Not only can this sort of damage render the blemished fruit unmarketable, but up to 50% crop reduction has been reported (Moore, 2003b). Unfortunately, no gumming and almost no infestation of eggs on fruit were recorded.

Spray trials conducted in 2003 showed all products tested to be effective in reducing egg laying and damage to fruit (Moore *et al.*, 2003). This included Mevinphos and DiPel. In similar trials conducted with Mevinphos and DiPel the following year (Moore & Kirkman, 2004), both of these products were again very effective in reducing larval and pupal infestation, but were not very effective in reducing the damage caused by the following generation. Although treated blocks consisted of 112 trees, reinfestation may still have occurred too easily.

Conclusion

Delta traps loaded with *P. citri* pheromone dispensers proved successful in trapping moths of this species. However, no correlation between trap catches and larval and pupal infestation of blossom clusters was evident. No egg laying was observed on lemon fruitlets. Only one parasitoid was recovered from *P. citri* larvae. Identification is still forthcoming. A spray trial showed mevinphos, methidathion and *Bacillus thuringiensis* all to control *P. citri* larvae. *Bt* was the most effective.

Future research

No further research is planned within this experiment. However, if justified by the industry as a priority, further work should be conducted to establish a trap related intervention threshold and to determine the relationship between blossom infestation and subsequent egg laying on fruit.

Technology Transfer

No technology transfer has been conducted from this experiment. However, both a scientific publication and a paper in the SA Fruit Journal are planned.

References cited

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- Moore, S.D. 2003b. The lemon borer moth: a new citrus pest. *SA Fruit Journal* 2(5): 37-41.
- Moore, S.D., Richards, G.I. & Kirkman, W. 2003. The status and control of new moth pests on lemons. In: *CRI Group Annual Research Report 2003*, pp. 73-75.
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3.4.6 **PROGRESS REPORT: Treatments for the control of leafhoppers on citrus** Experiment 942 (2008/9-2009/10) by Tim G Grout, Peter R Stephen and Charl Kotze (CRI)

Opsomming

Bladspringers kan ernstige kosmetiese plae wees, maar uitbrake is sporadies, wat moontlik die rede is hoekom daar nie chemiese bespuitings vir hul beheer geregistreer is nie. Mevinphos en methomyl is bekend om effektief te wees van vroeëre CRI proefwerk, maar meer inligting word vir registrasie doeleindes benodig. Alhoewel daar vir persele in die Noord-Kaap en Mpumalanga Hoëveld gesoek is, is slegs een perseel met 'n lae populasie digtheid van sitrus bladspringer gevind. 'n Proef wat daar gespuit is het bevestig dat mevinphos SL teen 30 of 45 ml/hl of methomyl SP teen 40 of 60 g/hl water effektief is vir die beheer van sitrus bladspringer met redelike lae volume bespuitings (5 000 l/ha). Wanneer persele beskikbaar kom sal verdere proewe uitgevoer word.

Summary

Leafhoppers can be serious cosmetic pests but outbreaks are sporadic so perhaps this is why no chemical sprays have been registered for their control. Mevinphos and methomyl are known to be effective from earlier CRI trial work but more data is required for registration purposes. Although sites were looked for in the Northern Cape and Mpumalanga Highveld, only one site was found with a low population density of citrus leafhopper. A trial was sprayed there which confirmed that mevinphos SL at 30 or 45 ml/hl or methomyl SP at 40 or 60 g/hl water were effective at controlling citrus leafhopper using moderately low volume sprays (5 000 l/ha). Further trials will be conducted when sites become available.

Introduction

Leafhoppers can cause cosmetic injury to oranges in particular between January and harvest. However, due possibly to their sporadic nature and the relatively small market for insecticides, no chemicals are registered for leafhopper control on citrus. Earlier research by Grout and Moore resulted in the comments in the Production Guidelines (Moore 2003) that low volume sprays of endosulfan, methomyl and mevinphos are effective against leafhoppers. However, GlobalGAP requirements are that only registered treatments should be used so some of these products need to be registered. Chlorpyrifos and methidathion may still be used late in the season for most markets and would probably be effective against leafhoppers, but their residual effect on parasitoids at this time of year may result in an increase in red scale. Emphasis has therefore been placed on acquiring more data on mevinphos and methomyl.

Materials and methods

Orchards were monitored in the Vaalharts area by Ms. J. Mathewson with yellow sticky traps, but no suitable infestations of leafhoppers were found in the 2008-9 season. Frequent communication with growers in the Groblersdal-Marble Hall region resulted in only a single suitable trial site in this report period.

Fruit in orchards used for leafhopper trials often already have blemishes and these pre-treatment blemishes are impossible to distinguish from post-treatment blemishes. Yellow sticky traps are therefore used for evaluating treatments. Leafhoppers occur on outside and inside fruit so medium cover sprays are required. However, because the insects are very mobile and readily come into contact with residues, treatments can be applied at low volume and at a moderate ground speed of ± 4 km/h. The single site used was at Schoeman Boerdery, Marble Hall where citrus leafhopper numbers appeared high enough for a trial. A block of 10-year-old Delta Valencias was used where, on 4 and 5 June 2008 each treatment was applied to 2 blocks of trees (8 rows by 12 trees per row). Treatments were randomly awarded to each block, according to average leafhopper counts prior to treatment. However, after 1 mm rain fell during spraying of treatment 4, the treatment was re-applied to two additional blocks allocated next to the existing replicates. Treatments were applied with a mistblower at a calculated 6.17 l/tree or 5 140 l/ha.

Speed:	3.4 km/h
Pressure:	15 bar
Gear:	Tortoise 3
Tractor:	B11
Spray machine:	B4
Nozzle disc:	D3
Whirler core:	35

One week prior to treatment, two yellow sticky traps [Chempac (Pty) Ltd, P. O. Box 516, Suider Paarl] were placed in the centre of each treatment block between rows four and five. The traps were removed for counting prior to application of treatments and then approximately one hour after treatment four fresh traps were placed per block.

Results and discussion

Only results for the brown citrus leafhopper (*Penthimiola bella*) are shown (Table 3.4.6.1) as the green leafhopper was present in very low numbers. The numbers of citrus leafhopper present were below the suggested intervention threshold of 35 per yellow trap per week (Moore 2003). Even with the use of 96 trees per replicate and the sprayed block being eight rows wide, post-spray infestation levels in the control declined slightly, due most likely to movement of leafhoppers into treated areas. However, all treatments reduced the citrus leafhopper population significantly and there were no significant differences between sprays. The effect of 1 mm rain on the methomyl 40 g/hl efficacy appeared to be negligible. The previously recorded efficacy of methomyl at 60 g or 270 ml/hl water (Moore 2003) was confirmed but it may be possible to reduce this concentration to 40 g or 180 ml/hl water (Table 3.4.6.1). The rate of mevinphos SL at 45 ml/hl water would also be adequate for this pest but the reduced rate of 30 ml/hl may prove inadequate at high population densities because the numbers of trapped citrus leafhoppers showed a non-significant increase at the relatively low populations experienced in this trial (Table 3.4.6.1).

Table 3.4.6.1. Mean numbers of citrus leafhopper per yellow sticky trap before and after spraying.

Treatments Applied 4 and 5 June 2009	Concentration (per 100 L water)	Pre-spray 5 June	1 week post-spray 11 June
Control	Unsprayed	11.8	7.3 a
Mevinphos 500 SL	30 ml (= 100 ml of 150 EC)	15.5	1.1 b
Mevinphos 500 SL	45 ml (= 150 ml of 150 EC)	10.0	0.5 b
Methomyl 900 SP	40 g (= 180 ml of 200 SL)	-	0.9 b
Methomyl 900 SP followed by 1 mm rain	40 g	14.0	0.5 b
Methomyl 900 SP	60 g (= 270 ml of 200 SL)	8.8	0.6 b

Means followed by the same letter are not significantly different ($P > 0.05$ SNK test)

Conclusion

Only one suitable site could be found for a spray trial and there the numbers of citrus leafhopper were below the intervention threshold. However, mevinphos SL at 30 or 45 ml/hl or methomyl SP at 40 or 60 g/hl water were all effective at controlling the leafhopper with moderately low volume sprays (5 000 l/ha).

Further objectives and work plan

As leafhoppers are sporadic pests and only one suitable site was found last season, this research will have to continue until adequate trial data has been gathered to allow for registration.

Technology transfer

After only one trial result it is premature for any technology transfer.

Reference cited

Moore, S.D. 2003. Leafhoppers and planthoppers. pp. 63-65. In: Integrated Production Guidelines for Export Citrus Volume III: Integrated pest and disease management. Citrus Research International, Nelspruit.

3.5 PROJECT: MEALYBUG AND OTHER PHYTOSANITARY PESTS

Project Coordinator: Sean Moore (CRI)

3.5.1 Project summary

Only two experiments were conducted within this project during the 2008/09 research cycle. The first study was carried out to determine the rate of development, survival and fecundity of oleander mealybug (3.5.2). It was determined that citrus mealybug exhibited lower developmental thresholds and higher rates of development than oleander mealybug at both 1st and 2nd instars. In the second experiment a laboratory culture of *Leptomastix* spp. was established and developed through a few generations (3.5.3). However, insufficient parasitoids emerged to conduct experiments on the biology of the parasitoids or augmentative release trials.

Projekopsomming

Net twee eksperimente is gedurende die 2008/09 navorsingssiklus op hierdie projek uitgevoer. Die eerste studie is uitgevoer om die ontwikkelingstempo, oorlewing en vrugbaarheid van die oleander-witluis te bestudeer (3.5.2). Dit is bepaal dat die sitruswitluis se ontwikkelingsdrumpel laer was en dat dit vinniger ontwikkel het as oleander witluis by beide die eerste en tweede stadiums. In die tweede eksperiment is 'n laboratorium kultuur van *Leptomastix* spp. Gestig, wat deur 'n paar generasies ontwikkel het (3.5.3). Ongelukkig het te min parasitoïede ontpop om hulle biologie te bestudeer of om vrylatingsproewe uit te voer.

3.5.2 PROGRESS REPORT: Biology of the oleander mealybug, *Paracoccus burnerae*

Experiment SU-E-2006 (January 2007 – December 2008): by JH Giliomee and Todd Johnson (SU)

Opsomming

In hierdie studie is die ontwikkelingstempo, oorlewing en vrugbaarheid van die oleander-witluis, *Paracoccus burnerae* by verskillende konstante temperature op sitrusplante bestudeer. Die tempo van ontwikkeling van hierdie spesie is met die van die sitruswitluis, *Planococcus citri*, vergelyk ten einde vas te stel waarom dit laasgenoemde in sekere dele van die Oos- en Weskaap uitkompeteer. Vyf konstante temperature en 'n 16L:8D lig/donker siklus is gebruik. Die tempo van ontwikkeling het versnel met 'n toename in temperatuur, terwyl die oorlewing afgeneem het. 'n Ontwikkelingsdrumpel van 13.6°C en 'n termiese konstante van 98.4 daggrade is vir die eierstadium beraam. Die vrugbaarheid van wyfies wat by konstante temperature geteel is, was laer as die van wyfies wat by wisselende temperature geteel is. Ontleding van die lewenstabel toon aan dat die reprodutiewe tempo 3.3 is en die gemiddelde generasietyd 78.25 dae is; die geslagsverhouding is 0.53:0.47 (manneling, wyfie). *P. burnerae* se dominansie kon nie aan die ontwikkelingstempo toegeskryf word nie aangesien die sitruswitluis se ontwikkelingsdrumpel laer was en dit vinniger ontwikkel het as *P. burnerae* by beide die eerste en tweede stadiums.

Summary

The study was carried out to determine the rate of development, survival and fecundity of the oleander mealybug, *Paracoccus burnerae*, at various constant temperatures on citrus plants. Its rate of development was compared with that of the citrus mealybug, *Planococcus citri*, to determine why it has out-competed the latter in certain parts of the Eastern and Western Cape. Development, fecundity and survival were investigated at five constant temperatures and a 16L: 8D light-darkness phase. Rate of development increased with an increase in temperature while survival decreased with an increase in temperature. A lower developmental threshold (T_0) of 13.6°C and thermal constant (K) of 98.4 degree-days were estimated for the egg stage. Fecundity of females raised at constant temperature was lower than that of females raised at varying temperature. A net reproductive rate (R_0) of 3.3, mean generation time of 78.25 days and sex ratio of 0.53:0.47 (male: female) were obtained from the life table. *P. burnerae*'s dominance could not be associated with development rate as the citrus mealybug, *P. citri* exhibited lower developmental thresholds and higher rates of development than *P. burnerae* at both 1st and 2nd instars.

Introduction

The oleander mealybug, *Paracoccus burnerae* (Brain) is indigenous to the southern and eastern Africa subregions. It was first described by Brain (1915) and redescribed by De Lotto (1967). This is a mealybug that belongs to the family Pseudococcidae comprising approximately 2000 species in 300 genera (Ben-Dov

1994) in Millar (2002). Twenty of these species are pests of cultivated plants in South Africa (Millar 2002). Five species of *Paracoccus* are known to occur in South Africa.

According to Bedford *et al.* (1998), *P. burnerae* only became markedly more prevalent during the early 1990s. Reasons for the increase in prevalence are not yet known. It is found in all citrus growing zones of southern Africa and Hattingh *et al.* (1994) in Bedford *et al.* (1998) describe it as being problematic in KwaZulu-Natal and Swaziland. Apart from that, it has also become a widespread and dominant pest of citrus in some parts of the Eastern and Western Cape of South Africa. Its presence on citrus fruits entails reduced quality of fruit bound for export to overseas markets. Due to phytosanitary concerns raised in the fruit export sector, *P. burnerae* has now become one of three species with a quarantine status (Wakgari and Giliomee 2003). It does not only affect citrus but is also known to attack plants in a wide range of families. (<http://www.sel.barc.usda.gov/scalenet/scalenet.htm>).

Although parthenogenesis is common in mealybugs, *Paracoccus burnerae* reproduces sexually. The adult female is neotenic and undergoes three nymphal instars in its development while the male has three. As an ectotherm and multivoltine insect species, *P. burnerae* populations survive low winter temperatures in the cracks of trees and inside curled leaves (Bedford *et al.* 1998). In ectotherms, temperature is the deciding factor affecting the development rate. When the temperature is low, development occurs at a much slower rate than at high temperature (Jarosik *et al.* 2004), and subsequently time spent in each developmental stage increases. In reality, the relationship between temperature and development in insects is often linear but can also be sigmoid. Linear models are good for estimation of the developmental thermal constant. Both thermal constant (K) and lower developmental threshold (T_0) are frequently employed to explain how insect development is dependent on temperature (Aysal *et al.* 2008). However, the adjusted Logan model depicts a nonlinear relationship between rate of development and temperature, and can be used to estimate a developmental threshold above which development occurs, optimum temperature for development and upper lethal temperature at which death transpires (Pilkington *et al.* 2007).

Life tables and developmental rates are essential tools for investigating and understanding the impact temperature has on growth, survival, reproduction and rate of increase of an insect population. Life tables are especially important in understanding age dynamics of adult populations studied under controlled laboratory conditions (Aysal *et al.*, 2008) and in tackling the issue of life expectancy when affected by environmental changes (Pilkington *et al.* 2007).

The objective of the present study was to determine the development rate, survival and fecundity of *P. burnerae* at a range of constant temperatures as well as to investigate why *P. burnerae* seems to be outcompeting the citrus mealybug, *Planococcus citri* in some parts of the Eastern and Western Cape.

Materials and methods

Laboratory cultures of oleander mealybug were obtained from a colony being reared on citrus in the greenhouse at the Botany and Zoology Department of the University of Stellenbosch.

Fecundity, development time and survival of *P. burnerae* were determined at 20, 22, 25, 27 and 30°C using growth chambers with humidity varying between 40 and 70% and a light darkness phase of L16:D8.

Ovipositing females were obtained from the greenhouse and introduced onto potted citrus seedlings. The seedlings were then incubated and the females were allowed at least 24 hours to lay eggs before being withdrawn. A maximum of 10 eggs from each female ovisac were retained on each of the five seedlings. The development of each individual was observed under the microscope and recorded daily. Survivorship of the cohort was followed and recorded until the last surviving individual died.

Fecundity of *P. burnerae* was only observed at a temperature of 25°C. When the females reached adulthood and there were no males available from the same cohort within the growth chamber, they were provided males from the greenhouse. Once mating was achieved and formation of ovisac observed, egg counting was then conducted daily until no further oviposition took place. The eggs were collected in small trays and counted under a stereomicroscope.

Pre-ovipositing adult females reared on citrus in the greenhouse were introduced onto potted seedlings. The seedlings were then incubated at 25°C. When the formation of an ovisac was observed, the eggs were counted every week until no further oviposition took place. Their fecundity was then compared with that of females entirely bred on citrus at a controlled temperature.

Results and discussion

Developmental times

The mean development time of the egg increased with a decrease in temperature from 6.3 days at 30°C to 15.0 days at 20°C ($F = 66.7$, $df = 3$, $p = 0.003$). Females developed faster than males at 25°C. Females took 40 days to develop from egg to adult and males took 42.6 days (Table 3.5.2.1). Development somehow took longer in the 1st instar at all the four temperatures (20, 22, 25 and 27°C) than did any growth stage thereafter.

Table 3.5.2.1. Developmental times in days (mean±SEM) for each stage of *Paracoccus burnerae* on citrus at five constant temperatures.

Developmental stage	Temperature (°C)				
	20	22	25	27	30
Egg	15.0 (0.41)	13.0 (0.65)	8.2 (0.37)	6.8 (0.58)	6.3(0.21)
First instar	29.2 (1.72)	27.0 (2.35)	12.7 (0.97)	12.0 (1.12)	-
Second instar	15.8 (1.4)	12.0 (4.0)	10.8 (0.73)	7.7 (0.56)	-
Third instar	31.0	30.0	8.4 (0.6)	23.0	-
Male pupa	19.0	*	11.0 (0.26)	8.3 (0.88)	-
Adult male	*	*	4.3 (0.26)	2.3 (0.67)	-
Adult female	*	*	34.6 (4.12)	*	-
Egg to adult (females)	*	*	40.1 (0.67)	49.5	-
Egg to adult (males)	*	*	42.7 (0.58)	34.8 (0.79)	-

Note: * denotes experiments which are still ongoing.

- denotes no development observed beyond this point.

A positive linear relationship was obtained when developmental rate was regressed on temperature. Developmental rate increased with an increase in temperature. The lower developmental threshold and thermal constant (K) obtained from the regression equation were 13.6°C and 98.4 degree-days respectively for the egg phase at all five temperatures (Table 3.5.2.2). Lower developmental thresholds for the 1st and 2nd instars were slightly higher than that for the egg although 30°C was excluded from the analysis (Figs. 3.5.2.1 & 3.5.2.2).

Table 3.5.2.2. Thermal constant values and regression coefficients for the immature stage development of *Paracoccus burnerae*.

Parameters	Egg	1 st instar	2 nd instar
T_0 (°C)	13.6	16.2	12.7
K (degree-days)	98.4	123.5	117.6
$y = a + bx$	$-0.1377 + 0.01016x$	$-0.1316 + 0.0081x$	$-0.1078 + 0.0085x$
R^2	0.957	0.913	0.901
P	0.004	0.045	0.051

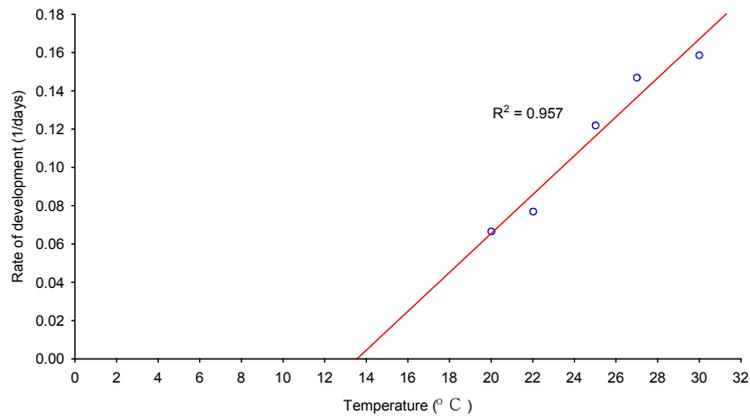


Fig. 3.5.2.1. Developmental rate of *Paracoccus burnerae* for the egg stage at five temperatures

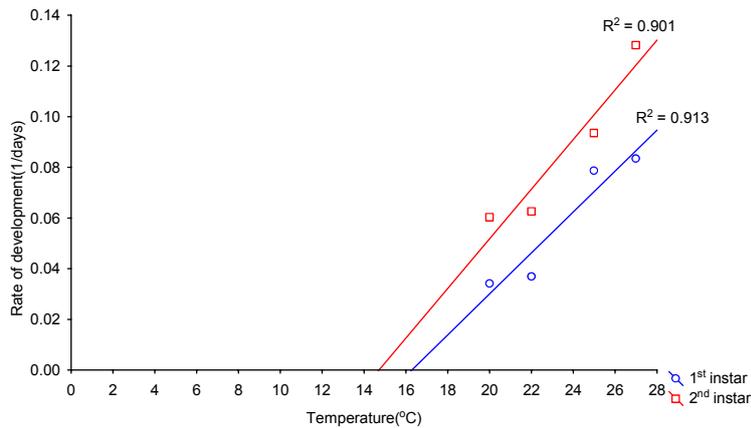
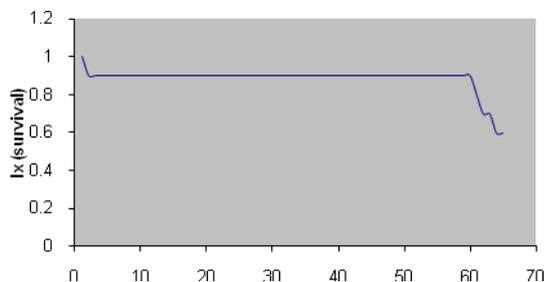


Fig. 3.5.2.2. Developmental rate of *Paracoccus burnerae* for the 1st and 2nd nymphal instars at four temperatures.

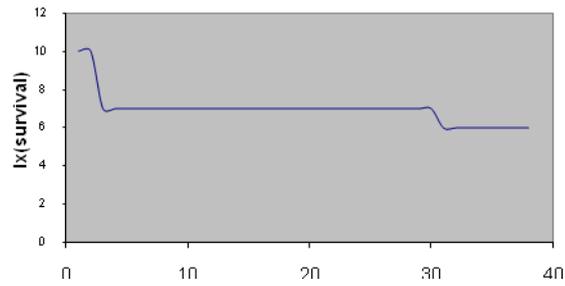
Survival and life table for *P. burnerae*

The survival of *P. burnerae* without any mortality was longest at 20°C. A noticeable decrease in longevity was observed with an increase in temperature (Fig. 3.5.2.3). At 30°C, nymphs were unable to moult beyond the first instar but those that resisted the high temperature survived for more than 60 days. At 25°C, a female had a total average life span close to 90 days (89.4 ± 15.1 days) and there was a 60% survival to the adult stage. Adult female survival was 26.7% and a total mortality of 73.3% was experienced to the adult female stage but no mortality was observed in the pupal stage. The net reproductive rate (R_0) at 25°C was 3.3 (Table 3.5.2.3) and an adult sex ratio of 0.53:0.47 (male: female) was obtained from the life table (Table 3.5.2.4).

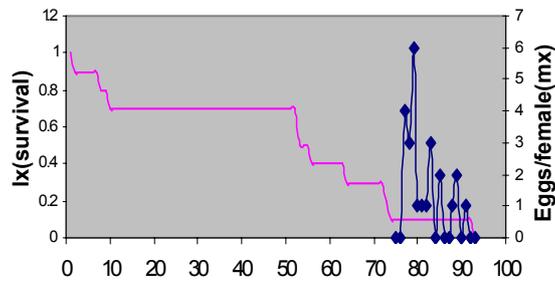
20° C



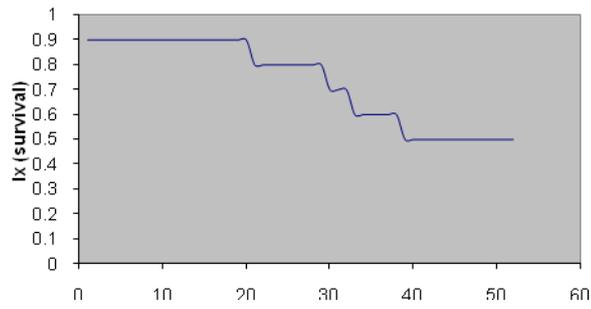
22° C



25° C



27° C



30° C

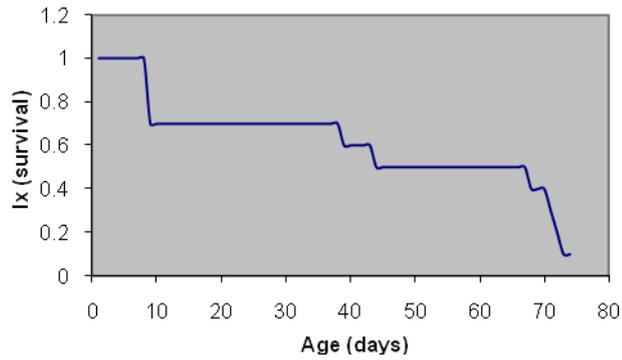


Fig. 3.5.2.3. Age specific survival (l_x) of *Paracoccus burnerae* at five temperatures.

Table 3.5.2.3. Population growth parameters for *Paracoccus burnerae* at 25°C.

Parameter	Value
Net reproductive rate (R_0)	3.3
Intrinsic rate of increase (r_m)	0.015
Doubling time (D)(days) $\ln 2/r_m$	46.21
Mean generation time (T)(days)	78.25

Table 3.5.2.4. Life table for *P. burnerae* at 25°C. Data based on a cohort of 30 individuals.

Stage	Initial no. of insects (nx)	No. dying	Mortality (dx)	Survival (lx)
Egg	30	8	0.267	0.733
Instar I-III	22	5	0.227	0.773
Pupa	17	0	0	1
Adults Sex ratio: (♂:♀) 0.53:0.47	17	9	0.529	0.471
Adult females	8			
		22	73.33	0.267

Fecundity

Oviposition at 25°C started 2.5 ± 0.58 days after mating and lasted for 12.3 ± 1.33 days. Post-oviposition lasted 2.75 ± 0.48 days. Greenhouse bred females induced to oviposit in growth chambers exhibited high fertility as they laid more eggs than females completely raised in the growth chamber at constant temperature. The former laid an average of 106.8 ± 51.0 while the latter laid 29.6 ± 8.93 eggs per female. A t-test analysis conducted on the fecundity of these females, revealed significant differences between the two means, with $t = 3.33$, $df = 8$ and $p < 0.02$ (Fig. 3.5.2.4).

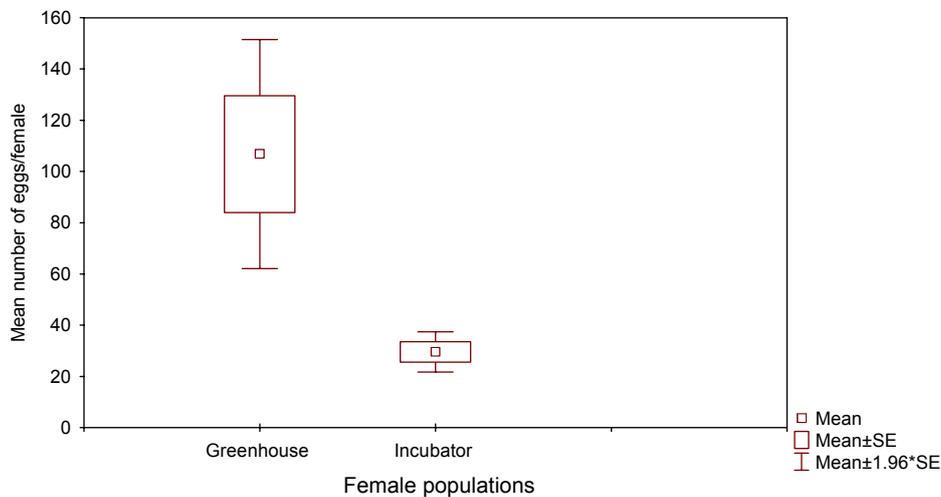


Fig. 3.5.2.4. Comparison of fecundity between *P. burnerae* females reared in greenhouse and laboratory (incubator) conditions.

In this study, temperature had an impact on the development, survival and fecundity of *P. burnerae*. Survival in the young stages was prolonged at low temperatures compared to high temperature. Nevertheless, the surviving 1st instar nymphs, unable to moult beyond the 2nd instar at 30°C, were able to persist for more than 60 days, a clear indication that this species is able to survive under harsh field conditions. The egg, first and second nymphal instars revealed a linear relationship between development rate and temperature. No upper developmental threshold was obtained, as there was no turning point in the graph. The development time decreased with an increase in temperature from 20°C to 30°C. At lower temperature this is expected because initially the nymphs convert less food into body tissue resulting in low growth and longer developmental times (Atkinson and Sibly, 1997).

First nymphal instars were observed to migrate from the leaves to the stem where they hid in cracks for a few days until the mealy waxy layer thickened before migrating back to the leaves to begin feeding. This might have been one way of avoiding the effects of desiccation soon after hatching.

Studies on the development of *P. burnerae* on citrus by Hattingh *et al.* (1998) at 27°C, revealed longer developmental times than those obtained in this study for the 1st and 2nd nymphal stages, though they found a shorter developmental time in the egg stage. This shorter developmental time in the egg stage could be attributed to incubating slightly older eggs. In this study, freshly laid eggs were used at all stages. When dark eggs, two or three days old were incubated at 27°C, a shorter development time was also obtained.

A comparison of the 1st and 2nd nymphal developmental rates of *P. burnerae* and *P. citri* at four temperatures (20, 22, 25 and 27°C) gives a clear indication that the citrus mealybug (*P. citri*) actually does better than the oleander mealybug (Fig. 3.5.2.5). *P. citri* exhibited lower developmental thresholds of 12.8°C and 8.91°C, compared to 16.2°C and 12.7°C for *P. burnerae* in both stages respectively (Table 3.5.2.5) (Hattingh *et al.*, 1998). Hattingh *et al.* (1998) found that the developmental rate of *P. burnerae* on citrus in the field was much slower than *P. citri*. Since *P. burnerae* is a tropical species, its lower developmental threshold is expected to be much higher than that of *P. citri*. Tropical species have been found to have lower developmental thresholds (Honek, 1996 in Charnov and Gillooly, 2003). If this is the case, *P. burnerae*'s dominance can therefore not be attributed to its development rate.

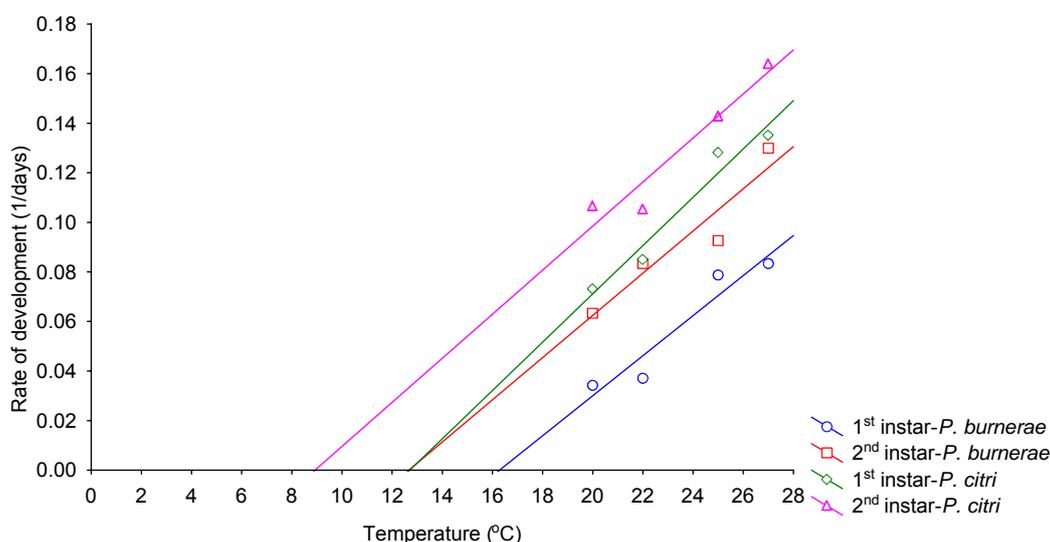


Fig 3.5.2.5 A comparison of the 1st and 2nd instar developmental rates and lower developmental thresholds for *P. burnerae* and *P. citri*. (Source: *P. citri* data - Arai, 1996)

Table 3.5.2.5. Comparison of thermal constant values and regression coefficients for the immature stages of *P. burnerae* and *P. citri*.

Parameters	<i>P. burnerae</i>		<i>P. citri</i>	
	1 st instar	2 nd instar	1 st instar	2 nd instar
T ₀ (°C)	16.2	12.7	12.8	8.91
K (degree-days)	123.5	117.6	103.1	112.4
y= a + bx	-0.1316+0.01016x	-0.1078+0.0081x	-0.1238+0.0097x	-0.0793+0.0089x
R ²	0.913	0.901	0.957	0.922
P	0.045	0.051	0.022	<0.04

Constant high temperature also seems to be detrimental to the fertility of *P. burnerae* females raised in growth chambers their entire life. The number of eggs they produced was much less than that of females raised under field conditions. When a life table was constructed, the net replacement rate at 25°C was found to be 3.3, suggesting that every adult female was being replaced by an average of three daughters (females) although the generation time was much longer (78.25 days). The low r_m of 0.015 suggests a low rate of increase in *P. burnerae* populations in the wild, which may not be case as the cause of this lowered fertility has been linked to constant temperature.

Although there was an increase in the development rate with temperature, an upper developmental threshold could not be estimated. Therefore, there is need to conduct further experiments at higher temperatures to determine when development of *P. burnerae* ceases. Complete data on the development rate at all five temperatures is necessary for estimation of the lower developmental threshold and degree-days for the whole period from egg to adult for *P. burnerae*.

In this study, it has been shown that *P. burnerae*'s development rate is slower than that of *P. citri*. Its dominance in some parts of South Africa is, therefore, not linked to development rate but maybe due to fecundity, natural enemies, pesticides and feeding habits. This uncertainty requires further investigation on the actual cause of this prevalence. Results on the effect of temperature on fecundity and longevity remain inconclusive. Only when the effect of temperature on fecundity and longevity in the remaining experiments is known and tested can correct conclusions be drawn.

Conclusion

Although the oleander mealybug can survive at constant high temperature, it was unable to develop beyond the 1st nymphal stage. *P. burnerae* was able to survive longer with less mortality at lower temperatures. Constant high temperature is unfavorable to the survival and reproductive potential of the oleander mealybug. A comparison of the development rates of both the citrus and oleander mealybugs suggest the latter's dominance is not due to development rate.

Further objectives (milestones) and work plan

In the next six months (from June 2009), we intend to complete the development, survival and fecundity of *P. burnerae* at five temperatures thus excluding 30°C. Its biology under South African climatic conditions in the field and the rearing of parasitoids are almost complete. The rearing of parasitoids will however continue up to January 2010. Experiments to identify suitable hosts for mass rearing of *P. burnerae* as well as to establish which stages parasitoids prefer for oviposition are ongoing and we hope to be through by December 2009.

Technology transfer

This information will be presented at the Entomological Congress to be held in Stellenbosch in July 2009.

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3.5.3 PROGRESS REPORT: *Leptomastix* spp. as biocontrol agents for control of mealybug, with particular reference to oleander mealybug, *Paracoccus burnerae*
Experiment 934 (April 2008 – March 2011): by Wayne Kirkman and Sean Moore (CRI)

Summary

This experiment proposes to attempt trapping and rearing of *Leptomastix* spp. parasitoids to investigate augmentative releases against oleander mealybug, *P. burnerae*, in citrus orchards. A healthy culture of *P. burnerae* was built up on sprouting potatoes. Attempts to rear *P. burnerae* on butternuts failed. Mealybug infested potatoes were placed in citrus orchards in an attempt to trap parasitoids. Several trappings were conducted in each of two orchards, one in Hankey and the other at the Citrus Foundation Block in Uitenhage. Few parasitoids were trapped, possibly due to the low occurrence of *P. burnerae* in citrus orchards at the time of trapping. A laboratory culture of *Leptomastix* spp. was established, and has developed through a few generations, which would indicate that the rearing method is sound. However, insufficient parasitoids have emerged to conduct experiments on the biology of the parasitoids or augmentative release trials. Trapping will continue in order to produce enough parasitoids to assess the efficacy of augmentative releases of *Leptomastix* spp. as a control method for *P. burnerae*.

Opsomming

Die doel van hierdie eksperiment was om *Leptomastix* spp. parasitoïede te vang, te teel en die effek van bykomende vrylatings van *Leptomastix* spp. teen oleander wiluis, *P. burnerae*, in sitrusboorde te bepaal. 'n Goeie kultuur van *P. burnerae* is op aartappelspruite opgebou. *P. burnerae* teel nie goed aan op botterskorsies nie. Aartappels, wat met *P. burnerae* besmet is, is in sitrusboorde geplaas in 'n poging om *Leptomastix* spp. te vang. Verskeie sulke pogings is aangewend in twee boorde, een in Hankey en die ander by die Sitrusgrondvesblok in Uitenhage. Min parasitoïede is gevang, waarskynlik as gevolg van min *P. burnerae* in dié boorde. 'n Laboratoriumkultuur van *Leptomastix* spp. is gevestig en 'n paar generasies is opeenvolgens geteel, wat wys dat die teelmetode doeltreffend is. Ongelukkig het te min parasitoïede ontpop om hulle biologie te bestudeer of om vrylatingsproewe uit te voer. Die vang van die parasitoïede sal voortgaan totdat groot genoeg getalle gekry word om die doeltreffendheid van vrylatings van *Leptomastix* spp., vir die beheer van *P. burnerae*, te ondersoek.

Introduction

From 2002 until recently, an experiment was conducted to investigate biocontrol agents of mealybug species, other than citrus mealybug (exp 692). This was chiefly targeted against oleander mealybug, *Paracoccus burnerae*. The chief justification of this study was the recent emergence of oleander mealybug, a phytosanitary pest, as the dominant mealybug species in a high percentage of orchards in a number of areas. Up to this point, the biocontrol status of *P. burnerae* was not clear, except that it was known that the commercially available parasitoid, *Coccidoxenoides perminutus*, was not adequately effective against this mealybug species (Hattingh & Tate, 1997). This study succeeded in establishing that *Leptomastix* spp. were probably the dominant parasitoid species. There were two *Leptomastix* species: an unidentified *Leptomastix* sp. and *Leptomastix thukumiensis* (Moore & Kirkman, 2006a). This experiment proposes to attempt rearing either or both of these species on either citrus or oleander mealybug and to investigate augmentative parasitoid releases for control of mealybug in citrus orchards.

Materials and methods

A culture of *P. burnerae* was built up and maintained on sprouting potato seedlings. The purpose of this was two-fold: to attract and capture *Leptomastix* spp. parasitoids in citrus orchards; and to rear these parasitoids in the laboratory. The culture was initially started with material from the National Department of Agriculture (NDA). Additional mealybug was sourced from Stellenbosch University and from field collections. Previously the mealybug culture was maintained on citrus seedlings, but with no greenhouse available, it was decided to rear them on sprouting potatoes.

Parasitoid trapping

Two sprouting potatoes, well infested with *P. burnerae*, were placed into an orange bag. One such bag was hung from a citrus tree in a mealybug infested orchards in an attempt to trap *Leptomastix* spp. parasitoids. These were placed in an orchard of Niewenhoud navel orange trees on Datenskraal Farm in the Hankey district, and in an orchard of rough lemon trees at the Citrus Foundation Block in Uitenhage. The bags containing the infested potatoes were hung from a citrus tree in such a way that no foliage or branches of the tree were in contact with the bag. Tangletrap, a sticky substance, was applied to the string which attached the bag to the tree, in order to prevent ants and predators from obtaining access to the mealybug, and to prevent the mealybug from escaping from the bag. These infested potatoes were left in the orchard for a period of two weeks, after which they were returned to the laboratory and placed in an emergence box. Glass vials were attached to the emergence boxes in order to capture the emerging parasitoids. Between October 2008 and the end of March 2009, several trappings were conducted at each site (Table 3.6.3.1).

Table 3.5.3.1. Dates of placement and removal of *P. burnerae*-infested sprouting potatoes at two sites, in attempts to trap *Leptomastix* spp. between October 2008 and March 2009.

Site	Date infested potatoes placed	Date infested potatoes removed
Citrus Foundation Block	14/10/08	29/10/08
Datenskraal Farm	22/10/08	05/11/08
Citrus Foundation Block	29/10/08	10/11/08
Datenskraal Farm	05/11/08	18/11/08
Citrus Foundation Block	10/11/08	25/11/08
Datenskraal Farm	18/11/08	30/11/08
Citrus Foundation Block	25/11/08	10/12/08
Datenskraal Farm	30/11/08	17/12/08
Citrus Foundation Block	17/12/05	05/01/09
Datenskraal Farm	17/12/08	05/01/09
Citrus Foundation Block	03/02/09	16/02/09
Datenskraal Farm	03/02/09	16/02/09
Citrus Foundation Block	16/02/09	02/03/09
Datenskraal Farm	16/02/09	02/03/09
Citrus Foundation Block	02/03/09	16/03/09
Datenskraal Farm	02/03/09	16/03/09
Citrus Foundation Block	16/03/09	30/03/09
Datenskraal Farm	16/03/09	30/03/09

Parasitoid rearing

Once parasitoids emerged from each trapping, they were captured in glass vials and moved into one communal rearing box containing mealybug-infested sprouting potatoes. Exact numbers of emerging parasitoids were not recorded, as the main aim of these trappings was to build the laboratory culture. New mealybug-infested sprouting potatoes were placed into the emergence box periodically, and the oldest ones removed as they started to decay.

Mealybug rearing improvements

Potatoes decay relatively fast and provide a relatively small substrate for mealybug. Consequently, new sprouting potatoes had to be added to the culture regularly. Butternuts have been used to rear citrus mealybug, *Planococcus citri*. In addition, butternuts are larger and don't decay as rapidly as potatoes. Therefore attempts were made to rear *P. burnerae* on butternuts. Mealybug infested sprouting potatoes were put among several butternuts on three different occasions, to see if the mealybug would move onto and develop on the butternuts. Some butternuts were left among the potatoes, while others were removed and placed in a separate rearing box once a few mealybug had crawled onto them.

Results and discussion

Sprouting potatoes proved to be a successful medium for rearing *P. burnerae*. No problems were experienced with contamination by other mealybug species. However, as successful as this method of rearing was, it was not ideal, as the potatoes decayed relatively quickly. The mealybug only infested the sprouts of the potatoes. These sprouts remained suitable for infestation even after the potatoes had decayed. When new fresh sprouting potatoes were added to the culture, mealybug did not immediately move across onto them. The decaying potatoes could therefore not be removed in time to prevent a soggy and odorous mess in the rearing box. To prevent this, sprouts were removed from the decayed potatoes and placed onto the fresh potatoes. However, several mealybug were damaged in this process. Several were also lost, as some fed at the base of the sprout and were left behind when the sprouts were transferred.

Parasitoid trapping

A few *Leptomastix* spp. parasitoids emerged from the mealybug exposed in citrus orchards, but numbers were disappointing. In previous years, the most successful *Leptomastix* spp. trappings were conducted in the Gamtoos River Valley, and so attempts were made to find suitable orchards in the same area. Difficulty was experienced in finding suitable orchards to trap parasitoids, as most of the growers were following an intensive chemical spray programme, which included treatments for mealybug. Although some mealybug were observed in the orchards where trappings were conducted, infestation was not high.

During the 1990s, the citrus mealybug was undeniably the dominant mealybug species throughout South Africa (Hattingh *et al.*, 1997 & 1998). *P. burnerae* was found to be the dominant species in the Eastern Cape in 2002 and 2003 (Moore & Kirkman, 2006b). During 2005, a survey was conducted in 10 different orchards of different cultivars from different citrus producing regions outside the Eastern Cape, in South Africa. *P. burnerae* was found in all but one of the orchards surveyed, and was the dominant species in the Citrusdal area (Moore & Kirkman, 2005). However, the demographics of the mealybug species complex appears to have changed. Although no official survey was conducted in 2008, very few *P. burnerae* were observed in orchards in the Eastern Cape during the period of attempted trappings (Dave Gerber, personal communication). This was probably the reason for the low numbers of *Leptomastix* spp. parasitoids being trapped. Similar trends have been observed in the other citrus producing areas, where citrus mealybug appears to be far more abundant than *P. burnerae* (Izak Bruwer, personal communication; Christo Breytenbach, personal communication).

A few generations of *Leptomastix* spp. parasitoids emerged from the laboratory culture, indicating that the rearing method is sound. However, insufficient parasitoids were trapped to provide enough breeding material to establish a strong culture. Parasitoid numbers have been insufficient to conduct any experiments on the biology of the parasitoids, and to assess their efficacy as an augmentative biocontrol agent for *P. burnerae*. Arrangements have been made to trap parasitoids in the Western Cape, and plans are in place to do the same in other areas outside of the Eastern Cape.

Rearing improvements

Attempts to rear *P. burnerae* on butternuts proved unsuccessful. Several mealybug moved from the potato sprouts onto the butternuts within a day or two, but moved back onto the sprouts shortly afterwards. Where the mealybug infested butternuts were removed from the potatoes and put into a separate rearing box, most of the mealybug died within a few days. One or two females formed egg sacs, but when the crawlers emerged they did not survive and a second generation was never completed. It is possible that there could have been detrimental chemical residues on butternuts. However, similarly poor results were observed with previous attempts at rearing *P. burnerae* on butternuts (Bruce Tate, personal communication).

Conclusion

Sprouting potatoes are a suitable medium for laboratory rearing of *P. burnerae* and *Leptomastix* spp. parasitoids, although their use is somewhat cumbersome. Citrus seedlings might be a better option if a greenhouse is available. Butternuts are not an option as a rearing medium for *P. burnerae*, even though citrus mealybug flourish on them. The *Leptomastix* spp. culture has developed through a few generations. However, parasitoid numbers were too low to conduct experiments on the parasitoids' biology and to conduct augmentative release trials. The main reason for the shortage of *Leptomastix* spp. parasitoids was the low occurrence of their host, *P. burnerae*, in the orchards where trapping was conducted. However, the population dynamics of the different mealybug species is likely to change again, as they have in the past, and *P. burnerae* could once again become dominant in citrus orchards in South Africa. It is therefore

imperative to continue trapping and to develop a strong culture of *Leptomastix* spp. parasitoids, in order to test the efficacy of augmentative releases for the control of *P. burnerae*.

Acknowledgments

Welma Pieterse (NDA), Jan Gilliomee and Todd Johnston (University of Stellenbosch) are thanked for their supply of *P. burnerae*. Citrus growers and the Citrus Foundation Block are thanked for making their orchards available and for assisting with the management of the trial sites.

Further objectives (milestones) and work plan

Trapping of *Leptomastix* spp. parasitoids will be continued in the Eastern Cape and other citrus producing areas in South Africa. Attempts will be made to rear *Leptomastix* spp. on *P citri*. Laboratory cultures will be built up in order to conduct biological studies on the parasitoid and to conduct augmentation trials with parasitoids.

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3.6 PROJECT: BIOCONTROL DISRUPTION

Project coordinator: Tim G Grout (CRI)

3.6.1 Project summary

Although biological control does not receive the emphasis that it did in the past due to the need to control some phytosanitary pests at unsustainable levels, it is still important to control ants to reduce unnecessary disruption of natural enemies of important pests such as mealybug. Further research on the development of a bait that will be effective against the two most important ants in citrus was therefore conducted (3.6.2) and has led to some positive results that now must be evaluated further on a large scale. The urgent need for an acaricide that can be used on fruit a few weeks before harvest has led to the development of RJU37PY which was tested for non-target effects against five key natural enemies (3.6.3). The product was found to be harmless to *Chilocorus nigritus*, *Aphytis coheni* and *Coccidoxenoides perminutus* and slightly harmful to *Trichogrammatoidea cryptophlebiae* and the predatory mite *Euseius citri*. It will therefore fit well in an IPM spray programme late in the season. Ant research and contract non-target effect bioassays will continue.

Projekopsomming

Alhoewel daar nie soveel klem op biologiese beheer soos in die verlede gelê is nie, weens die behoefte om sommige fitosanitêre plae teen nie-volhoubare vlakke te beheer, is dit nog steeds belangrik om miere te beheer om onnodige ontwinging van natuurlike vyande van belangrike plae soos witluis te verminder. Verdere navorsing op die ontwikkeling van 'n lokaas wat effektief teen die twee mees belangrike miere vir sitrus sal wees is dus uitgevoer (3.6.2) en het tot positiewe resultate gelei wat nou op 'n groter skaal verder geëvalueer moet word. Die dringende behoefte aan 'n mytdoder wat 'n paar weke voor oes op vrugte gebruik kan word, het tot die ontwikkeling van RJU37PY gelei, wat vir nie-teiken effekte teen vyf belangrike natuurlike vyande getoets is (3.6.3). Dit is gevind dat die produk skadeloos teen *Chilocorus nigritus*, *Aphytis coheni* en *Coccidoxenoides perminutus* is en effens skadelik teen *Trichogrammatoidea cryptophlebiae* en die predatoriese myt, *Euseius citri*. Dit sal dus goed in 'n GPB - program laat in die seisoen pas. Navorsing op miere en kontrak nie-teiken effek biotoetse sal voortgesit word.

3.6.2 PROGRESS REPORT: Development of ant baits and the use of bait stations Experiment 857 (2006/7-2008/9) by Tim Grout and Kim Stoltz (CRI)

Opsomming

Miere versteur natuurlike vyande in sitrusboorde en moet uit die bome gehou word. Ongelukkig word sommige produsente afgesit deur die arbeidsvereistes om effektiewe stamversperrings vir miere in stand te hou en hul sou verkies om die miere op die vloer van die boord dood te maak. Voorheen is 'n lokmiddel, bekend as Saga, ontwikkel wat beide die bruin huismier (*Pheidole*) en die malmier aangelok het. Die lokmiddel is nou met of fipronil of imidacloprid as gifstowwe gebruik. Alle skale van fipronil wat gebruik is, het geblyk om te hoog te wees met die laagste wat 0.006% a.b. was. Twee derdes van die neste wat in die proefwerk gebruik is, het egter uiteindelik onaktief geraak. Die lokmiddels het dus 'n langtermyn effek gehad. Verdere proefwerk moet fipronil teen 0.001% evalueer. Imidacloprid dosisse wat teen *Pheidole* gebruik was, was ook te hoog, maar was effektief teen die malmier wanneer dit teen 0.005% gebruik is. Hierdie dosis word in 'n kommersiële mier lokmiddel in ander lande gebruik en moet verder met Saga in grootskaalse proewe gebruik word.

Summary

Ants disturb natural enemies in citrus orchards and need to be kept out of trees. Unfortunately the labour requirements to maintain effective stem barriers for ants put some growers off and they would prefer to kill the ants on the orchard floor. Previously an attractant called Saga was developed that attracted both the brown house ant (*Pheidole*) and the pugnacious ant and this attractant was now used with either fipronil or imidacloprid as toxicants. All rates of fipronil used appeared to be too high with the lowest being 0.006% a.i. However, two-thirds of the nests used in the trial work ultimately became inactive so the baits were having a long-term effect. Further trial work should evaluate fipronil at 0.001%. Imidacloprid dosages used against *Pheidole* were also too high but when used at 0.005% against pugnacious ant it was effective. This dosage rate is used in a commercial ant bait in other countries and should be used further with Saga in large scale trials.

Introduction

Some mealybugs are phytosanitary pests and are largely being controlled by the use of organophosphates at the moment. As usage restrictions on these products increase, growers will need to depend on biocontrol more than at present as there are few chemical alternatives. In this scenario, cost effective ant control will be important because ants are attracted to honeydew-producing homopteran insects such as mealybugs (and the new woolly whitefly) and often protect these pests from their natural enemies. In addition, ant activity in trees can disrupt the natural enemies of non-honeydew-producing pests such as red scale *Aonidiella aurantii* and result in an increase in the population density of this pest. Control options for red scale are also threatened as the only corrective treatment available is methomyl. The use of trunk barriers requires the lower canopy of the tree to be skirt-pruned, weeds under the tree to be well managed and rejuvenation of the barrier every few months. Some growers would therefore prefer the low maintenance approach of using an ant bait. Siege (previously Amdro) has been registered for use in citrus for many years but is only effective against one of the two major ant pests, *Pheidole megacephala*.

In 2008 an attractant was developed that was effective for the pugnacious ant, *Anoplolepis custodiens*, and *Pheidole* called Saga (Grout and Stoltz 2008). Regent (fipronil 200 g/l SC) was first evaluated as a toxicant with Saga at 0.03% Regent by weight. This was equivalent to 0.006% a.i. compared with 1×10^{-5} % a.i. used by Hooper-Bui and Rust (2000) for *Linepithema humile*, 0.0001% a.i. used by Vega and Rust (2003) for the same ant, 0.0015% a.i. used by Collins and Callcott (1998) for *Solenopsis invicta* and the 0.05% used by Ulloa-Chacón and Jaramillo (2003) for *Tapinoma melanocephalum*. However, the results after six and seven days showed no significant impact (Grout and Stoltz 2008). Further evaluations of different dosages of fipronil were then tested against *Pheidole* and the pugnacious ant.

Materials and methods

The technique used for *Pheidole* was similar to that used before. Peanut butter was placed in small petri dishes close to *Pheidole* nests to determine ant activity (numbers feeding per minute after 30 min exposure) before selecting nests for a trial. Nests were always separated by at least 10 m. Bait mixtures were then placed in petri dishes (39 mm diameter) for 24 or 48 h with ant feeding being determined at the bait after this period. Later evaluations were conducted using peanut butter as an attractant in case ants that had received a sub-lethal dosage of toxicant were repelled.

The technique used for pugnacious ants differed in that the petri dishes were each placed on top of a vertical dowel rod (46 cm long), within 50 cm of a nest opening to prevent hyper-active ants from running into the bait and disturbing other ants. This meant that it took longer (numbers feeding per minute determined 2 h after setting up fish paste) for the ants to find the bait but that all ants that were visiting the bait were doing so for feeding purposes and not by chance. With pugnacious ants, fish paste was used before and after presenting the Saga mixture to the ants because these ants are not attracted to peanut butter.

The first small *Pheidole* trial was started on 22 April 2008 at 12 nests on the CRI – Nelspruit grounds. Dosages of Regent SC of 0.05, 0.1, 0.25, 0.5 and 1.0% by mass (0.01, 0.02, 0.05, 0.1 and 0.2% a.i., respectively) were used with two replicates each plus two controls that received Saga without Regent. Treatments were evaluated by counting ants visiting the bait per minute using Saga after 2 and 24 h to determine whether any of the higher dosages were repelling the ants. Maximum temperatures were in the low 20s°C. On 23 April a trial with pugnacious ant at the Lowveld Agricultural College was initiated using the same range of Regent dosages. After determining numbers of ants feeding on fish paste per minute, the Saga bait was placed and evaluated after 2.5 h and 24 h with two controls.

Another trial was started at CRI – Nelspruit against *Pheidole* on 12 May 2008 using Regent at 0.05 and 0.1% by mass (0.01 and 0.02% a.i.) and a control comprising Saga alone. Four replicates of each treatment were used. Evaluations of activity towards Saga per minute were conducted after 24 and 48 h and towards peanut butter after 7 and 14 d. A similar trial was conducted with pugnacious ant at the Lowveld Agricultural College starting on 13 May. However, although there was a lot of ant activity on the ground on this day, none of the ants visited the bait stations during the set up evaluation using fish paste. The baits were still placed but evaluations on Saga after 24 and 48 h still showed virtually no activity so the trial was discontinued. It was concluded that the night temperatures had become too cold for this species and it was no longer foraging. This was the last trial with pugnacious ant until summer.

On 26 May a further *Pheidole* trial was initiated at CRI in Nelspruit with the same design as the previous trial but with Regent at 0.25% and 0.5% by mass (0.05 and 0.1% a.i.). Activity was evaluated 30 min after setting up using peanut butter. Counts were made of the number of ants feeding per bait station per minute after 24 and 48 h on Saga bait, then after 7 and 14 d at peanut butter.

In July, two trials were conducted at CRI with Confidor WG as a toxicant in Saga against *Pheidole*. In the first trial the same technique was used as before with the use of peanut butter to evaluate nest activity, presentation of the baits for 48 h with evaluation of feeding activity after 24 and 48 h, then further evaluation of activity with peanut butter after 7 and 14 d. Confidor WG was used at 1.4 g/kg (0.14% formulated or 0.098% a.i.) in four replicates and at 0.14 g/kg (0.014% formulated or 0.0098% a.i.) in another four replicates. There were also four control replicates that received Saga alone. The trial was started on 1 July and the last evaluation conducted 14 d later.

The second trial with Confidor against *Pheidole* was started on 23 July but fewer active nests were available by this time so only a single rate of 3 g/kg (0.3% formulated or 0.06% a.i.) was compared with a control using three replicates each. The last evaluation was conducted 5 d later.

Activity at most *Pheidole* nests at CRI then declined and no further work was conducted there during 2008. However, due to the decline of the nests an investigation of all the treatments used at each nest was made to see whether the decline could be due to certain low dosages of Regent that had not appeared toxic at the time but may have had an effect over several weeks.

During summer 2008-9, Confidor was evaluated against pugnacious ant at two different locations (Litchi orchard and storm drain) at the Lowveld College of Agriculture near Nelspruit. Two dosages of Confidor WG in Saga were used and Saga alone as a control. The dosages were 0.071 g Confidor WG/kg Saga (0.005% a.i.) and 0.71 g Confidor WG/kg Saga (0.05% a.i.). Five replicates were used per dosage and control, using

dowel rods to raise the bait stations above the ground. An upward facing petri dish was placed on top of the rod as before with a hole or two to let water out. A roof over this dish formed by a larger petri dish was used to prevent rain from getting in the bait. A space of at least 1 cm between the bottom petri dish and the roof was left so that the small dish containing bait could be slipped in and removed easily and the ants had ready access.

Nests were identified and numbered that were at least 10 m apart. A rod was pushed into the ground approximately 50 cm from the nest opening. When the ants had calmed down, a small petri dish of known weight containing fish paste (6 g) was placed in the feeding chamber. After 1 h the number of ants feeding on the fish paste in 1 minute was determined. 22 h later, each bait dish was removed and weighed. The nests were ranked according to feeding and weight removal, then treatments assigned so that the average activities or bait removals were similar for each treatment. The weighed baits were then placed on the appropriate rods. A feeding count was conducted after 22 h when the baits were also weighed. The feeding chamber was then left empty and after 6 days a weighed fish paste bait dish was inserted and the number of ants feeding per minute after 1 h determined. The fish paste was left for 22 h, then removed and weighed. This was repeated 14, 21 and 28 d after placing the Saga bait mixtures. The days when the Saga bait mixtures were placed at these sites were 19 January in the litchi orchard and 27 January in the storm drain.

Two additional sites were monitored for four weeks in April but pugnacious ant activity was not suitable for further trials.

Results and discussion

All results proved difficult to interpret due to various factors influencing ant activity. This has been a continual challenge with all ant work because worker activity is influenced by needs in the nest. This was why an attractant had to be developed that satisfied both carbohydrate and protein requirements. However, rainfall results in nest repairing activity that may last for a few days. During this time ants do not feed much. The pugnacious ant is very sensitive to ground temperature. If it becomes too hot the ants stay in the nest. As the soil temperature cools in winter they also become less active until they stop foraging all together in May.

Confidor and Regent have been used by other researchers in ant baits and there are some commercial products available that use these toxicants. However, the concentrations of these toxicants in the commercial baits vary considerably depending on how much is consumed and the target ant species. Stringer et al. (1964) maintained that a suitable toxicant for an ant bait should cause less than 15% mortality after 24 h but more than 89% mortality after 20 d. However, by 7 d most suitable toxicants should be showing a significant degree of mortality. Ants could not be retrieved in these trials so actual mortality levels could not be determined. This meant that efficacy had to be determined by the numbers of ants foraging after exposure to the bait.

The first trial with *Pheidole* and Regent involved a range of dosages to see whether certain dosages would be repellent. This appeared to be the case with fewer ants feeding on the Saga bait mixes at the higher dosages after 24 h (Table 3.6.2.1). However, further trials with dosages in this range did not seem to have much effect in lowering ant numbers by 7 or 14 d after treatment (Table 3.6.2.1). Perhaps all these dosages are too high.

Table 3.6.2.1. Mean numbers of *Pheidole* feeding per minute after feeding on Saga bait with various dosages of Regent at CRI – Nelspruit.

Treatments	Trial started 22 April	Trial started 12 May	Trial started 26 May
	Mean ants on Saga bait per min 24 h after treatment	Mean ants on peanut butter per min 7+14 d after treatment	Mean ants per min 7+14 d after treatment
Saga only	1150 b	1300 a	464 a
Saga + Regent 0.05%	25 a	785 a	-
Saga + Regent 0.10%	20 a	451 a	-
Saga + Regent 0.25%	14 a	-	650 a

Saga + Regent 0.50%	6 a	-	463 a
Saga + Regent 1.0%	4 a	-	-

Means followed by the same letter in the same column were not significantly different ($P>0.05$ SNK)

Results of two trials with Regent against pugnacious ant in April and May 2008 were inconclusive (Table 3.6.2.2). In the second trial, although the ants appeared active, they were not attracted to the bait as they were probably preparing for winter.

Table 3.6.2.2. Number of pugnacious ants feeding per minute after feeding on Saga bait with various dosages of Regent at Lowveld College of Agriculture.

Treatments	Trial started 23 April	Trial started 13 May
	Mean ants per min 24 h after treatment	Mean ants per min 48 h after treatment
Saga only	43	1
Saga + Regent 0.05%	0	0
Saga + Regent 0.10%	6.5	0
Saga + Regent 0.25%	0.5	0
Saga + Regent 0.50%	0	0
Saga + Regent 1.0%	0	0

Table 3.6.2.3. Mean numbers of *Pheidole* feeding per minute after feeding on Saga bait with various dosages of Confidor WG for 48 h at CRI – Nelspruit.

Treatments	Trial started 1 July	Trial started 23 July
	Mean ants per min 7+14 d after treatment	Mean ants per min 48 h after treatment
Saga only	174 a	107 a
Saga + Confidor WG 0.014%	241 a	-
Saga + Confidor WG 0.14%	494 a	-
Saga + Confidor WG 0.3%*	-	233 a

There were no significant differences between treatments ($P>0.05$)

*This dosage was noticeably repellent at times.

Two trials with *Pheidole* and Confidor in Saga showed no apparent toxic effect of a range of dosages from 0.014 to 0.3% formulation (Table 3.6.2.3), although the 0.3% rate was noticeably repellent at times. After all these trials at CRI – Nelspruit, many of the *Pheidole* nests became inactive and could not be used in the last July trial. It is therefore possible that some of the treatments used ended up weakening the nests. Unfortunately, when it appeared that a treatment had no effect on ants from a specific nest, that nest was re-used in another trial. It is therefore difficult to determine which dosages ultimately had an effect or whether certain combinations of treatments were responsible for the collapse of these colonies (Table 3.6.2.4). One possible fault with the experimental design was the removal of the bait after 24 or 48 h. In the future the bait should be left in position and replenished as necessary with an alternative food source such as peanut butter or fish paste used for activity evaluations.

In the last two Confidor trials against the pugnacious ant in summer, the extra step of weighing the bait and evaluation food after 22 h provided a valuable back up for feeding activity. This should be used in further trials. These trials used an extra low dosage of Confidor and were evaluated for a long period. In the litchi orchard trial (Table 3.6.2.5) it appeared that the low rate of Confidor at 0.0071% formulated or 0.005% a.i. had an effect until 8 d after the 22 h feeding period (highlighted in table). The amount of Saga bait removed at T1 was not different from the control so the dosage was not repelling them in any way, in fact the higher concentration was removed more rapidly. After 2 d there were no ants feeding and the remaining test food was significantly more than the control or the other treatment. The numbers feeding after 7 d were still significantly lower than in the control and the remaining test food mass after 8 d was significantly higher.

Thereafter, no further treatment effect was noticeable. Unfortunately the results from the second Confidor trial with pugnacious ants in a storm drain (Table 3.6.2.6) were too variable and there were no significant differences. However, the mass of the Saga baits after feeding for 22 h were very similar for all three treatments so there was no evidence of repellency with these rates of Confidor.

While this research was being conducted, several commercial ant baits appeared on the market in other countries that contain either fipronil or imidacloprid as toxicants. Two liquid ant baits are produced by Bayer that probably have a sugar solution attractant. Maxforce for home use contains imidacloprid at 0.005% (as we concluded above for pugnacious ant) and Vitis for agricultural use contains imidacloprid at 0.001%. The latter product is aimed at Argentine ant but also has *Pheidole* on the label. These baits will not work for pugnacious ants because the attractant is sugar. Bayer also now produces a bait in a bait station called Maxforce FC that contains 0.01% fipronil while they also have a granular bait for fire ant (*Solenopsis invicta*) that contains only 0.00015% fipronil. Our dosages of fipronil that probably ultimately led to the collapse of two-thirds of the *Pheidole* nests that we used were at the upper end of this dosage range and did seem to cause some repellency (Table 3.6.2.1), so further large scale trials should be conducted with fipronil in Saga at 0.001%. As this active ingredient is still covered by a patent it may be easier to work with imidacloprid at 0.005% for commercialisation purposes.

Table 3.6.2.4. History of treatments applied near *Pheidole* nests at CRI and ant-feeding activity per minute.

NEST	25 Mar	AVG 6+7d	22 Apr	1 DAT	12 May	AVG 7+14 d	26 May	AVG 7+14d	1 Jul	AVG 7+14d	23 Jul	5d	Comments on nest
1	Regent 0.03%	500.5	Regent 1.0%	0	Regent 0.1%	625	Regent 0.5%	16	Control	35.5	Inactive	-	Possible effect of Regent 0.03, 1, 0.1 and 0.5%
2	Regent 0.03%	15.5	Regent 0.5%	7	Control	1600	Regent 0.25%	1100	Confidor 0.14%	400	Inactive	-	No effect Regent 0.5%
3	Regent 0.03%	193.5	Regent 0.25%	20	Regent 0.05%	1450	Control	800	Confidor 0.014%	50	Inactive	-	No effect Regent 0.05, 0.25%
4	Regent 0.03%	450	Control	1100	Regent 0.05%	1150	Control	451.5	Control	0	Inactive	-	Possible effect of Regent 0.03 and 0.05% only.
5	Regent 0.03%	97.5	Regent 0.25%	7	Regent 0.05%	140	Control	320	Confidor 0.014%	900	Inactive	-	No effect Regent 0.03, 0.25 and 0.05%
6	Regent 0.03%	400	Regent 0.05%	50	Regent 0.1%	450	Regent 0.25%	175	Control	60	Inactive	-	Regent 0.03, 0.05, 0.1, 0.25%
7	Regent 0.03%	50	Regent 0.1%	40	Control	98.5	Regent 0.5%	60	Confidor 0.014%	0.5	Confidor 0.3%	0	Low activity throughout
8	Regent 0.03%	350	Regent 0.05%	0	Regent 0.05%	400	Control	285	Confidor 0.014%	12.5	Inactive	-	Regent 0.03 and 0.05% followed by Confidor 0.014%.
9	Regent 0.03%	232.5	Regent 1.0%	8	Control	1750	Regent 0.5%	800	Confidor 0.14%	350	Confidor 0.3%	700	No effect Regent 0.03 or 1%
10	Regent 0.03%	192.5	Regent 0.5%	5	Regent 0.1%	500	Regent 0.25%	850	Confidor 0.14%	700	Control	70	No effect of Regent 0.03 or 0.5, 0.1 or 0.25%
11	Regent 0.03%	65.5	Regent 0.1%	0	Regent 0.1%	230	Regent 0.25%	475	Control	600	Inactive	-	No effect of Regent 0.25%
12	Regent 0.03%	654	Control	1200	Control	1750	Regent 0.5%	975	Confidor 0.14%	525	Confidor 0.3%	0	No effect of Regent 0.03 or 0.5%
Days between treatments and evaluations:													
	Treated			29 d		59 d		73 d					
			Treated			31 d		45 d					
					Treated			25 d		61 d			
							Treated			47 d			

Table 3.6.2.5. Pugnacious ants feeding per minute in a litchi orchard at the Lowveld College of Agriculture and the mean mass of fish paste remaining after 22 h when tested at various periods in days after 24 h exposure to Confidor bait.

Statistic	Treatments applied 19 Jan 2009		
	Control	Confidor 0.0071%	Confidor 0.071%
Ants feeding T1*	26.4 b	5.4 a	70 c
Mass of bait T1*	2.66 b	2.36 b	0.86 a
Ants feeding T2	162 c	0 a	27.2 b
Mass of bait T2	2.5 a	4.32 c	3.42 b
Ants feeding T7	60 b	0.2 a	27 ab
Ants feeding T8	10.6 a	2.8 a	13.2 a
Mass of bait T8	0.76 a	3.44 b	1.08 a
Ants feeding T15	17 a	13.4 a	30 a
Mass of bait T15	1.48 a	1.68 a	0 a
Ants feeding T22	2.2 a	2 a	6.2 a
Mass of bait T22	1.4 b	1.5 b	0 a
Ants feeding T29	9.8 a	3.6 a	23.4 a
Mass of bait T29	0.78 ab	1.98 b	0 a
Ants feeding T90	1.2 a	0.2 a	5.2 a
Mass of bait T90	1.88 a	0.92 a	1.32 a

*On Saga bait, whereas feeding after this was always on fish paste.

Means in the same row followed by the same letter were not significantly different ($P>0.05$ SNK).

Table 3.6.2.6. Pugnacious ants feeding per minute in a storm drain at the Lowveld College of Agriculture and the mean mass of fish paste remaining after 22 h when tested at various periods in days after an initial 24 h exposure to Confidor bait.

Statistic	Treatments applied 27 Jan 2009		
	Control	Confidor 0.0071%	Confidor 0.071%
Ants feeding T1*	9.4	0.2	3.6
Mass of bait T1*	4.42	4.58	4.82
Ants feeding T7	49.0	35.2	25.4
Mass of bait T7	1.18	1.58	2.08
Ants feeding T14	4.6	6.2	0.4
Mass of bait T14	1.46	No data	No data
Ants feeding T21	14.8	4.2	17.6
Mass of bait T21	0.6	0.06	0.66
Ants feeding T28	16.6	43.0	12.6
Mass of bait T28	0.34	0.96	0.24

Ants feeding T82	0.6	3.0	8.8
Mass of bait T82	1.62	2.18	1.7

*On Saga bait, whereas feeding after this was always on fish paste. No means in the same row were significantly different ($P > 0.05$ SNK).

Conclusion

Results with fipronil and Confidor have been difficult to interpret due to variability but this may largely be due to the dosage range being too high and there being some repellency. Positive results were obtained with imidacloprid in Saga at 0.005% against pugnacious ant and this dosage is used in a commercial liquid bait for ants in several other countries so this combination should now be used in semi-commercial evaluations where the bait is left in place for continued foraging.

Further objectives and work plan

Further research will be conducted with Saga and imidacloprid (and perhaps fipronil) on a semi-commercial basis.

Technology transfer

The Ant Bait Tube bait station was demonstrated at the Citrus Research Symposium. Other research results are not yet suitable for technology transfer.

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3.6.3 PROGRESS REPORT: Non-target effect testing for RJU37PY a new acaricide for citrus bud mite control

Experiment 916 (2007-2008/9) by Tim G Grout and Kim C Stoltz (CRI)

Opsomming

Daar is 'n dringende behoefte in die sitrusbedryf aan 'n mytdoder wat effektief teen sitrus knopmyt is en wat binne twee maande voor oes gebruik kan word om die beskerming van die nuwe groei in die lente te verbeter. RJU37PY is so 'n mytdoder en om die registrasie van hierdie produk te bespoedig het CRI die vereiste nie-teiken effek biotoetse gedoen. Die resultate het getoon dat die residue van RJU37PY 50 EC teen 150 ml/hl water, as skadeloos vir *Chilocorus nigritus*, *Aphytis coheni* en *Coccidoxenoides perminutus* geklassifiseer is, maar as effens skadelik vir *Trichogrammatoidea cryptophlebiae* en die predatoriese myt, *Euseius citri* is. Geen verdere nie-teiken effek toetse word vir RJU37PY benodig nie, maar sal op 'n kontrak basis vir ander chemikalieë, wanneer en waar nodig, uitgevoer word.

Summary

There is an urgent need in the citrus industry for an acaricide that is effective against citrus bud mite and can be used within approximately two months of harvest to optimize the protection of the spring growth flush. RJU37PY is such an acaricide and in order to expedite the registration of this product CRI conducted the required non-target effect bioassays. The results showed that residues from RJU37PY 50 EC at 150 ml/hl water were categorised as Harmless for *Chilocorus nigritus*, *Aphytis coheni* and *Coccidoxenoides perminutus* but were slightly harmful to *Trichogrammatoidea cryptophlebiae* and the predatory mite *Euseius citri*. No further non-target effect testing is required for RJU37PY but will be conducted on a contract basis for other chemicals when and if necessary.

Introduction

The potential impact of RJU37PY 50 EC and Lannate (methomyl) 900 SP was tested against five biocontrol agents of relevance to citrus in southern Africa. This was done in accordance with the system of non-target effect evaluations developed for incorporation into the Registration of Pesticides (Act 36 of 1947) for use on citrus (Hattingh et al. 2000).

Tests were conducted from October 2008 to March 2009 at the Citrus Research International facility in Nelspruit. The effects of exposure to aged pesticide residues were determined with appropriate life stages of each indicator species. An overall impact rating (I) was calculated for each species by combining the effects on various life stages with the pesticide residue age. The products were placed into one of five categories of total potential impact for each indicator species:

- 0) No interaction: where an exposure risk assessment indicates that negligible risk exists and therefore the test species is exempt from testing.
- 1) Harmless: where $I < 25\%$.
- 2) Slightly harmful: where $25\% < I < 50\%$.
- 3) Harmful: where $50\% < I < 75\%$.
- 4) Very harmful: where $I > 75\%$.

Pesticide application

RJU37PY 50 EC was mixed with water at the dosage rate of 150 ml/hl water. Lannate 900 SP was used as a standard at the dosage rate of 100 g/hl water. Both products were applied to potted citrus seedlings as film cover sprays to the point of run-off, using a knapsack sprayer. Seedlings were exposed to environmental conditions but moved under cover when rain was expected. Leaves were picked at various periods after spraying and the effect of exposing test organisms to their weathered residues was determined.

Effect on *Chilocorus nigritus*

Materials and methods

Source of beetles

Beetle larvae used in the bioassays were from a new culture established at CRI in October 2008 and maintained on Oleander scale (*Aspidiotus nerii*) on butternuts. Additional beetle adults were obtained from the DuRoi IPM insectary at Letsitele and added to this culture.

Effect on adult and larval mortality

Plants were sprayed on 17 November 2008. Ten test cells were set up per treatment group. Each cell consisted of a halved film canister that provided a closed test arena of 30 mm diameter and 10 mm high. The inside of each test arena was coated with Fluon [Polytetrafluoroethylene (PTFE) dispersion, grade GP1]. Ventilation holes were made on opposite sides of the test arena. Thin plastic tubing (3 mm internal diameter) was tightly fitted through the holes, with fine mesh material over the ends. Each leaf was placed on top of a damp filter paper, which in turn was placed on top of a 100 x 60 x 3 mm glass plate. The test arena was placed over the leaf surface and secured onto the glass plate with a rubber band. One side of each cell was connected to a pressurised plenum via the plastic tubing, so that air was passed through each cell at a rate of one volume exchange per minute.

In the adult beetle assessment, five beetles were inserted into each cell. To assess the effects on larvae, five first instar larvae were transferred onto the adaxial leaf surface in each cell, using a fine paintbrush.

The cells were held for 48 hours under laboratory conditions of 23°C before the number of live insects was determined. Treatment mortalities were corrected for control mortality, using Abbott's (1925) correction.

$$MA \text{ or } ML = ((MT - Mc)/(100 - Mc)) \times 100$$

Where: MA = % corrected mortality of adults
 ML = % corrected mortality of larvae
 MT = mortality in the treated group
 MC = mortality in the control group

Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality of the test product dropped below 25%.

Persistence

A persistence factor (P) was incorporated into the impact rating. The relevant factor was determined by the residue age at which the corrected effect dropped below 25%. The factors used for the residue age categories of 1, 7, 14, 28 and 42 days were 1.03, 1.07, 1.1, 1.2 and 1.4, respectively. A factor of 2.0 was used if the corrected effect was still greater than 25% at 42 days. The persistence factor was combined with the maximum corrected adult (MA) and larval (ML) mortalities and an additional factor of 0.5 was included to prevent the maximum possible combination of persistence factor and corrected mortality from exceeding 100%. Thus the impact of the product on adults (I_A) and the impact on larvae (I_L) were derived as follows:

$$I_A = M_A \times 0.5P \text{ and } I_L = M_L \times 0.5P$$

Overall impact rating

The adult and larval impacts, already adjusted for persistence, were combined to obtain a single impact rating (I):

$$I = 0.5 I_A + 0.5 I_L$$

Results

Effect on adult and larval mortality

Table 3.6.3.1. Effect of aged residues on *C. nigratus* adult and larval mortality after exposure for 48 hours.

Formulation	Dosage	Corrected % larval mortality after exposure to aged residues (days)			Corrected % adult mortality after exposure to aged residues (days)		
		1	7	14	1	7	14
RJU37PY 50 EC	150 ml/hl	39.1	20.9	-	17.0	14.6	-
Lannate 900 SP	100 g/hl	98.0	95.9	-	84.0	64.6	-

Persistence and overall impact

Table 3.6.3.2. The overall impact of aged residues on *C. nigritus*.

Formulation	Dosage	Impact on larval mortality adjusted for persistence (I _L)	Impact on adult mortality adjusted for persistence (I _A)	Overall impact rating (I)
RJU37PY 50 EC	150 ml/hl	20.9	8.8	14.9
Lannate 900 SP	100 g/hl	58.8	50.4	54.6

RJU37PY was categorized as being “Harmless” ($I < 25$) to *C. nigritus* whereas the Lannate was “Harmful” ($50 < I < 75$).

Effect on *Aphytis coheni*

Materials and methods

Source of parasitoids

Aphytis coheni (previously identified as *A. lingnanensis*) were obtained from a commercial insectary (Du Roi IPM) and used the next day in the bioassay.

Effect on adult mortality

Plants were sprayed on 5 November 2008. Ten test cells were used per treatment group, comprising 34 mm diameter petri dishes (10 mm high). The substrate for the cell comprised a leaf placed on filter paper dampened with distilled water, which in turn rested on a glass plate (100 x 60 x 3 mm). Each test arena was firmly held on the leaf surface by two spring-loaded clamps. Two screened ventilation holes (6 mm diameter) were made on top of each test arena. Streaks of honey were applied to the inside of each petri dish and a small piece of paper provided as a refuge. Thin plastic tubing (3 mm internal diameter) was tightly fitted through a hole in the wall of the petri dish, with fine mesh material over the end. This tubing was connected to a pressurised plenum so that air was passed through each cell at a rate of one volume exchange per minute. *Aphytis* were received from Du Roi IPM insectary in a small container with shredded paper and honey. The *Aphytis* were slowed down by placing the container on an ice pack. Approximately 50 to 100, 24 to 48 hour-old adults, were then transferred into each cell by cutting off pieces of shredded paper with *Aphytis* on them and placing them on top of each leaf. The *Aphytis* were exposed to the residues in the cells for 24 hours under laboratory conditions before the number of live insects was determined.

Treatment mortalities were corrected for control mortality using Abbott’s (1925) correction:

$$M_A = ((M_T - M_C)/(100 - M_C)) \times 100$$

Where: M_A = % corrected adult mortality

M_C = % mortality in control group

M_T = % mortality in treatment group

Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality dropped below 25%.

Overall impact rating

The corrected mortality figures were adjusted for persistence using the summation method. The corrected mortality figures (including zeroes for those after tests were stopped), obtained from different residue ages were each multiplied by persistence factors (Y_x) of 98.6, 90, 80, 60 and 40 for the residue ages (x) 1, 7, 14,

28 and 42 days, respectively, and summed. The resultant figure was divided by the sum of all persistence factors to obtain the overall impact rating (I):

$$I = \frac{\sum (Yx = - 1.429x + 100)(M_A)}{\sum (Yx = - 1.429x + 100)}$$

X = 1, 7, 14, 28, 42

Results

Table 3.6.3.3. Percentage mortality of *A. coheni* adults after exposure to aged residues for 24 hours

Formulation	Dosage	Days after treatment					Impact rating (I)
		1	7	14	28	42	
RJU37PY 50 EC	150 ml/hl	76.9	14.5	-	-	-	24.2
Lannate 900 SP	100 g/hl	100.0	100.0	100.0	98.6	83.6	96.9

RJU37PY was categorized as “Harmless” ($I < 25$) to *A. coheni*, whereas Lannate was “Very harmful” ($I > 75$) to *A. coheni*.

Effect on *Coccidoxenoides perminutus*

Material and methods

Source of parasitoids

Parasitoids were obtained from the commercial insectary, Du Roi IPM, in Letsitele.

Effect on mortality

Plants were sprayed on 9 October 2008. Ten test cells were set up per treatment group: each cell consisted of a 34 mm diameter petri dish lid (10 mm high). A 6 mm diameter hole was made on one side of the test arena and a 6 mm diameter hole was made on top of the test arena. Plastic tubing (3 mm internal diameter) was first wrapped with fine mesh material at one end then forced into the hole in the side of the arena. The other end of the tube was attached to a vacuum pump. A 1.5 mm diameter capillary tube was attached to an adaptor so that it fitted tightly in the 6 mm diameter hole on top of the test arena. Twelve pre-fed, 24-hour-old adults were aspirated into each cell through the capillary tube. Minute streaks of diluted honey were provided as food on the inside walls of the arena. Each test arena was attached to a pressurised plenum via the 3 mm internal-diameter plastic tube, so that air passed through each cell at a rate of one volume exchange per minute. Adult mortality was recorded after 24 hours of exposure. Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality dropped below 25%.

Treatment mortalities were corrected for control mortalities (Abbott, 1925):

$$MA = (MT - Mc)/(100 - Mc) \times 100\%$$

Where: MA = % corrected adult mortality
 MT = mortality in the treated group
 Mc = mortality in the control group

Persistence

The relevant persistence factor (P) for adult mortality was determined by the residue age at which the corrected adult mortality dropped below 25%. The factors used for the residue ages were categorised as before (section 3.1.3). The persistence factor was combined with the maximum corrected adult mortality (MA) as well as an additional factor of 0.5 to prevent the maximum possible combination of persistence factor and corrected mortality from exceeding 100%. Thus:

$$IA = MA \times 0.5P$$

Where: IA = maximum impact on adult mortality adjusted for persistence

Overall impact rating

The product's impact (I) was equivalent to IA.

Results

Effect on adult mortality

Table 3.6.3.4. Effect of aged residues on *C. perminutus* adult survival after exposure for 24 hours.

Formulation	Dosage	Corrected % adult mortality after exposure to aged residues (days)				
		1	7	14	28	42
RJU37PY 50 EC	150 ml/hl	3.1	-	-	-	-
Lannate 900 SP	100 g/hl	100.0	42.7	100.0*	52.3*	81.4*

*From a previous bioassay.

Persistence and overall impact

Table 3.6.3.5. The overall impact of aged residues on *C. perminutus*.

Formulation	Dosage	Overall impact rating (I = I _A)
RJU37PY 50 EC	150 ml/hl	1.6
Lannate 900 SP	100 g/hl	100.0

RJU37PY was categorized as being "Harmless" (I<25) to *C. perminutus* whereas Lannate was "Very harmful" (I>75).

Effect on *Trichogrammatoidea cryptophlebiae*

Materials and methods

Source of egg parasitoids

Parasitised FCM eggs on wax paper were obtained from a private research culture in Citrusdal. The parasitoids commenced eclosion one day after receiving the shipment (i.e., fresh FCM eggs had been exposed to adult parasitoids for 24 hours, seven days prior to shipment, then held at 25-26°C).

Effect on mortality

Plants were sprayed on 30 January 2009. Ten test cells were set up per treatment group. Each cell consisted of a small petri dish lid that provided a test arena of 34 mm diameter and 10 mm high. Ventilation holes were made on opposite sides of the test arena. Thin plastic tubing with fine mesh material over the ends was tightly fitted through pre-cut holes. Residue bearing leaves were placed on top of glass plates (100 mm x 65 mm x 3

mm). Fine streaks of diluted honey were applied to the inside walls of the arena as food. Each test arena was tightly clamped over the leaf surface using two spring-loaded clamps.

Sheets of wax paper containing parasitised FCM eggs were placed inside a plastic container (227 mm x 178 mm x 74 mm) that had been painted black. Twenty holes were punched in the lid of the container. Small glass Polytop vials were inserted into the holes to collect emerging parasitoids. Each vial was replaced as soon as 20 - 50 parasitoids had been collected. The parasitoids were randomly allocated to treatments. Each cell was attached to a pressurised plenum and air was passed through each test arena at approximately one volume exchange per minute.

The number of live adults was determined after 24 hours of exposure. Bioassays were conducted with progressively older residues (1, 7, 14, 28 and 42 days) until the corrected mortality dropped below 25%.

The treatment mortalities were corrected for control mortality (Abbott, 1925):

$$MA = (MT - Mc)/(100 - Mc) \times 100\%$$

Where: MA = % corrected adult mortality after exposure to x day old residues

MT = mortality in the treated group

Mc = mortality in the control group

Overall impact rating

The corrected mortality figures were adjusted for persistence using the summation method. The corrected mortality figures (including zeroes for those after tests were stopped), obtained from different residue ages were each multiplied by persistence factors (Yx) of 98.6, 90, 80, 60 and 40 for the residue ages (x) 1, 7, 14, 28 and 42 days, respectively, and summed. The resultant figure was divided by the sum of all persistence factors to obtain the overall impact rating (I):

$$I = \frac{\sum_{X=1,7,14,28,42} (Yx = -1.429x + 100)(M_A)}{\sum_{X=1,7,14,28,42} (Yx = -1.429x + 100)}$$

The product's impact (I) was categorised as before.

Results

Table 3.6.3.6. Percentage effect of aged residues on the mortality of *T. cryptophlebiae* after exposure for 24 hours.

Formulation	Dosage	Residue age (days)				Impact rating (I)
		1	9	14	28	
RJU37PY 50 EC	150 ml/hl	94.8	0.3	-	-	25.5%
Lannate 900 SP	100 g/hl	100.0	66.5	100.0*	92.3*	95.0%*

*Estimates used for Lannate as insufficient parasitoids to continue testing beyond 7 d

RJU37PY was categorized as "Slightly harmful" (25% < I < 50%) to *T. cryptophlebiae* and Lannate was categorized as "Very harmful" (I > 75%).

Effect on *Euseius citri*

Materials and methods

Source of predatory mites

Mites were collected from a citrus orchard at Crocodile Valley Citrus, 24 hours before initiating the bioassays, in order to provide them with food and give them an opportunity to mate. The substrate for the temporary colonies consisted of large inverted citrus leaves placed on floating sponge rafts. Each raft consisted of a 135 mm x 190 mm sponge, floating on a polystyrene raft (135 mm x 190 mm) in a water-filled container. Strips of cotton wool were placed on the edges of the leaf to ensure a continuous wet barrier. The mites were fed with pollen from *Typha capensis* by sprinkling the pollen on the leaf surface.

Effect on adult survival

Plants were sprayed on 2 March 2009. Treated and untreated leaves were collected one and three days after spraying, and thereafter at weekly intervals until corrected mortality dropped below 5% for a second time. Disks, 22 mm in diameter, were punched out of the leaves. Only the flattest disks were used and placed abaxial surface uppermost on water-saturated filter paper (Whatman 90) disks (25 mm diameter) on top of a floating sponge raft. Gaps between the filter paper disks on the sponge prevented movement of mites between disks. Care was taken to ensure continued contact between the filter paper and the entire ventral surface of the leaf disk to prevent the mites from going underneath the leaf disk. Where necessary, cotton wool was used to fill any gaps between the disks and the filter paper to maintain a continuous wet barrier.

Twenty leaf disks were used per treatment. Two disks of filter paper (6 mm diameter) were placed on each leaf disk to provide shelter for the mites and a fibre substrate for the attachment of eggs. Pollen was lightly dusted onto the leaf disks.

Five adult female mites were transferred to each leaf disk from the bioassay colonies. Each disk from each treatment received one mite before a second mite was placed on each disk. The bioassay trays were left for 48 hours under laboratory conditions before the numbers of live adult females were determined. Missing mites or mites found drowned in the sponge were considered dead.

Treatment mortalities were corrected for control mortality using Abbott's correction (1925):

$$MA = [(MT - MC)/(100 - MC)] \times 100$$

Where: MA = corrected adult mortality
MT = mortality in treatment
MC = mortality in control

Persistence

A persistence factor (P) was determined by the residue age at which the adult mortality dropped below 5% for a second time. The factors used for the categories 0-15 days, 16 -30 days, 31-45 days and more than 45 days were 1.1, 1.2, 1.4, and 2.0, respectively. The persistence factor was combined with the maximum corrected adult mortality (M_A) as well as an additional factor of 0.5 (to prevent the maximum possible combination of persistence factor and corrected mortality from exceeding 100%) to determine the impact of the product on adults (I_A). i.e.,

$$IA = MA \times 0.5P$$

Effect on adult fecundity, egg hatch and immature mortality (population increase)

Once the adult mortality remained below 5% for a second test, a "once-off" procedure was used to determine the effects on egg production, egg hatch and survival of immature life stages. The adult mites were removed from the last bioassay. The eggs were left to hatch and develop to adults and were fed with pollen every second day. The number of adults (offspring) surviving was determined nine days after the original females were placed on the disks. This number was expressed as a percentage (SF1) of the number of offspring in the untreated control.

$$SF1 = [(number\ of\ offspring\ in\ treatment)/(number\ of\ offspring\ in\ control)] \times 100 \%$$

Where: SF1 = survival percentage (including any effect on fecundity, egg hatch or immature mortality relative to the control)

The detrimental effect on reproduction, or mortality of the F1 generation (MF1) is therefore 100-SF1. This negative effect on reproduction (MF1) was then adjusted according to the potential contribution to the overall impact rating, giving:

$$I_{F1} = M_{F1}[(100-I_A)/100]$$

Where: I_{F1} = Impact on reproduction adjusted for potential to contribute to the overall impact rating.

Overall impact rating

The overall impact rating (I) was obtained as follows:

$$I = I_A + I_{F1}$$

Results

Adult survival

Table 3.6.3.7. Percentage corrected mortality of adult *E. citri* on aged residues.

Formulation	Dosage	Residue age (days)				Adult impact adjusted for persistence (I_A)
		1	3	7	14	
RJU37PY 50 EC	150 ml/hl	3.2	4.3	-	-	2.4
Lannate 900 SP	100 g/hl	63.6	11.1	-	-	35.0

Population increase

The number of offspring recovered was expressed as a percentage of the number of progeny in the untreated control.

Table 3.6.3.8. Effect of aged residues on the population increase of *E. citri*.

Formulation	Dosage	Survival % (S_{F1})	% progeny decrease (M_{F1})	I_{F1}
RJU37PY 50 EC	150 ml/hl	71.3	28.7	28.0
Lannate 900 SP	100 g/hl	36.0	64.0	41.6

Overall impact rating

Table 3.6.3.9. The overall impact of aged residues on *E. citri*.

Formulation	Dosage	Impact on adult mortality adjusted for persistence (I_A)	Further impact on progeny (I_{F1})	Overall impact rating (I)
RJU37PY 50 EC	150 ml/hl	2.4	28.0	30.4
Lannate 900 SP	100 g/hl	35.0	41.6	76.6

RJU37PY was categorized as "Slightly harmful" (25% < I < 50%) to *E. citri* and Lannate was categorized as "Very harmful" (I > 75%).

Summary tables

Table 3.6.3.10. Effect of aged residues on *Chilocorus nigritus*.

Formulation	Dosage	Impact on larval mortality adjusted for persistence (I _L)	Impact on adult mortality adjusted for persistence (I _A)	Overall impact rating (I)	Impact category
RJU37PY 50 EC	150 ml/hl	20.9	8.8	14.9	Harmless
Lannate 900 SP	100 g/hl	58.8	50.4	54.6	Harmful

Table 3.6.3.11. Effect of aged residues on *Aphytis coheni*.

Formulation	Dosage	Overall impact rating	Impact category
RJU37PY 50 EC	150 ml/hl	24.2	Harmless
Lannate 900 SP	100 g/hl	96.9	Very harmful

Table 3.6.3.12. Effect of aged residues on *Coccidoxenoides perminutus*.

Formulation	Dosage	Overall impact rating	Impact category
RJU37PY 50 EC	150 ml/hl	1.6	Harmless
Lannate 900 SP	100 g/hl	100.0	Very harmful

Table 3.6.3.13. Effect of aged residues on *Trichogrammatoidea cryptophlebiae*.

Formulation	Dosage	Overall impact rating	Impact category
RJU37PY 50 EC	150 ml/hl	25.5	Slightly harmful
Lannate 900 SP	100 g/hl	95.0	Very harmful

Table 3.6.3.14. Effect of aged residues on *Euseius citri*

Formulation	Dosage	Impact on adult mortality adjusted for persistence (I _A)	Further impact on progeny (I _{F1})	Overall impact rating (I)	Impact category
RJU37PY 50 EC	150 ml/hl	2.4	28.0	30.4	Slightly harmful
Lannate 900 SP	100 g/hl	35.0	41.6	76.6	Very harmful

Conclusion

Residues from RJU37PY 50 EC at 150 ml/hl water, were categorised as Harmless for *Chilocorus nigritus*, *Aphytis coheni* and *Coccidoxenoides perminutus* but were slightly harmful to *Trichogrammatoidea cryptophlebiae* (a very delicate parasitoid) and the predatory mite *Euseius citri*.

These results show that RJU37PY will be compatible with integrated pest management and the product is

unlikely to cause a resurgence of any pests due to a negative effect on their natural enemies. If releases of *T. cryptophlebiae* are planned for an orchard that is to be sprayed with RJU37PY, the releases should be made two weeks or more after the acaricide spray.

Future research

No further non-target effect research is required on this acaricide but will be conducted on other new products on a contract basis as required.

Technology transfer

Technology transfer will be delayed until RJU37PY is registered.

References cited

- Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.
- Hattingh, V., Ware, A.B. & Grout, T.G. 2000. The development of a non-target evaluation system for Southern African citrus. *Proc. Int. Soc. Citriculture*: 795-797.

3.7 PROJECT: PRODUCTION PESTS

Project coordinator: Tim G Grout (CRI)

3.7.1 Project summary

Red scale was the most important production pest for many years, especially around the time that citrus growers were learning to deal with organophosphate resistance. However, the status of this pest is currently low due largely to the availability of generic imidacloprid and pyriproxyfen which are often used on a preventive basis. An unusual production problem raised in 2007 was the need to stop bees from cross-pollinating seedless cultivars and causing the production of seed that drastically reduced the value of the crop. It seems that researchers have been searching for effective bee repellents since the early 1900s for various reasons, but none of these products seem to have worked in the field. The result of research conducted at Nelspruit with several chemicals that have appeared in publications as bee repellents was no different because none could prevent bees from visiting litchi flowers (3.7.2). Growers may therefore need to resort to nets or other strategies to prevent cross-pollination. Citrus psylla is a serious production pest but it is difficult to work with because growers do not tolerate it in their orchards. Some possible psyllid attractants were received late in the season for testing with the objective of using them in a mass trapping control strategy, but no trial site could be found with suitable numbers of psyllids (3.7.3). This work will be conducted if such an orchard is found in the future.

Projekopsomming

Vir baie jare was rooi dopluis die mees belangrike produksieplaag, veral gedurende die tydperk toe sitrusprodusente moes leer hoe om weerstand teen organofosfate te hanteer. Die status van hierdie plaag is nou laag hoofsaaklik as gevolg van die beskikbaarheid van generiese imidacloprid en pyriproxyfen wat dikwels op 'n voorkomende basis gebruik word. 'n Ongewone produksie probleem in 2007 was die behoefte om bye te verhoed om saadlose kultivars te kruisbestuif wat aanleiding tot die vorming van sade gegee het, wat drasties die waarde van die oes verminder het. Dit blyk dat navorsers reeds sedert die vroeë 1900's vir verskeie effektiewe afweermiddels vir bye gesoek het, maar geen van die produkte het geblyk om in die veld te werk nie. Die resultate van navorsing by Nelspruit met verskeie chemikalieë en in verskeie publikasies as afweermiddels vir bye verskyn het, was nie anders nie omdat dit nie die bye kon verhoed om litchi blomme te besoek nie (3.7.2). As gevolg hiervan sal dit dalk nodig wees vir produsente om van nete of ander strategieë gebruik te maak om kruisbestuiving te voorkom. Sitrus bladvooi is 'n ernstige produksieplaag, maar is moeilik om mee te werk, omdat produsente dit nie in hul boorde toelaat nie. 'n Paar moontlike bladvlooi lokmiddels is laat in die seisoen vir toetsing ontvang met die doel om hulle in 'n massa lokval beheerstrategie te gebruik. Geen proefperseel met geskikte getalle van bladvlooi kon gevind word nie (3.7.3). Hierdie werk sal gedoen word indien so 'n boord in die toekoms gevind kan word.

3.7.2 **FINAL REPORT: Development of a bee repellent to prevent cross-pollination of seedless cultivars**

Experiment 943 (2008/9) by Tim G Grout and Kim C Stoltz (CRI)

Opsomming

Party sitrusprodusente ondervind probleme met saad by kultivars, wat as saadloos bemark word, met die gevolg dat die inkomste vir die produk baie laer is. 'n Versoek is dus van produsente gekry dat die moontlikheid vir die gebruik van 'n afweermiddel om bye te verhoed om blomme tydens blomtyd te besoek ondersoek word. Verskeie chemikalieë of produkte wat in die literatuur gerapporteer is as afweermiddels vir bye is getoets teen bye wat heuningwater in petribakkies besoek het. Die vermoë van sekere afweermiddels om bye te verhoed om aangrensende litchi blomme te besoek, is ook geëvalueer. Nie een van die produkte het 'n betekenisvolle effek op die versoeking na kos relatief tot die kontrole gehad nie. Om kruisbestuiwing te verhoed sal die afweermiddel baie doeltreffend moet wees. Hierdie is heel waarskynlik nie moontlik nie en produsente sal daarom voldoende afstande tussen die relevante kultivars moet behou, of die gebruik van nette oorweeg om bome tydens die blomperiode teen bye te beskerm.

Summary

Some citrus growers experience problems with seed in cultivars that are marketed as seedless, resulting in a much lower return for the commodity. Growers have therefore requested that the possibility of using a repellent to prevent bees from visiting flowers during bloom be investigated. Several chemicals or products that have been recorded in the literature as bee repellents were tested against bees visiting honey-water in petri dishes and the ability of some repellents to prevent bees from visiting adjacent litchi flowers was also evaluated. None of the products had a significant effect on foraging relative to the control and in order to prevent cross-pollination the repellent will have to be extremely effective. This is therefore not considered possible and growers will have to maintain adequate distances between affected cultivars or consider the use of netting to protect trees from bees during bloom.

Introduction

Every so often, someone tries to develop a repellent for bees, most frequently to reduce poisoning from insecticides (Atkins et al. 1975; Johansen 1977). However, although many chemicals are reported to repel bees (Woodrow et al. 1965; Melksham et al. 1988) and even some surfactants (Moffett and Morton 1975) few have been able to provide practical levels of repellency (Atkins et al. 1975). As the plantings of seedless mandarins increase and the value of the crop increases, growers are increasingly frustrated by bees that cross-pollinate cultivars that are normally seedless when planted in isolated blocks with compatible pollen, resulting in seeds. This causes a drastic reduction in the price obtainable for the commodity. As cross pollination by bees and consequent seed formation are not wanted in citrus fruit, a repellent to prevent cross-pollination by bees during the three weeks of full bloom would be very valuable to growers experiencing this problem. It may be possible to spray the trees with a chemical that would repel the bees or to load dispensers with a strong smelling liquid and hang them in the trees during this time. The following research was conducted to determine whether any known bee repellents could be effective in preventing bee foraging.

Materials and methods

The first phase to compare different chemicals as possible repellents was conducted with bees from 14 hives near a litchi orchard at the Institute for Tropical and Subtropical Crops in Nelspruit. A 30% honey (Multiflora) solution in water was found to be more attractive than the same concentration of sugar water and more practical than higher concentrations of honey that caused the bees to become too aggressive. The first evaluation of possible repellents was conducted on 8 July 2008 using the following technique. Inverted lug boxes were covered with green shade cloth and used as a substrate on which a petri dish containing 5 ml honey-water (30%) was placed. There were five lug boxes which were placed at least 5 m apart and 15 m from the nearest bee hive. One of the five crates was used as a control and only had the petri dish with honey-water on top. The other four crates had an M3 fruit fly bait station impregnated with 5 ml of the prospective repellent hanging inside the crate and inaccessible to the bees, but underneath the petri dish with honey-water that the bees could feed on. The first four prospective repellents evaluated were Teepol, Creosote (both reported as repellent by Glynne-Jones (1952), Jeyes fluid and trimethylamine. Initially, honey-water was placed on each crate without any

repellents in position and 5 min later the number of bees feeding at each dish over a 2 min period was determined. Once the bees had finished the 5 ml honey-water and dispersed, the repellents were hung within each crate (except for the control) and another 5 ml honey-water placed in each petri dish. Another count of the numbers of bees feeding in a 2-min interval was then conducted approximately 15 min after the former count and the dishes left until the honey-water was consumed and the bees had dispersed. The repellents were then removed and 5 ml honey-water was again placed in each petri dish and the numbers of bees feeding per 2 min interval determined after 15 min. This last evaluation was conducted to determine whether the repellents would have any long-term effects. The trials were generally conducted between 09:30 and 14:00 each day at temperatures of between 16 and 26°C. A second and third replicate of the first trial were conducted on 9 and 10 July 2008, but the positions of the repellents were randomised.

In the following week (14-16 July 2008) a second trial was conducted using the same methodology but the following chemicals: phenol (Glynne-Jones 1952), cypermethrin (Melksham et al. 1988), methamidophos and trimethylamine. At the end of this trial the first litchi flowers opened and the bees were no longer attracted to the honey-water. As there were several different varieties of litchis present, blossom continued for a long time and we decided to try some repellents in the litchi trees to see whether we could stop bees from visiting flowers. The following chemicals were evaluated by soaking 5 ml into or applying 5 g paste onto M3 dispensers, then hanging one of these in the NE quadrant of a litchi tree approximately 1.3 m above ground. Peppermint oil (Melksham et al. 1988), methyl salicylate as wintergreen ointment (Mayer 1997; Henning et al. 1992), phenol and acetic acid (Glynne-Jones 1952) were used and treated trees were at least 15 m apart. A white PVC quadrat with sides 1 m was placed around the repellent device and the number of bees visiting the flowers in the quadrat in 1 min recorded after waiting about 10 min to allow the bees to get used to the quadrat. Three replicates per treatment were used in randomized blocks and the repellents were evaluated at around 12:00 each day for three consecutive days (2-4 September 2008).

Results and discussion

The results of the petri dish trials were extremely variable between replicates; perhaps due to the influence of temperature and other food sources. In the first trial (Table 3.7.2.1) there were fewer bees attracted to the honey-water alone when repellents were placed at the other feeding stations and this difference was greater when the repellents were removed from the other feeding stations. Teepol and trimethylamine generally appeared to have an attractant effect and perhaps were pulling bees away from the control. Apart from the control, creosote did attract fewer bees once the repellents were removed, but none of these differences were significant ($P>0.05$). In the second trial (Table 3.7.2.2) the numbers attracted to the control were higher when repellents were present at other treatments but there were no significant differences between the control and other treatments ($P>0.05$). In this trial trimethylamine appeared to be slightly repellent whereas it appeared attractive in the first trial. Cypermethrin was significantly attractive relative to methamidophos and phenol when the repellents were present (Table 3.7.2.2). This is contrary to Melksham et al. (1988) who listed cypermethrin, deltamethrin and permethrin as repellent to bees. Although phenol appeared to be the most consistently repellent it was not significantly different from the control and was not effective in keeping bees from litchi flowers (Table 3.7.2.3).

Table 3.7.2.1. Influence of repellents on bees visiting 30% honey solution before, during and after repellents were placed below feeding stations (Trial 1).

Treatments	Replicates with fewer bees than pretreatment when repellents present	Replicates with fewer bees than pretreatment after repellents removed	Mean % change with repellents present versus pretreatment ¹	Mean % change after repellents removed versus pretreatment ²
Teepol	1	1	27.8	87.3
Creosote	1	2	17.0	-22.1
Jeyes fluid	1	1	11.0	37.1
Trimethylamine	1	1	91.6	43.0
Control	2	3	-8.2	-38.1

¹F=0.73; df=4,10; P=0.589. ²F=1.39; df=4,10; P=0.306

Table 3.7.2.2. Influence of repellents on bees visiting 30% honey solution before, during and after repellents were placed below feeding stations (Trial 2)

Treatments	Replicates with fewer bees than pretreatment when repellents present	Replicates with fewer bees than pretreatment after repellents removed	Mean % change with repellents versus pretreatment	Mean % change after repellents versus pretreatment ¹
Phenol	3	3	-20.4 a	-38.5
Cypermethrin	0	1	66.8 b	14.2
Control	1	1	21.3 ab	-6.5
Methamidophos	2	1	-5.9 a	100.4
Trimethylamine	3	2	-33.1 a	-19.4

¹F=0.52; df=4,10; P=0.7213

The quadrat evaluation of repellents in litchi trees while they were flowering showed no repellent effects of any of the chemicals but a significant ($P < 0.05$) attractant effect of methyl salicylate (Table 3.7.2.3). Variable results have been found with this chemical before which is a common flower volatile. Henning et al. (1992) had hoped that it could be used as an attractant for alfalfa but it was found to be slightly repellent. Mayer (1997) found it was not effective in stopping bees from foraging on apple blossom and only repelled bees for a few hours from dandelions. There were no significant differences ($P > 0.05$) between the days on which the numbers of bees visiting the litchi flowers within the quadrats were determined.

Although much of the published research has involved feeding studies with dishes of honey-water or sugar-water as we did, or the use of olfactometers, some of the more repellent compounds have been used in field evaluations but these have always fallen short of the degree of repellency required to prevent foraging and may only repel at the most about 25% of the bees (Atkins et al. 1975; Moffett and Morton 1975; Mayer 1997). It is therefore unlikely that a chemical will ever be found that will prevent cross-pollination.

Table 3.7.2.3. Bees visiting litchi flowers within a 1 m quadrat containing a repellent on an M3 dispenser.

Repellents placed on 2 Sep 2008	Mean no. bees at flowers in quadrat
Untreated control	4.7 a
Peppermint oil	7.2 ab
Phenol	7.3 ab
Acetic acid	11.0 ab
Methyl salicylate	13.3 b

Means followed by the same letter were not significantly different ($P > 0.05$ SNK test)

Conclusion

None of the chemicals evaluated, most of which have been regarded as bee repellents in other publications, showed adequate repellency to prevent bees from foraging in flowers. As bees are known to prefer citrus flowers to some other flowers such as avocado it is highly unlikely that a chemical will be found that will protect most citrus flowers from pollination for a three week period. Growers should therefore consider using netting to exclude bees as used in California or ensure that cultivars that are susceptible to cross-pollination are planted far apart.

Future research

No further research is planned.

Technology transfer

An article on this research will be published in the S A Fruit Journal.

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3.7.3 PROGRESS REPORT: Psylla attractants

Experiment 944 (2008/9-2009/10) by Tim G Grout and Peter R Stephen (CRI)

Opsomming

In vroeër werk wat op lokmiddels vir valskodlingmot uitgevoer is, het Christo Smit aangedui dat sommige van die chemikalieë waarmee hy gewerk het, moontlik die sitrus bladvlou kan aanlok. Hierdie navorsing is voorgestel met die siening dat sommige van die chemikalieë evalueer kan word in 'n massa lokval beheertegniek. Ongelukkig is 'n paar monsters eers laat in die seisoen ontvang en 'n perseel met geskikte hoë bladvlou getalle vir vergelyking van lokmiddels kon nie gevind word nie. Geen navorsing was dus moontlik nie en tensy 'n verlate boord of 'n boord wat uitgetrek gaan word gevind kan word, sal dit moeilik wees om 'n perseel met genoegsame bladvlou in die toekoms te kry. Hierdie is omdat produsente normaalweg 'n zero toleransie vir hierdie plaag handhaaf.

Summary

In earlier work conducted on attractants for false codling moth, Christo Smit had indicated that some of the chemicals he was dealing with may be attractive to citrus psylla. This research was therefore proposed with a view to evaluating some chemicals for use in mass-trapping as a control technique. Unfortunately, a few samples were only received late in the season and a site with suitably high numbers of psyllids to allow for the comparison of a number of attractants could not be found. No research has therefore been possible and unless an orchard is found that has been abandoned or is due to be removed, it will be difficult to find a site in the future with sufficient psyllids because growers normally have zero tolerance for this pest.

4 PROGRAMME: DISEASE MANAGEMENT

4.1 PROGRAMME SUMMARY

By Paul H. Fourie (Programme Manager)

All projects in the Disease Management programme are showing very good progress and most grower priorities are addressed in experiments designed to meet certain short-, medium- and long-term strategic objectives. The progress of the 2008/9 reporting period is briefly summarised below.

Apart from pure research experiments, the Graft Transmissible Diseases project provided essential services for the Citrus Improvement Scheme through re-indexing of foundation block trees, shoot tip grafting and pre-immunisation of new entries. Virus elimination was successful in several entries that were submitted to the Citrus Foundation Block for multiplication. Citrus viroids were detected in certain cultivars and *ad hoc* surveys and experiments were conducted as part of the risk assessment involved in addressing this concern. Several experiments are under way to select and evaluate new mild CTV strains for pre-immunisation of grapefruit, soft citrus and Valencia oranges; one of these was successfully concluded and relevant recommendations were made. An *ad hoc* experiment was concluded to identify suitable CTV sources to be evaluated in field trials for cross-protection of soft citrus cultivars. The dynamics between VT, B165 and RB variant groups that were identified in the GFMS12 cross-protection source will be evaluated in mixed inoculations onto Marsh and Star Ruby grapefruit plants at a range of temperatures. "*Candidatus Liberibacter africanus subspecies capensis*" was again detected in several Cape Chestnut trees, but in no other Rutaceae plants surveyed. Cross-inoculation studies with this subspecies as well as the greening pathogen are under way to determine whether other *Citrus*-related trees can act as alternate hosts. To date, six clones were derived by rescuing embryos from healthy chimeras on greening-infected fruit and are being evaluated for greening resistance in glasshouse and orchard. Following promising results in pot trials, the bactericides being evaluated for greening control yielded disappointing results in the field. In order to address a potential market access concern, another *ad hoc* experiment was initiated to study the possible seed transmissibility of greening, following USA-reports pointing towards this unlikely phenomenon.

In the Soilborne Diseases Project, several contract trials were conducted. From these trials, invaluable information was obtained regarding the control of nematodes with alternative products. A trial site was identified for evaluation of several pre-plant nematicide treatments, including fumigation and biofumigation. This experiment supports the general focus in this project aiming at sustainable management of citrus soil and root health. Promising results were obtained with a proprietary product involving stimulation of nematode egg hatching, and several other softer and/or biological control options were evaluated. A thorough survey of *Phytophthora* species in SA citrus orchards identified *P. nicotianae*, *P. citrophthora*, *P. citricola*, *P. cinnamomi* and a putative new species, with *P. nicotianae* having the highest incidence followed by *P. citrophthora*. Trunk and branch cankers of Clementines, caused by *P. citrophthora*, were effectively inhibited through a late-winter foliar phosphonate application, followed by 3 trunk sprays (every 2 months) with a Sporekill and captan mixture during winter. Promising results were also obtained with alternative algacides. Among Navel cultivars evaluated, 'Royal Late' was the most tolerant cultivar, with 'Witkrans' and 'Powell Summer' moderate tolerant and 'Washington' highly susceptible. Residue analyses of potassium phosphonate levels in the roots following application as foliar sprays or through the irrigation system showed similar levels. An experiment was initiated in which the effect of compost, amended with beneficial organisms, on tree condition and general disease resistance is studied.

In the Citrus Black Spot project, different copper formulations were evaluated and found to be similarly effective and rainfast, although residue deposition (per amount of metallic copper applied) with the tested cuprous oxide product was remarkably better. New generic strobilurin fungicides were found to be effective in field trials. Replacement of oil with Sporekill was studied in a collaborative project between SA, Brazil and Argentina and showed good control at reduced rates of mancozeb or copper. A holistic approach aimed at CBS inoculum management through foliar sprays and accelerating leaf decomposition is under way. CBS epidemiology is being studied in the Eastern Cape Province through spore trapping and weather monitoring.

In the Fruit and Foliar Diseases Project, the control of Alternaria brown spot (ABS) was obtained with four strobilurins or phosphonate + mancozeb tank mix applications with either mineral spray oil or Sporekill, thereby saving growers 4-5 spray rounds. An ABS disease prediction model is being evaluated to assist growers in management of this disease. Research also focuses on improving spray application through optimal use of spray machines or adjuvants. Conventional and novel spray machines were evaluated in several orchard trials and it was shown that similar and even improved spray deposition can be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers.

Post Harvest Diseases remain a very high priority and several experiments were directly aimed at improving post harvest disease management in packhouses. Through identification of *Penicillium* species occurring at various stages in the supply chain, it was shown that postharvest contamination often occurs further down the export chain. On a local level, it was shown that other green mould causing *Penicillium* species, probably less sensitive to the fungicides used, have increased in prominence. Resistance against imazalil and guazatine resistance in *Penicillium* spp. was demonstrated and needs to be elucidated on a regional and packhouse level to further clarify the practical occurrence and implications. An audit of fungicide application systems indicated that imazalil residue loading was generally sub-optimal, which will result in loss of control and sporulation inhibition of especially the resistant strains. Several means of improving residue loading are being studied to develop specific recommendations for fungicide application in packhouses. In future, these recommendations should improve management of these systems, which at present were found to be sub-standard. Generic imazalil and pyrimethanil formulations in a dip treatment were evaluated against green mould and recommended for registration. A yeast antagonist was evaluated against green mould, and although it demonstrated some level of control, was found not to be effective as a stand-alone treatment. Further trials should focus on improved application and/or using it as part of a combined approach. Sodium bicarbonate alone or in combination with imazalil did not show the synergistic control that was anticipated. A study on biological control of green mould was concluded and a *Bacillus subtilis* strain was shown to be a good coloniser of citrus fruit and also controlled green mould relatively well. However, as it was found that this bacterium produced antibiotics as part of its mode of action, further studies will not be considered. Retain and Maxim were evaluated as potential replacements for 2,4-D sodium salts used for calyx retention and Retain again showed promising results. In order to improve technology transfer, a handbook explaining the most important postharvest citrus diseases and their control is being compiled as part of the concomitant updating of the production guidelines.

In the Diagnostic Centre (DC), Laura Huisman took early retirement after many years of sterling service. Laura was replaced by Wilma Bester and the DC continues to perform regular water and soil analyses for *Phytophthora* and nematode infestation for the citrus nurseries and researchers, as well as general citrus pest / disease diagnoses. The services are presently being expanded to offer the citrus industry a 'one-stop shop' for diagnostic and technical services.

In general, good progress was made in Disease Management. However, research progress is continually hampered by constant demands on the researchers' time by *ad hoc* crisis management and industry actualities. Unfortunately, the 'non-research' demands on the available human resources in technical support of the industry limit the quality and quantity of tangible research outputs and divert from consolidated and focused research. In an attempt to address this concern, research alliances with universities and other research service providers are used to broaden the research capacity, while sustaining service delivery to the industry. However, this alone will not address this concern and additional capacity building for technical support should be considered.

PROGRAMOPSOMMING

Al die projekte in die Siektebestuurprogram toon baie goeie vordering en die meeste produsente-prioriteite word aangespreek in eksperimente wat ontwerp word om sekere kort-, medium- en langtermyn strategiese doelwitte te bereik. Die vordering vir die 2008/9 verslagperiode word kortliks hieronder opgesom.

Die Ent-oordraagbare Siektes Projek het, afgesien van suiwer navorsingseksperimente, noodsaaklike dienste aan die Sitrus Verbeteringskema verskaf, deur her-indeksering van grondvesblokbome, groeipunt-enting en pre-immunisasie van nuwe kultivars. Virus-verwydering was suksesvol in verskeie kultivars wat in die Sitrus Grondvesblok vir vermeerdering ingedien is. Sitrusviroïede is in sekere kultivars opgespoor en *ad hoc* ondersoek en eksperimente is uitgevoer as deel van die risiko-bepaling betrokke in die aanspreek van hierdie probleem. Verskeie eksperimente is onderweg om nuwe matige CTV isolate vir pre-immunisasie van pomelos, sagtesitrus en Valencia lemoene te selekteer en te evalueer; een van hierdie is suksesvol afgesluit en relevante aanbevelings is gemaak. 'n *Ad hoc* eksperiment om geskikte CTV bronne te identifiseer om in veldproewe vir kruisbeskerming van sagtesitrus kultivars geëvalueer te word, is afgesluit. Die dinamika tussen VT, B165 en RB variant groepe wat in die GFMS12 kruisbeskermingsbron geïdentifiseer is, sal in gemengde inokulasies op Marsh en Star Ruby pomelo plante teen 'n reeks van temperature, geëvalueer word. "*Candidatus Liberibacter africanus subspecies capensis*" is weer in verskeie Kaapse Kastaiing bome waargeneem, maar in geen ander Rutaceae plante wat ondersoek is nie. Kruis-inokulasie-studies met hierdie subspesie, asook met die vergroeningspatogeen, is onderweg ten einde vas te stel of ander *Citrus*-verwante bome as alternatiewe gashere kan optree. Ses klone is tot op datum verkry deur embryos vanaf gesonde chimeras op vergroeningsgeïnfekteerde vrugte te red, en word in glashuise en boorde vir vergroeningsweerstand geëvalueer. Die bakteriesiedes wat vir vergroeningsbeheer geëvalueer is, het, volgende op belowende resultate in potproewe, teleurstellende resultate in die veld gelewer. Ten einde 'n

potensiële marktoegangsprobleem aan te spreek, is nog 'n *ad hoc* eksperiment geïnisieer om die moontlike saad-oordraagbaarheid van vergroening te bestudeer, volgende op VSA-verslae wat op hierdie onwaarskynlike verskynsel dui.

Verskeie kontrakproewe is in die Grondgedraagde Siektes Projek uitgevoer. Belangrike inligting rakende die beheer van nematodes met alternatiewe produkte, is vanuit hierdie proewe verkry. 'n Proefperseel is vir die evaluasie van verskeie vóór-plant nematisiedbehandelings geïdentifiseer, insluitende beroking en bio-beroking. Die eksperiment ondersteun die algemene fokus in hierdie projek wat gemik is op volhoubare bestuur van sitrusgronde en wortelgesondheid. Belowende resultate is verkry met 'n self-ontwikkelde produk betrokke in die stimulering van die uitbrou van nematode-eiers, en verskeie ander sagter en/of biologiese beheer opsies is geëvalueer. 'n Deeglike opname van *Phytophthora* spesies in SA sitrusboorde het *P. nicotianae*, *P. citrophthora*, *P. citricola*, *P. cinnamomi* en 'n moontlike nuwe spesie geïdentifiseer, met *P. nicotianae* wat die hoogste voorkoms gehad het, gevolg deur *P. citrophthora*. Stam- en takkankers van Clementines, veroorsaak deur *P. citrophthora*, is effektief deur 'n laat-winter blaar-fosfonaattoediening geïnhibeer, gevolg deur 3 stamtoedienings (elke 2 maande) met 'n Sporekill- en kapitanmengsel gedurende die winter. Belowende resultate is ook met alternatiewe algesiedes verkry. Van die Navel kultivars wat geëvalueer is, was 'Royal Late' die mees bestande kultivar, met 'Witkrans' en 'Powell Summer' matig bestand en 'Washington' hoogs vatbaar. Residu-analises van kaliumfosfonaatvlakke in die wortels, ná toediening as blaarspuit of deur die besproeiingsstelsel, het soortgelyke vlakke getoon. 'n Eksperiment is geïnisieer waarin die effek van kompos, aangepas met voordelige organismes, op boomtoestand en algemene siekteweerstand bestudeer word.

In die Sitrus Swartvlek Projek, is verskillende koperformulasies geëvalueer, en gevind om ewe effektief en reënvas te wees, hoewel residu-neerlegging (per hoeveelheid metaalkoper toegedien) met die getoetste koper-oksied produk merkbaar beter was. Nuwe generiese strobilurienfungisiedes was effektief in veldproewe. Vervanging van olie met Sporekill is in 'n gesamentlike projek tussen SA, Brasilië en Argentinië bestudeer en het goeie beheer teen verminderde dosisse van mankoseb of koper getoon. 'n Holistiese benadering gemik op SSV inokulum-bestuur deur blaartoedienings en versnelling in blaar-ontbinding, is onderweg. SSV epidemiologie word in die Oos-Kaap provinsie deur spoorvangstudies en weermonitering bestudeer.

In die Vrugte- en Blaarsiektes Projek, is die beheer van *Alternaria* bruinvlek (ABV) verkry met vier strobilurien of fosfonaat + mankoseb tenkmengseltoedienings, met óf minerale spuit-olie óf Sporekill, en hierdeur spaar die produsent 4-5 spuitronddes. 'n ABV siekte-voorspellingsmodel word geëvalueer om produsente in die bestuur van hierdie siekte te ondersteun. Navorsing fokus ook op die verbetering van spuittoediening deur optimale gebruik van spuitmasjiene of byvoegmiddels. Konvensionele en nuwe spuitmasjiene is in verskeie boordproewe geëvalueer en daar is getoon dat soortgelyke en selfs verbeterde spuitneerlegging teen laer spuitvolumes verkry kan word deur optimale gebruik van toerusting of deur die gebruik van meer effektiewe spuitmasjiene.

Na-oes Siektes bly 'n baie hoë prioriteit en verskeie eksperimente is direk gerig op die verbetering van na-oes siektebestuur in pakhuse. Deur die identifikasie van *Penicillium* spesies wat by verskillende stadiums in die verskaffingsketting voorkom, is aangetoon dat na-oes kontaminasie dikwels verder af in die uitvoerketting voorkom. Op 'n plaaslike vlak is aangetoon dat ander groenskimmel-veroorsakende *Penicillium* spesies, wat moontlik minder sensitief is vir die fungisiedes wat gebruik word, in voorkoms toegeneem het. Weerstand teen imazalil en guazatine in *Penicillium* spp. is gedemonstreer en moet op 'n streek- en pakhuisvlak uitgeklaar word ten einde die praktiese voorkoms en implikasies te bepaal. Nasien van fungisiedtoedieningsstelsels het aangedui dat imazalil residu-lading oor die algemeen sub-optimaal was, wat verlies in beheer en sporulasie-inhibisie van veral die weerstandbiedende isolate, tot gevolg sal hê. Verskeie maniere om residu-lading te verbeter, word bestudeer om spesifieke aanbevelings vir fungisiedtoediening in pakhuse te ontwikkel. Hierdie aanbevelings behoort bestuur van hierdie stelsels in die toekoms te verbeter, wat huidig onder-standaard is. Generiese imazalil en pyrimethanil formulasies in 'n doop-behandeling is teen groenskimmel geëvalueer en vir registrasie aanbeveel. 'n Gis-antagonis is teen groenskimmel geëvalueer, en hoewel dit 'n mate van beheer getoon het, is dit nie as 'n alleenstaande behandeling effektief gevind nie. Verdere proewe moet op verbeterde toediening en/of die gebruik daarvan as deel van 'n gekombineerde benadering fokus. Natrium-bikarbonaat alleen of in kombinasie met imazalil het nie die sinergistiese beheer getoon wat verwag is nie. 'n Studie op biologiese beheer van groenskimmel is afgesluit en 'n *Bacillus subtilis* isolaat is aangedui as 'n goeie koloniseerder van sitrusvrugte en het ook groenskimmel relatief goed beheer. Verdere studies word egter nie oorweeg, aangesien gevind is dat hierdie bakterium antibiotika as deel van sy werkswyse geproduseer het. Retain en Maxim is as potensiële vervangings vir 2,4-D natriumsoute vir blomkelk-behoud gebruik, en Retain het weereens belowende resultate getoon. Ten einde tegnologie-oordraging te verbeter, word 'n handboek saamgestel as deel van

die gepaardgaande opdatering van die produksie-riglyne, waarin die belangrikste na-oes sitrussiektes en hul beheer verduidelik word.

In die Diagnostiese Sentrum (DS), het Laura Huisman vroeg, ná baie jare van staatsmakerdiens afgetree. Laura is deur Wilma Bester vervang en die DS gaan voort om gereelde water- en grond-analises vir *Phytophthora* en nematode-infestasië vir die sitruskwekerie en navorsers te doen, asook algemene sitrus pes- of siektediagnoses. Die dienste word tans uitgebrei ten einde vir die sitrus-industrie 'n "one-stop-shop" vir diagnostiese en tegniese dienste te bied.

Goeie vordering is oor die algemeen in Siektebestuur gemaak. Navorsingsvoortgang word egter voortdurend gestrem deur konstante eise op die navorsers se tyd deur *ad hoc* krisisbestuur en industrie gebeurlikhede. Hierdie 'nie-navorsing' eise op die beskikbare menslike hulpbronne in tegniese ondersteuning van die industrie beperk ongelukkig die kwaliteit en kwantiteit van tasbare navorsingsuitsette en stuur weg van gekonsolideerde en gefokusde navorsing. In 'n poging om hierdie probleem aan te spreek, word navorsingsalliansies met universiteite en ander navorsingsdiensverskaffers gebruik om die navorsingskapasiteit te verbreed, terwyl dienslewering aan die industrie volgehou word. Dit alleen sal egter nie die probleem oplos nie, en addisionele kapasiteitsbou vir tegniese ondersteuning moet oorweeg word.

4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project coordinator: Gerhard Pietersen (CRI-UP)

4.2.1 Project summary

Amongst the graft transmissible diseases of Citrus, the two most important ones from a southern African perspective are tristeza and citrus greening, and experiments within this project are directed at improving various aspects of control of these two diseases. In the case of tristeza, experiments in sections 4.2.1 to 4.2.8 all aim at finding better *Citrus tristeza virus* (CTV) mild strains for pre-immunization of various citrus species. Steady progress is made in these long-term projects, two of which were concluded and final reports presented here. The response of different 7 red grapefruit cultivars to four CTV cross-protecting sources in the Malelane area is discussed in section 4.2.5. After 10 years, the overall reaction of the commercially grown cultivars with regard to various yield parameters and symptoms was very similar, although some interactions were observed, which might also be influenced by other climatic conditions. The reduction of tree size and yield efficiency of 'Star Ruby' and the general occurrence of more severe stem pitting with GFMS 12 confirmed the unsuitability of this CTV source. The reduction of production and crop value of 'Flame' by the GFMS 35 source causes concern as it is the current pre-immunizing source for all grapefruit in the Citrus Improvement Scheme. The most stable CTV source where the least cultivar interactions occurred was GFMS 67. This study furthermore revealed that it is not only essential to look for CTV cross-protecting sources for specific citrus types, but also within a type. In another concluded experiment, section 4.2.8, the possibility of improving fruit size by planting virus-free, rather than pre-immunized Clementine and Satsuma trees was investigated. Natural CTV infection of the virus-free trees, however, occurred and the experiment was terminated. The objective to improve fruit size by eliminating CTV is therefore not feasible in South Africa, and CTV cross-protecting sources should be evaluated to elucidate this problem. Such an experiment was initiated in April 2009.

In section 4.2.9, the cause of mild strain cross protection breakdown in grapefruit as well as the development of rapid sensitive detection methods to CTV strains is investigated. Two hypotheses are being tested: 1) that cross protection breakdown in GFMS 12 and GFMS 35 pre-immunized plants is due to "super-infection" with severe CTV strains, or 2) breakdown occurs through dominance by an inherent severe component of GFMS 12 under high temperature selection. Two main sequence types were shown to occur in a severely infected field source (pre-immunized with GFMS 12) while the glasshouse-maintained GFMS 12 population (used in citrus pre-immunization) have 3 main sequence types. Various single aphid isolates were characterised. Representative sub-isolate of VT-, B165- and RB-like groups were bark chip grafted in different combinations onto Marsh and Star Ruby grapefruit plants and maintained at 4 different temperature regimes in order to study the strain dynamics and interactions at different temperatures. A previously reported real-time RT-PCR protocol for CTV detection, using a TaqMan[®] hydrolysis probe, has been tested and optimised.

Experiments on greening are aimed at understanding its epidemiology, and to improve or find additional methods of control. Attempts to obtain greening-resistant plants were made by rescuing embryos from healthy chimeras on greening-infected fruit (section 4.2.10). The resulting plants have been exposed to field psylla and tested by PCR for Laf. Two clones look promising as they have remained asymptomatic since 2006, and they also test free of Laf by PCR despite being challenged by vector individuals testing Laf positive. This may suggest that the plants are resistant, and will be evaluated under field conditions. In section 4.2.11, two foliar applied systemic products were assessed with regard to effective control of Laf.

Promising results were observed for a second time in the pot trial, especially product B (a known bactericide). The field trial data were, however inconclusive and require repeating. Identification of potential alternate hosts to citrus of Laf will allow disease pressure reduction to be conducted more efficiently. In this study (section 4.2.12), indigenous plants mainly of the citrus family (*Rutaceae*) are evaluated for their capability to host the pathogen. Except for *Calodendrum capense* (cape chestnut), none of the indigenous plants tested positive for Liberibacters. Seventeen *C. capense* plants tested positive and were shown to be infected with “*Candidatus Liberibacter africanus*” subspecies *capensis*” (LafC). No symptoms were obtained on various indigenous rutaceous species following graft-transmission of Laf, but high Ct values in real-time PCR tests may be indicative of low concentrations of the bacteria and needs more time post-inoculation to be confirmed. The spread in recent years of three different citrus Liberibacters in different countries of the world required confirmation of the presence of only “*Candidatus Liberibacter africanus*” (Laf) in South Africa (section 4.2.13). Sequence analysis of a portion of the ribosomal protein (*rpl*) gene revealed that all samples were essentially identical irrespective of geographic location in South Africa. No instances of LafC on citrus were found. Reports emanating from several laboratories in the United States suggest that “*Candidatus Liberibacter asiaticus*” (Las) may be transmissible by seed. To determine if this is true for Laf (section 4.2.14), fruit was collected from greening symptomatic branches, which had leaves testing positive for Laf by PCR. The seeds were removed and planted under insect-free conditions. The seedlings (1570) from six Citrus species are currently being monitored for symptoms and will be tested by real-time PCR for Laf if any greening-like symptoms are noted.

Projekopsomming

Uit alle ent-oordraagbare siektes van sitrus is tristeza en vergroening die twee mees belangrikstes in 'n suidelike Afrika.. Eksperimente in hierdie projek is gemik op die verbetering van verskeie aspekte in die beheer van die twee siektes. In die geval van tristeza het eksperimente in afdelings 4.2.1 tot 4.2.8 almal ten doel om beter milde rasse van sitrus tristeza virus (CTV) te verkry vir die pre-immunisering van verskeie sitrus spesies. Bestendige vordering word gemaak in hierdie lang-termyn projekte, waarvan twee voltooi is en as finale verslae hier aangebied word. Die reaksie van 7 rooi pomelo kultivars op 4 CTV kruisbeskermingsrasse in die Malelane area is bespreek in afdeling 4.2.5. Na 10 jaar is die algemene reaksie wat opbrengs parameters van die kommersiële kultivars soortgelyk; al is daar interaksies verkry wat verder deur klimaatsomstandighede geaffekteer mag word. Die vermindering in boomgrote en opbrengs van “Star Ruby”, en die voorkoms van strawwe stamgleuf met GFMS 12 bevestig die onbruikbaarheid van die CTV bron. Die vermindering in produksie en oeswaarde van GFMS 35 op “Flame” is rede to kommer aangesien dit die huidige bron is wat gebruik word vir pre-immunisering van alle pomelo binne die Sitrus Verbeteringskema. Die mees stabiele CTV bron, met die minste kultivar interaksies, was GFMS 67. Hierdie studie het gewys dat CTV kruisbeskermingsbronne nie net vir sitrus-tipes geselekteer moet word nie, maar ook vir spesifieke kultivars. In 'n ander voltooide projek, afdeling 4.2.8, word die moontlikheid om groter vrugte in Clementine's en Satsuma te kry, ondersoek deur virus-vrye plante eerder as gepre-immuniseer te plant. Natuurlike besmetting van CTV het egter vinnig plaasgevind en wys dat hierdie benadering nie haalbaar in Suid-Afrika is nie en dat meer geskikte CTV kruisbeskermingsbronne gekry moet word. So 'n eksperiment is in April 2009 begin.

In afdeling 4.2.9 word die oorsaak van kruisbeskerming-verval in pomelo beskryf tesame met die ontwikkel van sensitiewe opsporingsmetodes vir CTV. Twee moontlikhede word ondersoek: 1) dat kruisbeskerming-verval in GFMS 12 en GFMS 35 deur 'n super infeksie van strawwe rasse veroorsaak word, en 2) dat oorkoming a.g.v. dominasie van 'n strawwe CTV ras, inherent in die GFMS 12 bron, onder warmer weersomstandighede is. Twee hoof nukleotied volgorde variante kom in 'n straf-besmette boom, wat met GFMS 12 gepre-immuniseer was, voor, terwyl die glashuisbron van GFMS 12 (wat vir pre-immunisering gebruik word) 3 variante bevat. Verskeie enkel plantluis ge-isoleerde bronne is gekarakteriseer. 'n Verteenwoordiger van VT-, B165-, en RB-agtige bronne is met bas-strook enting in verskillende kombinasies na Marsh en Star Ruby bome oorgedra en by vier verskillende temperatuur omstandighede gehou om die dinamika tussen hierdie bronne te ondersoek. 'n Gepubliseerde “real-time” tru-transkriptase PKR protokol vir CTV opsporing, m.b.v. 'n Taqman® peeler, is getoets en ge-optimeer.

Eksperimente op vergroening is daarop gemik om die siekte se epidemiologie beter te verstaan, en om verbeterde of nuwe beheer strategieë te verkry. Embrio's is vanuit gesonde chimera-sektore in vergroende vrugte te verkry in 'n poging om vergroening-bestande plante te kry (afdeling 4.2.10). Die gevolglike plantjies word aan boord-versamelde psilla's blootgestel en getoets met PKR vir “*Candidatus*” *Liberibacter africanus* (Laf). Twee klone lyk belowend aangesien hulle sedert 2006 simptoombloos bly en ook negatief toets vir Las, ten spyte dat hulle aan Laf positiewe vektor individue blootgestel was. Dit dui daarop dat hulle moontlik weerstandbiedend is, en sal in die veld getoets word. In afdeling 4.2.11 word blaartoedienings met twee sistemiese middels ge-evalueer vir hul vermoë om Laf te beheer. Belowende resultate is vir 'n tweede keer in die potproewe verkry, veral met produk B (n bakteriesied). Gevolgtrekkings kan egter nog nie uit die

resultate van die veldproef gemaak word nie en dit sal herhaal moet word. Identifisering van alternatiewe gashere tot sitrus vir Laf kan tot gevolg hê dat die inokulumdruk van die siekte meer doeltreffend bestuur kan word. Verskeie inheemse lede van die sitrus-familie (*Rutaceae*) word ge-evalueer vir hul vermoëns om as gasheer op te tree (Afdeling 4.2.12). Behalwe vir *Calodendrum capensis* (wilde kastaiing) was geen ander inheemse sitrus familie plante met Liberibacters besmet nie. Sewentien wilde kastaiings is positief vir “*Candidatus*” Liberibacter africanus spp. capensis (LafC) gevind. Geen simptome is op verskeie inheemse sitrus-familie plante na inokulering met Laf waargeneem nie, maar hoë Ct waardes met “real-time” PKR mag op lae konsentrasies van die bakterieë dui. Die verspreiding van drie Liberibacters oor die laaste paar jaar na nuwe lande het genoop dat daar bevestig word dat slegs Laf in suider Afrika op sitrus voorkom (Afdeling 4.2.13). Nukleotied volgordes binne ’n gedeelte van die ribosomale proteïen (*rpl*) geen was dieselfde vir alle bronne, ongeag geografiese oorsprong. Geen LafC besmetting van sitrus is gevind nie. Twee laboratoriums in die VSA het gerapporteer dat “*Candidatus*” Liberibacter asiaticus (Las) saadgedraagde is. Om te bepaal of dieselfde geld vir Laf (afdeling 4.2.14), is vrugte vanaf vergroende takke, positief vir Laf, versamel en die saad uitgeplant in insekvryst glashuise. Saailinge word gemonitor vir vergroening-agtige simptome en sal d.m.v. “real-time” PKR getoets word vir Laf.

4.2.2 PROGRESS REPORT: Cross-protection of Marsh and Star Ruby grapefruit using Beltsville sub-isolates of Nartia mild strain Experiment 679 (2003 - 2013) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

The Nartia source (GFMS 12), currently used to pre-immunise white grapefruit and pummelos, is contaminated by a severe strain of CTV. The GFMS12 source was replaced with GFMS 35 as a cross protecting source for red grapefruit during 1998 and during 2007 for all grapefruit. In the search for more suitable cross-protection sources, 20 sub-isolates were derived from two Nartia sources (A=GFMS 12, C=GFMS 14) and the Mouton source in Beltsville MD, USA, by single aphid transmissions and imported back to South Africa. Six of these sub-isolates showed potential as cross-protecting agents in glasshouse tests and their protecting abilities against severe strains had to be evaluated in the field. Virus-free Star Ruby and Marsh grapefruit trees were pre-immunised with the six Beltsville sub-isolates as well as two single aphid transmitted sub-isolates from the ITSC (GFMS 12/7, GFMS 12/9), GFMS 12 and GFMS 35. The control trees were left virus-free. Pre-immunisation was confirmed with ELISA. ELISA revealed that two of the Beltsville sub-isolates did not comply with traits of a good cross-protecting isolate as they transmitted poorly and multiplied and moved slowly in the plant. These two sub-isolates were excluded from further evaluations. The Marsh trees were planted at Riversbend in the Nkwaleni Valley and the Star Ruby trees at Tambuti Estates in Swaziland during 2003. Trees were evaluated for growth, yield and stem pitting. Some sub-isolates suppressed growth of the Marsh trees and are similar to the trees with GFMS 12, which carries a severe strain. Based on tree sizes, trees with B390/3 sub-isolate performed the best in Marsh grapefruit and trees with B389/4 sub-isolate performed the best in Star Ruby grapefruit. In terms of production (cumulative yield for three years), trees with GFMS 35, the current cross protecting source for all grapefruit and the trees that were planted with a virus free status performed the best with regards Marsh grapefruit and trees with sub-isolates B389/4 and GFMS 12/9 performed the best with regards Star Ruby grapefruit. The Marsh (white grapefruit) trees with GFMS 35, which was previously the only red grapefruit pre-immunising source, are better than trees with GFMS 12, which previously was the white grapefruit pre-immunising source. Once again it confirms that GFMS 12 is not a good pre-immunising source for Star Ruby. With time it will become clear if any of the sub-isolates are better than GFMS 35, the present pre-immunising source for grapefruit.

Opsomming

Daar is gevind dat die Nartia tristeza bron (GFMS 12) wat voorheen vir pre-immunisering in die suider Afrikaanse Sitrus Verbetering-skema gebruik is, met ’n strawwe *Citrus tristeza virus* (CTV) ras gekontamineer is. Die GFMS 12 bron is gedurende 1998 met GFMS 35 vervang as ’n kruisbeskerming- CTV bron vir rooi pomelos en gedurende 2007 vir wit pomelos en pompelmoese. In die soeke na meer geskikte kruisbeskermingsbronne, is 20 sub-isolate vanaf twee afsonderlike Nartia bronne (A=GFMS 12, C=GFMS 14) en die Mouton bron in Beltsville MD, VSA, deur middel van enkel plantluis oordragings voorberei. Ses van die 20 sub-isolate wat potensiaal as kruisbeskermingsagente getoon het nadat dit deur biologiese indeksering in die glashuis ge-evalueer is, is gebruik om hul beskermingsvermoëns teen strawwe rasse in die boord te bepaal. Virus-vrye Star Ruby en Marsh pomelo boompies is met die ses Beltsville sub-isolate ge-preïmmuniseer, twee enkel plantluis oordraging sub-isolate van die LNR-ITSG (GFMS 12/7, GFMS 12/9), GFMS 12 en GFMS 35 is ingesluit. Boompies wat aanvanklik virusvry is, is as kontrole gelaat. Preïmmunisasie is bevestig deur middel van ELISA. ELISA het uitgewys dat twee van die Beltsville sub-isolate nie voldoen aan sekere vereistes vir ’n goeie kruisbeskermingsbron nie, deurdat hulle ’n lae

persentasie oordraagbaarheid het, asook stadig vermeerder en beweeg in die plant. Hierdie twee sub-isolate word nie verder ge-evalueer nie. Die Marsh boompies is by Riversbend in die Nkwaleni Vallei uitgeplant, en die Star Ruby is by Tambuti landgoed in Swaziland gedurende 2003 uitgeplant. Die boompies se boomvolumes, stamgleufwaardes asook oesopbrengs is geneem 5 jaar na plant. In die vroeë stadium van die proef is die volgende waarnemings moontlik: i) GFMS 12 onderdruk groei en veroorsaak strawwe stamgleuf, ii) GFMS 35 presteer beter as GFMS 12 in Marsh en Star Ruby. Na aanleiding van die boomvolumes presteer sub-isolaat B390/3 die beste in Marsh en B389/4 die beste in Star Ruby. Met betrekking tot produksie (kumulatiewe opbrengs vir drie jaar), presteer sub-isolaat GFMS 35, die huidige kruisbeskermer vir alle pomelos en die virus vrye bome die beste in Marsh en B389/4 en GFMS 12/9 die beste in Star Ruby. Die Marsh virusvrye kontrole bome presteer goed in die vroeë stadium maar mag verander sodra hulle geïnfecteer word met natuurlike CTV rasse. Met tyd sal dit egter duidelik word of van die sub-isolate beter beskermers vir pomelo is as die huidige bron, GFMS 35.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by propagation material and various aphid species of which *Toxoptera citricida* is the most efficient. Many strains of CTV occur and may co-exist as mixtures in individual field trees and even in single buds on a tree. Symptoms induced by CTV, range from mild with no noticeable effect on the host to severe stem pitting and decline, resulting in uneconomic production (Marais *et al.*, 1996). The only practical means of controlling CTV at present is by mild strain cross-protection (van Vuuren *et al.*, 1993). A breakdown in the protection offered by the Nartia A (GFMS 12) source owing to the presence of a severe strain within the complex (van Vuuren *et al.*, 2000), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained from two Nartia sources (Nartia A = GFMS 12 and Nartia C = GFMS 14; van Vuuren *et al.*, 1993) and the Mouton source that was collected by Marais. In this study, the sub-isolates are being evaluated for mildness and their potential as cross-protecting sources in the field.

Materials and methods

The 20 SAT sub-isolates of the Nartia A and C sources (A=GFMS 12 and C=GFMS 14) as well as that from the Mouton source were prepared at the quarantine facility in Beltsville MD, USA and were imported back to South Africa. In a greenhouse experiment, they were bud-inoculated separately onto CTV sensitive Mexican lime indicator plants to differentiate the sub-isolates according to their effects on the host. Growth and stem pitting were determined and the virus titer was measured by means of enzyme-linked immunosorbent assay (ELISA) for each sub-isolate 6 months after inoculation. The four sub-isolates with the best potential (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) were used to pre-immunise virus-free Marsh and Star Ruby grapefruit on MxT rootstocks that were prepared under insect-free conditions in the greenhouse. They are compared with GFMS 12 (previous standard for white grapefruit), GFMS 35 (present standard for grapefruit), GFMS 12/7 and GFMS12/9 (ARC-ITSC single aphid transfer sub-isolates from GFMS 12) and trees that were planted with a virus-free status. Pre-immunisation has been confirmed by ELISA 6 months after inoculation. During 2003, the Star Ruby grapefruit trees were planted in Swaziland at Tambuti Estates and the Marsh grapefruit trees at Riversbend in the Nkwaleni Valley. The trees were planted according to a randomised block design with 5 replications. Tree size, were measured and the canopy volumes calculated according to Burger *et al.* (1970), which involves a formula where it is assumed that a citrus tree is composed out of a cylinder and a half sphere *viz.* volume = $R^2(\text{PIH}-1.046R)$, where R = the radius of the tree and H = the height of the fruit bearing part, production and tree health are monitored on an annual basis.

Results and discussion

The data of the two grapefruit cultivars cannot be directly compared since they are grown under different climatic conditions.

Tree size:

Marsh grapefruit. The canopy volumes of the Marsh trees are presented in Table 4.2.2.1. There are significant differences in plants pre-immunized with the original CTV sources and sub-isolates. At this stage, trees with sub-isolate B390/3 and GFMS 35, the current cross protecting source for grapefruit, grew the best. The increase in canopy volume between 2007 and 2008 is 21% and 27%, respectively. Some of the sub-isolates (B389/4, B390/5) retarded growth with an increase of only 1% and 11% respectively and the trees are similar to those with the GFMS 12 source.

Star Ruby grapefruit. The canopy volumes of the Star Ruby trees are presented in Table 4.2.2.3. The Star Ruby tree sizes are more even, indicating lower differences among the treatments. All the trees with the sub-isolates were significant larger than those with GFMS 12. There is no significant difference between the tree

canopies with the sub-isolates and those with GFMS 35, the current cross-protecting source for grapefruit. The increase in canopy volume between 2007 and 2008 were the highest in sub-isolate GFMS12/9 with 41% compared to the 19% of GFMS35.

There is some contradiction with the results of sub-isolate B389/4 in the two grapefruit cultivars. Trees with this sub-isolate performed the best in the Star Ruby trees but poorly in the Marsh trees. This, however, can be the influence of the host or due to climatic differences of the two sites.

Production

Marsh grapefruit. The average production per tree as well as the cumulative yield for each treatment for the Marsh trees is presented in Table 4.2.2.2. Trees with sub-isolate B389/1 and B390/3 produced the highest yield. There were significant differences when looking at the cumulative yield of the last three years. Trees with GFMS 35, the current cross protecting source for grapefruit, yielded the best followed by trees that were plant virus-free. Trees with sub-isolate GFMS 12/7 and GFMS 12/9 produced the poorest.

Star Ruby grapefruit. The average production per tree as well as the cumulative yield for each treatment for the Star Ruby trees is presented in Table 4.2.2.4. Trees with sub-isolate B389/4 had the highest yield. There were significant differences when looking at the cumulative yield of the last three years. Trees with B389/4 yielded the highest followed by trees with GFMS 12/9. Trees that were planted virus free yielded the worst.

This was the third year that the trees bear fruit at a commercial level and it is still too early to draw final conclusions, however, trends are starting to develop.

Tree health: The Marsh and Star Ruby trees were inspected for the occurrence of stem pitting and rated on a severity scale of 0 to 3, where 0 is a smooth trunk with no visible pits, and 3 is severe stem pitting accompanied by decline of twigs. The results are presented in Table 4.2.2.1 and Table 4.2.2.3 for the Marsh and Star Ruby trees, respectively. Both the Marsh and Star Ruby trees with GFMS 12 had a significant higher occurrence of stem pitting, which confirms that GFMS 12 is contaminated with a severe strain. Sub-isolate GFMS 12/7 in Star Ruby also had significant more stem pitting than the rest of the sub-isolates, but still falls in the acceptable range. No decline was observed.

Table 4.2.2.1. Tree size (canopy volume in m³) and stem pitting rating of Marsh grapefruit trees pre-immunised with different CTV sources and sub-isolates, 5 years after planting at Riversbend*.

Treatment	Canopy volume (m ³)			Stem pitting rating**
	2007	2008	% Increase	
B389/1	13.5 cd	15.4 c	12	0 a
B389/4	10.0 de	10.1 de	1	0 a
B390/3	19.1 a	24.1 a	21	0 a
B390/5	8.0 e	9.0 e	11	0 a
GFMS 12/7	10.7 de	11.6 de	8	0.4 b
GFMS 12/9	11.5 de	12.9 cd	11	0.1 ab
GFMS 12	9.4 e	10.0 de	6	1.5 c
GFMS 35	15.3 bc	21.0 ab	27	0 a
Virus-free	18.6 ab	19.4 b	4	0.1 ab

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Table 4.2.2.2. Average yield (kg/tree), the yield efficiency and cumulative yield over 3 years of Marsh grapefruit pre-immunised with different CTV sources and sub-isolates, 5 years after planting at Riversbend*.

Treatment	Yield Kg/tree	Efficiency Kg/m ³	Cumulative yield
B389/1	79.2	5.2	158.6 ab
B389/4	64.4	6.1	141.1 bc
B390/3	90.1	3.7	127.9 cd
B390/5	57.8	7.0	120.6 a
GFMS 12/7	44.5	4.0	117.0 d
GFMS 12/9	38.0	4.7	119.3 d
GFMS 12 (previous cross-protector)	58.7	6.3	133.6 cd
GFMS 35 (current cross-protecting source)	77.2	4.0	173.4 a
Virus-free (Control)	75.3	4.0	168.3 a

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.2.3. Tree size (canopy volume in m³) and stem pitting rating of Star Ruby grapefruit trees pre-immunised with different CTV sources and sub-isolates, 5 years after planting at Tambuti*.

Treatment	Canopy volume (m ³)			Stem pitting rating**
	2007	2008	% Increase	
B389/1	29.7 a	48.3 a	38	0 a
B389/4	33.0 a	49.7 a	34	0 a
B390/3	29.8 a	47.2 a	36	0 a
B390/5	26.2 a	41.7 a	37	0 a
GFMS 12/7	27.6 a	41.8 a	33	1.0 c
GFMS 12/9	24.8 a	43.3 a	41	0.4 b
GFMS 12	23.7 b	30.9 b	23	2.2 d
GFMS 35	29.0 a	36.2 a	19	0 a
Virus-free	32.5 a	46.1 a	30	0 a

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Table 4.2.2.4. Average yield (kg/tree), the yield efficiency and cumulative yield over 3 years of Star Ruby grapefruit pre-immunised with different CTV sources and sub-isolates, 5 years after planting at Tambuti.

Treatment	Yield Kg/tree	Efficiency Kg/m ³	Cumulative yield
B389/1	55.8	1.2	98.2 d
B389/4	81.2	1.7	153.7 a
B390/3	64.4	1.4	121.7 c
B390/5	72.1	1.7	121.2 c
GFMS 12/7	57.9	1.4	101.4 d
GFMS 12/9	69.9	1.7	133.4 b
GFMS 12 (previous cross-protector)	56.7	1.9	122.5 c
GFMS 35 (current cross-protecting source)	69.1	1.5	115.5 c
Virus-free (Control)	54.7	1.2	97.9 d

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

The trees are still young and therefore current differences should only be seen as preliminary indications:

- Sub-isolate 390/5 suppressed growth of the Marsh trees and are similar to the trees with GFMS 12, which is known to carry a severe strain;
- Based on tree sizes, trees with B390/3 sub-isolate grew the best in Marsh grapefruit and trees with B389/4 sub-isolate grew the best in Star Ruby grapefruit. The Marsh grapefruit trees with GFMS 35 had an increase in canopy volume of 27% between 2007 and 2008, which confirms previous findings that GFMS 35 is better than GFMS 12;
- Sub-isolate B389/4 yielded contradictory results in the two cultivars, with trees with this sub-isolate performing well in the Star Ruby trees with a 34% increase in canopy volume but poorly in the Marsh trees with only a 1% increase. If this is because of the climatic difference of the two sites, this sub-isolate will not be suitable for pre-immunisation since the vast variation of the grapefruit producing areas in southern Africa;
- According to production (cumulative yield for three years), trees with sub-isolates GFMS 35, the current cross protecting source yielded the best in Marsh grapefruit and trees with B389/4 yielded the best in Star Ruby grapefruit;
- Trees that were planted virus-free are growing well and it is an indication that challenge infections of natural strains by aphids have no influence yet.

Further objectives

Evaluate the horticultural performance of the trees over a 10-year period using the following parameters:

- Growth (canopy volume);
- Yield and fruit size;
- Tree health (stem pitting and decline).

Technology transfer

Talk at the 5th Citrus Symposium at the Champagne Sports resort, Drakensberg

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4.2.3 PROGRESS REPORT: Cross-protection of Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley

Experiment 738 (2004 - 2014) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

As a result of the presence of a severe strain in the Nartia (GFMS 12) CTV source, it was necessary to separate the strain population into pure sub-isolates by single aphid transmissions. These sub-isolates were obtained from two Nartia sources (A=GFMS 12, C=GFMS 14) and a Mouton source under quarantine at Beltsville, USA, and imported back to South Africa. After they were biologically indexed to differentiate between severe and mild sub-isolates, only four (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) showed potential for further evaluation. Two sub-isolates from the ITSC (GFMS 12/7, GFMS 12/9) were incorporated in the trial as well as GFMS 12 (previous standard for white grapefruit) and GFMS 35 (standard for red grapefruit). Virus-free Star Ruby trees were prepared in a glasshouse and were pre-immunised in the scions with the sources and sub-isolates. A virus-free treatment was included as a control. After confirming pre-immunisation by ELISA, they were planted in the Kakamas area during September 2004. Tree size was measured 4 years after planting and the trees were also harvested for the first time during the report period. Trees grew much slower than in other grapefruit production areas. Stem pitting evaluations were done and sub-isolate B389/1 started to develop stem pitting. It is still too early to draw any conclusions from these early results.

Opsomming

As gevolg van die teenwoordigheid van 'n strawwe ras in die Nartia *Citrus tristeza virus* (CTV) bron (GFMS 12) was dit nodig om die virus ras populasie in sub-isolate deur middel van enkel plantluis oordragings te verdeel. Hierdie sub-isolate is vanaf twee Nartia bronne (A=GFMS 12, C=GFMS 14) en 'n Mouton bron by die kwarantyn fasiliteit in Beltsville, VSA, voorberei en terug na Suid Afrika ingevoer. Nadat die sub-isolate deur biologiese indeksering ge-evalueer is om tussen die ligte en strawwe rasse te onderskei, is gevind dat slegs vier potensiaal het vir verdere evaluering (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5). Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG (GFMS 12/7, GFMS 12/9) is ingesluit in die proef asook GFMS 12 (vorige kruisbeskermingsbron) en GFMS 35 (huidige kruisbeskermingsbron). Virusvrye Star Ruby boompies is in 'n glashuis voorberei en met die bronne en sub-isolate gepre-immuniseer. 'n Virusvrye behandeling is as kontrole ingesluit. Omdat CTV deur die gasheer en klimaat beïnvloed word, is dit nodig om pre-immuniseringsbronne in die verskillende sitrus produserende streke te evalueer. Hierdie proef is 'n herhaling van die twee proewe in eksperiment 679 wat onderskeidelik in die Nkwali Vallei en Swaziland gedoen word. Nadat pre-immunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing gedurende September 2004 uitgeplant, en sal jaarliks vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte ge-evalueer word. Die boompies se grootte is gemeet 4 jaar na uitplant en is ook vir die eerste keer geoes en die verslag tydperk. Die boompies groei heelwat stadiger as boompies in die ander pomelo produserende streke. Stamgleuf evaluasies is gedoen en slegs sub isolaat B389/1 het begin stamgleuf ontwikkel. Dit is egter nog te vroeg om afleidings te maak vanaf die vroeë resultate.

Introduction

The severe effect of *Citrus tristeza virus* (CTV) on grapefruit production makes pre-immunisation with mild strains essential (Marais *et al.*, 1996). A breakdown in the CTV protection offered by the GFMS 12 (Nartia A)

source, owing to the presence of severe strains within the complex, motivated the separation of the strains in sources by single aphid transmission (SAT). SAT from two Nartia sources (A=GFMS 12, C=GFMS 14; van Vuuren *et al.*, 1993) and a Mouton source were prepared at the quarantine facility in Beltsville MD, USA. After re-importation to South Africa, these sub-isolates underwent biological evaluation to differentiate between the severe and mild forms. Some sub-isolates had no potential as cross protectors due to the development of unacceptably severe stem pitting, or the virus concentration and movement of the virus were poor (Breytenbach *et al.*, 2002). Four of the sub-isolates showed potential and are evaluated as cross-protectors. Promising SAT sub-isolates of GFMS 12 (Nartia A) obtained from the ARC-ITSC are also included in this experiment (van Vuuren *et al.*, 2000). As CTV exhibits host and geographical specificity, it is imperative that mild protecting isolates be tested in the different production areas (da Graça *et al.*, 1984).

Materials and methods

Virus-free Star Ruby budwood was budded to virus-free MxT rootstocks. When the scions had developed to approximately 5 mm thickness, they were bud inoculated with the sub-isolates of GFMS 14 or Mouton (B389-1, B389-4, B390-3, B390-5), ARC-ITSC sub-isolates (GFMS 12/7, GFMS 12/9), and compared to the two standards (GFMS 12, previously for white grapefruit; GFMS 35 for all grapefruit) as well as trees that were left virus-free. After pre-immunisation of the trees was confirmed by ELISA, they were planted in the Kakamas area according to a randomised block design with five replications. The trees are evaluated annually regarding their growth, production and tree health.

Results and discussion

Tree size: The heights and diameters of the 4-year-old trees were measured and the canopy volumes (m³) calculated. The results are presented in Table 4.2.3.1. Although there are significant differences in growth among trees with the different CTV sources and sub-isolates, it should be seen as trends since the trees are still young. At this stage, GFMS 12 (previous cross protector) performs the best and sub-isolate B389/4 the worst. The site of this experiment is not in a typical grapefruit area and the cooler winter climate will suppress growth compared to other optimal grapefruit production areas.

Tree health: The trees were inspected for the occurrence of stem pitting and rated on a severity scale of 0 to 3, where 0 is a smooth trunk with no visible pits, and 3 is severe stem pitting accompanied by decline of twigs. The results are presented in Table 4.2.3.1. Sub-isolate B389/1 developed significantly more stem pitting than the other isolates and sub-isolates.

Production: The average production per tree for each treatment is presented in Table 4.2.3.2. There were no significant differences between the treatments. This was, however, the first year that the trees were harvested on a commercial basis.

Table 4.2.3.1. Canopy volumes of Star Ruby trees pre-immunised with different CTV sources and sub-isolates, 4 years after planting in the Orange River Valley.

Treatment	Canopy volume (m ³)	Stem pitting rating**
B389/1	8.7 abc	0.9 B
B389/4	5.2 d	0 A
B390/3	8.4 abcd	0 A
B390/5	7.1 bcd	0 A
GFMS 12/7	9.0 abc	0 A
GFMS 12/9	9.5 Ab	0 A
GFMS 12 (previous cross-protector)	11.1 A	0 A
GFMS 35 (current cross-protecting source)	6.0 cd	0 A
Virus-free (control)	7.1 bcd	0 A

* Figures in the columns followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Table 4.2.3.2. Average yield (kg/tree) of Star Ruby trees pre-immunised with different CTV sources and sub-isolates, 4 years after planting in the Orange River Valley.

Treatment	Yield Kg/tree
B389/1	54.8 a
B389/4	42.8 a
B390/3	34.3 a
B390/5	58.6 a
GFMS 12/7	42.8 a
GFMS 12/9	46.0 a
GFMS 12 (previous cross-protector)	35.2 a
GFMS 35 (current cross-protecting source)	38.7 a
Virus-free (Control)	33.3 a

* Figures in the columns followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

The trees are still young and no conclusions can be made at this stage. The site of this experiment is not in a typical grapefruit area and the cooler winter climate will suppress growth compared to other optimal grapefruit production areas.

Further objectives

The horticultural performance i.e. growth (tree size), yield (kg/tree and fruit size) and tree health (stem pitting and decline), must be evaluated in future.

Technology transfer

None.

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4.2.4 PROGRESS REPORT: The effect of different CTV sources in Valencias on different rootstock combinations for the Orange River Valley

Experiment 739 (2004 - 2014) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

CTV is host and climate specific and it is therefore necessary to evaluate the different protective sources in the different citrus production areas. Mild sources derived from sweet orange trees (SM45, SM 46, SM 47, SM 48, SM 49) were used to pre-immunise virus-free Delta-, Midknight-, McClean seedless-, and Turkey Valencia on C35 citrange rootstocks. These sources will be compared with LMS 6 (standard for sweet oranges) and virus-free controls. Pre-immunisation has been confirmed by means of ELISA. The trees were planted at Karsten boerdery in the Kakamas area during September 2007. The trees will be evaluated annually for growth, production, fruit size, as well as tree health. The trees are still young and no conclusions can be made at this stage.

Opsomming

Omdat *Citrus tristeza virus* (CTV) gasheer en klimaat spesifiek is, is dit nodig om verskillende CTV bronne in die verskillende sitrus produserende streke te evalueer. Ligte CTV bronne wat oorspronklik vanaf soetlemoenbome versamel is (SM 45, SM 46, SM 47, SM 48, SM 49), is gebruik om virusvrye Delta-, Midnight-, McClean seedless-, en Turkey Valencia op C 35 citrange onderstam te pre-immuniseer. Hierdie bronne word met LMS 6 (die standaard vir soetlemoene) en boompies wat virusvry geplant is, vergelyk. Pre-immunisering is deur middel van ELISA bevestig waarna die boompies gedurende September 2007 by Karsten Boerdery in die Kakamas omgewing geplant is. Jaarlikse evaluasies word vir boomgrootte, vruggrootte, oes opbrengs, sowel as van hul gesondheidstoestand gedoen. Die boompies is steeds te jonk en geen gevolgtrekkings kan op hierdie stadium gemaak word nie.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by propagation material and by various aphid species of which *Toxoptera citricida* is the most abundant. Symptoms induced by CTV range from mild with no noticeable effect on the host to severe stem pitting and decline resulting in uneconomic production. As CTV exhibits host and geographical specificity, it is necessary that mild protective sources be evaluated in the different production areas. The only practical means of controlling CTV disease at present is by mild strain cross-protection. The objective of this experiment is to evaluate selected CTV sources in four different Valencia selections on C35 citrange rootstock in order to identify a suitable cross-protecting CTV source for specific scion selections.

Materials and methods

Four virus-free Valencia scions (Delta, Midnight, McClean Seedless, Turkey) were budded on C35 citrange rootstock. When the scions had developed sufficiently, each Valencia selection was bud-inoculated with one of five selected CTV sources originating from sweet orange (SM 45, SM 46, SM 47, SM 48, SM 49). Trees with these sources are compared to trees inoculated with LMS 6 (standard) and trees that were planted virus-free. Successful pre-immunisation was confirmed with ELISA where after the trees were planted during September 2007 at Karsten Boerdery in the Kakamas area. Horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline) will be evaluated on an annual basis.

Results

Tree size: The heights of the 1 year-old trees were measured and are presented in Table 4.2.4.1.

Table 4.2.4.1. Tree heights of the four Valencia scions pre-immunised with different CTV sources 1 year after planting in the Orange River Valley.

Treatment	Rootstocks			
	Delta	Midnight	McClean sl	Turkey
SM 45	1.1	0.9	0.9	0.9
SM 46	1.1	1.0	0.9	0.6
SM 47	1.0	1.0	0.7	0.7
SM 48	1.0	0.8	0.5	0.9
SM 49	1.2	0.8	0.7	0.9
LMS 6	1.1	0.9	1.1	1.0
Virus Free	1.0	1.0	1.0	0.9

Conclusion

The trees are still young and no conclusions can be made at this stage.

Further objectives

Evaluate horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline).

Technology transfer

None.

4.2.5 PROGRESS REPORT: Cross-protection of Marsh and Star Ruby by using the best field isolates collected in the different grapefruit production areas of southern Africa Experiment 742 (2004 - 2014) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

Budwood was cut in the different grapefruit production areas of southern Africa from 108 superior grapefruit trees that possibly harbour mild CTV sources. After the sources were established in the glasshouse, material was inoculated to virus-free Mexican lime indicator plants to evaluate the mildness of the CTV. After the first biological test, 19 were selected for further evaluation. These 19 sources were inoculated again to virus-free Mexican lime plants and compared to GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9, and the four best Beltsville sub-isolates (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5). The Mexican lime plants were evaluated for growth and stem pitting. Virus titre was determined by ELISA. The most promising of these 19 field sources (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwaleni Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), all free of citrus viroids, are being used as pre-immunising agents for virus-free Marsh and Star Ruby trees. These sources will be compared to GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit), as well as the four best Beltsville sub-isolates (B389-1, B389-4, B390-3, B390-5) and the ITSC sub-isolates (GFMS 12/7, GFMS 12/9). Pre-immunisation was confirmed by means of ELISA and the Star Ruby trees were planted at Bosveld Sitrus in the Letsitele area during February 2007, while the Marsh trees were planted at Riverside in the Malelane area during March 2007. The trees were evaluated during 2008 for growth and stem pitting. Although there are differences between the treatments, it is still too early to draw any conclusions. The trees will be evaluated annually for growth, production, fruit size, as well as tree health.

Opsomming

Enthout is vanaf 108 uitstaande pomelo bome, wat gesondheid en produksie betref, in die verskillende pomelo gebiede in suider Afrika versamel. Die bronne is op virusvrye onderstamme in die glashuis by CRI gevestig. Hierna is die verskillende bronne afsonderlik op Meksikaanse lemmetjie geïnkuleer (biologiese indeksering) om te bepaal of die bome moontlik ligte rasse van *Citrus tristeza virus* (CTV) huisves wat as kruisbeskermingsbronne kan dien. Na die eerste biologiese indeksering van 6 maande het slegs 19 bronne potensiaal getoon en is vir verdere evaluering gebruik. Hierdie 19 bronne is 'n tweede keer op Meksikaanse lemmetjie geïnkuleer en met bekende bronne GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9 en die vier beste Beltsville sub-isolate (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) vergelyk. Na 'n tydperk van 6 maande is die geïnkuleerde plante vir groei en voorkoms van stamgleuf asook die virus titer d.m.v. ELISA ge-evalueer. Die 4 mees belowendste bronne, wat vry is van viroïede, is Tabankulu 1 – versamel vanaf Star Ruby in Swaziland; New Venture 41/2 – versamel vanaf Star Ruby in die Nkwaleni Vallei; ORE 8 – versamel vanaf Marsh in die Hoedspruit gebied; en Tshipise 19/5 – versamel vanaf Marsh in Tshipise. Hierdie bronne is verder gebruik om virus-vrye Marsh en Star Ruby boompies vir boord evaluasie te pre-immuniseer. Die bronne word met GFMS 12 (vorige standard vir pomelos), GFMS 35 (huidige standard vir pomelos), asook die vier beste Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR-ITSG sub-isolate (GFMS 12/7, GFMS 12/9) vergelyk. Pre-immunisering is deur middel van ELISA bevestig voordat hulle geplant is. Die Star Ruby boompies is gedurende Februarie 2007 by Bosveld Sitrus in die Letsitele omgewing geplant en die Marsh boompies is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is gedurende 2008, een jaar na uit plant, vir groei en stamgleuf ge-evalueer. Alhoewel daar verskille is, is dit nog te vroeg om enige gevolgtrekkings te maak. Die bome sal jaarliks vir groei, produksie, vrug grootte en algemene boom gesondheid ge-evalueer word.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources (de Lange *et al.*, 1981). In South Africa, the potential benefit of optimum growth and production of virus-free trees cannot be utilized as a result of the abundance of the aphid insect vector of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings would get infected with various strains of CTV within a few years after planting. Many strains of CTV exist, and they usually occur as mixtures in a host (McClellan, 1963). Factors such as the type of strain, host and environment will determine dominance of a specific strain and it may change if any of the factors change *viz.* co-infection of a new strain or extreme temperatures (da Graca *et al.*,

1984; Oberholzer, 1959). The only method to overcome this problem is by cross-protection, a deliberate infection of virus-free material with a known CTV source. The first step in searching for mild sources for cross-protection purposes is to look for old trees that are healthy and produce good quality fruit (Müller & Costa, 1987).

This experiment is a follow-up of the glasshouse trial (experiment 49) where 108 CTV sources were collected in different grapefruit production areas from productive old grapefruit trees. After an initial screening in the glasshouse, 19 sources showed potential as cross protectors. These 19 sources were then compared to the present pre-immunising sources. The 4 best field sources, that are free of citrus viroids, are evaluated as cross-protecting agents in Star Ruby and Marsh grapefruit trees in the field.

Materials and methods

Virus-free Troyer citrange rootstocks were propagated and budded with virus-free Star Ruby and Marsh grapefruit budwood in a greenhouse. When the scions had developed to approximately pencil thickness, they were inoculated with the selected CTV sources in the scions. The following CTV sources were used: the four most promising sources selected from the original 108 field sources (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwaleni Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), the four best Beltsville sub-isolates (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) and two ARC-ITSC sub-isolates (GFMS 12/7, GFMS 12/9). Sources GFMS 12 (previous standard for grapefruit) and GFMS 35 (present standard for grapefruit) are used as standards and a treatment are left virus-free as a control. Three months after inoculation, ELISA confirmed positive pre-immunisation. The trees were then planted in two grapefruit production areas according to a randomised block design with five replicates. The Star Ruby trees were planted during February 2007 at Bosveld Citrus in the Letsitele area and the Marsh trees were planted during March 2007 at Riverside in the Malelane area. Growth, production and tree health will be monitored annually.

Results and discussion

The data of the two grapefruit cultivars cannot be compared directly since they are grown under different climatic conditions.

Tree size:

Marsh grapefruit: The canopy volumes of the Marsh trees are presented in Table 4.2.5.1. There are significant differences between the original sources and sub-isolates, but at this stage it is still too early to draw any conclusions

Star Ruby: The canopy volumes of the Star Ruby trees are presented in Table 4.2.5.2. There are no significant differences between the original sources and sub-isolates, but at this stage it is still too early to draw any conclusions

Tree health:

Marsh grapefruit: The trees were inspected for the occurrence of stem pitting and rated on a severity scale of 0 to 3, where, 0 is a smooth trunk with no visible pits and 3 is severe stem pitting accompanied by decline of twigs. The results are presented in Table 4.2.5.1. No stem pitting had developed at this stage.

Star Ruby: The trees were inspected for the occurrence of stem pitting and rated on a severity scale of 0 to 3, where, 0 is a smooth trunk with no visible pits and 3 is severe stem pitting accompanied by decline of twigs. The results are presented in Table 4.2.5.2. There were significant differences between the sources and the sub-isolates. At this early stage, GFMS 12 had significantly more stem pitting than the other treatments, which confirms previous findings that GFMS 12 not a suitable cross protector is for grapefruit. Sub-isolate GFMS 12/7 also showed some stem pitting symptoms, although not at statistically significant levels.

Table 4.2.5.1. Tree size (canopy volume in m³) and stem pitting rating of Marsh grapefruit trees pre-immunised with different CTV sources and sub-isolates, 1 year after planting at Riverside, Malelane*.

Treatment	Canopy volume (m ³)	Stem pitting rating**
389/1	4.1 ab	0
389/4	6.6 ab	0
390/3	3.6 ab	0
390/5	5.6 a	0
12/7	4.7 ab	0
12/9	2.6 b	0
Ore	2.8 b	0
Tshipise	3.0 ab	0
Tambankulu	2.5 b	0
New Venture	4.1 ab	0
GFMS 12	3.1 ab	0
GFMS 35	3.9 ab	0
Virus-free	5.1 ab	0

* Figures in the columns followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Table 4.2.5.2. Tree size (canopy volume in m³) and stem pitting rating of Star Ruby trees pre-immunised with different CTV sources and sub-isolates, 1 year after planting at Bosveld Sitrus, Letsitele*.

Treatment	Canopy volume (m ³)	Stem pitting rating**
389/1	1.5 a	0 a
389/4	1.4 a	0 a
390/3	1.4 a	0 a
390/5	1.6 a	0 a
12/7	1.9 a	0.5 ab
12/9	1.7 a	0 a
Ore	1.2 a	0 a
Tshipise	1.4 a	0 a
Tambankulu	1.5 a	0 a
New Venture	1.4 a	0 a
GFMS 12	1.5 a	0.8 b
GFMS 35	1.6 a	0 a
Virus-free	2.0 a	0 a

* Figures in the columns followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 0 = Smooth trunk; 1 = Mild pitting; 2 = Moderate pitting; 3 = Severe pitting.

Conclusion

The trees are still young and no conclusions can be made at this stage.

Future research

Evaluate horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline).

Technology transfer

None.

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4.2.6 FINAL REPORT: The response of different red grapefruit cultivars to *Citrus tristeza virus* Experiment 785 (1998 - 2008) by S.P. van Vuuren & J.H.J. Breytenbach (CRI)

Summary

Four mild *Citrus tristeza virus* (CTV) cross-protecting sources, GFMS 12, GFMS 35, GFMS 67 and GFMS 73 were evaluated as cross-protecting sources in seven red grapefruit cultivars, viz. 'Star Ruby', 'Rio Red', 'Henderson', 'nel Ruby', 'Flame', 'Ruben' and 'Oran Red' on 'Swingle' citrumelo rootstock in the Malelane area. After 10 years the overall reaction of the commercial grown cultivars ('Star Ruby', 'Rio Red', 'nel Ruby', 'Flame') to the various mild CTV regarding growth, yield (kg/tree), yield efficiency (kg/m³ canopy volume), cumulative production over a period of 3 years, crop value (production of large fruit) or the occurrence of stem pitting sources was very similar. However, there were interactions between some CTV sources with some grapefruit cultivars, resulting in retarded growth, lower production, small fruit and stem pitting. These interactions may change in other climatic conditions. Tree sizes of 'Star Ruby' and 'Rio Red' were reduced by GFMS 12 and GFMS 73 and that of 'Flame' by GFMS 35. Smaller trees are not necessarily a negative property and can be beneficial in high density plantings, providing the yield efficiency is not reduced and fruit size is acceptable. The reduction of tree size and yield efficiency of 'Star Ruby' and the general occurrence of more severe stem pitting where GFMS 12 was used confirm the unsuitability of this CTV source for cross-protection of red grapefruit cultivars. The reduction of production and crop value of 'Flame' by the GFMS 35 source causes concern since it is the present pre-immunising source for all grapefruit in the Citrus Improvement Scheme. During 2003 to 2008 only 30 000 buds were cut from 'Flame' mother trees in contrast to 208 000 and 64 000 buds for 'Star Ruby' and 'nel Ruby' respectively and therefore indicates that 'Flame' is currently not planted on a large scale. If the popularity of 'Flame' changes, an alternative CTV source, either GFMS 67 or GFMS 73, should be considered as a pre-immunising source. The most stable CTV source in the commercial cultivars, where the least interactions occurred, was GFMS 67. This study furthermore revealed that it is not only essential to look for CTV cross-protecting sources for specific citrus types, but also within a type.

Opsomming

Vier ligte *Citrus tristeza virus* (CTV) bronne, GFMS 12, GFMS 35, GFMS 67 en GFMS 73 is as kruisbeskerminingsbronne in sewe rooi pomelo kultivars, nl. 'Star Ruby', 'Rio Red', 'Henderson', 'nel Ruby', 'Flame', 'Ruben' en 'Oran Red' op 'Swingle' citrumelo onderstam in die Malelane gebied ge-evalueer. Na 10 jaar was die algemene reaksie van die kultivars, wat kommersiëel gekweek word ('Star Ruby', 'Rio Red', 'nel Ruby', 'Flame'), ten opsigte van boomgrootte, produksie (kg/boom), produksie-doeltreffendheid (kg/m³ kroonvolume), kumulatiewe produksie oor 'n tydperk van 3 jaar, oeswaarde (die produksie van groot vrugte) of die voorkoms van stamgleuf baie dieselfde. Nietemin was daar interaksies tussen sommige CTV bronne en sommige pomelo kultivars ten opsigte van boomgroei, produksie, teenwoordigheid van klein vrugte en

strawwe stamgleuf. Hierdie interaksies mag verander in ander klimaatstoestande. Boomgrootte van 'Star Ruby' en 'Rio Red' was kleiner waar GFMS 12 en GFMS 73 gebruik was en die van 'Flame' met die GFMS 35 bron. Kleiner bome is nie noodwendig 'n negatiewe eienskap nie en kan tot voordeel wees in hoëdigheidsaanplantings op voorwaarde dat produksie-doeltreffendheid en vruggrootte nie nadelig beïnvloed word nie. Die vermindering van boomgrootte en produksie-doeltreffendheid van 'Star Ruby' en die algemene hoër voorkoms van stamgleuf waar GFMS 12 gebruik was, bevestig die ongeskiktheid van hierdie bron as 'n kruisbekeringsbron vir rooi pomelo kultivars. Die verlaging van produksie- en oeswaarde van 'Flame' deur GFMS 35 veroorsaak kommer omdat GFMS 35 tans die kruisbeskeringsbron vir alle pomelos in die Sitrusverbeteringskema is. Slegs 30 000 ogies is gedurende 2003 tot 2008 vanaf 'Flame' moederbome gesny in teenstelling met 208 000 en 64 000 ogies repektiewelik vir 'Star Ruby' en 'nel Ruby' moederbome. Dit dui aan dat 'Flame' tans nie op groot skaal aangeplant word nie. Indien 'Flame' meer gewild word sal 'n alternatiewe CTV bron soos GFMS 67 of GFMS 73 as pre-immuniseringsbron gebruik moet word. Die mees stabiele kruisbeskeringsbron, waar die minste interaksies waargeneem is, was GFMS 67. Hierdie studie het weer eens gewys dat dit belangrik is om geskikte CTV kruisbeskeringsbronne vir spesifieke sitrustipes, maar ook binne tipes te vind.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources in South Africa (Fourie & van Vuuren, 1993). However, the benefit of optimum growth and production of virus-free trees cannot be attained because of the abundance of the brown citrus aphid vector, *Toxoptera citricida* (Kirkaldy), of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings will get infected with various strains of CTV within a few years after planting. Many strains of CTV exist and they usually occur as mixtures in a host (McClellan, 1963). Factors such as the type of strain, host plant and environment will determine dominance of a specific strain and it may change if any of the factors change *viz.* co-infection of a new strain or extreme temperatures (Oberholzer, 1959; da Graça *et al.*, 1984). The only method to overcome this problem is by cross-protection, a deliberate infection of virus-free material with a known CTV source (Müller and Costa, 1987).

Of the commercial citrus cultivars grown in southern Africa, grapefruit is the most sensitive to the CTV, which causes stem pitting, decline and production of small fruit. With the initiation of the southern African Citrus Improvement Scheme (CIS), all grapefruit selections are pre-immunised with the GFMS 12 CTV source (von Broembsen & Lee, 1988). This source originated from a, then 50-year-old 'hartia' ('Marsh' type) grapefruit tree in the Western Cape Province. Bud-wood source trees at the Citrus Foundation Block (CFB) at Uitenhage, which is the only source for propagating certified trees, are evaluated visually each year for decline and stem pitting symptoms. In addition, each tree of every selection is biologically indexed bi-annually to establish the CTV severity. When there are indications of severe CTV, such a tree is terminated as a bud-wood source.

In 1993, it was found that 6-year-old Star Ruby bud-wood mother trees varied in stem pitting development and fruit size (unpublished data). Testing the CTV stem pitting severity revealed that severe strains were dominating in four of the five bud-wood source trees. At first it was thought that GFMS 12 did not protect against co-infection of severe strains. However, subsequent research revealed the presence of a severe strain in the original source and that segregation of the strains, where the severe strain became dominant, might have been the cause of the problem (van Vuuren *et al.*, 2000). The unsuitability of GFMS 12 as a protector for Star Ruby was also confirmed in a field trial (van Vuuren & van der Vyver, 2000). Consequently, GFMS 35 (derived from a Rosé grapefruit tree) has been approved for the pre-immunisation of all red grapefruit until more suitable sources are identified (Luttig *et al.*, 2002).

The first step in searching for mild sources for cross-protection purposes is to look for old trees that are healthy and produce good quality fruit (Müller & Costa, 1987). The Star Ruby grapefruit industry in South Africa started in the late 1970's and therefore trees older than 15 years did not exist at the time. To overcome this problem, the best producing trees in the oldest plantings at Malelane, Mpumalanga Province, and Swaziland were selected. Sources from these trees were evaluated in glasshouse tests and those with the best potential were evaluated in the field.

The objective of this study was to evaluate new CTV sources in different red grapefruit cultivars to establish the best source for each cultivar.

Materials and methods

Seven red grapefruit cultivars *viz.* 'Star Ruby', 'Flame', 'Rio Red', 'nel Ruby', 'Henderson', 'Ruben' and 'Oran Red' were budded as scions on 'Swingle' citrumelo rootstocks. CTV sources GFMS 35, GFMS 67, GFMS 71

and GFMS 73 were evaluated in each scion and compared to the standard mild protecting strain of the time (GFMS 12), and a severe source (GFSS 5). After pre-immunisation (inoculation by buds) of the scions with the different CTV sources, ELISA confirmed infection where after they were planted in a randomised split plot (Rayner, 1967) with five replications at Malelane during December 1998.

The following data were taken annually: 1) Tree sizes were measured and the canopy volumes calculated according to Burger *et al.* (1970) which involves a formula where it is assumed that a citrus tree is composed out of a cylinder and a half sphere *viz.* volume = $R^2(\pi H - 1.046R)$, where R = the mean radius of the tree and H = the height of the fruit bearing part; 2) The fruit were harvested, graded into the different export sizes and weighed; 3) Tree health was rated according to the occurrence of stem pitting and decline. A rating of 1 was assigned when the trunk appeared smooth and no twig decline occurred; a rating of 2 was when occasional mild dents occurred on the trunk with no twig dieback; a rating of 3 refers to instances where moderate pitting occurred all over the trunk and no twig decline; a rating of 4 was when many pits (large and/or small) occurred on the trunk and decline started; and a rating of 5 was when severe pitting and severe decline occurred.

The following calculations were made from the data: 1) The cumulative yield over all the harvest seasons; 2) From the canopy sizes and total crop for the season the yield efficiency (kg/m³ canopy volume) was calculated; 3) The average value of the crop per tree in relation to fruit size was determined by calculating the average value per 15kg export box for each size (average market value over a period of ten years). The highest price equalled a value of 10 while the other values of the different sizes were calculated accordingly. Data was analysed according to a multifactor analysis of variance at the 5% confidence level (Statgraphics Plus 5.1, 2000).

Results and discussion

Tree size, yield, yield efficiency, cumulative yield, crop value and stem pitting ratings of the red grapefruit selections that were pre-immunised with different CTV sources are presented in Table 4.2.6.1, Table 4.2.6.2, Table 4.2.6.3, Table 4.2.6.4, Table 4.2.6.5 and Table 4.2.6.6, respectively.

Tree size: Overall, canopy volumes of trees that were pre-immunised with the different mild sources did not differ from each other but were significantly larger than the trees pre-immunised with a severe source. Of the selections, trees of 'nel Ruby', 'Flame' and 'Ruben' were the largest and were significantly larger than 'Henderson' and 'Oran Red' trees. The 'Oran Red' trees were the smallest and it is possible that Oran Red has a genetic dwarfing characteristic. It also appears that Ruben has some tolerance to CTV since the severe source affected tree size to a lesser extent (Table 4.2.6.1).

From Table 4.2.6.1 it is evident that some interactions between selections and some of the mild CTV sources (Rio Red with GFMS 12 and GFMS 73) exist. 'Henderson' and 'Oran Red' trees were generally smaller and 'Ruben' trees were generally less affected by the CTV sources including the severe source (Table 4.2.6.1). However, 'Ruben' fruit do not have a good internal red colour and is also known as 'Ruben Pink'. These 3 cultivars are not planted on a commercial scale in South Africa. Of the 4 commercial cultivars, of which 'Star Ruby' is by far the most popular, GFMS 67 had the smallest variation in canopy size. The other 3 CTV sources showed interactions with the cultivars where tree sizes were significantly reduced: GFMS 12 in 'Star Ruby' and 'Rio Red'; GFMS 35 in 'Flame'; GFMS 73 in 'Star Ruby' and 'Rio Red' (Table 4.2.6.1).

Production: Trees with the different mild CTV sources produced very similar amounts (Table 4.2.6.2). There is only 7 kg difference between the highest yield (trees with GFMS 73) and the lowest (trees with GFMS 35). Of the grapefruit cultivars, 'Rio Red' had the best yield, which was not significantly better than 'nel Ruby', 'Flame' and 'Ruben', but was significantly better than 'Henderson' and 'Oran Red'. The production of 'Star Ruby' was significantly less than the 'Rio Red' trees (Table 4.2.6.2). To summarise, of the commercially grown cultivars, 'Rio Red', 'nel Ruby' and 'Flame' produced a higher crop at 10 years of age, 19% better than 'Star Ruby'.

Table 4.2.6.2 demonstrates the significant reduced production of the following mild CTV source and cultivar combinations: GFMS 12 in 'Oran Red'; GFMS 35 in 'Henderson', 'Flame' and 'Oran Red'; GFMS 67 in 'Henderson'; GFMS 73 in 'Star Ruby' and 'Ruben' (Table 4.2.6.2). From a commercial point of view the use of GFMS 35 in 'Flame' causes concern since GFMS 35 is the current CTV source recommended for pre-immunisation of red grapefruit.

The yield efficiency of the trees with the different mild CTV sources did not differ from each other (Table 4.2.6.3). Yield efficiencies are usually affected by disease stress (as can be seen with the severe CTV

source, GFSS 5), dwarfing or compact growth where it is increased (tree size of Oran Red cultivar, Table 4.2.6.1) and vigour where it is decreased (large trees of 'nel Ruby' and 'Ruben' in Table 4.2.6.1).

The cumulative yield over the last 3 years shows a significant reduction in yield of trees pre-immunised with GFMS 35 as opposed to GFMS 67 (Table 4.2.6.4). In the previous year no significant difference was recorded. This may be a seasonal variation but it is possible that the trees of GFMS 35 is starting to decline, however, it is not apparent in Table 4.2.6.6 that trees with GFMS 35 are in a poorer condition. Of the cultivars, 'Rio Red', 'nel Ruby' and 'Flame' trees produced significantly better than the 'Star Ruby', 'Henderson' and 'Oran Red' trees. The lower production of the latter two cultivars may be ascribed to the smaller tree size (Table 4.2.6.1). The lower production of the Star Ruby trees may be due to lower yield efficiency (Table 4.2.6.3).

Apart from tree life, the symptom that makes CTV such an important disease, is the reduction in fruit size. This has a direct affect on the market value. Table 4.2.6.5 shows the calculated market value of the 2008 crop. There was no significant difference between trees which were pre-immunized with the different mild CTV sources but they all had significantly higher market values than the trees pre-immunized with the severe CTV source. The lower values of the 'Henderson' and 'Oran Red' trees can be attributed to small fruit caused by GFMS 12 ('Oran Red') and GFMS 35 ('Henderson') CTV sources. Table 4.2.6.5 illustrates the reduced crop value of 'Flame' by GFMS 35, and that of 'Ruben' by GFMS 73, mainly because of lower yields (Table 4.2.6.2).

Stem pitting: Overall, GFMS 12 caused significantly more severe stem pitting than the other three mild sources.(Table 4.2.6.6). The Ruben trees had the least stem pitting and displayed some tolerance to CTV. Generally the trunks of the trees were smooth with only occasional pits.

Table 4.2.6.1. Tree size (canopy volume = m³) of 10-year-old red grapefruit cultivars that were pre-immunised with different CTV sources.

Grapefruit Cultivars	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
'Star Ruby'	26.4	34.9	27.5	26.5	20.7	27.2 xy
'Rio Red'	23.6	35.0	30.1	26.2	16.4	26.3 xy
'Henderson'	25.8	21.5	21.7	24.1	21.1	22.8 yz
'nel Ruby'	35.6	35.9	32.7	37.5	19.0	32.1 w
'Flame'	33.5	24.5	28.9	33.6	21.3	28.4 wx
'Ruben'	39.7	34.2	32.6	29.9	26.7	32.6 w
'Oran Red'	18.8	20.4	22.3	21.4	15.9	19.8 z
Mean	29.1 a	29.5 a	28.0 a	28.5 a	20.2 b	

Means followed by the same letter do not differ significantly (P = 0.05). For the body of the table: LSD = 9.62 (P = 0.05).

Table 4.2.6.2. Production (kg/tree) of 10-year-old red grapefruit cultivars that were pre-immunised with different CTV sources.

Grapefruit cultivars	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
'Star Ruby'	197.4	208.2	206.1	186.9	108.2	181.3 yz
'Rio Red'	198.8	244.7	213.2	217.9	143.5	203.6 x
'Henderson'	193.9	140.7	182.6	192.7	165.7	175.1 z
'nel Ruby'	191.0	214.2	209.5	236.1	156.9	201.6 xy
'Flame'	227.3	178.5	194.7	230.8	175.3	201.3 xy
'Ruben'	226.0	207.8	200.8	162.9	202.0	199.9 xy
'Oran Red'	163.5	174.0	193.6	185.1	142.4	171.7 z
Mean	199.7 a	195.5 a	200.1 a	202.8 a	156.3 b	

Means followed by the same letter do not differ significantly (P = 0.05). For the body of the table: LSD = 46.07 (P = 0.05).

Table 4.2.6.3. Yield efficiency (kg/m³ canopy) of 10-year-old red grapefruit cultivars that were pre-immunised with different CTV sources^{*}.

Grapefruit cultivars	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
'Star Ruby'	4.8	5.0	5.1	6.5	4.3	5.1 z
'Rio Red'	7.9	6.2	5.9	8.0	7.1	7.0 x
'Henderson'	6.1	6.3	7.9	8.8	6.8	7.2 x
'nel Ruby'	5.1	4.6	5.6	6.5	7.8	5.9 yz
'Flame'	6.6	6.8	6.0	6.7	8.3	6.9 xy
'Ruben'	5.7	5.4	5.9	5.8	6.4	5.8 z
'Oran Red'	10.7	8.8	7.5	9.2	8.6	8.9 w
Mean	6.7 abc	6.2 c	6.3 bc	7.6 a	7.1 ab	

^{*} Means followed by the same letter do not differ significantly (P = 0.05). For the body of the table: LSD = 2.28 (P = 0.05).

Table 4.2.6.4. Cumulative yield over a 3-year period of 10-year-old red grapefruit cultivars that were pre-immunised with different CTV sources^{*}.

Grapefruit cultivars	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
'Star Ruby'	551.8	492.8	586.7	571.5	376.6	515.9 yz
'Rio Red'	577.6	638.2	585.7	678.4	404.5	576.9 x
'Henderson'	541.3	440.0	599.3	561.9	463.7	521.2 yz
'nel Ruby'	558.5	642.6	618.5	646.5	464.1	586.0 x
'Flame'	625.4	516.7	607.8	635.2	513.6	579.8 x
'Ruben'	646.4	591.9	596.2	453.4	494.9	556.6 xy
'Oran Red'	462.7	511.7	542.1	549.2	414.7	796.1 z
Mean	566.2 ab	547.7 b	590.9 a	585.2 ab	447.5 c	

^{*} Means followed by the same letter do not differ significantly (P = 0.05). For the body of the table: LSD = 101.77 (P = 0.05).

Table 4.2.6.5. The effect of different CTV sources on the average crop value per tree for the 2008 season of red grapefruit cultivars^{*}.

Grapefruit Cultivars	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
'Star Ruby'	106.7	116.9	122.0	108.4	51.4	101.1 yz
'Rio Red'	104.4	135.2	110.7	123.8	61.9	107.2 xy
'Henderson'	107.7	77.4	91.0	103.0	72.2	90.2 z
'nel Ruby'	107.3	124.0	128.4	127.3	72.8	111.9 xy
'Flame'	134.8	96.3	111.6	123.0	80.3	109.2 xy
'Ruben'	141.5	125.6	122.5	101.9	110.8	120.5 x
'Oran Red'	82.2	90.9	108.8	108.3	68.7	91.8 z
Mean	112.1 a	109.5 a	113.6 a	113.7 a	74.0 b	

^{*} Means followed by the same letter do not differ significantly (P = 0.05). For the body of the table: LSD = 28.49 (P = 0.05).

Table 4.2.6.6. The effect of different CTV sources on stem pitting rating^{**} of 10-year-old red grapefruit cultivars^{*}.

Grapefruit Cultivars	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
'Star Ruby'	3.0	2.6	2.1	2.0	3.0	2.5 yz
'Rio Red'	2.9	1.9	2.3	2.7	3.0	2.6 yz
'Henderson'	2.8	2.6	3.5	2.9	2.4	2.9 z
'nel Ruby'	3.0	2.5	1.7	3.0	2.3	2.5 xyz
'Flame'	3.0	2.7	2.5	2.6	2.5	2.7 yz
'Ruben'	3.0	1.7	2.7	2.3	1.9	2.3 xy
'Oran Red'	2.4	2.3	2.0	1.8	2.1	2.1 x

Mean	2.9 b	2.3 a	2.4 a	2.5 a	2.5 a	
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* Means followed by the same letter do not differ significantly ($P = 0.05$). For the body of the table: LSD = 0.86 ($P = 0.05$).

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

Overall, the reaction of the commercial grown cultivars ('Star Ruby', 'Rio Red', 'nel Ruby', 'Flame') to the various mild CTV sources was very similar. However, there were interactions between some CTV sources with some grapefruit cultivars, resulting in retarded growth, lower production, small fruit and stem pitting. These interactions may change in other climatic conditions.

GFMS 12 reduced the tree sizes of 'Star Ruby' and 'Rio Red' as well as the yield efficiency of 'Star Ruby'. Stem pitting development in trees with this CTV source was also significantly higher, confirming the unsuitability of GFMS 12 as a cross-protector for red grapefruit.

Tree size and production of 'Flame' were reduced by GFMS 35, the present pre-immunising CTV source. The lower crop value of this cultivar indicates small fruit. From a commercial point of view this causes concern. During 2003 to 2008 only 30 000 buds were cut from 'Flame' mother trees opposed to 208 000 and 64 000 for 'Star Ruby' and 'nel Ruby' respectively and therefore indicates that 'Flame' is currently not planted on a large scale. If the popularity of 'Flame' changes, an alternative CTV source, either GFMS 67 or GFMS 73, should be considered as a pre-immunising source.

The most stable CTV source in the commercial cultivars, where the least interactions occurred, was GFMS 67. Tree size and production of 'Star Ruby' as well as the tree size of 'Rio Red' were reduced by GFMS 73. Looking at the matter as a whole, smaller trees are not necessarily a negative property and can be beneficial in high density plantings, providing the yield efficiency is not reduced and fruit size is acceptable.

This study revealed that it is not only essential to look for CTV cross-protecting sources for specific citrus types, but also within a type.

Future research

Searching for better CTV sources for cross-protection is an ongoing process. Several trials are continuing in this respect viz. experiments 679, 738 and 742.

Technology transfer

S.P. van Vuuren, J.H.J. Breytenbach, B.Q. Manicom, J.G.J. Maritz and Nikki Combrink. Cross-protection to reduce the effect of *Citrus tristeza virus* (CTV) in Citrus production of southern Africa. Fifth Citrus Symposium, Drakensberg, Natal.

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4.2.7 **PROGRESS REPORT: Evaluation of *Citrus tristeza virus* sources in Valencia** Experiment 788 (2000 - 2009) by S.P. van Vuuren & J.H.J. Breytenbach (CRI)

Summary

The cross-protecting abilities of *Citrus tristeza virus* (CTV) sources (LMS 6 (standard), SM 34, SM 36, SM 41, SM 45 and SM 49) are being compared in three Valencia cultivars ('McClean', 'McClean' Seedless and 'Delta') on 'Troyer' citrange rootstock. Trees infected with a known severe CTV source, as well as trees that were planted with a virus-free status serve as controls. The trees were planted in 2000 at Malelane. Over the long term, there is little difference in effect among the mild CTV sources, with the exception of SM 45, which reduced tree size and production. The 'McClean' Seedless Valencia trees yielded the best over a 3-year period. The best CTV source at this stage is LMS 6, which is currently the approved pre-immunisation source for Valencias in the Citrus Improvement Scheme.

Opsomming

Die effek van *Citrus tristeza virus* (CTV) bronne (LMS 6 (standaard), SM 34, SM 36, SM 41, SM 45 en SM 49) word in drie Valencia kultivars ('McClean', 'McClean' Saadloos en 'Delta') op 'Troyer' citrange onderstam vergelyk om hul kruisbeskermingsvermoëns te evalueer. Bome geïnfekteer met 'n bekende strawwe CTV bron en bome wat as virusvry geplant is, dien as kontroles. Die bome is in 2000 in Malelane geplant. Daar is min verskil in die langtermyn effek van die verskillende CTV bronne met die uitsondering van SM 45 wat boomgrootte en produksie nadelig beïnvloed. Die 'McClean' Saadlose Valencia bome het die beste produksie gelewer oor 'n drie-jaar periode. Bome met LMS 6, tans die goedgekeurde CTV preïmmuniseringsbron vir Valencias in die Sitrus Verbeteringskema, presteer tans die beste.

Introduction

The failure of sour orange as a rootstock for citrus cultivars in South Africa in 1896, is probably the earliest recorded evidence for the presence of *Citrus tristeza virus* (CTV), although it does not necessarily mean that South Africa is the country of origin (Webber, 1925; Oberholzer, 1959). The sensitive sour orange rootstock was abandoned because of CTV quick decline, and replaced by tolerant rootstocks such as rough lemon (Marloth, 1938). This practice is not a solution for sensitive scion cultivars such as grapefruit, and cross-protection with mild strains is the most successful approach to reduce the effect of the disease (Müller & Costa, 1972; van Vuuren, *et al.*, 1993a, 1993b).

Since the establishment of the South African citrus industry on tolerant rootstocks, it was generally accepted that CTV has no effect on sweet oranges and mandarins. This situation can partly be ascribed to nurserymen who unwittingly applied cross-protection by selecting parent trees showing the best health and production. These trees carried virus that had the least effect on growth and production. With the implementation of shoot-tip grafting, the virus-free material is vulnerable to infection by various strains occurring in nature, which are transmitted by aphids. Evidence of the presence of severe strains that can affect sweet orange exist in foreign countries (Roistacher, 1988; Barkley, 1991) as well as in South Africa (Marais, 1994).

All citrus cultivars in the Southern African Citrus Improvement Scheme are freed from viruses by shoot-tip grafting (de Lange, *et al.*, 1981). The abundance of the most effective aphid vector, *Toxoptera citricida* (Kirk.), will result in virus-free trees becoming naturally infected with various strains (Schwarz, 1965) including virulent strains (Müller, *et al.*, 1968; Calavan, *et al.*, 1980; Barkley, 1991). It is therefore necessary to protect the virus-freed plants from severe CTV strains by deliberately infecting them with mild strains (de

Lange, *et al.*, 1981; von Broembsen & Lee, 1988). The interaction of mild CTV sources with regard to cross-protection is specific with regard to biological activity (Müller & Costa, 1987; Van Vuuren, *et al.*, 1993b) and therefore, mild CTV sources specifically for sweet orange cultivars should be identified.

The objective of this study is to evaluate promising CTV sources in three Valencia scions and identify suitable cross-protecting sources.

Materials and methods

'McClean', 'McClean' Seedless (SL) - and 'Delta' Valencia trees on 'Troyer' citrange rootstock were grown according to normal nursery practices under aphid-free conditions. When the scions developed to approximate 5 mm in diameter, they were inoculated with CTV sources derived from sweet orange and showed promise in glasshouse tests. The sources are LMS 6 (standard derived from 'Mexican' lime), SM 36, SM 41, SM 45 and SM 49. Trees with these sources are compared to trees with a severe source (SOSS 3) as well as un-inoculated virus-free trees.

Pre-immunised trees which tested CTV positive were planted at Malelane ARC-ITSC Experimental Farm in a split plot design with the CTV sources being the main plot and the scions sub-plots (Rayner, 1967). Each treatment was replicated five times and planted at a spacing of 6 X 4 m. The trees were planted during 2000 and normal farming practices were applied regarding irrigation, fertilization, pest and disease control. No additional sprays were applied to control aphids and the trees were therefore naturally challenged by CTV introduced by the aphids.

The effects of the CTV sources on tree size, yield, fruit size and tree health were determined annually. For tree size the heights and diameters of the tree canopies were measured, and tree volumes determined according to Burger *et al.* (1970), where the canopy was calculated as a cylinder and half sphere viz. volume = $R^2(PIH-1.046R)$, where R = the mean radius of the tree and H = the height of the fruit bearing part. At maturity, fruit from each tree were harvested separately according to normal farming practices and weighed. The trees were inspected annually for disease symptoms and growth abnormalities such as decline of twigs, bud-union crease and stem pitting. Data was analysed according to a multifactor analysis of variance at the 5% confidence level (Statgraphics *Plus* 5.1, 2000).

Results and discussion

Tree size. In general, the canopy volumes of 'McClean' SL- Valencia trees were significantly larger than that of the 'Delta', and 'McClean' Valencia trees. The 'McClean' Valencia trees were significantly smaller than the 'Delta' Valencia trees (Table 4.2.7.1). With the CTV sources, trees with the severe (SOSS 3) source were significantly smaller than all the other treatments. Trees with SM 36 were the largest but not statistically significantly larger than trees with LMS 6 (standard), SM 34, SM 41 and SM 49. However, they were significantly larger than trees that were planted virus-free. Of the sweet orange CTV sources, trees with SM 45 were the smallest and differed significantly from trees with SM 34, SM 36 and SM 49 (Table 4.2.7.1). The body of Table 4.2.7.1 shows severe reductions in canopy size where the known severe CTV source (SOSS 3) was used in all three cultivars. Of the mild sources SM 45 reduced canopy volumes of 'McClean' Valencia severely and to a lesser extent in the 'McClean' SL and 'Delta' Valencias.

Production. The production of the 'McClean' SL- and 'Delta' Valencia trees were significantly better than that of the 'McClean' trees (Table 4.2.7.2). The lower production of the 'McClean' trees was not only because the trees were smaller (Table 4.2.7.1) but also because of a significant lower yield efficiency (Table 4.2.7.3). Trees with LMS 6 (standard) produced the best but not significantly better than trees with SM 36, SM 41, SM 49 and those that were planted virus-free (Table 4.2.7.2). Trees with SM 34, SM 45 and the severe source (SOSS 3) produced significantly less. The yield efficiency of the different CTV sources did not differ (Table 4.2.6.3). The body of Tables 4.2.7.2 and 4.2.7.3 show a generally poor production and yield efficiency of 'McClean' trees with all the treatments except the standard (LMS 6). With the other two cultivars production was severely reduced by the severe CTV source and to a lesser extent by CTV sources SM 45 ('McClean' SL and 'Delta') and SM 41 ('Delta') (Table 4.2.7.2).

The long-term effect of the CTV sources on production that are shown in Table 4.2.7.4 reveals little difference among the CTV sources, with the exception of SM 45. The 'McClean' SL trees yielded the best over a 3-year period. The best CTV source at this stage is LMS 6, which is currently the pre-immunization source for Valencias in the Citrus Improvement Scheme. At this stage trees with SM 45 are the poorest.

Table 4.2.7.1. Tree size (canopy volume = m³) of three Valencia cultivars that were pre-immunised with different mild CTV sources, a severe source and trees that were planted virus-free, 8 years after planting*.

CTV source	Scions**			Mean
	McC	McC SL	Delta	
LMS 6	22.3	34.6	30.5	29.1 wx
SM 34	25.0	32.5	26.6	28.0 wx
SM 36	25.1	33.5	30.9	29.8 w
SM 41	26.7	27.1	27.4	27.1 wxy
SM 45	20.3	26.9	25.2	24.1 y
SM 49	25.1	30.2	28.3	27.9 wx
SOSS 3	11.2	15.5	14.8	13.8 z
Virus-free	23.8	28.2	27.7	26.5 xy
Mean	26.4 B	22.4 c	28.6 a	

* ** Means followed by the same letter are not significantly different (P = 0.05). The body of the table: LSD = 5.72 (P = 0.05).

** Scions: McC = 'McClellan' Valencia; McC SL = 'McClellan' Seedless Valencia; Delta = 'Delta' Valencia.

Table 4.2.7.2. The production (kg/tree) of three Valencia cultivars that were pre-immunised with different mild CTV sources, a severe source and trees that were planted virus-free, 8 years after planting*.

CTV source	Scions**			Mean
	McC	McC SL	Delta	
LMS 6	62.2	100.5	85.7	82.8 x
SM 34	50.4	76.6	74.0	67.0 y
SM 36	44.8	84.2	97.0	75.3 xy
SM 41	53.4	81.8	71.8	69.0 xy
SM 45	46.1	70.9	73.6	63.5 z
SM 49	55.9	87.2	69.1	70.8 xy
SOSS 3	18.9	53.5	42.0	38.2 z
Virus-free	53.6	89.6	67.9	70.4 xy
Mean	48.2 B	80.5 a	72.6 a	

* ** Means followed by the same letter are not significantly different (P = 0.05). The body of the table: LSD = 26.98 (P = 0.05).

** Scions as in Table 4.2.7.1.

Table 4.2.7.3. Yield efficiency (kg/m³) of three Valencia cultivars that were pre-immunized with different mild CTV sources, a severe source and trees that were planted virus-free, 8 years after planting*.

CTV source	Scions**			Mean
	McC	McC SL	Delta	
LMS 6	2.8	2.9	2.9	2.9 z
SM 34	2.1	2.3	2.8	2.4 z
SM 36	1.8	2.5	3.1	2.5 z
SM 41	2.0	3.1	2.6	2.6 z
SM 45	2.2	2.6	2.9	2.6 z
SM 49	2.3	2.9	2.4	2.6 z
SOSS 3	1.8	3.4	2.8	2.7 z
Virus-free	2.4	3.2	2.4	2.7 z
Mean	2.2 B	2.9 a	2.7 a	

* ** Means followed by the same letter are not significantly different (P = 0.05). The body of the table: LSD = 0.97 (P = 0.05). ** Scions as in Table 4.2.7.1.

Table 4.2.7.4. Cumulative yield (kg) over a 3-year period of 8-year-old Valencia cultivars that were pre-immunised with different mild CTV sources, a severe source and trees that were planted virus-free*.

CTV source	Scions**			Mean
	McC	McC SL	Delta	
LMS 6	210.4	163.9	163.9	179.4 w
SM 34	187.2	189.2	147.7	174.7 wx
SM 36	163.0	166.9	141.2	157.0 wx
SM 41	179.1	141.3	160.1	160.1 wx
SM 45	142.6	98.7	130.3	123.9 y
SM 49	209.5	145.8	111.7	155.7 wx
SOSS 3	69.8	94.2	89.5	84.5 z
Virus-free	163.6	139.6	139.5	147.6 xy
Mean	165.6 A	142.5 b	135.5 b	

* Means followed by the same letter are not significantly different ($P = 0.05$). The body of the table: LSD = 48.64 ($P = 0.05$).

** Scions as in Table 4.2.7.1.

Conclusion

In the short and long term, trees pre-immunised with the LMS 6 CTV source, which is the current cross-protecting source, produced the best and trees with SM 45 were the poorest.

Further objectives

This experiment will continue for another year. Evaluation of new CTV sources for Valencia is also continuing in Experiment 789.

Technology transfer

S.P. van Vuuren, J.H.J. Breytenbach, B.Q. Manicom, J.G.J. Maritz and Nikki Combrink. Cross-protection to reduce the effect of *Citrus tristeza virus* (CTV) in Citrus production of southern Africa. Fifth Citrus Symposium, Drakensberg, Natal.

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4.2.8 **PROGRESS REPORT: Identification of suitable *Citrus tristeza virus* sources for pre-immunizing Turkey Valencia**

Experiment 789 (2005 - 2015) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

Summary

It appears that Turkey Valencia is more sensitive to CTV than other Valencia types. Since Turkey Valencia is an early Valencia, it forms an important component of the citrus industry and it is therefore a high priority to identify a suitable CTV source for pre-immunisation of this cultivar. Virus-free Turkey Valencia on Troyer citrange rootstocks were prepared in the glasshouse and inoculated with different CTV isolates (LMS 6 (standard), SM 46, SM 47, SM 48, SM 49 (all collected from sweet orange) to identify the best source for cross-protection purposes. Trees inoculated with GFMS 12 and trees left virus-free will serve as positive and negative controls, respectively. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Riverside in the Malelane area during March 2007. The trees were evaluated during 2008 for growth. Although there are differences between the treatments, it is still too early to draw any conclusions. The trees will be evaluated annually for growth, production, fruit size, as well as tree health.

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) as ander Valencia tipes is (CRI Groep Navorsingsjaarverlag, 2003). Aangesien Turkey Valencia 'n vroeë Valencia is, vorm dit 'n belangrike deel van sitrus produksie in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte CTV pre-immunisasie bron vir Turkey Valencia te vind. Virusvrye Turkey Valencia op Troyer citrange onderstam is in 'n glashuis voorberei en met verskeie CTV bronne, LMS 6 (standaard), SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemoene versamel), geïnkuleer om die beste ligte CTV bron vir kruisbeskermingsdoeleindes te identifiseer. Bome wat met die GFMS 12 bron geïnkuleer is en bome wat virusvry gelaat is, dien as positiewe en negatiewe kontroles, onderskeidelik. Pre-immunisasie is deur middel van ELISA bevestig en die bome is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is gedurende 2008, een jaar na uit plant, ge-evalueer vir groei. Alhoewel daar verskille is, is dit nog te vroeg om enige gevolgtrekkings te maak. Die bome sal jaarliks ge-evalueer word vir groei, produksie, vrugsgrootte en algemene boom gesondheid.

Introduction

Cross protection to control *Citrus tristeza virus* (CTV) is specific with regard to the citrus type, i.e. the most effective protecting CTV isolate for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Scheme, all citrus, including sweet oranges, were pre-immunised with a CTV source originating from grapefruit until suitable sources are found for the different types (von Broembsen & Lee, 1988). Subsequently, a suitable source, LMS 6, has been identified for lime (van Vuuren *et al.*, 1993). LMS 6 contains a mild form of seedling yellows, which the grapefruit source does not have, and it was therefore approved to replace GFMS 12 as the pre-immunising source for sweet oranges (van Vuuren *et al.*, 2000).

The suitability of LMS 6 as a protector for sweet oranges and mandarins has not been confirmed and evaluations are currently being done in Clementine, navel and Valencia. Midseasons and other sweet oranges are known to be affected by a transmissible factor causing abnormal bud-union on Rough lemon rootstock (McClellan, 1974). The transmissible factor is not insect- or seed transmissible and was not correlated to any known citrus disease. It has been shown that it can be removed by shoot tip grafting (Navarro *et al.*, 1993).

Recently, it was found that Turkey Valencia trees on Rough lemon and Volckameriana rootstocks developed bud-union creasing symptoms (personal observation; Beeton *et al.*, 2000). Various sources of Turkey Valencia had stem pitting symptoms and it appears that this cultivar is more sensitive to CTV than other Valencia cultivars (CRI Group Annual Research Report, 2003). Since Turkey Valencia is an early cultivar, it forms an important part of citrus production, and therefore the identification of a suitable CTV source for cross-protection remains a high priority.

The objective of this study is to evaluate CTV sources to identify a suitable cross protecting source for Turkey Valencia.

Materials and methods

Virus-free Turkey Valencia scions on Troyer citrange rootstocks were prepared in the greenhouse according to normal nursery practices. When the scions have developed to approximately 5 mm, they were inoculated with different mild CTV sources by budding two buds containing the required CTV source into the scions (Table 4.2.8.1). After 3 months, the trees were tested for the presence of the CTV sources by means of ELISA. The trees were planted at Riverside in the Malelane area where they will be subjected to normal CTV challenges by aphids. Each treatment was replicated five times and uninoculated virus-free trees serve as controls. Evaluations will be done annually on growth, production and tree health.

Table 4.2.8.1 Treatments for Turkey Valencia on Troyer citrange rootstock to identify a suitable CTV source for pre-immunisation.

CTV sources	Origin and comments
LMS 6	Mexican lime, Tzaneen. Present pre-immunising source for sweet orange
SM 46	Shamouti Midseason, Messina
SM 47	Valencia, Grahamstown. Tree > 100 years old
SM 48	Midseason, Citrusdal. First planting of citrus in the area
SM 49	Valencia, Nelspruit. Induce some greening tolerance
GFMS 12	Nartia grapefruit. Positive control
Virus-free Control	Virus-free. Negative control

Results and discussion

The canopy volumes of the Marsh trees are presented in Table 4.2.8.2. There are significant differences between the sources, but at this stage it is still too early to draw any conclusions

Table 4.2.8.2. Tree size (canopy volume in m³) of Turkey Valencia trees pre-immunised with different CTV sources, 1 year after planting at Riverside, Malelane*.

Treatment	Canopy volume (m ³)
SM 46	2.7 ab
SM 47	2.8 a
SM 48	0.8 c
SM 49	1.6 abc
GFMS 12	1.7 abc
LMS 6	2.5 ab
Virus-free	1.6 abc

* Figures in the columns followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

The trees are still young and no conclusions can be made at this stage.

Further objectives

Evaluate horticultural performance i.e. growth (tree size), yield data (fruit size and kg/tree) and health (stem pitting, decline).

Technology transfer

None.

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4.2.9 FINAL REPORT: The effect of CTV pre-immunisation on the fruit size of Clementine and Satsuma

Experiment 816 (2005 - 2010) by S.P. van Vuuren (CRI), J.G. Maritz and N. Combrink (ITSC)

Summary

Fruit size is a big problem with Clementines in the Eastern and Western Cape. To assess the effect of CTV pre-immunisation on fruit size, 7 non-pre-immunised and pre-immunised Clementine cultivars ('Clemlate', 'Oronules', 'Esbal', 'Orogrande', 'Guillermina', 'hour', 'Clemenpons') and a satsuma selection ('Miho Wase') were compared at Addo Research Station. In most cases, no statistical differences were observed between pre-immunised trees and trees that were planted virus-free, but results from the two trials did not correlate. Results will, however, become increasingly inconclusive due to the fact that some trees that were initially planted as virus-free became naturally infected by CTV. The effect of natural CTV infection will become more apparent in the years to follow. The objective to improve fruit size by eliminating CTV can therefore not be achieved in South Africa and the experiment was terminated. The effect of various CTV cross-protection sources specifically on fruit size in these cultivars should be evaluated to elucidate this problem, as well as to potentially identify improved cross-protection sources.

Opsomming

Vruggrootte is 'n groot probleem by Clementines in die Oos- en Wes-Kaap. Om die invloed van pre-immunisering op vruggrootte te bepaal, is ongepre-immuniseerde en gepre-immuniseerde bome van sewe Clementine cultivars ('Clemlate', 'Oronules', 'Esbal', 'Orogrande', 'Guillermina', 'hour', 'Clemenpons') en een satsuma seleksie ('Miho Wase') op Addo Navorsingstasie vergelyk. In die meeste gevalle was daar geen betekenisvolle verskille tussen virus-vrye en gepre-immuniseerde bome, hoewel resultate uit die twee proewe nie gekorreleer het nie. Resultate sal egter meer verwarrend raak in die toekoms, siende dat van die virus-vrye bome natuurlik met CTV besmet raak. Die doel om vruggrootte te verbeter deur die uitsluiting van CTV kan dus nie in Suid Afrika gehaal word nie en die eksperiment is getermineer. Die effek van verskeie CTV kruisbeskermingsbronne spesifiek op vruggrootte in hierdie cultivars moet ondersoek word om sodoende hierdie probleem aan te spreek, asook moontlik meer geskikte kruisbeskermingsrasse te identifiseer.

Introduction

All citrus propagation material is pre-immunised with a mild source of *Citrus tristeza virus* (CTV). Cross-protection is specific with regard to the citrus type, i.e. the most effective protecting strain for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Southern Africa Citrus Improvement Scheme, all citrus, including mandarin types, was pre-immunised with a CTV source

originating from grapefruit until suitable sources were found for the different citrus types (von Broembsen & Lee, 1988). Subsequently, a suitable CTV source, LMS 6, has been identified for lime (van Vuuren *et al.*, 1993). LMS 6 contains a mild form of seedling yellows, which the grapefruit source does not possess, and it was therefore approved to replace GFMS 12 as the pre-immunising source for the mandarin types. At that stage the suitability of LMS 6 as a protector for Clementines had not been confirmed and evaluations were in progress at Addo research Station (van Vuuren & Maritz, 2002).

Fruit size of Clementine is a major problem in the Western and Eastern Cape citrus production regions. Production costs associated with cultural practices aimed at fruit size improvement are high. Since mandarins have a lower sensitivity to CTV, it may not be essential to pre-immunise mandarin cultivars to protect them against severe strains of CTV. The prospect to improve size of fruit produced on virus-free trees is investigated in this experiment.

Materials and methods

This trial was initiated by Prof. E. Rabe and was taken over by S.P. van Vuuren when Prof. Rabe left South Africa. Virus-free and LMS 6 pre-immunised trees of seven Clementine selections ('Clem late', 'Oronules', 'Esbal', 'Orogrande', 'Guillermina', 'hour', 'Clemenpons') and one Satsuma selection ('Miho Wase') were prepared on 'Swingle' citrumelo rootstock in a commercial nursery (rootstocks might have been infected with CTV prior to budding).

When the scions have developed they were planted at Addo Research Station according to a randomised block design in 2003. Since there was a variation in the number of trees available, they were split in three separate trials. Trial one consisted of the selections 'Clem late', 'Oronules', 'Esbal', 'Orogrande', 'Guillermina', 'hour', 'Clemenpons' and each treatment was replicated four times. Trial two consisted of the selections 'Clem late', 'Esbal', 'Orogrande', 'Guillermina', 'hour', 'Clemenpons' and each treatment was replicated five times. Trial three was 'Miho Wase' satsuma and each treatment was replicated eight times.

Tree canopies were measured and the volume calculated according to Burger *et al.* (1970) which involves a formula where it is assumed that a citrus tree is composed out of a cylinder and a half sphere *viz.* volume = $R^2(PIH-1.046R)$, where R = the mean radius of the tree and H = the height of the fruit bearing part. The fruit were harvested, graded into the different export sizes and weighed.

During 2007 the presence of CTV was confirmed in the trees that were planted virus-free in trial 1. In August 2008 leaf samples were taken from all the trees in all three trials and tested by ELISA to determine CTV infection.

Results and discussion

Tree size

The canopy volumes of the Clementine and Satsuma trees are presented in Table 4.2.9.1 (Clementine trial 1), Table 4.2.9.2 (Clementine trial 2) and Table 4.2.9.3 (Satsuma) respectively.

No statistical difference was observed between virus-free and pre-immunised trees of the various cultivars evaluated in trial 1, except for pre-immunised 'Oronules' and 'Clemenpons' trees that were significantly smaller than the virus-free trees (Table 4.2.9.1). Although the overall result showed that pre-immunised trees were smaller than unpre-immunised trees, this difference was attributed to the smaller tree sizes of the 'Oronules' and 'Clemenpons' cultivars that were significantly affected by pre-immunisation (body of Table 4.2.9.1).

The results in trial 2 did not support the results of trial 1. 'Clemenpons' trees were again the smallest but in this trial were significantly smaller than the 'Clem late' trees (Table 4.2.9.2). The reduction of the pre-immunised trees that occurred in trial 1 over unpre-immunised trees, did not occur in trial 2. There were also no differences between pre-immunised and unpre-immunised trees between each cultivar (body of Table 4.2.9.2).

Tree sizes of the pre-immunised and unpre-immunised satsuma trees did not differ significantly (Table 4.2.9.3).

Production

The fruit of the 3 trials were harvested, weighed and are presented in Table 4.2.9.4, Table 4.2.9.5 and Table 4.2.9.6, respectively.

No significant difference in production occurred between pre-immunised and unpre-immunised trees (Table 4.2.9.4) as well between the two treatments among the different cultivars (body of Table 4.2.9.4). Overall, the trees that were planted with a virus-free status did not produce better than the pre-immunised trees in both trials. A significant variation occurred among the Clementine selections with the lowest production by 'hour Tardif V15' in trial 1 and trial 2. 'Clemlate' (trial 1 and 2) and 'Clemenpons' (trial 2) also yielded significantly lower.

In trial 1 the 'Clemlate' and 'Orogrande' trees produced significantly better than the 'hour Tardif V15' and 'Esbal' trees but not better than the other cultivars (Table 4.2.9.4).

Results in trial 2 are similar to that in trial 1 except that the production of the cultivars differed significantly, this time 'Clemlate' and 'Guillermina' were better than the rest (Table 4.2.9.5).

There was no significant difference in production between the satsuma trees that were planted virus-free and those that were pre-immunised (Table 4.2.9.6).

Table 4.2.9.1. Canopy volumes (m³) of trees planted virus-free and pre-immunised Clementine selections in trial 1, 5 years after planting¹.

Cultivar name and number	Treatment		Mean
	Virus-free	Pre-immunised	
'Clemlate' 1163	10.4 abc	9.5 abcde	10.0 yz
'Orogrande' 1300	10.4 abc	12.2 ab	11.3 y
'Guillermina' 1331	9.6 abcd	9.1 bcde	9.3 yz
'hour Tardif V15' 1561	8.8 bcde	10.7 abc	9.7 yz
'Oronules' 1570	12.4 ab	5.7 de	9.1 yz
'Esbal' 1571	13.1 a	7.8 cde	10.4 yz
'Clemenpons' 1581	9.2 abcde	5.6 e	7.4 z
Mean	10.6 v	8.6 w	

¹ Figures in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.2. Canopy volumes (m³) of trees planted virus-free and pre-immunised Clementine selections in trial 2, years after planting¹.

Cultivar name and number	Treatment		Mean
	Virus-free	Pre-immunised	
'Clemlate' 1163	10.8 a	11.1 a	11.0 y
'Orogrande' 1300	9.5 a	10.5 a	10.0 yz
'Guillermina' 1331	8.6 a	9.6 a	9.1 yz
'hour Tardif V15' 1561	7.8 a	9.6 a	8.7 yz
'Esbal' 1571	10.9 a	8.9 a	9.9 yz
'Clemenpons' 1581	9.1 a	7.4 a	8.2 z
Mean	9.4 v	9.5 v	

¹ Figures in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.3 Canopy volumes (m³) of trees planted virus-free and pre-immunised satsuma trees in trial 3, 5 years after planting¹.

Cultivar name and number	Treatment	
	Virus-free	Pre-immunised
'Miho Wase' 983	8.9 a	7.4 a

¹ Figures in the row followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.4. Average yield per tree (kg) of virus-free planted and pre-immunised trees of Clementine selections in trial 1, 5 years after planting¹.

Cultivar name and number	Treatment		Mean
	Virus-free	Pre-immunised	
'Clemlate' 1163	54.0 ab	52.6 abc	53.3 X
'Orogrande' 1300	49.8 abc	57.0 a	53.4 X
'Guillermina' 1331	51.8 abc	29.6 abcd	40.7 xyz
'hour Tardif V15' 1561	23.6 cd	18.6 d	21.1 z
'Oronules' 1570	57.3 a	26.3 bcd	41.8 xy
'Esbal' 1571	30.3 abcd	35.0 abcd	32.6 yz
'Clemenpons' 1581	40.3 abcd	34.1 abcd	37.2 xyz
Mean	42.5 v	37.5 v	

¹ Figures in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.5. Average yield per tree (kg) of virus-free planted and pre-immunised trees of selections in trial 2, 5 years after planting¹.

Cultivar name and number	Treatment		Mean
	Virus-free	Pre-immunised	
'Clemlate' 1163	74.7 a	52.0 bcd	63.4 y
'Orogrande' 1300	35.2 d	48.7 cd	42.0 z
'Guillermina' 1331	60.8 abc	73.5 ab	67.2 y
'hour Tardif V15' 1561	32.7 d	43.2 cd	37.9 z
'Esbal' 1571	49.8 cd	44.3 cd	47.0 z
'Clemenpons' 1581	38.3 d	33.6 d	35.9 z
Mean	48.6 v	49.2 v	

¹ Figures in the body of the table as well as the means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.6. Average yield per tree (kg) of virus-free planted and pre-immunised satsuma trees of trial 3, 5 years after planting¹.

Cultivar name and number	Treatment	
	Virus-free	Pre-immunised
'Miho Wase' 983	76.6 a	73.6 a

¹ Figures in the row followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

In most cases, no statistical differences were observed between pre-immunised trees and trees that were planted virus-free. However, results will become increasingly inconclusive due to the fact that some trees that were initially planted as virus-free became naturally infected by CTV. The effect of natural CTV infection will become more apparent in the years to follow. The objective to increase fruit size by eliminating CTV is not feasible in South Africa and the only solution for the small fruit problem will be to identify better CTV sources for cross-protection.

Future research

The trees that were planted with a virus-free status initially, became naturally infected with CTV and therefore the aim of comparing virus-free and pre-immunised trees can no longer be done. This experiment was terminated. An experiment has been approved to evaluate new CTV sources for cross-protection of soft citrus (Experiment 968).

Technology transfer

None.

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4.2.10 PROGRESS REPORT: Dynamics of citrus tristeza virus mild and severe strains in mild strain cross-protection strategies

Experiment 885 (April 2007 – April 2011): by Katherine Stewart, David Read, Lara van der Westhuisen (UP) and Gerhard Pietersen (CRI-UP)

Summary

This study attempts to gain more insight into the cause of mild strain cross protection failure in grapefruit in South Africa. Two hypotheses are being tested: 1) that cross protection breakdown in GFMS 12 pre-immunized plants is due to “super-infection” with severe CTV strains, or 2) breakdown occurs through dominance by an inherent severe component of GFMS 12 under high temperature selection. Furthermore it is also being assessed whether GFMS 35 cross protection breakdown is due to “super-infection” with severe CTV strains. Cloning and sequencing of the PCR product of a variable region of CTV from a severely infected field source (pre-immunized with GFMS 12) has shown that 2 main sequence types occur, the VT strain being predominant. Clones of the glasshouse-maintained GFMS 12 population (used in citrus pre-immunization), however, have 3 main sequence types with the *Poncirus trifoliata* resistance breaking strain (RB) being predominant. Sequences from the A-region of the CTV genome suggest sub-isolates 12-4 and 12-1 are identical and similar to 12-9, which are all VT-like variants. Sub-isolate 12-8 was unique and a B165-like variant. Sub-isolate 12-1-2, 12-5 and 12-7 were similar to the RB variants. A representative sub-isolate from the VT, B165 and RB groups were selected and bark chip grafted in different combinations onto Marsh and Star Ruby grapefruit plants and placed in 4 different temperature conditions in order to study the strain dynamics and interactions at different temperatures. A previously reported real-time RT-PCR protocol for CTV detection, using a TaqMan[®] hydrolysis probe, has been tested and optimised for this purpose, as well as a potential replacement of ELISA for routine CTV tests. During development of a real-time PCR to detect the CTV generically and three strains specifically, sample preparation, means of reducing costs, relative sensitivity, optimisation of blotting, reverse transcription and amplification steps were assessed. A PCR against a constitutively expressed healthy citrus component has been established to serve as an internal control on RNA extractions for quantitative real-time PCR.

Opsomming

In hierdie studie word daar gepoog om meer insig in die oorsaak van milde ras kruisbeskerming afbraak in pomelos in Suid-Afrika te verkry. Twee hipotesisse word ondersoek: 1) dat kruisbeskerming afbraak in GFMS 12 gepre-immuniseerde plante is a.g.v. hiperinfeksie met strawwe rasse of 2) dat afbraak is as gevolg van dominasie van die strawwe ras binne GFMS 12 onder hoër temperature is. Verder word daar ook bepaal of GFMS 35 kruisbeskerming afbraak ook a.g.v. hiperinfeksie met strawwe rasse is. Klonering en volgordebepalings van die PCR produk van 'n variëerbare gedeelte van CTV vanuit 'n straf besmette veldbron (gepre-immuniseerd met GFMS 12) het getoon dat twee verskillende hoof nukleotied volgorde tipes bestaan. Die VT vorm is die dominante een. Hierteenoor het die glashuisbron van GFMS 12, wat tans vir pre-immunisering gebruik word, drie hoof nukleotied volgorde tipes bevat, waaronder die *Poncirus trifoliata* weerstandbiedendheid oorkomende (RB) ras, die dominante een is. Nukleotied volgorde bepaling van die A segment van die CTV genoom dui daarop dat sub-isolate 12-4 en 12-1 identies is en soortgelyk is aan 12-9, en dat aldiere soortgelyk is aan die VT-tipe rasse. Sub-isolaat 12-8 is uniek maar mees verwant aan B165-tipe isolate. Subisolate 12-1-2, 12-5 en 12-7 is soortgelyk aan RB isolate. Verteenwoordigende sub-isolate van VT-, B165- en RB-agtige isolate is geselekteer en in verskillende kombinasies met bas-stukkies op March en Star Ruby plante geënt. Herhalings van hierdie behandelings is dan in 4 beheerde temperatuurtoestande geplaas sodat die ras dinamiek onder verskillende temperature gestudeer kan word. 'n Reeds-gepubliseerde intydse omgekeerde transkriptase PCR ('real-time RT-PCR') protokol vir CTV is gevestig en ge-optimeer die om die rasdinamiek in die bogenoemde proef te kan bepaal sowel as om 'n alternatief vir ELISA binne

die Sertifiseringskema te kan optree. Gedurende ontwikkeling van die PKR protokolle om CTV generies en die drie rasse spesifiek op te kan spoor, is monster voorbereiding, koste besparing, relatiewe sensitiviteit, optimisering van klad, omgekeerde transkriptase en amplifiseringstappe geëvalueer. PKR teen 'n konstitutief uitgedrukte geen van gesonde sitrus is ook as 'n interne kontrol op RNA ekstraksies vir die huidige tyd PKR gevestig.

Introduction

Citrus tristeza virus (CTV) is an aphid-borne closterovirus and has ranked as one of the most important citrus diseases for the last sixty years (Bar Joseph *et al.*, 1989). CTV causes varying degrees of symptoms from none to very severe. Severe symptoms are mainly the decline of trees, stem-pitting, reduction in fruit size and a seedling yellows effect on young plants.

Within the southern African Citrus Improvement Scheme (CIS) all citrus is pre-immunized with one of a few mild strain population sources of CTV. This protects the plant in most cases from infection by severe forms of CTV. However, occasionally severe symptoms are still found. It is unknown whether this is due to: 1) super-infection of the plant with wild-type severe forms of the virus; 2) uneven distribution or segregation of CTV forms in different parts of the plants; 3) mutations within the mild population to severe forms or; 4) selection under specific host and environmental conditions for severe forms inherently present in the population and 5) specific strain competition dynamics changing the balance.

It was found that possible cross-protection breakdown occurred when the brown citrus aphid (BrCA) was introduced into Florida (Powell *et al.*, 2003). This suggests that the introduction of BrCA possibly accelerated the breakdown of cross-protection, but how this occurred is not understood as well as possible factors behind the breakdown.

In 2002, a study examined changes in the pre-immunized grapefruit trees in South Africa (van der Vyver *et al.*, 2002). Certain grapefruit cultivars pre-immunized with South African CTV isolates GFMS (Grapefruit mild strain) 12 and 35 exhibited changes in the level of protection and produced high percentages of small fruit. These isolates were biologically evaluated on CTV sensitive plants and examined by Single-Strand Conformation Polymorphism (SSCP) analysis. The biological data indicated that the cross-protecting isolates had not retained their original status since a seedling yellows (CTV-SY) as well as severe stem-pitting components were recorded. Other strains were also found in infected trees, which were not part of the original pre-immunization strains. It was postulated that super-infection occurred by other CTV strains introduced. There was no evidence in the SSCP patterns that segregation of strains within the original pre-immunization isolates occurred. The SSCP patterns of the additional severe strains introduced did not correspond to the SSCP profiles of the strains within the cross-protecting isolates. It was concluded that changes occurred in the viral RNA populations within trees but did not necessarily indicate cross-protection failure (van der Vyver *et al.*, 2002).

When plants are inoculated with complex isolates, strain separation can readily occur during systemic invasion (Moreno *et al.*, 1991). Hosts can influence the CTV strain balance as shown by passage through grapefruit, smooth Seville orange and Mexican lime (Moreno *et al.*, 1991). Differences in SSCP patterns were found in different sectors of individual plants strongly suggesting uneven distribution of the CTV strains within the tree, possibly due to multiple aphid introductions (Rubio *et al.*, 2000).

Cross-protection trials done on Marsh grapefruit over a 20-year period in Australia in two climatically distinct sites showed that there are definite effects of climate on tristeza symptom expression (Broadbent *et al.*, 1991) and the benefits of mild strain protection. After 20 years more than half the uninoculated (initially virus-free) control trees at Somersby (humid, coastal site) were unproductive with small fruit and severe trunk stem-pitting as were the severe control plants, whereas most trees that were pre-immunized with mild strains showed no deterioration/breakdown (Broadbent *et al.*, 1991). By comparison at Dareton (hot, dry inland site) trees inoculated with the severe strain remained in good health for 17 years before the production of small fruit became a problem (Broadbent *et al.*, 1991). There was no marked difference between uninoculated, mild strain protected and severe control trees in this 17-year period (Broadbent *et al.*, 1991).

The aim of this study was to gain more insight into the cause of mild strain cross protection failure in grapefruit in South Africa. Two hypotheses are being tested: 1) that cross protection breakdown in GFMS 12 pre-immunized plants is due to "super-infection" with severe CTV strains, or 2) breakdown occurs through dominance by an inherent severe component of GFMS 12 under high temperature selection. Furthermore it is also being assessed whether GFMS 35 cross protection breakdown is due to "super-infection" with severe CTV strains.

Materials and methods

PCR and sequencing

Sub-isolates were amplified and sequenced using A-region primers (Rubio *et al.*, 2001); p23 gene (Sambade *et al.*, 2002); and the Coat protein gene (Huang *et al.*, 2004). Sequencing was completed at the University of Pretoria sequencing facility.

Cloning of variable region of severe source from field (pre-immunized with GFMS 12)

Amplicons from a variable region of ORF1a of the source were amplified and cloned into pGEM-T Easy vector system (Promega, USA). Clones were selected and sequenced in the forward and reverse directions. Phylogenetic analyses were done using BioEdit and Mega 3.1 software.

Experiment to determine the effect of temperature on CTV strain dynamics.

Three CTV variants 12-7 (strain 1); 12-8 (strain 2); 12-9 (strain 3) were chosen for use in the biological trial from GFMS 12 sub-isolates. The plants were inoculated with 3 bark chips of the same size per strain, in different combinations onto (a) Star Ruby grapefruit and (b) Marsh grapefruit cultivars as follows: 1x2; 1x3; 2x3; 1x2x3 (1-3 refers to the 3 strains). Control plants included single strains of strain 1; strain 2; strain 3 and GFMS 12. Virus free controls were left un-inoculated. Plant treatments were performed in triplicate. Four different batches of the inoculated plants mentioned above were placed in 4 different growth rooms set at different temperature ranges: Room 1 (Max 35°C; Min 25°C); Room 2 (Max 30°C; Min 22.5°C); Room 3 (Max 25°C; Min 20°C) and Room 4 (Max 20°C; Min 15°C). Plants will be tested at 4 month intervals up to 12 months and the individual strain/s analyzed for strain competition dynamics and concentration at different temperatures with 2 different grapefruit cultivars.

Development of quantitative Real-time PCR system (courtesy of THRIP matching funding)

The primer pair and TaqMan[®] probe sequences used were from Bertolini *et al.* (2008). In the original protocol an Applied Biosystems TaqMan[®] Universal PCR Mastermix (Applied Biosystems, Foster City, CA) is used. Real-time PCR assays conducted at CRI@UP were carried out using a LightCycler 1.5 platform (Roche Molecular Diagnostics, Mannheim, Germany). The reaction mixture consisted of: 12.5 µl 1X Applied Biosystems TaqMan[®] Universal PCR Mastermix, 2.5 µl 3'UTR1 forward primer (10 µM), 2.5 µl 3'UTR2 reverse primer (10 µM), 0.75 µl of 181T TaqMan[®] probe (5 µM), 0.2 µl MultiScribe[®] Reverse transcriptase (Applied Biosystems, Foster City, CA), 0.25 µl RNase inhibitor (Applied Biosystems, Foster City, CA), 5 µl purified total RNA and 1.3 µl molecular grade water to a total reaction volume of 25 µl. PCR cycling conditions were performed as follows, 48°C for 30 minutes (reverse transcription), 95°C for 10 minutes (denaturation), 45 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. Data analysis was performed using LightCycler 4.05 software (Roche Molecular Diagnostics, Mannheim, Germany). A two step procedure in which cDNA synthesis from CTV RNA templates was performed according to Herron *et al.* (2005) were also conducted.

Samples of plant tissue harbouring GFMS 12 CTV populations were immobilized on either 0.45 µm nitrocellulose (Nitropure, MSI, Westboro MA) or nylon (Hybond-N+, Amersham Biosciences, Little Chalfont, UK) by pressing the petioles of five leaves and the cut end of thin branch material, with green bark, onto a 4 cm² block of membrane. After air-drying for approximately 30 minutes, the blots were cut out (or punched using a Harris punch) of the membrane blocks and placed into a 0.2 ml PCR tube containing 100 µl of CTV tissue print buffer, (Bertolini *et al.*, 2008), which consists of 0.1 M glycine, 0.05 M NaCl and 1 mM EDTA (ethylene diamine tetra-acetic acid). The tubes were incubated at 95°C for 10 minutes to lyse immobilized virus particles. Membranes were removed and the extracts vortexed. 5 µl of each extract was used as a template in real-time RT-PCR reactions. Minor variations in numbers of cut petioles used, cutting numbers and angles and blot tissue punches and extracts were assessed.

To assess the possibility of using cheaper reagents than in the original published protocol, Bioline *Taq* polymerase and its associated components (Bioline, London, UK), routinely used for conventional PCR reactions in our laboratory, was used. The reaction was optimised with regards dNTP and MgCl₂ concentrations. After optimisation, reactions were as follows (Bioline protocol): 2.4 µl of Bioline 10x NH₄ reaction buffer (160 mM (NH₄)₂SO₄, 670 mM Tris-HCl (pH 8.8 at 25°C), 0.1% stabilizer), 2.75 µl of 3.5 mM dNTP mix, 2.5 µl of 10 mM 3'UTR 1 primer, 2.5 µl of 10 mM 3'UTR 2 primer, 0.75 µl of 10 mM 181T TaqMan[®] probe, 0.2 µl MultiScribe[®] Reverse transcriptase (Applied Biosystems, Foster City, CA), 0.25 µl RNase inhibitor (Applied Biosystems, Foster City, CA), 3.5 µl of Bioline 50 mM MgCl₂, 0.5 µl of Bioline *Taq* DNA polymerase, 5 µl of RNA template and 4.65 µl of molecular grade water to a final volume of 25 µl.

To determine the relative sensitivity of the blotting protocol compared to commercial RNA extraction kits (Bertolini *et al.*, 2008), plant material was collected from Mexican lime trees harbouring populations of CTV

strains GFMS 12 and 12-5. These plants were chosen because a previous TAS-ELISA assay showed that they contained high virus titres, which was needed to produce conclusive results in this experiment. Low titre plants would have led to results having high C_t (threshold) values and thus making the results potentially difficult to interpret. Tissue blots of the collected material were prepared according to the protocol described above. RNA isolated with the SV Total RNA extraction kit (Promega, Madison, WI) was used as a comparison. The Bioline protocol was used to amplify the sample with the two extraction techniques.

To scale up the real-time PCR method, the usefulness of the Roche LightCycler® 480 instrument (Roche Molecular Diagnostics, Mannheim, Germany) was assessed. The use of this instrument would greatly increase the throughput capabilities of the existing protocol since the LightCycler® 480 instrument uses multi-well plates with the capacity to process 384 samples per experiment, compared to the 32 capillary system associated with the LightCycler 1.5 instrument. RNA extractions of GFMS 12, GFMS 35, CRI 1 4/12, Marsh 4/12, Star R 4/12 or blot extracts of viral isolates, 12-9, GFMS 12, 12-5, Croc positive, B389-3, SR12, GFMS 35, B390-3, B389-4, M12-9, SR35, 12-7 and SR1 were analysed. Parallel TAS-ELISA assays were conducted on the same material to compare the sensitivity of the two assays.

The 101 bp (base pair) amplicons, obtained through amplification of GFMS 12 template using the Bioline protocol were cloned in order to produce templates of defined concentration for standard curve production required to make the technique quantitative as described by Bertolini *et al.* (2008). The amplicon was ligated to the p-GEM-T Easy plasmid vector, according to the manufacturer's specifications (Promega, Madison WI). Competent *E. coli* JM 109 cells ($>10^8$ cfu/ μ g) (Promega, Madison WI) were transformed with recombinant plasmid. 2 μ l of the ligation reaction mix were added to 50 μ l of cells. The cells were incubated on ice for 20 minutes, followed by a 42°C heat-shock step for 50 seconds. Heat-shocked cells were incubated on ice for a further 2 minutes. A transformation efficiency control was included by following the same steps as the ligation transformation but adding 10 ng of uncut plasmid to 100 μ l of competent cells. 950 μ l of SOC medium, containing 2 M glucose and 2 M Mg^{2+} , was added to transformation reaction tube. The tubes were incubated at 37°C while shaking for 1.5 hours. 100 μ l of the transformation reaction mix was added to LB (Luria-Bertani) + amp (ampicillin) selective agar plates (Sambrook, 2001), in duplicate. 100 μ l of 100 mM IPTG and 20 μ l of 50 mg/ml X-gal was spread over the surface of each plate prior to spreading the transformation reaction mix. Recombinants were selected through blue/white screening for white colonies (Primrose and Twyman 2007). White colonies presumed to be recombinant were re-plated onto LB + amp agar plates. Real-time PCR, using the Bioline protocol was performed on colony blots of promising clones. Colony blots were prepared by placing a single colony in 100 μ l of molecular grade water followed by incubation at 95°C for 10 minutes. 5 μ l of the colony blot extract was used directly as template in real-time PCR reactions. Sequencing of the cloned inserts was done to confirm the presence of inserts of interest and that the positive results observed in the real-time PCR reactions were not due to non-specific amplifications. Two clones, showing the lowest threshold cross-over (C_t) values in the real-time PCR assay, were selected for sequencing. The colonies were named 3'-2 and 3'-6. Plasmid minipreps from colonies 3'-2 and 3'-6 were prepared by the protocol by Sambrook (2001). Forward and reverse sequencing reactions were conducted using primers specific to the T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') and SP6 (5'-ATT TAG GTG ACA CTA TAG AA-3') promoters, respectively. Each sequencing reaction mix was set up using the BigDye® Terminator mix v3.1 and associated components (Applied Biosystems, Foster City CA), as follows: 1 μ l of sequencing buffer, 1 μ l of primer (3.2 pmol/ μ l), 3 μ l of plasmid template (1 μ g/ μ l), 2 μ l BigDye Terminator mix (ddNTP's) and 3 μ l of molecular grade water to a final reaction volume of 10 μ l. Sequencing cycling conditions were carried as follows: 94°C for 1 minute (denaturation) followed by 25 cycles of 94°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes.

RNA transcripts were prepared to serve as calibration standards in standard curve production. Linearization of the 3'-2 plasmid DNA, isolated using the miniprep protocol (Sambrook, 2001) was carried out using the *Spe I* restriction enzyme. Restriction digest reactions were set up according to the manufacturer's specifications (Promega, Madison WI) and incubated for 4 hours at 37°C. The digestion was stopped by incubation at 65°C for 20 minutes. The concentration of the linearized plasmid was determined using the Nanodrop ND-1000 spectrophotometer (Thermo, Wilmington, DE). Production of RNA transcript through *in vitro* transcription was carried out using a T7 polymerase system (Promega, Madison WI) according to the manufacturer's protocol for synthesis of non-radiolabelled RNA. After the completion of the *in vitro* transcription step, DNase digestion of the plasmid template must be carried out (Bertolini *et al.*, 2008). RQ1 RNase-Free DNase (Promega, Madison, WI) was used and DNase reactions were prepared according to the manufacturer's specifications. Free rNTPs were removed from the *in vitro* transcription product using the protocol described in the Ambion MAXIscript® kit (Ambion, Austin TX) prior to RNA concentration determination using the Nanodrop (Thermo, Wilmington, DE) spectrophotometer. A real-time RT-PCR was done using 1 μ l of the *in vitro* transcription reaction product directly as template to ensure the success of the *in vitro* transcription to produced RNA copies of the target sequence. After *in vitro* transcription and removal

of free rNTPs, multiple Nanodrop readings per sample were taken to determine the RNA concentration. Conversion of μg of ssRNA to pmol was done by using the mathematical formula (Olmos *et al.*, 2005): $\text{pmol of ssRNA}/\mu\text{l} = \mu\text{g (of ssRNA)} \times (10^6 \text{ pg}/1\mu\text{g}) \times (1 \text{ pmol}/340 \text{ pg}) \times (1/N_b)$, where N_b represents the number of bases in the RNA transcript. A tenfold serial dilution series was produced by diluting the stock *in vitro* transcript product into molecular grade water. Dilutions containing $2.04 \times 10^{12} - 2.04 \times 10^6$ were used in triplicate. 1 μl of each of the dilutions was used directly as template. PCR conditions used as the same as those described above. The standard curve was generated using the LightCycler 4.05 software (Roche Molecular Diagnostics, Mannheim, Germany).

Results and discussion

Single Aphid transmissions

To date, 680 single aphid transmissions (SAT's) of the glasshouse maintained GFMS 12 pre-immunizing source plant have been completed. Testing SATs for positive CTV infection proved very difficult because the plants were very small and sampling would be too destructive. Testing with the real-time PCR protocol of Bertolini *et al.* (2008) is the method of choice for testing the plants once they have grown sufficiently, using a more sensitive mastermix (e.g. Roche Taqman kit) for detecting low titer CTV infected plants. To date, there are 22 sub-isolates that are CTV positive. All SATs will be re-tested towards the end of the study.

Cloning of GFMS 12 Citrus pre-immunizing source

The remaining few sequences of clones made from the A and F regions of the GFMS 12 source were completed and phylogenetic analysis completed. The A region clone sequences showed that the predominant variant (67% of the clones) was most similar to a *Poncirus trifoliata* resistance breaking type strain (RB). There was a clear predominance of the RB variant within this population of variants and significant variability within this group of clones suggesting the RB-like variants has undergone divergence. The other 30 % of the clones grouped with the B165 variant and 3% grouped with the VT variant.

Cloning of severe field source (GFMS 12 breakdown)

Collections were made from 5 severely infected CTV plants from orchards in Malelane and Nelspruit areas. Plants selected originally had GFMS 12 pre-immunization but now have severe stem-pitting, decline and small fruit size, signs of cross-protection breakdown in the field. RNA extractions and RT-PCR of the A region of ORF1a were performed on all severe samples. Sample UP@CRI 08-2001 was selected for use in cloning. 110 positive clones of the A region of the severely infected plant (pre-immunized with GFMS12) were selected and amplified. The products were purified and their concentration determined for use in sequencing. 70 Clones were sequenced using the pGEM-T vector primer T7 in one direction.

The SSCP technique was assessed to distinguish sequence variants before sequencing but the method could not be optimised sufficiently to be of use. The SSCP method gave problems in not being able to see the banding patterns clearly and gels often ran in a distorted gradient. Sequencing of clones in one direction was done as an alternative to SSCP.

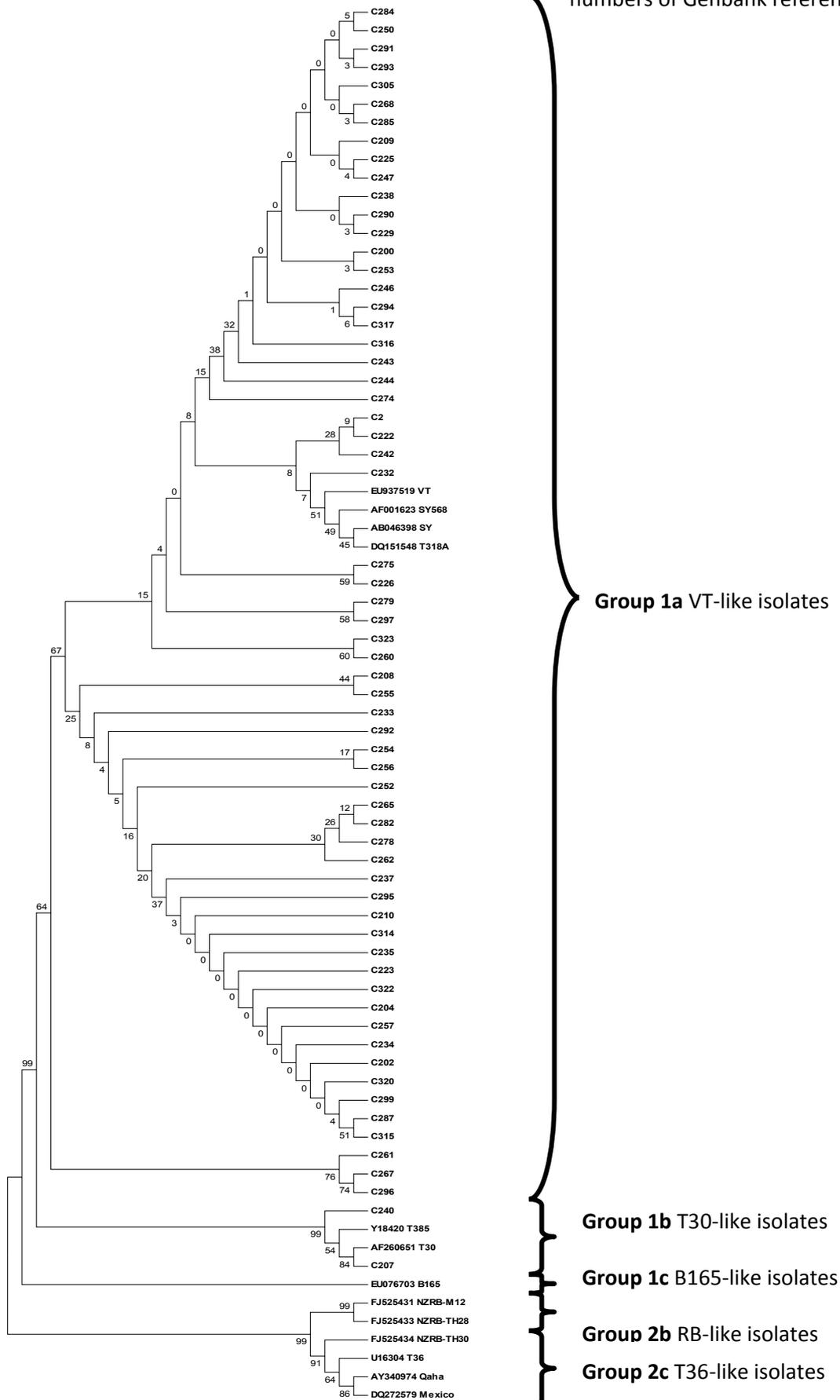
There are two main groups of sequences referred to as group 1 and 2. Group 1 is separated into sub-groups 1a, 1b and 1c. Sub-group 1a consists of the full genome sequences of isolates: VT (EU937519), T318A (DQ151548), Seedling yellows strain (AB046398); SY568 (AF001623) and 61 clones. This sub-group represents VT-like sequences and the clone sequences differ by 0.7–13.7% compared to the index isolate VT (EU937519). The 61 clones represent 97% of the total clones sequenced from this source. This represents the major predominant sequence type in the cloning of this severe source. The VT-like clone sequences within this sub-group have high variability. Sub-group 1b consists of the full genome sequence of isolates T385 (Y18420), T30 (AF260651) and 2 clones. This sub-group represents T30-like sequences. The 2 clones represent 3% of the total clones sequenced in this experiment. Sub-group 1c consists of the full genome sequence of isolate B165 (EU076703) and has no clones grouping with it.

Group 2 is separated into sub-groups 2a and 2b. Sub-group 2a consists of full genome sequences of the resistance-breaking isolates Sub-group 2b consists of full genome sequences of T36-like isolates: There were no clones within group 2. See Neighbourhood-joining tree constructed using Mega 3.1 with a bootstrap of 1000 (Figure 4.2.10.1).

There were not many clones that were similar between the original GFMS 12 source and the severe (pre-immunised GFMS 12) source. Only 2 GFMS 12 clones had more similarities to the clones of the severe source. There seems to be a general trend that the sequence variants found within the severe source introduced from the field have overridden the pre-immunizing sequence variants found within GFMS 12 or that the specific cultivar/environment has selected for differing sequence types to predominate under those set dynamics.

Four sub-isolates (12-7, 12-5, 12-1-2, 12-8) of GFMS 12 were identical or very similar to clones from the GFMS 12 source and not to clones derived from the field severe CTV source (pre-immunised with GFMS12) except for sub-isolates 12-9, 12-1 and 12-4 (See section below).

Fig 4.2.9.1. NJ tree of region A constructed using Mega 3 of Severe source clones. The accession numbers of Genbank reference isolates are included.



Characterization of GFMS12 pre-immunizing source sub-isolates

The A regions and p23 gene of sub-isolates 12-1, 12-3, 12-4, 12-5, 12-6, 12-7, 12-8, 12-9 (original sub-isolates from GFMS 12, supplied by S.P. van Vuuren) have been amplified, purified and sequenced. The

coat protein (CP) and F region of sub-isolates 12-1, 12-4, 12-6, 12-1-2 12-5, 12-7, 12-9 have been amplified and sequenced.

Sequences from the A-region suggest sub-isolates 12-4 and 12-1 are identical and similar to 12-9, which are all VT-like variants. Sub-isolate 12-8 was unique and a B165-like variant. Sub-isolate 12-1-2, 12-5 and 12-7 were similar to the resistance breaking variants (refer to Figure 4.2.10.2). A representative sub-isolate from the VT, B165 and RB groups were selected for use in a biological trial on strain dynamics.

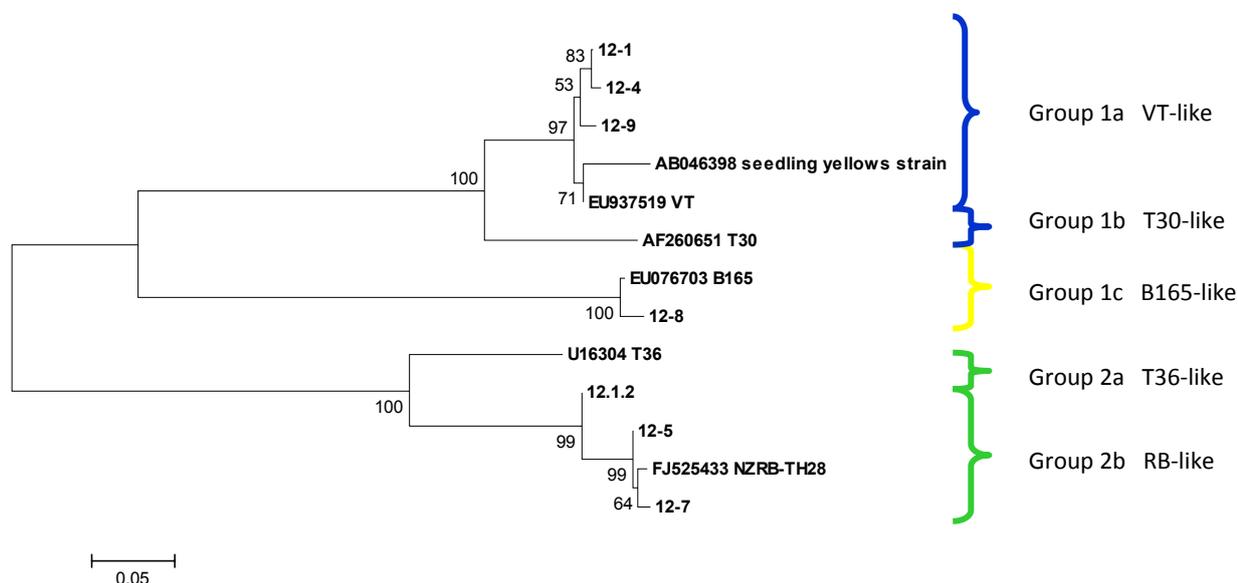


Fig 4.2.10.2. NJ tree of region A constructed using Mega 3 of GFMS 12 sub-isolates. The accession numbers of Genbank reference isolates are included.

PCR systems:

Experiment to determine the effect of temperature on CTV strain dynamics.

Only 3 bark chips did not take and these plants have been noted. The experiment continues as planned.

Development of Real-time PCR protocol

RNA extracted from plants harbouring pre-immunising mild-strain CTV isolates was successfully amplified, showing C_t values of below 40 when the real-time RT-PCR protocol of Bertolini *et al.* (2008) was done exactly as published using Applied Biosystems TaqMan® Universal Mastermix reagents. As it is envisaged that, amongst other uses, the protocol be used as a routine detection method for CTV, potentially resulting in the analysis of large numbers of samples, it would be desirable for the per reaction costs to be as low as possible, without compromising the sensitivity of the test. Biorline *Taq* polymerase (Biorline, London, UK) and its associated reagents, used in conventional PCR assays in our laboratory, was tested for its potential to replace the more expensive Applied Biosystems TaqMan® Universal Mastermix (AB Mastermix). The per reaction costs when using the Biorline *Taq* polymerase are approximately half those of the AB Mastermix. The Biorline *Taq* polymerase also proved to have greater efficacy in CTV detection, with much higher resultant fluorescence values and lower C_t values (Fig 4.2.10.3). While it is possible that the specific batch of AB Mastermix used was faulty, the efficacy of the Biorline *Taq* proved acceptable and it was selected as the reagent of choice for most further experiments, without re-testing a different batch of AB Mastermix.

When using tissue blotting for sample preparation, C_t values below 45 were obtained (Fig 4.2.10.4), indicating successful detection of CTV with either the AB mastermix or Biorline *Taq* polymerase, and shows that it can be used as an alternative to kit extractions of total RNA from infected plants. This makes the technique more useful as a routine diagnostic method because the cost of sample analysis can be reduced since RNA extraction kits are not required for blotting and increased sample throughput can be achieved as the blotting protocol is much less time consuming than RNA kit extraction protocols. However, Bertolini *et al.* (2008) described a loss of sensitivity of approximately 100 times when tissue-blot RNA extracts were used instead of commercial RNA extraction kits. To confirm this loss of sensitivity the two sample preparation protocols were compared. The C_t values for CTV GFMS 12 were ~14 and ~23 for the RNA extraction kit and blotting, respectively, and CTV 12-5 had C_t values of ~15 and ~22 for the RNA extraction kit and blotting, respectively. However this loss of sensitivity is probably acceptable because of the added advantages of a

significant reduction in costs and an increase in the potential throughput of samples and the fact that the technique remains more sensitive than the ELISA currently used (see below). Tissue-blot extractions have the added advantage when used in quantitative real-time PCR assays that the dRNA (defective RNA) and subgenomic RNA components of CTV (Ruiz-Ruiz *et al.*, 2006) are degraded by RNases present in the plant extracts and only gRNA (genomic RNA) copies are detected. This allows for more accurate absolute quantification of CTV within a given sample (Bertolini *et al.*, 2008).

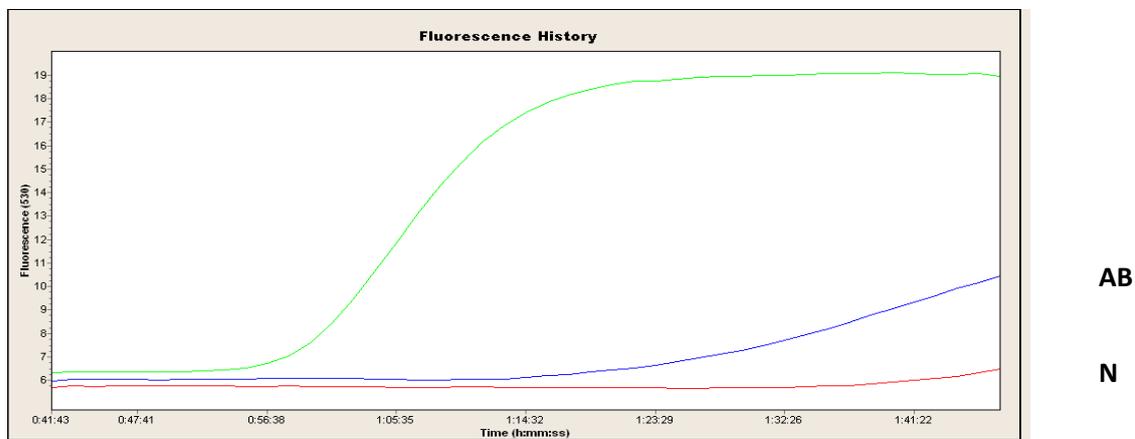


Figure 4.2.10.3. A comparison between the efficacy of the AB Mastermix and the Bioline mastermix. BL, AB and N represent the amplification curves of the samples using the Bioline mastermix, the Applied Biosystems mastermix and the negative control respectively. From this figure, it was determined that the efficacy of the Bioline mastermix is greater than the Applied Biosystems mastermix. The slight amplification in the negative control was probably due to a small contamination during mastermix preparation.

The CTV titres of eight Mexican lime trees (*Citrus aurantifolia*) were determined using TAS-ELISA to correlate the ELISA results with those obtained by real-time RT-PCR using tissue blotting and to determine the relative sensitivity of the tests. The photometric data obtained in the TAS-ELISA experiment is shown in Figure 4.2.10.5 and suggest that plants 08-0011 (harbouring CTV strain 389-3) and 08-0068 (harbouring CTV strain T30) were CTV free by TAS-ELISA. RNA from plants 08-0011 and 08-0068 was extracted using tissue blotting and used as template in a real-time RT-PCR assay and determined that they do in fact harbour CTV, since amplification curves of the tissue-blotting template with C_t values of less than 40 were obtained (Fig. 4.2.10.6). Therefore, the real-time RT-PCR assay, using Bioline (London, UK) protocol and coupled to tissue-blot extractions, was more sensitive than the TAS-ELISA assay because it was able to amplify CTV template from plants that were considered CTV free when using the TAS-ELISA assay. Bertolini *et al.* (2008) compared the sensitivities of DAS- (double antibody sandwich) ELISA and real-time RT-PCR for CTV detection by using a serial dilution of a CTV infected sweet orange viral extract into healthy plant total RNA. They demonstrated that the DAS-ELISA assay was able to detect virus to a dilution of $1:10^3$, while the real-time RT-PCR assay was able to detect dilutions of up to $1:10^9$. Therefore, depending on the reagents used, the real-time RT-PCR assay of Bertolini *et al.* (2008) can be up to 10^6 times more sensitive than an equivalent ELISA assay.

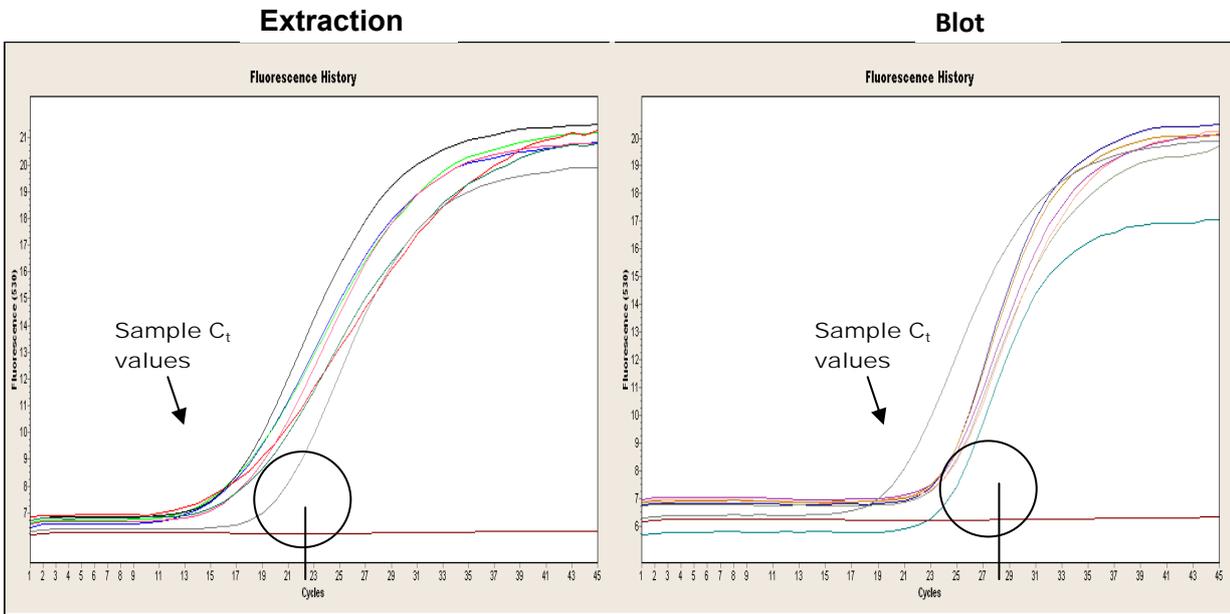


Figure 4.2.10.4. Comparison of the sensitivity of real-time RT-PCR experiments using commercial RNA kit and tissue-blot extractions. The images are the amplification curves produced when RNA from a kit extraction and tissue blotting respectively were used as template. The same material was used to produce the tissue blot and commercial kit extractions. The region where sample C_t values can be found is illustrated on the figure by circles. The vertical lines extending down to the x axes show the approximate mean C_t values for the samples tested. The mean C_t values for the extraction and blot experiments were 17 and 23, respectively.

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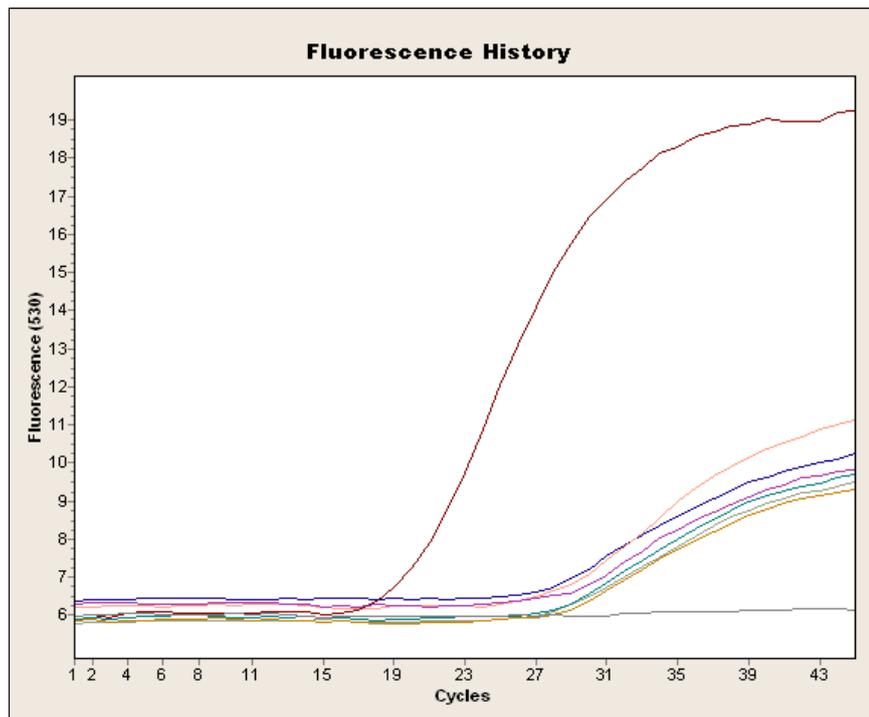
09 May 2008 11:24:15                               zELISA2005                               Page 1
Instrument      : Multiskan Ascent V1.24 354-00973T
Software version : Version 1.3.1
Measurement time : 09 May 2008 11:23:51
Type of plate   : 96
Measurement mode : Stepping
Measurement type : End point
Measurement filter : 405 nm
Reference filter : 492 nm
Start temperature : 30.0 C

PROCESSED DELTA ABSORBANCE DATA MATRIX

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	1	2	3	4	5	6	7	8	9	10	11	12
A	0.011	0.009	0.011	0.010	0.008	0.009	0.008	0.009	0.008	0.008	0.009	0.009
B	0.008	1.040	0.432	0.399	1.498	1.406	0.333	0.348	0.352	0.318	0.336	0.009
C	0.009	1.021	0.392	0.434	1.455	1.325	0.316	0.345	0.357	0.297	0.330	0.009
D	0.010	0.902	0.367	0.382	1.192	1.233	0.296	0.281	0.296	0.244	0.332	0.008
E	0.009	0.339	0.847	0.907	1.204	0.355	0.276	0.297	0.301	0.397	0.373	0.011
F	0.010	0.364	0.806	0.927	1.129	0.331	0.310	0.301	0.296	0.367	0.351	0.009
G	0.009	0.364	0.832	0.958	1.160	0.354	0.275	0.283	0.305	0.403	0.333	0.009
H	0.009	0.009	0.017	0.010	0.008	0.009	0.008	0.009	0.009	0.009	0.009	0.010

Figure 4.2.10.5. Photometric data for TAS-ELISA. Samples were analysed in triplicate. The co-ordinate values of this data set correlate to the following plant accession numbers: 2B-D → 08-0026 (positive control), 2E-G → Virus free, 3B-D → 08-0011, 3E-G → 08-0005, 4B-D → 08-0068, 4E-G → 08-0022, 5B-D → 08-0009, 5E-G → 08-0024 and 6B-D → 08-0039. The remainder of the data set was not relevant to this study. The values of 08-0011 and 08-0068 are in a similar range to that of the virus free samples, suggesting that these two plants are CTV free.



P

Blotted samples
of 08-0011 and
08-0068

N

Figure 4.2.10.6. Amplification curves of a real-time PCR experiment, using RNA extracted from plants 08-0011 and 08-0068, using tissue blotting. The curve labelled P is the positive control, which used RNA from GFMS 12 extracted using the Promega SV Total RNA extraction kit. N is the curve representing the no template negative control. A previous TAS-ELISA assay suggested that these plants were CTV free. It was determined that these plants are in fact harbouring strains of CTV, since C_t values of less than 40 were obtained when amplifying the RNA obtained from tissue-blot.

A sensitivity analysis performed by Saponari *et al.* (2007) demonstrated their real-time RT-PCR assay was 10^3 times more sensitive than a standard ELISA protocol. Real-time RT-PCR has gained popularity as a detection technique in plant virology, which is able to detect virus from field samples when the titres are too low to be detected by serological assays (Saponari *et al.*, 2007). The specificity of serological assays may also suffer from a loss of specificity when CTV isolates from various geographic and biological origins are analysed because of coat protein variability between isolates, which may not be detected by certain specific monoclonal antibodies (Saponari *et al.*, 2007). Real-time RT-PCR detection suffers less from the effects of geographic and biological variability and assays can be made even more tolerant by introducing degeneracy amongst primer pairs and probes (Saponari *et al.*, 2007).

Use of the LightCycler® 480 instrument (Roche Molecular Diagnostics, Mannheim, Germany) offers the advantage of high sample throughput by using the 384-well plates and halved reaction costs, as the cost of the plates versus the capillaries used by the LightCycler 1.5 instrument is lower. In an assay where GFMS 12, GFMS 35, CRI 1 4/12, Marsh 4/12 and Star R 4/12 RNA extractions were used as templates, a number of samples yielded amplification curves proving that it would be possible transfer the currently used protocol to the LightCycler® 480. However, the positive control samples remained negative in the real-time RT-PCR assay, which was run in parallel with a TAS-ELISA assay (data not shown). This rendered the experiment a failure, which may have been due to an enzyme or reagent component that was not functioning optimally, such as the reverse transcriptase. The experiment was not repeated because of the many disadvantages experienced with the use of the LightCycler® 480 instrument. These include the facts that 1) reagent and template loading into the 384 well plate can be very confusing, 2) the inherent design of the plate lends itself to extensive potential cross-over contamination problems due to template aerosols, 3) difficulties in keeping the plate with loaded reagent mix cool during preparation, which could result in enzyme damage, 4) difficulty in data analysis of a fully loaded 384-well plate and 5) the need to use a whole 384-well plate even if only a few samples need testing. Furthermore, the LightCycler® 1.5 instrument does not suffer from these disadvantages but has the following advantages over the LightCycler® 480 instrument, such as the facts that capping of each capillary after template addition reduces carry-over contamination risk enormously, less samples per run means more manageable data analyses and capillary set-up leads to better reproducibility of replicates. Automated dispensing of reagents into the LightCycler® 480 Multiwell plates (Roche Molecular Diagnostics, Mannheim, Germany) may alleviate many of the problems experienced. However, this type of facility is not available to our laboratory. For the purposes of this study and the development of the routine protocol as a whole, the capabilities of the LightCycler® 1.5 instrument are sufficient. The samples tested by

the Southern African Citrus Improvement Scheme are in the range of 200-300 per year and the throughput capacity of the LightCycler® 1.5 is adequate for this volume of analysis.

The advent of real-time PCR analysis has provided diagnostic laboratories with the ability to quantify target nucleic acids and has subsequently become an important part of any quality real-time PCR assay (Mackay *et al.*, 2002). There is currently two ways of performing quantification using real-time PCR assays, namely relative and absolute quantification. Relative quantification is less laborious and is easier to develop and involves measuring the changes in the amount of a target sequence and compares this with the changes in amount of a reference target (Mackay *et al.*, 2002). Absolute quantification is technically more demanding and involves determining the exact number of nucleic acid targets present in a sample (Mackay *et al.*, 2002). Absolute quantification of CTV gRNA targets in a sample requires the cloning of and subsequent *in vitro* transcription a specific region of the gRNA (Ruiz-Ruiz *et al.*, 2007, Saponari *et al.*, 2007). The *in vitro* transcript is then serially diluted and used as a template in a real-time RT-PCR experiment (Bertolini *et al.*, 2008).

Cloning of the PCR amplicon was done to create a DNA template from which *in vitro* transcription could be carried out, for the purposes of viral quantification. Ligation of the fragment to the plasmid vector and the transformation of *E. coli* JM 109 carried out according to the protocol described above. Ten putative recombinant colonies were selected through blue/white screening and re-plated. When the re-plated colonies were analysed using the colony blot assay, all samples showed C_t values of less than 40 when amplified with primers 3'UTR1 and 3'UTR2 and it was deduced that the putatively recombinant colonies harboured plasmids containing the sequence of interest. Sequence data recovered for the plasmid of clone 3'-6 was not useable. However, the expected 101 bp of the sequence of interest was recovered from the 3'-2 sequence data, which was then used in a BLAST (Basic Local Alignment Search Tool) search and showed 100% homology with three other CTV sequences within GenBank (accession numbers AY170468.1, AY340974.1 and AF260651.1). Plasmid harboured by clone 3'-2 was therefore chosen for use in subsequent *in vitro* transcription experiments. RNA produced in the *in vitro* transcription experiment yielded C_t values below 8 when used in a real-time RT-PCR experiment, demonstrating that the *in vitro* transcription experiment successfully produced full-length RNA copies of the cloned insert. The reverse transcriptase lacking control showed no amplification and demonstrated that no DNA template was present in the *in vitro* transcription product present after DNase digestion. Presence of any DNA template would skew the data produced during standard curve production (Saponari *et al.*, 2007).

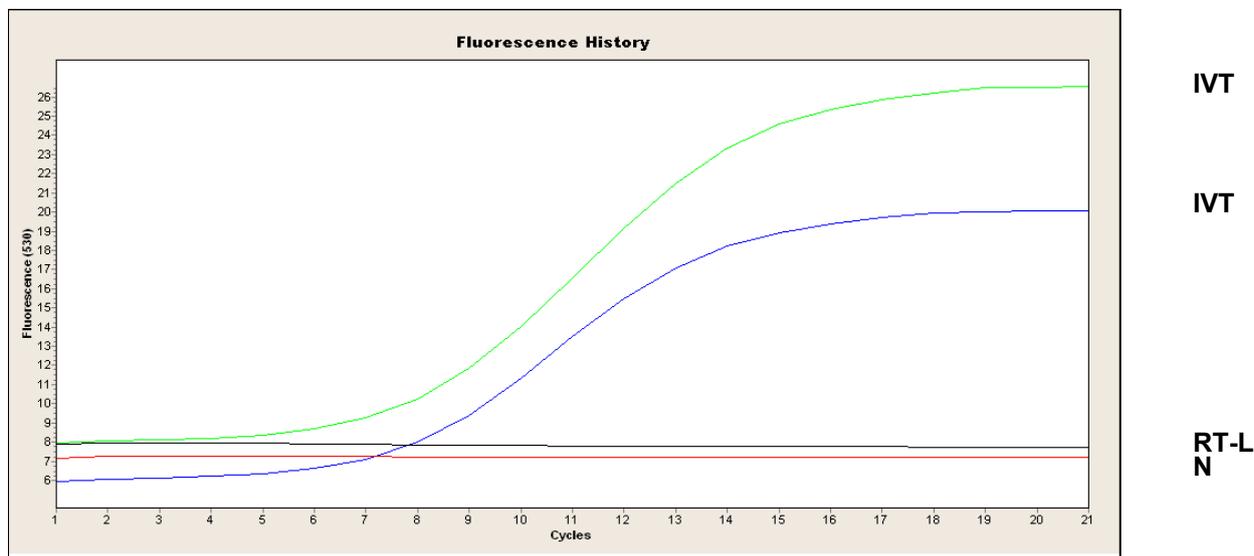


Figure 4.2.10.7. Amplification curves of a real-time RT-PCR experiment, using RNA produced in the *in vitro* transcription of the recombinant 3'-2 plasmid, containing the sequence of the CTV amplicon. Curves labelled IVT are replicate samples, which used RNA produced by the *in vitro* transcription experiment. The amplification of the transcript shown in the figure demonstrates that the *in vitro* transcription of the recombinant plasmid was successful. The curve labelled RT-L represents the control, which lacked reverse transcriptase. The fact that no amplification occurred in this control demonstrates that no residual DNA template was present in the *in vitro* transcript. The curve labelled N represents the no template negative control.

A standard curve is prepared in order to calculate the number of gRNA copies in different samples (Ruiz-Ruiz *et al.*, 2007). The current study has yielded reliable data, in terms of C_t values, for a transcript number

as low as 2.04×10^7 . The inability to amplify transcript number greater than these numbers is probably due to the inefficiency of the Bionline *Taq* polymerase. Further experiments, using more efficient reagents would be able to expand the range of template concentrations that this assay is able to detect. However, the Bionline protocol is a compromise between a loss of sensitivity and financial feasibility and the standard curve that has been produced using this protocol will serve the purposes of this study. Other studies have shown the ability to detect dynamic ranges of for template concentrations for the purposes of standard curve production for CTV quantification. Bertolini *et al.* (2008) and Ruiz-Ruiz *et al.* (2007) obtained C_t value data for template numbers as low as 1.7×10^2 and 1.0×10^3 , respectively.

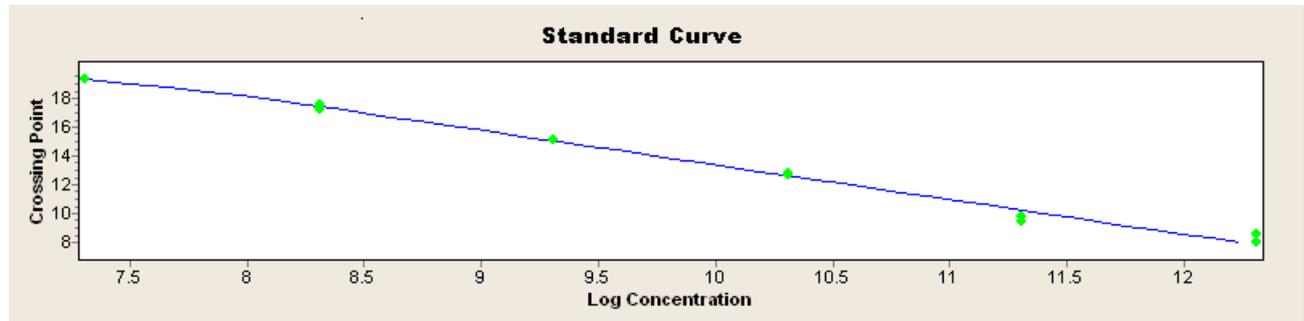


Figure 4.2.10.8. Standard curve for CTV quantification produced using a 10-fold serial dilution of the in vitro RNA transcripts as template. The curve will allow for the absolute quantification of CTV genomic RNA copies in any plant sample being tested. The curve was obtained by plotting the crossing point values (C_t) against the logarithm of the number of starting RNA transcripts. The C_t values plotted are for each of dilutions used and are the means of three sample replicates.

In order to make the real-time PCR specifically useful to the experiment in which the effect of temperature on the dynamics and interactions of CTV strains will be studied, the following aspects were also addressed:

1. Blotting was optimised: CTV samples were blotted onto nitrocellulose paper as described by Bertolini *et al.* (2008), but modifications made include: (1) using only 5 leaf petioles, cutting each petiole twice and blotting; (2) using 3 punches of each blot with a Harris micro-punch (Sigma); and (3) eluting in 75 μ l blot extraction buffer (Bertolini *et al.*, 2008). The RNA concentration of all elutes was checked using a Nanodrop spectrophotometer.
2. A two step procedure of a separate reverse transcriptase and amplification step was assessed. 12 μ l of the blot extract is used to make cDNA using the A-reverse primer as described by Herron *et al.* (2005). In the PCR, 2 μ l of cDNA was used, together with 4 μ l TaqMan mastermix (Roche) and the PCR conditions performed as described by Rubio *et al.* (2001). By using more than 2 μ l of cDNA there were problems with PCR inhibition.
3. A control PCR was established allowing standardisation of RNA extracts during quantitative real-time PCR analyses using a plant RNA specific control (Osman *et al.*, 2007). The primers and probe are based on the 18S rRNA of several plant species. Citrus CTV samples were blotted and eluted (Bertolini *et al.*, 2008). The C_t value of the internal control of the different samples (with replicates) ranged from around 12-14. This test will be used to validate if there are any false negatives and to ensure that the starting RNA from the plant is within the acceptable range preventing skewed/bias data.
4. Hydrolysis probe were designed to differentially detect the strains used in the temperature dependence experiment: A few probe options were designed for each of the 3 strain variants and will be checked and verified. Design parameters taken into account were: (1) the 5' nucleotide being other than a guanosine (G); (2) probe T_m of 8-10 degrees higher than the primers; (3) no dimer formations; (4) no repeats at the end of the probe; (5) fewer G's than C's in the probe; (6) the forward PCR primer and probe should be as close as possible to the probe.

Conclusion

Cloning of the severe CTV source (pre-immunized with GFMS 12) showed a predominance of the VT strain, whereas the GFMS 12 clones had a predominance of a resistance strain (RB). It is possible that the VT strain could have been introduced from the field and has overridden the other strains or the VT strain inherent to GFMS 12 has become dominant in this cultivar, and under those field conditions and possibly caused cross-protection breakdown. Sub-isolates of GFMS 12 consist of 3 strain types, which were selected for use in a biological trial. The strains were inoculated onto Marsh and Star Ruby grapefruit plants and kept

at 4 different temperature conditions. A quantitative real-time PCR has been developed to detect the 3 strains, but will need further optimizing.

This study has led to the successful implementation of a published real-time RT-PCR assay for the detection and quantification of CTV. The protocol has been slightly modified from that of the published article to accommodate the use of less expensive reagents, which adds to the attractiveness of the assay. When compared with ELISA, the assay has been shown to have greater sensitivity as well as being much more rapid and also has the added advantage of being quantitative. For these reasons, the implemented real-time RT-PCR assay has great potential for the replacement of ELISA as the method of CTV detection within the CIP certification program.

Further objectives and work plan

1. Fully sequence selected isolates from (June – Dec 2009).
2. Cloning sub-isolates to check purity and produce a standard curve (April-June 2009)
3. Analyse competition of severe and mild isolates obtained by inoculation onto Marsh and Star Ruby grapefruit hosts in different combinations, time of inoculation and challenge and under different temperature regimes, Monitor CTV strain concentration and distribution within the plant using quantitative PCR (June 2009 - March 2010).
4. Biological indexing of sub-isolates (June – March 2010)
5. A further assessment on whether grapefruit mild strain cross protection breakdown is due to “super-infection” by severe strains will be conducted using GFMS35 pre-immunizing strain too, and field-evaluated samples with mild and severe symptoms. Samples will be taken from the ARC experimental block Malelane H7 from plants 1A (GFMS 12 on Star Ruby), 1B (GFMS 12 on Flame), 5A (GFMS 35 on Star Ruby) and 5B (GFMS 35 on Flame), and analysed with regards to the viral populations present. CTV strains in glasshouse maintained GFMS 35 population will also be characterised and compared to the field samples (Jan 2009 to Dec. 2010)

Technology transfer

Scott, K.A. and Pietersen, G., 2009. Strain differentiation by PCR and DNA microarray of Citrus Tristeza virus (CTV) isolates in South Africa. 46th congress of the South African Society for Plant Pathology, 25-28 January, 2009 Gordons Bay, South Africa.

Scott, K.A., and Pietersen, G., 2008. Strain differentiation of CTV isolates by PCR and DNA Microarray in South Africa. 5th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 3-6 August, 2008.

Read, D., Scott, K.A., and Pietersen, G., 2008. Detection of Citrus tristeza virus using real-time RT-PCR. 5th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 3-6 August, 2008.

Van der Westhuizen, L., Scott, K.A., and Pietersen, G., 2008. Sequence characterization of selected genes in four Citrus tristeza virus (CTV) mild sources potentially useful in mild strain protection of soft citrus cultivars. 5th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 3-6 August, 2008.

An article was submitted to “Virus Research” journal.

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4.2.11 PROGRESS REPORT: Recovering and evaluation of embryo rescued plants for genetic greening resistance

Experiment 815 (2006 - 2015) by S.P. van Vuuren (CRI)

Summary

Attempts are being made to obtain greening resistance by rescuing embryos derived from healthy chimeras on greening-infected fruit and growing them on artificial medium. The plantlets that are generated are micro-grafted on vigorous rootstocks. These clones are multiplied on healthy rootstocks and exposed to field psylla. After the insects have fed for 7 days on the plants, they are removed and tested by polymerase chain reaction (PCR) for "*Candidatus Liberibacter africanus*" (Laf), the organism responsible for greening in South Africa, to establish if they were infectious. After three months, the plants are also evaluated for the presence of greening by visual inspections and tested by PCR for Laf. Symptomless plants, exposed to positive psylla insects, and free of Laf, may suggest that the plants are resistant or, if they contain Laf without symptom expression they may be tolerant. Two clones, GTC-E2 and GTC-T2 were still asymptomatic in 2006 after exposure to the vector. PCR confirmed that they were free of Laf. Various batches of psylla that were used for challenging the plants were also confirmed infected with Laf. These two clones have been multiplied on virus-free rootstocks and pre-immunised separately with two CTV sources whereafter they were planted in an orchard for field evaluations. Four clones that were obtained in 2007 were multiplied on rootstocks for exposure to the vector. Due to low populations of psylla insects, challenges were only partly done. No new clones were generated.

Opsomming

Daar word gepoog om vergroening weerstandbiedendheid te verkry deur embryo's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Die plantjies wat genereer word, word op groeikragtige onderstamme deur middel van mikro-enting gevestig. Hierdie klone word op onderstamme vermeerder, en aan boord versamelde sitrus bladvlooië, die vektor van vergroeningsiekte, blootgestel. Nadat die insekte vir 'n week op die plante gevoed het, word hulle verwyder en deur middel van polimerase ketting reaksie (PKR) getoets om te bevestig dat hulle met "*Candidatus Liberibacter africanus*" (Laf), die oorsaak van vergroening, besmet was, en sodoende dat die plantjies blootgestel is aan Laf. Na 3 maande word die plante ge-evalueer vir die voorkoms van vergroeningsimptome, en getoets vir die teenwoordigheid van Laf d.m.v. PKR. Daar word sodoende bepaal of simptoomblose plante wat aan positiewe sitrus bladvlooië blootgestel was, vry is van Laf (weerstandbiedend) en of Laf teenwoordig is sonder dat simptome ontstaan (verdraagsaamheid of toleransie). Twee klone, GTC-E2 en GTC-T2, is in 2006 as simptoombloos na blootstelling aan die vektor geïdentifiseer. PKR het getoon dat hulle vry van Laf is. Dit is ook deur PKR bevestig dat die sitrus bladvlooië wat vir oordraging deur Laf gebruik is, besmet was. Die twee klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* isolate ge-preïmmuniseer en is in die boord vir verdere evaluasie uitgeplant. Vier klone wat in 2007 verkry is, is op onderstamme vermeerder vir blootstelling aan sitrus bladvlooië. As gevolg van lae bevolkings van die insek vektor is blootstellings net gedeeltelik gedoen. Geen nuwe klone is gegeneer nie.

Introduction

Huanglongbing (HLB) (syn. greening disease) in South Africa, caused by the gram-negative bacterium "*Candidatus Liberibacter africanus*" (Laf) (Garnier *et al.*, 2000), remains the most destructive disease in the cooler production areas of South Africa. The disease has occurred in the country since 1928 (Oberholzer *et al.*, 1965) and was first thought to be caused by a toxicity (van der Merwe & Andersen, 1937). In 1973, it was shown that greening in South Africa was caused by a gram-negative bacterium (Moll & Martin, 1973). Crop losses of 30 to 100% were recorded in some areas due to the lack of marketability of infected fruit as well as fruit falling prematurely (Schwarz, 1968). During this time HLB was not observed in the Eastern Cape province but occurred sporadically in the Western Cape province despite the presence of the psylla insect vector, *Trioza erytrae* (Del G) (Oberholzer *et al.*, 1965; Schwarz, 1968). In the Western Cape, the origin of infected trees could be traced to nurseries in infected areas in the northern provinces (Schwarz, 1968). Subsequently the movement of citrus material from infected areas to uninfected areas in South Africa was prohibited. Despite this, and the present control measures of using certified healthy planting material, insect control by systemic insecticides and the eradication of infected plant material (Buitendag & von Broembsen, 1993), the disease was detected in the Western Cape in 1995 (Garnier *et al.*, 2000) and is still invading formerly HLB-free areas (Pretorius *et al.*, 2006). The ultimate control measure will be the use of resistant plant material.

Attempts in South Africa to obtain greening resistant citrus cultivars by conventional breeding were unsuccessful (de Lange *et al.*, 1985). The main obstacle being the absence of proven sweet orange (*Citrus sinensis* (L.) Osb.), mandarin (*C. reticulata*), grapefruit (*C. paradisi* Macf.) or lemon (*C. limon* (L.) Burm.f.)

resistance to greening. Thousands of seedlings with pollen parents of Palestine sweet lime (*C. aurantifolia* (Chrism.) Swing), citron (*C. medica* L.), shaddock (*C. grandis* (L.) Osb.) and sour orange (*C. aurantium* L.) were planted among greening infected trees and none showed any resistance or tolerance. In another attempt, clones were collected by Dr. C.H. Buitendag from “healthy” twigs growing out from infected branches in heavily infected orchards. None of these showed tolerance or resistance in subsequent field evaluations (unpublished data, Citrus Research International, 2 Baker Street, Nelspruit 1200).

Chimera development on citrus fruit is a genetic disorder and occurs quite often. Some cultivars are more prone to this disorder than others. Greening affected fruit with chimeras are observed on a regular basis on diseased branches. Affected fruit often display “healthy looking” sectors in contrast to the affected part of the fruit. Seeds can be removed aseptically and regenerated on artificial medium. Possibly resistant plants may be generated from the “abortive” and normal seeds in these healthy fruit sections by means of embryo rescue. Artificial challenging of regenerated plants with Laf by means of the psylla insect vector *T. erytrae*, and using molecular techniques (Hocquellet *et al.*, 1999; Irey *et al.*, 2006; Li *et al.*, 2007) for evaluation after challenges, may prove a rapid approach to identify truly resistant or tolerant clones.

The objective of this study was to recover embryos from healthy chimeras on Laf infected fruit and to screen the recovered clones for genetic resistance or tolerance to greening before planting promising clones in the field for evaluation under commercial conditions.

Materials and methods

Embryo rescue

‘Boschhoek’ navel and ‘Olinda’ Valencia (*Citrus sinensis* L. Osb.) fruit with greening symptoms, displaying healthy chimeras, were collected in two orchards at harvest during winter. Wide healthy chimeras (>30°) were preferred as this should enhance the chances of obtaining ovules for embryo rescue in the healthy part. Each fruit was surface sterilised in the laboratory on a flow bench by dipping for 20 min in a 0.5% sodium hypochlorite solution containing 0.1% Tween-20. “Abortive” and healthy seeds were dissected aseptically from the healthy sectors of diseased fruit and cultured on modified Murashige & Tucker (M&T) (1969) medium containing 500 mg per l malt extract. As a control, a culture was also made from seeds from a segment of the symptomatic part of the fruit. Cultures were allowed to develop for four weeks in continuous dark at 30°C and then were transferred to a growth room at 28°C and with 18 h light.

Establishing clones of embryo rescued plants

When shoots of a clone had developed to 1 to 2 cm on the artificial medium, they are micro-grafted to healthy rough lemon (*C. jambhiri* Lush) rootstocks in the greenhouse. When the grafts had developed approximately 15 to 20 mature buds, each clone is multiplied for challenging with Laf via the psylla insect vector.

Artificial challenge with Laf by the insect vector

After the multiplication buds of 10 replications per clone have grown for 1 to 2 cm, the clones were challenged with Laf by means of psylla insects collected in infected orchards. The plants of four clones as well as that of the control ‘Ponkan’ (*C. reticulata*) mandarin (Table 4.2.11.1) were exposed to psylla insects caught during September and November 2008. Five insects were confined in a small plastic cage on the young shoot of each clone for seven days (van Vuuren & van der Merwe, 1992). All psylla insects, alive and dead, were recovered from the challenged plants after the feeding period and stored separately at -20°C until PCR was done. After removal of the insects, the plants of each clone were sprayed with a suitable insecticide to kill all psylla eggs. They were then transferred to a greenhouse at 26°C and 18 h light for three months. During this time greening symptom development was monitored and then PCR was done to confirm Laf infection.

PCR

DNA isolation and detection of Laf in plants: Leaf petioles were collected and the Wizard miniprep DNA purification kit (Promega) was used to obtain total DNA from infected plants for PCR amplification (Jagouix *et al.*, 1996). PCR amplification of part of the β ribosomal protein operon was carried out with *Liberibacter*-specific primers A2J5 (Hocquellet *et al.*, 2000) with the following programme: Initial denaturation at 94°C for 60 s, 35 cycles of 92°C for 20 s, 62°C for 20 s, 72°C for 40 s, extending the extension time by 2 s per cycle, and a final 5 min at 72°C, using Taq DNA polymerase and buffer from Promega.

DNA isolation detection of Laf in psylla: Psylla insects collected from each plant as alive or dead were processed separately. In initial validation trials, PCR using the above protocol on psylla insects produced no samples positive for Laf. An improved extraction and hybridisation protocol was developed as follows, and used for all psylla insect assessment. One to five psylla were placed in a 500 μ l eppendorf tube and

squashed with a sterile pipette tip after which 50 µl 5X SSPE buffer (0.9 M NaCl, 50 mM NaH₂PO₄, 5 mM EDTA, pH 7.4) was added. An equal volume of phenol:chloroform:isoamylalcohol (25:24:1) was added to the extract and vortexed for 1 min. The layers were separated by centrifugation for 5 min and the supernatant transferred to a new tube. The DNA was denatured for 10 min in a boiling bath and chilled for 5 min on ice. The amplicon obtained from infected plants was used to prepare a non-radioactive probe by PCR using the DIG labelling and detection kit (Roche Molecular Biochemicals, Germany). The direct PCR of psylla DNA was conducted as described above.

Field trial for natural challenge

Clones that showed potential resistance or tolerance to Laf were multiplied on virus-free rough lemon rootstocks in replicates of 10. As a control, virus-free 'Midnight' Valencia was used as the standard commercial cultivar. Five replicates of the virus-free clones as well as the control were pre-immunised with the standard LMS 6 *Citrus tristeza virus* (CTV) source (Van Vuuren *et al.*, 2000) and the other five with CTV source SM 49 [GX1] (Van Vuuren *et al.*, 2000) previously reported to give some protection against greening (Table 4.2.12.2). ELISA was done on all the plants before they were planted in the field in a greening area in a randomised block design. Normal orchard practices are being followed to control psylla and Laf infection will be monitored on a regular basis and confirmed by PCR.

Results and discussion

Embryo rescue

Nine 'Boschhoek' navel fruit with suitable chimeras were found of which a large number of small embryos from healthy and diseased sectors were transferred to artificial medium. None developed callus or shoots. Eleven 'Olinda' Valencia fruit with suitable chimeras were found. Embryos were transferred to M&T medium from the healthy chimeras as well as the opposite greening symptomatic side as controls. Callus formation was good from 15 embryos but shoot development was poor. No shoots were micro-grafted yet.

Artificial challenge of embryo rescued plants with Laf by the insect vector

The clones that were generated during 2007 (O/03/30-RA2, O/03/30-GB, OC4/10-RA, OC4/11-RA, OC4/15-RA2) were multiplied to 10 replications for the Liberibacter challenge. Low populations of the vector occurred but 42 challenges were done during September and October 2008. The clones that were challenged are listed in Table 4.2.11.1. As soon as insects are available, challenges will continue.

Greening leaf symptoms of plants that were challenged during 2008.

Yellow veins and mottling started to develop on the leaves of 2 plants 8 weeks after the challenge with Laf using psylla. Assessment of infection by symptom expression of plants was terminated 3 months after the challenges.

Detection of Laf in plants and psylla.

Laf was detected by PCR in Wizard extracts from 2 plants infected with Laf and agreed with the presence of leaf symptoms (Table 4.2.11.1). Six batches (1 to 5) of psylla out of 42 tested positive by PCR (Table 4.2.11.1). The PCR results of the plants were not always complimented by PCR results of the psylla (Table 4.2.11.1). Several factors can contribute to this result but since the overall transmission rate was low, any conclusion will be speculative and therefore challenges of the negative plants will be repeated.

Table 4.2.11.1. Symptom development of plants and PCR results of embryo-rescued clones that were challenged by batches of 1 to 5 psylla collected at the end of the challenge period.

Clones	Greening symptoms	PCR	
	Positive/challenged	Psylla batches Positive/Tested	Plants Positive/Tested
Ponkan	0/8	0/8	0/8
OC 4/11 RA	1/7	2/7	1/7
OC/ 4/15 RA	0/9	1/9	0/9
O/03/30/ RA2	0/9	2/9	0/9
OC 2/11 G1	1/9	1/9	1/9

Field evaluation

Plants of three clones, as well as a field clone, were multiplied on rough lemon rootstock for field evaluation. They were positively pre-immunised with two CTV sources (LMS 6 and SM 49) before being planted in the field (Table 4.2.11.2). The trees were inspected regularly and no adult psylla or signs that psylla insects were present (eggs, nymphs or psylla nymph marks on leaves) were observed. The trees will be inspected

for possible greening symptoms during winter. Fruit developed on some trees and the first horticultural evaluation will be possible when the fruit ripens.

Table 4.2.11.2. Summary of treatments in the field trial to evaluate greening resistance or tolerance under commercial conditions.

Cultivar or clone	Pre-immunising CTV source
Midnight Valencia (Control 1)	LMS 6 (Standard) (Van Vuuren <i>et al.</i> , 2000)
Midnight Valencia (Control 2)	SM 49 (Van Vuuren <i>et al.</i> , 2000)
GTC-E2	LMS 6
GTC-E2	SM 49
GTC-T2	LMS 6
GTC-T2	SM 49
GTC-14 ^a	SM 49
GTC-CV	Carrying original CTV source

^a This clone had a low % infection in the laboratory test during 2005. CTV source SM 49 may increase its greening tolerance.

Conclusions

A total of four clones developed into suitable material for micro-grafting, multiplication and challenging with Laf by the psylla insect vector. Three of these were from the healthy sectors of chimeras while only one was derived from a greening infected sector. Psylla numbers were low during the past year and challenges could not be completed. Promising clones were planted in the field during 2007. Some trees have fruit on and the first horticultural evaluations can be done during winter.

Further objectives

Continue with embryo rescue to obtain clones for evaluation against *Liberibacter* infection. Repeat challenges of clones with Laf in the laboratory. Monitor for HLB symptoms in the field experiment and evaluate fruit for horticultural characteristics.

Technology transfer

S.P. van Vuuren. 2008. Searching for resistance in the battle against greening. Fifth Citrus Symposium, Drakensberg, Natal.

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4.2.12 **PROGRESS REPORT: Eradication of citrus greening infections in existing orchards** Experiment 818 (2006 - 2010) by M.C. Pretorius (CRI)

Summary

Huanglongbing, commonly called citrus greening in South Africa, is a serious bacterial disease of all citrus cultivars. The causative agent locally is "*Candidatus Liberibacter africanus*" (Laf). The purpose of this trial was to investigate a new approach to effectively control the greening bacteria in citrus trees by reducing the bacterium with two foliar applied systemic products. Two trials were conducted: a re-evaluation of a pot trial at CRI, and a field trial at Crocodile Valley Citrus Co. The foliar applied bactericides were applied at various times and rates during the season for a period of four months. The results of the foliar applied systemic products in the pot trials again showed promising results especially Product B (Bactericide), where only one tree from five infected and treated trees tested positive. The field trial data were, however, disappointing. A final PCR analysis will indicate the way forward with regards to the use of systemic bactericides for the control of citrus greening in South Africa.

Opsomming

Huanglongbing, wat meer algemeen as sitrusvergroening bekend staan in Suid-Afrika, is 'n ernstige bakteriese siekte wat alle sitrus kultivars affekteer. *Candidatus Liberibacter africanus* (Laf) is die siekte veroorsakende spesie. Die doel van hierdie proef was om 'n nuwe benadering tot sitrusvergroeningsbeheer te evalueer deur die bakteriese inokulum in reeds besmette plantmateriaal (in die veld asook in potte) deur met sistemiese blaar bespuitingsprodukte te verminder. 'n Potproef by CRI asook 'n veldproef is uitgelê om die effektiwiteit van die twee bakteriesiede te evalueer. Die produkte is teen verskillende dosisse en tye toegedien. Die sistemiese blaar bespuitingsbehandelings in die potte het weereens belowende resultate gelewer, veral Produk B wat vier keer in die seisoen met 2-maandelikse intervalle toegedien is, slegs een boom uit vyf wat positief getoets het. Die veldproef se resultate was teleurstellend alhoewel daar gehoop word dat die Julie 2009 PCR resultaat 'n beter resultaat sal oplewer. Hierdie uitslag sal die toekoms van die gebruik van hierdie sistemiese produkte vir die beheer van vergroening bepaal.

Introduction

Citrus Huanglongbing (HLB), commonly called citrus greening in South Africa, is considered the most serious disease of citrus worldwide (Halbert, *et al.*, 2004). Greening disease has been known in China for more than 100 years. It was initially reported by Reinking in 1919 and it was named Huanglongbing, meaning yellow shoot disease (Garnier and Bové, 1993). In 1937, a disease with similar symptoms was described in South Africa and was called greening disease because of the fruit that remains green during ripening (Van der Merwe, *et al.*, 1937). Before these diseases were identified as being identical, the disease was also described as likubin (decline) in Taiwan, dieback in India, leaf mottle in the Philippines and vein phloem degeneration in Indonesia. Subsequently it became clear that all these were similar diseases and the commonly accepted word describing the disease was greening (da Graça, 1991). The disease has long

been present in Asia, Africa, the Indian subcontinent, Mascarene Islands and the Arabian Peninsula (da Graca, 1991). It was recently found in South and North America. All commercial citrus species and cultivars worldwide are sensitive regardless of the rootstocks (Bové, 2006).

The causal agent of the disease is a Gram-negative phloem-limited bacteria belonging to the alpha sub-division of the Proteobacteriaceae and has not been cultured (Jagoueix, *et al.*, 1994). The bacterium was named *Candidatus Liberibacter*. Various species were named, *Liberibacter africanus* (Laf), (causing the disease in Africa), *Liberibacter asiaticus* (Las) and *Liberibacter americanus* (Lam) (causing the disease in Asia and America) (Texeira, *et al.*, 2005). The disease is mainly transmitted from tree to tree by citrus psyllid insect vectors: *Diaphorina citri* in Asia and the Americas and *Trioza erytrae* in Africa (Bové, 2006). Only Laf is currently present in Africa.

Common symptoms of the disease are yellowing of the veins and adjacent tissues, followed by yellowing or mottling of the entire leaf, although the disease syndrome to some extent differs according to citrus variety. Advanced or chronically infected trees show yellowing of the entire canopy and have sparse foliage and twig die back. Diseased trees produce small, lopsided fruit that tend to remain mostly green in colour even when mature, have undeveloped seed and impart an objectionable bitter-salty flavour (McClellan, *et al.* 1970; da Graca, 1991). The Asian greening symptoms are more severe than the African strain. They can clearly be distinguished on the basis of temperature tolerance. With the African greening, severe symptom expression was obtained in glasshouse conditions at 22°C whereas no symptoms appeared at 27-30°C. In contrast, the Asian greening is pronounced at both temperatures (Schwarz, 1972).

It was demonstrated by McClellan (1965) that greening was graft-transmissible. There are no curative methods to control greening. The only control measure effective in preventing the disease is to prevent the trees from becoming infected. Control measures known to be effective against greening disease consist of the following: (i) to prevent the spread of the bacteria by restricting the movement of plant material from infected regions to uninfected regions; (ii) to provide the industry with disease-free propagation material; and (iii) to control the vector effectively and eliminate the inoculum by removing infected trees and infected branches.

Antibiotic control by trunk injections of tetracyclines was investigated and although promising results were obtained this idea was abandoned because of ecological reasons but essentially because tetracycline is bacteriostatic, rather than bactericidal, and treatments had to be repeated each year (Bové, 2006; Van Vuuren, 1977). According to Schwarz (1967) it was noticed during a survey that greening symptoms were less severe in hot, low-lying areas than in the cool, high-lying areas. It appeared that heat and high temperatures, do have a direct effect on symptom expression. Schwarz and Green (1972) then demonstrated in trials using an index of total centigrade degree-hours above 30°C (DH/30) that the incidence of greening can be reduced or totally inhibited when trees are exposed to heat applications in excess of 1 500 DH/30.

The purpose of this experiment was to evaluate new approaches for the control of the greening bacteria in citrus trees. In previous seasons, infected trees were covered with plastic sheets to increase temperature in an attempt to observe whether thermotherapy might kill the bacterium in trees. This approach was, however, unsuccessful and impracticable. In this report year, two bactericides were re-evaluated in a greening infected orchard and in potted plants. These products are systemic and were applied as a foliar spray at different rates and times during the season.

Materials and methods

Field trial

A field trial was laid out at Crocodile Valley Citrus Co. in a 15 year old Delta Valencia orchard. Forty-five visually greening-infected trees were randomly selected throughout the orchard. Product A (80 ml /100 l water) and Product B (100 ml /100l water) were applied at different times as foliar applications. The trial consisted of 9 treatments replicated 5 times (Table 4.2.12.1). The products were applied with a trailer-mounted, high volume, high-pressure (2500 to 3000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of runoff. All the trees were treated twice per season (September and December), with imidacloprid (Confidor®) to restrict Psylla damage/feeding and reduce the re-introduction of the greening organism. The first imidacloprid application in 2007 was only applied in November after PCR results indicated the status of the tested trees with regards to greening, however at Croc Valley imidacloprid was applied during the spring flush in September 2007 as a normal cultural practice. The trees were visually inspected on a regular basis throughout the season for any phytotoxic reaction due to the foliar application. Fallen fruit was collected and

counted on a fortnightly basis from December to April during the 2007/08 as well as 2008/09 seasons (Table 4.2.12.4). The occurrence of fallen fruit after the normal November drop could be due to the presence of greening bacterium in the trees.

Thirty leaves were collected per tree prior to the first application of the products (during November 2007) to confirm the presence of Laf. During the cooler winter months (July 2008) a second set of leaf samples were collected, six months after the last application of the bactericides. The samples were sent to UP for PCR analysis to determine the HLB status in each tree. The leaf samples from all the trees were tested with species-specific primers for Laf and Las and PCR according to a protocol slightly adapted from the original technique. This PCR implemented by CRI at UP has been optimised and tested against specific local and international pathogen isolates. Templates for PCR were prepared by extracting total DNA on pooled selections of leaf midrib and petiole of the samples submitted. The PCR was performed twice on the DNA extracts of each sample. Positive and negative controls (using water instead of template DNA) were included.

Pot trial

The pot trial consisted of nine treatments with five single tree replications per treatment. Products A and B were applied at different dosages and times during the season. The foliar sprays were done by means of a knapsack sprayer and a full cover spray was applied to the point of runoff. The treatments and times of application are shown in Table 4.2.12.2. Leaf samples were collected during the cooler months at the end of winter in 2006, prior to the first applications of the sprays and again in the winter months in 2007. The final sampling was done during July 2008 as shown in Table 4.2.12.5. Thirty leaves per tree of each tree were collected and sent to the University of Pretoria for PCR analysis. The same PCR procedure as described for the field trial was used to do the pot trial evaluations.

All the trees were treated with imidacloprid (Confidor®) to restrict Psylla damage/feeding and reduce the re-introduction of the greening organism. The trees were visually inspected on a weekly basis to determine whether any phytotoxic reaction was visible as a result of the foliar applications.

Results and discussion

Field trial

The initial PCR analysis of samples, collected prior to the first application, confirmed the presence of Laf in all the trees. The results obtained from the PCR analysis collected during winter of 2008, six months after the last foliar applications were done, are presented in Table 4.2.12.3. The results indicate that all the leaf samples tested positive for Laf. The initial field results of the foliar applied bactericides did not reduce the greening bacterium in the trees and the results are therefore disappointing. It is, however, necessary to keep in mind that the trees have originally been planted in a high pressure greening infected area and these trees were also exposed to a number of Psylla infestations over the years. The first applications were applied in October one month after the spring flush. The spring flush is very susceptible to Psylla infections and it is believed that the first applications should have been applied earlier to protect the trees from the beginning of the new season. The first imidacloprid applications should be applied at least two weeks before the spring flush to prevent any re-infestations by psylla.

The trial was repeated during the 2008/09 season and the next set of PCR results will be collected during July 2009 and should give a better indication of the effect of these products on the Laf in the trees. No phytotoxic reaction was visible on leaves of any of the sprayed trees.

The average numbers of fallen fruit collected per treatment on a two-weekly basis during the 2007/08 and 2008/09 seasons for the periods December to April are presented in Table 4.2.12.4. The occurrence of fallen fruit after the normal November drop could be ascribed to the presence of the greening bacterium in the trees. The 2007/08 results showed no significant differences between the treatments possibly due to a large variation within the treatments. However the results clearly indicate that an average of 15.4 fallen fruit per tree was recorded for treated trees compared to the untreated control where 35.2 fallen fruit were recorded. The 2008/09 results showed the same tendency, although not significantly between all treatments, less fruit fell from the treated trees compared to the untreated control trees. Treatment 9 with an average of 11.2 fallen fruits (Treatment 9) had significantly less fruit drop if compared to the untreated control with an average of 35 fruits (Treatment 1). These results show that the bactericides and especially Product B (treatment 9) prevented the loss of fruit. The reason for this result is unclear, but could be ascribed to the some inhibitory effect of the bactericides on the greening bacterium in the infected trees. It was originally anticipated that in the event of the foliar applications being successful, the treated trees would have less fallen fruit and possibly a higher yield, and although the bacterium was not eliminated from trees, some

inhibition might have occurred. Further studies will be necessary to determine the effect (mode of action) of the bactericides with regards to the reduction in fallen fruit on treated trees.

No phytotoxic reaction was visible on any of the sprayed trees.

Pot trial

The PCR results showed that Product B generally appeared more effective than Product A (Table 4.2.12.5). It is clear that treatment 9 (Product B, applied once every 2 months – 4 applications during the season) was the most effective application resulting in only one tree still infected with the greening bacterium. Treatment 2 (Product A, single application 2 X the dosage) resulted in only two infected trees and therefore this been the most effective treatment of Product A. One tree tested negative in treatment 1 (Product A, single application). The results are still very inconsistent if compared to last season's data, but the effect of the bactericides that were applied, especially Product B, are very promising. Further studies will be necessary to determine the most effective dosage as well as the mode of action of these products on the greening bacterium. The results obtained by Product B complimented the fruit drop results of the field trial.

Conclusion and future research

The results obtained in the pot trial indicate that at least one of the bactericides show potential in possibly reducing the incidence of the bacterium in infected trees. The initial field results regarding the PCR analysis were disappointing, but the reduction in fallen fruits indicate that the applied product did prevent some fruit loss after the normal fruit drop in November. The follow-up PCR analysis to be done in July/August 2009 will give a better indication of the effect of the bactericides on the greening status in the trial due to the fact that these trees were treated in two consecutive seasons. Further studies will be necessary following the final PCR result in 2009 if shown to be effective in reducing the number of infected trees in the trial.

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Table 4.2.12.1. Treatments and dosage rates of two bacteriacides applied at Crocodile Valley Citrus Co. as foliar sprays during 4 months in 2007 and 2008.

Treatment	Dosage/ 100 ℓ water	Oct		Nov		Dec		Jan	
1. Control	-	-	-	-	-	-	-	-	-
2. Product A	80 ml	X							
3. Product A	80 ml	X		X					
4. Product A	80 ml	X		X		X			
5. Product A	80 ml	X		X		X		X	
6. Product B	100 ml	X							
7. Product B	100 ml	X		X					
8. Product B	100 ml	X		X		X			
9. Product B	100 ml	X		X		X		X	

Table 4.2.12.2. Foliar applied systemic products at different times and rates to potted trees for the control of HLB for the periods November 2006 to April 2007.

Treatment	Dosage/10 ℓ water	Period
Untreated Control	-	-
Product A	1 ml	1 application only
Product A	2 ml	1 application only
Product A	1 ml	Every two weeks
Product A	1 ml	1 x month
Product A	1 ml	1 x every 2 months
Product B	1 ml	Every 2 weeks
Product B	1 ml	1 x month
Product B	1 ml	1 x every 2 months

Table 4.2.12.3. PCR analysis conducted on leaf samples collected from the field trial prior to the commencement of the foliar applications and 6 months after the last foliar application.

Treatment	Dosage/ 100ℓ water	Aug 07 # Tested / # +	Aug 08 # Tested / # +
1. Untreated Control	-	5/5	5/5
2. Product A	80 ml	5/5	5/5
3. Product A	80 ml	5/5	5/5
4. Product A	80 ml	5/5	5/5
5. Product A	80 ml	5/5	5/5
6. Product B	100 ml	5/5	5/5
7. Product B	100 ml	5/5	5/5
8. Product B	100 ml	5/5	5/5
9. Product B	100 ml	5/5	5/5

Table 4.2.12.4. Amount of fallen fruit collected on a 2-weekly basis at Croc Valley Citrus Co. for a five month period from November to April for the 2007/08 and 2008/09 seasons.

Treatment	Dosage/ 100ℓ water	Total fallen fruit collected 2007/08	Total fallen fruit collected 2008/09
1. Untreated Control	-	35.2 a*	35 c
2. Product A	80 ml	23.8 a	22.8 abc
3. Product A	80 ml	14.8 a	27.8 abc
4. Product A	80 ml	18.4 a	29 bc
5. Product A	80 ml	23 a	21.6 abc
6. Product B	100 ml	15.6 a	15.4 ab
7. Product B	100 ml	19.4 a	28.6 abc
8. Product B	100 ml	17 a	19.2 abc
9. Product B	100 ml	15.4 a	11.2 a

* Means in a column followed by the same letter are not significantly different ($P>0.05$) according to Fisher's LSD test.

Table 4.2.12.5. PCR analysis conducted on leaf samples collected prior to the commencement of pot trial 2 and during two successive seasons.

Treatment	Dosage / 10 ℓ water	Time of application	Aug 06 # Tested / # +	Aug 07 # Tested / # +	Aug 08 # Tested / # +
1. Untreated Control	-	-	5/5	5/5	5/4
2. Product A	1 ml	1 application only	5/5	5/2	5/4
3. Product A	2 ml	1 application only	5/5	5/0	5/2
4. Product A	1 ml	Every two weeks	5/5	5/2	5/5
5. Product A	1 ml	1 x month	5/5	5/5	5/5
6. Product A	1 ml	1 x every 2 month	5/5	5/5	5/5
7. Product B	1 ml	Every 2 weeks	5/5	5/3	5/4
8. Product B	1 ml	1 x month	5/5	5/3	5/3
9. Product B	1 ml	1 x every 2 month	5/5	5/3	5/1

4.2.13 PROGRESS REPORT: Epidemiology of greening disease – alternate hosts and spread

Experiment 886 (April 2007 – April 2011): by Baby Phahladira (UP) and Gerhard Pietersen (CRI-UP)

Summary

Determining potential alternate hosts to citrus of "*Candidatus Liberibacter africanus*" (Laf) amongst the indigenous plants of South Africa will aid in the development of an integrated control strategy for the greening disease by making disease pressure reduction more efficient. In this study, indigenous plants mainly of the citrus family (*Rutaceae*) were evaluated for their capability to host the pathogen. During the report period further symptomatic and non-symptomatic leaf and petiole samples of numerous indigenous Rutaceous species were collected from areas near citrus orchards, in natural habitats, or from botanical gardens. The presence of Laf was determined in these by DNA extraction and a published conventional PCR protocol, and a newly developed multiplex PCR containing an internal control directed at a healthy plant component. Except for *Calodendrum capense* (Cape chestnut) none of the indigenous plants yielded *Liberibacter* specific bands following PCR. Seventeen *C. capense* plants, however, tested positive for the presence of *Liberibacter* DNA. Direct DNA sequencing of the PCR products from these plants confirmed the presence of "*Candidatus Liberibacter africanus*" subspecies *capensis*" (LafC). The host range for Laf was also studied amongst a number of plants of the family *Rutaceae* by graft-transmission tests. For a second season, these plants still did not display any symptoms. High Ct values in real-time PCR tests, possibly indicative of low concentrations of the Laf, were obtained from a number of these inoculated plants. While these tests must be redone at a later stage post-inoculation, this may be an indication that these rutaceous plants can host the bacterium naturally and play a role in the epidemiology of the disease in South Africa.

Opsomming

Om te bepaal watter inheemse lede van die *Rutaceae* in Suid-Afrika kan dien as alternatiewe gashere tot sitrus vir "*Candidatus Liberibacter africanus*" (Laf) sal help om 'n meer doeltreffende beheer van siekte druk

daar te stel, aangesien die verwydering van besmette plantmateriaal (in sitrus tans), meer volledig kan geskied. In hierdie studie word inheemse plante, hoofsaaklik van die Sitrus-familie (Rutaceae) ge-evalueer vir hul vermoëns om as gasheer vir die patoog op te tree. Beide simptomatiese sowel as asimptomatiese blaar en blaarstingel materiaal van verskeie inheemse Rutaceae-lede is vanaf areas naby aan sitrus boorde, in hul natuurlike habitat, en uit botaniese tuine versamel. Die teenwoordigheid van Laf is d.m.v. DNA ekstraksie gevolg deur PCR met 'n gepubliseerde metode, sowel as 'n nuut-ontwikkelde PCR wat 'n interne kontrole teen gesonde plantmateriaal bevat, bepaal. Behalwe vir *Calodendrum capense* (wilde kastaiing), het nie een van die inheemse plante positief vir Liberibacter getoets nie. Sewentien *C. capense* plante was egter positief vir Liberibacter DNA. Direkte volgordebepalings op die PCR produkte het getoon dat die plante met "*Candidatus Liberibacter africanus* subsp. *capensis*" (LafC) besmet is. Behalwe vir die versameling van inheemse plante, is die gasheer-reeks van Laf ook bepaal deur verskeie lede van die Rutaceae te inokuleer met Laf-besmette sitrus ogies. Hoë Ct waardes, in ander woorde baie lae konsentrasie van bakterieë, is vir verskeie plante met huidige-tyd PCR verkry. Hierdie mag daarop dui dat die bakterieë in hierdie plante gevestig het en dat hierdie plante 'n rol in die epidemiologie van vergroening mag speel. Hierdie resultate moet egter deur verdere toetse wanneer die bakterieë tot hoër vlakke vermeerder het, bevestig word.

Introduction

Citrus greening is a destructive disease of citrus (Bové, 2006) and is caused in South Africa by a fastidious bacterium "*Candidatus Liberibacter africanus*" (Laf). The disease has been reduced to manageable levels through stringent vector control strategies, but remains a problem in cooler citrus production areas of South Africa. Perpetuation of the disease, in areas where it is managed in citrus by infected tree and branch removal, may be due to the presence of other hosts, which may serve as reservoirs of the disease. In this study, we studied the possibility that other hosts of the bacteria exist. Such studies done in the past have relied on symptoms and biological indexing by grafting to detect the disease. These were time-consuming techniques, which required graft or vector transmission of the bacterium and which did not allow the analysis of large numbers of samples. Furthermore, graft transmission may be difficult or not possible at all between genetically incompatible plants while vector transmission studies may have been affected by differing feeding preferences of the insects. The aim of this study was to identify possible hosts of Laf amongst Rutaceae plants of South Africa. The aim will be achieved by collecting indigenous rutaceous and other plants from their natural environment in regions with high greening infection pressure and by graft inoculating selected rutaceous seedlings with a known Laf infected source. These plants will be tested for the presence of Laf using conventional PCR, a multiplex PCR with an internal plant-directed control and real-time PCR.

Materials and methods

Samples

Leaf and petiole tissue, irrespective of the presence of symptoms, were collected from indigenous non-citrus Rutaceous potential hosts. These have been collected from Vredefort, Pretoria, Nelspruit at the Lowveld National Botanical Gardens, ARC-ITSC, Crocodile Valley and Fredenheim Experiment station, Kirstenbosch National Botanical Gardens in Cape Town, and Rhenosterpoort, Alma. The samples include native "buchu" species *Agathosma*, *Acmadenia*, *Adenandra*, *Coleonema*, *Diosma*, *Euchaetis* and the most common indigenous rutaceous tree species *Calodendrum*, *Clausena*, *Murraya*, *Techlia*, *Toddalia*, *Toddaliopsis*, *Vepris* and *Zanthoxylum*.

Liberibacter detection

- 1) Total DNA extraction:
Total DNA extraction was performed using the CTAB extraction method by Doyle and Doyle (1991) with modifications according to Fundecitrus.
- 2) PCR detection:
A real-time PCR published by (Li *et al.*, 2006) modified for Laf-specific detection by using Taqman probe HLBpr and primers HLBafr/HLBbr was used to detect Laf in various experiments including those described below. This aim is made possible through the supplemental matching funding from THRIP, which has allowed for the purchase of a real-time thermocycler and funds for implementation of the apparatus. This technique will compliment the multiplex PCR with an internal control, developed in this study, with primers pairs targeting the β ribosomal proteins of Liberibacter DNA and ubiquitous ribulose biphosphate carboxylase oxygenase (Rubisco) gene (Nassuth *et al.*, 2000), and the A2/J5 conventional PCR (Hocquellet *et al.*, 1999) to test DNA extracts from collected samples for the presence of Laf or "*Candidatus Liberibacter asiaticus* (Las). A LafC-specific PCR, based on primers Cal1 and J5 (Garnier *et al.*, 2000) was also established. The Cal1 primer targets a 25bp insert specific to LafC.

Graft transmission

Symptom expression has been monitored since September 2007 when graft transmission was done on 10 Rutaceae seedlings of *Agosthema capensis*, *A. ciliaris*, *Calodendrum capensis*, *Clausena anasita*, *Vepris lanceolata* and *Zanthoxylum capense* using three bark patches of confirmed Laf infected citrus material (UPCRI 06-0150, 06-0195 and 06-0280). Symptoms were also monitored on two citrus seedlings inoculated with the same infected sources to serve as positive controls and on single seedlings of each Rutaceous and citrus host which were not inoculated and are used as negative controls. Samples from inoculated plants were tested for Laf or Las three months, six months and a year post inoculation, ending in September of 2008. Tests were with multiplex, conventional and real-time PCR using Taqman probe HLBpr and primers HLBafr/HLBr specific for Laf (Li *et al.*, 2006).

Surveys

Citrus groves will be monitored on the tree-for-tree basis for greening symptoms annually in winter by a team of people trained in greening symptom detection. Trees are assigned a row/plant position coordinate and infected and missing trees are plotted and analysed to establish greening spread.

Write up MSc. dissertation

This study is primarily to be used for the awarding of a MSc. degree. Miss Phaladira must therefore prepare a thesis and draft publication using a prescribed format based on the studies conducted within this project.

Establish Trioza erytreae colony

Trioza erytreae individuals were collected from Citrus groves, not subjected to insecticide treatment. These were establish in two colonies on greening free, insecticide-free lemon seedlings, maintained within insect-cages under natural light conditions (UP) or artificial light (PPRI). Seedlings were replaced on a regular basis with seedlings with young, not fully expanded leaves.

Results and discussion

Develop a sensitive detection method for Laf

A real-time PCR using Taqman probe HLBpr and primers HLBafr/HLBr (Li *et al.*, 2006) was modified from the published multiplex PCR to be specific for Laf and was used to detect Laf in various experiments including those described below.

A LafC-specific PCR, based on primers Cal1 and J5 (Garnier *et al.*, 2000) was also established, optimized and utilised during this study. The Cal1 primer targets a 25 bp insert specific to LafC and detects only this subspecies and not Laf.

Search for Liberibacters in hosts other than Citrus

A further 26 *Calodendrum capense* plant samples were collected during 2008 to make a total of 193 indigenous non-citrus specimens collected during the course of this study. Amongst the 28 *Calodendrum capensis* plants collected in and around Pretoria, 14 tested positive by conventional PCR. The PCR products obtained, which were slightly larger than those expected for Laf were sequenced directly (without cloning) and analyses thereof revealed high similarity to that of LafC. This subspecies of Liberibacter has been detected previously on *C. capensis* only in the Stellenbosch region (Garnier *et al.*, 2000). The detection of LafC from *C. capensis* plants located in three provinces Gauteng, Limpopo and Mpumalanga shows an unexpected widespread nature of this bacterium in this host in South Africa.

Do experimental transmission of Laf to hosts other than citrus

None of the *Calodendrum capensis*, *Clausena anasita*, *Vepris lanceolata* and *Zanthoxylum capense* samples inoculated with Laf showed symptoms associated with greening over a period longer than one-year since inoculation. Both the conventional and multiplex PCR was used to test DNA extracts of the inoculated seedlings but no Liberibacter DNA was detected. Due to its sensitive nature, real-time PCR was then used to test DNA extracts of these plants. High Ct values were obtained from inoculated seedlings of *C. capense*, *C. anisata*, *V. lanceolata* and *Z. capense* potentially indicative of presence of low concentrations of the inoculated Laf. This needs to be confirmed using a different test.

Search for evidence of natural spread from indigenous vegetation.

One of the orchards in which these studies were initiated (Union Homestead) was unfortunately removed due to roadworks this season and further monitoring of it will no longer be possible. At Kingstonsvale, Nelspruit, amongst 692 plants (various Navels) monitored, only two trees (additional to 2007) with symptoms were noted, and confirmed positive with PCR. Three trees recorded as positive based on symptoms in 2007 did not show symptoms in 2008 and tested negative by PCR. The slow spread of greening in this orchard suggests that disease pressure is not very high in the immediate vicinity of this orchard and therefore it is not a good region in which to try to determine the effect of potential indigenous reservoirs. Furthermore, the

inconsistency of symptoms (recovery, partial position on tree) and interpretation by different personnel (some new students helped to monitor the orchards in 2007 than in 2008) makes it extremely difficult to follow the spread of this disease. This was also experienced in the Schoemanskloof orchard, where the inconsistency of symptom expression (and interpretation) was severe, and very little correlation with 2007 data was possible. In order to do these studies it is clearly important that only one (trained) person monitors an orchard, or that tree samples with symptoms about which uncertainty exists (by relatively untrained personnel viz. students) be taken for PCR tests. In view of the cost of monitoring the orchards in this way and the lack of useful information gained thus far, this approach will be abandoned from 2009, and the presence of *Liberibacters* will be monitored in indigenous Rutaceae in areas where a high incidence of greening occurs in proximal citrus orchards, without trying to determine the presence of disease gradients in them.

Writing up of dissertation

A first draft of the entire dissertation has been prepared, submitted and returned with some corrections. Final submission is imminent.

*Establish *Trioza erytreae* colony*

About 100 *Trioza erytreae* individuals were collected from a lemon orchard on the ARC-ITSC experimental station in Nelspruit. These were transported to Pretoria where they were immediately placed on actively flushing lemon seedlings in two separate insect-cages. The one cage was transferred to ARC-PPRI while the second one was maintained at UP. In both colonies, *T. erytreae* eggs could be detected on the leaf margins of young seedlings within a few days. However, adult numbers declined steadily over a one-month period and no nymphs were noted. Due to the lateness of the season, no more adults were present in the field when attempts were made to re-establish the insect.

Conclusion

This study has found *Calodendrum capense* trees from widely distributed areas infected with the LafC variant, and may indicate that this host is endemically infected with this bacterium. The role of the subspecies *capensis* bacterium in the epidemiology of citrus greening as well as potential of a number of other rutaceous plants that can serve as hosts of Laf must still be assessed in future studies.

Technology transfer

Two presentations on this study were made in the report period:

Phahladira, M.N.B. and Pietersen, G., 2008. Identification of alternative hosts to Citrus of *Candidatus Liberibacter africanus* amongst indigenous rutaceous plants of South Africa. 5th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 3-6 August, 2008.

Pietersen, G., Kotze, A., Phahladira, M.N.B. and Schwerdtfeger, M. 2008. Survey for "*Candidatus*" *Liberibacter* species in South Africa. HLB International Conference, Orlando, Florida, USA 1-5 December, 2008.

Further objectives (milestones) and work plan

Preparation and submission of final version of MSc. dissertation

Develop sensitive detection method for Laf

Keep abreast of literature for new innovations in detection methods. Modify and apply where relevant, mainly through THRIP additionality. Plan to design primers and fluorescent-labelled probe for use in real-time PCR specific detection of LafC. Establish this PCR for routine use. Using new sequence information on *Liberibacters* (including those of Solanaceous plants) look for conserved nucleotide sequences to target for generic *Liberibacter* primers (THRIP additionality project).

*Search for *Liberibacters* in host other than Citrus*

In order to detect the bacteria in indigenous plants probably not showing symptoms much larger numbers of plants need to be tested than has been possible thus far. Therefore further samples of indigenous Rutaceae will be collected from various sites in South Africa. Test for *Liberibacters* using real-time PCR (Also retrospectively test DNA extracts collected in previous years by real-time PCR). Establish citrus and other rutaceous seedlings for graft inoculations. Graft inoculate *Liberibacter* positive samples onto Citrus/original host seedlings. Re-collect symptomatic *Zanthoxylum* in Kirstenbosch. Test with real-time PCR, and low-

stringency conventional PCR with conserved primers. Determine identity of Liberibacters from indigenous plants by sequencing. Test inoculated samples for Liberibacters by real-time PCR.

Do experimental transmission to host other than citrus

Confirm presence of Laf in rutaceous plants inoculated in 2008 using a second round of real-time PCR tests. Identify, characterize and determine transmission and if Citrus can serve as a host, of any Liberibacters detected in plants other than Citrus: (to serve as start of PhD study for Miss Phahladira)

- 1) Establish and maintain *Trioza erytreae* colony, confirm colony is Liberibacter free. Do inoculation feeds and *Trioza erytreae* transmission tests of LafC to *Calodendrum* and citrus. Graft transmit LafC to citrus. Test for LafC in grafted and experimental vector transmission plants.
- 2) Establish citrus and *Calodendrum* seedlings for graft and vector inoculations of LafC. Establish a (insecticide-free) source(s) of LafC in the glasshouse by graft inoculation to *C. capensis* seedlings grown from seed and not subjected to Confidor treatment. Test whether LafC was established in *Calodendrum* seedlings.
- 3) THRIP Additionality: If funding is secured from THRIP, real-time PCR, specific to LafC will be developed as well as a real-time PCR to universally detect Liberibacters, based on primers to conserved regions of known Liberibacters.
- 4) Investigate sequencing strategy to amplify and sequence novel portion of Laf and a similar sequencing strategy to LafC novel regions.

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4.2.14 PROGRESS REPORT: Epidemiology of greening disease - variability

Experiment 887 (April, 2007 – April, 2011): by Aletta Kotze, Marius Schwerdtfeger (UP), and Gerhard Pietersen (CRI-UP)

Summary

The detection in recent years of three different citrus Liberibacters in different countries of the world required confirmation of the presence of only "*Candidatus Liberibacter africanus*" (Laf) in South Africa. This was confirmed by conducting a survey for citrus Liberibacters in all greening affected areas in South Africa. Confirmation of the identity of the bacteria detected was achieved through sequence analysis of a portion of the ribosomal protein (*rpl*) gene amplified by PCR. Consensus sequence data of two runs from this part of the genome was essentially identical in all citrus sources tested, comprising at least one source from every greening infected citrus production region in South Africa. No instances of "Ca." *Liberibacter africanus* spp. *capensis* (LafC) (known to occur on *Calodendrum capensis* in South Africa) on citrus was found. It remains possible that other strains or subspecies of Laf may exist in citrus, with some biological and serological evidence of this having been reported. To identify potential strains for use in challenge experiments of putatively greening resistant cultivars, the sequence variability within the outer membrane protein (*omp*) gene, previous shown to be variable in "*Candidatus Liberibacter asiaticus* (Las), was determined. Based on un-replicated, single sequence runs, no sequence differences have thus far been found. Sequence data to a new region of the genome, the RNA polymerase B' subunit (*rpoB*) was generated through international collaboration. This was used to design primers to amplify part of the *rpoB* locus.

Opsomming

Die onlangse opsporing van drie verskillende sitrus Liberibacters in verskeie lande van die wêreld het genoop dat die teenwoordigheid van slegs "*Candidatus Liberibacter africanus*" (Laf) in Suid-Afrika bevestig word. Dit is bevestig deur 'n opname vir sitrus Liberibacters in alle sitrus vergroenings-geteisterde areas van Suid-Afrika. Die identiteit van bakteriële verkry is deur nukleotiedvolgordebepalings van 'n gedeelte van die ribosomale proteïengeen (*rpl*) bevestig. Konsensus nukleotiedvolgorde data op PCR produkte vanaf twee volgordebepalingslopië per sitrusbron was grootliks identies in alle sitrusbronne wat getoets is, wat ten minste een bron per vergroeningsarea in Suid-Afrika insluit. Geen voorvalle van infeksie deur "Ca." *Liberibacter africanus* spp. *capensis* (LafC, bekend op *Calodendrum capensis* in Suid-Afrika) is gevind nie. Dit bly egter moontlik dat rasse of subspecies van Laf voorkom, en daar bestaan reeds biologiese en serologiese bewyse daarvoor. Om rasse te identifiseer vir gebruik in uitdagingsproewe op moontlike vergroenings weerstandbiedende sitrusbronne, is die nukleotiedvolgorde variasie bepaal binne die buitekanste membraan proteïen geen (*omp*), wat varieer in "*Candidatus Liberibacter asiaticus*" (Las). Gebaseer op ongerepliseerde enkel nukleotiedvolgorde lopië, is geen verskille tot dusvêr gevind nie. Nukleotiedvolgorde data is vir die RNA polimerase B' subeenheid (*rpoB*) d.m.v. internasionale samewerking gegeneer. Hierdie data is gebruik om voorvoeders te ontwerp sodat 'n gedeelte van die *rpoB* area vermeerder kan word m.b.v. PCR.

Introduction

Three pathogens have been discovered that cause citrus greening or huanglongbing (HLB), namely "*Candidatus Liberibacter asiaticus*" (Las), "*Candidatus Liberibacter africanus*" (Laf) and "*Candidatus Liberibacter americanus*" (Lam). A subspecies of Las, namely "*Ca. L. africanus* subsp. *capensis*" (LafC), has been described only in the Western Cape region of South Africa from an indigenous Rutaceous host, *Calodendrum capensis*. Only Laf and LafC are known to occur in South Africa. Both Las and Lam have recently been detected in regions of the world previous free of any Liberibacters, and it has become important to unequivocally determine the presence of only Laf and its *capensis* subspecies in South Africa. A survey for citrus Liberibacters was conducted in 2006. While no Lam amplicons were obtained, amplicons of Laf and Las differed too little in size to be differentiated with certainty on the agarose electrophoresis gels used. In this project these amplicons were therefore sequenced to identify the bacterial template found. This will also allow differentiation of Laf and LafC.

Furthermore, as resistance to greening is considered the most desirable control strategy, an experiment to select resistant trees by embryo-rescue from fruit chimeras lacking the symptoms is currently being performed (Exp. 815). Prior to general release of such plants with some form of resistance, they must be challenged with greening in order to assess its ability to be resistant to the disease. Such challenges must be performed with the range of variants to which the trees will be exposed in the field. The sequence variability within specific regions of the Liberibacter genome will be used to determine the variability of South African sources of Laf, and allow selection of variants for use as challenge sources in the resistance trials.

Chemical control of greening must also be assessed. Antibiotic use was evaluated and implemented in the past in South Africa, but is no longer used due to environmental concerns and the re-occurrence of the disease after treatment was stopped. New antibacterial compounds have been discovered in recent years, and a number of compounds exist which stimulate the systemically acquired resistance of plants (SAR). These may have an effect on Liberibacter infection and need to be assessed. In this study, a greenhouse trial, and possible subsequent field trial to test the efficiency of a harpin compound on the control of Laf is also planned. The effect of application of the compound on the bacterial concentration, symptoms of the disease and the health of the trees in general will be assessed.

Materials and methods

Samples

A total of 249 samples with greening-like symptoms were collected with the aid of French, Brazilian and South African experts from 57 orchards within most major citrus production areas in South Africa during an October-November 2006 collection trip. Total DNA extracts from each of these sources were used in this study. Samples throughout greening affected areas in South Africa are also submitted on an *ad hoc* basis for Liberibacter diagnosis to CRI@UP and some are included in this study.

DNA extraction and PCR

DNA extraction was by the CTAB extraction method of Doyle & Doyle (1990). Amplification was carried out in a 35 µl reaction mixture with the *rpl* A2 and *rpl* J5 (A2/J5) primer pair of Hocquellet *et al.*, 1999 (A2: 5' TAT AAA GGT TGA CCT TTC GAG TTT – 3' and J5: 5' AGA AAA GCA GAA ATA GCA CGA ACA A – 3'). This

PCR is capable of detecting both Laf and Las with only a relatively small difference in size of amplicons obtained for the two species. The PCR reaction was carried out for 35 cycles each consisting of 20 s at 92°C, 20 s at 62°C and 45 s at 72°C. The reagents used in the amplification were prepared according to the protocol of Hocquellet *et al.* (1999). The PCR products were electrophoresed in a 1% agarose gel at 100 V for 35 minutes in 1x Sodium boric acid (SB) electrophoresis buffer (0.2 M NaOH and 0.73 M boric acid, pH 8). 15 µl of sample was loaded into the wells after mixing 15 µl of sample with 2 µl of loading buffer. The size of the amplicon was determined with the aid of a molecular DNA marker (200 bp marker, Fermentas, Hanover, Md.) with 5 µl (20 ng) of the marker loaded on the gel. After electrophoresis the agarose gels were post-stained with EtBr-SB buffer (1 mg/ml) for 10 min, washed twice with water and viewed on an UV transilluminator (UVP, Model M-15). The DNA fragments were photographed using a digital camera mounted on a hood over the transilluminator (Vilber Lourmat, France).

Samples positive in the A2/J5 PCR were subjected to a further PCR using a nested system (Bastianel *et al.*, 2005), modified for local conditions, directed at the outer membrane protein gene (*omp*) for sequence analysis. The PCR reaction mixture contained: 25 µl 2x PCR Master Mix (Fermentas, Hanover, Md.), 1 µl template, 1 µl of 10 mM forward and reverse primer. The final volume of the reaction was 25 µl. The primers used in the first round of amplification were HP1inv and OMP8inv, while the primers used in the nested PCR reaction were Hp1inv seq and OMP8inv seq (Bastianel *et al.*, 2005). The thermal cycling programme involved a precycle at 92°C for 2 minutes followed by 35 cycles of denaturation (94°C, 1 minute), annealing (55°C, 1 minute) and extension (94°C, 2 minutes). The final extension cycle was set at 72°C for 10 minutes. The PCR was performed using the GeneAmp 2700 thermocycler (Applied biosystems, Warrington, United Kingdom). Control included a positive plant control, negative control (buffer) and a healthy plant control. Amplicons were visualized by electrophoresis and post staining as described above.

Sequence analysis

To determine if variation occurred in the A2/J5 primer or *omp* (Hp1inv seq and OMP8inv seq) amplified regions, the PCR products were sequenced. Bands of the expected size were cut out of the gel and purified with the Wizard SV gel and PCR clean-up system (Promega, Madison, USA) according to the manufacturer's protocol. The purified products were subjected to electrophoresis to determine the concentration of the amplicon for sequencing. The reaction mixture used for sequencing contained: 2 µl Big Dye v3.1 (Applied Biosystems, Warrington, United Kingdom), 1 µl sequencing buffer (Applied Biosystems, Warrington, United Kingdom), 1 µl of 3.2 pmol primer, 100 ng template and molecular grade water (Sigma, Missouri, USA) up to 10 µl. The thermal cycling programme consisted of an incubation step at 94°C for 1 minute followed by 25 cycles of denaturation (94°C, 10 seconds), annealing (50°C, 5 seconds) and extension (60°C, 4 minutes). Sequencing was done in the forward and the reverse orientations with the primers used for amplification. Precipitation of the sequencing reactions was performed at Inqaba Biotech using column-based purification (ZR-96 DNA sequencing clean-up kitTM). Sequence analysis was conducted at the Inqaba Biotech sequencing facility using the ABI PRISM® 3100/3130 genetic analyser. Sequence trace files obtained were analyzed utilizing BLAST and DNAMAN (Lynnon Biosoft, Quebec, Canada).

Results and discussion

Determine variability of nucleic acid sequences within the ribosomal protein gene amplicons generated by rpl A2 and rpl J5 (A2/J5) primers of Laf isolates in South Africa

197 citrus sources out of 249 sources collected in 2006 have yielded amplicons in the conventional A2/J5 – GB1/GB3 multiplex PCR utilised for Laf, Las and Lam detection. Of these 112 were successfully used as templates in 2007 to generate further amplicons for un-replicated forward- and reverse-primer mediated direct sequencing reactions using the *rpl* A2 and *rpl* J5 (A2/J5) primer pair of Hocquellet *et al.* (1999). Nucleotide sequence data was analysed using an automated sequencer at the sequencing facility of UP or at Inqaba, Pretoria. Analysis of sequence data generated from all sources in 2008 indicates the presence of only "Ca." *L. africanus*. Sequence data from 84 sources were of sufficient quality that forward and reverse consensus regions could be established. Multiple alignment of these sequences during the current report period suggests that all local sources were essentially identical. The small amount of variation existing within this region may reflect true differences in single nucleotides amongst sources but may also be due to errors in bases introduced through PCR amplification or sequencing amplification. Studies to characterize these differences fully were not considered worthwhile as differences were very small but they would require cloning of PCR amplicons and sequencing of at least three clones. Lower amplicon yielding sources, did not yield greater sequence variability as expected and lower yields obtained are therefore not likely to be due to less efficient primer/template binding associated with nucleotide changes. None of the citrus sources were infected with LafC, formerly known to occur in *Calodendrum capensis* in the Western Cape, and it is possible that this subspecies does not play any role in citrus.

An article detailing the above aspects of this study has been submitted for publication in a peer-reviewed journal, and this aspect of the study is now concluded. Plants inoculated with the local *Liberibacter* sources, and maintained since 2006 were tested using a real-time PCR technique (established through THRIP additionality funding). A total of 50 samples yielded positive results, albeit often with high crossover threshold (C_t) values indicative of very low target template concentrations. Plants with low *Liberibacter* concentrations will be re-tested to determine if concentrations will rise.

Establishment of PCR to the outer membrane protein (omp) gene, and generation of sequence information to this region

PCR directed at the outer membrane protein (*omp*) gene, known to be variable in *Laf* (Bastianel *et al.*, 2005) was utilized to identify possible sequence variants amongst local *Laf* sources, following discovery of the lack of variability in the A2/J5 primer amplicons region of the ribosomal protein gene. This particular PCR had numerous problems initially and required significant time to modify, optimize and get it to work consistently. Ultimately, modified primers and completely different PCR reagents (see detail presented under material and methods) were required to get the PCR to work against the South African DNA samples. Total DNA extracts from known *Laf* infected sources (as tested by the A2/J5 system) could then be used as templates from which amplicons could be obtained. Twenty-four (24) samples yielded no amplicon even though they had yielded amplicon with the A2/J5 primers. While 74 samples did yield amplicons with the *omp* PCR, yields were low and PCR on these samples had to be repeated in replicate in order to obtain sufficient amplicons for sequencing. These were then purified and subjected to direct sequencing. A total of 45 useful sequences with the OMP8inv seq (forward) and 20 useful sequences using the HP1inv seq (reverse) primers were generated. Multiple alignments performed on the 45 forward sequences (with the OMP8inv seq primer) showed no sequence variation.

Design primers for PCR to target newly sequenced region of Laf, establish and optimise the resulting PCR and generate sequence data for this region of the genome.

RNA polymerase B' subunit (*rpoB*) locus sequence data generated from *Laf* source UPCRI 06-0071 by Dr. Hong Lin, USDA, supplied to him in terms of a collaborative agreement has been deposited at Genbank (EF438154). Primers were designed and synthesized in order to establish PCR to the new (*rpoB*) sequence region of the *Laf* genome. Establishment and optimisation of the relevant PCR to use for variability studies in this portion of the genome could not be conducted due to the premature termination of the PhD studies of the student involved. DNA of a further 20 sources of *Laf* representing 4 widely separated collection sites and 5 samples within each site were sent to Dr. Lin for further sequence studies. Variant specific PCR/real-time PCR will be developed if sequence variants are obtained.

Glasshouse and Field trials to assess effect of harpin-like compound on Laf concentration in treated plants

Laf real-time PCR test (established as part of additionality THRIP-funded tasks), but required for this aspect of the project, has been shown to work reliably. This technique uses Taqman probe HLBpr and primers HLBafr/HLBr (Li *et al.*, 2006) and was modified from the published multiplex PCR in order to specifically detect *Laf*. A field trial site for the harpin-treatment studies was identified near Nelspruit, and *Liberibacter* infection of trees confirmed by PCR. The premature termination of the PhD studies of the student involved, have prevented further studies on this aspect in the report period, and as this aspect was specifically introduced as part of her studies, it will not be continued with.

Challenge putatively Laf-resistant plants with various Liberibacter strains

As no sequence variability has been found amongst local "*Ca.*" *Liberibacter africanus* sources, there has been no need to challenge infected plants with sources other than those routinely used (field collected *Trioza erytaeae* from greening infected lemon orchards in Nelspruit).

Further objectives (milestones) and work plan

1. *Determine variability of nucleic acid sequences of various parts of Laf genome*
Finalize studies on *omp* gene sequence data generation and analysis. Write publication.
2. *Screen embryo-rescued plants for resistance by challenging them with Laf variants*
Obtain a pure source (lacking CTV) of a representative source of *Laf*. Propagate. Do graft transmission and/or controlled *Trioza erytaeae* transmission challenge on embryo-rescued, putative resistant lines. Test challenged plants for *Laf* using quantitative real-time PCR (developed in THRIP project).
3. *Screen for the presence of phytoplasma in plants with greening-like symptoms testing negative for Laf*
Establish and modify phytoplasma universal real-time PCR. Test samples previously negative for *Laf* by conventional PCR with real-time PCR. Screen samples which were real-time and conventional

PCR negative for “Ca.” *L. africanus* for phytoplasma. Determine if phytoplasma, if found, were transmitted to plants in greenhouse. Discard all negative plants.

Technology transfer

Scientific articles:

Doddapaneni, H., Liao, H., Lin, H., Bai, X., Zhao, X., Civerolo, E.L., Irey, M., Coletta-Filho, H., and Pietersen, G., 2008. Comparative phylogenomics and multi-gene cluster analyses of the Citrus Huanglongbing (HLB)-associated bacterium *Candidatus Liberibacter*” BMC Research Notes 1(75).

Pietersen, G., Arrebola, E., Bové, J.-M., Breytenbach, J.H.J., Korsten, L., Le Roux, H.F., La Grange, H., Lopes, S.A., Meyer, J.B., Pretorius, M.C., Schwerdtfeger, M., Van Vuuren, S.P., and Yamamoto, P. 2009. Survey for “*Candidatus*” *Liberibacter* species in South Africa confirms the presence of only *Ca. L. africanus* in commercial Citrus. (Submitted to Plant Disease).

Presentations:

Pietersen, G., Schwerdtfeger, M., and Kotze, A.C., 2008. Survey for *Candidatus liberibacter* species on citrus in South Africa. 5th Citrus Research Symposium, Champagne Sports Resort, Drakensberg, 3-6 August, 2008.

Pietersen, G., Kotze, A., Phahladira, M.N.B. and Schwerdtfeger, M. 2008. Survey for “*Candidatus*” *Liberibacter* species in South Africa. HLB International Conference, Orlando, Florida, USA 1-5 December, 2008.

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Li, W., Hartung, J.S., and Levy, L.. 2006. Quantitative real-time PCR for detection and identification of *Candidatus Liberibacter* species associated with citrus Huanglongbing. *J. Microbiol. Meth.* **66**:104-115.

4.2.15 PROGRESS REPORT: Epidemiology of greening disease – seed transmission

Experiment 967 (April 2008 – April 2010): by Gerhard Pietersen (CRI-UP) and Fanie van Vuuren (CRI)

Summary

Citrus greening is a destructive disease of citrus and is caused in South Africa by a fastidious bacterium “*Candidatus Liberibacter africanus*” (Laf). Unpublished, preliminary reports emanating from two laboratories in the United States contend that the related bacterium “*Candidatus Liberibacter asiaticus*” (Las) may be transmissible by seed. In order to assess whether this may also be the case for Laf, fruit was collected from greening symptomatic branches, tested for Laf, the seeds removed and planted under insect-free conditions. The seedlings were monitored for symptoms and were tested by real-time PCR for Laf. 1570 seedlings were obtained from six different Citrus species or cultivars with greening symptoms. Tests for Laf will be conducted on any symptomatic seedlings observed on an ongoing basis.

Opsomming

Sitrus vergroening is 'n vernietigende siekte en word in Suid Afrika veroorsaak deur die onkweekbare bakterie, “*Candidatus Liberibacter africanus*” (Laf). Voorlopige, ongepubliseerde verslae vanuit twee onafhanklike laboratoriums in die VSA beweer dat die verwante bakterie “*Candidatus Liberibacter asiaticus*” (Las) is moontlik saadgedraag. Om te bepaal of dit ook die geval is met Laf, is vrugte vanaf takke met vergroening simptome wat vir Laf getoets is, gepluk, die saad verwyder en gegroei onder insek-vrye toestande. Die saailinge word deurgaans gemonitor vir simptome en word deur middel van “real-time” PKR

vir Laf getoets. 1570 saailinge is vanaf ses verskillende sitrus spesies of kultivars met vergroenings-simptome verkry en toetse vir Laf sal op enige simptomatiese plante op 'n deурlopende basis gedoen word.

Introduction

Citrus greening is a destructive disease of citrus and is caused in South Africa by a fastidious bacterium "*Candidatus Liberibacter africanus*" (Laf). Unpublished, preliminary reports emanating from two laboratories in the United States contending that the related bacterium "*Candidatus Liberibacter asiaticus*" (Las) is transmissible by seed have prompted the USDA to prohibit the movement of seed from Florida (where the disease occurs) to other citrus-growing states in the USA. Without scientific evidence to this effect, the USA has extended such restrictions on other countries having the Liberibacter species other than Las, including South Africa. Should this restriction be expanded to fruit, this will have severely deleterious consequences in terms of market access for the citrus export industry. It is therefore imperative that tests be conducted to determine whether Laf is seed-transmitted. In this project, fruit from Laf infected branches of five citrus species will be collected and the seed harvested. Growing-on tests will be done and seedlings tested for the bacteria by real-time PCR.

Materials and methods

Sampling

Fruit of various Citrus species and cultivars were collected from branches displaying greening symptoms. Leaf and petiole tissue was taken from the same branches and submitted for Laf-specific PCR tests in order to confirm the presence of Laf.

DNA extraction and PCR

DNA extraction was by the CTAB extraction method of Doyle & Doyle (1990), using petiole and midrib tissue from plants as starting material. Through supplemental matching funding of THRIP, a real-time thermocycler was purchased and a published real-time PCR (Li *et al.*, 2006) modified for *Ca. L. africanus*-specific detection by using Taqman probe HLBpr and primers HLBafr/HLBr. DNA extracts (0.5 µl) were used as templates after being diluted 1:10 in distilled water.

Preparation of seed and planting

Seed were removed from fruit and categorized as either normal-looking or abortive. Seeds were planted in seedling trays and grown under insect-free conditions in the tunnel at CRI, Nelspruit, under natural light conditions. Seedlings were transplanted into small planting bags once sufficiently developed to do so.

Results and discussion

Seeds were collected from six different Citrus species or cultivars (Table 4.2.15.1), often from different source trees. Each tree, fruit from the tree, seeds and seedlings were treated as separate samples. Table 4.2.15.1 describes the number of seedlings obtained from each greening source, the category of seed and real-time PCR test results on the original plant leaf material. In total, 1570 seedlings were obtained. A batch of 66 samples (each of 4 pooled seedlings) were submitted for real-time PCR testing (accession numbers 08-0298 through to 08-0363) but DNA extracts could not be prepared in the period directly after harvesting the leaves due to other commitments and it was decided to re-harvest leaves of these samples at a later date. The delay proved fortuitous as new information gleaned by attending (G. Pietersen) the International Huanglongbing (HLB) Conference, 1-5 December, 2008, held in Orlando, Florida suggested that pooling of samples would have rendered the tests meaningless. A number of research groups in the US presented data on seed transmission experiments of Las during the conference. Results obtained were ambiguous, with real-time PCR results suggestive of extremely low concentrations of Las in the seedlings and a lack of clear HLB symptoms obtained in any of the seedlings. The general consensus is that seed-transmission is still not proven, and in the author's opinion, contamination of seedlings from infected mother tissue is not completely eliminated. However, some groups are proposing a theory that some unknown component of what is now being thought of as the "Las complex" is being seed transmitted. The apparent extremely low concentration of Las detected in seedlings are at the detection limit of the real-time PCR technique utilised, and pooling of samples, containing some potentially healthy would potentially dilute any positive samples to below detection thresholds. As it is likely that this would also hold true for Laf, pooling of samples for real-time PCR tests can therefore not be performed. As individual tests on all 1570 seedlings in this study is not feasible, a change in the methodology of this project was implemented whereby seedlings are grown out over a much longer period under vector-free conditions and monitored for symptoms. Only plants with greening-like symptoms will then be analysed by real-time PCR for Laf. Currently some seedlings are showing some abnormalities (but may be due to the seedlings being heterozygotes) and these will be tested for Laf.

Conclusion

At this stage no conclusions can be made regarding seed transmissibility of Laf.

Technology transfer

None.

Further objectives (milestones) and work plan

Maintain seedlings under vector free conditions. Monitor plants for greening-like symptoms. Test individual symptomatic seedlings for Laf on an ongoing basis by real-time PCR.

References cited

None.

Table 4.2.15.1. Summary of fruit samples collected, source tree cultivar, PCR results and seeds and seedlings derived for seed transmission studies on “Ca.” *Liberibacter africanus*.

Source cultivar	PCR #	PCR Result	# of Seed		# of Seedlings					
			Normal	Abortive	Min	Prem	Clan	Troy	Lem	Olin
Min	08-0176	+	2	18	6					
	08-0177	+	0	0	0					
	08-0178	+	8	0	0					
	08-0181	+	0	8	12					
Prem	08-0184	+	26	32		40				
	08-0186	+	36	27		32				
Clan	08-0189	+	10	77			39			
	08-0190	+	45	196			83			
Troy	08-0192	-	70	7				110		
	08-0193	-	89	12				159		
	08-0194	-	45	3				57		
	08-0195	-	27	14				53		
	08-0196	-	55	0				70		
	08-0197	-	386	18				468		
Lem	08-0198	+	74	1					61	
	08-0199	+	6	0					7	
Olin	08-0201	+	80	66						96
	08-0202	+	14	66						48
	08-0203	+	12	19						26
	08-0204	+	7	61						28
	08-0205	+	7	25						5
	08-0206	+	29	14						43
	08-0207	+	51	30						56
	08-0208	+	11	63						32
	08-0209	+	8	44						27
	08-0210	+	5	42						12
TOTAL					18	72	122	917	68	373

Cultivars: Min = Minneola tangelo; Prem = Premier midseason sweet orange; Clan = Clanor midseason sweet orange; Troy = Troyer citrange; Lem = Eureka lemon; Olin = Olinda Valencia sweet orange.

4.3 **PROJEK: VRUG- EN BLAARSIEKTES** Projek Koördineerder: G.C. Schutte (CRI)

4.3.1 **Projekopsomming**

Resultate van Alternaria bruinvlek proewe wat in die somerreënvalstreek uitgevoer is toon dat drie strobilurin en minerale spuitolie bespuitings met óf mancozeb óf Sporekill, goeie beheer van Alternaria bruinvlek gegee het wat kwekers 2 spuitronddes kan spaar. Beide die standaard maandelikse toedienings (8 in totaal) van koperhidroksied en mancozeb het goed teen Alternaria bruinvlek gevaar. 'n Nuwe suspensie konsentraat formulاسie van kopersulfaat (Coflo Super) het goed teen dosisse van 270 en 540 ml/100 l water presteer. In 'n nuwe benadering waarin daar gekyk word na die afwisseling van koperhidroksied met 'n fosfonaat plus Sporekill (twee-maandeliks x 4 toedienings), het die programme uitstekend vir die beheer van Alternaria bruinvlek gewerk. Proewe is in twee verskillende boorde gedoen en die resultate was dieselfde. Alhoewel die fosfonaat op sy eie (sonder Sporekill) ook goed gewerk in die program het, is ons huiwering om dit so aan te beveel as 'n enkele bespuiting. Hierdie programme waar koperswamdoders in plaas van mancozeb gebruik word, sal geskik wees vir boere in die Wes-Kaap omrede hulle nie mancozeb vir vrugte wat na die VSA toe uitgevoer word, mag gebruik nie (4.3.2)..

Aanvanklik is probleme met weerstasies ondervind wat gebruik word om Alternaria siektevoorspelling te doen, en is op 'n laat stadium waartydens die proef uitgevoer is, uitgesorteer. Die siektevoorspelling is ook nie met die ware voorkoms van die siekte in die veld gekontroleer waar die weerstasie geïnstalleer was nie en die proef sal dus herhaal moet word alvorens die model kommersialiseer kan word (4.3.3).

Sitrus blaar- en vrugsiektes word meestal deur hoë-volume swamdoderspuitte beheer. Hierdie spuite lei meestal tot groot mates van afloop. Aanvanklike resultate dui duidelik daarop dat kwantitatiewe en kwalitatiewe bedekking asook biologiese effektiwiteit van spuite afneem met toenemende afloop. Navorsing in hierdie projek sal dus op optimisering van spuittoediening fokus om voldoende bedekking met minimale afloop te verseker. Konvensionele en nuwe-tegnologie spuitmasjiene is in verskeie boordproewe evalueer. Die voorlopige resultate van boordproewe dui daarop dat die hoogste kwantitatiewe bedekking teen die beste uniformiteit tussen blare met hoër spuitvolumes behaal is. Desnieteenstaande moet dit beklemtoon word dat die fluoriserende pigmentdosis dieselfde was met vergelyking van verskillende spuitmasjiene en kalibrasie-verstellings teen verskillende spuitvolumes. Dieselfde of selfs beter spuitbedekking kon dus deur die optimale gebruik van masjiene of deur meer effektiewe masjiene behaal word, veral as die dosis per hektaar gestandariseer word. Spuitproewe moet herhaal word en veral die drempelwaardes vir biologiese effektiwiteit moet bepaal word om die interpretasie van resultate te ondersteun (4.3.4).

Project summary

Results from a field trial conducted in the summer rainfall region showed that three applications of strobilurins and mineral spray oil with either mancozeb or Sporekill, gave good control of Alternaria brown spot hereby saving growers 2 spray rounds. Both the standard copper hydroxide and mancozeb spray programmes sprayed at monthly intervals (8 applications), performed well at registered rates against Alternaria brown spot. A new suspension concentrate formulation of copper sulphate (Coflo Super) performed well at rates of 270 and 540 ml/100 l water. Excellent control of Alternaria brown spot was obtained in a new approach where copper hydroxide was alternated with a phosphonate plus Sporekill (every second month x 4 applications). Although the phosphonate performed well on its own without Sporekill as part of the tank mixture, are we reluctant to recommend it as a stand-alone treatment at this stage. The programmes where copper fungicides, instead of mancozeb, were tested, will be favoured by the growers in the Western Cape because they are prohibited from exporting mancozeb-treated fruit to the USA (4.3.2).

Initial problems experienced with weather stations required to conduct Alternaria disease forecasting were solved at a late stage of the experiment. The disease forecasts were also not validated in accordance with the biological infections in the orchard where the weather station was installed. The trial should be repeated before we commercialise the model (4.3.3).

Fruit and foliar diseases of citrus are mostly controlled by means of high volume fungicide application, often leading to excessive levels of run-off. However, it was clear that quantitative and qualitative deposition as well as biological efficacy declined with increased run-off. Research in this experiment focuses on optimising application to ensure adequate deposition of the active ingredient with minimal run-off. Conventional and novel spray machines were evaluated in several orchard trials. From the results obtained to date from orchard spray trials, it was clear that the highest quantitative deposition per leaf at the best uniformity between leaves was generally obtained with higher spray volumes. However, a fluorescent pigment dosage

of 1× was used when comparing all the different sprayers and calibration settings, even though spray volumes differed. Hence, the dosage per hectare differed substantially between treatments. Similar and even improved spray deposition can thus be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers, especially if dosage per hectare is equated between treatments. Orchard spray trials must be repeated and especially the benchmarks for biological efficacy need to be satisfactorily determined to support conclusive interpretation of the results (4.3.4).

4.3.2 **PROGRESS REPORT: Evaluation of new spray programmes for the control of *Alternaria* brown spot in the summer rainfall regions of South Africa**

Experiment 750 (September 2004 – June 2010) by G.C. Schutte and C. Kotze (CRI)

Opsomming

Drie behandelings bestaande uit drie strobilurin en minerale spuitolie met óf mancozeb óf Sporekill, het goeie beheer van *Alternaria* bruinvlek gegee wat kwekers 2 spuitronddes kan spaar. Beide die standaard maandelikse toedienings (8 in totaal) van koperhidroksied en mancozeb het goed gevaar teen *Alternaria* bruinvlek. 'n Nuwe suspensie konsentraat formulاسie van kopersulfaat (Copflo Super) het goed presteer teen dosisse van 270 en 540 ml/100 l water. In 'n nuwe benadering waarin daar gekyk word na die afwisseling van koperhidroksied met 'n fosfonaat plus Sporekill (twee-maandeliks x 4 toedienings), het die programme uitstekend gewerk vir die beheer van *Alternaria* bruinvlek. Proewe is in twee verskillende boorde gedoen en die resultate was dieselfde. Alhoewel die fosfonaat op sy eie (sonder Sporekill) ook goed gewerk het in die program, sal dit weer getoets moet word om te kyk of dieselfde tipe resultate verkry kan word. Hierdie programme waar koperswamdoders in plaas van mancozeb gebruik word, sal geskik wees vir boere in die Wes-Kaap omrede hulle nie mancozeb vir vrugte wat na die VSA toe uitgevoer word, mag gebruik nie.

Summary

Three applications consisting of three strobilurins and mineral spray oil with either mancozeb or Sporekill, gave good control of *Alternaria* brown spot hereby saving growers 2 spray rounds. Both the standard copper hydroxide and mancozeb spray programmes sprayed at monthly intervals (8 applications), performed well at registered rates against *Alternaria* brown spot. A new suspension concentrate formulation of copper sulphate (Copflo Super) performed well at rates of 270 and 540 ml/100 l water. In a new approach where copper hydroxide was alternated with a phosphonate plus Sporekill (every second month x 4 applications), control of *Alternaria* brown spot was excellent. Although the phosphonate performed well on its own without Sporekill as part of the tank mixture, this spray programme will need to be re-tested to establish whether we can obtain the same results. The programmes where copper fungicides, instead of mancozeb, were tested, will be favoured by the growers in the Western Cape because they are prohibited from exporting mancozeb-treated fruit to the USA.

Introduction

Alternaria brown spot (ABS) is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa. Susceptibility to ABS is a dominant trait that is transferred from 'Dancy' mandarin to its progeny (Dalkilic *et al.*, 2005). Dancy mandarin hybrids and some cultivars of unknown origin, such as 'Murcott', 'Emperor' and 'Ponkan', are affected by the disease. The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with Dancy tangerine such as the 'Nova', 'Minneola' and 'Mor'.

The causal agent of ABS was designated originally as *Alternaria citri* Ellis & N. Pierce (Pegg, 1966) and later renamed *A. alternata* (Fr.:Fr.) Keissl. pv. *citri*, based on the production of a toxin specific to mandarin fruit (Solel, 1991). Later, eight species were described among *Alternaria* isolates pathogenic to mandarins based on morphological and biochemical traits (Andersen *et al.*, 2005; Simmons, 1999). However, all small-spored *Alternaria* spp. from citrus are closely related by molecular analysis and they have been placed into a single phylogenetic species, *A. alternata* (Peever *et al.*, 2004 & 2005).

ABS attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. ABS sporulates most abundantly on lesions on mature leaves remaining in the canopy (Reis *et al.*, 2006) The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop and twig dieback. On more mature fruit, lesions may vary from small necrotic spots to large, sunken pockmarks. Leaves are susceptible until they are fully expanded and hardened. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit that are unacceptable to the consumer. Leaves are susceptible to infection from the time of formation until they are fully expanded

and hardened, and fruit are susceptible from petal fall until harvest. In the USA, however, fruit are only susceptible from petal fall until they reach about 5 cm in diameter.

Cultural measures, such as wider tree spacing and pruning to allow air movement and dry-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards. However, fungicide applications are essential for disease control and production of blemish-free fruit. In South Africa, it is important to protect fruit and flushes of cultivars such as tangerines and their hybrids with fungicides from September to April/May, often requiring 8+ spray applications. This number of sprays and the products being used are not economically sustainable and may result in unacceptable residues on fruit. Our aim is to evaluate the three strobilurins using three applications only with 8-week intervals during the high disease pressure period from October to January to establish whether less fungicides can be used and that fungicides like the strobilurins with longer lasting residues for the control of brown spot rather be used. Also to evaluate new copper hydroxide WG formulations as well as a new SC mancozeb formulation will also be evaluated.

Materials and methods

Ten single-tree plots per treatment were randomly selected in a 'Nova' orchard at Belmont 50 km west of Nelspruit. The trees were 16 years old and planted in 2x5 m tree spacing in rows that ran from North to South. Trees were selected for uniformity in canopy density and tree size. Guard trees were located between plots within rows. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2500-3000 kPa) sprayer with two hand-held spray guns on the dates mentioned in Table 4.3.2.1. and Table 4.3.2.2. The weather was fine and dry on all occasions with minimal wind. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. At fruit maturity in June, *Alternaria* brown spot severity was rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no brown spot lesions; 1 = one to five brown spot lesions per fruit; and 2 = six and more brown spot lesions per fruit. Data were analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

Results and discussion

Strobilurins and spray oil in tank mixtures with either mancozeb or Sporekill

Three strobilurin applications sprayed at 60-day intervals in a spray programme preceded with Copstar and Sporekill and ended with 2 more mancozeb treatments gave good control of *Alternaria* brown spot (Table 4.3.2.4). Results showed that tank mixtures of strobilurins with mineral spray oil with either mancozeb or Sporekill, all performed well in the control of *Alternaria* brown spot. There were no significant differences ($P < 0.05$) between any of these spray programmes (6 applications in total) and the standard mancozeb and Copstar (8 applications in total) treatments with regards to the amount clean exportable fruit with no *Alternaria* brown spot lesions. All these treatments resulted between 91.4 and 97 % clean exportable fruit. The same applies for the other two criteria showing that there were no significant differences between these treatments. The only exception was the Cabrio treatment that had significant more *Alternaria* brown spot of fruit with 1 – 5 brown spot lesions than the mancozeb and the Flint, mancozeb and oil spray programme. The disease pressure was high as the untreated control resulted only in 48.6% clean exportable fruit (Table 4.3.2.4).

Copflo Super (Copper sulphate)

Results from both field trials conducted at Belmont and at Eugene Kruger (Table 4.3.2.5) showed that there were no significant differences ($P < 0.05$) between the standard registered mancozeb (except for the Eugene Kruger field trial) and Copstar (copper hydroxide) treatments and Copflo Super. In both field trials, the 2x rate of Copflo Super only resulted in about 3% more clean exportable fruit. The trees of Eugene Kruger is generally in a poor condition and the disease pressure was also extremely high during the previous year and was carried over to the new season resulting in only 14.4% clean exportable fruit. The standard mancozeb treatment resulted in only 80.6% clean exportable fruit which could be ascribed due to wash-off from rain following one of the applications. With regards to the criteria fruit with one to five *Alternaria* brown spot lesions fruit with six and more *Alternaria* brown spot lesions, showed that there were no significant differences between the treatments (table 4.3.2.4.).

Alternating copper hydroxide and copper sulphate with Fighter and Sporekill

Results from the field trial where this new concept was tested at Belmont and Eugene Kruger (Tables 4.3.2.3 & 4.3.2.6) show that there were no significant differences ($P < 0.05$) between the standard registered

mancozeb and Copstar treatments and all the alternated spray programmes where the phosphonates (with or without Sporekill) were sprayed bi-monthly for the control of *Alternaria* brown spot. In those tank mixtures where Sporekill was added to Fighter rates of 570 ml and 400 ml/100 l water, Sporekill contributed 4.6 and 0.2% more clean fruit, respectively. No significant differences were observed in both field trials where Copflo Super was sprayed instead of Copstar in the spray programmes with Fighter (400 ml/100 l water) and Sporekill.

Conclusion

Three strobilurin and mineral spray oil applications in tank mixtures with either mancozeb or Sporekill followed with two mancozeb treatments sprayed with 60 day intervals, gave good control of *Alternaria* brown spot versus eight of the other spray programmes that consisted mainly of contact fungicides. This type of spray programme will save the growers two spray rounds if compared with the contact/preventative type of spray programme that one has to spray with monthly intervals. Sporekill can be recommended as a replacement for mancozeb. As the strobilurins have a systemic or local systemic mode of action, their long lasting residual action play an important role for the good fungicidal action against *Alternaria* brown spot (Häuser-Hahn, Pontzen & Baur, 2003). Furthermore, the strobilurin, Flint, has a mesostemic mode of action whereby it has a high affinity for the plant's waxy layer and is thus stored there very effectively. This results in a fungicide reservoir from which the active ingredient penetrates continuously into the deep-lying tissue of the plant. Due to this reservoir, a continuous protective effect is exerted against fungal attack (Krieg, Weile & Göhlich, 2003). A late April application of mancozeb will be necessary for future trials if these types of spray programmes will be registered in the future.

Both the registered copper hydroxide and mancozeb spray programmes, sprayed at monthly intervals, performed well at registered rates against *Alternaria* brown spot; Copflo Super at 270 and 540 ml/100 l (1x and 2x) also performed well. As expected, some copper stippling was observed at the 2x or 570 ml/100 l rate. This can be overcome if copper fungicides are alternated with mancozeb.

With the new concept of alternating copper fungicides with phosphonates plus DDAC's, will the growers not only benefit from foliar disease control such as *Alternaria*, but also *Phytophthora* root rot and brown rot at the same time. The bi-monthly applications of the phosphonates, which started in September, coincides with the root rot recommendations (Schutte *et al.*, 1991). Boosting the phosphonates (which was sprayed at 400 ml/100 l water instead of 570 ml/100 l water) with DDAC (Sporekill) proved successful as no phytotoxic problems were observed. In one field trial the DDAC contributed in 4.6% more clean exportable fruit. Phosphonates are cheap and can serve as an alternative to mancozeb especially in the Western Cape where growers are not permitted to export fruit treated with mancozeb to the USA. As the copper fungicides were alternated with other fungicides, no copper stippling occurred.

Future objectives (milestones) and work plan

Any new fungicides in spray programmes of different modes of action have to be evaluated in the new season.

Technology transfer

This research will be included in the annual research report to be distributed to citrus growers and will be included in various talks to citrus growers. It also formed part of the 2008 CRI Citrus Symposium in the Drakensberg.

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Table 4.3.2.1. Monthly application of different fungicides during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Belmont, near Schagen during 2007 and 2008.

19 September 2007	16 October 2007	13 November 2007	11 December 2007	8 January 2008	5 February 2008	5 March 2008	2 April 2008
Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g
Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml
Kontrole	Kontrole	Kontrole	Kontrole	Kontrole	Kontrole	Kontrole	Kontrole
Copstar 350 ml	Fighter 570ml	Copstar 350 ml	Fighter 570ml	Copstar 350 ml	Fighter 570ml	Copstar 350ml	Mancozeb 200g
Copstar 350 ml	Fighter + Sporekill 570ml +100ml	Copstar 350 ml	Fighter + Sporekill 570ml+100ml	Copstar 350 ml	Fighter +Sporekill 570ml+100ml	Copstar 350ml	Mancozeb 200g
Copstar 350 ml	Fighter + Sporekill 400ml +100ml	Copstar 350 ml	Fighter + Sporekill 400ml+100ml	Copstar 350 ml	Fighter +Sporekill 400ml+100ml	Copstar 350ml	Mancozeb 200g
Copstar 350 ml	Fighter 400ml	Copstar 350 ml	Fighter 400ml	Copstar 350 ml	Fighter 400ml	Copstar 350ml	Mancozeb 200g
Copflo Super 270 ml	Fighter + Sporekill 400ml +100ml	Copflo Super 270 ml	Fighter + Sporekill 400ml +100ml	Copflo Super 270 ml	Fighter + Sporekill 400ml +100ml	Copflo Super 270 ml	Mancozeb 200g
Copflo Super 270 ml	Fighter + Sporekill 570ml +100ml	Copflo Super 270 ml	Fighter + Sporekill 570ml +100ml	Copflo Super 270 ml	Fighter + Sporekill 570ml +100ml	Copflo Super 270 ml	Mancozeb 200g
Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml
Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml

Table 4.3.2.2. Monthly application of different strobilurin fungicides in tank mixtures with either mancozeb or Sporekill during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Belmont, near Nelspruit in S.A. during 2007 and 2008.

19 September 2007	16 October 2007	27 November 2007	8 January 2008	19 February 2008	18 March 2008
Mancozeb 200g	Cabrio +oil+Sporekill 10ml+250ml+100ml	Cabrio +oil+Sporekill 10ml+250ml+100ml	Cabrio +oil+Sporekill 10ml+250ml+100ml	Mancozeb 200g	Mancozeb 200g
Copstar +Sporekill 300ml+100ml	Cabrio +Mancozeb+Oil 10ml+150g+250ml	Cabrio +Mancozeb+Oil 10ml+150g+250ml	Cabrio +Mancozeb+Oil 10ml+150g+250ml	Mancozeb 200g	Mancozeb 200g
Copstar +Sporekill 300ml+100ml	Flint +oil+Sporekill 10g+250ml+100ml	Flint +oil+Sporekill 10g+250ml+100ml	Flint +oil+Sporekill 10g+250ml+100ml	Mancozeb 200g	Mancozeb 200g
Copstar +Sporekill 300ml+100ml	Flint +Mancozeb+Oil 10g+150g+250ml	Flint +Mancozeb+Oil 10g+150g+250ml	Flint +Mancozeb+Oil 10g+150g+250ml	Mancozeb 200g	Mancozeb 200g
Copstar +Sporekill 300ml+100ml	Ortiva+oil+Sporekill 20ml + 250ml + 250ml	Ortiva+oil+Sporekill 20ml + 250ml + 250ml	Ortiva+oil+Sporekill 20ml + 250ml + 250ml	Mancozeb 200g	Mancozeb 200g
Copstar +Sporekill 300ml+100ml	Ortiva+Mancozeb+Oil 20ml + 150g + 250ml	Ortiva+Mancozeb+Oil 20ml + 150g + 250ml	Ortiva+Mancozeb+Oil 20ml + 150g + 250ml	Mancozeb 200g	Mancozeb 200g

Table 4.3.2.3. Monthly application of different fungicides during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Eugene Kruger, Alkmaar during 2007 and 2008.

19 September 2006	24 October 2006	21 November 2006	19 December 2006	16 January 2007	13 February 2007	12 March 2007	9 April 2007
Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g
Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml	Copstar 350ml
Kontrole	Kontrole	Kontrole	Kontrole	Kontrole	Kontrole	Kontrole	Kontrole
Copstar 350 ml	Fighter + Sporekill 400ml +100ml	Copstar 350 ml	Fighter + Sporekill 400ml+100ml	Copstar 350 ml	Fighter +Sporekill 400ml+100ml	Copstar 350ml	Mancozeb 200g
Copflo Super 270 ml	Fighter + Sporekill 400ml +100ml	Copflo Super 270 ml	Fighter + Sporekill 400ml +100ml	Copflo Super 270 ml	Fighter + Sporekill 400ml +100ml	Copflo Super 270 ml	Mancozeb 200g
Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml	Copflo Super 270ml
Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml	Copflo Super 540ml

Table 4.3.2.4. Evaluation of strobilurins and mancozeb in tank mixtures with either mineral spray oil or Sporekill during the high disease pressure period from October for *Alternaria* brown spot control on 'Novas' from September to April during 2007 and 2008 for the control of *Alternaria alternata* on 'Nova' mandarins at Belmont, Schagen.

Treatment	Concentration (g/ml product/100 litre water)	Percentage of fruit in each class ^x		
		Lesions/fruit		
		0	1-5	≥6
Mancozeb ^y	200 g	97.0 a	2.2 a	0.8 a
Flint+mancozeb+oil ^z	10 g+150 g+250 ml	95.2 a	3.0 a	1.8 a
Copstar ^y	350 ml	94.6 a	3.6 ab	1.8 a
Ortiva+mancozeb+oil ^z	20 ml+150 g+250ml	93.4 a	5.0 ab	1.6 a
Ortiva+Sporekill+oil ^z	10 g+100 g+100 ml	93.2 a	4.8 ab	2.0 a
Flint+Sporekill+oil ^z	20 ml+100 g+100 ml	92.6 a	5.4 ab	2.0 a
Cabrio+mancozeb+oil ^z	10 ml+150 g + 250ml	91.8 a	7.2 b	3.6 a
Cabrio+Sporekill+oil ^z	10 ml+100 g+100 ml	91.4 a	5.0 ab	3.6 a
Control		48.6 b	15.0 c	36.4 b

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 19 September 2007, 16 October 2007, 13 November 2007, 11 December 2006, 8 January 2008, 5 February 2008, 4 March 2008, 1 April 2008.

^z Spray dates were 16 October 2007, 27 November 2007 and 8 January 2008.

Table 4.3.2.5. Evaluation of Copflo Super during the high disease pressure period from October for *Alternaria* brown spot control on 'Novas' from September to April during 2007/2008 for the control of *Alternaria alternata* on 'Nova' mandarins at Belmont, Schagen and at Eugene Kruger, Alkmaar.

Treatment ^y	Concentration (g/ml product/100 litre water)	Belmont, Schagen			Eugene Kruger, Alkmaar		
		Percentage of fruit in each class ^x			Percentage of fruit in each class ^x		
		Lesions/fruit			Lesions/fruit		
		0	1-5	≥6	0	1-5	≥6
Mancozeb	200 g	97.0 a	2.2 a	0.8 a	80.6 b	9.8 a	9.6 a
Copstar	200 g	94.6 a	3.6 ab	1.8 a	90.4 a	5.2 ab	4.4 a
Copflo Super	540 ml	96.0 a	3.2 a	1.0 a	93.8 a	3.6 b	2.6 a
Copflo Super	270 ml	93.0 a	6.2 ab	7.6 a	90.4 a	6.4 ab	3.2 a
Control		48.6 b	15.0 c	36.4 b	14.4 c	8.2 ab	77.4 b

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 19 September 2007, 16 October 2007, 13 November 2007, 11 December 2006, 8 January 2008, 5 February 2008, 4 March 2008, 1 April 2008.

Table 4.3.2.6. Evaluation of four Copstar (copper hydroxide) and Copflo Super (copper sulphate) alternated with four Fighter and Sporekill applied as bi-monthly applications during the high disease pressure period from September to April 2007/2008 for the control of *Alternaria* brown spot control on 'Novas' at Belmont, Schagen and at Eugene Kruger, Alkmaar.

Treatment ^y	Concentration (g/ml product/100 litre water)	Belmont, Schagen			Eugene Kruger, Alkmaar		
		Percentage of fruit in each class ^x			Percentage of fruit in each class ^x		
		Lesions/fruit			Lesions/fruit		
		0	1-5	≥6	0	1-5	≥6
Mancozeb	200 g	97.0 a	2.2 a	0.8 a	80.6 b	9.8 a	9.6 a
Copstar	350 ml	94.6 a	3.6 a	1.8 a	90.4 a	5.2 a	4.4 a
(Copstar alternated with Fighter) x 4	350 ml / 570 ml	90.8 a	5.8 a	3.4 a	ND	ND	ND
(Copstar alternated with Fighter + Sporekill) x 4	350 ml / 570 ml + 100 ml	95.4 a	1.8 a	2.8 a	ND	ND	ND
(Copstar alternated with Fighter) x 4	350 ml / 400 ml	94.6 a	4.2 a	1.2 a	ND	ND	ND
(Copstar alternated with Fighter + Sporekill) x 4	350 ml / 400 ml + 100 ml	94.8 a	3.6 a	1.6 a	90.0 ab	5.8 a	4.2 a
(Copflo Super alternated with Fighter+Sporekill) x4	270 ml / 570 ml + 100 ml	93.8 a	5.6 a	0.6 a	ND	ND	ND
(Copflo Super alternated with Fighter+Sporekill) x4	270 ml / 400 ml + 100 ml	93.0 a	5.8 a	1.2 a	91.6 a	5.6 a	2.8 a
Control		48.6 b	15.0 c	36.4 b	14.4 c	8.2 ab	77.4 b

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 19 September 2007, 16 October 2007, 13 November 2007, 11 December 2006, 8 January 2008, 5 February 2008, 4 March 2008, 1 April 2008.

4.3.3 PROGRESS REPORT: Evaluation of a Spanish prediction model for *Alternaria* brown spot of mandarins

Experiment 917 (September 2008 – June 2011) by G.C. Schutte and C. Kotze (CRI)

Opsomming

Die Metos weerstasie was eers operasioneel in November nadat tegniese probleme wat sedert September ondervind is, uitgesorteer is. Dit is krities om die eerste infeksieperiode te bepaal omrede dit 'n nadelinge invloed kan hê op die eerste groeistuwing en die klein vruggies as hulle nie beskerm is nie. Daar was 28 infeksie gebeurtenisse van November 2008 tot Februarie 2009 voorspel. Volgens die model het 2 ander gebeurtenisse nie in infeksie ontaard nie, omrede die temperatuur te laag was.

Summary

The Metos weather station was operational only in November after we had technical problems with the weather station since September. It is critical to establish the first infection period as it will have a devastating effect on the first flush and small fruit if they are not protected. There were 28 infection events from November 2008 to February 2009. According to the model, 2 other events did not result in infection because the temperature was too low.

Introduction

Alternaria brown spot (ABS) is caused by *A. alternata* pv *citri* and is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa. ABS is described in more detail in Exe 750. Certain aspects regarding its epidemiology are however, pertinent to this investigation. Release of conidia of *A. alternata* pv *citri* on citrus is triggered by rainfall events and by sudden changes in relative humidity. The optimum conditions for infection are temperatures from 23 to 27°C and 8 to 12 h of continuous leaf wetness. Fungicide applications are essential for disease control and production of blemish-free fruit. Growers may apply up to eight fungicide sprays during the course of a growing season, depending on the disease history and variety planted. Considerable research has been conducted on *Alternaria* diseases of potato and tomato and a number of predictive models such as EPIDEM, FAST and some newer models have been developed such as Alter-Rater for *Alternaria* brown spot prediction in the USA. Another version was also developed and tested in Spain and this model will be evaluated in South Africa under the summer rainfall conditions and can later be extended to include the winter rainfall region as well. If sprays can be timed according to accurate predictions, growers will be able to save on spray costs and might save on up to 5 applications per year. In the USA, growers could save up to \$1316/ha by using the Alter-rater model.

Materials and methods

A Metos weather station was installed in a 'Nova' orchard at Belmont 50 km west of Nelspruit to monitor the RH, temperature, rainfall and leaf wetness. Data was withdrawn by means of SMS from the weather station and processed. Infection periods were calculated by means of the prediction model. Ideal weather conditions for *Alternaria* brown spot infection was determined using the following score sheets:

Average daily temperature (°C)	Score
≤10	a
>10 ≤ 15	b
>15 ≤ 20	c
>20 ≤ 25	d
>25	e

Average daily RH (%)	Score
<70	1
>70 ≤ 80	2
>80	3

Leaf wetness hours per day	Score
<9	0
≥9	1

Daily risk score			
	1	2	3
a	0	0	0
b	0	0	1.5
c	0	0	2
d	0	1	2
e	0	1	2

Daily infection risk scores for *Alternaria* brown spot were determined by collecting weather data from the Metos weather station. Weekly orchard inspections to monitor outbreaks of the disease will be conducted by visual inspection of 50 arbitrarily selected fruit per unsprayed tree.

Results and discussion

After initial problems we had with the weather station at Belmont, the system was up and running for the last part of the season. Temperature, RH and leaf wetness duration are the main drivers of this model, with at least 9 h of leaf wetness required for infection. Chances for infection (daily risk scores) increase with an increase in temperature and RH. Results showed that there were 29 infection events from November 2008 to February 2009 (Table 4.3.3.2). According to the prediction model only one or two other events did not result in infection because the temperature was too low (Fig. 4.3.3.1 to 4). Adequate periods of leaf wetness were in all cases associated with rainfall events and it seems as if leaf wetness as a result of dew formation alone was not long enough to support successful infection.

Unfortunately the disease prediction for *Alternaria* brown spot could not be verified in the field. In general, it is also difficult to use this data if a proper weather forecast for at least 3 days can not be operated at the same time for growers to plan spray treatments. This is, however, not possible with the Metos weather stations.

Conclusion

The forecasting system was operational only in November after we had technical problems with the weather station since September. It is critical to establish the first infection period as it will have a devastating effect on the first flush and small fruit if they are not protected. The infection events as determined with the model must be verified in the field to see if it coincides with actual infections. A dedicated person is also needed to see how the disease would progress from hourly/daily data collected from the weather station and to verify it as soon as possible. The model is a useful tool and can save growers a lot of money in spray costs as unnecessary applications can be excluded and only sprayed if suitable conditions for infection are predicted.

Future objectives (milestones) and work plan

More development work is necessary to validate the model and to validate it in a susceptible orchard before it can be commercially implemented as a prediction model.

Technology transfer

This research will be included in the annual research report to be distributed to citrus growers and will be included in various talks to citrus growers. It also formed part of the 2010 CRI Citrus Symposium in the Drakensberg.

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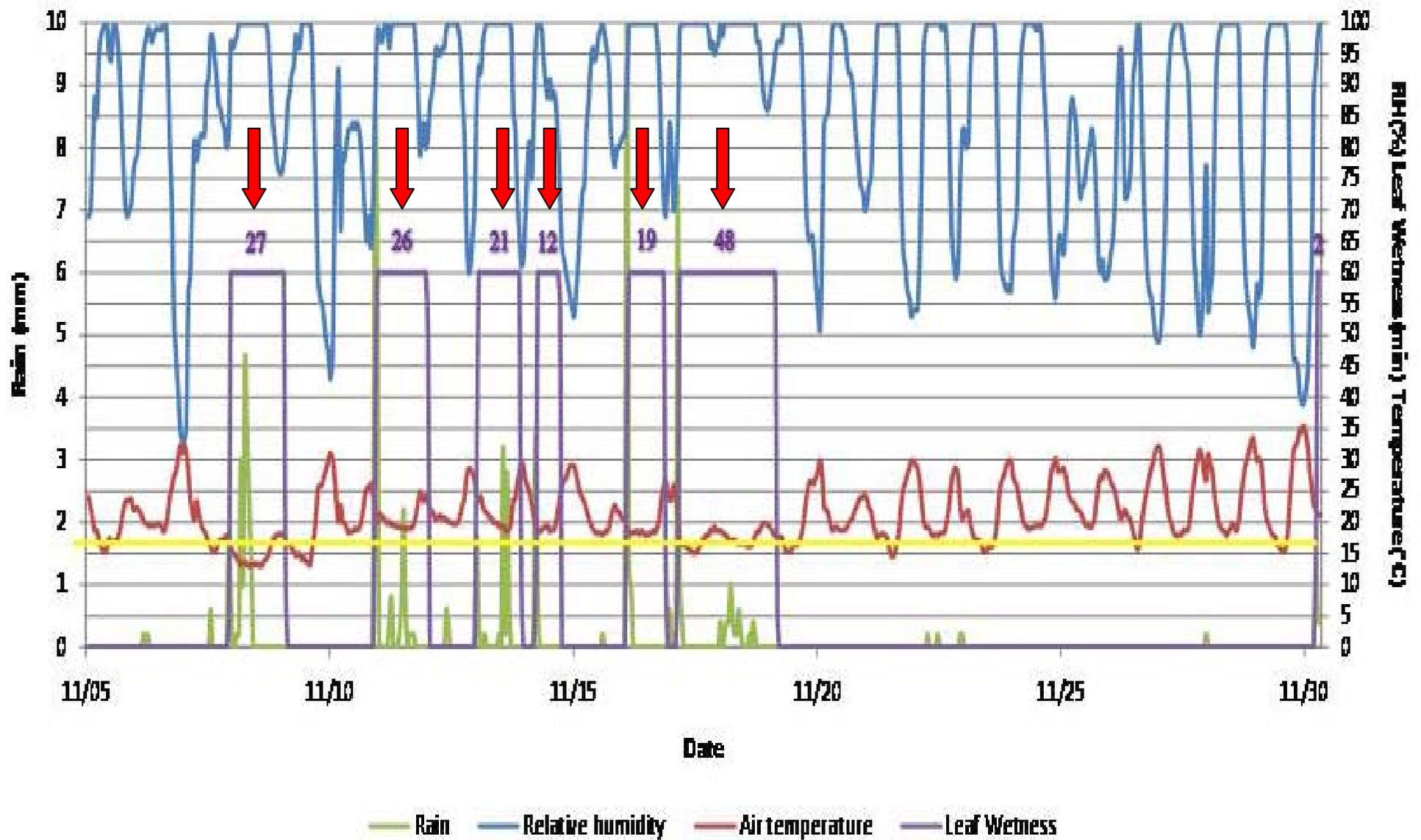


Fig. 4.3.3.1. *Alternaria* brown spot infection events (←) as determined from weather conditions for November 2008 monitored at Belmont, Schagen

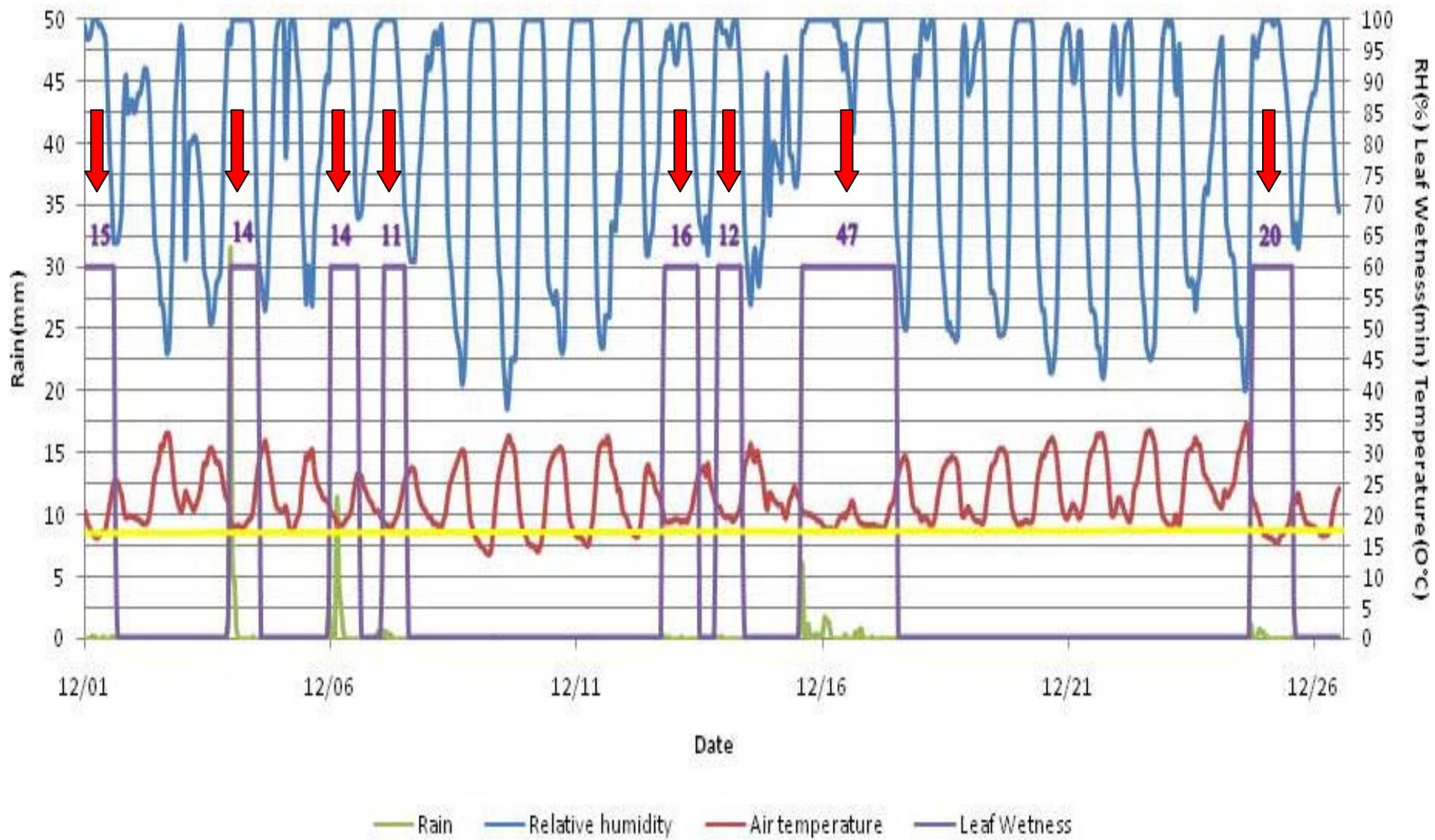


Fig. 4.3.3.2. Alternaria brown spot infection events (←) as determined from weather conditions for December 2008 monitored at Belmont, Schagen

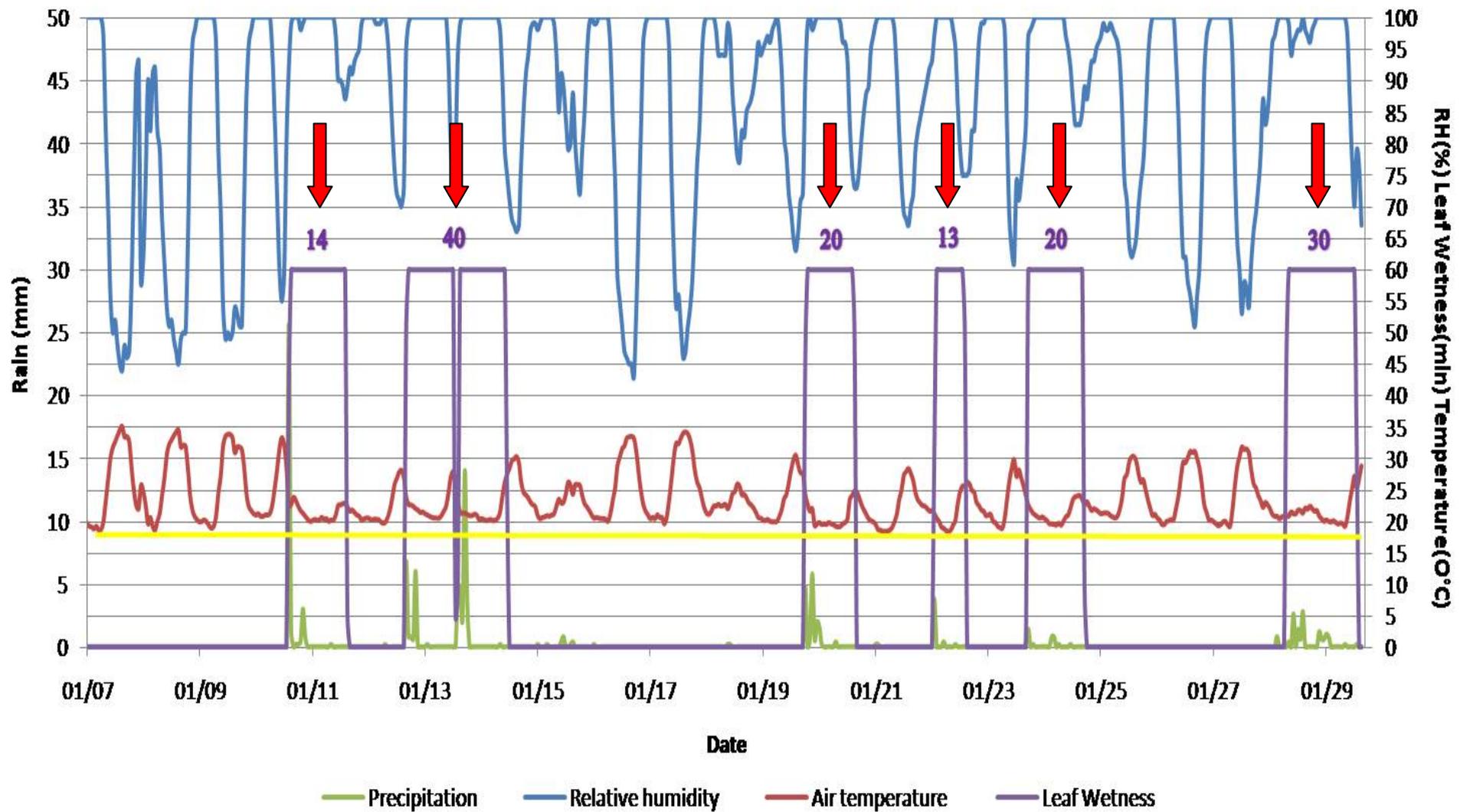


Fig. 4.3.3.3. *Alternaria* brown spot infection events (←) as determined from weather conditions for January 2009 monitored at Belmont, Schagen

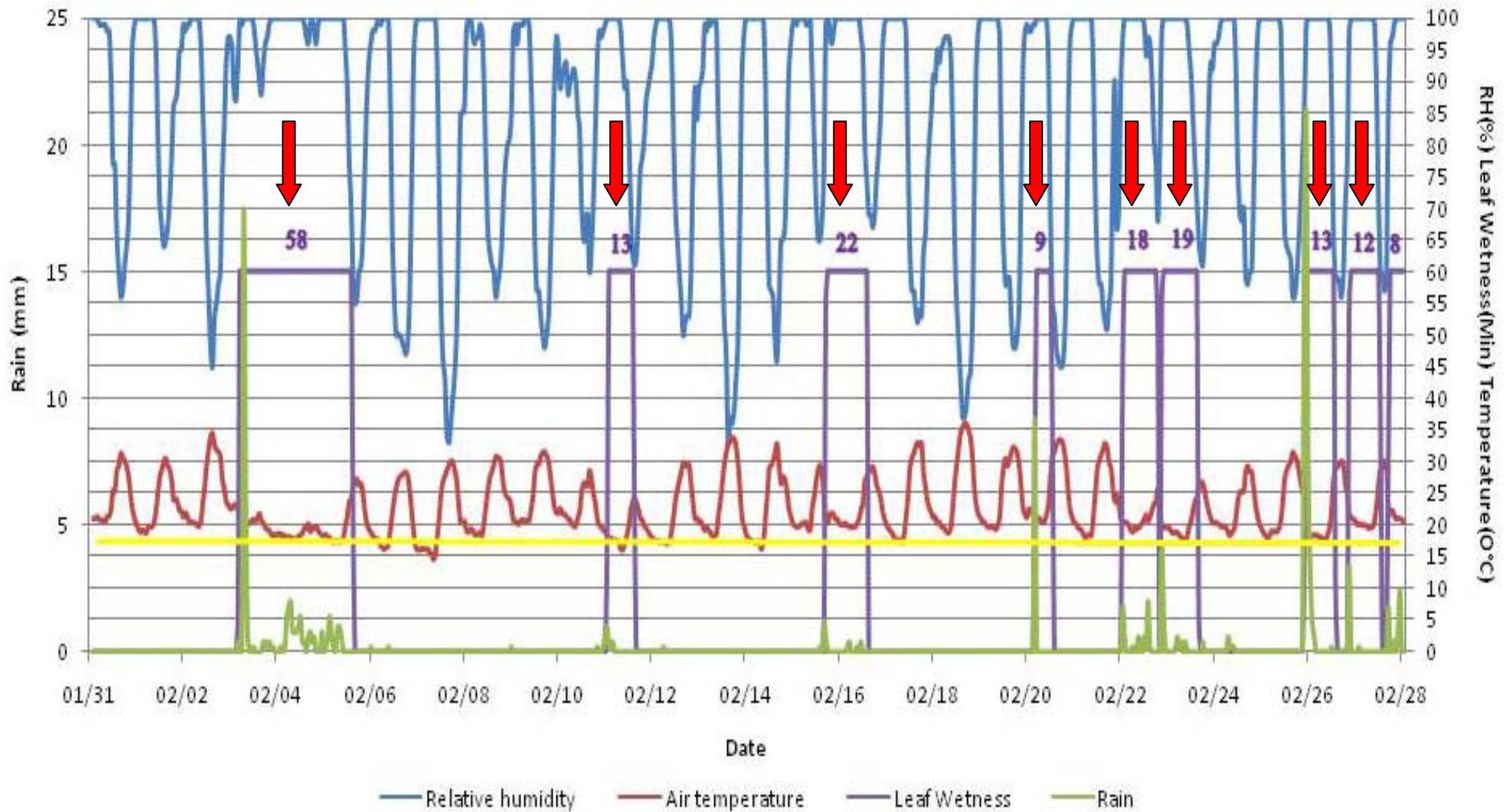


Fig. 4.3.3.4. Alternaria brown spot infection events () as determined from weather conditions for February 2009 monitored at Belmont, Schagen

Table 4.3.3.2. Risk scores of *Alternaria* brown spot infection events monitored at Belmont, Schagen from November 2008 to February 2009.

Date	Avg minimum temperature per event (°C)	Avg RH per event (%)	Leaf wetness per event (hours)	Risk Score
09-Nov	14.9	93.5	27	1.5
11-Nov	20.7	95.0	26	2
13-Nov	22.0	92.8	21	2
15-Nov	19.7	89.9	12	2
17-Nov	19.6	95.0	19	2
18-Nov	17.5	97.4	48	2
01-Dec	19.4	90.8	15	2
04-Dec	20.4	93.9	14	2
06-Dec	21.2	92.4	14	2
07-Dec	20.7	91.5	11	2
13-Dec	20.3	93.2	16	2
14-Dec	20.7	96.1	12	2
15-Dec	19.6	95.9	47	2
24-Dec	18.6	92.6	20	2
10-Jan	21.5	97.3	14	2
12-Jan	22.1	96.7	40	2
19-Jan	20.9	95.5	20	2
22-Jan	21.1	93.2	13	2
23-Jan	21.4	95.5	20	2
28-Jan	21.5	96.1	30	2
04-Feb	19.3	97.0	58	2
11-Feb	18.6	89.9	13	2
16-Feb	21.2	97.1	22	2
21-Feb	22.7	91.6	9	2
23-Feb	20.8	94.7	18	2
24-Feb	19.9	94.0	19	2
26-Feb	20.0	93.8	13	2
27-Feb	21.2	95.1	12	2
28-Feb	21.3	97.4	8	0

4.3.4 PROGRESS REPORT: Optimisation of fungicide spray applications in citrus orchards
Experiment PPL 891 (April 2007 - March 2010): by Paul Fourie (CRI at SU)

Opsomming

Sitrus blaar- en vrugsiektes word meestal deur hoë-volume swamdoderspuitte beheer. Hierdie spuite lei meestal tot groot mates van afloop. Aanvanklike resultate dui duidelik daarop dat kwantitatiewe en kwalitatiewe bedekking asook biologiese effektiwiteit van spuite afneem met toenemende afloop. Navorsing in hierdie projek sal dus op optimisering van spuittoediening fokus om voldoende bedekking met minimale afloop te verseker. Konvensionele en nuwe-tegnologie spuitmasjiene is in verskeie boordproewe evalueer. Die voorlopige resultate van boordproewe dui daarop dat die hoogste kwantitatiewe bedekking teen die beste uniformiteit tussen blare met hoër spuitvolumes behaal is. Desnieteenstaande moet dit beklemtoon word dat die fluoriserende pigment dosis dieselfde met vergelyking van verskillende spuitmasjiene en kalibrasie-verstellings teen verskillende spuitvolumes was. Dieselfde of selfs beter spuitbedekking kon dus

deur die optimale gebruik van masjiene of deur meer effektiewe masjiene behaal word, veral as die dosis per hektaar gestandariseer word. Spuitproewe moet herhaal word en veral die drempelwaardes vir biologiese effektiwiteit moet bepaal word om die interpretasie van resultate te ondersteun.

Summary

Fruit and foliar diseases of citrus are mostly controlled by means of high volume fungicide application, often leading to excessive levels of run-off. However, it was clear that quantitative and qualitative deposition as well as biological efficacy declined with increased run-off. Research in this experiment focuses on optimising application to ensure adequate deposition of the active ingredient with minimal run-off. Conventional and novel spray machines were evaluated in several orchard trials. From the results obtained to date from orchard spray trials, it was clear that the highest quantitative deposition per leaf at the best uniformity between leaves was generally obtained with higher spray volumes. However, a fluorescent pigment dosage of 1× was used when comparing all the different sprayers and calibration settings, even though spray volumes differed. Hence, the dosage per hectare differed substantially between treatments. Similar and even improved spray deposition can thus be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers, especially if dosage per hectare is equated between treatments. Orchard spray trials must be repeated and especially the benchmarks for biological efficacy need to be satisfactorily determined to support conclusive interpretation of the results.

Introduction

Several economically important fungal diseases (such as citrus black spot and *Alternaria* brown spot) and insect pests (such as false codling moth, mealybug, red scale and citrus thrips) are primarily controlled by means of regular fungicide or insecticide sprays. At present, full cover fungicide/insecticide spray application to citrus trees in South Africa involves applications of 10 000 to 16 000 L/ha (Grout, 1997). However, mature citrus trees are reported to hold sprays to a maximum of 2 300 L/ha only (Cunningham and Harden, 1998, 1999). As much as 85% of the excessive spray volume is therefore lost to endo- and exodrift, which results not only in considerable environmental pollution of soils and air, but also increased run-off, reduced spray cover and therewith reduced spray efficacy (Furness *et al.*, 2006ab; Landers and Farooq, 2004). Moreover, excessively high spray volumes are not time and cost effective. Scope for improvement of the current spray application in southern Africa certainly exist as growers of citrus for processing in Florida (USA) apply 1 500 L/ha to mature trees (Pete Timmer, pers. comm.), while the use of novel spray applicators allowed a reduction in spray volumes to below 6 000 L/ha in Australia (Furness *et al.*, 2006b).

In order to study the optimisation of spray application on grape vineyards, researchers at Stellenbosch University's Plant Pathology department (USPP) have developed a spray assessment protocol using fluorometry, photomicrography and digital image analyses (Brink *et al.*, 2004, 2006). Following the determination of benchmark levels for biologically effective spray deposits, they clearly demonstrated that the current best-practice spray applications in table and wine grape vineyards did not result in biologically effective spray deposits. One method of improving the *status quo* was to use spray applicators within specific optimal volume output ranges. USPP's research has shown that optimal use for an air shear machine (Cima™) in table or wine grape vineyards was between 250 and 500 L/ha, compared with the standard 1,000-1,500 L/ha. Biologically effective spray deposits on leaves and bunches were effected by increasing the fungicide concentration relative to the decrease in volume (2- or 4-fold).

A similar study is herewith proposed for the citrus industry, with ultimate aims to optimise spray application in citrus orchards and to improve cost and time effectiveness, without compromising biological efficacy.

The following objectives are proposed for this study:

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas.
2. Determine benchmarks for biological efficacy of copper hydroxide against *Alternaria* brown spot.
3. Characterisation of spray deposition with current spray application methods.
4. Evaluate methods for optimisation of spray application with commonly-used applicators.
5. Evaluate methods for optimisation of spray application with novel applicators.
6. Development and validation of a user-friendly calibration system.
7. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards.
8. Use of adjuvants for improved spray deposition on citrus leaves and fruit [reported as separate experiment].

Materials and methods

Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas

A comprehensive questionnaire comprising all aspects of spray application in citrus orchards was compiled. This questionnaire was handed out after grower study group meetings in various citrus growing areas. The questionnaire was also circulated on CRInet. The data will be summarised to accurately reflect the current status of spray application in the citrus industry, which is essential for conceptualisation of following experimentation. The information will furthermore prove invaluable when future changes to the *status quo* are negotiated with growers, the agrichemical industry and the Registrar for Agricultural Remedies.

Determine benchmarks for biological efficacy of copper hydroxide against *Alternaria* brown spot

Spray deposition on leaves and fruit

This aspect has extensively been reported on in the 2007/8 report. A scientific article was published: PH Fourie, M du Preez, JC Brink and GC Schutte. 2008. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. *Australasian Plant Pathology* 38: 173-182.

The protocol for spray deposition assessment has been changed to ensure portability, improve throughput and allow assessment on whole leaves and fruit, and therefore this objective had to be repeated.

Upper surfaces of individual young leaves of an *Alternaria* brown spot (ABS) sensitive mandarin cultivar 'Nova' were sprayed with 0.5 mL of a mixture of the SARDI Yellow Fluorescent Pigment (1 mL/L) and copper oxychloride (WP, Villa Crop Protection; 200 g/hL) by means of a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products, 195 Internationale Blvd, Glendale Heights IL 60139 USA) with a fluid nozzle tip of 1.5 mm in diameter at 1.85 bar pressure. As spray volume has a pronounced effect on spray run-off and therewith quantitative deposition (Fourie *et al.*, 2008), volume could not be used to accurately manipulate deposition. Therefore, the various treatments involved a fixed spray volume at dosages ranging from 0 to 2 times the recommended dosages. Spraying was conducted in a spray chamber, which consists of a steel framework (800 × 1410 × 660 mm; L × H × W) with leaves slanted at a 60° angle. The spray gun was mounted onto the spray chamber at a distance of 60 cm from the target with a spray angle of 90° relative to the target. After spraying, leaves were carefully removed and hung vertically from petioles, and the deposited spray mixture was allowed to dry. Leaves were illuminated using a Labino Mid-light (UV-A; ≈365 nm) and digital photos were taken using a Canon EOS 40D camera equipped with a 50 mm macro lens.

Spray deposition assessment involved digital image analyses with Image-Pro Plus version 6.2 software (Media Cybernetics, www.mediacy.com) to determine quantitative and qualitative deposition per leaf of the fluorescent pigment particles. Similar to the methodology used in Fourie *et al.* (2008), quantitative deposition per leaf analysis involved the measurement of the area covered by pigment particles, but as a percentage of total leaf area (presented as %FP). The qualitative deposition per leaf assessment protocol was changed quite significantly from the version used in Fourie *et al.* (2008). The leaf-area of the ≈30 MB *.tiff image was divided into equally-sized 30×30-pixel squares. Depending on the leaf size, this amounted to anything from 300 to in excess of 3000 individual squares per leaf, of which the percentage area covered by fluorescent pigment particle was determined for each square. The relative standard deviation of the mean value of all the blocks analysed per leaf (%RSD = Standard deviation / Mean × 100) indicated the qualitative deposition per leaf.

Upper surfaces of sprayed leaves were subsequently spray-inoculated with 0.3 mL of a 1×10⁵ spores/mL suspension of a virulent isolate of *Alternaria alternata* pv. *citri*, the causal agent of ABS. Sprayed and inoculated leaves were immediately incubated at high relative humidity (>95%) at 25°C for 48-65 h, depending on the rate of symptom development. Following sufficient symptom development on the control leaves, the midribs of leaves were removed and the inoculated leaf sides digitally photographed under white light. The percentage symptomatic area per leaf was determined by means of image analysis in Image Pro-Plus version 6.2.

For the purpose of this report, data are summarised as means, but once these trials have been repeated enough times, data will be subjected to suitable regression analyses in order to determine the benchmark values for biological control.

Characterisation of spray deposition with current spray application methods (Objectives 3-5)

Spray trials were conducted in Addo, Hoedspruit and Letsitele, using SARDI Yellow Fluorescent Pigment (100 mL/hL) as spray mixture.

Addo. A novel multi-fan spray machine, the BSF-Multiwing was developed by Meyer Boshoff from Hoedspruit. Similar multi-fan spray technology was demonstrated to perform better and at higher tractor speeds in Australian citrus orchards (Geoff Furness, SARDI-Loxton Australia, personal communication). This machine was compared with a grower-owned Volcano oscillating boom mistblower in a mature Navel orchard at various settings.

Hoedspruit. The BSF-Multiwing was compared with a conventional oscillating boom mistblower, the BSF-Extreme, at various calibration settings in a mature Valencia orchard.

Letsitele. The BSF-Multiwing was compared with various conventional oscillating boom mistblowers, viz. the Ultima, Bateleur (1-sided ability only) and BSF-Extreme, as well as the Cima air-shear (1-sided ability only) sprayer and ESS electrostatic sprayer (1-sided ability only). Various calibration settings for the different sprayers effecting spray volumes ranging from 300 to 12,000 L/ha were evaluated.

Trial layout and sampling

Trials were conducted in a uniform section of the selected orchard. For each treatment combination, a row-section with 6 to 10 trees was sprayed from both sides. Two buffer rows were left unsprayed between treatments. For logistical reasons, spray deposition on leaves was determined instead of deposition on fruit. However, in another experiment using similar methodology (CRI 918), we observed an 80% correlation between fluorescent pigment deposition on leaves and deposition on fruit. A 76% and 90% correlation was observed between the copper residue analysed and the quantitative fluorescent pigment measurements on leaves and fruit, respectively, which supports this methodology as an effective tool for spray deposition assessment. As replications, 3 uniform trees were selected from each sprayed section from which leaves were sampled for spray deposition analysis. At least 12 intact leaves were sampled from each of various positions in the canopy: inner and outer canopy, and top, middle and bottom part of each tree. Leaves from these 6 positions were collected separately in plastic bags and transported in cool, dry conditions to the laboratories at Stellenbosch University, where it was cool-stored at 4°C until further analysis. Quantitative and qualitative spray deposition analyses of upper and lower leaf surfaces were conducted as described previously from 12 leaves per position.

Data were subjected to analysis of variance and Student's T-test for least significant difference ($P = 0.05$). The variation (%RSD) in the mean quantitative deposition per leaf values was indicative of general spray uniformity between leaves. Spray efficiency was expressed as the mean quantitative deposition per leaf value per 1000 L of spray volume.

Results and discussion

Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas

To date, 61 questionnaires have been received. Data have been transferred to an Excel spread sheet and will be collated to determine the status of spray application in South Africa. This aspect is ongoing.

Determine benchmarks for biological efficacy of copper hydroxide against Alternaria brown spot

The copper oxychloride biotest was repeated 4 times; twice at 0, 2.5, 0.5, 0.75, 1, 1.5 and 2× the recommended dosage of 200 g/hL and twice at 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1× the recommended dosage. Despite maintaining similar methodology between repetitions, variable symptom expression was observed between the different leaf sizes/ages. Data were summarised by infection and deposition means over dosage and are presented in Figure 1. Linear and quadratic regression analyses over means of deposition and infection data, respectively, yielded excellent linear fit for deposition data ($y = 6.53x - 0.51$; $R^2 = 0.9784$) and fairly good quadratic fit for infection data ($y = 17.40x^2 - 50.59x + 43.76$; $R^2 = 0.6095$). Outlier data points were not removed from this preliminary analysis (for example higher infection values at dosage 0.8), which is the most likely reason for the relatively poor fit for infection. At the recommended dosage (1×), quantitative deposition of fluorescent pigment was predicted at 6.02% FP and infection at 10.57%. At 0.5× the recommended dosage, deposition was predicted at 2.76% FP and infection at 22.81%. This is in agreement with Fourie *et al.* (2008) who predicted quantitative deposition of 2.99% FP was needed to reduce infection

from 100% to 20% with sprays of copper hydroxide on 'Nova' leaves. It should be stressed that different dosages were used in this trial to manipulate quantitative deposition of a fixed spray volume, and these laboratory findings do not indicate that lower dosages of fungicide can be advocated for ABS control in commercial citrus orchards. Moreover, aspects such as residual activity and rainfastness could not be evaluated in the laboratory study. This objective is ongoing and following additional repetitions, the data will be consolidated to remove outliers and analysed by means of suitable regression.

Characterisation of spray deposition with current spray application methods (Objectives 3-5)

Qualitative deposition per leaf assessment of the captured images is in progress, and these results could not be included in this report. However, it is clear from initial results that the quality of spray deposition on individual leaves decreased with increased spray volume and the concomitant spray run-off, which supports the findings of Fourie *et al.* (2009) following laboratory spray trials.

Addo. The Navel trees in the Addo orchard were on average 3.7 m tall, 4.4 m wide with 0.7 m skirt. Canopy density was rated as fairly sparse, with an index-rating of 2, with 5 being a very dense canopy. Row spacing was 7×4 m. Analysis of variance indicated a significant 3-factor interaction for treatment, leaf side and canopy position or canopy height ($P < 0.05$). The treatment × leaf side × canopy position results from the Addo spray trial are summarised in Figure 4.3.4.2.

The Volcano sprayer (operating at 20 bar pressure) was used exactly according to the grower's calibration settings, which should result in a spray volume of 8436 L/ha at the spray speed of 1.5 km/h. It should be noted, however, that the alternating nozzle setup of hollow and full cone nozzles had not been serviced in 12 months. Quantitative deposition per leaf on upper and lower leaf surfaces in the outer canopy was ≈5% FP, but a relatively large variation between leaves (%RSD; indicative of spray uniformity) was observed, especially on the upper leaf surfaces (56.4% RSD). Quantitative deposition per leaf values for inner canopy leaves were lower than those observed for the outer canopy (4.3 and 2.3% FP for upper and lower leaf surfaces, respectively). Interestingly, the variation between leaves was markedly lower with the %RSD <30%. For the Volcano sprayer at 8436 L/ha, the spray efficiency was poor with an average of 0.49% FP (quantitative spray deposition per leaf) per 1000 L of spray volume.

The BSF-Multiwing sprayer (operating at 10 bar pressure) was equipped with either disc-core D4 or D2 hollow cone nozzles (45 whirler type). When using the D4 hollow cone nozzles at tractor speed of 1.3 km/h, a spray volume of 6767 L/ha was obtained. This was 20% lower spray volume than with the Volcano, but higher quantitative spray deposition per leaf values were retained on outer and inner canopy leaves (6.4-6.0 and 4.6-3.6% FP, respectively). Variation in quantitative spray deposition between leaves was also lower (<≈30% RSD), except for lower leaf surfaces of inner canopy leaves (43.4% RSD). Spray efficiency at an average of 0.76% FP per 1000 L was 55% better than that observed for the Volcano. At a tractor speed of 4.0 km/h, spray volume was reduced to 2261 L/ha with the D4 hollow cone nozzles. Quantitative spray deposition per leaf was comparable to what was observed with the Volcano, but slightly lower than those observed with the Multiwing with the same nozzle setup at slower tractor speeds. Variation in quantitative spray deposition between leaves following spraying at this lower spray volume was slightly higher on upper leaf surfaces (39.6% RSD) than on lower leaf surfaces (<30% RSD), but spray efficiency was markedly improved (average of 1.79% FP per 1000 L). As spraying with the D2 hollow cone nozzles resulted in several blockages of the nozzle tips, we realised that this setup will not be practicable. Hence, the results for the D2 nozzle setup will not be discussed.

Hoedspruit. The Midnight Valencia trees in the Hoedspruit orchard were on average 3.9 m tall, 3.7 m wide with 0.9 m skirt. Canopy density was rated as fairly dense, with an index-rating of 4. Row spacing was 6×2 m. Analysis of variance indicated a significant 3-factor interaction for treatment, leaf side and canopy position or canopy height ($P < 0.05$). The treatment × leaf side × canopy position results from the Hoedspruit spray trial are summarised in Figure 4.3.4.3.

The BSF-Extreme (operating at 15 bar pressure) was set up with alternating Jacto J4 full cone and J5 hollow cone nozzles, which effected spray volumes of 7033, 3624, 2349 and 1554 L/ha at tractor speeds of 1.5, 3.0, 4.6 and 6.9 km/h, respectively. At tractor speeds of 4.6 and 6.9 km/h, visual observation of fluorescent pigment deposition on trees clearly indicated an uneven deposition, which was visible as 0.75 to 1 m vertical segments of canopy with visibly variable deposition, as a result of incompatible oscillation and tractor speeds. At 1.5 km/h, the Extreme deposited 5.9 and 7.3% FP on upper and lower leaf surfaces of the outer canopy at a very low variation between leaves of 13.2 and 22.8% RSD, respectively. On the inner canopy of these dense Valencia trees, quantitative deposition per leaf was lower (4.1 and 2.1% FP, respectively for upper and lower leaf surfaces) and variation between leaves was also markedly higher (46.7 and 47.2% RSD, respectively). Spray efficiency was calculated at an average of 0.69% FP per 1000 L. At 3.0 km/h,

spray volume was reduced to 3525 L/ha and quantitative spray deposition per leaf was also lower with 4.8 and 5.3% FP deposited on upper and lower leaf surfaces of the outer canopy leaves at a variation between leaves of 23.8 and 38.2% RSD, respectively. Quantitative deposition per leaf on inner canopy leaves was lower (3.3 and 2.1% FP, respectively for upper and lower leaf surfaces) and of markedly higher variation between leaves (64.3 and 96.1% RSD, respectively), which is indicative of reduced spray penetration at higher tractor speed and/or reduced spray volume. Spray efficiency was, however, markedly better at 1.06% FP per 1000 L.

The BSF-Multiwing sprayer (operating at 10 bar pressure) was again evaluated using the D2 hollow cone nozzles at 1.3 and 2.6 km/h, which resulted in spray volumes of 4421 and 2279 L/ha, respectively. The impracticability of this nozzle selection, in terms of frequent blockages, was supported by the relatively poor penetration observed in terms of quantitative deposition per leaf and variation between leaves, especially at the faster tractor speed. With D4 full cone nozzles (56 whirler type), spray volume was 12632, 6512 and 4221 L/ha at 1.3, 2.6 and 4.0 km/h, respectively, while the comparative spray volumes with D4 hollow cone nozzles were 7895, 4070 and 2638 L/ha, respectively. Quantitative spray deposition per leaf was the highest at 1.3 km/h tractor speed (and higher spray volumes) and fairly similar when comparing the D4 hollow (5.2-2.6% FP) and full cone (5.8-2.6% FP) nozzles. Variation between leaves was, however, slightly lower with the hollow cone nozzles (average 26.9 vs. 29.4% RSD) and spray efficiency was better (average 0.55 vs. 0.36% per 1000L). At 2.6 km/h, quantitative spray deposition per leaf with the D4 full cone nozzles was slightly lower (5.3-1.5% FP) than that of the conventional BSF-Extreme at 1.5 km/h (7.3-2.1% FP), but variation between leaves and spray efficiency were comparable (38.5 vs. 32.5% RSD and 0.53 vs. 0.69% per 1000 L, respectively). The D4 hollow cone nozzles showed reduced penetration at 2.6 km/h as was clearly demonstrated by the increased variation between quantitative deposition values on inner canopy leaves (70.3-95.4% RSD). Likewise, penetration at 4 km/h with the D4 full and hollow cone nozzles was reduced, hence the high variation between upper (48.3 and 49.5% RSD, respectively) and especially lower (85.8 and 91.7% RSD, respectively) surfaces of inner canopy leaves.

Letsitele. The Du Roi Valencia trees in the Letsitele orchard were on average 4.3 m tall, 4.2 m wide with no skirt. Canopy density was rated as fairly dense, with an index-rating of 4. Row spacing was 7×3.5 m. Analysis of variance indicated a significant 3-factor interaction for treatment, leaf side and canopy position or canopy height ($P < 0.05$). The treatment × leaf side × canopy position results from the Letsitele spray trial are summarised in Figure 4.3.4.4.

The Ultima, using a 18×D3-full and 12×D4-hollow cone nozzle setup at 20 bar pressure, was evaluated at 1.5 and 2.3 km/h, which resulted in spray volumes of 12258 and 7748 L/ha, respectively. At these tractor speeds, quantitative deposition per leaf measured a mean of 12.2 and 11.1% FP on outer canopy leaves and 9.6 and 8.2% FP on inner canopy leaves, respectively, with not much difference between upper and lower leaf surfaces. Spray uniformity at 1.5 km/h was relatively good at 38.5 and 42.4% RSD for outer and inner canopy leaves, respectively, and 38.9 and 51.7% RSD at 2.3 km/h. Given the higher spray volume at 1.5 km/h, spray efficiency was relatively low at 0.89% FP per 1000 L, but was markedly better at 2.3 km/h (1.25% FP per 1000 L).

The Bateleur (operating at 20 bar pressure) was used with alternating 14×D4-hollow cone + 14×D3-full cone nozzle setup at 1.5 km/h (11507 L/ha; Bat-i and Bat-ii), and 19 nozzles of each (10 additional nozzles on a fixed boom without wind to spray the skirt) at 2.3 km/h (9870 L/ha). The Bat-ii setup at 1.5 km/h (tower angled more diagonally downward) resulted in poorer penetration of the canopy, and the results will not be discussed. At 1.5 km/h using the Bat-i setup, the Bateleur retained significantly less fluorescent pigment (7.1 and 7.7% FP for outer and inner canopy at a variation between leaves of 51.0 and 37.0% RSD, respectively) than the Ultima at both tractor speeds. At 2.3 km/h, spray deposition was slightly better at 8.5 and 8.3% FP with a variation between leaves of 37.8 and 44.2% RSD, respectively. A larger number of nozzles was used at the faster tractor speed, which did not change the volume delivery that much, but spray efficiency was improved (0.85 vs. 0.64% FP per 1000 L) by the lower spray volume and improved deposition.

The BSF-Extreme (operating at 15 bar pressure) setup differed from what was evaluated at Hoedspruit, specifically in a change from the Jacto J5-2 hollow cone nozzles to green Albus hollow cone nozzles with similar volume delivery but a wider swath angle. This setup resulted in spray volumes of 6933 and 4382 L/ha at 1.5 and 2.3 km/h, respectively. At the slower tractor speed, spray deposition on inner and outer canopy leaves were fairly similar at an average of 6.5% FP (34.6% RSD). At 2.3 km/h, deposition on outer canopy leaves was still similar [8.4% FP (41.7% RSD)], but penetration to inner canopy leaves was slightly reduced as was evident from less quantitative deposition on lower leaf surfaces and more variation between leaves [4.2% FP (91.7% RSD)] compared with upper leaf surfaces [(7.3% FP (55.1% RSD))]. Nonetheless, the BSF-Extreme proved to be relatively efficient at 0.94 and 1.61% FP per 1000 L at these tractor speeds. The BSF-Extreme was also evaluated at 3.5 km/h (2926 L/ha), which effected fairly good deposition values

on outer canopy leaves [5.0% FP (54.0% RSD)], but relatively poor penetration of the inner canopy [2.6% FP (86.1% RSD)]. Spray efficiency at an average of 1.31% FP per 1000 L was not as good as was observed at 2.3 km/h. Reduced penetration at faster tractor speeds might possibly have been accentuated by use of the wide-swath green Albus nozzles, as the latter nozzles also adversely affected the penetration ability of the BSF-Multiwing.

The BSF-Multiwing (operating at 10 bar pressure) was evaluated with either J4-full cone nozzles or green Albus hollow cone nozzles (D4-equivalent). It was also tested using the same nozzle setup as the BSF-Extreme. With the full cone nozzles at 1.5 km/h, the spray volume was 4956 L/ha, comparable to the BSF-Extreme at the same tractor speed. Deposition on the outer canopy [9.6% FP (52.7% RSD)] was significantly more than the Extreme and similar to the Ultima and Bateleur, although at somewhat higher variation between leaves. Upper leaf surface deposition of inner canopy leaves [6.7% FP (51.8% RSD)] was comparable to that following application with the Extreme at the same tractor speed, but deposition on lower leaf surfaces was significantly lower (4.4% FP) and with more variation between leaves (89.3% RSD). At 2.3 km/h and 3958 L/ha, the Multiwing deposited statistically similar quantities of fluorescent pigment on the inner canopy leaves than at 1.5 km/h, albeit at higher variation between leaves (81.8% vs. 70.5% RSD). However, the spray efficiency at 2.3 km/h was markedly better at 2.33% FP per 1000 L, compared with 1.53% FP at 1.5 km/h. At 3.5 km/h and 2643 L/ha, the spray efficiency was further improved to 2.62% FP per 1000 L, markedly better than the Extreme at the same tractor speed. However, variation between leaves was relatively high at an average of 65.0 and 98.5% RSD for outer and inner canopy leaves, respectively. The use of the green Albus hollow cone nozzles alone or in combination with the J4 full cone nozzles at 2.3 km/h and \pm 3900 L/ha proved to be inefficient on the dense canopies and wide row spacing as penetration of the inner canopy was relatively poor in terms of quantitative deposition and variation between leaves compared with the complete set of J4 full cone nozzles. These findings were confirmed when comparing the full cone and hollow cone nozzles at 3.5 km/h.

The Cima air shear sprayer was evaluated at 1.5 km/h, but at different pressure settings of 2.5 – 1 bar, which resulted in spray volumes of 3000, 2000 and 1000 L/ha. At 3000 L/ha, the Cima retained markedly less fluorescent pigment and at more variation between leaves (3.8% FP at 92.3% RSD on outer canopy and 3.0% FP at 99.3% RSD on inner canopy leaves) than the BSF-Extreme and BSF-Multiwing (using full cone nozzles) at 3.5 km/h and \pm 2-3000 L/ha. At lower spray volumes, the deposition quantity and uniformity with the Cima declined in a linear fashion. It should be noted, however, that poor sprayer setup by the company representative might have contributed to the relatively poor results. Whilst spraying, it was noted that the tops of the canopies were not covered by the spray plume of the top fishtails. This resulted in significantly reduced deposition in the tops of canopies (results not shown) and would have contributed to the large variation between leaves. Spray efficiency with the Cima ranged from 1.14 to 1.67% FP per 1000 L.

The ESS electrostatic sprayer was used at 1.5, 2.3 and 3.5 km/h, resulting in spray volumes of 490, 310 and 207 L/ha. Given the very low spray volumes, the pigment concentration was increased to 400 mL/hL (4 \times the concentration used for the other sprayers). Application of active ingredient per hectare therefore roughly equals that of the Cima sprayer at 2000 and 1000 L/ha and despite comparable quantitative deposition per leaf values, variation between leaves was very high at an average of 132.1% RSD for all treatments.

Conclusion

From the results obtained to date, it was clear that the highest quantitative deposition per leaf values at the lowest variation between leaves was generally obtained with higher spray volumes. However, it was obvious that the dispersion quality of pigment deposition on individual leaves declined with increasing spray volumes due to more run-off, which might also have a detrimental effect on biological efficacy. It should furthermore be stressed that the fluorescent pigment dosage of 1 \times was used when comparing all the different sprayers and calibration settings (except for the ESS sprayer tested at 4 \times), even though spray volumes differed. Hence, the dosage per hectare differed substantially between treatments. In relative terms, spray efficiency (expressed as quantitative deposition per leaf per 1000 L of spray volume) in combination with spray uniformity (expressed by the variation in quantitative deposition between leaves) are therefore the parameters that should be used when comparing sprayers and calibration settings. Similar and even improved spray deposition can thus be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers, especially if dosage per hectare is equated between treatments.

In the sparse canopies of the Navel orchard in Addo, the BSF-Multiwing performed more efficiently and spray deposition was more uniform at similar or faster tractor speeds than with the grower's Volcano sprayer. Remarkably, spray efficiency with this multi-fan tower sprayer at 4 km/h (with D4 hollow cone nozzles) was 365% better than with the Volcano at 1.5 km/h, while uniformity was also improved (average 31.8% RSD). Sprays at lower volumes per hectare and/or faster tractor speed can result in massive savings in chemical,

water, fuel and labour cost, and/or substantially improved time efficiency. Additionally, the BSF-Multiwing operated at 10 bar pump pressure compared with the 20 bar of the Volcano, and was substantially more power efficient as it used only 23 kW of tractor power. This would amount to a considerable fuel saving (as much as 50%) as a smaller tractor with less power usage can be used to spray with this machine.

As could be expected, spray uniformity was generally poorer in more dense canopies, especially on inner canopy leaves. Canopy management through pruning practices should therefore aim to reduce canopy density. This was especially pertinent should lower spray volumes and/or faster tractor speeds be used for spray application. Nonetheless, it was clear from the Letsitele results that optimised application could result in improved and more efficient application. For example, the Ultima and Bateleur at faster tractor speeds and lower spray volumes deposited similar quantities of pigment at similar uniformity levels, but at markedly improved efficiency. In these denser canopies, the BSF-Extreme and BSF-Multiwing retained on average comparable quantities of pigment per leaf, but used lower spray volumes and therewith markedly better efficiency. However, a concomitant reduction in spray uniformity between leaves was observed, and can be attributed to reduced spray penetration of the inner canopy. At higher spray volumes (7000-13000 L/ha) and slow tractor speeds (1.5 km/h) in dense canopies at Hoedspruit, these machines showed improved uniformity ($\pm 30\%$ RSD). In these dense orchards, the Multiwing proved to as effective at faster tractor speeds.

The results with the Cima sprayer were disappointing when compared with those of the BSF-Extreme and BSF-Multiwing at similar spray volumes. Trials with this machine, however, will be repeated as poor sprayer setup undoubtedly contributed to poor performance. The ESS electrostatic sprayer, evaluated at 4 \times the pigment dosage used for the other sprayers, showed relatively poor results in terms of quantitative deposition per leaf and variation between leaves. From the sampled leaves, it was obvious that excellent qualitative deposition at reasonable quantities was obtained on some leaves, but with almost no deposition on other. The absence of any form of oscillation might have contributed to leaf shingling and poor penetration; a detriment which the electrostatically charged spray droplets seemed unable to overcome.

In terms of tractor speed, it seems that 3 km/h should be the upper limit for medium cover spray application; defined as adequate cover of outer and inner canopy leaves (and fruit), without emphasis on film-wetting of the trunk and branches as would be required for full cover application. Faster tractor speed reduced spray penetration and spray uniformity, especially in denser canopies. This effect was more pronounced when using hollow cone nozzles. Nozzle selection is important and could result in reduced sprayer performance as was seen when the green Albus nozzle (wide swath angle) were used with the BSF-Extreme and -Multiwing in a dense canopy orchard with wide row spacing.

From the findings to date, it seems clear that medium cover sprays can be adequately delivered with 2-sided sprayers. This holds a big advantage over 1-sided sprayers as work rates are improved, costs reduced and orchard traffic and concomitant soil compaction are also halved.

This research is still work in progress, and the conclusions drawn from the results obtained thus far should be viewed in this context. Most spray trials will be repeated to confirm the initial findings. Additionally, the benchmarks for biological efficacy need to be satisfactorily determined to support conclusive interpretation of the results.

Technology transfer

- Extension
 - Orchard spray demonstration (Addo, Hoedspruit).
 - Study group meeting and orchard spray demonstration (Constantia-Letsitele).
 - *Ad hoc* lecture to Western Cape growers on study tour in Hoedspruit.
 - PH Fourie, M du Preez, JC Brink and GC Schutte. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. Talk at CRI Citrus Research symposium (Drakensberg, Aug 2008).
 - JC Brink, GC Schutte and PH Fourie. Development of selected adjuvants for application in Southern African citrus orchards. Poster presentation at CRI Citrus Research symposium (Drakensberg, Aug 2008).
- Society meetings
 - Fourie PH, Brink JC, van Zyl S, Schutte T. 2008. Improving fungicide application: reality, options and impact. Invited presentation at Western Cape branch symposium of SASPP, Stellenbosch (8 May 2008).

- Fourie PH, Brink JC, van Zyl S, Schutte T. 2008. Improving fungicide application: reality, options and impact. Invited presentation at Northern branch symposium of SASPP, Pretoria (29 May 2008).
- P.H. Fourie, J.C. Brink, G.C. Schutte & T.G. Grout. 2009. Efficiency and uniformity of fungicide spray deposition in citrus orchards. Oral presentation at 46th Congress of the SASPP, Gordon's Bay (25-28 January 2009).
- J.C. Brink, G.C. Schutte & P.H. Fourie. 2009. Influence of selected adjuvants on fungicide spray retention on Satsuma Mandarin leaves. Oral presentation at 46th Congress of the SASPP, Gordon's Bay (25-28 January 2009).
- Scientific paper published
 - PH Fourie, M du Preez, JC Brink and GC Schutte. 2008. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. *Australasian Plant Pathology* 38: 173-182.

Further objectives (milestones) and work plan

1. Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas.
2. Determine benchmarks for biological efficacy of copper hydroxide against *Alternaria* brown spot.
3. Characterisation of spray deposition with current spray application methods.
4. Evaluate methods for optimisation of spray application with commonly-used applicators.
5. Evaluate methods for optimisation of spray application with novel applicators.
6. Development and validation of a user-friendly calibration system.
7. Develop a guideline for optimal use of commonly-used and novel spray applicators in citrus orchards.
8. Use of adjuvants for improved spray deposition on citrus leaves and fruit. [Note that this objective is subject to contractual buy-in from selected companies. Project commenced in January 2008 and is reported on in a separate experiment]

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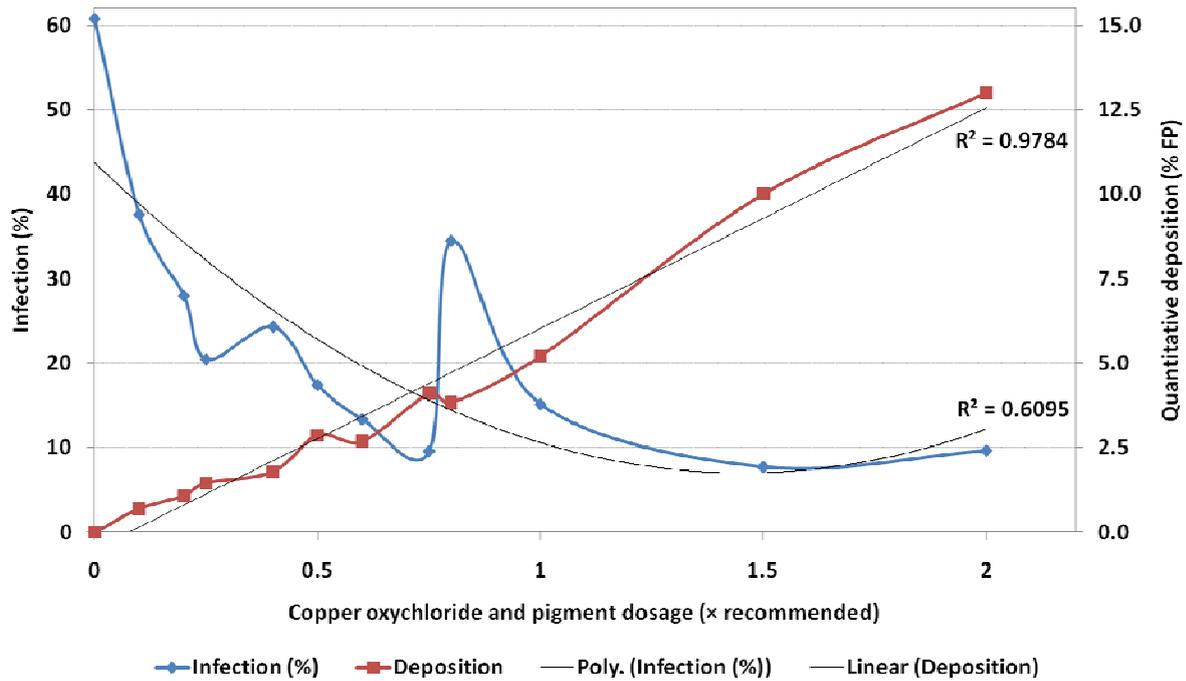


Figure 4.3.4.1. Mean quantitative deposition of fluorescent pigment following spray application to individual ‘Nova’ leaves with a spray mixture of SARDI Yellow Fluorescent Pigment (1 mL/L) and copper oxychloride (200 g/hL) at 0 to 2× the recommended dosage and mean leaf infection (total lesion size as percentage of leaf area) following subsequent spray inoculation with 0.3 mL of a 1×10^5 spores/mL suspension of *Alternaria alternata* pv. *citri*. Linear and quadratic regression lines were fitted for deposition and infection means, respectively.

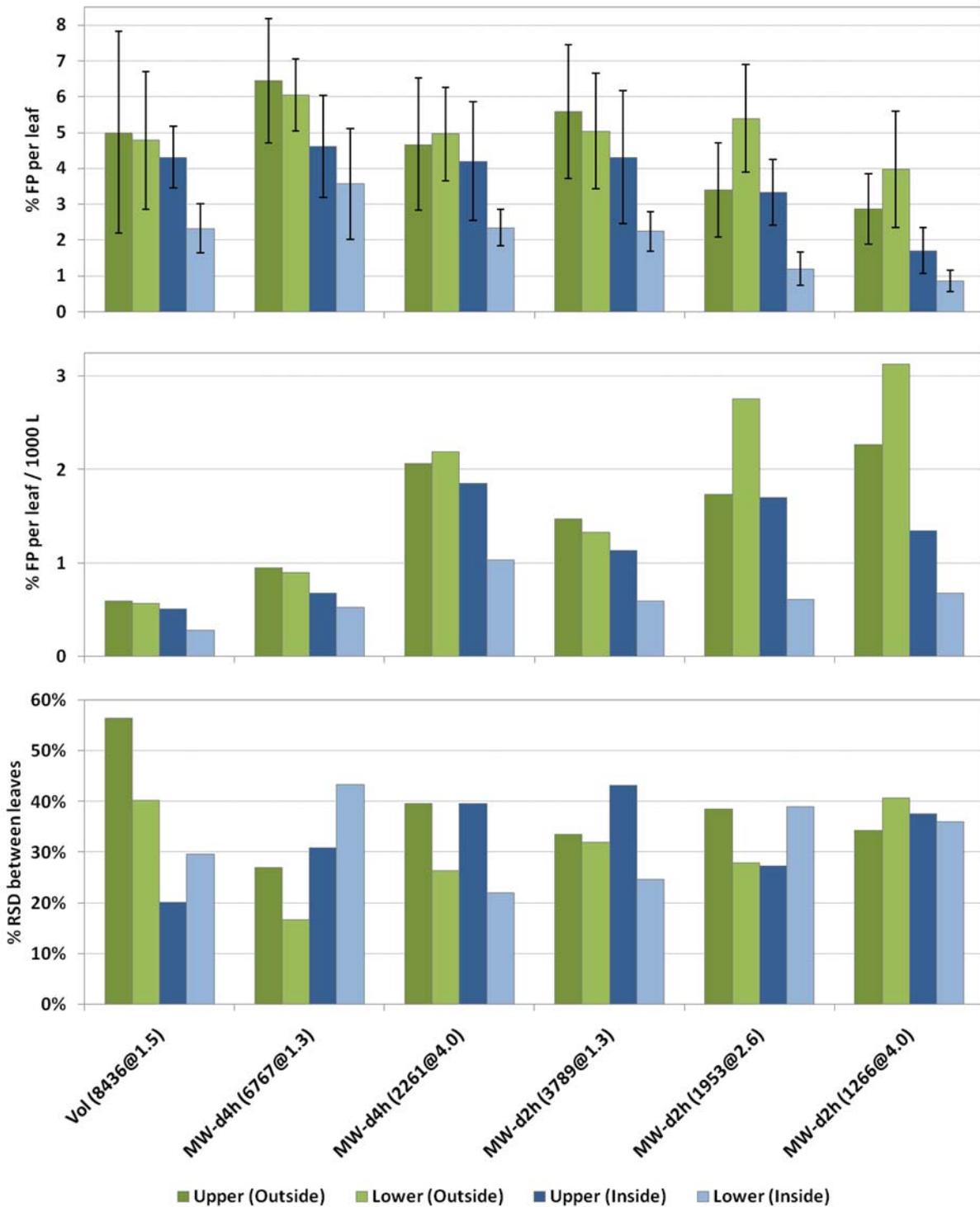


Figure 4.3.4.2. Mean quantitative spray deposition per leaf (% FP), variation between leaves (%RSD) and spray efficiency (expressed as % FP per leaf / 1000 L) on upper and lower leaf surfaces, which were sampled from top, middle and bottom sections of the inner and outer canopy, following spray application with SARDI Yellow Fluorescent Pigment (1 mL/hL) in a mature Navel orchard in Addo with a Volcano oscillating boom mistblower (Vol) and BSF-Multiwing sprayer (MW) with various nozzle selection and tractor speeds [legend = Sprayer; nozzle type (D4- or D2-hollow cone); spray volume in L/ha; tractor speed in km/h].

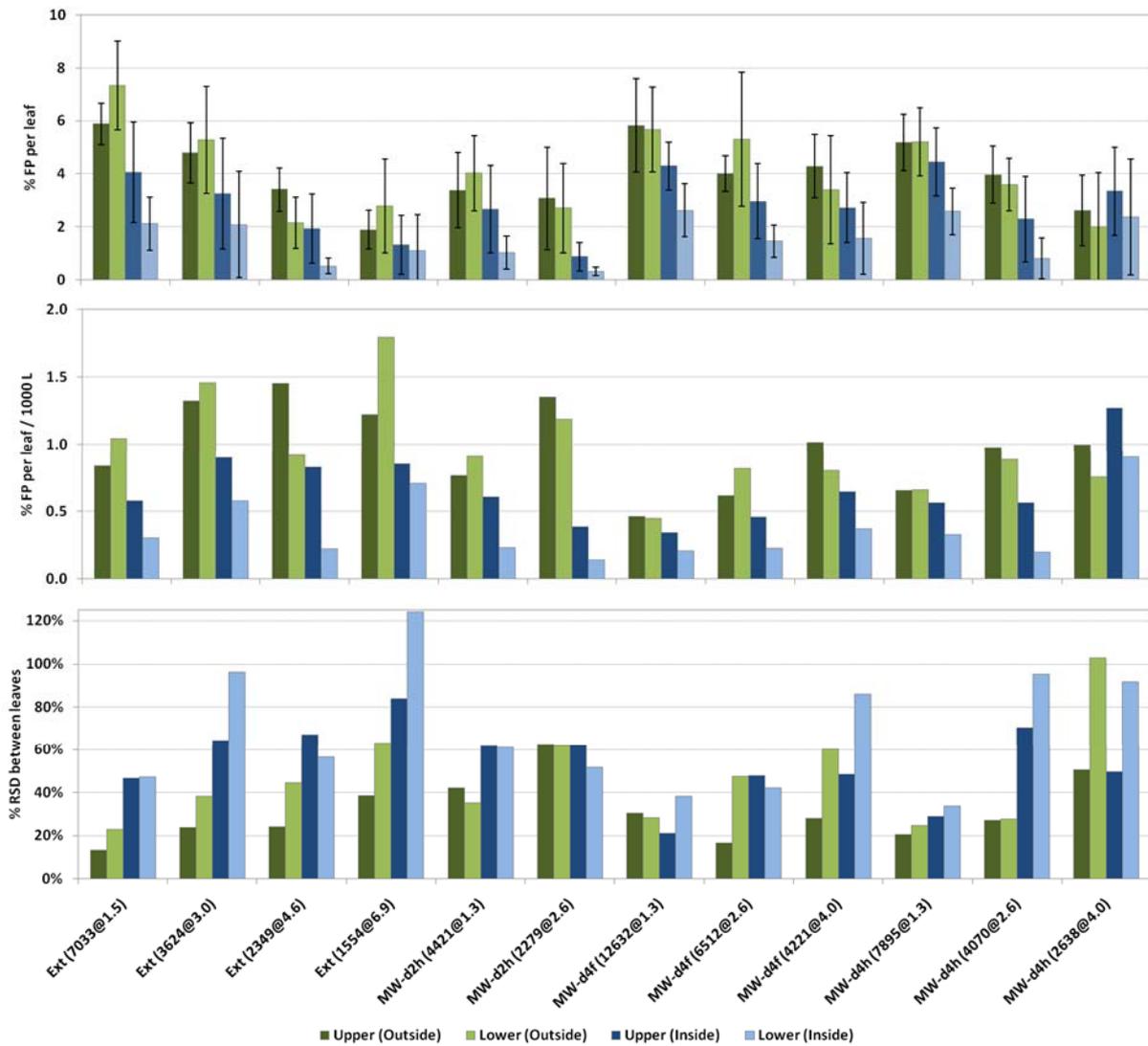


Figure 4.3.4.3. Mean quantitative spray deposition per leaf (% FP), variation between leaves (%RSD) and spray efficiency (expressed as % FP per leaf / 1000 L) on upper and lower leaf surfaces, which were sampled from top, middle and bottom sections of the inner and outer canopy, following spray application with SARDI Yellow Fluorescent Pigment (1 mL/hL) in a mature Valencia orchard in Hoedspruit with a BSF-Extreme oscillating boom mistblower (Ext) and BSF-Multiwing sprayer (MW) with various nozzle selection and tractor speeds [legend = Sprayer; nozzle type (D4-hollow or full cone or D2-hollow cone); spray volume in L/ha; tractor speed in km/h].

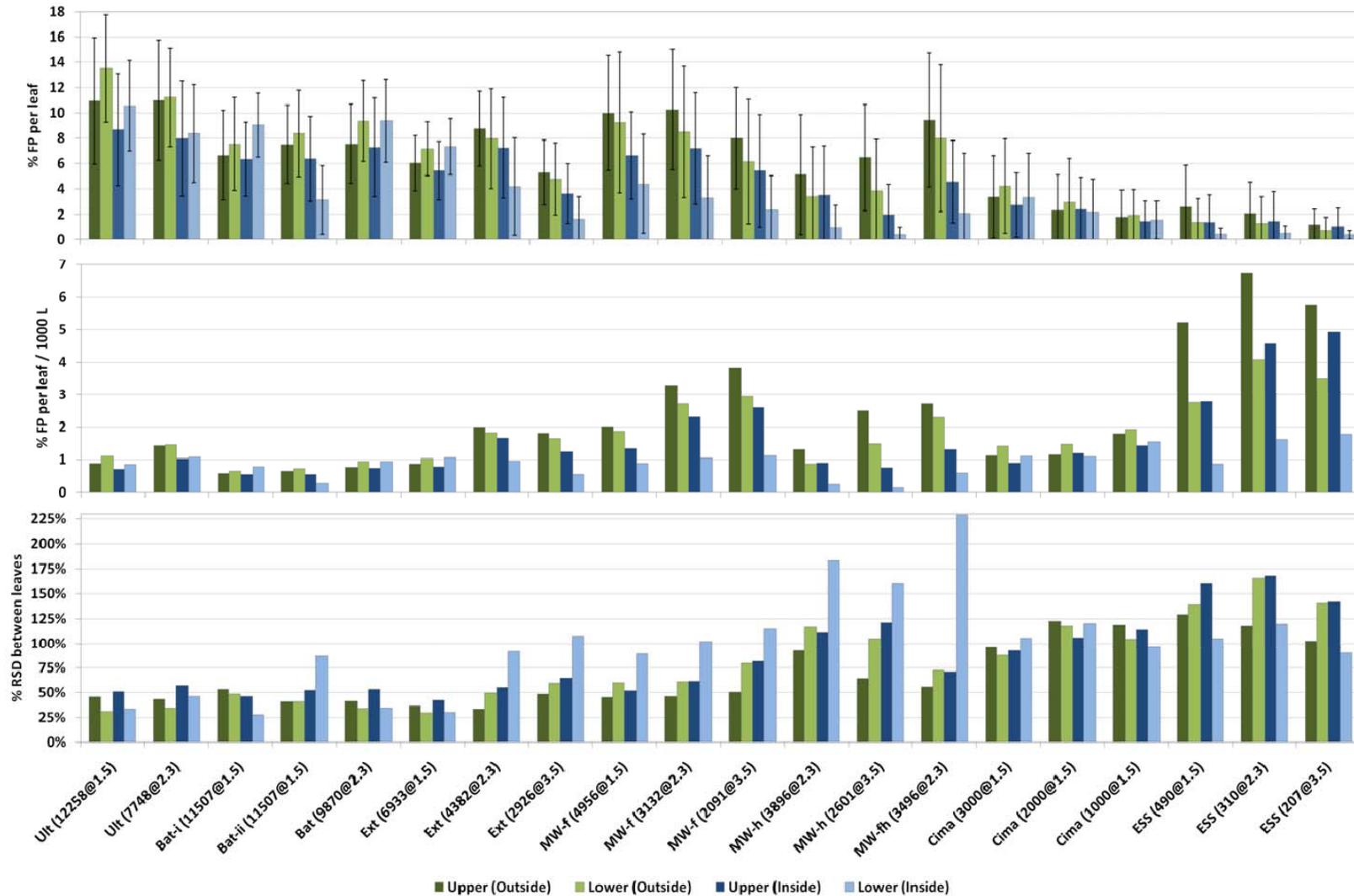


Figure 4.3.4.4. Mean quantitative spray deposition per leaf (% FP), variation between leaves (%RSD) and spray efficiency (expressed as % FP per leaf / 1000 L) on upper and lower leaf surfaces, which were sampled from top, middle and bottom sections of the inner and outer canopy, following spray application with SARDI Yellow Fluorescent Pigment (1 mL/hL) in a mature Valencia orchard in Letsitele with Ultima (Ult), Bateleur (Bat) and BSF-Extreme (Ext) oscillating boom mistblowers, BSF-Multiwing sprayer (MW), Cima air shear sprayer (Cima) and ESS electrostatic sprayer (ESS; used at 4× dosage of fluorescent pigment) with various nozzle selection and/or tractor speeds [legend = Sprayer; nozzle type (hollow or full cone); spray volume in L/ha; tractor speed in km/h].

4.4 PROJEK: GRONDGEDRAAGDESIEKTES

Projekkoördineerder: M.C. Pretorius (CRI)

4.4.1 Projekopsomming

Die sitrusaalwurm, *Tylenchulus semipenetrans*, is wêreldwyd die mees algemene aalwurm wat ekonomiese verliese in sitrusboorde veroorsaak. Toksiese aalwurmdoders het 'n groot effek op die omgewing asook lewende organismes, en al meer en meer druk teen die gebruik van die middels word deur markagente, verbruikers asook omgewingsbewuste groepe wêreldwyd op produsente geplaas. In die verlede is hoofsaaklik van chemiese produkte gebruik gemaak om die probleem in 'n kits op te los. CRI het 'n reeks voor-plant behandelings voorgestel om die effek van die produkte oor 'n lang termyn op herplant gronde te evalueer. Die voordeel van so 'n behandeling, indien effektief, sal die gebruik van toksiese aalwurmdoders, verminder. 'n Boord in die Karino omgewing is geoormerk vir die proef en sal in September 2009 behandel word. Twee maatskappye stel belang om hul produkte in die proef in te sluit, nl. Arysta LifeScience asook Dow AgroScience (4.4.2). Tans word die karbamate en organofosfaat aalwurmdoders algemeen gebruik vir die beheer van sitrusaalwurm in sitrusboorde. Internasionale druk om die gebruik van hierdie uiters toksiese middels te verminder neem toe en alternatiewe beheermaatreëls word ondersoek. 'n Verskeidenheid van produkte is ge-evalueer om hul effektiwiteit te bepaal nl. Abamectin, Nontox – Silica (silica), Bio-Neem (*Azadirachta indica*), 'n eierstimulant, 'n eierstimulant gekombineer met 'n aalwurmdoder, Mocap (ethoprophos) asook NemaCur (fenamiphos) en Rugby Gr (cadusafos). Voorlopige resultate lyk uiters belowend. Die eierstimulant gekombineer met 'n aalwurmdoder was uiters effektief en na slegs twee toedienings is 'n afname van 76 % behaal. Opvolg toedienings behoort vir nog een seisoen gedoen te word om die langtermyn effek van die alternatiewe produkte te bepaal (4.4.3). 'n Studie is geloods om vas te stel (1) watter *Phytophthora* spesies kom in verskillende Suid Afrikaanse sitrus produksie areas voor, (2) of *P. palmivora* in sitrusboorde in Suid Afrika teenwoordig is, (3) wat die patogenisiteit van vier *Phytophthora* spesies op sitrussaailinge en vrugte is en (4) wat die populasie struktuur van *P. citrophthora* in geselekteerde boorde is. Die hoof *Phytophthora* spesies wat in boorde landswyd geïdentifiseer is, was: *P. nicotianae*, *P. citrophthora*, *P. citricola*, *P. cinnamomi* en 'n potensiële nuwe spesie. *P. nicotianae* het die hoogste voorkoms gehad, gevolg deur *P. citrophthora*. Die *P. citrophthora* isolates kon in twee molekulêre groepe (G1SA and G2SA) verdeel word. Die isolate word tans morfologies verder gekarakteriseer. *Phytophthora palmivora* is nie geïdentifiseer in enige van die boorde nie. Patogenisiteitstoetsing met vier *Phytophthora* spesies op sitrus saailinge was nog nie suksesvol nie. In die *P. citrophthora* populasie studie is slegs een potensiële RXLR effektor geen gevind wat moontlik gebruik sal kan word (4.4.4). Beheer van *Phytophthora* stamkanker op 'nules' Clementines met 'n enkele stambehandeling van verskillende algdoders (fungisiede) teen verskillende konsentrasies is ge-evalueer. 'n Nuwe kwantitatiewe evaluasietegniek is ook ontwikkel om die algdoders te toets. Resultate toon dat al die algdoders effektief is behalwe die laagste dosis van chlorothalonil (100 ml/ 100 L water). Stambehandelings met beide die kontakdoders asook 'n sistemiese produk het goed gewerk teen die siekte wat toon die infeksie baie oppervlakkig is. Opvolgbespuitings is ook nodig omrede van die behandelde bome weer geïnfecteer raak. Die Captan en Sporekill tankmengsel is in die proses van registrasie. Evaluasies op verskillende nawel kultivar kwekeryboompies se vatbaarheid toon dat 'Royal Late' die mees tolerante nawel kultivar is, 'Witkrans' en 'Powell Summer' matig tolerant en 'Washington' hoogs vatbaar (4.4.5). Die is bekend dat fosfonaat blaarbespuitings fitotoksies mag wees en daarom is die toediening van kalium-fosfonaat deur die besproeiingstelsel ge-evalueer. Residu analises het gelyke kalium-fosfonaatvlakke in die wortels vir beide konvensionele blaarbespuiting en grondtoediening getoon. Die toediening van fosfonate het by sommige observasies 'n opbrengs- en vruggrootheid-toename getoon (4.4.6). Chemiese bemestingsprodukte kan ongunstige grondtoestande veroorsaak wat kan lei tot ongesonde wortels en plante. Die effek van verskillende komposprogramme / kompos-verwanteprogramme op grondkondisies, plant gesondheid, opbrengs en die plant se vermoë om siektes te beveg, word ge-evalueer. Geen resultate is tans beskikbaar nie (4.4.7). Aalwurm kontraknavorsing met nuwe alternatiewe middels is vir drie oorsese maatskappye gedoen nl; 'n VSA, Israeliese en Belgiese maatskappye (4.4.8, 4.4.9 and 4.4.10).

Project summary

The citrus nematode infects citrus worldwide and is the most abundant and frequent plant-parasitic nematode in citrus groves. International pressure to reduce the use of highly toxic compounds, such as the nematicides, is increasing and alternative measures need to be investigated. Pre-plant treatments that include the incorporation of non-toxic nematicides and safer soil fumigants will be investigated. A suitable orchard was identified in the Karino area and the treatments will start in September 2009. Two companies are interested in including their soil fumigants, Arysta LifeScience and Dow AgroScience (4.4.2). Currently the carbamate or organophosphate nematicides are the most popular means of nematode control. Increase in market pressure to reduce the use of these toxic compounds increased the search for alternative control measures. The following alternative products were evaluated:

Abamectin, Nontox – Silica (silica), Bio-Neem (*Azadirachta indica*), an egg stimulant, an egg stimulant + nematicide, Mocap (ethoprophos), Nemacur (fenamophos) + two Rugby Gr (cadusafos) applications. Preliminary results indicate a reduction of nematode populations by all the alternative products. The combined, egg stimulant+nematicide treatments were very effective and were able to reduce females in the roots by 76% after only two applications. Further research to confirm these results is necessary (4.4.3). A study was undertaken to determine (1) which *Phytophthora* species are present in different South African citrus production regions, (2) whether *P. palmivora* is present in citrus orchards in South Africa, (3) the pathogenicity of four *Phytophthora* species on citrus seedlings and fruits and (4) the population structure of *P. citrophthora* in selected orchards. The species identified during the survey included *P. nicotianae*, *P. citrophthora*, *P. citricola*, *P. cinnamomi* and a putative new species, with *P. nicotianae* having the highest incidence, followed by *P. citrophthora*. *Phytophthora palmivora* was not identified. Pathogenicity studies on citrus seedlings using four species have thus far not been successful. The *P. citrophthora* population study showed that only one putative RXLR effector gene showed some promise and will be investigated further (4.4.4). Control of *Phytophthora* trunk canker on 'hules' Clementines was evaluated through a single spray treatment consisting of different algicides (fungicides) at different rates. A new quantitative evaluation technique was developed to test the algicides. Results showed that all the selected algicides performed well, except for the lowest rate of chlorothalonil (100 ml/ 100L water). All selected trunk algicides (except Fighter) are contact treatments, showing that this superficial disease can be treated effectively. Follow-up treatments are necessary because treated trees can be re-infected and at least 4 treatments with 3-monthly intervals per year with a mixture of captan and Sporekill is recommended. Different Navel orange nursery trees tested for their susceptibility, showed that the 'Royal Late' was the most tolerant cultivar, with 'Witkrans' and 'Powell Summer' moderate tolerant and 'Washington' highly susceptible (4.4.5). Foliar applications of phosphonates can be problematic due to minor phytotoxic damage. The efficacy of the phosphonates when applied through the irrigation system was therefore evaluated in the Letsitele area. Residue analyses showed that potassium phosphonate levels in the roots for conventional foliar applications were similar to levels obtained for potassium phosphonates applied through the irrigation system. Some observations showed an increase in yield as well as fruit size (4.4.6). Chemical fertilizer can cause unfavourable soil condition for soil microbes that can lead to unhealthy roots and plants. The effects of compost / compost derivative programmes on overall soil conditions, plant health and ability to resist attack or infections and yield are being monitored. No results are currently available (4.4.7). Nematode contract research was done for three international companies in the USA, Israel and Belgium (4.4.8, 4.4.9 and 4.4.10).

4.4.2 **PROGRESS REPORT: To evaluate alternative nematode control products, i.e. Biofumigants, as part of an integrated nematode control approach in citrus replant situations** Eksperiment 762 (2007–2014) by M.C. Pretorius (CRI)

Opsomming

Die soektog na alternatiewe beheermaatreëls teen die sitrusaalwurm is 'n prioriteit vir navorsers wêreldwyd. Die gebruik van hoogs toksiese aalwurmdoders wat 'n groot effek het op die omgewing asook lewende organismes, word al meer en meer veroordeel deur markagente, verbruikers asook omgewingsbewuste groepe regoor die wêreld. 'n Ge-integreerde beheerbenadering sal in die toekoms meer aandag moet ontvang vanaf die produsente om te verseker dat die beheer van die aalwurm probleem nie agter weë gelaat word nie. In die verlede is hoofsaaklik van chemiese produkte gebruik gemaak om die probleem in 'n kits op te los. CRI het 'n reeks van voor–plant behandelings voorgestel om die effek van die produkte oor 'n lang termyn op herplant gronde te evalueer. Die voordeel van so 'n behandeling indien effektief sal die gebruik van toksiese aalwurmdoders, wat huidiglik ook baie duur is, verminder. 'n Boord in die Karino omgewing is geormerk vir die proef en sal in September behandel word. Twee maatskappye stel belang om hul produkte in die proef in te sluit nl. Arysta LifeScience asook Dow AgroScience. Beide maatskappye sal grondberokingsmiddels beskikbaar stel.

Summary

The search for alternative control methods for the effective control of nematodes is a priority by all researchers worldwide. A more integrated approach is recommended that will require a new way of thinking by producers when planning to control nematodes. Alternative control methods could include: pre-plant treatments that include the incorporation of non-toxic nematicides and safer soil fumigants. A suitable orchard was identified in the Karino area and the treatments will be done in September 2009. Two companies were interested in including their soil fumigants, i.e. Arysta LifeScience and Dow AgroScience.

Introduction

The search for alternatives to soil fumigants and very toxic nematicides is a priority at research stations and by researchers worldwide. Producers will have to change their way of thinking from a one shot control

strategy they have become accustomed to, to a more integrated approach such as host plant resistance, bio-fumigation, alternative chemicals and cultural practices. By focusing on alternative control strategies, e.g. by incorporating bio-fumigants into traditional control strategies, could reduce the usage of toxic nematicides polluting the environment. A combination of bio-fumigants, alternative non-toxic chemicals, Biocontrol agents, rootstock choices and cultural practices should be implemented as a new approach of pest control in the soil against known soil borne diseases. This trial was initiated due to the economic impact, high treatment costs, as well as to reduce use of the toxic nematicides. Alternative measures should therefore be available to producers to keep their replant orchards nematode free for as long as possible before a post-plant nematicide treatment is necessary, if at all.

Materials and methods

The idea is to include pre-plant treatments of different products at different rates and times. The effect of the pre-plant treatments will then be monitored by means of soil and root analyses to determine the nematode population status. A visual evaluation will also be done on an annual basis. This trial will have to be monitored for at least eight years.

The trial layout will consist of eight treatments (ten trees per treatment), and will be repeated four times. The treatments will include an untreated control, Vapam, Telone, Biofumigation product A, Biofumigation product A + Plastic (soil solarisation), nematode egg stimulating product A, nematode egg stimulating product + nematicide and a furfural. A suitable replant site with nematode female population numbers in excess of 6000 females/10g roots will be ideal for this trial.

Results and conclusion

It was very difficult to find a suitable site to commence with this trial. Various options were utilised to find a suitable trial site, it included the following; ask the extension department for assistance, send a request for help to the private consultants in the different regions, collect information from nurseries to establish if growers are planning to remove orchards in the different regions, lastly an request was sent to all the study group forums via the CRI net. A grower from Karino contacted CRI informing us that he is planning to remove an old orchard. Samples were collected from the orchard and sent to the Diagnostic Centre at CRI for analyses. Female count in excess of 5000 /10g root were recorded. The original removal of the orchard was scheduled for November 2008 however, farm management decided to keep the orchard for one more season. The next scheduled removal date is August 2009. Arista LifeScience, a Durban, KwaZulu-Natal based company, who is involved in many different agricultural crops specialising in soil fumigants are currently involved with the deciduous fruit industry with pre-plant treatments of the soil to reduce the incidence of soil pathogens with the main aim of reducing the use of post plant chemicals. A planning meeting at their offices was attended and it was decided that three formulations of Midas soil fumigant (Iodomethane:chloropicrin) will be included in our trial at Karino. Dow AgroSciences are also interested to participate in this trial by providing Telone as a soil fumigant.

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4.4.3 Progress report: An integrated approach to ensure root and plant health Experiment 910 (2008–2010) by MC Pretorius (CRI)

Opsomming

Die sitrusaalwurm, *Tylenchulus semipenetrans*, is wêreldwyd die mees algemene aalwurm wat ekonomiese verliese in sitrusboorde veroorsaak. Aalwurms is uiters effektief beheer met die DBCP (dibromo-3-chloropropane) produkte wat tans nie meer beskikbaar is nie. Weens die onttrekking van die DBCP produkte is die karbamate en organofosfaat aalwurmdoders in gebruik geneem. Internasionale druk om die gebruik van hierdie uiters toksiese middels te verminder het navorsers wêreldwyd genoop om alternatiewe beheermaatreëls te ondersoek. 'n Verskeidenheid van produkte is ge-evalueer om hul effektiwiteit vir die beheer van sitrusaalwurm te bepaal. Die volgende produkte is ge-evalueer: Abamectin, Nontox – Silica (silica), Bio-Neem (*Azadirachta indica*), 'n eierstimulant, 'n eierstimulant gekombineer met 'n aalwurmdoder, Mocap korrels (ethoprophos) en een Namacur (fenamiphos) toediening opgevolg met twee Rugby Gr (cadusafos). Voorlopige resultate blyk uiters belowende te wees met die alternatiewe produkte. Die eierstimulant gekombineer met 'n aalwurmdoder was uiters effektief en na slegs twee toedienings is reeds 'n afname van 76% behaal. Die chemiese standaarde het weereens getoon dat hulle ook steeds uiters effektief is. Opvolg toedienings behoort vir nog een siesoen gedoen te word om die langtermyn effek van die alternatiewe produkte vir die beheer van die sitrusaalwurm op sitrus te bepaal.

Summary

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus worldwide and is the most abundant and frequent plant-parasitic nematode in citrus groves. Yield losses are estimated at about 10% worldwide. In the past, the nematicide 1,2 dibromo-3-chloropropane (DBCP) was widely used with great success but is no longer available. The non-fumigant post-plant nematicides (carbamate or organophosphates) were introduced. Due to safety, environmental concerns and market pressure the continued use of these toxic compounds is increasingly doubtful. Developing alternatives to chemical nematicides are therefore essential. The following products were evaluated in the trial: Abamectin, Nontox – Silica (silica), Bio-Neem (*Azadirachta indica*), an egg stimulant, an egg stimulant + nematicide, Mocap (ethoprophos) and a single Namacur (fenamiphos) + two Rugby Gr (cadusafos) applications. Preliminary results indicate that all the alternative products reduced the nematode populations in both the soil and roots. The combined, egg stimulant+nematicide treatments were very effective, after only two applications, and a reduction of female populations of up to 76% were recorded in one of the treatments. This concept showed much potential and will revolutionise the citrus nematode control approach if proved to be cost effective. It is clear from these results that the chemical nematicides are still very effective. Further research to confirm these results and a better understanding of the products mode of action and residual activity are necessary before these kinds of products could be utilised by the South African citrus growers.

Introduction

Nematodes are a diverse group of invertebrates, abundant as parasites or free living forms in soil, freshwater and marine environments. Soils are a particularly rich environment for nematodes, with about 26% of described genera inhabiting soil as bacterivores, fungivores, omnivores, predators or plant parasites (McSorley, 2005).

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus worldwide (Van Gundy and Meagher, 1977; Heald and O'Bannon, 1987) and is the most abundant and frequent plant-parasitic nematode in citrus groves. Yield losses are estimated at about 10% worldwide. The citrus nematode is associated with poor growth of young citrus trees planted in infested groves and with poor performance of mature citrus trees. The host range of *T. semipenetrans* includes all Citrus species and most hybrids of citrus with other members of the rutaceous family such as trifoliolate orange (*Poncirus trifoliolate* L.Raf). Non – rutaceous plants such as grape (*Vitis vinifera*, L), olive (*Olea europea*, L) and persimmon (*Diospyrus spp.*) are also hosts (Verdejo – Lucas, 2002).

The citrus nematode has a relatively simple life cycle consisting of the egg, four larval stages and the adult male and female. Under suitable conditions, the eggs hatch and new larvae emerge to complete the life cycle within 4-8 weeks depending on temperature. Citrus nematode females become semi-endoparasitic and sedentary following infection of fibrous roots of susceptible rootstocks (Cohn, 1965b). The citrus nematode male appears to complete its life cycle without feeding (Van Gundy, 1958).

Damage thresholds, nematode population densities that suppress tree growth and yield, are influenced by several factors, including aggressiveness of the nematode population, soil type, rootstock, other diseases

and grove management practices (Garabedian *et al.* 1984). Threshold values in South African have been set at 10 000 juveniles/ 250 cc soil and a 1000 females/10 g root in samples.

T. semipenetrans migrates very slowly on its own power and therefore does not readily spread from tree to tree in existing orchards. Infestation of new orchards occurs mainly through infested planting material and contaminated irrigation water (Tarjan, 1971; Baines, 1974). It is recorded that the sheath nematode, *Hemicycliophora* spp. occurs in combination with the citrus nematode in certain citrus producing countries in the world (Van Gundy, 1959) but the effect of the nematode on yields is not known. The sheath nematode was also detected in certain citrus producing regions in South Africa (L. Huisman, personal communication, CRI Diagnostic Centre, Nelspruit, 2007).

In the past, the nematicide 1,2 dibromo-3-chloropropane (DBCP) was widely used in the irrigation water against this nematode with great success. The nematode was effectively controlled while yields were also substantially increased (O'Bannon *et al.*, 1963; Philis, 1969). The nematicide was used every 3 to 4 years, thus reducing treatment cost to a great extent. Post-plant fumigants such as DBCP are no longer available to reduce nematode populations to undetectable levels. The latter chemical is a volatile compound with a short residual activity in soil. Control was thus achieved by reducing nematode populations through the initial action of DBCP, and not through residual activity (Baines *et al.*, 1966). From the recovery rate reported by O'Bannon *et al.*, (1967) it is clear that DBCP not only killed juveniles and adult stages of the nematode, but also prevented eggs from hatching. This activity on eggs is the most important difference between the soil fumigants used to control nematodes earlier this century and today's non-fumigant chemicals. Following the withdrawal of DBCP, non-fumigant post-plant nematicides (carbamate or organophosphate acetylcholinesterase inhibitors) were introduced. These chemicals however, could not eliminate, or greatly reduce, nematode populations even if applied every year. Aldicarb and fenamiphos are translocated systemically in the vascular system of plants, whilst the other nematicides are non-systemic and reduce nematode populations through their initial contact action only. This explains the quick recovery of nematode populations once the nematicide has been degraded in soil and emphasises the adverse effect of enhanced degradation, as eggs hatching after the nematicide has been degraded can continue the nematode's life-cycle. South African citrus growers traditionally relied mainly on the postplant fumigant DBCP for controlling *T. semipenetrans* in existing orchards. With the banning of this compound they had to adjust to using granular postplant nematicides. The following nematicides currently registered on citrus in South Africa are aldicarb, cadusafos, fenamiphos, terbufos, ethoprophos and fosthiazate (Nel *et al.*, 2002). When multiple nematicide applications was introduced on a commercial scale to citrus orchards in South Africa, situations occurred where growers were not successful in disrupting the nematode's life cycle despite adhering strictly to prescribed procedures. In an investigation to determine the efficacy of cadusafos in soil where aldicarb and fenamiphos failed as a result of accelerated degradation, it was found that in the absence of sufficient irrigation water none of the nematicides were distributed thoroughly through the soil profile and they consequently failed to eliminate the citrus nematode (Le Roux *et al.*, 1998).

Due to safety, environmental concerns and market pressure, only a few registered chemical nematicides are available worldwide for utilization by farmers, and the use of these products is highly restricted. Developing alternatives to chemical nematicides is essential and a great concern to researchers worldwide. Recent attempts to develop alternative methods to manage plant-parasitic nematodes include the use of entomopathogenic nematodes and various biologically derived nematicides and other organic compounds. The aim of this experiment is to: (1) evaluate less toxic compounds for the control of the citrus nematode as well as for the control of *Phytophthora* spp. in citrus orchards, (2) the re-evaluation of alternative control products for the control of the citrus nematode and (3) to investigate the implementation of a multi-variate Statistical programme (Environmental Data Analysis, ade4), which was developed by researchers in France to manage tree health.

In this report, results of the re-evaluation (screening) of a range of alternative products such as insecticides, non-toxic and organic compounds for the control of the citrus nematode will be discussed. International pressure from various market organisations and Governments to reduce the use of highly toxic and environmentally unfriendly products justifies this approach. If this approach is found to be effective, it would provide local producers with possible alternative products in the event of South Africa losing the use of the registered nematicides.

Materials and methods

1. Evaluate less toxic compounds for the control of the citrus nematode as well as for the control of *Phytophthora* spp. in citrus orchards

No new compounds have been found with potential nematocidal or *Phytophthora* control properties but, the re-evaluation of the alternative control products continued as discussed in this document.

2. Re-evaluation of alternative control products for the control of the citrus nematode

A nematode-infested citrus orchard with nematode female counts in excess of 5500 females per 10 g of roots was identified. This was regarded as a suitable trial site, as the standard threshold value of 1000 females per 10 g of root was exceeded. The 12-year old citrus orchard with a 10 m² drip zone is situated east of Nelspruit at Crocodile Valley Citrus Co. The liquid formulated products were applied by means of a 10 litre watering can to ensure an even distribution of the products under the drip zone of the trees. The granular formulations were applied by hand. Cadusafos, ethoprophos and fenamiphos served as the standard chemical control. Protective clothing was used to protect the researcher and staff when applying these products. Single tree plots were randomly selected and replicated six times. The following products were evaluated in the trial: Abamectin, Nontox – Silica (silica), Bio-Neem (*Azadirachta indica*), nematode egg stimulating product, a combination of a nematode egg stimulating product with a nematicide, and the standard nematicide applications which consisted of a combination of a single NemaCur (fenamiphos) followed by two Rugby (cadusafos) applications and Mocap (ethoprophos). The different dates of applications and dosages are presented in Table 4.4.3.1. All the applications were executed in good weather conditions with an average day temperature of 32°C.

The trees were sampled before the January 2009 applications and again in March 2009 before the third applications. The third set of samples collected two months after the last application in March 2009 were collected during May 2009. The nematode population analyses in the soil and roots were conducted by the Diagnostic Centre in Nelspruit. The second stage larvae in the soil was determined according to the method of Whitehead and Hemming (1965) and the female populations in the roots were determined according to the method of Van der Vegte (1973). Only the first (January 2009) and second (March 2009), sampling dates data will be discussed at this time (Table 4.4.3.2 and 4.4.3.3). The final report with all data included will be presented in next year's annual report.

3. Implementation of a multi-variate statistical programme (ADE-4) to manage citrus tree health

Two potential decline orchards will be identified once the literature study has been completed. A number of parameters will be monitored and the results will be analyzed by utilizing the Multi-variate statistical program with the help of Prof. Patrice Cadette of France. The different parameters will be identified once the literature study is completed.

Results and discussion

1. Evaluate less toxic compounds for the control of the citrus nematode as well as for the control of *Phytophthora* spp. in citrus orchards

No alternative *Phytophthora* control products could be found to evaluate for possible inclusion in a holistic rootrot control programme. A chemical company did, however, indicate that they are investigating a range of possible alternative products for the control of *Phytophthora* in the soil. The industry desperately needs a contact compound to reduce the initial inocula in the soil. No products were received during the season but negotiations are ongoing. As soon as the company makes them available these products will be included in glasshouse and field trials.

2. Re-evaluation of alternative control products for the control of the citrus nematode

Tables 4.4.3.2 and 4.4.3.3 present the results obtained from the root and soil samples that were collected during January and March 2009.

All the products had an initial (January 2009), effect by reducing the larvae counts in the soil with the exception of Bio-neem (Treatment 5) and Silica ([400g/l] Treatment 6). The March 2009 data showed that only the Bio-neem (Treatments 4 and 5) and egg stimulating products (Treatments 8,9,10 & 11) did not reduce the larvae counts in the soil. All the other treatments effectively decreased the larvae counts in the

soil with the largest reduction by the egg stimulation / nematicide combination (Treatment 13), a significant reduction of 76% when compared to the untreated control (Table 4.4.3.2).

The initial female count results (January 2009), represented in Table 4.4.3.3, clearly showed that all the products applied in December 2008 reduced the female counts in the roots. One ethoprophos (Treatment 16) application significantly reduced the female count by 67% if compared to the untreated control. The results obtained during the March 2009 sampling showed promising results from all the treatments. The abamectin treatments (2 & 3) were able to reduce the root counts significantly by 40 and 50% respectively. The Bio-neem treatments (4 & 5) only reduced the numbers by 28 and 25%. The silica treatments (6 & 7) showed a 34% reduction in female counts. Surprisingly, all the nematode egg stimulating product treatments (8, 9 10 & 11), with the exception of treatment 10 with a population increase of 20%, reduced the female counts in the roots. In contrast, the same product was previously possible to increase the female counts in the roots. The reduction of females in the roots could be attributed to the very high rainfall recorded during January to April 2009.

The combined product, egg stimulant + nematicide treatments (12, 13 & 14) decreased the female population counts significantly by 76, 71 & 50% respectively. The standard nematicides were also effective and reduced the female populations by 65% (Cadusafos, treatment 15), 74% (Ethoprophos, treatment 16) and by 71% (Fenamiphos + Cadusafos, treatment 17).

The final set of data, which will represent the effect of the different treatments on the female populations on the roots, will be collected during May 2009. These results supported the data obtained from Experiment 893 that was terminated during the previous season.

3. Implementation of a multi-variate statistical program (ADE-4) to manage citrus tree health

The investigation with regards to the implementation of a multi-variate statistical program (Environmental Data Analysis, ade4), were discussed with Dr. Patrice Cadette (France) and Prof. Nico Labuschagne (UP). Shortcomings with the contents of the statistical program with regards to the layout of the trial and the interpretation of the data based on annual crop data, had to be addressed. The program was originally developed with great success on annual crops but was never tested on perennial crops. Two potential orchards have been identified. A literature study with regards to citrus decline will commence during the winter months of 2009 at the University of Pretoria.

Conclusion

It was essential to re-evaluate a range of alternative products for the control of the citrus nematode to reduce the use of highly toxic chemical compounds such as the registered nematicides. It is clear from the preliminary results that the combined egg stimulant + nematicide treatment was highly effective in reducing the female population counts in the roots after only two applications. These results compared well with the standard nematicide treatments and the long-term effect of these combined applications should be monitored. If shown to be effective the idea to stimulate as many citrus nematode eggs as possible to hatch and to have a toxic nematicidal compound immediately available in the soil when the eggs hatch will revolutionise the citrus nematode control approach in all citrus-producing regions worldwide. The results of the other alternative products are also effective and showed promising results. These products will in future assist growers to have an alternative approach in place in the event of them losing the use of the registered toxic nematicides due to international market pressure. However, it is not certain whether registration of these products for the control of nematodes will occur.

Futher objectives

Research in obtaining promising results with the less toxic products as well as the combined egg stimulant + nematicide should continue for one more season to determine the long term effect of these products on the nematode populations in the soil. This approach would enable researchers to make a final conclusion with regards to a new alternative approach for citrus nematode control on citrus in South Africa. The search for Phytophthora control alternatives will continue and the ADE Statistical program will be implemented after the literature study is completed.

Technology transfer

Results were presented at the 19th Bi-annual Symposium of the Nematological Society of Southern Africa at Hazyview, Mpumalanga, South Africa during May 2009. Data will also be presented at the next bi-annual CRI Symposium in August 2010.

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Table 4.4.3.1. Dosages and dates of application of the different products applied to determine the effect of these treatments on the citrus nematode populations at Crocodile Valley Citrus Company.

	Treatment	Dosage/10m ² tree	25 Nov 08	Dec 08		Jan 09	Feb 09		Mar 09	Apr 09	
				9/12	23/12	21/1	04/02	18/02	19/03	02/04	15/04
1	Untreated control	-	-	-	-	-	-	-	-	-	-
2	Abamectin	10 ml	X	X	X		X		X		
3	Abamectin	40 ml	X	X	X		X		X		
4	Neem	15 ml	X		X				X		
5	Neem	30 ml	X		X				X		
6	Silica (400 g/l)	250 ml	500	250	250	500	250		250	250	
7	Silica (400 g/1/2l)	250 ml	500	250	250	500	250		250	250	
8	DL (30 g/m ²)	300 ml	X	X	X		X		X		X

9	DL (60 g/m ²)	600 ml	X			X			X	
10	DL (90 g/m ²)	900 ml	X			X			X	
11	DL (30 g/m ²)	300 ml	X			X			X	
12	DL+	75 g	X	X		X	X		X	
13	DL+	75 g	X			X			X	
14	DL+	75 g	X						X	
15	Rugby	150 g	X			X			X	
16	Mocap	75 g	X			X			X	
17	Fen + 2 Rugby	200 ml	X			X			X	

* Products applied as a soil application by means of a 10l watering can.

Table 4.4.3.2. The effect of various soil – applied, toxic, non - toxic, organic and nematode egg stimulating range of products applied at different rates and dates at Crocodile Valley Citrus Company for the control of citrus nematode larvae populations in the soil collected during January and March 2009.

No.	Treatments ^x	Dosage/10m ² tree canopy	January 09 ^y		March 09 ^y	
			L2/250 cc soil	% Increase(+)/ Decrease(-) vs Control	L2/250 cc soil	% Increase(+)/ Decrease(-) vs Control
1	Untreated control	-	4933 bcd	-	7100 abcd	-
2	Abamectin	40ml	2733 abcd	- 45	4683 abcd	- 34
3	Abamectin	10 ml	4433 bcd	- 10	5416 abcd	- 24
4	Bio-neem	15 ml	4033 abcd	- 18	8416 bcd	19
5	Bio-neem	30 ml	5616 d	14	8983 cd	27
6	Silica (400 g/l)	25 ml	5416 cd	10	2100 ab	- 70
7	Silica (800 g/l)	25 ml	3283 abcd	- 33	5783 abcd	- 19
8	DL	300g	2500 abcd	- 49	10683 d	50
9	DL	600g	4000 abcd	- 19	7550 abcd	6
10	DL	900g	3816 abcd	- 23	10000 cd	41
11	DL	300g	3700 abcd	- 25	9166 cd	29
12	DL+	75g	2133 ab	- 57	4083 abc	- 42
13	DL+	75g	2006 ab	- 59	1683 a	- 76
14	DL+	75g	3933 abcd	- 20	4166 abcd	- 41
15	Cadusafos	150 g	1833 ab	- 63	4650 abcd	- 35
16	Ethoprophos	75g	916 a	- 81	4083 abc	- 42
17	Fen + Cadusafos	50g + 150g	21416 abc	- 51	2350 ab	- 67

^x Application dates for treatment 2 and 3: 25 November 2008, 25 December 2008, 21 January 2009 and 18 February 2009.

Application dates for treatment 4, 5, 10, 11, 13, 15, 16 and 17: 25 November 2008 and 21 January 2007.

Application dates for treatment 6 and 7: 25 November 2008, 9 & 23 December 2008, 21 January 2009 and 4 February 2009.

Application dates for treatment 8 and 12: 25 November 2008, 23 December, 21 January 2009 and 18 February 2009.

Application dates for treatment 9: 25 November 2008 and 21 January 2009.

Application date for treatment 14: 25 November 2008.

^y Nematode sampling dates

^z Means in a column followed by the same letter are not significantly different (P>0.05) according to Fisher's LSD test.

Table 4.4.3.3. The effect of various soil – applied, toxic, non- toxic, organic and nematode egg stimulating range of products applied at different rates and dates at Crocodile Valley Citrus Company for the control of citrus nematode female populations in the roots, collected during January and March 2009.

No.	Treatments ^x	Dosage/10m ² tree canopy	January 09 ^y		March 09 ^y	
			♀ / 10g roots	% Increase(+)/ Decrease(-) vs Control	♀ / 10g roots	% Increase(+)/ Decrease(-) vs Control
1	Untreated control	-	6900 D	-	7000 gh	-
2	Abamectin	40mℓ	4500 Abcd	-35	4166 bcde	- 40
3	Abamectin	10 mℓ	5866 Cd	-15	3366 abcd	- 52
4	Bio-neem	15 mℓ	3800 Abc	-45	5066 defg	- 28
5	Bio-neem	30 mℓ	4833 Bcd	-30	6266 efgh	- 25
6	Silica (400 g/ℓ)	25 mℓ	5333 Bcd	-23	4633 cdef	- 34
7	Silica (800 g/ℓ)	25 mℓ	3800 Abc	-45	4600 cdef	- 34
8	DL	300g	3300 Ab	-52	5533 defg	- 21
9	DL	600g	4133 Abc	-40	6466 fgh	- 8
10	DL	900g	5366 Bcd	-22	8383 h	20
11	DL	300g	5066 Bcd	-27	6766 fgh	- 3
12	DL+	75g	3533 Abc	-49	1666 a	- 76
13	DL+	75g	5066 Bcd	-27	2033 ab	- 71
14	DL+	75g	3166 Ab	-54	3466 abcd	- 50
15	Cadusafos	150g	4500 Abcd	-35	2433 abc	- 65
16	Ethoprophos	75g	2300 A	-67	1800 a	- 74
17	Fen + Cadusafos	50 g + 150g	4800 Bcd	-30	2000 b	- 71

^x Application dates for treatment 2 and 3: 25 November 2008, 25 December 2008, 21 January 2009 and 18 February 2009.

Application dates for treatment 4, 5, 10, 11, 13, 15, 16 and 17: 25 November 2008 and 21 January 2007.

Application dates for treatment 6 and 7: 25 November 2008, 9 & 23 December 2008, 21 January 2009 and 4 February 2009.

Application dates for treatment 8 and 12: 25 November 2008, 23 December, 21 January 2009 and 18 February 2009.

Application dates for treatment 9: 25 November 2008 and 21 January 2009.

Application date for treatment 14: 25 November 2008.

^y Nematode sampling dates

^z Means in a column followed by the same letter are not significantly different ($P>0.05$) according to Fisher's LSD test.

4.4.4 PROGRESS REPORT: Characterization of *Phytophthora* species from various South African citrus production regions

Experiment US1/07 (April 2008–May 09) by J. Meitz (SU), M.C. Pretorius, T. Schutte, L. Huisman, E. Carstens (CRI), W.J. Botha (ARC) & A. McLeod (SU)

Opsomming

Die hoof doelwitte van die studie was om vas te stel (1) watter *Phytophthora* spesies kom voor in verskillende Suid Afrikaanse sitrus produksie areas, (2) of *P. palmivora* teenwoordig is in sitrusboorde in Suid Afrika, (3) wat die patogenisiteit van vier *Phytophthora* spesies op sitrussaailinge en vrugte is en (4) wat die populasie struktuur van *P. citrophthora* in geselekteerde boorde is. *Phytophthora* isolate (n = 182) is in 46 sitrus boorde en kwekerie in Suid Afrika versamel. Die isolate is geïdentifiseer tot op spesievlak deur gebruik te maak van PCR-RFLP van die interne getranskribeerde spasiëerder area van die rRNA geen (ITS), en daarna is die DNA basispaar volgorde van 'n paar isolate bepaal. Die spesies wat geïdentifiseer was sluit in *P. nicotianae*, *P. citrophthora*, *P. citricola*, *P. cinnamomi* en 'n potensieële nuwe spesie. *P. nicotianae* het die hoogste voorkoms gehad, gevolg deur *P. citrophthora*. Die *P. citrophthora* isolates kon in twee molekulêre groepe (G1SA and G2SA) verdeel word. Hierdie twee groepe het op die ITS DNA vlak ooreengestem met die G1 en G2 groepe wat geïdentifiseer is deur Cohen *et al.* (2003) in Corsica. Alhoewel filogenetiese analise van die ITS area getoon het dat die G2 and G2SA groepe eerder aan *P. colocasiae* verwant is, het die beta-tubulin en cytochrome oxidase 1 filogenieë getoon dat die isolate eerder aan die *P. citrophthora* G1SA isolates verwant is. Die isolate word tans morfologies verder gekarakteriseer. *Phytophthora palmivora* is nie in enige van die boorde deur isolasies of "real-time" PCR geïdentifiseer nie. Patogenisiteitstoetsing van vier spesies (*P. nicotianae*, *P. citrophthora*, *P. palmivora* van laventel en *P.*

arecae van palms) op sitrus saailinge was nog nie suksesvol nie. Die *P. citrophthora* populasie studie is al begin, waar die eerste stap was om merkers te identifiseer wat geskik is vir gebruik in populasie studies. Alhoewel baie geen areas en iso-ensieme ondersoek is, is slegs een potensiële RXLR effektor geen gevind wat moontlik gebruik sal kan word.

Summary

The main objectives of the study were to determine (1) which *Phytophthora* species are present in different South African citrus production regions, (2) whether *P. palmivora* is present in citrus orchards in South Africa, (3) the pathogenicity of four *Phytophthora* species on citrus seedlings and fruits and (4) the population structure of *P. citrophthora* in selected orchards. *Phytophthora* isolates (n = 182) were collected from 46 citrus orchards and nurseries in South Africa. The isolates were identified to the species level using PCR-RFLP of the internal transcribed spacer of rRNA genes (ITS), followed by sequence analysis of a subset of the isolates. The species that were identified included *P. nicotianae*, *P. citrophthora*, *P. citricola*, *P. cinnamomi* and a putative new species, with *P. nicotianae* having the highest incidence followed by *P. citrophthora*. Among the *P. citrophthora* isolates, two molecular groups (G1SA and G2SA) were identified which corresponded at the ITS level to the G1 and G2 groups identified by Cohen *et al.* (2003) in Corsica. Although phylogenetic analyses of the ITS region showed that the G2 and G2SA isolates grouped with *P. colocasiae*, the beta-tubulin and cytochrome oxidase 1 phylogenies rather grouped these isolates with *P. citrophthora* G1SA isolates. The isolates are currently being characterized further morphologically. *Phytophthora palmivora* was not identified in any of the orchards using conventional isolation methods and real-time PCR. Pathogenicity studies on citrus seedlings using four species (*P. nicotianae*, *P. citrophthora*, *P. palmivora* from lavender and *P. arecae* from palms) have thus far not been successful. The *P. citrophthora* population study has been initiated by first identifying markers suitable for use in a population study. Among all the investigated gene sequence areas and isozymes, only one putative RXLR effector gene showed some promise for revealing polymorphisms among isolates, and will be investigated further.

Introduction/Objectives of the study

1. Characterise *Phytophthora* species associated with citrus seedlings and trees (nurseries and orchards) in different production regions of South Africa (August 2007 to December 2008).
2. Determine if *Phytophthora palmivora* is present in South African citrus production regions (August 2007 to December 2008).
3. Determine the susceptibility of different citrus rootstocks and fruits to *Phytophthora* species associated with citrus (September 2008 to March 2010).
4. Development of a real-time PCR detection method for *P. palmivora* (February 2009 to December 2010).
5. Investigating the population genetic structure of *P. nicotianae* (September 2008 to March 2010).

Materials and methods

1. Characterise *Phytophthora* species associated with citrus seedlings and trees (nurseries and orchards) in different production regions of South Africa

Soil samples were collected from 46 citrus orchard and nursery soils, and isolations were made for *Phytophthora*. Citrus leaf discs were used to bait *Phytophthora* isolates from a soil-water slurry, which were plated onto *Phytophthora* semi-selective media (PARPH, Tsao *et al.*, 1983). Hyphal growth from all *Phytophthora* isolations was subcultured for DNA extraction. *Phytophthora* species were identified using PCR amplification with *Phytophthora* ITS specific primers, followed by restriction fragment length polymorphism (RFLP) analyses (PCR-RFLP) (Drenth *et al.*, 2006). The ITS region of a group of isolates representing the different PCR-RFLP groups were sequenced in order to confirm the PCR-RFLP identifications. Since some *Phytophthora* species have identical ITS sequences, the beta-tubulin gene (Kroon *et al.*, 2004) was also sequenced for these isolates. Furthermore, since two different putative *P. citrophthora* groups were identified among the isolates based on ITS sequence data, these isolates were also sequenced for the cytochrome oxidase 1 gene area (Schena *et al.*, 2006).

2. Determine if *Phytophthora palmivora* is present in South African citrus production regions

This objective was conducted simultaneously with objective one, where all *Phytophthora* isolates were identified to the species level, to determine if *P. palmivora* was present.

3. Determine the susceptibility of different citrus rootstocks and fruits to *Phytophthora* species associated with citrus

Four *Phytophthora* species including *P. nicotianae*, *P. palmivora* (from lavenders), *P. citrophthora* and *P. arecae* (from palms and possibly con-specific with *P. palmivora*) were selected for pathogenicity tests on citrus seedlings (Rough lemon and Carrizo), two isolates per species. Two trials were conducted using 10 seedlings per treatment and two different inoculation methods. In the first trial a sand-bran medium was used for inoculating soil, whereas in the second trial millet seed was used to inoculate soil. The trials were evaluated by determining seedling height at planting and at the end of the trial, as well as root rot. Re-isolations from the soil were conducted 3 months after planting, in order to determine whether the inoculation method was successful.

4. Development of a real-time PCR detection method for *P. palmivora*

In order to ascertain that the presence of the quarantine pathogen *P. palmivora* did not go undetected in citrus soils due to its slow growth compared to *P. nicotianae* and *P. citrophthora* on the semi-selective medium, species specific primers were developed for use in qPCR directly from the leaf discs used in soil baitings. The DNA was extracted from approximately 60mg citrus leaf discs using the Phenol-Chloroform extraction method described by Goodwin *et al.* (1992). The extracted DNA was purified with PVPP columns and diluted 1/10. A standard dilution series of *P. palmivora* DNA extracted from mycelia were included in all real-time PCR analyses and all samples were duplicated within a real-time PCR run. DNA extracted from leaf discs from an artificial soil inoculation experiment with *P. palmivora* were used as positive control. qPCR conditions were optimized using the SensiMix dT kit (Quantase, London, UK) that contains SYBR® Green I. The qPCR reaction contained 1x Sensimix, 50nM forward primer (PpalF1, 5'ATCAAACCTTAGTTGGGGGGTCTCT) and 150nM reverse primer (PpalR2, 5'TGAAGAAATATTCAATAAGCGTC 3'). Amplification was performed on a Rotagene machine with 40 cycles of 95°C for 10s, 58°C for 10s and 72°C for 30s and a melt step from 72°C to 95°C.

5. Investigating the population genetic structure of *P. nicotianae* – This aim has been changed to a characterization study on *Phytophthora citrophthora* and closely related species associated with citrus branch cankers

The initial aim of the study was to investigate the role of sexual reproduction and genotypic diversity within *P. nicotianae* populations, and thus the evolutionary potential of the pathogen. Furthermore, gene flow and population structure would have been investigated that may provide information on inoculum sources and disease spread. Although this study is important, through the course of our study it became clear that *P. citrophthora* is currently of greater concern for growers than *P. nicotianae* and that more information and characterization studies are required for this species in light of the identification of two molecular groups (see point 4). Consequently, this aim has been changed to investigating the characteristics of *P. citrophthora* and closely related species isolated from citrus branch cankers.

Currently, there are no polymorphic co-dominant markers available for population genetic studies in *P. citrophthora* and closely related species, which are ideal for population studies. Therefore, several markers were investigated in order to identify polymorphic markers suitable for population studies. These markers include sequence data of (a) housekeeping genes, (b) elicitor and putative RXLR-effectors and (c) isozymes.

(a) Published housekeeping gene areas

The regions that were selected included the triosephosphate isomerase/glyceraldehyde-3-phosphate dehydrogenase (tigA) gene (Unkles *et al.*, 1997; Blair *et al.*, 2008), mitochondrial ATP synthase gene, mitochondrial intergenic spacer (IGS), ras related protein (YPT) (Schena *et al.*, 2006), Enolase gene (Blair *et al.*, 2008) and the small GTPase gene (Armstrong *et al.*, 2006). PCR conditions for the amplification of all these genes had to be optimized for *P. citrophthora*. The amplified PCR products were sequenced to determine if polymorphisms were present within *P. citrophthora* isolates.

(b) Effector genes and elicitor-like genes

Proteins that are secreted by *Phytophthora* and that are thought to play a role in the recognition of the pathogen by the host plant or suppression of the host plant response are referred to as elicitors and effectors, respectively, although these terms are often used interchangeably. *Phytophthora* effectors have recently been shown to contain an RXLR motif and signal peptide for secretion. Since genes that encode these proteins may be highly variable, a few candidates for amplification and sequencing were selected. The first was an Elicitor-like protein 6 precursor *ctp6* found in Genbank (DQ821142). Potentially secreted RXLR

gene candidates (encoding signal peptides, <http://www.cbs.dtu.dk/services/SignalP>) were also investigated by screening two root infection expression libraries (PhyrootSr1 and PhyrootSw1; Forment *et al.*, 2005) from the Citrus Functional Genomics Project database consisting of 2845 genes (<http://bioinfo.ibmcp.upv.es/genomics/cfgpDB>) using a bioinformatics approach.

(c) Isozymes

The variability of the isolates was also investigated using glucose-6-phospho-isomerase (*Gpi*) analyses as previously described (McLeod *et al.*, 2001).

Results and discussion

1. Characterisation of *Phytophthora* species associated with citrus seedlings and trees (nurseries and orchards) in different production regions of South Africa

In total, 182 *Phytophthora* isolates were collected from 46 orchards and nurseries in South Africa (Table 4.4.4.1). The ITS-PCR-RFLP method of Drenth *et al.* (2006) identified five different restriction patterns among these isolates that corresponded to *P. nicotianae* (130 isolates), *P. citrophthora* (46 isolates), *P. citricola* (1 isolate) and *P. cinnamomi* (1 isolate) and an unidentified *Phytophthora* species (2 isolates). Sequencing of the ITS region of a subset (n = 25) of these isolates representing the different PCR-RFLP patterns confirmed that most of the species identifications were correct, except for the *P. citrophthora* isolates that could be divided into two groups based on their ITS sequences. The ITS sequences of the first group (*P. citrophthora* G1SA) had 99% similarity to the *P. citrophthora* sequence (AF266785) of Cooke *et al.* (2000) and the second group (*P. citrophthora* G2SA) had 99% similarity to the *P. colocasiae* sequence of Cooke *et al.* (2000). The ITS sequence of the two *Phytophthora* isolates that had a PCR-RFLP pattern of unknown identity had 96% similarity to a potential *P. asparagi* sequence (EU301114) from Australia (Burgess *et al.*, 2009).

Since the ITS region of some *Phytophthora* species may be similar, the beta-tubulin gene was also sequenced for a subset of isolates that represented the different species. Sequence data from these regions confirmed the species identification of *P. nicotianae*, *P. citricola* and *P. cinnamomi*. The beta-tubulin sequences of the *P. asparagi*-like isolates (PCT010, PCT011) are still in the process of being determined.

The phylogenetic position of the two *P. citrophthora* groups (G1SA and G2SA) were investigated further through phylogenetic analyses of three gene areas (ITS, beta-tubulin and *cox1*), also including Genbank sequences with close similarity to the South African *P. citrophthora* sequences. Phylogenetic analyses of the ITS region showed that the South African *P. citrophthora* G2SA isolates with homology to *P. colocasiae* grouped with the ITS sequences of citrus isolates of Cohen *et al.* (2003) to which they refer to as *P. citrophthora* G2, that causes branch canker in Corsica (Fig. 4.4.4.1). The *P. citrophthora* G1SA isolates grouped with the Cohen *et al.* (2003) *P. citrophthora* G1 isolate sequences and *P. citrophthora* sequences of Kroon *et al.* (2004) and Drenth *et al.* (2004). Phylogenetic analysis of the beta-tubulin and *cox1* gene areas showed that the South African *P. citrophthora* G1SA and G2SA isolates grouped together with the *P. citrophthora* sequence of Kroon *et al.* (2004), rather than with the *P. colocasiae* sequences of Kroon *et al.* (2004) (Fig. 4.4.4.2 and 4.4.4.3). Therefore, the South African *P. citrophthora* G2SA isolates may be a subgroup of *P. citrophthora*, but this will need confirmation through morphological characterisation and possibly further molecular characterisation studies. Morphological identification studies are currently underway. The possibility that the G2SA isolates may represent an unreported species can currently not be excluded. Thus far, the ITS sequence of only 16 South African isolates with a *P. citrophthora* ITS-PCR-RFLP pattern have been sequenced. Among these isolates, only three isolates (PCT183, PCT149 and PCT151) were identified as belonging to the *P. citrophthora* G2SA group, the remainder were of the G1SA group. The three G2SA isolates were obtained from three orchard soils in the Citrusdal area. The remaining isolates are currently being sequenced for the ITS region.

Since the *P. citrophthora* G1 and G2 isolates identified in Corsica have different modes of reproduction, with G1 isolates mainly being sterile and G2 isolates being of the A2 mating type (Cohen *et al.*, 2003), this could hold implications for the survival of the pathogen and potential for the generation of genotypic diversity through sexual reproduction. It should be noted that in the study of Cohen *et al.* (2003) it was not determined whether the *P. citrophthora* isolates or the tester strain was able to produce oospores. Mchau & Coffey (1994) have previously identified a few *P. citrophthora* isolates in world-collections of isolates that were able to induce oospore formation in A1 mating type tester isolates, i.e. were A2 mating types, but were not able to produce oospores themselves. The G1 and G2 isolates from Corsica has also been shown to differ in their virulence against root and scion cultivars (Verniere *et al.*, 2004).

Table 4.4.4.1. *Phytophthora* isolates collected from 2005 to 2008 from citrus orchards in South Africa and identified to the species level using the PCR-RFLP method of Drenth *et al.* (2002).

Province	Area	# Orchards and nurseries	# Isolates 2005	# Isolates 2006	# Isolates 2007	# Isolates 2008	Total # Isolates
Eastern Cape	Fort Beaufort, Kirkwood, Patensie, Sunland	10	1 Pc	1 Pc	1 Pc, 4 Pn, 2 Pasp	20 Pn, 4 Pc	24 Pn, 7 Pc, 2 Pasp
Kwazulu-Natal	Melmoth, Pongola	2	-	-	-	4 Pn	4 Pn
Limpopo	Baltimore, Hoedspruit, Mokopane, Letsitele	5	1 Pn	4 Pn, 2 Pc	11 Pn	8 Pn, 8Pc	24 Pn, 10 Pc
Mpumalanga	Barberton, Karino, Malelane, Marblehall, Nelspruit, Ohrigstad	11	-	-	27 Pn, 1 Pc	17 Pn, 3 Pc, 1 Pcin	44 Pn, 4 Pc, 1 Pcin
North-West	Brits	1	-	-	4 Pn	-	4 Pn
Western Cape	Buffelsjagrivier, Citrusdal, Swellendam, Knysna, Wildernis	14	15 Pc	1 Pc	1 Pcit	8 Pc, 24 Pn	24 Pc, 19 Pn, 1 Pcit
Northern Cape	Hartswater	1	-	-	-	3 Pn	3 Pn
Swaziland	Jeppes Reef, Big Bend	2	-	-	-	8 Pn, 1 Pc	8 Pn, 1 Pc
Total		46	1 Pn, 16 Pc	4 Pn, 4 Pc	46 Pn, 2 Pc, 2 Pasp, 1 Pcit	79 Pn, 24 Pc, 1 Pcin	130 Pn, 46 Pc, 2 Pasp, 1 Pcin, 1Pcit

Pc = *Phytophthora citrophthora*, Pn = *Phytophthora nicotianae*, Pcit = *Phytophthora citricola*, Pasp = unidentified *Phytophthora* sp. with ITS sequence similarity to *P. asparagi*; ND= not determined

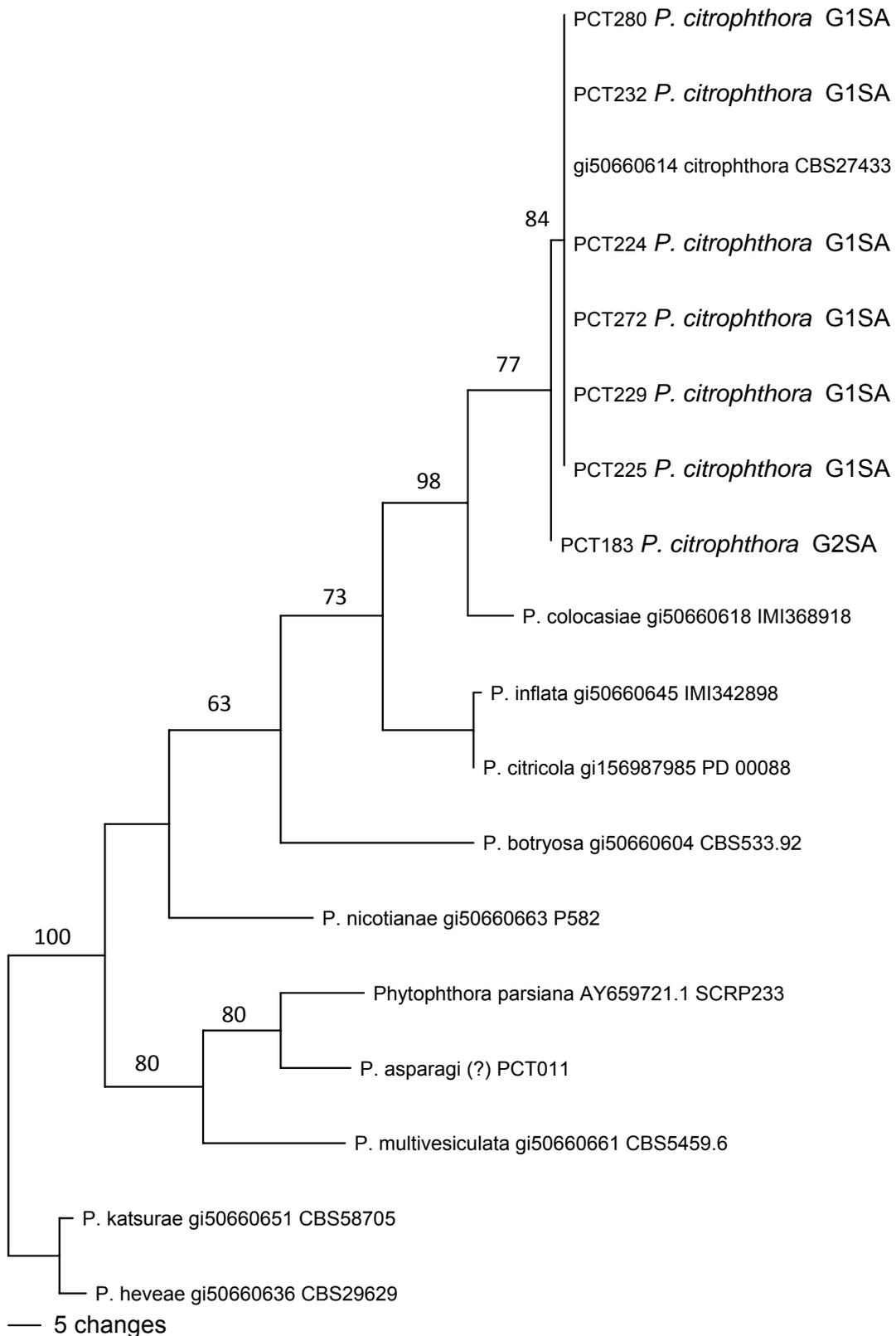


Figure 4.4.4.2. Phylogeny of *Phytophthora* species based on the beta tubulin gene. *Phytophthora* isolates from South African citrus soils have "PCT" codes. The South African *Phytophthora citrophthora* group 1 (G1SA) and group 2 (G2SA) isolates had ITS sequences that corresponded with the *P. citrophthora* group 1 (G1) and group 2 (G2) isolates of Cohen *et al.* (2003).

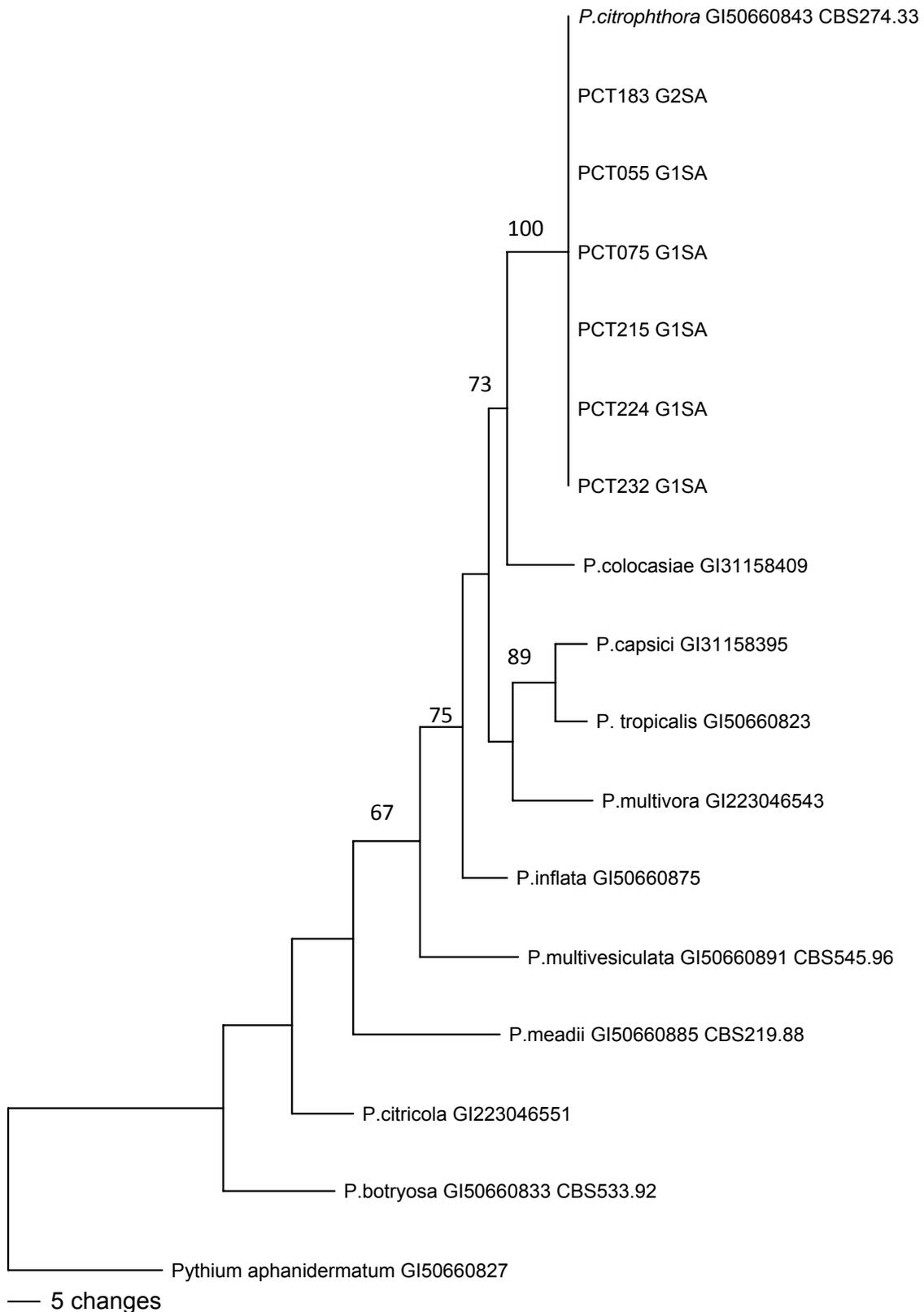


Figure 4.4.4.3. Phylogeny of *Phytophthora* species based on the cytochrome oxidase 1 gene. *Phytophthora* isolates from South African citrus soils have “PCT” codes. The South African *Phytophthora citrophthora* group 1 (G1SA) and group 2 (G2SA) isolates had ITS sequences that corresponded with the *P. citrophthora* group 1 (G1) and group 2 (G2) isolates of Cohen *et al.* (2003).

2. Determine if *Phytophthora palmivora* is present in South African citrus production regions

None of the *Phytophthora* isolates obtained from South African citrus soils (see point 4) had the PCR-RFLP pattern of a *P. palmivora* reference strain. Thus, *P. palmivora* is absent from citrus soils in South Africa.

3. Determine the susceptibility of different citrus rootstocks and fruits to *Phytophthora* species associated with citrus

The first pathogenicity trial where sand-bran colonized by *Phytophthora* was used for inoculating soil, was not successful, since no symptoms (stunting and root discoloration) were observed after 3 months of growth on any of the inoculated seedlings. This is most likely due to the fact that the inoculum did not establish in soil, since soil baiting did not yield *Phytophthora* growth from leaf disks for any of the inoculated species.

In the second trial where millet seed colonized by *Phytophthora* was used to inoculate soil, the inoculation method was successful since analyses of the soil after 3 months revealed high inoculum (all leaf disks yielded *Phytophthora*) levels in the inoculated soil. However, due to over-watering of seedlings along with a breakdown of the air conditioning system that resulted in high temperatures, the trial had to be terminated without results.

A new seedling inoculation trial will be established as soon as seedlings can be obtained. Inoculation of fruits will be conducted in August and September 2009.

4. Development of a real-time PCR detection method for *P. palmivora*

A real-time PCR method was optimised that only detected *P. palmivora*. Only some leaf disks from the different citrus soils were available for real-time PCR analyses. Eighteen leaf discs from 13 citrus orchards (at least one from each province (Eastern Cape, Northern Cape, Western Cape, Limpopo, Mpumalanga) were tested using the real-time PCR method, which confirmed that *P. palmivora* was absent from these orchards, since only the positive control consisting of genomic DNA of *P. palmivora* or leaf disks artificially inoculated with *P. palmivora* yielded amplification.

5. Investigating the population genetic structure of *P. nicotianae* – This aim has been changed to a characterisation study on *Phytophthora citrophthora* and closely related species associated with citrus branch cankers

(a) Published housekeeping gene areas

Sequence analyses of the ATP synthase gene in five *P. citrophthora* isolates showed polymorphisms, but only between the two main *P. citrophthora* groups (G1SA and G2SA) and not within the groups. More isolates will be sequenced to determine if this gene is polymorphic enough for population studies.

The IGS, YPT, tigA, enolase and small GTPase gene did not reveal polymorphisms between or within the *P. citrophthora* G1SA and G2SA isolates.

(b) Isozymes

Gpi analyses of 15 isolates from the two *P. citrophthora* groups (G1SA and G2SA) revealed two genotypes among the isolates (Fig. 4.4.4.3). The one genotype corresponded to isolates of the G1SA group and the other to the G2SA group. *P. nicotianae* isolates had a *Gpi* genotype that differed from that of *P. citrophthora* (Fig. 4.4.4.3).

(c) Elicitin-like genes and effector genes

Amplification with primers targeting the elicitin-like protein 6 precursor (*ctp6*) resulted in the amplification of a 587 bp PCR product. Sequencing of the amplified product showed that there were multiple copies of this gene within the amplified product. Further optimisation with more stringent conditions is required to determine if only one copy of the gene can be amplified for sequencing.

All 2845 expressed sequence tags (ESTs) in the two infected citrus root EST libraries were assessed for the presence of signal peptides and RXLR motifs. 475 sequences were identified that encoded an RXLR motif, but only 20 also encoded a signal peptide of which 17 had homology to plant genes. This was expected since plants also contain proteins with RXLR motifs and therefore these sequences were not analysed further. Only three sequences were found that encoded predicted signal peptides and RXLR motifs with no homology to plant proteins. Subsequent attempts to amplify these three genes from *P. citrophthora* genomic DNA using newly designed primers, were only successful for one of the ESTs. The amplified product of this

RXLR-EST is currently being sequenced to determine whether it is indeed a RXLR effector, which is polymorphic between isolates.

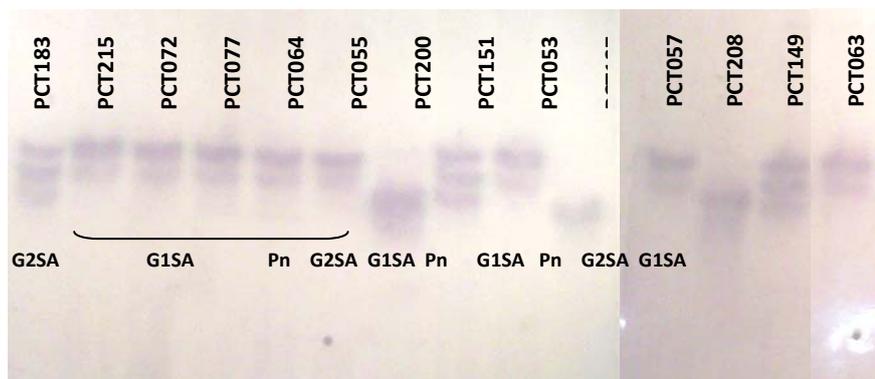


Figure 4.4.4.4. GPI isozyme analysis of *Phytophthora* isolates (PCT codes) from citrus soils in South Africa. Two groups of *P. citrophthora* of G1SA and G2SA, which were identified with sequence data, each had a distinct *Gpi* genotype that were different from the *Gpi* genotype of *P. nicotianae* (Pn) isolates.

Conclusion

The most commonly found *Phytophthora* species in South African citrus orchards are *P. nicotianae* and *P. citrophthora*. Among the *P. citrophthora* isolates two groups (G1SA and G2SA) were identified based on ITS sequence data and *Gpi* analyses. Based on the ITS sequence data these two groups correspond to two *P. citrophthora* groups (G1 and G2) identified as causing citrus branch canker in Corsica by Cohen *et al.* (2003). Whether the *P. citrophthora* G2 isolates of Cohen *et al.* (2003) represents variation within this species, or is a potential new species, requires morphological characterisation.

The investigation of sequence data of several gene areas and allozyme analyses have thus far failed to reveal polymorphisms within *P. citrophthora* G1SA and G2SA isolates. However, *Gpi* and ATP synthase gene sequence data may be useful for differentiating the two groups. The one RXLR-EST sequence that was amplified successfully may show potential for revealing polymorphisms within groups. The lack of polymorphism in gene sequence areas may suggest that isolates within the two groups may only consist of a few clonal lineages, as suggested by Cohen *et al.* (2003) using Randomly Amplified Microsatellites (RAMS).

Phytophthora palmivora was not identified in any of the investigated South African citrus soils, using either conventional isolation studies or real-time PCR with *P. palmivora* specific primers.

Further objectives

1. A survey will be conducted in citrus orchards where *Phytophthora* cankers occur in order to establish which of the two *P. citrophthora* groups are responsible for causing branch cankers. Isolations will be made directly from cankers. The two *P. citrophthora* groups will be identified using ITS sequencing and *Gpi* analyses.
2. The mating type or sterility of the *Phytophthora* isolates obtained from citrus cankers will be investigated through mating type studies.
3. The existence of clonal lineages within the two *P. citrophthora* groups will be investigated using randomly amplified polymorphic DNA (RAPDs) and RAMS.
4. If enough time is available, representative *Phytophthora* isolates obtained from citrus cankers will be evaluated for their pathogenicity and virulence using inoculation of detached shoots, and measuring lesion lengths. The method of inoculation of detached shoots may need optimisation since Alvarez *et al.* (2009) recently showed using only one *P. citrophthora* isolate that several environmental factors can influence lesion size. Should the RXLR-EST sequence data prove to be variable, sequence data from this region along with RAMS and RAPD genotyping will be used to group isolates in order to select a representative subset of isolates for virulence testing.

Technology transfer

Posters presented at conferences

1. Julia Meitz, MC Pretorius, Wilhelm Botha, Laura Huisman, Elma Carstens, Adele McLeod (2008) Characterization and detection of citrus *Phytophthora* species in South Africa. CRI Citrus Research Symposium, August 2008.
2. Meitz, J., Pretorius, M.C., Z. Buhlungu, Z, Botha, W.J., Huisman, L., Langenhoven, S. and McLeod, A. (2009) A survey of *Phytophthora* species on citrus in South Africa, and the development of a real-time PCR method for detection of citrus *Phytophthora* species from soil. January 2009, South African Plant Pathology meeting, Gordon's Bay.

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4.4.5 PROGRESS REPORT: Control of *Phytophthora* trunk and branch canker on Clementines in the Western Cape

Experiment 836 (2006–2008) by G.C. Schutte, C. Kotze and M.C. Pretorius (CRI)

Opsomming

’n Enkele stambehandeling van verskillende algdoders (fungisiede) teen verskillende konsentrasies is ge-evalueer vir die beheer van *Phytophthora* stamkanker op ’nules’ Clementines. ’n Nuwe kwantitatiewe evaluasietegniek is ontwikkel om die algdoders te toets teen die siekte. Resultate toon dat al die algdoders goed gewerk het teen die siekte behalwe die laagste dosis van chlorothalonil (100 ml/ 100 L water) wat getoets is. Dit kan toegeskryf word aan die formulering van die produk. Die stambehandelings toon dus dat al die geselekteerde algdoders waarvan almal (behalwe Figther) kontakdoders is, goed werk teen die siekte wat toon die infeksie baie oppervlakkig is. Opvolgbespuitings is ook nodig omrede van die behandelde bome weer geïnfekteer is. Studies in Spanje toon dat die siekte dwarsdeur die jaar voorkom en sodoende moet die spuitprogramme (4 bespuitings met 3-maandelikse intervalle) ook sodanig toegedien word. Captan en Sporekill tenkmengsel wat as die standaardbehandeling vir jare al toegedien word, is in die proses van registrasie. Verskillende nawel kultivar kwekeryboompies is ook getoets vir hulle vatbaarheid vir stamkanker. Resultate toon dat ‘Royal Late’ die mees tolerante nawel kultivar was, ‘Witkrans’ en ‘Powell Summer’ matig tolerant en ‘Washington’ hoogs vatbaar. Al die ander kultivars was ook vatbaar tesame met die ’nules’ Clementine wat as vergelykende kontrole gebruik is.

Summary

A single spray treatment consisting of different algicides (fungicides) at different rates was evaluated for the control of *Phytophthora* trunk canker on ’nules’ Clementines. A new quantitative evaluation technique was developed to test the algicides. Results showed that all the selected algicides performed well against the disease, except for the lowest rate of chlorothalonil (100 ml/ 100L water) which can be ascribed to the formulation of the product. All selected trunk algicides (except for Fighter) are contact treatments, showing that this superficial disease can be treated effectively. Follow-up treatments are necessary because treated trees can be re-infected. Studies in Spain showed that the disease occurred right through the year and spray programmes (4 treatments with 3 monthly intervals) should therefore be applied accordingly. Captan and Sporekill tank mixture that has been applied as the standard trunk application for a couple of years, is in the process of registration. Different Navel orange nursery trees were also tested for their susceptibility for the disease. Results showed that the ‘Royal Late’ was the most tolerant cultivar, with ‘Witkrans’ and ‘Powell Summer’ moderately tolerant and ‘Washington’ highly susceptible. All the other cultivars were also susceptible when compared with the ’nules’ Clementine that served as comparative control.

Introduction

South Africa cultivates more than 57 000 ha citrus trees and is the world’s second largest exporter of citrus as it exports more than 90 million cartons (15 kg) of citrus worldwide. Clementine mandarins comprises of 2 289 ha of these plantings or 1.7 million trees were planted in South Africa with an average of 743 trees /ha (Anonymous, 2007). South African citrus growing areas are scattered all over South Africa in the winter and summer rainfall regions of the country, but 70% of all the Clementine trees are planted in the Western Cape province of South Africa, which is subjected to a Mediterranean climate.

Gum diseases of citrus trees worldwide are associated with *Phytophthora* spp. that can affect roots, trunk, branches, fruits and shoots (Klotz, 1950). However, the most widespread and important are *P. nicotianae* Breda de Haan (syn. *P. parasitica*) and *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian (Erwin & Ribeiro, 1996). They have distinct temporal and climatic requirements, so that their relative distribution and influence also vary in different production areas (Matheron *et al.*, 1997). *P. nicotianae* is more common in subtropical areas of the world and causes foot rot and root rot and occasionally attacks aerial parts of the tree and causes a brown rot of fruit (Graham & Menge, 2000). In South Africa, *Phytophthora nicotianae* var. *parasitica*

(Dastur) Waterhouse and *P. citrophthora* are the most common organisms and were isolated from a third of citrus soils (Martin, 1960).

P. citrophthora causes gummosis and root rot in Mediterranean climates and is the most common cause of brown rot in these areas as well. Concomitantly, foot rot and gummosis occur when *Phytophthora* propagules are splashed onto susceptible trunks near ground level, infect through wounds or growth cracks and produce lesions which extend down to the bud union (Graham & Menge, 1999). Recently, *P. citrophthora* was identified to be the predominant species in orchard soils in Spain as well as the causal organism for branch cankers on Clementine mandarins (Alvarez *et al.*, 2006, 2008).

Fawcett (1936) described *Phytophthora* spp. that affected all parts of grapefruit trees from the crown roots to the topmost branches in the Western Cape province of South Africa. A *Phytophthora* sp. was isolated from cankers on trunks and branches of citrus trees in a preliminary survey of this new syndrome that was conducted in some Spanish citrus-growing areas (Vicent *et al.*, 2004). Although 10 *Phytophthora* species have been reported from diseased trees around the world, three species cause the most serious disease, stem gummosis, as well as root and fruit rot: *P. citrophthora* (R.E. Sun & E.H. Sun) Leonian 1906), *P. nicotianae* (syn *P. parasitica*) and *P. palmivora* (Erwin & Ribeiro, 1996; Graham & Menge, 2000). They have distinct temporal and climatic requirements, so that their relative distribution and influence vary in the different production areas (Matheron, Porchas & Matejka, 1997). *P. citrophthora* is extremely sensitive to high temperatures of above 33°C and this explains why *P. citrophthora* is so active in the mediterranean type of climate experienced along the Eastern and Western Cape coastline.

Rootstocks like the sour orange (*Citrus aurantium*), which appeared to be resistant to *Phytophthora* following the mid-1800s gummosis epidemics in the Mediterranean area (Laviola, Somma & Evola, 1990), was later shown to be highly susceptible to other pathogens such as the citrus tristeza virus (CTV) (Bar-Joseph, Roistacher, Garnsey & Gumpf, 1981), nematodes and 'mal secco' (Laviola *et al.*, 1990). Replacing the sour orange rootstock with resistant rootstocks such as Troyer citrange, Cleopatra mandarin and Carrizo citrange helped to curb the disease in countries such as Corsica. But there has been a resurgence of *Phytophthora* in Corsican groves probably due to the change in soil and climatic conditions or changing cultural practices or the adaptation of the *Phytophthora* to the new rootstocks (Cohen, Allasia, Venard & Notter, 2003).

Fungicides such as metalaxyl or fosetyl-Al control *P. citrophthora* (Davis, 1982) but require several applications and must be timed correctly (Davino, Gamberini, Areddia, Aldaresi, 1990). Other management practices include irrigation management, foliar and trunk application of fungicides and fumigation. A registered fungicide (Fighter) effective for use against *Phytophthora nicotianae*, have been selected for the field trial using them at their registered rates and times of application. In the USA, 0.06 g / l water Captan and copper fungicides are required to attain 100% inhibition of *Phytophthora* (Timmer, 1977). Captan, registered in Argentina for use against gummosis at a rate of 200 g/hl water, was also included in this trial. It is reported from Argentina that this fungicide is not that effective against the disease and it was therefore decided to boost it with Sporekill to be used as a trunk application during the winter period.

The aim was to see how Acrobat and Captan would perform in a spray programme to be sprayed during optimal growth conditions for the algae.

Materials and methods

Algicidal treatments

Four rows in a 'nules' orchard at Frankenhof Estates east of Swellendam were selected (31 May 2008). Three trees within each row with visible infections were randomly selected for treatments. The trunks or branches with visible lesions were surface scratched with a chisel to partially expose three regions (5 x 5 cm) that were also 5 cm apart of the outer borders of the visible lesion. This was done to determine the efficacy of the trunk treatment that would follow as well as for the final evaluation at a later stage (6 July 2008). This procedure was done to see if there was any growth or not of the canker lesions of the non-exposed parts by joining the previously exposed parts two months later (Fig. 4.4.5.1). Algicides were applied at different rates with a knapsack sprayer. Rates of the different algicides are presented in Table 4.4.5.1.

Determining the susceptibility of different Navel orange cultivars to *P. citrophthora*

Ten different Navel orange nursery trees consisting of different cultivars (Cara Cara, Witkrans, Washington, Royal Late, Powel Summer, Palmer, Lina, Glen Ora Late, Cal Lane Late, Bahianinha) were obtained from the CFB. They were transplanted into plastic pots at CRI. An isolate of *P. citrophthora* on PDA was cut into 1 cm² pieces that were used to inoculate the trunks of these Navel nursery trees. Before inoculation, the

surface of each tree was sterilised with ethanol. The bark was aseptically removed with a sterilised scalpel. The 1 cm² agar plugs were placed onto these exposed parts and wrapped with Parafilm®. The trees were kept in a room at 25°C that was not exposed to direct sunlight. The Parafilm® was removed after 2 weeks. The lesions were exposed with a chisel and the outer circumference was drawn onto transparency sheets.

The sheets were sent to US where the lesion size of each redrawn lesion was scanned and images were calibrated and surface area of lesions was determined using Image Pro-Plus software. Data were analysed by analysis of variance (ANOVA) and Fisher's Least Significance Difference (LSD) test ($P = 0.05$).

Results and discussion

Algicidal treatments

All the algicide treated trees in the 'nules' orchard at Frankenhof recovered significantly ($P < 0.05$) from further die-back when compared with the non-treated control (Fig. 4.4.5.1). Only chlorothalonil at a rate of 100 g /100 L water resulted in further development of *P. citrophthora* canker. This treatment was, however, not significantly different from the control (Table 4.4.5.1). No callus formation took place in any of the partially exposed lesions but all the other treatments did result in callus formation. It was previously reported that trees with a less dense canopy (<6/10) of which the trunks are also infected (>60%), should not be treated. These types of trees were excluded from our initial tree selection before treatments commenced.

Determining the susceptibility of different Navel oranges to canker

'Royal Late' was significantly ($P < 0.05$) more tolerant against *P. citrophthora* trunk canker when compared with 'nules' (comparative control), 'Glen Ora Late', 'Cara Cara', 'California Lane Late' and the 'Washington' navel. The mean lesion size of the 'Royal Late' was 12.48 cm² in comparison with the 27.37 cm² of the 'Washington' navel. "Royal Late", 'Witkrans' and 'Powell Summer' were the only cultivars that were significantly different from the 'Washington'.

Conclusion

All selected algicides were effective against *Phytophthora* canker except for chlorothalonil tested at a rate of 100 ml/ 100 L water. Chlorothalonil (Bravo) is an excellent preventative fungicide belonging to the nitrile group and is registered for the control of late blight on tomatoes and potatoes, but we have experienced poor application or sticking properties due to the experimental formulation that we have tested. The trial should be repeated with a better formulation.

Fighter, Sporekill and Captan on their own gave excellent control, showing that contact algicides are also effective in the superficial *P. citrophthora* infections. Captan in tank mixtures with Sporekill (standard treatment) again gave effective control. The trial will be repeated.

Snails and ants can also serve as dispersal vectors of *Phytophthora* spp. (El-Hamalawi & Menge, 1996). In South Africa, brown snails [*Helix aspersa* (Müller)] and dune snails [*Theba pisana* (Müller)], were identified in Clementine mandarin trees and their role as possible vectors are proposed as targets for future research.

Technology transfer

Orchard demonstration (Swellendam).

Study group meetings (Western and Eastern Cape) (M.C. Pretorius – April 2009).

Citrus Research symposium (1 talk and 1 poster) (2008).

Southern African Society for Plant Pathology biennial congress (2009).

Presented a talk entitled: "*Phytophthora citrophthora*, the cause of trunk and branch canker of Clementine mandarins in South Africa and Spain" at the 46th SAPP congress, Gordons Bay, South Africa.

Presented a poster entitled: "Identification and control of *Phytophthora citrophthora*, the cause of a new trunk disease of Clementines in South Africa" at the International Congress for Plant Pathology Turin, Italy.

Future objectives (milestones) and work plan

More control programmes consisting of different fungicides with different modes of action should be investigated and the possibility that snails can serve as a vector should also be investigated. New evaluation methodology will be implemented.

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Table 4.4.5.1. Residual activity of algicide treatments on trunks of Clementine mandarins for the control of *Phytophthora citrophthora* trunk canker after 60 days.

Algicide	Rate/100 L water	Lesion length (cm) ^z
Sporekill (120 SC)	100 ml	0 a
	300 ml	0 a
	500 ml	0 a
Chlorothalonil (500 SC)	100 ml	1.8 ab
	200 ml	1.0 a
	300 ml	0 a
Captan (500 WP)	100 g	1.0 a
	200 g	0 a
	300 g	0 a
Figthet (555 SL)	400 ml	0 a
	570 ml	0 a
	710 ml	0 a
Captan + Sporekill (standard)	200 g + 100 ml	0 a
Nontreated control		3.4 b

^z Numbers within column followed by the same letters are not significant different (LSD at $P \leq 0.05$).

Table 4.4.5.2. Susceptibility of different Navel oranges for *Phytophthora citrophthora* trunk canker after artificial trunk inoculation.

Navel orange cultivar	^z Mean lesion size (cm) ^z
Royal Late	12.4799 a
Witkrans	16.6086 ab
Powell Summer	17.9812 ab
Bahianinha	18.3454 abc
Palmer	20.2593 abc
Lina	21.0011 abc
Nules (comparative control)	21.0810 bc
Glen Ora Late	23.1974 bc
Cara Cara	24.4178 bc
Californian Lane Late	24.9919 bc
Washington	27.3705 c

^z Numbers within column followed by the same letters are not significant different (LSD at $P \leq 0.05$).



Fig. 4.4.5.1. Partial lesion exposure of a 'nules' Clementine trunk (5 x 5 cm) with a chisel before the application of algicides (A). Two months later, the lesions will be joined (yellow arrows) and lesion size measured to determine the increase in lesions size (red brackets) of *Phytophthora citrophthora* trunk canker (B).

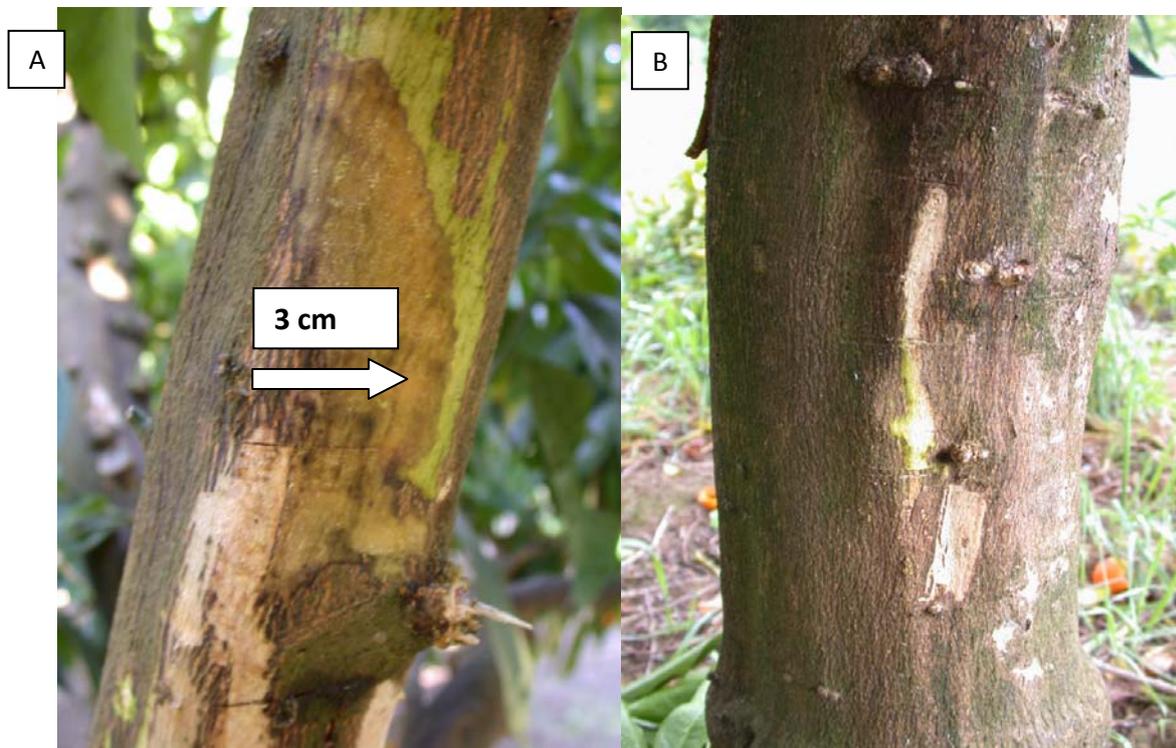


Fig. 4.4.5.2. *Phytophthora citrophthora* canker development on the trunk of an untreated 'nules' Clementine (A). Sporekill (100 ml/100 L water) resulted in no canker development after two months (B).

4.4.6 **FINAL REPORT: Determine the efficacy of phosphonates applied through the irrigation systems in citrus for control of *Phytophthora* root rot**
Experiment QMS 07 / WvdP 2 (2005–2008) by W. van der Pypekamp (QMS)

Opsomming

Die toediening van fosfonate as 'n blaarbespuiting of stam-verf is problematies as gevolg van die potensiële fitotoksiese risiko met eersgenoemde en die arbeidsintensiewe vereistes van laasgenoemde metode. Die doel van die projek was om die effektiwiteit van kalium-fosfonaat, toegedien deur die besproeiingstelsel vir die beheer van wortelvrot te bepaal, en of die metode konvensionele toediensmetodes se tekortkominge kan oorkom. Projekte is uitgevoer om die effek van verskillende toediensmetodes, boomouderdom en dosis, die tipe besproeiingssisteem en grondeienskap op die toediening van kalium-fosfonaat deur die besproeiingstelsel te bepaal. Residu analyses het gelyke kalium-fosfonaatvlakke in die wortels vir beide konvensionele blaarbespuiting en grondtoediening getoon. Die toediening van fosfonate het by sommige waarnemings 'n opbrengs- en vruggrootheid-toename getoon. Geen duidelike patroon kon egter rakende boomouderdom en dosis toegedien bepaal word nie. Indikasies het egter getoon dat die dosis teen die 1x konsentrasie toegedien effektief was, maar moontlik te laag was vir 7-10 jaar ouderdomsgroep. Resultate was moontlik deur faktore soos wortelstok- weerstandbiedendheid, wortel-entstok kombinasies, boomkondisies en verskillende bestuurspraktyke beïnvloed.

Summary

The application of phosphonates as a foliar spray or trunk paint is problematic due to the potential phytotoxic risk involved with the former method and the intensive labour requirements of the latter. The aim of this project was to determine the efficacy of root rot control when potassium phosphonates are applied through the irrigation system, and if this method of application would reduce shortcomings of conventional application methods. Experiments to determine the role of tree age, irrigation type, soil type, and type of application (foliar, trunk or irrigation) were conducted in the Letsitele area. Residue analyses showed that potassium phosphonate levels in the roots for conventional foliar application were similar to levels obtained for potassium phosphonates applied through the irrigation system. Some observations showed an increase in yield as well as fruit size due to phosphonate applications but no clear trends could be determined with regards to tree age and dosage applied. Indications are that most dosages at the 1x concentration tested are effective, but that dosages for the 7-10 year age group might be too low. Results were possibly influenced by factors such as tolerance of rootstocks, rootstock/scion combinations, tree condition, and many other production practices.

Introduction

Production costs, especially the cost of labour and fuel, as well as efficacy of treatments are very important factors that should be considered in disease control strategies. The application of phosphonates as a foliar spray or trunk paint is problematic due to the potential phytotoxic risk on fruit and the cost and labour intensiveness of applying sufficient trunk paints to be effective. The application of potassium phosphonate through the irrigation system under commercial conditions seems to be very effective for the control of *Phytophthora* root and collar rot (S. H. Swart; personal communication). Applying phosphonate products through the irrigation system reduced the cost component of labour and also greatly reduced the amount of product necessary, especially for some tree age groups compared to current registered application methods.

The aim of this project was firstly to compare the efficacy, and determine optimal dosages when phosphonates were applied through different types of irrigations systems (drip vs. micro) and then to determine the effect of soil texture and different dosages when phosphonates are applied to trees of different age groups. The effect of foliar, trunk or soil application was also compared.

Materials and methods

The effect of tree age, phosphonate dosages and type of irrigation system on production

The influence of tree age, dosages applied and type of irrigation system on production, when potassium phosphonates are applied through the irrigation system, was determined in citrus orchards. The effect on production was evaluated by determining yield and fruit size at harvest. The trial was conducted in the Letsitele area, Limpopo province, over three growing seasons (2005/6, 2006/7 and 2007/8). Table 4.4.6.1 depicted potassium phosphonate dosages applied on different tree age groups and types of irrigation systems. Applications were done three times per growing season, in October / November, December / January and February / March. Plots in the tree age group > 15, received an extra application per season.

Tree age groups included in this trial were, <2 years on micro and drip irrigation, 3 - 6 years on micro and drip irrigation, 7 - 10 years on micro irrigation only and > 15 years on micro and drip irrigation. Each treatment consisted of 3-tree plots replicated 10 times in randomised block designs.

The effect of soil type (sand vs. clay) and dosage on production

The effect of soil types on production when applying potassium phosphonates through the irrigation system was investigated. The trial was carried out on two similar Valencia plots in the Letsitele area. The selected plots were both in the age group 7 – 10 years under micro irrigation, planted in close proximity and at similar tree spacing. Plot E-7 had sandy soils, and plot E-10, clay soils. The different treatments and dosages applied are depicted in Table 4.4.6.2.

The effect of application methods on production

The effect of different potassium phosphonate application methods on production was determined on different tree age groups. The age groups studied were 7-10 years (plot LQ-28), and > 15 years (plot LQ – 28). Treatments and dosages applied are depicted in Table 4.4.6.3. The dosages for trunk and foliar application are according to labels for product registration and dosages for irrigation application as shown in Table 4.4.6.3.

The effect of treatments on phosphorous acid residue levels in the roots

The effect of tree age groups, soil type, dosage and irrigation types, on phosphorous acid residue levels in the roots of trees were determined (Tables 4.4.6.4, 4.4.6.5 and 4.4.6.6). The effect of different application methods on phosphorous acid residue levels in the roots was also determined. The analysis of roots for phosphonates levels was very expensive, and 15 samples were analysed each season at a cost of more than R17000 per season. Due to the high cost of analyses, a limited number of samples could be analysed, and plots applied at 1x concentration were mostly used for evaluations. Previous publications suggested that phosphorous acid levels in the roots of citrus should be above 30 mg/kg to ensure protection against *Phytophthora* root rot pathogens (Schutte *et al.*, 1991), therefore this levels was used as reference point for sufficient levels.

Data collection

The effect of treatments on production was determined by stripping all the fruit from data trees (centre tree of 3-tree-plots) for each of the 10 replicates per treatment. Yield was determined by weighing the fruit of each replicate separately. A representative sub-sample was taken from each replicate, consisting of approximately 20 kg of fruit, pooled separately for each treatment, and sized with a commercial “rope-and-roller” sizer to determine size distribution. Residue levels was determined by taking samples approximately 21 days after the last seasonal application for some of the treatments and pooling roots obtained from each of the 10 replicate trees. Residue analysis was done by the South African Bureau of Standards (SABS), Chromatographic Services in Pretoria.

The presence of *Phytophthora* was confirmed with a lemon leaf baiting technique. *Phytophthora* presence was determined for all treatments by taking two soil samples close to irrigation points of each replicate. Although the leaf baiting technique is not suitable for quantitative analysis, the number of infected leaf discs per sample was recorded to give some indication of the distribution and size of the pathogen population present at each trial site.

Results and discussion

The effect of tree age, dosages applied and type of irrigation system on *Phytophthora* detection, production and residue levels in roots

<2 Years: No *Phytophthora* could be detected in the orchard under micro irrigation (RS-22), but was detected in the orchard under drip irrigation (RS-21) for this age group (Table 4.4.6.4). No yield data could be obtained for this age group since fruit are removed at an early stage to promote vegetative growth. The level of phosphonate acid detected in the roots of micro irrigated trees in this age group showed sufficient levels above the 30 mg / kg level for effective control of *Phytophthora* root rot. Results in terms of phosphorous acid levels in the roots (21 days after last application) showed significantly higher levels in trees under micro irrigation than in drip irrigation during both seasons. A possible explanation for low residue levels in the orchard under micro irrigation might be the fact that *Phytophthora* was detected in this orchard,

and therefore root rot and lack of feeder roots could have been the main reason for inability to take up the phosphonates and not necessarily the method of application. The presence of root rot and/or feeder roots was not covered in this study and should possibly also be included in future studies of this nature. Observations in the field over many years showed improved tree condition when phosphorous acid was applied through either drip or micro irrigation systems under conditions of severe root rot caused by high levels of *Phytophthora*. The implications of this result must be investigated further in order to determine why the difference occurred and whether it would impact on tolerance to *Phytophthora* infection and production.

Age group 3-6: In this group the status of *Phytophthora* was positive in the micro irrigation plot (RS-11) and negative in the drip plot (J-40, Table 4.4.6.4). There was no significant effect on yield compared to the untreated control when phosphonates were applied through micro irrigation or drip irrigation for either of the two years evaluated. However, results showed an increase in yield for all treatments (42 – 73 %) and the untreated control (53 %) during the second year of evaluation when phosphonates were applied through a micro irrigation (RS-11) system (Table 4.4.6.4). Fruit size also increased for all treatments and the untreated control, but more significantly for the phosphonate applications, especially the x and 2x applications. Trees under micro irrigation only showed phosphorous acid levels above 30 mg / kg during the second year of evaluation, indicating that sufficient potassium phosphonates were taken up by the roots during the second season (2007 / 2008). Trees under drip irrigation showed phosphorous acid levels above 30 mg / kg during both years of evaluation, indicating that sufficient potassium phosphonates were taken up by the roots. Production might be useful for comparison of dosages in a single block, but not for comparing application methods by using two different blocks of trees where other production practices and or climatic conditions might have a more significant effect than the factor that are studied. No data was taken on root rot and feeder root condition and the difference in phosphonate levels in roots between seasons for the micro irrigation plot and with regards to the drip irrigation plot might also be related to the presence of root rot and possible recovery of root systems during the second year, ensuring more effective uptake.

Age group 7–10: Only trees under micro-irrigation (D18) were studied for this age group due to lack of finding a suitable plot in this age group under drip irrigation in the area (Table 4.4.6.4). *Phytophthora* could not be detected in this plot and there were no statistical difference with regards to either yield or fruit size improvement for treated and untreated trees. The fact that residue levels were relatively low during both seasons can not be explained for this trial site, since *Phytophthora* was not detected and root rot was possibly absent.

Age group >15: *Phytophthora* was detected in plots under micro and drip irrigation systems (Table 4.4.6.4). Trees under micro irrigation, treated with phosphonates showed an increase in production during the second year of evaluation (14–29 %). The untreated control also showed an increase in yield of 24%. Fruit size increased for all treatments (20–25 %) and the untreated control (10%) during the second year when phosphonates were applied through a micro irrigation (RS-1) system. Although yield increased for treated and untreated trees, the fruit size for treated trees increased 10 % if compared to the untreated control. Trees under drip irrigation, treated with phosphonates ($\frac{1}{2}$ x and x) showed a statistically significant higher average yield per tree compared to the untreated control during the second season of evaluation. Fruit sizes for treated trees were also slightly higher compared to the untreated control. Trees under micro irrigation showed phosphorous acid levels above 30 mg/kg during both years of evaluation, indicating that sufficient potassium phosphonates were taken up by the roots. Trees under drip irrigation showed phosphorous acid levels slightly lower than 30 mg/kg during both years of evaluation, indicating possible suboptimal uptake of potassium phosphonates or other reasons such as transportation from the roots to other plant parts. Phosphonates are transported effectively in both the xylem and phloem of plants and possible depletion in the roots might also be a reason for variable results with regards to phosphonate levels detected in roots 21 days after application and should also be taken into account when results are analysed.

Sand vs Clay (Age group 7–10): In this group the status of *Phytophthora* was negative for the plot with sandy soils (E-7) and positive for the plot with clay soil (E-10, Table 4.4.6.5). The influence of treatments on production in E-7 (sandy soils) could possibly not be credited to the treatments alone due to the fact that *Phytophthora* was not detected in this plot. Results showed an increase in yield between 40 and 62% for treated and 40% for untreated trees with no reduction or difference for fruit size.

Trees planted in clay soils under micro irrigation and treated with phosphonates showed a decrease in production during the second year of evaluation between 5 and 13%, compared to the average yield per tree for untreated trees, decreasing by 10%. Fruit from treated trees were generally larger than fruit from untreated trees (size 72 vs. 80). Trees under micro irrigation on sandy and clay soils showed phosphorous acid levels above 30 mg/kg during both years of evaluation, except for plot E-7 (sandy soil) with levels less than 30 mg/kg during the 2007/2008 season. The reason for this decrease in phosphorous levels in roots of trees on both sites during the second season can not be explained at this stage. However, it must be noted that trees in the 7–10 age group under micro irrigation where dosages were studied, also showed relatively low residue levels 21 days after application. Unfortunately only roots where 1x concentration was applied could be analysed, due to high cost and no information are available on residue levels where 2x was applied.

Distribution of phosphonates through the trees and other reasons such as the 1x dosage being too low for this age group must be investigated.

Application methods, (Age group 7–10): *Phytophthora* was detected in plot LQ-28 where the effect of different application methods was studied on young trees (Table 4.4.6.6). The average yield increased slightly during the second evaluation year for trees treated with phosphonates as a trunk paint application and for trees in the untreated control, but decreased if applied as soil application. Fruit size increased for all treatments and the untreated control during the second evaluation year, therefore yield and fruit size were not influenced by phosphonate treatments alone in this trial. Phosphorous acid levels were above the 30 mg/kg level for foliar application methods during the two years of evaluation, however, lower levels were observed for the micro irrigation application (7 mg/kg) and trunk paint (25 mg/kg) during the 2006/2007 and 2007/2008 growing season, respectively. Phosphonate levels were higher than 30 mg/kg in trees treated with soil applications during the second year of evaluation.

Age group >15: *Phytophthora* was detected in plot LQ-21 where the effect of different application methods was studied on older, larger trees (Table 4.4.6.6). No production values were available during both years of evaluation due to fruit being harvested before data were recorded. Phosphorous acid levels were highest when applied to foliage of large trees and above the 30 mg / kg level for foliar and soil application methods during the two years of evaluation, however, slightly lower levels were observed for the trunk paint application (28 mg/kg and 25 mg/kg during the 2006/07 and 2007/08 growing season, respectively).

Phytotoxic evaluation

The foliar application had a statistically significantly higher percentage fruit with phytotoxic symptoms, with 88 % of fruit affected compared to 0% for Treatment 2, 3 and 4 (Table 4.4.6.7, Figure 4.4.6.1).

Conclusion

Variable results with regard to yield, fruit size and residue levels in roots of trees due to application dosages and tree age were obtained in several field trials over 3 years of study. Some observations showed an increase in yield as well as fruit size due to phosphonate applications but no clear trends could be determined with regard to tree age and dosage applied. Indications are that most dosages at the 1x concentration tested were effective, but that dosages for the age group 2–10 years might be too low. The effect of applying potassium phosphonates through irrigation systems for reducing the influence of *Phytophthora* root rot on production where the pathogen was present in the soil was only evident in plot G-1.11 (Tree age group >15 years, drip). Results were possibly influenced by factors such as tolerance of rootstocks, rootstock/scion combinations, tree condition, and many other production practices.

Results showed higher phosphorous acid (PO₃) residue levels in the roots of plants receiving a foliar application compared to soil or trunk paint applications. Phosphorous acid levels in the roots of plants receiving potassium phosphonate through irrigation systems were higher than trunk paint application except for one instance (Plot LQ-28, 2006/07 growing season). The phosphorous acid levels in the roots for potassium phosphonates applied through the irrigation system were in most plots (52.3%), higher than 30 mg/kg. Thus potassium phosphonates can be applied through an irrigation system for sufficient uptake to reduce the effect of *Phytophthora* root rot. The reason why the phosphorous acid levels in the roots of plant in certain plots were not higher than 30 mg/kg could not be determined in this trial and further research is needed to investigate factors influencing uptake, distribution and detection. Factors such as rootstock/scion combination, physiological status of the tree and various other production factors might also have an influence on dispersal in the plant detectability in the roots. In this study residue samples were taken 21 days after the last application. Therefore, the effect of timing of sampling on resultant residue levels must also be investigated.

The possibility of phytotoxicity when potassium phosphonates are applied to the foliage and fruit on trees was again confirmed in this trial, and enhances the emphasis on the need for alternative application methods.

Future research

Investigate the effect of application methods and dosages in more areas under different production and climatic conditions. Find trees that are affected by *Phytophthora* in order to have a realistic comparison of efficacy with regards to application

Yield and fruit size can be used for comparing treatments but all other factors affecting these parameters must be similar for all treatments in a trial. However, due to natural variation occurring between individual trees it might be more useful to do semi-commercial trials on a larger scale over several years in order to demonstrate the efficacy of soil applications

Using phosphonate residue levels as a parameter for comparison should be studied more carefully, especially with regards to factors affecting uptake (foliage, roots and trunk), distribution in the plant and detection of residues.

Technology transfer

Technology transfer will take place at biannual CRI symposium and presented at relevant study groups.

References cited

Shutte, C.G., Bezuidenhout, J.J., & Kotzé, J.M., 1991. Timing of application of phosphonate fungicides using different application methods as determined by means of Gas-Liquid-Chromatography for *Phytophthora* root rot control of Citrus. *Phytophylactica* 23:69-71.

Table 4.4.6.1. Treatments applied to determine influence of tree age, irrigation types and dosages on the effect of potassium phosphonates, applied through irrigation systems.

Group	Plot number	Treatment description	Tree age group (years)	Application method	Phytex dosage (ml / tree)
1	RS-22	M < 2, Ptx (½x)*	< 2	Micro	10.5
		M < 2, Ptx (1x)			21
		M < 2, Ptx (2x)			42
		Untreated control			-
2	RS-21	D < 2, Ptx (½x)*	< 2	Drip	10.5
		D < 2, Ptx (1x)			21
		D < 2, Ptx (2x)			42
		Untreated control			-
3	RS-11	M 3-6, Ptx (½x)	3 – 6	Micro	21
		M 3-6, Ptx (1x)			42
		M 3-6, Ptx (2x)			84
		Untreated control			-
4	J-40	D 3-6, Ptx (½x)	3 – 6	Drip	21
		D 3-6, Ptx (1x)			42
		D 3-6, Ptx (2x)			84
		Untreated control			-
5	D-18	M 7-10, Ptx (½x)	7 – 10	Micro	35.5
		M 7-10, Ptx (1x)			71
		M 7-10, Ptx (2x)			142
		Untreated control			-
6	RS-1	M > 15, Ptx (½x)	> 15	Micro	35.5
		M > 15, Ptx (1x)			71
		M > 15, Ptx (2x)			142
		Untreated control			-
7	G-1.11	D > 15, Ptx (½x)	> 15	Drip	21
		D > 15, Ptx (1x)			42
		D > 15, Ptx (2x)			84
		Untreated control			-

* Ptx = Phytex, M = micro irrigation, D = drip irrigation.

The first application started in October 2005, with 3 applications per season (between September and March) except for age group >15 years with an extra application per season.

Table 4.4.6.2. Treatments applied to plot E-7 and E-10 to determine influence of soil types and dosages on the effect of potassium phosphonates.

Group	Plot number & soil type	Treatment description	Tree age group (years)	Application method	Soil type	Phytext dosage (mℓ / tree)
1	E-7, sandy soil	M 7-10, Ptx (½x)	7 – 10	Micro	Sandy	35.5
		M 7-10, Ptx (1x)				71
		M 7-10, Ptx (2x)				142
		Untreated control		-		
2	E-10, clay soil	M 7-10, Ptx (½x)	7 - 10	Micro	Clay	35.5
		M 7-10, Ptx (1x)				71
		M 7-10, Ptx (2x)				142
		Untreated control		-		

* Ptx = Phytext, M = micro irrigation.

Table 4.4.6.3. Treatments applied to plot LQ-21 and LQ-28 to determine the effect of different application methods of potassium phosphonates on residue levels in the roots.

Group	Plot number	Treatment description	Tree age group (years)	Application method	Dosage (/ 100 ℓ)	Volume mixture applied per tree
1	LQ-28	Phosguard 400 SL FL	7 – 10	Foliar	500 mℓ	3.8 ℓ
		Phosguard 400 SL TP		Trunk paint	1:1 diluted	250 mℓ
		Phosguard 400 SL IR		Micro	350 mℓ	10 ℓ
		Untreated control		-	-	-
2	LQ-21	Phosguard 400 SL FL	> 15	Foliar	500 mℓ	7.1 ℓ
		Phosguard 400 SL TP		Trunk paint	1:1 diluted	250 mℓ
		Phosguard 400 SL IR		Micro	350 mℓ	10 ℓ
		Untreated control		-	-	-

* FL = foliar application, TP = trunk paint, IR = micro irrigation application.

Table 4.4.6.4. The influence that irrigation type, tree age and dosage of phosphonate applications has on yield, fruit size, phosphorous acid residue levels and *Phytophthora* infection.

Group	Plot number	Treatment description	Average yield per tree (kg)		Average fruit size		Phosphorous acid root residue levels (mg/kg)		Phy* status
			2006 / 2007	2007 / 2008	2006 / 2007	2007 / 2008	2006 / 2007	2007 / 2008	
1	RS-22	*M < 2, Ptx (½x)	Non bearing trees						-
		M < 2, Ptx (1x)					45	32	-
		M < 2, Ptx (2x)							-
		Untreated control							-
2	RS-21	*D < 2, Ptx (½x)	Non bearing trees						+++++*
		D < 2, Ptx (1x)					4	8	+++++*
		D < 2, Ptx (2x)							+++++*
		Untreated control							+++++*
3	RS-11	M 3-6, Ptx (½x)	66	94	67	60			+++++*
		M 3-6, Ptx (1x)	58	95	66	59	7	52	+++++*
		M 3-6, Ptx (2x)	63	109	67	58			+++++*
		Untreated control	65	99	65	60			+++++*
4	J-40	D 3-6, Ptx (½x)	88	70	64	48			-
		D 3-6, Ptx (1x)	83	75	65	50	36	52	-
		D 3-6, Ptx (2x)	78	66	66	49			-
		Untreated control	73	64	65	50			-
5	D-18	M 7-10, Ptx (½x)	96	74	76	61			-
		M 7-10, Ptx (1x)	96	72	79	60	17	13	-
		M 7-10, Ptx (2x)	103	56	77	62			-
		Untreated control	89	66	80	62			-
6	RS-1	M > 15, Ptx (½x)	146	177	74	59			+++++*
		M > 15, Ptx (1x)	144	187	79	59	91	114	+++++*
		M > 15, Ptx (2x)	135	155	81	60			+++++*
		Untreated control	141	175	76	68			+++++*
7	G-1.11	D > 15, Ptx (½x)	263	271 a	83	80			+++++*
		D > 15, Ptx (1x)	277	268 a	79	78	17	13	+++++*
		D > 15, Ptx (2x)	255	259 b	81	81			+++++*
		Untreated control	245	238 c	81	83			+++++*

* M = micro irrigation, D = drip irrigation, Ptx = Phytex, "+" = Indicate the amount of positive *Phytophthora* count out of a total of 5 leaf pieces. Leaf bating technique is not a quantitative method. *Phy.* = *Phytophthora*.

Table 4.4.6.5. The influence of soil types and dosage of phosphonate applications on yield, fruit size, phosphorous acid residue levels and *Phytophthora* recovery.

Group	Plot number & soil type	Treatment description	Average yield per tree (kg)		Average fruit size		Phosphorous acid root residue levels (mg/kg)		Phy* status
			2006 / 2007	2007 / 2008	2006 / 2007	2007 / 2008	2006 / 2007	2007 / 2008	
1	E-7,	M 7-10, Ptx (½x)	113	183	67	67			-
	sandy	M 7-10, Ptx (1x)	132	185	65	70	50	5	-
	soils	M 7-10, Ptx (2x)	123	189	66	67			-
		Untreated control	117	164	68	68			-
2	E-10,	M 7-10, Ptx (½x)	138	125	68	75			+++++*
	clay	M 7-10, Ptx (1x)	141	133	69	72	48	18	+++++*
	soil	M 7-10, Ptx (2x)	134	116	71	72			+++++*
		Untreated control	153	137	70	80			+++++*

* M = micro irrigation, Ptx = Phytex, "+" = Indicate the amount of positive *Phytophthora*-count out of a total of 5 leaf pieces. Leaf bating technique is not quantitative method. *Phy.* = *Phytophthora*.

Table 4.4.6.6. The influence of different phosphonate application methods has on yield, fruit size, phosphorous acid residue levels and *Phytophthora* infection.

Group	Plot number & soil type	Treatment description	Average yield per tree (kg)		Average fruit size		Phosphorous acid root residue levels (mg/kg)		Phy* status
			2006 / 2007	2007 / 2008	2006 / 2007	2007 / 2008	2006 / 2007	2007 / 2008	
1	LQ-28	Phosguard 400 SL FL*	86	85	81	57	46	36	+++++*
		Phosguard 400 SL TP*	98	118	75	56	41	25	+++++*
		Phosguard 400 SL IR*	105	96	74	57	7	31	+++++*
		Untreated control	103	109	73	59	0	0	+++++*
2	LQ-21	Phosguard 400 SL FL*	No data				70	86	+++++*
		Phosguard 400 SL TP*					28	25	+++++*
		Phosguard 400 SL IR*					56	31	+++++*
		Untreated control					0	0	+++++*

* FL = Foliar application, TP = trunk paint, IR = micro irrigation, "+" = Indicate the amount of positive *Phytophthora*-count out of a total of 5 leaf pieces. Leaf bating technique is not quantitative method. *Phy.* = *Phytophthora*.

Table 4.4.6.7. The percentage fruit showing phytotoxic symptoms for trial sprayed in the Letsitele area during the 2006/2007 growing season.

Treatment number	Treatment description	Plot number	Tree age group	Application method	Phytotoxicity (% fruit affected)
1	Phosguard 400 SL FL	LQ-28	7 - 10 years	Foliar application	88 b
2	Phosguard 400 SL SP			Trunk paint application	0 a
3	Phosguard 400 SL IR			Irrigation application	0 a
4	Untreated control			-	0 a



Figure 4.4.6.1. Phytotoxic symptoms observed on fruit in a trial sprayed in the Letsitele area during the 2006/2007 growing season.

4.4.7 PROGRESS REPORT: The effect of compost, amended with beneficial organisms, applied as soil treatments, on tree condition and general disease resistance
 Experiment 08QMS WvdP 01, (2008 – 2010) by W. van der Pypekamp (QMS)

Opsomming

Chemiese bemestingsprodukte kan ongunstige grondtoestande veroorsaak wat tot ongesonde wortels en plante kan lei. Ongesonde plante kan meer vatbaar wees vir infeksies en aanvalle deur insekte. Die doel van die navorsingsprojek is om te bepaal wat die effek van verskillende komposprogramme / komposverwanteprogramme op grondkondisies, plant gesondheid, opbrengs en die plant se vermoë om siektes beveg is. Die projek is in die Letsitele-area uitgevoer. Geen resultate is tans beskikbaar nie, met die eerste resultate moontlik beskikbaar aan die einde van die eerste seisoen (einde van Augustus 2009).

Summary

Chemical fertilizer can cause unfavourable soil conditions for soil microbes that can lead to unhealthy roots and plants. Unhealthy plants can be more prone to attack by pests and more susceptible to infection. The aim of this study was to determine the effects of compost / compost derivative programmes on overall soil conditions, plant health and ability to resist attack or infections and yield. The trials were conducted in the Letsitele area. No results are currently available, with possible results being available at the end of the first growing season (end of August 2009).

Introduction

The ability of plants to resist infection by microbial organisms can be enhanced by improving general plant health. Plants are more prone to fungal and bacterial infection when stressed. Tree condition or plant health is mostly influenced by root health and, therefore, can be affected by a multitude of factors e.g. soil compaction, organic content, microbial diversity, irrigation, nutrition, etc. Improving some of these factors can improve tree condition to an extent, but ultimately soil condition must be improved for sustainable improvement of tree condition. The main objective of this project is to improve general soil conditions and microbial diversity by re-introducing beneficial organisms and organic matter in the form of compost that will stimulate root growth, tree condition and enhance resistance to diseases.

Materials and methods

The trial was conducted at two localities, Bosveld Citrus and Laeveld Citrus in the Letsitele area, Limpopo Province, on five orchards. Orchard D-25 and DVK-3, both are cultivar Valencia, age group 7 – 10 years with micro and drip irrigation respectively. Orchards J-16 and DVK-3 are cultivar Valencia, age group 1 – 2 years with micro and drip irrigation respectively. And orchard DVK-10, cultivar Valencia, age group > 15 years with micro irrigation. Each treatment consisted out of 9-tree blocks, replicated 5 times in a randomised block design. Specific dosages applied were per manufacturer's specification as a soil application and are depicted in Table 4.4.7.1. A technical sheet of all products utilised in each program will be included in final report. Programme 1 (Agrilibruim) and Program 3 (Local Compost) was combined with standard producer applied fertilizer programme; amendments will be made to each programme in future if necessary.

During this season, all programmes were applied according manufactures recommendations. Soil and leaf samples were sampled individually for each program and orchard in March / April of 2009 to determine amendments to specific fertilizer programs. The effects of programs on tree condition and root condition would be determined at the end of the first growing season in August of 2009. A tree rating and root rating would be given to each program in a specific orchard and compared to the following seasons. *Phytophthora* and nematode counts were sampled for certain orchards at the beginning of the first growing season, with follow-up counts being done at the first seasons' end in August of 2009. The effect of programmes on tree condition would be further determined by evaluating each programme's trees separately for black spot in August of 2009. Yield and fruit size would also be determined for each programme separately at the end of a growing season to determine the effect on production.

Preliminary results

The first growing seasons' end is in August / September 2009, depending of harvest dates of specific orchards according to relevant producers. No results are available, with possible results being available at the end of the first growing season. The initial *Phytophthora* and nematode count for different programs and orchards are depicted in Table 4.4.7.2.

Conclusion

No conclusions can be made at this stage. The evaluating of the different parameters continues.

Table 4.4.7.1. Programmes applied to determine the effect of different compost / compost derivative on tree condition, yield and resistance to specific pests and diseases.

Prog-no.	Program description	Type of fertilizer	Orchard number & age group	Product 1 (volume / tree)	Application dates	Product 2 (volume / tree)	Application dates	Product 3 (volume / tree)	Application dates
1	Agrilibrium*	Compost & Microbes	DVK-4, <2	10kg, com-A*	Aug	10ml, QCM 360	Aug, Sep, Oct, Nov		
			J-16, <2	10kg, com-A	Aug	10ml, QCM 360	Aug, Sep, Oct, Nov		
			DK-3, 7-10	20kg, com-A	Aug	10ml, QCM 360	Aug, Sep, Oct, Nov		
			D-25, 7-10	20kg, com-A	Aug	8ml, QCM 360	Aug, Sep, Oct, Nov		
			DVK-10, >15	40kg, com-A	Aug	17ml, QCM 360	Aug, Sep, Oct, Nov		
2	I.C.F (International Carbon Fertilizer)	Organic carbon	DVK-4, <2	90 ml, 13:3:5	Aug to Feb	23ml, CAL 2	Oct - Feb		
			J-16, <2	90ml, 13:3:5	Aug to Feb	23ml, CAL 2	Oct - Feb		
			DVK-3, 7-10	240 ml, 13:1:5	Aug, Sep	40ml, CAL 3	Sep, Oct, Nov	120ml, 6:0:10+Ca+Mg	Oct, Nov, Dec
			D-25, 7-10	240 ml, 13:1:5	Aug, Sep	40ml, CAL 3	Sep, Oct, Nov	120ml, 6:0:10+Ca+Mg	Oct, Nov, Dec
3	Local Compost* (Bosveld Citrus)	Compost	J-16, <2	10kg, com-B	Aug				
			DVK-3, 7-10	20kg, com-B	Aug				
			D-25, 7-10	20kg, com-B	Aug				
			DVK-10, >15	30kg, com-B	Aug				
4	Agron	Organic carbon	J-16, <2	125ml, NPK 10-1-2	Aug to Feb	125ml, CalMagN 10-2-8	Aug to Feb	250g, Bio-soil-Blend	Sep
			D-25, 7-10	1.25l, Hume 4-1-10	Sep, Oct	2kg, Bio-Soil-Blend	Sep	1.85l, CAAN+B+Mo	Oct
5	Producer program	Chemical	DVK-3&4, J-16, D-25, DVK-10	-	-	-	-	-	-

*Programs also receiving producer applied fertilizer program, Com = compost, Aug = August, Sep = September, Nov = November, Dec = December, Jan = January, Feb = February.

Table 4.4.7.2. Initial *Phytophthora* and nematode count for programmes applied in the Letsitele area.

Programme number	Program description	Orchard number & tree age group	<i>Phytophthora</i> counts (Oct 2008)	Nematode counts (Oct 2008)
1	Agrilibrium	DVK-4, <2	+++	960
2	I.C.F.	DVK-4, <2	+++++	0
5	Control (Producers' program)	DVK-4, <2	-	0
1	Agrilibrium	DVK-3, 7-10	-	3840
2	I.C.F.	DVK-3, 7-10	+++++	2640
3	Local Compost	DVK-3, 7-10	++++	1680

5	Control (Producers' program)	DVK-3, 7-10	-	2880
1	Agrilibrum	DVK-10, >15	+++++	1200
3	Local Compost	DVK-10, >15	+++++	1920
5	Control (Producers' program)	DVK-10, >15	+++++	720
1	Agrilibrum	J-16, <2	+	0
2	I.C.F.	J-16, <2	+	240
3	Local Compost	J-16, <2	-	480
4	Agron	J-16, <2	-	240
5	Control (Producers' program)	J-16, <2	-	0
1	Agrilibrum	D-25, 7-10	++	3360
2	I.C.F.	D-25, 7-10	+++++	2400
3	Local Compost	D-25, 7-10	++	960
4	Agron	D-25, 7-10	+++++	1680
5	Control (Producers' program)	D-25, 7-10	+++++	0

“+” = Indicate the amount of positive *Phytophthora*-count out of a total of 5 leaf pieces. Leaf bating technique is not quantitative method. Nematode = Mean female count in 10g roots for 5 replicates.

4.4.8 Evaluation of a new biological control product for the control of the citrus nematode Experiment 894 by M.C. Pretorius (CRI)

Opsomming

Registrasie proewe is vir Desert King, 'n VSA gebaseerde maatskappy, op 'n kontrakbasis uitgevoer om 'n nuwe biologiese beheer produk te evalueer vir die beheer van die sitrusaalwurm. 'n Proef wat op Friedenheim Sitrus Landgoed in 'n 12 jaar oue Delta Valencia boord asook in die Wes Kaap by ALG Boerdery, Citrusdal uitgelê is, is oor twee seisoene gemonitor. Toedienings is op beide proewe gedurende die seisoen gedoen en drie stelle monsternemings het plaasgevind. 'n Vorderingsverslag is aan Desert King gestuur. Die maatskappy het CRI versoek om die proef vir nog 'n seisoen te herhaal.

Summary

Desert King, a USA based company, approached CRI to conduct registration trials to establish the efficacy of a biological control product for the control of the citrus nematode on a contract basis. The one trial was laid out at Friedenheim Citrus Estate. The trial site was selected on 12-year-old Delta Valencia trees with ± 6000 ♀/10 g roots and the second trial was laid out at ALG Boerdery in Citrusdal. The product was applied and the sites were sampled three times during the season. A progress report was sent to Desert King for the work conducted during the 2007 season. Desert King suggested that the trial should be repeated this season.

4.4.9 Evaluation of a new nematicide for the control of the citrus nematode Experiment 950 by M.C. Pretorius (CRI)

Opsomming

'n Evaluasie proef is vir Makhteshim, Israel, uitgevoer met 'n nuwe nematisied. Hierdie produk is nog slegs in 'n proef formaat met 'n beperkte hoeveelheid produk beskikbaar gestel. 'n Proef is op Croc Valley uitgelê in 'n 12-jaar-oue Delta Valencia boord met aalwurmwyfie telings van >6000 wyfies/10 g wortels. Aanvanklike resultate was belowend gewees en opvolg proefwerk is deur CRI voorgestel. 'n Finale verslag is aan Makhteshim gegee.

Summary

A contract trial was laid out at Croc Valley on a 12-year-old Delta Valencia orchard with ± 6000 ♀/10 g roots. A new nematicide formulation as supplied by Makhteshim, Israel was evaluated. Initial results were promising and follow-up trial work was suggested by CRI. A Final report was given to Makhteshim.

4.4.10 Evaluation of a new safer nematicide for the control of the citrus nematode Experiment 951 by M.C. Pretorius (CRI)

Opsomming

Registrasie proewe is vir 'n Belgiese maatskappy, DevGen, uitgevoer op Crocodile Valley Citrus Co. in 'n 12-jaar-oue Delta Valencia boord met aalwurmwyfietellings van meer as 5000 wyfies / 10 g wortels. 'n Tweede proef is ook in die Wes-Kaap in Citrusdal gemonitor. Toedienings is op beide proewe gedurende die seisoen gedoen en drie stelle monsternemings het plaasgevind. 'n Vorderingsverslag is aan DevGem gestuur.

Summary

DevGem, a Belgium based company, approached CRI to conduct registration trials on a contract basis to establish the efficacy of a new softer nematicide formulation for the control of the citrus nematode. One trial was laid out at Crocodile Valley Citrus Co. on 12-year-old Delta Valencia trees with > 5000 ♀/10 g roots and a second trial was laid out in Citrusdal. The product was applied and the sites were sampled three times during the season. A progress report was sent to DevGem for the work conducted during the 2008 season.

4.5 PROJECT: POST-HARVEST PATHOLOGY

Project coordinator: K.H. Lesar (CRI)

4.5.1 Project summary

Pyrimethanil 400 SC demonstrated good control of the citrus pathogen *Penicillium digitatum* (sensitive and resistant strains) infection on navel and Valencia oranges and lemons, in dip treatments at both ambient and 30°C, compared to the standard recommended Fungazil sulphate 750 WSP. Results on the yeast antagonist, submitted by Plant Health Products for screening in a simulated packhouse total loss brush-on application against postharvest pathogen infection, demonstrated that the yeast did not provide any significant level of control in any of the trials. The generic imazalil sulphate 750 WSG submitted by Volcano Agrosience demonstrated good control of postharvest infection by the citrus pathogen, *P. digitatum*, compared to the standard recommended Fungazil sulphate 750 WSG. The organic sanitiser/biocide compounds, OrganoKare and KannarKare, did neither effectively sanitise a simulated citrus packhouse dump tank washing system, nor inhibit infections on fruit by the post-harvest citrus pathogen *P. digitatum* thereby not demonstrating any fungicidal properties.

The repeat evaluation of the plant growth regulator Retain, and the screening of a new synthetic auxin, Maxim, as possible alternatives to 2,4-D, was conducted on Valencia oranges. Results confirmed previous findings that Retain can be used as a possible alternative to 2,4-D.

The efficacy of the GRAS chemical, sodium bicarbonate, was evaluated at concentrations of 1 and 2%, alone and in combination with the standard 500 ppm imazalil sulphate and the reduced concentration of 250 ppm in a simulated hot water dip treatment against postharvest infections by imazalil-sensitive and -resistant *P. digitatum* (green mould) spores. The anticipated synergistic effect between imazalil and sodium bicarbonate was not observed.

Practical guidelines addressing proper practices and procedures for handling of citrus fruit during harvesting and packing are needed to aid producers and packhouses in ensuring that a disease-free, quality product is delivered into the markets. These are being compiled in combination with updating of the production guidelines.

Imazalil application methods in citrus packhouses varied considerably between packhouses and divergence from recommended guidelines was often observed. Imazalil residue loading could not be related to single factors, but rather seemed to be an interaction between exposure time, bath temperature, pH and/or fruit type. Optimisation studies will continue in order to improve methods of IMZ residue loading onto different citrus fruit types.

A diverse range of *Penicillium* spp. including the citrus blue and green mould pathogens *P. italicum* and *P. digitatum*, were isolated in facilities in the citrus supply chain. This emphasises the importance of adhering to stricter hygiene standards throughout the supply chain in an effort to reduce high inoculum loads as fruit handling and high *Penicillium* inoculum levels are important contributing factors to the high incidence of postharvest decay in the export market.

A bacterial isolate was successfully isolated, identified as *Bacillus subtilis* PPCB002 and analysed as a potential biocontrol agent for the control of postharvest diseases of citrus. This product could provide an organic alternative for the industry since this *Bacillus* strain occurs naturally on citrus fruit surfaces.

Penicillium isolates were obtained from decaying fruit at various packhouses in citrus producing regions of South Africa and evaluated for resistance against imazalil and guazatine. Many of the *Penicillium* spp. that were tested during this study showed sensitivity towards these fungicides, however *Penicillium* spp. with a higher level of resistance and pathogenic abilities were also identified.

Projekopsomming

Pyrimethanil 400 SC het goeie beheer van infeksie deur die sitruspatogeen *Penicillium digitatum* (sensitiewe and bestande rasse) op nawel and Valencia lemoene en suurlemoene, in doopbehandelings teen beide kamertemperatuur en 30°C, in vergelyking met die standaard aanbevole Fungazil sulfaat 750 WG, gewys. Die evaluering van die gis antagonist, vanaf Plant Health Products, in 'n gesimuleerde pakhuis totale verlies aanborsel aanwending teen na-oes patogeen infeksie, het nie noemenswaardige vlakke van beheer teen infeksie gewys nie. Die generiese imazalil sulfaat 750 WSG vanaf Volcano Agroscience het goeie beheer van *P. digitatum* na-oes infeksie, in vergelyking met die standaard aanbevole Fungazil sulfaat 750 WSG gewys. Die organiese sanitasie-middels, OrganoKare en KannarKare, het nie die gewenste ontsmetting van 'n gesimuleerde sitruspakhuis dompelbad getoon nie, en ook nie die besmetting van beseerde vrugte deur die bad voorkom nie. Die produkte het ook nie swamdodende eienskappe getoon nie.

Die herhaling van die evaluasie van die plantgroeireguleerder, Retain en 'n nuwe sintetiese oksien, Maxim, as alternatief vir 2,4-D, is op Valencia lemoene uitgevoer. Resultate het weer eens getoon dat Retain moontlik as plaasvervanger vir 2,4-D gebruik kan word.

Die effektiwiteit van die GRAS chemikalie, natrium bikarbonaat, is teen konsentrasies van 1 en 2%, alleen en in kombinasie met die standaard 500 dpm imazalil sulfaat en die verlaagde konsentrasie van 250 dpm in 'n gesimuleerde warm water doopbehandeling teen na-oes infeksies deur imazalil-sensitiewe en -bestande *P. digitatum* (groenskimmel) spore ge-evalueer. Die verwagte sinergistiese werking is nie waargeneem nie.

Praktiese riglyne wat die goeie praktyke en prosedure vir die hantering van sitrus tydens pluk en verpakking aanspreek is nodig om produsente en pakhuisse te help om seker te maak dat 'n siektevrye produk van goeie gehalte by die markte afgelewer word. Hierdie riglyne word opgestel, en daarmee saam ook die opdatering van die produksie-riglyne.

Imazalil aanwending in sitruspakhuisse het aansienlik verskil tussen die pakhuisse en afwyking van aanbevole riglyne is gereeld waargeneem. Imazalil residulading kon nie aan enkele faktore toegeskryf word nie, maar het ieder voorgekom as 'n samewerking van blootstellingstyd, bad temperatuur, pH en/of vrug tipe. Optimaliseringsstudies op verskillende vrugtipies sal voortgaan om IMZ-residulading te verbeter.

'n Diverse verskeidenheid *Penicillium* spp., insluitende die sitrus patogene *P. italicum* en *P. digitatum*, die oorsaak van blou en groen skimmel, is in verskeie fasiliteite in die uitvoerketting geïsoleer. Die data versterk ons teorie dat besmetting later in die ketting kan plaasvind en dit lê die klem op die belangrikheid vir alle rolspelers in die uitvoerketting om aan strengere higiëne standaarde te voldoen.

'n Bakteriese isolaat is geïsoleer, geïdentifiseer as *Bacillus subtilis* PPCB002 en geëvalueer vir moontlike biologiese beheer van na-oes siektes op sitrus. Dié produk sal vir die bedryf 'n organiese alternatief voorsien, aangesien dat hierdie *Bacillus* op sitrus vrugoppervlakte natuurlik voorkom.

Penicillium isolate is vanaf bederwe vrugte by verskeie pakhuisse in die sitrus-produiserende areas van Suid Afrika geïsoleer, en vir bestandheid teen imazalil en guazatine getoets. Heelwat *Penicillium* spp. wat geïsoleer is tydens hierdie studie was sensitief vir hierdie swamdoders, alhoewel *Penicillium* spesies met 'n hoër vlak van weerstand en patogeeniese eienskappe ook geïdentifiseer is.

4.5.2 PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided
Experiment 123 (Ongoing) by K.H. Lesar (CRI)

Opsomming

Verskeie proewe is in hierdie eksperiment hanteer:

Die na-oes swamdoder pyrimethanil is teen die beheer van infeksies op sitrus, veroorsaak deur imazalil sensitiewe en bestande *Penicillium digitatum* (groenskimmel) rasse, ge-evalueer. Die besmette vrugte is deur pyrimethanil in doop behandelings in warm water teen 30°C en teen kamer temperatuur behandel. Pyrimethanil het die na-oes infeksies, in vergelyking met die standaard imazalil 750 SG, goed beheer. Hierdie data is saam met vrugmonsters vir residuontledings ingedien vir registrasie.

'n Gis antagonis vanaf Plant Health Products (Pty) Ltd. is teen natuurlike groenskimmel infeksies op Valencia en nawel lemoene en mandaryne ge-evalueer. Die produk is in 'n gesimuleerde pakhuis totale verlies aanborseling stelsel aangewend vir moontlike registrasie as 'n alleen behandeling op uitvoer sitrus. Die resultate in hierdie proewe het gewys dat die gis geen noemenswaardige mate van beheer na gesimuleerde lang verskeping in vergelyking met imazalil getoon het nie. Die inhibisie deur die antagonis het 'n mate van wisselvalligheid, in vergelyking met imazalil, getoon.

Die imazalil 500 EC formulاسie, ingestuur deur Volcano Agrosience (Arysta LifeScience Group), het goeie beheer van die na-oes sitrus patogeen *P. digitatum* (groenskimmel), in vergelyking met die standaard Fungazil 800 EC formulاسie, gewys.

Twee Kannar organise middels is in gesimuleerde pakhuisproewe ge-evalueer, maar het nie die gewenste ontsmetting van 'n pakhuis dompelbad getoon nie, en ook nie die besmetting van beseerde vrugte deur die bad voorkom nie. Die produk het ook nie swamdodende eienskappe getoon nie.

Summary

Various trials were conducted in this experiment:

The post-harvest fungicide pyrimethanil was evaluated in hot water (30°C) and ambient dip treatments for the control of infections on citrus fruit caused by imazalil sensitive and resistant *Penicillium digitatum* (green mould) strains. Pyrimethanil demonstrated good control of the postharvest infections compared to the standard imazalil 750 SG. This data, together with residue samples were submitted for registration.

A yeast antagonist from Plant Health Products (Pty) Ltd. was screened against natural green mould infections on Valencia and navel oranges and mandarins. The product was applied in a simulated packhouse total loss brush-on application for possible registration for commercial use as a stand-alone treatment on export citrus fruit. The degree of inhibition of natural infections by the antagonist, after simulated long storage shipping conditions, did not compare favourably with the standard imazalil sulphate. The results demonstrated that the yeast did not provide any significant level of control in any of the trials. The incidence inhibition by the antagonist in these trials demonstrated a degree inconsistency when compared with imazalil.

The imazalil 500 EC formulation, submitted by Volcano Agrosience (Arysta LifeScience Group) demonstrated good control of the postharvest citrus pathogen *P. digitatum*, compared to the standard Fungazil 500 EC formulation.

Two Kannar organic compounds were evaluated in simulated packhouse trials. Neither of the products demonstrated the desired disinfestation of a packhouse dumptank washing system, nor the prevention of infection of any injured fruit moving through the bath. Neither of the products demonstrated any fungicidal properties.

Trial 1. The evaluation of the postharvest fungicide pyrimethanil for the control of postharvest infections by imazalil sensitive and resistant *Penicillium digitatum* (citrus green mould) isolates after harvest (October–December 2008)

Introduction

It is important to control post-harvest diseases of citrus to ensure the good quality and maintain the shelf life of citrus fruit, given the distance of the Southern African citrus producers from the markets. Green mould, *Penicillium digitatum* (Pers.:Fr.) Saccardo and blue mould, *Penicillium italicum* Wehmer are the most economically important post-harvest diseases of citrus fruits in South Africa, responsible for 90% of all the losses caused by the post-harvest pathogens (Christ, 1966).

In South African citrus packhouses, citrus fruits are treated with the post-harvest fungicides, imazalil, thiabendazole (TBZ) and guazatine to control *Penicillium* decay. Sodium ortho-phenylphenate (SOPP) and prochloraz, also registered for use for the control of the *Penicillium* moulds, have not been used in citrus packhouses for the last two decades. The fungicides imazalil, thiabendazole and guazatine are being used in a manner highly conducive to the selection and proliferation of fungicide resistant isolates of the *Penicillium* moulds.

Green and blue mould resistance to TBZ has been in existence in South Africa for the last three decades. The same scenario exists in California for both SOPP and TBZ (Eckert, 1987; Eckert et al., 1994; Kuramoto, 1976). Imazalil was introduced into the Californian and South African citrus industries in the early 1980s as a successful treatment for the TBZ-resistant biotypes. However, 5 years after the introduction of imazalil as a commercial treatment in California packhouses, imazalil-resistant biotypes were detected and have been widely reported (Eckert et al., 1994). Random *in vivo* screening of 160-200 *Penicillium* spore samples from 2001-2005 in the South African citrus industry revealed 20 samples with imazalil resistance. (Reported, not published). Many of these spore samples were taken in citrus packhouses from fungicide-treated culled fruit and fruit for processing that was allowed to rot within the confines of the packhouses.

These reports of the existence of widespread fungicide resistance in the *Penicillium* moulds (particularly *P. digitatum*) make the development of new post-harvest decay control treatments for citrus fruit important (Smilanick et al., 2006). For this reason several new fungicides have been proposed for approval for postharvest use on citrus in California (Adaskaveg et al., 2005) and Florida (Zhang, 2003). Janssen Pharmaceutica (Belgium) formulated two post-harvest fungicides, imazalil and pyrimethanil into a single mixture compound named Philabuster. Imazalil and pyrimethanil have different modes of action against *P. digitatum*. Pyrimethanil is classified as a “new chemistry reduced risk” compound and does not share a mode of action with any of the other citrus post-harvest fungicides currently being used in the South African citrus industry.

With the introduction of new fungicides, effective mixture and rotation programs can be designed that could delay the onset of resistance development against these new compounds. In addition, the seasonal increase in the incidence of resistance against the older compounds (J.E. Adaskaveg, *unpublished*) might be delayed. Thus, imazalil and TBZ will remain important management tools and can be incorporated into management programs. Ideally, integrated management programs using all the postharvest fungicides should be developed for each packhouse based on monitoring of fungicide sensitivity in *P. digitatum* populations and rotations of mixtures of products with different modes of action (Kanetis et al., 2007)

The aim of this study was to evaluate the efficacy of pyrimethanil for the control of *P. digitatum* (citrus green mould) post-harvest infections by imazalil sensitive and resistant spores. Pyrimethanil was applied in an ambient water dip treatment and also a hot water dip treatment at 30°C.

Materials and methods

In vivo evaluation trials were conducted with the ICA International Chemicals pyrimethanil 400 SC formulation (Batch Nr. AC 093) on lemons, navels and Valencia oranges that were inoculated with an imazalil sensitive and resistant strain of *P. digitatum* for the ambient dip and hot water treatments. Pyrimethanil was compared with the standard Fungazil sulphate 750 WG (Janssen Pharmaceutica) at the recommended commercial rate of 67 g/100 l giving a treatment concentration of 500 ppm imazalil in the dip treatments. Pyrimethanil was evaluated at the rates of 0.25% (1/2×), 0.5% (×) and 1.0% (2×), giving treatment concentrations of 500, 1000 and 2000 ppm, respectively, in the dip treatments.

Spore suspensions of *P. digitatum* were made up by suspending the spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were spectrophotometrically adjusted to a concentration of 1×10^6 spores/ml (Eckert et al., 1986; Morris et al., 1978).

Untreated navel and Valencia oranges (from Crocodile Valley Estate) and lemons (from Larten Estates) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit were selected. The fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides, giving a total of 60 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 µl of spore suspension to each injury site using a micropipette. The inoculated fruit was incubated for 12 hours at ± 23°C (to simulate a 12-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated.

Ambient and hot water dip treatments

Inoculated fruit was divided into 3 replicates of 10 fruit per treatment for the ambient and hot water dip treatments. All the treatments were immersed in a 3 minute dip at ambient (18°C) and at 30°C. Treatments on navels, Valencias and lemons with imazalil sensitive and resistant *P. digitatum* spores at 18°C and 30°C:

1. Untreated control - water dip
2. Standard treated control – Fungazil WG 67g /100 l (500 ppm imazalil)
3. Pyrimethanil – 0.25% (500 ppm)
4. Pyrimethanil – 0.5% (1000 ppm)
5. Pyrimethanil – 1.0% (2000 ppm)

After treatment, the fruit was incubated in paper packets at 23°C for 7 days or until such time as the untreated controls exhibited sufficient green mould. The treatments were then evaluated by counting the number of infected wounds per treatment and the results were recorded as percentage decay inhibition.

Results

Pyrimethanil 400 SC demonstrated good control of the citrus pathogen *P. digitatum* (sensitive and resistant strains) infection on navel and Valencia oranges and lemons, at both treatment temperatures, compared to the standard recommended Fungazil sulphate 750 WSP (Tables 4.5.2.1-6). On navel oranges pyrimethanil, at the recommended rate of 0.5% (1000 ppm) for registration of the product, demonstrated 96.7 and 93.3% inhibition of infection by the imazalil-sensitive and resistant *P. digitatum* strains, respectively, at 18°C, and 100% inhibition of infection by both strains at 30°C, compared to 100% inhibition of the sensitive strain and only 23.3% and 16.7% inhibition of the resistant strain by the standard recommended imazalil 750 WG at 500 ppm at treatment temperatures 18°C and 30°C, respectively (Tables 4.5.2.1 and 4.5.2.2). On Valencia oranges, pyrimethanil at 0.5% achieved 100% and 96.7% control of infection by the imazalil-sensitive and resistant *P. digitatum* strains respectively at 18°C, and 100% inhibition of infection by both strains at 30°C, compared to 100% inhibition of the sensitive strain at both treatment temperatures and only 16.7 and 26.7% inhibition of the resistant strain by the standard recommended imazalil 750 WG at 500 ppm at treatment temperatures 18°C and 30°C, respectively (Tables 4.5.2.3 and 4.5.2.4). On lemons, pyrimethanil at 0.5%, achieved 100% control of infection by both *P. digitatum* strains at both treatment temperatures, compared to 100% inhibition of the sensitive strains at both treatment temperatures and only 26.7 and 23.3% inhibition of the resistant strain by the standard recommended imazalil 750 WG at 500 ppm at treatment temperatures 18°C and 30°C, respectively (Tables 4.5.2.5 and 4.5.2.6).

Table 4.5.2.1 (Trial 1). Percentage inhibition of green mould on navel oranges that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 12 hours before dip-treatment in ambient water (18°C) with 0.25%, 0.5% and 1.0% pyrimethanil and 67 g/100l Fungazil 750 WG.

Treatments	% Inhibition ^a	
	Navels (18°C)	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	6.7 b	0.0 c
2. Treated control- Fungazil (67 g/100 l)	100.0 a	23.3 b
3. Pyrimethanil (0.25%)	93.3 a	93.3 a
4. Pyrimethanil (0.5%)	96.7 a	93.3 a
5. Pyrimethanil (1.0%)	100.0 a	100.0 a

^a Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; P ≤ 0.05).

Table 4.5.2.2 (Trial 1). Percentage inhibition of green mould on navel oranges that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 12 hours before dip-treatment in water (30°C) with 0.25%, 0.5% and 1.0% pyrimethanil and 67 g/100ℓ Fungazil 750 WG.

Treatments	% Inhibition ^a	
	Navels (30°C)	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	6.7 b	3.3 c
2. Treated control- Fungazil (67 g/100 ℓ)	100.0 a	16.7 b
3. Pyrimethanil (0.25%)	96.7 a	96.7 a
4. Pyrimethanil (0.5%)	100.0 a	100.0 a
5. Pyrimethanil (1.0%)	100.0 a	100.0 a

^a Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.3 (Trial 1). Percentage inhibition of green mould on Valencia oranges that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 12 hours before dip-treatment in water (18°C) with 0.25%, 0.5% and 1.0% Pyrimethanil and 67 g/100ℓ Fungazil 750 WG.

Treatments	% Inhibition ^a	
	Valencias (18°C)	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	0.0 c	0.0 d
2. Treated control- Fungazil (67 g/100 ℓ)	100.0 a	16.7 c
3. Pyrimethanil (0.25%)	93.3 b	90.0 b
4. Pyrimethanil (0.5%)	100.0 a	96.7 a
5. Pyrimethanil (1.0%)	100.0 a	100.0 a

^a Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.4 (Trial 1). Percentage inhibition of green mould on Valencia oranges that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 12 hours before dip-treatment in water (30°C) with 0.25%, 0.5% and 1.0% pyrimethanil and 67 g/100ℓ Fungazil 750 WG.

Treatments	% Inhibition ^a	
	Valencias (30°C)	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	0.0 b	0.0 c
2. Treated control- Fungazil (67 g/100 ℓ)	100.0 a	26.7 b
3. Pyrimethanil (0.25%)	93.3 a	93.3 a
4. Pyrimethanil (0.5%)	100.0 a	100.0 a
5. Pyrimethanil (1.0%)	100.0 a	100.0 a

^a Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.5 (Trial 1). Percentage inhibition of green mould on lemons that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 12 hours before dip-treatment in water (18°C) with 0.25%, 0.5% and 1.0% pyrimethanil and 67 g/100ℓ Fungazil 750 WG.

Treatments	% Inhibition ^a	
	Lemons (18°C)	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	0.0 c	0.0 d
2. Treated control- Fungazil (67 g/100 ℓ)	100.0 a	26.7 c
3. Pyrimethanil (0.25%)	90.0 b	93.3 b

4. Pyrimethanil (0.5%)	100.0 a	100.0 a
5. Pyrimethanil (1.0%)	100.0 a	100.0 a

^a Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.6 (Trial 1). Percentage inhibition of green mould on lemons that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 12 hours before dip-treatment in water (30°C) with 0.25%, 0.5% and 1.0% pyrimethanil and 67 g/100l Fungazil 750 WG.

Treatments	% Inhibition ^a	
	Lemons (30°C)	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	6.7 b	0.0 d
2. Treated control- Fungazil (67 g/100 l)	100.0 a	23.3 c
3. Pyrimethanil (0.25%)	93.3 a	90.0 b
4. Pyrimethanil (0.5%)	100.0 a	100.0 a
5. Pyrimethanil (1.0%)	100.0 a	100.0 a

^a Values represent the means of 3 replicates of 10 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Conclusions and discussion

Pyrimethanil demonstrated that it was a very effective compound for the management of citrus green mould infections in packhouses. Pyrimethanil is classified as a "new chemistry reduced risk" compound and does not share a mode of action with any of the other citrus post-harvest fungicides currently being used in the South African citrus industry. This makes the product of particular value because it is effective in controlling *P. digitatum* isolates that are resistant to imazalil and also guazatine and thiabendazole as well.

Effective mixture and rotation programs can be designed that could delay the onset of resistance development against any new compounds. In addition the seasonal increase in the incidence of resistance against the older compounds (J.E. Adaskaveg, *unpublished*) might be delayed.

For this purpose, it is recommended that pyrimethanil be registered at a dosage rate of 0.5% (1000 ppm) in a standard packhouse water dip application for use in an integrate mixture and rotation program for preventing the onset of resistance to any new compounds and the current generation of postharvest fungicides.

Future research

The trials for the registration of Pyrimethanil in the drench, total loss brush-on and wax applications will also be conducted.

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Trial 2. The screening of a yeast antagonist in a citrus packhouse total loss application against natural postharvest *Penicillium digitatum* (green mould) infections (August-November 2008)

Introduction

Green mould (*Penicillium digitatum* Saccardo), blue mould (*Penicillium italicum* Wehmer) and sour rot (*Geotrichum candidum* (Link ex Pers.) are three of the most economically important post-harvest diseases of citrus, responsible for severe economic losses worldwide (Bancroft et al., 1984). The primary mode of infection by these pathogens is through wounds on fruit inflicted during harvest and subsequent handling, and these infections must be eradicated to achieve acceptable levels of control (Eckert and Brown, 1986). Currently, post-harvest citrus decay in South Africa is controlled by application of the post-harvest fungicides thiabendazole, imazalil, guazatine and sodium ortho-phenylphenate (SOPP).



***Penicillium digitatum* (green mould)**

Consumers are concerned about chemical products that are applied to perishable commodities during post-harvest treatments. During the last 10-15 years there has been continuous pressure exerted from various quarters, political and regulatory, health groups and certain markets, to discontinue the use of fungicides or decrease the residue levels of these compounds on citrus fruit. Eventually, this might result in discontinuation of many chemical treatments for the control of post-harvest diseases. Without the use of these treatments, producers and suppliers of many commodities could face serious threats of increased spoilage and economic losses. An additional practical issue that has placed the continued use of fungicides at risk is the problem of resistance development in pathogen populations to post-harvest fungicides (Eckert et al., 1994).

It has thus become necessary to screen safer chemicals or antagonists for the control of the citrus pathogens in order to find new, safe compounds that could assist in overall decay control as well as preventing or delaying the development of fungicide resistance.

Biological control using microbial antagonists has received a great deal of attention as a promising alternative to chemicals. Many organisms, such as *Pseudomonas* spp. (Smilanick et al., 1992), *Debaryomyces hansenii* (Chalutz et al., 1990), *Pichia guilliermondii* (Droby et al., 1993) etc. have been isolated to protect wounds from postharvest pathogens on citrus fruits. One yeast and two bacterial products are currently commercially available in USA.

Attempts to continuously select and develop new microbial antagonists with improved efficacy are an ongoing process. The challenge remains consistent product performance with biocontrol applied in an integrated way so as to ensure optimum performance under various conditions and with different postharvest practices.

A yeast biocontrol agent was supplied to CRI by Plant Health Products (Pty) Ltd. for screening in a simulated packhouse total loss brush-on application against postharvest pathogen infection, for registration for commercial use in the Southern African citrus industry.

The aim of these trials was to treat three citrus cultivars with the yeast antagonist, applied in a total loss application system on a citrus packline, for the purpose of evaluating the antagonist against the degree of inhibition of natural *Penicillium digitatum* (citrus green mould) infections, after storage of the treated fruit under simulated shipping conditions.

Materials and methods

Evaluation trials were conducted with the Plant Health Products yeast antagonist on Empress mandarins and navel and Valencia oranges on the CRI packline in the spray on/brush-on application system. The yeast antagonist was compared with the standard Fungazil sulphate 750 WG (Janssen Pharmaceutica) at the recommended commercial rate of 67 g/100 ℓ giving a treatment concentration of 500 ppm imazalil in a dip treatment. The yeast antagonist was applied by dissolving a vacuum packed sachet of the formulated antagonist in 100 ℓ of water giving an approximate concentration of 1×10^6 spores/ml in a total loss spray-on/brush-on application.

Untreated navel and Valencia oranges (from Crocodile Valley Estate) and Empress mandarins (from Terblanche Boerdery Schagen) were obtained in bulk. Prior to treatment, blemish free, sound fruit was selected. The fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was allowed to dry prior to treatment.

The Valencia oranges were divided into 5 replicates of 20 fruit per treatment, the mandarins into 4 replicates of 20 fruit per treatment and the navels into 4 replicates of 15 fruit per treatment. All the treatments were exposed to the water (untreated control) and the antagonist on the brushes for 3 minutes in the total loss application. The imazalil-treated fruit was immersed in a 3 minute dip at ambient (18°C).

After treatment of the fruit with the antagonist and the standard imazalil, half the treatments were waxed and the other half were left unwaxed. A Carnuba wax was used for the wax applications.

Treatments for Valencias, navels and mandarins

1. Untreated control – wax application.
2. Standard treated control – Fungazil WSP 67g /100 ℓ (500 ppm imazalil) + wax.
3. Yeast antagonist in total loss application + wax.
4. Untreated control – water dip application (without wax).
5. Standard treated control – Fungazil WSP 67g /100 ℓ (500 ppm imazalil) (without wax).
6. Yeast antagonist in total loss application (without wax).

After treatment the fruit was dried in the drying tunnel on the packline. Treatments 1, 2 and 3 were then waxed on the packline and dried in the drying tunnel.

Storage

After treatment the fruit was stored as for long shipping storage for 4-10 weeks at 4,5°C and 10°C + 7 days at 20°C.

The fruit was evaluated after the normal shipping protocol (4 weeks) and no natural *P. digitatum* infections were observed. After a further 2 weeks storage (6 weeks) results were obtained for the navels. The mandarins and Valencias were stored for a further 4 weeks before the final results were recorded. The treatments were evaluated by counting the number of infected fruit per treatment and the results were recorded as percentage decay.

Results

The results recorded in the three trials below did not demonstrate any significant statistical differences between the incidence of natural *P. digitatum* infections on the yeast antagonist-treated fruit and the untreated control. However, the incidence of decay on untreated control fruit and the fruit treated with the antagonist differed significantly from fruit treated with the standard recommended Fungazil sulphate 750 WG on waxed and unwaxed fruit, after simulated shipping storage at 4.5 and 10°C (Tables 4.5.2.1-6). The degree of natural infections on yeast antagonist-treated fruit for **waxed fruit** was 16.6 to 30% (Untreated control, 20 to 29%) on Valencia and navel oranges (Tables 4.5.2.11-4), and 27.5 to 37.5% (Untreated control, 27.5 to 45%) on Empress mandarins (Tables 4.5.2.5 and 6), compared to 5 to 8.3% for imazalil-treated Valencias and navels and 6.3 to 12.5% for imazalil-treated mandarins, respectively. The degree of natural infections on yeast antagonist-treated fruit for **unwaxed fruit** was 12 to 20% (Untreated control, 16 to 21.7%) on Valencia and navel oranges (Tables 4.5.2.1-4), and 47.5 to 55% (Untreated control, 50 to 67.5%) on Empress mandarins (Tables 4.5.2.5 and 6), compared to 4 to 6% for imazalil-treated Valencias and navels and 15 to 27.5% for imazalil-treated mandarins, respectively.

Table 4.5.2.1 (Trial 2). Percentage inhibition of natural green mould infections on waxed and unwaxed Valencia oranges that were stored under simulated shipping conditions at 4.5°C for 4-10 weeks after packhouse treatments with a yeast antagonist and the standard 500 ppm imazalil sulphate.

Treatments	% Decay ^a
	Valencias (4.5°C)
1. Untreated control – wax application	29.0 a
2. Standard treated control – Fungazil (500 ppm imazalil) + wax	8.0 b
3. Yeast antagonist in total loss application + wax	22.0 ab
4. Untreated control – water dip (without wax)	18.0 a
5. Standard treated control – Fungazil (500 ppm imazalil) (without wax)	4.0 b
6. Yeast antagonist in total loss application (without wax)	14.0 a

^a Values represent the means of 5 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.2 (Trial 2). Percentage inhibition of natural green mould infections on waxed and unwaxed Valencia oranges that were stored under simulated shipping conditions at 10°C for 4-10 weeks after packhouse treatments with a yeast antagonist and the standard 500 ppm imazalil sulphate.

Treatments	% Decay ^a
	Valencias (10°C)
1. Untreated control – wax application	29.0 a
2. Standard treated control – Fungazil (500 ppm imazalil) + wax	8.0 b
3. Yeast antagonist in total loss application + wax	30.0 a
4. Untreated control – water dip (without wax)	16.0 a
5. Standard treated control – Fungazil (500 ppm imazalil) (without wax)	6.0 b
6. Yeast antagonist in total loss application (without wax)	12.0 ab

^a Values represent the means of 5 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.3 (Trial 2). Percentage inhibition of natural green mould infections on waxed and unwaxed navel oranges that were stored under simulated shipping conditions at 4.5°C for 4-10 weeks after packhouse treatments with a yeast antagonist and the standard 500 ppm imazalil sulphate

Treatments	% Decay ^a
	Navels (4.5°C)
1. Untreated control – wax application	20.0 a
2. Standard treated control – Fungazil (500 ppm imazalil) + wax	5.0 b
3. Yeast antagonist in total loss application + wax	16.6 ab
4. Untreated control – water dip (without wax)	21.7 a
5. Standard treated control – Fungazil (500 ppm imazalil) (without wax)	5.0 b
6. Yeast antagonist in total loss application (without wax)	20.0 a

^a Values represent the means of 4 replicates of 15 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.4 (Trial 2). Percentage inhibition of natural green mould infections on waxed and unwaxed navel oranges that were stored under simulated shipping conditions at 10°C for 4-10 weeks after packhouse treatments with a yeast antagonist and the standard 500 ppm imazalil sulphate

Treatments	% Decay ^a
	Navels (10°C)
1. Untreated control – wax application	21.7 a
2. Standard treated control – Fungazil (500 ppm imazalil) + wax	8.3 b
3. Yeast antagonist in total loss application + wax	21.7 a
4. Untreated control – water dip (without wax)	21.7 a
5. Standard treated control – Fungazil (500 ppm imazalil) (without wax)	5.0 b
6. Yeast antagonist in total loss application (without wax)	18.3 a

^a Values represent the means of 4 replicates of 15 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.5 (Trial 2). Percentage inhibition of natural green mould infections on waxed and unwaxed Empress mandarins that were stored under simulated shipping conditions at 4.5°C for 4-10 weeks after packhouse treatments with a yeast antagonist and the standard 500 ppm imazalil sulphate

Treatments	% Decay ^a
	Mandarins (4.5°C)
1. Untreated control – wax application	27.5 a
2. Standard treated control – Fungazil (500 ppm imazalil) + wax	6.3 b
3. Yeast antagonist in total loss application + wax	27.5 a
4. Untreated control – water dip (without wax)	67.5 a
5. Standard treated control – Fungazil (500 ppm imazalil) (without wax)	27.5 b
6. Yeast antagonist in total loss application (without wax)	55.0 a

^a Values represent the means of 4 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.6 (Trial 2). Percentage inhibition of natural green mould infections on waxed and unwaxed Empress mandarins that were stored under simulated shipping conditions at 10°C for 4-10 weeks after packhouse treatments with a yeast antagonist and the standard 500 ppm imazalil sulphate

Treatments	% Decay ^a
	Mandarins (10°C)
1. Untreated control – wax application	45.0 a
2. Standard treated control – Fungazil (500 ppm imazalil) + wax	12.5 b
3. Yeast antagonist in total loss application + wax	37.5 ab
4. Untreated control – water dip (without wax)	50.0 a
5. Standard treated control – Fungazil (500 ppm imazalil) (without wax)	15.0 b
6. Yeast antagonist in total loss application (without wax)	47.5 a

^a Values represent the means of 4 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Conclusions and discussion

Plant Health Products (Pty) Ltd. submitted the antagonist to CRI for screening in a simulated packhouse total loss brush-on application against postharvest pathogen infection, for registration for commercial use as a possible stand-alone treatment on export citrus fruit in the Southern African citrus industry.

The above results demonstrated that the yeast did not provide any significant level of control in any of the trials. Significant statistical differences were, however, observed in natural *P. digitatum* infection between untreated and yeast antagonist-treated fruit and the standard recommended Fungazil sulphate 750 WG-treated fruit on the three citrus cultivars after simulated long storage shipping conditions at 4.5 and 10°C. No clear trends in infections between **waxed and unwaxed** fruit were observed.

In spite of a large volume of research published about postharvest biocontrol of citrus rots, the commercial use of these products was and remains limited and accounts for only a very small fraction of the potential market (Palou et al., 2008). As discussed in several reviews, the main shortcoming of the use of postharvest biocontrol products has been inconsistency in their performance, especially when used as a stand-alone product to replace synthetic fungicides (Droby et al., 2001; Wisniewski et al., 2001).

Successful commercial control of postharvest diseases of fruits must be extremely efficient, in the range of 95-98%, unlike the control of tree, field crop or soilborne diseases. Consistent performance to such levels of control cannot presently be achieved by alternatives to fungicides as stand-alone treatments. Strategies where alternatives to fungicides should be combined and effective integrated control systems need to be investigated (Palou et al., 2008).

In previous evaluations with the PHP yeast, it was applied in dip treatment (Mike Morris, personal communication), which would not be practicable on commercial scale as these high-volume dip-baths are infrequently replaced. In this trial, the yeast was applied as a total-loss spray-on application, which might have negatively influenced the performance of the yeast through reduced deposition levels and/or penetration of wounds. Based on previously reported levels of performance (Mike Morris, personal communication), it is recommended that the yeast antagonist from Plant Health Products (Pty) Ltd. needs to be screened further to improve application methods and possibly for incorporation into an effective integrated control strategy for postharvest disease control on citrus fruit. Further screening with pathogen- and antagonist-inoculated fruit could also be conducted.

Future research

The screening of biological control agents, against postharvest pathogen infections, and possible strategies for the incorporation of such compounds in integrated postharvest disease control treatments, will be conducted on an ongoing basis, when such compounds become available.

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Trial 3. The evaluation of a new 500 EC formulation of imazalil against post-harvest diseases for the purpose of registration (October 2008)

Introduction

A sample of imazalil sulphate 750 WSG (**Batch Nr. R1753**) was submitted to Citrus Research International (CRI) by Volcano Agrosience (Arysta LifeScience Group) for evaluation of efficacy against post-harvest infection of citrus. The Arysta imazalil sulphate was screened against the post-harvest citrus pathogen *Penicillium digitatum* (green mould) to determine the efficacy of the product in inhibiting infection caused by this pathogen.

Materials and methods

An *in vivo* evaluation of the Arysta imazalil sulphate was conducted against the post-harvest citrus pathogen *Penicillium digitatum* (green mould) to determine the efficacy of the product in inhibiting infection caused by

this pathogen. This product was compared with the standard Fungazil sulphate 750 WSP (Janssen Pharmaceutica) at the recommended commercial rate of 67 g/100 l giving a treatment concentration of 500 ppm imazalil. The Arysta imazalil sulphate was evaluated at the rates of 250 g/kg ($\frac{1}{2}x$), 500 g/kg (x) and 1000 g/kg (2x).

Untreated navel and Valencia oranges (Crocodile Valley Estate) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. All the fruit was surface sterilised by washing on the CRI experimental packline in the recirculating spray-on brush washing system with a suitable quaternary ammonium compound for 2 minutes. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation.

The fruit was divided into 3 replicates of 20 fruit per treatment for the dip treatments.

A spore suspension of *P. digitatum* was made up by suspending the spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were spectrophotometrically adjusted to a concentration of 1×10^6 spores/ml (Eckert et al., 1986; Morris et al., 1978).

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette.

All the treatments, with the relevant chemical being evaluated, were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse.

The inoculated fruit was incubated for 4 hours at $\pm 23^\circ\text{C}$ (to simulate a 4-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated. Each treatment was immersed in the water bath for 3 minutes.

Treatments

1. Untreated control (*P. digitatum*) – water dip.
2. Treated control – Fungazil WG (500 g/kg).
3. Arysta imazalil – 250 g/kg ($\frac{1}{2}x$).
4. Arysta imazalil – 500 g/kg (1x).
5. Arysta imazalil – 1000 g/kg (2x).

Two trials were conducted with Valencia oranges and one trial with navel oranges. All concentrations designated g/kg refer to the a.i. of imazalil. After treatment, the fruit was incubated in paper packets at $\pm 23^\circ\text{C}$ for 7 days or until such time as the untreated controls exhibited sufficient green mould. The treatments were then evaluated by counting the number of infected wounds per treatment and the results recorded as percentage decay.

Results

The results in Tables 4.5.2.1-3 below indicate that the Arysta imazalil sulphate 750 WSG submitted by Volcano Agrosience demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard recommended Fungazil sulphate 750 WSP.

Table 4.5.2.1 (Trial 3). The effect of Arysta imazalil on *P. digitatum* infections on Valencia oranges, applied in a hot water dip treatment (40°C) and compared to the standard imazalil sulphate 750 WG.

Valencias 1	
Treatments	% Decay^a
1. Untreated Control (water dip)	100.0 a
2. Treated Control – 500 g/kg Fungazil	0.0 b
3. Arysta imazalil – 250 g/kg	1.7 b
4. Arysta imazalil – 500 g/kg	0.0 b
5. Arysta imazalil – 1000 g/kg	0.0 b

^a Values represent the means of 3 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Table 4.5.2.2 (Trial 3). The effect of Arysta imazalil on *P. digitatum* infections on Valencia oranges, applied in a hot water dip treatment (40°C) and compared to the standard imazalil sulphate 750 WG.

Valencias 2	
Treatments	% Decay ^a
1. Untreated Control (water dip)	100.0 a
2. Treated Control – 500 g/kg Fungazil	0.0 b
3. Arysta imazalil – 250 g/kg	0.0 b
4. Arysta imazalil – 500 g/kg	0.0 b
5. Arysta imazalil –1000 g/kg	0.0 b

^a Values represent the means of 3 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; P≤ 0.05).

Table 4.5.2.3 (Trial 3). The effect of Arysta imazalil on *P. digitatum* infections on navel oranges, applied in a hot water dip treatment (40°C) and compared to the standard imazalil sulphate 750 WG.

Navels	
Treatments	% Decay ^a
1. Untreated Control (water dip)	100.0 a
2. Treated Control – 500 g/kg Fungazil	0.0 b
3. Arysta imazalil – 250 g/kg	6.7 b
4. Arysta imazalil – 500 g/kg	0.0 b
5. Arysta imazalil –1000 g/kg	0.0 b

^a Values represent the means of 3 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; P≤ 0.05).

Conclusion

The imazalil sulphate 750 WSG submitted by Volcano Agrosience demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard, recommended Fungazil sulphate 750 WSP. CRI recommends the registration of this formulation of imazalil at a dosage rate of 67 g/100l (500 pm) in a packhouse dip treatment.

Future research

No specific research on generic formulations of the current postharvest citrus fungicides is planned. However, when a chemical company requests the evaluation of a generic formulation of one of these compounds for registration purposes, the product/s will be screened on a contract basis.

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Trial 4. The evaluation of two Kannar organic products in a citrus packhouse dumptank washing system as a sanitising agents against post-harvest disease, and *in vivo* against post harvest infections (December 2008)

Introduction

Two organic sanitiser/biocide compounds, Kannar OrganoKare KOR 100 and KannarKare KE 200 (from Kannar Earth Science) were evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The products were compared with the standard recommended Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation. The two products were also evaluated for fungicidal properties against *P. digitatum* infection in fruit and were compared with the standard recommended imazalil sulphate 750 WSP.

Materials and methods

Evaluation as sanitising agent. A spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10^8 spores/ml. Good, sound, untreated Valencia oranges from Crocodile Valley Citrus Co. were obtained in bulk. All the fruit was surface sterilised by washing on the CRI experimental packline in the recirculating spray on brush washing system with a suitable quaternary ammonium compound for 2 minutes. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation. The fruit was divided into lots of 10 fruit per treatment

A clean water dump tank washing system in a packhouse was simulated. The dump tank water was maintained at ambient temperature for this trial. The clean, surface sterilised Valencia oranges were injured by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured three times, twice equatorially on opposite sides and once at the stylar end of the fruit, giving a total of 30 injury sites per treatment.

The 10 fruit of the untreated control treatment were dipped in water in the clean dump tank (10 l volume) for 3 minutes. The system was then seeded with the 1×10^8 spores/ml concentration, giving a final concentration of 1×10^4 spores of *P. digitatum*. Untreated, injured fruit dipped in the contaminated water for 3 minutes (untreated, inoculated control). The contaminated dump tank was then sanitised with concentrations both Kannar products at 10 ml and 100 ml per 10 l for 10 minutes. Injured fruit was dipped in each of the "sanitised" systems for 3 minutes. In a similar manner, injured fruit was also exposed to the standard recommended quaternary ammonium compound Sporekill at concentrations of 1 l and 2 l/1000 l for 3 minutes.

To summarise, treatments involved the following:

1. Untreated control - injured fruit dipped in clean water.
2. Untreated, inoculated control – injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml.
3. Injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml + 10 ml Kannar OrganoKare.
4. Injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml + 100 ml Kannar OrganoKare.
5. Injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml + 10 ml KannarKare.
6. Injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml + 100 ml KannarKare.
7. Untreated Control as in 1.
8. Untreated, inoculated control as in 2.
9. Injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml + 1 l/1000 l Sporekill.
10. Injured fruit dipped in water + 1×10^4 *P. digitatum* spores / ml + 2 l/1000 l Sporekill.

All the treated fruit were placed in paper packets and incubated for 7-10 days at 23°C. After incubation the fruit was evaluated for decay and the results were recorded as percentage decay (number of infected wounds).

In vivo evaluation for fungicidal properties. For this aspect of the experiment, Valencia oranges (obtained and pre-treated as above) were wounded with a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides giving 20 infection sites per treatment. Each fruit was then infected with the pathogen by applying 35 μ l of a 1×10^6 *P. digitatum* spore suspension to each injury site using a micropipette.

After 4 hours incubation at $\pm 23^\circ\text{C}$, the following treatments were conducted:

1. Untreated control (clean water).
2. Treated control 500 ppm imazalil sulphate WSP.
3. 100 ppm Kannar OrganoKare.
4. 200 ppm Kannar OrganoKare.
5. 500 ppm Kannar OrganoKare.
6. 100 ppm KannarKare.
7. 200 ppm KannarKare.
8. 500 ppm KannarKare.

Each treatment involved an immersion in a water bath at ambient temperature for 3 minutes. After treatment, the fruit was incubated in paper packets at 20°C for 7-10 days or until such time as the controls had sufficiently rotted. The treatments were then evaluated and the results recorded as percentage decay (number of infected wounds).

Results

Evaluation as sanitising agent. All the fruit in the Kannar-treated dump tank were infected with green mould, indicating that the two Kannar products did not effectively sanitise a citrus packhouse simulated dump tank washing system at the tested concentrations (Table 4.5.2.1). The standard recommended quaternary ammonium compound Sporekill at 1 l and 2 l/1000 l reduced green mould decay incidence to 20% and 16%, respectively.

Table 4.5.2.1 (Trial 4). The percentage decay incidence recorded on Valencia fruit that were injured and dip-treated in a simulated dump tank system with clean water, or water with 1×10^4 *P. digitatum* spores that had been treated with different concentrations of Kannar OrganoKare, KannarKare and Sporekill.

Treatments	% Decay
1. Untreated control	Nil
2. Inoculated control	85
3. 10 ml OrganoKare	100
4. 100 ml OrganoKare	100
5. 10 ml KannarKare	100
6. 100 ml KannarKare	100
7. Untreated control	Nil
8. Inoculated control	90
9. Sporekill 1.0 l / 1000 l	20
10. Sporekill 2.0 l / 1000 l	16

In vivo evaluation for fungicidal properties. The results (Table 4.5.2.2) indicate that both Kannar formulations did not inhibit infections by the pathogen *P. digitatum* at the concentrations of 100, 200 and 500 ppm compared to the standard recommended imazalil sulphate WSP. This demonstrates that neither of the Kannar formulations exhibit any fungicidal properties.

Table 4.5.2.2. The percentage decay incidence recorded on Valencia oranges that were injured and inoculated with $35 \mu\text{l}$ of a 1×10^6 *P. digitatum* spore suspension to each injury site and subsequently treated with clean water, 500 ppm imazalil sulphate WSP or the two Kannar formulations (100, 200 and 500 ppm).

Treatments	% Decay
1. Untreated Control (clean water)	100
2. Treated control 500 ppm imazalil sulphate WSP	Nil
3. 100ppm OrganoKare	100
4. 200ppm OrganoKare	100
5. 500ppm OrganoKare	100
6. 100ppm KannarKare	100
7. 200ppm KannarKare	100
8. 500ppm KannarKare	100

Conclusion

The organic sanitiser/biocide compounds, OrganoKare and KannarKare did not effectively sanitise a simulated citrus packhouse dump tank washing system at concentrations of 10 ml and 100 ml compared to the standard recommended quaternary ammonium compound Sporekill. Moreover, OrganoKare and KannarKare did not inhibit infection by the post-harvest citrus pathogen *P. digitatum* thereby not demonstrating any fungicidal properties. These trials will be repeated with two reformulated Kannar organic compounds.

Future Research

Pilot trials on two reformulated Kannar organic products will be conducted. If the products demonstrate any significant activity as effective sanitisers or indicate fungicidal properties against postharvest infections on citrus fruit, further larger scale trials will be conducted on a contract basis.

4.5.3 **PROGRESS REPORT: The screening of potential alternative products as replacements for 2,4-D as a post-harvest treatment in citrus packhouses**
Experiment 754 (April 2008 – March 2009) by K.H.Lesar (CRI)

Opsomming

Die verdere evaluasie van die plantgroeireguleerder, Retain, en die evaluasie van 'n nuwe sintetiese ouksien, Maxim, is op Valencia lemoene uitgevoer. Die twee produkte is in water doopbehandelings aangewend vir blomkelk-behoud op Valencias na gesimuleerde verskeping. Goeie blomkelk-behoud deur Retain teen konsentrasies van 250 en 500 dpm, in vergelyking met die standaard aanbevole 2,4-D (Deccomone), is waargeneem. 'n Laer mate van blomkelk-behoud deur Maxim, teen die drie konsentrasies, in vergelyking met die standaard aanbevole Deccomone, is aangeteken.

Summary

The repeat evaluation of the plant growth regulator Retain, and the screening of a new synthetic auxin, Maxim, was conducted on Valencia oranges. The products were applied to Valencia oranges at different concentrations in dip treatments and evaluated for calyx retention on the fruit after simulated shipping. Fairly good calyx retention by Retain at both concentrations of 250 and 500 ppm was recorded compared to the standard Deccomone. Conversely, however, low rates of calyx retention were recorded by Maxim at the three concentrations, compared to the standard recommended Deccomone.

Introduction

Postharvest disease of citrus fruit can cause significant losses when environmental and fruit conditions are conducive to pathogen infections and disease development. *Diplodia* stem-end rot and anthracnose are two major decays related to ethylene degreening and subsequent inopportune calyx abscission due to the harsh effect of degreening on early harvested fruit. High temperatures and humidity in certain areas often result in early internal fruit maturation and often this early fruit colour is not fully developed. Early harvested fruit subjected to ethylene degreening. This treatment and the conditions experienced in the degreening chamber is harsh on the fruit, thereby promoting increased ageing of the fruit, premature calyx abscission (Fig. 4.5.3.2), as ethylene stimulates the formation of an abscission zone between the fruit and the button, and consequent increased risk of infection by these latent citrus pathogens. Button abscission on fruit serves as a port of entry for these pathogens and then subsequent infections after packing and in transit to the market place (Zhang, et al., 2002).



Figure 4.5.3.1. Live retained calyx.



Figure 4.5.3.2. Premature calyx abscission.

The use of plant-growth regulators, notably 2,4-D has been recommended in South Africa for citrus as early as 1953 especially for the control of *Diplodia* and anthracnose decay. The principle effect is that the button is treated preventing button abscission (Fig.1) and thereby reducing the risk of infection by these two pathogens (Kriel et al., 1953).

Various communications between the Citrus Growers Association, Citrus Research International and the European Union residue committee during the mid-portion of the 2003 South African citrus season led to a harmonised EU MRL of 1.0 ppm for 2,4-D being considered. In the interim, the UK set a national MRL at 1.0 ppm, but the other member states have retained the 0.05 ppm MRL until a national MRL of 1.0 ppm is set.

In the eventuality of 2,4-D being discontinued as a post-harvest treatment in the not too distant future, there is currently no other alternative product registered as a post-harvest treatment on citrus for the preservation of fruit buttons (calyx).

In the eventuality of 2,4-D being discontinued as a post-harvest treatment, there is currently no other alternative product registered as a post-harvest treatment on citrus for the preservation of fruit buttons. Therefore it is imperative to evaluate new safe products that could prevent calyx abscission given the distance of the South African fruit from the markets. Button abscission on citrus fruit could possibly expose the fruit to infestation by one or more of the latent citrus pathogens, viz. Anthracnose, *Diplodia* and *Alternaria*, as was evident in the 2003 production season. Button abscission also leads to scruffy fruit arriving at the market.

In these trials the two plant growth regulators (PGR's), Retain (repeat evaluation) and Maxim were evaluated for calyx retention. Retain (aminoethoxyvinylglycine) and Maxim, a synthetic auxin (3,5,6 trichloro-2-pyridiloxycetic acid) were compared with the standard 2,4-D sodium salt formulation, Deccomone.

Materials and methods

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Company) were obtained in bulk. For the purpose of this trial, blemish-free, sound fruit was selected and randomised. The fruit was washed and surface sterilised on the packline at CRI in a high-pressure spray using the sanitising agent Prasin (a quaternary ammonium compound). The fruit was then dried in the drying tunnel on the packline. All the fruit for this trial was selected with live, firm green buttons (calyxes). All the treatments were immersed in water dip solutions for 3 minutes. Each treatment consisted of 4 replicates of 12 fruit each.

Treatments:

1. Untreated control – water dip.
2. Treated control - 250 ppm 2,4-D (Deccomone).
3. Treated control - 500 ppm 2,4-D (Deccomone).
4. Retain – 250 ppm.
5. Retain – 500 ppm.
6. Maxim – 20 ppm.
7. Maxim – 40 ppm.
8. Maxim – 60 ppm.

After dipping, the treated fruit was allowed to dry overnight and then all the treatments were placed into paper packets and stored under simulated shipping conditions: 1 week at 20°C; 6 weeks at 4.5°C; 1 week at 20°C. After the simulated shipping period, the treatments were evaluated and the results recorded as percentage button retention. The fruit was also evaluated for stem-end infections, caused by any of the latent pathogens.

Results

Retain demonstrated fairly good calyx retention in a repeat evaluation on Valencia oranges at both concentrations, compared to the standard recommended Deccomone, after simulated shipping storage at 4.5°C. Maxim, on the other hand, did not attain good button retention at all three concentrations, compared to Deccomone. Retain achieved 79.15 and 83.3% button retention at concentrations 250 and 500 ppm respectively, compared to 83.3 and 95.85% by the standard Deccomone at the same concentrations. Maxim only attained low rates of calyx retention of 14.57, 16.67 and 22.92% at concentrations of 20, 40 and 60 ppm, respectively (Table 4.5.3.1). No stem-end infections by any of the latent pathogens were observed on the fruit evaluated.

Table 4.5.3.1 (Trial 4). Mean percentage button retention on Valencia oranges following dip-treatment with various concentrations of Deccomone, Retain and Maxime after 8-week storage under simulated shipping conditions.

Treatments	Mean Calyx Retention (%) ^a
1. Untreated Control	22.92 c
2. Treated Control 250 ppm 2,4-D (Deccomone)	83.32 b
3. Treated Control 500 ppm 2,4-D (Deccomone)	95.85 a
4. Retain 250 ppm	79.15 b
5. Retain 500 ppm	83.30 b

6. Maxim 20 ppm	14.57 c
7. Maxim 40 ppm	16.67 c
8. Maxim 60 ppm	22.92 c

^a Values represent the means of 4 replicates of 12 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \leq 0.05$).

Conclusion

The ongoing screening of these products as possible alternatives to the 2,4-D has once again revealed the product (Retain) with good activity against calyx retention, compared to the standard 2,4-D. Maxim, on the other hand, did not attain good button retention at all three concentrations screened, compared to Deccomone.

Future research

The screening of Maxim will need to be repeated at higher rates and on different citrus cultivars and the ongoing screening of other new products will continue.

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4.5.4 PROGRESS REPORT: The synergistic use of GRAS chemicals for citrus post-harvest disease control

Experiment 907 (April 2008 – March 2009) by K.H.Lesar (CRI)

Opsomming

Die effektiwiteit van natrium bikarbonaat is, teen konsentrasies van 1 en 2%, alleen en in kombinasie met verlaagde konsentrasies van imazalil in 'n gesimuleerde warmwaterbad behandeling teen imazalil sensitiewe en bestande *P. digitatum* (groenskimmel) infeksies ge-evalueer. Navel lemoene is gewond en met *P. digitatum* (sensitiewe and bestande spore) ge-inokuleer, 8 ure voor 'n 1 en 2% natrium bikarbonaat warmwater doopbehandeling alleen en in kombinasie met 250 en 500 dpm imazalil sulfaat. Die resultate het nie goeie beheer van na-oes infeksie deur imazalil-sensitiewe en -bestande *P. digitatum* (groenskimmel) spore deur albei 1 en 2% natrium bikarbonaat in hierdie gesimuleerde warmwaterbad proef gewys nie. Die hoër konsentrasie het nietemin 'n mate van statistiese betekenisvolle beheer getoon. Die 250 en 500 dpm imazalil sulfaat oplossings het albei 'n pH van 3.6. Natrium bikarbonaat het die oplossings teen 'n pH van om en by 8.0 gebuffer, afgesien van die byvoeging van imazalil of nie. Die titrasie vir imazalil konsentrasie het gewys dat albei imazalil konsentrasies, in kombinasie met 1% natrium bikarbonaat, nie beïnvloed is nie, maar wel só in kombinasie met 2% natrium bikarbonaat.

Summary

The efficacy of sodium bicarbonate was evaluated at concentrations of 1 and 2% alone and in combination with reduced concentrations of imazalil in a simulated hot water dip treatment against postharvest infections by imazalil-sensitive and -resistant *P. digitatum* (green mould) spores. Navel oranges were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 8 hours before dip-treatment in hot water (40°C) with 1 and 2% sodium bicarbonate, alone and in combination with 250 and 500 ppm imazalil SO₄. The results recorded in this simulated hot water bath trial did not demonstrate good control of postharvest infections by imazalil-sensitive and -resistant *P. digitatum* (green mould) spores by both 1 and 2% sodium bicarbonate, although the higher concentration exhibited some statistically significant level of control. The 250 and 500 ppm imazalil sulphate solutions each had a pH of 3.6, while sodium bicarbonate buffered solutions at a pH of around 8.0, irrespective of whether imazalil was added or not. The titration results indicate that the both the

concentrations of imazalil were not affected by the sodium carbonate at 1%. Both the imazalil concentrations were, however, affected, in combination with 2% sodium bicarbonate.

Introduction

Green mould (*Penicillium digitatum* Saccardo) and blue mould (*Penicillium italicum* Wehmer) are two of the most economically important post-harvest diseases of citrus, responsible for severe economic losses worldwide (Bancroft et al., 1984). The primary mode of infection by these pathogens is through wounds on fruit inflicted during harvest and subsequent handling, and these infections must be eradicated to achieve acceptable levels of control (Eckert and Brown, 1986). Currently, post-harvest citrus decay in South Africa is controlled by application of the post-harvest fungicides thiabendazole, imazalil, guazatine and sodium ortho-phenylphenate (SOPP).



***P. digitatum* (green mould)**

Consumers are concerned about chemical products that are applied to perishable commodities during post-harvest treatments. During the last 10-15 years there has been continuous pressure exerted from various quarters, political and regulatory, health groups and certain markets, to discontinue the use of fungicides or decrease the residue levels of these compounds on citrus fruit. Eventually, this might result in discontinuation of many chemical treatments for the control of post-harvest diseases. Without the use of these treatments, producers and suppliers of many commodities could face serious threats of increased spoilage and economic losses. An additional practical issue that has placed the continued use of fungicides at risk is the problem of resistance development in pathogen populations to post-harvest fungicides (Eckert et al., 1994).

It has thus become necessary to screen new chemicals, such as GRAS compounds for possible fungicidal properties against the citrus pathogens in order to find new, safe compounds that could assist in overall decay control as well as preventing or delaying the development of fungicide resistance.

The GRAS compounds offer much promise in post-harvest technology. Bicarbonates and carbonates are common food additives and they have been shown to control many plant pathogens. Potassium bicarbonate has been shown to reduce post-harvest decay development on bell pepper fruits (Fallik et al., 1997). Brief immersion of citrus fruit in solutions of sodium bicarbonate or sodium carbonate has been shown to reduce the subsequent incidence of post-harvest green mould (Smilanick et al., 1999). This practice is inexpensive, poses a minimum risk of injury to the fruit, and can be a useful tool in the management of fungicide resistant isolates which have become particularly problematic (Eckert et al., 1994). Its effectiveness compares favourably with that of fungicides employed for this purpose and in general is superior to other treatments that are proposed as alternatives to fungicides, such as heat (Houck, 1967) or biological control (Smilanick and Denis-Arrue, 1992). Sodium carbonate controls green mould even when applied long after inoculation. The incidence of infections from wounds on lemons inoculated 48 h before treatment was reduced more than 90% (Smilanick et al., 1995).

The aim of this study was to evaluate the efficacy of sodium bicarbonate at 1 and 2% alone and in combination with reduced concentrations of imazalil in a simulated hot water dip treatment against

postharvest infections by imazalil sensitive and resistant *P. digitatum* (green mould) spores. An important issue when using imazalil and sodium bicarbonate together in a mixture is the role of pH and the efficacy of imazalil. Imazalil functions efficiently at a pH of 7.0, therefore pH readings were also taken of the different combinations during this study. The management of the concentration of imazalil used alone in the bath is measured by titration. The affect of the titration of imazalil concentration, in combination with sodium bicarbonate, was evaluated.



Hot Water Fungicide Bath

Materials and methods

Untreated navel oranges (Crocodile Valley Citrus Company) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was divided into 3 replicates of 20 fruit per treatment, washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was allowed to dry prior to inoculation.

Spore suspensions of *P. digitatum* (sensitive and resistant spores) were made up by suspending the spores in sterile deionised water containing the wetting agent, Tween 20. Both spore suspensions were spectrophotometrically adjusted to an absorbance of 0.1 at 420 nm, which relates to a concentration of 1×10^6 spores/ml (Morris and Nicholls, 1978). This concentration is recommended for in vivo evaluations of post-harvest treatments against these pathogens (Eckert and Brown, 1986).

Wounding of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides and drop-inoculated with 35 μ l of spore suspension to each injury site, thereby giving a total of 120 inoculation sites per pathogen \times treatment combination. In order to simulate a short delay after harvesting prior to packhouse treatments, inoculated fruit were incubated at 23°C for 8 hr until further treatment.

The infected navel oranges were treated in a hot water bath at 40°C, thereby simulating a heated fungicide bath in the packhouse. The 20 inoculated fruit involved in each pathogen \times treatment \times repetition combination were immersed in a hot water bath containing each chemical for 3 minutes. After treatment, the fruit was incubated in paper packets at 23°C for 7-10 days. The treatments were evaluated and the results recorded as percentage infected green mould wounds.

The following treatments were conducted for both imazalil-sensitive and imazalil-resistant spores:

1. Untreated control – water dip.
2. Treated control – 250 ppm imazalil SO₄.

3. Treated control – 500 ppm imazalil SO₄ (standard recommendation).
4. 1% NaHCO₃.
5. 2% NaHCO₃.
6. 1% NaHCO₃ plus 250 ppm imazalil SO₄.
7. 1% NaHCO₃ plus 500 ppm imazalil SO₄.
8. 2% NaHCO₃ plus 250 ppm imazalil SO₄.
9. 2% NaHCO₃ plus 500 ppm imazalil SO₄.

After treatment, the fruit was incubated in paper packets at 23°C for 7-10 days, or until such time as the controls had grown. The treatments were evaluated and the results recorded as percentage infected wounds. The pH readings of imazalil at the two treatment concentrations and in combination with sodium carbonate were recorded. These mixtures of imazalil alone and in combination with sodium carbonate were titrated and recorded.

Results

The results recorded in this simulated hot water bath trial did not demonstrate good control of postharvest infections by imazalil-sensitive and -resistant *P. digitatum* (green mould) spores by both 1 and 2% sodium bicarbonate, although the higher concentration exhibited some statistically significant level of control. There was no significant difference between the 1 and 2% sodium bicarbonate in the control of decay by the imazalil-sensitive spores. The same applied to the 1 and 2% sodium bicarbonate, in the control of decay by the imazalil-resistant spores, although the 2% concentration yielded significantly lower levels of decay. Decay levels caused by imazalil-sensitive spores were significantly lower when the standard and reduced rate of imazalil was added to these treatments. There was also no statistically significant difference in the control of decay of infections by imazalil-resistant spores between the reduced rate of imazalil, the lower rate of sodium bicarbonate and these two applied in combination, at both 1 and 2% (Table 4.5.4.1).

The 250 and 500 ppm imazalil sulphate solutions each had a pH of 3.6, while sodium bicarbonate buffered solutions at a pH of around 8.0, irrespective of whether imazalil was added or not (Table 4.5.4.2). The titration results indicate that the concentration of imazalil was not affected by the sodium carbonate at 1% in combination with imazalil at 250 and 500 ppm, compared to the concentration of imazalil alone at the same concentrations. Both the imazalil concentrations were, however, affected, in combination with 2% sodium bicarbonate (Table 4.5.4.3).

Table 4.5.4.1 (Trial 4). Percentage *P. digitatum* infected wounds on navel oranges that were wounded and inoculated with *P. digitatum* (sensitive and resistant spores) 8 hours before dip-treatment in hot water (40°C) with 1 and 2% sodium bicarbonate, alone and in combination with 250 and 500 ppm imazalil SO₄.

Treatments	Infected wounds (%) ^a	
	Navels	
	Imazalil - sensitive spores	Imazalil - resistant spores
1. Untreated control	96.7 a	100.0 a
2. Treated control- 250 ppm imazalil SO ₄	13.3 c	93.3 ab
3. Treated control- 500 ppm imazalil SO ₄	0.0 c	53.3 cd
4. 1% NaHCO ₃	76.7 ab	90.0 ab
5. 2% NaHCO ₃	63.3 b	70.0 bcd
6. 1% NaHCO ₃ plus 250 ppm imazalil SO ₄	10.0 c	83.3 ab
7. 1% NaHCO ₃ plus 500 ppm imazalil SO ₄	0.0 c	46.7 d
8. 2% NaHCO ₃ plus 250 ppm imazalil SO ₄	6.7 c	76.7 abc
9. 2% NaHCO ₃ plus 500 ppm imazalil SO ₄	0.0 c	50.0 d

^a Values represent the means of 3 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; P ≤ 0.05).

Table 4.5.4.2 (Trial 4). pH readings of imazalil and sodium bicarbonate alone and in combination at the different concentrations.

Treatments	pH Readings
Imazalil 250 ppm	3.60
Imazalil 500 ppm	3.56
1% NaHCO ₃	8.22
2% NaHCO ₃	8.19
1% NaHCO ₃ plus 250 ppm imazalil SO ₄	8.03
1% NaHCO ₃ plus 500 ppm imazalil SO ₄	8.03
2% NaHCO ₃ plus 250 ppm imazalil SO ₄	8.18
2% NaHCO ₃ plus 500 ppm imazalil SO ₄	8.17

Table 4.5.4.3 (Trial 4). Titration results of imazalil and sodium bicarbonate alone and in combination at the different concentrations.

Treatments	Titration Results (ppm)
Imazalil 250 ppm	249.6
Imazalil 500 ppm	511.1
1% NaHCO ₃ plus 250 ppm imazalil SO ₄	249.6
1% NaHCO ₃ plus 500 ppm imazalil SO ₄	487.3
2% NaHCO ₃ plus 250 ppm imazalil SO ₄	202.1
2% NaHCO ₃ plus 500 ppm imazalil SO ₄	297.2

Conclusions

The decrease in infected wounds by imazalil alone (250 and 500 ppm) and imazalil in combination with sodium bicarbonate (1 and 2 %) was not significant. This did not indicate any synergism between imazalil and sodium bicarbonate.

The adjustment of the pH of imazalil from acidic to alkaline by sodium bicarbonate needs to be evaluated further on the effect of imazalil residue loading on the fruit.

The effect of the concentration of imazalil in a mixture with 2% sodium bicarbonate is significant enough to indicate a risk on the retention of an effective imazalil residue on the fruit, in such a mixture, thereby jeopardising the effective management of this important fungicide.

Future research

There are still too many unknowns as far as the combined use of these GRAS compounds and postharvest fungicides are concerned. These trials will need to be repeated and further trials will be conducted on different citrus cultivars together with the inclusion of other GRAS compounds, as other researchers have demonstrated the efficacy of GRAS compounds in controlling postharvest decay on citrus.

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4.5.5 **PROGRESS REPORT: Practical guidelines for good post-harvest handling of citrus fruit** Experiment 908 (April 2008 – March 2009) by K.H.Lesar (CRI)

Opsomming

’n Begrip van waar die na-oes sitrus siektes vandaan kom, hoe die siektes die vrugte besmet en die identifikasie van die verskillende soorte na-oes bederf, wat gewoonlik na pluk en pak van die uitvoervrugte ontwikkel, is noodsaaklik vir die beplanning van navorsing en die akkurate terugvoering na voorligting en uitvoerders te verseker. Die doel van hierdie verduidelikende riglyne is om die vruginspekteurs, in die sitruspakhuse en by inspeksie persele by beide die plaaslike en oorsee se hawens, te help om die verskeie siektes te identifiseer. Dit sal dan die akkurate terugvoering van informasie aan die plaaslike bemakingsagente verseker en sorg dat opvolg en regstellende stappe vroegetydig geneem word.

Summary

An understanding of where the postharvest citrus diseases originate, how they infect citrus fruit and identification of the different types of postharvest decay, which usually develop after harvesting and packing of the fruit for export, is fundamental to research planning and ensuring accurate feedback to extension and exporters alike. The purpose of these illustrated guidelines is to assist fruit inspectors in citrus packhouses and inspection depots at the local ports, and ports of arrival overseas, to correctly identify the various maladies, thereby ensuring the accurate feedback of information to the local marketing agents whereby timeous follow up and corrective action can be taken.

Introduction

The control of post-harvest citrus diseases occurs within a system, from A to Z i.e. from the orchard to the market. Within this system are a number of critical control points, which have to be managed efficiently so as to prevent waste from developing.

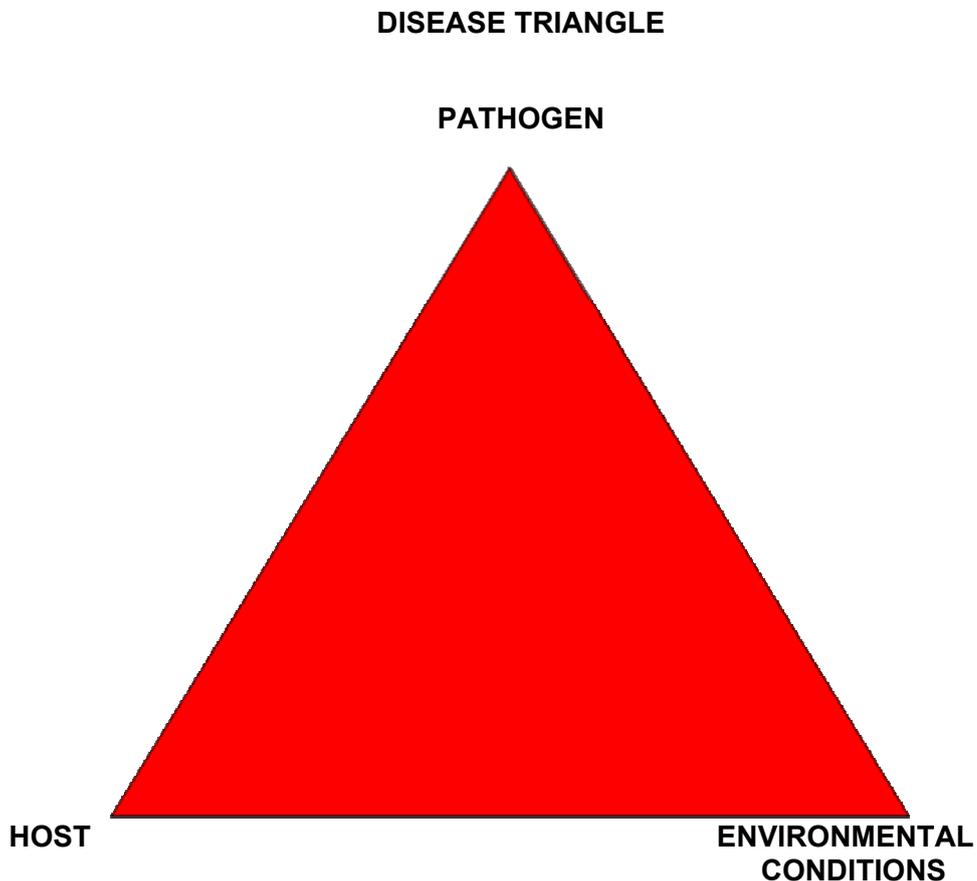
The first, and most important critical control point in this system is the **orchard**. This is where the postharvest pathogens survive and proliferate. Here the correct cultural practices need to be well managed in order to ensure the delivery of good, sound, healthy fruit to the citrus packhouse. If these cultural practices are diligently managed then “sick” (less vigorous) fruit will be delivered to the packhouse, and then even the most efficiently controlled procedures in the packhouse will often not be able to prevent waste from developing. Citrus packhouses are, far too often, blamed for the poor quality product and subsequent financial losses experienced in the markets. Packhouses can certainly contribute to the problems by not having adequate checks and balances in place. However, the problems start in the orchards, not in the packhouses. It is the producer’s duty to deliver a sound product to the packhouse and not a “sick” product and expect the packhouse to sort out the problems.

“The packhouse is not a hospital for “sick” fruit”

The postharvest citrus pathogens all survive and proliferate, under ideal conditions, in the orchard. The occurrence and severity of the diseases are determined by the virulence of the pathogen, the susceptibility of the host (fruit) and the environmental conditions. This is schematically indicated in the well known “Disease Triangle” (Fig. 4.5.5.1).

The three variables, the pathogen, the host (the fruit) and the environmental conditions will ultimately determine the incidence of disease development. However, if these variables are managed correctly the disease incidence can be maintained at a low to zero level. Two of these variables, the pathogen/s and the host can certainly be managed and controlled correctly by means of proper cultural practices. The pathogen

load can be maintained at a low level by reducing spore load, through effective sanitation by removing fallen sound fruit and decayed fruit from the orchard, removal of dead wood in trees, proper harvesting and handling procedures etc., thereby preventing a carry-over of a high spore load on the harvested fruit into the packhouses. The fruit on the other hand can be maintained in a vigorous state, treating the fruit as a perishable commodity with a minimum number of injuries to the fruit during harvest and during handling and transport to the packhouse, thereby ensuring the delivery of a healthy crop for treatment and packing and delivery in a sound condition to the markets. The third variable, environmental conditions cannot be controlled during the orchard phase.



POSTHARVEST DISEASES

The citrus industry has to contend with 12-14 different types of postharvest fungal diseases. A small percentage (23%) of the pathogens consistently contribute to major economic losses (80-90%) caused by the post-harvest pathogens in the industry (Christ, 1965 and 1966). The remaining proportion of postharvest pathogens contributes to economically important losses from time to time.

The major postharvest citrus diseases can be divided into two groups. These are the **wound** pathogens and the **latent** pathogens.

Descriptions and photos of the postharvest citrus diseases

1. Wound Pathogens

Green mould (*Penicillium digitatum*)

Infection begins through any injury to the rind as a water-soaked, soft area easily punctured by pressure of the finger. A wrinkly, pasty white mycelium appears and later olive-green powdery spore masses appear in the centre of the white mycelium (Fig. 4.5.5.2). In advance of the white band of mycelium, an indefinite band of water-soaked tissue with an ill-defined margin is found.

Infection takes place solely through wounds caused by insect attack and injuries caused during picking, transportation and packing. Incidence of green mould can be reduced by careful handling (to minimize

injuries), sanitation in orchard and packhouse (to minimize spore load in air and on the fruit), insect control, disinfection of fruit washing systems by the use of chlorine or the quaternary ammonium compounds, and by fungicide treatments in the packhouse.

Infection may also spread, in packed cartons of export fruit, from infected fruit to sound, uninjured fruit, causing “nests” of decay and soilage in the cartons (Fig. 4.5.5.1).



Figure 4.5.5.1. Infection spread and soilage.

Figure 4.5.5.2. White mycelium - leading edge of infection

Blue mould (*Penicillium italicum*)

Decay starts from injuries in the rind or spreads through contact from infected to sound fruit. At first, small water-soaked areas, softer than in the case of green mould, and enlarging more slowly, form on the rind surface. Blue spore masses appear sooner on the white mycelium than in the case of green mould, resulting in a narrower band of white mycelium surrounding the blue spore masses. Ahead of the white mycelium a water-soaked area is found, wider than in the case of green mould and with a well-defined margin. With age the blue spores change to a brownish-olive colour. Green mould often starts after blue mould and soon surrounds the area where blue mould started (Fig. 4.5.5.3). Where the two fungi are intermingled in the rind, a pinkish to reddish colour may be found under the surface. Blue spores may also be found inside the fruit.

Mode of infection and control measures are the same as for green mould. As with green mould, blue mould infection may also spread in packed cartons of export fruit from infected fruit to sound, uninjured fruit, causing “nests” of decay and soilage.



Figure 4.5.5.3. Blue and green mould.

Sour rot (*Geotrichum candidum*)

The initial symptoms of sour rot are similar to those of green and blue mould. Lesions first appear as water-soaked, light to dark yellow, slightly raised spots. The cuticle is more easily removed from the epidermis than it is in lesions formed by green mould and blue mould. Highly active extra-cellular enzymes produced by the sour rot fungus degrade the rind, segment walls and juice vesicles, causing the fruit to disintegrate into a slimy, watery mass. Spore-laden watery debris from infected fruit spread to healthy fruit in packed containers, causing nests of decay, hence the progressive, destructive nature of the fungus (Fig. 4.5.5.4). The sour odour associated with the advanced stages of sour rot attracts vinegar flies (*Drosophila* spp.), which can spread the fungus causing other injured fruit to become infected.

The fungus is widespread and is present in the soil of all citrus producing areas. The spores are spread to the fruit by dust and by water splashing from the soil onto low-hanging fruit. Injuries penetrating to the albedo (white portion of the peel) are necessary for infection to take place. Such injuries are most often caused by insect damage and by incorrect snap-picking. All varieties are susceptible, especially the mandarin hybrids.

Fruit become more susceptible to sour rot as they increase in maturity, when the moisture content of the rind is high and under conditions of high relative humidity. As the fungus develops most rapidly at temperatures above 27°C it is important to store susceptible fruit at low temperatures and to maintain the cold chain.



Figure 4.5.5.4. Sour Rot.

Trichoderma brown rot (*Trichoderma viride*)

Trichoderma viride caused a firm, pliable, leathery rot. At first the colour of the affected area is unchanged, but then turns to pinkish-brown (lemons and grapefruit), brown (oranges and Tangers), beige, beige-brown and finally dark-brown (lemons). The albedo becomes beige to beige-brown with a water-soaked appearance. In most cases the rot remains firm and leathery, but may become soft on occasion. The rot may spread evenly, but sometimes in streaks along the segments (especially in mandarin-type citrus fruit) similar to classical *Diplodia* stem-end rot symptoms. A white mycelium may appear in tufts, producing dark-green, and sometimes yellow, spore masses (now powdery and dry like blue and green mould). The decay, which has a characteristic coconut-like odour, is accompanied by browning of the veins on the segments but the pulp itself does not change in colour. It spreads from infected to sound fruit by contact, making good growth on wrappers and therefore often appears as nests of rotten fruit (Fig. 4.5.5.5).

The fungus is common soil inhabitant. Initial infection depends on injuries to the fruit rind. The occurrence of *Trichoderma* brown rot can be limited by not allowing fruit to come into contact with soil, not packing fruit that had fallen either to the ground during harvesting or to the packhouse floor, and by not packing over-mature fruit. Cooling fruit to below 10°C after packing effectively limits the development and spread of this rot.



Figure 4.5.5.5. *Trichoderma* brown rot.

2. Latent Pathogens

Anthracnose rot (*Colletotrichum gloeosporioides*)

Anthracnose rot may start from the stem- or stylar-end, from insect punctures or other injuries or anywhere on the fruit surface. The affected area may at first be brown to dark brown, and firm to semi-pliable. Later brick-coloured to orange spore masses may occur on the lesions (Fig. 4.5.5.6). The colour of the spore masses may fade when exposed to the air. The lesion may then appear as a soft, pliable dark-brown area with scattered dark-brown to black dots. Underneath the lesion the albedo (white part of the rind) becomes brown to grey with a sponge-like consistency (Fig. 4.5.5.7). The pulp dries out and is at first white to pink, later becoming grey to black. In some cases, e.g. in mandarin-type or Washington navel oranges, the affected areas have a silvery-grey appearance, later turning light brown (Figs. 4.5.5.8 & 9). The degreening of citrus fruit, especially navels, favours the expression of these symptoms.

This fungus occurs widespread in citrus orchards. Spores are produced on dry twigs and dead tissue from where they are spread by wind, rain and insects to the young fruitlets. The fungus penetrates the rind and then remains latent until conditions are favourable for further growth, e.g. over-maturity or injuries to the fruit. Occasionally young fruit still on the tree may be attacked by the fungus growing from infected, dying twigs into the fruit. All citrus species are susceptible but especially mandarin types.

The incidence of anthracnose rot may be reduced by cultural practices which result in vigorous trees and fruit of high vitality, by avoiding an accumulation of dry twigs in the tree, harvesting at optimum maturity and by avoiding long storage.



Figure 4.5.5.6. Anthracnose – typical brick-coloured to orange spore mass.



Figure 4.5.5.7. Anthracnose – light brown lesions.



Figure 4.5.5.8. Anthracnose – silvery gray lesions.
***Diplodia* stem-end rot (*Diplodia natalensis*)**

The first sign of *Diplodia* stem-end rot is an abnormal pliability around the stem-end. The area then becomes translucent and watery, and, depending on how rapidly the rot develops, may turn brown to dark brown and eventually dark olive-green to black (Fig. 4.5.5.9). The decay sometimes develops more rapidly on the rind corresponding to the divisions of segments, giving the impression of fingers of decay progressing down the fruit from the stem- to the stylar-end. When *Diplodia* rot develops rapidly, the whole rind becomes watery and slightly discoloured and the cuticle can be rubbed off easily. The whole fruit becomes very soft and when broken open, the segment walls are found to be dissolved leaving the segments of a similar appearance to canned segments. In this form, the rot can easily be confused with sour rot, except that sour rot results in a complete destruction of the pulp and rind. When *Diplodia* stem-end rot develops more slowly, the decay progresses much more rapidly down the core than in the pulp or rind and the stylar-end may show a brown discolouration long before the entire rind is affected. The fruit remains much firmer and, upon squeezing, an amber coloured sticky juice will appear at the stem-end. The juice vesicles sometimes become a greenish colour. A strong, sour to fermented odour can usually be detected.

The fungus forms spores on dead bark and twigs where they survive from season to season. During rainy weather the spores are washed onto the young fruitlets where the fungus penetrates the stem and button tissue. Normally no further development of the infection takes place, until after harvest when loosening of the button results in natural openings, through which the fungus then penetrates.

The incidence of *Diplodia* stem-end rot may be reduced by removing dead twigs and by avoiding conditions that will lead to abscission of the button such as injuries, over-maturity, degreening of fruit with ethylene, high temperature during transit of packed fruit and long storage.



Figure 4.5.5.9. Diplodia stem-end rot.

Phomopsis stem-end rot (*Phomopsis citri*)

The first sign of the disease is simply a pliableness around the stem-end with no initial discolouration. Thereafter a very slight off-colour and eventually a tan to brown or almost black colour develops. The infected tissue shrivels somewhat causing a shoulder or ridge to form between decayed and healthy tissue (Figs. 4.5.5.10 & 4.5.5.11). The infected tissue remains leathery and pliable and is not easily punctured by finger pressure. White surface growth is occasionally seen. The decay progresses more quickly down the core than in the rind or pulp but does not reach the stylar-end until two thirds of the external surface is decayed. The pulp becomes mushy but no discolouration develops. An unpleasant rancid odour develops.

Phomopsis completes its life cycle as a saprophyte on dead bark and twigs. Spores (conidia) are dispersed by rain and establish quiescent (latent) infections in necrotic tissue in and around the button or on the rind of the fruit. Further development of the infection takes place after harvest when loosening of the button results in natural openings, through which the fungus then penetrates.

The incidence of phomopsis stem-end rot may be reduced by cultural practices which result in vigorous trees and fruit of high vitality, by avoiding an accumulation of dry twigs in the tree, avoiding conditions that will lead to abscission of the button such as injuries, over-maturity, degreening of fruit with ethylene, high temperature during transit of packed fruit and long storage.



Figure 4.5.5.10. Ridge between decayed and healthy tissue



Figure 4.5.5.11. Shrivelled infected tissue

Alternaria rot (*Alternaria citri*)

Four general types of *Alternaria* rot can be distinguished: (1) a core rot; (2) a soft brown to black stem- or stylar-end rot; (3) a dry, black navel-end rot of Washington navel oranges; and (4) a brown to black rot developing from insect punctures or other injuries.

The core rot develops after the button starts abscising. A pinkish to light brown discolouration proceeds along the vascular tissues of the core and to some extent in the flavedo of the rind, without at first visibly affecting the exterior surface. A light-brown, pinkish-brown to pitch black rot develops in the centre of the fruit and at this stage the fruit can be easily crushed, although it may appear sound from the exterior. Later a soft brown to black stem-end rot may develop (Fig. 4.5.5.14).

The stem- or stylar-end rot starts as a gradual browning of the exterior and interior. At first the affected tissue is leathery and pliable, becoming quite soft. The internal rot advances only slightly faster than the external rot. The exterior colours are usually shades of brown (olive brown to chestnut brown) or may be iron grey to olivaceous black.

The navel-end rot of Washington navels takes the form of a dry, black internal discolouration accompanied sometimes by a brown external colour, usually to one side of the navel-end. The affected fruits normally have a deep orange to red rind colour.

A black or dark-brown rot caused by *Alternaria* may develop from insect punctures or other injuries and may develop extensively in the pulp (Figs. 4.5.5.12, 13 & 14).

The spores of the fungus infect the flower tissue and when the fruit is more mature, the fungus is already established in the button tissue, deep in the navel cavity of Washington navels and on the stylar scar – in most cases out of reach of fungicides. *Alternaria* rot may also develop from injuries to the rind, either caused mechanically or by insects.

Mature fruit and fruit of low vitality, produced under unfavourable weather conditions such as prolonged low temperatures or frost, dry, hot winds, low humidity or extreme heat, are very prone to attack by *Alternaria*, as well as fruit showing sunburn, or, in the case of lemons, affected by endoxerosis.

Control measures consist of cultural procedures that will produce fruit of high vitality, and the culling of fruit showing sunburn, cracks at navel or stylar-ends, signs of endoxerosis, e.g. fruit having high colour or no buttons.



Figure 4.5.5.12. Rind infection – injuries.



Figure 4.5.5.13. Rind infection – insect sting.



Figure 4.5.5.14. Internal black core rot and black rot from injuries on the rind.

Brown rot (*Phytophthora* spp.)

This is a very firm, leathery rot. The affected tissue does not cave in. The colour of the lesions vary from medium brown, drab to greenish brown on oranges, and yellowish brown to drab on lemons and grapefruit (Fig. 4.5.5.15). Infected rind often features islands of apparently normal rind. Under moist conditions, the whole fruit surface may be covered by a layer of white, fluffy mycelium, which quickly disintegrates on exposure to less humid conditions, revealing a brown, somewhat sticky rind surface. This decay is accompanied by a characteristic sweetly pungent, aromatic, rancid odour. The fungus spreads easily from infected to sound fruit and nests of infected fruit are commonly found in packed cartons of citrus fruit.

Phytophthora is present in the soil of all citrus producing areas. Infection of fruit takes place during rainy weather when spores are splashed onto the fruit from the soil surface or when fruit is in contact with wet soil. The incidence of *Phytophthora* brown rot can be reduced by avoiding contact of the fruit with soil and by protecting low hanging fruit by means of pre-harvest copper and other fungicidal sprays.



Figure 4.5.5.15. *Phytophthora* brown rot.

Conclusion

The most important postharvest diseases on South African citrus fruit have been illustratively described in this report. These descriptions and photos of the economically important pathogens will aid an essential group of role players in the industry to correctly identify these infections on export fruit both locally, on

inspection of export consignments in the packhouses and at the inspection and repacking facilities at the ports, prior to shipping, and especially at the ports of arrival overseas, prior to the fruit being moved into the markets for sale.

The marketing agents and researchers in the South African citrus industry more often than not receive reports back from fruit inspectors overseas for citrus consignments being “flagged” for high waste due reported waste types such as “wet” mould, “white” mould, “brown” mould etc. The correct identification and reporting of the decay on “flagged” or “reject” fruit will thereby ensure the accurate feedback of information to the local marketing agents whereby timeous corrective action can be taken.

Future objectives

The updating of the postharvest handling guidelines, in an illustrative form, is ongoing and will be included in the “Citrus Production Guidelines”.

References cited

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4.5.6 PROGRESS REPORT: Optimisation of fungicide application in citrus packhouses Experiment 936 (April 2008 – March 2010): by Paul Fourie (CRI – SU)

Opsomming

Penicillium digitatum veroorsaak groenskimmel en is verantwoordelik vir groot na-oesverliese van suider Afrikaanse uitvoer sitrusvrugte. Imazalil (IMZ) word algemeen in pakhuisse toegedien en, hoewel dit groenskimmel effektief beheer, is die siekte steeds 'n beduidende probleem. Maksimum toelaatbare residuvlakke (MRL) vir imazalil op sitrusvrugte is op 5 µg/ml vasgestel, terwyl 2-3 µg/ml as 'n biologiese effektiewe residuvlak wat ook sporulasie sal inhibeer, gereken word. Gereelde residu-analise van uitvoer sitrusvrugte uit die meerderheid pakhuisse het getoon dat residuvlakke in die algemeen laer as 1 µg/ml is. Swak IMZ-residulading by pakhuisse kan verder aanleiding gee tot verswakte beheer van groenskimmel en kan vererger word deur IMZ-weerstandbiedende isolate van *P. digitatum*. Die doel van hierdie studie is om imazalil toediening in kommersiële sitruspakhuisse te ondersoek, om die residuvlakke vir infeksiebeheer en sporulasie-inhibisie van IMZ-sensitiewe en -weerstandbiedende isolate te bepaal, en huidige en nuwe toedieningsmetodes te ondersoek en te optimaliseer. Blootstellingstyd, temperatuur en pH is faktore van die swamdoderbaddens wat ondersoek is. Uitstekende genesende en beskermende beheer van IMZ-sensitiewe isolate op Nawel en Valencia lemoene is verkry deur IMZ. Aan die anderkant was die genesende beheer van IMZ-weerstandbiedende *Penicillium* isolate aansienlik swakker en totale verlies aan beskermende beheer is gevind, selfs wanneer dubbel die geregistreerde dosis aangewend is; sporulasie inhibisie was ook algeheel afwesig. Die pH van IMZ-sulfaat baddens kon tot pH 8 gebuffer word met hoë konsentrasies (2%) van natrium bikarbonaat en het ook aanleiding gegee tot dramatiese hoër IMZ-residulading, maar ongelukkig was die MRL vêr oorskry in sekere gevalle. Verder het IMZ-sulfaat in water oplossing gedissosieër na 'n oliërige vrybasis vorm in sekere pH omstandighede (>7) wat dalk aanleiding kon gee tot die uitermatig hoë residulading. Die aanwendingsmetodes van IMZ het aansienlik variëer tussen pakhuisse en afwykings van industrie aanbevelings was algemeen. In terme van IMZ-residulading, wil dit voorkom asof daar 'n interaksie tussen blootstellingstyd, badmengsel-temperatuur, badmengsel-pH en/of vrug tipe is, aangesien nie een van hierdie faktore alleen met IMZ residuvlakke gekorreleer het nie. Optimaliseringsstudies op verskillende vrugtipies sal voortgaan om IMZ-residulading te verbeter. Veilige (d.w.s. sonder om die MRL te oorskry) en effektiewe aanwending van IMZ (2-3 ppm) mag dalk nie moontlik wees deur slegs 'n enkeltoediening van IMZ nie, en 'n dubbel aanwending (byvoorbeeld bad + waks) sal oorweeg moet word om IMZ-residulading en die beheer van groenskimmel te verbeter.

Summary

Green mould caused by *Penicillium digitatum* is responsible for substantial losses of South African export citrus fruit. Imazalil (IMZ) is commonly applied in packhouses and has been shown to effectively control green mould, yet the disease still causes significant losses. Maximum residue level (MRL) for IMZ on citrus fruit is set at 5 µg/ml, whereas 2-3 µg/ml is regarded as a biologically effective residue level that should at least inhibit green mould sporulation. Standard compliance auditing of residue levels of citrus fruit, however, indicated that citrus fruit from the majority of packhouses have residue levels below 1 µg/ml. Poor control of

green mould as a result of insufficient residue loading might further be compounded by the presence of IMZ-resistant strains of *P. digitatum* in packhouses. This study was therefore conducted to determine the adequate residue levels needed for control and sporulation inhibition of IMZ sensitive and resistant strains, to investigate IMZ application and resultant residue levels in commercial citrus packhouses, and to study optimisation of IMZ application in citrus packhouses. Exposure time, solution temperature and solution pH in the fungicide bath was studied. On Navel and Valencia oranges, IMZ exhibited excellent curative and protective control of *Penicillium* strains sensitive to IMZ. However, curative control of IMZ resistant strains was substantially reduced and protective control was lost, even at double the recommended dosage, nor was sporulation inhibited. High concentrations of sodium bicarbonate (2%) buffered the pH of IMZ sulphate baths at 8, and also improved residue loading dramatically, but unfortunately exceeded the MRL of 5 ppm in some cases. Moreover, in some high pH (>7) instances, IMZ sulphate in aqueous solution dissociated to its oily freebase form, which might have contributed to the excessive residue loading observed. IMZ application methods varied considerably between packhouses and divergence from recommended guidelines was often observed. However, IMZ residue loading could not be related to single factors, but rather seemed to be an interaction between exposure time, bath temperature, pH and/or fruit type. Optimisation studies will continue in order to improve methods of IMZ residue loading onto different citrus fruit types. Safe (i.e. not exceeding MRL) and effective application of IMZ (2-3 ppm) might not be obtainable through a single application only, and split applications (for example bath + wax) should be considered to improve IMZ residue loading and green mould control.

Introduction

Excessive financial losses occur every year due to decay caused by post harvest pathogens such as *Penicillium digitatum* (causal agent of citrus green mould). Excellent fungicides such as imazalil are available and effective to control diseases such as citrus green mould. An investigation was conducted to determine why this fungicide is not as effective as it should be, the main focus was on the application method and the subsequent residue loading in practice. Laboratory studies were conducted to study ways and methods in an effort to optimise fungicide application. If fungicide applications could be effective less resistance and waste would occur.

Management of post-harvest diseases of citrus involves several fungicide applications in packhouses, such as in drenches, dips, sprays and wax applications. Biological efficacy of the fungicides is, however, directly related to the adequacy of deposition of the active ingredient on the hydrophobic citrus fruit surfaces. By using green mould caused by *Penicillium digitatum* and the fungicide imazalil sulphate (IMZ) as model system optimisation of fungicide application in citrus packhouses was studied. Maximum residue level (MRL) for IMZ on citrus fruit is set at 5 µg/ml, whereas 2-3 µg/ml is regarded as a biologically effective residue level that should at least inhibit green mould sporulation. Standard compliance auditing of residue levels of citrus fruit, however, indicated that citrus fruit from the majority of packhouses have residue levels below 1 µg/ml. Poor control of green mould as a result of insufficient residue loading might further be compounded by the presence of IMZ-resistant strains of *P. digitatum* in packhouses. The aims of this study were to determine the adequate residue levels needed for control and sporulation inhibition of IMZ-sensitive and -resistant strains, to investigate IMZ application and resultant residue levels in commercial citrus packhouses, and to study optimisation of IMZ application in citrus packhouses. Factors that were studied include application type (spray vs. dip), exposure time, bath temperature, wound size, solution temperature and solution pH on the control of *Penicillium digitatum* with IMZ.

The following aims were defined in the 2008/9 project proposal and will be reported on below:

1. Improve the coverage assessment protocol for use on mature citrus fruit.
2. Determine benchmark values for biologically effective fungicide deposition [Imazalil sulphate and *Penicillium digitatum* (green mould) will be used as the model system].
3. Correlate deposition values as measured with the spray assessment protocol and the corresponding values following residue analyses.
4. Evaluate deposition as effected by drench, dip, spray and wax applications in citrus packhouses.
5. Optimise fungicide deposition in terms of biological efficacy, MRLs and fruit quality.

Materials and methods

1. Improve the protocol for use on mature citrus fruit

Initially, it was anticipated that the spray assessment protocol as developed in CRI-891 would be used in this experiment, but since IMZ is much more lipophilic and systemic than the inert fluorescent pigment, we realised that it would not be an effective tracer. This objective was therefore changed to development of an

'in-house' residue analysis protocol to reduce the cost of routine analysis of large number of samples required for research.

Since the University of Stellenbosch has a Liquid Chromatography Mass Spectrophotometer (LCMS), which is regarded as a very accurate analytical tool for determination of IMZ residues in fruit samples, it was decided to optimise and validate a published protocol (Kearney *et al.*) for 'in-house' use. The proprietary protocol is in the final stages of development, and will be validated as part of a ring-test involving commercial and government analytical laboratories.

For the ring test, 3 replicate samples of 3 different imazalil (IMZ) concentrations of 100 g each were prepared for each participating laboratory. The different IMZ concentrations were 0.7, 2.7 and 7.7 ppm. The participating laboratories were AgChem Africa, Department of Agriculture - Pretoria, Department of Agriculture - Stellenbosch, Hearshaw and Kinnes Analytical Laboratory, SABS, Synexa life sciences and University Department of Plant Pathology. A 1 l stock solution of 1000 ppm IMZ was prepared. To prepare the pulp Valencia oranges were cut into pieces and blended individually for 2 minutes. The blended pulp of each fruit was added together and again mixed with an electrical mixer for 2 minutes. An aliquot of 200 g of the pulp was transferred into a mixing bowl and 0.14, 0.54 and 1.54 ml of the 1000 ppm IMZ stock solution was added and mixed for 2 minutes in order to prepare IMZ concentrations, in the pulp, of 0.7, 2.7 and 7.7 ppm, respectively. All the samples of each specific IMZ concentration were again transferred to a mixing bowl to accumulate a mass of 2800 g and mixed for 2 minutes. Aliquots of approximately 100 g each were transferred to plastic honey jars and deep frozen at -20°C before the samples were couriered to the partaking laboratories.

2. Determine benchmark values for biologically effective fungicide deposition

An IMZ-resistant isolate of *Penicillium digitatum* was obtained from CRI Nelspruit and an IMZ-sensitive isolate was obtained from the Satsuma orchard on the Stellenbosch University experimental farm, Welgevallen. The species identity of the two isolates was confirmed as being *P. digitatum*, since BLAST analyses in GenBank showed that (1) the ITS sequence of both isolates had 100% similarity to the *P. digitatum* sequence (AY373910) of Haugland *et al.* (2004) and (2) the B-tubulin sequence of both isolates had 99.8% similarity to the *P. digitatum* sequence (AY674405) of Samson *et al.* (2004). In order to determine IMZ sensitivity, plugs from a 10- to 14-day-old culture that was grown on potato dextrose agar (PDA) (potato extract 4.0 g/L, dextrose 20.0 g/L and agar 15 g/L, Biolab, Wadeville, Gauteng) medium in a Petri dish were plated on PDA medium amended with 0, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 ppm IMZ (Imzacure). As expected the IMZ-sensitive isolate only grew on the un-amended PDA medium. The IMZ-resistant isolate grew on all the amended media except the 10.0 ppm.

In order to obtain inoculum for biotests, the isolates were grown at ambient temperature on PDA medium in Petri dishes and were replated in 2-week cycles. Conidia were harvested from 10 to 14 days old cultures. The surface of the culture was covered with sterile deionised water treated with Tween at a concentration of ± 0.01 ml/l. After 60 s, the water with conidia were transferred into a 100 ml Scotts bottle containing ± 50 ml sterile water with Tween at a concentration of ± 0.01 ml/l. The conidial suspensions were amended to a concentration of 1×10^6 spores/ml by means of a haemocytometer one hour before each trial commenced. The conidia suspensions were placed on magnetic stirrers to maintain a homogenic suspension of spores for the duration of the trials.

Untreated export quality Navel and Valencia oranges were collected from two citrus packhouses in Wellington and Citrusdal, respectively. The fruit were stored at 7°C for ± 3 days. A day before the trial the fruit were removed from cold storage and left at ambient in order for the fruit temperature to reach ambient and to allow any possible condensation to evaporate. Fruit were treated curatively and protectively to evaluate the efficacy of varying dosages of IMZ against an IMZ sensitive and IMZ resistant isolate of *P. digitatum*. The fruit destined for the curative treatment were inoculated 4 to 6 hours before treatment with IMZ. Fruit were wounded with a triple wound inducer, which consisted out of three insect needles placed in a needle clamp to create small wounds of 0.5 mm wide and 2 mm deep at a triangular distance of 1.5 mm apart. Wounding and inoculation were conducted simultaneously by dipping the wound inducer into a spore suspension of *P. digitatum* (1×10^6 spores per ml) immediately prior to wounding. Four wounds were induced on each fruit around the stem-end and 36 fruit were wounded per treatment combination. Fruit destined for protective treatment were first treated with IMZ, left to dry and inoculated as above.

Fruit were treated with IMZ concentrations of 0, 250, 500 and 1000 ppm (Imzacure, imazalil sulphate, 750 g/kg SG a.i., ICA International Chemicals, Stellenbosch), alone or in combination with 2% sodium bicarbonate. IMZ sulphate used alone resulted in a pH of ≈ 3 , whereas 2% sodium bicarbonate (Alkalinity

Plus, Pool Perfect) buffered the IMZ solutions at a pH of ≈ 8 . The solution temperature was amended to 20°C and the fruit were dipped in the various treatments for 60s. A fresh treatment solution was prepared for each treatment. From each treatment, 6 extra fruit were sampled for IMZ residue analysis, which were done through LCMS/MS by Hearshaw and Kinnes Analytical Laboratory (Pty) Ltd., Cape Town.

After treatment the fruit were stored at 7°C for 21 days plus a subsequent 7 days at 23°C. Infection was evaluated after 3, 7, 10, 14, 18, 21 and 28 days by noting the number of infected wounds on each fruit. At similar time intervals, sporulation inhibition was evaluated for each fruit by means of a rating index of 0 to 5, where 0 was no sign of disease and 5 was fruit totally green of sporulating citrus green mould. For reporting purposes, data from the 28 days rating were summarised as percentages of infected and/or sporulating fruit, where a sporulation rating 3 to 5 was used for the sporulating fruit.

3. Correlate deposition values as measured with the spray assessment protocol and the corresponding values following residue analyses.

For reasons mentioned above (1), this objective will not be done.

4. Evaluate deposition as effected by drench, dip, spray and wax applications in citrus packhouses

Current application techniques were evaluated by mean of a thorough survey of southern African packhouses. The survey included a detailed description of the pack line, IMZ concentration, pH and temperature of all solutions where IMZ was used, as well as IMZ residue analyses from fruit sampled prior to and after each IMZ treatment.

The temperatures of the bath solutions in the operational packhouses were measured by means of thermo probe connected to a Sentry ST642 infrared thermometer. Additionally, the fruit surface temperature was measured before and after the bath, before the wax application and after the last hot air tunnel by means of the infrared thermometer. The exposure time of the fruit to the bath solution or the wax application was measured by wrapping some of the fruit in aluminium foil and measuring the exposure time of these marked fruit in the bath or the wax applicator. The fruit temperature and exposure time measurements were replicated 12 times. The number of brushes after the fungicide bath and under the wax applicator was recorded. The wax application rate of some of the packhouses that used atomisers was determined by measuring the amount of wax coming out of an atomiser for 30 s; the answer was calculated to ml/s.

A 250 ml sample of the drench and bath solution was taken where imazalil (IMZ) was part of the solution. The samples were deep frozen and stored at -20°C. Titration of thawed samples was done by a two-phase titration method (Jansen Pharmaceutica, Belgium). Sodium lauryl sulphate (90- 91%, UniLab, Krugersdorp) is titrated into 25 ml of the IMZ solution to which 10 ml of 1M sulphuric acid (Synthon, Netherlands), 25 ml dichloromethane (99.8%, Sigma-Aldrich, Germany) and 12 drops of indophenol blue indicator (Sigma-Aldrich, USA) have been added. When the end point is reached the amount of sodium lauryl sulphate (ml) used is noted as "A". The same procedure is followed with a blank solution of water and the end point (ml) is noted as "B". The IMZ concentration (ppm) is calculated as $(A \text{ ml} - B \text{ ml}) \times 0.1 \times 40 \times 297.18$. The pH of solutions was measured by means of a Jenway 3310 pH meter in the laboratory.

After each IMZ application, i.e. drench, fungicide bath or wax application, 6 fruit were sampled for IMZ residue analysis. All fruit samples were deep frozen and stored at -20°C. At a later stage, the whole fruit samples were chopped and blended by means of a Salton Elite food processor. Approximately 250 g of the blended fruit samples were sent to Hearshaw and Kinnes Analytical Laboratory (Pty) Ltd. for IMZ residue analysis.

5. Optimise fungicide deposition in terms of biological efficacy, MRLs and fruit quality.

As most packhouses apply IMZ primarily in a fungicide bath, initial optimisation studies were focussed on this application.

Exposure time. Untreated, export quality Valencia oranges were collected from a citrus packhouse in Wellington. The fruit were stored at 7°C for ± 3 days. A day before the trial the fruit were removed from cold storage and left at ambient in order for the fruit temperature to reach ambient and to allow possible condensation to evaporate. Fruit were treated in a 500 ppm solution of IMZ sulphate alone, or in combination with 2% sodium bicarbonate in municipal water. As mentioned previously, the pH of these solutions were *circa* 3 and 8, respectively. The bath temperature was adjusted to 20 or 35°C and exposure time periods were 15, 45, 90, 180 and 540 s. A fresh treatment solution was prepared for each

temperature/pH combination. After treatment, fruit were left to dry before it was sent to Hearshaw and Kinnes for IMZ residue analysis. Six fruit per treatment were used and the trial was repeated once. Prior to the residue analysis, the fruit were stored and prepared as described for the packhouse survey.

Results

1. Improve the protocol for use on mature citrus fruit. Imazalil residue analyses ring test of commercial and public analytical laboratories

The most accurate results for 0.7, 2.7 and 7.7 ppm came from laboratories A and D, A and B, and A, respectively (Table 4.5.6.1). It was obvious, however, that the variation between the different replicates could prove to be a source of concern. Overall it appears that all the laboratories were more accurate with the middle concentration of 2.7 ppm and variation increased and accuracy declined with the higher concentration of 7.7 ppm.

Table 4.5.6.1. Results of imazalil concentrations of the various samples sent to the different partaking commercial and public laboratories for a ring test on imazalil residue analyses.

Imazalil concentration of spiked sample (ppm)	Analytical laboratory [#]			
	A	B	C	D
0.7	0.57 (14.36%)*	0.46 (2.17%)	0.10 (0.00%)	0.61 (4.15%)
2.7	2.62 (10.58%)	2.96 (21.58%)	2.19 (13.48%)	1.90 (8.97%)
7.7	6.77 (9.30%)	11.74 (14.28%)	10.48 (4.50%)	4.50 (15.21%)

[#]Results from 3 participating laboratories are still pending.

*Average of 3 samples and relative standard deviance (% RSD; (Standard deviance/mean)*100).

2. Determine benchmark values for biologically effective fungicide deposition

The imazalil (IMZ) residue levels of Navels oranges treated in an unbuffered (IMZ sulphate alone) IMZ solution of 0, 250, 500 and 1000 ppm were 0.00, 0.28, 0.35 and 0.81 ppm, respectively. In the IMZ solutions buffered with 2% sodium bicarbonate the residue levels were 0.00, 0.86, 2.10 and 7.10 ppm, respectively. Back-up samples were retained and will also be analysed to duplicate these residue results. The addition of 2% sodium bicarbonate and buffering of the pH at 8 therefore resulted in substantially improved IMZ residue loading.

Mean infection and sporulation percentages on Navel orange fruit are summarised in Figure 1. In general, curative treatment proved to be more effective than protective treatment. Some curative control failure (Fig. 4.5.6.1A) and loss of sporulation inhibition of infections (Fig. 4.5.6.1B) by the IMZ sensitive isolate was observed at 250 ppm, but this isolate was successfully controlled and sporulation inhibited at curative treatments in 500 ppm IMZ alone. In 250 ppm IMZ + 2% sodium bicarbonate, curative control of the sensitive strain was slightly improved and sporulation inhibited by *circa* 50%. The resistant strain was, however, not controlled by 500 or 1000 ppm, nor was sporulation inhibited in the case of 500 ppm IMZ, and only 60% inhibited at 1000 ppm. Curative treatment of fruit in 500 and 1000 ppm IMZ + 2% sodium bicarbonate resulted in complete control and sporulation inhibition of the resistant strain. In protective treatments in IMZ-alone baths, complete control and sporulation inhibition of the sensitive strain was lost in all but the 1000 ppm treatment. Almost no protective control over the resistant strain was observed in any of the IMZ alone treatments. In the IMZ + 2% sodium bicarbonate treatments, the sensitive strain was completely controlled, while the resistant strain was adequately controlled at 500 ppm IMZ, although sporulation was not inhibited at this concentration, and only at 1000 ppm.

The IMZ residue measured on the Valencia orange fruit was 0.31, 1.46, 0.66 and 1.67 mg/kg for fruit treated with 0, 250, 500 and 1000 ppm respectively. For fruit treated with IMZ + sodium bicarbonate the IMZ residue was 0.06, 1.26, 2.8 and 8.25 ppm for the same treatments. Back-up samples were retained and will also be analysed to duplicate and/or confirm these residue results; especially in the questionable cases as was observed on the Valencia fruit. Similar results were observed, but IMZ + sodium bicarbonate treatments were able to curatively inhibit citrus green mould infection and sporulation to lower levels than on Navels.

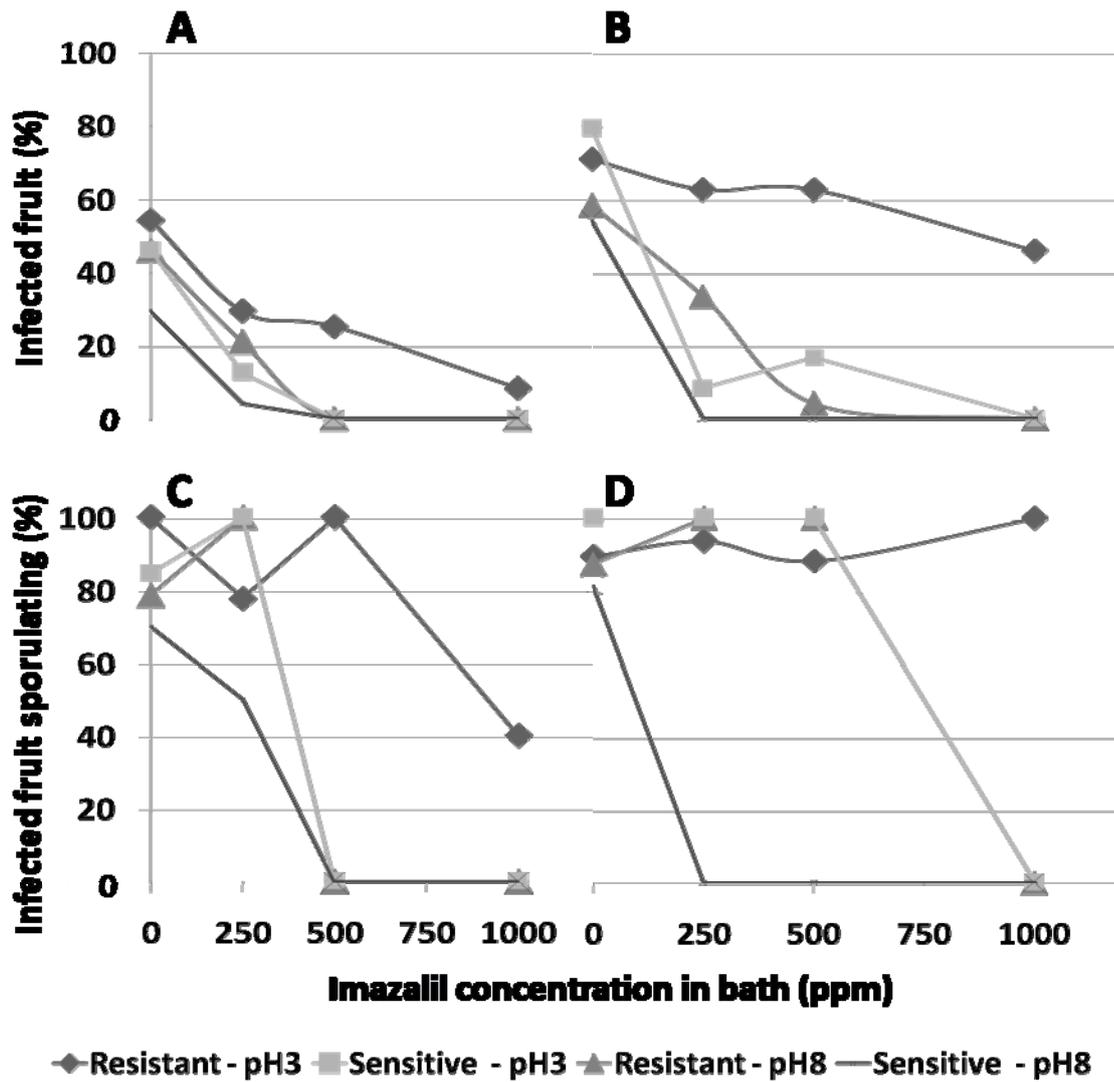


Figure 4.5.6.1. Mean percentages infected Navel fruit and sporulating infected fruit that were inoculated with imazalil-sensitive and -resistant strains of *Penicillium digitatum*, dip-treated for 60 s curatively (A and C, respectively) or protectively (B and D, respectively) with 0, 250, 500 and 1000 ppm imazalil sulphate alone (pH 3) or in combination with 2% sodium bicarbonate (pH 8) and cold stored for 21 days at 7°C plus an additional shelf life period of 7 days at 23°C.

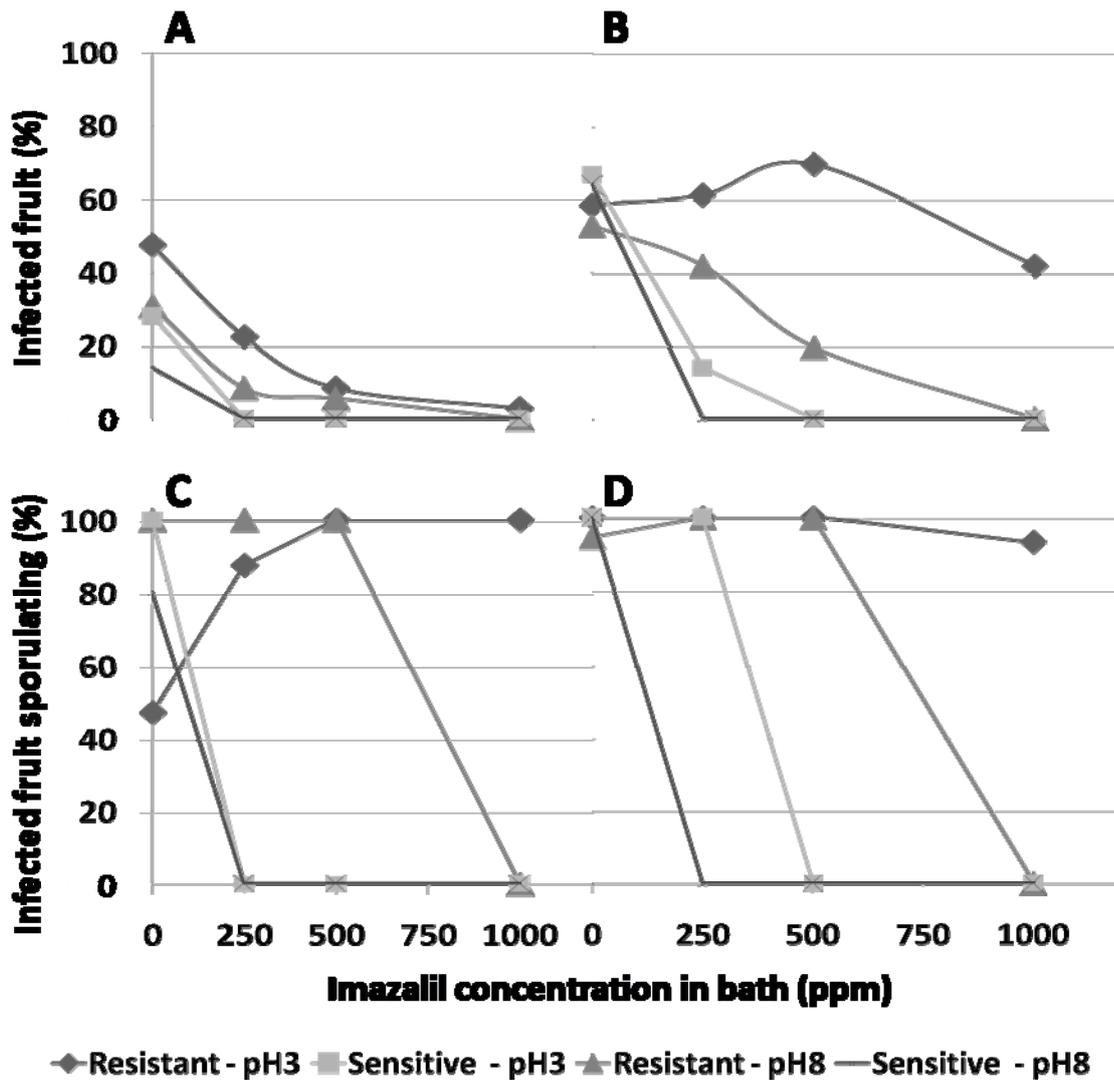


Figure 4.5.6.2. Mean percentages infected Valencia fruit and sporulating infected fruit that were inoculated with imazalil-sensitive and -resistant strains of *Penicillium digitatum*, dip-treated for 60 s curatively (A and C, respectively) or protectively (B and D, respectively) with 0, 250, 500 and 1000 ppm imazalil sulphate alone (pH 3) or in combination with 2% sodium bicarbonate (pH 8) and cold stored for 21 days at 7°C plus an additional shelf life period of 7 days at 23°C.

- Evaluate deposition as effected by drench, dip, spray and wax applications in citrus packhouses

Packhouse survey

During the 2008/9 season, 37 packhouses were visited throughout South Africa (Table 4.5.6.2). The results from the survey are summarised below.

Table 4.5.6.2. Packhouse names, areas and dates visited during the packhouse survey conducted in 2008.

Packhouse	Date Visited	Packhouse	Date Visited
Western Cape		Eastern Cape	
Middelpos	4 July 2008	Hankey	12 August 2008
Cedarpack	16 July 2008	Patensie Sitrus Beperk	12 August 2008
ALG	16 July 2008	Papillon	12 August 2008
Citrus Select	16 July 2008	Katco	13 August 2008
Noordhoek	16 July 2008	Riverside	13 August 2008
Groenkloof	22 July 2008	SRCC Hermitage	13 August 2008
Oudam	22 July 2008	Unifrutti	14 August 2008
Goede Hoop Citrus	22 July 2008	Suncitrus	14 August 2008

Thornlands	3 September 2008	Sitrusrand	14 August 2008
Swellenfruit	3 September 2008	Limpopo	
Kwazulu Natal		Ambrosia	18 August 2008
Katope	7 August 2008	Unifruitti	19 August 2008
Riverbend	7 August 2008	Richmond	19 August 2008
Thulwane	7 August 2008	Canyon	19 August 2008
Rhino Packers	7 August 2008	Bavaria	19 August 2008
Bedlane	7 August 2008	C.P. Minnaar	20 August 2008
Mpumalanga		Groep 91	20 August 2008
Croc Valley	21 August 2008	Laparisa	20 August 2008
Karino Sitrus	21 August 2008		

Drench application

Of the packhouses surveyed, 51% did not use a drench at all, 35% used a drench but not for IMZ (Philabuster) application and 14% used a drench to apply IMZ (Philabuster).

Wash application

A total of 78% of the surveyed packhouses used a wet dump method to load the fruit on the packline and 22% used a dry dump method. Products used in the wash solution varied from chlorine, chlorine and Sporekill combination, Sporefix, Harvest Wash, Agrine, and Deccosol. Chlorine alone was used by 73% of the packhouses and Sporekill alone or Sporekill in combination with chlorine were each used by 8% of the packhouses. The other products mentioned above were each used by only one packhouse respectively.

Fungicide bath

One packhouse had a fungicide bath, but did not use it for IMZ application, 19% of the packhouses did not have a fungicide bath and 78% used a fungicide bath to apply IMZ. The majority of the fungicide baths which contained IMZ had a protocol to prepare the IMZ solution to a concentration of 500 ppm. Packhouse 32, 22 and 29 prepared the IMZ concentration to 700, 1000 and 1119 ppm, respectively. The actual IMZ concentrations of the different fungicide baths at the time of each specific survey were determined through titration. The majority of the fungicide baths (59.09%) had IMZ concentrations below 400 ppm, with only 4 packhouses (40.91%) with concentration above 400 ppm and below the registered and recommended 500 ppm. Five packhouses had IMZ concentrations of higher than 500 ppm (Fig. 4.5.6.3).

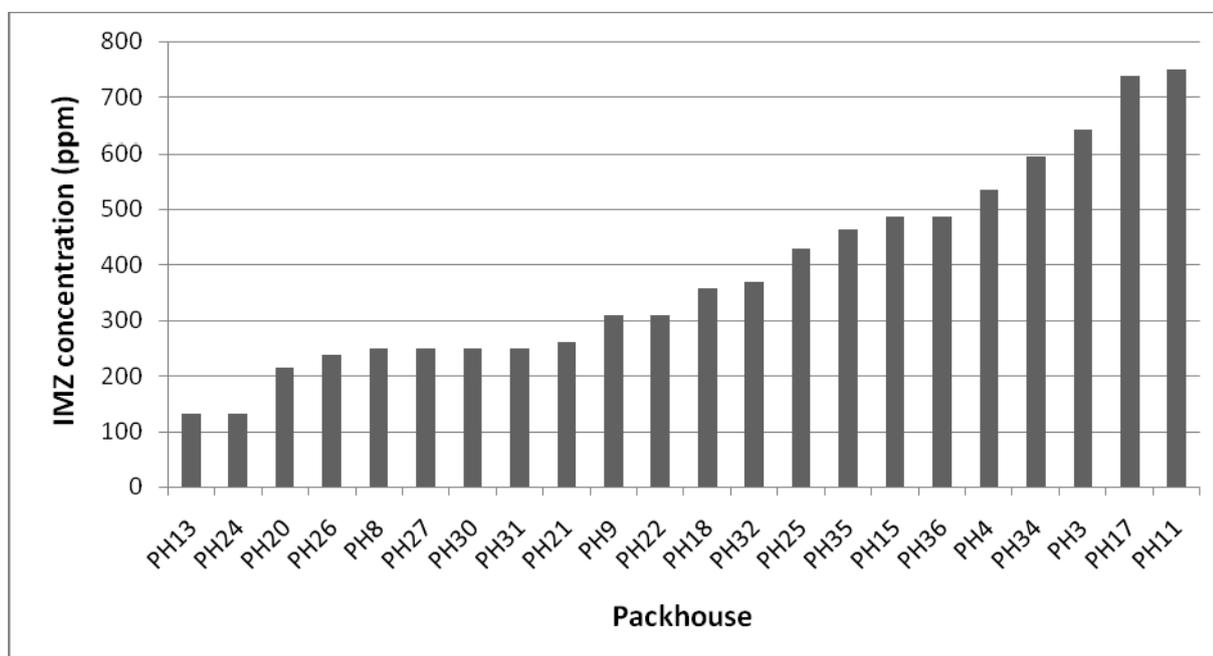


Figure 4.5.6.3. The actual imazalil concentration measured through titration of the different fungicide baths of packhouses surveyed in 2008.

The majority (92%) of the packhouses had an IMZ top-up protocol, and 8% did not have a top-up protocol. The top-up protocols and frequencies are summarised in Table 3. The frequency of packhouses starting with a freshly prepared IMZ solution in the fungicide bath was variable with 4% starting every 3 days, 52% every 7 days, 28% every 14 days, 8 every 21 days, 4% every 28 days and 4% every 60 days.

IMZ was combined with other products in the fungicide bath in 76% of the packhouses, while 24% applied only IMZ. Products applied with IMZ in the fungicide bath ranged from Exterminator, 2,4 D, guazatine, and Sporekill.

The majority (97%) of the packhouse had brushes directly after the fungicide bath of which 24% had less than 11 brushes, 38% between 11 and 20, 19% between 21 and 30, 14% between 31 and 40 and 5% between 41 and 50. The effect of the number of brushes on IMZ residue loading will be studied in 2009/10.

The bath solution temperatures (Figure 4.5.6.4) varied from 11.5 to 44.6°C, and 70% of the fungicide baths had a temperature of 30°C and higher. The pH (Figure 4.5.6.5) of the fungicide bath solution ranged from 3.34 to 8.02 with 61% higher than 5.0. Bath length (Figure 4.5.6.6) varied from 1.5 to 10 m, with the majority average being 3.50 m. Exposure time (Figure 4.5.6.6) of fruit in the fungicide baths varied from 15.5 to 106.8 s. Sixty eight percent (68%) of the fruit through these baths was exposed to the fungicide/s solution for less than 60 s. Interestingly, the exposure time correlated with the bath length, in 57% of the packhouses.

Fruit surface temperature was increased in 85% of the packhouses when the fruit had gone through the fungicide bath and in 54% of the packhouses the increase was more than 10°C. The fruit surface temperature after the baths ranged from 8.32 to 42.22°C, 50% were lower than 30°C and 23% higher than 35°C.

Table 4.5.6.3. Top-up protocol and frequency for each packhouse surveyed in 2008.

Packhouse number	Top-up protocol	Top-up frequency
PH3	1 l Philabuster per 50 bins	continuous
PH17	3000 g IMZ sulphate	Per 70 t
PH2	5 g IMZ sulphate per t	2 times per day
PH11	5 g IMZ sulphate per t	Once a day
PH21	5 g IMZ sulphate per t	n/a
PH33	5 g IMZ sulphate per t	Per 65 t
PH34	5 g IMZ sulphate per t	Every second day
PH35	5 g IMZ sulphate per t	n/a
PH36	5 g IMZ sulphate per t	n/a
PH29	6 g IMZ sulphate per t	n/a
PH20	Amended IMZ concentration in bath according to titration results	Per 27 t
PH22	Top bath up to level with 1000 ppm IMZ solution	Per 50 t
PH12	Top bath up to level from 1000 l tank with 1000 ppm IMZ solution	Once a day
PH15	Top bath up to level from 1000 l tank with 500 ppm IMZ solution	continuous
PH18	Top bath up to level from 200 l tank with 500 ppm IMZ solution	Per 2 hours
PH25	Top bath up to level with 500 ppm IMZ solution	continuous
PH27	Top bath up to level with 500 ppm IMZ solution	3 times per day
PH31	Top bath up to level with 500 ppm IMZ solution	continuous
PH10	Top-up with 300 l from 600 l tank with 1666.67 ppm	Once a day
PH24	n/a	Per 160 bins

PH30	n/a	Once daily
PH16	n/a	Per 30 t

*n/a = information not available.

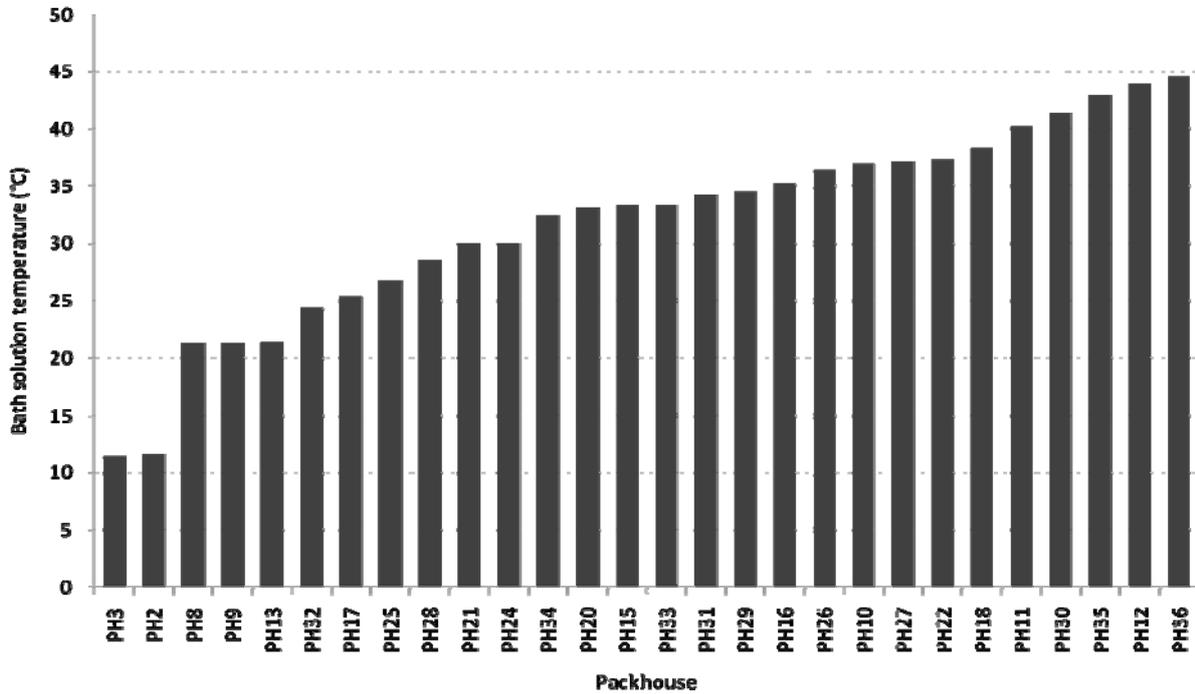


Figure 4.5.6.4. Bath solution temperature measured from the different fungicide baths of packhouses surveyed in 2008.

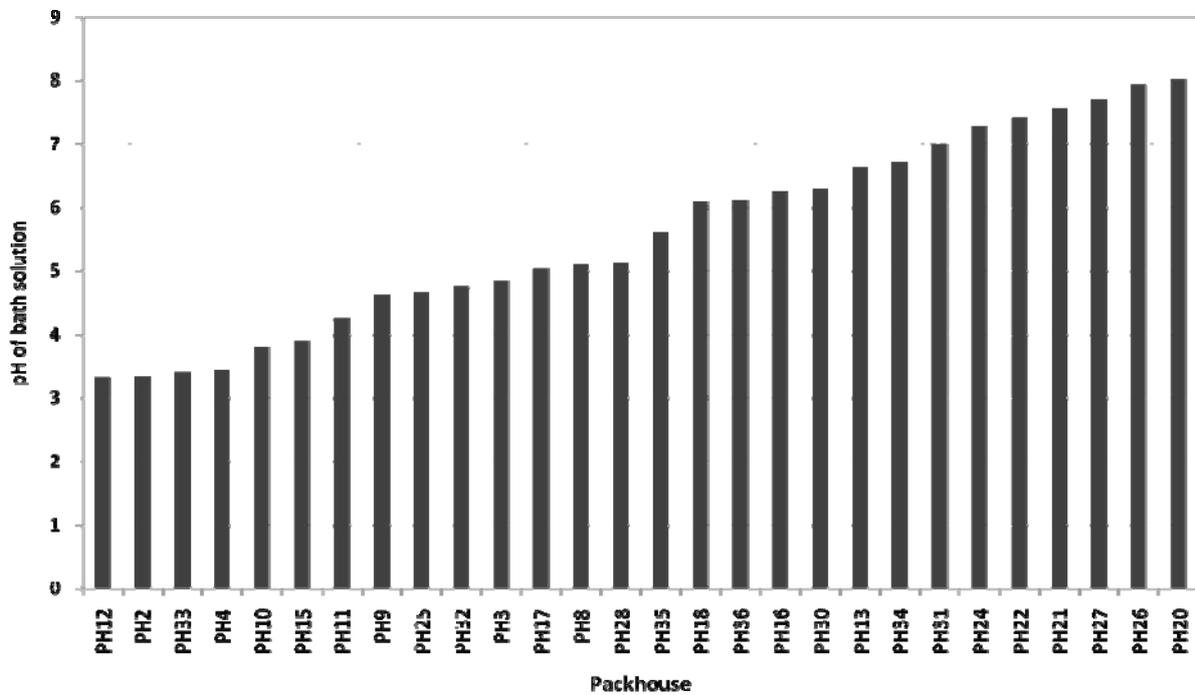


Figure 4.5.6.5. Bath solution pH measured from the different fungicide baths of packhouses surveyed in 2008.

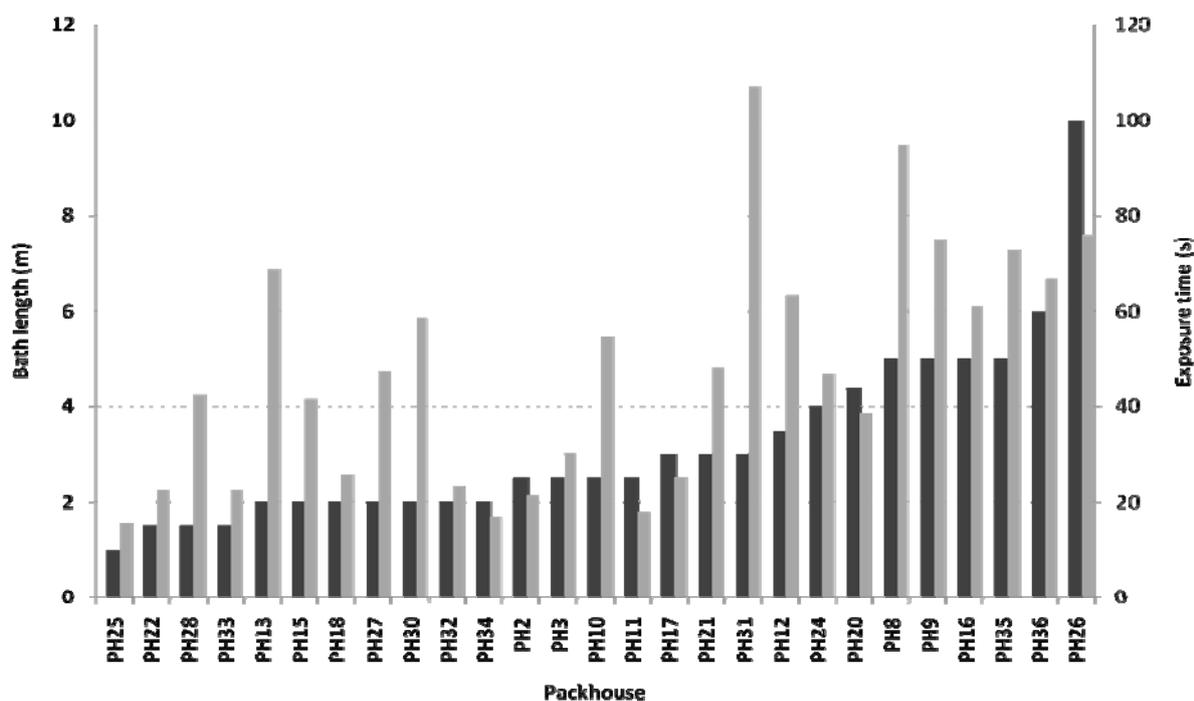


Figure 4.5.6.6. Bath length and fruit exposure time in the different fungicide baths of packhouses surveyed in 2008.

Wax application

Of the packhouses surveyed, 65% applied IMZ with the wax and 58% of these packhouses applied other products in combination with IMZ in the wax. Other products applied with IMZ were 2.4D and thiabendazole. In the majority (56%) of the packhouses the fruit exposure time under the wax applicators was less than 15 s. The exposure time varied from 7.45 to 34 s. The number of brushes in the wax applicators varied between 0 and 13 and 72% had 10 or more brushes. Three different wax applicators were used; pulse spray, atomiser and dripper and the percentage packhouses that used these systems were 48, 43 and 9%, respectively. The wax application rate was measured at 8 packhouses that used atomisers. The flow rate ranged from 0.29 to 3.17 ml/s with a mean of 1.45 ml/s. The fruit surface temperature in 74% of the packhouses showed an increase after the last hot air drying tunnel. There was a fruit temperature increase of higher than 10°C in 10% of these packhouses. The fruit surface temperatures ranged from 15.86 to 44.52°C after the hot air tunnel, 10% were higher than 35°C.

Imazalil residue loading

Of the packhouses surveyed, 49% applied IMZ once, 46% twice and 5% three times. The majority (78.38%) applied IMZ in the bath, 62.16% in the wax, 13.51% in the drench and 2.70% in either a spray-on or “drench-type” system on the packline.

The IMZ residue measured after drench application (4 packhouses) ranged from 0 to 0.86 ppm with a mean of 0.41 ppm. Thirty eight percent of the packhouses applying IMZ in the fungicide bath used the fungicide bath as the only IMZ application. Imazalil residue on fruit treated in the various fungicide baths varied from 0.24 to 2.57 ppm. The IMZ residue in 18% of the packhouses was higher than 2 ppm (Figure 4.5.6.7).

One packhouse applied IMZ in the drench and bath, the IMZ residue increased from 0 to 3.85 ppm. Another packhouse applied IMZ in the drench and through a spray applicator, and the IMZ residue increased from 0.30 to 0.65 ppm.

One packhouse applied IMZ in a “drench-type” system on the packline and the IMZ residue was measured on fruit treated with a 3-day-old solution and fruit treated with a 4-hour-old solution. Residues measured 0.08 and 0.23 ppm for the respective treatments. It should be noted that IMZ was not detectable in the 3-day-old solution, while a concentration of 665.68 ppm was detected by means of titration in the 4-hour-old solution. After an additional application of IMZ in the wax the IMZ residues were 0.26 and 0.73 ppm, respectively.

Three (8%) of the packhouses applied IMZ in the wax only and the respective IMZ residues measured were 1.08, 2.78 and 3.07 ppm (mean of 2.31 ppm). Another 3 packhouses (8%) applied IMZ as a double application in the drench and wax, the respective IMZ residues measured at the end of the line were 1.60, 1.03 and 1.47 ppm (mean of 1.37 ppm). Two packhouses (3%) applied IMZ as a triple application one in the drench, spray and wax and the other in the drench, bath and wax the respective IMZ residues measured at the end of the line were 1.55 and 3.65 ppm. Thirty eight percent of the packhouses applied IMZ as a double application in the bath and the wax of which 36% loaded a residue of higher than 2 ppm and 14% lower than 1 ppm (Figure 4.5.6.8).

There appears to be no correlation between IMZ residue loading and bath solution temperature, bath pH or fruit exposure time in the bath. This could be due to a relatively small sample size. It remains to be explored whether there is an interaction between these 3 factors. Other factors such as clean bath solutions, wet fruit entering the fungicide bath, the cleanliness of fruit entering the bath and fruit cultivar may also influence IMZ residue loading.

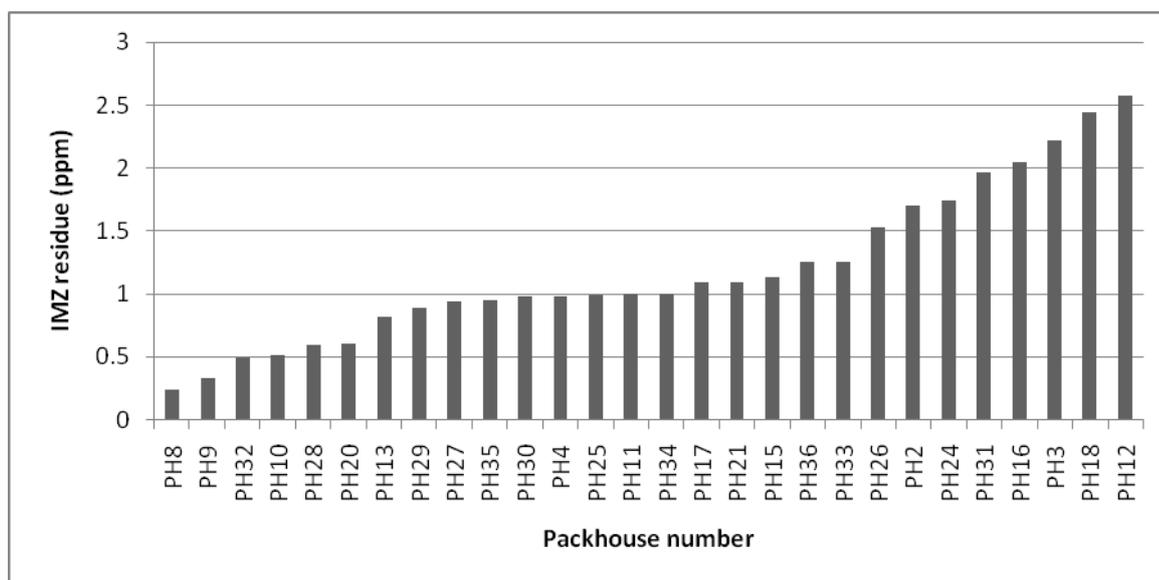


Figure 4.5.6.7. Imazalil residue (ppm) measured from fruit treated in fungicide baths at various packhouses surveyed in 2008.

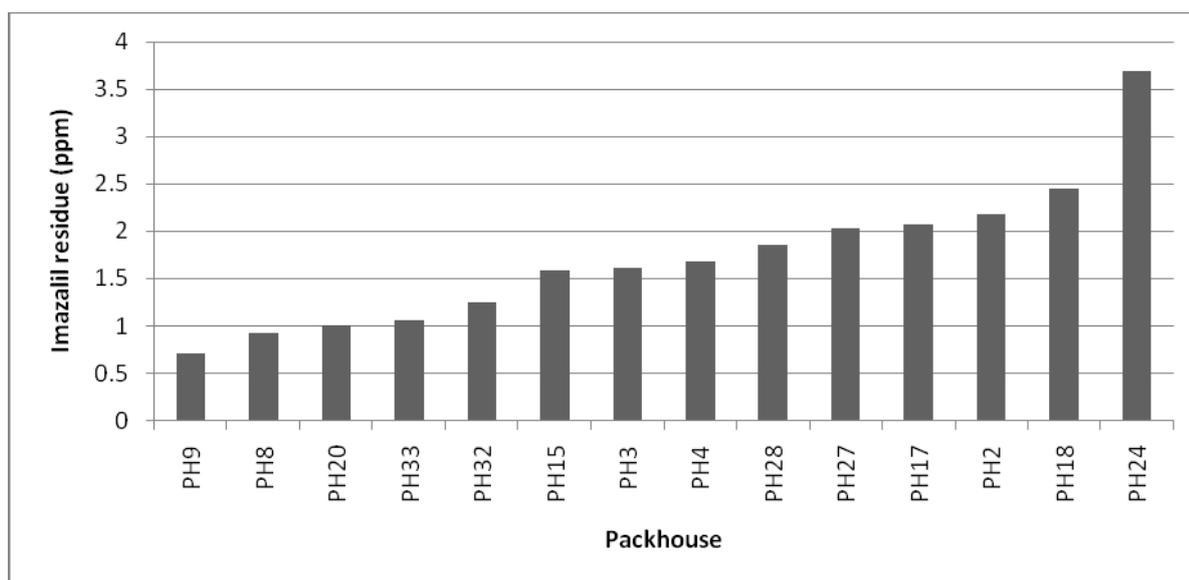


Figure 4.5.6.8. Imazalil residue measured from fruit treated with a double application of imazalil in the bath and the wax of packhouses surveyed in 2008.

5. Optimise fungicide deposition in terms of biological efficacy, MRLs and fruit quality

Exposure time

IMZ residues of fruit treated in a cold or warm IMZ sulphate solution did not exceed 2.14 ppm (Figure 4.5.6.9A). The IMZ residue of fruit treated in a cold IMZ + sodium bicarbonate solution reached a level of 3.93 ppm after 45 s exposure and exceeded the maximum residue level (MRL) of 5 after less than 90 s exposure (Figure 4.5.6.9B). Fruit treated in a heated IMZ + sodium bicarbonate solution reached an IMZ residue level of 4.73 ppm after 15 s exposure and exceeded the MRL after less than 45 s exposure. IMZ residues on fruit treated in cold and warm IMZ + sodium bicarbonate solutions exceeded 30 and 50 ppm, respectively after 9 min. Logarithmic regression lines were fitted on the IMZ residue data from fruit treated with IMZ in a heated and cold solution with an R-square of 0.70 and 0.79, respectively. R-squares of 0.998 for both the heated and cold treatments were calculated when linear regression lines were fitted the IMZ residue data for fruit treated with IMZ + sodium carbonate. These regression lines can be used in future bioassay studies to determine the effect of specific IMZ residues on the control of green mould. Smilanick *et al.* (2005) reported increased IMZ residue loading at higher pH, but not to the levels reported here. In this trial 2% sodium bicarbonate was used, which buffered the IMZ solution at pH 8. However, substantially lower sodium bicarbonate concentrations (*circa* 0.05%) are needed to buffer IMZ solution at this pH level and the pronounced residue loading with IMZ + 2% sodium bicarbonate solutions might therefore be attributed to the high pH of 8, and possibly also the high sodium bicarbonate concentration. Furthermore, at pH levels higher than 6, IMZ sulphate can dissociate to its oily free-base form (depending on the water quality), which is highly lipophilic and might have contributed to the very high residue levels obtained.

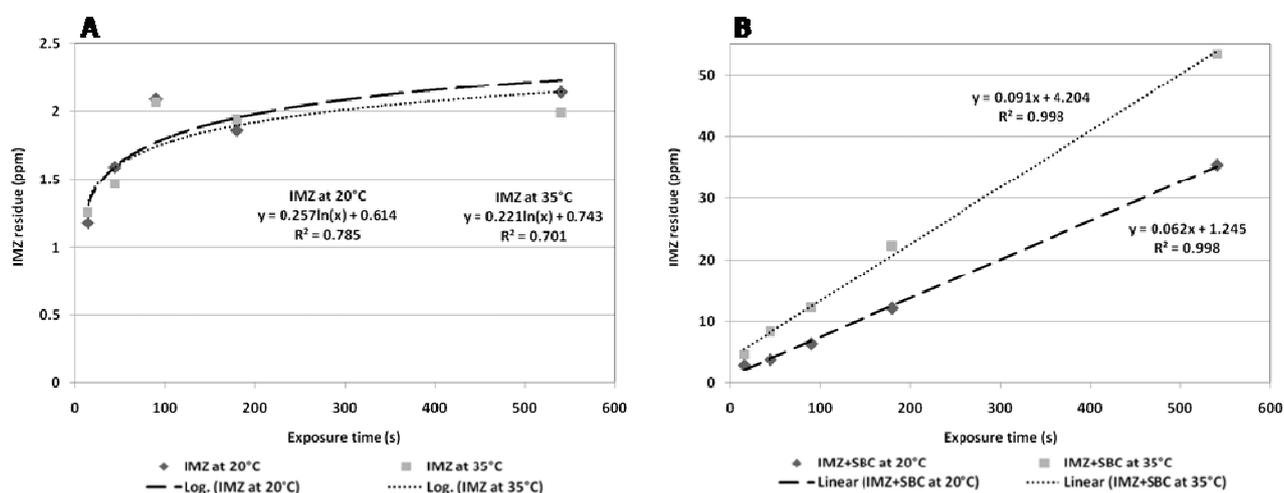


Figure 4.5.6.9. Mean imazalil residue levels on Valencia oranges that were dip treated in an (A) imazalil solution of 500 ppm or (B) imazalil (500 ppm) and sodium bicarbonate (2%) for 15, 45, 90, 180 and 540 at temperatures of 20 and 35°C, and the respective logarithmic or linear regression lines.

Conclusion

A proprietary in-house IMZ residue analysis protocol is being developed in this study and will be validated in ring tests with other laboratories. IMZ residue analysis of treated fruit is a very good indication of the effectiveness of IMZ application methods in packhouses and should be highly recommended. From the ring test results obtained thus far, the variation in results is a concern, also indicating that conclusions should not be drawn from individual sample tests alone. Trends from repeated measurements should be considered and/or samples should be analysed at least in duplicate to overcome this problem.

Imazalil demonstrated excellent curative and protective control of sensitive isolates at IMZ residue levels of 1 ppm, although sporulation inhibition was compromised at lower residue levels. Insufficient residue loading might lead to resistance development against IMZ and/or loss of control. This was clearly demonstrated in the biotests as the IMZ-resistant strain could not be adequately controlled at similar residue levels as the sensitive strain, and >2 ppm residue levels was required for curative and protective control as well as sporulation inhibition. Sporulation inhibition of resistant strains is of utmost importance, as its failure will rapidly lead to increased resistance frequency in packhouses (largely from IMZ treated and subsequently discarded fruit). This situation will be even further compounded when poor sanitation is practiced in packhouses. Treatments resulting in IMZ residue levels of <2 ppm, as was commonly observed for fruit from SA packhouses, might therefore lead to loss of sporulation inhibition of resistant strains.

Pre-packhouse drench applications in 4 packhouses resulted in mean IMZ residue levels of 0.41 ppm. At these residue levels in the biotests, sensitive strains were partially controlled, but sporulation was not completely inhibited, while resistant strains were not controlled nor was its sporulation inhibited. Therefore, application of IMZ in the pre-packhouse drench is highly imprudent in terms of IMZ resistance management in citrus packhouses. It also seems that adequate (> 2 ppm) IMZ residue levels might not be obtained through this application system, but future research will investigate this further.

Most packhouses applied IMZ in baths, but only 18% obtained IMZ residue levels >2 ppm. Inadequate residue loading in baths can be attributed to poor management of IMZ concentration in baths (almost 60% of baths tested had <400 ppm IMZ), low temperature (30% of packhouses' baths were <30°C), low pH (39% of baths were < pH 5) and short exposure times (68% was <60 s). IMZ residue loading in baths can be improved through increased bath temperature (ideal = 35°C), adjustment of pH (ideal = 6) and increased exposure times (ideal = 1 min).

High concentrations of sodium bicarbonate (2%) buffered the pH of IMZ sulphate baths at 8, and also improved residue loading dramatically, but unfortunately exceeding the MRL of 5 ppm in some cases. Moreover, in some high pH (>7) instances, IMZ sulphate in aqueous solution dissociated to its oily freebase form, which might have contributed to the excessive residue loading observed. Safe application of this GRAS chemical will be studied further as it also demonstrated some green mould control, while the improved IMZ residue loading dramatically improved control of sensitive and resistant strains of *P. digitatum*.

IMZ was loaded more successfully on the fruit in the wax application, although this application was observed as being less curative than the aqueous IMZ application. However, wax application provided good sporulation inhibition (Brown and Dezman, 1990; Smilanick *et al.*, 1997). In SA packhouses, the best residue levels were obtained through the double application of IMZ in the bath and subsequently in the wax (only 14% of cases <1 ppm, and 36% > 2 ppm).

IMZ application methods in citrus packhouses varied considerably between packhouses and divergence from recommended guidelines was often observed. However, IMZ residue loading could not be related to single factors, but rather seemed to be an interaction between exposure time, bath temperature, pH and/or fruit type. Optimisation studies will continue in order to improve methods of imazalil residue loading onto different citrus fruit types. Safe (i.e. not exceeding the MRL of 5 mg/kg) and effective application of imazalil (2-3 ppm) might not be obtainable by means of a single application only. Split applications (for example bath + wax) should be considered to improve imazalil residue loading and green mould control.

Technology transfer

- Citrus Research symposium (1 poster).
- Southern African Society for Plant Pathology biennial congress (1 talk).
- Presentation at Citrus Rind Condition workshop.
- Presentations at the various regional packhouse workshops.

Further objectives (milestones) and work plan

1. Improve the protocol for use on mature citrus fruit.
Imazalil residue analyses ring test of commercial and public analytical laboratories

There are still outstanding results from 2 commercial laboratories. The imazalil extraction protocol to be conducted at the Stellenbosch University, Department Plant Pathology is in its final stages of development and will participate in the ring test for validation purposes.

2. Determine benchmark values for biologically effective fungicide deposition

Fruit from various citrus types will be loaded with different IMZ levels and following biological efficacy trials benchmark values for effective control of sensitive and resistant strains will be statistically determined.

3. Evaluate deposition as effected by drench, dip, spray and wax applications in citrus packhouses

More packhouses will be surveyed in 2009 to compliment the survey of 2008.

4. Optimise fungicide deposition in terms of biological efficacy, MRLs and fruit quality

Exposure trials in fungicide baths will be conducted on other cultivars, but with additional treatments to clarify the effect of pH as well as sodium bicarbonate concentration on IMZ residue loading. Similar trials will also be conducted to evaluate wax and drench application.

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4.5.7 **PROGRESS REPORT: Occurrence of *Penicillium* spp. in the citrus supply chain and improving the citrus export chain through determining critical control points** Experiment PPL 20 (April 2008 – March 2009): by R. Jacobs and L Korsten (UP)

Opsomming

Ons het die sitrusuitvoerketting gevolg vanaf die vrugpluk stadium op die plaas tot by die Europese eindbestemming op die oorsese winkelrak. Mikrobiologiese monsters is geneem van oppervlaktes van Suid Afrikaanse pakhuse en koelkamers en oorsese herverpakkingseenhede en distribusie-sentrums se koelstore. In alle gevalle is oppervlak monsters geneem van mure en vloere en lugmonsters van die fasiliteite en, indien van toepassing, verpakkingsvoerbelle. Monsters ook geneem van hande van vrug hanteerders (plukkers en pakkers). Die doel was om die vlak van algemene fasiliteit en persoonlike higiëne te bepaal regdeur die hele sitrusuitvoerketting. Volgens ons bevindinge kan vrughantering en die hoë vlakke van inokulum waaraan vrugte in die uitvoerketting blootgestel word, baie tot die ontwikkeling van na-oes bederf tydens die uitvoerfase van die ketting bydra, wat veroorsaak dat vrugte verrottingssimptome sal wys teen die tyd dat dit by die winkel handelaar aankom. Ons glo hierdie besmetting het plaasgevind terwyl die vrugte in die ketting was en die oorsaak lê nie noodwendig by die plaas nie. 'n Diverse verskeidenheid *Penicillium* spp., insluitende die sitrus patogene *Penicillium italicum* en *P. digitatum*, die oorsaak van blou en groen skimmel, is in sekere fasiliteite in die uitvoerketting geïsoleer. Die data versterk ons teorie dat besmetting later in die ketting kan plaasvind en dit lê die klem op die belangrikheid vir alle rolspelers in die uitvoerketting om aan strenger higiëne standaarde te voldoen. Beter higiëne sal veroorsaak dat die inokulum druk van die na-oes patogene verlaag word en dus tot minder na-oes bederf lei. By afhandeling van hierdie studie sal riglyne beskikbaar gestel word rakende kritiese areas om te monitor vir inokulumlading en faktore wat tot na-oes bederf kan bydra en meer effektief beheer sal moet word. *Penicillium* spesie diversiteitsdata en moniteringsriglyne sal verskaf word om die industrie te help met die identifikasie van patogene wat vir sitrus bederf verantwoordelik is en hopelik tot beter siektebestuur en minder verliese sal lei.

Summary

We have followed the citrus export chain from on-farm fruit picking to European destinations and to the end of the retail chain. Microbiological surface samples were taken from packhouse and coldroom as well as repacking and distribution coldstore facilities including walls and floors and from conveyer belts when applicable. Hands of fruit handlers (pickers and packers) were also sampled as well as air from all the facilities to determine what level of hygiene was maintained. Our findings were that fruit handling and the high *Penicillium* inoculum levels that fruit are exposed to during export could be an important contributing factor to the high incidence of postharvest decay on the export market. A diverse range of *Penicillium* spp. including the citrus blue and green mould pathogens *P. italicum* and *P. digitatum*, were isolated in some facilities. This emphasises the importance of adhering to stricter hygiene standards throughout the supply

chain in an effort to reduce high inoculum loads. On completion of this study, guidelines concerning the critical areas to monitor for inoculum levels and factors which may contribute to postharvest decay will be recommended. *Penicillium* species diversity data and monitoring guidelines will be recommended to assist the citrus industry in identifying the causal agents of postharvest decay. This will align future research to focus on the species to which citrus is generally exposed to during export. Improved control of these species will ensure longer shelf life and reduced losses on exported citrus fruit.

Introduction

Penicillium is a well-known fungal genus with most species having the ability to colonise almost any surface (Hamada and Fujita, 2002). These species are commonly encountered in most indoor environments with the main purpose of degrading decaying organic matter. Species associated with this genus includes common food and feed spoilers as well as industrial starter cultures used in the fermentation industry (Pitt, 1991). For this reason, a number of *Penicillium* species have been isolated pre- and postharvestly from various types of fruit since harvested fruit is switching from a physiologically growth stage to a stage of senescence prior to decay with a reduction in chemical and physical defence barriers.

The ability of *Penicillium* spores to easily and effectively attach to almost any surface because of the sticky nature of the spores and the ease of the lightweight, aerodynamic spores to move through the air propelled by slight air movements makes hygiene management difficult. It is therefore extremely difficult to manage indoor and outdoor environments and keep surfaces clean from spores produced by these species (Pasanen *et al.*, 1992; Hamade and Fujita, 2002; Fallah *et al.*, 2004). *Penicillium* spores are more prevalent in intermittent wet and humid environments and surfaces. This is generally the case in cold rooms and fruit packhouses. High inoculum levels of these spores in any environment represent a serious contamination risk for fresh fruit that is handled and stored in contaminated facilities. Postharvest spoilage by *Penicillium* spp. results in increased postharvest decay, reduced shelf life and ultimately contributes to major economic losses for the fruit industry.

This study focuses on evaluating the effectiveness of hygiene standards throughout the citrus supply chain from the farm to the fork. The main objective of this study was therefore to determine critical control points that should in future be managed more effectively to reduce decay and improve quality and shelf life. *Penicillium* isolates obtained will be used as hygiene indicators for environmental monitoring and determining the cause of postharvest decay during export. In addition, the study will focus on species dominance and inoculum levels in various environments along the supply chain. The ultimate goal is to develop a rapid PCR-based diagnostic *Penicillium* assay for regular monitoring of hygiene levels and screening *Penicillium* decay pathogens in the citrus export industry.

Materials and methods

Over the past two citrus seasons, prior to the onset of the current study, citrus cold chain studies have been conducted by our research group. The first chain sampling focussed mainly on international hygiene indicators to determine possible cross-contamination points during export. The second sampling focussed more on local sampling for comparative purposes between local and internationally obtained *Penicillium* spp. Different environments along the cold chain such as packhouse and coldroom walls and floors, container walls and floors, fruit handlers' hands and gloves, various surfaces in retail centres and harbours etc. were sampled to detect the presence of *Penicillium* spp. that may serve as contamination points along the supply chain. During 2008, we focussed on late seasonal fruit and the effectiveness of the citrus cold chain. The reason for this was to determine the full extent of *Penicillium* inoculum loads in the chain to which the South African fruit is exposed during the end of the export season when increased losses associated with postharvest decay have been reported.

1. Local sampling

In this trial, local hygiene monitoring and sampling commenced during July 2008 where two local citrus export fruit producing farms in Tzaneen (Mpumalanga) were sampled. Trans-swab samples were taken in the orchard of fruit pickers' hands and bags as fruit destined for export was being picked and transported to the packhouses. As soon as the fruit went through the dip process, swab samples were taken of the packhouse walls, floors, packers and sorters hands, conveyer belts and sorting bins. Taps at the packhouse entrance where hands are washed prior to entering the packhouse were also sampled. Sufficient swabs were taken per sampling point for statistical analysis. All swabs were sealed and labelled for traceability purposes. The fruit that went through the packhouse at the time of sampling were packed into three experimental pallets per farm and normal inspection of pallets for export purposes were conducted. After passing inspection, two pallets per farm (four in total) were transported to the harbour for export and one pallet per farm (two in total) were sent to the University of Pretoria to simulate export conditions without

submitting fruit to the export process. This was done for comparative purposes of fruit quality deterioration during normal export vs. no handling. Fruit quality evaluation of the locally kept pallets and exported pallets was standardised at six and eight weeks after fruit packing.

At all local and international destinations, the relative humidity, temperature inside and outside of facilities, cold storage and general storage areas and containers were determined. Active and passive air sampling was performed to determine the *Penicillium* inoculum loads in the air. Once the pallets had been prepared, data recorders were placed inside the stacked unit to measure the fluctuation in temperature during export. When the fruit was repacked in the European Union, these devices were removed to download the temperature recordings.

2. International sampling

Sampling was performed at the port of Rotterdam which was the entry point of the fruit into Europe. When 10 containers of South African citrus fruit, including our experimental pallets, were opened to be inspected and stored for dissemination throughout Europe, swab sampling was performed in the containers on all wall and the floor areas. Cold room walls and floors at the Rotterdam distribution centre were sampled. If blue or green mould was found on inspected citrus fruit, swab sampling was done. A distribution centre in Antwerp was also sampled in the same way as the other cold store facilities because fruit is often first moved through this centre prior to further distribution. At every point of sampling throughout the export chain, location maps that pin point the sampling points were drawn. Critical observations were recorded at all sampling points and facilities regarding hygiene standards, handling practices and general quality management.

The experimental pallets were followed further down the supply chain and one pallet from each farm of origin was sent to a distribution centre in Hamburg and Luxembourg respectively. At these destinations, walls, floors and surfaces of cold rooms and fruit ripening rooms, distribution and storage areas, repack facilities and conveyer belts (where applicable), were swab sampled. Active and passive air sampling was performed to determine the *Penicillium* inoculum load in the air which could contribute to contamination. As fruit moved through the repack process, swab sampling was done on bags and fruit handlers' hands, where applicable.

In Germany (Hamburg and Stelle), large and small retailers and distribution centres were swab sampled as described previously. At Luxembourg only one retailer and distribution centre could be sampled. The South African fruit was followed to the crates or display tables in the retail centres where swab sampling was performed on these surfaces as well as on the floors. Active and passive air sampling was performed in these locations, which is the end of the supply chain when the consumer buys the fruit.

3. Sample processing

All swab and air samples were sent to the University of Pretoria for analysis. Swabs and plates were kept at 4°C prior to processing. A dilution series was performed on all swab samples where the swab was added to 9 ml of Ringers solution (Merck), agitated and 1 ml transferred to another 9 ml Ringers solution test tube. The dilutions up to 10^{-4} were plated on malt extract agar (MEA) and incubated at 25°C and 37°C for fungal and bacterial total counts respectively. Only the fungal counts will be referred to in this study.

Single *Penicillium* isolates were collected from MEA plates and plated again on MEA for seven days at 25°C to obtain pure cultures. Colony characteristics and exudate production of these isolates were assessed and compared to one another after all isolates were separated into morphological groups. A morphological group is a group of isolates representing the same morphological or physical characteristics such as colony colour, texture, conidia formation, exudate production and the colour of the exudates produced etc. These characteristics are essential for *Penicillium* spp. identification (Pitt, 1991; Samson and Pitt, 2000). All isolates that were obtained anywhere in the citrus supply chain more than once were allocated a species specific group number and a few representative isolates from each group were identified to represent the isolates in further morphological and molecular studies to reduce the thousands of isolates to a workable few hundred. If very slight variations were observed in cultural morphology, isolates were divided into different morphological groups. More than one morphological group may therefore represent the same *Penicillium* species. All of these isolates were preserved in sterile water and glycerol for future use. The verticillate nature of the representative isolates was determined microscopically.

4. Isolate identification

Single spore representative isolates were prepared for molecular identification of isolates. DNA extraction was performed on all representative isolate using the DNeasy Plant DNA extraction kit (Qiagen, USA). For the identification of isolates, both the ITS and β -tubulin gene regions were amplified with the primers ITS 1 and ITS 4 (White et al., 1990) as well as Bt 2A and Bt 2B (Glass and Donaldson, 1995). Components of the PCR reaction were 25 ng of genomic DNA, 200 mM of each of the four dNTPs, 100nM of each

oligonucleotide primer (ITS1/ITS4 or Bt2A/Bt2B), 1 U of *Taq* DNA polymerase and the manufacturer's specified buffer.

Previously developed PCR-RFLP profiles were used for initial screening and identification of isolates with various restriction enzymes (Johnston et al., 2008). For identity confirmation, isolates were subjected to a post-PCR clean up step with MSB Spin PCRapace Kit (Invitex Berlin Germany) and a sequencing PCR was performed. Sequencing of amplicons was conducted with a Big Dye sequence terminator kit (Applied Biosystems, USA). Post-PCR cleanup was done and homology studies were carried out using the NCBI program BLAST (Zhang et al. 2000).

Results and discussion

Thousands of *Penicillium* isolates were obtained from various areas along the cold chain. The largest number of isolates was obtained from active and passive air sampling plates. The aerial microbial load reflects poor hygiene management systems and high levels of surface contamination. This can contribute to postharvest *Penicillium* decay post export. The aerial microbial loads have been a factor of great concern for many years (Pasanen et al., 1992; Hamade and Fujita, 2002; Fallah et al., 2004), which may hold a threat to human and fruit health. As cold storage rooms and facilities along the export chain are cleaned, often with steam only, *Penicillium* spores which have very resistant survival structures are dislodged and freely move around the facility. These conidia attach to micro-droplets in the air and settle on surfaces floors and walls in these facilities. With the high moisture content generally maintained, it makes for the perfect breeding ground for *Penicillium* spp. (Samson and Pitt, 2000; Hamade and Fujita, 2002; Fallah et al., 2004). Controlling aerial microbial loads improves hygiene management and can reduce the risk associated with *Penicillium* contamination during export to a great extent.

Emphasis was placed on the hygiene or contamination levels in containers arriving at the port of entry during this study. Containers were sampled on opening and unloading thereof, and the highest number of isolates obtained in this study from a single focus area of isolation came from container walls followed by container floors. Upon inspection of the pallets, we found that some pallets were covered in areas with *Penicillium* mould. The inoculum is therefore brought into the export market with the fruit in such a case. Very moist conditions with a high relative humidity were generally measured in the problem containers and some fruit were already showing signs of decay starting upon arrival in Europe. Containerisation has great advantages over regular shipment of fruit such as reduced handling and improved temperature and humidity regulation. However, if diseased fruit is placed in a container or *Penicillium* spores are already present, container conditions will promote *Penicillium* spp. attachment, spread, infection and disease development. Greater emphasis should be placed on hygiene management of containers by disinfecting the unit and monitoring for the absence of *Penicillium* inoculum loads. Monitoring the adequacy of cleaning the air-conditioning system is further important from a hygiene management point of view and to prevent condensation in the container.

Distribution centres seem to have a high diversity of *Penicillium* species. This is to be expected since it was often observed that fruit and vegetables from all over the globe are stored side by side in these facilities. Separate cold rooms are assigned for citrus, however lower quality or diseased citrus fruit was stored on the outside of these rooms and in certain areas decaying fruit covered with *Penicillium* spores was left on the floor of the cold room. This contributes to increasing the inoculum load in the facility. Mishandling of fresh produce is very common due to the time constraints placed on the distribution centres and repack facilities to adhere to minimal turnaround time. Mishandling causes wounding and wounds are the main entry point for *Penicillium* species. Greater focus should in future be placed on adhering to hygiene standards and handling practices.

At most of the facilities sampled, it was evident that not enough attention is given to wall, floor and air conditioning system hygiene. A large number of isolates obtained during this study originated from facility floors and especially walls. *Penicillium* species are well-known for their ability to attach to and colonise almost any surface (Hamada and Fujita, 2002). With the moving air currents the spores that form on these surfaces get dislodged and settle down on stored fruit, possibly causing blue or green mould and making the fruit non-marketable. Developing and implementing hygiene standards that addresses wall and floor hygiene as well as more effective air conditioning cleaning is essential in decreasing decay levels by reducing inoculum loads in the export chain.

Almost five thousand *Penicillium* isolates were obtained during the 2008 survey. The most dominant *Penicillium* species on surfaces along the supply chain and in the air were determined and the inoculum level for each species isolated, from all areas sampled, is being analysed. *Penicillium glabrum*, *P. crustosum*, *P. commune*, *P. solitum* *P. brevicompactum*/*P. biourgeianum* and many others including the well-known pathogenic species *P. italicum* and *P. digitatum* were isolated in certain areas of the chain. This data corresponds well with previous supply chain studies conducted by Jacobs et al. (2004). These species are

differentiated from one another through the development of a PCR-RFLP method focusing on the β -tubulin and ITS gene regions. High species relatedness and conserved gene regions between very closely related species such as *P. crustosum*, *P. commune* and *P. solitum* is currently limiting the use of the PCR-RFLP system for such groups. Verification of species identity is currently being confirmed with sequence data. The PCR-RFLP system developed during this study may serve as a precursor for the development of a PCR-RFLP database. However, the method still requires validation against a few type-strains and additional *Penicillium* species which were not found in the supply chain. This system will prove valuable in the fight for fit fruit for increased competitiveness on the export market.

Conclusion

Penicillium spp. are known to be commonly responsible for major postharvest losses in the citrus fruit industry. Citrus export has increasingly been affected by poor handling and cold chain management systems contributing to increased losses and reduced shelf life. Citrus remains one of South Africa's most valuable and economically important export commodities. When the citrus fruit enters the export chain, the South African citrus farmer has little control over the hygiene and handling of the fruit. Similarly, the farmer can not enforce appropriate hygiene standards on various role players along the supply chain even though it is expected of him to implement international food safety standards. In this study it was found that poor hygiene along the supply chain may contribute to increased postharvest decay. Conclusions regarding the inoculum load, handling practices and hygiene standards in various sectors of the citrus supply chain can now be drawn. In this study we have been able to identify critical areas that should be managed more effectively to reduce inoculum levels and improve hygiene. Further the most commonly encountered *Penicillium* species dominant in the citrus supply chain have been identified and provides an insight into the microbial dynamics on a global scale.

Areas with repeatedly high inoculum levels can now be identified as critical control points that should be monitored regularly. Recommendations to the exporting bodies and distribution centre management can be made regarding the handling and 'best practice standards to be implemented in these facilities in order to reduce inoculum levels, minimize postharvest decay and maximize profits. With improved fruit quality from farm to fork, local producers and exporters can increase profit margins and be more competitive on the international export markets.

Technology transfer

Some of the findings of this study have been presented at international conference prior to the citrus industry funding this project by Prof. Lise Korsten and more recently at the CRI's research symposium in 2008. The results will also be presented at the 2010 CRI symposium and 2011 SASPP conference. At least one article will be published in a peer reviewed journal when the currently conducted species identification and identity confirmation is completed.

Further objectives (milestones) and work plan

- Finalise the species identification and identity confirmation for the 2008 season isolates.
- Draw concrete conclusions regarding the critical areas that should be controlled or standards that should be implemented to improve the citrus supply chain.
- Conduct the citrus supply chain again during the late citrus season in 2009; however focus will only be placed on critical areas which were identified during the previous study.
- Follow the citrus export chain and monitor environments for *Penicillium* inoculum loads, contamination points and obtain global isolates for the population genetic studies. Swab samples will be taken at various facilities including walls and floors, packlines, hands of fruit handlers and containers as before. Air samples will also be taken.
- Selected dominant *Penicillium* reference groups to be amplified using PCR and sequenced for species identification purposes.
- Primer development where applicable.
- Determine and re-evaluate critical control points along the citrus supply chain.
- Evaluate and set up standards for export facilities hygiene and cold chain practices.

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4.5.8 FINAL REPORT: Development of alternative disease control products

Experiment PPL 21 (January 2007 - March 2009): by E. Arrebola-Diez, K. Zeeman and L. Korsten (UP)

Opsomming

Tydens 'n poging om nuwe en alternatiewe beheer middels vir die beheer van na-oes sitrus patogene te ontwikkel, is die bakteriese antagonis PPCB002 vanaf sitrus geïsoleer. Ons het die antagonis PPCB002 geïdentifiseer as 'n *Bacillus subtilis* spesie. *Bacillus* spesies is goed gedokumenteer vir die biobeheer van patogene van 'n verskeidenheid vars produkte. PPCB002 is op biochemiese en molekulêre vlak geanaliseer en gevind dat dit ekso-ensieme, antibiotikums, fenoliese agense en vlugtige verbindinge produseer wat betrokke kan wees in die biobeheer werking teen na-oes sitrus patogene. Die lipopeptied bacillomycin is moontlik die hoof inhibeerder wat vir die biobeheer aktiwiteit van PPCB002 verantwoordelik is. Die aktiwiteit van die antagonis is ook as 'n voorkomende en genesende middel getoets. In die studie is gevind dat die voorkomende toediening van PPCB002 die beste opsie is om groen en blou skimmel van sitrus te beheer. Die antagonis vorm ook 'n biofilm rondom die vrug wat die beskermende oppervlak area vergroot vir meer effektiewe beheer. Hierdie antagonis kan dus moontlik na-oes op sitrus vrugte toegedien word indien toetse op kommersiële skaal effektief in die beheer van blou en groen skimmel is.

Summary

In an attempt to develop new and alternative disease control agents for the control of postharvest citrus pathogens, the bacterial antagonist PPCB002 was isolated from citrus and identified as *Bacillus subtilis*. *Bacillus* species are well documented for their use in biological control of various fresh produce. PPCB002 was biochemically and molecularly analysed and it was found to produce exo-enzymes, antibiotics, phenolics and volatile compounds which are known biocontrol mechanisms. The lipopeptide bacillomycin was identified as the most likely principal inhibitor responsible for the biocontrol activity of PPCB002. The activity of the antagonist was tested as a preventative and curative postharvest control treatment on citrus fruit and the preventive application of PPCB002 was the most effective against green and blue moulds. The antagonist also formed a biofilm around the fruit which increases the protective surface area for increased efficacy. This antagonist can possibly be applied to the citrus fruit postharvestly if commercial scale trials prove to be effective in controlling citrus blue and green mould.

Introduction

Often, up to 25% of the total production of harvested fruit is subject to fungal attack in both industrialised and developing countries and the damage is often higher, even exceeding 50% of total fruit production (Spadaro and Gullino, 2004). Citrus fruit are prone to attack by a few postharvest pathogens and some of the most important fungal diseases are green or blue mould. Green mould is caused by *Penicillium digitatum* Sacc. and blue mould by *P. italicum* Wehmer. Infection generally occurs through wounding during picking or handling, which may result in decay onset during storage or marketing.

Synthetic fungicides are the primary means of controlling postharvest diseases (Eckert, 1990). They are used alone, in combination with other products applied separately in sequence (Ismail and Zhang, 2004). However, several fungicides have not been re-registered according to new European Union regulations and is therefore no longer available for use in the postharvest environment. In addition, repeated use of certain systemic fungicides in packhouses has led to the appearance of fungicide-resistant populations (Holmes and Eckert, 1999; Kinay et al., 2007). The need for alternative disease control approaches has encouraged research in the field of biological control resulting in the registration of some biofungicides (Mercier and Jimenez, 2004).

Biocontrol, or the use of microorganisms and their secretions to prevent plant disease, is eco-friendly, safe, and may provide long-term protection to the crop (San-Lang et al., 2002; Fernando et al., 2005). Some saprotrophic bacteria like *Bacillus* spp., can serve as excellent biocontrol agents against plant pathogens. *Bacillus* species, including *Bacillus subtilis*, a ubiquitous soil bacterium, is important in the degradation of organic polymers in soil (Emmert and Handelsman, 1999). They produce spores that are resistant to desiccation, heat, UV irradiation, and organic solvents. *Bacillus* spp. have shown promise in controlling a wide range of fungi that cause decay, operating as an antagonist to plant pathogen growth through their production of antibiotics (e.g. iturin, surfactin, fengycin), enzymes that degrade fungal structural polymers and antifungal volatiles (Fiddaman and Rossall, 1993; Knox et al., 2000; Jiang et al., 2001; Pinchuk et al., 2002; Leelasuphakul et al., 2006). The potential of *B. subtilis* to control postharvest decay was first introduced by the work of Pusey and Wilson (1984) on brown rot of stone fruit. In addition, *B. subtilis* has been recommended by the United States Food and Drug Administration (US FDA) as one of the GRAS (Generally Recognized As Safe) organisms (Denner and Gillanders, 1996) for use in the food industry. *B. subtilis* endospores and its active vegetative products have shown promising activities against citrus postharvest pathogens, therefore the aim of this study was to isolate, identify and test possible bacterial antagonists for its efficacy in controlling postharvest diseases of citrus such as blue and green mould and study its mode of action.

Material and methods

1. Postharvest fungal pathogens tested

All the fungi used in this study are detailed in Table 4.5.8.1. The fungal isolates were taken from the Fungal Collection of Plant Pathology Laboratories, Department of Microbiology and Plant Pathology, University of Pretoria or the Agricultural Research Council (ARC), Plant Protection Research Institute (Biosystematics Division: Mycology). They were routinely grown on malt extract agar (MEA, Merck) or Potato Dextrose Agar (PDA, Merck). Inoculum was prepared by adding 5 ml of sterile deionised water with 0.02% of Tween 80 on the plate where the fungus was grown. Spores were re-suspended by agitating it with a sterile glass rod and the spore suspension was filtered through two layers of cheesecloth. The spore concentration of 10^6 spores/ml was determined by using a haemocytometer for each fungus.

Table 4.5.8.1. Postharvest citrus pathogens used in this study for screening the antagonist PPCB002.

Fungus	Isolate information		Reference
	Isolate code	Host Isolation	
<i>Alternaria citri</i> (Penz.) Mussat.	PPCF001	Isolated from citrus	UP ^a Fungus Collection
<i>Colletotrichum gloeosporioides</i> (Penz.) Penz. & Sacc.	PPRI179	Isolated from citrus	ARC
<i>Penicillium digitatum</i> Sacc	DIC	Isolated from citrus	UP Fungal Collection
<i>Penicillium italicum</i> Wehmer	PPRI5997	Isolated from citrus	ARC
<i>Penicillium crustosum</i> Thom	PPCF101	Isolated from citrus	UP Fungal Collection

UP: University of Pretoria

2. Isolation, identification and screening of antagonistic properties

Bacterial and yeast isolates were obtained from citrus fruit and were tested for their antagonistic properties against fungal postharvest pathogens. One bacterial and yeast isolate was identified to be used in further screening, and the other initially considered as potential isolates were not tested further. In addition although two yeasts were initially identified as very promising and one were further effectively screened in small scale trials the isolate could not be revived after preservation between seasons The bacterial antagonist isolate numbered PPCB002, which was originally isolated from the surface of Valencia oranges (Letaba Estates, Limpopo Province, South Africa) that was not treated with any fungicide were the best performing and used in all subsequent tests. Positive controls were further included for comparative purposes and included

commercial registered isolates such as avogreen. Bacterial isolates were tested for its antifungal properties using a dual culture assay (Yoshida et al. 2001) with common postharvest citrus pathogens. The antagonist was identified by 16S-rRNA sequence comparison. DNA was isolated from antagonist strains using the Illustra™ Bacterial genomicPrep Mini Spin kit (GE-Healthcare, UK). Molecular identification of bacterial isolates were based on analysis of the 16S rRNA region after PCR amplification with primer 41F (5'-GCT CAG ATT GAA CGC TGG CG-3'), and 1486R-P (5'-GCT ACC TTG TTA CGA CTT CGT CCC-3') specific for Gram positives bacteria (Stackebrandt and Goodfellow 1991). PCR products were cleaned using the MSB Spin PCRapace Kit (Invitex Berlin Germany), and the clean PCR product was used for sequencing with the BigDye® Terminator Kit (Applied Biosystems, USA). The resulting PCR products were purified and used directly for sequencing. Homology studies were carried out using the NCBI program BLAST (Zhang et al. 2000). Some of the other *Bacillus* spp were included as controls in certain trials.

3. Enzyme production test

Chitinase activity

The enzyme production was tested using a medium of Frändberg and Schnürer (1994), which was modified to contain 4 % chitin instead of 1.5 % w/v. The medium contained the following: 4 % w/v colloidal chitin from crab shell; 8.6 mM K₂HPO₄; 11.0 mM KH₂PO₄; 2.8 mM MgSO₄·7H₂O; 8.6 mM NaCl; 6.7 mM KCl (Saarchem); 0.9 mM CaCl₂·2H₂O (Fluka, Sigma-Aldrich); 0.05 % w/v yeast extract (Biolab); and 2 % w/v bacteriological agar (Biolab). The pH was adjusted to 6.6. The medium was autoclaved for 15 minutes at 121 °C. The plates were inoculated with the dual culture technique as previously described, incubated at 25 °C and the presence or absence of growth on the minimal medium was noted after seven days. Results were compared qualitatively in relation to the presence or absence of growth.

Extra-cellular amylases

To test for extracellular amylase activity, Petri dishes containing starch medium (Skinner and Lovelock, 1979) were prepared. The medium contained: 50 ml Czapek solution A (94.1 mM NaNO₃; 26.8 mM KCl; 0.2 mM MgSO₄·7H₂O); 50 ml Czapek solution C (23 mM K₂HPO₄ (Saarchem)); 1 ml zinc solution (3.5 mM ZnSO₄·7H₂O (AnalaR, British Drug Houses (BDH)); 1 ml copper solution (2 mM CuSO₄·5H₂O (Pro Analysi, Merck)); 50 ml starch solution (20% w/v starch (Biolab) in distilled water; heated slowly to 70 – 80°C and slowly added to the rest of the media); 1.2% w/v bacteriological agar (Biolab); and 850 ml distilled water. The medium was sterilized for 30 min at 121°C. Plates were inoculated as previously described, incubated at 25°C for three days and then covered with Gram's iodine (Sigma). The presence or absence of clear zones in the agar surrounding the bacterial and fungal growth zone was noted. Results were compared qualitatively in relation to the presence or absence of clear zones.

Lipase activity

To test for lipase activity, Petri dishes containing Tween-80 medium (Skinner and Lovelock, 1979) was prepared. The medium contained: 1% w/v peptone (Biolab); 8.6 mM NaCl (Saarchem); 0.7 mM CaCl₂·2H₂O (Fluka); 0.05 mM bromocresol purple (Pro Analysi) and 1.5 % w/v biological agar (Biolab). The pH was adjusted to 5.4. A 10% v/v Tween-80 stock solution was made by adding Tween-80 (Sigma) to distilled water that was heated to 65 ± 5°C. Both medium and Tween-80 stock solution was autoclaved for 10 minutes at 121°C. The final medium contained 10 ml of the Tween-80 stock solution and 90 ml of the medium before plates were poured. Plates were inoculated as previously described, incubated at 25°C for four days and monitored for change in colour of the medium from yellow to purple-blue. Results were compared qualitatively in relation to whether the medium colour changed or not.

Proteinase activity

To test for proteinase activity, Petri dishes containing casein hydrolysis medium (Skinner and Lovelock, 1979) was prepared. The medium contained: 7.3 mM KH₂PO₄; 6.7 mM KCl; 0.8 mM MgSO₄·7H₂O (Saarchem); 0.7 mM CaCl₂·2H₂O (Fluka); 1% w/v glucose (Sigma); 2.5% v/v skim milk and 1.2% w/v bacteriological agar (Biolab). The pH was adjusted to 5.4 and the medium was autoclaved for 30 min at 121°C. The plates were inoculated as previously described, incubated at 25°C and monitored for four days for the formation of clear zones surrounding growth. The presence or absence of clear zones in the agar was noted. Results were compared qualitatively in relation to the presence or absence of clear zones.

4. Determination of antibiotics produced by the antagonist

Bacterial cultures were grown on medium optimum for lipopeptide production (MOLP) (Ahimou et al. 2000) at 37°C for five days. After five days of incubation, cells were removed by centrifugation at 2500 g for 10 min and the supernatants were extracted with n-butanol (Yazgan et al. 2001). Once the butanol layer completely evaporated, the residue was dissolved in methanol for further chemical analysis. The presence of antifungal

compounds in the antagonists supernatants were determined first by direct inhibitory test using Watman filter discs soaked by 10 µl of enriched lipopeptide extracts against citrus postharvest fungi. Secondly, the methanolic extracts were analysed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis (Romero et al. 2007). The methanolic fractions were analysed by silicon TLC (Razafindralambo et al. 1993) with direct viewing using distilled water spraying as well as bioautography, where the inhibitive activity was displayed by a thin agar layer containing the fungal isolate as indicator organism. Thereafter, analysis was done by reverse phase HPLC (RP-HPLC) using an analytical reverse phase C18 column ultrasphere, (4.6-mm diameter and 150-mm long) (Supelco, Bellefonte, PA 16823, USA) and solutions of 0.05% trifluoroacetic acid in acetonitrile and milliQ water, with a flow rate of 1 ml/min.

5. Determination and quantification of phenolics produced by the antagonist

The antagonist strains and controls were plated out on antibiotic production media (APM) broth (McKeen et al. 1986) and were incubated at 37°C for five days with Niorbiting movement at 100 rpm. The cultures were centrifuged at 2500 g for 5 min and filtrated using Whatman filter paper No 1. The supernatant pH was adjusted to 2.5 by adding acetic acid at 20% final concentration. The phenolic extraction was performed using one volume of diethyl-ether. The upper layer was recovered and evaporated. The precipitates were dissolved in methanol for further analysis. The quantification of phenolics concentration was calculated using the Folin Ciocalteu's reaction (Swain and Hillis 1959, Harborne 1984). Distilled water was used as blank. The visual display of different phenolics produced was assayed on silicon TLC using Ethyl-acetate: methanol: water (8:1:1) as solvent with exposure of the TLC plate under UV light at 254 nm. Phenolic residues dissolved in 10% acetonitrilo were used in HPLC analysis (Department of Plant Production, University of Pretoria).

6. Antagonist and fungal interactions via volatile organic compounds

The antagonist and the strains used as controls were grown on nutrient agar (NA) and incubated at 37°C for 24 h before each assay to obtain fresh cultures. The antagonist strains were plated out on APM (McKeen et al. 1986) as minimal medium using sterile cotton buds and covering the surface completely with spores. Fungal spore suspension (10 µl) adjusted to 6×10^6 spore/ml were applied in the centre of PDA plates. The two base plates were sealed together with Parafilm and incubated at 25°C. APM media plates without bacterial growth were used as the blank control.

7. Biocontrol agent biofilm formation and quantification

The biofilm formation of strain PPCB002 was determined by direct observation using scanning electron microscopy (SEM). The fruit was disinfected by washing it in 0.5% sodium hypochlorite followed by two rinses in water. Two wounds per orange were made on two opposite sides by scrubbing the skin using sterile steel wool. The antagonist culture was grown in nutrient broth, incubated at 37°C for 24 h with a 150 rpm agitation, washed afterwards and re-suspended in the same volume in a biological sterile sticker (Citrosol) dissolved in water 1:400. Thereafter the antagonist was applied by spray, covering the fruit surface completely. Spore suspensions of both *Penicillium spp.* were applied directly to the wounded areas on fruit using sterile cotton buds. For the determination of biofilm formation, inoculated fruit were collected for SEM visualisation after 12h, 24h, 36h, 48h, 3days, 5days and 7days for controls and 1, 2, 3, 5 and 7 days for treatments. Three fruit were removed and two samples per fruit were taken for visualisation at every time interval.

For the quantification of PPCB002 and control strains, an overnight liquid culture of antagonist strains were incubated at 37°C and dilutions containing approximately 10^8 cfu ml⁻¹ were made. The biofilm experiments were carried out following the specifications of Peeters et al. (2008). For biofilm fixation, 100 µl of ethanol was added in each well. After 15 min, the ethanol was removed and the 96-well microtiter plate was air-dried. The quantification of biofim formation was made using the crystal violet assay (Peeters et al. 2008). The absorbance was measured at 595 nm and the quantification was estimated by comparison of absorbance intensity.

8. Application of antagonist to artificially inoculated fruit

Curative and preventive treatments were done on oranges cv. Valencia. For the curative application, the pathogen was applied 24 hours before the antagonist PPCB002 and in the preventive treatment the biological agent was applied 24 hours before the pathogen application. A hundred and twenty oranges were washed with 0.5% sodium hypochlorite for 3 min followed by two rinses in water. The fruit was prepared for

inoculation by inflicting a 1 mm deep wound on opposite sides of a fruit. The bacteria were grown on nutrient broth for 24 hours at 37°C with a 150 rpm agitation. Cultures (10^8 cfu/ml) were centrifuged for 5 min at 2500 g and re-suspended in the same volume of sterile water with a natural sticker diluted 1:400. The bacterial suspensions were sprayed onto the fruit covering the fruit completely. *Penicillium* isolates were grown on PDA plates for 10 days at 25°C whereafter spores were collected and suspended in Ringer with 0.02% Tween-80. The spore concentrations were calculated using a haemocytometer (10^6 sp/ml) and 10 μ l of the suspension was added to every wound. The inoculated fruit were packed into plastics bags containing a wet filter paper to maintain the moisture and lesion measurements and observations were taken after seven days of incubation at 25°C.

9. Statistical analyses

Analysis of variance among averages was performed using the ANOVA statistics test by SPSS 8.0 software for Windows (SPSS Inc., Chicago, IL, USA). Thereafter, applying the least significant difference (LSD) test, differences of $P < 0.05$ were considered to be significant.

Results

1. Antagonist identification and postharvest pathogen inhibition screening

The antagonist 16S sequence comparison with NCBI data base showed a sequence identity of higher than 99% with *B. subtilis* (Table 5.1.1). The bacterial isolate coded PPCB002, which was identified as a possible antagonist during this study, was identified as a *B. subtilis* strain. Moreover the dual culture of PPCB002 against postharvest citrus pathogens listed in Table 4.1.1 has displayed a general inhibitory property against all the fungi listed, presenting PPCB002 as a good applicant as a biological agent.

Table 4.5.8.1. Analysis of 16S gene amplification sequence to determine the antagonist PPCB002 specie.

Strain	Sequence (bp)	Identity (%)	NCBI Microorganism	NCBI Reference	Specie identified
PPCB002	915	99.78	<i>Bacillus subtilis</i>	F121112	<i>Bacillus subtilis</i>

2. Antagonist mode of action

Exo-enzymes

The enzyme secretion (such as chitinase, amylase, lipase and protease) from the biological agent during the biocontrol mode of action has been studied. Of the four different enzymes tested, only amylase and protease production was found.

Antibiotics (Lipopeptides)

The production of lipopeptides from *B. subtilis* has been extensively studied by other authors, therefore the lipopeptide production from PPCB002 and *B. subtilis* strain ATCC55466 (used as control since this strain is found in Avogreen® currently registered as a biocontrol treatment) was analysed in the present study. When extracts enriched in lipopeptides produced by PPCB002 were tested against citrus postharvest pathogens, the inhibition of fungal growth in close proximity to PPCB002 lipopeptide extracts was observed (Figure 4.5.8.1). The HPLC analysis of extracts enriched with lipopeptides showed the presence of two main groups of compounds eluded at 22.3 - 27.8 min and 87.3 - 101.6 min, which were identified as bacillomycin and surfactine respectively by comparison with retention times reported by Romero et al. (2007). To know which lipopeptides produce the fungal inhibition, bioautography on silica TLC was performed (Figure 4.5.8.2). For that, *Penicillium crustosum* was used as a hygiene indicator micro-organism. The results showed an inhibition area from PPCB002 which was identified as bacillomycin by direct observation and Rf comparison with the controls UMAF6614 and UMAF6639, which are producers of bacillomycin and iturin A respectively (Romero et al. 2007).

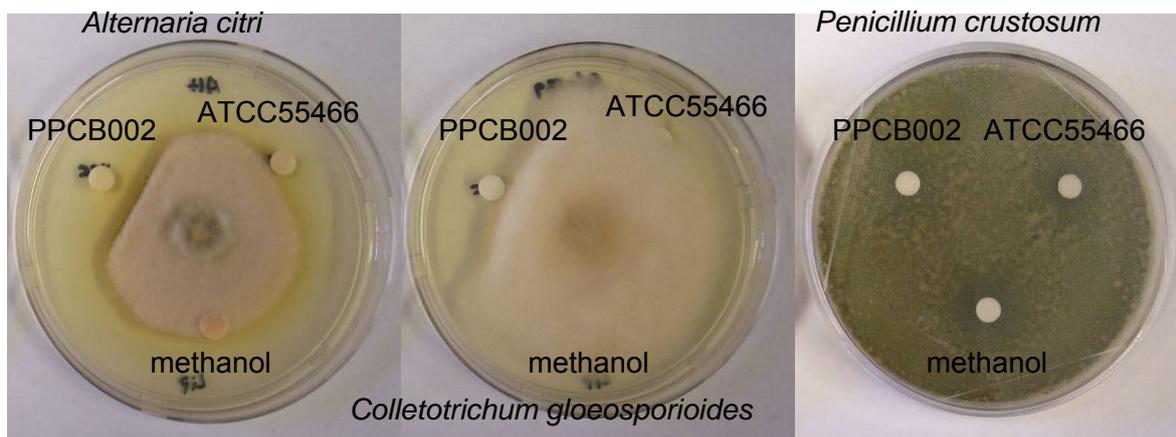


Figure 4.5.8.1. Inhibitory properties of extracts enriched with lipopeptides from *Bacillus subtilis* PPCB002 and *B. subtilis* ATCC55466, used as control, against citrus postharvest fungi. Discs soaked in methanol were used as additional negative controls.

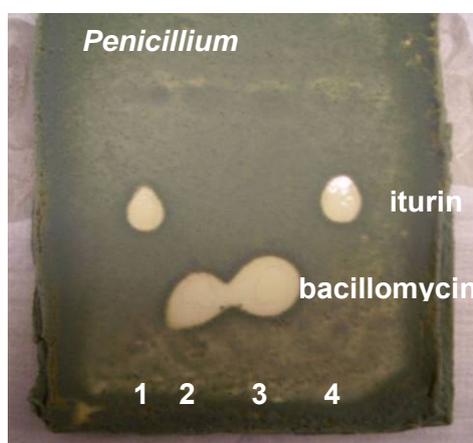


Figure 4.5.8.2. Absence of mycelial growth of *Penicillium crustosum* over silica TLC plate (bioautography) at the spot $R_f = 0.4$ that corresponds to iturin produced by other *Bacillus* spp. such as *B. amyloliquefaciens* PPCB004 (1) and *B. subtilis* UMAF6639 (4). $R_f = 0.3$ corresponds to bacillomycin produced by *Bacillus subtilis* PPCB002 (2) and UMAF6614 (3).

3. Phenolics

Some phenolic compounds could act as inhibitors in biocontrol mode of action, thus the total phenolic production was extracted and analysed in the current study. In the same procedure as lipopeptide production, the antagonist phenolic extracts were compared with ATCC55466 phenolic extracts as control. The phenolic test against postharvest citrus pathogens showed a light inhibition from PPCB002 extracts, displaying *Alternaria citri* as the most sensitive fungus and *Penicillium crustosum* as the least sensitive (Figure 4.5.8.1). Silica TLC displayed the presence of several spots corresponding to different kinds of phenolic compounds when it was exposed to UV light (Figure 5.3.2). However, the bioautographies done on these TLCs did not show any inhibition area to identify which kind of compound or group of compounds could be the inhibitors (data not presented). Finally, HPLC analysis confirmed the presence of different phenolics in the PPCB002 extracts (Figure 4.5.8.3), however, not all could be identified due to a lack of available standards for these compounds.



Figure 4.5.8.1. Inhibitory properties of extracts enriched with phenolics from *Bacillus subtilis* PPCB002 and ATCC55466 used as controls against citrus postharvest fungi. Discs soaked in methanol were used as additional negative controls.

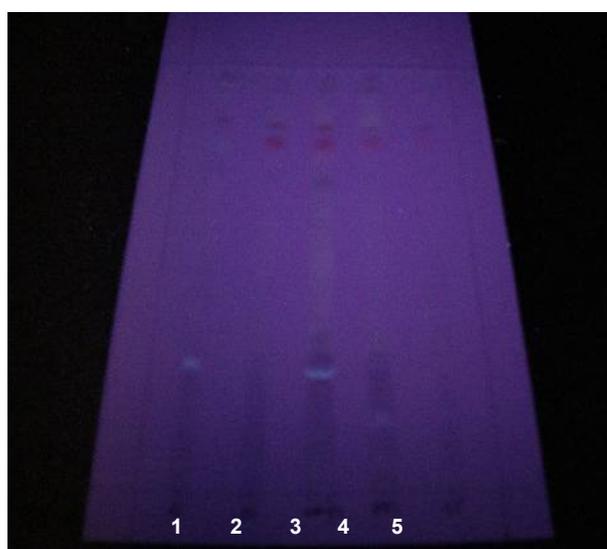


Figure 4.5.8.2. Chromatography displayed on silica TLC plate at 256 nm UV light of phenolic extracts from *Bacillus subtilis* PPCB001 (1), ATCC55466 (3) and PPCB002 (5); *Bacillus amyloliquefaciens* PPCB004 (2) and *Bacillus licheniformis* B251 (4).

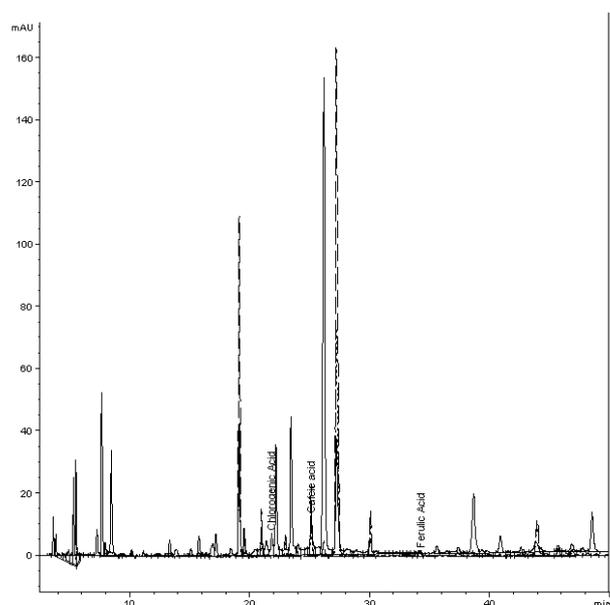


Figure 4.5.8.3. High Performance Liquid Chromatography (HPLC) of *Bacillus subtilis* PPCB002 phenolic extracts.

4. Volatile compounds

The volatile compounds' activity was analysed against citrus postharvest pathogens (Table 4.5.8.1). The comparison of radial growth percentage in presence and absence of PPCB002 gases presented non-significant differences. Therefore the volatile production from PPCB002 did not seem to have a major effect on fungal growth control.

Table 4.5.8.1. Postharvest fungal pathogens growth (mm) during exposure to volatile compounds produced by the antagonist PPCB002.

Fungi	Control	PPCB002
<i>Alternaria citri</i>	100	78.9±5.7a
<i>Colletotrichum gloeosporioides</i>	100	74.8±21.9ab
<i>Penicillium digitatum</i>	100	100±0.0a
<i>Penicillium crustosum</i>	100	77.6±22.0ab
<i>Penicillium italicum</i>	100	87.9±7.9a

5. Biofilm formation

To determine the ability of PPCB002 to form a biofilm, it was quantified and compared to the biofilm formed by the *B. subtilis* strain ATCC55466 (currently used as commercial biocontrol agent), and another *B. licheniformis* strain (B251) (Figure 4.5.8.1). The results showed the biofilm formed by PPCB002 contained more cells than the biofilm produced by the other two *Bacillus* strains, which are known for its biofilm formation. It is therefore possible to conclude that PPCB002 could produce the most dense and thick biofilm protective layer over the fruit. Figure 4.5.8.2 shows *P. italicum* and *P. digitatum* infection in the presence of the biofilm formed by PPCB002 on orange fruit within two days of incubation. The fungal growth development progressed slower or was even curbed when the PPCB002 biofilm was formed.

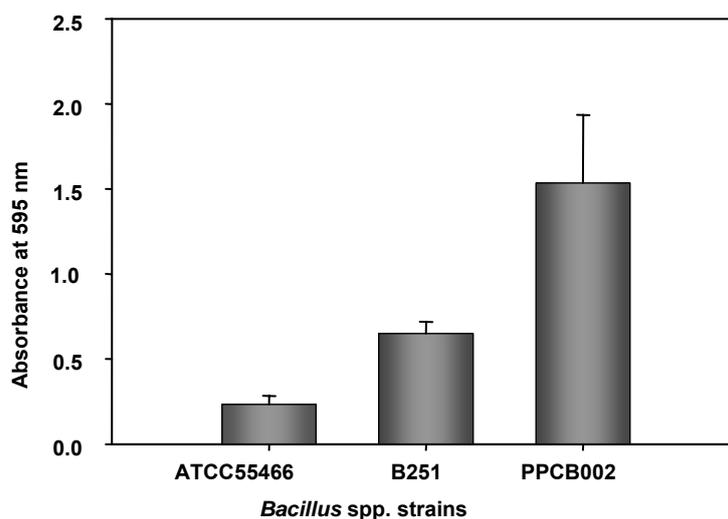


Figure 4.5.8.1. Average absorbance obtained with crystal violet assay on biofilm for *Bacillus subtilis* ATCC55466 and PPCB002, as well as *Bacillus licheniformis* B251. Error bars indicate standard deviations.

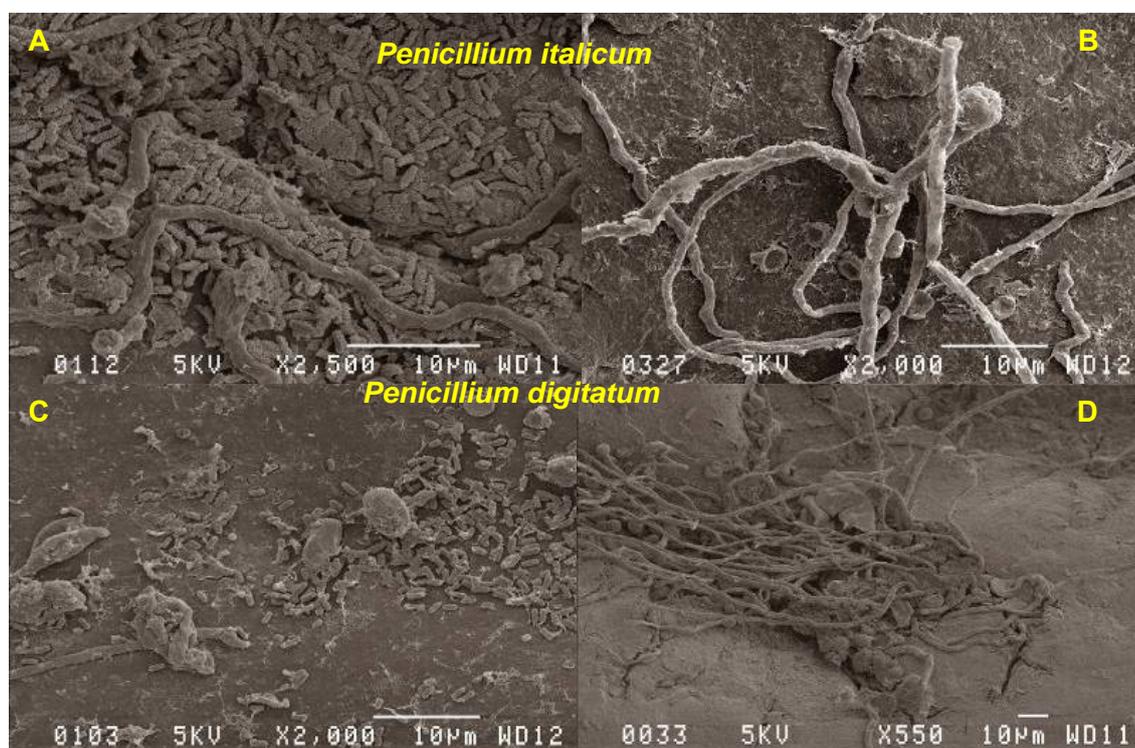


Figure 4.5.8.2. Scanning Electronic Microscopy (SEM) observation of *Penicillium italicum* (A and B) and *P. digitatum* (C and D) development on citrus peel in the presence (A and C) and absence (B and D) of *Bacillus subtilis* PPCB002 biofilm after two days of incubation.

6. Biocontrol application on citrus fruit

The data from an *in vivo* fruit trial showed an essential difference between curative and preventive treatments of citrus fruit with PPCB002 (Figure 4.5.8.3). *Penicillium digitatum* and *P. italicum* colonisation seems to have progressed without any problem in curative biocontrol treatments, with percentages being higher than 80% of disease incidence in every application which was not much different from the control (Table 4.5.8.2). The preventive treatments showed promising results. Even with the natural sticker control containing no bacteria it could reduce the disease incidence by 40-50%. The antagonist *Bacillus subtilis* PPCB002 displayed the best disease control with only 30-20% of disease incidence when the antagonist was applied as a preventative treatment (Table 4.5.8.2).

Table 4.5.8.2. Percentage of disease incidence in oranges treated with *Bacillus* spp. spore suspensions in curative and preventive applications.

Strains	<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
	Treatments		Treatments	
	Curative	Preventive	Curative	Preventive
Non-treated	100a	100a	100a	100a
Control	100a	58b	83a	50b
B251	93.7a	43.7c	100a	56.3b
PPCB001	100a	68.7b	81.3a	31.25c
PPCB002	100a	31.25d	100a	18.7d

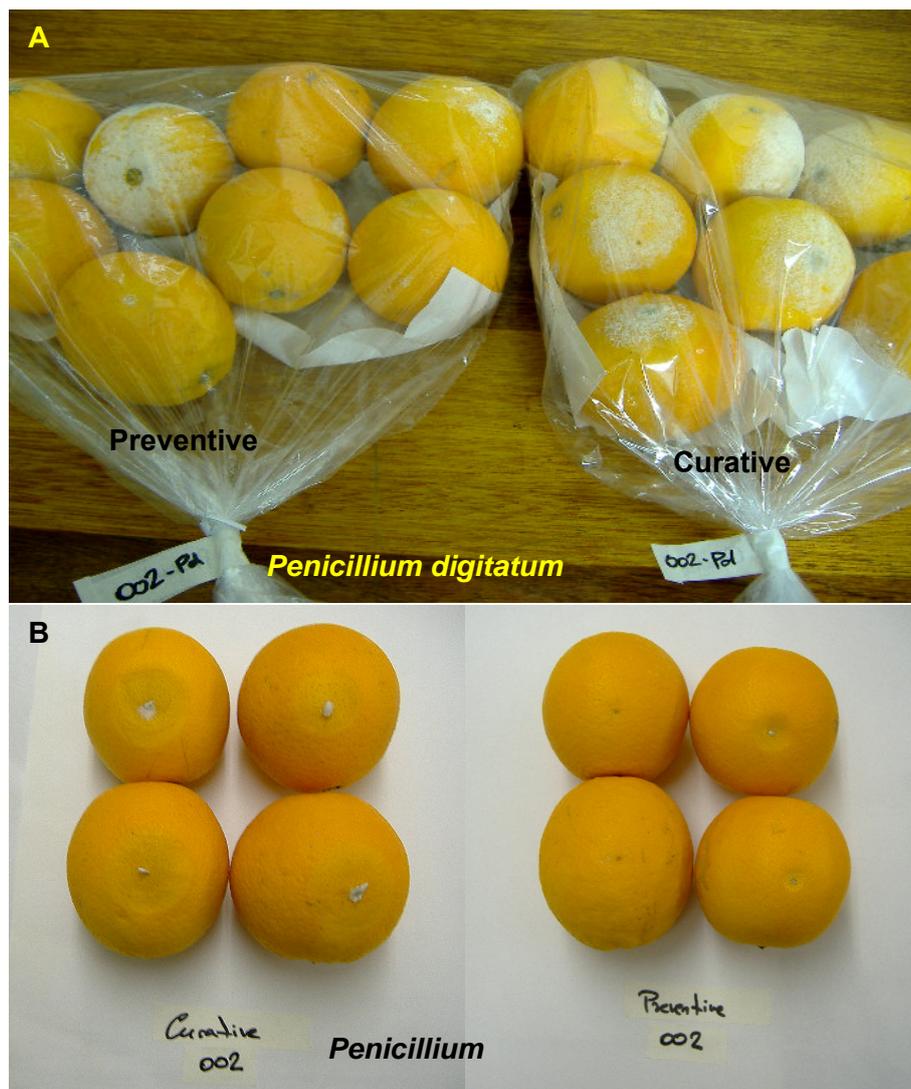


Figure 4.5.8.3. Preventive and curative application of the antagonist PPCB002 in the presence of *Penicillium digitatum* and *P. italicum*. Citrus fruit inoculated with *Penicillium digitatum* (A) and *Penicillium italicum* (B). Curative: PPCB002 applied 24 hrs after fruit inoculation; Preventative: PPCB002 applied 24 hrs prior to fruit inoculation.

Discussion

During the current study, an antagonist PPCB002, was isolated from a citrus fruit surface and it was identified as a *Bacillus subtilis* strain, which drew the attention to research on its application as a biocontrol agent on citrus fruit. The majority of biological control agents that were developed for plant and postharvest disease control have received considerable attention as alternatives to chemical pesticides. Several *B. subtilis* strains have a suppressive effect on certain pathogens and are often used as biocontrol agents

(Ohno et al 1993, Yu et al 2002). *Bacillus subtilis* is considered a potential biocontrol agent due to its high spore production and with spores being more commonly resistant to desiccation, heat, UV radiation and organic solvents (Huang et al. 1992; Romero et al. 2007). These characteristics are essential in the consideration of a biocontrol agent regarding field application and survival on various surfaces. *B. subtilis* was reported to control the growth of a number of plant pathogens through antagonism, and displaying multiple modes of action such as production of antibiotics (iturin, surfactin, fengycin), enzymes that degrade fungal structural polymers (chitinase, β -1,3 glucanase), and antifungal volatiles (Jiang et al. 2001; Pinchuk et al. 2002; Leelasuphakul et al. 2006).

The determination of exo-enzymes produced by PPCB002 has shown the presence of amylase and protease, exo-enzymes normally not associated with fungal cell wall degradation. However, the antagonist is a producer of three families of lipopeptides namely surfactins, bacillomycins D, and fengycins (data not presented). These are well-known secondary metabolites with mainly antifungal activity. The TLC experiments carried out in this study showed that only the lipopeptide bacillomycin has an inhibitory effect on the postharvest pathogens tested. The phenolics produced by PPCB002 seems to inflict a slight toxic effect on fungal pathogens when it was assayed on paper discs, however the chromatographies could not detect which compound was responsible of the toxic effect. Finally the experiments carried out to determine the effect of volatile compound production in the mycelial growth of fungi failed to show inhibition over 25%, therefore it has not been considered as the main mode of action of PPCB002. The main mode of action presented by *B. subtilis* PPCB002 on postharvest fungal pathogens of citrus is therefore determined to be caused by the production of lipopeptides and in particular bacillomycin.

Bacillus spp. forms biofilms, which are multicellular matrices of bacteria surrounded by extracellular polysaccharides called a glycocalyx, on the fruit surface. The glycocalyx acts as a physical barrier and is strongly anionic thereby protecting the microcolony from external agents (Jeyasekaran et al, 2000). Bacteria seem to initiate biofilm formation in response to specific environmental cues, such as nutrient and oxygen availability. These biofilms undergo dynamic changes during its transition from free-living organisms to sessile biofilm cells, including the specific production of secondary metabolites and a significant increase in the resistance to biological, chemical, and physical changes in the environment. Recent studies have suggested that the surfactine production could act positively in biofilm formation, and its mode of action is important for the bacterium's ability to act as a biocontrol agent (Bais et al., 2004). During this study, it was observed by crystal violet assays that the biofilm formed by PPCB002 is organised with more cells than for the other two strains included as controls. Thus, the capacity of attachment is higher in *B. subtilis* PPCB002 and the SEM proved that PPCB002 cells had colonised the fruit surface sufficiently after only two days, providing a protective biofilm barrier on the fruit surface. The biofilm formation and production of antifungal compounds are therefore essential contributing factors to the delay in *Penicillium* spp. mycelia growth and disease development.

The application of PPCB002 as a preventive or curative treatment on artificially infection fruit showed that *P. digitatum* and *P. italicum* development was reduced when the antagonist was already established prior to infection. This is in agreement with other biofilm study results, since the production of an effective biofilm prior to infection could act as a physical and also chemical (bacillomycin production) barrier against fungal infection. The denser the biofilm, the higher the production of lipopeptides which forms an almost impenetrable defence barrier.

Health and safety problems of introducing biological control agents into the human diet has been a factor of great concern. Public acceptance of this technology is generally problematic however, postharvest biocontrol agents are generally isolated epiphytically from fruit and vegetables and are indigenous to that agricultural commodity. Humans are exposed to these agents when consuming fresh fruit and vegetables. Even though these antagonists are introduced in larger volumes to the surface of a commodity, they survive and grow only in very restricted sites on the fruit surface (e.g. surface wounds). After their introduction on intact fruit surfaces, antagonist populations usually diminish to the level of natural epiphytic microflora within a short period of time (Droby et al. 2009). The biocontrol agent PPCB002 studied in the current research has showed great potential as a biocontrol application to control postharvest fungal pathogens. The Environmental Protection Agency (EPA) and European agencies demand basic toxicological tests on the formulated product and efficacy data, including semi-commercial and commercial tests. This is considered as future focus areas of research on this strain.

Conclusion

We have been able to successfully isolate, identify and analyse a bacterial isolate *B. subtilis* PPCB002 as a potential biocontrol agent for the control of postharvest diseases of citrus. The main mode of action of

PPCB002 is its capacity to colonise the fruit surface and produce an effective biofilm and the production of a potent and natural fungicide such as bacillomycin. The chemical production of bacillomycin and the physical biofilm barrier formation, together with the fact that *Bacillus* spores are very resistant to desiccation, heat and UV exposure, makes this antagonist a perfect candidate to be tested further on a commercial scale for its postharvest application as an alternative to chemical control of citrus fruit.

Future research

Due to the potential of this *Bacillus subtilis* strain (PPCB002) it could be important to test it further for eventual use as a biological control agent. We propose that this research should be extended further to include trials on a small and semi-commercial scale and with additional research focussing on formulation and application methods as well as combinations with other products. The product should be tested further as a postharvest dip or spray application to form a protective barrier on the fruit during long term storage or transportation to the export markets. If the product proves to be effective on a semi-commercial scale, it must be tested on a commercial scale at three localities over two seasons for product registration. In addition the secondary metabolites produced by this organism should be further investigated to ensure likelihood of registration. Once commercialised this product could provide an organic alternative for the industry since this *Bacillus* strain occurs naturally on citrus fruit surfaces.

Technology transfer

This work will be presented at the next citrus conference and during a postharvest disease workshop to be held in March 2009.

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4.5.9 PROGRESS REPORT: Screening of South African *Penicillium* isolates from citrus producing regions for resistance to the postharvest fungicides imazalil and guazatine.

Experiment PPL 23 (April 2008 - March 2009): by R. Jacobs and L Korsten (UP)

Opsomming

Die risiko dat 'n patogeen weerstand teen na-oes swamdoders met enkelsetel werking kan ontwikkel, veral in die geval waar die produk vir 'n geruime tyd reeds in die bedryf gebruik word, is groot. Aangesien daar min alternatiewe vir hierdie produkte is, is dit belangrik dat patogeen populasies gereeld vir swamdoder weerstandsontwikkeling getoets moet word. In hierdie studie is *Penicillium* isolate vanaf bederwe vrugte by

verskeie pakhuisse in sitrus produserende areas van Suid Afrika geïsoleer. Isolate van verskillende *Penicillium* spp. is getoets vir sensitiwiteit teen die swamdoders imazalil and guazatine wat algemeen in gebruik is in die sitrus industrie. Isolate is in morfologiese groepe geplaas en patogenisiteit van isolate in die verskillende groepe is bepaal. Heelwat *Penicillium* spp. wat geïsoleer is tydens hierdie studie was sensitief vir hierdie swamdoders, alhoewel *Penicillium* spesies met 'n hoër vlak van weerstand en patogeniese eienskappe ook geïdentifiseer is.

Summary

The risk associated with pathogen resistance development to postharvest fungicides with single-site modes of action, which have been in use for extended periods of time, is high and is an important factor to consider due to the lack of suitable alternative products. As a result of the continuous or excessive use of fungicides, pathogen populations have to be tested regularly for resistance development. During the current study, *Penicillium* isolates were obtained from decaying fruit at various packhouses in citrus producing regions of South Africa. Isolates representing different *Penicillium* spp. were tested for their sensitivity to the fungicides imazalil and guazatine, which are commonly used in the citrus industry. Isolates were separated into morphological groups and the pathogenic ability of isolates in the different groups was determined. Many of the *Penicillium* spp. that were tested during this study showed sensitivity towards these fungicides, however *Penicillium* spp. with a higher level of resistance and pathogenic abilities were also identified.

Introduction

Citrus fruit, once harvested, are known to undergo various chemical and enzymatic changes with the secretion of water and cellular components. This creates the optimal acidic environment for *Penicillium* conidial attachment, germination and colonisation of fruit surfaces (Amiri *et al.*, 2005; Hamada and Fujita, 2002; Brown *et al.*, 2000; Pitt, 1991). *Penicillium* decay is therefore a common phenomenon in the citrus industry. Postharvest blue and green mould of citrus are caused by the *Penicillium digitatum* Sacc. and *P. italicum* Wehm., respectively (Amiri *et al.*, 2005; Hamada and Fujita, 2002; Brown *et al.*, 2000; Pitt, 1991; Cohen, 1989). Blue and green mould contribute to major export and financial losses in the industry annually.

Postharvest disease control is generally limited to the use of chemical fungicides (Eckert, 1990). These fungicides are used separately, in mixtures or applied during consecutive applications on a regular basis. *Penicillium* postharvest decay of citrus is controlled by the fungicides imazalil or guazatine (Zhu *et al.*, 2006; Ismail and Zang, 2004). The development of pathogen resistance to certain fungicides is a well known fact and requires careful management and use of these products, especially given continuous or excessive use of the same chemical fungicide/s. Strong selection pressure and the repeated use of the fungicide can lead to the development of resistant pathogen populations (Zhu *et al.*, 2006; Baraldi *et al.*, 2003; Gubbins and Gilligan, 1999). In order to effectively manage resistance, preventative programmes with key fungicides require continuous monitoring for possible build-up of pathogen resistance and more effective use of these chemicals, and the implementation of new or alternative measures (Holmes and Eckert, 1999; Kinay *et al.*, 2007).

The objective of this study was therefore to identify *Penicillium* species associated with citrus fruit decay and screen *Penicillium* isolates obtained from diseased fruit at various citrus packhouses in citrus producing regions of Southern Africa. Selected species were screened *in vitro* for resistance to the commercially used fungicides, imazalil and guazatine. The pathogenic ability of these *Penicillium* species was also determined.

Materials and methods

1. Isolation

For the first facet of this project, 319 potato dextrose agar (PDA) (Merck) slants containing *Penicillium* isolates were received from KATCO. These isolates were obtained from citrus packhouses during a previous study conducted by KATCO. Upon receipt of the samples, 53% of the isolates were plated onto malt extract agar (MEA) (Merck) amended with chloramphenicol. For the second facet of this project, trans-swabs (five to six per packhouse) were sent in protective envelopes to 250 packhouses in the major citrus producing regions of southern Africa. A method describing aseptic sampling from fruit lesions was included in the envelopes to ensure, as far as possible, that only *Penicillium* spp. was isolated and, the risk of contamination was minimised. All the swabs received were processed by dilution plating of the 10^{-3} to 10^{-6} swab dilutions containing Ringers solution (Merck, SA) onto MEA to ensure that all possible species could be detected and isolated.

2. Morphological and molecular identification

All isolates were grouped into morphological groups based on identical cultural characteristics. Morphological identification of representative isolates was done on three different *Penicillium* species-specific media by comparing microscopic and cultural growth characteristics at different incubation temperatures (Pitt, 1991; Samson and Pitt, 2000). Each morphological group represents a *Penicillium* species with varying morphological characteristics. If a slight variation in cultural characteristics was observed, isolates were divided into another morphological group, therefore more than one morphological group could be representative of the same *Penicillium* species. A representative isolate was chosen randomly from all identical isolates of each morphological group to represent the characteristics of the group in further studies. If a morphological group contained a large number of isolates, more than one representative isolate was chosen to represent that group.

Single conidial isolates were produced from the morphological group representative isolates and were used for molecular identification purposes. Isolates were identified using the PCR-based identification system which focuses on the 16S rRNA gene region (using primers ITS1 and ITS4) and the beta-tubulin gene region (using primers Bt2A and BT2B) (White et al, 1990; Glass and Donaldson, 1995). Isolate identity was confirmed using sequence homology with the program NCBI Blast (Zhang et al. 2000). All isolates were purified and preserved on MEA culture plates, MEA slants and in sterile water.

3. Fungicide sensitivity screening

Penicillium isolates were cultured on MEA from which conidia were obtained to prepare a spore suspension for each isolate, at a concentration of 10^6 spores/ml (determined with a haemocytometer) for inoculation purposes. A 10 μ l droplet was inoculated onto the centre of the Petri dish containing either imazalil or guazatine at the following concentrations: 0 ppm fungicide (control); 0.05 ppm; 0.1 ppm; 0.5 ppm; 1.0 ppm or 2.5 ppm. These plate inoculations were performed in triplicate per fungicide concentration for each of the 28 dominantly isolated morphological groups, including *P. italicum* and *P. digitatum* control plates. Cultures were incubated for eight days at 25°C and culture growth measurements were taken in duplicate on each Petri dish every second day to determine growth inhibition or growth rate in the presence of the fungicide over time. The fungicide screening trial was repeated.

4. Pathogenicity

For the pathogenicity trials, the isolates obtained during the South African citrus packhouse survey was used. One or two isolates were chosen from each group for further trial purposes. Control isolates confirmed as *P. digitatum* and *P. italicum* were included in the trial for comparative purposes. Five citrus fruit (cv. Valencia) were used per isolate tested. Fruit were disinfected by dipping and agitating for 5 minutes in a 0.1% sodium hydroxide solution after which fruit was left to air dry prior to wounding. Fruit were injured by inflicting a 2 x 2 mm artificial wound on opposite sides at the equator of the fruit. Each injury site on each fruit was inoculated by adding 10 μ l of a 10^6 spores/ml suspension (determined with a haemocytometer) for each of the morphological group representative isolates tested. After inoculation, wounds were left uncovered for two hours to ensure spore suspension penetration into the wounded area, after which wounds were covered with Parafilm to prevent contamination. Fruit was left for seven days at ambient temperatures after which infection severity was evaluated by measuring lesion diameters. This trial was repeated twice.

5. Statistical analysis

EC50 values were calculated using culture growth or germination inhibition after 8 day of incubation at 25°C. Statistical analysis was performed using pairwise testing with Fisher's Protected Least Significant Difference (PLSD) test at a $P = 0.01$ significance level, Pathogenicity trials were also statistically analysed using statistical analysis as mentioned above (M. Smith, Head: Agricultural Research Council, ARC-Biometry Unit). All trials were analysed separately. Data of repeated trials were also combined and were analysed together. Where combined data did not differ from data of separate trials, combined data sets were used in this study.

Results

1. Isolation

During a countrywide survey of South African citrus packhouses 486 pure culture isolates were obtained. Thirty four packhouses participated in the latter part of the study. A representative isolate was chosen randomly from all identical isolates of the 94 morphological groups identified. Each isolate chosen to represent the group in resistance studies displayed identical cultural morphology and characteristics.

2. Identification and pathogenicity

All isolates were identified morphologically, using the PCR-based identification system and sequence identity confirmation. The *Penicillium* species identity of the isolates used for resistance testing and pathogenicity

trials is presented in table 2.1. Twelve species representing 88% of the total number of isolates obtained were positively identified at species level (Table 4.5.9.1). The remaining isolates were from single isolates obtained which could not positively be identified both morphologically and molecularly to species level with a high confidence level. The major dominating species obtained were *P. digitatum* (26%), *P. italicum* (23.9%) and *P. crustosum* (18.1%).

All isolates were divided into either a non-pathogenic group which produced no or very small lesions, a virulent pathogenic group and a highly virulent pathogenic group (Table 4.5.9.1). Half of all the isolates tested proved to be pathogenic and virulent or highly virulent on wounded citrus fruit (Table 4.5.9.1). Most of the virulent or highly virulent isolates included the species that were most often isolated namely *P. digitatum*, *P. italicum* and *P. crustosum*. Fruit that were inoculated with the highly virulent species were generally soft and collapsed upon touch (Fig 4.5.9.1 B and D). Isolates that were dominantly isolated or highly virulent on wounded citrus fruit were chosen for further chemical resistance trials, although some non-virulent isolates were also included for comparative purposes (Table 4.5.9.1).

Table 4.5.9.1. Citrus packhouse survey isolate identity and virulence level on wound infected citrus fruits.

<i>Penicillium</i> species	Total number of isolates	Highly virulent	Virulent	Non-pathogenic
<i>P. brevicompactum</i>	21 (4.4%)	0	0	21
<i>P. citrinum</i>	25 (5.2%)	0	10	15
<i>P. crustosum</i>	88 (18.1%)	40	37	11
<i>P. crysogenum</i>	5 (1%)	0	5	0
<i>P. digitatum</i>	126 (26.0%)	115	9	2
<i>P. expansum</i>	1 (0.2%)	1	0	0
<i>P. glabrum</i>	36 (7.4%)	5	0	31
<i>P. italicum</i>	116 (23.9%)	59	45	12
<i>P. olsoni</i>	1 (0.2%)	0	0	1
<i>P. paneum</i>	3 (0.6%)	1	0	2
<i>P. riasrickii</i>	5 (1%)	0	1	4
<i>P. ulaiense</i>	1 (0.2%)	0	1	0
<i>Penicillium</i> sp.	58 (12%)	2	3	55

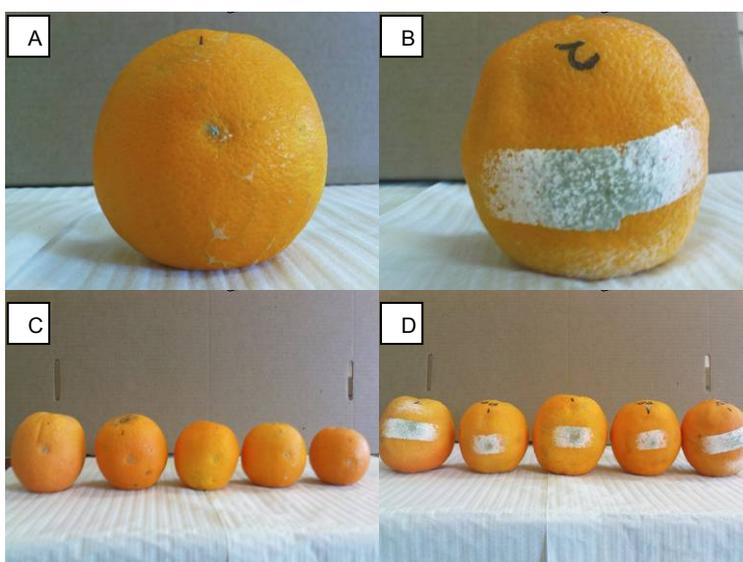


Figure 4.5.9.1. Pathogenicity trials with *Penicillium* species inoculated into wounded citrus fruit. A and C: Non-pathogenic *Penicillium* isolate inoculated into an artificial wound. C and D: Five replicate fruit of the same inoculated isolate. B and D: Growth of a wound inoculated pathogenic *Penicillium* spp.

3. Fungicide sensitivity screening

Penicillium spp. growth on the amended fungicide plates were compared with the control plates to determine growth rate inhibition (Fig 4.5.9.1). EC50 values were also determined to evaluate sensitivity or resistance to the fungicides (Table 4.5.9.1).

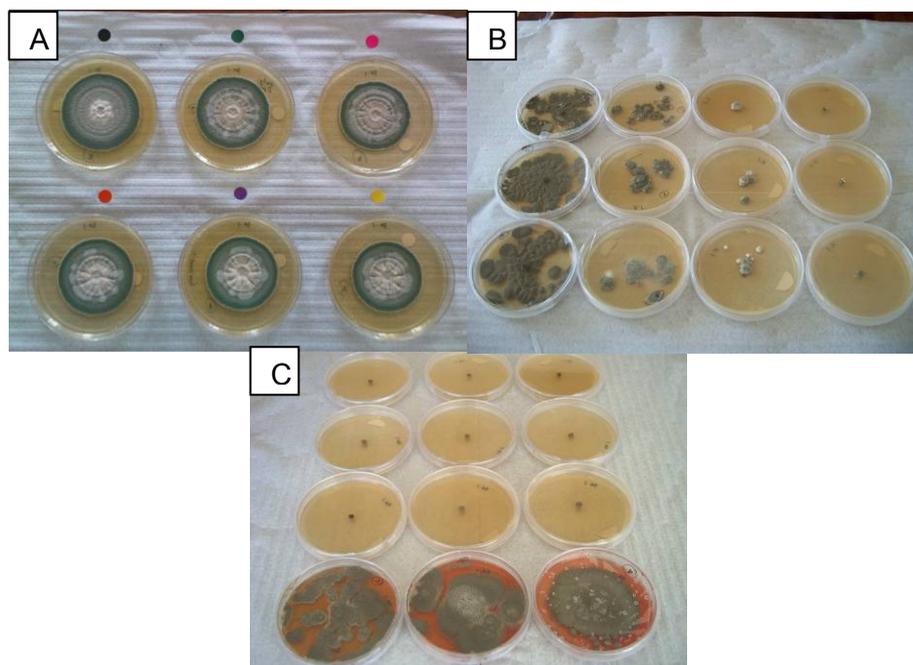


Figure 4.5.9.1. Growth of three different *Penicillium* isolates inoculated onto malt extract agar amended with different fungicide concentrations after eight days incubation at 25°C. A: No growth inhibition in the presence of any of the fungicide concentrations. B: Exponential growth inhibition from the highest fungicide concentration (right) to the lowest (left). C: Total growth inhibition in the presence of all fungicide concentrations. The bottom 3 plates are controls with no added fungicide.

Fungicide sensitivity differed between different species and within a species therefore, average EC50 values were determined for the species tested (Table 4.5.9.1). A higher average level of resistance to both fungicides was found for the species *P. brevicompactum* (Table 4.5.9.1). *Penicillium* species resistance or sensitivity to guazatine and imazalil was determined for the total number of isolates selected per species to conduct the trials. These results are summarised in Table 4.5.9.2.

Table 4.5.9.1. Average EC50 values of dominantly isolated *Penicillium* species sensitivity to the fungicides imazalil and guazatine.

Fungicide	<i>P. italicum</i>	<i>P. digitatum</i>	<i>P. crustosum</i>	<i>P. brevicompactum</i>
Guazatine	0.2054	0.1782	0.2749	1.026
Imazalil	0.2311	0.1312	0.473	4.494

Table 4.5.9.2. Percentage isolates tested in each dominantly isolated species that are sensitive or resistant to the chemicals imazalil and guazatine.

<i>Penicillium</i> species	Imazalil Sensitive	Imazalil resistant	Guazatine sensitive	Guazatine resistant
<i>Penicillium digitatum</i>	80	20	60	40
<i>P. italicum</i>	47.6	52.4	57.1	42.9
<i>P. crustosum</i>	0	100	100	0
<i>P. brevicompactum</i>	33.3	66.6	66.6	33.3

Penicillium digitatum, the most dominantly isolated species seems to be more sensitive to both chemical fungicides. However *P. italicum* displays an approximate 50% resistance in the population tested to both fungicides. *Penicillium crustosum* displayed a high level of resistance to the fungicide imazalil with no resistance to guazatine. This data can, however, only be confirmed when a larger subset of isolates are screened in this species group as was the case for *P. digitatum* and *P. italicum*. *P. brevicompactum* was included as a non-pathogenic control to see the resistance effect and this species, although not pathogenic to citrus, and it displayed a high level of resistance to imazalil and guazatine in all repeat trials.

Discussion

Twelve *Penicillium* species were positively isolated and identified during the current study. These species are the well known citrus pathogens, *P. digitatum* and *P. italicum*, as well as *P. crustosum*, *P. crysogenum*, *P. citrinum*, *P. glabrum*, *P. paneum*, *P. olsoni*, *P. ulaiense*, *P. expansum*, *P. raistrickii* and *P. brevicompactum*. Most of these species are commonly isolated in outdoor or indoor environments such as *P. glabrum*, *P. brevicompactum*, *P. citrinum* and *P. crysogenum* (Green *et al.*, 2005; Fallah *et al.* 2004; Pitt, 1999) and others such as *P. expansum* are pathogenic to other types of fresh produce such as apples and pears (Amiri *et al.*, 2005; Chen *et al.*, 2004; Pitt, 1999; Wilson and Nuovo, 1973). The three dominantly isolated *Penicillium* species include *P. digitatum* representing 26%, *P. italicum* 23.9% and *P. crustosum* representing 18.1% of the total number of isolates obtained during this study. These species dominance may, however, fluctuate slightly with different seasonal stages in the packhouse and seasonal inoculum build-up (Medrela-Kuder, 2003).

Fungicide resistance at population level is not well studied and understood. However, it is of practical importance in the long term efficacy of the product (Gubbins and Gilligan, 1999). Resistance development of *Penicillium* species to other chemical fungicides such as thiabendazole has been reported extensively (Baraldi *et al.*, 2003). Imazalil or guazatine resistant *Penicillium* species have also been reported from citrus sectors globally (Ghosoph *et al.*, 2007; Zhu *et al.*, 2006; Cohen, 1989). Based on statistically obtained EC50 values for *Penicillium* spp. sensitivity to imazalil and guazatine, it was found that the *P. digitatum* isolates were generally sensitive to the imazalil (80%) and guazatine (60%). Some level of *P. digitatum* resistance does therefore exist in citrus packhouses locally. Approximately 50% of the *P. italicum* population tested showed some degree of resistance to both fungicides tested. A very high resistance level to both fungicides was found in *P. brevicompactum*; however, this species is non-pathogenic to citrus and should not pose a treat to the industry unless population shift becomes a factor of concern. Regional fungicide sensitivity profiling is therefore the next step in identifying where the dominantly resistant isolates are more present and to protect regions with lower levels of resistant isolates from moving into an exponential resistance increase over time.

Although *P. digitatum* and *P. italicum* are currently the known pathogens on citrus fruit, results from this study indicate that one of the most commonly found *Penicillium* species in the environment has the ability to cause postharvest decay of citrus. *Penicillium crustosum* is a commonly encountered environmental *Penicillium* species (Pitt, 1999). These isolates were obtained from symptomatic fruit and were shown to be pathogenic during repeat inoculation studies. It was further found that the virulence level of most of the isolates in this group tested was at the same level as that of *P. digitatum* and *P. italicum*. This could pose a potential threat if the resistance to the currently applied fungicide imazalil is a true reflection of population resistance in the South African packhouses. The fungicide sensitivity testing of this species was not an objective of this study, however by analysing the current data, a larger subset of *P. crustosum* isolates should be tested in the near future for species population resistance profiling.

The high inoculum level of *P. digitatum*, *P. italicum* and especially *P. crustosum* in the citrus industry should be of some concern to the industry (Gubbins and Gilligan, 1999). It is therefore important that this aspect be further investigated. We also have to identify problem regions or packhouses where *Penicillium* species have already developed resistance to the fungicides imazalil or guazatine. This may pose a serious threat to citrus fruit production and explain the high postharvest decay levels despite commercial use of fungicides. *Penicillium crustosum* seem to fit this profile and should as a matter of urgency be studied further to determine its actual role and importance in the citrus industry.

Conclusion

We have been able to identify the major sources of postharvest citrus fruit decay in South African packhouses. *Penicillium digitatum*, *P. italicum* and a commonly found species *P. crustosum* was identified as the dominant species, with all species being pathogenic to citrus fruit. In all three species groups, resistance to currently used fungicides were detected, although resistance was often not detected in more than 50% of the isolates tested. Fungicide resistance profiling in species is becoming increasingly important since the dynamics of competition between fungicide resistant and fungicide sensitive strains determine whether or not resistance becomes established to such a degree that it leads to major crop losses (Gubbins and Gilligan, 1999). The fungicide sensitivity and resistance to currently applied and newly developed fungicides should be tested on a larger as well as a commercial scale to determine the continued use and efficacy of the fungicides in citrus postharvest applications. Results of this study should raise questions regarding the future of imazalil and guazatine fungicides in citrus postharvest disease control.

Technology transfer

This work will be presented at the citrus conference and at least one peer reviewed publication will result from this work.

Further objectives (milestones) and work plan

Selected species will be given to the CRI and this will form part of the *in vivo* study to be conducted on a commercial scale. We propose that a larger scale packhouse isolate survey be conducted by focussing only on the pathogenic *Penicillium* species and the resistance within these populations.

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4.6 **PROJEK: SWARTVLEK**
Projek Koördineerder: G.C. Schutte (CRI)

4.6.1 Projekopsomming

Koperoksied wat teen die geregistreerde dosis van 90 g/100 l water met 5- (standaard), 6- en 7-week intervalle gespuit is, was effektief vir die beheer van swartvlek (SSV). Koperoksied wat teen 'n laer dosis van

75 g/100 ℓ water met dieselfde interwalle gespuit is, was ook effektief teen SSV. Vier Nanogreen (koperoksichloried SK-formulasie) toedienings van 100 ml /100 ℓ water en meer, asook spuitprogramme wat uit twee nuwe strobilurin swamdoders soos SYPZ071 and SYP1620, in tenkmengsels met mancozeb en minerale spuitolie (voorafgegaan en afgeluit met 'mancozeb behandeling in Oktober en Januarie, soortgelyk aan die standaard Flint, Cabrio and Ortiva spuitprogramme), het ook uitstekende beheer van SSV gegee. Koperquinolaat se SK formulasie teen 'ndosis van 50 ml /100 ℓ water saam met 0.5% minerale olie het slegs 89.2% skoon uitvoerbare vrugte opgelewer en was die beste van al die koperquinolaat behandelings, wat die BP formulasie insluit. Die BP formulasie se dosisse sal drasties verhoog moet word (>100 g /100 ℓ water) om teen SSV te werk (4.6.2).

Afwisselende toedienings van tenkmengsels bestaande uit Sporekill met óf koperoksichloried óf mancozeb, het goeie beheer van sitruswartvlek (SSV) tot gevolg gehad. In Suid Afrika is benzimidazole soos carbendazim geregistreer in tenkmengsels met mancozeb en minerale spuitolie. Waar mancozeb en spuitolie vervang is met Sporekill, het dit ook tot goeie beheer van SSV tot gevolg gehad. Omdat minerale spuitolie 'n invloed op vrugkleur het, sal minder toedienings daarvan vir kultivars van vroeg ryp word tot groot voordeel wees. In Argentinië en Brasilië waar Sporekill i.p.v. minerale spuitolie in sekere spuitprogramme in kombinasie met verlaagde koper swamdoders vir die beheer van SSV en sitruskanker gebruik was, het dit ook goeie beheer tot gevolg gehad. Dit blyk ook 'n algemene probleem in Suid Amerika te wees om nie by die geregistreerde spuitintervalle te bly nie en word dit gestrek tot ver verby die maksimum van 28 dae (4.6.3).

Vergelykings tussen koperoksied, koperoksichloried en koperhidroksied toon dat koperoksied, wat teen 'n geregistreerde dosis van 90 g/hℓ water gespuit is, 23.58 g metalliese koper per boom (indien dit teen 33 ℓ per boom gespuit word) of 675 dele per miljoen (dpm) per boom behels. Dit is 33 and 3.5% minder metalliese koper per boom in vergelyking met koperoksichloried en koperhidroksie onderskeidelik. Nogtans het die onderskeie koperfungisiede soortgelyke residu-vlakke op vrugte gelaat. Residu-analise oor die 56 dae na toediening op 'Valencia' bome het soortgelyke reënvastheid van die drie koperformulasies getoon en ook dat die geregistreerde spuitinterval van 35 dae behou moet word. 'n Korrelasie van onderskeidelik 76% en 90% tussen koper residu en kwantitatiewe fluoriserende pigment meetings op blare en vrugte is waargeneem, wat hierdie metodologie ter bepaling van spuitbedekking en moontlik ook reënvastheid ondersteun. Die eksperiment word met klein veranderinge aan monsterneming-strategie en data-analise herhaal om sodoende beter interpretasie van die resultate, spesifiek in terme van die afwas en herdistribusie hipotese, moontlik te maak. Reënvastheid en 'ntoename in vrugoppervlak het 'n positiewe bydrae tot die afname in koperresidue gelewer en was meer waargeneem op vrugte as blare. Die hoë koperkonsentrasies (± 150 dpm) wat steeds teenwoordig was na 56 dae toon dat blare as reservoirs mag dien en dat die skielike toename in koperkonsentrasies na 35 dae a.g.v. die afwas van koperresidue vanaf die boonste dele van die blaredak toegeskryf kan word. Die gemiddelde koperkonsentrasie van al die koperformulasies was in die omgewing van 10 dpm na 42 dae (4.6.4).

Daar was probleme met die monitering en lees van die glasskyfies wat gebruik was in die inokulum-monitor by Katco. Inokulum monitering van plase in die Sondagsriviervallei (SRV) toon dat meer SSV op blare in die Addo gebied voorkom as in die ander streke in die SRV. 'n Spoorvanger is in die Addo omgewing geïnstalleer. Weerstoestande en askosporvystellings wat gemonitor is, toon dat slegs een betekenisvolle spoorvystelling met 'n hoë moontlikheid vir infeksie gedurende 11 – 15 November 2008 gemonitor is, asook een met 'n lae infeksie-moontlikheid teen die einde Februarie, begin Maart in 2009. In die algemeen het die Oos Kaap 'n baie droë seisoen beleef, vandaar die lae voorkoms van askosporvystellings (4.6.5).

In 'n veldproef te Letaba waar op die vermindering van inokulumvlakke as deel van 'n holistiese beheerprogram gefokus is, is verskeie produkte ge-evalueer vir hul potensiaal om blaarafval af te breek asook die gevolglike effek op inokulumproduksie. In 'n boord is die effek van verskeie programme wat voor vrugset gespuit is, blaarafval wat behandel is en swamdoderprogramme om vruginfeksie te verhoed, vergelyk. Voorlopige resultate toon dat nie een van die middels wat getoets is, beter afbreek van blare tot gevolg gehad het nie. Die studie toon ook dat carbendazim-bespuittings wat voor vrugset toegedien is, en/of strobilurien bespuittings om vrug infeksie te verhoed, wel inokulum verminder het. Behandeling van blaarafval met Breakdown All + Compost Aid het wisselvallige resultate opgelewer (4.6.6).

Project Summary

Cuprous oxide sprayed at the registered rate of 90 g/100 ℓ water with 5- (standard), 6- and 7-week intervals was effective for the control of CBS. Cuprous oxide sprayed at even a lower rate of 75 g/100 ℓ water with 5- (standard) and 6-week intervals was also effective for the control of CBS. Four Nanogreen (copper oxychloride SC formulation) applications of 100 ml /100 ℓ water and more, as well as spray programmes consisting of two new strobilurin fungicides, viz., SYPZ071 and SYP1620, in tank mixtures with mancozeb and mineral spray oil (preceded and ended with standard mancozeb treatments in October and January,

similar to the standard Flint, Cabrio and Ortiva recommendations), gave excellent control of CBS. Copper quinolate as a SC formulation performed the best against CBS at a rate of 50 ml /100 l water with 0.5% mineral oil. It resulted only in 89.2% clean exportable fruit. This is however not acceptable in a zero tolerance market. The WP formulation performed poorly in the trial and a drastic increase (>100 g /100 l water) in the rates should be considered in new field trials (4.6.2).

Alternated tank mixtures applications consisting of Sporekill with either copper oxychloride or mancozeb (both at reduced rates), proved to be effective for the control of CBS. In South Africa, benzimidazoles such as carbendazim, is registered in tank mixtures with mancozeb and mineral spray oil. When mancozeb and spray oil is replaced with Sporekill, the latter also resulted in excellent control of CBS. Because mineral spray oil has an influence on fruit colour, less oil applications will be beneficial to early maturing cultivars. In Argentina and Brazil, certain spray programmes where Sporekill was used in combination with reduced rates of copper and mancozeb instead of mineral spray oil for the control of CBS and citrus canker, also provide excellent control. It seems to be a common problem in South America not to stick to registered spray intervals as they are stretched beyond the maximum of 28 days (4.6.3).

Comparisons between cuprous oxide, copper oxychloride and copper hydroxide formulations used in this experiment, showed that the registered rate of cuprous oxide at 90 g/hl water resulted in 23.58 g metallic copper per tree (if it is sprayed at a rate of 33 l per tree) or 677 ppm. This is 33% and 3.5% less metallic copper per tree than for copper oxychloride and copper hydroxide, respectively. However, similar residue levels were recorded on the fruit for the respective copper fungicides. On leaves, cuprous oxide deposited significantly more copper residues than copper oxychloride and copper hydroxide. Copper residue analyses over the period of 56 days as sprayed on 'Valencia' trees showed that none of the copper fungicides were more rainfast than the other, as copper residues decreased at the same tempo. Based on these results, it also seems that the registered 35-day interval for copper fungicides should be maintained. A 76% and 90% correlation was observed between the copper residue analysed and the quantitative fluorescent pigment measurements on leaves and fruit, respectively, which supports this methodology as an effective tool for spray deposition assessment and potentially also as rainfastness assessment. The trial will be repeated for another year on another cultivar with some changes to sampling strategy and data analyses to allow better interpretation of the results, specifically with regard to the wash-off and redistribution hypotheses. Rainfall and the increase in fruit size had an influence on the decrease in copper residue over the same period, more so from fruit than from leaves. The high concentration of copper on the leaves (\pm 150 ppm) still left on day 56 in all the treatments shows that they might act as a reservoir and that the sudden increase in copper levels on the leaves after 35 days of the copper treatments might be due to the wash-off of copper residues from higher up in the canopy after rain. On the other hand, the mean copper residues of all the copper fungicides left on the fruit on day 42, were in the region of 10 ppm (4.6.4).

There was some difficulty in the monitoring and reading of the glass slides used in the inoculum monitor at Katco. Inoculum monitoring of farms in the SRV with an inoculum-monitor, showed that there was more CBS in the Addo region. A spore trap was installed in the Addo region of the Sunday's River Valley. Weather and ascospore releases were monitored. Only one significant ascospore release with a high probability for infection was recorded in Addo and took place from 11-15 November 2008; another one with a lower infection probability was recorded at the end of February and the beginning of March 2009. In general, the Eastern Cape experienced an extremely dry summer with little rainfall and therefore very little ascospore releases took place (4.6.5).

A study focused on reducing CBS inoculum levels as part of a holistic management program instead of relying on fruit protection alone. Several products were evaluated for their ability to degrade leaf litter and for effects on inoculum production. In an orchard trial, several programmes applied before fruit set, treatment of leaf litter and standard fungicide programmes to prevent fruit infection were studied. Preliminary results showed that no difference in degradation of leaf litter existed for the products evaluated, but several factors contributed to large variation within treatments. The results furthermore showed that pre-fruit set carbendazim, and/or strobilurin applications to protect fruit, might reduce inoculum, while variable results were obtained with Breakdown All + Compost Aid (4.6.6).

4.6.2 FINAL REPORT: Evaluation of a newly developed fungicide, new copper and mancozeb formulations, tank mixtures of strobilurins with copper fungicides and several different adjuvants for the control of citrus black spot on Valencias

Experiment 880 (September 2006 – June 2008): by G.C. Schutte and C. Kotze (CRI)

Opsomming

Koperoksied teen die geregistreerde dosis van 90 g/100 l water en gespuit met 5- (standaard), 6- en 7-week intervalle was effektief vir die beheer van swartvlek (SSV). Koperoksied wat teen 'n later dosis van 75 g/100 l water gespuit is met dieselfde intervalle, was ook effektief teen SSV. Vier Nanogreen (koperoksichloried SK-formulasie) toedienings van 100 ml /100 l water en meer asook spuitprogramme wat uit twee nuwe strobilurin swamdoders soos SYPZ071 and SYP1620, in tank mengsels met mancozeb en minerale spuitolie wat voorafgegaan en afgeluit is met 'n enkele standaard mancozeb behandeling in Oktober en Januarie (soortgelyk soos die standaard Flint, Cabrio and Ortiva spuitprogramme), het ook uitstekende beheer van SSV gegee. Koperquinolaat se SK formulasie teen 'n dosis van 50 ml /100 l water saam met 0.5% minerale olie het slegs 89.2% skoon uitvoerbare vrugte opgelewer en was die beste van al die koperquinolaat behandelings wat die BP formulasie insluit. Die BP formulasie se dosisse sal drasties verhoog moet word (>100 g /100 l water) om teen SSV te werk.

Summary

Cuprous oxide sprayed at the registered rate of 90 g/100 l water with 5- (standard), 6- and 7-week intervals was effective for the control of CBS. Cuprous oxide sprayed at an even lower rate of 75 g/100 l water with 5-(standard) and 6-week intervals, was also effective for the control of CBS. Four Nanogreen (copper oxychloride SC formulation) applications of 100 ml /100 l water and more, as well as spray programmes consisting of two new strobilurin fungicides, viz., SYPZ071 and SYP1620, in tank mixtures with mancozeb and mineral spray oil which were preceded and ended with standard mancozeb treatments in October and January (similar to the standard Flint, Cabrio and Ortiva recommendations), gave excellent control of CBS. Copper quinolate as a SC formulation performed the best against CBS. Promising results were achieved where mineral spray oil was added to the tank with the SC formulation at a rate of 50 ml /100 l water with 0.5% mineral oil, resulted only in 89.2% clean exportable fruit. This is, however, not acceptable in a zero tolerance market. The WP formulation performed poorly in the trial and a drastic increase (>100 g /100 l water) in the rates should be considered in new field trials.

Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa), affects all commercial citrus cultivars only in the summer rainfall regions of the world. Control of the disease is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in the southern hemisphere. The most important inoculum source of CBS is airborne ascospores. Pseudothecia of the fungus develop on dead infected leaves on the orchard floor within 40-180 days after leaf drop, depending on the temperature and frequency of wetting. Once mature, ascospores are discharged during rain spells. Ascospores are dependent on converging currents and favorable environmental conditions to reach a suitable host substrate, since the maximum vertical distance of ascospore ejection from a pseudothecium is 10-12 mm and the horizontal disease dispersion occurs at distances below 24.7 m. When protective fungicides such as copper and dithiocarbamates are used to control CBS, spray applications have to be carefully timed to coincide with the critical infection period. Spore trapping with an Interlock volumetric spore trap® and sampler is used to determine the first onset of ascospore release in South Africa.

A four-spray programme of copper fungicides used for CBS control can result in rind stippling and darkening of blemishes. However, alternating copper fungicides with mancozeb in a four-spray programme, solved this problem. Protective fungicides became less popular after the release of post-infection benzimidazole fungicides such as benomyl. In 1971, the introduction of a single benomyl application in a tank mixture with mancozeb and mineral spray oil came as a breakthrough as it replaced copper and dithiocarbamates that must be applied in a four-spray protective schedule (9). Since the detection of *G. citricarpa* resistance to benomyl in South Africa in 1981, emphasis has shifted back to the use of contact fungicides for disease control. Field evaluations using strobilurins for the control of CBS in 1993 also came as a breakthrough. Two applications of kresoxim-methyl and azoxystrobin at respective rates of 0.10 and 0.075 g a.i./liter in tank mixtures with mancozeb (1.2 g a.i./liter) and mineral oil (0.5% [vol/vol]/liter of water) were initially recommended. The possibility that CBS may develop resistance to the strobilurins, justifies the incorporation of two additional mancozeb before and after the strobilurin applications in October and January.

Since the registration of strobilurins in South Africa in 1999, no new fungicides have been registered for use against CBS. Testing of novel control measures against CBS is therefore regarded as a priority even if it includes tank mixtures with current registered fungicides. The aim is to evaluate any new potential fungicides for the control of CBS.

Materials and methods

Three orchards (Brits 1, 2A and 2B) were selected at Croc Valley Citrus Co. to do the evaluations. A randomised design with 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Spray volumes will vary according to the size and canopy density of the tree but all trees will be sprayed to the point of run-off. Certain treatments will commence in mid-October as previously recommended, depending on the climatological information required for infection during the critical infection period. Trees in both groves were selected for uniformity in canopy density and tree size. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data.

At fruit maturity in July or August, CBS severity will be rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportional data will be analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

Results and discussion

a) Avima

i) Efficacy of cuprous oxide (Nordox) at different spray intervals

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.6.2.1) show that there were no significant differences ($P < 0.05$) between all the criteria used for evaluation and the standard registered mancozeb, cuprous oxide and copper hydroxide spray programmes and the spray intervals. All these treatments were sprayed during the susceptible period from October to January for CBS. Disease pressure was high as the untreated control resulted in only 30% clean exportable fruit. No statistical significant differences were observed between any of the fungicide treatments in the other two criteria, but they were, however, significant different from the untreated control.

ii) Efficacy of cuprous oxide at lower than registered rates

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.6.2.2) show that there were no significant differences ($P < 0.05$) between all the criteria used for evaluation and the standard registered mancozeb and copper hydroxide spray programmes and the registered spray intervals. The same scenario was observed with the reduced rate of cuprous oxide (75 g/100 l water) sprayed at 5 and 6 week intervals as well as cuprous oxide sprayed at a higher rate of 150 g/100 l water). All these treatments were sprayed during the susceptible period from October to January for CBS. No statistical significant differences were observed between any of the fungicide treatments in the other two criteria, but they were however significant different from the untreated control.

b) Tsunami

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.5.2.3) show that there were no significant differences ($P < 0.05$) between all the criteria used for evaluation and the standard registered mancozeb and copper hydroxide and all the spray programmes.

i) Nanogreen

Nanogreen (copper oxychloride SC) tested at the highest rate of 200 ml/100 l water, resulted in 99.4% clean exportable fruit. The other two rates of 150 and 100 ml/100 l water resulted in 98.4 and 97.2 % clean exportable fruit, respectively, and was not significant different from the highest rate of 200 ml/100 l water (Table 4.6.2.3). Nanogreen should be retested for the control of CBS to see if similar results can be obtained.

ii) SYPZ071

Interesting results were obtained showing that irrespective of the rate used, all spray programmes resulted in >96.6% clean exportable fruit with the best treatment and also the lowest rate (20 ml) resulting in 99% clean

exportable fruit. They were also not significant different ($P < 0.05$) from each other (Table 4.6.2.3). This new strobilurin should be retested.

iii) SYP1620

The same scenario was observed with this new strobilurin fungicide as described above. All the SYP1620 rates (80 ml, 100 ml, 120 ml, 200 ml/100 l water) in the spray programmes tested were also not significant different from each other (Table 4.6.2.3). This new strobilurin should also be retested.

iv) Syllit

Syllit (dodine; 40% SC) was not significant different from the standard mancozeb treatment, and a differences of 6.4% in the amount of clean exportable fruit was recorded between these two treatments. On the other hand, Syllit was however significant different from the copper hydroxide standard treatment (Table 4.6.2.3).

v) Trimangol

Trimangol (maneb/zinc oxide 43.5/4,7 g/l SC) was used as a replacement for mancozeb as the first and last application (4th) in the spray programme where Ortiva plus mancozeb and mineral spray oil was used. It showed a lack of control, which can be ascribed to the early onset of the first summer rain as spray interval of 28 days could not protect the fruit successfully (Table 4.6.2.3).

c) Quimico

i) Copper quinolate (WP & SC)

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.6.2.4) show that there were no significant differences ($P < 0.05$) between the criteria clean exportable fruit as well as 4 and more CBS lesions/fruit used for evaluation and the standard registered copper hydroxide (Kocide 2000) treatment, mancozeb and the copper quinolate SC formulations. Copper hydroxide was, however, significant different from the copper quinolate WP formulation tested at rates of 35, 50 and 100g/100 l water. Both copper quinolate SC formulations sprayed alone (75 ml/100 l water) and in combination with mineral spray oil (50 ml + 250 ml/100l water) were not significant different from the copper quinolate WP formulation tested at rates of 50 and 100 g/100l water. They were both significant different from the copper quinolate WP formulation tested at 35 g/100 l water.

The copper quinolate WP formulation showed a dosage response with the highest rate of copper quinolate resulting in 76.8% clean exportable fruit. No statistical significant differences were observed between any of the fungicide treatments in the criterion fruit with 1 to 3 CBS lesions. Disease pressure was high as the untreated control resulted in only 30% clean exportable fruit and all treatments were significant different from the untreated control.

Conclusion

Cuprous oxide (90 g/100 l water) sprayed with 5- (standard), 6- and 7-week intervals were effective for the control of CBS. Although not significant, a 4-5% difference in clean exportable fruit was observed between the 5- and 7-week intervals with both copper fungicides. This is, however, significant with regards to export markets where they require a zero tolerance towards citrus black spot. On the other hand, when growers do run into difficulty in protecting their trees from citrus black spot infection during the onset of the rainy season, then they can extent the spray intervals to 6 weeks to give them enough time to proceed with their spray programmes. It will be interesting to see if this finding will correspond with the residue and fluorometry data from Experiment 918.

Cuprous oxide (75g/100 l water) sprayed with 5- (standard) and 6-week intervals were effective for the control of CBS. Although not significant, a 6-7% difference in clean exportable fruit was observed between the 5- and 6-week intervals with both copper fungicides. This is, however, significant with regards to export markets where they require a zero tolerance towards citrus black spot.

Four Nanogreen (copper oxychloride SC) applications and spray programmes consisting of two new strobilurin fungicides, viz., SYPZ071 and SYP1620, in tank mixtures with mancozeb and mineral spray oil, which were preceded and ended with standard mancozeb treatments in October and January (similar to the standard Flint, Cabrio and Ortiva recommendations), gave excellent control of CBS and can be considered

for registration if repeated field trials yield similar results. Syllit was only tested at one rate and should be retested at higher rates to see if an improvement in control can be achieved. Trimangol (maneb) showed a lack of control, which can be ascribed to the early onset of the first summer rain and a spray interval of 28 days could not protect the fruit successfully.

Copper quinolate as a SC formulation performed the best against CBS resulting in between 87 - 89% clean exportable fruit. This is, however, not acceptable in a zero tolerance market. Promising results were achieved where mineral spray oil was added to the tank with the SC formulation and should be investigated further. New trials should be planned where higher rates of the SC formulation should be tested. The same applies for the WP formulation. In this case, a drastic increase in the rates should be considered in new field trials.

Future research

There is a constant need to evaluate new and old fungicide formulations as well as fungicides that may possess activity against citrus black spot (CBS). Chemical companies frequently modify and upgrade their old products to possess new characteristics such as rain fastness and particle size and they need to be re-evaluated for efficacy. Searching for new fungicides or fungicides with new characteristics as well as some new ideas how we can alter aspects of old fungicide spray programmes to be included in effective spray programmes and to cope with fungal resistance strategies at the same time. Searching for and experimenting with cheaper and more effective fungicides will contribute a lot to reducing production costs and be more environmentally friendly and sustainable with regard to resistance development.

Technology transfer

Talks at study groups. Results will be presented on the bi-annual CRI Symposium in August 2010.

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Table 4.6.2.1. Evaluation of cuprous oxide and copper hydroxide at different spray intervals from October and January during 2007 and 2008 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Spray interval	Treatment (rate/100 ℓ water)	Percentage of fruit in each class ^w		
		Lesions/fruit		
		0	1-3	≥4
5 weeks	Cuprous oxide (90 g) ^x	99.6 a	0.2 a	0.2 a
5 weeks	Copper hydroxide (200 g) ^x	98.2 a	1.4 a	0.4 a
4 weeks	Mancozeb (200 g) ^w	96.4 a	2.6 a	1.0 a
6 weeks	Cuprous oxide (90 g) ^y	96.4 a	0.8 a	2.8 a
6 weeks	Copper hydroxide (200 g) ^y	96.0 a	0.4 a	3.6 a
7 weeks	Cuprous oxide (90 g) ^z	95.0 a	1.0 a	4.0 a
7 weeks	Copper hydroxide (200 g) ^z	94.6 a	1.4 a	4.0 a
	Control	30.0 b	12.4 b	57.6 b

^y Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^w Spray dates were 15 October 2007, 12 November 2007, 10 December 2007, 7 January 2008 (4 weeks)

^x Spray dates were 15 October 2007, 19 November 2007, 21 December 2007 and 28 January 2008 (5 weeks)

^y Spray dates were 15 October 2007, 26 November 2007 and 7 January 2008 (6 weeks)

^z Spray dates were 15 October 2007, 3 December 2007 and 21 January 2008 (7 weeks)

Table 4.6.2.2. Evaluation of cuprous oxide (at lower than registered rates), mancozeb and copper hydroxide at different spray intervals from October and January during 2007 and 2008 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Spray interval	Treatment (rate/100 ℓ water)	Percentage of fruit in each class ^w		
		Lesions/fruit		
		0	1-3	≥4
5 weeks	Cuprous oxide (150 g) ^y	99.2 a	0.4 a	0.4 a
5 weeks	Cuprous oxide (75 g) ^y	99.2 a	0.8 a	0.0 a
5 weeks	Copper hydroxide (200 g) ^y	98.2 a	1.4 a	0.4 a
4 weeks	Mancozeb ^x	96.4 a	2.6 a	1.0 a
6 weeks	Cuprous oxide (150 g) ^z	94.8 a	1.8 a	3.4 a
6 weeks	Cuprous oxide (75 g) ^z	92.6 a	2.2 a	5.2 a
	Control	30.0 b	12.4 b	57.6 b

^w Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^x Spray dates were 15 October 2007, 12 November 2007, 10 December 2007, 7 January 2008 (4 weeks)

^y Spray dates were 15 October 2007, 19 November 2007, 21 December 2007 and 28 January 2008 (5 weeks)

^z Spray dates were 15 October 2007, 26 November 2007 and 7 January 2008 (6 weeks)

Table 4.6.2.3. Evaluation of various fungicides sprayed at different spray intervals from October and January during 2007 and 2008 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment	Rate/100 ℓ water	Application dates						Percentage of fruit in each class ^z		
		15 Oct 2007	12 Nov 2007	10 Dec 2007	21 Dec 2007	7 Jan 2008	4 Feb 2008	Lesions/fruit		
								0	1-3	≥4
Mancozeb	200 g	x	x	x		x		96.4 ab	2.6 a	1.0 a
Copper hydroxide ^x	200 g	x	x	x		x		98.2 a	1.4 a	0.4 a
Nanogreen	200 ml	x	x	x		x		99.4 a	0.6 a	0.0 a
Nanogreen	150 ml	x	x	x		x		98.4 a	1.6 a	0.0 a
Nanogreen	100 ml	x	x	x		x		97.2 ab	0.6 a	2.2 a
MZ/ SYPZ071+MZ+oil/	200g / 20 ml + 150 g + 500 ml	x			x		x	99.0 a	0.4 a	0.6 a
MZ/ SYPZ071+MZ+oil	200 g/ 30 ml +150 g + 500 ml	x			x		x	96.6 ab	1.2 a	1.4 a
MZ/ SYPZ071+MZ+oil	200 g/ 60 ml +150 g + 500 ml	x		x			x	99.0 a	0.4 a	0.6 a
MZ/ SYP1620+MZ+oil	200 g/ 80 ml+150 g + 500 ml	x			x		x	95.4 ab	3.2 a	1.4 a
MZ/ SYP1620+MZ+oil	200 g/ 100 ml +150 g + 500 ml	x			x		x	96.6 ab	2.0 a	1.4 a
MZ/ SYP1620+MZ+oil	200 g/ 120 ml +150 g + 500 ml	x			x		x	96.8 ab	1.6 a	1.6 a
MZ/ SYP1620+MZ+oil	200 g/ 200 ml+150 ℓ + 500 ml	x			x		x	91.4 ab	1.6 a	7.0 a
Syllit ^x	120 ml	x	x	x		x		90.0 b	3.0 a	7.0 a
Trimangol/ Ortiva+MZ+ oil	200 ml/ 20 ml +150 g + 500 ml	x			x		x	83.0 c	7.6 b	9.4 b
Control								30.0 d	12.4 c	57.6 c

^z Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test. MZ = mancozeb

Table 4.6.2.4. Evaluation of copper quinolate WP and SC formulations sprayed during the susceptible period from October to January during 2007 and 2008 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment ^y	Rate/100 ℓ water	Percentage of fruit in each class ^z		
		CBS lesions/fruit		
		0	1-3	≥4
Copper hydroxide	200 g	98.2 a	1.4 a	0.4 a
Mancozeb	200 g	96.4 ab	2.6 a	1.0 ab
Copper quinolate (SC) + oil	50 g + 250 mℓ	89.2 abc	1.8 a	9.0 abc
Copper quinolate (SC)	75 mℓ	87.2 abcd	4.2 a	8.6 abc
Copper quinolate (WP)	100 g	76.8 bcd	0.6 a	22.6 bc
Copper quinolate (WP)	50 g	69.0 cd	4.0 a	27.0 c
Copper quinolate (WP)	35 g	68.4 d	1.6 a	30.0 c
Control		30.0 e	12.4 b	57.6 d

^y Spray dates were 15 October 2007, 12 November 2007, 10 December 2007, 7 January 2008 (4 weeks)

^z Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

4.6.3 FINAL REPORT: Further developments of spray programmes consisting of registered fungicides in tank mixtures with didecyldimethylammonium chloride (DDAC) for the control of citrus black spot in South Africa, Brazil and Argentina

Experiment 881 (September 2007 – June 2008): by G.C. Schutte (CRI), Beatriz Stein (EEAOC, Tucuman, Argentina), Fernando Azevedo (Brazil)

Opsomming

Afwisselende toedienings van tenkmengsels bestaande uit Sporekill met óf koperoksichloried óf mancozeb, het goeie beheer van sitruswartvlek (SSV) tot gevolg gehad. In Suid Afrika is benzimidazole soos carbendazim geregistreer in tenkmengsels met mancozeb en minerale spuitolie. Waar mancozeb en spuitolie vervang is met Sporekill, het dit ook goeie beheer van SSV tot gevolg gehad. Omdat minerale spuitolie 'n invloed op vrugkleur het, sal minder toedienings daarvan tot groot voordeel wees vir kultivars van vroeg ryp word. In Argentinië en Brasilië waar Sporekill i.p.v. minerale spuitolie in sekere spuitprogramme in kombinasie met verlaagde koper swamdoders vir die beheer van SSV en sitruskanker gebruik is, het dit ook goeie beheer tot gevolg gehad. Dit blyk ook 'n algemene probleem in Suid Amerika te wees om nie by die geregistreerde spuit intervale te bly nie en word hulle gestrek tot verby die maksimum van 28 dae.

Summary

Alternated applications of tank mixtures consisting of Sporekill with either copper oxychloride or mancozeb (both at reduced rates) proved to be effective for the control of CBS. In South Africa, benzimidazoles, such as carbendazim, are registered in tank mixtures with mancozeb and mineral spray oil. When mancozeb and spray oil is replaced with Sporekill, the latter also resulted in excellent control of CBS. Because mineral spray oil has an influence on fruit colour, less oil applications will be beneficial to early maturing cultivars. In Argentina and Brazil, certain spray programmes where Sporekill was used in combination with reduced rates of copper and mancozeb instead of mineral spray oil for the control of CBS and citrus canker, also provided excellent control. It seems to be a common problem in South America not to adhere to registered spray intervals as they are stretched beyond the maximum of 28 days.

Introduction

There is an urgent need for new chemicals to control citrus black spot (and citrus bacterial canker in countries where it occurs). Results from field trials the past three seasons have led to the registration of a sanitizing agent, Sporekill, in tank mixtures with mancozeb and copper fungicides where both groups were used at half their registered rates (Expt. 799). The first aim the past season was to see if one can expand the spray intervals from four weeks to six weeks using the same rates. There is also a need to replace expensive mineral spray oils in strobilurin spray programmes with cheaper stickers or spreaders. Sporekill has proven the past season to fulfil that role when it was mixed with the strobilurin, Ortiva, in a tank mixture with mancozeb (and perhaps copper fungicides as well). The aim (in South Africa and Brazil) was to see if one can replace mancozeb and mineral spray oil with Sporekill in a tank mixture with a benzimidazole and a strobilurin. In Argentina, the aim was to see how tank mixtures with copper oxychloride with either Sporekill

or mineral spray oil will perform for the control of *Guignardia mangiferae* (so-called “reddish spot”) and citrus canker (*Xanthomonas campestris* pv. *citri*).

Materials and methods

a) South Africa

Three orchards (Brits 1, 2A and 2B) were selected at Croc Valley Citrus Co. to do the evaluations. A randomised design with 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500 - 3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree, but all trees will be sprayed to the point of run-off. Certain treatments will commence in mid-October as previously recommended, depending on the climatological information required for infection during the critical infection period. Trees in both groves were selected for uniformity in canopy density and tree size. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. Fungicides that were tested are Dithane M45, 800 g/kg WP (mancozeb, Dow AgroSciences, L 2914), Bendazid 500 g/l SC (carbendazim, Plaaskem, L 6961), Ortiva 250 g/l SC (azoxystrobin, Syngenta, L 5968) and Demildex, 850 g/l WP (copper oxychloride, Delta Chemicals, L 5094). At fruit maturity in July or August, CBS severity was rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportional data was analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

b) Argentina

An 8-year old lemon orchard on P.T. Flying Dragon rootstock with planting distance of 6 x 3 m was sprayed with a high volume, sprayer machine with a hand held gun. Each tree received a spray volume of 14 l per tree. Spray volumes also varied according to the size and canopy density of the tree, but all trees were sprayed to the point of run-off. A randomized block design with 4 replications (12 plants per replicate) was used. Treatments commenced in mid-October as previously recommended. Evaluations were done in April and July 2008 for canker and July 2008 for Reddish spot (*Guignardia mangiferae*). Evaluation was performed on 200 lemon fruit exceeding size 138 (60 mm) harvested from 8 trees of the middle of each block.

c) Brazil

An experiment was carried out in a 16-year old Valencia orchard at the Centro APTA Citrus Sylvio Moreira, the Agronomic Institute, located in Cordeirópolis, State of Sao Paulo, near Conchal, a region with a high incidence of citrus black spot. The planting distance was 7 x 4 m. A randomized block design with 4 replications was used. A total of five monthly applications were performed from October to February. From June 2008, monthly assessments were performed, which extended until the end of November, to quantify the incidence (% fruits with the presence of symptoms (Fig. 4.6.3.1)) and severity (% of injured surface of the fruit) of disease in fruit, using a diagrammatic scale of photographs (Fig. 4.6.3.1). The data were analyzed statistically by comparison of means test (Tukey, 5%).

Results and discussion

a) South Africa

Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.6.3.1) show that there were no significant differences ($P < 0.05$) between all the different spray programmes the criterion clean exportable fruit with no CBS lesions used for evaluation and the standard registered mancozeb and copper oxychloride spray programmes and the spray intervals. All these treatments were sprayed during the susceptible period from October to January for CBS. Disease pressure was high as the untreated control resulted in only 44.8% clean exportable fruit. No statistical significant differences were observed between any of the fungicide treatments in the other two criteria, but they were, however, significant different from the untreated control (Table 4.6.3.1).

b) Argentina

“Reddish spot” as it is called in Tucuman, Argentina is caused by *Guignardia mangiferae*. This *Guignardia* species is of a larger prevalence in lemon in Tucuman province than *Guignardia citricarpa*. Reddish spot symptoms occur in July on yellow mature lemon fruits only and therefore the evaluation for “reddish spot” was performed only when the quiescent symptoms were observed on the unsprayed control treatment. It is

not common to see a high infestation of 98.3% of fruit with CBS in the Tucuman region. According to the protocol, the first application should have been applied in October, but the first application was only applied on 7 November. During October they have received 85 mm of rain and this is conducive to high levels of high infection. No spore traps were operational so the first ascospore release could not be determined. The fruit was not protected when the first summer rain fell, which resulted in poor Reddish spot control with all the contact fungicide tank mixtures with Sporekill (Table 4.6.3.4). On the 19th December 2007, a hail storm occurred and the trees were resprayed on the 20th because wounds created by hail will contribute to heavy citrus canker infections. Therefore the results from Tucuman were disappointing with regards to reddish spot control due to the untimeliness of the first application. With regards to citrus canker control, monthly applications of copper oxychloride at the recommended rate of 200 g/hℓ water performed significantly better than reduced rates of copper oxychloride with either mineral spray oil or Sporekill. For the control of reddish spot, the spray programme consisting of copper oxychloride + Sporekill (200 g + 100 ml/hℓ water) (November and December) and followed with a copper oxychloride + pyraclostrobin + oil (December and January) performed significantly better than the same programme with a lower rate of copper (100 g/hℓ water). This might be attributed to the reduced copper rate. On the other hand, these two spray programmes were equally effective for the control of citrus canker. The spray programme consisting of copper oxychloride alternated with the strobilurin, was also not significant different from the 5 x copper oxychloride + Sporekill (200 g + 100 ml/hℓ water). All the copper fungicides with mineral spray oil also performed better than the copper fungicides with Sporekill. Copper was included in all the applications and should have resulted in effective control of citrus canker. Trials should be repeated. In general, the high incidence of reddish spot can be attributed to the late application of the first fungicide application (Table 4.6.3.2).

c) Brazil

There was an increase in the CBS incidence (or % fruit with CBS symptoms) as the fruit matured (from 17 July to 21 August 2008), which is expected and underlines the fact that Valencia fruit should not remain on the trees for too long after maturity. The evaluation on 17 July 2008 showed that there were no significant differences between the treatments. The same was observed on the 21 August 2008, although all treatments differed significantly from the untreated control. In this case the 5 x mancozeb + Sporekill and the standard treatment (Copper oxychloride + Carbendazim/Mancozeb + Copper oxychloride + Pyraclostrobin/Mancozeb + Carbendazim; all sprays with oil) had the lowest incidence of CBS (7.50 and 3.13%, respectively). In comparison, 5x copper oxychloride (at 100g/hℓ water) + Sporekill, as well as the programme of copper oxychloride (at 100g/hℓ water) + Sporekill (3x) and the alternated with mancozeb (100g/hℓ water) + Sporekill (2x), resulted in the highest incidence of fruit with CBS (20% and 19.38%, respectively) on the 21 August rating. In all treatments where mineral spray oil was replaced with Sporekill, results were not significant better or worse (Table 4.6.3.3). Sporekill can therefore be used as a replacement for mineral spray oil.

The results could have been much better if the researcher adhered to the internationally acceptable spray intervals. For instance, the spray interval from 24 October to 24 November was extended by 3 days; from 23 December to 31 January 2008 was extended by 11 days, and this during the critical period for infection. Trials should be repeated.

Conclusion

Alternating sprays with either copper oxychloride or mancozeb in tank mixture with Sporekill in a spray programme, proved to be effective for the control of CBS. Benzimidazoles such as carbendazim are registered in tank mixtures with mancozeb and mineral spray oil, but if mancozeb and spray oil is replaced with Sporekill alone, the latter also gave good control of CBS. However, in terms of fungicide resistance management strategy towards the at-risk strobilurin and benzimidazole fungicides, this will not be recommended, as a mix partner with some effective residual activity against the pathogen is required. Mineral spray oil does not contribute in anything for the control of CBS and serves only as an effective sticker for these fungicides, whereas Sporekill was proven in previous experiments to have an effect on its own on CBS. Moreover, such a strategy will be considered if citrus canker will manifest itself in future in South Africa or any other bacterial disease of citrus.

Certain spray programmes where Sporekill was used in combination with reduced rates of copper and mancozeb as sprayed for CBS and citrus canker in Argentina and Brazil did provide good control if compared with their standard treatments. However, it seems to be a common problem in South America not to stick to registered 28 day spray intervals. In both Argentina and Brazil they also started too late with their first spray application.

Future research

There is a constant need to evaluate new and old fungicide formulations as well as fungicides that may possess activity against citrus black spot (CBS). Chemical companies frequently modify and upgrade their old products to possess new characteristics such as rain fastness and particle size and they need to be re-evaluated for efficacy. Searching for new fungicides or fungicides with new characteristics as well as some new ideas how we can alter old fungicidal spray programmes to be included in effective spray programmes and to cope with fungal resistance strategies at the same time. Searching for and experimenting with cheaper and more effective fungicides will contribute a lot to reducing production costs and be more environmentally friendly and sustainable with regard to resistance development. For instance, Sporekill has proved to be more effective in a tank mixture with carbendazim for the control of post bloom fruit drop caused by *Colletotrichum acutatum* in Brazil than those treatments where carbendazim was sprayed alone.

Technology transfer

Talks at study groups and results will be presented on the bi-annual CRI Symposium in August 2008.

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Table 4.6.3.1. Evaluation of spray programmes consisting of tank mixtures of different fungicides with Sporekill sprayed from October and January during 2007 and 2008 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Fungicide	Rate/100L water	Application dates				Percentage of fruit in each class ^z		
		15 Oct 2007	12 Nov 2007	10 Dec 2007	7 Jan 2008	Lesions/fruit		
						0	1-3	≥4
Mancozeb + Sporekill	100 g + 100 ml	x	x	x	x	91.4 a	4.4 ab	4.2 a
Copper OCl + Sporekill	100 g + 100 ml	x	x	x	x	99.6 a	0.2 a	0.2 a
Copper OCl + Sporekill & Mancozeb + Sporekill	100 g + 100 ml 100 g + 100 ml	x	x	x	x	95.0 a	2.0 ab	3.0 a
Copper OCl + Sporekill & Ortiva + Sporekill	100 g + 100 ml 20 ml + 100 ml	x	x	x	x	98.0 a	0.6 ab	1.4 a
Mancozeb + Sporekill & Ortiva + Sporekill	100 g + 100 ml 20 ml + 100 ml	x	x	x	x	98.8 a	0.8 ab	0.4 a
Copper OCl + Sporekill & Carbendazim + Sporekill	100 g + 100 ml 57ml + 100 ml	x	x	x	x	92.0 a	3.8 ab	4.2 a
Mancozeb + Sporekill & Carbendazim + Sporekill	100 g + 100 ml 57 ml + 100 ml	x	x	x	x	96.6 a	2.4 ab	1.0 a
Copper hydroxide	200 g	x	x	x	x	96.0 a	0.4 a	3.6 a
Mancozeb	200 g	x	x	x	x	96.4 a	2.6 ab	1.0 a
Control						44.8 b	9.6 c	45.6 b

^z Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test. Copper OCl = copper oxychloride.

Table 4.6.3.2. Evaluation of spray programmes consisting of tank mixtures of different fungicides with Sporekill sprayed from November to January during 2007 and 2008 for the control of reddish spot (*Guignardia mangiferae*) and citrus canker (*Xanthomonas campestris* pv. *citri*) on lemons at Tucuman, Argentina.

Fungicide	Rate/100L water	Application date					Percentage of fruit in each class ^x	
		7 Nov 2007	4 Dec 2007	20 Dec 2007	14 Jan 2008	12 Feb 2008	Lesions/fruit	
							Reddish spot	Citrus canker
Copper oxychloride + Sporekill	100g + 100ml	x	x	x	x	x	72.3 d	57.2 bcd
Copper oxychloride + Oil	200g + 100ml	x	x	x	x	x	41.0 ab	45.7 a
Copper oxychloride + Sporekill	100g + 100ml	x		x		x	71.8 d	59.6 bcd
Copper oxychloride+Mancozeb +Sporekill	100g + 100g + 100ml		x		x			
Copper oxychloride + Sporekill	200g + 100ml	x	x	x	x	x	59.5 cd	54.13 ab
Copper oxychloride + Sporekill	100g + 100ml	x		x		x	57.8 bcd	65.5 d
Copper oxychloride + pyraclostrobin + Sporekill	100g + 15ml + 100ml		x		x			
Copper oxychloride + Sporekill	200g + 100ml	x	x			x	29.0 a	64.8 cd
Copper oxychloride + pyraclostrobin + Oil	200g + 15ml + 100ml			x	x			
Copper hydroxide + Sporekill	100g + 100ml	x	x	x	x	x	61.8 d	49.8 ab
Copper hydroxide + Oil	150g + 100ml	x	x	x	x	x	35.0 a	46.1 a
Control							98.3 e	84.6 e

^x Means in a column, based on 4 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

Table 4.6.3.3. Evaluation of spray programmes consisting of tank mixtures of different fungicides with Sporekill sprayed from October to January during 2007 and 2008 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Mogi Mirim, Conchal, Sao Paulo, Brazil.

Fungicide	Rate/100L water	Application date					Percentage incidence of fruit with CBS in each period of evaluation	
		24 Oct 2007	24 Nov 2007	23 Dec 2007	31 Jan 2008	29 Feb 2008	17 July 2008	21 August 2008
Mancozeb + Sporekill	100 g + 100 ml	x	x	x	x	x	0.00 a	7.50 b
Copper oxychoride + Sporekill	100 g + 100 ml	x	x	x	x	x	0.00 a	20.00 b
Copper oxychoride + Sporekill & Mancozeb + Sporekill	100 g + 100 ml	x		x		x	2.50 a	19.38 b
	100 g + 100 ml		x		x			
Mancozeb + Sporekill & Pyraclostrobrin + Sporekill	100 g + 100 ml	x		x		x	0.00 a	8.75 b
	15 ml +100 ml		x		x			
Copper oxychoride + Sporekill & Pyraclostrobrin + Sporekill	100 g + 100 ml	x		x		x	1.25 a	13.13 b
	15 ml + 100 ml		x		x			
Copper oxychoride + Sporekill & Carbendazim + Sporekill	100 g + 100 ml	x		x		x	1.25 a	11.25 b
	50 ml + 100 ml		x		x			
Mancozeb + Sporekill & Carbendazim + Sporekill	100 g + 100 ml	x		x		x	0.00 a	11.25 b
	50 ml + 100 ml		x		x			
Copper oxychoride + oil & Carbendazim + Mancozeb + oil & Copper oxychoride + oil & Pyraclostrobrin + Mancozeb +oil & Carbendazim + oil	200 g + 500 ml	x					0.00 a	3.13 b
	50ml + 100g + 500 ml		x					
	200 g + 500 ml			x				
	15 ml + 100g + 500 ml				x			
	50 ml + 500 ml					x		
Control							0.62 a	45.0 a

^x Means in a column, based on 4 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Tukey's least significant difference test

Table 4.6.3.4. Rainfall recordings for Tucuman, Argentina during the 2007/2008 season.

Month	Rainfall (mm)
September	0
October	85
November	50
December	232
January	350
February	164
March	230
Total	1 111

4.6.4 **PROGRESS REPORT: Determining the persistency of copper fungicides as sprayed under natural conditions for the control of citrus black spot in South Africa**
Experiment 918 (September 2007 – June 2008): by G.C. Schutte, C. Kotze and P.H. Fourie (CRI)

Opsomming

Vergelykings tussen koperoksied, koperoksichloried en koperhidroksied toon dat koperoksied, wat teen 'n geregistreerde dosis van 90 g/h ℓ water gespuit is, 23.58 g metalliese koper per boom (indien dit teen 33 ℓ per boom gespuit word) of 675 dele per miljoen (dpm). Dit is 33 and 3.5% minder metalliese koper per boom in vergelyking met koperoksichloried en koperhidroksie, onderskeidelik. Nogtans het die onderskeie koperfungisiede soortgelyke residu-vlakke op vrugte gelaat. Residu-analise oor die 56 dae na toediening op 'Valencia' bome het soortgelyke reënvastheid van die drie koperformulasies getoon en ook dat die geregistreerde spuitinterval van 35 dae behou moet word. 'n Korrelasie van onderskeidelik 76% en 90% tussen koper residu en kwantitatiewe fluoriserende pigment meetings op blare en vrugte is waargeneem, wat hierdie metodologie ter bepaling van spuitbedekking en moontlik ook reënvastheid ondersteun. Die eksperiment word met klein veranderinge aan monsterneming-strategie en data-analiese herhaal om sodoende beter interpretasie van die resultate, spesifiek in terme van die afwas en herdistribusie hipotese, moontlik te maak.

Summary

Comparisons between cuprous oxide, copper oxychloride and copper hydroxide used in this experiment showed that the registered rate of cuprous oxide sprayed at 90 g/h ℓ water resulted in 23.58 g metallic copper per tree (sprayed at a rate of 33 ℓ per tree) or 677 ppm. This is 33 and 3.5% less metallic copper per tree than for copper oxychloride and copper hydroxide, respectively. However, similar residue levels were recorded on the fruit for the respective copper fungicides. On leaves, cuprous oxide deposited significantly more copper residues than copper oxychloride and copper hydroxide. Copper residue analyses over the period of 56 days as sprayed on 'Valencia' trees showed that none of the copper fungicides were more rainfast than the other, as copper residues decreased at the same tempo. Based on these results, it also seems that the registered 35-day interval for copper fungicides should be maintained. A 76% and 90% correlation was observed between the copper residue analysed and the quantitative fluorescent pigment measurements on leaves and fruit, respectively, which supports this methodology as an effective tool for spray deposition assessment and potentially also as rainfastness assessment. The trial will be repeated for another year on another cultivar with some changes to sampling strategy and data analyses to allow better interpretation of the results, specifically with regard to the wash-off and redistribution hypotheses.

Introduction

Copper fungicides are used for the control of CBS since the 1960's and various new formulations were recently registered. However, copper stippling is one of the largest restrictions on the continuous use of these highly effective fungicidal groups which are registered against a large variety of organisms such as algae, fungi and bacteria. Nordox, a new cuprous oxide formulation was registered in South Africa during 2007. The rate registered for CBS was 90 g/h ℓ water but in screening trials, rates as low as 50 g/h ℓ water was also effective. Questions arose such as: Is the registered rate of 90g/h ℓ water not an over-kill? If we stick to the same rate, can we not expand the spray interval beyond 35 days? What about copper hydroxide and copper oxychloride? Are we not applying too much copper and can we extent the intervals using the same rates? Can we save the growers money?

Coppers have the unique characteristic that it can be redistributed by rain, and as the fruit grows, the gaps created can therefore be covered by copper-residues. In order to study the optimisation of the copper spray application on citrus, researchers at Stellenbosch University's Plant Pathology department (USPP) have developed a spray assessment protocol using fluorometry, photomicrography and digital image analyses (Brink *et al.*, 2004, 2006; Fourie *et al.*, 2009). Spray retention is traced through the use of SARDI Yellow Fluorescent Pigment (400 g/l, EC; South Australian Research and Development Institute, Loxton SA 5333 Australia; Furness *et al.* 2006a). Microscopic measurements have indicated that particle size in the pigment ranged from 0.5 to 10 µm (JC Brink, unpublished results), which is equivalent to that of certain copper hydroxide formulations (Orbovic *et al.*, 2007).

This pigment and deposition assessment protocol will be used to determine the cuprous oxide, copper oxychloride and copper hydroxide persistence on citrus leaves and fruit under natural conditions. Concomitantly, the copper residue-analysis and fluorescent pigment deposition will also be correlated with the average increase of fruit surfaces over time and with the dilution or wash-off effect caused by rain over the experimental period. The efficacy of extended spray intervals for the control of CBS will be determined when the fruit ripens.

Materials and methods

a) Field application of coppers for CBS control

One orchard was selected at Croc Valley Citrus Co. (Brits 1) in the Nelspruit region to do the evaluations. A randomised block design with 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree and all trees were sprayed to the point of run-off. Trees were selected for uniformity in canopy density and tree size. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. The fungicides to be tested include copper hydroxide (Kocide 2000, 53.8% WG), copper oxychloride (Demildex, 85% WP) and cuprous oxide (Nordox, 85% WG). At fruit maturity in July or August, CBS severity will be rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportional data will be analysed by ANOVA, using Fisher's LSD test ($P=0.05$).

b) Cu-persistence under field conditions

The same spray application protocol was followed as described above except that there will be no follow-on treatments after the first and only application in October. Registered rates of 90 g for Nordox and 200 g /100 l water for both Kocide and Demildex were used, although these resulted in markedly differing metallic copper rates (Table 4.6.4.1). Treatments were subdivided in two groups for each of the three proposed Cu-rates and sprayed with the addition of 100 ml /100 l Yellow Fluorescent Pigment to the spray mixture. Twenty fruit and leaf samples were drawn randomly on the outside circumference from each of the three replicates on a bi-weekly and weekly basis, respectively, for eight weeks. Fluorescent pigment deposition analyses were done firstly at CRI after which the same samples were couriered to SGS in Somerset-West for copper residue analysis as described below.

Leaves and fruit were sprayed in the field according to the spray protocol as described above. The Yellow Fluorescent Pigment® (400 g/l, EC; South Australian Research and Development Institute) at 100 ml /100 l water was added to each application and samples were taken on days, 0, 7, 14, 21, 28, 35, 42, 49 and 56. Sprayed leaves and fruit were illuminated using a Labino Mid-light (UV-A; ≈365 nm) and digital photos were taken of upper and lower surfaces of 20 leaves and fruit using a Canon EOS 40 D camera equipped with a 50 mm macro lens. Spray deposition assessment involved digital image analyses with Image-Pro Plus version 6.2 software to determine quantitative and qualitative deposition per leaf and fruit of the fluorescent pigment particles. Quantitative deposition per leaf and fruit analysis involved the measurement of the area covered by pigment particles, but as a percentage of total fruit and leaf area. For qualitative deposition assessment on each fruit and leaf (i.e. uniformity), the fruit and leaf area of each ≈30 MB *.tiff image was divided into equally-sized 30×30-pixel squares. Depending on the leaf and fruit size, this amounted to anything from 300 to in excess of 3000 individual squares per fruit and leaf of which the percentage area covered by fluorescent pigment particle was determined for each square. The relative standard deviation of the mean value of all the blocks analysed per leaf and fruit (%RSD = standard deviation / mean x 100) indicated the qualitative deposition per fruit and leaf. Data were subjected to analysis of variance and Student's T-test for least significant difference ($P = 0.05$). The variation (%RSD) in the mean quantitative deposition per fruit and leaf values was indicative of general spray uniformity between leaves.

Copper residue analysis was done by SGS Agricultural Services in Somerset-West. External residues were removed immediately from the flavedo of all the fruit using a grater. Leaves samples were individually dried in paper bags in an oven at 70°C for 12 h, bulked and sent to SGS for copper residue analysis. From each fruit sample, 2 g fresh weight was dried in an oven for 8 h at 500°C. Each dried fruit and leaf sample was dissolved in 5 ml nitric acid (52%) and boiled on a hot plate until it was dry. Another 5 ml nitric acid was added to each sample and increased to 100 ml with distilled water. Copper residues (milligrams per kilogram) were determined with an inductive coupled plasma spectrophotometer (Jobin Yvon, JY 50 P) into which 0.5 ml of each sample was injected and copper residues determined (parts per million).

The same fruit that were used for fluorescent pigment deposition analyses were measured using a calliper and fruit surface area calculate by using the formula: $4\pi r^2$. The average increase in fruit surface area was correlated with the distribution pattern of copper residues on the fruit from the fluorescent pigment deposition results.

Deposition, residue and fruit size data were subjected to analyses of variance (ANOVA), Student's T-test for least significant difference ($P = 0.05$), Pearson's correlations and appropriate regression statistics using SAS software.

Results and discussion

a) Field application of coppers for CBS control

Results were presented in Experiment 880. Cuprous oxide (90 g/100 l water) sprayed with 5 (standard), 6 and 7 week intervals were effective for the control of CBS. Although not significant, a 4-5% difference in clean exportable fruit was observed between the 5 and 7 week intervals with both copper fungicides.

b) Cu-persistence under field conditions

i) Leaves

ANOVA indicated treatment \times day interaction ($P < 0.0001$) for copper residue and quantitative deposition assessment of the fluorescent pigment (Fluo%). At Day 0, mean copper residue on 'Valencia' orange leaves showed that cuprous oxide had significantly more ($P < 0.05$) copper residue than copper hydroxide and copper oxychloride (374.3 vs. 285.7 and 253.7 ppm, respectively; Table 4.6.4.2). Moreover, at 35 days, this is the registered spray interval, leaves from trees sprayed with cuprous oxide also revealed statistically more copper residues than copper hydroxide and copper oxychloride (247.0 vs. 174.3 and 160.0 ppm, respectively). On day 56, cuprous oxide, copper hydroxide and copper oxychloride had 144.33, 160.33 and 118.67 ppm copper residues left, respectively, which were not significantly different (Table 4.6.4.2). The high concentration of copper (± 150 ppm) still left on day 56 in all the treatments shows that they might act as a reservoir and that the sudden increase in copper levels of the copper treatments were due to the wash-off of copper residues from higher up in the canopy after rain.

Regression analysis of foliar residue data over time revealed reasonably good linear fits for cuprous oxide ($y = 336.13 - 3.48(x)$; $R^2 = 0.74$), copper hydroxide ($y = 250.27 - 1.85(x)$; $R^2 = 0.59$) and copper oxychloride ($y = 223.67 - 2.06(x)$; $R^2 = 0.79$). Statistical analysis of the linear coefficients ($y = A + B(x)$) indicated a significantly higher A-value for cuprous oxide, which indicates the predicted residue at day 0, compared with copper hydroxide and copper oxychloride, with no significant difference between the latter formulations. On the contrary, significantly steeper negative slope (B-value) was predicted for cuprous oxide, compared with copper hydroxide and copper oxychloride, with no significant difference between the latter formulations. These lines are plotted in comparison with similar regression lines for the quantitative fluorescent pigment coverage (fluo%), indicated fairly good correlation between the two measurements (Figure 4.6.4.1). Pearson's correlation coefficients between leaf copper residue and fluo% of 0.76, indicating 76% congruence between these measurements.

ii) Fruit

On fruit, ANOVA again indicated significant copper treatment \times time \times copper residues or fluo% interaction ($P < 0.0001$). At day 0, copper residue loading onto fruit were not statistically different (Table 4.6.4.2). However, at day 28, cuprous oxide residue levels were significantly higher than those of copper hydroxide and copper oxychloride, with no significant difference between the latter formulations (27.7 vs. 19.0 and 17.7 ppm, respectively), but at 42 days after application, residue levels were not significantly different (8-8.7 ppm). All the copper fungicides still had some residues left on the fruit on day 56, but if > 4 ppm is still effective against CBS or any other foliar citrus disease has to be determined.

Regression analysis of fruit residue data over time revealed very good linear fits for cuprous oxide ($y = 49.47 - 0.87(x)$; $R^2 = 0.92$) nor copper hydroxide ($y = 43.73 - 0.79(x)$; $R^2 = 0.90$) and copper oxychloride ($y = 45.07 - 0.80(x)$; $R^2 = 0.86$) was more rain fast than the other over a period of 56 days (Table 4.6.4.2; Fig. 4.6.4.1). Statistical analysis of the linear coefficients ($y = A = B(x)$) indicated a significantly higher A-value for cuprous oxide compared with copper hydroxide, with copper oxychloride not differing significantly from either formulation. No significant difference was observed between the predicted negative slopes (B-value) indicating similar rates of wash-off or persistence. These lines are plotted in comparison with almost identical regression lines for the quantitative fluorescent pigment coverage (fluo%), indicated very good correlation between the two measurements (Figure 4.6.4.2). Pearson's correlation coefficients between fruit copper residue and fluo% measured on fruit was 0.90, indicating 90% congruence between these measurements.

Although ANOVA of fruit surface area data indicated a significant interaction between treatment and day, this was ascribed to statistical, but minor differences between treatments on specific days after treatment, and not indicative of any growth inhibitory or promoting effects (Fig. 4.6.4.3). Fruit growth rate were therefore regarded as a constant variable and not included in analysis and interpretation of the residue and deposition results.

Rain events were recorded at regular intervals throughout the 56-day period (Fig. 4.6.4.4). Pearson's correlation indicated fairly good correlation between cumulative rainfall and fruit copper residues ($r^2 = -0.79$) and fruit fluo% ($r^2 = -0.60$), which might be indicative of wash-off. The respective correlation coefficients on leaves were poor ($r^2 = -0.35$ and -0.21), and might be attributed to redistribution of the copper or pigment between leaves. However, the wash-off and redistribution hypothesis should be viewed in light of similar correlations observed between these parameters and day after treatment (Fruit: $r^2 = -0.80$ and -0.60 ; Leaves: $r^2 = -0.35$ and -0.19), indicating the effect of time should also be considered. The random sampling strategy employed in this study unfortunately do not allow us to make any distinction in residue and pigment deposition on leaves or fruit at various vertical canopy positions, which would have elucidated the wash-off and redistribution hypothesis to some extent. This aspect will be addressed in the repeat trials.

Conclusions

Comparisons between the copper formulations used in this experiment, showed that the registered rate of cuprous oxide sprayed at 90 g/h ℓ water resulted in 23.58 g metallic copper per tree (if it is sprayed at a rate of 33 ℓ per tree) or 677 ppm. This is 33 and 3.5% less metallic copper per tree than for copper oxychloride and copper hydroxide, respectively. However, similar residue levels were recorded on the fruit for the respective copper fungicides. On leaves, cuprous oxide deposited significantly more copper residues than copper oxychloride and copper hydroxide.

An interesting observation was that the mean copper rate was 82% higher on the leaves than on the fruit surface and even the leaves of the untreated control had 5 times more copper than the untreated fruit. This might be attributed to differences in the analytical process, especially regarding the amount of biomass relative to surface area sampled for extraction purposes.

Copper residue analyses over the period of 56 days as sprayed on 'Valencia' trees showed that none of the copper fungicides were more rain fast than the other as copper residues decreased at the same tempo. Based on these results, it also seems that the registered 35-day interval for copper fungicides should be maintained.

A 76% and 90% correlation was observed between the copper residue analysed and the quantitative fluorescent pigment measurements on leaves and fruit, respectively, which supports this methodology as an effective tool for spray deposition assessment and potentially also as rainfastness assessment.

Future research

The trial will be repeated for another year on another cultivar. The sampling strategy will be amended to allow comparison in pigment deposition at various vertical canopy positions. More in-depths data analyses will include distinction between pigment deposition on upper and lower leaf surfaces as well as navel and button end-'hemispheres' of fruit. The above-mentioned changes in methodology will allow us to more accurately interpret the results, as well as to possibly elucidate the wash-off and redistribution hypotheses.

Technology transfer

Talks at study groups and results will be presented on the bi-annual CRI Symposium in August 2010.

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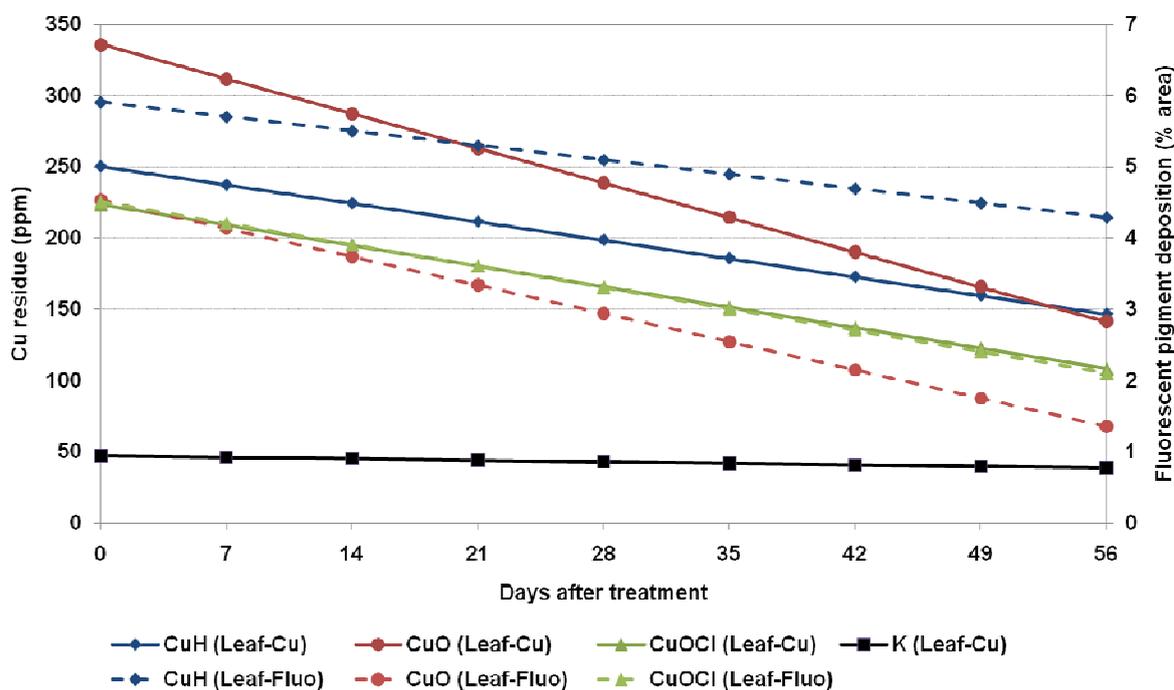


Fig. 4.6.4.1. Linear regression lines for copper residue in 'Valencia' leaves and fluorescent pigment deposition as determined 0, 7, 14, 21, 28, 35, 42, 49 and 56 days after spray application with copper hydroxide (RSQ value 0.5895), cuprous oxide (RSQ value 0.7358) and copper oxychloride (RSQ value 0.7903) each with SARDI Yellow Fluorescent Pigment.

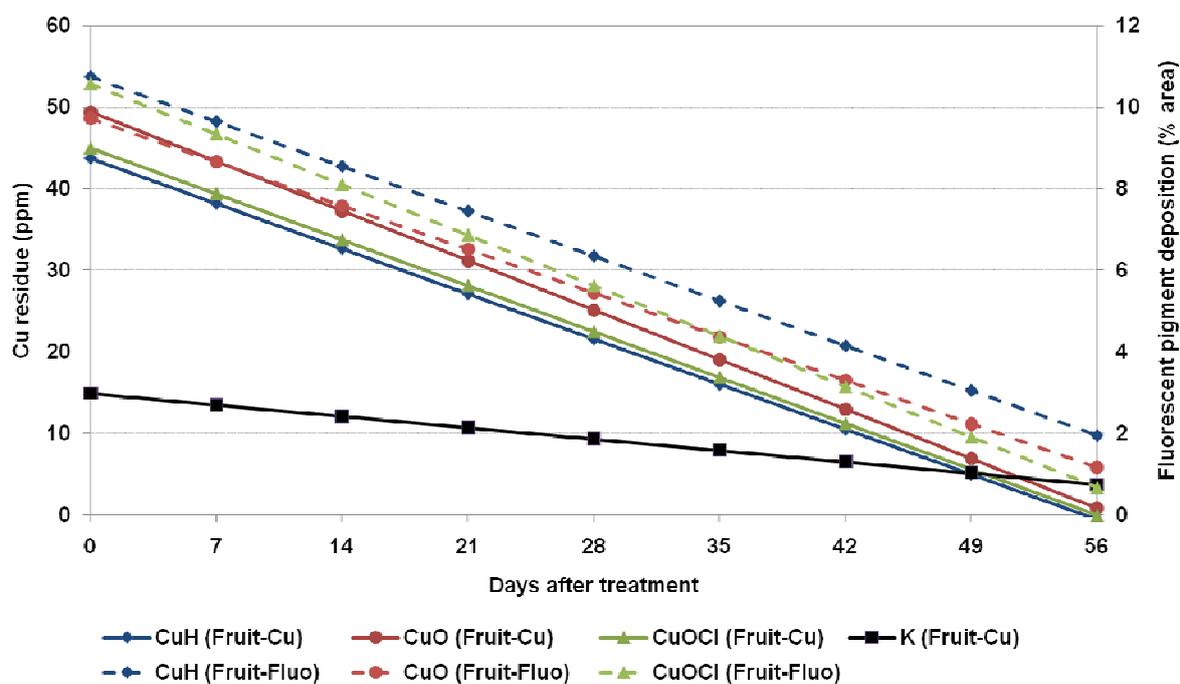


Fig. 4.6.4.2. Linear regression lines for copper residue from ‘Valencia’ fruit and fluorescent pigment deposition as determined 0, 14, 28, 42, and 56 days after spray application with copper hydroxide (RSQ value 0.9045), cuprous oxide (RSQ value 0.9200) and copper oxychloride (RSQ value 0.8618) each with SARDI Yellow Fluorescent Pigment.

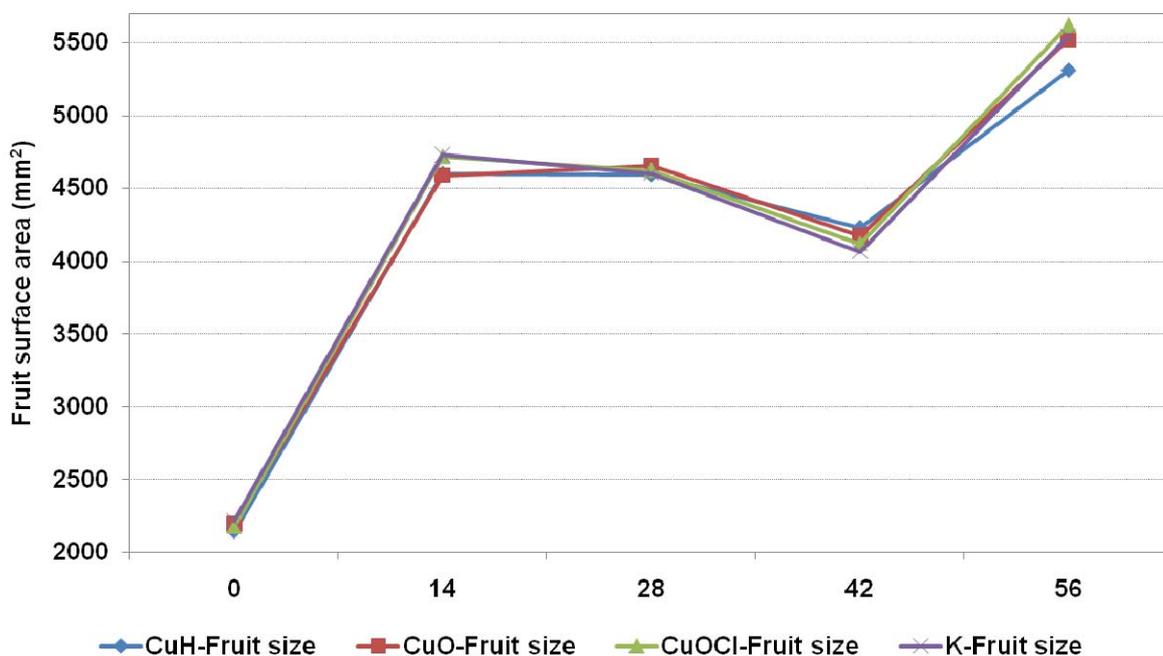


Fig. 4.6.4.3. Increase in fruit surface (mm^2) over a period of 56 days as determined from the same fruit used for fluorescent pigment deposition and copper residue analyses.

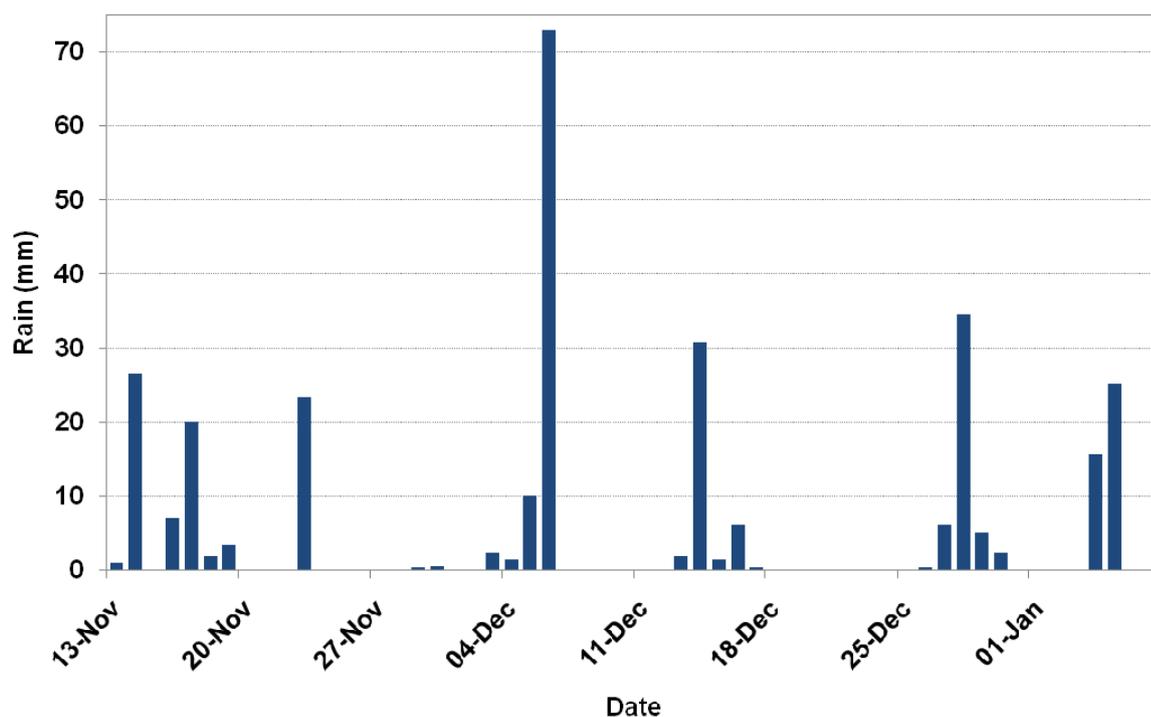


Fig. 4.6.4.4. Rainfall recorded during the 56-day trial period following treatments on 13 November 2008.

Table 4.6.4.1. Retention of cuprous oxide, copper oxychloride and copper hydroxide on 'Valencia' orange fruit and leaves on day 0 after an orchard application with hand-guns.

Treatment	Registered rate /100 ℓ water	Metallic copper rate per tree (@33 ℓ /tree)	Copper rate (ppm) on day of application
Cuprous oxide	90 g	22.28 g	675
Copper oxychloride	200 g	33.33 g	1000
Copper hydroxide	200 g	23.10 g	700

Table 4.6.4.2. Retention of copper hydroxide, cuprous oxide and copper oxychloride on 'Valencia' orange leaves and fruit over a period of 56 days.

Treatment	Day	Mean copper residue (ppm) ^z	
		Leaves	Fruit
Copper hydroxide	0	285.67 bc	50.7 a
	7	252.33 cd	
	14	202.00 efg	26.0 b
	21	177.00 ghij	
	28	150.67 ijkl	19.0 c
	35	174.33 ghijk	
	42	194.00 fgh	8.0 ef
	49	176.67 ghij	
	56	160.33 ijkl	4.3 f
Cuprous oxide	0	374.33 a	55.0 a
	7	297.00 b	
	14	257.67 cd	29.7 b
	21	226.67 def	
	28	186.67 ghi	27.7 b
	35	247.00 d	
	42	230.67 de	8.7 ef
	49	195 e fgh	
	56	144.33 jkl	4.7 f
Copper oxychloride	0	253.67 cd	53.7 a
	7	197.33 efg	

	14	168 ghijk	26.7 b
	21	166.33 ghijk	
	28	140.00 kl	17.7 c
	35	160.00 ghij	
	42	149.33 jkl	8.0 ef
	49	123.33 l	
	56	118.67 l	6.7 f
Control	0	53.33 m	15.0 dc
	7	58.33 m	
	14	38.67 m	10.0 def
	21	35.33 m	
	28	39.33 m	13.7 cde
	35	35.33 m	
	42	43.33 m	4.0 f
	49	43.67 m	
	56	40.00 m	4.0 f
t-LSD		36.458	6.3203

² Means in a column (based on three replicates) followed by the same letter are not significant different from each other ($P < 0.05$)

4.6.5 Monitoring ascospore releases in the Eastern Cape to determine the critical period for CBS infection

Experiment 919 (September 2007 – June 2008): by G.C. Schutte (CRI) and K. Serfontein (QMS)

Opsomming

Inokulum monitering van plase in die Sondagsriviervallei (SRV) toon dat meer sitruswartvlek op blare in die Addo gebied voorkom as in die ander streke in die SRV. 'n Spoorvanger is daarom in die Addo omgewing geïnstalleer. Weerstoestande en askospor vrystellings is gemonitor. Slegs een betekenisvolle spoorvrystelling met 'n hoë moontlikheid vir infeksie is gedurende 11 – 15 November 2008 waargeneem, asook een met 'n lae moontlikheid teen die einde Februarie, begin Maart in 2009. In die algemeen het die Oos-Kaap 'n baie droë seisoen beleef, vandaar die lae voorkoms van askospor vrystellings. Daar was probleme met die monitering en lees van die glasskyfies wat gebruik was in die inokulum- monitor by Katco, en hierdie deel van die studie is gestaak.

Summary

Inoculum monitoring of farms in the SRV with an inoculum-monitor, showed that there was more CBS in the Addo region. A spore trap was therefore installed in the Addo region of the Sunday's River Valley. Weather and ascospore releases were monitored. Only one significant ascospore release with a high probability for infection was recorded in Addo, which took place from 11-15 November 2008, and one with a lower probability at the end of February and the beginning of March 2009. In general, the Eastern Cape experienced an extremely dry summer with little rainfall and therefore very little ascospore releases took place. There was some difficulty in the monitoring and reading of the glass slides used in the inoculum monitor at Katco, and this part of the study was terminated.

Introduction

During the 2006-2007 and 2007-2008, seventeen and thirteen interceptions have taken place from lemons, oranges and Clementines infected with CBS in the Kat-river and Addo areas, respectively. This included areas around Adelaide for the first time. Rainfall patterns were studied from 1927 and it seems that the rainfall takes place a bit earlier than the northern areas of South Africa on which current control programmes are based. Therefore, if the critical period for infection can be determined for these regions, then control programmes have to be adjusted accordingly. The average rainfall for the Fort-Beaufort area is about 650 mm per year, which is double what the Sundays River Valley (SRV) receives annually. There are within the SRV also differences in inoculum distribution from the Kirkwood area (with a low CBS incidence) to the Addo area (with a high CBS incidence). However, the whole SRV is subjected to a spray programme consisting of spraying lower number of applications than what is registered if compared with the northern regions of South-Africa. The commercial approach this season for both the SRV and Katco is to start their spray programmes earlier (mid-October) with the strobilurins followed by the same application 42 days later. Last year, one mancozeb (October) followed by a strobilurin (+ mancozeb) in November were the only applications done by the SRCC with good effect, thereby saving the growers 2 spray rounds. Luckily it was a

dry season but the scenario can change if they have a wet season during 2007/8. For the northern areas, monitoring of the annual ascospore releases was done during the 1960s by Kotze and McOnie to determine the critical infection periods for infection. If duplicated for the Eastern Cape regions, this will be the only means to understand and predict the disease pattern for the Eastern Cape and to control the disease successfully.

Materials and methods

SRCC

Before the onset of the season, leaves were collected from growers in the Sundays River Valley and sent to QMS where they were placed in an inoculum-monitor. The amount of ascospores was recorded from each sample. A spore trap was installed on the farm of Dave Gerber in a lemon orchard close to the technical office of SRCC (Fig.4.6.5.1). An automatic weather station (Adcon) linked with Plant Plus in Holland, is already installed in the same orchard and operated by the SRCC. Spore trap discs were sent to QMS in Letsitele for analyses on a regular basis. Ascospore releases were correlated with the weather patterns experienced during the monitoring period.

Katco

Lynn Trollope monitored the ascospore potential in orchards also using an inoculum-monitor.

Results and discussion

SRCC

Leaves collected from different growers in the Sunday's river valley showed that there was only ascospore release from 2 growers (Table 4.6.5.1). Both farms were located in the Addo region, and the spore trap was therefore placed in a lemon orchard in Addo. Initially, no ascospores were recorded in the spore trap. In one case, this was because one disc was smeared too much for ascospores to be counted and a second because there was ideal weather conditions, no ascospores were counted also. Extra leaves were collected from an orchard known to be infected with CBS and placed around the spore trap to ensure sufficient inoculum in the immediate vicinity of the spore trap. Only then were ascospores recorded. Results showed that a spore release took place with extremely favourable weather conditions from the 11-15 of November 2008 (61 hours wetness followed by 7 hours dry and another 15 hours of wetness) (Fig. 4.6.5.2).

After that, the Sundays river valley experienced a very dry period during December, January and February with the first meaningful rain falling only in March. A low amount of ascospores were released and ideal weather conditions did occur from 2-3 March (and 13 hours of leaf wetness) (Fig. 4.6.5.2). This is, however, very late and should not have caused infection.

Katco

Lynne Trollip struggled with the inoculum-monitor and she eventually gave up. No further ascospore monitoring was done at Katco.

Conclusions

Only one significant ascospore release was recorded, which took place from 11-15 November 2008. No further rain fell during summer and only at the end of February and the beginning of March 2009, the next meaningful rain fell with some ascospore releases. Weather conditions in the Eastern Cape were not favourable for any further CBS infections. The monitoring service will continue.

Future research

It is suggested that more spore traps should be monitored in the Sunday's river valley, with weather stations, and monitoring must be done from September until the end of March for 2 - 3 more seasons. That would be the only means of reliably determining the inoculum dispersal patterns of CBS in the Eastern Cape.

Technology transfer

Talks at study groups and results will be presented on the bi-annual CRI Symposium in August 2008.

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Table 4.6.5.1. Appearance of *Guignardia spp.* ascospores as sampled with an inoculum-monitor from leaves from the Sundays river valley during 2008.

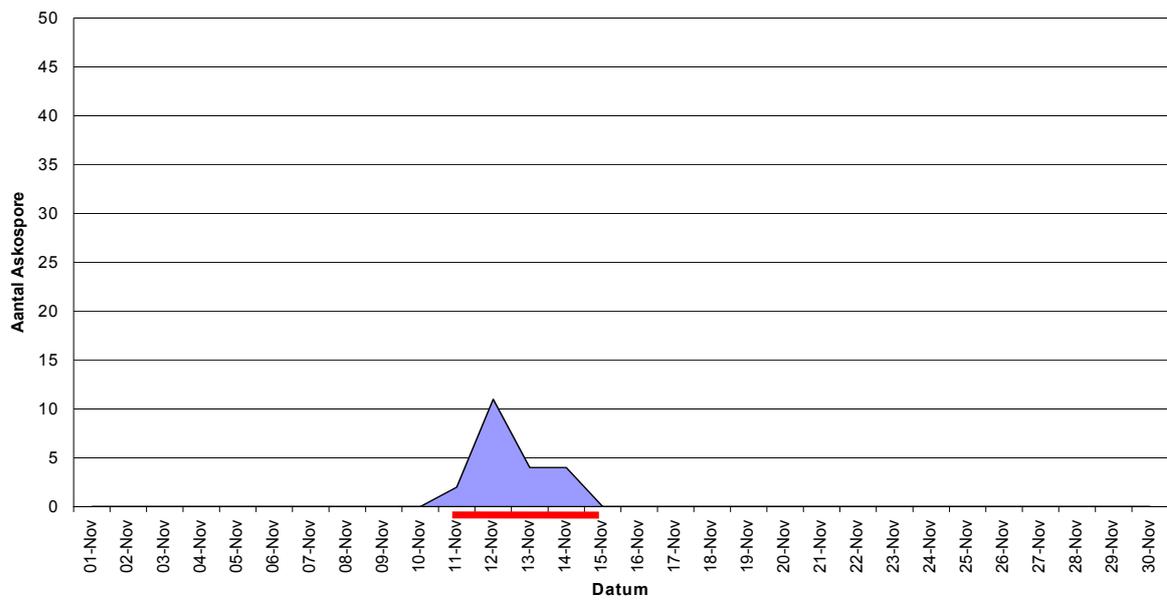
Sample no.	Farm / area	Producer	Orchard	Variety	<i>Guignardia spp.</i>
1	Addo	DJ Bouwer	Lemon 3	Lemon	6
2	Addo	Dave Lloyd	Lemon 2	Lemon	0
3	Addo	Keith Finnemore	Lemon 11	Lemon	0
4	Coerney	HHJ Potgieter	Lemon 17	Lemon	0
5	Addo	P Senekal	Lemon 13	Lemon	2
6	Dunbrody	SRFT Eendracht	Lemon 18	Lemon	0
7	Addo	W Kruger	Lemon 1	Lemon	0
8	Kirkwood	R Krause	Lemon 1	Lemon	0
9	Kirkwood	R Krause	Midnight 9	Midnight	0
10	Kirkwood	JC Botha	Navel 21	Navel	0
11	Kirkwood	JC Botha	Valencia 12	Valencia	0

Table 4.6.5.2. Ascospore releases and prevailing climatological conditions during the susceptible period from October 2008 to March 2009 as monitored in Addo in the Eastern Cape.

Date	Hours wet (dry)	Temp (°C)	Climatological pressure	Ascospore infection period	Infection potential
11-15 Nov	61(7 dry)15	21	Very high	21	Extremely favourable
08-09 Feb	24	24	Low	?	Favourable Favourable
13-15 Feb	± 72	20	High	?	Favourable – extremely favourable
24 Feb-25 Feb	22	23	Low	4	Probable
27 Feb-28 Feb	41	22	Medium	8	Somewhat favourable
02 Mar-03 Mar	13	20	Very low	8	Probable



Fig. 4.6.5.1. Position of the automatic weather station and spore trap relative to the SRCC technical office in the Sunday's River Valley.



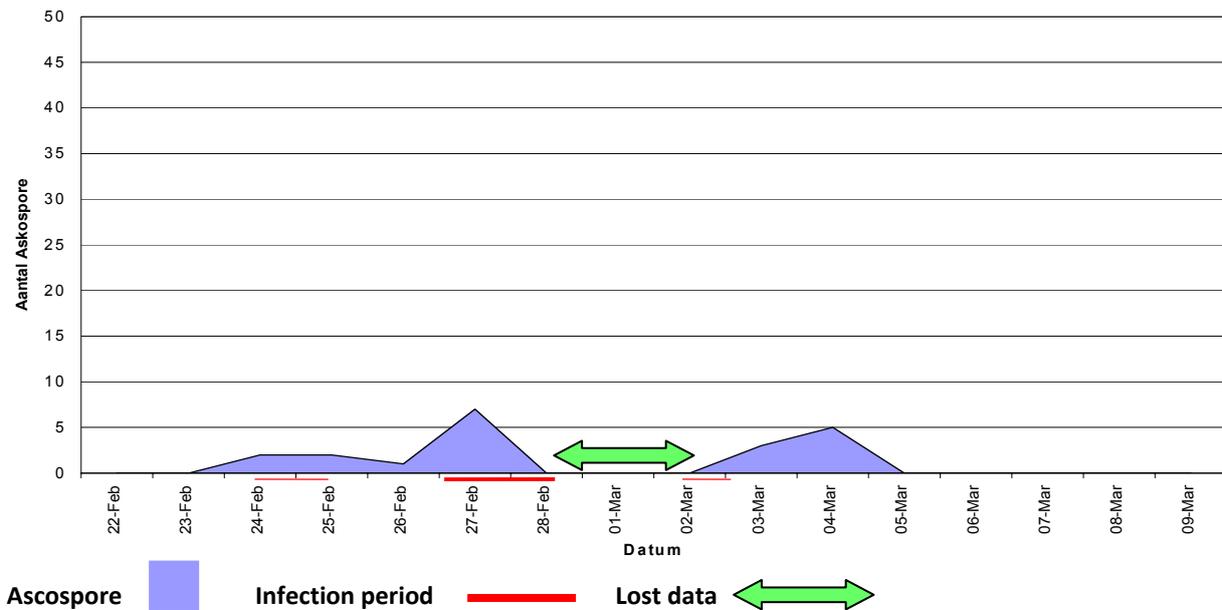


Fig. 4.6.5.2. Ascospore release and possible infection period of *Guignardia* spp. at Addo in the Eastern Cape for the period 2008-2009.

4.6.6 **PROGRESS REPORT: A holistic approach for the reduction of *Guignardia citricarpa* ascospore inoculum**
Project 08 CRI FS2 (March 2008 – 2010): by S H Swart (QMS Agri Science)

Opsomming

Hierdie studie het gefokus op vermindering van inokulumvlakke, as deel van 'n holistiese beheerprogram, in plaas van die algemene praktyk om slegs die vrugte teen infeksie te beskerm. Verskeie produkte is geëvalueer vir hul potensiaal om blaarafval af te breek en gevolglike effek op inokulumproduksie. In 'n boord is die effek van verskeie programme, wat voor vrugset gespuit is, die behandeling van blaarafval en swamdoderprogramme om vruginfeksie te verhoed, vergelyk. Resultate toon dat nie een van die middels wat getoets is, kompostering van blare betekenisvol beter kon aanhelp nie. Die studie toon ook dat carbendazim-bespuittings wat voor vrugset toegedien is, en/of strobilurien bespuittings om vrug infeksie te verhoed, wel inokulum verminder het. Behandeling van blaarafval met Breakdown All + Compost Aid het wisselvallige resultate opgelewer.

Summary

This study focussed on reducing CBS inoculum levels as part of a holistic management program, in stead of relying on fruit protection alone. Several products were evaluated for their ability to degrade leaf litter and for effects on inoculum production. In an orchard trial, several programmes applied before fruit set, treatment of leaf litter and standard fungicide programmes to prevent fruit infection were studied. Preliminary results showed that no difference in degradation of leaf litter existed for the products evaluated, but several factors contributed to large variation within treatments. The results furthermore showed that pre-fruit set carbendazim, and/or strobilurin applications to protect fruit, might reduce inoculum, while variable results were obtained with Breakdown All + Compost Aid.

Introduction

Citrus black spot is considered as a major threat to citrus producing countries where the disease do not occur and strict phytosanitary control measures have been implemented by several countries including the European Union, Japan and USA. Controlling the disease with fungicide spray programmes to prevent fruit infection are usually very effective, but due to a wide spectrum of factors that can influence efficacy, poor control is sometimes achieved with a direct negative economical impact to the producer and an increasing threat to exportation of citrus from certain production areas in South Africa to sensitive markets.

The severity of plant disease is determined by the susceptibility of the host, the availability and fitness of pathogen's inoculum levels and climatic conditions that usually also favour production and dispersal of inoculum, infection and lesion development. Agricultural disease management mainly focuses on the host for identification and development of genetic resistance, and the pathogen, where biocides are used to reduce inoculum, prevent infection, and inhibition of lesion development. Very little can be done with regards to climatic conditions, especially if requirements for optimum production and disease development are similar. Currently, citrus producers in South Africa are dependent only on the use of fungicides to protect fruit from infection for 6 months, between October and February, to ensure disease free fruit. With current practices of extensive pruning, leaf litter and the production of inoculum on leaf litter on the orchard may increase the threat of black spot disease.

The concept of reducing inoculum levels to control apple scab disease have been studied and was successful in a number of trials (Jespersion, 1995; Beresford, *et al.*, 2000; Carisse *et al.*, 2000; Sutton, *et al.*, 2000; Mondal & Timmer, 2003). By reducing inoculum levels in orchards the spread of disease to new orchards can be slowed down, disease pressure in orchards will decrease, resulting in a decrease in the number of successful infections and also the tempo of resistance development to certain fungicides groups. Therefore, the effects of current disease control and other production methods on inoculum levels were studied in order to find a holistic approach to citrus black spot disease control where all efforts are utilised to reduce risk of infection and disease development.

Materials and methods

Effect of degradation products

In a trial at Letaba Estates, Breakdown All + Compost Aid (Ba + Ca), Biomax and Agrilibrum products (QCM + Biocarb) have been applied on the 21st of November 2008 to 3-tree plots replicated 5 times in a randomised block design in 10 l of water/tree, i.e. approximately 5000 l of water/ha. QCM was prepared by adding 1 l of activator to 20 l of QCM. After 24 hr, 5 l of Biocarb were mixed with 5 l of activated QCM. Products used and rates are shown in Table 1. One square meter blocks of leaves under the tree canopies of each replicate were covered with shading net (Fig. 4.6.6.1). Leaves under the nets were gathered and weighed as well as evaluated with a Kotzé Inoculum Monitor (KIM) in February 2009 in order to determine the most effective treatment to enhance the degradation process of leaf litter and possible reduction of ascospore inoculum.

Effect of pre-fruit set applications, fungicide programmes and chemical applications to leaf litter

A trial to evaluate the effect of several treatments such as degradation of leaf litter under the trees, application of a pre-blossom carbendazim or urea application, and standard spraying programmes to protect fruit from infection with either carbendazim or strobilurin products, were conducted in the Letsitele area in a 31-year-old Valencia orchard with a history of citrus black spot disease. Trees were divided into 21 sub-plots containing approximately 50 trees per treatment combination (7 rows by 8 trees). Benomyl (500 g/kg, WP) at 50 g + 300 ml BP medium oil/100 l water was applied, or spray urea at 1000 g/100 l water, was applied to some trees as a pre-fruit set application on the 10th of September 2008. Either a mancozeb program, consisting of 6 applications of 200 g mancozeb (800 g/kg, WP)/100 l water, at 24-day intervals, or a strobilurin program, consisting of 3 applications of 10 ml pyraclostrobin (500 g/l, EC) + 150 g mancozeb (800 g/kg, WP) + 300 ml BP medium oil/100 l water, at 6-week intervals, were applied to specific blocks. Treatment numbers and description of applications are shown in Table 4.6.6.2. All treatments were randomly replicated twice except for treatments 10 (- Ma -) and 12 (- St -) where only 1 replicate was possible due to lack of trees. Applications commenced on the 15th of October 2008. Pruning was only completed late in October 2008 and therefore, leaf litter could only be treated with Break-down All + Compost Aid on the 5th of November 2008. Leaf analysis with a Kotzé Inoculum Monitor (KIM) was done to determine the effect of treatments on ascospore inoculum production on the remaining leaf litter during March 2009. The percentage fruit with black spot lesions will be determined in July/August 2009.

Results and discussion

Effect of degradation products

Results showed no statistical difference between products with regards to the average weight of remaining leaf litter or the number of ascospores trapped with the KIM (Table 4.6.6.3). Lack of differences could be attributed to large variances within replicates possibly due to the fact that 2 replicates of the control treatment and one of Biomax was vandalised as well as possible difference in the initial weights of leaf litter under trees due to irregular distribution as well as difference in microclimate under trees due irregular irrigation patterns. This trial should be repeated with pre-weighed batches of leaf litter and possibly with more than 5 replicates per treatment in order to reduce variance within treatments.

Effect of pre-fruit set applications, fungicide programmes and chemical applications to leaf litter

When evaluated in January 2009, leaf litter under trees from programmes with benomyl, applied to foliage before fruit set, in general had the lowest ascospore numbers (treatments 1 – 4 with between 68 and 452 and a total of 971 ascospores) when evaluated with the KIM (Table 4.6.6.4). With urea and no applications very high and very low numbers were obtained (treatments 5 – 8 with between 65 and 26 617 and a total of 36 964 ascospores and treatments 9 – 12 with between 0 and 26 135 and a total of 42 306 ascospores). These large variations were probably caused by other effects than the urea or no application. When analysing the effect of mancozeb or strobilurin programmes it gets even more complicated with the general trend showing low numbers where strobilurin was used (treatments 3, 4, 7, 8, 11, 12 with between 3 and 144 ascospores and one exception with 26 235 ascospores) and large variation where mancozeb was used (treatments 1, 2, 5, 6, 9, 10 with 2 treatments with more than 300 ascospores and 3 treatments with more than 10 000 ascospores and 1 exception where 0 ascospores could be found). The application of Breakdown All and Compost Aid (treatments 1, 3, 5, 7, 9, 11) resulted in higher ascospore availability in January with a total of 69 560 ascospores trapped compared to untreated leaves (treatments 2, 4, 6, 8, 10, 12) where only 10 681 ascospores were trapped.

When evaluated in March 2009 results showed that leaf litter under trees from all programmes had generally low numbers of ascospores compared to the January evaluation (Table 4.6.6.4). Samples from leaves under trees treated with benomyl (treatments 1 – 4) produced between 7 and 135 ascospores with a total of 265 for all treatments. Samples from leaves under trees treated with urea (treatment 5 – 8) produced between 8 and 148 ascospores with a total of 269, while samples from leaves under trees with no treatments (treatments 9 – 12) produced between 0 and 121 ascospores with a total of 183 for all for treatments. The difference for ascospores under trees receiving strobilurin applications (treatments 3, 4, 7, 8, 11, 12 with 105 ascospores in total) and mancozeb (treatments 1, 2, 5, 6, 9, 10 with 612 ascospores in total) were more dramatic. Where leaf litter was treated with Breakdown All and Compost Aid (treatments 1, 3, 5, 7, 9, 11) resulted in higher ascospore availability in January with a total of 462 ascospores trapped compared to untreated leaves (treatments 2, 4, 6, 8, 10, 12) where only 255 ascospores were trapped.

Conclusion

Infected leaves can remain on trees for 2 – 3 years and therefore, initial results might not show the true effect of all treatments. At this stage the pre-fruit set application of benomyl showed a general trend to reduce the production of ascospores on leaf litter especially for the January evaluation. No clear advantage was observed during the March evaluation, probably due to the masking effects of other treatments. The addition of a strobilurin programme for protection of fruit against infection showed a constant advantage over a mancozeb programme during both evaluations. No positive results were obtained with Compost Aid + Breakdown All during either of the evaluations.

Further objectives (milestones) and work plan

Programmes will be repeated in 2009/2010 season and hopefully the pruning can be completed just after harvest in August this production season. Variable results obtained with KIM and other methods for comparison of treatment efficacy will have to be investigated, but increasing the number of replicates for leaf litter samples might reduce variation. Hopefully more positive results will be obtained with lesion evaluation on fruit in July/August 2009. We expect larger differences between treatments during the 2nd and 3rd year of evaluation due a carry over effect from one season to the next, especially with regards to infected leaves that can remain in trees for a 2 – 3 year period.

Technology transfer

No conclusive results are available.

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Table 4.6.6.1. Products and dosages applied to leaf litter on Letaba Estates.

Treatment no	Products	Dosage
1	Breakdown all + Compost aid	750 ml + 1 kg / ha
2	Biomax	750 ml / ha
3	QCM + Biocarb	5 l + 5 l / ha
4	Control	-

Table 4.6.6.2. Products and treatments applied to different plots in an orchard trial.

Treatment no	Program description*	Products applied		
		Pre-fruit set application	Preventative fruit protection	Treatment of leaf litter
1	Be Ma Ba	Benomyl	Mancozeb	Ca + Ba
2	Be Ma -	Benomyl	Mancozeb	-
3	Be St Ba	Benomyl	Strobilurin	Ca + Ba
4	Be St -	Benomyl	Strobilurin	-
5	Ur Ma Ba	Urea	Mancozeb	Ca + Ba
6	Ur Ma -	Urea	Mancozeb	-
7	Ur St Ba	Urea	Strobilurin	Ca + Ba
8	Ur St -	Urea	Strobilurin	-
9	- Ma Ba	-	Mancozeb	Ca + Ba
10	- Ma -	-	Mancozeb	-
11	- St Ba	-	Strobilurin	Ca + Ba
12	- St -	-	Strobilurin	-

* Be = Benomyl, Ma = Mancozeb, Ba = Breakdown All, Ur = Urea, Ca = Compost aid, Ba = Breakdown All

Table 4.6.6.3. Results of leaf litter degradation and number of ascospores on remaining litter.

Treatment no.	Products	Average weight of leaf litter (g) ^z	Number of ascospores trapped in KIM ^z
1	Compost aid + Breakdown all	53 a	0 a
2	Biomax	97 a	0 a
3	QCM + Biocarb	62 a	0.2 a
4	Control	49 a	0.4 a

^z Results in a column (based on mean of 5 replicates) followed by the same letter are not significant different from each according to Fisher's t-test (P = 0.05)

Table 4.6.6.4. Results of pre-fruit set applications, fungicide programmes and chemical applications to leaf litter.

Treatment no	Program description*	Mean Number of ascospores trapped in KIM	
		Jan 2009	Mar 2009
1	Be Ma CaBa	307	103
2	Be Ma -	452	135
3	Be St CaBa	68	20
4	Be St -	144	7
5	Ur Ma CaBa	26 817	148
6	Ur Ma -	9 964	105
7	Ur St CaBa	65	8
8	Ur St -	118	8
9	- Ma CaBa	16 068	121
10	- Ma -	0	0
11	- St CaBa	26 235	62
12	- St -	3	0

Be = Benomyl, Ma = Mancozeb, CaBa = Compost aid + Breakdown All, Ur = Urea



Fig. 4.6.6.1. Increased leaf litter due to pruning and evaluation of different products with regards to degradation of leaf litter.

4.7 **CRI DIAGNOSTIESE SENTRUM** deur Laura Huisman, Wilma Bester en Timothy Zulu (CRI)

Ontleding	Sitrus Kwekerye	Kommersiële monsters	Ander gewasse	Navorsings monsters
Nematode: Wortels		493	3	524
Grond		13	18	492
<i>Phytophthora</i> : Grond	1268	556	86	663
Kwekery water	70	2	2	
Swartvlek		16		
Rooïdopluis				
Sitrusvergroeningsiekte (UP)				

Sitrus Geakkrediteerde Kwekerye

Dit is verpligtend vir al die sitruskwekerye wat aan die Sitrus Verbeteringskema deelneem om kwartaalliks monsters te laat ontleed vir *Phytophthora*. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. Eenduisend-tweehonderd-agt-en-sestig monsters is deur die diagnostiese sentrum vir *Phytophthora* ontleding ontvang, waarvan 5.7% positief getoets het.

Kommersiële monsters

Monsters is uit die volgende sitrusverbouingsareas ontvang: Wes Kaap, Mpumalanga, Limpopo, Oos Kaap, Noord Kaap en KwaZulu-Natal. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Sewe-en-veertig persent van die aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wifies per 10g wortels gehad. Aalwurmdoderbehandelings is aanbeveel. Twee-en-veertig persent van die monsters wat vir *Phytophthora* ontleed is, het positief getoets.

Ander Gewasse

Aalwurmtellings is op grond- of wortelmonsters van grenadella, ruscus, mielies, piesang, mango en tamaties gedoen. Grenadella, ruscus, tamatie, soetrissie, mielie, papaja, pekan, granaat, denneboom, avokado en macadamia monsters is vir *Phytophthora* en *Pythium* ontleed. Die macadamia industrie het met 'n kwekery verbeteringskema begin, soortgelyk aan die Sitrus Verbeteringskema. Die diagnostiese sentrum het 25 monsters vanaf macadamia kwekerye en 43 monsters vanaf avokado kwekerye vir *Phytophthora cinnamomi* ontleed.

Navorsings Monsters

Aalwurm en *Phytophthora* ontledings is op 'n groot aantal monsters afkomstig vanaf navorsingsprojekte, om omgewingsvriendeliker aalwurm- en swamdoders te toets, gedoen.

5 PROGRAMME: CROP AND FRUIT QUALITY MANAGEMENT

5.1 PROGRAMME SUMMARY

By Tim G. Grout (Manager: Research & Technical, CRI)

Research in the Crop and Fruit Quality programme continued to be as challenging as ever but some great advances were made and years of fundamental research on rind breakdown in Clementines finally bore fruit in showing that the accumulation of K, Mg, Ca and both reducing and non-reducing sugars in the flavedo, is influenced by canopy position. This helps to explain why fruit that have received less light inside the canopy are more prone to rind breakdown and led to the demonstration that sprays of Ca and Mg reduced this condition in Clementines. Some similarities exist between these results and research on creasing because shaded, inside fruit are more prone to creasing than outside fruit, but a correlation with albedo nutrient and mineral levels could not be found. Neither Messenger nor AVG were found to reduce creasing but both Maxcel and CPPU reduced it by 20%. Chilling injury was once again investigated from a number of angles and known treatments which included water at 53°C were shown to be effective. Rind sucrose levels were found to be correlated with chilling injury which may provide the opportunity for susceptibility predictions. Waxes with high solids were shown to decrease chilling injury but their ability to increase peteca spot could not be confirmed. Peteca spot research was once again frustrating in that sites that had produced high levels of the disorder in the past had very little this season and it could not be induced with rough handling and over-brushing. Hand thinning of Nules Clementines and Mihowase Satsuma mandarins showed some slight benefits but good management and timing will be critical. Applications of 2,4-D ester during bloom were shown to significantly reduce the size of the navel opening in navel oranges without causing other detrimental effects. Apart from having some horticultural benefits, smaller navel openings will reduce the chance of insects such as mealybugs and the grain chinch bug from hiding there. A new drought stress spectral index for citrus was developed and remote sensing was shown to be beneficial in predicting crop load. Towards the end of the report period Teunis Vahrmeijer was appointed as CRI nutritionist and he is being hosted by the University of Pretoria. Last year it was mentioned that Dr Malcolm Dodd had been asked to coordinate and conduct research in the area of Cold Chain and Packaging but with him leaving PPECB he did not conduct any of his own research during the report period so no report is included. Research on the carbon footprint of fruit industries was funded by CRI and other industries and outputs will be launched later in 2009. However, a summarised report is included in the Fruit Production and Quality project.

Programopsomming

Navorsing in die Oes- en Vrugkwaliteitsprogram het voortgegaan om so uitdagend soos altyd te wees, maar 'n paar groot deurbreke is gemaak en jare van fundamentele navorsing op skilafbraak by Clementines het uiteindelik vrugte afgewerp deurdat dit gewys het dat K, Mg, Ca en beide reduserende en nie-reduserende suikers in die flavedo deur die posisie van die blaredak beïnvloed word. Dit help om te verduidelik hoekom vrugte, wat minder lig aan die binnekant van die blaredak ontvang het, meer geneig is tot skilafbraak en het ook gelei tot die demonstrasie dat bespuitings van Ca en Mg hierdie toestand by Clementines verminder. Sommige ooreenkomste bestaan tussen hierdie resultate en navorsing op kraakskil omdat vrugte aan die binnekant wat in die skadu is, meer geneig is tot kraakskil as vrugte wat aan die buitekant is, maar 'n korrelasie met voedingstowwe en mineraalvlakke in die albedo kon nie gevind word nie. Nie Messenger of AVG is gevind om kraakskil te verminder nie, maar beide Maxcel en CPPU het dit met 20% verminder. Koueskade is weereens vanuit verskillende hoeke ondersoek en bekende behandelings, wat water teen 53°C insluit, was effektief. Sukrose vlakke in die skil is gevind om met koue skade te korreleer wat die geleentheid vir voorspellings vir gevoeligheid mag bied. Wakse met hoë vastestowwe het getoon om koueskade te verminder, maar hul vermoë om peteka kol te verhoog kon nie bevestig word nie. Peteka kol navorsing was weereens frustrerend omrede persele waar hoë vlakke van die skildefek in die verlede ondervind is, hierdie seisoen baie min opgelewer het en dit kon nie met rowwe hantering en oormatige borseling geïnduseer word nie. Uitdunning met die hand van Nules Clementines en Mihowase Satsuma mandaryne het geringe voordele getoon, maar goeie bestuur en tydsberekening sal krities wees. Toedienings van 2,4-D ester tydens blomtyd het getoon om die grootte van die nawel opening van nawel lemoene betekenisvol te verklein sonder om ander nadelige effekte te veroorsaak. Afgesien daarvan dat dit oor 'n paar hortologiese voordele beskik, sal kleiner nawel openinge ook die kans dat insekte soos wiluise en die stink graankewer daarin kan skuil verminder. 'n Nuwe droogte spanning spektrum indeks vir sitrus is ontwikkel en afstandsmeting (remote sensing) het getoon om voordelig in die voorspelling van die oeslading te wees. Teen die einde van die verslagperiode is Teunis Vahrmeijer as CRI se voedingskundige aangestel en hy word by die Universiteit van Pretoria gehuisves. Laas jaar is dit genoem dat Dr Malcolm Dodd gevra is om die navorsing in die Koueketting en Verpakkingsarea te koördineer en uit te voer, maar omdat hy PPECB verlaat het, het hy geen van sy navorsing gedurende hierdie periode uitgevoer nie en geen verslag is

ingesluit nie. Navorsing op die vrugtebedrywe se koolstof voetspoor is deur CRI en ander bedrywe befonds en uitsette sal later in 2009 bekend gemaak word. 'n Samevattende verslag is egter in die Vrugproduksie en Gehalte projek ingesluit.

5.2 PROJECT: RIND CONDITION

Project coordinator: J.P. Bower (UKZNP)

5.2.1 Project summary

Once again, the focus of the rind condition project was in three primary areas, creasing, chilling injury and rind breakdown disorders (rind breakdown, pitting and peteca spot). The creasing work studied a number of aspects. Although GA₃ is known to decrease the incidence of creasing, it is considered to delay colour development: work in section 5.2.2 reports on the effects of earlier sprays. Applications were made between late November and mid-January. The product Messenger® was tested at the same time. All GA₃ applications significantly decreased creasing but not Messenger®. Fruit colour was worse with later chemical applications, but not unacceptably so. The work on the effect of bearing position (5.2.3) indicated that fruit subject to more shade (inside, lower canopy or southern side of trees) had worse creasing. Although albedo nutrient levels varied with bearing position, there was no correlation with creasing at harvest. Future work is aimed at determining whether earlier measurements show correlation. A further extension of this work was aimed at determining the physiological role of light incidence on creasing. Manipulation of light levels, and carbohydrates are reported in 5.2.4. Lower light levels, as induced by covering fruit with a paper bag, increased creasing. Despite also increasing N, K and Mn, no significant correlation of minerals at harvest was again found. Based on the assumption that increased cell division may decrease creasing, two cytokinin products and an anti-ethylene (decrease senescence) compound AVG, were applied after physiological drop to extend cell division (5.2.5). MaxCel and CPPU both significantly decreased creasing (20%) without affecting fruit size or rind thickness. Neither AVG nor the products combined with chelated calcium had any effect. In order to stimulate root growth, production of cytokinins and uptake of minerals, soil amendments such as humates, fulvates, compost and chicken manure will be tested (5.2.6). No results are as yet available. Chilling injury has been considered from a number of angles. The use of hot water and other known treatments such as salicylic acid and methyl jasmonate were tested (5.2.7) and 53°C as well as the other treatments were effective. The physiology behind the results showed anti-oxidants were enhanced. Rind sucrose content (energy source) was significant, which may result in chilling injury potential forecasting being possible. The use of high solids waxes (5.2.8) also decreased chilling injury on 'Oroblanco' and satsumas, From a rind disorder (breakdown) aspect peteca spot again produced erratic results. In 5.2.9 the use of 1-MCP indicated that internal ethylene plays some role, but further work will be needed. Packhouse procedures such as rough handling, over brushing and heavy waxing provided no results (5.2.10). Paul Cronjé made great strides in our understanding of rind breakdown of Nules Clementines. He found that canopy position of the fruit affected accumulation of K, Mg, Ca and both reducing and non-reducing sugars in the flavedo. He confirmed that rind breakdown is worse where fruit receive less light and showed that foliar sprays of Mg and Ca reduce this condition (5.2.11).

Projekopsomming

Weereens, het die skil gehalte projek drie primêre focus areas gehad, kraakskil, koueskade en skil afwykings (skil afbraak in verskeie vorms en peteka kol). Die kraakskil werk het verskeie aspekte bekyk. Alhoewel GA₃ bekend is om kraakskil te verminder, is dit ook bekend om kleur verandering te vertraag. Die werk by 5.2.2 het na vroeë aanwendings bekyk. Die GA₃ is vanaf laat November to middle Januarie gespuit. Die produk Messenger® is ook getoets. Al die GA₃ behandelings het wel kraakskil beheer, maar nie Messenger® nie. Vrug kleur was wel meer vertraag met later aanwendings, maar nie tot 'n onaanvaarbare vlak nie. Die werk op vrug posiesie in die boom (5.2.3) het aangedui dat kraakskil erger was in vrugte in die skaduwee, die binnekant van die boom en die suidelikke kant. Al het die mineraal inhoud van die albedo ook met vrug posisie verander, was daar geen verband met kraakskil nie. Toekomstige werk sal na mineral inhoud van vrugte vroeg in die seisoen kyk om te sien of daar enige korrelasie met kraakskil is of nie. Verdere werk op die fisiologiese efek van lig op induksie van kraakskil is ook gedoen (5.2.4). Verandering van lig dmv die toemaak van vrugte en blare in papier sake het ook koolhidrate verander. Al het dit ook minerale soos N, K en Mn verander, was daar weereens geen korrelasie met kraakskil nie, maar die verminderde lig intensiteit het wel kraakskil vererger. Gebaseer op die moontlikheid dat verhoogde of verlengde seldeling kraakskil kan verminder, is twee sitokiniene en die produk AVG wat etileen (en dus veroudering) verminder, getoets (5.2.5). MaxCel en CPPU het kraakskil betekenisvol (20%) verminder, maar AVG nie, en al die produkte saam met 'n kalsium kelaat het ook nie gewerk nie. 'n Nuwe projek (5.2.6) om wortel stimulasie en dus verhoogde sitokiniene en mineraal opname is aangepak met die gebruik van humate, fulvate kompos en hoendermis. Daar is nog geen resultate nie. Koueskade is deur verskeie eksperimente ondersoek. Die

gebruik van warm water en ander behandelings wat bekend is om koueskade te verminder, is getoets (5.2.7). Water temperatuur van 53°C sowel as metieljasmonaat en salisiensuur het wel 'n positiewe impak gehad. Die fisiologie agter die resultate het bewys dat die vlak van teenoksidante belangrik is, en deur die behandelings verhoog was. Die vlak van die suiker sukrose (die energie bron) het 'n betekenisvolle voorspelling van koueskade gehad. Die gebruik van waks met hoë vastestowwe (5.2.8) het koueskade van 'Oroblanco' and satsumas verminder. By skilafbraak (peteka vlek) was resultate gereeld wisselend. Die gebruik van 1-MCP (5.2.9) het aangedui dat etileen wel 'n rol speel. Verdere werk sal moet gedoen word. Pakhuis behandelings soos tiepe waks, rowwe hantering en oor-borseling het geen resultate gelewer nie (5.2.10).

5.2.2 PROGRESS REPORT: Evaluation of alternative means of controlling creasing (albedo breakdown)

Experiment 849 (October 2005 - March 2009): Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

Opsomming

Kraakskil is 'n groot probleem in die Oos- en Weskaap, veral op Navel en Valencia lemoene. Gibberelliensuur (GA_3) word kommersieel gebruik om kraakskil te verminder met later toedienings wat beter werk, maar kan somtyds lei tot 'n vertraging in die kleurontwikkeling. Die doel van die studie was om die beste tyd van GA_3 toediening te bepaal sonder die negatiewe effek op kleurontwikkeling. Palmer Navel lemoenbome in Citrusdal is gebruik in 'n gerandomiseerde blok ontwerp met ag enkelboom herhalings per behandeling. GA_3 teen 10 ppm is toegedien op 26 Nov. 2007, 11 Des. 2007, 20 Des. 2007, of 14 Jan. 2008. Messenger® (30 g/100 L), a harpin protein, is toegedien op al vier die bogenoemde datums. GA_3 het die graad van kraakskil betekenisvol verminder en kraakskil voorkoms met 7-12% verminder, met geen verskil tussen die behandelings nie, ongeag die tyd van toediening. Al die behandeling tye het kleurontwikkeling vertraag, met 26 Nov. toediening die minste en 14 Jan die meeste. Messenger® het geen effek op kraakskil voorkoms gehad nie.

Summary

Creasing is a major problem in the Eastern and Western Cape especially on Navels and Valencias. Gibberellic acid (GA_3) is used commercially to reduce the incidence of creasing with later applications being more effective, but sometimes resulting in a negative effect on fruit colour. The objective of this study was to determine the most effective time for GA_3 spray application without the negative colour effect. Palmer Navel trees in Citrusdal were used in a randomized complete block design with eight single tree replicates per treatment. Gibberellic acid (GA_3) at 10 ppm was applied on 26 Nov. 2007, 11 Dec. 2007, 20 Dec. 2007, or 14 Jan. 2008. Messenger® (30 g/100 L), a harpin protein, was applied at all four dates mentioned above. GA_3 significantly reduced creasing severity and reduced creasing incidence by 7-12%, with no differences among the treatments, irrespective of the time of application. All treatment times significantly delayed colour development, with 26 Nov. resulting in the better coloured fruit and later application (14 Jan.) in the worst coloured fruit. Messenger® had no effect on creasing incidence.

Introduction

Creasing is a pre-harvest physiological disorder usually observed at post-colour break (Bar-Akiva, 1975). Early onset of senescence (Monselise et al., 1976) or expression of rapidly progressing senescence (Monselise, 1973) is associated with the development of creasing. Hence, creasing is characterized as one of the rind disorders associated with ageing (Coggins, 1973). Creasing incidence is normally reduced by pre-harvest application of Gibberellic acid (GA_3) (Coggins, 1973; Monselise, 1973, 1979). GA_3 appears to impede the initial development of creasing via its action of decreasing the pectin methyl esterase activity (Jona et al., 1989) which is unusually high in affected fruits (Jones et al., 1967) and increases the firmness and strengthens the rind of the fruit (Coggins, 1969; Gambetta et al., 2000).

The effectiveness of GA_3 as a control measure for creasing is dependent on the correct concentration, spray solution pH and timing of application. The most effective concentration was found to be 10 ppm and 20 ppm depending on the location, time of application and severity of creasing. Tugwell et al. (1996) observed that a high volume application of 20 ppm GA_3 was effective in controlling creasing under South Australian conditions. Gilfillan et al. (1981) recommended a concentration of 10 ppm on Navels under South African conditions. Monselise et al. (1976) recommended 20 ppm on Valencias under Israeli conditions.

Application of GA_3 when fruitlets are 30 - 55 mm in diameter were observed to reduce creasing incidence (Bevington et al., 1973; Gambetta et al., 2000; Gilfillan et al., 1980, 1981; Monselise et al., 1973; Monselise, 1973; Tugwell, 1996). Later applications when fruitlets were 65 mm had a negative effect on colour

development (Gambetta et al., 2000; Gilfillan and Stevenson, 1974; Gilfillan et al., 1980, 1981; Monselise et al., 1976; Monselise, 1979) but were observed to be more effective than the earlier sprays in South Africa (Gilfillan et al., 1980, 1981). Therefore, the evaluation of the best application timing of GA₃ with the least negative effect on colour development is required. The objective of the study was to pinpoint the timing of GA₃ application without a negative effect on colour development. Messenger® contains a harpin protein (Wei, 2004) and was also evaluated for its effect on creasing incidence and severity. It is believed that the harpin protein triggers a natural hyper-sensitive reaction within plants and activates the pathways that stimulate certain growth and stress-defense responses. This leads to increased defense secondary metabolites, reduces tissue senescence and improves cell wall formation.

Materials and methods

Plant material. Palmer navel trees were used for this study in the 2007/2008 season, in a commercial orchard located in Citrusdal in the Western Cape, South Africa. This site has a history of severe creasing incidence. The field trial consisted of a randomized complete block design with six treatments and eight single tree replicates per treatment.

Treatments. Gibberellic acid (GA₃) at 10 ppm was applied at four different application timings. Breakthru was added as a wetter at 5 ml/100 L water. Treatments were applied at the end of November (26 Nov. 2007), at the beginning of December (11 Dec. 2007), at the end of December (20 Dec. 2007), and in mid-January (14 Jan. 2008). Messenger® (30 g/100 L) was applied at all four dates mentioned above.

On-tree evaluation. An on-tree evaluation of creasing incidence presence and fruit colour was carried out at harvest time (14 May 2008). Forty fruit per single tree were scored for creasing incidence and colour. Ten fruit per single-tree replicate were evaluated at random from the outside of the tree at eye-level on each of four quadrants of the tree; north, south, east and west. Creasing severity was evaluated on a score of 0-4; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, if a single sphere was creased, a one. Fruit colour was determined based on the CRI colour chart for oranges (Set No: 34), with a range between one and eight, one being completely orange and eight being green. Creasing incidence was expressed as a percentage of total fruit evaluated that developed the disorder.

Laboratory analysis. Fruit were sampled on 14 May 2008 from the same site. Ten fruit per single tree replicate were sampled randomly from the outside of the tree at eye-level. The sampled fruit were assessed for creasing, colour, fruit diameter and peel thickness. Creasing and fruit colour were evaluated in the same way as the on-tree evaluation. The fruit diameter of each fruit was measured using an electronic calliper.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan's multiple range test at P= 0.05 was used to test the treatment effects.

Results

Creasing incidence (<25%) and severity (≤ 0.50) was relatively low in this study. Creasing incidence was reduced by up to half of the control by application of GA₃ at any time (Table 5.2.2.1), although it was not significantly different from the control. Creasing severity was significantly reduced by application of GA₃, irrespective of the timing of application. (Table 5.2.2.1). The earlier mid-November application (26 Nov. 2007) was as effective as the December applications (11 Dec. 2007; 20 Dec. 2007) and the later mid-January application (14 Jan. 2008). Messenger® did not reduce creasing incidence or severity compared to the control (Table 5.2.2.1). Colour was significantly delayed by all GA₃ treatments as well as Messenger. Fruit sprayed with GA₃ on 14 January 2008 had the worst colour development at harvest (Table 5.2.2.1 and Fig.5.2.2.1). However, colour development was not severely retarded by any of the treatments. There was a weak relationship between fruit colour and creasing incidence (R = -0.3). The diameter and peel thickness of fruit used in the laboratory assessment was not significantly different from the control, although large treatment differences (but not significant) were observed for creasing severity and incidence in these fruit (Table 5.2.2.2). Creasing incidence and severity was more pronounced in fruit on the north side of the trees compared to the south side (Table 5.2.2.3). Creasing severity was ranked from the highest to the lowest as follows: north > south > east = west and creasing incidence was ranked: north > south = east = west (Table 5.2.2.4). Colour was also significantly different amongst the different sides and was ranked from the best to worse as follows: south > east > north > west (Table 5.2.2.3).

Discussion

GA₃ either applied alone or in combination with other mineral nutrients, has been investigated extensively and is used commercially to ameliorate the incidence of creasing in most citrus producing countries (Coggins., 1969; Bevington, 1973; Monselise et al., 1973; Embleton et al., 1973; Gilfillan et al., 1980, 1981; Tugwell et al., 1996 and Gambetta et al., 2000). As expected, creasing incidence was reduced by the application of GA₃, although it was not significant in this study. The effectiveness of mid December to mid January (SH) applications usually when fruit were 30 – 55 mm in diameter have already been reported (Bevington, 1973; Monselise et al., 1976; Gilfillan et al., 1980, 1981; Tugwell, 1996 and Gambetta et al., 2000). Monselise et al. (1973) also suggested that application of GA₃ at the period when fruitlet growth consists essentially of peel growth effectively reduce creasing. Gilfillan et al. (1981) in South Africa reported that mid-November applications were less effective than later applications in controlling creasing, however, our data for the mid-November application could not confirm these findings.

Mid-November sprays gave a good colour at harvest as observed by Gilfillan et al. (1981). Messenger had no effect on creasing incidence.

Conclusion

GA₃ significantly reduced creasing severity and reduced creasing incidence by 7-12%, with no differences among the treatments, irrespective of the time of application (26 Nov., 11 Dec., 20 Dec, 14 Jan). All treatment times significantly delayed colour development, with 26 Nov. resulting in the better coloured fruit (2.89 vs 2.53) and later application (14 Jan.) in the worst coloured fruit (3.12 vs.2.53). Messenger® (harpin protein) applied on 26 Nov. (petal drop), 11 Dec., 20 Dec. and 14 Jan had no effect on creasing incidence.

Further objectives and workplan

The research is ongoing and will be repeated in the 2008/2009 season with the same objectives.

Technology transfer/Tegnologie oordrag

Phiri, Z.P., Verreyne, J.S. Studies in citrus creasing/albedo breakdown. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

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Table 5.2.2.1. On-tree evaluation of GA₃ (10 ppm) application timing on creasing severity, creasing incidence and colour of Palmer navel oranges in Citrusdal.

Treatment	Creasing severity	Creasing incidence	Colour
	--0-4 ^x --	--%-	--1-8 ^y --
1. Control	0.52 a ^z	24.37	2.53 d
2. GA ₃ (26 Nov. 2007)	0.32 b	15.48	2.89 c
3. GA ₃ (11 Dec. 2007)	0.30 b	17.18	3.07 ab
4. GA ₃ (20 Dec. 2007)	0.28 b	12.81	3.08 ab
5. GA ₃ (14 Jan. 2008)	0.36 b	15.31	3.12 a
6. Messenger (26 Nov. 2007, 11 Dec. 2007, 20 Dec. 2007 and 14 Jan. 2008)	0.42 ab	20.63	2.91 bc
P-value	0.0055	0.1360	0.0001

^zMeans with the same letter are not significantly different at the 5% level (Duncan).

^y1-8 (1 = orange, 8 = green) CRI colour chart (Set No: 34) for oranges.

^x0-4 (0 = 0%, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100%).

Table 5.2.2.2. Laboratory evaluation of the effects of GA₃ (10 ppm) application timings on creasing severity, creasing incidence, colour, diameter and peel thickness of Palmer navel oranges in Citrusdal.

Treatment	Creasing severity	Creasing incidence	Colour	Diameter	Thickness of peel
	--0-4 ^x --	--%-	--1-8 ^y --	--mm--	--mm--
1. Control.	0.46	25.00	2.82	67.34	5.72
2. GA ₃ (26 Nov. 2007)	0.15	16.67	2.95	68.95	5.81
3. GA ₃ (11 Dec. 2007)	0.27	8.33	3.11	66.83	5.60
4. GA ₃ (20 Dec. 2007)	0.13	5.00	3.03	67.23	5.76
5. GA ₃ (14 Jan. 2008)	0.38	18.33	2.91	68.19	5.84
6. Messenger (26 Nov. 2007, 11 Dec. 2007, 20 Dec. 2007 and 14 Jan. 2008)	0.33	16.67	2.98	68.84	5.95
P-value	0.1158	0.5497	0.5537	0.0884	0.5315

^y1-8 (1 = orange, 8 = green) CRI colour chart (Set No: 34) for oranges.

^x0-4 (0 = 0%, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100%).

Table 5.2.2.3. The relationship of the bearing position of fruit on a tree and the severity of creasing, creasing incidence and colour of Palmer navel oranges in Citrusdal.

Treatment	Creasing severity	Creasing incidence	Colour
	--0-4 ^x --	--%--	--1-8 ^y --
1. North	0.62 a ^z	25.42 a	3.17 b
2. South	0.38 b	18.33 b	2.29 d
3. East	0.24 c	13.62 b	2.90 c
4. West	0.22 c	13.12 b	3.38 a
P-value	0.0043	0.1112	0.0001

^zMeans with the same letter are not significantly different at the 5% level. (Duncan)

^y1-8 (1 = orange, 8 = green) CRI colour chart (Set No: 34) for oranges.

^x0-4 (0 = 0%, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100%).

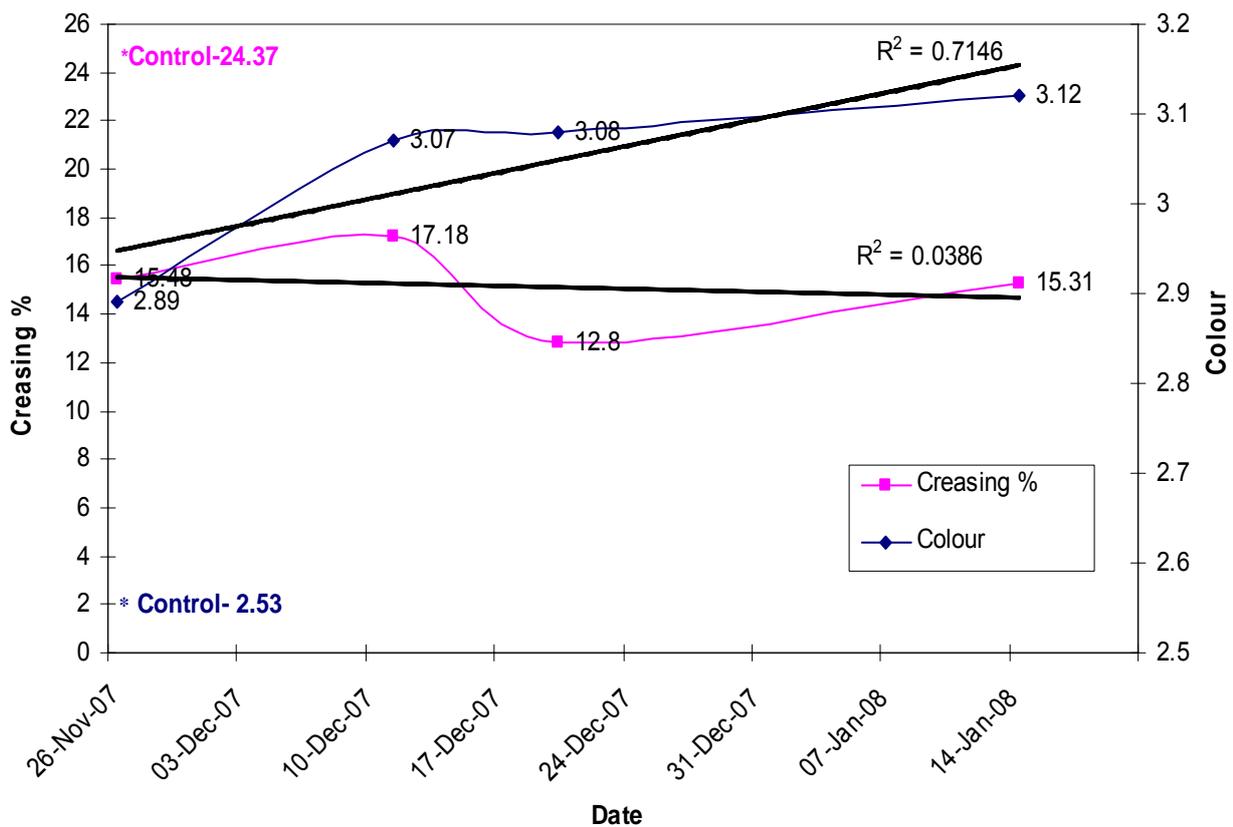


Fig 5.2.2.1. The effect of GA₃ application timing on creasing percentage and colour of Palmer navel oranges in Citrusdal.

5.2.3 PROGRESS REPORT: Relationship of bearing position of fruit on a tree and creasing incidence

Experiment 863 (April 2006 - March 2009): Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

Opsomming

Alhoewel die bydraende faktore van kraakskil bekend is, is die verband tussen draposisie in die boom en kraakskil voorkoms nog nie in detail bestudeer nie. Die doel van die studie was om te bepaal of kraakskil voorkoms bepaal word deur die draposisie van vrugte in die boom en of hierdie verskille in kraakskil voorkoms in die boom verband hou met verskille in makro- en mikroelement konsentrasies in die albedo van vrugte by oestyd. Ag Palmer navel lemoenbome in Addo is gebruik. Elke boom is in vier sektore ingedeel, noord, suid, oos en wes. In elke sektor is vrugte van 4 sub-sektore gemonster, binne en buite in die boonste gedeelte van die boom, en binne en buite in die onderste gedeelte van die boom. Kraakskil voorkoms is waargeneem in volgorde van hoogste tot laagste soos volg: onder binne>onder buite>bo binne>bo buite. Kraakskil voorkoms was hoër in die suidekant van die boom as in die noordekant. Binne vrugte het 'n hoër kraakskil voorkoms as buitevrugte en die skadukant van buitevrugte het meer kraakskil as die sonkant gehad. Geen makro- of mikroelemente in die albedo weefsel by oestyd het dieselfde patroon as kraakskil voorkoms gevolg in terme van draposisie in die boom nie en slegs swak korrelasies tussen elemente en kraakskil voorkoms is waargeneem. Dus kan ons aanneem dat minerale element konsentrasies in albedo weefsel by oestyd nie 'n rol speel in kraakskil ontwikkeling en voorkoms nie.

Summary

Although, the contributing factors of creasing are known, the relationship of bearing position of a fruit on a tree in relation to creasing incidence has not been studied in detail. The aim of the study was to determine if creasing incidence is influenced by the position of fruit on a tree and if these differences in the creasing

incidence are related to differences in macro and micro nutrient concentration in the albedo tissue of fruit at harvest. Palmer navel orange trees in Addo were used. Each of eight trees was divided into four sectors, viz. north, south, east and west. In each sector, fruit were harvested from four different sub-sectors, the inside and outside of the top part of the canopy and the inside and outside of the bottom part of the canopy. Creasing incidence was ranked from highest to lowest as follows: bottom inside > bottom outside > top inside > top outside and was higher on the south side than on the north side of the tree. Inside fruit had greater creasing incidence than outside fruit with a tendency of the shady side of fruit to be more creased than the sunny side of fruit from outside sub-sectors. None of the micro or macronutrients in the albedo tissue at harvest followed the pattern as for creasing incidence in relation to bearing position and there were only weak correlations between nutrients and creasing incidence. Therefore mineral nutrient concentrations in albedo tissue at harvest does not seem to be involved in creasing development and incidence.

Introduction

Creasing is a physiological disorder caused by cell to cell separation in the middle lamellae of albedo tissues (Treeby et al., 2000) resulting in fractures in the albedo and collapse of the flavedo showing creases on the surface of the fruit (Treeby et al., 1995). Although, the contributing factors are known, the relationship of bearing position of fruit on a tree and the incidence of creasing has not been studied in detail. Previous workers (Coggins, 1969; Fourie and Joubert, 1957; Gilfillan et al., 1981; Jones et al., 1967; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous., 1938) have commented on the effect of bearing position on creasing incidence.

Jones et al. (1967) reported that the creasing severity was higher on the south side of the tree than on the north side of the tree (NH). On the contrary, Coggins (1969) observed a higher incidence on the north side of the tree (NH), which was attributed to the shading effect. In the southern hemisphere (SH) Gilfillan et al. (1981) observed a higher creasing incidence on the south side of the tree canopy compared to the north side. Accordingly, creasing is usually greater on the shady side of the fruit (Fourie and Joubert, 1957; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous., 1938) with a tendency of the part of the fruit facing towards the trunk being more creased compared to the sun-exposed part of fruit (Jones et al., 1967; Gambetta et al., 2000).

Creasing is also associated with low Ca concentrations (Gambetta et al., 2000; Jones et al., 1967; Nagy et al., 1982; Storey and Treeby, 2000; Storey et al., 2002; Treeby et al., 2000; Treeby and Storey, 2002), low concentrations of K (Gambetta et al., 2000; Jones et al., 1967; Storey et al., 2002), high concentrations of P (Gambetta et al., 2000) and high concentrations of N (Jones et al., 1967) in the peel of whole fruit at harvest. Less Mg and Na was also found in the rind of creased fruit at the end of the season (Jones et al., 1967; Storey et al., 2002). Based on mineral analysis of fruit at four weeks post petal fall, Bower (2004) reported that Ca concentration was not a good indicator of creasing development and that the elements Mo, S and Zn were involved in creasing development.

In general, macro and micronutrient distribution in the albedo tissue of fruit varies depending on position of fruit within the canopy (Kruger, 2005). Storey et al., 2002 also reported on the variations in nutrient concentration in the albedo tissue of non creased and creased fruit collected from different positions in a tree without reporting on the incidence of creasing on the different positions. Hence, the aim of the study was to determine if creasing incidence is influenced by the position of fruit on a tree and if these differences in the creasing incidence are related to differences in macro and micro nutrient concentration in the albedo tissue of fruit at harvest.

Materials and methods

Plant material. Palmer navel trees were used for this study in the 2006/2007 season. A commercial orchard located in Addo in the Eastern Cape was used. The tree spacing was 6 m between rows and 4 m in rows and the row direction of the orchard was north to south. This site has a history of severe creasing incidence. Each tree replicate was divided into four sectors, viz. north, south, east and west. In each sector, fruit was harvested from four different sub-sectors, from the inside and outside of the top part of the tree canopy as well as the bottom inside and outside part of the tree canopy. These sixteen positions were replicated eight times.

Creasing severity, colour, fruit diameter, and peel mineral analysis. The fruit were sampled at commercial harvest on 5 June 2007. Six fruit were picked from each of the sixteen positions. Creasing severity was evaluated on a score of 0-4 for each fruit; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, if a single sphere was creased, a one and so on. Fruit

colour was determined based on the CRI colour chart (Set No: 34) for oranges, with a range between one to eight, one being completely orange and eight being wholly green. The diameter of each fruit was measured using an electronic caliper. The albedo tissue of the inside and outside of each fruit from the outside sub-sectors was removed, dried and stored in small vials for a complete mineral nutrient (macro and micro) analysis.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan multiple range test at $P= 0.05$ was used to test the treatment effects. The correlation between peel mineral content and creasing incidence or severity was demonstrated with the Pearson's correlation coefficients determined by the SAS (Statistical Analysis System) Enterprise Guide 3 and only R values ≥ 0.5 were considered physiologically significant.

Results

Creasing incidence and creasing severity. Creasing incidence was generally high (>50%) in this study. Although there were no significant differences in creasing incidence among sub-sectors, in general, bottom inside fruit had a greater creasing incidence than bottom outside fruit and top inside fruit were more creased than top outside fruit (Table 5.2.3.1). On average, inside fruit had significantly greater creasing incidence than outside fruit. On average, creasing incidence can be ranked from highest to lowest as follows: bottom inside > bottom outside > top inside > top outside. There were no differences between fruit sampled from the west and east side of trees and between fruit sampled from the top versus bottom part of trees and creasing incidence was significantly higher on the south side than on the north side of the tree (Table 5.2.3.1). Creasing incidence amongst the sides of the tree can be ranked from the highest to the lowest as follows: south > west > east > north. A similar trend was observed with creasing severity. The exception was that fruit sampled from the west side of trees had a greater creasing severity than fruit sampled from the east side of trees (Table 5.2.3.1).

Diameter. There were no significant differences in the fruit diameter from fruit sampled in the different sub sectors (Table 5.2.3.1). Accordingly, weak correlations between fruit diameter and creasing incidence or severity were observed (Table 5.2.3.2).

Macronutrients. Although, there were significant differences in the concentration of macronutrients in the albedo from fruit sampled from different sub-sectors of the tree (Table 5.2.3.3), there were no consistent trends as were shown for creasing incidence (Table 5.2.3.1) and none of the macronutrients showed any strong correlations between albedo concentrations at harvest and creasing incidence or severity. P, K and Mg concentrations in the albedo were significantly different in fruit sampled from north vs. south and west vs. east sides of the tree. N and K concentrations in the albedo were significantly greater on the inside vs. the outside of the tree. N, K, Ca and Mg concentrations in the albedo were significantly different between fruit sampled from the top vs. the bottom part of the tree.

Micronutrients. Sodium (Na), manganese (Mn), zinc (Zn) and boron (B) concentrations in the albedo were significantly different in fruit sampled from the north vs. the south side of the tree (Table 5.2.3.4). Significant differences were also observed with Na, Cu and B albedo concentration in fruit sampled from the west vs. the east side of the tree (Table 5.2.3.4). Na, Mn, Cu, and B concentration in the albedo were significantly different in fruit sampled from the top vs. the bottom part of the tree (Table 5.2.3.4). Mn concentrations in the albedo were significantly higher in outside fruit than in inside fruit. However, no general trends in the micronutrient concentrations in the albedo at harvest were observed as for creasing incidence (Table 5.2.3.1). Also, micronutrient concentrations in the albedo at harvest and creasing incidence or severity showed weak correlations (Table 5.2.3.2).

Sunny versus shaded part of fruit. Creasing severity was significantly greater on the shady side of the fruit than on the sunny side of the fruit in 7 out of the 8 outside sub-sectors evaluated (Table 5.2.3.5). Na (Table 5.2.3.8), Mn and Fe (Table 5.2.3.9) and Cu (Table 5.2.3.10) concentrations in the albedo were not significantly different between the shady side of the fruit and the sunny side of the fruit in any of the outside sub-sectors evaluated, whereas P (Table 5.2.3.6), Ca (Table 5.2.3.7), Mg (Table 5.2.3.8) and Zn (Table 5.2.3.10) concentrations in the albedo were significantly different between the shady side of the fruit and the sunny side of the fruit in only 1 of the 8 outside sub-sectors evaluated. N (Table 5.2.3.6) and K (Table 5.2.3.7) concentrations in the albedo were significantly higher in the shady side of the fruit than in the sunny side of the fruit in 2 of the 8 outside sub-sectors evaluated, and B (Table 5.2.3.11) concentrations in the albedo were significantly different in the shady side of the fruit and the sunny side of the fruit in 3 of the 8 outside sub-sectors evaluated. However, very weak correlations between creasing severity and mineral

nutrient concentrations in the albedo of the sunny or shady sides of the fruit, respectively, were observed (Table 5.2.3.12).

Discussion

Creasing incidence was higher on the south side than on the north side of the tree although it was not significant. This is an expected trend in the Southern Hemisphere (SH) since the south side is considered to be the shady side (SH) and creasing incidence is higher on the shaded side (Le Roux and Crous, 1938; Fourie and Joubert, 1957; Jones et al., 1967 and Holtzhausen, 1981). This was also confirmed by the tendency of inside fruit to show significantly greater creasing incidence than outside fruit. Moreover, creasing incidence was greater on the shady side than on the sunny side of the fruit in almost all the outside sub-sectors.

Differences in fruit diameter from fruit sampled in the different sub sectors were not observed in our study, hence the differences in creasing incidence were not due to fruit size. However, in general, the creasing incidence is more pronounced on smaller fruit (Le Roux and Crous, 1938; Jones et al., 1976; Holtzhausen, 1981 and du Plessis and Maritz, 2004).

Mineral element concentrations in the albedo tissue showed significant differences amongst all the sub sectors investigated. Therefore, mineral concentration in the albedo tissue is affected by the position of fruit in the tree. Differences in mineral distribution of fruit sampled at different positions in the tree canopy were reported earlier (Kruger, 2005; Storey et al., 2002). However, macronutrient concentrations did not show general trends as shown for creasing incidence and showed very weak correlations with creasing incidence at harvest. Hence, it can be concluded that these differences in the creasing incidence were not related to differences in macronutrient concentration in the albedo tissue of fruit at harvest. Nonetheless, macronutrients (K, N, P, Ca and Mg) have been implicated in creasing development in the past (Gambetta et al., 2000; Jones et al., 1967; Nagy et al., 1982; Storey and Treeby, 2000; Treeby et al., 2000; Treeby and Storey, 2002). Similarly, no general trends as observed for creasing incidence could be established with micronutrient concentration in the albedo and therefore differences in the creasing incidence were not related to the differences in micronutrient concentrations in the albedo tissue of fruit at harvest. However, Bower (2004) reported the importance of Mo, S and Zn in creasing development.

Conclusion

None of the micro or macronutrients in the albedo tissue at harvest followed the pattern as for creasing incidence or severity in relation to bearing position and there were only weak correlations between nutrients and creasing severity or incidence. Therefore nutrient concentrations in albedo tissue at harvest does not seem to be involved in creasing development and is not a good indicator thereof.

Further objectives and workplan

The research is ongoing and in the 2008/2009 season we will determine if mineral concentration in albedo tissue earlier in the season plays a role in creasing incidence later on.

Technology transfer

Phiri, Z.P., Verreynne, J.S. Studies in citrus creasing/albedo breakdown. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

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Table 5.2.3.1. Fruit diameter, creasing severity and incidence at harvest in different positions (sub-sectors) of Palmer navel trees in Addo.

POSITION WITHIN TREE		Diameter	Creasing severity	Creasing incidence
		--mm--	--0-4--	--%--
North	Top outside	69.9	0.9 d ²	52.3
	Top inside	72.9	1.4 bcd	64.1
	Bottom outside	67.9	1.4 bcd	64.3
	Bottom inside	69.9	1.1 cd	64.3
South	Top outside	68.7	1.7 abcd	69.3
	Top inside	69.9	1.9 ab	81.0
	Bottom outside	63.6	1.8 abc	78.4
	Bottom inside	68.1	2.3 a	83.3
East	Top outside	71.1	0.9 d	47.4
	Top inside	68.7	1.1 bcd	55.7
	Bottom outside	68.6	1.7 abcd	83.3
	Bottom inside	69.8	1.7 abcd	78.6
West	Top outside	69.6	1.5 abcd	73.9
	Top inside	68.8	1.8 abc	71.6
	Bottom outside	68.8	2.0 ab	66.7
	Bottom inside	70.4	2.3 a	80.1
	P-value SE	0.1861 1.6492	0.0019 0.2715	0.1106 9.1072
Average	Top outside	69.8	1.3	60.7
	Top inside	70.1	1.6	68.1
	Bottom outside	67.2	1.7	73.2
	Bottom inside	69.6	1.9	76.6
Source:	Df			
Treatment	15			
North vs. South	1	0.0282	0.0001	0.0109
West vs. East	1	0.7990	0.0078	0.2553
Outside vs. Inside	1	0.0574	0.0050	0.0207
Top vs. Bottom	1	0.1117	0.0992	0.2542

²Means in each column with the same letter are not significantly different at the 5% level (Duncan).

Table 5.2.3.2. Relationship between creasing severity or creasing incidence (%) and fruit diameter, macro- or micro nutrients in the albedo tissue at harvest of Palmer navel fruit sampled in Addo.

Fruit diameter vs. creasing %, creasing severity			
		R	P-value
Diameter	Creasing %	-0.09	0.3538
Diameter	Creasing severity	-0.06	0.5918
Creasing % vs. mineral nutrient in albedo			
Creasing %	N	0.11	0.2716
Creasing %	P	0.05	0.6268
Creasing%	K	0.12	0.1958
Creasing%	Ca	-0.14	0.1322
Creasing%	Mg	0.06	0.5190
Creasing %	Na	-0.02	0.7976
Creasing%	Mn	0.11	0.2675
Creasing %	Fe	0.10	0.3052
Creasing %	Cu	0.01	0.9156
Creasing %	Zn	0.06	0.5096
Creasing %	B	-0.20	0.0360
Creasing severity vs. mineral nutrients in albedo			
Creasing severity	N	-0.06	0.5765
Creasing severity	P	-0.27	0.0068
Creasing severity	K	-0.23	0.0270
Creasing severity	Ca	-0.07	0.5154
Creasing severity	Mg	-0.13	0.2058
Creasing severity	Na	-0.03	0.7353
Creasing severity	Mn	0.15	0.1539
Creasing severity	Fe	0.06	0.5618
Creasing severity	Cu	-0.27	0.0084
Creasing severity	Zn	-0.10	0.3191
Creasing severity	B	-0.18	0.0781

Table 5.2.3.3. Macronutrient concentrations in the albedo tissue from fruit sampled at harvest from different positions (sub-sectors) from Palmer navel trees in Addo.

POSITION WITHIN TREE		N	P	K	Ca	Mg
-----percentage-----						
North	Top outside	0.73 abcde ^z	0.032 bcd	0.28 bcd	0.50 ab	0.056 ab
	Top inside	0.86 a	0.039 a	0.36 a	0.38 d	0.062 a
	Bottom outside	0.76 abcd	0.034 abc	0.33 ab	0.49 abc	0.057 ab
	Bottom inside	0.81 abc	0.034 abc	0.34 ab	0.44 bcd	0.057 ab
South	Top outside	0.65 de	0.031 bcd	0.21 efg	0.54 a	0.046 cd
	Top inside	0.81 abc	0.029 d	0.24 cdefg	0.38 d	0.044 cd
	Bottom outside	0.72 bcde	0.034 abc	0.25 cdef	0.49 abc	0.050 bcd
	Bottom inside	0.83 abc	0.031 bcd	0.30 abcd	0.40 d	0.056 ab
East	Top outside	0.72 bcde	0.032 bcd	0.25 cdef	0.54 a	0.053 bc
	Top inside	0.40 f	0.036 ab	0.31 abc	0.43 dc	0.056 ab
	Bottom outside	0.70 cde	0.035 abc	0.26 cde	0.52 a	0.050 bcd
	Bottom inside	0.85 ab	0.034 abc	0.29 abcd	0.43 bcd	0.053 bc
West	Top outside	0.62 e	0.031 bcd	0.18 g	0.55 a	0.041 d
	Top inside	0.74 abcde	0.030 cd	0.22 efg	0.45 bcd	0.046 cd
	Bottom outside	0.61 e	0.029 d	0.19 fg	0.55 a	0.044 cd
	Bottom inside	0.71 cde	0.030 cd	0.24 defg	0.48 abc	0.048 bcd
	P-value	0.0001	0.0007	0.0001	0.0001	0.0001
	SE	0.0419	0.0017	0.0209	0.02193	0.0030

Average	Top outside	0.68	0.032	0.23	0.53	0.049
	Top inside	0.70	0.033	0.28	0.41	0.052
	Bottom outside	0.70	0.033	0.26	0.52	0.050
	Bottom inside	0.80	0.032	0.29	0.44	0.050
Source:	df					
Treatment	15					
North vs. South	1	0.1653	0.0015	0.0001	0.9354	0.0001
West vs. East	1	1.0000	0.0003	0.0001	0.0961	0.0003
Outside vs. Inside	1	0.0060	0.8749	0.0262	0.6308	0.3048
Top vs. Bottom	1	0.0027	0.6277	0.0001	0.0001	0.0363

²Means in each column with the same letter are not significantly different at the 5% level (Duncan).

Table 5.2.3.4. Micronutrient concentrations in the albedo tissue from fruit sampled at harvest from different positions (sub-sectors) of Palmer navel trees in Addo.

POSITION WITHIN TREE		Na	Mn	Fe	Cu	Zn	B
-----mg/kg-----							
North	Top outside	141 bcdef ^z	0.79 d	52.4 b	2.29 ab	6.57 abc	24.2 ab
	Top inside	245 a	4.00 ab	11.9 b	1.71 cd	7.29 ab	24.3 ab
	Bottom outside	199 abcd	0.43 d	47.7 b	2.43 a	8.36 a	24.6 ab
	Bottom inside	218 abc	3.86 ab	42.0 b	1.57 cde	6.57 abc	24.0 ab
South	Top outside	113 def	0.07 d	32.5 b	1.79 bcd	5.29 bc	24.4 ab
	Top inside	129 cdef	2.72 c	10.6 b	1.29 de	4.71 c	24.7 c
	Bottom outside	106 def	0.21 d	35.8 b	2.36 a	6.57 abc	24.5 ab
	Bottom inside	169 abcde	3.71 ab	16.0 b	2.00 abc	5.79 bc	23.6 ab
East	Top outside	99 ef	0.57 d	26.5 b	2.00 abc	5.71 bc	24.8 ab
	Top inside	216 abc	4.00 ab	21.3 b	1.71 cd	7.29 ab	23.9 ab
	Bottom outside	146 bcdef	0.50 d	42.6 b	2.07 abc	5.57 bc	25.1 a
	Bottom inside	227 ab	4.29 a	63.9 b	1.29 de	5.71 bc	24.3 ab
West	Top outside	65 f	0.29 d	175.8 a	1.42 de	7.36 ab	23.6 ab
	Top inside	83 ef	3.43 b	9.4 b	1.42 de	5.29 bc	23.0 ab
	Bottom outside	88 ef	2.14 c	38.6 b	1.14 e	5.86 bc	24.2 abc
	Bottom inside	109 def	3.57 ab	15.3 b	1.29 de	5.42 bc	22.7 b
	P-value	0.0001	0.0001	0.0209	0.0001	0.0176	0.0024
	SE	30.182	0.2406	27.781	0.7198	0.6785	0.6428
Average	Top outside	105	0.43	71.8	1.89	6.23	24.2
	Top inside	169	3.43	13.2	1.53	6.15	23.9
	Bottom outside	135	0.82	41.2	1.99	6.59	24.6
	Bottom inside	181	3.86	34.3	1.54	5.87	23.6
Source:	df						
Treatment	15						
North vs. South	1	0.0014	0.0008	0.5000	0.2432	0.0012	0.0334
West vs. East	1	0.0001	0.9166	0.2835	0.0004	0.8528	0.0165
Outside vs. Inside	1	0.1296	0.0038	0.6893	0.4692	0.8956	0.1181
Top vs. Bottom	1	0.0006	0.0001	0.0238	0.0001	0.2394	0.0007

²Means in each column with the same letter are not significantly different at the 5% level (Duncan).

Table 5.2.3.5. Differences in creasing severity between the outside (sunny) and inside (shady) part of fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Creasing severity		
		Sun	Shade	P-value
		-----0-2-----		
North	Top outside	0.21	0.65	0.0215 ^z
	Bottom outside	0.43	0.88	0.1825
South	Top outside	0.59	1.22	0.0235 ^z
	Bottom outside	0.66	1.19	0.0001 ^z
East	Top outside	0.13	0.68	0.0049 ^z
	Bottom outside	0.36	1.38	0.0292 ^z
West	Top outside	0.31	1.33	0.0017 ^z
	Bottom outside	0.61	1.13	0.0241 ^z

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.6. Differences in N and P concentrations between the outside (sunny) and inside (shady) part of the fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Nitrogen(N)			Phosphorus (P)		
		Sun	Shade	P-value	Sun	Shade	P-value
		-----percentage-----			-----percentage-----		
North	Top outside	0.73	0.74	0.6360	0.031	0.034	0.1723
	Bottom outside	0.73	0.78	0.0439 ^z	0.034	0.034	-
South	Top outside	0.65	0.65	0.4618	0.031	0.031	0.1723
	Bottom outside	0.70	0.73	0.0668	0.031	0.037	0.3632
East	Top outside	0.71	0.72	0.8353	0.031	0.030	0.3559
	Bottom outside	0.68	0.72	0.1596	0.036	0.033	0.0300 ^z
West	Top outside	0.61	0.62	0.5275	0.030	0.034	0.3739
	Bottom outside	0.60	0.62	0.0264 ^z	0.029	0.032	-

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.7. Differences in K and Ca concentrations between the outside (sunny) and inside (shady) part of the fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Potassium (K)			Calcium (Ca)		
		Sun	Shade	P-value	Sun	Shade	P-value
		-----percentage-----			-----percentage-----		
North	Top outside	0.28	0.28	0.7152	0.53	0.48	0.0347
	Bottom outside	0.33	0.33	0.8641	0.51	0.47	0.0029
South	Top outside	0.09	0.21	0.0141 ^z	0.54	0.54	0.1375
	Bottom outside	0.25	0.26	0.2150	0.50	0.49	0.0172
East	Top outside	0.23	0.25	0.0388 ^z	0.55	0.52	0.8049
	Bottom outside	0.27	0.25	0.2695	0.55	0.50	0.2919
West	Top outside	0.16	0.19	0.1087	0.56	0.52	0.2552
	Bottom outside	0.08	0.23	0.0583	0.58	0.47	0.0017 ^z

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.8. Differences in Mg and Na concentrations between the outside (sunny) and inside (shady) part of the fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Magnesium (Mg)			Sodium (Na)		
		Sun	Shade	P-value	Sun	Shade	P-value
		-----percentage-----			-----mg/kg-----		
North	Top outside	0.056	0.054	0.3559	139.43	142.71	0.8592
	Bottom outside	0.057	0.054	0.1723	202.00	197.57	0.7182
South	Top outside	0.044	0.046	0.0082 ^z	107.71	118.86	0.4355
	Bottom outside	0.049	0.049	0.6109	106.14	97.43	0.4468
East	Top outside	0.046	0.050	0.3559	93.86	103.57	0.2118
	Bottom outside	0.049	0.048	1.0000	141.14	112.17	0.4117
West	Top outside	0.037	0.044	0.3739	61.86	75.20	0.5533
	Bottom outside	0.041	0.043	0.6109	91.29	93.50	0.2821

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.9. Differences in Mn and Fe concentrations between the outside (sunny) and inside (shady) part of the fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Manganese (Mn)			Iron (Fe)		
		Sun	Shade	P-value	Sun	Shade	P-value
		-----mg/kg-----			-----mg/kg-----		
North	Top outside	0.86	0.71	0.6036	37.43	67.43	0.3260
	Bottom outside	0.43	0.43	1.0000	42.71	40.71	0.8456
South	Top outside	0.00	0.14	0.1723	31.43	33.57	0.5171
	Bottom outside	0.29	0.14	0.3632	28.57	35.71	0.4098
East	Top outside	0.43	0.71	0.3559	27.57	25.14	0.8557
	Bottom outside	0.57	0.16	0.3559	50.14	27.83	0.6004
West	Top outside	0.50	0.20	0.6042	301.29	57.20	0.2445
	Bottom outside	1.29	3.50	0.0580	55.14	9.17	0.1347

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.10. Differences in Cu and Zn concentrations between the outside (sunny) and inside (shady) part of the fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Copper (Cu)			Zinc (Zn)		
		Sun	Shade	P-value	Sun	Shade	P-value
		-----mg/kg-----			-----mg/kg-----		
North	Top outside	2.14	2.14	1.0000	6.43	6.71	0.4571
	Bottom outside	2.57	2.29	0.1723	10.57	6.14	0.2540
South	Top outside	1.71	1.86	1.0000	5.00	5.57	0.5686
	Bottom outside	2.43	2.29	0.3632	7.00	6.14	1.0000
East	Top outside	2.00	2.00	0.6036	5.86	5.57	0.0300 ^z
	Bottom outside	2.00	2.17	0.6891	5.57	5.50	0.5546
West	Top outside	1.29	1.80	0.1778	9.43	5.20	0.1321
	Bottom outside	1.14	1.17	-	6.71	4.67	0.2805

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.11. Differences in B concentrations between the outside (sunny) and inside (shady) part of the fruit sampled at harvest from different positions from the outside sub-sectors of Palmer navel trees in Addo.

POSITION WITHIN TREE		Boron (B)		
		Sun	Shade	P-value
		-----mg/kg-----		
North	Top outside	24.86	23.57	0.0781
	Bottom outside	25.43	24.00	0.0465 ^z
South	Top outside	24.71	21.14	0.0488 ^z
	Bottom outside	24.14	24.86	0.0067 ^z
East	Top outside	24.14	25.43	0.2308
	Bottom outside	26.14	23.33	0.3341
West	Top outside	23.71	23.00	0.8149
	Bottom outside	24.14	23.83	0.8220

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (T-test)

Table 5.2.3.12. Relationship between creasing severity and macro or micronutrient concentrations in the albedo tissue of Palmer navel fruit in the outside (sunny) and inside (shady) part of fruit sampled at harvest from different positions from the outside sub-sectors.

Creasing severity vs. mineral nutrients in the albedo in the sunny part of fruit			
		R	P-value
Creasing severity(0-2)	N	0.00	0.9902
Creasing severity	P	-0.16	0.2323
Creasing severity	K	0.06	0.6250
Creasing severity	Ca	-0.20	0.1335
Creasing severity	Mg	0.11	0.4191
Creasing severity	Na	0.02	0.8624
Creasing severity	Mn	-0.06	0.6396
Creasing severity	Fe	0.21	0.1214
Creasing severity	Cu	0.06	0.6484
Creasing severity	Zn	0.02	0.8639
Creasing severity	B	-0.37	0.0053
Creasing severity vs. mineral nutrients in the albedo in the shady part of fruit			
Creasing severity(0-2)	N	-0.07	0.6231
Creasing severity	P	-0.01	0.9284
Creasing severity	K	-0.12	0.3912
Creasing severity	Ca	-0.09	0.5476
Creasing severity	Mg	-0.26	0.0658
Creasing severity	Na	-0.09	0.5401
Creasing severity	Mn	-0.04	0.7720
Creasing severity	Fe	-0.16	0.2468
Creasing severity	Cu	-0.10	0.5027
Creasing severity	Zn	0.05	0.7301
Creasing severity	B	-0.19	0.1758

5.2.4 PROGRESS REPORT: Effect of the manipulation of light, carbohydrate and mineral nutrient allocation in the tree on creasing incidence

Experiment 864 (April 2006- March 2009): Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

Opsomming

Alhoewel die bydraende faktore tot kraaskil ontwikkeling bekend is, weet ons baie min van die manganisme hoe dit ontwikkel. Om die fisiologie van kraaskil beter te verstaan, is die effek van sekere lig manipulasie tegnieke bv. toemaak van blare agter die vrugte met skadunet, snoei, toemaak van vrugte met bruin papiersak en koolhidaat allokasie tegnieke soos die verwydering van blare agter vrugte op 'n tak, ringelering, en handuidtun, op die voorkoms van kraaskil geëvalueer. Washington navel lemoenbome in Citrusdal in 'n

gerandomiseerde blok ontwerp met ag enkelboom herhalings per behandeling is gebruik. Tien vrugdraende takkies per boom is behandel. Die toemaak van vrugte met 'n bruin papiersak het die graad van kraakskil en kraakskil voorkoms vermeerder, skildikte verminder en N en K konsentrasies in die albedo by oestyd verhoog. Daarteenoor het die toemaak van blare met skadunet en die verwydering van blare agter die vrugte, kraakskil voorkoms met 10% verminder. Die toemaak van blare met skadunet het N, P en K konsentrasies in die albedo by oestyd verhoog. Daar was swak korrelasies tussen al die minerale elemente in die albedo met oestyd en kraakskil voorkoms. Dus is minerale element konsentrasies in die albedo by oestyd nie 'n goeie indikator van kraakskil ontwikkeling nie. Die graad van kraakskil was betekenisvol hoër in die skadukant van vrugte teenoor die sonkant in al die behandelings. Dus ligvlakke waaraan beide vrugte en blare blootgestel is speel 'n belangrike rol in kraakskil ontwikkeling.

Summary

Although, the contributing factors are known, the mechanism of creasing development is unresolved. To further understand the physiology of creasing, light manipulation techniques viz. covering leaves behind fruit with a shade cloth, pruning, covering fruit with bags and carbohydrates allocation manipulations such as removing leaves from behind fruit, girdling and hand thinning were assessed. Washington navel orange trees in Citrusdal in a randomized complete block design with eight single tree replicates per treatment were used. Ten fruit bearing shoots per tree were treated. Covering fruit with a brown bag increased creasing severity and incidence, decreased peel thickness and increased N and K concentrations in the albedo at harvest time. In contrast, covering leaves with shade cloth and removing leaves behind fruit reduced creasing incidence by 10%. Covering leaves with shade cloth resulted in increased N, P and K concentrations in the albedo at harvest time. There were weak relationships between all mineral nutrients in the albedo at harvest time and creasing incidence. Therefore nutrient concentrations in the albedo at harvest time are not good indicators for creasing development. The shady side of fruit in all the treatments had a significantly higher creasing severity than the sunny side of the fruit. Therefore light levels received by fruit and leaves seem to play a major role in creasing development.

Introduction

Creasing is a pre-harvest physiological disorder of citrus of which the contributing factors are known but the mechanism of creasing development is unresolved. However, the potential of the albedo cells to expand and accommodate cell enlargement after cell division has ceased (Holtzhausen, 1981; Storey and Treeby, 1994) and weaknesses in cell wall connections result in creasing development (Storey and Treeby, 1994). Weaknesses in the middle lamellae were thought to be associated with low levels of the mineral elements (Mg, S, Zn) involved in the formation of pectic fractions in the albedo tissue (Bower, 2004) and low levels of Ca involved in bonding of the pectin chains (Bower 2004; Treeby et al., 2002). Hence, the formation and bonding of pectins is critical in creasing development and carbohydrates are thought to be related to the metabolism and the formation of pectins (Bower, 2004).

Therefore, source-sink imbalances which can be induced by different treatments i.e. removing leaves behind fruit on bearing shoots, girdling and hand thinning, can provide insight in the role of carbohydrates in the development of creasing. Thus, removal of leaves from behind fruit on bearing shoots results in manipulation of sugars normally allocated to the fruit from the leaves. In general girdling at or after the 'June drop' during active fruit growth (Goren et al. 2003; Erner et al., 2004; Mataa et al., 1998) removes competition from roots (Cohen, 1981; Li et al., 2003; Mataa et al., 1998; Wright, 2000) and increases allocation of sugars to the tree canopy. Hand thinning is also important in carbohydrate allocation because carbohydrate availability to any particular fruit is dependent upon the carbohydrate sources as well as the number of competitive sinks (Erner et al., 2004).

The initial development of creasing is also influenced by the internal temperature gradient across the fruit (Jones et al., 1967) and light since creasing severity is usually high on the shady side of the tree (Fourie and Joubert, 1957; Holtzhausen, 1981; Jones et al., 1967; Le Roux and Crous., 1938). Moreover, the part of the fruit facing towards the trunk was observed to have more creasing than the sun-exposed side of fruit (Gambetta et al., 2000; Jones et al., 1967). A higher concentration of immobile nutrients (Ca, Mg, Zn, Mn, Fe, and B) was observed on outside fruit than inside fruit. Mobile nutrients (N, P, and K) occur at a higher concentration in inside fruit than outside fruit (Kruger et al., 2005). Therefore, light manipulation techniques i.e. covering leaves behind fruit or pruning shading leaves and covering fruit with bags after physiological fruit drop should influence occurrence of creasing and the albedo mineral composition.

The aim of the study was to provide an insight into the role of light or temperature and the effect of manipulation of carbohydrate production and allocation on creasing incidence and the albedo mineral content.

Material and methods

Plant material. The research was conducted in a commercial orchard in Citrusdal on Washington navel orange trees. The experiment was laid out as a randomized complete block design with each block represented by a single tree. Eight single tree replicates per treatment were used. Ten fruit bearing shoots per tree were tagged randomly around the tree and treated.

Treatments. Treatments consisted of light manipulation techniques viz. covering leaves behind fruit with a green shade cloth, pruning shading leaves to improve light distribution around fruit and covering fruit with brown paper bags and carbohydrates allocation manipulations such as removing leaves from behind fruit on bearing shoots, girdling scaffold branches and hand thinning. All treatments were carried out after physiological fruit drop on 29 November 2007.

Creasing severity, colour, fruit diameter, peel thickness and mineral analysis. Treated fruit were harvested on 14 May 2008. Creasing severity was evaluated on a score of 0-4; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, if a portion or entire single sphere was creased, a one, therefore a four meant 100 percent creasing. Fruit colour was determined based on the CRI colour chart (Set No: 34) for oranges, with a range between one and eight, one being completely orange and eight being green. The fruit diameter of each fruit was measured using an electronic caliper. The albedo of the inside of the fruit was removed dried and stored in small vials for complete mineral analysis. Peel thickness was measured on the outside (sunny side) of treated fruit. Pearson's correlation coefficients reported were determined by the SAS statistical program. Only R values > 0.5 were considered physiologically significant.

Results

Creasing incidence and creasing severity. In general, creasing incidence was low in this orchard (Table 5.2.4.1). The severity of creasing was significantly increased by covering fruit with brown bag (Table 5.2.4.1). Covering leaves with shade cloth and removing leaves behind the fruit reduced creasing incidence by about 10%, although creasing incidence was not significantly different from the control in any of the treatments.

Colour. Fruit which had their leaves covered behind the fruit with shade cloth and fruit covered with a brown bag had significantly greener fruit than the control fruit at harvest (Table 5.2.4.1).

Diameter and peel thickness. Fruit from pruned trees, fruit which had their leaves covered and fruit covered with bags were significantly smaller at harvest (Table 5.2.4.1). Fruit covered with a paper bag and fruit which had their leaves covered behind the fruit with shade cloth had significantly thinner peels at harvest.

Shady vs. sunny side. The shady side of fruit in all the treatments had a significantly higher creasing severity than the sunny side of the fruit (Table 5.2.4.2).

Macronutrients. N and K concentration in the albedo tissue was significantly higher in fruit covered with a brown bag and fruit which had their leaves covered with shade cloth (Table 5.2.4.3). Covering leaves behind fruit with shade cloth significantly increased P and K concentrations in the albedo. Ca and Mg were not significantly affected by any of the treatments.

Micronutrients. Covering fruit with brown paper bags increased Mn concentrations in the albedo (Table 5.2.4.4). None of the other nutrients were significantly affected by the treatments (Table 5.2.4.4). Negative weak correlations between fruit diameter and creasing incidence or severity and between peel thickness and creasing incidence or severity were observed (Table 5.2.4.5).

Peel mineral content. Very weak correlations between creasing severity or creasing incidence and macronutrient concentrations in the albedo and between creasing severity or creasing incidence and micronutrient concentrations in the albedo were observed (Table 5.2.4.5).

Discussion

The severity of creasing was significantly increased by covering fruit with a brown bag. Hence, light or temperature is involved the development of creasing. This was also confirmed by the tendency of the shady

side of fruit to have a significantly higher creasing severity than the sunny side. This shading effect on creasing incidence was earlier observed by previous researchers (Le Roux and Crous., 1938; Fourie and Joubert, 1957; Jones et al., 1967 and Holtzhausen, 1981).

Removing leaves behind the fruit had the lowest creasing incidence, although it was not significantly different from the control. A reduction in the incidence of creasing may be due to an increased allocation of plant growth regulators (especially cytokinins and gibberellins) and nutrients to the fruit. Fruit from pruned trees, fruit which had their leaves covered and fruit covered with bags were significantly smaller at harvest. This could be an indirect involvement of light resulting in reduced photosynthetic activity in the fruit and leaves resulting in smaller fruit.

The N, K concentration in the albedo tissue was significantly higher in fruit covered with a brown bag and fruit which had their leaves covered with a shade cloth. P concentration was also higher in fruit which had their leaves covered with a shade cloth and Mn concentration in the albedo tissue was significantly higher in fruit covered with a brown bag. Although, the relationship between nutrients and creasing incidence did not show strong correlations, the involvement of the nutrient elements N, K and Mn in creasing development cannot be excluded since bagging fruit with brown bag increased concentrations of N, K and Mn and increased creasing severity. In contrast, covering leaves with shade cloth reduced creasing severity and incidence (not significantly), but also resulted in increased N and K concentrations in the albedo at harvest. Ca and Mg concentrations were not significantly affected by either the light or carbohydrate allocation manipulation techniques. However, it was reported that accumulation of calcium by fruit may be influenced by the fruit or tree microclimate (Storey et al., 2002). None of the other micronutrient concentrations were significantly affected by the treatments.

Conclusion

Covering fruit with a bag increased creasing severity and incidence, decreased peel thickness and increased N and K concentrations in the albedo. In contrast covering leaves with shade cloth and removing leaves behind fruit reduced creasing incidence by 10%. Covering leaves with shade cloth resulted in increased N, P and K concentrations in the albedo. Therefore light levels received by fruit and leaves seem to play a major role in creasing development. The results indicate that nutrient concentrations in the albedo at harvest time are not good indicators for creasing development, nutrient concentrations in the albedo earlier in the season may however play a role.

Further objectives and workplan

The research is ongoing and will be repeated in the 2008/2009 season.

Technology transfer

Phiri, Z.P., Verreyne, J.S. Studies in citrus creasing/albedo breakdown. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

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Table 5.2.4.1. The effect of light and carbohydrates allocation manipulation techniques on creasing severity, creasing incidence, colour, fruit diameter and peel thickness of Washington navel oranges in Citrusdal.

Treatment	Creasing severity	Creasing incidence	Colour	Diameter	Peel thickness
	--0-4 ^x --	--%-	--1-8 ^y --	--mm--	--mm--
1. Control	0.58 b	31.50	3.53 c	65.65 a	4.96 a
2. Covering leaves with shade cloth	0.29 b	21.67	4.15 a	59.47 b	4.51 b
3. Removing leaves from behind fruit	0.30 b	19.25	3.77 bc	65.25 a	4.98 a
4. Girdling scaffold branches	0.47 b	36.75	3.42 c	65.08 a	5.00 a
5. Pruning	0.54 b	35.13	3.51 c	61.49 b	5.12 a
6. Hand thinning	0.68 b	38.00	3.54 c	65.59 a	4.90 a
7. Covering fruit with brown bag	1.12 a	37.38	3.94 ab	60.22 b	4.36 b
P-value	0.0005	0.6932	0.0001	0.0001	0.0009

^zMeans with the same letter are not significantly different at the 5% level (Duncan).

^y1-8 (1 = orange, 8 = green) CRI colour chart (Set No: 34) for oranges.

^x0- 4 (0 = 0%, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100%).

Table 5.2.4.2. Differences in creasing severity between the outside (sunny) and inside (shady) part of fruit exposed to different light and carbohydrate allocation manipulation techniques on Washington navel trees harvested in Citrusdal area.

Treatment	Sun	Shade	P-value
	----0-2----		
1. Control	0.12	0.50	0.0001 ^z
2. Covering leaves with shade cloth	0.02	0.27	0.0036 ^z
3. Removing leaves from behind fruit	0.02	0.28	0.0004 ^z
4. Girdling scaffold branches	0.07	0.39	0.0001 ^z
5. Pruning	0.06	0.49	0.0001 ^z
6. Hand thinning	0.10	0.57	0.0001 ^z
7. Covering fruit with brown bag	0.19	0.56	0.0001 ^z

^zMeans for the parameter in each horizontal row are significantly different at $P \leq 0.05$ (t-test).

Table 5.2.4.3. Macronutrient concentrations in albedo tissue of fruit sampled at harvest from Washington navel trees exposed to different light and carbohydrate allocation manipulation techniques in Citrusdal.

Treatment	N	P	K	Ca	Mg
	-----percentage-----				
1. Control	0.48 b	0.030 bc	0.24 b	0.48	0.061
2. Covering leaves with shade cloth	0.56 a	0.035 a	0.34 a	0.48	0.068
3. Removing leaves from behind fruit	0.45 b	0.030 bc	0.25 b	0.51	0.065
4. Girdling scaffold branches	0.46 b	0.029 bc	0.22 b	0.52	0.069
5. Pruning	0.44 b	0.028 bc	0.23 b	0.48	0.069
6. Hand thinning	0.46 b	0.026 c	0.25 b	0.45	0.060
7. Covering fruit with brown bag	0.55 a	0.031 ab	0.32 a	0.47	0.070
P-value	0.0001	0.0072	0.0001	0.1870	0.0750

^zMeans in each column with the same letter are not significantly different at the 5% level (Duncan).

Table 5.2.4.4. Micronutrient concentrations of albedo tissue of fruit sampled at harvest from Washington navel oranges exposed to different light and carbohydrate manipulation techniques harvested in Citrusdal.

Treatment	Na	Mn	Fe	Cu	Zn	B
	-----mg/kg-----					
1. Control	244.37	5.86 bc	104.8	1.42	6.29	21.21
2. Covering leaves with shade cloth	255.91	6.94 ab	153.6	1.49	7.22	20.02
3. Removing leaves from behind fruit	263.97	6.45 abc	138.4	1.43	6.92	21.37
4. Girdling scaffold branches	267.57	5.74 bc	98.7	1.21	6.11	20.05
5. Pruning	257.21	4.79 c	33.5	1.19	6.02	19.99
6. Hand thinning	261.91	6.67 abc	196.2	1.43	5.87	19.50
7. Covering fruit with brown bag	250.79	8.43 a	292.2	1.60	6.25	21.74
P-value	0.3165	0.0146	0.0778	0.1519	0.1422	0.5569

²Means in each column with the same letter are not significantly different at the 5% level (Duncan).

Table 5.2.4.5. Relationship between creasing severity and creasing incidence (%), peel thickness, fruit diameter and macro- or micro nutrients in the albedo tissue of Washington navel fruit exposed to different light and carbohydrate allocation manipulation techniques harvested in Citrusdal.

Peel thickness vs. creasing severity, creasing %		R	P-value
Peel thickness	Creasing severity	-0.21	0.1341
Peel thickness	Creasing %	-0.24	0.0853
Fruit diameter vs. creasing severity, creasing % and peel thickness			
Diameter	Creasing severity	-0.14	0.3152
Diameter	Creasing %	-0.12	0.3945
Diameter	Peel thickness	0.33	0.0141
Creasing % vs. mineral nutrient in albedo			
Creasing %	N	-0.07	0.6059
Creasing %	P	-0.07	0.6267
Creasing %	K	0.00	0.9904
Creasing %	Ca	-0.22	0.1161
Creasing %	Mg	0.12	0.3879
Creasing %	Na	0.25	0.0708
Creasing %	Mn	-0.02	0.8878
Creasing %	Fe	0.03	0.8341
Creasing %	Cu	-0.11	0.4386
Creasing %	Zn	0.04	0.7928
Creasing %	B	0.17	0.2270
Creasing severity vs. mineral nutrients in albedo			
Creasing severity	N	0.01	0.9270
Creasing severity	P	-0.04	0.7557
Creasing severity	K	0.15	0.2674
Creasing severity	Ca	-0.26	0.0595
Creasing severity	Mg	-0.04	0.7974
Creasing severity	Na	0.19	0.1766
Creasing severity	Mn	0.01	0.9710
Creasing severity	Fe	0.04	0.7602
Creasing severity	Cu	-0.12	0.3823
Creasing severity	Zn	-0.06	0.6495
Creasing severity	B	0.16	0.2309

5.2.5 PROGRESS REPORT: The evaluation of alternative methods of uptake of products and plant growth regulators to reduce the incidence of creasing

Experiment 883 (April 2007- March 2009): Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

Opsomming

Kraaskil is 'n groot probleem in lemoen boorde veral op Navels en Valencias. Die doel van die studie was om die effek van gelokaliseerde toediening van Aminoethoxyvinylglycine (AVG) en die sitokiniene, CPPU and MaxCel, alleen of in kombinasie met kalsium op die kraaskil voorkoms van Bahianinha navel lemoene in Citrusdal te toets. Behandelings is toegedien na die fisiologiese vrugval periode. Daar word verwag dat sitokiniene Fase I van vruggroei, wanneer die meeste seldeling in die albedo plaasvind, sal verleng. Sitokiniene kan moontlik selverdeling stimuleer in die albedo, wat skille kan verdik en kraaskil verminder. Ag enkel boom herhalings per behandeling is gebruik in 'n gerandomiseerde blok ontwerp. Tien vrugte per boom rondom die boom is behandel. Maxcel en CPPU (sitokiniene) het die graad van kraaskil betekenisvol verlaag and kraaskil voorkoms is met 20% verminder, met geen effek op vruggroei of vrugdeursnit nie. Toediening van AVG en CPPU in kombinasie met geheleerde kalsium het geen effek op kraaskil voorkoms gehad nie. Geen behandeling het 'n effek op skildikte gehad nie.

Summary

Creasing is a problem in citrus orchards especially, with Navels and Valencias. The objective of this study was to evaluate the effect of localized application of Aminoethoxyvinylglycine (AVG) and the cytokinins, CPPU and MaxCel, alone and in combination with calcium on the incidence of creasing on Bahianinha navel orange trees in Citrusdal. Treatments were applied after physiological fruit drop. It is expected that cytokinins would extend Stage I of fruit growth, when the majority of cell division in the albedo takes place. Cytokinins may increase cell division in the albedo which may increase peel thickness and reduce creasing. Eight single tree replicates per treatment were used in a randomized complete block design. Ten fruit per tree were tagged randomly around the tree and treated. Maxcel and CPPU (cytokinins) reduced creasing severity significantly and creasing incidence by about 20%, with no effect on fruit growth or fruit diameter. Application of AVG and CPPU in combination with chelated calcium had no effect on creasing incidence. None of the treatments had an effect on peel thickness.

Introduction

Creasing is a physiological disorder that affects the albedo of citrus fruits and it is also known as albedo breakdown. It is normally detectable at maturity (Gambetta et al., 2000; Jona et al., 1989) or post colour break (Storey et al., 2002) and tends to worsen as fruit matures (du Plessis and Maritz, 2004; Nagy et al., 1982). The potential of the albedo cells to expand and accommodate enlargement, after cell division has ceased in the albedo and weaknesses of the middle lamella of adjacent daughter cells in the albedo normally results in creasing development (Storey and Treeby, 1994). Weaknesses in the middle lamellae were thought to be associated with the early onset of senescence or expression of rapidly progressing senescence (Monselise, 1973; Monselise et al., 1976) or the malnutrition or genetic weakness or moisture stress or water logging resulting to increased fluctuating pressure in the albedo tissue (Holtzhausen, 1981). Thus, extending the period of cell division and strengthening the cohesion of the middle lamellae of adjacent albedo cells or a combination thereof could be a key in the control of creasing. All the naturally occurring cytokinins have the ability to stimulate cell division (Staden and Cook, 1986; Salisbury, and Ross, 1992) and also enhance sink strength in developing fruits (Erner et al., 2004; Talon et al., 1997). Equally, ethylene inhibiting products could have a potential to reduce creasing by their ability to delay senescence which was observed to be unusually early in fruits with a creasing problem (Monselise et al., 1976). Calcium is known to form calcium pectates in the middle lamella of the cell plate that forms between daughter cells (Hopkins and Huner, 2004; Taiz and Zeiger, 2002) and thus it has the ability to strengthen the middle lamella and thus reduce the occurrence of creasing (Storey and Treeby, 2002 and Storey et al., 2002).

CPPU (N-(2-chloro-4pyridyl)-N-phenyl urea) and Maxcel (6-benzyladenine) exhibit cytokinin-like properties when applied to plants. Aminoethoxyvinylglycine (AVG) is a known inhibitor of ethylene (Clayton et al., 2002 and Vizzotto et al., 2002) and has been used to retard preharvest drop in apples (Bramlage et al., 1980; Byers, 1997; Brackmann and Waclawovsky, 2001; Greene, 2000; Williams, 1980;), peach (Vizzotto et al., 2002; Kim and Choi, 2004) and pear fruit (Clayton et al., 2002 and Khan et al., 2002). In citrus preliminary studies showed that preharvest application of AVG reduces preharvest fruit drop and also the incidence of creasing (Gonzalez and Lovatt, 2004).

The objective of this study was to evaluate the effect of localized application of AVG and cytokinins alone and in combination with calcium on the incidence of creasing. It is expected that cytokinins would extend Stage I of fruit growth, when the majority of cell division in the albedo takes place. Cytokinins may increase cell division in the albedo which may increase peel thickness and reduce creasing. Uptake of foliar applied cytokinins and most Ca formulations are not sufficient, therefore the use of alternative methods of application such as spray bottle applications and fruit dips were investigated.

Materials and methods

Plant material. The research was conducted in a commercial orchard in Citrusdal in the Western Cape, South Africa on Bahianinha navel orange trees. The experiment was laid out in a randomized complete block design with each block represented by a single tree. Eight single tree replicates per treatment were used. Ten fruit per tree were tagged randomly around the tree and treated.

Treatments. Treatment solutions of the cytokinins CPPU [N-(2-chloro-4-pyridyl)-N-phenylurea] and MaxCel (6-Benzyladenine), AVG (aminoethoxyvinylglycine) and calcium were used. Treatment solutions consisted of CPPU (10 ppm), MaxCel (190 ppm), AVG (38 ppm) and a mixture of CPPU (10 ppm) and chelated calcium (84 g/L). Tagged fruit were individually sprayed using a spray bottle. All treatments were applied after physiological fruit drop on 30 November 2007. Cytokinin was applied to stimulate cell division in fruitlets while calcium was also added to strengthen cell wall connections in the albedo tissue. AVG was applied to inhibit ethylene synthesis and thereby, delaying the normal ageing of the peel.

Fruit growth. Fruit diameter of each tagged fruit was measured on 30 November 2007, 17 January 2008, 23 February 2008, 28 March 2008 and 10 May 2008 using an electronic caliper.

Creasing severity, colour, fruit diameter and peel thickness. The fruit were harvested on 10 May 2008. Creasing severity was evaluated on a score of 0-4; the orange was divided into four equivalent imaginary spheres. If no sphere was creased it was designated a zero, if a portion or an entire single sphere was creased, a one, etc. Creasing incidence is expressed as the percentage of fruit affected by the disorder. Fruit colour was determined based on the CRI colour chart (Set No: 34) for oranges, with a range between one and eight, one being completely orange and eight being wholly green. The fruit diameter of each fruit was measured using an electronic caliper. Peel thickness was measured on each fruit.

Statistical analysis. Analysis of variance was performed using the computer program SAS (Statistical Analysis System) Enterprise Guide 3. Duncan's multiple range test at $P=0.05$ was used to test the treatment effects. Repeated measures analysis of variance was used to test treatment effects on fruit growth.

Results

Creasing incidence (50.38%) and severity (1.18) were fairly high in this study. Creasing severity was significantly reduced by all the treatments, except AVG (Table 5.2.5.1) with CPPU and MaxCel giving the best results (Table 5.2.5.1). Creasing incidence was also reduced by both CPPU and MaxCel, although it was not significantly different from the untreated control (Table 5.2.5.1). Therefore, in this study CPPU and MaxCel applied alone were the most effective in reducing creasing incidence and severity. In general, fruit from this orchard had poor colour development at harvest (Table 5.2.5.1). MaxCel and CPPU in combination with calcium resulted in a significant delay in fruit colour development (Table 5.2.5.1). In general, fruit from this orchard were very small at harvest (<55 mm). Trees were water stressed at one occasion during the course of the experiment. Surprisingly, fruit treated with the mixture of CPPU and calcium resulted in significantly smaller fruit at harvest (Table 5.2.5.2) and fruit growth during the season was significantly reduced on fruit sprayed with CPPU in combination with calcium (Table 5.2.5.2 and Fig. 5.2.5.1). Peel thickness was not significantly affected by any of the treatments (Table 5.2.5.1).

Discussion

CPPU and Maxcel reduced creasing incidence, although not significantly. It is known that cytokinin-like products act by stimulating additional cell division (Salisbury and Ross, 1992; Talon et al., 1997; Stern et al., 2003 and Erner et al., 2004). Hence, the effectiveness of the cytokinins in controlling creasing could be associated with its role in stimulating cell division, since the potential of the albedo cells to expand and accommodate cell enlargement after cell division has ceased (Holtzhausen, 1981; Storey and Treeby, 1994) in the albedo is critical in creasing development. Preharvest application of AVG did not reduce creasing incidence, which was contrary to what was observed in preliminary trials in California (Gonzalez and Lovatt, 2004).

Spray treatment of 1% or 2% Ca(NO₃)₂ or CaCl₂ throughout fruit development in Australia resulted in a reduction in creasing incidence (Storey et al., 2002 and Treeby and Storey, 2002). The application of CPPU alone reduced creasing incidence, but CPPU in combination with chelated calcium did not reduce creasing incidence. In general, fruit from this orchard had poor colour development at harvest. However, MaxCel and CPPU in combination with calcium resulted in a significant delay in fruit colour development with the worst colour development on fruit sprayed with the mixture of CPPU and calcium.

The primary physiological effect of CPPU is the increase in fruit size in citrus (Erner et al., 2004). Hence, an increase in fruit size or peel thickness was expected. On the contrary, fruit in this orchard were very small at harvest and peel thickness was not affected by any of the treatments. This may be due to water stress; trees were water stressed at one occasion during the course of the experiment and since cell division alone does not increase size (Salisbury and Ross, 1992), it resulted in smaller fruit at harvest.

Conclusion

Maxcel and CPPU (cytokinins) reduced creasing severity significantly and creasing incidence by about 20%, with no effect on peel thickness and fruit growth or fruit diameter. It is assumed that localized spray bottle application on fruit will result in better uptake than whole tree foliar application, in which poor results were previously obtained. Unfortunately, due to the water stress the trees received during the experiment, interpretation of the results is very difficult.

Further objectives and workplan

The research is ongoing and will be repeated in the 2008/2009 season with the same objectives.

Technology transfer

Phiri, Z.P., Verreynne, J.S. Studies in citrus creasing/albedo breakdown. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

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Table 5.2.5.1. The effects of CPPU, MaxCel, Aminoethoxyvinylglycine (AVG) and calcium on the incidence of creasing of Bahianinha navels in Citrusdal.

Treatment	Creasing severity	Creasing incidence	Colour	Diameter	Peel thickness
	--0-4 ^x --	--%-	--1-8 ^y --	--mm--	--mm--
1. Control	1.18 a ^z	50.38	4.59 cd	55.18 a	4.98
2. MaxCel	0.55 c	31.25	4.88 ab	55.35 a	4.93
3. CPPU	0.45 c	30.00	4.86 bc	53.75 ab	5.10
4. AVG	1.01 ab	54.50	4.56 d	55.06 a	5.04
5. CPPU + Chelated calcium	0.77 bc	42.25	5.14 a	52.57 b	4.95
P-value	0.0001	0.1018	0.0001	0.0016	0.8271

^zMeans in the same column with the same letter are not significantly different at the 5% level (Duncan).

^y 1-8 (1 = orange, 8 = green) CRI colour chart (set No: 34) for oranges.

^x 0- 4 (0 = 0%, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100%).

Table 5.2.5.2. Fruit diameter of Bahianinha navel fruit treated with CPPU, MaxCel, Aminoethoxyvinylglycine (AVG) and calcium.

Treatment	30-Nov-07	17-Jan-08	23-Feb-08	28-Mar-08	10-May-08
	-----mm-----				
1. Control	27.58	43.53 ab ^z	49.93 ab	53.30 a	55.59 a
2. MaxCel	27.22	42.21 bc	49.45 ab	53.08 a	55.35 a
3. CPPU	28.21	42.24 bc	48.35 bc	51.34 bc	53.67 ab
4. AVG	28.57	43.93 a	50.14 a	52.68 ab	55.04 a
5. CPPU + Chelated calcium	28.02	41.21 c	47.72 c	50.74 c	52.73 b
P-value	0.1666	0.0007	0.0077	0.0041	0.0039

^zMeans in the same column with the same letter are not significantly different at the 5% level (Duncan).

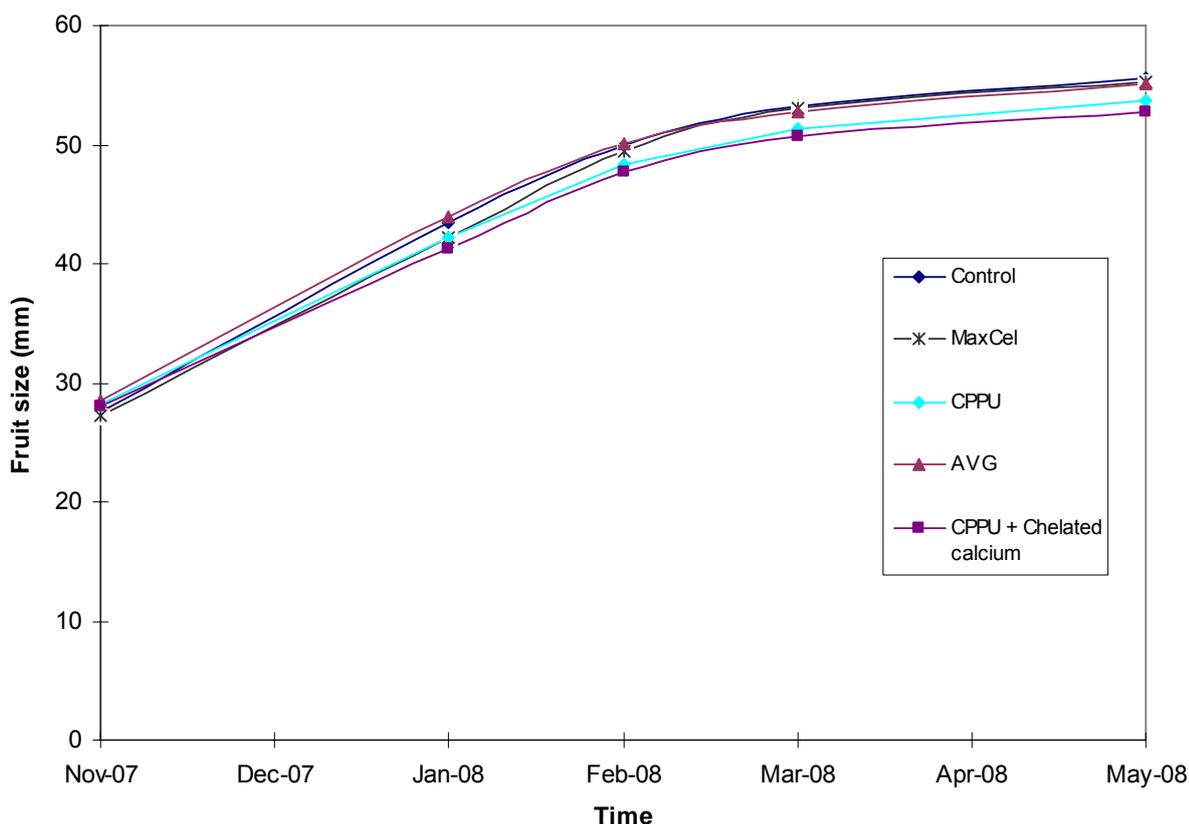


Fig. 5.2.5.1. Fruit growth of Bahianinha navel fruit, treated with CPPU, MaxCel, Aminoethoxyvinylglycine (AVG) and calcium.

5.2.6 PROGRESS REPORT: Effect of different products on root activity and creasing incidence
 Experiment 884 (August 2008- March 2009): Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

Summary

The involvement of Ca in creasing incidence has been speculated by the Australians. The uptake of Ca in most formulations is poor. Some soils have very high Ca availability, but low root activity and pH prevents the uptake of Ca. Potential benefits of humates and fulvates include improving the uptake of essential nutrients (also in foliar applied nutrients), stimulating beneficial microbial activity and root uptake of nutrients. Other treatments in the study include compost and chicken manure. The objective of this study is to stimulate root activity which in turn should result in a greater uptake of the available Ca and stimulate cytokinin production in the roots and the transport thereof to the fruit for the stimulation of cell division, which can result in larger fruit, thicker peels and less creasing. This trial is ongoing and results are not yet available.

Opsomming

Volgens die Australiërs speel kalsium 'n groot rol in kraakskil voorkoms. Die opname van kalsium tot in die vrug is swak met die meeste formulasies. Sekere gronde het 'n baie hoë kalsium beskikbaarheid, maar swak wortel aktiwiteit en pH voorkom die opname van Ca. Potensiële voordele van humate en fulvate sluit in die verbetering van die opname van essensiële elemente (ook by blaartoegedende elemente) en stimulering van voordelige mikrobe aktiwiteit en wortelopname van elemente. Ander behandelings in die studie sluit kompos en hoendermis in. Die doel van die studie is om wortelaktiwiteit te stimuleer wat dan tot 'n verbeterde opname van Ca kan lei en sitokien produksie kan stimuleer in die wortels en die transport na die vrug om selverdeling te stimuleer, wat kan lei tot groter vrugte, dikker skille en minder kraakskil. Die studie is aan die gang, maar resultate is nog nie beskikbaar nie.

Technology transfer

Phiri, Z.P., Verreyne, J.S. Studies in citrus creasing/albedo breakdown. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

5.2.7 PROGRESS REPORT: Hot water dip treatments to prevent chilling injury (CI) on early season harvested lemons, grapefruit and Oroblancos exported to Japan

Experiment 869 (April 2008 – March 2009) by K.H.Lesar (CRI) and J P Bower (UKZN)

Opsomming

Skil beskadiging as gevolg van koueskade veroorsaak groot verliese, veral as suurlemoene en pomelos onder kouesterilisering verskeep word. Hitteskok en warm water doping is al bewys as waardevol om koueskade te verminder. Werk op suurlemoene by UKZN het bewys dat warm water met molibdeen bygevoeg laat hoer temperatuur water sonder skade toe, en veroorsaak die vorming van teenoksidante wat die vrugte teen koueskade beskerm. Gedurende die navorsingstydperk, werk is in Nelspruit gedoen om die effek van warm water behandelings swamdoders, salisiensuur en metieljasmonaat te evalueer. By UKZN, is die fisiologie van suurlemoene wat met warm water, molibdeen, salisiensuur en metieljasmonaat sowel as die effek van oorkoms ondersoek. Koueskade van 'Oroblanco' is deur warm water by 53°C verminder, veral waar swaar waks toegedien was. Werk sal weer moet gedoen word om die resultate te bevestig, en 'Star Ruby' sal ook moet ondersoek word. Die werk by UKZN het bewys dat verskille in koueskade as gevolg van oorsprong van vrugte as gevolg van teenoksidante is. Die is verder verhoog deur die warm water behandeling, salisiensuur en metieljasmonaat. 'n Hoogs betekenisvolle negatiewe korrelasie tussen die suiker sucrose in die flavedo en koueskade is ook gevind, wat die vrug oorsprong effek verduidelik, en dit moontlik maak om koueskade voorspellings te maak.

Summary

Rind injury due to chilling injury (CI) is especially costly when fruit, in particular lemons and grapefruit, are stored at low temperature such as required for cold sterilisation, for long periods. Heat shock and hot water dips have previously been shown to be useful in reducing CI. Work on lemons at UKZN has shown that molybdenum (MO) added to the hot water dip allows higher temperatures to be used without rind damage, and the physiological effects of the treatment were demonstrated to be related to production of anti-oxidants and free radical scavengers. During the period under review, further work was done on grapefruit and grapefruit types in Nelspruit, where hot water treatments were combined with fungicides and salicylic acid. At UKZN, lemons were used in the study, where the physiology of fruit treated with hot water, MO, methyl jasmonate and salicylic acid, plus different fruit origins were investigated. CI of oroblanco was significantly reduced by hot water at 53°C plus waxing with high solids wax. CI was also significantly reduced by TBZ and salicylic acid applications. No decay was recorded in these trials. Work will need to be repeated to verify results as well as determine the effects on lemons and Star Ruby grapefruit. Work at UKZN showed that fruit origin is important in CI potential due to fruit rind anti-oxidant levels. MO, salicylic acid and methyl jasmonate all enhanced the anti-oxidant levels, enabling fruit to withstand potentially damaging temperatures. A highly significant negative correlation was also found between rind sucrose content at harvest and chilling injury potential, helping to explain site effects and allow for easy prediction of chilling injury potential.

Introduction

Producers and exporters alike, lose millions of Rands every year due to chilling injury (CI) on lemons and grapefruit exported to Japan. Citrus fruit can incur rind damage due to CI if stored for extended periods at sub-optimal temperatures as occurs with lemons and grapefruit, exported to Japan, during the cold disinfestation (sterilisation) of the fruit against fruit fly. Research conducted by CRI in 2001 on the conditioning of grapefruit at 16 and 20°C and the heat shock treatment (35°C for 3 days in a hot room) of lemons and grapefruit, prior to the cold disinfestation treatment, demonstrated promising results in the reduction of CI. Research conducted by other researchers on hot water dip treatments, with and without fungicides (specifically thiabendazole), prior to sub-optimal temperature storage, have also demonstrated promising reduction of CI on lemons and grapefruit. The addition of salicylic acid to peaches in a 5 minute dip treatment reduced the incidence of CI on peaches under cold storage at 0°C for 28 days. (Wang, et al., 2006). A 2 minute fruit dip treatment with hot water (50-53°C) and a hot thiabendazole dip (1000 mg/l) or hot benomyl dip (500 mg/l) at the same temperatures significantly reduced chilling injury on navels and Marsh grapefruit stored at 1°C. for up to 15 weeks. Damage (98.4% CI) was most severe in controls dipped in water at 18°C. (Wild, 1990). Pre-storage hot water dips (53°C for 2-3 minutes) significantly reduced CI damage on Marsh grapefruit, lemons and Oroblancos (Rodov et al., 1995). Pre-storage hot water dips (47-53°C for 1-3 minutes) significantly reduced CI on eureka lemons stored at 1°C. for 28 or 42 days (McLauchlan, et al., 1997). Storing of citrus fruit in low O₂ or very high CO₂ concentrations (e.g. 10%) reduces CI. Waxing reduces CI depending on the gas permeability of the wax and the CO₂ build up on the surface of the fruit. Waxes that restrict gas exchange (e.g. shellac, highTS) and allow the build up of CO₂, reduce CI (Ritenour, 2002).

Factors that cause CI are still largely unknown. Methods to reduce CI, especially during cold disinfestation, must be afforded high priority.

The aim of these trials was to evaluate the early season cultivars under cold disinfestation conditions exported to Japan, and other markets at longer storage under further shipping conditions, if necessary, to determine if hot water treatments with or without certain chemicals, and also different citrus wax treatments could inhibit the development of chilling injury.

Materials and methods

Fruit was treated in Nelspruit and at UKZN. The following citrus cultivars were obtained for the trials in Nelspruit:

- Early (green T7-T5) lemons harvested at Larten Estates in the Karino area.
- Early Oroblancos harvested at TSB Hectorspruit; and
- Star Ruby grapefruit from the Strydom block.

The three cultivars were separately divided up into 4 reps of 20 fruit per treatment. All the fruit was treated on the CRI experimental packline in Nelspruit. The fruit was washed in the high pressure spray with a suitable sanitising agent (Prasin). After washing the three cultivars were treated in the hot water bath for 3 minutes exposure at ambient (18°C) or at 53°C and thereafter dried in the drying tunnel. The waxed treatments were done in the wax applicator on the citrus packline and the chemical treated fruit (thiabendazole and salicylic acid) was treated in dip applications for three minutes. The fruit was allowed to dry overnight before storage.

Treatments

1. Untreated Control.
2. Treated Control - Ambient dip at 18°C for 3 minutes.
3. Hot water dip at 53°C for 3 minutes **unwaxed**.
4. Hot water dip at 53°C for 3 minutes - carnauba tropical – 10.6% total solids.
5. Hot water dip at 53°C for 3 minutes - carnauba tropical – 26.0% total solids.
6. 1000 ppm TBZ in ambient water dip for 3 minutes.
7. 1000 ppm TBZ in 53°C water dip for 3 minutes.
8. Condition fruit for 7 days + hot water dip at 53°C for 3 minutes.
9. 50 ml Sentinal (salicylic acid) in ambient dip (22°C) for 3 minutes.
10. 50 ml Sentinel (salicylic acid) in 53°C dip for 3 minutes.

Storage

After treatment the fruit was stored as follows:

Cold disinfestation

24 days at -0.6°C + 7 days at 20°C.

Long shipping storage

5 weeks at 4,5°C + 7 days at 20°C.

The fruit investigated at UKZN came from two sources, a commercial lemon farm in the KZN midlands and the Ukulinga experimental farm. After hot water and other treatments, fruit was waxed, and stored at -0.5°C for 28 days. After each 7 days fruit was removed for cold injury determination and flavedo analysis after fruit was allowed to remain at room temperature for 7 days before cold injury was determined. The flavedo was removed, and total anti-oxidant capacity determined by the FRAP assay. Rind sugars were determined by HPLC.

Results and discussion

A three minute hot water dip treatment at 53°C alone and also after the application of a high solids Carnauba-Shellac wax (26.0% TS) significantly reduced the incidence of CI on the Oroblancos (Fig. 5.2.7.1). There was also a significant reduction in the incidence of CI on the fruit where both TBZ (thiabendazole) and salicylic acid was included in both the ambient and hot water dip treatments. Trials that were done in the South African citrus industry in 2002-3 on the role of citrus waxes, with and without chemicals, on CI on Marsh grapefruit that were stored under cold disinfestation and longer low temperature storage, demonstrated that TBZ in the wax significantly reduced the incidence of CI on the grapefruit. No results

were recorded for CI damage on the early season lemons and the Star Ruby grapefruit in these trials after cold disinfestation and extended shipping storage. No decay was recorded in these treatments either. However results in trials in 2006 indicated a high incidence of *Diplodia* stem end rot on Oroblancos after cold disinfestation treatment. Previous years' research results have indicated that fruit stored at low temperatures, with a high degree of this quiescent pathogen, have a higher risk of infection by this pathogen than fruit stored at higher temperatures. This is because the fruit stored at the low temperatures is stressed somewhat and this promotes the development of the infection.

Table 5.2.7.1. Hot water treatments with and without chemicals compared for the inhibition of CI damage on Oroblancos after conventional shipping conditions vs cold disinfestation conditions during export to Japan.

Treatments	% Chilling Injury Inhibition ^a
1. Untreated Control	20.0 e
2. Treated Control- ambient water dip	53.75 d
3. Hot water dip at 53°C	71.25 bcd
4. Hot water dip at 53°C + 10.6% total solids wax	57.5 cd
5. Hot water dip at 53°C + 26.0% total solids wax	71.25 bcd
6. 1000ppm TBZ in ambient water dip	75.0 abc
7. 1000ppm TBZ in 53°C water dip	80.0 ab
8. Condition fruit for 7 days + 53°C water dip	8.75 e
9. Salicylic acid in ambient dip	91.25 a
10. Salicylic acid in 53°C water dip	83.5 ab

^aValues represent the means of 3 replicates of 10 fruit each. Means followed by the same letter are not significantly different (Fisher's Unprotected LSD; P > 0.05).



Fig. 5.2.7.1. Chilling injury symptoms on Oroblancos.

In the case of the UKZN fruit, it was clearly shown that the hot water treatment enhanced the total anti-oxidant content of the rind, confirming preliminary results. Molybdenum appeared to ensure that the anti-oxidant levels also did not decrease as rapidly as the untreated fruit, where the levels decreased after about 14 days in cold storage. Salicylic acid and methyl jasmonate also appeared to enhance the levels. Of further interest, there was a strong negative correlation between the rind sucrose level and chilling injury.

Conclusions and further objectives

These trials will be repeated on early season Star Ruby and/or Marsh grapefruit and lemons. The trials on 'Oroblancos' will also need to be repeated to verify these results. The physiological basis for chilling injury or resistance to damage has been demonstrated. It is suggested that sucrose may form the energy basis for production of the anti-oxidants. If this is the primary determining factor, methods of enhancing sucrose levels and the ability to measure sucrose levels for prediction of sensitivity of fruit to chilling injury need to be considered.

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5.2.8 FINAL REPORT: The influence of citrus waxes on rind defects on sensitive citrus cultivars during shipping Experiment 947 (April 2008 – March 2009): by K.H.Lesar (CRI)

Opsomming

Dit is geglo dat skil defekte soos skilafbraak is deur verskeie voor-en naoes faktore geinduseer. Een van die naoes faktore is moontlik die tiepe waks wat aangewend is. Die doel van die eksperiment was om die rol van wakse op die Suid Afrikaanse mark is, op die induksie van skil defekte te ondersoek. Satsumas, Oroblancos, Star Ruby en Valencias is gebruik. 'n Reeks wakse is gebruik en vrugte teen -0.6°C vir 24 dae en verlengde opberging van 6 weke by 4.5°C , elk gevolg deur opberging by 20°C vir 7 dae. Vir Oroblancos and Satsumas die shellac-resin waks met die hoogste vastestowwe (26%) het die laagste skade (koueskade) gehad.

Summary

It is considered that rind disorders such as rind breakdown, may be caused by a number of pre-and postharvest factors. One of the postharvest factors is thought to be the type of wax applied. The objective of this experiment was to investigate the role of waxes available on the South African market on the development of rind disorders. Satsumas, Oroblancos, Star Ruby and Valencias were used. A range of waxes were applied, and fruit stored at -0.6°C for 24 days or extended storage of 6 weeks at 4.5°C . Each was followed by 7 days at 20°C . For Oroblancos and Satsumas the shellac-resin formulated wax with the highest total solids level (i.e. 26% TS) gave the lowest incidence of rind damage (CI).

Introduction

Citrus fruit are very prone to develop physiological rind disorders, manifested by a diversity of morphological symptoms during postharvest handling and storage. A large number of disorders have been described in citrus species and cultivars. Distinction and etiological classification of these disorders is often erratic since several indicative factors may lead to similar symptoms, but how they may be related to each other is still unknown. Many physiological disorders may occur under preharvest conditions and been considered to be of either nutritional (creasing, boron deficiency) or related to environmental conditions (zebra skin, sunburn, freezing injury etc.). Other preharvest disorders affecting fruit quality are related to fruit maturity (Lafuente et al. 2006). Fruits of many citrus cultivars may develop chilling injury (CI) when exposed to low non-freezing temperatures (Schiffman-Nadel et al 1971).

Citrus waxes play an important role in maintaining the quality of export citrus fruit during storage and shipping to the various markets. Citrus waxes ensure that the fruit arrives in the market place as a marketable commodity by maintaining a good shine, colour and texture on the fruit, and at the same time acting as a barrier to rapid moisture loss and consequent ageing of the fruit and also as a barrier to fungal pathogen infections on the fruit surface. The long term storage of a wax is the only true evaluation of the quality of the wax (i.e. the quality of the formulation of the wax and the role the wax plays in maintaining the quality of the fruit) during shipping. Citrus waxes on the other hand, by virtue of their formulation and application, can also either prevent or promote various **rind defects** on citrus cultivars. Citrus waxes play an important role in the fruit respiration and depending on the formulation of the waxes (i.e total solids levels, shellac, wood rosins etc.) could restrict (slow down) or speed up fruit respiration and thereby either prevent or promote **rind defects** (eg. puffy fruit, pitting, peteca spot, rind breakdown, chilling injury etc.). Poor wax application (under or over waxing) could have the same effect on **rind defects**.

Exposure of fruit to high levels of CO₂ concentrations (e.g. 10%) reduces CI on citrus fruit. Fruit respiration uses O₂ and gives off CO₂. Covering citrus fruit with a wax coating slows the movement of O₂ into the fruit and CO₂ out of the fruit so that internal O₂ concentration decreases while CO₂ levels rise. The extent of tolerance that wax coated fruit have to CI is related to the coating's gas permeability. Lower gas permeability results in higher internal CO₂ levels and a reduced tendency to develop CI. Thus, waxes that form a stronger barrier to gas exchange (e.g. shellac) reduce CI more than do waxes that "breathe" more (e.g. carnauba). However, too little gas exchange leads to off flavours (anaerobic respiration) and the development of other physiological disorders such as postharvest rind pitting (Ritenour et al. 2005).

This influence of a range of South African citrus waxes on rind defects on sensitive citrus cultivars during shipping needs to be evaluated.

The aim of these trials was to evaluate the early season cultivars under cold disinfestation conditions, and at longer storage under further shipping conditions, if necessary, to determine what influence the different citrus waxes have on the prevention or development of any physiological disorders on the fruit under storage conditions.

Materials and Methods

The following citrus cultivars were obtained for these trials:

- Early season satsumas harvested at Burgersfort ex Naranja Packhouse. (03/08).
- Early Oroblancos harvested at TSB Hectorspruit (18/03/08).
- Star Ruby grapefruit in the Strydomblock from Dirk Horn (6/05/08); and
- Valencia oranges harvested at Crocodile Valley in the Nelspruit area (09/08).

The Satsumas were divided up into 4 reps of 15 fruit per treatment and the other three cultivars were divided up into 4 reps of 20 fruit per treatment. All the fruit was treated on the CRI experimental packline in Nelspruit. The fruit was washed in the high pressure spray with a suitable sanitising agent (Prasin). After washing the four cultivars were dried in the drying tunnel, prior to wax application. The waxed treatments were done in the wax applicator on the citrus packline and each cultivar was waxed with the following citrus waxes:

- | | |
|-----------------------------|----------------------------------|
| (i) Carnauba Tropical wax | 10.6% total solids |
| (ii) Carnauba Tropical wax | 14% total solids |
| (iii) Carnauba Tropical wax | 15.5% total solids |
| (iv) Carnauba Tropical wax | 18% total solids |
| (v) Sta-fresh 890 HS | 26% total solids (shellac-resin) |

The waxed fruit was dried in the drying tunnel, prior to packing and storage.

Treatments

1. Untreated Control – ambient water dip for 3 minutes.
2. Carnauba Tropical wax 10.6% total solids
3. Carnauba Tropical wax 14% total solids
4. Carnauba Tropical wax 15.5% total solids
5. Carnauba Tropical wax 18% total solids
6. Sta-fresh 890 HS 26% total solids (shellac-resin)

Storage

After treatment the fruit was stored as follows:

Cold disinfestation

24 days at -0.6°C + 7 days at 20°C.

Extended shipping storage

6 weeks at 4.5°C + 7 days at 20°C.

Results and discussion

Waxes that form a stronger barrier to gas exchange (e.g. shellac) reduce CI more than do waxes that “breathe” more (e.g. carnauba) (Ritenour et al. 2005). The trials conducted with both Oroblancos and Satsumas demonstrated, in both cases, that the **shellac-resin** formulated wax with the highest total solids level (i.e. 26% TS) gave the lowest incidence of CI. This is what was expected as waxes with high total solids levels form a stronger barrier to gas exchange and allow the build up of high levels of CO₂ concentrations thereby reducing the incidence of CI on citrus fruit. The trial results below indicate that the incidence of CI on the Oroblancos was limited to 17.5% by the high solids wax compared to the 73.7% CI on the untreated control fruit and the incidence of CI on the Satsumas was limited to 34.9% compared to the 70.0% CI on the untreated control fruit.

Table 5.2.8.1. The influence of citrus waxes on physiological rind defects on Oroblancos stored under cold disinfestation conditions and under extended shipping storage.

Treatments		% Chilling Injury Inhibition ^a
Untreated Control - ambient water dip		26.25 c
Carnauba Tropical wax	10.6% total solids	42.5 bc
Carnauba Tropical wax	14% total solids	57.5 ab
Carnauba Tropical wax	15.5% total solids	55.0 ab
Carnauba Tropical wax	18% total solids	70.0 ab
Sta-fresh 890 HS	26% total solids (shellac-resin)	82.5 a

^a Values represent the means of 3 replicates of 10 fruit each. Means followed by the same letter are not significantly different (Fisher’s Unprotected LSD; P > 0.05).

Table 5.2.8.2. The influence of citrus waxes on physiological rind defects on Satsumas stored under cold disinfestation conditions and under extended shipping storage.

Treatments		% Chilling Injury Inhibition ^a
Untreated Control - ambient water dip		30.0 d
Carnauba Tropical wax	10.6% total solids	50.0 bc
Carnauba Tropical wax	14% total solids	44.97 c
Carnauba Tropical wax	15.5% total solids	58.32 ab
Carnauba Tropical wax	18% total solids	55.02 abc
Sta-fresh 890 HS	26% total solids	65.02 a

^a Values represent the means of 3 replicates of 10 fruit each. Means followed by the same letter are not significantly different (Fisher’s Unprotected LSD; P > 0.05).

Conclusions and further objectives

These trials need to be repeated on these cultivars to verify these results and the trials will be continued using other sensitive citrus cultivars and a larger variety of formulated citrus waxes for screening against a range of postharvest physiological rind defects.

Further objectives and work plan

This project (Exp. 947) has been discontinued. Nevertheless these trials on the influence of citrus waxes on rind defects on sensitive citrus cultivars under simulated shipping conditions will be repeated under this Experiment Nr: 869: Other sensitive citrus cultivars, such as soft citrus cultivars, lemons, grapefruit, Bennie Valencias etc. need to be included in these evaluations.

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5.2.9 **PROGRESS REPORT: Peteca spot of lemons**

Experiment 833 (April 2006 – July 2009): Paul Cronje (CRI)

Opsomming

Gedurende 2008 is daar 'n reeks proewe uitgevoer waartydens vrugte aan hoë CO₂ (1 en 5 %) en etileen (5 dpm) gas blootgestel is om die invloed op peteka kol te toets. Daar is ook van 1-MCP (500, 1000, 2000 en 4000 ppb) as 'n watertoediening gebruik gemaak om as etileen blokker te dien om sodoende die impak op peteka te bepaal. As gevolg van die uiters wisselvallige voorkoms van die skildefek tydens 'n seisoen asook tussen areas, het slegs 1 van die 4 proewe enige noemenswaardige resultate opgelewer. Die data bevestig egter dat die interne etileen metabolisme wel 'n rol speel en blyk dit dat 'n seker minimum vlak van etileen in die skil benodig word om sensitiwiteit te verminder. Daar sal in 2009 verdere proewe in verskeie areas gedoen word om die hipotese verder te toets.

Summary

A set of postharvest experiments, testing the influence of CO₂ (1 and 5 ppm), ethylene (5ppm) and 1-MCP on the occurrence of peteca spot of lemons, were done during 2008. Due to the erratic occurrence and variation of this rind disorder within one season and between areas, only 1 of the 4 experiments using a concentration range of 1-MCP (500, 1000, 2000 and 4000 ppb) provided any significant data. The data do, however, add support to the hypotheses that a certain level of internal ethylene is required for the reduction of the occurrence of peteca spot. This line of investigation will be carried forward to the 2009 season as it could potentially offer some practical management tools.

Introduction

Peteca spot of lemons is a postharvest physiological disorder resulting in the collapse of the oil gland. Subsequently the oil leaks into the adjacent tissue and causes darkened depressions or sunken areas (Cronje, 2007). The occurrence can be severe resulting in substantial economical losses without any known pre- or postharvest practises that could be implemented to reduce the incidence. Peteca spot occurs in all production areas of South Africa and is thought to be the result of the immature rind being subjected to postharvest stress, such as water loss and increased respiration under high CO₂ conditions which lead to the oil gland collapse. Peteca has also been linked in literature to applications of pre-harvest mineral oils, lack of pruning and postharvest fruit waxing (Wild, 1990). Although earlier reports linked peteca spot to an imbalance of calcium in the rind this hypothesis is currently not universally accepted (Khalidy *et al.*, 1969; Storey and Treeby, 2002). Peteca spot incidence has been shown to increase under higher temperatures during cold storage conditions, with 3°C resulting in significantly less peteca than 11°C (Undurraga *et al.*, 2007). This result was probably due to increased respiration and water loss. Weather conditions prior to harvest were also suspected to increase the incidence and Undurraga *et al.* (2006) collected data illustrating that peteca decreases if the days after a rainfall event and harvest are extended.

Materials and methods

During 2008 a number of experiments were done in the Eastern and Western Cape Provinces to test the influence of ethylene metabolism on the occurrence of peteca spot. However, only 1 out of 4 experiments had results, due to the lack of peteca spot development. The experiment looked at the involvement of CO₂ and ethylene gas on peteca spot. Fruit were subjected to continual gas treatments of ethylene (5 ppm), CO₂ (1 and 5%) and air as the control for 7 days at 20°C. The fruit were evaluated after 7 days for peteca spot incidence. In an additional treatment, fruit were dipped for 2 minutes in an aqueous solution of 1-MCP (500, 1000, 2000 and 4000 ppb) prior to storing for 14 days in plastic bags before evaluation. The 1-MCP mixed into 5 litres of water contains no chlorine. Fruit were wind dried after treatments and 8 replications of 10 fruit each were used in all instances.

Results and discussion

Data could only be generated from one of the 1-MCP treatment experiments that showed an incidence of peteca spot (Fig 5.2.9.1). The 500 ppb treatment resulted in no peteca symptoms but the low incidence of peteca in the controls (6%) did not result in a significant difference. The higher values in the 1000 to 4000 ppb treatments gave an indication that this chemical could have a negative action if used at too high a concentration.

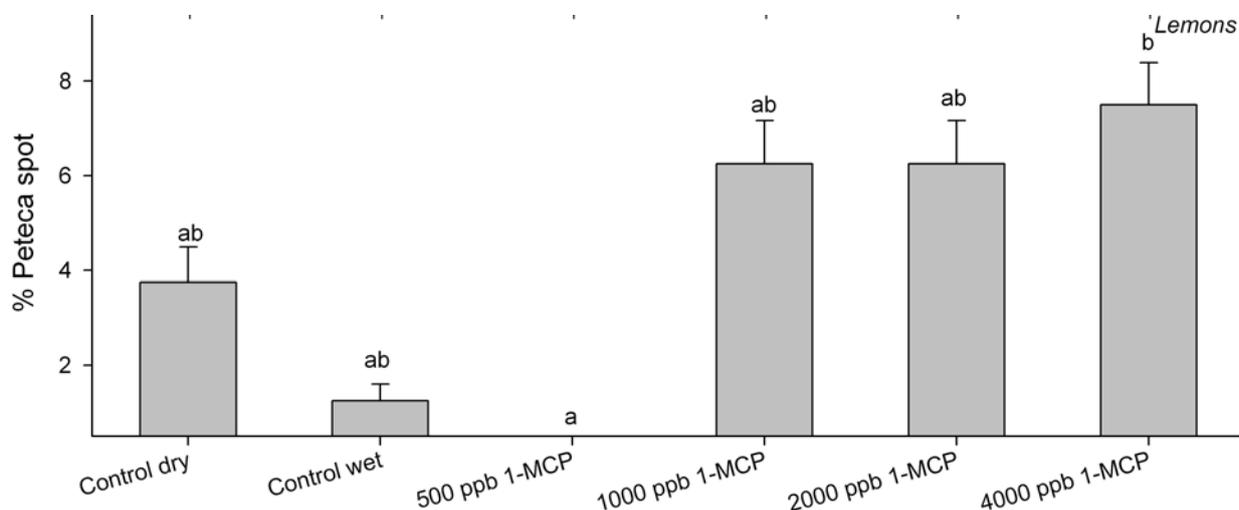


Figure 5.2.9.1. The effect of 1-MCP at a concentration range prior to storage in closed plastic bags on the occurrence of peteca spot of lemons. Different letters between bars denote significant differences according to Fishers LSD test ($P \leq 0.05$).

Conclusion

The results of 2008 give further support, although vague, to the reasoning that internal ethylene metabolism is involved in the development of peteca spot. However, the 2007 data give a better indication that the rind needs some level of internal ethylene to reduce the incidence of peteca spot (CRI Annual report 2008). The low levels of peteca as seen in Fig 5.2.9.1 do not give an adequate opportunity to test the various treatment effects.

Further objectives and work plan

During the 2009 season, the above treatments will be used on fruit from diverse climatic zones *viz.* Limpopo, Eastern and Western Cape in order to test the hypothesis that ethylene metabolism is involved in peteca spot development.

Technology transfer

Citrus Cold Chain Forum: Packhouse workshop. Influence of packhouse practises on rind condition.4 -29 Feb, Gordons Bay, Western Cape; Letsitele, Limpopo; Loskopdam, Mpumalanga; Nkwaleni Valley, KwaZulu-Natal.

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5.2.10 **PROGRESS REPORT: The evaluation of postharvest operational issues and the effect of different citrus wax applications on the development of peteca spot on lemons**

Experiment Nr: 795 (April 2008 – March 2009): by K.H.Lesar (CRI)

Opsomming

Die oorsaak van peteca vlek is not onbekend. Beide voor-en naes faktore kan moontlik 'n rol speel. Vrugte is onder nat en koue toestande, wat moontlik 'n rol kan speel, gepluk. Die vrugte was dan deur hoë temperatuur water gewas, oorgeborsel, en swaar waks toegedien. Vrugte was dan by -0.6°C vir 24 dae opgeberg, gevolg met 7 dae by kamer temperatuur. Geen peteca is gevind nie. Verdere navorsing sal moet gedoen word.

Summary

The causes of peteca spot are still unknown. However, both pre- and postharvest conditions are thought to be implicated. In this work, fruit was harvested under cold and wet conditions, thought to favour the disorder, and subjected to high temperature washing, over-brushing and heavy wax applications before storage at -0.6°C for 24 days and ambient storage for 7 days. No peteca developed, and research will need to continue.

Introduction

Peteca spot is a physiological disorder that appears as deep pits on the peel surface of lemons, usually after packing (Fig. 1). Peteca is particularly prevalent when the trees undergo water stress alternating with periods of freely available water in the period two to three months prior to harvest. Other cultural practices, that have been reported to increase the incidence of peteca spot, are late heavy pruning practices, late application of Nitrogen and late oil sprays.

Erratic environmental conditions appear to play a major role in predisposing lemons to the development of peteca. i.e. sudden changes from long periods of hot dry conditions followed by colder weather.



Fig. 5.2.10.1. Peteca Spot.

Peteca seems to be more prevalent after the harvesting of lemons during cold, moist or wet conditions. The rough handling of lemons, especially the more sensitive greener fruit, during picking, transport to the packhouse and operational processes in the packhouse, also seem to predispose the fruit to the development of peteca.

The operational processes in the packhouse that have triggered the development of peteca spot on lemons are the rough handling of the fruit, as already mentioned, over brushing and too high brush speeds, too high a temperature in the hot water fungicide bath and in the drying tunnel, and most importantly the waxing of the fruit. Trials done on 'Meyer' lemons in Australia demonstrated that peteca was aggravated by citrus wax application and fruit brushing. It was found that increasing brushing time increased the incidence of peteca and polyethylene waxes induced more peteca than carnauba wax formulations (Wild, 1991). Reasons for the difference between the two waxes is unclear, but it could be due to the stress on the fruit produced by the increased CO₂ concentration associated with wax application (Vines et al., 1968). Waxing of the fruit is one of the major critical control processes in the packhouse. Choosing the right wax for lemons, specifically peteca-prone lemons, is critical. It has been reported that the use of heavy waxes should be avoided.

Polyethylene waxes with high solid levels (18% and higher) and/or increased shellac or wood rosin levels are classified as heavy waxes. The natural waxes i.e. Carnauba waxes with lower solid levels (16% and lower) and with not too high shellac levels or without shellac are classified as lighter waxes and are reported to be the preferred waxes for peteca-prone lemons.

The application rate of the wax used for lemons is also vitally important. Even though a light wax may be used for peteca-prone lemons, the over application thereof could also induce the development of peteca. The slight under application, but a good uniform coverage of a light wax is by far the desired application of a light wax to lemons reported to reduce the risk of peteca development. However the slight under application, but erratic non-uniform application of a light wax could also predispose the fruit to loss of quality and cold damage during shipping.

In these trials early season green lemons from peteca-prone orchards at Larten (Karino) were harvested early in the morning during cold moist conditions, which are typical conditions conducive to the development of peteca spot. Early season green lemons were also obtained from Schoeman Boerdery. Schoeman boerdery had reported to CRI that they were experiencing a high incidence of peteca on their early lemons. A consignment of these lemons from the same orchards at Schoeman where they had had peteca were also requested for inclusion in these trials.

All these lemons were exposed to a few of the operational issues, that had been reported to contribute to peteca spot, and were treated with a range of citrus waxes with different solid levels to determine the effect of these operational issues and the waxes on the possible development of peteca spot after the cold disinfestation (sterilisation) treatment.

Materials and methods

Twenty lug boxes of green to colour break (T7-T6) lemons were harvested at Larten, Karino and transported to CRI Nelspruit during the last week in March 2007. These lemons were harvested from the same orchards where a high incidence of peteca spot was experienced on lemons in the 2004 and to a lesser extent during the 2005 season.

Twenty lug boxes of the same colour lemons, as above, were also harvested at Schoeman Boerdery and delivered to CRI for inclusion in these trials.

The lemons were treated on the packline by first washing the fruit in the high pressure spray with the quaternary ammonium compound, Prasin. The lemons were then exposed to temperatures of 30, 40, and 50°C in the hot water bath for 2 minutes. A temperature of 40°C is not recommended as being ideal for green to colour break lemons with **sensitive** rinds. All the packline treated lemons were roughly handled during dumping prior to washing and also after drying of the fruit. The lemons were then dried in the packline drying unit for 2 minutes and 10 minutes to simulate "normal" and **over brushing** of the fruit. After drying, both sets of lemons were divided up into 6 replicates x 20 fruit each per treatment. The lemons were then waxed, on the CRI packing line in the waxing unit, with the following citrus waxes:

(i)	Carnauba Tropical wax	10.6% total solids
(ii)	Carnauba Tropical wax	14% total solids
(iii)	Carnauba Tropical wax	15.5% total solids
(iv)	Carnauba Tropical wax	18% total solids
(v)	Carnauba Citrosol	18% total solids
(vi)	Sta-fresh 890 HS	26% total solids (shellac-resin)
(vii)	Sta-fresh 875 HS	26% total solids (carnauba-shellac)
(viii)	Polyethylene	18% total solids

After waxing the lemons were dried in the drying tunnel on the packline.

Treatments

1. Untreated Control
- 2(a). Treated Control – washed in the bath at ambient (16°C) and dried on brushes for 2 minutes in the drying tunnel.
- 2(b). Treated Control - washed in the bath at ambient (16°C) and dried on brushes for 10 minutes in the drying tunnel.
3. Hot water bath at 30°C and drying in tunnel for 2 minutes.
4. Hot water bath at 40°C and drying in tunnel for 2 minutes.
5. Hot water bath at 50°C and drying in tunnel for 2 minutes.
6. As in 4 then waxed with wax (i).
7. As in 4 then waxed with wax (ii).
8. As in 4 then waxed with wax (iii).
9. As in 4 then waxed with wax (iv).
10. As in 4 then waxed with wax (v).
11. As in 4 then waxed with wax (vi).
12. As in 4 then waxed with wax (vii).
13. As in 4 then waxed with wax (viii).

After drying, 3 reps x 20 fruit were placed in paper bags and 3 reps in plastic bags for storage purposes.

The reason for storing in plastic bags is based on results obtained in other trials where peteca symptoms were evident on lemons stored in plastic bags. During the respiration of the lemons in the plastic bags high levels of CO₂ were measured which resulted in the development of peteca spot.

Storage

After treatment the fruit was stored the following day under simulated cold disinfestation conditions at -0.6°C for 24 days + 7 days at 20°C. After the fruit stood at ambient (20°C) for 7 days the treatments were evaluated for any peteca spot symptoms.

Results

No peteca spot symptoms were observed on the lemons in both the paper packets and the plastic bags. The treatments were then stored for a further 6 weeks at 2°C to possibly induce the development of peteca spot/CI (chilling injury) symptoms. After this storage the treatments were stored for a further 7 days at ambient before finally being evaluated. Still no symptoms were observed after extended storage and thus there were no results to report.

Conclusions

There are still far too many unknowns, both pre- and post-harvest, that lead to the development of peteca spot on lemons. The occurrence of peteca spot on lemons over the last 10 years has been very erratic and this has resulted in inconsistent research being conducted on this disorder. Nevertheless the research will continue and these trials will be repeated until answers are found.

Further objectives and work plan

The role of the operational processes in the packhouse and the type of citrus waxes applied to lemons have been reported to play a role in the promoting of peteca spot on lemons. These issues need to be investigated on an ongoing basis until the answers are found. The research will continue.

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5.2.11 FINAL REPORT: The effect of light and competition on the development of rind disorders
Experiment 758 (April 2003–March 2008): Paul Cronje (CRI), Graham Barry and Marius Huysamer (SU)

This experiment forms part of Paul Cronje's PhD thesis. Sections 1 & 2 are available in the completed thesis. Sections 3 to 7 are reported on in this annual report.

1. Introduction.
2. Literature review.
3. Accumulation of K, Mg and Ca during 'Nules Clementine' mandarin flavedo development as influenced by canopy position.
4. Canopy position affects reducing and non-reducing sugar accumulation in the flavedo of 'Nules Clementine' mandarin fruit.
5. The incidence of rind breakdown of 'Nules Clementine' mandarin fruit is influenced by exposure to sunlight.
6. Foliar application of Mg and Ca reduces the incidence of rind breakdown of 'Nules Clementine' mandarin fruit.
7. Postharvest pigment and carbohydrate changes in relation to the incidence of rind breakdown of 'Nules Clementine' mandarin fruit.

5.2.11.3 Accumulation of K, Mg and Ca during 'Nules Clementine' mandarin flavedo development as influenced by canopy position

Opsomming

Dit is welbekend dat die genoegsame hoeveelhede, asook verhouding van K, Ca en Mg benodig word in vrugte vir die ontwikkeling van selwande en membrane en indien dit nie die geval is nie daar na-oes fisiologies defekte ontwikkel. In die 'Nules Clementine' mandaryn boom is daar a.g.v. die digte blaredak heelwat mikroklimaat variasie in fotosintetiese aktiewe radiasie (PAR) en temperature. Die doel van die proef was om te bepaal of hierdie mikroklimaat variasie sal lei tot 'n verandering in die minerale samestelling van die flavedo, veral ten opsigte van K, Mg en Ca gedurende fase II en III van vrug groei. Bykomend is die vrugmassa, lengte en breedte, skil kleur bepaal om die verskil tussen binne en buitvrugte vas te stel. Daar was betekenisvolle verskille gevind tussen binne en buitvrugte in die akkumulاسie patroon van mineraal elemente. Die binnevrugte se flavedo's het betekenisvol minder Ca en Mg asook meer K in vergelyking met die buitvrug flavedo. Die akkumulاسie van die minerale elemente volg nie die bekende patrone vir vrugte nie maar is meer die van blare en dit kan dui dat die flavedo alle minerale elemente direk van die xileem ontvang en nie deur floëem her-translokasie in die geval van K en Mg nie. Die verlaging in transpirasie tempo van die binnevrugte kan dus tot die afname in Ca en Mg lei en wat 'n verswakking van die skilkondisie teweeg kan bring.

Summary

Adequate K, Mg and Ca supply are importance to develop well-structured and functional cell walls and membranes in fruit and insufficient levels or imbalances of these minerals are known to be involve in various postharvest disorders. Microclimatic variation exists in the 'Nules Clementine' mandarin tree canopy and results in a reduction of PAR and temperature as well as an increase in humidity inside the tree canopy. The aim of the experiment was to determine the impact of this variation on accumulation patterns of K, Ca and Mg in the flavedo of the fruit rind during stages II and III of fruit development. Fruit mass, dimensions, rind colour development and mineral composition of the flavedo were measured to describe the outside and inside fruit. The data showed canopy position influenced mineral nutrient accumulation patterns in the flavedo. Outside fruit flavedo accumulated significantly higher concentrations of Ca and Mg in all three seasons (2005 - 2007). In contrast, inside fruit flavedos (shaded fruit) accumulated significantly higher levels of K compared with outside fruit. The accumulation pattern of K and Ca differed from that of kiwifruit and apple in that Ca concentration increased and K decreased towards maturity. This result suggests that xylem, as in citrus leaves, is the main vasculative supply conduit to the fruit flavedo. The reduction of transpiration potential by low temperatures and high humidity inside the canopy could be responsible for the reduced accumulation of Ca and Mg. The high K concentration of inside fruit flavedo is suggested to be a stress response to maintain osmotic potential in the shaded rind tissue, but this imbalance could possibly lead to a reduction in rind condition. Canopy position affects reducing and non-reducing sugar accumulation in the flavedo of 'Nules Clementine' mandarin fruit.

Introduction

The citrus tree develops a dense canopy of leaves that results in microclimatic variation within the canopy, e.g. temperature (Barry et al., 2000), VPD (vapour pressure deficit) (Syvertsen and Albrigo, 1980) and PAR (photosynthetically active radiation) (Green and Gerber, 1967; Jahn, 1979). These factors are also known to influence water movement and fruit tissue water content (Kauffman, 1970) as well as photosynthesis (Jahn, 1979) in citrus trees. In addition, a relationship exists between areas in the canopy with high light and temperature values and fruit with high juice and soluble sugars content (Reitz and Sites, 1948; Fallahi and Moon, 1989; Barry et al., 2000; Morales et al., 2000). The higher juice and soluble sugar content of exposed fruit are thought to be the result of a more rapid rate of development compared to partially shaded fruit (Barry et al., 2000). Although higher air temperature in the exposed canopy will lead to an increased sugar content, it is thought that the additional effect of higher PAR, although not quantified (Sites and Reitz, 1949; 1950), and water stress (Syvertsen and Albrigo, 1980) of exposed fruit will in particular play an important role in causing difference in juice soluble sugar content between canopy positions (Barry et al., 2000).

However, canopy microclimate not only impacts on differences in fruit internal quality, but also on differences in accumulation of mineral nutrients in leaves and whole fruit (Koo and Sites, 1956; Fallahi and Moon, 1989). It can be concluded from these studies that leaves and fruit positioned inside the dense citrus canopy have higher levels of N, P and K compared with leaves and fruit borne on the outside. In contrast outer, more exposed leaves and fruit, have higher Ca, and to a lesser extent, Mg levels. This variation of mineral nutrient distribution (and other solutes) within the citrus tree canopy could be argued to depend on microclimatic factors, e.g. temperature, light and relative humidity within the canopy, as these factors influence water movement - the mobile phase of all solutes - in the xylem and phloem vascular bundles. Water movement in the xylem, and the associated mineral nutrient transport, is generally accepted to occur due to evaporative water loss from the leaves, driven by VPD gradient pulling water and therefore solutes towards the transpiring leaf surface (Fisher, 2001).

Transpiration rate can also be influenced by fruit anatomical changes, e.g. declining conductance in apple fruit stalks (*Malus domestica* Borkh.) (Lang and Ryan, 1994) and xylem discontinuation in some grape cultivars (*Vitis vinifera* L.) (Coombe and McCarty, 2000). In *Citrus* fruit the possible plugging of stomata by the development of surface wax, as well as the reduction in stomata per unit surface area, are thought to influence fruit transpiration (Albrigo, 1972). However, fluctuations in transpiration rate do not influence all mineral nutrients equally and, whereas Ca movement is significantly reduced, K and Mg movement are not drastically affected by a reduction in transpiration rate (Marschner, 1995).

Phloem facilitated transport of mineral nutrients follow Munch-pressure flow dynamics between the sources (leaf) and sinks (fruit) (Patrick, 1997). However, the difference in mobility of mineral nutrients in the phloem results in high concentrations of K and Mg and very low Ca and B being transported (Bukovac and Wittwer, 1957). This occurrence is suggested to be due to the variation in element solubility in the phloem sap (Tromp, 2005).

A fruit mineral concentration can therefore be a function of either xylem or phloem supply, although a shift from xylem towards phloem transport is thought to occur in apple fruit (Lang, 1990). In contrast to most other fruits, citrus fruit consists of a fleshy pulp and a leathery rind which is hydrolytically separated (Koch and Avigne, 1990). The accumulation of mineral nutrients in citrus fruit pulp follows, to a large extent, the same pattern as apple (Ferguson and Watkins, 1983) and kiwifruit (*Actinidia deliciosa* Chev.) (Clark and Smith, 1988), whereby K and Mg concentrations increase and Ca decreases towards fruit maturity. In contrast, citrus rind has a high Ca concentration and lower K at maturity. This pattern is thought to be due to different contributions by the xylem and phloem towards these fruit parts (Storey and Treeby, 2000).

Mineral nutrient status and accumulation of especially Ca, K and Mg in various fruits have been closely associated with postharvest disorders, although not necessarily as the underlying mechanism in avocado (*Persea americana* Mill.) (Van Rooyen and Bower, 2005), apple (Ferguson and Watkins, 1983), tomato (*Lycopersicon esculentum* Mill.) (Del Amor and Marcelis, 2006) and kiwifruit (Ferguson et al., 2003). The influence of position within the tree or vine has also been suggested to determine fruit sensitivity to physiological disorders due to influence on mineral nutrient composition in plums (*Prunus salicina* Lindl.) (Taylor et al., 1993) and kiwifruit (Thorpe et al., 2003).

'Nules Clementine' mandarin fruit (*Citrus reticulata* Blanco.) develop a progressive rind disorder, called rind breakdown (RBD), related to the collapse of the oil gland in the flavedo 3 to 5 weeks after harvest which results in a "leopard spot" pattern. This disorder has been suspected to have a higher prevalence in fruit flavedo developing in low light conditions (Van Rensburg et al., 1995; Khumalo, 2006; Cronje, 2007). The

aim of this experiment was to determine if K, Mg and Ca accumulation in the flavedo of 'Nules Clementine' mandarin fruit is influenced by canopy position (sun exposed vs. shaded) during the period after physiological fruit drop which coincides with developmental stages II and III (Bain, 1958). It is hypothesised that the citrus flavedo receives most of its mineral nutrients via the xylem and consequently the accumulation rate will be affected by microclimatic factors impacting on transpiration rate.

Materials and methods

Sites and plant material

The experiment was conducted in two orchards of 'Nules Clementine' mandarin budded on Carrizo citrange {*Poncirus trifoliata* (L.) Raf. x [*Citrus sinensis* (Osb.) L.]} rootstock. In the 2004, 2005 and 2007 seasons, fruit were sampled from the orchard at the University of Stellenbosch experimental farm, Western Cape Province, South Africa, whereas in 2006, a commercial orchard in the Paarl area, 20 km from the experimental farm was used. This was necessary due to severe alternate bearing in the experimental farm, orchard. Both these orchards were planted with a North–South row orientation. The Stellenbosch orchard was planted in 1991 at a spacing of 4.5 x 2.5 m, and the Paarl orchard in 1993 at a spacing of 5 x 3 m.

Fruit sampling

The experimental design was a randomized complete block design with eight single tree replications. Fruit were sampled at regular monthly intervals coinciding with stages II and III of fruit development (Jan.–May) and commenced after physiological fruit drop (Bain, 1958). From these eight trees, 25 fruit were from each canopy position, i.e. outside (90–100% of full sunlight) or inside (< 80% of full sunlight).

To quantify the microclimate in the canopy, light profile measurements were recorded during 2005 in the top, middle and below the leaf canopy of 10 trees. The measurements were done between 10:00 and 12:00 AM on a clear day on 21 Jan. 2005 using a light meter (Li-250 light meter with a Li-190SA quantum meter, Li-COR, Lincoln, Neb., USA), which took point measurements integrated over 15 seconds. The 80 cm long probe, consisting of 80 individual light meters, was divided into eight 10-cm zones from which the average values were plotted. Two TempTale4/Humidity data loggers (Sensitech Inc., Beverly, Ma., USA), measuring temperature and humidity were placed in one tree in an outside and inside position to quantify the climatic variables in the canopy.

Rind colour of each fruit was determined using a chromameter (Minolta NR 4000, Osaka, Japan). Fruit dimensions (length and diameter) and weight were measured prior to removing the flavedo. During 2006, the pedicel diameter of each fruit was measured at each monthly sampling (except in Mar.). The flavedo of the 25 fruit per replicate was pooled to ensure that these was enough material for analysis. The flavedo was frozen in liquid nitrogen whereafter it was freeze dried (VirTis Freezemobile 25ES, The VirTis Company, Gardiner, NY, USA) and stored at -80°C.

The dried flavedo material was supplied to a commercial analytical laboratory for mineral analysis (Bemlab (Pty) Ltd., Strand, South Africa). The standard preparation and analysis of samples was followed using the ICP-OES (Inductively Coupled Plasma – Optical Emission spectrometer) (Varian PRX-OEX, Varian, Inc. Corporate, Palo Alto, CA, USA) procedure and a nitrogen analyser (LECO FP528 Nitrogen analyser, LECO cooperation, St. Joseph, Michigan, USA) [W.A.G. Kotzé, Bemlab (Pty), Ltd., Strand, South Africa; personal communication]. The results from the mineral analyses are expressed as per mmol·g⁻¹ dry weight (DW) of each mineral. All macro and microelements were analysed but only the K, Ca and Mg data are presented.

Statistical analysis

The correlation procedure (PROC CORR) (SAS v. 6.12, SAS institute, Cary, NC, USA) was used to determine significance of correlations between mineral nutrient concentrations (K, Ca and Mg) and fruit weight. Differences between fruit position effects on rind colour and fruit size were analysed with PROC GLM, and from the analysis of variance (ANOVA) significance differences between treatments were determined and means were separated by Fishers's least significant differences (LSD).

Results

Variation in microclimate within the tree canopy

The available light in a 'Nules Clementine' mandarin tree decreases within the first 10 cm into the canopy, illustrating that the dense leafy canopy of citrus trees only allows low levels of PAR to penetrate into the inner canopy (Fig. 5.2.11.3.1), as reported by Greene and Gerber, (1967). Temperature and humidity measured

over the sampling period concurred with those previously reported (Barry et al., 2000; Morales et al., 2000), and showed that the outside position to be warmer, as well as having a lower relative humidity compared to the inside position (Fig. 5.2.11.3.2), which would result in a higher transpiration rate driven by atmospheric evaporative demand. It is clear that significant differences exist between the microclimate of the inside and outside of the canopy and these would influence key plant physiological processes such as photosynthesis and transpiration (Jones, 1983).

Fruit development

Position within the canopy significantly influenced the fruit growth and rind colour development in all three seasons. Only the 2005 data are shown to avoid repetition. Inside fruit were consistently smaller (diameter and length) and lighter compared with outside fruit (Figs. 5.2.11.3.3 and 5.2.11.3.4). The positional effect also resulted in a difference in rind colour development of the flavedo. During the green, immature stage (Jan – Mar/Apr) the inside fruit had a lighter and less green colour (lower chroma values) compared with the outside fruit (Fig. 5.2.11.3.5). After colour break (between Mar and Apr) the outside fruit developed a more intense orange colour (lower hue angle) compared with the more yellow coloured inside fruit at harvest in May.

Pedicle growth

The pedicle diameter of outside fruit was not only significantly greater than the inside fruit pedicle in all four months, but also showed a difference in rate of development (Fig. 5.2.11.3.6). Whereas the variation between pedicles from inside and outside fruit was ± 0.1 mm in January, this difference increased to ± 0.5 mm at harvest, indicating a higher degree of vascular development in the pedicles of outside fruit nearer to maturity.

Flavedo mineral nutrient accumulation

During the sampling period (after physiological fruit drop), significant differences were seen in the accumulation patterns of K, Mg and Ca in the flavedo of inside and outside fruit (Fig. 5.2.11.3.7). In all 3 years, more K accumulated in the flavedo of the inside fruit compared with the outside fruit. However, both inside and outside flavedo showed a reduction of K towards harvest date. In contrast, Ca accumulation was higher in the outside fruit flavedo (except during Mar. and Apr. 2007) and increased towards harvest date in all three seasons. Magnesium accumulation differed significantly between flavedo of outside (a higher concentration) and inside fruit. A less clear pattern exists, however, when considering all seasons, the data suggest an increase towards Mar. whereafter a reduction occurs coinciding with rind colour development.

K, Ca and Mg accumulation vs. fruit weight

In all three seasons, a negative correlation, at a high level of significance, existed between K accumulation and fruit weight, as denoted by the high Pearson's correlation values (r). It is evident that during all seasons the flavedo from inside fruit start and end at higher K concentrations compared to the outside fruit (Figs. 5.2.11.3.8 to 5.2.11.3.10).

Calcium accumulation in the flavedo generally had a significant, positive correlation with fruit weight increase except for the flavedo from outside fruit during the 2006 season. The Ca concentration tends to level off in the outside fruit between Mar. and May coinciding with a reduction in rate of fruit growth. This was not apparent in the inside fruit, which showed an increase in Ca and fruit weight until harvest, although at a lower rate compared with the outside fruit (Figs. 5.2.11.3.8 to 5.2.11.3.10).

Magnesium accumulation did not consistently correlate with fruit weight (Figs. 5.2.11.3.8 to 5.2.11.3.10). However, a slight trend towards a reduction in Mg content is evident in all three seasons and positions towards fruit maturity, but the most drastic increase and reduction occurred in the outside fruit flavedo in 2005. This dramatic reduction (to half of the previous levels) occurred after colour break in April which could possibly be ascribed to a reduction of chlorophyll content at this stage. It is possible that dividing Mg accumulation into two stages, i.e. pre- and post- colour break (coinciding with increased carotenoids and a reduction in Mg-containing chlorophyll) could result in a correlation with Mg concentration.

Ratios of K, Mg and Ca accumulated in the flavedo

The K/Ca ratio followed the same pattern during the sampling period in all three seasons, with inside fruit flavedo having significantly higher ratios compared with outside fruit (Fig. 5.2.11.3.11 abc). A decreasing trend was evident and the ratio values were in the same range in all seasons and were a function of the increase in Ca and decrease in K (Figs. 5.2.11.3.8 to 5.2.11.3.10).

The K/Mg ratio followed a less clear, but also decreasing trend in all seasons due to variation in Mg concentrations between seasons (Fig. 5.2.11.3.11 def). However, the ratio of the inside fruit flavedo was

significantly higher than that of the outside fruit samples. The more pronounced decline in the K/Mg ratio of the outside fruit indicates a marked reduction in the K and increase in Mg accumulation (Figs. 5.2.11.3.8 to 5.2.11.3.10) which is also seen in Fig. 5.2.11.3.7. In 2006 and 2007 the Mg concentration did not vary to a large extent between positions and this is mirrored in the constant difference in the ratio of Mg to K.

The Mg/Ca values followed a different pattern from the other ratios in that the flavedo from outside fruit, at the onset of sampling (Jan. to Feb.) had values lower than those of the inside fruit. However, during the Feb. to Mar. period the inside ratio value decreased at a lesser rate compared to the outside and resulted in a higher value at harvest in May (Fig. 5.2.11.3.11 ghi).

The (K + Mg)/Ca ratio followed much the same pattern as the K/Ca ratio due to the small changes in Mg concentration, and illustrated that K and Ca were the most influential elements in this ratio (Fig. 5.2.11.3.10 jkl). Inside fruit had a higher ratio during the sampling period of all three seasons due to the higher K and lower Ca concentration compared with the outside flavedo (Fig. 5.2.11.3.8 to 5.2.11.3.10).

Discussion

The accumulation patterns of K, Ca and Mg in the flavedo of 'Nules Clementine' mandarin fruit differ from the mineral nutrient accumulation patterns found in the pulp, but show some similarities to flavedo and albedo of 'navel' orange [(*Citrus sinensis* (L.) Osb.)] (Storey and Treeby, 2000) and 'Valencia' orange (Sinclair and Bartholomew, 1944). Whereas the citrus pulp accumulation pattern is similar to that of fleshy fruit (increasing K and decreasing Ca) towards fruit maturity (Quinlan, 1969; Clark and Smit, 1988), the flavedo behaves inversely. The mineral nutrient accumulation pattern seen in the 'Nules Clementine' mandarin fruit flavedo (increased Ca and reduced K) more closely resembles the mineral accumulation of orange leaves than fruit during their development (Jones and Parker, 1950; 1951; Embleton et al., 1973) and supports the notion that the flavedo is a modified leaf rather than a fleshy fruit (Schneider, 1968).

The accumulation pattern in apple fruit is attributed to a shift from xylem to phloem transport as fruit progress towards maturity (Lang, 1990), and result in phloem immobile elements, viz. Ca, decreasing in fruit concentration (Mengel and Kirkby, 2001) after the cell division stage. The data presented do not follow this pattern and the decrease in K and increase in Ca seem to indicate the opposite situation with regard to vascular connectivity. Therefore, a decreasing phloem component or more probably an increased xylem supply towards the flavedo of the 'Nules Clementine' mandarin fruit could be the reason for the increase in Ca. Support for this argument can be found in various anatomical and physiological studies documented in literature. It is known that in the citrus fruit pedicel that the xylem continues to differentiate for 15 days longer than the phloem and constitutes 42 – 46% of the final pedicel diameter (Garcia-Luis et al., 2002). Scott and Barker (1947) described the citrus rind (albedo and flavedo) as leaf-like due to the three main vasculature veins branching off into the flavedo, ending in a knot of spiral tracheids while "bracketing" the oil glands, as in a leaf. It is therefore evident that xylem is present in the flavedo tissue, although it is known that the vascular bundles become amphicribal (phloem encircling the xylem) when they enter the fruit and that the xylem content is reduced as distance from the calyx increases, often resulting in a single vessel strand completely surrounded by phloem (Goldschmidt and Koch, 1996). However, the degree of connectivity between the rind and shoot xylem is illustrated by daily xylem backflow from the rind (and not pulp) towards leaves (Chaney and Kozlowski, 1971; Elfving and Kaufmann, 1972).

It can therefore be argued that the flavedo will continue to accumulate Ca after the cell division stage (as in a leaf), via the xylem as it progresses towards maturity. At maturity of the flavedo (Apr./May in outside flavedo), coinciding with colour break and a decrease in fruit growth rate, the accumulation of Ca remains constant after 7–8 months as seen in 'Valencia' orange leaves (Jones and Parker, 1950; 1951; Embleton et al., 1973). The same pattern exists in *Phaseolus vulgaris* leaves, which do not accumulate more Ca after they reach maturity even at high transpiration rates (Koontz and Foote, 1966). The continuing Ca accumulation and increasing weight of inside fruit suggests that the flavedo lags in maturity behind that of the outside fruit flavedo. The reduction in K in the flavedo mirrors the situation in a leaf (Jones and Parker, 1950; 1951), which could be an indication that the K, accumulated in the flavedo via xylem influx, is phloem transported outwards from the flavedo, as in the leaf, towards a K sink.

The slight increase in accumulation of Mg from Jan. until Feb.–Mar. and the reduction thereafter is also seen in 'Valencia' orange leaves, which decrease after 6–9 months of leaf growth (Jones and Parker, 1950; 1951; Embleton et al., 1973). This reduction could be related to the colour change of the flavedo and degradation of the Mg-containing chlorophyll molecules by Mg-dechelataase. It could be argued that a reduced call for Mg exists as less chlorophyll is synthesised at this stage of fruit development and this phloem mobile-element

could be transported out of the flavedo as seen in senescent soybean leaves [*Glycine max* (L.) Merrill cv. Anoka] (Belma et al., 1978).

The flavedo, as a modified leaf, possesses actively transpiring stomata (Blanke, 1996) and the physiological responses would be affected by climatic variations of temperature, humidity and light, as it would in a leaf (Syvertsen and Albrigo, 1980). Flavedo of inside fruit are subjected to a lower VPD due to the reduced temperature and higher humidity compared with outside fruit. These conditions would result in a lower transpiration rate towards the inside fruit (Jones, 1983), and a reduced accumulation of xylem transported mineral nutrients, i.e. Ca and Mg, in the flavedo, as unloading of mineral nutrients from the xylem occurs predominantly at sites of highest evaporation (Fisher, 2000; Kochian, 2000; Mengel and Kirkby, 2001). However, fluctuations in transpiration rate do not increase the transport of all mineral nutrients by the same order of magnitude. As a rule, increased transpiration affects transport of uncharged molecules to a greater extent than that of ions. A lower transpiration rate leads to a decrease in Ca content in fruit, but the effect is less severe for Mg and negligible for K (Marschner, 1995). These variations among nutrients in transport rate are due to cations reacting to the negatively charged groups in the xylem cell wall. This exchange of cations can vary by physicochemical and metabolic means, depending on passive and active exchange processes along the xylem (Wolterbeek, 1987; Schurr, 1999; Sattelmacher, 2001).

The surprisingly higher K concentration of the inside fruit could be due to two possible reasons. Firstly, K transport could be less severely affected by the reduction in transpiration flow compared with Ca, as discussed above. The second reason is a probable plant stress response which is not fully understood, but documented in literature as it occurs in shaded parts of plants of 'Shiraz' grape (*Vitis vinifera*), C_4 tropical grass (*Dichanthium aristatum* Pior.) and cotton (*Gossypium hirsutum* L.) (Smart et al., 1985; Rojas-Lara and Morrison, 1989; Cruz, 1997; Zhao and Oosterhuis, 1998). A simultaneous reduction of carbohydrates in all these studies led Mpelasoka et al. (2003) to hypothesise that the increase in K translocation is a mechanism to maintain osmotic potential and turgor in the shaded plant part and thereby decrease the impact of reduced growth due to a reduction in carbohydrates and dry matter accumulation in shaded fruit and leaves.

The phenomenon whereby one cation depresses another in plant tissue (Hoagland and Martin, 1933) has been shown to exist in bitter orange leaves (*C. aurantium* L.) between cations in the following order $K > Ca > Mg$ (Salardini and Khossussi, 1972), and offers an additional explanation for the high K and low Ca and Mg concentrations in the inside fruit flavedo. The single layer tonoplast is thought to be the site of this mechanism responsible for the cation antagonism, due to its low selectivity between cations (Barkla and Pantoja, 1996), which would result in the oversupplied cation being preferentially treated in transport from the cytoplasm to the vacuole. Potassium competes very effectively with Mg and Ca due to its high uptake ability into the cytoplasm, and during high K supply the levels of the other two cations are depressed. The inverse situation has also been shown to be true (Leggett and Gilbert, 1969).

The tonoplast antagonistic mechanism, favouring K uptake at the expense of Mg and Ca, could negatively influence loading and long-distance transport of these cations in the phloem and xylem (Diem and Godbold, 1993), and would suggest cation antagonism to be an active controlling process to maintain ionic balance in a plant. A number of studies on cation antagonism, focusing on the impact on plant physiology and growth, have shown that even though one cation can be replaced by another, in the short term, a negative physiological impact will eventually develop (Salardini and Khossussi, 1972; Jensen, 1982; Diem and Godbold, 1993). The significant and negative correlation between K and Ca, except in 2006 inside fruit, could be explained by this cation antagonism phenomenon to have occurred in the inside fruit flavedo, where K inhibited Ca uptake, whereas the reverse happened in outside fruit flavedo.

In apple fruit, the K/Ca and $(K + Mg)/Ca$ ratios follow an increasing curve, due to the higher supply via phloem at the expense of xylem, and therefore relatively lower Ca supply (Tromp and Wertheim, 2005). This same increase of K/Ca was reported by Storey and Treeby (2000) on 'navel' orange pulp, as well as a sharp decrease of the rind K/Ca during stage I, whereafter it stayed steady or increased only slightly during stages II and III. The ratios in 'Nules Clementine' mandarin flavedo do not correspond with the citrus fruit pulp or other fleshy fruit patterns. However, the same pattern was reported in the 'navel' orange rind, but occurred at different stages when the K/Ca-ratio decreased, indicating a possible difference in mineral nutrient requirements between 'navel' orange and 'Clementine' mandarin. Increases in K/Ca ratio have been used in grape literature to indicate increased phloem transport (Hrazdina et al., 1984; Rogiers et al., 2000). This argument also supports the hypothesis that the xylem is the main supply of mineral nutrients to the flavedo during stages II and III of 'Nules Clementine' mandarin growth.

The concern about high K and Mg and low Ca concentration in apple fruit stems from the antagonistic effect of K and Mg on Ca, and that fruit with high K/Ca and Mg/Ca ratios are more prone to bitter pit development

as well as *Gloeosporium* rot (Sharples, 1980). It is therefore suggested that it is not only desirable to have high Ca levels, but also low K and Mg [and therefore low (K + Mg/Ca) and K/Ca ratios] to avoid bitter pit and senescent breakdown (Wertheim, 2005). No such information is available on postharvest rind disorders of citrus fruit. However, the higher susceptibility of inside fruit flavedo to rind breakdown (Van Rensburg et al., 2004; Cronje 2007), which was shown to have higher K/Ca and (K + Mg/Ca) ratios, suggest that this hypothesis could also be valid in citrus flavedo.

Imbalances of mineral nutrients and specifically K, Ca and to a lesser extent Mg are contributing factors to postharvest physiological disorders in various fruits, e.g. apple (Ferguson and Watkins, 1989), kiwifruit (Ferguson et al., 2003) and avocado (Van Rooyen and Bower, 2005). Microclimatic variation within the kiwifruit vine is thought to contribute to the development of mineral imbalances and increased sensitivity to postharvest pitting of 'Hayward' kiwifruit (Thorp et al., 2003; Montanaro et al., 2006). It has been shown that, in addition to delaying senescence by stabilising cell walls and membranes Ca is also part of the intercellular signal transduction in cell metabolism (Poovaiah, 1988). Magnesium has also been shown to add an additional senescence delaying action to Ca application on apples (Lieberman and Wang, 1988). It is therefore not surprising that the flavedo of inside developing fruit, which are more susceptible to rind breakdown, had significant differences in K, Mg and Ca concentrations. These imbalances could contribute to impaired formation of cellular structures as well as metabolic activities that need adequate levels of Ca and Mg and could, therefore, lead to a sub-optimal rind condition. The higher Mg and Ca content in the flavedo of outside fruit could therefore play a role in preventing a process of premature senescence in the rind during postharvest storage.

In conclusion, it is hypothesised that the flavedo, as part of the 'Nules Clementine' mandarin fruit, accumulates K, Ca and Mg during fruit developmental stages II and III via the xylem. The flavedo showed a leaf-like mineral nutrient accumulation pattern which was responsive towards microclimatic variation within the canopy. The flower of inside fruit, which experienced reduced light levels, lower temperatures and higher humidity (hence, lower VPD), accumulated Ca and Mg at reduced concentrations compared with the flavedo of outside fruit probably due to the reduced transpiration rate experienced inside the citrus canopy. The high K accumulation in the inside fruit is thought to be a stress response to maintain osmotic potential of the flavedo and in which cation antagonism could play a role in suppressing especially Ca uptake into the flavedo. This imbalance of K, Ca and Mg is evident in the comparative K/Ca, (K + Mg)/Ca and K/Mg ratios, and could be an indicator of an increased susceptibility to postharvest rind breakdown of 'Nules Clementine' mandarin fruit.

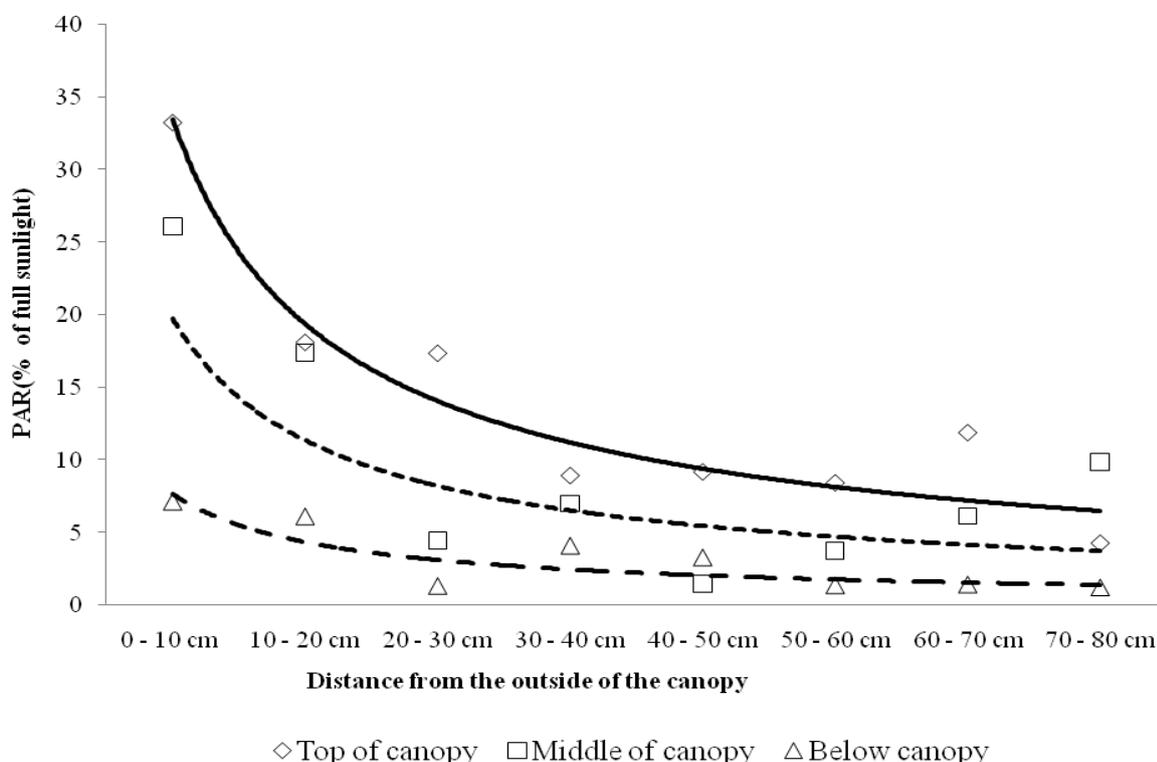


Figure 5.2.11.3.1. Light distribution in the canopy of a 'Nules Clementine' mandarin tree (measured from the

outside of the canopy) and expressed as % of full sunlight on a clear summer day in January (middle of summer). Measurements were taken below the canopy (—, Δ), in the middle of the canopy (± 1.5 m from ground) (—, \square) and in the top half (± 2.5 m from the ground) (—, \diamond).

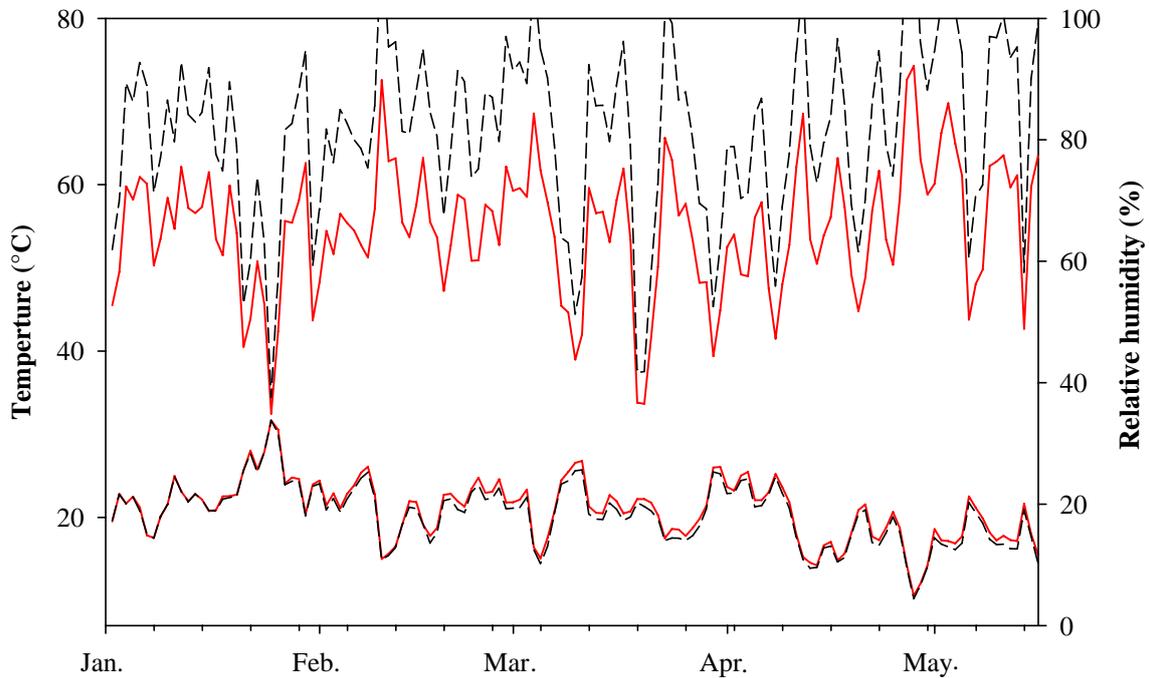


Figure 5.2.11.3.2. Effect of position (inside vs. outside) in the canopy of a 'Nules Clementine' mandarin tree, during stages II and III of fruit development on temperature ($^{\circ}\text{C}$) and relative humidity (%). The data presented are the average values for 24 hours. The solid (grey) lines denote the outside and broken lines (black) the inside position.

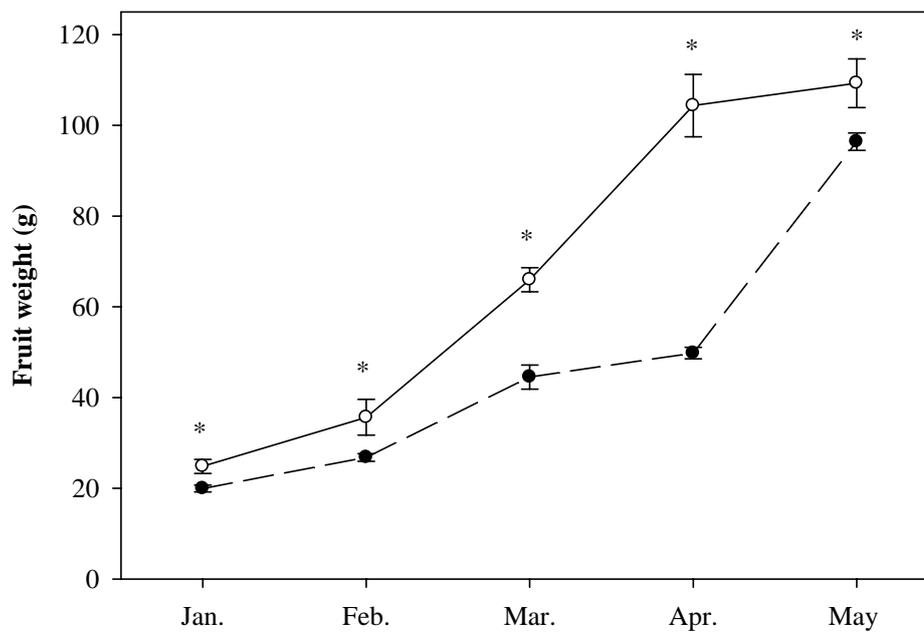


Figure 5.2.11.3.3. Increase in fruit weight of inside (\bullet) and outside (\circ) fruit during stages II and III of fruit growth of 'Nules Clementine' mandarin during 2005. Values are means ($n = 8$) with standard errors. A * above two data points indicate significant differences according to Fishers least significant difference test ($P \leq 0.05$).

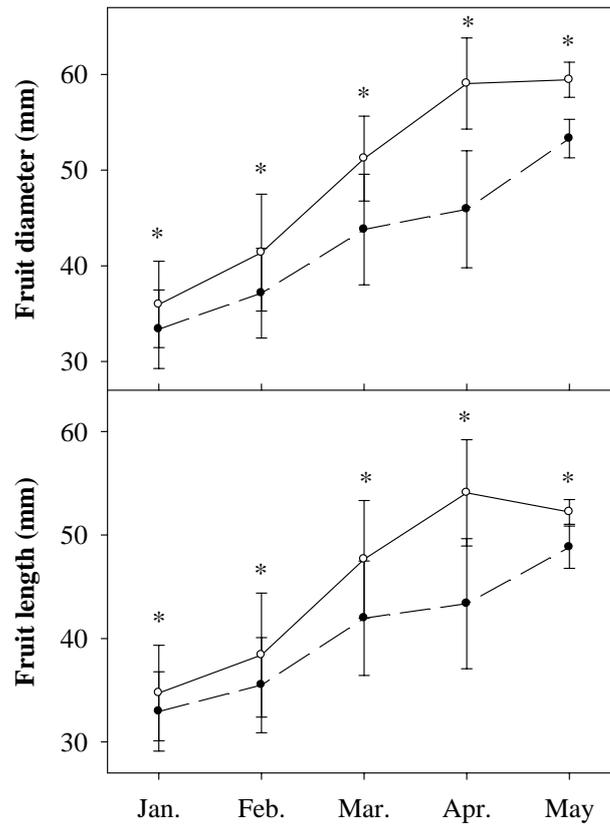


Figure 5.2.11.3.4. Average in fruit length and diameter of inside (●) and outside (○) fruit during stages II and III of fruit growth of 'Nules Clementine' mandarin during 2005. Values are means ($n = 8$) with standard errors. A * above two data points indicate significant differences according to Fishers least significant difference test ($P \leq 0.05$).

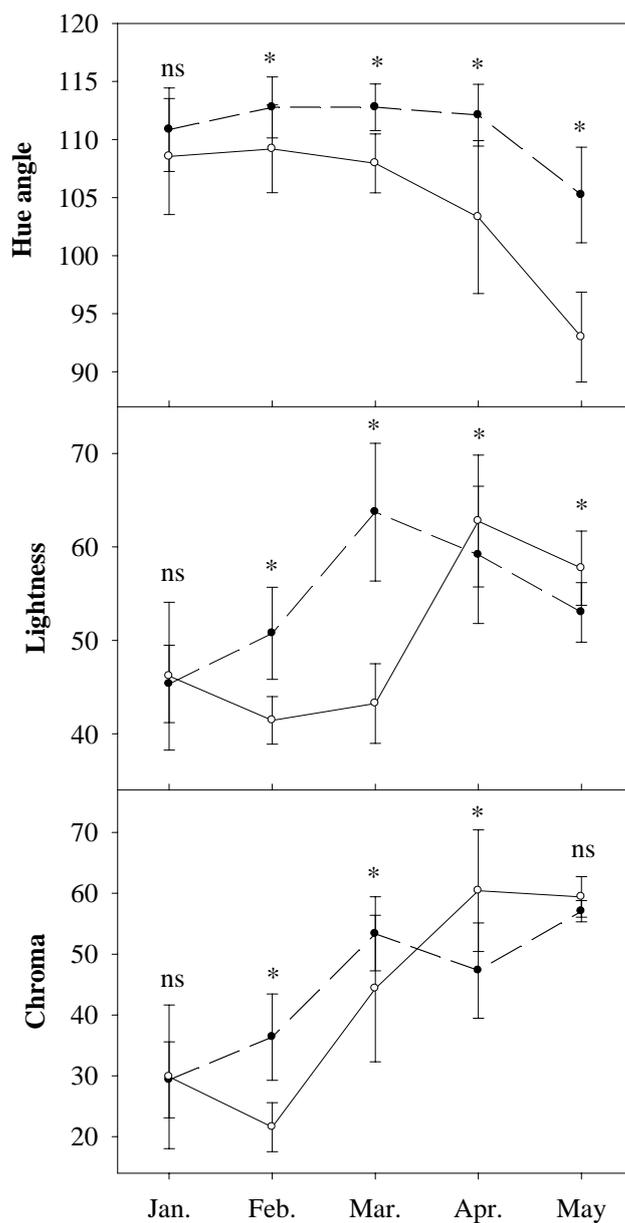


Figure 5.2.11.3.5. Changes in rind colour of inside (●) and outside (○) fruit flavedo during stages II and III of fruit growth of 'Nules Clementine' mandarin during 2005. Values are means ($n = 8$) with standard errors. High lightness values indicate a less dark colour, a high hue angle indicates a more yellow fruit whereas a lower value a more orange colour. Chroma represents the vividness of the colour and therefore a high value represents a more intense colour. A * above two data points indicate significant differences according to Fishers least significant difference test ($P \leq 0.05$).

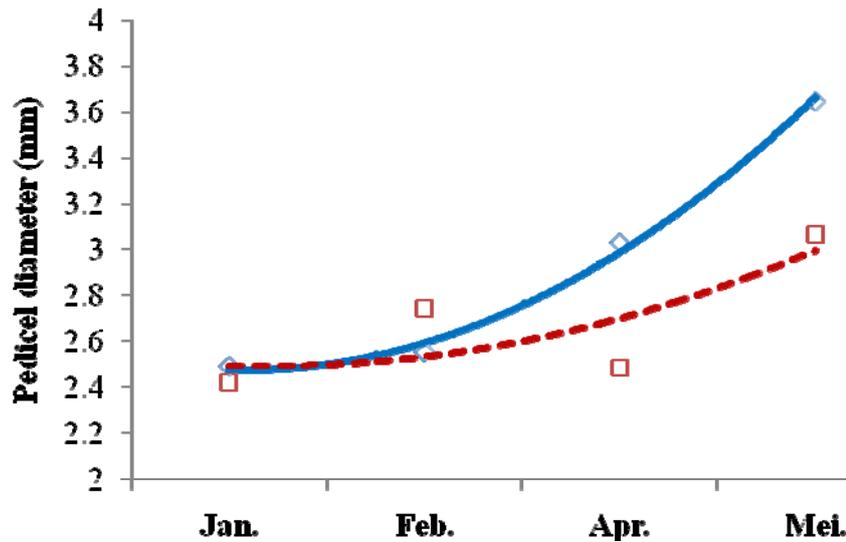


Figure 5.2.11.3.6. Pedicel diameter (mm) of inside (broken line and \square) and outside (solid line and \diamond) 'Nules Clementine' mandarin fruit during stages II and III of fruit development. Polynomial regression lines were fitted through the data points ($n = 8$).

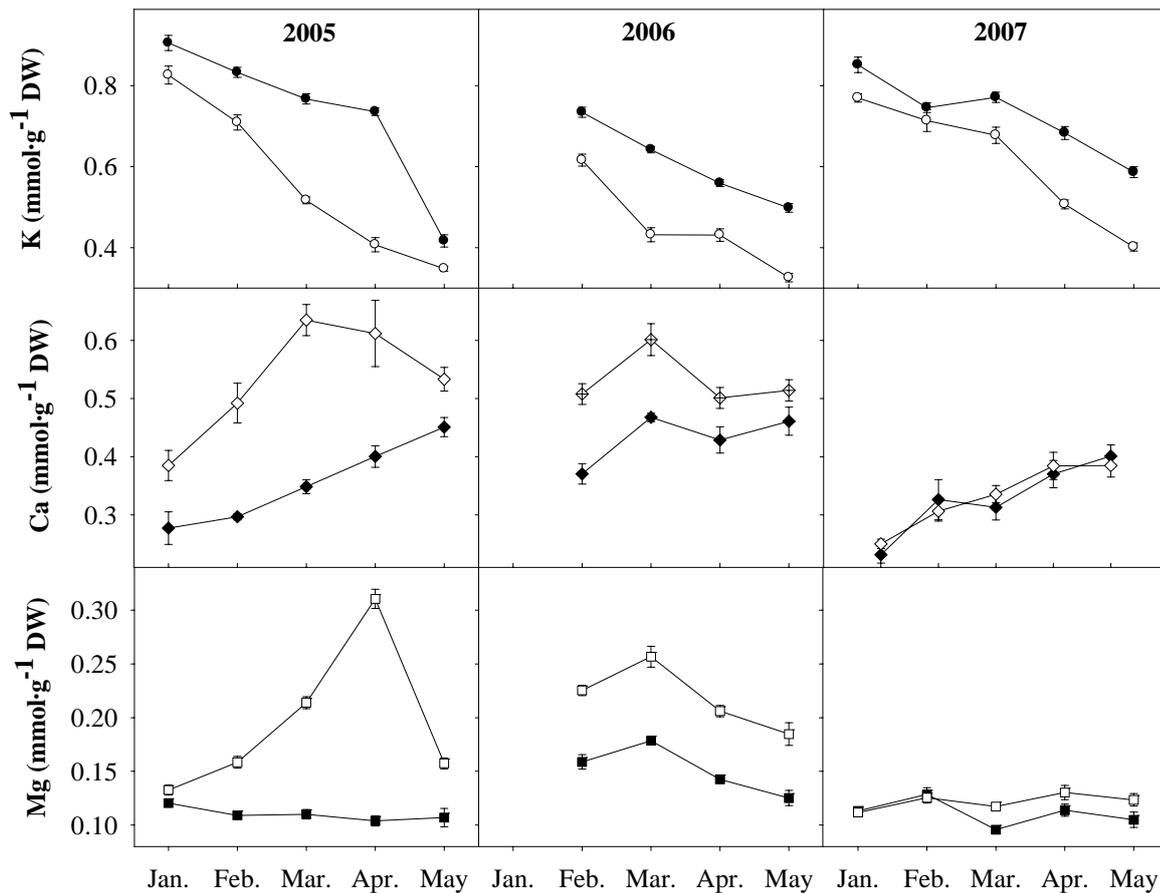


Figure 5.2.11.3.7. Accumulation of K (\circ outside; \bullet inside), Ca (\diamond outside; \blacklozenge inside) and Mg (\square outside, \blacksquare inside) in the flavedo of inside and outside fruit of 'Nules Clementine' mandarin during the period after physiological fruit drop (stages II and III). Vertical bars indicate LSD at $P = 0.05$ ($n = 8$).

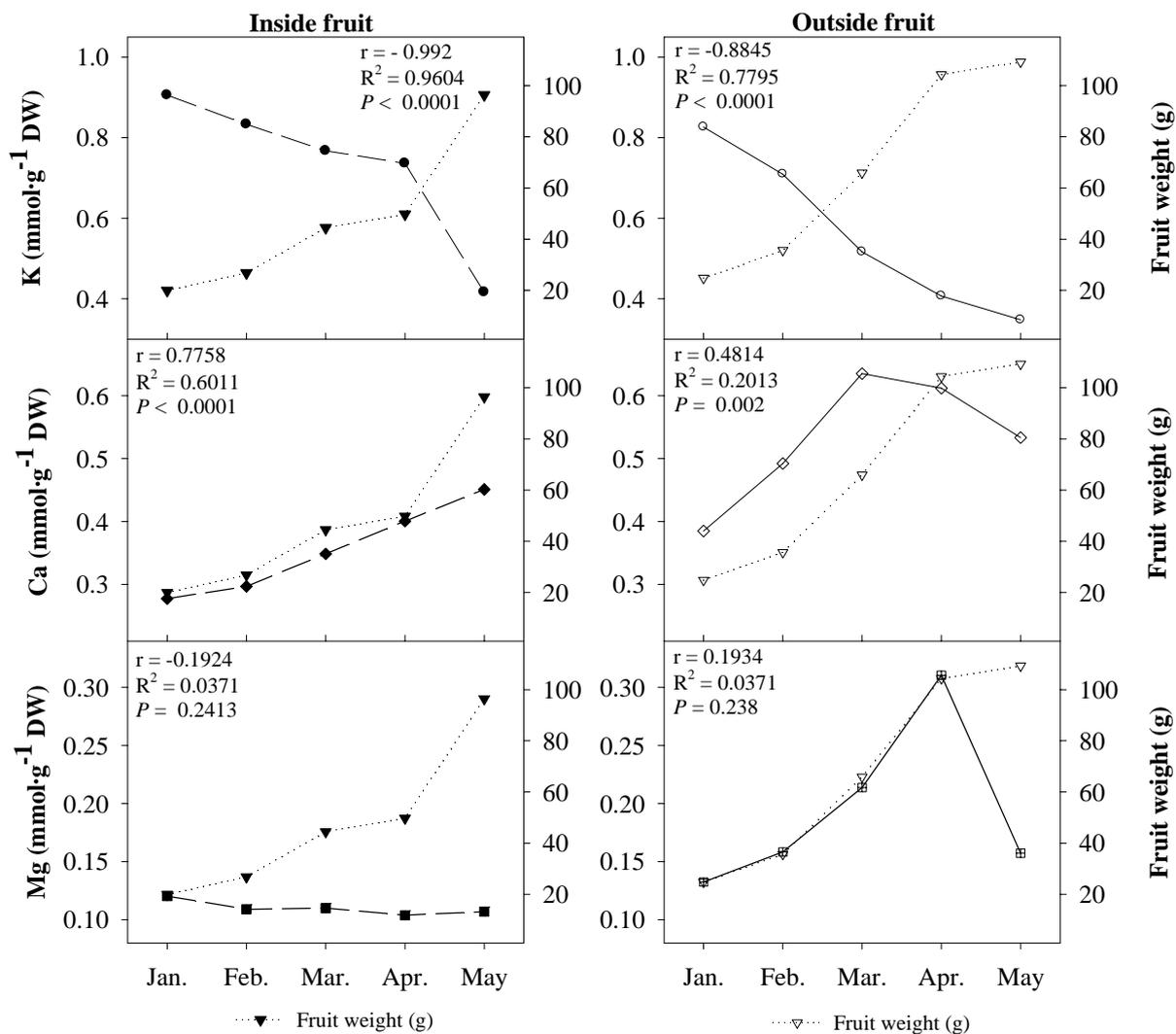


Figure 5.2.11.3.8. Accumulation during 2005 of K, Ca and Mg (mmol·g⁻¹ DW) correlated with increase in fruit weight (g) of inside and outside fruit flavedo sampled after physiological fruit drop (stages II and III). Open symbols represent the outside fruit mineral nutrient content and there fruit weight and closed symbols the inside fruit. The results from Pearson correlations between each mineral nutrient and fruit weight are represented in each relevant figure (n = 8).

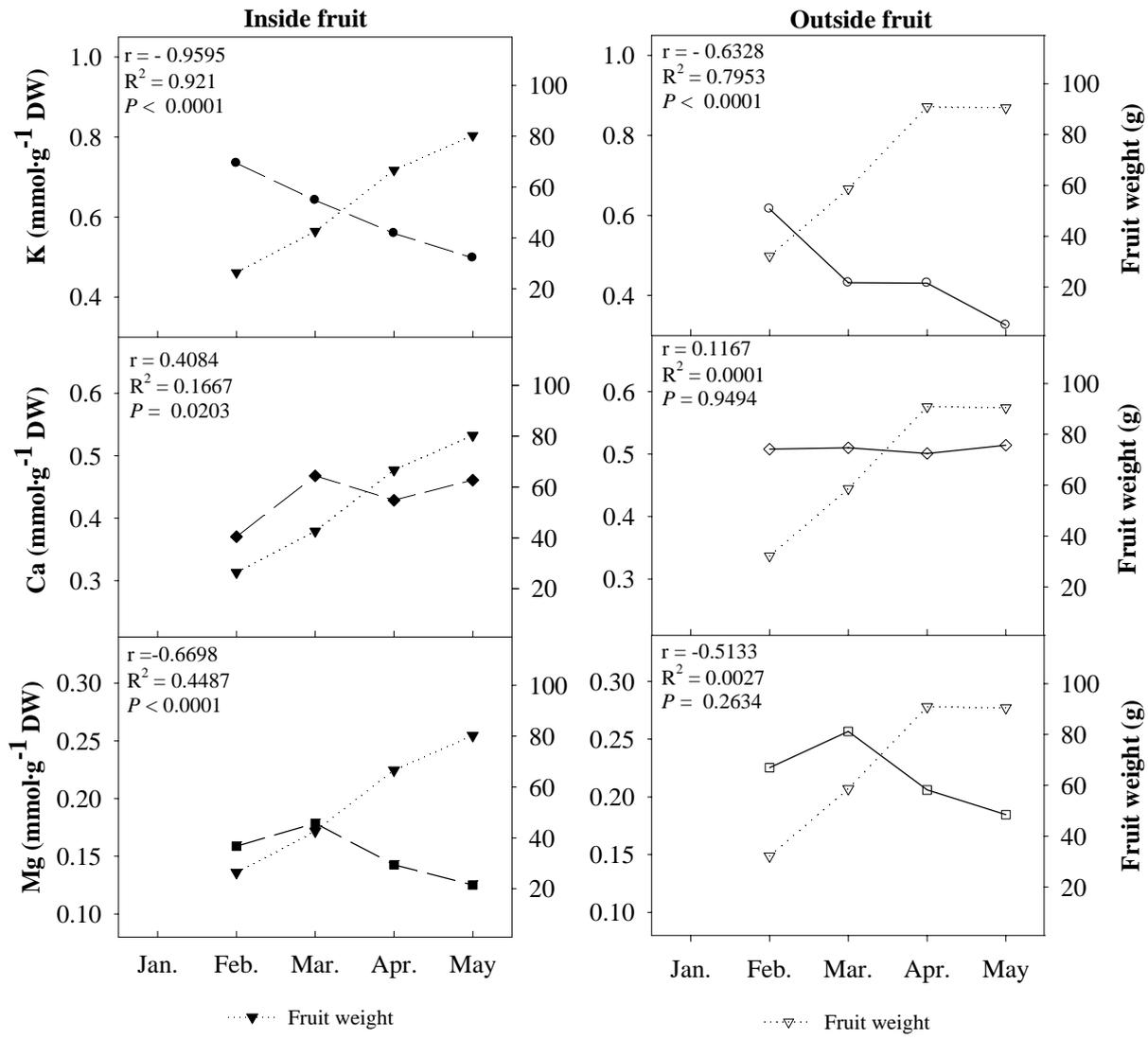


Figure 5.2.11.3.9. Accumulation during 2006 of K, Ca and Mg (mmol·g⁻¹ DW) correlated with increase in fruit weight (g) of inside and outside fruit flavedo sampled after physiological fruit drop (stages II and III). Open symbols represent the outside fruit mineral nutrient content and their fruit weight and closed symbols the inside fruit. The results from Pearson correlations between each mineral nutrient and fruit weight are represented in each relevant figure (n = 8).

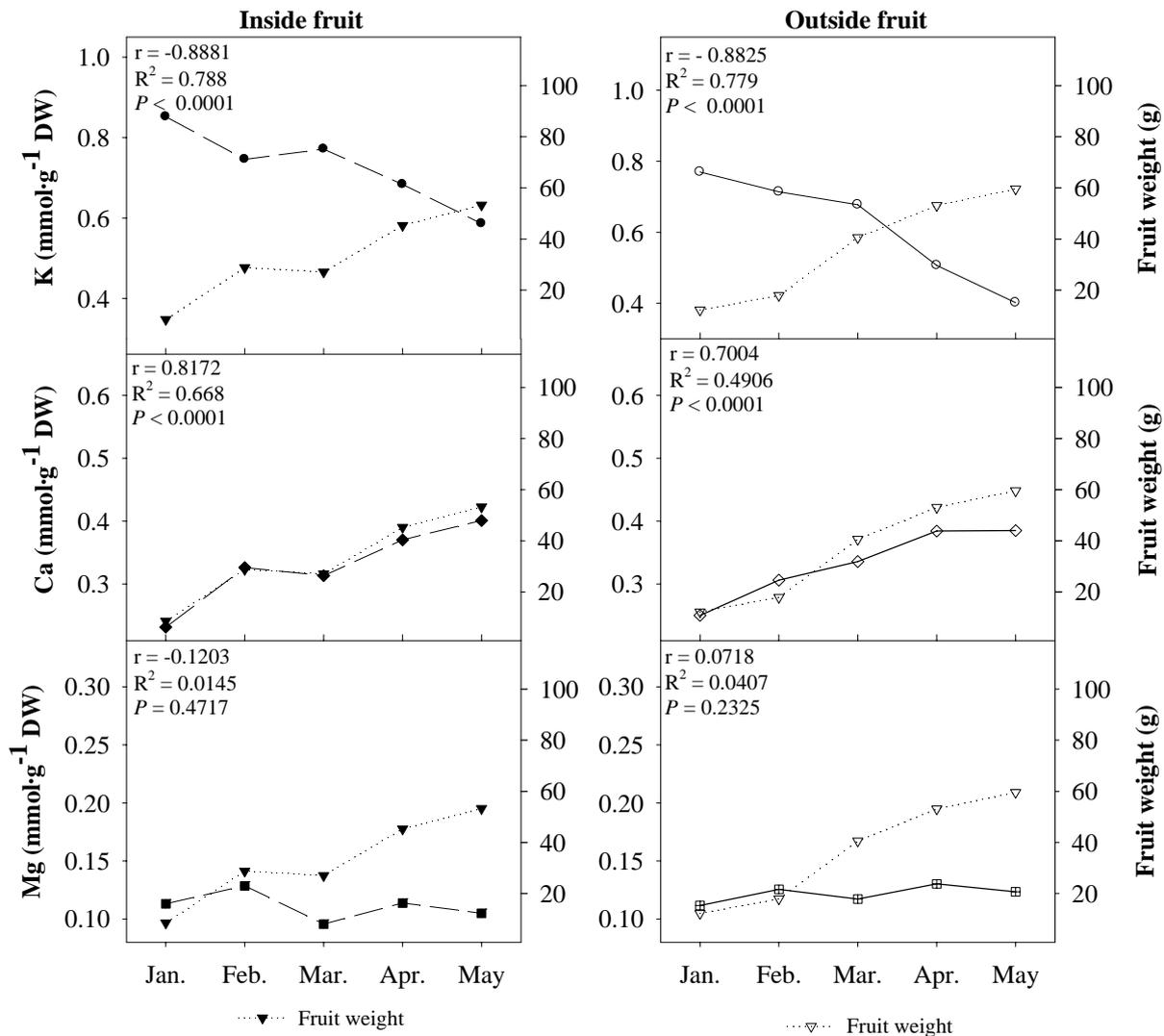


Figure 5.2.11.3.10. Accumulation during 2007 of K, Ca and Mg (mmol·g⁻¹ DW) correlated with increase in fruit weight (g) of inside and outside fruit flavedo sampled after physiological fruit drop (stages II and III). Open symbols represent the outside fruit mineral nutrient content and their fruit weight and closed symbols the inside fruit. The results from Pearson correlations between each mineral nutrient and fruit weight are represented in each relevant figure (n = 8).

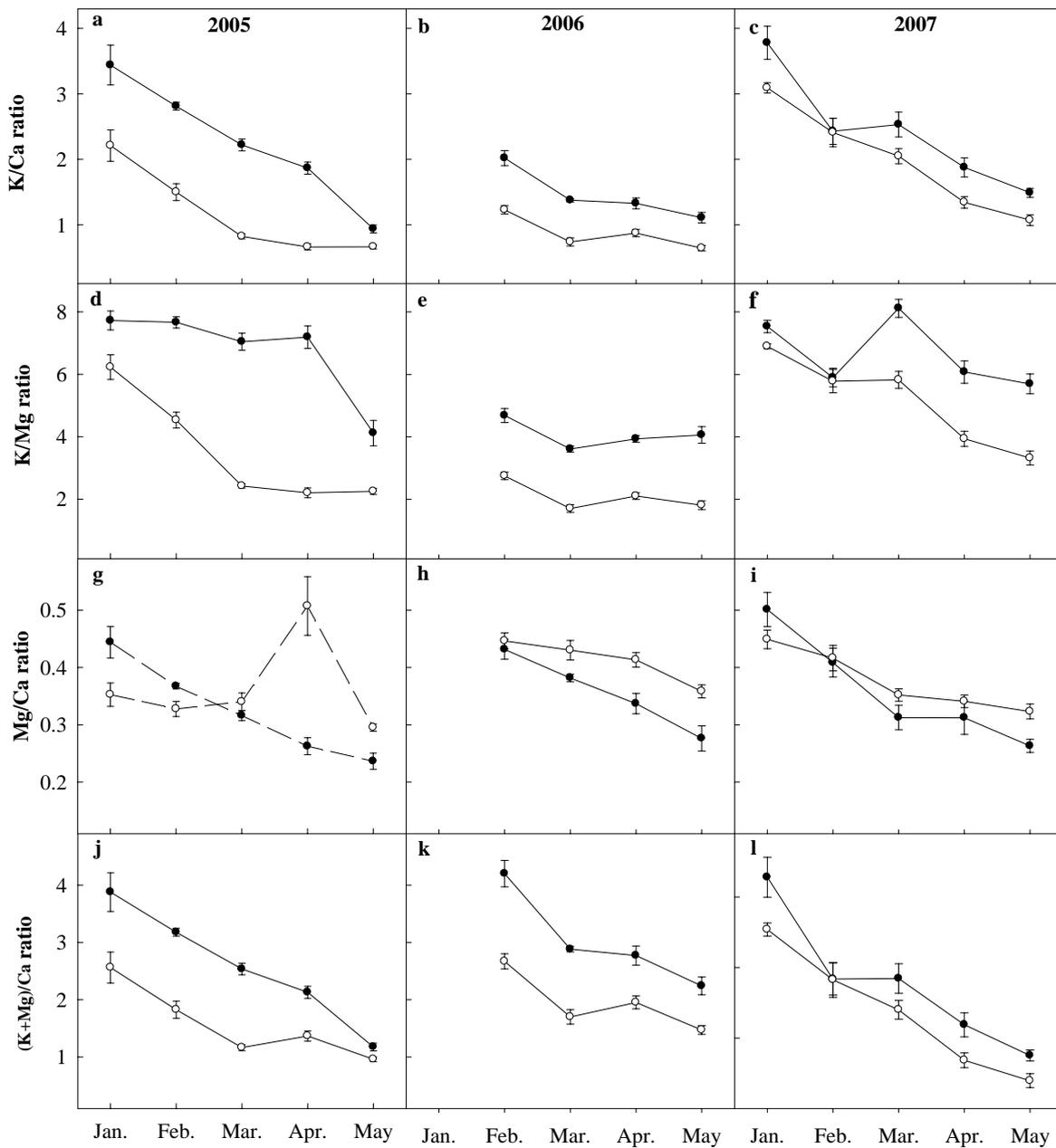


Figure 5.2.11.3.11a-l. Difference in ratios of K, Ca and Mg contents in the flavedo of 'Nules Clementine' mandarin in the period after physiological fruit drop (stages II and III), for the 2005 to 2007 seasons. The broken line and closed symbol (●) denote inside fruit flavedo and the solid line and open symbol (○) outside fruit flavedo. Vertical bars indicate LSD at $P \leq 0.05$ ($n = 8$).

Table 5.2.11.3.1. Results of the Pearson correlation (*r*) of mineral nutrient accumulation (K, Ca and Mg) in the flavedo of inside and outside fruit sampled after the physiological fruit drop (stage II and II of fruit development).

Correlation		2005		2006		2007	
		Inside	Outside	Inside	Outside	Inside	Outside
K vs. Ca	<i>r</i>	-0.471	-0.785	0.039	-0.534	-0.701	-0.703
	<i>P</i>	0.003	<.0001	0.831	0.002	<.0001	<.0001
	<i>R</i> ²	0.222	0.616	0.002	0.286	0.489	0.495
K vs. Mg	<i>r</i>	-0.429	0.128	0.212	0.553	-0.348	0.066
	<i>P</i>	0.006	0.439	0.245	0.001	0.035	0.693
	<i>R</i> ²	0.184	0.016	0.045	0.306	0.121	0.004
Mg vs. Ca	<i>r</i>	-0.132	0.027	0.074	0.093	0.593	0.221
	<i>P</i>	0.422	0.871	0.687	0.612	0.0001	0.182
	<i>R</i> ²	0.017	0.001	0.006	0.009	0.351	0.049

5.2.11.4 Canopy position affects reducing and non-reducing sugar accumulation in the flavedo of 'Nules Clementine' mandarin fruit

Opsomming

Fotosintese verskaf die benodigde suiker wat as premier bron dien vir alle metaboliese prosesse in plantselle en so ook vir respirasie gedurende vrugontwikkeling. Dit is dus belangrik dat 'n plant orgaan toegang het tot 'n bron van suikers om sterk selstrukture (selwande en membrane) te ontwikkel asook reserwes op te bou vir onderhouds respirasie gedurende opberging. In die geval van die sitrusvrug is die aktiewe fotosintetiserende flavedo, wat optree as 'n gemodifiseerde blaar, byvoegend tot die van blare in die koolhidraat poel. Die flavedo gesintetiseerde koolhidrate bly egter in die flavedo en albedo en word nie na die pulp vervoer nie. So ook word die koolhidrate gesintetiseer in die blaar eers na kleurbreuk na die skil getranslokeer waar dit voor die chlorofil afbraak slegs na die pulp beweeg. Die proef het gepoog om te bepaal of die lae PAR binne-in die 'Nules Clementine' mandaryn blaardak die koolhidraat vlakke in die flavedo kan beïnvloed. Die binnevrugte het oor 3 seisoen getoon dat hulle flavedo's laer vlakke van sukrose, fruktose en glukose in die flavedo het as die buitevrugte wat in die son ontwikkel. Daar is ook fotosintese meetings van binne en buitevrugte geneem, onder natuurlike lig om te bepaal of daar 'n verskil in die prosesse bestaan. Daar was gevolglik 'n hoër CO₂ fiksering en respirasietempo in die buitevrugte gemeet as die binnevrugte. Die swakker kleur van binne vrugte (minder groen voor-oes en oranje na oes) hou vermoedelik verband met die laer vlakke van koolhidraat sintese. Die laer koolhidrate vlakke asook sekondêre metaboliete soos karoteen in die binnevrugte het vermoedelik bygedra tot die swakker skilkondisie van die binnevrugte wat hulle dan meer sensitief maak vir die ontwikkeling van skilafbraak.

Summary

Since photosynthesis provides the required carbohydrates for fruit development, and respiration releases the stored energy from these carbon compounds during postharvest storage, it is therefore important that fruit structures have adequate carbohydrate content at the start of the postharvest period in order to ensure long storage life. In addition to photosynthate supply from leaves, the rind (flavedo and albedo) of citrus fruit has the ability to fix CO₂ via its own photosynthetic system in the chlorophyll-containing flavedo. In this experiment, from 2004 to 2007, the three main sugars (sucrose, glucose and fructose) were quantified in the flavedo of 'Nules Clementine' mandarin during stages II and III of fruit development. The flavedo was sampled from fruit borne on the inside (low light intensity) or outside (high light intensity) of the tree canopy. In 2007, the photosynthetic- and respiration rates of fruit borne in the two canopy positions were measured pre- and post-colour break. Sucrose content increased constantly from initial sampling in February until harvest (May), whereas glucose and fructose contents increased significantly only during the last month of fruit development. The flavedo of inside fruit that had developed under low light conditions, was less well coloured (higher Hue°) and had lower carbohydrate content than the flavedo which developed under high light conditions (outside fruit), and this could be attributed to the higher photosynthetic rate and greater sink strength of the outside fruit. Increased susceptibility of inside fruit to the progressive postharvest

physiological disorder, rind breakdown, is thought to be related to the lower carbohydrate content in the rind compared with those from the outside of the canopy.

Introduction

The carbohydrate content of the citrus rind flavedo is thought to affect rind condition. Sugar levels in the flavedo have been shown to not only be involved in the determination of chilling sensitivity (Purvis and Grierson, 1982; Purvis and Rice, 1983; Holland et al., 2002), but also in chloroplast-chromoplast conversion responsible for rind colour change (Huff, 1984). However, it Holland et al. (2005) interpreted that the flavedo carbohydrate content of 'navelate Navel' orange [*Citrus sinensis* (L.) Osbeck] was not involved in the non-chilling physiological disorder, rind staining.

The carbohydrate content in the rind (flavedo and albedo) and pulp increases steadily during fruit maturation (Tadeo et al., 1987; Koch and Avigne, 1990), but differs in specific carbohydrates. For example, the carbohydrate content of 'Satsuma' mandarin rind (*C. unshiu* Marc.) (30% sucrose, 32% fructose and 38% glucose) differs from the pulp (65% sucrose, 20% fructose and 14% glucose) (Komatsu et al., 1999; 2002).

Citrus fruit have a relatively constant supply of leaf-produced photosynthates, except when inhibited by low winter temperatures (Syvertsen and Lloyd, 1994). In contrast to the citrus fruit pulp, carbohydrate supply to the flavedo and albedo not only occurs via the phloem from leaves (Kock, 1984; Purvis and Yelenosky, 1983), but also from the fixation of CO₂ in the flavedo itself, via photosynthesis and refixation of fruit respiratory CO₂ (Bean and Todd, 1960; Huang et al., 1992). The aforementioned processes in the rind (acting as both a sink and a source) and pulp (acting as a sink) are evident from the activity of carbohydrate metabolising enzymes, viz. sucrose phosphate synthase (SPS; E.C. 2.3.1.14), sucrose synthase (SS; E.C. 2.4.1.13) and acid invertase (EC; 3.2.1.96), in these tissues. However, these enzymes remain active in the flavedo during fruit maturation, resulting in not only conversion of sucrose into reducing sugars (via the increase in SS and invertase activity), but also in sucrose synthesis (via SPS) occurring until harvest (Lowell et al., 1989; Holland et al., 1999; Komatsu et al., 1999).

Rind carbohydrate supply from leaf photosynthesis was calculated by Koch (1984) to be 35% from leaves, concurring with Purvis and Yelenosky (1983) who showed that defoliation and girdling reduced rind carbohydrate content. Holland et al. (1999) supported these data and stated that the maintenance of sucrose levels in the flavedo of 'Fortune' mandarin (*C. reticulata* Blanco) is related to sucrose import rather than synthesis by SPS (which decreases in activity towards maturity), since chlorophyll content is reduced after rind colour development. Sucrose phosphate synthase is known to be more active in tissue containing chlorophyll (Lingle and Dunlap, 1987).

Photosynthesis of orange and lemon (*C. lemon* (L.) Burm. f.) fruit, which takes place in the chlorophyll-containing flavedo, was initially considered of minor importance in fruit development and only compensated for respiration, even at maximum photosynthesis rates (Bean et al., 1960; 1963; Todd et al., 1961). Nevertheless, Moreschet and Green (1980), for the first time measured photosynthesis of fruit on the citrus tree, considered the flavedo as self-sustaining in carbohydrate synthesis. Further supporting data were presented by Vu et al. (1985), Yen and Koch (1990) and Huang et al. (1992) pertaining to extensive CO₂ fixation occurring from flowering until fruit maturation and even during the high respiration rates of early fruit development. Yen and Koch (1990) reported that grapefruit (*C. paradisi* Macf.) leaf photosynthates are supplied primarily to the pulp, whereas the rind photosynthates remain mostly in the flavedo and to a lesser extent in the albedo, with an insignificant amount translocated to the juice vesicles. Evidence therefore suggests that flavedo photosynthesis contributes substantially to the rind carbohydrate content, therefore enabling the rind to act neither as a classical carbohydrate source (for the pulp) nor a sink (from the leaves) during rind development (Yen and Koch, 1990; Huang et al., 1992; Goldschmidt and Koch, 1996).

Canopy microclimate is recognised for its importance in fruit production and positive relationships exist between light levels [(photosynthetically active radiation, (PAR; $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)], temperature and fruit carbohydrate accumulation (Reitz and Sites, 1948; Sites and Reitz, 1949; Barry et al., 2000; Morales et al., 2000). Comparable physiological responses to temperature and light levels were reported in citrus fruit photosynthesis in comparison with leaves by Moreschet and Green (1980). However, as opposed to Bean et al. (1960), Moreschet and Green (1980) did not find a light saturation point for fruit photosynthesis, unlike in the 'Valencia' orange leaves studied. Moreschet and Green (1980) concluded that the CO₂ uptake of the flavedo is not only dependent on light levels but also fruit size (relating to stomatal density), chlorophyll content, as well as the epidermal conductance, which has an important regulatory function. The CO₂ assimilation rate of 'Valencia' orange fruit was calculated as being 50-75% lower than that of leaves. This could be attributed to the 30-40% lower stomatal frequency and responsiveness in fruit flavedo (Blanke and

Lenz, 1989; Blanke, 1996). In addition, the plugging of stomata by the developing wax layer on the fruit surface (Turrell and Klotz, 1940) and the reduction of chlorophyll content towards maturity (Bean et al., 1960; Moreshet and Green, 1980) would also reduce the fruit photosynthetic rate.

Refixation of respiratory produced CO₂ can contribute significantly to the fruit carbohydrate balance, and Huang et al. (1992) calculated grapefruit flavedo to refix 30–100% of the respiratory produced CO₂ during fruit development. This refixation of CO₂ also avoids excessive water loss and the prevention of anoxic conditions due to avoidance of excessive internal CO₂ levels (Ashcan and Pfanz, 2003).

In view of the above, the first objective of this study was to determine the pattern of sucrose, fructose and glucose accumulation in the rind after physiological fruit drop during stages II and III of 'Nules Clementine' mandarin fruit development. The second objective of this study was to determine whether differences occur in flavedo carbohydrate accumulation of fruit positioned in full (outside the tree canopy) or less than 80% sunlight (inside the tree canopy). The final objective was to determine whether photosynthetic rate differed for fruit developing under these two light conditions, before and after colour break of the rind. Studies were conducted using 'Nules Clementine' mandarin fruit which is known to develop a progressive postharvest rind disorder related to the collapse of the oil gland in the flavedo 3-5 weeks after harvest, resulting in a "leopard spot" pattern. This disorder has been proposed to have a higher prevalence in fruit flavedo which developed under low light conditions (Van Rensburg et al., 1995; Van Rensburg, 2004; Khumalo, 2006). It is hypothesised that the flavedo of fruit borne inside the canopy (low PAR exposure) have a reduced carbohydrate content in comparison with flavedo from outside borne fruit (high PAR exposure) and that a lower photosynthetic rate of inside fruit is at least partly responsible for this difference in carbohydrate content. In addition, this lower carbohydrate content of inside fruit flavedo is thought to be an underlying cause of rind breakdown development.

Materials and methods

Sites and plant material

This experiment was conducted in two orchards of 'Nules Clementine' mandarin budded on Carrizo citrange [*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (Osb.) L.] rootstock. In the 2004, 2005 and 2007 seasons, fruit were sampled from the orchard at the University of Stellenbosch experimental farm, Western Cape province, South Africa, whereas in 2006, a commercial orchard in the Paarl area was used. This was necessary due to severe alternate bearing in the experimental farm orchard. Both these orchards were planted with a North – South row orientation. The Stellenbosch orchard was planted in 1991 at a spacing of 4.5 x 2.5 m, and the Paarl orchard in 1993 at a spacing of 5 x 3 m.

Pre-harvest fruit sampling

The experimental design was a randomized complete block design with eight single tree replicates. Fruit were sampled at monthly intervals coinciding with stages II and III of fruit development (Jan until May) and commenced after physiological fruit drop (Bain, 1958). From each of these eight replicate trees, 25 fruit were collected from two, distinct canopy positions, viz. outside (90-100% of full sunlight) and inside (< 80% of full sunlight).

To quantify canopy microclimate, light profile measurements were recorded during 2005 from the top, middle and lower sections of each leaf canopy for eight uniform trees. The measurements were taken between 10:00 and 12:00 AM on a clear, cloudless day on 21 January 2005 using a light meter (Li-250 with a Li-190SA quantum meter, Li-COR, Lincoln, NEB., USA), which took integrated point measurements over 15 seconds. The 80-cm long probe, consisting of 80 individual light sensors, was divided into eight 10-cm zones from which the average values were plotted. Two TempTale4/Humidity data loggers (Sensitech Inc., Beverly, MA., USA), measuring temperature and humidity were placed in one tree in both the outside and inside canopy positions to quantify these climatic variables within the canopy.

Rind colour of each sampled fruit was measured using a chromameter (Minolta NR 4000, Osaka, Japan). Fruit dimensions (length and diameter) and weight were determined prior to removing the flavedo. The flavedo of the 25 fruit per replicate was pooled to ensure that there was enough material for analysis. The flavedo was frozen in liquid nitrogen whereafter it was freeze dried (VirTis Freezemobile 25ES, The VirTis Company, Gardiner, NY, USA) and milled to a fine powder for storage at -80°C.

At the commercial harvest date in May 2004 and 2007, fruit were picked according to these two light classes. In both seasons the fruit were transported to a commercial packhouse where they were drenched, (thiabendazole 1000 mg·L⁻¹; guazatine 500 mg·L⁻¹; 2,4-D sodium salt 250 mg·L⁻¹; Sporekil[®] 1000 mg·L⁻¹) and degreened (3 days at 3 µL·L⁻¹ ethylene, >90 % RH and 20 to 22 °C) before receiving all standard commercial

packhouse treatments, [thiabendazole, 500 mg·L⁻¹; imazalil, 500 mg·L⁻¹; 2,4-dichlorophenoxyacetic acid, 125 mg·L⁻¹, and polyethylene citrus wax application (Citrushine[®], Johannesburg, South Africa)]. Afterwards, the fruit were separated into replicates of 25 fruit. In 2004 and 2007 the fruit from the inside and outside of the canopy were stored at either 7°C or -0.5°C for 14 weeks, during which time the fruit were scored for RBD incidence every second week.

Rind pigment analysis

To determine the chlorophyll and carotenoid contents a 0.2 g sub-sample of the freeze-dried and finely milled, powdered flavedo was added to 10 ml of 95% (v/v) aqueous ethanol solvent containing butylated hydroxytoluene (BHT) (100 mg·L⁻¹) and diethyldithiocarbamate (DC) (200 mg·L⁻¹) antioxidants to prevent carotenoid degradation. The samples were vortexed for two 1-minute intervals and stored at 4°C for 1.5 hours to extract the pigments. Thereafter, the solution was poured through ashless filter paper (Schleicher & Schuell, Dassel, Germany) to remove rind particles. The extracts were poured into disposable plastic cuvettes, placed in a spectrophotometer (Cary 50 conc UV-visible spectrophotometer, Varian Australia (Pty.) Ltd, Mulgrave, Victoria, Australia) and readings were taken at 470, 646 and 664 nm. A cuvette filled with the ethanol/antioxidant solvent was used as a standard to calibrate the spectrophotometer. Absorbance values were calculated to determine chlorophyll a (C_a), chlorophyll b (C_b), total chlorophylls (C_{a+b}) and total carotenoids (C_{x+c}) concentrations. All of these were calculated as mg·g⁻¹ DW, using the Lichtenthaler equations (Lichtenthaler, 1987).

$$C_a = 13.36 A_{664.2} - 5.19 A_{648.6}$$

$$C_b = 27.43 A_{648.6} - 8.12 A_{644.2}$$

$$C_{a+b} = 5.24 A_{664.2} + 22.24 A_{648.6}$$

$$C_{x+c} = (1000 A_{470} - 2.13 C_a - 97.64 C_b) / 209$$

Fruit photosynthesis

The determination of fruit photosynthesis was conducted during 2007. The experiment was laid out as a complete, randomised block design with eight single tree replications. Measurements of net CO₂ assimilation rate (A) of attached fruit were made before and after colour break, on 18 March and 17 April, respectively, using one fully sun exposed (outside) and shaded (inside) fruit per tree. The same trees were used during both measurement dates, however, the fruit used were removed at each measurement time to determine fresh weight for calculation of CO₂ exchange. The CO₂ assimilation rate of attached fruit was determined with a Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NEB., USA) using a conifer chamber attachment (Li-6400-05, Li-Cor, Lincoln, NEB., USA). The system is based on the equations derived by Von Caemmerer and Farquhar (1981) to calculate net CO₂ assimilation rate (A), transpiration rate (E) and stomatal conductance (g_s). Since the conifer chamber does not have its own light source, incident light levels (ambient sunlight) were used and PPFD (photosynthetic photon flux density) was measured using a quantum sensor (Li-190SA, Li-Cor, Lincoln, NEB., USA) attached to the conifer chamber. A black photographic cloth was used to block out all light in the chamber to determine dark respiration rate (R_d) of the fruit. Cuvette CO₂ concentration was controlled at 380 μmol·mol⁻¹ using the CO₂ injection system (LI-6400-01, Li-Cor, Lincoln, NEB, USA) and compressed CO₂ cylinders. The light-saturated net CO₂ assimilation rate (A_{max}), dark respiration rate (R_d) and light-saturated photosynthetic rate (P_{max}, the difference between A_{max} and R_d) are reported.

Determination of flavedo carbohydrate content

For each treatment date, eight replications were extracted and analysed for all treatments and seasons. However, only one extraction per replication was performed. Sugars were extracted and purified from the flavedo by a modification of the method used by Koch and Avigne (1990). This method, using a solution of methanol, chloroform and water (MCW), was necessary to eliminate the vast number of pigments and lipids in the flavedo. An additional step of filtering the extract through a C18 column (preparative C18 125Å, 55 – 105 μm WAT 0250594, Waters corporation, Milford, MA, USA) was deemed necessary, to remove phenolic compounds extracted from the flavedo and to extend the life of the HPLC column.

Extraction of sugars

Sucrose, glucose and fructose were extracted from 0.1 g flavedo with a 5 ml solution of 60% methanol, 25% chloroform and 15% deionised water (MCW). Water was deionised through a Millipore water filtration system (RiOs/Elix SynergyPak™ system, Millipore SAS, Molsheim, France). The sample and MCW solution were both vortexed for 2 minutes and left for 16 hours at ambient temperatures (18 ± 2°C). The extraction mixture was centrifuged (3 000g_n, 10 min, 20±1°C) and the supernatant was collected. One ml MCW was added to the residue, vortexed and thereafter centrifuged (3 000 g_n, 10 min, 20 ± 1°C). The clear supernatant was again collected and added to the initial supernatant. To the pooled MCW-extract, 1 ml of chloroform was first added, followed by 1 ml deionised water. The tube was shaken after each addition and finally centrifuged (3 000 g_n, 10 min, 20 ± 1°C) to separate the layers. The top, aqueous layer, containing the

sugars and phenolics, was collected and evaporated to dryness under a rotary vacuum centrifuge (SC 210 A Speed Vac® Plus, Thermo Savant, Holbrook, NY, USA).

The dried residue was dissolved in 5 ml of deionised water. The C18 cartridge (2 g) was conditioned, first with methanol, then with four portions of 5 ml deionised water under vacuum (VacMaster Sample Processing Station, International Sorbent Technology Ltd, Glamorgan, UK). In a preliminary investigation 2 g C18 was found to be adequate to remove the phenolic compounds from the aqueous sugar extract. One ml of the sugar solution was then purified by C18 cartridge under vacuum into a 10-ml volumetric flask. Each cartridge was furthermore washed with another four aliquots of 2 ml deionised water. The eluate was made up to a final volume of 10 ml, by adding deionised water. The eluate was finally filtered through a 0.45µm filter paper (Millex-HV Hydrophilic PVDF, Millipore Corporation, Billerica, MA, USA) into a vial for HPLC analysis.

HPLC carbohydrate analysis

Carbohydrate analysis was performed using high performance liquid chromatography (Agilent 1100 Series HPLC, Agilent, Waldbronn, Germany) with an auto sampler (110 Serie; Hewlett Packard, Waldbronn, Germany) operated by HP ChemStation software (LC Rev.A.06.03 [509], Hewlett Packard, Waldbronn, Germany). A Transgenomic™ ion exchange (stainless steel) column for analysis of sugars and organic acids (3000 x 7.8 mm) (model ICsep ICE-99-9850); Transgenomic, Omaha, NE, USA) was used with a Transgenomic™ guard column (model ICsep-ICE-GC-801, Transgenomic Inc., San Jose, CA, USA) and was maintained at 30°C. Sugars were separated using 17 mM H₂SO₄ at a flow rate of 0.5 ml·min⁻¹. A refractive index detector (model G1352A, Agilent, Waldbronn, Germany) was used to detect the separated sugars. An injection volume of 30 µl was used per sample. The sugar content of the freeze dried flavedo was expressed as mg·g⁻¹ DW.

Statistical analysis

Differences in rind colour, fruit size and carbohydrate content (sucrose, fructose and glucose) were analysed using PROC GLM (SAS v. 6.12, SAS Institute, Cary, NC, USA). The significance of treatments was determined from the analysis of variance (ANOVA) and means were separated by least significant difference (LSD) using the Statistica Program (Statistica 7, Statsoft, Tulsa, OK, USA, 2005). Fruit photosynthesis data were analysed using a one way ANOVA (SAS v. 6.12, SAS Institute, NC, USA) to test the significance ($P \leq 0.5$) of canopy position. A multiple comparison test (Fisher, $P \leq 0.05$) was also performed to separate means.

Results

Variation in canopy microclimate within the tree canopy

The available light in the 'Nules Clementine' mandarin tree canopy decreased within the first 10 cm of the canopy, illustrating that the dense leaf canopy of citrus trees only allows low levels of PAR to penetrate into the inner canopy (Fig. 5.2.11.4.1), as reported by Greene and Gerber (1967). Temperature and humidity measured over the sampling period revealed that the outside position was marginally warmer and significantly less humid than the inside position (Fig. 5.2.11.4.2). It is therefore clear that differences exist between the prevailing microclimate of the inside and outside of the canopy, influencing key plant physiological processes such as photosynthesis and transpiration (Jones, 1983).

Fruit development

Tree canopy position influenced fruit growth, rind colour development and pigment content in all three seasons, however, only the 2005 data are presented to avoid repetition (Figs. 5.2.11.4.3 to 5.2.11.4.6). Inside fruit were consistently smaller (diameter and length) and lighter compared to outside fruit (Figs. 5.2.11.4.3 and 5.2.11.4.4). The positional effect also translated into a difference in rind colour development of the flavedo. During the green stage (Jan to Mar/Apr) the inside fruit were lighter, more intensely coloured (higher chroma values) and greener (higher hue angle) compared to the outside fruit (Fig. 5.2.11.4.5). After colour break (between Mar and Apr) the outside fruit developed a more intense orange (lower hue angle) compared with the more yellow coloured inside fruit at harvest in May. These colorimeter values are mirrored by the changes in chlorophyll and carotenoid pigment contents in the inside and outside fruit flavedo during fruit development (Fig. 5.2.11.4.6). The outside fruit flavedo not only had higher levels of chlorophyll pigments (Jan to Mar), but also of carotenoids (Jan to May), compared with the inside fruit flavedo. The reduction in chlorophyll between Mar. and Apr. resulted in an unmasking effect and is the main reason for the change in hue angle. However, the dramatic colour change between Apr. and May, as expressed by hue angle, would largely be the result of the sudden increase in carotenoid pigments.

Fruit CO₂ fixation

The on-tree fruit photosynthetic measurements recorded during two clear days, pre- and post-colour break (Mar and Apr), showed significant differences ($P > 0.05$) between the CO₂ exchange rates of the fruit borne on the inside and outside (A_{\max} , R_d and P_{\max} in Fig. 4.7). Light-saturated net CO₂ assimilation rate (A_{\max}) of fruit borne on the outer canopy was significantly higher than the inner canopy, whereas the dark respiration rate (R_d) of fruit in that position was significantly lower in both Mar. and Apr. The light saturated photosynthetic rate (P_{\max} , from the difference between A_{\max} and R_d), also differed significantly between canopy positions, with outside fruit having a much higher CO₂ fixation rate. The light-saturated net CO₂ assimilation rate (A_{\max}) and dark respiration rate (R_d) values became less negative, whereas the light saturated photosynthetic rate (A_{\max}) decreased from Mar. to Apr., coinciding with the rind colour break in the flavedo (Fig. 5.2.11.4.7).

Flavedo carbohydrate accumulation

Carbohydrate content (sucrose, glucose and fructose) increased in the flavedo during stages II and III of fruit development in all four seasons (Fig. 5.2.11.4.8). Sucrose content was higher at the start of the sampling period than that of glucose and fructose, with the glucose and fructose contents noticeably increasing during the last month of fruit development. Sucrose content (on average) reached $\pm 4 \text{ mg}\cdot\text{g}^{-1}$ between Feb and Mar, whereas the two reducing sugars only attained that level between Apr. and May (close to harvest), coinciding with a marked colour change of the flavedo (Figs. 5.2.11.4.5 and 5.2.11.4.6).

Fruit position, and therefore exposure to high (outside) or low (inside) light levels in the canopy, affected the flavedo content of all three sugars during fruit development. In the seasons 2004 to 2006, the flavedo from fruit borne on the outside of the canopy, had significantly higher sucrose content than the fruit borne inside the canopy. The same trend was observed in 2007, albeit not statistically significant. The difference in glucose and fructose content between flavedo from inside and outside fruit was not as evident as that of sucrose. However, during 2004 and 2005 the flavedo from the outside fruit had significantly higher glucose and fructose contents than the flavedo from inside and outside. This trend was not evident in 2006, where a confusing interchange occurred between highest and lowest glucose and fructose values from the two canopy positions. The glucose and fructose contents during 2007 did not differ significantly, although the mean values were generally higher in the flavedo from the outside.

The ratio of reducing to non-reducing sugars, calculated as $[(\text{Fru} + \text{Glu})/\text{Suc}]$ was generally higher for the flavedo from outside fruit compared to the inside flavedo. However, the 2006 fluctuations of the glucose and fructose values between flavedo from the inside and outside resulted in the same indiscernible pattern as for the separate sugars. No explanation could be found for the interchange between the inside and outside glucose and fructose values during 2006.

Rind breakdown incidence during cold storage

The incidence of rind breakdown increased during both seasons of data collection (2004 and 2007) along a progressive pattern (Fig. 5.2.11.4.9 A and B). Canopy position during fruit development (inner and outer canopy) influenced the rind condition and resulted in higher incidence of rind breakdown of the inside fruit compared with the outside fruit in both seasons and storage temperatures. However, the fruit stored at 7°C (non-chilling temperature) had a higher incidence of the disorder in both seasons compared with fruit stored at -0.5°C (chilling temperature).

Discussion

This study shows that the flavedo continues to accumulate carbohydrates (reducing and non-reducing) during both stages II and III of fruit development. This finding agrees with previous reports in the literature of not only increased carbohydrate content, but also increased carbohydrate metabolism until fruit maturity (Holland et al., 1999; Komatsu et al., 1999; 2002). It is known that accumulated carbohydrates perform the function of stored energy reserves and are part of the structural framework of the cells during development (Dennis and Blakely, 2000; Kays and Paull, 2004). In addition, the balance between the sucrose metabolising pathways is hypothesised to be an essential factor in plant development systems as it influences carbon allocation, and hexose-based sugar signals in sink organs and these processes are known to be highly responsive to internal and external environmental conditions (Koch, 2004).

During fruit development the sucrose content in the flavedo would increase due to three processes, viz. photosynthesis in the flavedo, sucrose influx from the leaves and the cycling transformation of sucrose and hexose sugars in the cell (Huber, 1989). During photosynthesis, sucrose will be synthesised as the primary product in the cytosol by SPS, before being stored in the vacuole. Increases in glucose and fructose in the flavedo would be due to the breakdown of sucrose, either via SS or invertase. However, these reducing

sugars can be recycled into sucrose. Sucrose is not only the primary product of photosynthesis, but also the main transport form of carbohydrate in the phloem of plants and could be phloem transported from the leaves to the fruit rind due to its non-reducing characteristic (Goodwin and Mercer, 1983; Lunn and Furbank, 1999).

The accumulation of carbohydrates in the flavedo of the rind of 'Nules Clementine' mandarin fruit was influenced by fruit position in the tree's canopy. Fruit from the outside of the canopy (receiving higher PAR and temperature), had a higher pigment (chlorophyll pre-harvest and carotenoid postharvest), carbohydrate content (sucrose, glucose and fructose) in the flavedo compared with the inside fruit. The inside fruit flavedo, receiving low PAR levels coupled with the lower chlorophyll content in the flavedo, could account for the lower carbohydrate content. The colour change of the flavedo from green to yellow is a very important quality aspect of citrus fruit rind, and adequate sugar accumulation in the flavedo is necessary to supply energy required for the chloroplast-to-chromoplast conversion (Huff, 1984). In addition to the higher market value of well coloured citrus fruit, carotenoids play an essential role in scavenging reactive oxygen species, and at relatively low concentrations are effective in protecting membrane lipids from oxidation (Larson, 1988; Allan and Fluhr, 2007). The higher hue angle value (yellower) of the inside fruit flavedo could therefore be construed as a visual symptom, indicative of the suboptimal PAR and carotenoid synthesis and the generally lower carbohydrate content, indicative of a reduced rind condition and a contributing factor to the higher incidence of rind breakdown during 2004 and 2007.

In contrast, Purvis (1980) found no difference in total or non-reducing sugar content of grapefruit rind between the interior and exterior position in the canopy. A possible explanation could be the different bearing habits of grapefruit and mandarins. Mandarin fruit in a well managed tree are more exposed than grapefruit, which hang primarily within the leafy canopy out of direct sunlight, and would therefore have higher carbohydrate content in the flavedo. Carbohydrate content of the flavedo and albedo could play a major role in determination of rind condition. Insufficient carbohydrate content does not only result in lower internal fruit quality but is also suspected to be involved in increased susceptibility to chilling injury of the rind (Purvis 1980; Purvis and Grierson, 1982; Purvis and Rice, 1983; Holland et al., 2002). However, levels of carbohydrates alone were argued to not be solely responsible for the canopy positional effect on fruit susceptibility to chilling injury (Purvis, 1989).

The ratio of reducing to non-reducing sugars [(Fru + Gluc)/Suc] can be seen as an indication of carbohydrate metabolism in a sink organ and the cycling of hexose sugars. An increasing ratio would indicate the cleaving of sucrose into glucose and fructose, whereas increased sucrose content will lead to a reduction of the ratio. In this study, it was found that the ratio only dramatically increased after the glucose and fructose contents started to increase between Apr and May. Sucrose cleaving occurs either via hydrolysis by invertase in the cell walls and vacuoles or by SS in the cytosol. Both these routes produce hexose sugars (glucose-6-phosphate, fructose-6-phosphate and glucose-1-phosphate) which equilibrate before fructose-6-phosphate enters glycolysis for energy release (Hill, 2007). However, the sucrose content did not decline and showed a steady increase until harvest. It is, therefore, possible that the activity of sucrose cleaving enzymes (SS and invertase) had to increase towards maturity and sucrose would be imported as discussed above.

The results from the on-tree fruit photosynthetic measurements concur with previous studies which led Moreshet and Green (1980) to speculate that "the CO₂ uptake by fruit could support the growth of the flavedo cell layer when exposed to light". This higher CO₂ fixation rate of the outside fruit offers support to the notion that adequately exposed fruit have the ability to synthesise carbohydrates necessary for flavedo development and thereby reduce the sensitivity to rind breakdown. However, it should be noted that the inside fruit, as opposed to outside fruit, were not exposed to saturated light during measurements, and it could be argued that they may have the ability to fix CO₂ at the same rate if exposed to similar levels of PAR. On the other hand, the inside fruit was constantly exposed to low light conditions during development and therefore lacks the chlorophyll content vital for fruit photosynthesis to provide adequate CO₂ fixation for optimal flavedo development (Moreshet and Green, 1980).

The reduced photosynthesis would be largely the result of the reduction in chlorophyll content in the flavedo during this developmental stage (Ikoma et al., 2001) and is in accordance with the literature (Bean et al., 1963; Moreshet and Green, 1980). The reduction of the chlorophyll content coincides not only with the colour change in the rind (Apr to May) and the reduction of P_{max} (nett photosynthetic rate), but also the increase of reducing and non-reducing carbohydrate contents. The increase is, therefore, not only of the glucose and fructose (which would originate from sucrose), but also of sucrose, possibly being imported from the leaves as fruit photosynthesis decreases at this stage. This argument concurs with Chen et al. (2008) who reported fruit photosynthesis of fully mature 'Satsuma' mandarin fruit to approach zero, and that at this stage the rind becomes the major sink of leaf photosynthates. The reduction in SPS activity in the late stage

of 'Fortune' mandarin fruit rind development also indicates that the maintained sucrose levels in the flavedo are probably due to the importation from leaves and not synthesis of sucrose in the rind (Holland et al., 1999).

The reduction in respiration rate from Mar to Apr (before and after colour break) is normal in citrus fruit as they progress towards maturity (Bain, 1958; Todd et al., 1961) and occurred in both inside and outside fruit. The flavedo is known to respire at twice the rates of the albedo and three times the rate of the pulp (Hussein, 1944). The higher respiration rate of the outside fruit could, therefore, be an indication of higher fruit and fruit rind growth rate (metabolic activity) resulting in not only increased fruit size, but also possibly a better developed rind structure in the outside fruit. However, the flavedo is thought to be able to re-fix 30-100% of the CO₂ produced by fruit respiration (Huang et al., 1992). The reduction in respiration rate (Rd) from Mar. to Apr. and the increase in sucrose, glucose and fructose contents could indicate that a pool of hexose is synthesised and stored at this stage, without being used in respiration and could then serve as respiration substrate for subsequent usage during postharvest storage.

To conclude, the content of the three sugars measured (sucrose, glucose and fructose) in the flavedo increased during stages II and III of fruit development. However, the glucose and fructose lagged behind the sucrose content and only increased one month before harvest. The flavedo sampled from the inside and outside of the canopy (high and low sun exposure) contained different levels of these reducing and non-reducing sugars, with the inside flavedo possessing, in total, a lower sugar content. Photosynthesis measurements of inside and outside fruit showed the outside fruit to have a higher positive carbon budget, which could supply carbohydrates to the flavedo cell structure during development. The reduced carbohydrate content in the inside fruit flavedo is also suspected to contribute to the reduction of the rind condition as seen in higher rind breakdown incidence and lower colour development of the inside fruit rind.

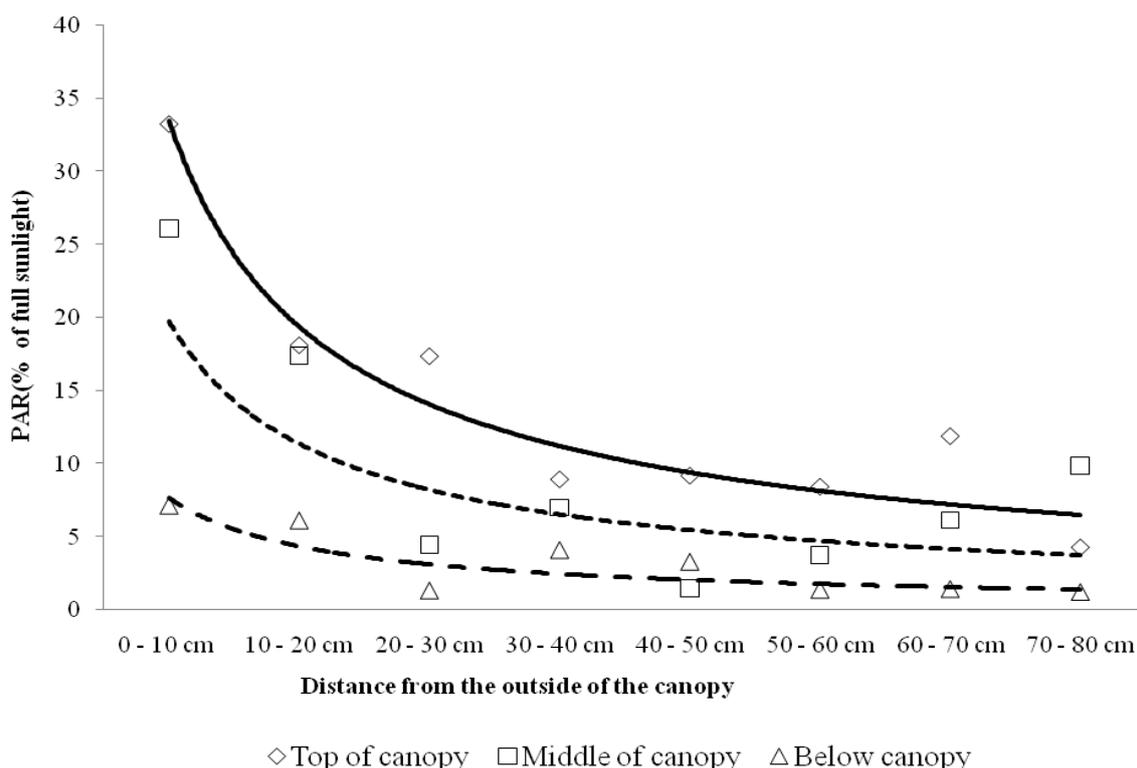


Figure 5.2.11.4.1. Light distribution in the canopy of a 'Nules Clementine' mandarin tree (measured from the outside of the canopy) and expressed as % of full sunlight on a clear day in January (middle of summer). Measurements were taken below the canopy (— · —, Δ), in the middle of the canopy (\pm 1.5 m from ground) (— —, \square) and in the top half of the canopy (\pm 2.5 m from the ground) (—, \diamond).

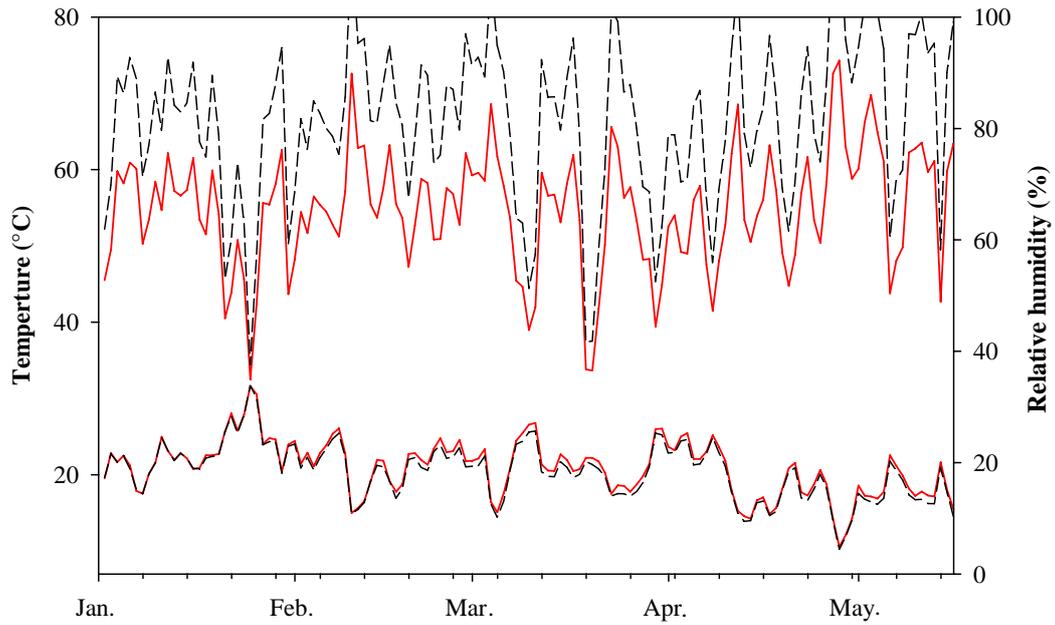


Figure 5.2.11.4.2. Effect of position (inside vs. outside) in the canopy of a ‘Nules Clementine’ mandarin tree, during stages II and III of fruit development on temperature (°C) and relative humidity (% RH). The data presented are the average values over a 24 hour period. The solid lines (grey) denote the outside and broken lines (black) the inside position.

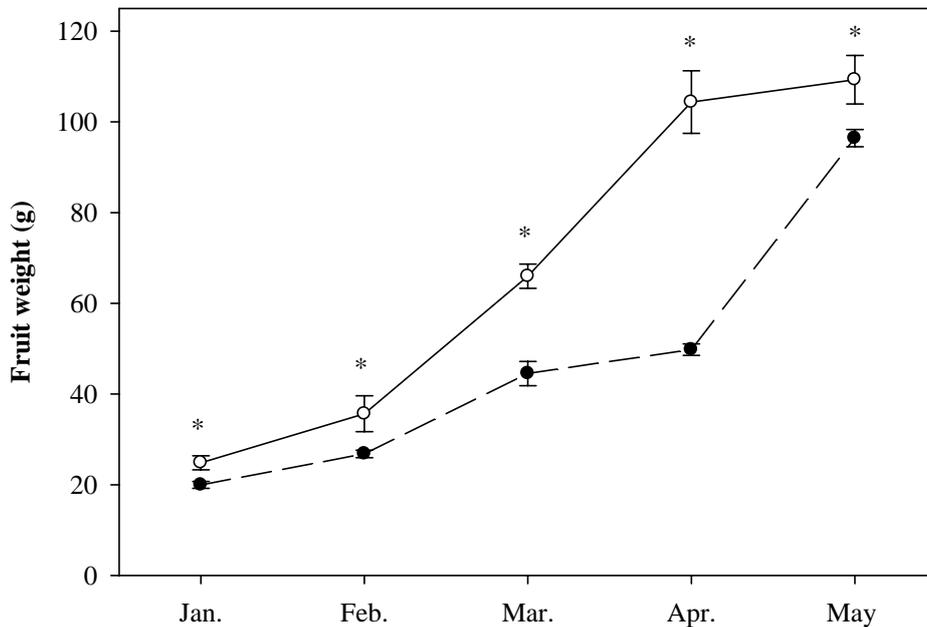


Figure 5.2.11.4.3. Increase in fruit weight of inside (●) and outside (○) fruit during stages II and III of fruit development of ‘Nules Clementine’ mandarin during 2005. Values are means (n = 8) with standard errors. A * above two data points indicates significant differences according to Fisher’s least significant difference test ($P \leq 0.05$).

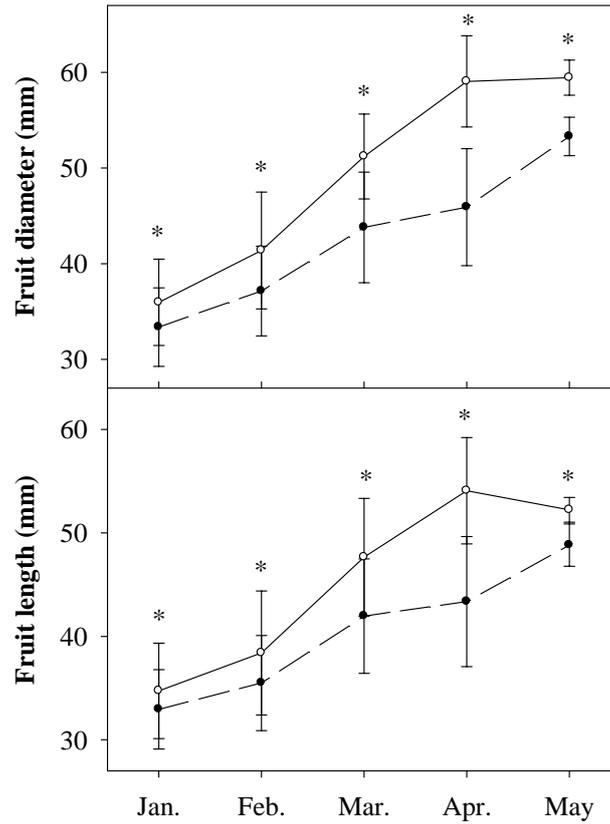


Figure 5.2.11.4.4. Average fruit length and diameter of inside (●) and outside (○) fruit during stages II and III of fruit development of 'Nules Clementine' mandarin during 2005. Values are means ($n = 8$) with standard errors. A * above two data points indicates significant differences according to Fisher's least significant difference test ($P \leq 0.05$).

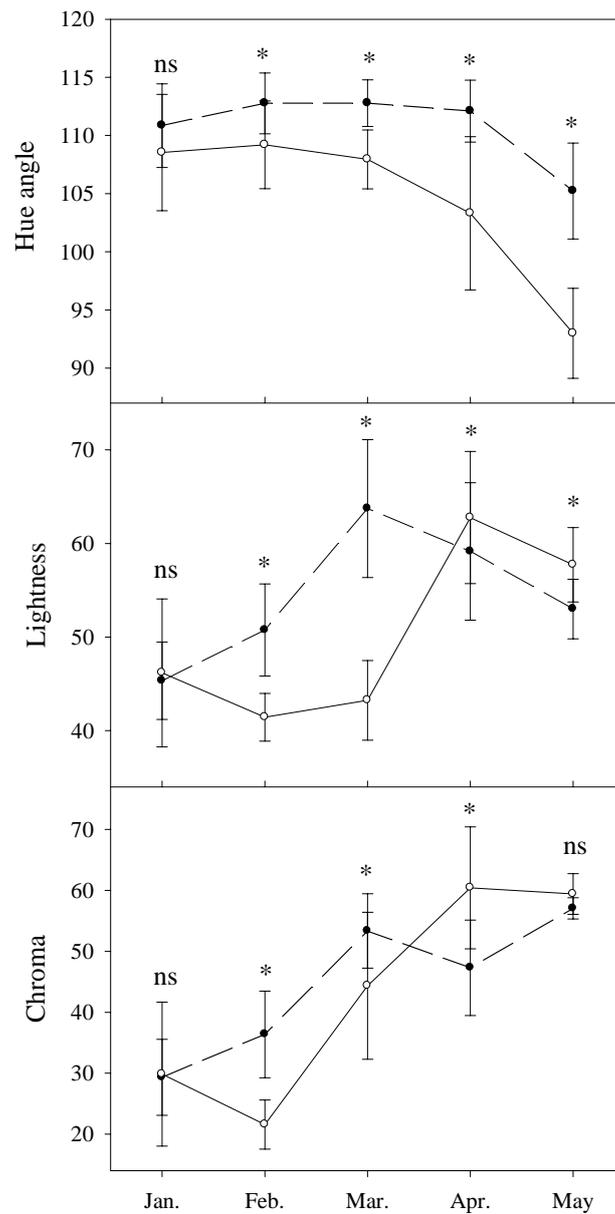


Figure 5.2.11.4.5. Change in fruit rind colour of inside (●) and outside (○) fruit flavedo during stages II and III of fruit development of 'Nules Clementine' mandarin during 2005. Values are means (n = 8) with standard errors. A * above two data points indicates significant differences according to Fisher's least significant difference test ($P \leq 0.05$). High hue angle indicates a more yellow fruit whereas a lower value indicates a more orange colour, and high lightness values indicate a less dark colour. Chroma represents the vividness of the colour, and therefore a high value represents a more intense colour.

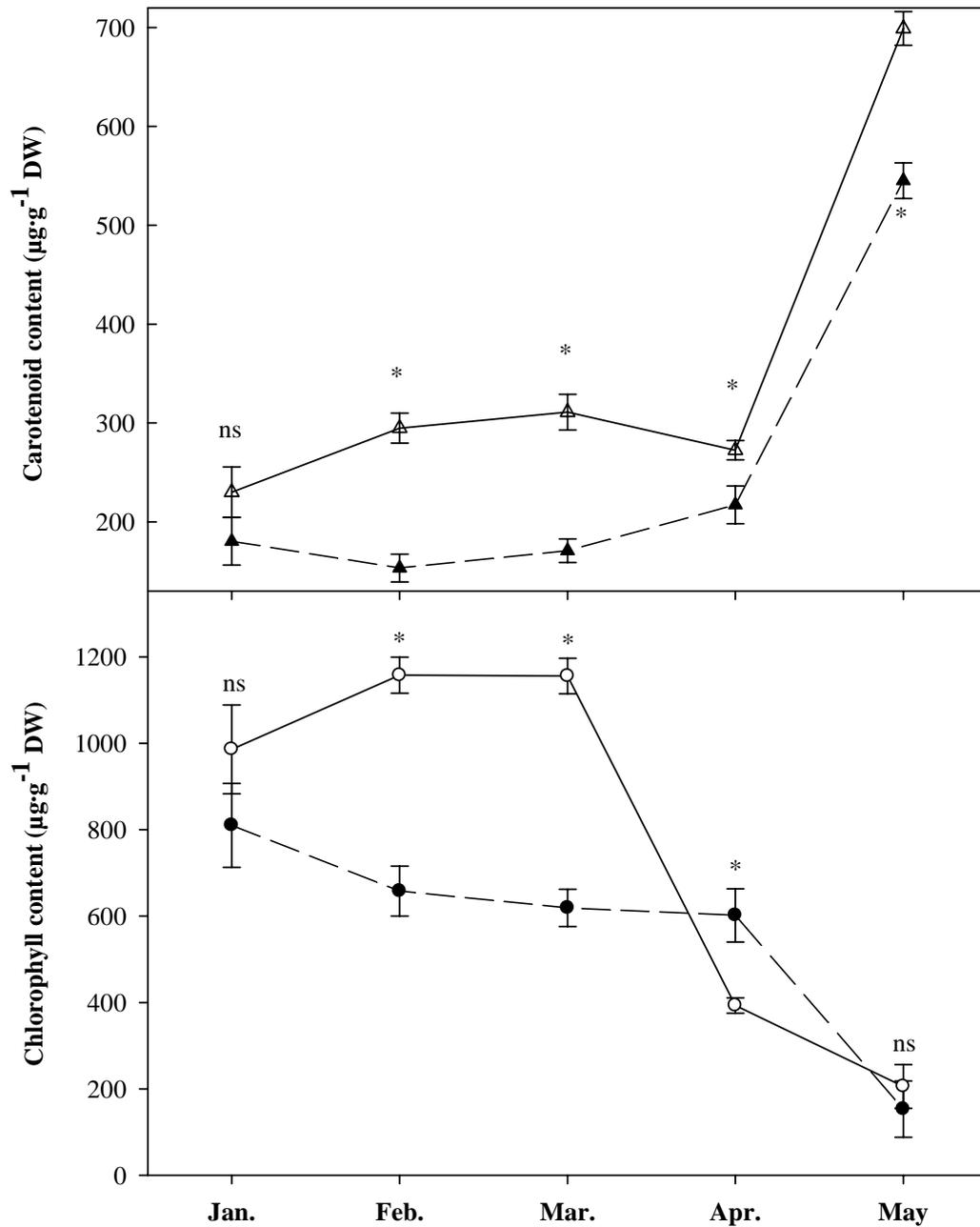


Figure 5.2.11.4.6. Change in fruit rind pigment contents during stages II and III of fruit development of 'Nules Clementine' mandarin for 2005. The broken lines and solid symbols denote the values for inside fruit flavedo [chlorophyll (●) and carotenoid (▲)] and the solid lines and empty symbols those for outside fruit flavedo [chlorophyll (○) and carotenoid (Δ)]. Values are means (n = 8) with standard errors. A * above two data points indicates significant differences according to Fisher's least significant difference test ($P \leq 0.05$).

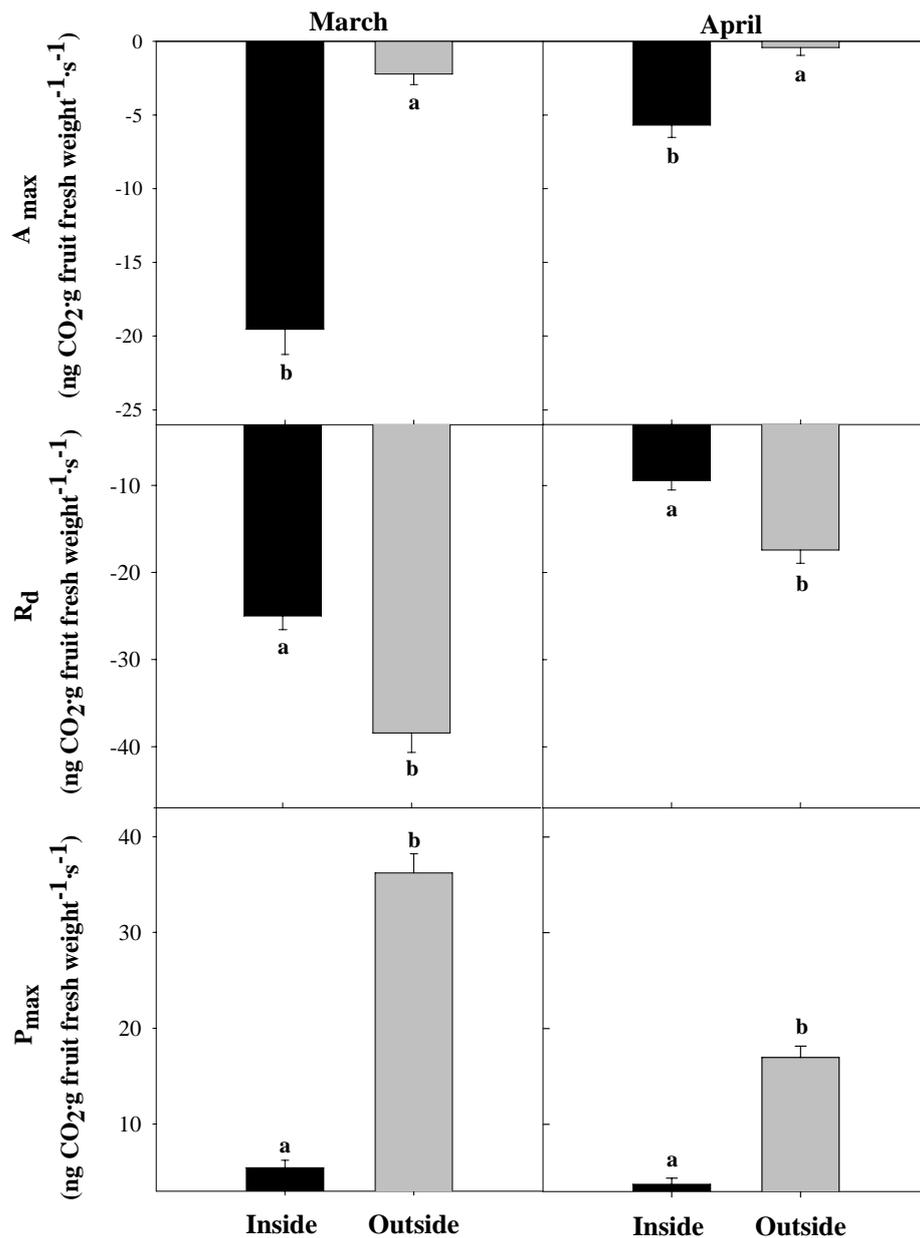


Figure 5.2.11.4.7. Light-saturated net CO₂ assimilation rate (A_{max}), dark respiration rate (R_d) and light-saturated photosynthetic rate (P_{max} , the difference between A_{max} and R_d) for 'Nules Clementine' mandarin fruit measured before (March) and after (April) rind colour break. Values are means ($n = 8$) with standard errors on the bars. Different lettering on bars indicates significant differences according to Fisher's least significant difference test ($P \leq 0.05$).

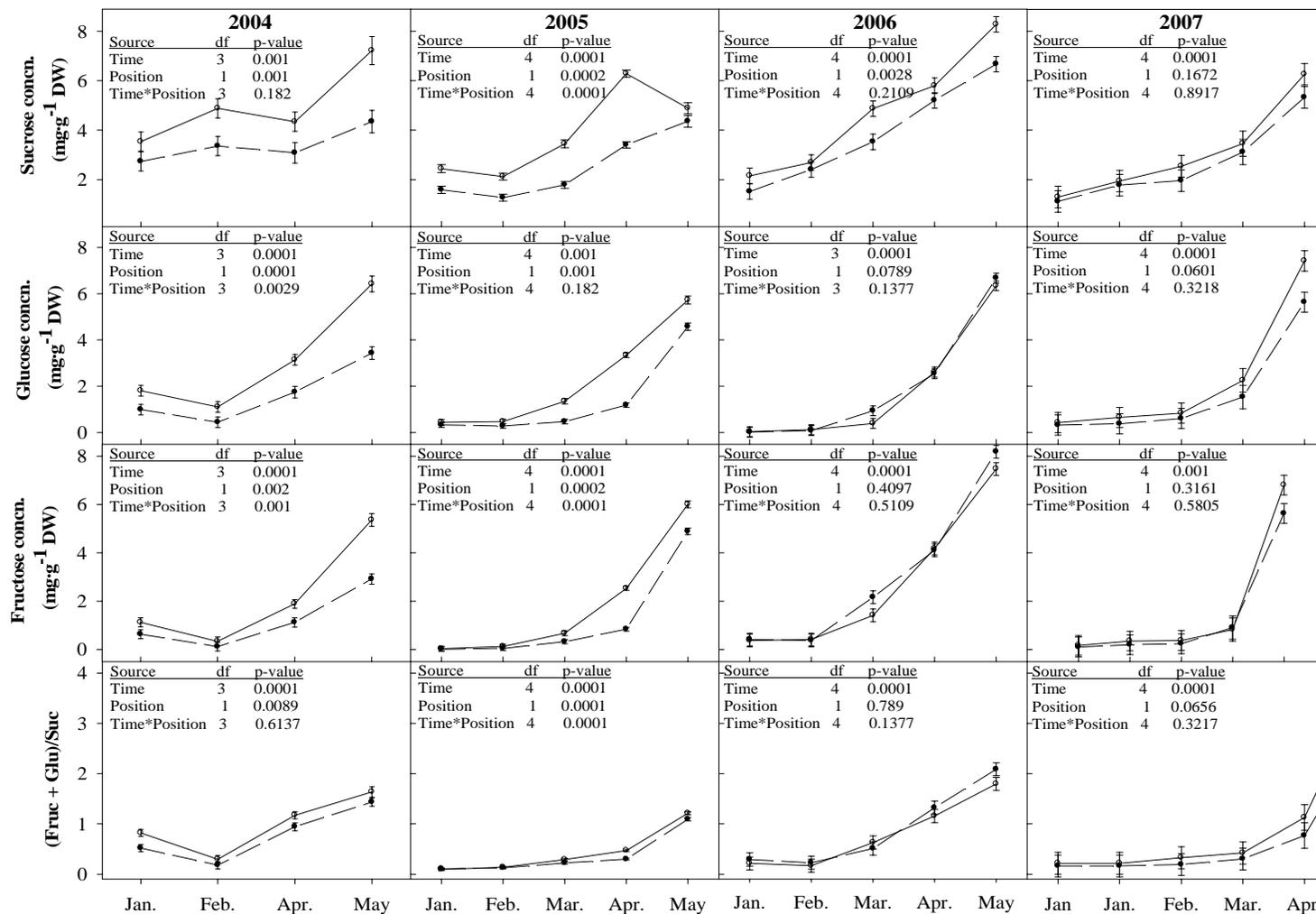


Figure 5.2.11.4.8. The accumulation in the flavedo of sucrose, glucose and fructose as well as the reducing to non-reducing sugar ratio during stages II and III of 'Nules Clementine' mandarin fruit development in 2004 – 2007. Analysis of variance of each sugar is shown. Values are means ($n = 8$) with SE bars. Values for the inside fruit (●) flavedo are denoted by broken lines whereas those for outside fruit (○) flavedo are solid lines.

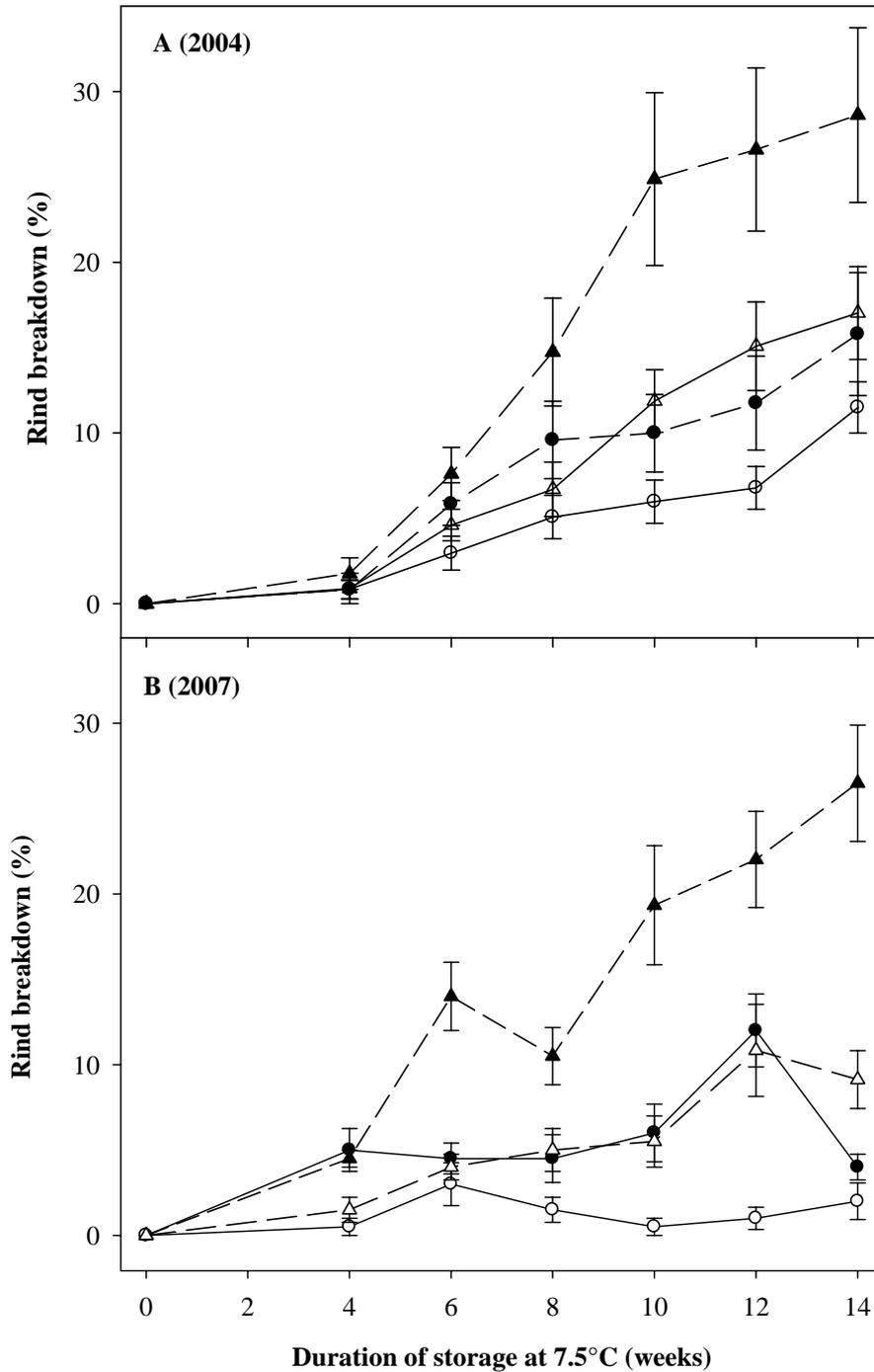


Figure 5.2.11.4.9. Percentage rind breakdown incidence of 'Nules Clementine' mandarins cold stored during 2004 (A) , and 2007 (B) at 7.5°C or -0.5°C. Values for the inside fruit (▲ for 7.5°C and ● for -0.5°C) are denoted by broken lines whereas those for outside fruit (Δ for 7.5°C and ○ for -0.5°C) are solid lines. Values are means (n = 8) with SE bars.

5.2.11.5 The incidence of rind breakdown of 'Nules Clementine' mandarin fruit is influenced by exposure to sunlight

Opsomming

Daar bestaan 'n bekende positiewe verhouding tussen PAR en die akkumulering van suiker in die vrugpulp van sitrus en die fiksering van CO₂ kan gekortwiek word deur swak ligindringing in die blaardak. 'Nules Clementine' mandaryne vrugte wat binne-in die blaardak ontwikkel is meer geneig om skilafbraak te ontwikkel en het ook lae vlakke van fotosintese en respirasie asook gevolglike laer koolhidratvlakke in die flavedo. Die doel van die poef was om te bepaal of direkte son gedurende groeifases II en III verantwoordelik was vir die swak skilkondisie. Om dit te bepaal is daar vrugte van dieselfde grootte met bruin papiersakkie bedek 4-, 3-, 2-, 1- en 0 maande (kontrole) voor oes in 2005. Gedurende 2006 is daar ook in 'n addisionele behandeling die eerste 30 cm blare agter 'n vrug bedek met 80% skadunet om fotosintese te keer. Die vrugte het al dieselfde na-oes behandelings ontvang voor opberging by 7.5°C waantydens hulle elke 2 weke vir skilafbraak voorkoms geïnspekteer is. Koolhidraat en pigment inhoud van die flavedo is in 2005 en 2006 gedoen asook die bepaling van K, Ca en Mg in 2005. Daar is gevind dat om die vrug in die skadu te plaas vanaf 3 tot 4 maande voor oes sal lei tot hoër skilafbraak. Dit is vermoedelik veroorsaak deur die lae koolhidraat en mineraal (Mg en Ca) akkumulering in die skil wat lei tot 'n swakker skilkondisie. Daar is dus aanduidings dat direkte sonlig gedurende fase II van vergroei krities belangrik is in skilontwikkeling.

Summary

Photosynthetically active radiation is a fundamental requirement in citrus fruit production due to the positive relationship with sugar accumulation in the pulp. In addition, the fixing of CO₂ by the photosynthetically active citrus fruit flavedo is known to be impeded by insufficient light levels within the canopy. Fruit which develops inside the canopy (low light) is thought to be more susceptible to the postharvest physiological disorder rind breakdown of 'Nules Clementine' mandarin. The aim of the study was to determine the influence of direct sunlight on the fruit during stage II and III of fruit development. Fruit positioned on the outside of the canopy were covered with paper bags 4-, 3-, 2-, 1- and 0 month (control) before harvest in 2005. During 2006, an additional treatment of covering leaves on the first 30 cm behind the fruit with 80% shade cloth, at the same intervals was done. In both seasons the fruit received all commercial packhouse treatments prior to being stored at 7.5°C during which the incidence of rind breakdown was noted at two weekly intervals. Carbohydrate and pigment content of the flavedo were determined during 2005 and 2006 as well as the K, Mg and Ca contents in the 2005 season. Removing the light from the fruit 3-4 months prior to harvest resulted in a higher incidence of rind breakdown. The light is thought to influence the mineral nutrient balance as well as the carbohydrate accumulation and metabolism of the fruit rind. It was concluded that direct light on the flavedo during stage II of fruit growth is important to development of a good rind condition. Quality of the rind condition includes both the cellular structure and amount of reserve energy needed to withstand postharvest stress and reduce the incidence of rind breakdown.

Introduction

'Nules Clementine' mandarin (*C. reticulata* Blanco) fruit produced in South Africa develops a progressive postharvest physiological disorder associated with the collapse of oil glands 3 to 5 weeks after harvest. The symptoms, called rind breakdown (RBD), develop into unsightly dark lesions of the flavedo and a randomly spread "leopard spot" pattern (Van Rensburg et al., 1995; Khumalo, 2006; Cronje, 2007). Preliminary industry studies led to the conclusion that fruit borne inside the dense 'Nules Clementine' mandarin canopy and out of direct sunlight, are more prone to this progressive disorder (Van Rensburg et al., 1995; 2004). Subsequently, it was determined that the flavedo of fruit from inside the canopy have lower carotenoid, Ca, Mg and carbohydrate contents coupled with higher levels of K than fruit borne in the outer canopy (see Sections 5.2.11.3 and 5.2.11.4). This difference in flavedo chemical composition was thought to be the result of the microclimate influence, viz. photosynthetically active radiation (PAR), temperature and relative humidity, on transpiration and photosynthetic rate of the fruit rind.

The characteristically dense canopy of leaves of citrus trees results in variation in light levels, with around 80% of PAR being intercepted by the outer 1 m of canopy (Greene and Gerber, 1967; Jahn, 1979; Section 5.2.11.3). This microclimatic variation in the citrus canopy translates into a positive relationship between light levels and soluble solids content (Sites and Reitz, 1949; 1950; Morales et al., 2000; Barry et al., 2000), rind colour of citrus fruit (see Section 5.2.11.4), as well as anatomical, physical and biochemical differences in shaded citrus leaves (Syvertsen and Smith, 1984).

The photosynthetic ability of the citrus fruit rind (Bean et al., 1960) adds another dimension to the need for good light distribution in the canopy. The photosynthetic ability of fruit rind was initially thought to be of minor

importance for fruit development (Bean et al., 1960). However, subsequent studies, measuring on-tree fruit photosynthesis, show a high degree of CO₂ fixation occurring from flowering to fruit maturity (Moreshet and Green, 1980; Huang et al., 1992; Vu et al., 1995). The flavedo-synthesised photosynthates remain mostly in the flavedo and to a lesser extent in the albedo, with insignificant amounts being translocated into the fruit pulp (Yen and Koch, 1990). The effect of different light levels in the citrus canopy between inside and outside positioned fruit are evident in their photosynthetic rate. Inside fruit have a marked reduction of CO₂ fixation compared to outside fruit, even though outside fruit have a higher respiration rate. Low light levels inside the canopy have, therefore, been shown to reduce the flavedo carbohydrate content of 'Nules Clementine' mandarin fruit, compared to sun exposed fruit flavedo (see Section 5.2.11.4). It is only during the colour development phase, when both chlorophyll breakdown and carotenoid synthesis occurs, that leaves become the main carbohydrate source to the rind (Chen et al., 2008).

The carbohydrate content of a fruit is determined prior to harvest and therefore adequate light levels in the tree canopy facilitating CO₂ fixation are vital, not only during developmental stages for growth respiration, but also to ensure adequate surplus carbohydrate levels during the postharvest period for maintenance respiration (Salisbury and Ross, 1992). Insufficient carbohydrate levels at harvest can lead to postharvest disorders, such as leaf blackening of various *Protea* cultivars, which has been correlated to a depletion of sugars in the leaves (McConchie et al., 1991; Stephens et al., 2001). In addition, the senescence processes that fruit undergo during the postharvest period are energy-demanding and reactions such as chlorophyll catabolism and carotenoid synthesis, occurring in citrus flavedo, are ATP-consuming (Huff, 1984; Buchanan-Wollaston, 1997; Dangl et al., 2000). Therefore, the increase in respiration rate associated with senescence could, in an already carbon-deficient fruit tissue, lead to premature senescence and the development of progressive postharvest disorders.

Position of fruit within the tree affects not only carbohydrate content of developing fruit, but also mineral nutrient balance, water relations, as well as the fruit's response to temperature extremes (Ferguson et al., 1999). These factors have been identified to play important roles in disorders such as bitter pit of apple (*Malus domestica* Borkh.) (Ferguson and Watkins, 1989), mealiness and flesh browning in peaches [*Prunus persica* (L.) Batsch] (Crisosto et al., 1997), watercore of apple (Bowen and Watkins, 1997), gel breakdown of 'Songold' plum (*Prunus salicina* Lindl.) (Taylor et al., 1993) and pitting of 'Hayward' kiwifruit (*Actinidia deliciosa* Chev.) (Thorpe et al., 2003; Montanaro et al., 2006). Mineral nutrient imbalances, specifically the balance of K, Mg and Ca, have been identified in the above-mentioned physiological disorders due to their direct involvement in cell wall and membrane structure and functioning, and therefore fruit cell viability during storage (Bramlage et al., 1980; Poovaiah et al., 1998; Rato et al., 2008).

In addition to mineral and carbohydrate content of the fruit, anti-oxidant species or the lack thereof, have been implicated in various postharvest physiological disorders, thereby limiting shelf-life, product quality and nutritional content of fresh produce (Hodges, 2003). Temperature and light levels are known to significantly influence ascorbic acid and vitamin A content in vegetables (Klein and Perry, 1982; Rosales et al., 2007). Ascorbic acid content and light levels have a strong positive correlation (although not causally linked), due to the carbohydrates (glucose and fructose) serving as the energy source for ascorbic acid synthesis, leading to higher levels in more sun-exposed fruit (Harris, 1975; Lee and Kader, 2000; Rosales et al., 2006; Carrari and Fernie, 2006). Low light levels in the 'Nules Clementine' mandarin canopy had, in addition to lower carbohydrates, lower carotenoid content in the flavedo compared to the outside fruit flavedo (Khumalo, 2006; see Section 5.2.11.4).

In contrast to the previously mentioned positive aspects of light, an excessive high light level can negatively affect fruit quality. In combination with high temperature, it is known to lead to sunburn on fruit surfaces (Rabinowitch et al., 1974; Wand et al., 2006). In 'Kiyomi' tangor (*Citrus unshiu* Marcs. x *C. sinensis*) small pitting lesions, called 'Kohansho', develop due to high irradiance and temperature build-up in the rind ($\pm 10^{\circ}\text{C}$ above ambient), which cause a three-fold increase in transpiration (Hasegawa and Yuno, 1992; Chikaizumi, 2003). These conditions could also affect respiration rate of the overexposed fruit rind and result in a depletion of carbohydrate reserves in the flavedo.

The aim of this study was to determine whether RBD incidence is due to direct sunlight on the fruit surface or to the fruit being in an advantageous position between photosynthesising and solute exporting source leaves. It is hypothesised that the fruit flavedo should receive direct sun exposure to ensure a good rind condition, viz. high Ca, Mg, pigment and carbohydrate contents, which would reduce its sensitivity to physiological disorders. This hypothesis was tested by using paper bags to cover individual fruit, to manipulate the duration of direct sun exposure to a fruit from after physiological fruit drop. This technique has not previously been used in the induction of citrus rind disorders.

Materials and methods

Sites, plant material

To avoid repetition see section 5.2.11.3 (Materials and methods) for details.

Pre-harvest fruit sampling and treatments

During 2004, 'Nules Clementine' mandarin fruit were harvested at commercial maturity on 16 May, according to canopy position, i.e. outside (full sunlight exposure) or inside (less than 80% of full sunlight, Section 5.2.11.3 Material and methods). The fruit received normal commercial postharvest treatments as described in the next section.

During January 2005, five treatments were allocated in a randomised complete block design of eight single-tree replications per treatment. On each of these trees (constituting one replication), 10 exposed fruit of the same size, positioned in the outer and eastern section of the canopy, were selected and tagged. The selected fruit were covered (shaded), with a brown paper bag, without removing or covering of subtending leaves at monthly (30 day) intervals, starting after physiological fruit drop (in December) from January (4 months before maturity), until April (1 month before maturity). Control treatment comprised of unbagged fruit, i.e. bagging 0 months before maturity, which were also selected and marked in January. At maturity (18 May), labelled fruit were picked. The bagging treatment did not permit any light to pass through and after logging the temperature and relative humidity over a 4-day period (Sensitech Inc., Beverly, Ma., USA), it was shown that no drastic alteration of the heat and humidity profile around the bagged fruit occurred, which could influence rind condition.

The experiment was repeated in 2006 with two additional monthly treatments, viz. covering only the 30 cm of leaves behind a tagged, but uncovered fruit with an 80% shade cloth (net) or covering these leaves behind a bagged fruit (bag + net). At commercial harvest date (14 May), these fruit were coded according to the time of the shading treatment [4-, 3-, 2-, 1-, and 0 months (control)], treatment (bag, net and bag + net), replication (1-8) and fruit number (1-10), prior to receiving the postharvest treatments as described below.

Postharvest handling, cold storage and data collection

After harvest, the fruit were transported to a commercial packhouse where they were drenched, (thiabendazole 1000 mg·L⁻¹; guazatine 500 mg·L⁻¹; 2,4-D sodium salt 250 mg·L⁻¹; Sporekil® 1000 mg·L⁻¹) and degreened (3 days at 3 µL·L⁻¹ ethylene, >90 % RH and 20 to 22 °C) before receiving all standard commercial packhouse treatments, [thiabendazole, 500 mg·L⁻¹; imazalil, 500 mg·L⁻¹; 2,4-dichlorophenoxyacetic acid, 125 mg·L⁻¹, and polyethylene citrus wax application (Citrushine®, Johannesburg, South Africa)]. Afterwards, the coded fruit were resorted into treatments and replications before being placed in cold storage at 7.5°C, a temperature which is known to cause the highest degree of RBD incidence (Khumalo, 2006).

During cold storage, fruit were scored for RBD incidence every second week for the duration of the experiment. Fruit rind colour was determined with a chromameter on the side of the fruit with the highest colour development (Minolta NR 4000, Osaka, Japan). Fruit dimensions (length and diameter) were measured prior to removing the flavedo of the fruit, which was pooled per replicate to ensure enough material for the various analyses. The flavedo was frozen in liquid nitrogen whereafter it was freeze dried (VirTis Freezemobile 25ES, The VirTis Company, Gardiner, NY, USA) and stored at -80°C. These samples were milled to a fine powder which was used for pigment (2005–2006), mineral nutrient (2005) and carbohydrate (2005–2006) analysis.

Rind pigment analysis

To avoid repetition see section 5.2.11.4 (Material and methods) for details of pigment analysis.

Mineral nutrient analysis

To avoid repetition see section 5.2.11.3 (Materials and methods) for details of mineral nutrient analysis.

Determination of flavedo carbohydrate content

To avoid repetition see section 5.2.11.4 (Material and methods) for details of carbohydrate extraction and analysis.

Statistical analysis

Differences between treatment effects on rind breakdown, and carbohydrate and mineral nutrient contents were analysed with PROC GLM. From the analysis of variance (ANOVA), significant differences between treatments were determined and means were separated by Fisher's least significant differences (LSD). The correlation procedure (PROC CORR) was used to determine significance of correlations between mineral

nutrient concentrations (K, Ca and Mg), carbohydrates, pigments (2005–2006), fruit diameter (2005) and rind breakdown of treated fruit (SAS v. 6.12 SAS Institute Inc., NC, USA).

Results

Canopy position (2004)

The fruit harvested from the inside of the 'Nules Clementine' mandarin fruit tree canopies had a significantly higher incidence of RBD after 10 weeks of storage at 7.5°C compared with the outside fruit (Fig. 5.2.11.5.1). Rind breakdown increased progressively in both the inside and outside fruit during the storage period, but at a higher rate in the inside fruit.

Fruit bagging (2005)

Bagging (shading) of the fruit at monthly intervals for 4 months (January) until 1 month before harvest altered the incidence of RBD in fruit stored at 7.5°C (Fig. 5.2.11.5.2). Despite all fruit having been sampled from the outer canopy position, early (4- or 3-month period) covering of fruit to exclude sun light resulted in a higher RBD incidence after 10 weeks in storage. On the other hand, shading for 2 months or less did not result in significantly higher incidence of RBD than the unbagged control fruit.

Shading of fruit by bagging affected rind colour; fruit shaded for a longer period had significantly higher hue angle and lower chroma than unbagged fruit and fruit bagged for a shorter period (Table 5.2.11.5.1). Before degreening and storage, both chlorophyll and carotenoid contents in the rind of fruit bagged 4 months before harvest were approximately half the concentration of unbagged fruit, based on the non-statistical data from pooled flavedo samples. After cold-storage, carotenoids concentration was lower the longer the fruit were bagged. The expected increase in carotenoids and decrease in chlorophyll contents in the flavedo during storage are illustrated in their vastly different values of the pre- and post- storage analysis.

Shading of fruit for 4 months resulted in smaller fruit at maturity than shading for shorter periods. Potassium levels of the rinds of fruit that were bagged at least 2 months before harvest, were significantly higher than K levels of unbagged fruit and fruit bagged only 1 month before harvest (Table 5.2.11.5.2). Calcium levels were not affected by bagging. Magnesium levels tended to be lower the longer that fruit were shaded, with a sudden drop in Mg levels at harvest, coinciding with colour break.

The carbohydrate content (sucrose, fructose and glucose) as well as the ratio of reducing to non-reducing sugars [(fructose+glucose)/sucrose] in the flavedo of the bagged fruit after 16 weeks storage of 7.5°C did not differ among treatments (Table 5.2.11.5.2). However, all three carbohydrates showed an increasing trend from 4 months before maturity until maturity. It is also evident that at this stage of fruit maturity and after 16 weeks of cold-storage, reducing sugars (especially fructose) were at higher levels compared with the non-reducing sucrose.

Correlation analyses between the factors discussed above and RBD incidence after 16 weeks storage at 7.5°C, showed that K and Ca contents in the flavedo are correlated with RBD, although K was positively (0.46) and Ca was negatively (-0.39) correlated. None of the carbohydrates in the flavedo were significantly correlated with rind breakdown, however, fruit diameter and height had a negative correlation with RBD (Table 5.2.11.5.3). However, even though there were significant correlations the very low R² values indicate no individual factor accounted for the majority of the variation in RBD.

Fruit bagging, leaf shade netting and the combination treatments (2006)

Rind breakdown incidence after 14 weeks storage at 7.5°C was affected by the various pre-harvest manipulations (Fig. 5.2.11.5.3). The 2006 season was characterised by very low RBD incidence in fruit from the orchard used (e.g. zero for control fruit) and only the fruit bagging and leaf netting treatments resulted in RBD incidence. These treatments of "inducing" RBD by limiting sunlight exposure to fruit and leaves were effective until 2 months before harvest.

Rind colour was reduced (higher hue angle and lightness and lower chroma values) by the fruit bagging and leaf netting treatments applied 4 (Jan) and 3 months (Feb) before harvest (Fig. 5.2.11.5.4). These colour measurements after 14 weeks storage at 7.5°C were accompanied by analysis of chlorophyll and carotenoid contents in the flavedo and, as expected, the carotenoid contents in the flavedo were high at this stage of fruit maturity (Fig. 5.2.11.5.5) (compared with the pre- and post-storage values in Table 5.2.11.5.1). At this stage of fruit maturity, carotenoid content gives a better representation of rind colour development than chlorophyll content, which could be too low at this late period to give an indication of treatment differences. However, the effect of fruit bagging alone and in combination with leaf netting on pigments were evident after 14 weeks storage and resulted in lower carotenoid content. In contrast, by only applying netting to leaves, the carotenoid content was not reduced, even if applied 4 months prior to harvest (Jan) (Fig. 5.2.11.5.5).

Carbohydrate analysis (sucrose, glucose and fructose contents) of fruit flavedo in 2006, after 14 weeks of storage at 7.5°C, showed that the bagging and netting treatments influenced the carbohydrate content compared with the control (Fig. 5.2.11.5.6). However, against expectations the application of treatments 3 (Feb) and 1 month (Apr) before harvest resulted in higher sucrose content (except for netting in Feb). The bagging treatment 4 to 2 months before harvest (Jan–Mar) resulted in a higher glucose content compared with other treatments. However, the combination of bagging and netting did not give the same result in the same months. The fructose content was higher in 4 (Jan), 3 (Feb) and 1 month before harvest (Apr) treatments, but again the combination treatment differed from the individual treatments. The sucrose values in all the bagging and netting treatments were higher than those determined at the end of the growing season even though the values of the control were still within the range of 5–8 mg.g⁻¹ DW as shown in Section 5.2.11.4. The same increase in glucose content in the flavedo is evident, but to a lesser extent. The ratio of the reducing to non-reducing sugars [(fructose+glucose)/sucrose] in the control treatment were higher compared with all the other shading treatments. This result gives a strong indication that these treatments changed the accumulation and metabolism of these carbohydrates in the fruit flavedo.

Rind breakdown at week 14 had a weak negative correlation with carotenoid content. Carbohydrates (sucrose, fructose and glucose) were not correlated with RBD (with the possible exception of fructose, $P = 0.063$). However, the ratio of reducing vs. non-reducing sugars had a significant positive correlation, albeit weak, with RBD (Table 5.2.11.5.4).

Discussion

A higher incidence of RBD was found in fruit from the shaded (inside) part of the canopy, supporting the notion that if the light levels around an individual fruit, positioned in a high light environment, were drastically reduced, i.e. by shading during stage II and III, a reduction in rind condition would occur. In this study the treatments were applied after physiological fruit drop had occurred in December, since the vast number of abscising fruit would make a treatment involving individual fruit impractical. Subsequently, by eliminating sunlight from a fruit in an advantageous position, i.e. by shading (bagging) fruit borne outside of the canopy, the incidence of RBD development was increased. This was the first treatment (pre- or postharvest) of which the author is aware which “induced” this particular progressive postharvest disorder in ‘Nules Clementine’ mandarin. In addition, it is evident that the negative impact of bagging fruit is not realised by shading fruit in the last 1 to 2 months of fruit development (stage III), compared to the 3 to 4 months prior to harvest (stage II). These findings were in accordance with other physiological disorders such as bitter pit of apple (Ferguson and Watkins, 1989), gel breakdown of ‘Songold’ plum (Taylor et al., 1993) and pitting of ‘Hayward’ kiwifruit (Thorp et al., 2003; Montanaro et al., 2006) where position in the canopy influenced the occurrence of these disorders.

Citrus fruit, however, differ from these examples in that the fruit pulp and rind function to a large extent independently, and the rind (flavedo and albedo) can develop a physiological disorder without affecting internal quality. The autonomy of these fruit parts is due to the very low level of transport (water or solutes) occurring between the rind and pulp (Kaufmann, 1970), as well as the different mineral nutrient accumulation patterns (Jones and Parker, 1950; 1951; Embleton et al., 1973). These aspects support the notion that the flavedo is a modified leaf rather than a fleshy fruit (Schneider, 1968), and growth conditions detrimental to leaf development could impact negatively on the condition of the flavedo. Rind development primarily occurs during stages I and II of fruit development, whereas the accumulation of solutes and water in the pulp during stage III is responsible for the dramatic increase in fruit size (Bain, 1958).

By shading the flavedo and limiting photosynthesis and transpiration, a reduction in the flavedo’s physiological condition was achieved by reducing the fruit sink strength, as illustrated by the reduction of fruit size as well as mineral nutrient and carbohydrate accumulation in the flavedo (see Sections 5.2.11.3 and 5.2.11.4). It could be speculated that by shading the fruit rind, the tissue loses its ability to maintain its sink strength via synthesis and export of auxin from the rind as the correlative signal in the seedless ‘Nules Clementine’ mandarin fruit (Bangerth, 1989; Bangerth and Grubber, 2000; Verreyne, 2006). The negative correlation between RBD and fruit size, even though citrus fruit size is mainly a function of solute and water import into the pulp, supports the argument that a fruit with low sink capabilities, and therefore low solute accumulation, will be more susceptible to RBD development. However, by aiming to increase the sink strength of a rind with hormones such as auxin, this hypothesis could be further developed.

The citrus flavedo is thought to be self-sufficient in carbohydrate synthesis from flowering until late in stage III of fruit development, when the conversion of chloroplasts to chromoplasts occurs (Moreschet and Green, 1980; Vu et al., 1985; Huang et al., 1992). Chen et al. (2008) showed that shading of ‘Satsuma’ mandarin fruit (*C. unshui* Marc. cv. Miyagawa Wase) resulted in more leaf photosynthate being allocated to the rind if the treatment was applied before colour break. At the same time, the allocation of sugars to the juice sacs

declines by 20% and 15% before and after colour break, respectively (Chen et al., 2008). This higher sugar allocation to the shaded fruit was thought to be due to the elevated activity of sucrose-metabolising enzymes in the rind at the cost of the pulp (Chen et al., 2008).

The carbohydrate content in the flavedo during this study was only determined after a prolonged postharvest storage period and therefore provided a snapshot of the situation at a given point in time. This added to the complexity in interpreting the results. However, Chen et al., (2008) showed that shaded fruit flavedo could be supplied of leave carbohydrates in detriment of the pulp. It could therefore be possible that under these shading conditions, the leave supplied sucrose augment the existing flavedo supply and thereby reducing the impact of potentially low carbohydrate content.

Carotenoid synthesis is directly related to sucrose levels, as the energy source for the chloroplast-chromoplast conversion (Huff, 1984). The lower level of carotenoid pigments in the flavedo, determined at the same time as the carbohydrates, i.e. 14 weeks after harvest, add support to the argument that hindrance of the rind's photosynthetic ability during rind development by shading influences the rind carbohydrate balance negatively. The same reduction in pigments and carbohydrate content of the rind was seen in fruit from the inside of the canopy receiving less than 80% of PAR (see Section 5.2.11.4). The low carotenoid content could also be an indication of the reduced anti-oxidative capacity of the flavedo (Khumalo, 2006), since carotenoids are thought to play an important role in the protection of cellular membranes (Sies and Stahl, 1995; Laurie, 2003).

Therefore, it is possible that part of the detrimental influence of fruit shading during stage II on rind condition (and increased incidence of RBD) occurred due to the alteration of carbohydrate metabolism, during preharvest fruit growth as well as during postharvest respiration. Low light levels inside the citrus canopy have been shown to have lower levels of sugar accumulation in the fruit rind due to lower CO₂ fixation and in addition, the inside fruit had a lower respiration rate (see Section 5.2.11.4). Leaves and flowers are more subject to insufficient carbohydrate reserves during the postharvest period as they are not commonly known as storage sites of sugars. This lack of adequate reserves necessary for maintenance respiration could possibly lead to the postharvest leaf blackening seen in *Protea* flowers (Stephens et al., 2001). This could possibly be part of the reason for the higher incidence of RBD in bagged fruit as well as those from the shaded parts of the canopy (see Section 5.2.11.4).

The second important aspect influenced by fruit shading is the mineral nutrient content in the flavedo. As in a previous experiment (see Section 5.2.11.3), where the low PAR conditions within the canopy resulted in higher K and lower Mg (and Ca) in the flavedo, the bagging of fruit in this experiment resulted in what can be interpreted as an imbalance of these minerals. The higher K content in shaded plant parts are thought to be an osmotic adjustment mechanism to serve as a replacement for the lower osmotic potential due to the reduced sucrose content (Mpelasoka et al., 2003). The reduced Mg content in the fruit rind, shaded for 3 to 4 months, could be due to the breakdown of chlorophyll and re-distribution of Mg as a result of the lack of photosynthetic activity. The reduction in Mg seen between the month before harvest treatment and the control follows the same pattern as seen in Section 5.2.11.3 (Fig. 5.2.11.3.7), and could be as a result of natural colour development, which results in the Mg-containing chlorophyll pigments breaking down and releasing Mg for redistribution (Belma et al., 1978; Dorenstouter et al., 1985). Imbalances of K, Ca and Mg due to fruit canopy position have been linked to various postharvest disorders, viz. gel breakdown of 'Songold' plums and pitting of 'Hayward' kiwifruit (Taylor et al., 1993; Thorp et al., 2003; Montanaro et al., 2006). In both instances the imbalance of mineral nutrients, such as K, Ca and Mg, were thought to develop in the fruit due to being in the shaded canopy and is one of the causative factors even if not being the sole cause responsible for the disorder.

The shading of the photosynthetically active flavedo could, in addition to influencing various chemical characteristic, viz. pigments, carbohydrates and mineral nutrients, also result in anatomical modifications of the flavedo. It has been shown that the citrus leaf adapts to shading by concentrating the pigments more in the upper leaf tissue, while the leaf becomes thicker and denser with less chlorophyll (Syvertsen and Smith, 1984). This corresponds to changes in shaded beech leaves (*Fagus sylvatica* L.) which develop more stomata and a single layer of more loosely arranged palisade parenchyma cells (Lichtenthaler, 1981; 1984). It is therefore possible that the shaded flavedo's anatomical structure differs from a sun-exposed flavedo as suggested by the pigment content at harvest, which could make the shaded flavedo more susceptible to postharvest stress.

In conclusion it can be reasoned that the reduction of the fruit's photosynthetic ability during stage II of fruit development, and probably earlier, will be detrimental to rind condition, and can lead to its physiological breakdown. Even though these biochemical changes measured in the flavedo (pigments, carbohydrate and mineral nutrients) indicate correlations with the rind's physiological response, i.e. RBD incidence, no single

factor could be identified as the main underlying cause of RBD, as indicated by the low R^2 values. This further indicates the complexity of identifying the underlying mechanism of this progressive rind disorder which develops over an extended storage period. Studies on postharvest disorders such as rind breakdown of 'Nules Clementine' mandarin are hampered by their erratic incidence varying significantly between seasons (15-20% in 2004, 20% in 2005 and 0% in 2006). This fact, indicative of an overarching climatic influence, complicates the testing of a causative hypothesis on this physiological disorder. The successful "induction" of RBD by bagging the fruit preharvest should therefore be seen as a major advance on its own, even though all biochemical changes in the flavedo, e.g. carbohydrate content, cannot be fully explained. These data serve as a start in unravelling the factors predisposing the citrus fruit rind to a progressive postharvest disorder associated with the collapse of the oil glands.

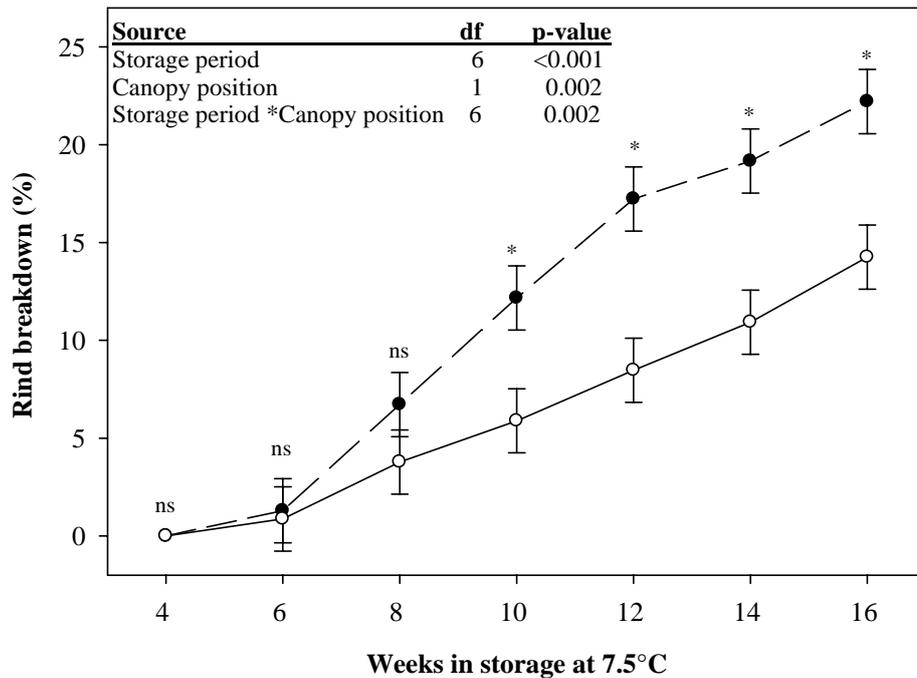


Figure 5.2.11.5.1. The influence of canopy position percentage on rind breakdown of 'Nules Clementine' mandarin during storage at 7.5°C in 2004. Values for the inside fruit (●) are denoted by a broken line and those for the outside fruit (○) by a solid line. Values are means ($n = 8$) \pm SE. Different lettering indicates significant differences among treatments according to Fisher's least significant difference test ($P \leq 0.1$).

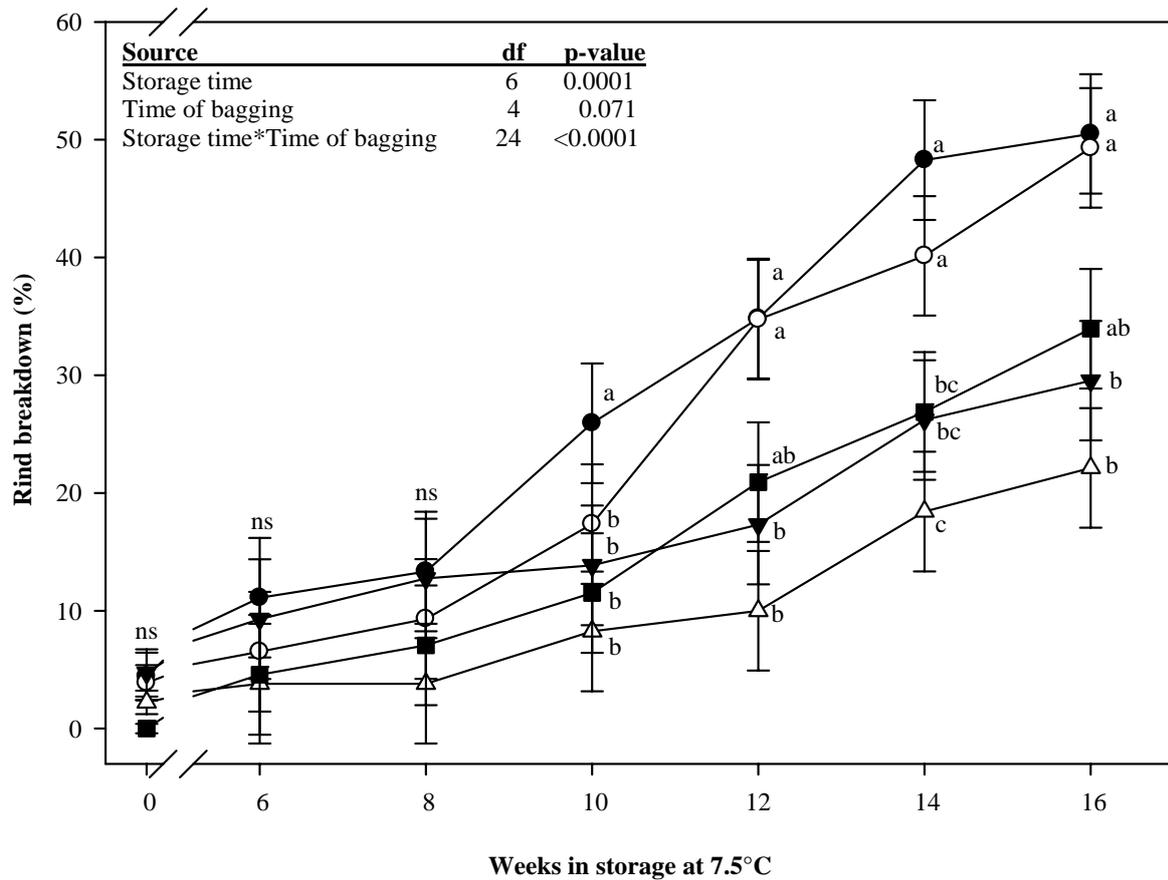


Figure 5.2.11.5.2. The effect of fruit bagging for different period on percentage rind breakdown of 'Nules Clementine' mandarin fruit in 2005. Fruit were bagged in January (4 months, ●), February (3 months, ○), March (2 months, ▼), April (1 months, △) and control (0 months, ■) before harvest. The fruit were stored at 7.5°C and scored for rind breakdown incidence every second week for a 16-week period. Values are means (n = 8) ± SE. Different lettering indicates significant differences among treatments according to Fisher's least significant difference test ($P \leq 0.1$).

Table 5.2.11.5.1. The effect of monthly bagging (shading) of 'Nules Clementine' mandarin fruit on rind colour (hue angle, lightness and chroma) and flavedo pigment content before degreening and cold-storage as well as after 16 weeks of storage at 7.5°C during 2005.

Months of bagging (shading)	Pre-degreening and cold-storage					Post cold-storage				
	Hue°	Lightness	Chroma	----- $\mu\text{g}\cdot\text{g}^{-1}\text{DW}$ -----		Hue°	Lightness	Chroma	----- $\mu\text{g}\cdot\text{g}^{-1}\text{DW}$ -----	
				Chlorophyll content ^z	Carotenoid content ^z				Chlorophyll content	Carotenoid content
0 (May)	66.1 c	67.4 ns	71.0 a	1237.3 ns	355.5 ns	59.8 c	60.8 ns	66.3 ns	13.0 ab	1624.23 a
1 (Apr)	69.3 b	66.3 ns	67.9 b	1222.2 ns	345.5 ns	61.0 bc	62.2 ns	65.4 ns	5.7 b	1606.77 a
2 (Mar)	71.6 ab	67.6 ns	65.5 c	1069.5 ns	293.4 ns	61.3 b	61.7 ns	65.4 ns	17.6 a	1585.42 a
3 (Feb)	70.6 b	68.4 ns	65.3 c	1171.9 ns	305.3 ns	62.5 b	62.9 ns	68.1 ns	24.9 a	1312.22 b
4 (Jan)	73.1 a	69.5 ns	65.5 c	571.7 ns	161.8 ns	65.1 a	64.4 ns	64.7 ns	20.0 a	987.25 c

Values in the same column followed by different letters are indicates significantly different according to the Fisher's least significant test ($P \leq 0.05$). Values are means (n = 8).

^z No statistical analysis was done due to the data being a single value point of pooled samples.

Table 5.2.11.5.2. The effect of monthly bagging (shading) of 'Nules Clementine' mandarin fruit on size, flavedo mineral nutrient and carbohydrate content after 16 weeks of storage at 7.5°C during 2005.

Months of bagging (shading)	Fruit size (mm)		-----mmol·g ¹ DW-----			Post cold storage			
			K	Ca	Mg	-----µg·g ¹ DW-----			[(Fru+Glu)]/Suc
	Length	Diameter				Sucrose content	Fructose content	Glucose contnet	
0 (May)	53.4 b	60.5 ab	0.32 b	0.90 ns	0.08 c	3.84 ns	6.20 ns	4.30 ns	2.38 ns
1 (Apr)	54.5 b	63.5 ab	0.31 b	0.99 ns	0.11 a	4.71 ns	7.77 ns	5.32 ns	3.21 ns
2 (Mar)	52.9 a	64.3 a	0.41 a	0.99 ns	0.10 ab	4.48 ns	6.92 ns	5.08 ns	2.67 ns
3 (Feb)	52.8 a	62.8 ab	0.42 a	0.94 ns	0.09 bc	3.81 ns	5.63 ns	3.26 ns	2.39 ns
4 (Jan)	50.6 a	58.5 c	0.42 a	0.93 ns	0.08 c	3.84 ns	5.52 ns	4.01 ns	2.58 ns

Values in the same column followed by different letters are indicates significantly different according to the Fisher's least significant test ($P \leq 0.05$). Values are means (n = 8).

Table 5.2.11.5.3. Results from correlation analysis among various factors measured in the flavedo after 16 weeks of storage at 7.5°C and the occurrence of rind breakdown of 'Nules Clementine' mandarin fruit, after being bagged from Jan to Apr in 2005.

Correlation		Correlation value r (Spearman)	p-value	R ²
Rind breakdown vs.	K	0.46	0.001	0.129
	Ca	-0.39	0.001	0.218
	Mg	-0.08	0.616	0.006
Rind breakdown vs.	Sucrose	-0.14	0.376	0.019
	Fructose	-0.23	0.142	0.053
	Glucose	-0.13	0.428	0.017
Rind breakdown vs.	Diameter Length	-0.49	0.001	0.240
		-0.48	0.001	0.230

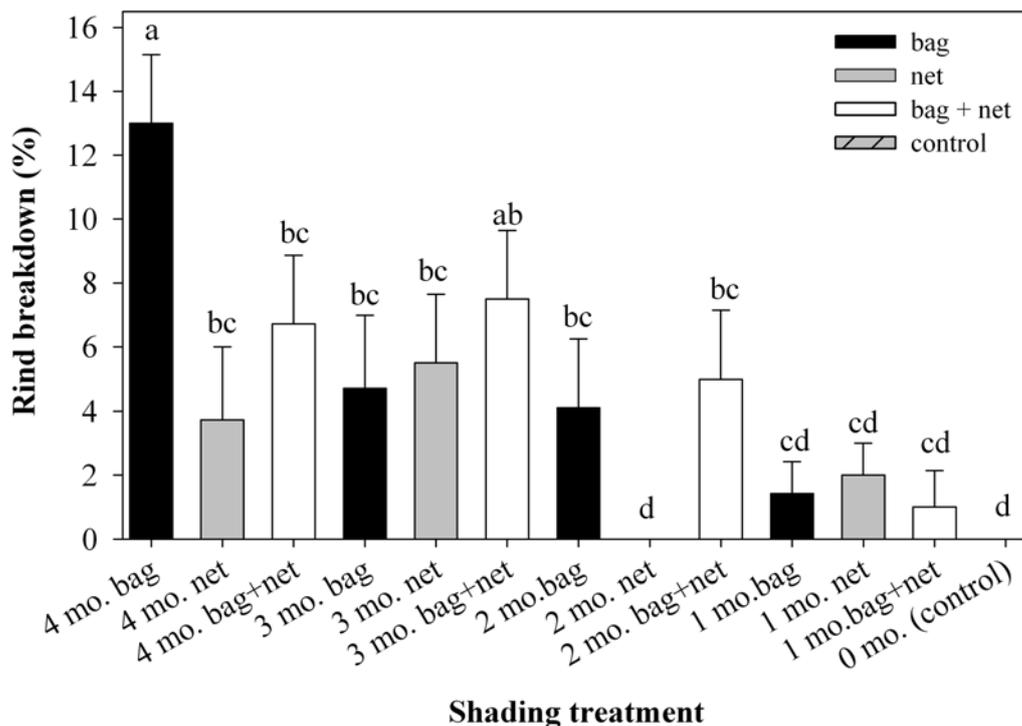


Figure 5.2.11.5.3. The influence of fruit bagging, leaf covering with shade-netting and the combination of these treatments at monthly intervals (i.e. 4 months of shading for the January fruit and 1 month for the April fruit) during fruit developmental stage III on percentage rind breakdown of 'Nules Clementine' mandarin stored for 14 weeks at 7.5°C during 2006. Different lettering indicates significant differences among treatments according to Fisher's least significant difference test ($P \leq 0.05$). Values are means ($n = 8$) \pm SE.

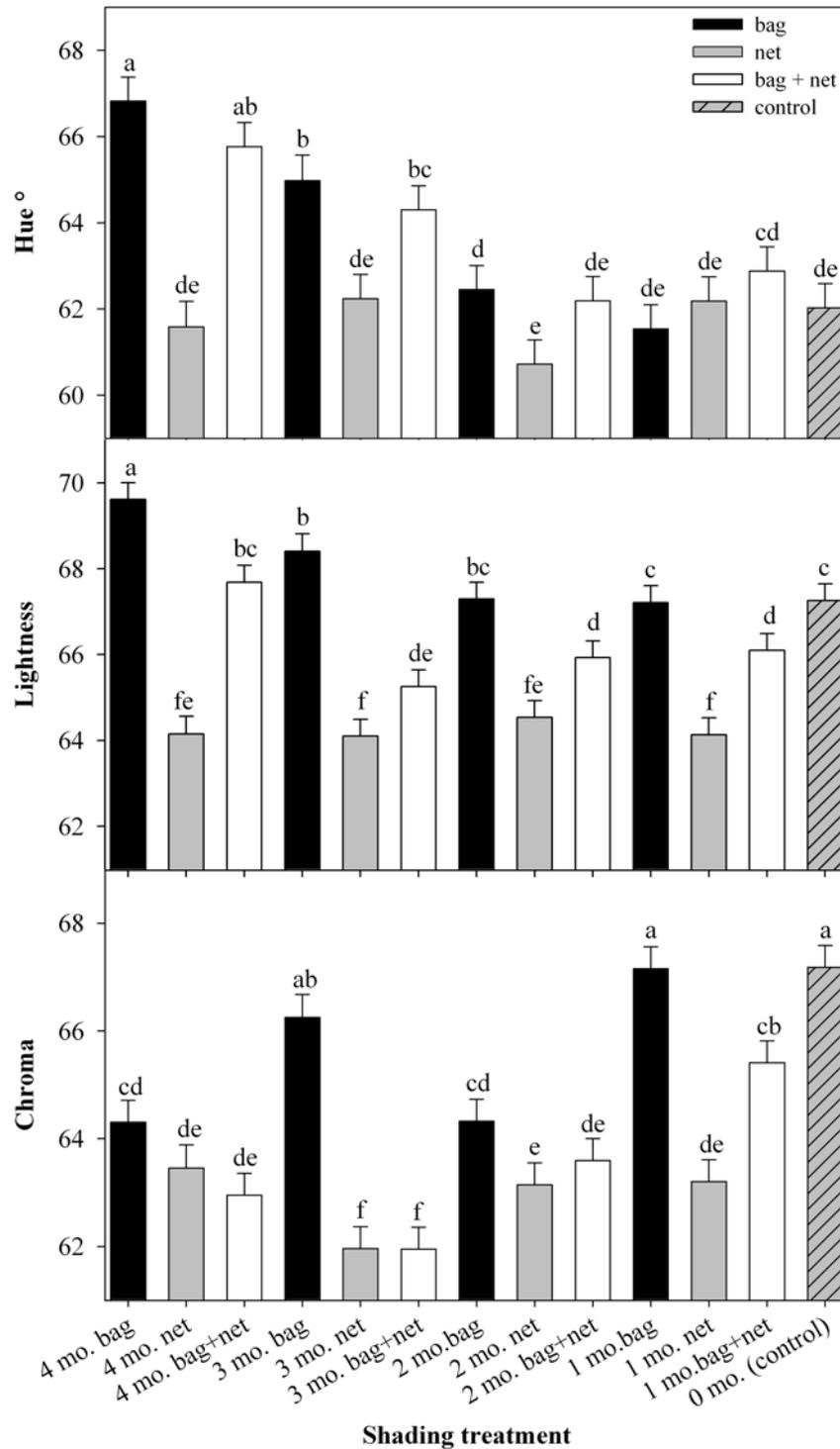


Figure 5.2.11.5.4. The influence of fruit bagging, leaf covering with shade-netting and the combination of these treatments at monthly intervals (i.e. 4 months of shading for the January fruit and 1 month for the April fruit) during fruit developmental stage III on hue angle, lightness and chroma values of 'Nules Clementine' mandarin flavedo during 2006. Different lettering indicates significant differences among treatments according to Fisher's least significant difference test ($P \leq 0.05$). Values are means ($n = 8$) \pm SE.

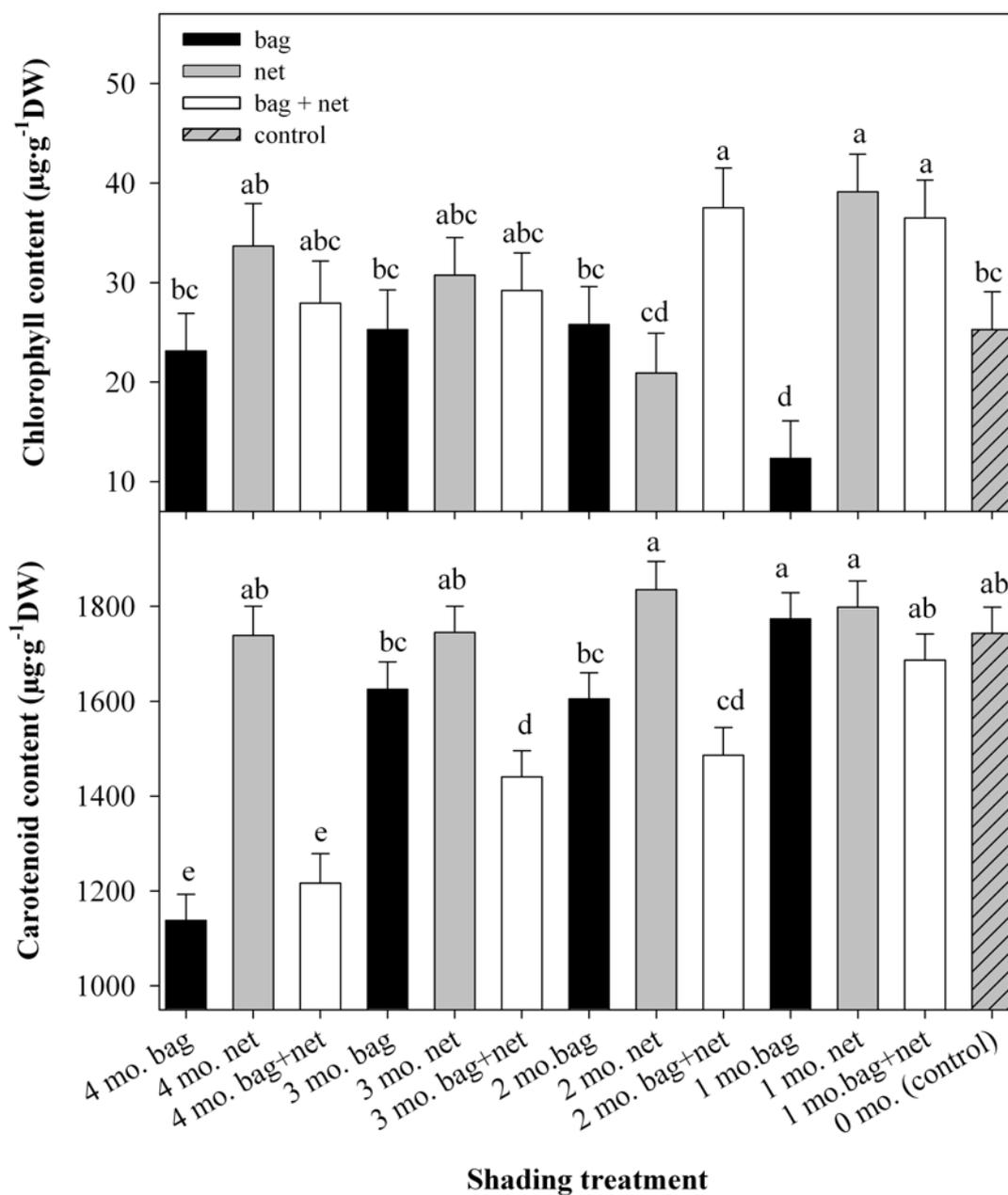


Figure 5.2.11.5.5. The influence of fruit bagging, leaf covering with shade netting and the combination of these treatments at monthly intervals (i.e. 4 months of shading for the January fruit and 1 month for the April fruit) during fruit developmental stage III on chlorophyll and carotenoid ($\mu\text{g}\cdot\text{g}^{-1}\text{DW}$) content of 'Nules Clementine' mandarin flavedo during 2006 after 14 weeks storage at 7.5°C . Different lettering indicates significant differences among treatments according to Fisher's least significant difference test ($P \leq 0.05$). Values are means ($n = 8$) \pm SE.

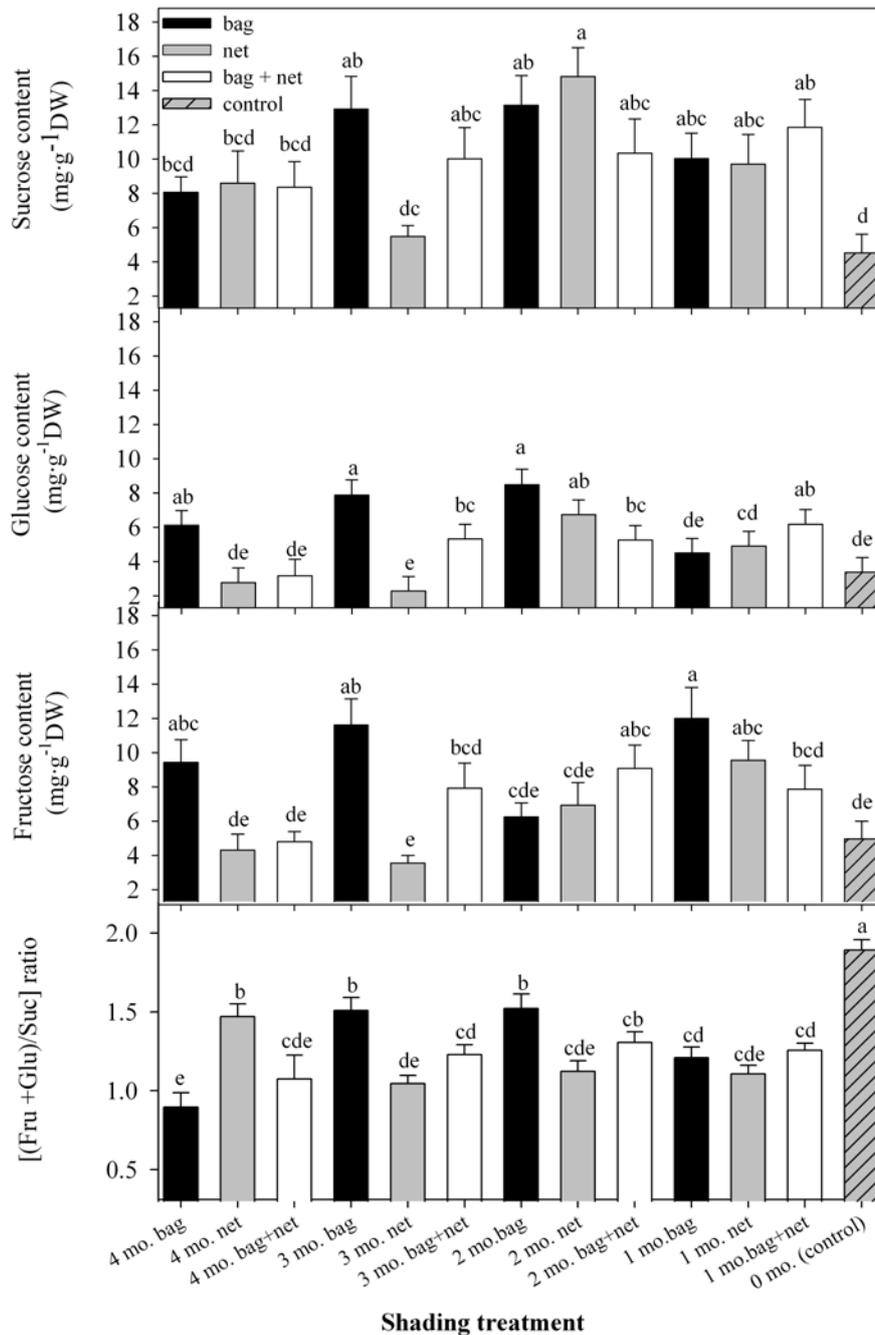


Figure 5.2.11.5.6. The influence of fruit bagging, leaf covering with shade-netting and the combination of these treatments at monthly intervals (i.e. 4 months of shading for the January fruit and 1 month for the April fruit) during fruit developmental stage III on sucrose, glucose, fructose contents (mg·g⁻¹DW) and the ratio [(Fru+Glu)/Suc] in the flavedo of 'Nules Clementine' mandarin during 2006. The carbohydrates were determined after a 14 week storage period at 7.5°C. Different lettering indicates significant differences among treatments according to Fisher's least significant difference test ($P \leq 0.05$). Values are means ($n = 8$) \pm SE.

Table 5.2.11.5.4. Results from correlation analysis between rind breakdown and various factors (chlorophyll, carotenoid and carbohydrates contents) measured in the flavedo of 'Nules Clementine' mandarin after 14 weeks of storage at 7.5°C. The preharvest treatments the fruit received were bagging, leaf covering with shade-netting and the combination at monthly intervals (Jan to Apr) during fruit developmental stage III of 2006.

Correlation	Correlation value r (Spearman)	p-value	R ²
Rind breakdown vs. Total chlorophylls	-0.07	0.451	0.005
Total carotenoids	-0.33	0.001	0.011
Rind breakdown vs. Sucrose	-0.07	0.421	0.005
Glucose	0.12	0.202	0.015
Fructose	0.17	0.063	0.029
[(Fruc+Gluc)/Suc]	0.18	0.049	0.034

5.2.11.6 Foliar application of Mg and Ca reduces the incidence of rind breakdown of 'Nules Clementine' mandarin fruit

Opsomming

Blaarbespuitings word algemeen in vrugproduksie gebruik om regstellings te maak in kritiese groeistadiums. Sulke toedingings kan effektief wees om bekende na-oes fisiologies defekte soos bitterpit van appel asook "blossom end-rot" van tamatie te beperk. Mikroklimate variasie in die Nules Clementine' mandaryne boom lei tot wanbalanse in K, Ca en Mg en daar is gepoog om deur blaarbespuitings die Ca en Mg tekort reg te stel. Daar is vanaf blomblaarval elke maand (6x) blaarbespuitings gedoen van 2% en 6% KNO₃, 2% en 6% Mg(NO₃)₂ en 1% en 4% Ca(NO₃)₂. Tydens oes is binne en buitevrugte gepluk en opgeberg teen 7.5°C en vir die voorkoms van skilafbraak gemoniteer. Die koolhidraat inhoud van die vrugte se flavedo's asook die minerale inhoud is na 8 weke opberging bepaal. Die 2% en 6% Mg asook 4% Ca en 2% K het die voorkoms van skilafbraak betekenisvol verminder in veral die binnevrugte. Die blaarbespuitings het ook die flavedo koolhidraat vlakke verander van die vrugte en het die opname van katione volgens die bekende antagonistiese meganisme beïnvloed. Die verlaging van skilafbraak word vermoed het te doen met die hoër Ca en Mg wat tot 'n algemene beter skilkondisie gelei het.

Summary

Foliar mineral nutrient application is often used in fruit production to apply specific nutrients to the plant on a timely basis during critical stages of fruit growth. These applications have been successfully used to reduce the incidence of postharvest disorders such as bitter pit of apple and blossom-end rot of tomato. Microclimatic variation within the dense leaf canopy of the 'Nules Clementine' mandarin tree causes imbalances of K, Mg and Ca in the flavedo of the rind during development. This is thought to contribute to the fruit flavedo being more susceptible to the development of physiological disorders. The aim of this study was to test the impact of foliar applied mineral nutrients (K, Ca and Mg) on the incidence of the physiological postharvest disorder, rind breakdown. Six monthly foliar sprays, starting at petal fall and continuing until one month before harvest, were applied with 2% and 6% KNO₃, 2% and 6% Mg(NO₃)₂ and 1% and 4% Ca(NO₃)₂. Fruit from the inside and outside of the tree's canopy were harvested at commercial maturity, stored at 7.5°C and evaluated for rind breakdown every second week for an 8 week period, before carbohydrate and mineral contents were determined. The 2% and 6% Mg, 4% Ca and 2% K reduced the incidence of rind breakdown in the inside and outside fruit. The foliar treatments altered the carbohydrate content of the flavedo, and influenced the uptake of these cations in antagonistic fashion. The reduction of rind breakdown by some of these treatments is thought to be the result of an increase in rind condition and specifically the carbohydrate, Mg and Ca contents in the flavedo.

Introduction

Mineral nutrient imbalances that develop during fruit growth have been identified as an important pre-harvest factor affecting the occurrence of various postharvest disorders. Even new fertigation technologies, claiming nearly optimal fertiliser application, are failing to decrease economically damaging levels of postharvest

disorders in various horticultural crops. Considering the wide range of physiological defects it is interesting to note that, to a large extent, the same elements, viz. nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg) and boron (B), are generally thought to play a role in defects of avocado (*Persea americana* Mill.) (Van Rooyen and Bower, 2005), apple (*Malus domestica* Borkh.) (Bangerth, 1979), tomato (*Lycopersicon esculentum* Mill.) (Del Amor and Marcelis, 2006), kiwifruit (*Actinidia deliciosa* Chev.) (Ferguson et al., 2003) and citrus [*Citrus sinensis* (L.) Osbeck] (Zaragoza et al., 1996; Bower, 2000). Whereas earlier research focused on localised mineral deficiencies in fruit, more recently the distribution and allocation of these mineral nutrients in the whole plant are receiving more attention (Saure, 2001; 2005; Ho and White, 2005; Del Amor and Marcelis, 2006).

The long distance transport of a mineral nutrient in the xylem and phloem and the accumulation during fruit development are influenced by canopy microclimate (temperature, relative humidity and light levels), as well as endogenous factors within the tree (sink strength, mobility of the element in the transport pathway and connectivity of the transport vessels to the fruit). Mineral nutrients are transported in the xylem by the transpiration stream, the rate of which is determined by the vapour pressure deficit (VPD) between the plant surface and external atmosphere (Tyree, 1997; Fisher, 2000). The rate of mineral nutrient transport in the xylem vessels could be influenced by fruit physiological and anatomical developments and by climatic factors such as temperature and humidity (Bangerth, 1979; Lang and Ryan, 1994; Coombe and McCarthy, 2000; Moreschet and Green, 1980).

Fluctuation in transpiration rate does not affect the transport of all mineral nutrients by the same order of magnitude. As a rule, increased transpiration affects transport of uncharged molecules to a greater extent than that of ions. For example, a lower transpiration rate leads to a decrease in Ca content in fruit, but the effect is less severe for Mg and negligible for K (Marschner, 1995). Unloading of the mineral nutrients from the xylem occurs predominantly at sites of highest evaporation due to water flow to these areas. The mobile elements in the phloem are passed at these sites from the apoplast to the phloem symplast for further distribution (Fisher, 2000; Kochian, 2000).

Solute movement in the phloem depends on a Münch pressure flow between the source and sink organs. The pressure difference is created by the loading of osmotically active solutes, e.g. sucrose, K and Mg, into the phloem sieve tubes (low solute potential and corresponding high turgor) and offloading at the sink (with high solute potential), ensuring a pressure gradient to drive the flow of the phloem sap (Patrick, 1993; 1997). This process is highly regulated by correlative dominance, a phenomenon which determines the organ sink strength (Bangerth, 1989; Minchin and Thorpe, 1996). Mineral nutrients differ substantially in their phloem sap mobility, e.g. high mobility for K, Mg and P, but low mobility for Ca and B (Bukovac and Wittwer, 1957), which is suggested to be due to element solubility in the phloem sap (Tromp, 2005).

The light environment within the fruit canopy has been shown to influence Ca accumulation of kiwifruit, apple and grape (*Vitis vinifera* L.) (Hopping 1977; Jackson et al., 1977; Giuseppe et al., 2006; Montanaro et al., 2006). In contrast to Ca, low light levels within a plant canopy result in higher K accumulation in leaves and berries of shaded 'Shiraz' grapes (Smart et al., 1985; Rojas-Lara and Morrison, 1989). These effects appear not to be related to shading of the leaves, but, to a larger extent, to shading of the grape bunch during the initial stages of berry growth (Archer and Strauss, 1989; Dokoozlian and Kliewer, 1996).

Light quantity and quality are major factors influencing plant vegetative and reproductive growth, yield, internal fruit quality (Sites and Reitz, 1949; 1950; Barry et al., 2000) and fruit colour (Jackson et al., 1977; see Section 5.2.11.4). In addition, several postharvest physiological disorders are thought to be associated with canopy position and the influence of variation in light levels on mineral nutrient allocation, predisposing fruit to develop disorders during storage (Ferguson et al., 1999). These include bitter pit of apple (Volz et al., 1994), mealiness and flesh browning in peaches [*Prunus persica* (L.) Batsch] (Crisosto et al., 1997), gel breakdown of 'Songold' plums (*Prunus salicina* Lindl.) (Taylor et al., 1993), pitting of 'Hayward' kiwifruit (Thorp et al., 2003; Montanaro et al., 2006) and rind breakdown of 'Nules Clementine' mandarin (see Section 5.2.11.4).

Some mineral nutrient cations, viz. K, Ca and Mg, have a competitive or antagonistic behaviour associated with transport and accumulation in plant organs, and the single layer tonoplast is thought to be the site of this mechanism due to the low selectivity between cations (Barkla and Pantoja, 1996; Mengel and Kirkby, 2001). Salardini and Khossussi (1972) reported cation antagonism in 'Bitter' orange (*C. aurantium*) leaves and summarised the strength of the antagonistic action of K, Mg and Ca on each other: for Mg: K>Ca, for K: Ca>Mg and for Ca: Mg>K. It has been shown that even though one cation can be replaced by another, without a problem in the short term, a negative impact will develop later by favouring K uptake at the expense of Mg and Ca (Salardini and Khossussi, 1972; Jensen, 1982; Diem and Godbold, 1993).

'Nules Clementine' mandarin (*C. reticulata* Blanco) fruit produced in South Africa develops a postharvest physiological disorder associated with the collapse of the oil glands 3–5 weeks after harvest. The symptoms, called rind breakdown (RBD), are dark lesions in the flavedo of the rind, in a randomly spread "leopard spot" pattern (Van Rensburg et al., 1995; Khumalo, 2006). Preliminary industry studies concluded that fruit borne inside the dense 'Nules Clementine' mandarin canopy and out of direct sunlight are more prone to this progressive disorder (Van Rensburg et al., 1995; 2004). It was determined that the flavedo of fruit from inside the canopy have lower Ca, Mg and carbohydrate content coupled with higher levels of K (see Section 5.2.11.3). This difference in mineral nutrient accumulation and final content of the flavedo was thought to be the result of the microclimate influence, viz. temperature and relative humidity, on transpiration rate and mineral nutrient transport (see Section 5.2.11.3).

The accumulation of mineral nutrients in the flavedo of citrus fruit follows the same pattern as the leaves and not the fleshy pulp, i.e. increased Ca and decreased K accumulation towards maturity (Sinclair and Bartholomew, 1944; Storey and Treeby, 2000; see Section 5.2.11.3). Foliar mineral nutrient application is often used in fruit production to apply specific nutrients to the plant on a timely basis during critical stages of fruit growth, because mineral nutrients are taken up more rapidly by the plant after foliar sprays than if applied to the soil. Foliar Ca applications have been successfully used to reduce the incidence of postharvest disorders such as bitter pit of apple and blossom-end rot of tomato. However, the effect of mineral nutrient application is only transient and lasts only as long as it takes for the targeted growth to mature (Obreza et al., 2008).

The aim of this study was to test the impact of foliar applied mineral nutrients (K, Ca and Mg at two concentrations each) during fruit development on the occurrence of RBD incidence of 'Nules Clementine' mandarin fruit.

Materials and methods

Sites, plant material and treatments

The experiment was conducted in two orchards of 'Nules Clementine' mandarin budded on Carrizo citrange [*Poncirus trifoliata* (L.) Raf. x [*Citrus sinensis* (Osb.) L.]] rootstock and planted in 1991 at a spacing of 4.5 x 2.5 m. After 80% petal fall in October 2006, i.e. at the start of the 2006–7 season, foliar mineral nutrients were sprayed on eight single tree replicates in a complete randomised block design on the University of Stellenbosch experimental farm, Western Cape Province, South Africa. A guard tree was used at the end of each row, and between treatments. The foliar nutrients were applied as complete canopy coverage by a handheld spray gun to the point of runoff. The foliar nutrients were applied at 30-day intervals until 1 month before harvest in May (six applications). The mineral nutrients were applied at the following concentrations: Ca(NO₃)₂ at 1 and 4%, Mg(NO₃)₂ at 2 and 6% and KNO₃ at 2 and 6%. The nitrate form of these mineral nutrients was chosen as it is accepted in horticultural production and due to its generally good uptake ability (Mengel, 2002). No additional foliar fertilisation was applied to the orchard including the untreated controls.

Fruit sampling, postharvest handling, cold storage and data collection

The fruit were picked at commercial harvest maturity on 14 and 15 May 2007. Fruit were picked separately from the inside of the canopy (<80% sun) (see Section 5.2.11.3) and outside (full sunlight exposure) from each replicate tree. The fruit were transported to a commercial packhouse where they were drenched, (thiabendazole 1000 mg·L⁻¹; guazatine 500 mg·L⁻¹; 2,4-D sodium salt 250 mg·L⁻¹; Sporekil® 1000 mg·L⁻¹) and degreened (3 days at 3 µL·L⁻¹ ethylene, >90 % RH and 20 to 22 °C) before receiving all standard commercial packhouse treatments, [thiabendazole, 500 mg·L⁻¹; imazalil, 500 mg·L⁻¹; 2,4-dichlorophenoxyacetic acid, 125 mg·L⁻¹, and polyethylene citrus wax application (Citrushine®, Johannesburg, South Africa)]. During cold storage at 7.5°C, fruit were scored for rind breakdown incidence every second week for 8 weeks. Fruit dimensions (length and diameter) were measured prior to removing the flavedo. The flavedo of the 25 fruit per replicate were pooled to ensure that enough material was available for the various analyses. The flavedo was frozen in liquid nitrogen, freeze dried (VirTis Freezemobile 25ES, The VirTis Company, Gardiner, NY, USA) and stored at -80°C. These samples were milled to a fine powder which was used for rind pigment, mineral nutrient and carbohydrate analyses.

Rind pigment analysis

To avoid repetition see section 5.2.11.4 (Materials and methods) for details of rind pigment analysis.

Mineral nutrient analysis

To avoid repetition see section 5.2.11.4 (Materials and methods) for details of mineral nutrient analysis.

Determination of flavedo carbohydrate content

To avoid repetition see section 5.2.11.4 for details of carbohydrate extraction and analysis.

Statistical analysis

Differences between fruit position effects on rind breakdown, carbohydrate and mineral nutrient contents were analysed with PROC GLM, and from the analysis of variance (ANOVA) significance differences between treatments were determined and means were separated by Fisher's least significant differences (LSD). The correlation procedure (PROC CORR) of SAS was used to determine significance of correlations between mineral nutrient concentrations (K, Ca and Mg) of inside and outside fruit flavedo (SAS v. 6.12 SAS Institute Inc., NC, USA).

Results

Foliar application of K, Mg and Ca, affected rind breakdown (RBD) occurrence of the inside fruit as well as the outside fruit (although to a lesser extent) (Fig. 5.2.11.6.1). Differences in RBD incidence between treatments were evident from week 4 to 8 for the inside fruit but only at week 8 for the outside fruit. The treatment effect on the incidence of RBD in the inside fruit can arbitrarily be separated into a higher and lower group at week 4, with the control and 6% K falling in a higher group (although not differing significantly). These two groups separate further into three at week 6 and 8 (6% K, 1% Ca and control as the highest RBD incidence, 2% Mg, 4% Ca and 2% K as intermediate and 6% Mg as the lowest). The outside fruit also had a higher and lower RBD incidence group from week 4 (low incidence grouping of 2 and 6% Mg, 4% Ca, 2 and 6% K). In addition the foliar nutrient treatments increased fruit size (expressed as diameter and length), specifically for the inside fruit of the 6% Mg treatment (Fig. 5.2.11.6.2).

The six foliar sprays altered the K, Ca and Mg content in the fruit flavedo of both the inside and outside fruit (Fig. 5.2.11.6.3). In the flavedo of fruit inside the canopy, the K content was reduced by only the 6% Mg treatment. In the outside fruit, the 2% and 6% K treatments as expected increased the K content of the flavedo. Calcium content was reduced in the inside fruit flavedo by the 2% Mg, 2 and 6% K applications, with the same pattern in the outside fruit flavedo, except that the 2% K treatment did not result in a reduction of the Ca content. Magnesium content was reduced in the inside fruit by the 4% Ca, 2 and 6% K and unexpectedly the 2% Mg. The same pattern was evident in the outside fruit flavedo, with the 2% Mg, 1 and 4% Ca, 2 and 6% K foliar treatments resulting in a reduce Mg content in the flavedo.

Carbohydrate content of the flavedo, determined as sucrose, fructose and glucose contents, after 8 weeks of storage was affected by foliar application of Mg, Ca and K compared to the control treatments in both inside and outside fruit (Fig. 5.2.11.6.4). Specifically, 2% Mg and 1% Ca increased the sucrose, fructose and glucose levels of the inside fruit flavedo. In the outside fruit flavedo, the carbohydrate content of the nutrient treatments did not differ from the control, although there were differences between foliar treatments, e.g. 2 and 6% K application resulted in higher fructose content than the 1% Ca treatment. In addition to the change in carbohydrate content after the Mg, Ca and K treatments, the ratio of reducing to non-reducing sugars [(fructose + glucose)/sucrose] in the inside fruit flavedo was higher than the control in all treatments (except 6% K), but not significantly so. No significant changes in the ratio of reducing to non-reducing sugars occurred in the outside fruit flavedo.

Correlation analysis between RBD, and mineral nutrient and carbohydrate contents in the flavedo as well as fruit size (diameter and length) show that mineral nutrient contents in the flavedo (K, Mg and Ca) had a low and non-significant correlation with RBD. In contrast, carbohydrates, as well as fruit diameter and length had a significant negative correlation with RBD, except the [(fructose + glucose)/sucrose] ratio in the outside fruit flavedo (Table 5.2.11.6.1).

Further correlations were evaluated between K, Mg and Ca content in the flavedo of the foliar treatments, to test for cation antagonism (Table 5.2.11.6.2). Significant correlations were found in the control (K vs. Mg), 6% Mg (Ca vs. Mg), 4% Ca (K vs. Mg) and 6% K (K vs. Mg). The results indicate that Ca and Mg had positive correlations, whereas K correlated negatively with Mg and Ca in the flavedo of inside and outside flavedo.

Discussion

The difference in RBD incidence between inside ($\pm 25\%$ in control) and outside ($\pm 8\%$ in control) fruit indicates that the position within the 'Nules Clementine' mandarin canopy plays a critical role in sensitivity of the flavedo towards RBD during postharvest storage. This is in accordance with other physiological disorders such as bitter pit of apple (Ferguson and Watkins, 1989), gel breakdown of 'Songold' plums (Taylor

et al., 1993) and pitting of 'Hayward' kiwifruit (Thorp et al., 2003; Montanaro et al., 2006), which have been linked to position in the fruiting canopy. The interplay between mineral nutrient distribution, as influenced by fruit position in the canopy, is thought to be part of the main underlying causes that make fruit sensitive to RBD and these other disorders mentioned (Ferguson et al., 1999).

Transpiration rate, and therefore the supply of mineral nutrients via the xylem, can in addition to climatic factors, viz. temperature and humidity, be influenced by physiological and anatomical developments during fruit development. During the cell division stage, fruitlets and young leaves have low transpiration rates, which are thought to contribute to localised Ca deficiency (Bangerth, 1979). In the later stages of maturity, the transpiration rate can be reduced by developments such as a reduction in xylem differentiation of new vessels, as well as declining hydraulic conductance, as reported in apple fruit stalks (Lang and Ryan, 1994) and some grape cultivars. This results in a significant reduction in xylem transport of water and nutrients to the fruit (Coombe and McCarthy, 2000). In citrus, the transpiration rate of the fruit is less than that of the leaves since the fruit have 30–40% fewer stomata. The number of viable stomata may also decrease as a result of wax layer build-up and subsequent plugging of the stomata (Albrigo, 1972; 1977; Moreshet and Green, 1980).

The dense canopy of leaves, which is characteristic of citrus trees, is thought to change the microclimate within the tree canopy, viz. temperature, relative humidity and PAR (Jahn, 1979; Syvertsen and Albrigo, 1980; Greene and Gerber, 1987; Barry et al., 2000) and affect transpiration rate and therefore mineral nutrient accumulation (Patrick, 1997; Fisher, 2001). Positional differences, viz. inside and outside the leaf canopy, resulted in a different accumulation pattern of K, Mg and Ca in the flavedo of fruit sampled from the inside and outside of the canopy (Koo and Sites, 1959; Section 5.2.11.3). In Section 5.2.11.3, it is reported that the inside fruit flavedo had lower Ca and Mg and higher K compared with the outside, sun-exposed fruit flavedo. It is thought that light-exposed fruit have an increased vasculature development in the fruit pericarp and pedicel, although more in the phloem and to a lesser extent the xylem bundles, leading to higher levels of Ca and Mg compared with shade-grown fruit (Biasi and Altamura, 1996). This higher rate of vascular development in higher light environments is thought to be associated with basipetally exported auxin (Montanaro et al., 2006). Auxin has been shown to stimulate Ca uptake in avocado (Cutting and Bower, 1989), tomato (Banuelos et al., 1987; Brown and Ho, 1993) and apple (Stahly and Benson, 1970; Bangerth, 1976). Mpelasoka et al. (2003) argued that the increase in K in shaded plant parts is, in almost all cases, coupled with a reduction in sugar levels and is a stress reaction to maintain osmotic potential and turgor in grapes by decreasing the impact of reduced growth due to a reduction in carbohydrates and dry matter accumulation in the shaded fruit. The lower carbohydrates and higher K content in the inside fruit flavedo (see Section 5.2.11.4.) support this argument.

Applications of foliar mineral nutrients, specifically Ca, are widely used as a timely correction of a mineral nutrient imbalance during specific growth stages (Obreza et al., 2008). Foliar mineral nutrient applications can be used to improve fruit quality (Crisosto et al., 2000) and prevent the development of physiological disorders such as bitter pit in apples (Lötze et al., 2008) and blossom-end rot (BER) of tomato (Saure, 2001). The reduction of RBD incidence in the inside, and to a lesser extent the outside fruit, after 4% Ca, 2 and 6% Mg is therefore in agreement with the known importance of these elements on fruit quality. The less dramatic effect of the foliar treatments on the RBD incidence of the outside fruit could be due to an already adequate and more balanced mineral nutrient composition, viz. Mg and Ca, in their flavedo (see Section 5.2.11.3).

The improvement fruit size of inside fruit by the foliar mineral treatment, can be argued to be as a result of these applied mineral nutrients countering the negative effect of the inside fruit's weak sink capabilities. The outside fruit size did not improve markedly, since these fruit probably received adequate mineral nutrients due to their dominant position and therefore stronger sink strength. Fruit size of inside fruit may therefore be limited by inadequate mineral nutrient supply, whereas outside fruit size is limited by genetic potential rather than nutrient supply.

A mineral nutrient deficiency in citrus leaves normally produces chlorotic symptoms (Chapman, 1968) and is associated with chloroplast structural damage. Such deficiencies in the citrus rind, although being a modified leaf, are unfortunately less evident. However, these deficiencies during fruit development could reduce rind condition, i.e. susceptibility to physiological disorders, by not only impacting on the cellular structure, but also the carbohydrate levels in the flavedo. The photosynthetic ability (Bean and Todd, 1960) and carbohydrate accumulation of the flavedo (see Section 5.2.11.4) would be hindered by mineral nutrient deficiencies in the same way as in a leaf, viz. reduction of chlorophyll content and of sucrose transport (Shaul, 2002). Potassium deficient leaves of tomato (Wall, 1939), wheat (*Triticum aestivum* L.) (Ward, 1960) and soy bean (*Glycine max* L. Merr.) (Huber, 1984) have been shown to have an increased accumulation of soluble sugars, whereas Ca and Mg deficiencies in bean (*Phaseolus vulgaris* L.) leaves (Fischer and Bremer, 1983)

and tomato and spinach (*Spinacia oleracea* L.) plants showed increased starch content (Vesk et al., 1966). In citrus leaves, deficiencies of K, Mg and Ca reduced starch content, although K increased sugar accumulation. In addition Ca deficiency also resulted in a loss of chlorophyll, total proteins and ribulose 1,5-bisphosphate carboxylase activity (Lavon, 1999), which could indicate that Ca deficiency would increase the rate of leaf senescence as Ca has been shown to delay leaf senescence (Poovaiah and Leopold, 1973; Poovaiah, 1988).

The vital role of Mg in photosynthesis (Krause, 1977) and activation of Rubisco (Portis, 1992) during carbohydrate synthesis could be improved by Mg foliar sprays. In addition, Mg is important in the transport of sucrose from the leaves through Mg-dependent H⁺ ATPase (Marschner, 1995). This effect was illustrated by the increase of starch in Mg-deficient leaves of bean, tomato and spinach as discussed above. The increase of carbohydrates in the flavedo of the inside fruit after 2%, 6% Mg and 1% Ca applications gives an indication that these nutrients improved the CO₂ fixation ability of the inside flavedo. On the other hand, CO₂ fixation was probably functioning optimally in the outside fruit and therefore mineral nutrient application did not result in a significant increase in carbohydrate levels. Deficiencies in Mg and K of adjacent leaves could also contribute to the lower carbohydrate content of the inside fruit as both of these elements are important in sucrose transport from source to sink (Mengel and Haeder, 1977; Shaul, 2006). Chen et al. (2008) showed that in the weeks after colour break and prior to harvest most carbohydrates are imported and not synthesised in the flavedo. Potassium nutrition is known to impact on rind quality aspects of citrus fruit. At high levels it results in larger fruit, with reduced colour development, increased rind thickness, and possibly other physiological disorders (Grierson, 1965; Bar-Akiva, 1975; Bower, 2000; Treeby et al., 2000; Alva et al., 2006).

Calcium is involved in a number of cellular functions, the best known of which is its key role as a structural component of macromolecules, through coordination of reversible linkages in the cell wall (apoplast) and membranes (symplast) (Wyn Jones and Lunt, 1967). Adequate Ca levels are known to delay leaf senescence (Poovaiah, 1988) and Ca deficiency is argued to enhance senescence, leading to the accumulation of starch (Lavon et al., 1995). Therefore the increase in carbohydrate content after the 4% Ca sprays could indicate a slower senescence process in the flavedo leading to a more active chloroplast and Rubisco, involved in carbohydrate synthesis. This relationship between carbohydrates and K, Mg and Ca still remains speculative. However, the separate contribution of these solutes in the flavedo as well as an interaction influencing rind condition seems likely.

Following the application of K, Mg and Ca, cation antagonism becomes evident in the inside and outside fruit flavedo. Mg and Ca generally had positive correlations with each other indicating the mutual improvement of uptake after treatments. This is not in accordance with Schimanski (1981) and Mengel and Kirkby (2001), who thought that Mg deficiency could result from not only K but also Ca antagonism. However, their argument focused more on the uptake into the plant and transport than accumulation in the fruit. The negative correlation between K and Mg is in agreement with Mengel and Kirkby (2001) and Salardini and Khossussi (1972). This could be interpreted as an indication that the higher Ca levels (due to the foliar sprays), enabled Mg content to increase instead of K.

These data as well as the results in Section 5.2.11.3, suggest that mineral nutrient imbalances of especially K, Mg and Ca, could be more important than the absolute levels of the respective minerals in the flavedo. It has been suggested that imbalances of minerals can contribute to postharvest disorders of apple (Ferguson and Watkins, 1983), kiwifruit (Ferguson et al., 2003) and avocado (van Rooyen and Bower, 2005). Wertheim (2005) argued that the ratio of these minerals is important and it is not only desirable to have a high Ca level but also a low K and Mg to avoid bitter pit and senescent breakdown of apples. The initial studies from a postharvest perspective on Ca shows that this cation can delay senescence in various plant tissues by stabilizing the cell walls and membranes (Poovaiah and Leopold, 1973; Liebermann and Wang, 1982; Ferguson, 1984). However, its role has evolved further as the importance of intercellular Ca and the effect on signal transduction and cell metabolism was realised (Poovaiah, 1988). It is possible that the less RBD-susceptible outside fruit, with the higher Ca content in the flavedo (see Section 5.2.11.3) could thus not only develop stronger cellular structure, but also be better equipped to avoid premature senescence as a result of postharvest stress. By incubating apple slices with Ca and Mg, Lieberman and Wang (1982) slowed the senescence process by stabilising the cellular membranes. They argued that Mg applied in addition to Ca provided an additional senescence delaying action. It is therefore possible that the higher Mg content in the outside fruit rind (see Section 5.2.11.3), could play a role in addition to preharvest carbon fixation (see Section 4) in the determination of rind condition during the postharvest period after being released by the Mg-dechelataase from the chlorophyll molecule (Dangl et al., 2000).

In conclusion, it is proposed that the application of Ca and Mg foliar nutrient sprays can reduce the incidence of rind breakdown of 'Nules Clementine' mandarins. This cultural practice is thought to adjust the mineral nutrient levels and in particular the imbalances within the flavedo of inside fruit to levels less conducive to RBD development. The physiological action of these mineral nutrients is thought to involve better cellular structure and functioning, specifically carbohydrate accumulation during development, which better maintains sound physiological integrity during a prolonged postharvest period.

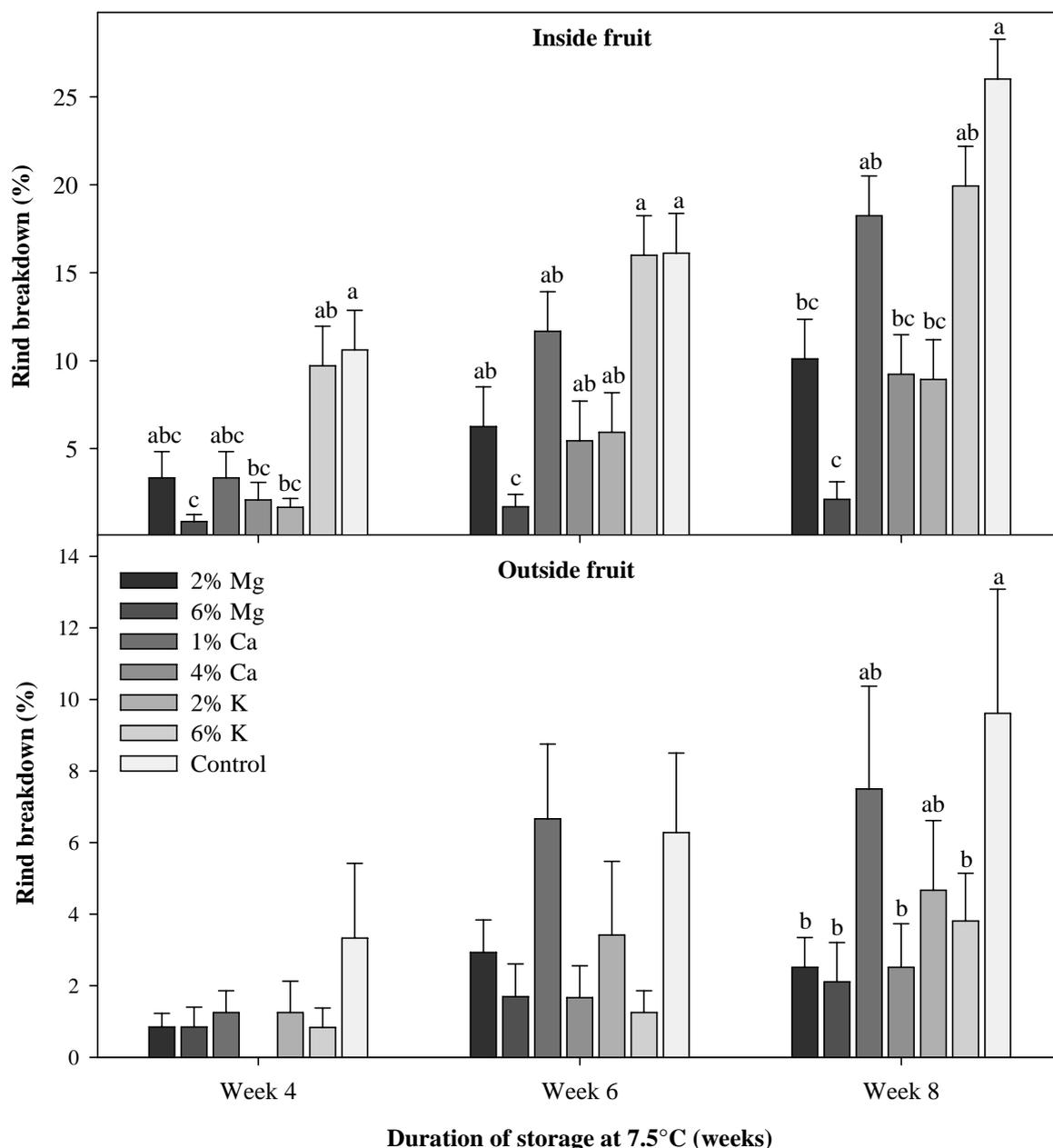


Figure 5.2.11.6.1. Effect of monthly applied (Oct. to Mar.) foliar mineral sprays on percentage rind breakdown of 'Nules Clementine' mandarin fruit from both inside and outside the canopy during 8 weeks of storage at 7.5°C. Values are means (n = 8) with standard error bars. Different letters indicate significant differences among foliar application treatments within a canopy position (inside and outside) for each sampling time according to Fisher's least significant difference test ($P \leq 0.05$).

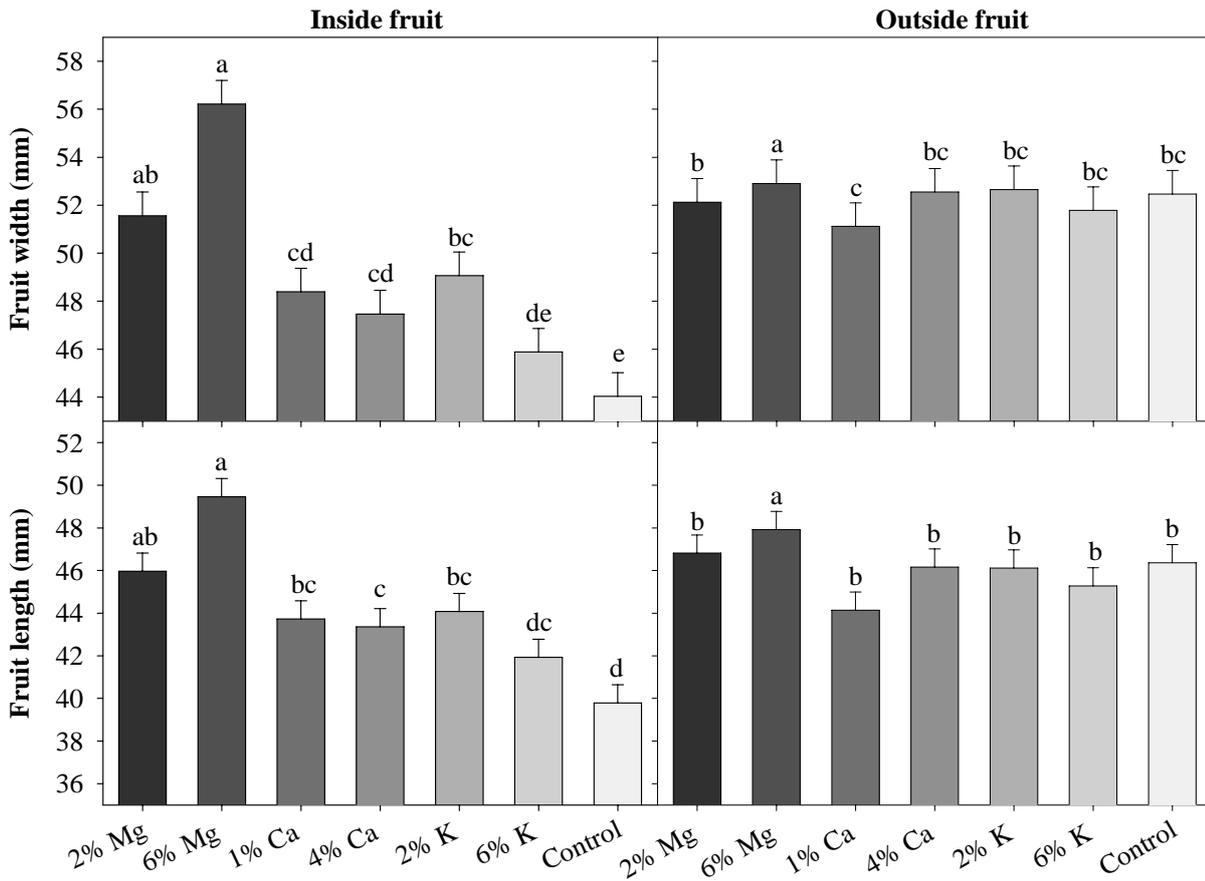


Figure 5.2.11.6.2. Effect of monthly applied (Oct. to Mar.) foliar mineral sprays on fruit size (diameter and length) of 'Nules Clementine' mandarin fruit from both inside and outside the canopy. Values are means ($n = 8$) with standard error bars. Different letters indicate significant differences among foliar application treatments within a canopy position (inside and outside) according to Fisher's least significant difference test ($P \leq 0.05$).

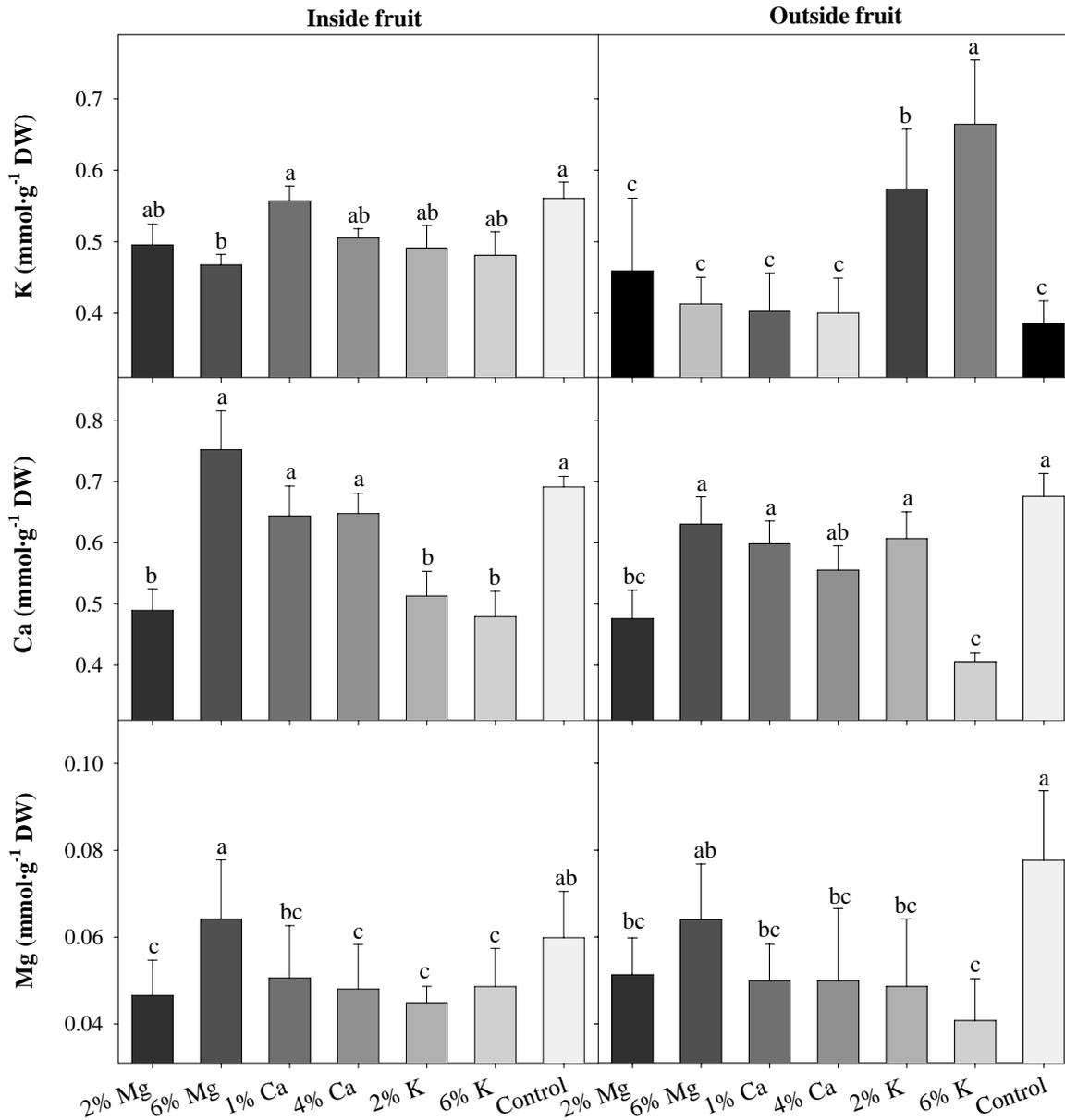


Figure 5.2.11.6.3. Effect of monthly applied (Oct. to Mar.) foliar mineral sprays on K, Ca and Mg content in the flavedo of 'Nules Clementine' mandarin fruit from inside and outside the canopy. Values are means ($n = 8$) with standard error bars. Different letters indicate significant differences among foliar application treatments within a canopy position (inside and outside) according to Fisher's least significant difference test ($P \leq 0.05$).

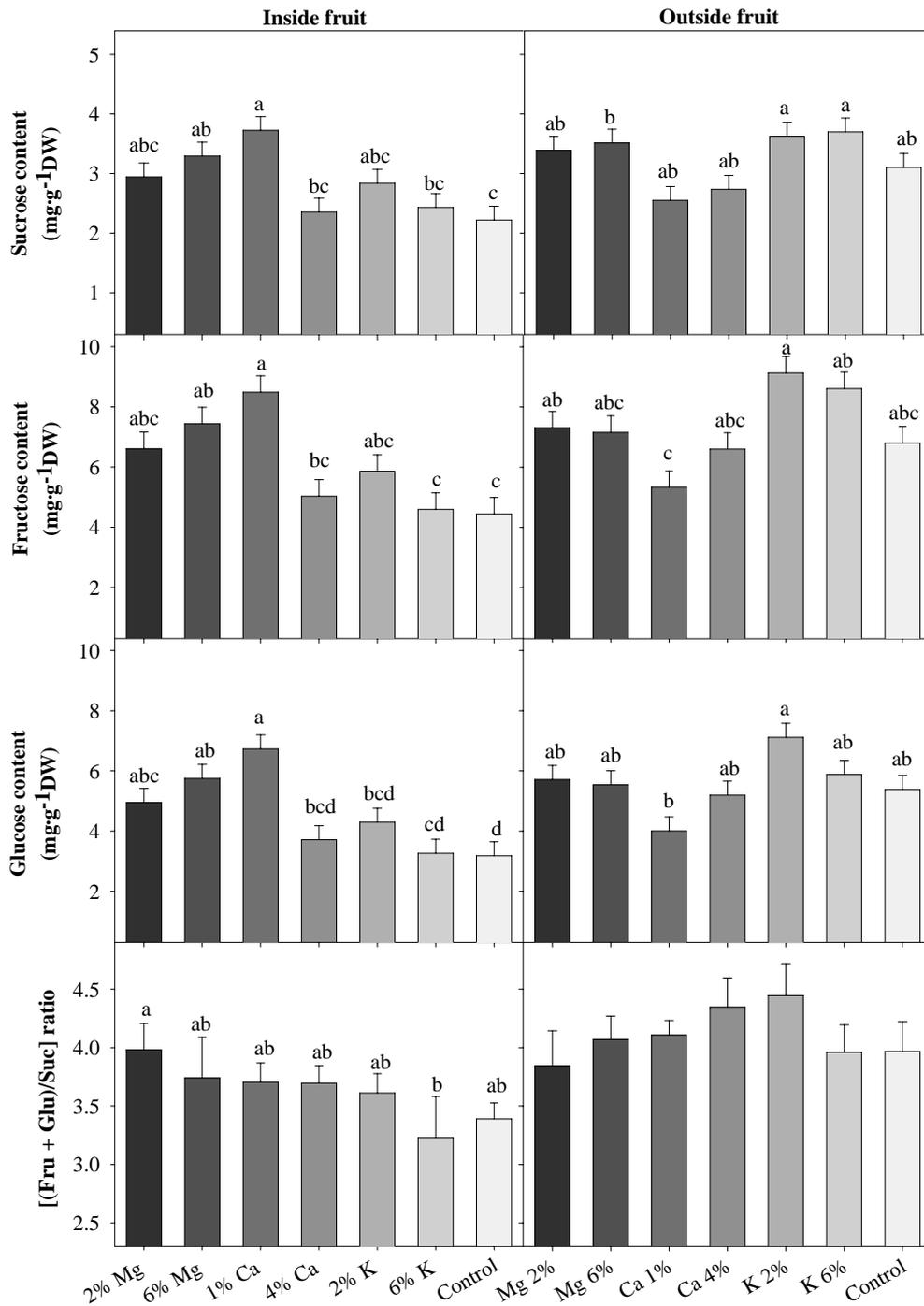


Figure 5.2.11.6.4. Effect of monthly (Oct. to Mar.) applied foliar mineral sprays on the carbohydrate content (sucrose, glucose and fructose) in the flavedo of 'Nules Clementine' mandarin fruit from inside and outside the canopy after 8 weeks of storage at 7.5°C. Values are means ($n = 8$) with standard error bars. Different letters indicate significant differences among foliar application treatments within a canopy position (inside and outside) according to Fisher's least significant difference test ($P \leq 0.05$).

Table 5.2.11.6.1. Spearman correlations between rind breakdown, mineral nutrients (K, Mg and Ca) and carbohydrate (sucrose, glucose and fructose) contents in the flavedo as well as fruit dimensions after application of mineral nutrients (Mg, Ca and K) during 2007 on 'Nules Clementine' mandarin.

Correlation	Correlation value r (Spearman)	p-value	R ²
Rind breakdown vs. K	0.12	0.22	0.014
Mg	0.13	0.17	0.016
Ca	-0.05	0.60	0.002
Rind breakdown vs. Sucrose	-0.27	0.001	0.073
Glucose	-0.38	0.001	0.144
Fructose	-0.32	0.001	0.102
[Fru+Glu]/Suc (Inside fruit)	-0.44	0.001	0.194
(Outside Fruit)	0.05	0.701	0.003
Rind breakdown vs. Diameter	-0.60	0.001	0.361
Length	-0.61	0.001	0.371

Table 5.2.11.6.2. Results from Pearson correlation analysis between K, Mg and Ca in the inside and outside fruit flavedo of 'Nules Clementine' mandarins, after mineral nutrient application (Mg, Ca and K) during 2006/7 season.

Foliar Application treatment	Correlation	Inside fruit flavedo			Outside fruit flavedo		
		Correlation value r (Pearson)	p-value	R ²	Correlation value r (Pearson)	p-value	R ²
Control	K vs. Mg	-0.72	0.041	0.518	0.16	0.728	0.026
	K vs. Ca	0.09	0.824	0.008	0.04	0.914	0.002
	Ca vs. Mg	0.17	0.690	0.029	0.25	0.596	0.063
2% Mg	K vs. Mg	0.65	0.115	0.423	-0.14	0.768	0.020
	K vs. Ca	-0.11	0.829	0.020	-0.18	0.706	0.032
	Ca vs. Mg	-0.58	0.228	0.336	0.65	0.075	0.410
6% Mg	K vs. Mg	0.07	0.877	0.005	-0.68	0.093	0.348
	K vs. Ca	0.12	0.805	0.012	-0.46	0.245	0.212
	Ca vs. Mg	0.96	0.001	0.922	0.39	0.390	0.325
1% Ca	K vs. Mg	-0.66	0.075	0.436	-0.47	0.245	0.221
	K vs. Ca	-0.35	0.436	0.123	-0.16	0.707	0.026
	Ca vs. Mg	0.69	0.082	0.476	0.60	0.113	0.360
4% Ca	K vs. Mg	-0.73	0.042	0.533	-0.66	0.077	0.436
	K vs. Ca	-0.31	0.452	0.096	-0.49	0.217	0.240
	Ca vs. Mg	0.55	0.159	0.303	0.96	0.001	0.922
2% K	K vs. Mg	-0.11	0.789	0.012	-0.65	0.080	0.423
	K vs. Ca	0.43	0.291	0.185	-0.70	0.051	0.490
	Ca vs. Mg	0.48	0.232	0.230	0.85	0.007	0.723
6% K	K vs. Mg	-0.63	0.091	0.397	-0.80	0.054	0.640
	K vs. Ca	-0.37	0.371	0.137	-0.42	0.410	0.176
	Ca vs. Mg	0.88	0.004	0.774	-0.11	0.211	0.010

5.2.11.7 **Postharvest pigment and carbohydrate changes in relation to the incidence of rind breakdown of 'Nules Clementine' mandarin fruit**

Opsomming

Skilafbraak van 'Nules Clementine' mandaryn ontwikkel 3-5 weke na oes en die voorkoms van die fisiologiese skildefek is hoër in vrugte wat binne in die blaredakontwikkel, en veral oorskadu word gedurende groeifases I en II. Hierdie "binnevrugte", wat meer geneig is om skilafbraak te ontwikkel, het swakker skille agv die laer Ca, Mg koolhidrate asook pigmente tesame met 'n verhoogde K inhoud in die flavedo. Na-oes etileen benadeling (ontgroening), 'n vertraging in tyd voor verkoeling, verkoelings tempertuur (7.5°C), asook

tydperk van opberging het almal 'n negatiewe rolle in die voorkoms van skilafbraak. Die pigmente, asook koolhidraat inhoud in die flavedo speel vermoedelik 'n rol in voorkoming van die defek en goedgekleurde vrugte ontwikkel betekenisvol minder van die skildefek. Die resultate in die eksperiment dui egter daarop dat enige faktor bekend vir die verhaasting van die verouderingsproses in vrugte kan tot verhoogde skilafbraak lei.

Summary

The progressive disorder referred to as rind breakdown (RBD) of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco) starts to develop during storage approximately 3 to 5 weeks after harvest. Fruit developing inside the tree canopy and shaded from direct sunlight exposure until stage III of fruit development, is known to be more sensitive to RBD. These fruit have a weaker rind condition which is ascribed to lower carbohydrate, pigment, Mg and Ca contents, and coupled with a higher K content in the flavedo. Postharvest ethylene degreening treatment, as well as storage temperature (chilling and non-chilling conditions) and the storage duration have been tested on the incidence of RBD. Two experiments were conducted in the current study. In the first experiment, fruit were subjected to ethylene degreening and a delay in commencement of cold storage. In the second experiment, fruit from the inside and outside of the canopy were cold-stored at -0.5°C or 7.5°C during two seasons (2005 and 2007). The rind pigment and carbohydrate contents as well as rind colour and RBD incidence were recorded during a prolonged storage period. These treatments are known to promote senescence and the flavedo, a modified leaf, reacted negatively to all treatments with an increase in RBD incidence, except for storage at -0.5°C which resulted in a lower occurrence of RBD. Overall, results indicate that the incidence of RBD can be aggravated by senescence-promoting factors during the postharvest handling of fruit and this is thought to lead to a premature senescence of the flavedo. Anatomical studies supported this conclusion.

Introduction

'Nules Clementine' mandarin (*Citrus reticulata* Blanco) is a widely-planted cultivar in the citrus producing regions of the Eastern and Western Cape provinces of South Africa. The $\pm 1\,700$ ha of plantings produce around 2.3 million 15 kg export cartons annually (i.e. $\pm 35\,000$ tons), accounting for 35 % of all soft citrus exports from southern Africa, and it is therefore considered to be an important cultivar (Barry and Rabe, 2004; CGA, 2008). However, 'Nules Clementine' mandarin fruit are prone to developing a progressive postharvest physiological disorder with symptoms becoming visible 3 to 5 weeks after harvest. Symptomatic development of this disorder coincides with the commercial shipping period and is therefore extremely problematic as the disorder can lead to tremendous financial losses at this stage of the logistical supply chain. This disorder is commonly referred to as rind breakdown (RBD) in the South Africa citrus industry. Rind breakdown manifests itself in the flavedo of the rind as randomly distributed dark/brown spots, locally described as a "leopard spot" pattern. The darkened areas are associated with the collapse of oil glands (Fig. 5.2.11.7.1) (Van Rensburg et al., 1995; 2004; Cronje, 2007).

This disorder is not thought to be associated with low temperature storage, unlike pitting of grapefruit (*C. paradisi* Macf.) in Florida (Schiffmann-Nadel et al., 1971), although similarities exist in the visual symptom development (Petracek, 2005). However, it is known that 'Nules Clementine' mandarin fruit have a higher propensity to develop RBD when stored at $\pm 7^{\circ}\text{C}$ than 4 or 11°C (Khumalo, 2006). Initial industry studies could not elucidate the causal factors triggering RBD, but identified preharvest growing conditions which influence fruit rind sensitivity. The single-most important factor identified in these studies was the role of the fruit's position within the canopy. It was concluded that fruit shaded from direct sunlight exposure were more prone to develop RBD (Van Rensburg et al., 1995; 2004). These RBD sensitive fruit were observed to have a paler/lighter orange-coloured rind. This raised the question of whether low rind pigment content contributed to RBD development, or if the poor colouring was the result of RBD symptom development. This question was addressed in Sections 4 and 6 of this dissertation, where it was concluded that the flavedo of fruit borne on inside canopy positions had lower pigment content (chlorophyll preharvest and carotenoid postharvest), in addition to lower carbohydrate content. Adequate sugar levels are needed to facilitate citrus rind colour development (chloroplast to chromoplast conversion) (Huff, 1984), and are also required for pre- and postharvest respiration critical to fruit developmental and cellular maintenance processes. The flavedo of fruit borne on inside canopy positions also had higher K and lower Ca and Mg contents during development and these factors are thought to contribute, together with reduced carbohydrate and pigment contents, to a weakened rind condition and therefore a higher RBD susceptibility (see Sections 5.2.11.3 and 5.2.11.4).

Postharvest conditions (storage temperature and duration) have been suggested to influence the incidence of RBD in 'Nules Clementine' mandarins (Khumalo, 2006) and are known to influence the incidence of

various citrus rind disorders. The superficial flavedo necrosis of 'Shamouti' orange (*C. sinensis* Osbeck.), known as noxan in Israel, was reduced if the fruit were stored at ca. 5°C in 97–99% relative humidity (RH) (Ben Yehoshua et al., 2001). Postharvest rind staining in 'navel' orange fruit, caused by rind breakdown, is characterised by irregular colourless depressed areas in the rind and the oil glands appear to be intact (Agustí et al., 2001; Alférez et al., 2003). Previous microscopic studies have shown that cellular damage, seen as a reduced cytoplasm and irregular cell walls, occurs in the zone where the flavedo and albedo connect. Over time the volume of collapsed cells progresses further into the outer flavedo and inner albedo, but without affecting the cuticular and wax layer (Agustí et al., 2001). An alteration of rind water status by transferring fruit from 45 to 95% RH, at a constant temperature, was shown to be a critical factor in the incidence of rind breakdown of navel oranges (Alférez et al., 2005). This dehydration and rehydration effect was shown to be the causal factor in postharvest rind pitting of 'Marsh' grapefruit. Furthermore, in the case of waxed fruit, a drastic change in RH prior to waxing, enhanced the severity of this condition, but would not be the causal factor of the lesions and resulting rind breakdown of 'navel' oranges (Alférez and Burns, 2004). The increased electrolyte leakage triggered by the shift from low to high RH was argued by Alférez et al. (2008) to be an indication of the loss of membrane function and organization. They speculated that an increase of phospholipase A2 (PLA2) in 'Fallglo' mandarin a hybrid between 'Bower' mandarin [*C. reticulata* × (*Citrus paradisi* Macf. × *C. reticulata*)] and 'Temple' tangor (*C. reticulata* × *C. sinensis*), and 'navel' orange plays a prominent role in the symptomatic development of pitting. The application of shellac-based wax on white grapefruit led to a higher incidence of pitting compared to carnauba or polyethylene based waxes. Petracek et al. (1998) suggested that this influence of postharvest pitting by the wax type was probably caused by a differential reduction of internal fruit O₂ levels.

Ethylene degreening is widely used as a postharvest practice in the citrus industry to ensure adequate rind colour development. However, the effectiveness of using this senescence-promoting hormone not only depends on the fruit rind physiological stage, but also on the specific cultivar and can lead to the development of various rind defects (Krajewski and Pittaway, 2002). Ethylene, known to stimulate senescence in plant organs, may also protect plant tissue against stress (Yang and Hoffmann, 1984), as reported for *Penicillium* sp. infection (Marcos et al., 2005), rind staining (Lafuente and Sala, 2002), chilling injury (Porat et al., 1999; Lafuente et al., 2001; 2004) and non-chilling rind pitting (Cajuste and Lafuente, 2007). Although additional unknown defences are suspected by Cajuste and Lafuente (2007) in the reduction of non-chilling pitting, part of the resistance that was triggered by ethylene application involved phenolic metabolism.

The aims of this study were firstly, to investigate the influence of two postharvest practices on RBD incidence, viz. ethylene treatment (degreening) and time before commencing cold storage. Secondly, to investigate the difference between the sensitivity of fruit from the inside (low light) and outside (high light) of the tree canopy on RBD development during prolonged storage at a chilling (-0.5°C) and non-chilling temperature (7.5°C). Thirdly, to supplement the recording of the visual RBD symptom development of cold stored fruit, the carbohydrate and pigment contents of the flavedo were determined. Fourthly, a comparative microscopic investigation was conducted on mature fruit flavedo with and without RBD symptoms after 14 weeks of storage.

Materials and method

Sites, postharvest handling and data collection

Experiments were conducted using fruit from an orchard of 'Nules Clementine' mandarin budded on Carrizo citrange [*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (Osb)L.] rootstock and planted in 1991 at a spacing of 4.5 x 2.5 m on the University of Stellenbosch experimental farm Welgevallen, Western Cape Province, South Africa.

The fruit were picked at commercial harvest maturity on 18 May 2004, 14 and 15 May 2005 and 16 May 2007. Fruit picked during 2005 and 2007 were from the inside (<80% sun) and outside (full sunlight exposure) of the canopy of selected trees (see Section 5.2.11.3). However, in 2004 no distinction was made between inside and outside fruit, as two separate bins of fruit were harvested. One of these bins received a degreening treatment as described below. Harvested fruit were transported to a commercial packhouse each year where they were drenched (thiabendazole 1000 mg·L⁻¹; guazatine 500 mg·L⁻¹; 2,4-D sodium salt 250 mg·L⁻¹; Sporekil® 1000 mg·L⁻¹) and degreened (3 days at 3 µL·L⁻¹ ethylene, >90 % RH and 20 to 22 °C) before receiving all standard commercial packhouse treatments, [thiabendazole, 500 mg·L⁻¹; imazalil, 500 mg·L⁻¹; 2,4-dichlorophenoxyacetic acid, 125 mg·L⁻¹, and polyethylene citrus wax application (Citrushine®, Johannesburg, South Africa)].

After packing in 2003, the fruit were separated into eight replicates of 25 fruit per replicate. The fruit were kept at ambient conditions (± 20°C) in a shaded packhouse. The fruit were put into 7.5°C cold storage after

7, 11 or 14 days, whereafter the cumulative RBD incidence was recorded every second week. After packing, in 2005 and 2007, the inside and outside fruit were further sub-divided into two lots for storage at -0.5°C or 7.5°C . For each treatment (e.g. inside vs. outside fruit stored at -0.5°C or 7.5°C), eight replicates of 25 fruit each were pre-designated for each evaluation date before being put into cold storage and rated for RBD incidence every second week for the duration of the experiment. Fruit rind colour was determined on these dates, using a chromameter (Minolta NR 4000, Osaka, Japan) on the side of the fruit with the highest colour development. Fruit dimensions (length and diameter) were measured prior to removing the flavedo of the fruit, which was pooled for each replicate, ensuring adequate flavedo material for the various analyses. The flavedo was frozen in liquid nitrogen whereafter it was freeze dried (VirTis Freezemobile 25ES, The VirTis Company, Gardiner, NY, USA) and stored at -80°C . These samples were milled to a fine powder which was then used for rind pigment and carbohydrate analyses.

Rind pigment analysis

To avoid repetition see Section 5.2.11.4 (Materials and methods) for details of rind pigment analysis.

Determination of flavedo carbohydrate content

To avoid repetition see Section 5.2.11.4 (Materials and methods) for details of carbohydrate extraction and analysis.

Determination of fruit respiration rate

Respiration rate ($\text{mg CO}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was repeatedly measured from eight replications of five fruit of each of the treatments. The fruit were put into each of the eight airtight 1–L jars and closed for 30 min before a gas sample was taken from each jar, using an airtight syringe for injection into a gas chromatograph (Model N6980, Agilent Inc., Wilmington, U.S.A.) fitted with a PorapakQ and a Molsieve packed column. The volume of free space in the jar as well as the mass of the fruit was used to calculate CO_2 production rates.

Microscopic preparation

Rind samples (5 x 5 mm) from fruit stored for 14 weeks, with or without RBD symptoms, were fixed in a 1:1 (v/v) solution of 2.5% glutaraldehyde and 2.5 % formaldehyde in 0.075 M phosphate buffer (NaPO_4), pH 7.4 for 1 to 2 h at room temperature. The material was rinsed three times for 10 min in the 0.075 M buffer before being fixed in 0.5% aqueous osmium tetroxide (OsO_4) for 1 to 2 h. Thereafter, the samples were rinsed three times with distilled water, before dehydration in an ethanol concentration series [30%, 50%, 70%, 90% and 100% (repeated three times)]. The material was stored in 100% ethanol before infiltration for transmission electron microscope (TEM) and light microscopy with 30% Quetol in ethanol for 1 h, followed by 60% Quetol for 1 h and pure Quetol for 4 h prior to polymerisation at 60°C for 39 h. The material was cut into 0.5–1 μm sections with a Reichert ultracutE ultra-microtome (Reichert AG., Vienna, Austria) before being transferred onto water droplets on a specimen slide and stained with Toluidine blue for light microscopic images. Light microscope sections were viewed with a Zeiss Axiovert 200 (Zeiss, Gottingen, Germany) microscope fitted with a Nikon digital camera DXM 1200 (Nikon Insteck 10, Kanagawa, Japan). The TEM ultrathin sections were made using a Reichert ultracutE ultra-microtome and contrasted in 4% aqueous uranyl acetate (10 min) before being rinsed with water and followed by 2 min exposure to Reynolds lead citrate and rinsed in water (Reynolds, 1963). TEM sections were viewed with a Phillips EM3001 transmission electron microscope (Phillips, Eindhoven, Netherlands) set at 200 kV.

The material for the scanning electron microscopic (SEM) images was collected directly after the above-mentioned dehydration step and critical point drying with liquid CO_2 before being mounted on a stub and splattered with gold (Biorad E3000, Pobrón, West Sussex, UK) (Van der Merwe and Peacock, 1999). The samples were viewed with a JSM840 Joel SEM (Joel, Tokyo, Japan) at 5 kV and a working distance of 12 mm.

Statistical analysis

Differences in RBD, carbohydrate and mineral nutrient contents between fruit canopy position effects were analysed with PROC GLM, and from the analysis of variance (ANOVA) significance differences between treatments were determined and means were separated by least significant differences (LSD) (SAS v. 6.12, SAS Institute, Cary, NC, USA).

Results

Rind breakdown incidence

The incidence of RBD (Fig. 5.2.11.7.1) during 2003 followed a progressive pattern as the storage period increased (Fig. 5.2.11.7.2). A delay in cooling of the fruit resulted in increased RBD, which became evident

after only 2 weeks of cold storage. Furthermore, ethylene treatments accentuated the incidence of RBD when storage was delayed by 7 and 11 days. However, no noticeable difference occurred when storage was delayed by 14 days with or without degreening.

The RBD incidence recorded during 2005 and 2007 followed the same pattern as observed in 2003, whereby the incidence of RBD increased during storage (Fig. 5.2.11.7.3 A to F). There was a difference in RBD incidence of fruit from the two different canopy positions in both the 2005 and 2007 seasons, with the inside fruit being more susceptible to the development of RBD (Fig. 5.2.11.7.3 A and B). The possibly mitigating influence of low storage temperature (-0.5°C) on RBD was not as evident in the 2005 season as in 2007, where significantly higher incidence of RBD occurred in the fruit stored at 7.5°C (Fig. 5.2.11.7.3 C and D).

Rind colour and pigment content

Fruit rind colour, expressed as hue angle, lightness and chroma values, was influenced by canopy position as well as subsequent storage temperature during both seasons (Fig. 5.2.11.7.4). In 2007 the measurements started at harvest (week 0), whereas measurements started in week six in 2005. Fruit borne on the outside of the trees canopy had lower hue angle and higher lightness and chroma, which resulted in visibly more orange fruit compared to a pale yellow rind of the inside fruit (Fig. 5.2.11.7.4). Although the inside fruit rind colour improved during storage, the rind colour remained poorer (higher hue angle) than the outside fruit. The outside fruit stored at -0.5°C developed a better rind colour (lower hue angle) after 6 weeks in 2005 and 8 weeks in 2007 than the inside fruit stored at 7.5°C (Fig. 5.2.11.7.4).

The flavedo chlorophyll content declined drastically after harvest and degreening, and is probably inconsequential in determining the colour of the rind during postharvest storage (Fig. 5.2.11.7.4). However, it is interesting to note that in 2007 the inside fruit at harvest (week 0) had higher chlorophyll content in the rind than the outside fruit. In contrast to the chlorophyll content in the flavedo, the carotenoid content increased in 2007 from $600\text{--}800\ \mu\text{g}\cdot\text{g}^{-1}\text{ DW}$ at harvest to as much as $1600\ \mu\text{g}\cdot\text{g}^{-1}\text{ DW}$ after prolonged storage. There is a noticeable difference of carotenoid content in the rind between inside and outside fruit from the 2005 and 2007 seasons, except in the 7.5°C treatment in 2005 in which the same pattern was only evident after week 10. Although the above-mentioned differences between canopy positions remained, at 7.5°C storage, the carotenoid content increased until week 12 in 2005 whereafter it inexplicably decreased, whereas it continued to increase until week 14 in 2007. However, in both seasons, -0.5°C storage resulted in a gradual reduction of carotenoid content in both the inside and outside fruit flavedo (Fig. 5.2.11.7.4).

Rind carbohydrate content and respiration rate

The carbohydrate content, measured as sucrose, fructose and glucose contents, in the flavedo of the stored fruit did not change drastically during the prolonged storage periods in 2005 and 2007 (Fig. 5.2.11.7.5). However, there were noticeable differences between storage temperatures (-0.5°C vs. 7.5°C), as well as fruit bearing position (inside vs. outside fruit) in the individual sugars, which resulted in differences between the reducing and non-reducing sugar ratio [(fructose + glucose)/sucrose]. In the 2005 and 2007 seasons, glucose and fructose contents were similar, in contrast sucrose contents in all treatments were higher in 2005 compared to 2007. The sucrose content during 2005 was influenced by storage temperature, with fruit at -0.5°C having higher levels compared with fruit stored at 7.5°C . However, there were no consistent canopy position treatment effects. During the 2007 season, canopy position effect was more evident and the sucrose content, of the inside fruit at both storage temperatures, had lower values than the outside fruit. Glucose and fructose contents were more strongly influenced by storage temperature than canopy position during 2005 with the -0.5°C treatment resulting in lower values. In contrast, canopy position was more influential in 2007, resulting in higher carbohydrate levels in the outside fruit. The ratio of reducing to non-reducing sugars shows a clear separation due to storage temperature in both seasons, with the higher temperature resulting in a higher value. In 2007, this trend only became evident after 8 weeks in storage. The fruit canopy position did not have an effect on this ratio, except in 2005 at 7.5°C , when outside fruit flavedo had higher values from 8 weeks onwards.

Respiration rate measured during 2007 showed a reduction from the initial values (week 0) after commencing with cold storage (Fig. 5.2.11.7.6). At harvest (week 0), the inside fruit had a higher respiration rate than the outside fruit, although not significantly so. This difference was still evident until 4 weeks of storage at 7.5°C , whereafter the outside fruit generally had a higher respiration rate. The low storage temperature (-0.5°C) tended to result in a lower respiration rate ($\pm 10\ \text{mg CO}_2\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) from 6 weeks onwards.

Microscopic cellular structure

In the light microscopic images, cellular damage was evident in the sub-epidermal cell layer of the RBD-affected flavedo (Fig. 5.2.11.7.7 A) as well as in the area adjacent to and above the oil gland (arrows). The unaffected flavedo in Fig. 5.2.11.7.7 K shows the differences in cell size, shape and cell wall thickness between the various citrus oil gland cell layers as labelled in Fig. 5.2.11.7.7 A, viz. sheath and epithelial cells as well as the oil filled lumen. Cellular damage occurred in the hypodermal and sheath cells in the RBD tissue.

When comparing the epidermal cell layers of the RBD affected and unaffected flavedo with TEM, it was evident that this cellular layer was not affected by RBD (Fig. 5.2.11.7.7 B and L). In this layer the vacuole does not fill the entire cell and dark osmiophilic droplets were visible in the cytoplasm. There was also evidence of various cellular organelles in these cells.

In both the sub-epidermal layer as well as in the sheath cells of the RBD affected tissue, symptoms of cellular collapse were observed (Fig. 5.2.11.7.7 C and D). In these cells, the vacuole occupies the majority of the cell volume. The symptoms of cellular collapse include plasma membrane damage (stars in Fig. 5.2.11.7.7 D), the cell walls of the sheath cells displayed a "jagged" shape and the disintegration of the multi-membranous myelin-like structure (Fig. 5.2.11.7.7 C). In the unaffected flavedo, various cellular organelles, namely the mitochondria, Golgi-apparatus (with the Golgi stacked and associated trans-Golgi network) and smooth endoplasmic reticulum (SER), were observed in the cytoplasm of the sup-epidermal layer, which was more numerous compared with the RBD-affected tissue (Fig. 5.2.11.7.7 C and M). In these unaffected sheath cells, an intact plasma membrane was visible with the cytoplasm closely pressed towards the cell walls by the large vacuole with bulbous bending shapes of the plasma membrane and cytoplasm occurring in these particular cells (see star in Fig. 5.2.11.7.7 N). The epithelial cells around the lumen had thin cell walls which were bent into undulating shapes (Fig. 5.2.11.7.7 E and O), resulting in three to five cells being pressed together into the multicellular wall of the lumen (Fig. 5.2.11.7.7 Piii). In the RBD-affected tissue (Fig. 5.2.11.7.7 E), disruption of the plasma membrane was visible in two adjacent cells (stars). However, in the epithelial cell layer of the unaffected flavedo (Fig. 5.2.11.7.7 O), the large vacuoles filled most of the cell volume, with some cellular organelles present in the cytoplasm.

In all the previously described cell layers (epidermal, hypodermal, sheath and epithelial cells), a multi-membrane structure was detected. However, these myelin-like structures, as named by Bosabalidis and Tsekos (1984a), were more evident in the hypodermal layer of the unaffected tissue (Fig. 5.2.11.7.7 Pi-iii). In the RBD-affected tissue these structures were seldom found, and in the instance shown in Fig. 5.2.11.7.7 F (an enlargement of C) they were in a disintegrating state.

Recognisable differences were evident with SEM between RBD affected and unaffected flavedo tissue in the SEM images (Fig. 5.2.11.7.8 G–J and Q–T). When comparing Fig. 5.2.11.7.8 G and Q the result of the collapse of the hypodermal tissue surrounding the oil glands on the flavedo topography can be seen. This collapse of the hypodermal tissue is thought to be caused by the collapse of an oil gland (arrow) (Fig. 5.2.11.7.8 H) leading to hypodermal cellular damage in Fig. 7.8 I (arrow), as well as to the sheath cells between oil glands (star in Fig. 5.2.11.7.8 J) by the phytotoxic essential oil which was released on rupturing of the oil gland.

Discussion

The incidence of rind breakdown (RBD) of 'Nules Clementine' mandarin, a physiological disorder of the fruit rind, was affected by the various postharvest treatments in this study. The treatments, viz. ethylene degreening, delay in cooling and increase storage duration, all increased the occurrence of RBD. Rind breakdown of 'Nules Clementine' mandarin develops neither due to physical damage, such as oleocellosis of citrus fruit (Knight et al., 2002), nor to exposure to a chilling injury-inducing temperature (-0.5°C) during storage (Lafuente and Zacarias, 2006; Khumalo, 2006), as RBD was more prevalent at 7.5°C than at -0.5°C in this study.

The deleterious effect of drastic changes in relative humidity (45 to 90% RH) in the week after harvest, has not been tested as a causative triggering mechanism in RBD of 'Nules Clementine' mandarin and as reported in non-chilling rind pitting of grapefruit (Alferez and Burns, 2004) and rind staining of 'navel' orange (Alferez et al., 2003). However, there is a similarity of cellular collapse in the hypodermal cells, but unlike rind breakdown of navel orange, RBD of 'Nules Clementine' mandarin differs in a few substantial aspects which could influence symptom development. The first difference is the time of symptom development where rind breakdown or staining of navel orange occurs after 1 week, whereas RBD only starts to develop

progressively after 3 to 5 weeks in storage. The second difference is that even under fully developed RBD symptoms, the epidermal cell layers do not seem to be damaged as in rind breakdown of 'navelate' orange (Agustí et al., 2001). The third difference (from a fruit rind anatomical point of view), which could be the reason for the different symptomatic developments and causes of these physiological rind disorders, is that the mandarin rind has a much thinner albedo compared to a 'navel' orange or grapefruit. Therefore, the mechanism of cellular collapse, proposed to be caused by the dehydration at low RH (45%) and re-hydration at high RH (90%), which leads to water potential differences and tension in the flavedo-albedo interface of oranges and grapefruit (Alferez et al., 2003), could differ in the reticulated mandarin rind.

The progressive developmental pattern of RBD could be interpreted as being similar to a senescence related physiological process, in which a series of controlled cellular changes result in the death of these cells or tissue and can be attributed to the eventual progressive loss of membrane integrity or their regulatory control (Legge et al., 1986; Palma et al., 1995; Dangl et al., 2000; Kays and Paull, 2004). The process of leaf senescence and cellular death expends energy, supplied by the mitochondria, which along with the nucleus and plasma membrane are normally the last organelles to be maintained until final collapse and cell death. Senescence, a physiological process in plant tissue, is responsive to any sub- or supra-optimal environmental condition of which temperature, water deficit and gaseous concentrations (O_2 , CO_2 and ethylene) in the postharvest environment are known to act as primary senescence promoting agents (Dangl et al., 2000). The citrus rind is regarded as a modified leaf as it consists of the same anatomical features and physiological abilities as a citrus leaf (Schneider, 1968). Therefore, as in leaves, the mineral nutrient [especially Ca and Mg known to influence senescence (Lieberman and Wang, 1982)], carbohydrate and pigment contents of the rind, are strongly influenced by microclimatic conditions within the canopy, such as light levels (see Sections 5.2.11.3, 5.2.11.4 and 5.2.11.6).

The most visible symptom of leaf senescence, viz. chloroplast degradation, also occurs in the citrus flavedo and is called "colour break" whereby the previously green rind turns more yellow/orange. This process of chlorophyll catabolism is the earliest and most significant senescence-related change in leaf cells and is thought to take place in order to recycle the $\pm 70\%$ of total protein (and therefore N) in the leaf from these organelles (Gan and Amasino, 1997). However, the degradation of chlorophyll unmasks the existing carotenoid pigments and is followed by the additional synthesis of carotenoids via the isoprenoid pathway (Gross et al., 1983). For chloroplast-to-chromoplast conversion to take place during ripening, the citrus rind requires light (Goldschmidt 1988), ethylene exposure (Eaks, 1977), low temperatures and high sugar levels, while high or late N applications inhibit the conversion of chloroplast to chromoplast (Huff, 1984; Takagi et al., 1989).

Rind colour of citrus fruit is commercially improved by exposing fruit for 3 days at 1 to 3 $\mu L \cdot L^{-1}$ ethylene gas, resulting in chlorophyll breakdown; however this practice can also lead to various rind defects (Krajewski and Pittaway, 2002). Ethylene is thought to be necessary for the senescence in *Arabidopsis* leaves, acting as a modulating factor or influencing the time of the process, possibly via the enhanced expression of senescence-associated genes and suppression of photosynthesis-associated genes (Grbić and Bleecker, 1995). The increase in RBD of 'Nules Clementine' mandarin fruit after ethylene exposure is, therefore, not surprising. In contrast, the application of ethylene for 4 days at 2-10 $\mu L \cdot L^{-1}$ was shown to reduce rind staining of 'navelina' and 'navelate' oranges (Lafuente and Salla, 2002; Cajuste and Lafuente, 2007). In comparison, this rind disorder develops in a shorter time span (± 1 week after harvest) than RBD, and could therefore differ in causal mechanism from RBD, which develops progressively from 3 to 5 weeks in storage.

Temperature management, in the non-chilling range ($>4^\circ C$ for citrus), is the primary approach taken during postharvest storage of fruit to maintain product quality by decreasing all physiological processes, including the rate of senescence (Paull and Kays, 2004). The increase in RBD due to delays in commencement of cooling of the fruit after packing is therefore consistent with basic postharvest principles, and leads to an implementable practice to avoid or reduce the occurrence of this rind disorder. The higher incidence of RBD of the fruit stored at $7.5^\circ C$ compared with $-0.5^\circ C$, further substantiates the fact that temperature influences the rate of senescence in the flavedo. The consistent pattern of increased RBD development as storage duration progresses, could furthermore be seen as the clearest indication that this physiological disorder is associated with an accelerated senescence process in the more RBD-prone inside fruit, leading to cellular breakdown.

Senescence is an energy-requiring process whereby carbohydrates are catabolised during the various metabolic processes involved in senescence. The finite source of carbohydrates in the flavedo, however, does not seem to be a limiting factor during storage, as none of the three sugars measured showed a dramatic reduction in quantity during storage. It is known that sugar content affects the growth and development of plant cells, but, in addition to their role as energy source, sugars act as a signal to activate

various gene-regulated processes, such as pigment synthesis and senescence (Graham et al., 1992; Knight et al., 1994; Jang et al., 1997). However, the lower carbohydrate content at onset of postharvest storage of the more RBD-prone inside fruit could be an indication that resources for developmental respiration were lacking and that those fruit, under deficit conditions, could not tolerate exposure to stresses during postharvest handling. Fruit respiration rate, indicative of a fruit's postharvest physiological condition, was influenced not only by the storage temperature, as expected, but also by canopy position in the 7.5°C treatment. The higher respiration rate and ratio of reducing to non-reducing sugars in the less RBD-prone outside fruit, is seen as an indication of adequate carbon metabolism to facilitate maintenance respiration needed to repair existing cellular structures (Salisbury and Ross, 1992).

The cellular damage visible in the microscopic images is thought to be due to the collapse of an oil gland leading to the phytotoxic oil leaking into the adjacent cellular structure. This collapse of cells and damage to the flavedo tissue as seen in RBD of 'Nules Clementine' mandarin differs significantly from the cellular collapse due to oleocellosis (Knight et al., 2002) or rind pitting /staining/breakdown (Petracek and Davies, 2000; Agustí et al., 2001; Alférez and Burns, 2004). Whereas oleocellosis is known to develop at harvest after a physical pressure or damage of the rind, leading to the leakage of oil onto and into the epidermal layers, RBD develops during prolonged storage without any physical damage (Knight et al., 2002). The release of oil in oleocellosis, probably through the oil gland stalk which could be the weakest point, damages both the epidermal and hypodermal cells. However, with RBD the epidermal cells remain intact, but all cell layers adjacent to the oil gland tend to be severely damaged.

The difference in cellular structures and organelles between a RBD-affected and unaffected rind, adds support to the argument that this physiological disorder maybe due to premature senescence. Not only do the damaged plasma membrane and the multi-membranous myelin-like structure (Bosabalidis and Tsekos, 1982) suggest a reduced physiological condition, but also to a large extent the lack of mitochondria, Golgi-apparatus and ER in the cells of the flavedo with RBD. The higher respiration rate of the non-RBD sensitive outside fruit stored at 7.5°C further show that the mitochondria in these fruit are still effective in supplying energy for maintenance respiration, as there does not seem to be a lack of respiratory carbon. The multi-membranous structures, called myelin-like by Bosabalidis and Tsekos (1982), and reported by Thomson et al. (1966), but not Bennici and Tani (2004), was referred to by Gross et al. (1984) as uncommon types of extensive "achlorophyllous membranes" which were typically arranged in concentric circles in grapefruit flavedo. These membranous structures are thought to play a role in carotenoid synthesis during fruit ripening of *Capsicum annuum* L. cv. Yolo Wander fruit (Camara and Brangeon, 1981). The low carotenoid content in the inside flavedo and the very low occurrence of these structures, some of which were damaged, could support the hypothesis of their involvement in colour development in citrus rinds.

To conclude, the incidence of RBD of 'Nules Clementine' mandarin was increased by postharvest treatments known to promote the rate of leaf senescence, viz. exposure to ethylene and relatively high storage temperature ($\pm 7.5^\circ\text{C}$). It is thought that the progressive nature of this physiological disorder is indicative of a premature senescence process in those fruit rinds with a higher propensity for RBD. The higher carbohydrate and carotenoid contents, and resulting better colour at the onset of postharvest storage, are an indication of the importance of rind condition during pre-harvest development as a good rind condition translated into a significant reduction in the occurrence of RBD. The anatomical evidence suggests that this disorder is not due to any physical stress, but rather due to a lack of cellular organelles and disruption of cellular structures. The reaction of the fruit to the postharvest treatments used, offers some implementable practices to prevent this disorder, even though the causative mechanism is not fully elucidated.



Figure 5.2.11.7.1. Rind breakdown (RBD) of 'Nules Clementine' mandarin fruit. The two top fruit were stored at 7.5°C and show severe (left) and less severe incidence (right) of RBD. The bottom fruit were stored at -0.5°C. Typical differences in symptom development due to low temperature storage can be seen between the bottom and top fruit. The low temperature leads to a more watery lesion although still randomly situated in the flavedo.

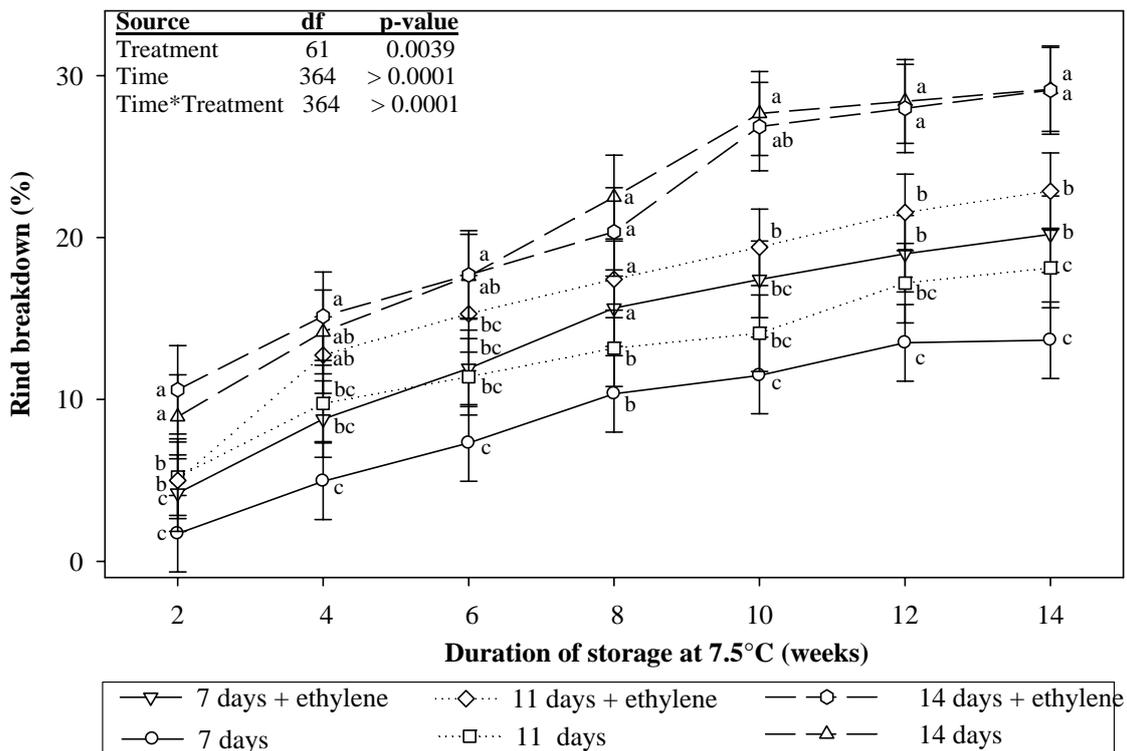


Figure 5.2.11.7.2. The influence of postharvest ethylene degreening (72 hours at $3 \mu\text{L}\cdot\text{L}^{-1}$ ethylene, >90% RH and 20 to 22°C) and a delay in commencement of cold storage on rind breakdown incidence during 2003. After being either degreened or not, 'Nules Clementine' mandarin fruit were kept at ambient temperature for 7, 11 or 14 days before cold storage at 7.5°C for 14 weeks. Values are means ($n = 8$) \pm SE. Different lettering at each measurement stage indicates significant differences among treatments at this time, according to Fisher's least significant difference test ($P \leq 0.05$).

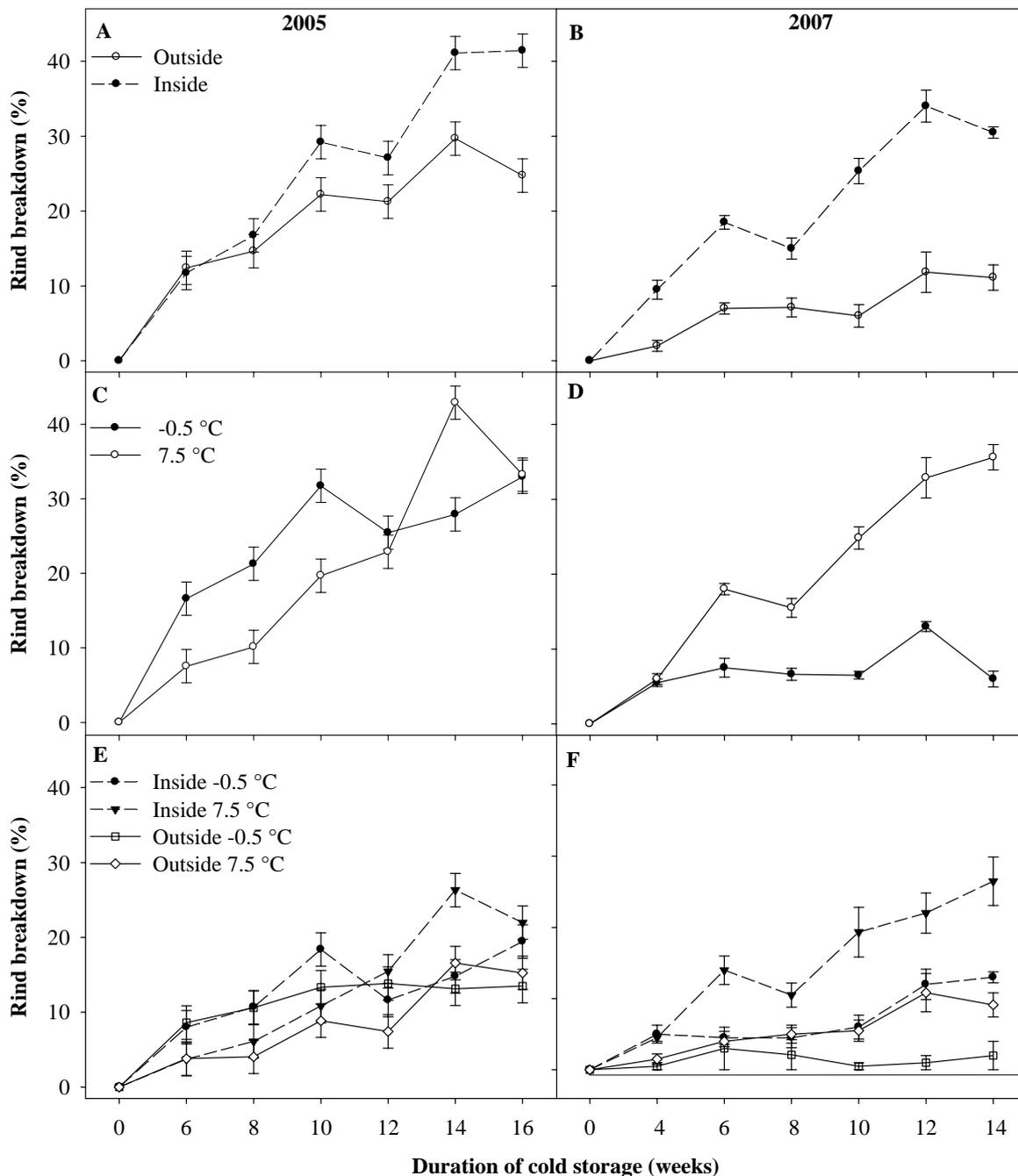


Figure 5.2.11.7.3. Incidence of rind breakdown of 'Nules Clementine' mandarin fruit during 2005 and 2007 of fruit sampled from two positions within the tree canopy, viz. inside (A) and outside (B), during cold storage at 7.5°C or -0.5°C (C and D). The combined data are presented in E and F. Values are means ($n = 8$) \pm SE.

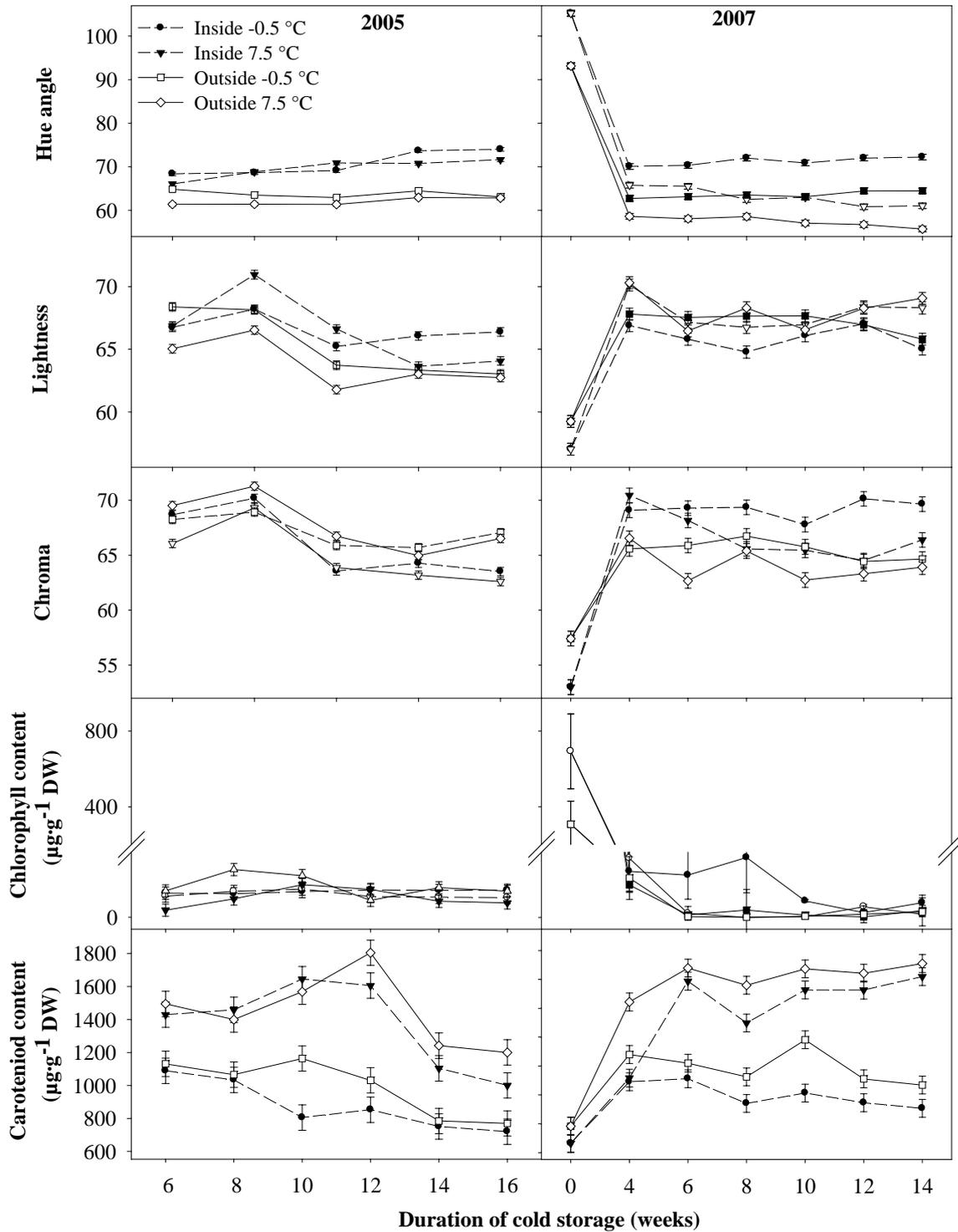


Figure 5.2.11.7.4. Postharvest changes in rind colour (hue angle, lightness and chroma values), chlorophyll and carotenoid content of 'Nules Clementine' mandarin flavedo during postharvest storage at -0.5°C and 7.5°C. Values for the inside fruit (●, -0.5°C and ▼ 7.5°C) are denoted by broken lines and those for the outside fruit (□, -0.5°C and ◇, 7.5°C) by a solid line. Values are means (n = 8) ± SE.

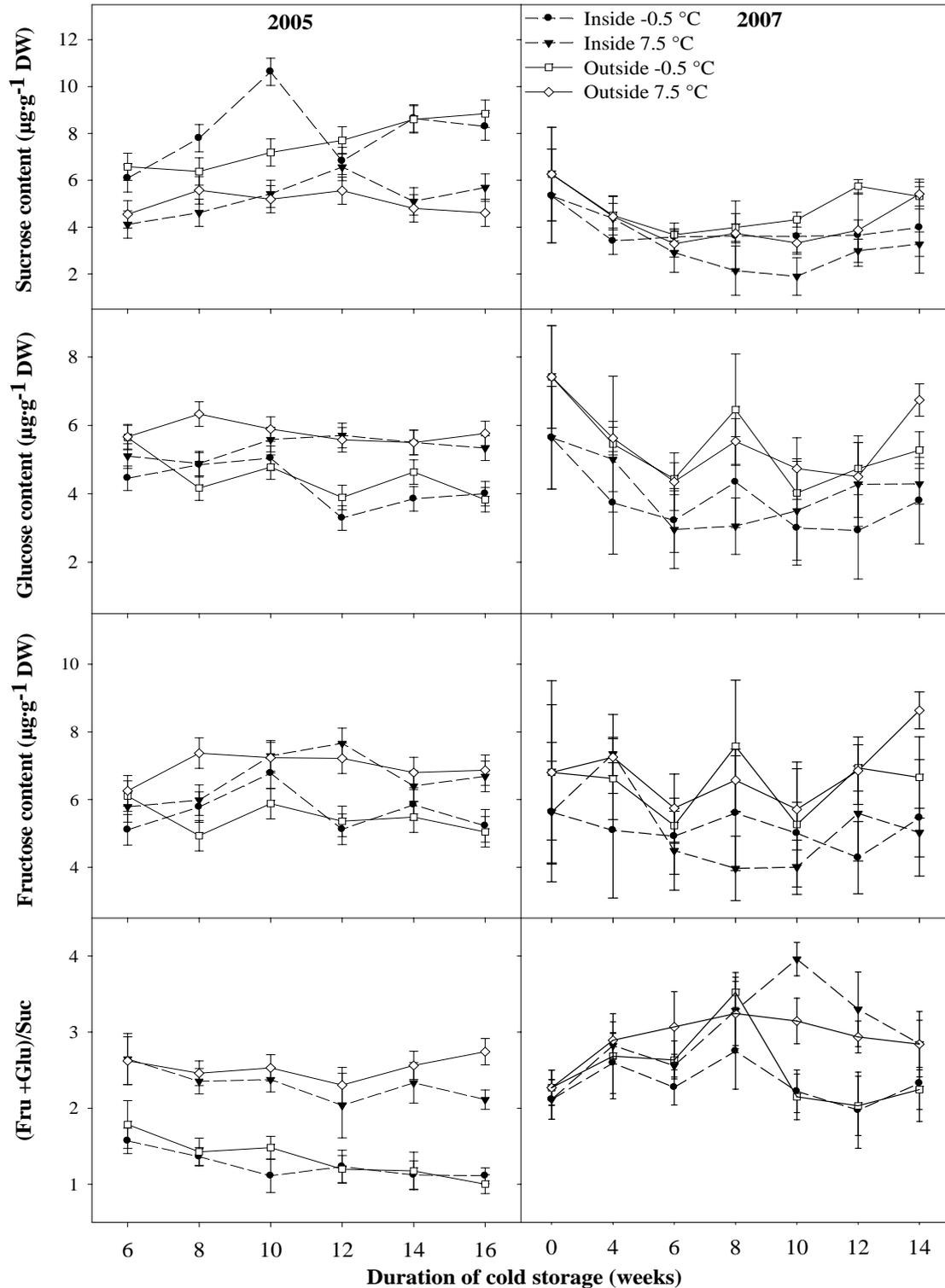


Figure 5.2.11.7.5. Sucrose, glucose and fructose contents of 'Nules Clementine' mandarin flavedo during postharvest storage of fruit positioned inside and outside the tree canopy, at a non-chilling (7.5°C) and chilling (-0.5°C) temperature. Values for the inside fruit (●, -0.5°C and ▼ 7.5°C) are denoted by broken lines and those for the outside fruit (□, -0.5°C and ◇, 7.5°C) by a solid line. Values are means ($n = 8$) \pm SE.

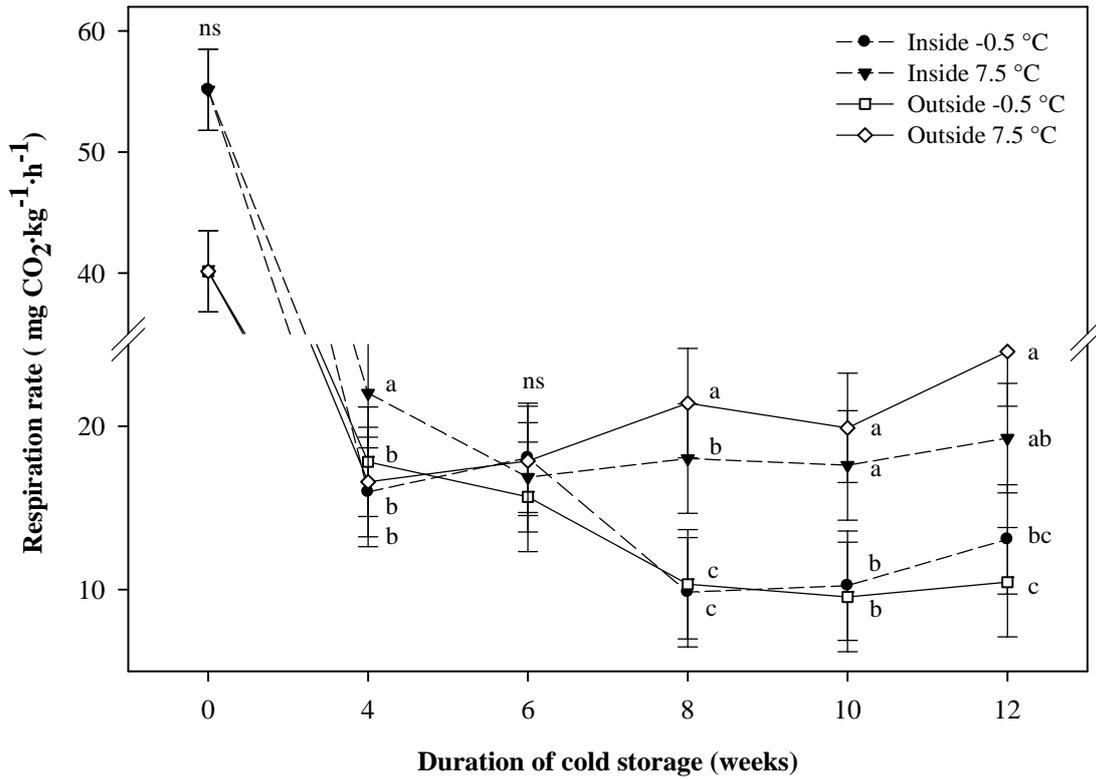


Figure 5.2.11.7.6. Respiration rate during cold storage of 'Nules Clementine' mandarin fruit during 2007. Values for the inside fruit (●, -0.5°C and ▼ 7.5°C) are denoted by broken lines and those for the outside fruit (□, -0.5°C and ◇, 7.5°C) by a solid line. Values are means (n = 8) ± SE. Different lettering on bars indicate significant differences according to Fisher's least significant difference test ($P \leq 0.05$).

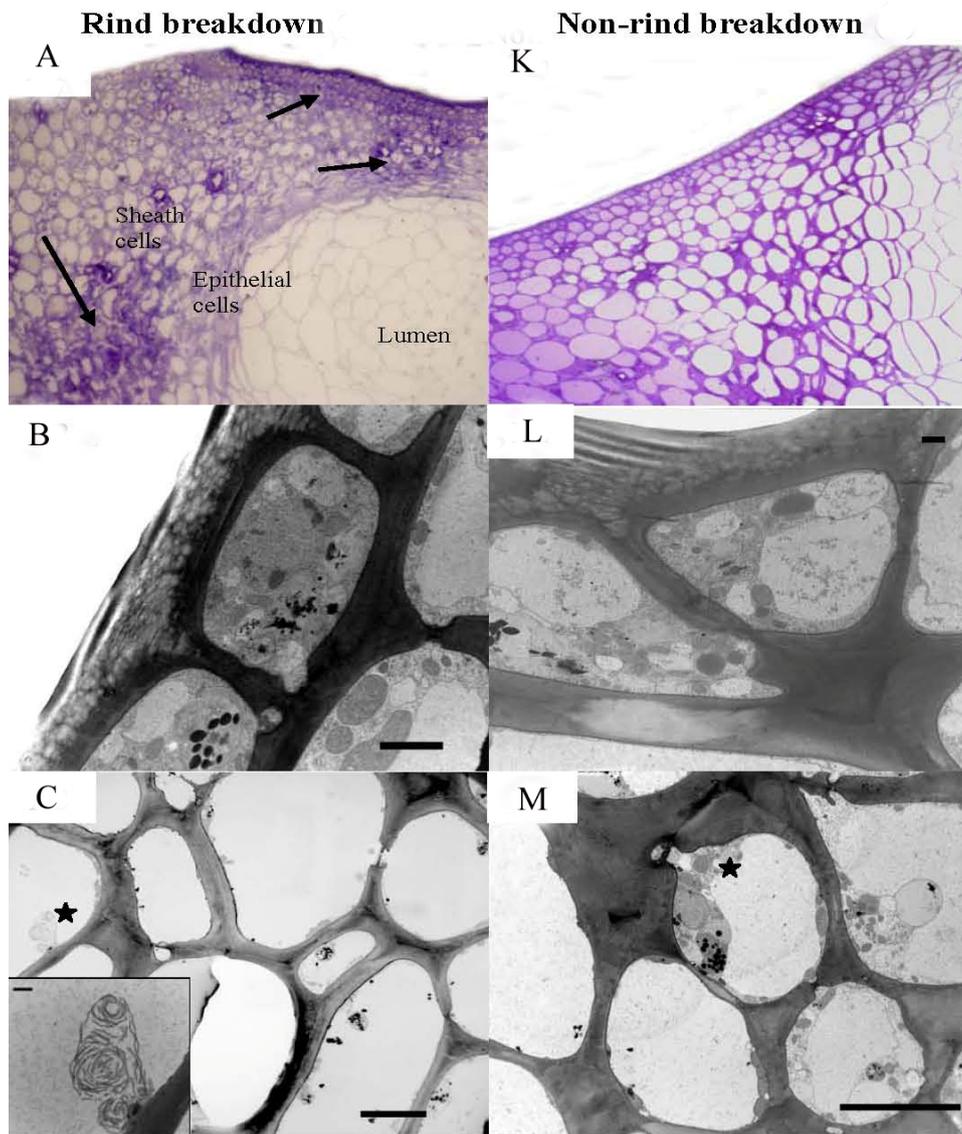


Figure 5.2.11.7.7. Comparative images of different cellular layers of 'Nules Clementine' mandarin fruit rind with RBD (A–F) and without RBD (K–P) after 14 weeks of storage at 7.5°C. Profile images with a light microscope (A and K, x10 mag.) show the cellular damage of the hypodermal and sheath cells in the RBD tissue (A). In B (RBD) and L no cellular damage occurred in the epidermal layer as evidenced by TEM. C; images of the hypodermal cell layer and membrane damage (star) to the multi-membrane structure, less cytoplasm and cellular organelles than in M (non-RBD). Bar = 1 μm.

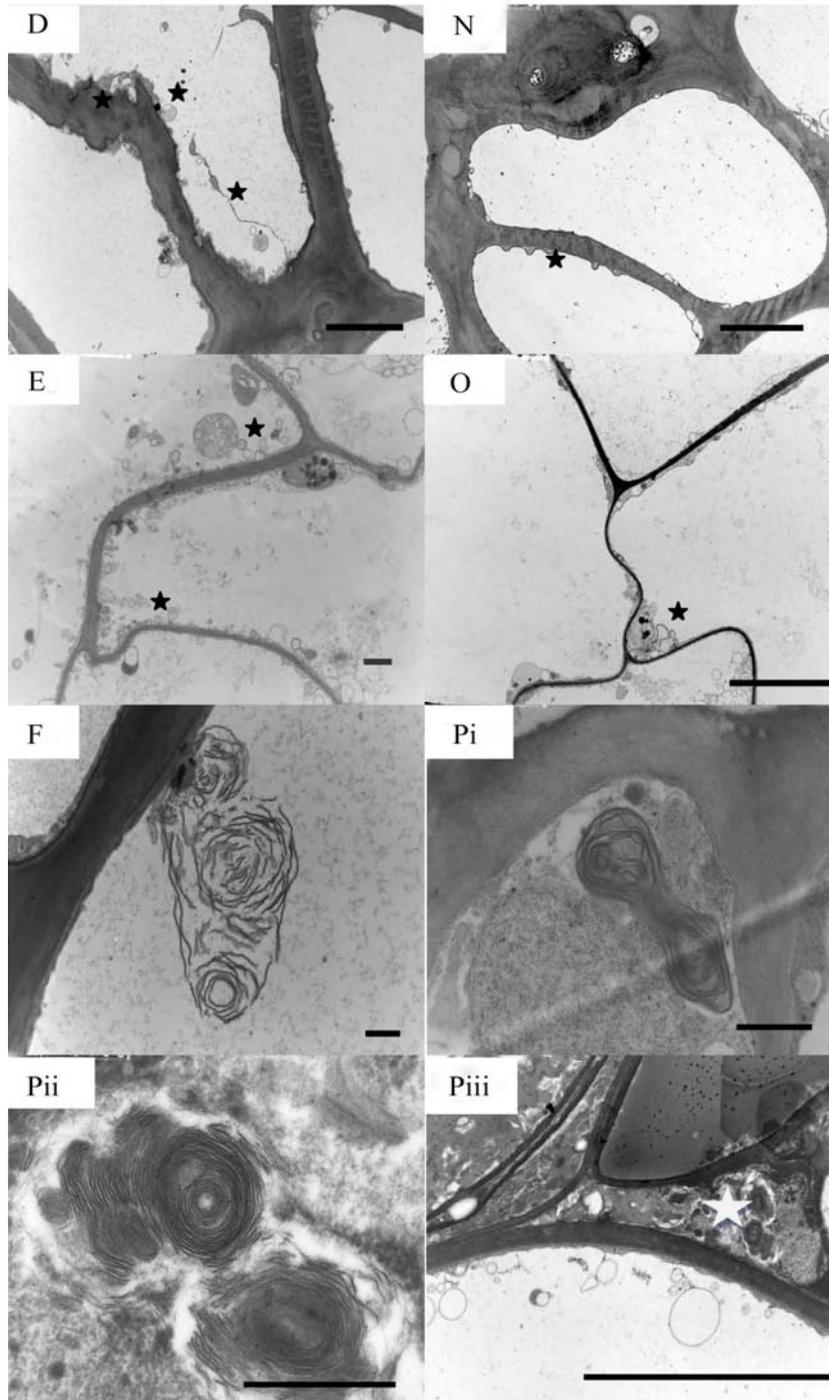


Figure 5.2.11.7.7 (continued). D and N, sheath cells; damage to plasma membrane and cell walls in D (stars) and no damage in N with intact plasma membrane and bulbous protrusion of the cytoplasm into the large vacuole (star). E and O, epithelial cells; disruption of plasma membrane and cytoplasm in two adjacent cells of tissue with RBD (stars), O has undamaged cells with osmiophilic droplets in the cytoplasm. F and Pi-iii, multi-membranous structure; F shows the breakdown of the structure, and Pi-iii the different forms of the structure. Bar = 1 μm .

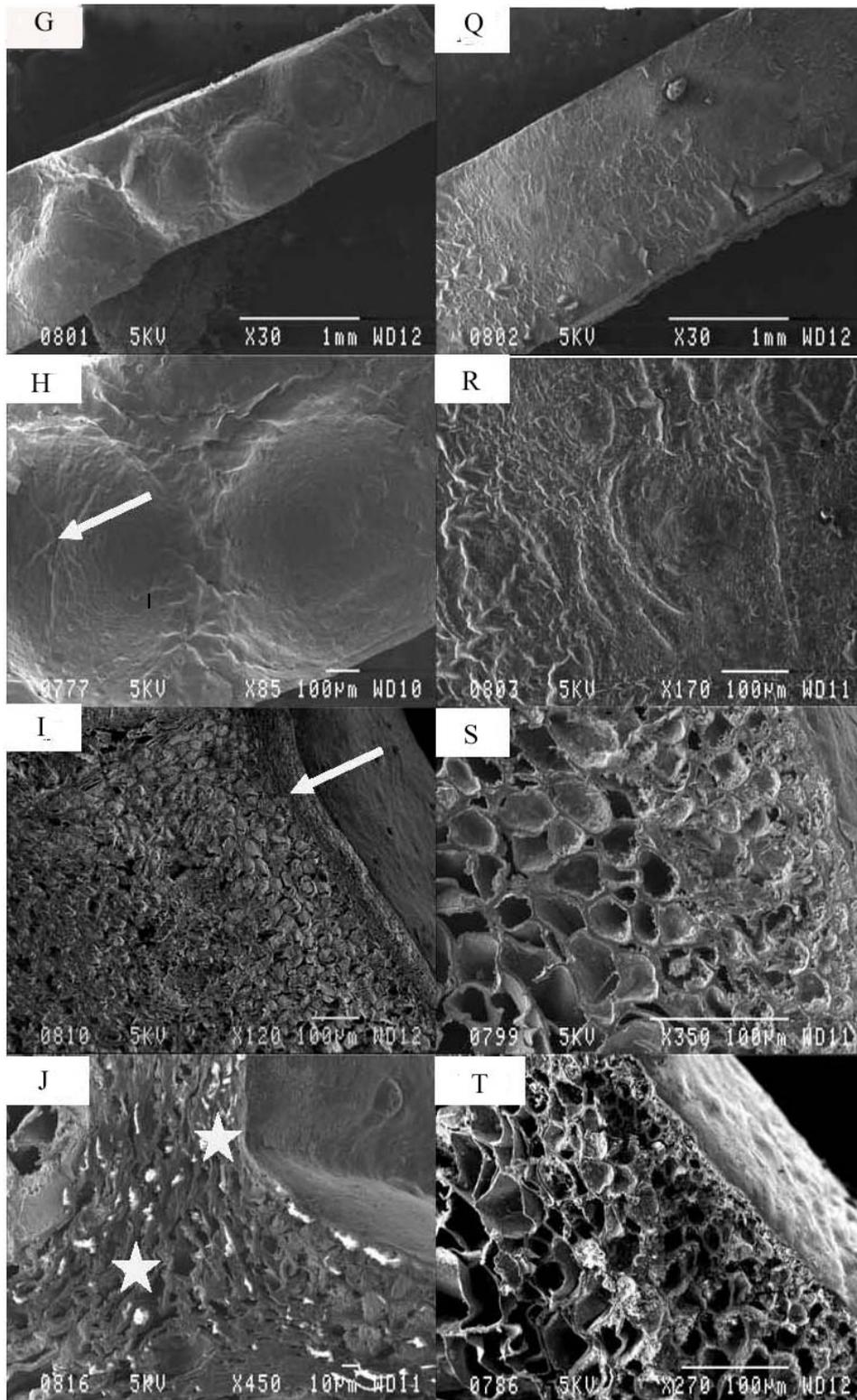


Figure 5.2.11.7.8. SEM images of 'Nules Clementine' mandarin rind after 14 weeks of storage at 7.5°C with (G to J) and without RBD (Q to T). G–H and Q–R; topographic changes after the collapse of an oil gland (H, arrow). I–J and S–T; profile images of the RBD damage (arrow I, star J) in tissue adjacent to an oil gland.

Discussion and conclusion

The postharvest physiological disorder rind breakdown (RBD) of 'Nules Clementine' mandarin fruit develops after 3 to 5 weeks of cold-storage and causes severe financial losses to South African Clementine producers. This progressive postharvest rind disorder is thought to be related to the collapse of the oil gland within the flavedo. From preliminary studies it was surmised that fruit from the inside of a tree's canopy have a "weak rind" and are more susceptible to this postharvest physiological disorder. However, what this relationship between canopy position and rind condition actually meant in physiological terms was unknown. This study therefore aimed at determining the physiological changes during fruit development, specifically subsequent to physiological fruit drop, and postharvest storage as influenced by light levels within the tree canopy. To describe rind condition, mineral nutrient, carbohydrate and pigment contents of the flavedo were determined pre- and postharvest. These variables were selected as they are known for their cause visible changes (pigments), and myriad of actions on a cellular level (mineral nutrients and carbohydrates) and therefore, could be involved in the determination of the physiological condition of the flavedo and its eventual susceptibility to RBD.

The main finding in this study was the significant influence that variation of photosynthetically active radiation (PAR) within the tree canopy has on rind condition and the fruits' propensity to develop RBD. This \pm 80% difference in PAR between fruit sampled from the inside and outside of the tree canopy resulted in differences in pigment, carbohydrate and mineral nutrient contents of the fruit flavedo. In addition, the metabolism (respiration and photosynthesis rates) of these fruit was found to differ, all of which is thought to contribute to the significant difference in RBD incidence between fruit from high and low PAR conditions during development. This impact of PAR on the rind was dramatically illustrated by shading individual fruit and thereby limiting the exposure of the photosynthesising flavedo to light during stages II and III of fruit development, which resulted in the first successful technique to induce RBD. Adequate PAR, as perceived in the outside of the tree's canopy leads to a cascade of physiological responses in the rind, viz. higher pigment, Ca, Mg and carbohydrate contents due to the net positive carbon fixation. Fruit exposed to high PAR had visibly darker green flavedo during growth and also developed a darker orange colour rind colour during storage. This aspect, viz. good rind colour development, stood out in all experiments as a consistent indication of low RBD susceptibility. In general, it can be concluded that high PAR results in high Mg and Ca allocation via the xylem and increased chlorophyll synthesis and activity in the flavedo, leading to improved carbohydrate synthesis. These different aspects give a clear indication of the flavedo's leaf-like behaviour and reaction to environmental constraints, which in this instance was PAR. From another perspective the flavedo's photosynthetic ability could supply the required extra resources to ensure a "good rind condition" and low RBD susceptibility by not being a carbon sink in the tree canopy, as was the case in the inside fruit. Therefore, optimising light penetration in the orchard system would be a low technology solution to a complex physiological problem. This hypothesis that rind photosynthesis contributes significantly to rind condition could be further tested on cultivars with different bearing habits e.g. 'Star Ruby' grapefruit which develops less chilling susceptible fruit in the shaded part of the canopy.

The measurement of postharvest carbohydrate content did not indicate a sudden lack thereof during storage. This was expected, as citrus fruit respiration tends to be low and is the sum of the difficult to separate rind and pulp tissues. However, carbohydrate metabolism reflected in the ratio of reducing to non-reducing sugars, indicated differences in RBD susceptibility of fruit, and further studies at the enzyme level as influenced by PAR levels and postharvest temperature and ethylene treatments could supply important information on the carbon balance of the rind. A shortcoming in this study was that the carbohydrate content of the shaded fruit, which developed significantly higher levels of RBD, was only determined at the final evaluation date and not throughout storage along with the recording of RBD incidence. A clearer correlation between RBD incidence and flavedo carbohydrate levels could possibly have been established if the experiment was repeated in such a manner.

Fruit flavedo exposed to direct sunlight had higher Ca and Mg and lower K contents compared to shaded fruit. This leaf-like mineral nutrient accumulation pattern suggests that the xylem supplies the majority of mineral nutrients, and that the same bio-physiological constraints regulating leaf mineral accumulation rate, such as vapour pressure deficit (VPD) and pedicel diameter, would also determine accumulation in the flavedo. The phloem is thought to play less of a role in mineral nutrient accumulation in the flavedo, but it could be involved in re-allocation, as is suggested by the decrease in Mg content after Mg-dechelatase releases Mg from the chlorophyll molecule during colour break. By employing radioactive or having isotope marker techniques, the possible re-translocation of Mg from chlorophyll could be traced. Additionally, it would be interesting to determine if part of the released Mg-ions are re-translocated intra-cellularly to other organelles. The possible negative effect of the high K concentrations in the inside fruit flavedo, which is thought to be a stress reaction to rectify osmotic potential necessary for growth, should be further studied.

This is an especially important aspect considering the mounting evidence of the involvement of rind water balance in the various postharvest physiological disorders such as rind pitting and stem-end rind breakdown. By using K-transport inhibitors, the influence of K levels on rind condition and physiological processes, such as carbohydrate content, water potential and possibly the susceptibility to RBD, could be elucidated.

From a horticultural perspective, the mineral nutrient accumulation pattern of the flavedo should be documented in unison with the fruit pulp and leaves. In hindsight, this aspect was lacking in the study. In general fertilisation practices and research in citriculture have focused primarily on yield and fruit size, but in the evermore discerning markets the focus should include the optimisation of rind mineral nutrients to improve postharvest rind condition. Additional aspects not to be ignored in this regard are the ever enlarging cultivar basket and shift towards “easy peeler” mandarin type fruit, which are more susceptible to postharvest physiological defects than ‘Valencia’ oranges. In this regards, the significant reduction in RBD achieved with certain foliar nutrient applications in this study offers a fairly low cost, low technology solution to RBD-associated losses.

The tentative correlation between carbohydrate and mineral nutrients, as suggested in this study, is a fascinating physiological aspect in plants. However, it is not well documented, especially in fruit tree crops. This is probably due to the fact that these two factors are directly or indirectly involved in all primary and secondary cellular metabolism thereby mask the measured response. However, the same reason makes it logical to suspect that there is a cause and effect between mineral nutrients (K, Ca and Mg in this study) and carbohydrate content, as optimum PAR would not result in optimum carbohydrate levels if the xylem supply of mineral nutrients was inadequate. By measuring the photosynthetic ability of fruit after application of specific mineral nutrient foliar sprays, e.g. Mg, it would be possible to explain the relationship between mineral nutrients and carbohydrate levels.

The difficulty of “finding” RBD during storage after extensive preharvest manipulation was illustrated in the 0% RBD incidence recorded during 2006. The experimental design of 25 fruit per replication, used to ensure at least some fruit developed RBD, unfortunately have the disadvantage of masking of the physiological signals as there were always fruit in the replicates which did not show any symptoms. It could be advantageous to use single-fruit replications in future research with detailed experiments, such as K-transport inhibitors, the effect of auxins and auxin inhibitors (to test the influence of sink strength) and fruit photosynthesis.

The suggestions made above regarding new avenues of research focus on improving rind condition or elucidating the mechanism involved in RBD. However, the data gathered in this study paves the way for other opportunities. The close link established between RBD susceptibility, canopy position and eventual carbohydrate content in the flavedo could be determined by using NIR (near infra red) spectroscopy in a packing line sorting technique. NIR technology is able to measure carbohydrate content in plant tissue and, by focusing on the flavedo, the distortion of the signal would be avoided compared to the measurement of pulp sugar content. As previously stated rind colour was consistently a good indicator of RBD susceptibility and by using existing colour grading, along with size grading technology (the other most consistent factor influencing RBD), the occurrence of RBD in the market place could be significantly reduced.

During this study an hypothesis was developed as a more complete picture of those factors involved in the determination of RBD incidence was evolving. Senescence, a process involving the ordered disassembly of cellular components in plant tissue, can be initiated and accelerated by various environmental, hormonal or developmental signals. Even though no single factor stood out in this study as the primary cause of RBD, all could be fitted into an hypothesis related to premature senescence of the flavedo. The first step in this hypothesis of premature flavedo senescence involved the development of a RBD sensitive fruit flavedo under low PAR which led to low pigment, Ca, Mg and carbohydrate contents. Secondly, an environmental (temperature) or hormonal (ethylene) signal could initiate and promote the senescence process, perceived as chlorophyll degradation, and could lead to high RBD incidence in fruit with lower Ca, Mg, pigments and carbohydrates (inside fruit) necessary to delay senescence or maintain rind integrity. This hypothesis not only serves to unify the complex factors which interact and affect RBD, but also opens new avenues of research from practical, i.e. implementation of postharvest protocols, to fundamental studies into the gene expression patterns associated with senescence, to move towards rejecting or accepting this hypothesis.

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Future research

The study served as a scientific confirmation of what was seen in the industry, i.e. inside fruit being more susceptible towards rind breakdown. However, by determining factors that influence rind conditions such as carbohydrate and mineral nutrients we moved closer to explaining the occurrence of this disorder as well as serving as a starting point to further investigate the nutrient balance from a rind quality perspective. It is clear that the pulp and rind are separate (although interlinked) entities of the citrus fruit, however those practices developed over the last few years to optimise yield should be re-looked at as to their influence on rind disorders. Further research should look at the influence of micro-climatic variability on carbon fixing of the flavedo and the contribution that the rind makes to the carbon pool. Mineral nutrient values for the flavedo should also be correlated with leaves and pulp in order to see if contradictory practises exist that give preferential treatment to the pulp at the detriment of the rind. Postharvest handling practises such as ethylene treatments and cold storage should be re-evaluated.

Technology transfer

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5.3 **PROJECT: FRUIT PRODUCTION AND QUALITY** Project coordinator: Stephan Verreyne (CRI at SU)

5.3.1 **Project summary**

The project consisted of 3 studies, handthinning of Satsumas and Clementines, methods to reduce the navel end size reduction and the potential application of remote sensing in citrus orchards.

In section 5.3.2. the benefits of hand thinning over two seasons were evaluated. Nules Clementine trees and Mihowase Satsuma trees were used and fruit estimated to be <55 mm at harvest were removed after the physiological fruit drop. In the first season, hand thinning on Nules Clementine increased fruit growth and fruit size and hand thinning reduced the harvest time. In the second season handthinning had no effect on fruit growth, fruit size and yield for both Satsumas and Clementines due to irrigation problems and later thinning compared to the first season.

In section 5.3.3 the efficacy of 2,4-D in reducing navel end size of different navel orange cultivars at different sites was evaluated by applying 2,4-D (ester) at different concentrations (0-45 ppm) and different timings (from full bloom until 4 weeks after petal drop). 2,4-D at full bloom caused an increase in the percentage of closed navel ends and significantly reduced the navel end size, with no differences among the different concentrations at full bloom. There were no negative effects on yield, fruit number and fruit internal or external quality. The petal drop applications seem to be ineffective.

The potential application of hyperspectral remote sensing for detecting drought stress, nutrient stress and yield was investigated on Satsuma mandarin trees in section 5.3.4. A new drought stress spectral index for citrus was developed which could accurately predict the leaf water content for all stresses. Crop load can be determined from hyperspectral remote sensing and fruit color is not an important factor in predicting citrus yield with remote sensing. Canopy colour change due to leaf nitrogen deficiency could be detected from the canopy spectra.

Projekopsomming

Die projek bestaan uit 3 studies, handuitdun van Satsumas en Clementines, metodes om die navelent opening van navel lemoene te verklein en die potensiele toepassing van afstandsmeting in sitrusboorde.

In seksie 5.3.2 is daar gepoog om te bepaal of handuitdun enige voordele inhou. Nules Clementine bome en Mihowase Satsuma bome is gebruik en vrugte met geskatte grootte <55 mm is verwyder na fisiologiese vrugval. In die eerste seisoen het handuitdun op Nules Clementine vruggroei en vruggrootte verbeter en handuitdun het die oestyd versnel. In die tweede seisoen het handuitdun geen effek op vruggroei, vruggrootte en oeslading gehad op beide Satsuma en Clementine nie a.g.v. besproeiings probleme en omdat uitdunning later as in die eerste seisoen gedoen is.

In seksie 5.3.3 is die effektiwiteit van 2,4-D om die navelent opening op verskillende navel kultivars te verklein in verskillende areas ge-evalueer deur 2,4-D (ester) by verskillende konsentrasies (0-45 ppm) en op verskillende tye (volblom tot 4 weke na blomblaarval) toe te dien. 2,4-D by volblom het die persentasie toe navelente verhoog en het die navelent opening betekenisvol verklein, met geen verskille in die konsentrasie

wat by volblom toegedien is nie. Daar was geen negatiewe effekte op oeslading, aantal vrugte per boom en eksterne en interne kwaliteit nie. Die blomblaarval behandelings was oneffektief.

Die potensieële toepassing van hiperspektrale afstandmeting om droogtestres, voedingstres en oeslading te bepaal is geëvalueer op Satsuma mandaryn bome in seksie 5.3.4. 'n Nuwe droogtestres spektrale indeks vir sitrus is ontwikkel wat die blaar waterinhoud vir alle stresse akkuraat voorspel. Oeslading kan met hiperspektrale afstandmeting bepaal word en vrugkleur is nie belangrik in oeslading voorspelling deur afstand meting nie. Boom kleur verandering a.g.v. stikstoftekort in die blare is waargeneem deur die boom spektra.

5.3.2 FINAL REPORT: Economic benefit of hand thinning

Experiment 865 (December 2006 - March 2009): Stephan Verreyne (CRI at SU), Willem van Kerwel (SU)

Opsomming

'n Premie word betaal vir groter vrugte en die inkomste uit klein vrugte is meestal minder as die pluk en transport kostes. Die doel van die studie was om te bepaal of handuitdun enige voordele inhou oor twee seisoene, al veroorsaak dit nie groter vrugte nie. Nules Clementine bome in die Porterville area is gebruik in die eerste seisoen en vrugte <21 mm is op 7 Desember 2006 verwyder. In die tweede seisoen is Nules Clementine en Mihowase Satsuma bome in Stellenbosch gebruik en vrugte <22 mm is op 19 Desember 2007 by Satsuma en vrugte <23 mm op 17 Januarie 2008 by Clementine verwyder om alle vrugte met geskatte grootte < 55 mm by oestyd te verwyder. In die eerste seisoen het handuitdun op Nules Clementine vruggroei en vruggrootte verbeter en die oes met 11% verlaag. Die totale tyd geneem om uit te dun en te oes was dieselfde vir die twee behandelings, dus het handuitdun die oestyd versnel. In die tweede seisoen het handuitdun geen effek op vruggroei, vruggrootte en oeslading gehad op beide Satsuma en Clementine nie, maar het 16 minute (Satsuma) of 22 minute (Clementine) langer geneem in totaal om uit te dun en te oes as onuitgedunde bome. In die tweede seisoen het besproeiings probleme opgeduik, nadat uitdunning reeds gedoen is en uitdunning is ook baie later as in die eerste seisoen gedoen. Daar is egter situasies waar produsente steeds voordeel kan trek uit handuitdunning, maar alle bestuurspraktyke, veral besproeiing, moet optimaal wees en uitdunning moet gedoen word so vroeg as moontlik na die fisiologiese vrugval periode.

Summary

An economic premium is paid for larger fruit and the income from the smaller fruit is often less than the picking and transport costs. The objective of this study was to determine the benefits of hand thinning over two seasons, even if it doesn't result in an increased fruit size. Nules Clementine trees in Porterville were used in the first season where fruit <21 mm on 7 December 2006 were removed. In the second season Nules Clementine trees and Mihowase Satsuma trees in Stellenbosch were used and fruit <22 mm on 19 December 2007 for Satsuma and fruit <23 mm on 17 January 2008 for Clementine were removed in order to remove all fruit estimated to be < 55 mm at harvest. In the first season, hand thinning on Nules Clementine increased fruit growth and fruit size and resulted in a 11% yield reduction. The total time taken to thin and harvest was the same for the two treatments, therefore hand thinning reduced the harvest time. However, in the second season of the study, handthinning had no effect on fruit growth, fruit size and yield for both Satsumas and Clementines, but took 16 minutes (Satsumas) or 22 minutes (Clementines) longer in total to thin and harvest than unthinned trees. In the second season, irrigation problems were encountered after thinning was done and thinning was also done later compared to the first season. However, there may be situations where growers can benefit from handthinning, but all management practices, especially irrigation, should be optimal and thinning should be done as early as possible after the physiological fruit drop period.

Introduction

Fruit size is very important in determining marketable yield. Returns to the grower for small-sized citrus fruit are marginal, due to consumer preference for larger sizes (Gilfillan, 1987). An economic premium is paid for larger fruit and the income from the smaller fruit is often less than the picking and transport costs (Guardiola and Garcia-Luis, 2000). Therefore, an economic premium is usually obtained through an increase in fruit size even at the expense of a reduction in yield (Guardiola and Garcia-Luis, 2000).

For Clementines, fruit size at time of harvest can be fairly accurately estimated (Koch *et al.*, 1996) and damaged and unmarketable small fruit or fruit in clusters can be removed early to prevent fruit overload, therefore alternate bearing and it results in less unmarketable fruit to pick at harvest time. For deciduous

fruit growers it is common to handthin after chemical thinning. Fruit thinning usually causes a certain reduction in total fruit yield, although the smaller yield may be of higher commercial value, therefore the increase in fruit size can compensate for the yield reduction (Galliani et al., 1975).

Since fruit growth rate is dependent on the number of source leaves (Gilfillan, 1987), hand thinning is aimed at varying the leaf:fruit ratio to obtain an optimal ratio for regular yield and fruit size. Hand thinning is a time-consuming, labour-intensive operation. The effect of thinning on the increase in fruit size, is probably due to a reduction in the competition between fruit, resulting in a higher growth rate of the remaining fruit (Zaragoza et al., 1992). However, for the reduction of the competition effect to be evident, thinning must be severe, 20-30% of a heavy set in oranges should be removed to obtain an improvement in fruit size. (Zaragoza et al., 1992). Fruit size improvement is also not as effective on a medium to a low crop load. Rabe (1991), Zaragoza et al. (1992) and Harty and Sutton (1992) also found a good relationship between the severity of thinning and fruit size improvement, but the most severe treatments reduced yields too much. Harty and Sutton (1992) found that lighter thinning levels were ineffective.

Timing of thinning is also very important. The sooner the hand thinning can commence after the end of 'november drop' until 21 days thereafter in oranges (30-50 mm), the better the results will be (Rabe, 1991). This coincides with the end of phase I (cell division) of fruit growth. It is usually not very practical to hand thin earlier in mandarins, since the fruitlets are so small. Removal of flowers was, however, ineffective to increase final fruit size (Zaragoza et al., 1992). Rabe (1991) found that late thinning treatments provided no fruit size benefit, but resulted in a yield reduction.

The objective of this study was to determine the benefits of hand thinning over two seasons, even if it doesn't result in an increased fruit size.

Materials and methods

Plant material and treatments

2006/2007

Nules Clementine trees on Troyer citrange rootstock in Porterville were used in the study. Trees were treated with the synthetic auxin, Corasil E®, earlier in the season. The following treatments were replicated on 12 single trees in a completely randomized block design: 1) control and 2) hand thin all fruit <21 mm on 7 December 2006. Thinning was done as a normal commercial practice on the farm. The number of fruit removed with the thinning treatment and the time taken to thin each tree was recorded. Fruit on all tree replicates were tagged for monthly fruit size measurements. Total yield (kg/tree) and fruit size per tree were determined at harvest on 8 and 23 May 2007.

2007/2008

Nules Clementine trees and Mihowase Satsuma trees in Stellenbosch were used in the study. Trees were treated with the synthetic auxin, Corasil E®, earlier in the season. The following treatments were replicated on 10 single trees in a completely randomized block design: 1) control and 2) hand thin all fruit <22 mm on 19 December 2007 for Satsuma and all fruit <23 mm on 17 January 2008 for Clementine in order to remove fruit estimated to be < 55 mm at harvest. The number of fruit removed with the thinning treatment and the time taken to thin each tree was recorded. Fruit on all tree replicates were tagged for monthly fruit size measurements. Total yield (kg/tree) and fruit size per tree were determined at harvest.

Statistical analysis

Analyses of variance were performed using the GLM (General Linear Models) procedure in the SAS (Statistical Analysis System) computer program.

Results and discussion

2006/2007

Thinning removed on average 494 fruit per tree and took on average 21 minutes for one person per tree (Table 5.3.2.1). Thinning had no significant effect on total yield (kg/tree), but an 11% yield reduction occurred due to thinning. The time taken to harvest unthinned trees was 17 minutes longer than thinned trees but there were no differences in the total time to thin and harvest a tree between the two treatments. Thinning also did not cause a significant shift in the timing of harvest, but 5% less fruit were harvested in the first harvest from thinned trees (Table 5.3.2.2). Handthinning resulted in 8% more marketable fruit >55 mm, but only 3% more marketable fruit >51 mm (Table 5.3.2.3). Hand thinning also resulted in a major shift towards larger fruit sizes compared to the control (Fig. 5.3.2.1). The control trees peaked at size 2 (59-64 mm) while thinned trees peaked at X (68-72 mm) to 2X (72-78 mm). Thinning also increased fruit growth

significantly from the 2nd measuring date until harvest as indicated by the significant P-values at the 5% level for each measuring date after thinning was applied (Fig. 5.3.2.2).

2007/2008

Satsumas

Thinning removed on average 204 fruit per tree and took on average 19 minutes for one person per tree (Table 5.3.2.4). Thinning had no significant effect on total yield (kg/tree), but resulted in a 2% yield increase. The time taken to harvest unthinned trees was only 3 minutes longer than thinned trees, but the total time to thin and harvest a tree was 16 minutes longer for thinned trees. Thinning also had no significant effect on average fruit weight and total fruit number per tree. (Table 5.3.2.5). In general, fruit were small due to irrigation problems on the farm. Handthinning resulted in 16% more marketable fruit >55 mm and 22% more marketable fruit >51 mm (Table 5.3.2.6). Hand thinning did not result in a major shift towards larger fruit sizes compared to the control, but had a higher percentage of fruit in all the larger fruit size categories compared to the control. (Fig. 5.3.2.3). Thinning also resulted in a reduction in small fruit <48 mm from 45 % to 23%. Similarly, thinning had no effect of fruit growth (Fig. 5.3.2.4).

Clementines

Thinning removed on average 218 fruit per tree and took on average 33 minutes for one person per tree (Table 5.3.2.7). Thinning had no significant effect on total yield (kg/tree), but resulted in a 5% yield decrease. The time taken to harvest unthinned trees was 11 minutes longer than thinned trees, but the total time to thin and harvest a tree was 22 minutes longer for thinned trees. Thinning also had no significant effect on average fruit weight and total fruit number per tree. (Table 5.3.2.8). In general, fruit were small due to irrigation problems on the farm. Although non-significant, handthinning resulted in only 4% more marketable fruit >55 mm and 8% more marketable fruit >51 mm (Table 5.3.2.9). Hand thinning did not result in a major shift towards larger fruit sizes compared to the control, but had higher percentages of fruit in size categories 3, 4, and 5 compared to the control and resulted in a reduction in small fruit <48 mm from 31 % to 21% (Fig. 5.3.2.5). Similarly, thinning had no effect of fruit growth (Fig. 5.3.2.6).

Conclusion

In the first season on Clementines, hand thinning increased fruit growth and fruit size and had no significant effect on yield, although it resulted in a 11% yield reduction. The total time taken to thin and harvest was the same for the two treatments, but harvest took 17 minutes longer in the unthinned control trees. However, in the second season of the study, handthinning had no effect on fruit growth, fruit size and yield for both Satsumas and Clementines, but took 16 minutes (Satsumas) or 22 minutes (Clementines) longer in total to thin and harvest than unthinned trees, without the benefits obtained in the first season. The irrigation problems encountered after thinning was done resulted in a reduction in fruit growth compared to normal conditions and therefore smaller fruit, therefore an increase in the percentage of unmarketable fruit. Thinning was also done in the second season on both Satsumas and Clementines later than in the first season. Therefore although handthinning is very time consuming, there may be situations where growers can benefit, if the synthetic auxin used didn't provide the degree of thinning required or where thinning with synthetic auxins are not permitted. Therefore, all management practices, especially irrigation, should be optimal and thinning should be done as early as possible after the physiological fruit drop period to have the desired benefits of removing small unmarketable fruit earlier in the season to make harvesting faster and possibly reduce alternate bearing.

Future research

The study was terminated, but can be extended to other cultivars for instance grapefruit, where fruit size improvement using synthetic auxins is not recommended for the majority of the export markets.

Technology transfer

Verreyne, J.S., van Kerwel. W. The benefits of hand thinning Nules Clementine mandarins (Poster). Proceedings of the 11th International Citrus Congress. Wuhan, China, 26-30 October 2008. (In press).

Presentations (Abstracts in proceedings)

Verreyne, J.S., van Kerwel. W. The benefits of hand thinning Nules Clementine mandarins (Poster). 5th Citrus Research Symposium, Citrus Research International, Drakensberg, South Africa, 3-6 August 2008.

Verreyne, J.S., van Kerwel. W. The benefits of hand thinning Nules Clementine mandarins (Poster). 11th International Citrus Congress. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

Grower Bulletins-Cutting Edge

J.S. Verreyne, Fruit size management strategies on Citrus. Cutting Edge No 72.

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Zaragoza, S., Trenor, I., Alonso, E., Primo-Millo, E. and Agusti, M., 1992. Treatments to increase the final fruit size on Satsuma 'Clausellina'. Proc. Int. Soc. Citric. 2, 725-728.

Table 5.3.2.1. The effect of hand thinning on total yield and the time taken to thin and harvest Nules Clementine trees in 2007.

Treatment	Removed	Time taken	Yield	Time taken	Total time Taken for thin and harvest	Yield reduction
	-no./tree-	--minute ^y --	-kg/tree	--minute--	--minute--	--%--
Control	--	--	187.6	137	137	--
Handthin, 7 Dec 2006, ≤ 21 mm	494	21	167.2	120	141	11%
P-value			0.1433	0.2153	0.8423	

^yminute indicate the time taken for one person to thin or harvest one tree.

^zMeans in a vertical column followed by different letters are significantly different at the 5% level.

Table 5.3.2.2. Effect of hand thinning on the shift in harvest time of Nules Clemetine trees in 2007.

Treatment	1 st harvest	% of total yield	2 nd harvest	Total yield
	-kg/tree-	--%--	-kg/tree-	-kg/tree-
Control	119.6	63.4	62.1	187.6
Handthin, 7 Dec 2006, ≤ 21 mm	97.3	58.5	69.9	167.2
P-value	0.1620	0.3578	0.8280	0.1433

There were no significant differences at the 5% level.

Table 5.3.2.3. The effect of hand thinning on the percentage of marketable fruit > count 3 (55 mm) or > count 4 (51 mm) on Nules Clementine trees in 2007.

Treatment	>55 mm	Marketable yield	>51 mm	Marketable yield
	--%--	--kg--	--%--	--kg--
Control	90.4 b	169.6	96.3	180.7
Handthin, 7 Dec 2006, ≤ 21 mm	98.1 a	164.0	99.5	166.4
P-value	0.0413	--	0.0874	--

Means in a vertical column followed by different letters are significantly different at the 5% level.

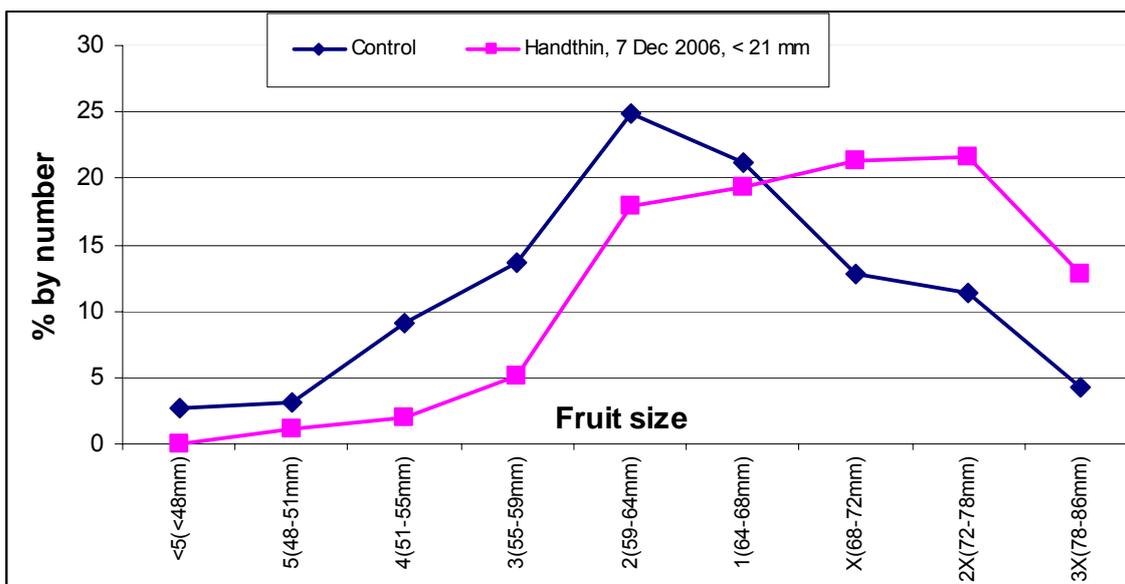


Figure 5.3.2.1. The effect of handthinning on the fruit size distribution at harvest of Nules Clementine trees in 2007.

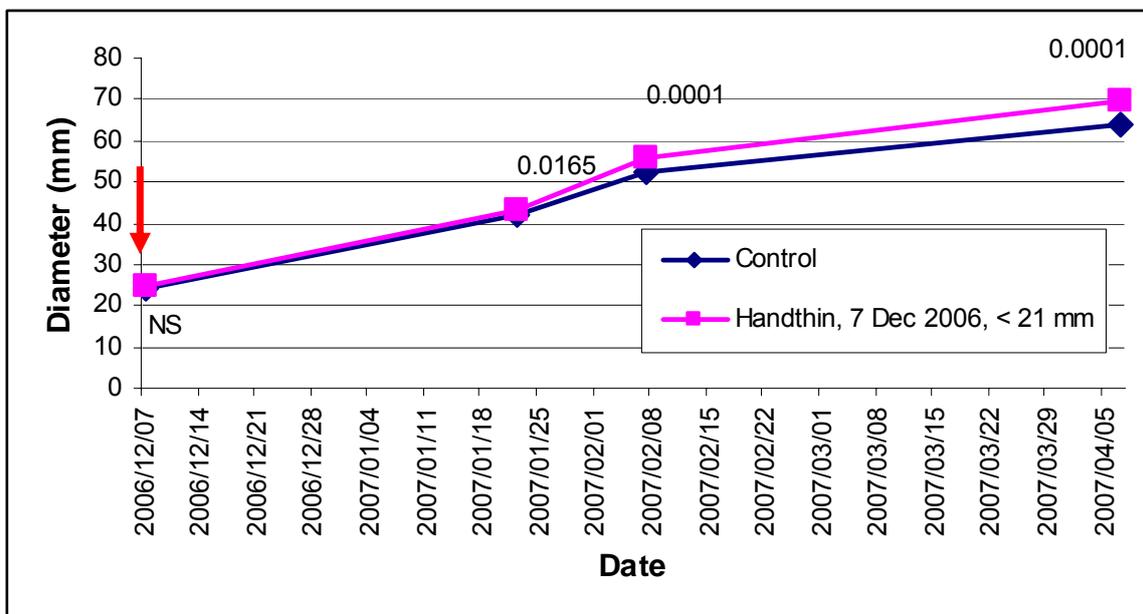


Figure 5.3.2.2. The effect of handthinning on fruit growth throughout the season of Nules Clementine fruit in 2007.

Table 5.3.2.4. The effect of hand thinning on total yield and the time taken to thin and harvest Miho Wase Satsuma trees in 2008.

Treatment	Removed	Time taken	Yield	Time taken	Total time taken for thin and harvest	Yield increase
	-no./tree-	--minute ^y --	-kg/tree	--minute--	--minute--	--%--
Control	--	--	44.6	45	45 b ^z	--
Handthin, 19 Dec 2007, ≤ 22 mm	204	19	45.4	42	61 a	2%
<i>P</i> -value			0.8736	0.2930	0.0076	

^yminute indicate the time taken for one person to thin or harvest one tree.

^zMeans in a vertical column followed by different letters are significantly different at the 5% level.

Table 5.3.2.5. Effect of hand thinning on the average fruit weight and number of fruit of Miho Wase Satsuma trees in 2008.

Treatment	Average weight of fruit	Number of fruit	Total yield
	--g--	--no.--	-kg/tree
Control	73.7	633	44.6
Handthin, 19 Dec 2007, ≤ 22 mm	82.6	559	45.4
<i>P</i> -value	0.2843	0.3976	0.8736

There were no significant differences at the 5% level.

Table 5.3.2.6. The effect of hand thinning on the percentage of marketable fruit > count 3 (55 mm) or > count 4 (51 mm) on Miho Wase Satsuma trees in 2008.

Treatment	>55 mm	Marketable yield	>51 mm	Marketable yield
	--%--	--no. of fruit--	--%--	--no. of fruit--
Control	17.3 b	102 b	34.2 b	207 b
Handthin, 19 Dec 2007, ≤ 22 mm	33.4 a	175 a	56.3 a	303 a
<i>P</i> -value	0.0105	0.0069	0.0006	0.0021

Means in a vertical column followed by different letters are significantly different at the 5% level.

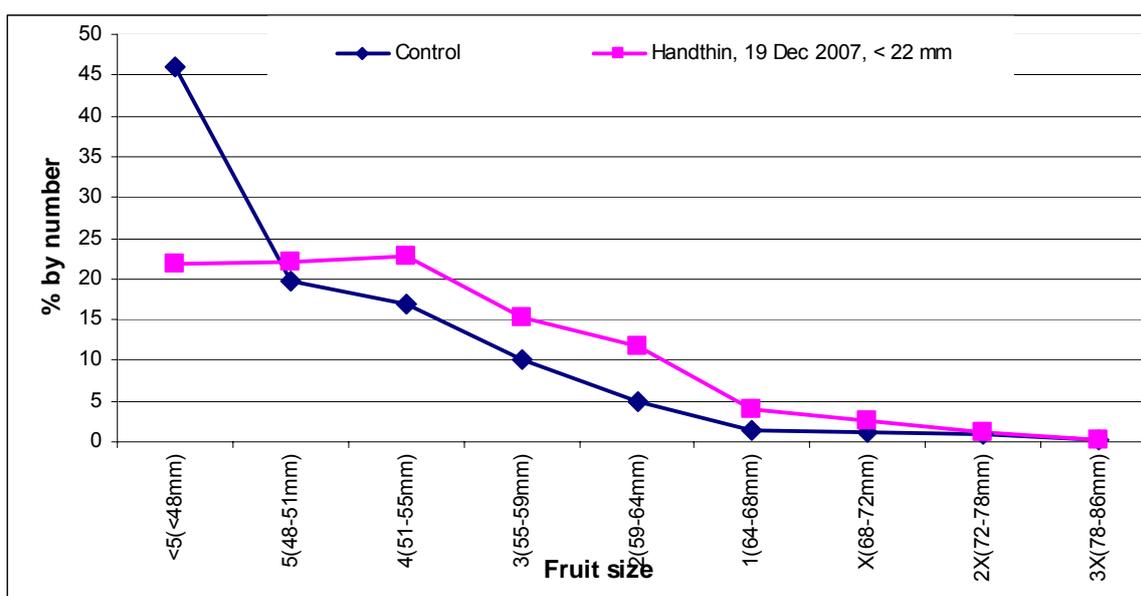


Figure 5.3.2.3. The effect of handthinning on the fruit size distribution at harvest of Miho Wase Satsuma trees in 2008.

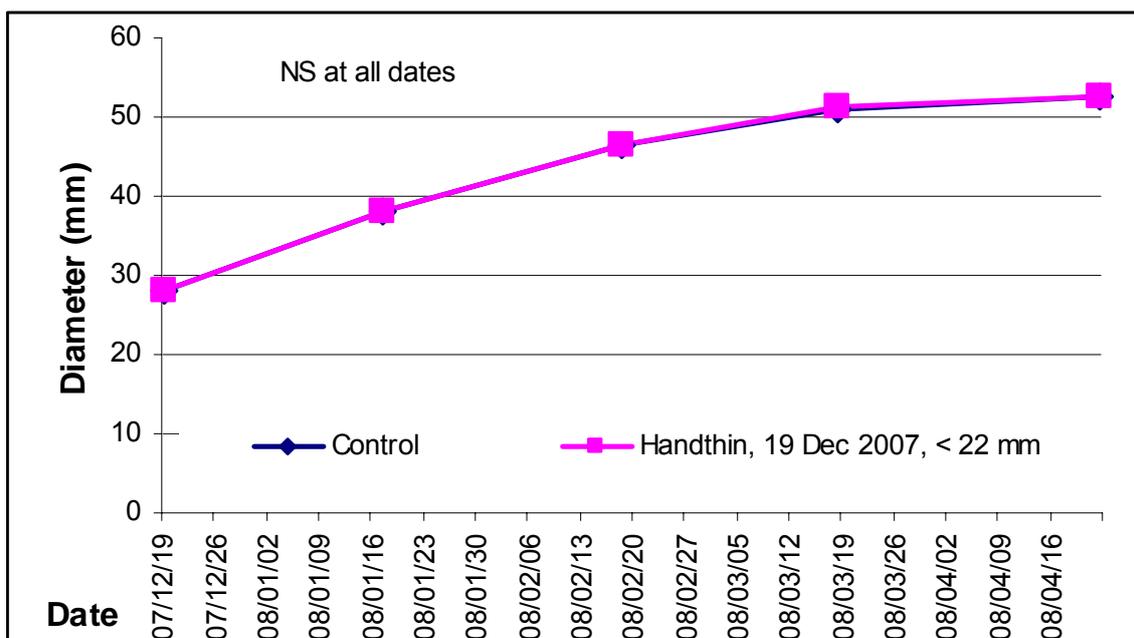


Fig.5.3.2.4. The effect of handthinning on fruit growth throughout the season of Miho Wase Satsuma fruit in 2008.

Table 5.3.2.7. The effect of hand thinning on total yield and the time taken to thin and harvest Nules Clementine trees in 2008.

Treatment	Removed	Time taken	Yield	Time taken	Total time taken for thin and harvest	Yield decrease
	-no./tree-	--minute ^y -	-kg/tree	--minute--	--minute--	--%--
Control	--	--	45.3	68	68	--
Handthin, 17 Jan 2008, <23 mm	218	33	42.9	57	90	5
<i>P</i> -value		--	0.7472	0.4979	0.1735	

^yminute indicate the time taken for one person to thin or harvest one tree. There were no significant differences at the 5% level.

Table 5.3.2.8. Effect of hand thinning on the average fruit weight and number of fruit of Nules Clementine trees in 2008.

Treatment	Average weight of fruit	Number of fruit	Total yield
	--g--	--no.--	-kg/tree
Control	55.8	830	45.3
Handthin, 17 Jan 2008, <23 mm	58.8	754	42.9
<i>P</i> -value	0.5658	0.6335	0.7472

There were no significant differences at the 5% level.

Table 5.3.2.9. The effect of hand thinning on the percentage of marketable fruit > count 3 (55 mm) or > count 4 (51 mm) on Nules Clementine trees in 2008.

Treatment	>55 mm	Marketable yield	>51 mm	Marketable yield
	--%--	--no. of fruit--	--%--	--no. of fruit--
Control	34.7	292	52.5	438
Handthin, 17 Jan 2008, <23 mm	39.3	294	60.8	454
P-value	0.4792	0.9802	0.3019	0.8571

There were no significant differences at the 5% level.

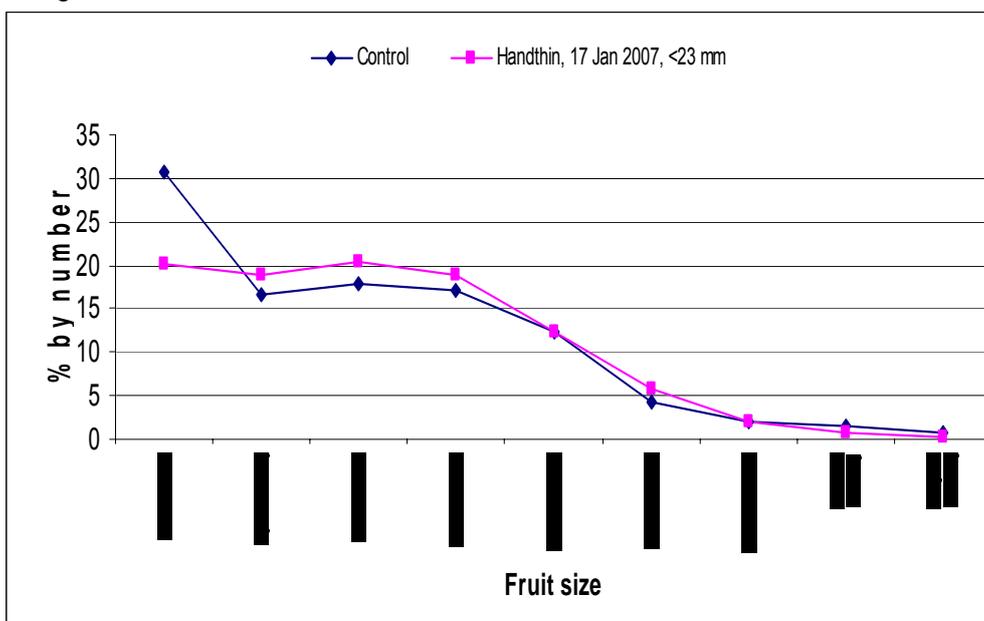


Figure 5.3.2.5. The effect of handthinning on fruit growth throughout the season of Nules Clementine fruit in 2008.

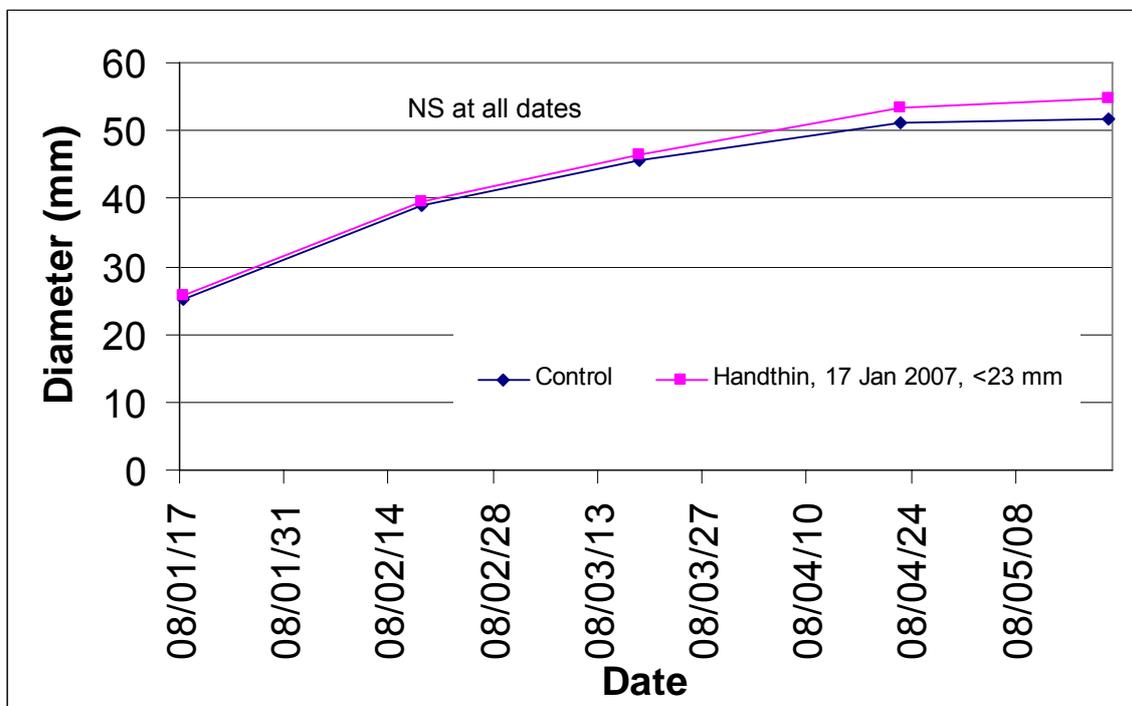


Figure 5.3.2.6. The effect of handthinning on the fruit size distribution at harvest of Nules Clementine in 2008.

5.3.3 PROGRESS REPORT: Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges

Experiment 935 (October 2007 - March 2009): Stephan Verreyne (CRI by SU), Giverson Mupambi (SU)

Opsomming

Groot, uitstaande navel ente by navel lemoene is 'n groot uitskot faktor in die pakhuis. Om die effektiwiteit van 2,4-D, met die doel om die navelent opening te verklein, onder Suid-Afrikaanse toestande te toets, is navel lemoenbome behandel met 2,4-D by verskillende konsentrasies en verskillende tye. Washington, Newhall en Navelina navel lemoenbome in Citrusdal, Robyn navelbome in Clanwilliam en Palmer navelbome in Addo is behandel met kombinasies van 15, 20, 25, 30, 35 of 45 ppm 2,4-D (ester) by volblom (FB), blomblaarval (PD), 2 weke na blomblaarval (2WAPD) of 4 weke na blomblaarval (4WAPD). By oestyd is navelent opening, oeslading, eksterne en interne kwaliteit geëvalueer. Toediening van 2,4-D by volblom het die persentasie toe navelente verhoog en het die navelent opening betekenisvol verklein, met geen verskille in die konsentrasie wat by volblom toegedien is nie. Daar was geen negatiewe effekte op oeslading, aantal vrugte per boom en eksterne en interne kwaliteit nie. Die blomblaarval behandelings was oneffektief, in kontras met die vorige seisoen se resultate. 2,4-D toon baie potensiaal as 'n behandeling om navelente te verklein by navel lemoene.

Summary

Large, protruding navel ends of navel oranges are a major cull factor in the packhouse. To examine the efficacy of 2,4-D in reducing navel end size under South African conditions navel trees were treated with 2,4-D at different concentrations and different timings. Washington, Newhall and Navelina navel trees in Citrusdal, Robyn navel trees in Clanwilliam and Palmer navel trees in Addo were treated with combinations of 15, 20, 25, 30, 35 or 45 ppm of 2,4-D (ester) at full bloom (FB), petal drop (PD), 2 weeks after petal drop (2WAPD) or 4 weeks after petal drop (4WAPD). At harvest, navel end size, yield, external and internal fruit quality were evaluated. Application of 2,4-D at full bloom caused an increase in the percentage of closed navel ends and significantly reduced the navel end size, with no differences among the different concentrations at full bloom. There were no negative effects on yield, fruit number and fruit internal or external quality. The petal drop applications seem to be ineffective, in contrast to results obtained the previous season. 2,4-D shows a lot of potential as a treatment to reduce navel end size of navel oranges.

Introduction

Large, protruding navel ends of navel oranges are a major cull factor in the packhouse. A smaller navel end would not only reduce the percentage culled fruit due to large navels, but would possibly lead to reduced insect damage, caused by mealybugs, bud mite or false codling moth and reduce the occurrence of insects hiding in the navel end opening.

Previous work on Lane Late navels suggested that the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), sprayed at full bloom reduces the size of the navel end (Gardiazabal, 2006). Five to 20 ppm was evaluated with 20 ppm giving the best results. 20 ppm resulted in 49% closed navel ends compared to 3% in the control and a navel end size of 4.8 mm compared to 12 mm in the control. 2,4-D had no effect on the number of fruit per tree and yield. In the following season, 15 ppm applied on Lane Late navel trees resulted in smaller navel ends (2.5 vs. 7.6 mm) and a greater percentage closed navel ends (82 vs. 22%) with no effect on yield or fruit size (Gardiazabal, 2006).

In a separate study, comparing the synthetic auxins 2,4-D (20 ppm at full bloom), 2,4-DP (50 ppm at 26 or 27mm fruit diameter) and 3,5,6-TPA (15 ppm at 26 or 27mm fruit diameter), only 2,4-D increased the percentage fruit with closed navels, decreased the percentage fruit with split navels and reduced navel end size, with no differences in yield, fruit size or fruit shape compared to the control, with the other auxins giving no significant results (Saavedra, 2006). 2,4-D decreased juice percentage (38 vs. 41%) and titratable acidity (TA), had no effect on total soluble solids (TSS), and increased the TSS:TA ratio. All auxins applied increased rind coarseness, but had no effect on external colour, fruit shape or rind thickness (Saavedra, 2006).

The effect of 2,4-D on fruit size is well documented (Duarte et al., 1996; Erickson and Richards, 1955; Erner et al., 1993; Guardiola, 1996; Stewart et al. 1951, 1952; Stewart and Klotz, 1947; Stewart and Parker, 1954). 2,4-D applied at full bloom or slightly later normally results in a slight thinning effect resulting in no effect on yield due to a reduced number of larger fruit (Duarte et al., 1996; Erickson and Richards, 1955; Stewart et al.

1951). Some negative treatment effects on fruit quality reported include decreased juice percentage and TSS, increased TA, delayed colour development, thicker pedicels in relation to fruit diameter and a thinning effect. None of the studies using 2,4-D to hang fruit late reported a negative effect on internal or external fruit quality.

The objective of this study was to determine the efficacy of 2,4-D in reducing navel end size under South African conditions as well as to determine any detrimental effects on internal fruit quality, external fruit quality and vegetative growth.

Materials and methods

Treatments and plant material

Washington

Healthy Washington navel trees of the same size on rough lemon rootstock were used for this trial. The orchard was situated in the Citrusdal area of Western Cape, South Africa and was planted in 1954. Tree spacing was 6 m between the rows and 6 m within the rows. Trees used in the trial were selected for consistency in size and health status. Row direction of the orchard was east-west. Trees were treated with 15, 25 or 35 ppm 2,4-D (ester) respectively, at full bloom (FB) or at petal drop (PD). The trial consisted of a randomized complete block design with eight single-tree replicates per treatment.

Robyn

Healthy Robyn navel trees of the same size on rough lemon rootstock were used for this trial. The orchard was situated in the Clanwilliam area of Western Cape, South Africa and was planted in 1987. Tree spacing was 6 m between the rows and 4 m within the rows. Trees used in the trial were selected for consistency in size and health status. Row direction of the orchard was north-south. Trees were treated with 20 or 25 ppm 2,4-D (ester) respectively, at full bloom (FB) or at petal drop (PD) and with 25 ppm 2,4-D 2 weeks after petal drop (2WAPD). The trial consisted of a randomized complete block design with eight single-tree replicates per treatment.

Newhall

Healthy Newhall navel trees of the same size on rough lemon rootstock were used for this trial. The orchard was situated in the Citrusdal area of Western Cape, South Africa and was planted in 1993. Tree spacing was 5 m between the rows and 2 m within the rows. Trees used in the trial were selected for consistency in size and health status. Row direction of the orchard was north-south. Trees were treated with 25 ppm 2,4-D (ester) at full bloom (FB), 20 ppm 2,4-D or 25 ppm 2,4-D in combination with 10 ppm GA at petal drop, 25 ppm 2,4-D 2WAPD or 25 ppm 2,4-D 4WAPD. The trial consisted of a randomized complete block design with eight single-tree replicates per treatment.

Navelina

Healthy Navelina navel trees of the same size on rough lemon rootstock were used for this trial. The orchard was situated in the Citrusdal area of Western Cape, South Africa and was planted in 1993. Tree spacing was 5 m between the rows and 2 m within the rows. Trees used in the trial were selected for consistency in size and health status. Row direction of the orchard was north-south. Trees were treated with 15, 25 or 35, or 45 ppm 2,4-D (ester) or with 35 ppm 2,4-D (ester) in combination with 10 ppm GA at petal drop (PD). The trial consisted of a randomized complete block design with eight single-tree replicates per treatment.

Palmer

Healthy Palmer navel trees of the same size on carrizo rootstock were used for this trial. The orchard was situated in the Addo area of Eastern Cape, South Africa and was planted in 2001. Tree spacing was 6 m between the rows and 4 m within the rows. Trees used in the trial were selected for consistency in size and health status. Row direction of the orchard was north-south. Trees were treated with 15, 20, 25 or 30 ppm 2,4-D (ester) at petal drop (PD). The trial consisted of a randomized complete block design with eight single-tree replicates per treatment.

Measurements

For the Newhall, Navelina and Robyn navels all fruit was harvested and weighed and counted on a digital scale to determine total yield per tree (kg). At commercial harvest of each cultivar the following measurements were done: Approximately 20 kg from each tree was harvested for evaluations. Fruit diameter and the size of the navel end were measured on each fruit using an electronic caliper to determine the percentage closed navel ends. A subsample of 12 average sized fruit was taken per tree. The subsample was used to determine external and internal fruit quality. External fruit quality was determined by

measuring fruit diameter, fruit height and peel thickness using an electronic calliper, fruit colour based on the no. 34 CRI colour chart for oranges, with eight being dark green and one being fully coloured (orange), and rind coarseness based on the no. 20 CRI skin texture chart for oranges with eight being coarse and one being smooth. Colour at the navel end was scored as four being green and one being orange. The pedicel diameter was measured using electronic calipers. Fruit were scored for creasing severity with a score of 0-4; the orange was divided into four equivalent spheres. If no sphere was creased it was designated a zero, if a single sphere was creased, a one etc. Each fruit was cut in half and scored for the presence of granulation. Peel thickness was measured using an electronic caliper. Juice was extracted to determine internal fruit quality, using a citrus juicer. Juice was then strained through a layer of muslin cloth and the percentage juice content was determined by dividing the weight of the juice with the total fruit weight. The total soluble solids (TSS) of the juice was determined by an electronic refractometer. Titratable acidity (TA), expressed as citric acid content, was determined by titration against 0.1 N sodium hydroxide, using phenolphthalein as an indicator. The TSS:TA ratio was calculated by dividing the TSS values by the TA values.

Statistical analysis

Statistical analysis of variance (ANOVA) was carried out using generalized linear models (GLM) method in Statistical Analysis System (SAS) computer software (SAS Inc., 1990).

Results and discussion

Washington

The application of 2,4-D at full bloom significantly reduced the average navel end size and increased the percentage of closed navel ends significantly compared to the control (by about 24%), with no difference among the different concentrations (Table 5.3.3.1). 2,4-D applied at petal drop, irrespective of the concentration applied (15, 25 or 35 ppm), had no effect on the average navel end size or the percentage of closed navel ends. None of the treatments applied at full bloom or petal drop effected fruit diameter significantly. Full bloom applications resulted in a delay in styler abscission as well as delayed petal drop by about two weeks. None of the treatments had an effect on fruit colour, pedicel diameter, colour at the navel end or creasing incidence (Table 5.3.3.2). Similarly, none of the treatments had a significant effect on juice percentage, total soluble solids (TSS), titratable acidity (TA) or the TSS:TA ratio (Table 5.3.3.3).

Robyn

Some treatments had significantly larger fruit compared to the control, but there was no clear trend (Table 5.3.3.4). Both full bloom treatments (20 and 25 ppm) had significantly smaller navel end size than the control, whereas the late treatment (4 weeks after petal drop) had bigger navel end size than the control. Similarly, both full bloom treatments significantly increased the percentage of closed navel ends (by about 24%). There were no significant differences in yield compared to the control. There were no significant difference in fruit colour, colour at the navel end and creasing severity compared to the control (Table 5.3.3.5). 2,4-D applied at 25 ppm at FB, 25 ppm applied 2WAPD, and 25 ppm applied 4WAPD significantly reduced pedicel diameter compared to control fruit. Later applications of 2,4-D applied at 25ppm at PD, 2WAPD or 4WAPD significantly reduced juice percentage, with earlier applications having no effect (Table 5.3.3.6). None of the treatments had an effect on TSS or TA, but 2,4-D at 20 ppm or 25 ppm both at PD increased the TSS:TA ratio.

Newhall

There were no significant difference in fruit diameter, total fruit number per tree at harvest and yield (kg/tree) compared to the control (Table 5.3.3.7). All the treatments, except for 2,4-D at 25 ppm applied at 4WAPD significantly reduced the average navel end size, but only 2,4-D applied at 25 ppm at FB (by about 24%) and the combination of 2,4-D (25 ppm) and GA (10 ppm) (by about 12%) both applied at PD significantly reduced the percentage of closed navel ends compared to the control. None of the treatments had a significant effect on fruit colour, colour at the navel end or creasing severity, but 2,4-D at 25 ppm applied at PD, 2WAPD, 4WAPD and the combination of 2,4-D and GA at PD significantly reduced pedicel diameter compared to the control (Table 5.3.3.8). 2,4-D applied at 25 ppm at FB, PD, and 4WAPD significantly reduced juice percentage compared to the control, but none of the treatments significantly affected TSS, TA or the TSS:TA ratio (Table 5.3.3.9).

Navelina

There were no significant differences in fruit diameter, navel end size, the percentage of closed navel ends, total fruit number per tree at harvest and yield per tree (kg/tree) compared to the control (Table 5.3.3.10). 2,4-D at 25 ppm applied at PD significantly delayed colour development and none of the treatments had an effect on the colour at the navel end (Table 5.3.3.11). The combination of 2,4-D and GA at PD and 2,4-D at 35 ppm at PD significantly increased pedicel diameter compared to the control. 2,4-D at 15 ppm at PD

significantly increased creasing severity whereas 2,4-D at 45 ppm at PD significantly decreased creasing severity compared to the control. Although the combination of 2,4-D and GA applied at PD, and 2,4-D applied at 35 ppm at PD significantly reduced juice percentage, none of the treatments had an effect on TSS, TA or the TSS:TA ratio (Table 5.3.3.12).

Palmer

There were no significant differences in fruit diameter, navel end size or the percentage of closed navel ends compared to the control (Table 5.3.3.13). There were no significant differences in fruit colour, pedicel diameter and fruit shape (height/diameter) compared to the control, but all the treated fruit had significantly greater peel thickness than the control (Table 5.3.3.14). Although 2,4-D applied at 20 ppm at PD significantly reduced juice percentage and 2,4-D applied at 25 ppm at PD significantly increased juice percentage compared to the control, there were no significant differences in TSS, TA and the TSS:TA ratio compared to the control (Table 5.3.3.15).

Conclusion

Application of 2,4-D at full bloom caused an increase in the number of closed navel ends and significantly reduced the navel end size. There were no differences in navel end size or percentage closed navels among the different concentrations applied at full bloom. There were no negative effects on yield and fruit internal or external quality. Therefore, the petal drop applications seem to be ineffective and full bloom applications gave the best results. This is contradictory to the results obtained in the previous season. Climate and/or crop load or cultivar differences may be the result of these contradictory findings between the two seasons. Also, the timing of applications seems to be more important than the concentration applied, since even the lowest concentration at full bloom gave very good results. Although the mode of action by which 2,4-D reduces the size of the navel end opening is still unclear, the delay in style abscission on treated fruit may play a role. 2,4-D shows a lot of potential as a treatment to reduce navel end size of navel oranges. Also, the optimal timing and concentration of application of 2,4-D on different navel cultivars with different crop loads under different climatic conditions needs to be determined.

Additionally, the following observations were made throughout the season on all the cultivars. Some of the styles were still attached to fruit at a late stage of fruit development. Leaf damage comprising of curled and distorted leaves occurred on the fruit bearing flush (spring flush) of treated trees, but the damage was not obviously visible at harvest time. No attached styles were observed on fruit from treated trees at harvest time.

Further objectives and work plan

Future research includes extending the study to cover more climatic areas, Swellendam etc, to study the effect of 2,4-D on fruit split, to use different wetters/spreaders to increase the effectiveness and to study the effect of using different spray volumes, etc. low volumes vs. high volume.

Technology transfer

- JS Verreynne. Extension Briefs, Crop and fruit quality management, South African Fruit Journal 7(2):42, Apr/ May 2008.
- JS Verreynne. Extension Briefs, Crop and fruit quality management, South African Fruit Journal 7(3):31, June/ July 2008.
- JS Verreynne. Extension Briefs, Crop and fruit quality management, South African Fruit Journal 7(4):83, Aug/ Sept 2008.
- JS Verreynne. Extension Briefs, Crop and fruit quality management, South African Fruit Journal 7(5):60, Oct/Nov 2008.
- JS Verreynne. Extension Briefs, Crop and fruit quality management, South African Fruit Journal 7(6):63, 64, Dec 2008/Jan 2009.
- JS Verreynne. Extension Briefs, Crop and fruit quality management, South African Fruit Journal 8(1):64, Feb/March 2009.

Popular Journals

- JS Verreynne. Factors affecting sheeppnose incidence in grapefruit, South African Fruit Journal 7(3):16-18, June/ July 2008.

Conference/symposium presentations
Presentations (Full-length Proceedings)

Verreyne, J.S. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges - A preliminary study. Proceedings of the 11th International Citrus Congress. Wuhan, China, 26-30 October 2008. (In press).

Presentations (Abstracts in proceedings)

Verreyne, J.S. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges - A preliminary study. 5th Citrus Research Symposium, Citrus Research International, Drakensberg, South Africa, 3-6 August 2008.

Mupambi, G., Verreyne, J.S.: Studies to reduce the size of the navel end opening. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.

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Table 5.3.3.1. Effect of 2,4-D on fruit diameter, navel end size and percentage closed navels of Washington navel oranges.

Treatments	Fruit diameter	Navel end size	Closed navels
	--mm--	--mm--	--%--
Control	71.25	3.71 a ^z	28.36 bc
2,4-D at 15 ppm at FB	70.69	2.07 bc	53.33 a
2,4-D at 25 ppm at FB	73.28	2.05 bc	49.87 a
2,4-D at 35 ppm at FB	72.16	1.79 c	54.23 a
2,4-D at 15 ppm at PD	70.43	3.24 ab	26.72 bc
2,4-D at 25 ppm at PD	70.87	3.08 ab	39.69 ab
2,4-D at 35 ppm at PD	71.38	3.67 a	19.76 c
P- value	0.3799	0.0071	0.0005

^zMeans in a vertical column followed by different letters are significantly different at the 5% level (LSD).

FB (full bloom).

PD (petal drop).

Table 5.3.3.2. Effect of 2,4-D on external fruit quality of Washington navel oranges.

Treatments	Colour ^y	Pedical diameter	Colour at navel end ^x	Creasing ^w
Control	1.55	2.94	0.88	0.77
2,4-D at 15 ppm at FB	2.05	2.78	1.01	0.90
2,4-D at 25 ppm at FB	1.86	3.08	0.83	0.67
2,4-D at 35 ppm at FB	1.73	2.73	0.95	0.76
2,4-D at 15 ppm at PD	1.45	2.70	0.47	1.02
2,4-D at 25 ppm at PD	1.61	3.17	1.01	1.23
2,4-D at 35 ppm at PD	1.83	2.92	0.75	1.42
<i>P- value</i>	0.1676	0.1899	0.1851	0.6166

^y1-8 on colour chart, 1-orange, 8-green.

^x0-4, 0-orange, 4-green.

^w0-4, 0-no creasing, 4 whole fruit creased.

FB (full bloom).

PD (petal drop).

Table 5.3.3.3. Effect of 2,4-D on the internal fruit quality of Washington navel oranges.

Treatments	Juice %	TSS	TA %	TSS:TA
	--%--		--%--	
Control	42.63	12.06	0.87	13.93
2,4-D at 15 ppm at FB	41.93	11.38	0.81	13.92
2,4-D at 25 ppm at FB	42.19	11.38	0.92	12.53
2,4-D at 35 ppm at FB	41.49	12.08	0.84	14.36
2,4-D at 15 ppm at PD	42.21	11.88	0.84	14.14
2,4-D at 25 ppm at PD	39.04	12.30	0.84	14.65
2,4-D at 35 ppm at PD	42.06	11.78	0.82	14.37
<i>P- value</i>	0.3908	0.1214	0.5763	0.1886

FB (full bloom).

PD (petal drop).

Table 5.3.3.4. Effect of 2,4-D on fruit diameter, navel size, percentage closed navels and yield of Robyn navel oranges.

Treatments	Fruit diameter	Navel size	Closed navels	Yield
	--mm--	--mm--	--%--	--kg/tree--
Control	71.70 c	2.27 bc	44.05 bcd	132.85
2,4-D at 20 ppm at FB	71.58 c	1.32 d	68.12 a	147.17
2,4-D at 25 ppm at FB	74.29 ab	1.35 d	67.26 a	125.59
2,4-D at 20 ppm at PD	71.95 bc	1.71 bcd	53.37 abc	133.09
2,4-D at 25 ppm at PD	71.16 c	1.48 cd	62.16 ab	131.28
2,4-D at 25 ppm at 2WAPD	72.48 abc	2.39 b	35.13 cd	142.73
2,4-D at 25 ppm at 4WAPD	74.37 a	3.41 a	30.68 d	139.08
<i>P- value</i>	0.0352	0.0001	0.0030	0.8834

Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

FB (full bloom).

PD (petal drop).

WAPD (weeks after petal drop).

Table 5.3.3.5. Effect of 2,4-D on the external fruit quality of Robyn navel oranges.

Treatments	Colour ^y	Pedicle diameter	Colour at navel end ^x	Creasing ^w
Control	2.32	3.67 ab	1.27	0.39
2,4-D at 20 ppm at FB	2.07	3.55 bc	1.69	0.44
2,4-D at 25 ppm at FB	1.96	3.28 cd	1.59	0.32
2,4-D at 20 ppm at PD	2.08	3.66 ab	1.48	0.49
2,4-D at 25 ppm PD	1.98	3.85 a	1.31	0.51
2,4-D at 25 ppm at 2WAPD	2.01	3.09 d	1.43	0.55
2,4-D at 25 ppm at 4WAPD	1.87	3.33 cd	1.71	0.16
<i>P</i> -value	0.5375	0.0001	0.2827	0.2826

Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

^y1-8 on colour chart, 1-orange, 8-green.

^x0-4, 0-orange, 4-green.

^w0-4, 0-no creasing, 4 whole fruit creased.

FB (full bloom).

PD (petal drop).

WAPD (weeks after petal drop).

Table 5.3.3.6. Effect of 2,4-D on the internal fruit quality of Robyn navel oranges.

Treatments	Juice	TSS	TA %	TSS:TA
	--%--		--%--	
Control	48.92 a	9.63	0.96	9.96 bc
2,4-D at 20 ppm at FB	47.84 a	9.73	1.01	9.62 c
2,4-D at 25 ppm at FB	47.71 a	9.93	0.94	10.45 abc
2,4-D at 20 ppm at PD	48.54 a	10.18	0.93	11.02 a
2,4-D at 25 ppm at PD	45.74 b	10.48	0.93	11.28 a
2,4-D at 25 ppm at 2WAPD	44.70 b	9.95	0.92	10.77 ab
2,4-D at 25 ppm at 4WAPD	45.53 b	10.27	0.96	10.65 ab
<i>P</i> -value	0.0001	0.0795	0.3307	0.0263

Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

FB (full bloom).

PD (petal drop).

WAPD (weeks after petal drop).

Table 5.3.3.7. Effect of 2,4-D on fruit diameter, navel end size, percentage closed navels, total fruit number and yield of Newhall navel oranges.

Treatments	Fruit Diameter	Navel end size	Closed navels	Total fruit	Yield
	--mm--	--mm--	--%--	--no.--	--kg/tree--
Control	74.93	7.31 a	12.93 c	595	135.91
2,4-D at 25 ppm at FB	75.57	4.73 c	36.24 a	561	124.07
2,4-D at 25 ppm at PD	75.72	5.41 bc	20.02 bc	554	124.16
2,4-D (25 ppm) + GA (10 ppm) at PD	75.74	5.35 c	24.41 b	464	110.12
2,4-D at 25 ppm at 2WAPD	74.09	5.82 bc	17.63 bc	533	119.83
2,4-D at 25 ppm at 4WAPD	74.20	6.76 ab	14.42 c	574	127.93
<i>P</i> -value	0.8293	0.0057	0.0001	0.3395	0.2452

²Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

FB (full bloom).

PD (petal drop).

WAPD (weeks after petal drop).

Table 5.3.3.8. Effect of 2,4-D on the external fruit quality of Newhall navel oranges.

Treatments	Colour ^y	Pedicel diameter	Colour at navel end ^x	Creasing ^w
Control	1.73	3.37 a	0.67	0.21
2,4-D at 25 ppm at FB	1.56	3.19 ab	0.91	0.22
2,4-D at 25 ppm at PD	1.27	2.92 c	0.73	0.21
2,4-D (25 ppm) + GA (10 ppm) at PD	1.90	2.89 c	0.70	0.21
2,4-D at 25 ppm at 2WAPD	1.61	2.65 d	0.71	0.36
2,4-D at 25 ppm at 4WAPD	1.48	3.02 bc	0.68	0.21
<i>P- value</i>	0.0627	0.0001	0.2228	0.8178

Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

^y1-8 on colour chart, 1-orange, 8-green.

^x0-4, 0-orange, 4-green.

^w0-4, 0-no creasing, 4 whole fruit creased.

FB (full bloom).

PD (petal drop).

WAPD (weeks after petal drop).

Table 5.3.3.9. Effect of 2,4-D on the internal fruit quality of Newhall navel oranges.

Treatments	Juice	TSS	TA %	TSS:TA
	--%--		--%--	
Control	45.07 a	9.89	0.81	12.16
2,4-D at 25 ppm at FB	42.58 c	9.98	0.83	12.03
2,4-D at 25 ppm at PD	42.27 c	10.03	0.84	11.96
2,4-D (25 ppm) + GA (10 ppm) at PD	44.74 ab	10.00	0.85	11.88
2,4-D at 25 ppm at 2WAPD	45.28 a	9.75	0.82	11.88
2,4-D at 25 ppm at 4WAPD	42.89 bc	9.83	0.85	11.53
<i>P- value</i>	0.0065	0.9544	0.8789	0.8407

Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

FB (full bloom).

PD (petal drop).

WAPD (weeks after petal drop).

Table 5.3.3.10. Effect of 2,4-D on fruit diameter, navel end size, percentage closed navels and yield of Navelina navel oranges.

Treatments	Fruit diameter	Navel end size	Closed navels	Total fruit	Yield
	--mm--	--mm--	--%--	--no.--	--kg/tree--
Control	75.05	6.37	21.14	317	74.94
2,4-D at 15ppm at PD	75.70	5.68	30.36	375	91.07
2,4-D at 25ppm at PD	76.36	6.27	30.42	392	101.85
2,4-D (25ppm) + GA (10ppm) at PD	76.92	7.47	32.24	317	85.72
2,4-D at 35ppm at PD	77.64	7.45	24.44	327	81.69
2,4-D at 45ppm at PD	76.39	6.65	21.12	352	86.97
<i>P- value</i>	0.8528	0.1847	0.4621	0.7451	0.5698

PD (petal drop).

Table 5.3.3.11. Effect of 2,4-D on the external fruit quality of Navelina navel oranges.

Treatment	Colour ^y	Colour at navel-end ^x	Pedicle Diameter	Creasing ^w
Control	1.62 b	0.77 ab	3.07 b	0.22 b
2,4-D at 15ppm at PD	1.91 ab	0.94 a	2.94 b	0.46 a
2,4-D at 25ppm at PD	2.12 a	0.95 a	3.22 b	0.16 cb
2,4-D (25ppm) + GA (10ppm) at PD	1.81 ab	0.74 ab	3.68 a	0.19 cb
2,4-D at 35ppm at PD	2.00 ab	0.81 ab	4.04 a	0.15 cb
2,4-D at 45ppm at PD	1.78 ab	0.63 b	3.29 b	0.04 c
<i>P- value</i>	0.3770	0.1332	<.0001	0.0008

Means in a vertical column followed by different letters are significantly different at the 5% level (LSD).

^y1-8 on colour chart, 1-orange, 8-green.

^x0-4, 0-orange, 4-green.

^w0-4, 0-no creasing, 4 whole fruit creased.

PD (petal drop).

Table 5.3.3.12. Effect of 2,4-D the internal fruit quality of Navelina navel oranges.

Treatments	Juice	TSS		TSS:TA
	--%--		--%--	
Control	43.28 a ^z	9.30	0.79	11.85
2,4-D at 15ppm at PD	42.84 a	8.85	0.77	11.48
2,4-D at 25ppm at PD	42.68 a	9.37	0.75	12.72
2,4-D (25ppm) + GA (10ppm) at PD	39.76 b	9.73	0.77	12.63
2,4-D at 35ppm at PD	39.24 b	9.37	0.74	12.65
2,4-D at 45ppm at PD	42.12 a	9.06	0.71	12.70
<i>P- value</i>	0.0016	0.3799	0.1308	0.2677

^zMeans in a vertical column followed by different letters are significantly different at the 5% level (LSD).

PD (petal drop).

Table 5.3.3.13. Effect of 2,4-D on fruit diameter, navel end size and percentage closed navels of Palmer navel oranges.

Treatments	Fruit diameter	Navel end size	Closed navels
	--mm--	--mm--	--%--
1. Control	80.26	4.60	16.45
2. 2,4-D at 15 ppm at PD	82.99	4.04	25.52
3. 2,4-D at 20 ppm at PD	82.38	3.89	23.97
4. 2,4-D at 25 ppm at PD	81.90	3.60	29.18
5. 2,4-D at 30 ppm at PD	83.80	3.91	29.31
<i>P- value</i>	0.0760	0.4418	0.1512

PD (petal drop).

Table 5.3.3.14. Effect of 2,4-D on the external fruit quality of Palmer navel oranges.

Treatments	Colour ^y	Pedical diameter	Peel thickness	Fruit shape (height/diam.)
		--mm--	--mm--	
1. Control	2.05	3.21	4.10 c ^z	1.02
2. 2,4-D at 15 ppm at PD	1.99	3.39	5.14 ab	1.02
3. 2,4-D at 20 ppm at PD	1.91	3.34	4.84 b	1.04
4. 2,4-D at 25 ppm at PD	1.97	3.26	4.74 b	1.02
5. 2,4-D at 30 ppm at PD	2.10	3.13	5.49 a	1.01
P-value	0.8491	0.0941	0.0001	0.1960

^zMeans in a vertical column followed by different letters are significantly different at the 5% level (LSD).

^y1-8 on colour chart, 1-orange, 8-green.
PD (petal drop).

Table 5.3.3.15. Effect of 2,4-D on the internal fruit quality of Palmer navel oranges.

Treatments	Juice	TSS	TA	TSS:TA
	--%--		--%--	
1. Control	45.60 b ^z	11.10	0.90	12.59
2. 2,4-D at 15 ppm at PD	46.94 ab	11.36	0.86	13.23
3. 2,4-D at 20 ppm at PD	40.74 c	11.34	0.90	12.75
4. 2,4-D at 25 ppm at PD	49.09 a	10.94	0.86	12.69
5. 2,4-D at 30 ppm at PD	44.41 b	11.16	0.86	13.61
P-value	0.0001	0.4770	0.3909	0.4110

^zMeans in a vertical column followed by different letters are significantly different at the 5% level (LSD).

PD (petal drop).

5.3.4 PROGRESS REPORT: Monitoring irrigation, nitrogen stress and phenology of citrus trees using physiological and remote sensing approaches

Experiment 946 (April 2008 – March 2009): Sebinasi Dzikiti (SU) and Stephan Verreyne (CRI at SU)

Opsomming

Die potensieële toepassing van hiperspektrale afstandmeting (100 golfbande) om droogtestres, voedingstres en oeslading te bepaal is geëvalueer op Satsuma mandaryn bome in potte en in 'n 10 jaar oue Satsuma boord. Bestaande droogtestres spektrale indekse voospel slegs plant waterinhoud akkuraat maar nie waterpotensiaal nie. Plant waterpotensiaal word meer algemeen gebruik in besproeiingsbestuur as waterinhoud. 'n Nuwe droogtestres spektrale indeks vir sitrus (Simple ratio 5 met weerkaatsing by 1030 en 1380 nm) is ontwikkel in die studie deur regressiestatistiek op 25 Satsuma bome in potte onder verskillende stresstoestande te gebruik. Die data bestaan uit metings van blaarweerkaatsing, waterinhoud, middag blaar en stam waterpotensiaal oor verskillende benatting en uitdroging siklusse. Hierdie indeks voorspel akkuraat die blaar waterinhoud vir alle stresse. Voorspellings van stam waterpotensiaal verbeter slegs as die data van erg gestresde bome bygevoeg word, iets wat ook by die bestaande indekse gesien word. Vastelling van ligte stres is belangrik in besproeiingsbestuur. Alternatiewelik is 'n model ontwikkel wat blaarweerkaatsing data (400-2500nm, in 1-15 nm golfbande) met transpirasie en grondwaterpotensiaal integreer om stam waterpotensiaal te voorspel en om die model suksesvol te verklaar vir nie gestresde bome in potte. Die verandering van die model vir gebruik vir boordbome is in wording. Meting van boomspektra by oestyd op 5 bome waar vrugte geleidelik geoes word toon duidelike veranderinge in die spektra in die naby infrarooi deel. Dit dui aan dat oeslading met hiperspektrale afstandmeting bepaal kan word deur die effek op boom waterinhoud en boomstruktuur (verandering in die oriëntasie van blare a.g.v. die gewig van vrugte). Geen veranderinge in weerkaatsing is waargeneem in die sigbare spektrum nie, wat aandui dat vrugkleur nie belangrik is in oeslading voorspelling deur afstand meting nie. In kontras, boomkleur verandering a.g.v. stikstofekort in die blare is waargeneem deur die boom spektra omdat blaar eienskappe die boom spektra domineer. Addisionele resultate van die effek van stikstof en water stress op fotosintese en produktiwiteit van Satsuma mandaryn bome word bespreek.

Summary

The potential application of hyperspectral remote sensing (100s of wavebands) for detecting drought stress, nutrient stress and yield was investigated on potted Satsuma mandarins and in a 10 year old Satsuma orchard. Existing drought stress spectral indices only accurately predict plant water content but not the water potential. Plant water potential is more commonly used for irrigation management than water content. A new drought stress spectral index for citrus (Simple Ratio 5: ratio of reflectance at 1030 and 1380 nm) was developed in this study using regression statistics on data from 25 potted Satsumas under different stress regimes. The data comprised measurements of leaf reflectance, water content, midday leaf and stem water potentials over several wetting and drying cycles. This index accurately predicted the leaf water content for all stresses. Predictions of stem water potential only improved when data from severely stressed trees was included, a result which is consistent with the performance of existing indices. However, detecting low stresses is crucial in irrigation management. In an alternative approach, we then developed a model which integrates leaf reflectance data (350 – 2500 nm, in 1-15 nm wavebands) with transpiration and soil water potential to predict the stem water potential and successfully validated the model for non stressed potted trees. Upscaling of this model to field grown trees is in progress. Measurements of canopy spectra at harvest on 5 trees when fruit were gradually harvested showed clear changes in the spectra in the near infrared region. This suggested that crop load can be determined from hyperspectral remote sensing through its effects on canopy water content and canopy structure (e.g. change in orientation of the leaves under the weight of fruit). No changes in reflectance occurred in the visible spectrum implying that fruit color is not an important factor in predicting citrus yield with remote sensing. In contrast, canopy color change due to leaf nitrogen deficiency could be detected from the canopy spectra since leaf attributes dominate the canopy spectral signature. Additional results of the effect of nitrogen and water deficiency on photosynthesis and productivity of Satsuma mandarins are also presented.

Introduction

Timely detection of stress is crucial to minimize loss of yield. Traditionally citrus growers detect drought stress using the water balance and soil moisture – based approaches. Many studies have shown that with these indirect methods, plants get stressed well before the stress thresholds have been reached, leading to yield loss (Jones, 1990; Bacon, 2004). According to Naor and Cohen (2003) and Jones (2004), direct sensing of the tree's response to water deficit is a better indicator of irrigation need. This is because many features of the plant's physiology respond directly to changes in water status of the plant's tissues rather than to changes in the bulk soil water status. Few, plant – based diagnostic tools are being used in practice to schedule irrigation because of practical difficulties. In this study we investigate the potential application of hyperspectral remote sensing to monitor drought stress, nutrient stress and yield of citrus trees. Hyperspectral sensing involves the detection of hundreds of data points, normally of reflectance or transmittance of light by a target which in our study was either a citrus leaf or a tree. Such information on the spectral responses of citrus will be useful in the context of recent novel initiatives on precision satellite based citrus production monitoring systems e.g. the In Situ – HyperSpectral project (IS – HS). The IS – HS project is a sub component of the South African/ Flemish ZaSat II satellite design project focusing on the development of a crop production module with citrus as the test crop (Copin, 2007). Satellite based remote sensing measurements of a target (orchard) has larger spatial resolutions and thus sampling problems associated with *in situ* sensors are minimized. To create contrasts in the target characteristics, citrus trees were subjected to different water and nitrogen stress regimes. The water stress and nitrogen stress data are interpreted and documented both from a remote sensing and a physiological context in this study.

Materials and methods

Detecting citrus tree water status with hyperspectral measurements

Experimental set up

Experiments were set up with 25 young Satsuma mandarin trees grown in pots ~35 cm x 30 cm (diameter x depth) at the Agronomy Department, Stellenbosch University. The trees were planted in a standard pot mixture medium comprising bark, sand, clay, peat and other constituents in varying proportions and maintained on a drip irrigation system. We started with potted trees to clearly understand the relationship between changes in leaf spectral properties and leaf water stress given that very little is known about these for citrus. The results would then be scaled up to orchard conditions.

Intensive measurements of leaf reflectance, predawn leaf water potential, midday leaf and midday stem water potentials, leaf water content (g cm^{-2}), tree sap flow and soil water potential were taken at selected intervals in December 2008, February and March 2009. At each of these dates, the above quantities were measured at daily intervals excluding cloudy or rainy days on 5 trees subjected to a drying cycle lasting

about 2 weeks. At the end of each drying cycle, the trees would be severely stressed with the soil water potential measured with a WP4 potentiometer (Decagon Devices, USA) reaching -3.5 MPa. An additional 5 trees would be controls. Water potential was measured with a Scholander pressure bomb on at least 4 healthy leaves for each of the water potential variables i.e. the predawn leaf, midday leaf and midday stem water potentials for the stressed and non stressed trees. After each water potential measurement, at least 2 reflectance spectra were measured on a 3.5 cm disc of each sampled leaf using the spectroradiometer (Analytical Spectral Devices, Boulder, USA) against a white spectralon background. Leaf discs were stored in ice and immediately transported to the lab for fresh weight measurements. Samples were oven dried at 70°C for at least 3 days for water content determination.

Data analysis

To establish the relationship between the leaf reflectance spectra, leaf water content and the water potential in response to the stress regimes, a principal component analysis was done using The Unscrambler software 9.2 (CAMO, Norway). The water content data were normalized with the area of each leaf disc to give a leaf equivalent water thickness (EWT). In addition, the performance of major plant water stress indices derived from other plant species was also evaluated on the citrus dataset namely:

- 1) Normalized Difference Water Index (NDWI) $(R_{860} - R_{1240}) / (R_{860} + R_{1240})$,
- 2) Maximum Difference Water Index (MDWI): $(R_{\max 1500-1750} - R_{\min 1500-1750}) / (R_{\max 1500-1750} + R_{\min 1500-1750})$
- 3) Water Index (WI): R_{900} / R_{970} and
- 4) Simple Ratio 2 (SR2): R_{1070} / R_{1340} where **R** is the reflectance at the given wavelength.

Water and nitrogen stress treatments

Experiments to investigate the effect of water and nitrogen stress on the productivity of Satsuma mandarins were set up at the University of Stellenbosch Orchard on 10 yr old trees. Each control tree received 112 g of nitrogen for the 2008/09 season. Treatments comprised three N application levels at 66%, 33% and 0% of the control trees and each treatment had 5 single tree replicates. Trees were irrigated by drip with the control trees receiving 6 L/h irrigation. Three other irrigation levels were set up with trees receiving 4, 2, 0 L/h irrigation. Five single-tree replicates of each treatment were also established. However, irrigation treatment effects were affected by severe hot weather late December requiring irrigation levels to be adjusted. Photosynthetic rates were measured using the LI – 6400 Infrared Gas Analyzer (Li –Cor, Lincoln, USA) only in the nitrogen stress treatments on 5 healthy and fully expanded leaves on selected clear days.

Crop load estimation with canopy level hyperspectral measurements

The canopy reflectance spectrum of 5 Satsuma mandarin citrus trees was measured on cloudless days at a height of about 1.7 m above the canopy from the top of a scaffold using the bare optic fiber of the ASD spectroradiometer. This height enabled most of the canopy to be viewed by the sensor. Spectral measurements were first taken before fruit had been removed. Then 100 fruit were harvested from each of the 5 trees and canopy spectral measurements repeated. Then another 200 fruit were picked and the procedure repeated. The third picking removed all the remaining fruit before the last round of canopy spectral measurements. After each picking the fruit were weighed.

Results and discussion

Water stress detection

Figure 5.3.4.1 shows a typical reflectance spectrum of an intact citrus leaf at 3 water stress levels, i.e. – 1.25; -2.50 and – 4.00 MPa. The spectrum shows massive absorption of the visible light (400 – 700 nm) mainly by leaf pigments e.g. chlorophylls and caretonoids. In the near infrared (700 – 1300 nm), reflection is controlled by structural features of the leaf e.g. the arrangement of air spaces and cell boundaries and also by water. In the middle infrared (1300 – 2500 nm) absorption by water is the dominant feature of the spectrum.

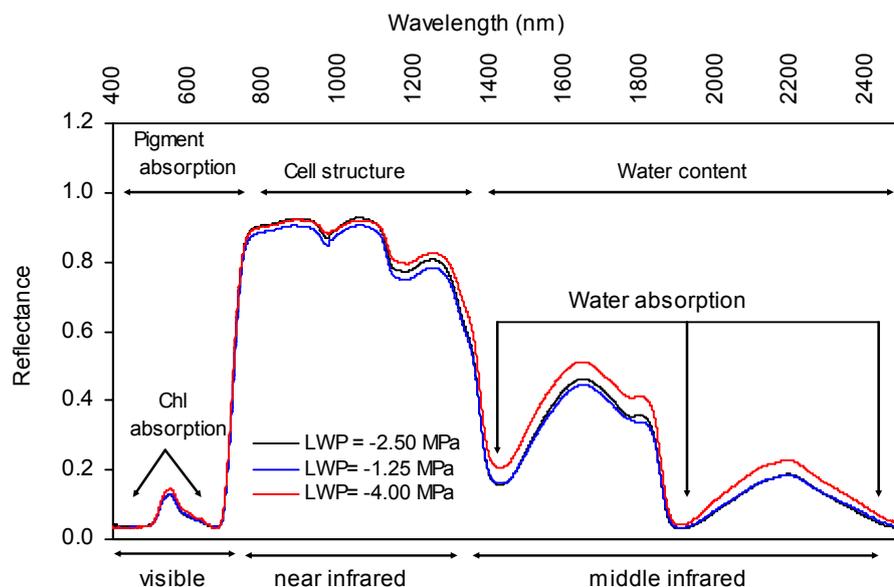


Figure 5.3.4.1. The reflectance spectrum of a citrus leaf at 3 different water stress levels.

Analysis of the data of the midday leaf water potential and the corresponding water content (EWT) only from the stressed Satsuma trees revealed a strong regression between the leaf spectra and both EWT and water potential at 1030 and 1380 nm. This simple ratio (R_{1030}/R_{1380}), which we called simple ratio 5 (SR5) in Table 5.3.4.1, accurately predicted the water content (EWT) of both the stressed and non-stressed trees. Water potential predictions for stressed trees were also high, but the index performed poorly when applied to non-stressed trees.

Applying spectral indices developed on other species to the citrus dataset showed that generally these indices predicted the water content (EWT) fairly accurately, but they also failed to give good estimates of the water potentials particularly with data from non-stressed trees (Table 5.3.4.1).

By integrating hyperspectral measurements of leaf water content with a physiological model (Dzikiti et al., 2008) to take into account the physiological relationship between water content and water potential we obtained improved estimates of the stem water potential for the potted Satsuma mandarins in Fig 5.3.4.2. This scheme is being scaled up to mature field grown trees.

Table 5.3.4.1. Performance of existing water spectral indices on stressed and non-stressed Satsuma mandarins showing R^2 values between measured water potential, water content (EWT) values and changes in leaf spectral characteristics. The SR5 was developed for citrus in this study.

Index		Including stressed trees			Excluding stressed trees	
		Predawn	Midday leaf	Midday stem	Midday leaf	Midday stem
NDWI	EWT	0.85	0.54	0.46	0.64	0.54
	WP	0.60	0.13	0.21	0.05	0.04
MDWI	EWT	0.69	0.50	0.69	0.76	0.62
	WP	0.69	0.57	0.57	0.08	0.08
WI	EWT	0.87	0.87	0.83	0.80	0.72
	WP	0.65	0.57	0.57	0.10	0.005
SR2	EWT	0.92	0.93	0.82	0.91	0.66
	WP	0.67	0.42	0.51	0.07	0.002
SR5	EWT	0.86	0.86	0.85	0.94	0.71
	WP	0.77	0.57	0.60	0.09	0.03
PROSPECT	EWT	0.89	0.86	0.80	0.87	—

WP = water potential, EWT = equivalent water thickness (water content per cm^2 of leaf area).

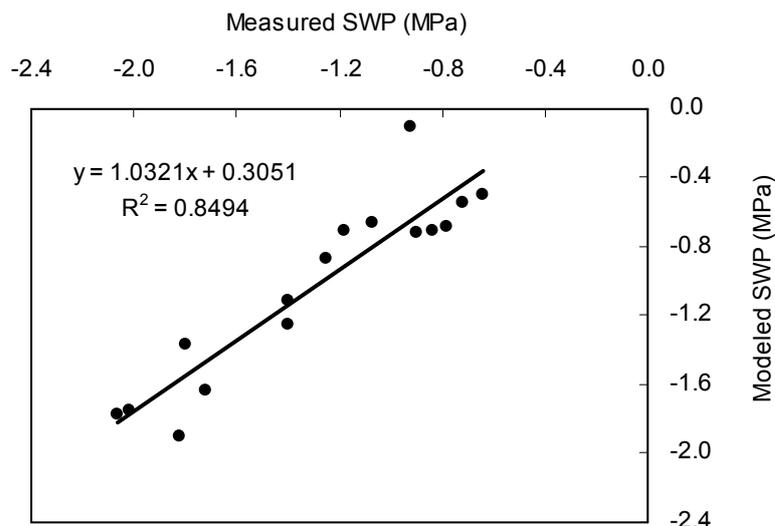


Figure 5.3.4.2. Validation results for a model integrating hyperspectral measurements of leaf reflectance with physiological information to predict the stem water potential of Satsuma mandarins.

Water and nitrogen treatment effects on canopy spectra and productivity of Satsuma mandarins

Differences in canopy appearance were particularly evident between the 0% N and the control treatments. The 33 and 66% N treatments showed only mild differences relative to the control trees in terms of canopy greenness.

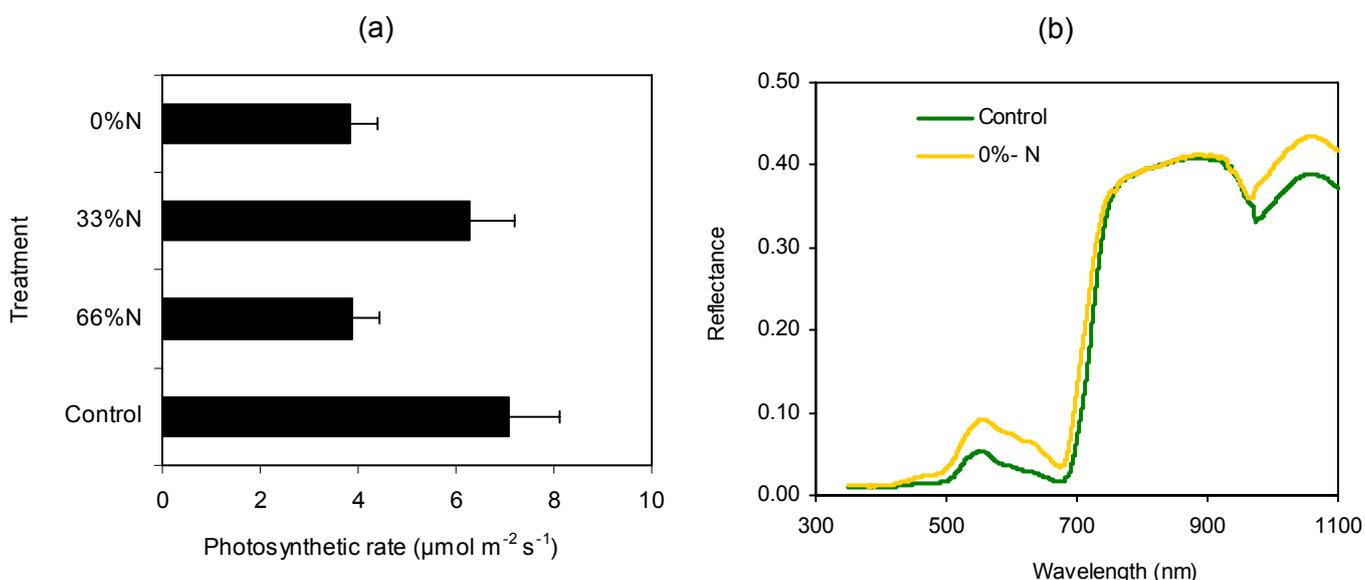


Figure 5.3.4.3 a) Effect of different nitrogen application levels on the leaf photosynthetic rate of mature Satsuma mandarin citrus trees. **b)** Canopy reflectance spectrum of a nitrogen stressed and non stressed Satsuma canopy.

Severe nitrogen stress reduced the photosynthetic rate (Fig 5.3.4.3a) although treatment effects were not consistent. For instance, the 66% N treatment had lower photosynthetic rates than the 33% N and similar to 0% N for unclear reasons. This trend in the photosynthetic response was confirmed by the yield data (Table 5.3.4.2) where the 66% N treatment still gave lower yields than the 33% N suggesting that this trend was sustained throughout the season. The reduced photosynthetic rate under the 66% N was probably a result of lower stomatal conductances probably due to an undetected water deficit. Figure 5.3.4.3b shows the difference in the canopy reflectance spectrum of a nitrogen stressed and a non – stressed Satsuma canopy. The nitrogen stressed trees showed a high reflectance in the visible and a less steep slope at the red edge while the reflectance of the 33% N and 66% N was similar to that of the controls.

Table 5.3.4.2. Summary of yield data showing the effects of water and nitrogen deficiency treatments on yield per tree and fruit quality attributes. (H = water treatment, N = nitrogen treatment).

Treatment	Yield (kg/tree)	Mean fruit diameter (mm)	Color	%Juice	Brix
ControlH	47.99	66.5	5.2	49.0	10.1
66%H	48.50	65.4	6.2	50.6	9.2
33%H	37.08	67.3	5.8	48.3	9.7
0%H	42.48	60.5	5.5	47.8	9.7
ControlN	56.77	67.1	6.3	51.5	8.5
66%N	47.52	66.2	6.0	51.0	8.5
33%N	57.44	70.2	5.8	50.1	8.7
0%N	41.73	64.8	5.7	49.5	9.3

Water stress treatment effects were masked by the effects of the steep slope such that severe stress conditions were unachievable. This is the reason why yield in the non-irrigated treatments were similar to the irrigated ones, although fruit size and juice percentage were smaller.

Crop load estimation

Figure 5.3.4.4 clearly illustrates that it is possible to determine crop load of Satsuma mandarin citrus trees with hyperspectral remote sensing from the changes in the near infrared wavebands. This result is consistent with the observations by Somers et al. (2009) on Valencia oranges. In the near infrared, canopy structural changes, especially changes in the orientation of the branches and hence the leaves under the weight of fruit rather the fruit themselves seem to be an important factor. The panels at the top of Fig. 5.3.4.4 give an idea of the amount of fruit present at each stage of measurement. Analysis of the data to establish specific combinations of the spectral wavebands to give quantitative yield information is still in progress.



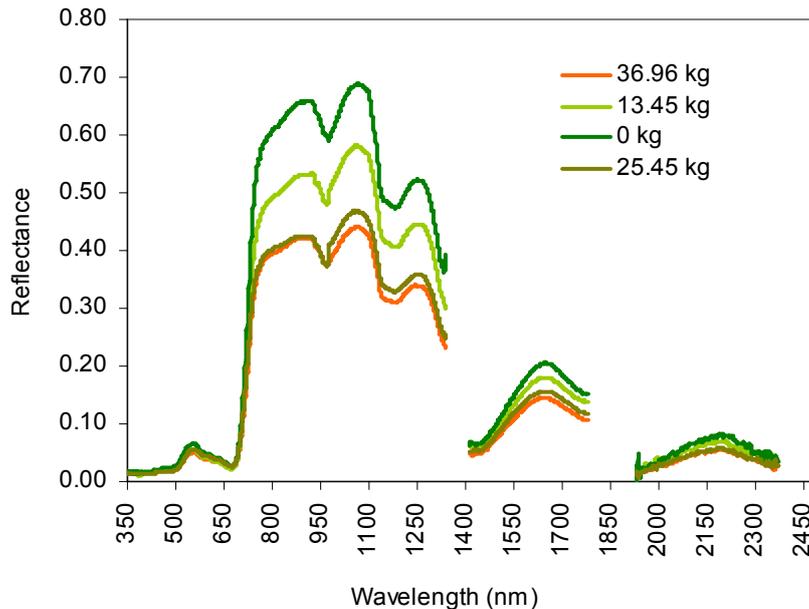


Figure 5.3. Effect of gradual fruit removal on the canopy reflectance spectrum of Satsuma mandarin citrus trees. The panel at the top shows a pictorial view of fruit density while the actual mass of fruit in the whole tree is shown in numbers.

Conclusions

This study shows that it is possible to monitor citrus water and nitrogen status and yield with hyperspectral remote sensing. However, direct detection of plant water potential with remote sensing only is still difficult, especially for managing non-severely stressed plants. We therefore propose combining remote sensing with physiological approaches which take into account the capacity of the plant leaves and hence the canopy to store water and the hydraulic resistances to water transport to achieve improved estimates of plant water potential for non – severely stressed trees. Growers manage irrigation between the non–stressed and mildly stressed regimes without allowing severe stress to set in and this management range is not yet taken care of by existing spectral indices including the one we derived for citrus. Crop load can potentially be estimated with hyperspectral remote sensing in the near infrared but we are still working on a specific predictive index that can be used.

Further objectives and work plan

Further objectives are as follows:

April – September 2009

- Upscaling of the model for predicting stem water potential integrating remote sensing and *in situ* data to field grown citrus trees.
- Further refining the water potential model so that it uses only readily available climate data and canopy spectra as inputs.
- Validation of this model at canopy level on midnight Valencia oranges.
- Search for robust spectral indices for direct sensing of plant water potential continues.
- Application of an inversion of the leaf radiative transfer model PROSPECT to predict leaf chlorophyll and carotenoid concentrations and to upscale to canopy level.

October 2009 – March 2010

- Investigations of the seasonal dynamics of canopy structural, water content and mineral composition in relation to changes in crop load and its influence on the canopy spectral properties.
- Derivation of robust spectral indices for the estimation of citrus yield using hyperspectral remote sensing on Satsuma mandarins and validated on Valencia oranges.

Technology transfer

Results from this study will be presented at the International Geosciences and Remote Sensing Symposium in Capetown on 16 July 2009.

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5.3.5 Confronting Climate Change



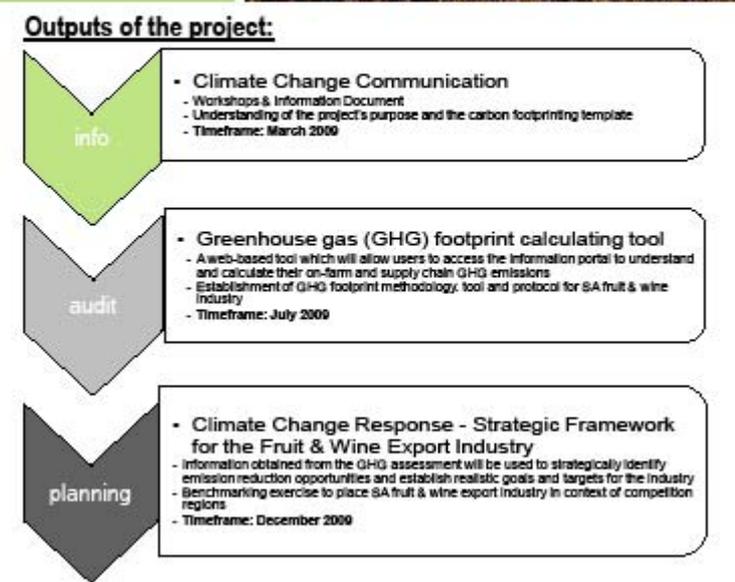
To preserve South Africa's competitive position in the global fruit & wine export markets, the industry needs to make a unified response to climate change – informed by sound science and based on local information.

Introduction:
Climate change is predicted to directly impact South Africa's mean annual temperature and rainfall ranges, influencing pest & disease distribution, flowering and fruiting seasons, and ground water resources. Climate change also impacts indirectly, through the growing awareness amongst consumers and the corresponding demand for carbon-efficient business processes. The agricultural sector is a large source of greenhouse gases (GHGs) through activities such as land-use change, agrochemical application and fossil fuel use. Through conservative energy technologies and sustainable farming practices, substantial emission reduction opportunities exist which can improve efficiency and reduce costs throughout the supply chain.

- Aims of the project:**
- To highlight and communicate climate change issues, opportunities and threats to the agricultural sector
 - To create an industry standard for GHG auditing within the fruit and wine sector, and ensure a standardised measurement, reporting and comparison of individual farm emissions that highlights emission reduction opportunities.
 - To enable informed and authoritative comment, debate and negotiation by stakeholders and policy-makers
 - To guide short and long term strategy formulation by decision-makers across the industry



How to get involved:
The success of this project relies on the active involvement of the industry. Please do not hesitate to contact the project team, or your industry representative.
Project Co-ordinator: Hugh Campbell
Tel: 021 882 8470, email: hugh@dipresearch.co.za
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This industry project is a joint initiative supported by the local fruit and wine industries, government (NAMC & PHI) and donor funding (ComMark Trust)



'n Webgebaseerde inligtingsportaal vir klimaatverandering en landbouvraagstukke wat verband hou met die vrugte- en wynuitvoerbedryf, insluitend 'n aanlyn-koolstofvoetspoorberekeningswerktuig.

Om Suid-Afrika se mededingende posisie op die wêreldwye vrugte- en wynuitvoermarkte te behou, moet die bedryf 'n verenigde reaksie ten opsigte van klimaatverandering toon – ingelig deur grondige wetenskap en gebaseer op plaaslike inligting.

Inleiding:

Daar word voorspel dat klimaatverandering 'n direkte impak op Suid-Afrika se gemiddelde jaarlikse temperatuur en reënvalverspreiding sal hê en gevolglik plaag- en siekteverspreiding, die blom- en vrugdaseisoen, asook grondwaterhulpbronne sal beïnvloed. Klimaatverandering het ook 'n indirekte impak vanweë toenemende bewuswording onder verbruikers en die ooreenkomstige aandrag op koolstof-doeltreffende sakeprosesse. Die landbousector is 'n groot bron van kweekhuiskasse (KHG's) as gevolg van bedrywighede soos grondgebruiksverandering, landbouchemiese toedoening en fossielbrandstofgebruik. Deur middel van konserwatiewe energietechnologieë en volhoubare boerderypraktyke bestaan daar groot vrystellingsverminderingseleenthede wat doeltreffendheid kan verbeter en koste deur die hele verskaffingsketting kan verminder.

Doelwitte van die projek:

- Om klimaatveranderingsvraagstukke, -geleentehede en -bedreigings op landbougebied op die voorgrond te plaas en bekend te maak
- Om binne die vrugte- en wynsektor 'n bedryfstandaard vir KHG-ouditering te skep en om 'n gestandaardiseerde meting, verslagdoening en vergelyking van individuele plaasvrystellings wat vrystellingverminderingseleenthede op die voorgrond te plaas.
- Om ingeligte en gesaghebbende kommentaar, debatvoering en onderhandeling deur belanghebbendes en beleidsmakers moontlik te maak.
- Om leiding te gee aangaande kort- en langtermynstrategieformulering deur besluitnemers dwarsdeur die bedryf.



Hoe om betrokke te raak:

Die sukses van hierdie projek hang af van die aktiewe betrokkenheid van die bedryf. Moenie huiwer om met die projekspan of u bedryfsverteenwoordiger te skakel nie.

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Uitkommste van die projek:



Hierdie Industriële projek is 'n gesaamentlike inisiatief geborg deur die plaaslike vrugte en wyn industrie, regering (NAMC& PHI) asook geborge finansiering (ComMark Trust).

6 **PROGRAMME: CULTIVAR AND ROOTSTOCK EVALUATION**
Andrew T.C. Lee (Manager Cultivar Development)
Johan Joubert, Richard Fenwick and Zongezile Zondi (Cultivar Evaluators)

6.1 **PROGRAMME SUMMARY**

The long term competitiveness of the southern African citrus producer is an essential process that requires ongoing awareness. The production of a wide range of citrus cultivars of superior quality provides a means to achieve this objective. A further step in this direction is the evaluation of a series of new rootstock cultivars to improve all aspects of production.

CRI's Cultivar Development division (CD) aims to facilitate the availability of promising new citrus cultivars that will meet market requirements as rapidly as possible. In addition CD aims to provide impartial and objective recommendations on all available citrus cultivars to augment grower decision making.

To achieve these objectives CD will be actively involved in the identification and evaluation of promising scion and rootstock cultivars.

PROGRAMOPSOMMING

Die langtermyn mededingendheid van die suider Afrika sitrus produsent is 'n essensiële proses wat konstante bewusmaking vereis. Die produksie van 'n wye reeks sitrus kultivars van uitstaande kwaliteit voorsien 'n metode om hierdie doelwit te bereik. 'n Verdere stap in hierdie rigting is die evaluering van 'n reeks onderstam kultivars om alle aspekte van produksie te verbeter.

CRI se Kultivar Ontwikkelings afdeling (KO) behels die fasilitering van beskikbare, belowende nuwe sitrus kultivars wat aan die markte se vereistes so spoedig moontlik kan voldoen. Addisioneel streef KO om neutrale en objektiewe aanbevelings op alle beskikbare sitrus kultivars uit te voer en sodoende die produsent se besluitneming te verbreed.

Om hierdie doelwitte te bewerkstellig sal KO aktief betrokke wees by die identifikasie en evaluasie van belowende bo- en onderstam kultivars.

6.2 **PROJECT: CULTIVAR EVALUATIONS**

6.2.1 **Project summary**

Satsumas: The objective of this project is to find suitable, high quality, early maturing and early colouring selections for the early marketing season and to overcome production peaks by extending the harvest season both earlier and later. Satsuma x Nova (Sonet) looks promising as an early maturing selection, but is still at the experimental stage. Primosole is early maturing, but needs intensive production and irrigation controls to ensure quality fruit production. The commercial Dobashi Beni trees are only now starting to come into production. Two trial sites with all the late maturing selections have been established.

Clementines: The aim of this work is to flatten out existing midseason Clementine production peaks by extending the harvest period both earlier and particularly later and to provide selections of superior external colour, internal quality and larger fruit size. Clemenpons is early maturing with good quality but only medium fruit size. Trunks have galls and tree size is variable. The long term effect of the galls needs to be established and the tree size variability is cause for concern. A full report has been published on the later maturing Clementine selections, viz. Nour, LL, Sidi Aissa, Clementarde and Ain Taoujdate, in the SA Fruit Journal. Tardif de Janvier I, Tardif de Janvier II, and Tardivo have not performed well and new selections are needed to further the Clementine range of selections. Information on these selections is limited.

Mandarins: The objective of the mandarin hybrid project is to find high quality, well coloured, seedless selections with good production and fruit size, with special emphasis on extending the soft citrus season earlier and particularly later. The Murcott x Clementine semi commercial block bore its second crop with large fruit size, good quality and seedy fruit, maturing early July. Hadas had good production and fruit size but consistently high acid like an Ellendale. Winola had poor production and medium fruit size and excessive acid at the CFB. This is an excellent quality selection similar to Page in flavour, but is seedless being a triploid, has larger fruit size and is very late maturing (end September). Nectar is also a neglected selection that needs to be tested thoroughly in suitable areas in South Africa. The TDE series, Gold Nugget and Tango selections from UCR are soon to be released for experimental purposes in South Africa. Trials

are being planned and the first trees will be established in spring 2009 in locations throughout SA. These selections will be included in existing trials or established as completely new trials where necessary.

Navels: The aim is to find cultivars to spread the navel season both earlier and later and to minimise production peaks, also to improve rind colour, particularly at the commencement of the season and with improved fruit set potential in the required fruit size range. Fukumoto is early maturing, similar, to slightly earlier than Lina/Newhall, developing a deep orange/red rind colour. The incompatibility problem of Fukumoto on citrange hybrid rootstocks reported from California has not been a major factor here to date. A survey is being conducted and a preliminary report on findings to date is included in this report. Dream matures after Lina/Newhall, but is still substantially earlier than Palmer and Washington. Fisher is another very promising earlier maturing navel that fits between the earlies and Palmer/Washington. Cambria (CFB material) had good production and fruit size, round to elongated fruit shape and very good internal quality. Summer Gold is late maturing. The Witkrans (old selection) has performed well in the SRV and is slightly earlier than Cambria, so these selections are a good complementary choice. Glenora Late is late maturing and a vigorous tree and had good quality and yields in the Citrusdal mountain region. A comparison between the Autumn Gold, Powell, Chislett, Barnfield and Californian Lane Late showed only slight differences between the selections. Further evaluations of all the selections are necessary as some of the trees are still young. Navel trials are being conducted in the Cape areas as well as Marble Hall and Burgersfort. New developments at Orighstad will include trials of selections considered suitable for this climate.

Midseasons: The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless. Tarocco trials using available selections are planned for the East Cape Midlands as the other citrus production areas are not cold enough to develop internal pigmentation in this cultivar. The Maltaise selections and Raratonga trial have been discontinued and will be published in due course. Further evaluations on the Clara and Tacle are necessary.

Valencias: The aim of the Valencia project is to optimise profitability by finding early, mid and late maturing Valencia selections that have improved productivity and internal fruit quality (seedlessness, fibre toughness, juice quality), have large fruit size and with improved fruit set ability, have improved rind colour, external appearance and peelability compared with the existing range of selections. Various new selections were evaluated at the CFB. Limpopo seedless is the earliest to mature with acceptable quality from young trees and seedless without cross pollination. G5 is also seedless where not cross pollinated and has acceptable to good quality. Portsgate had borderline quality and was not outstanding in any way except for virtual seedlessness in a mixed block. McClean Seedless looks similar to Valencia Late with fairly high acid and is virtually seedless. Rietspruit had smallish fruit size, poor quality, high acid and odd seed. Bend 8A2 production and quality was poor with smallish fruit size and virtually seedless where no cross pollination. Delicia had good production and fruit size, meeting standards in August.

In the Onderberg area there was good potential between most of the selections that were evaluated. Alpha and Turkey were the best selections for this season. Ruby Valencia seemed to have an off season, producing small green fruit up to harvest time. There are still no signs of incompatibility with Turkey on CC, and the bud union looks healthy.

Valencias (Swaziland): The trees are still young and this was the first evaluation conducted. The production and quality of the fruit will improve with time, including the average fruit size. Alpha seems promising, with most of the other selections not complying with the minimum export standards.

Knysna area: This area is not suitable for the production of most of the commercial citrus cultivars available. Sweet Spring is one cultivar that has performed reasonably well with good crops of acceptable fruit size. The search for suitable cultivars for this region is continuing.

Lemons: To develop cold hardy, thornless, seedless lemon selections with acceptable fruit size which are compatible with a wide range of rootstocks; to extend the picking period to ensure continuity of supply from March to September, i.e. to develop early and late maturing selections; to reduce the problems associated with protracted flowering; to maintain high fruit quality (colour, rind thickness, juice content). Tree characteristics and performance of new cultivars were compared with the commercially grown Eureka in light of the above objectives. Villafranca still produces the lowest seed count per fruit followed by Verna. We determined the production per tree for this season. Limoneira produced the best yield on the trees with 151 kg, followed by Fino at 49. Evaluations will stop. Eureka SL (ARC) remains the best seedless lemon selection available, keeping in mind that Limoneira produced the best crop on the trees but with high numbers of seed per fruit.

Projekopsomming

Satsumas: Die doel van hierdie projek is om geskikte, hoë gehalte seleksies te vind wat vroeg in die seisoen opkleur en die vroeë mark kan binnedring, asook om produksie pieke, beide vroeër en later, te voorkom. Satsuma x Nova (Sonet) lyk belowend as 'n vroeg rypwordende seleksie, maar is nog steeds eksperimenteel. Primasole word ook vroeg ryp, maar benodig steeds intensiewe produksie en besproeiings beheer om kwaliteit vrugproduksie te verseker. Die kommersiële Dobashi Beni bome begin nou in produksie kom. Twee proef persele met al die laat rypwordende seleksies is reeds gevestig.

Clementines: Die doel van die projek is om 'n breë verskeidenheid kultivars met hoër gehalte, goeie skilkleur en met goeie vruggrootte te verskaf om die pieke in die middel Clementine seisoen af te plat deur die oestyd beide vroeër en veral later te verleng. Clemenspons word vroeg ryp met goeie kwaliteit vrugte, maar produseer slegs medium vruggrootte. Die stam vorm galle en boom volume varieer. Die langtermyn effek van die galle moet vasgestel word en die variasie in boom volume is rede tot kommer. 'n Volledige verslag is gepubliseer op die laat rypwordende Clementine seleksies, bv. Nour, LL, Sidi Aissa, Clementarde en Ain Taoujdate, in die SA Vrugte joernaal. Tardif de Janvier I, Tardif de Janvier II and Tardivo het nie goed presteer nie en nuwe seleksies word benodig om die Clementine seleksie reeks uit te brei. Inligting oor hierdie seleksies is beperk.

Mandaryne: Die doel van die mandaryn projek is om hoër gehalte, goed gekleurde, saadlose mandaryn seleksies te ondersoek. Hulle moet ook goeie produksie en vruggrootte hê en die sagte sitrus seisoen kan versprei, beide vroeër en veral later. Die Murcott x Clementine semi kommersiële blok het sy tweede oes geproduseer met groot vruggrootte, goeie interne kwaliteit en vrugte met saad, wat vroeg Julie rypword. Hadas toon goeie produksie en vruggrootte, maar met hoë sure soortgelyk aan 'n Elendale. Winola het swak produksie getoon met medium vruggrootte en oormatige suur vlakke by die CFB. Hierdie is 'n uitstekende kwaliteit seleksie soortgelyk aan Page wat smaak aanbetref, maar is saadloos want dis 'n triploïed, produseer groter vruggrootte en word werklik laat ryp (einde September). Nectar is ook 'n afgeskepte seleksie wat deeglik getoets moet word in geskikte areas in Suid Afrika. Die TDE reeks, Gold Nugget en Tango seleksies van die UCR word binnekort vrygestel vir eksperimentele evaluasies regdeur geskikte areas in Suid Afrika. Proewe word beplan en die eerste bome sal September 2009 aangeplant word regdeur SA. Hierdie seleksies sal ingesluit word in bestaande proewe of gevestig word as totale nuwe proewe waar nodig.

Nawels: Die doel van die proef is om kultivars te bekom om die seisoen vroeër en later te versprei en produksie pieke te verminder, asook om seleksies te vind met meer gevorderde vrugkleur, veral aan die begin van die seisoen en vrugte te kry met verbeterde vrugset wat binne die gesogte vruggrootte reeks val. Fukumoto word vroeg ryp, soortgelyk, tot selfs effens vroeër as Lina/Newhall, met 'n diep oranje/rooi skil kleur. Die onverenigbaarheids probleme van Fukumoto op citrange hibried onderstamme wat in California aangemeld is, blyk nie hier 'n groot probleem nie. 'n Opname word huidiglik gedoen en resultate tot op datum word in die verslag weergegee. Dream word na Lina/Newhall ryp, maar is steeds betreklik vroeër as Palmer en Washington. Fisher is ook 'n baie belowende vroeg rypwordende nawel wat tussen die vroeë seleksies en Palmer/Washington inpas. Cambria (CFB materiaal) toon goeie produksie en vruggrootte, met ronde tot langwerpige vrugte en baie goeie interne kwaliteit. Summer Gold word laat ryp. Witkrans (ou seleksie) het goed presteer in die SRV en is effens vroeër as Cambria, albei seleksies is 'n goeie komplementerende keuse. Glen Ora Late word laat ryp en die boom groei aggressief, met goeie kwaliteit en produksie in die bergagtige Citrusdal omgewing. 'n Vergelyking tussen Autumn Gold, Powell, Chislett, Barnfield en Californian Lane Late toon slegs geringe verskille tussen hierdie seleksies. Verdere evaluasies van al die seleksies is nodig omdat sekere van die bome nog jonk is. Nawel proewe word in die Wes-Kaap, sowel as in die Marble Hall en Burgersfort omgewing uitgevoer. Nuwe ontwikkelings by Orighstad sal proewe van seleksies insluit wat moontlik aangepas is vir hierdie klimaat.

Midseisoene: Die doel van die proef is om midseisoen seleksies, wat beter in die koeler streke aangepas is in terme van vruggrootte, gepigmenteerde vleis en saadloosheid, te vind. Tarocco proewe met beskikbare seleksies word beplan vir die Oos-Kaap, want die ander sitrus produksie areas is nie koud genoeg om die interne pigmentasie te ontwikkel nie. Die Maltaise seleksies en Raratonga proef is gestaak en sal binnekort opgeskrif word. Toekoms evaluasies op die Clara en Tacle is noodsaaklik.

Valencias: Die doel van die Valencia projek behels optimale winsgewendheid deur vroeër, mid en laat rypwordende seleksies met verbeterde produktiwiteit en interne kwaliteit (saadloosheid, vesel taaiheid, sap kwaliteit), met groot vruggrootte en verbeterde vrugset vermoë, verbeterde skilkleur en skikbaarheid in vergelyking met die bestaande reeks seleksies te lewer. Verskeie nuwe seleksies is by die CFB ge-evalueer. Limpopo saadloos word die vroegste ryp met aanvaarbare kwaliteit van jong en saadloos sonder kruis bestuwing. G5 is ook saadloos waar die seleksie nie kruisbestuif word nie met aanvaarbare tot goeie

kwaliteit. Portgate produseer grensgeval kwaliteit en is nie uitstaande behalwe vir saadloosheid in 'n gemengde blok nie. McClean saadloos lyk soortgelyk as Valencia Late met betreklik hoe sure en is feitlik saadloos. Rietspruit produseer kleinerige vruggrootte, swak kwaliteit, hoe sure en soms sade. Bend 8A2 se produksie en kwaliteit was swak met klein vruggrootte en feitlik saadlose vrugte sonder kruisbestuiwing. Delicia toon goeie produksie en vruggrootte, en voldoen aan standaardde in Augustus.

In die Onderberg area was daar goeie potensiaal tussen meeste van die seleksies wat ge-evalueer word. Alpha en Turkey was die beste seleksies vir hierdie seisoen. Ruby Valencia toon 'n moontlike af seisoen, met klein groen vrugte tot en met oestyd. Daar is steeds geen tekens van onverenigbaarheid tussen Turkey en CC nie, en die entlas verbinding lyk gesond.

Valensias (Swaziland): Die bome is nigg jonk en hierdie seisoen was die eerste evaluasie. Doe produksie en kwaliteit van die vrugte sal verbeter met tyd, ingesluit die gemiddelde vruggrootte. Alpha lyk belowend, met meeste van die ander seleksies wat nie aan die uitvoer standaardde voldoen nie.

Knysna area: Hierdie area is nie geskik vir die produksie van meeste van die kommersiele kultivars wat beskikbaar is nie. Sweet Spring is een kultivar wat redelik goed gevaar het met goeie produksie en aanvaarbare vruggrootte. Die soektog vir geskikte kultivars vir hierdie area gaan voort.

Suurlemoene: Ontwikkel koue geharde, doringlose, saadlose suurlemoen seleksies met aanvaarbare vruggrootte wat verenigbaar is met 'n wye reeks onderstamme; wat die pluk periode verleng en konstante produksie van Maart tot September kan verseker, maw. om vroeg en laat rypwordende seleksies te ontwikkel; om die probleme met verlengde blom te beperk; behou goeie vrugkwaliteit (kleur, skil dikte, sap inhoud). Boom eienskappe en prestasie van nuwe kultivars word vergelyk met kommersieel aangeplante Eureka in die lig van bogenoemde doelwitte. Villafranca het steeds die laagste saad tellings per vrug geproduseer gevolg deur Verna. Ons het die produksie per boom vir hierdie seisoen bepaal. Limoneire het die beste oes met 151kg per boom gelewer, gevolg deur Fino 49. Evaluasies sal gestop word. Eureka SL (LNR) bly steeds die beste saadlose suurlemoen seleksie beskikbaar, hou in gedagte dat Limoneira die beste oes op die bome geproduseer het met hoe saadtellings per vrug.

Abbreviation	Rootstock
C35	Citrance 35
X639	Cleopatra x Trifoliata
SC	Swingle citrumelo
RxT	Rangpur x Troyer
RL-C	Rough lemon cairn
CC	Carrizo citrange
TC	Troyer citrange
Volk	Volkameriana
KC	Koethen citrange
TB	Terrabella citrumelo
Rangpur	Rangpur Lime
Tri x SO	Trifoliata x Sweet Orange
J.Sitroen	J Citroen
BC	Benton citrange

Oranges		Grapefruit		Lemons		Easypealers	
Count	Diameter (mm)	Count	Diameter (mm)	Count	Diameter (mm)	Count	Diameter (mm)
36	95 - 100	27	100	64	72	1XXX	78
40	90 - 96	32	97	75	69	1XX	72
48	86	36	92	88	66	1X	68
56	82	40	87	100	63	1	64
64	78	48	84	113	59	2	59
72	73	64	76	138	56	3	55
88	68			162	54	4	51
105	65			189	51	5	48
125	62			216	48		

6.2.2 Evaluation of Valencia selections in the inland areas (Onderberg)

Experiment 75 A by J. Joubert (CRI)

Opsomming

Wingsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sappehalte) te verbeter; beter boomproduksie en vrug grootteverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skikbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies vir warm streke. Turkey het die hoogste suiker inhoud vir hierdie proef geproduseer, gevolg deur McClean SL. Lavelle and Rietspruit het ook goeie kwaliteit vrugte gelewer, gevolg deur Ruby en Benny. Die laagste saadinhoud per vrug is deur McClean SL, Midnight en Rietspruit geproduseer. Evaluasies sal voortgaan in die volgende seisoen.

Summary

To optimise profitability by improving internal fruit quality (seedlessness, fibre toughness, juice quality); improving productivity and fruit size distribution (count 48, 56 and 72); improving rind colour, external appearance and peelability; extending the picking period by developing early (June / mid July) and late maturing (September / October) selections for hot areas. Turkey produced the highest sugar content for this trial, followed by McClean SL. Lavelle and Rietspruit also performed well, producing good quality fruit, followed by Ruby and Benny. The lowest seed count per fruit was produced by McClean SL, Midnight and Rietspruit. Evaluations will continue the next season.

Objective

To find suitable Valencia selections for the hot inland citrus production areas with superior characteristics.

Materials and methods

Field evaluations and laboratory analyses were conducted on Benny, Lavelle, McClean SL, Midnight, Rietspruit, Ruby and Turkey (control) at Esselen Nursery, Malelane, Mpumalanga.

Table 6.2.2.1. Internal fruit quality minimum export requirements for Valencia types.

Variety	Juice %	TSS	Min Acid	Max Acids	Ratio	Colour
Valencia	48	9.75	0.68	1.6%	8.5:1	Colour plate 3 of set no. 34
Midnight	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
*Turkey	50	10.0	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

*Interim internal fruit quality standards.

Table 6.2.2.2. List of Valencia selections evaluated at Esselen Nursery (Malelance) during 2008.

Selection	Rootstock	Year Planted	No. of trees
Benny	CC	2003	1
Lavelle	CC	2000(Top*)	3
McClellan SL		2001	5
Midnight	C35	2001	1
Rietspruit	CC	1996	1
Ruby	SC	2003	1
Turkey	C35	1998	1

* Topworked

Results and discussion

Benny

Benny produced an excellent yield with medium to large (count 72-56) fruit size on the trees. Internally the fruit was soft, low rag, and juicy (56.5% juice) with a deep yellow colour. Benny complied with the minimum export standards and the sugar content tested 10.8 Brix although seed counts were relatively high at 4.5 per fruit. Maturity is estimated from mid to end of June.

Lavelle

Trees were evaluated at Esselen nursery (Malelance) during the 2008 season. Seed counts were low and in this trial fruit compared well with McClellan SL, Midnight and Rietspruit. Lavelle produced an average yield in comparison with the other selections producing a good yield this season. The fruit size varied from medium to very large (count 72-36). Externally the rind texture was fairly coarse, but internally the fruit performed well with 11° Brix, 59.4% juice and 1.45 acid, complying with the minimum export standards. Lavelle produced the highest average juice content at 58.3% in comparison with the other selections evaluated. Maturity end of July.

McClellan SL

McClellan SL produced the second highest sugar content for this trial evaluated with 12.2° Brix. The trees produced good yields and medium to large (count 88-56) fruit size. The rind was fairly smooth, and internally the fruit was deep yellow in colour. Maturity middle of July.

Midnight

Internally the fruit complied with the minimum export standards producing 59.1% juice, 10.9° Brix and 0.99 acid. The fruit size on the trees varied from medium, large to very large fruit (count 88-36). Midnight outperformed most of the other selections with a Brix/acid ratio of 11.0, the second highest ratio in this trial evaluation. Maturity end of June.

Rietspruit

Rietspruit produced medium to large (count 88-48) fruit on the trees with a good yield. The rind texture was fairly coarse and oily. Internally the fruit produced 55.4% juice, 11.9° Brix and 1.45 acid, complying with the minimum export standards. The fruit had thin rinds and was a deep yellow internal colour. Maturity middle of July.

Ruby

Ruby produced an elongated fruit shape with small, medium and large fruit size (count 105-48). The internal quality of the fruit was above the minimum export standards, however this selection was considered too seedy to be classified as a superior selection (4.7-5.8 seeds per fruit) Maturity middle of July.

Turkey

Turkey produced the highest sugar content in this trial with 13° Brix, and a Brix:acid ratio of 12.3. Yields were excellent and the fruit size varied from medium to large (count 88-65). Externally the rind texture was smooth with some similarity to the mid seasons. Seed counts were the highest of all the selections evaluated in this trial. Maturity end May to mid of June.

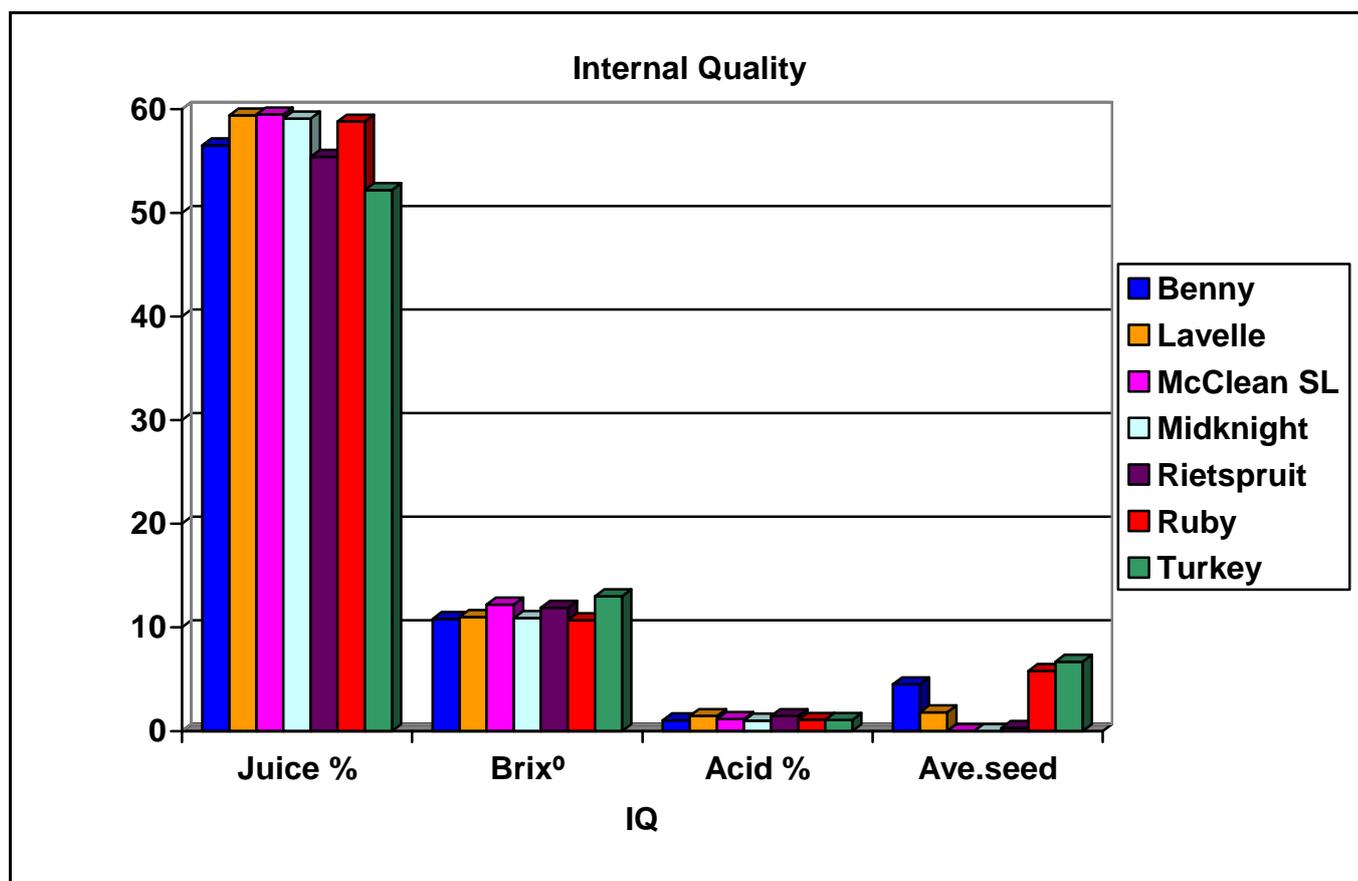
Conclusions and recommendations

All the selections evaluated complied with the export standards. McClellan SL performed the best on average, followed by, Midnight, Lavelle and Rietspruit. Turkey, Benny and Ruby performed well this season

with good internal quality, but seed counts were high ranging from 4.5 to 6.7 seeds per fruit. Evaluations will continue.

Table 6.2.2.3. Internal fruit quality data for Valencia and late orange selections at Esselen Nursery (Malelane) during the 2008 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Benny	C35	26/06/2008	73-85	56-72	56.5	10.80	1.04	10.4	4.5	T1
Lavelle	CC	26/06/2008	81-97	36-64	57.2	10.50	1.50	7.0	0.0	T2-3
Lavelle	CC	16/07/2008	74-92	40-72	59.4	11.00	1.45	7.6	1.8	T1-2
McClellan SL		26/06/2008	79-88	48-64	56.1	11.30	1.11	10.2	0.1	T1
McClellan SL		16/07/2008	72-82	56-88	59.5	12.20	1.15	10.6	0.0	T1
Midnight	C35	26/06/2008	82-97	36-56	54.8	10.80	1.53	7.1	0.2	T1-2
Midnight	C35	16/07/2008	72-85	56-88	59.1	10.90	0.99	11.0	0.0	T1
Rietspruit	CC	26/06/2008	76-86	48-72	53.0	11.60	1.60	7.3	0.2	T1-3
Rietspruit	CC	16/07/2008	72-88	48-88	55.4	11.90	1.45	8.2	0.3	T1-2
Ruby	SC	26/06/2008	71-87	48-88	56.3	10.00	1.14	8.8	4.7	T1
Ruby	SC	16/07/2008	70-84	56-88	58.8	10.70	1.08	9.9	5.8	T1-2
Turkey	C35	26/06/2008	72-78	64-88	52.2	13.00	1.06	12.3	6.7	T1



6.2.3 Evaluation of Valencia selections in the inland areas (Letsitele) Experiment 75 B by J. Joubert (CRI)

Opsomming

Wingsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sappehalte) te verbeter; beter boomproduksie en vruggrootteverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skikbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke).

Die verskillende seleksies is vir die 1ste keer hierdie seisoen geoes om 'n moontlike indikasie van vruggrootte, drag en interne kwaliteit weer te gee. Die bome is nog baie jonk en verdere evaluasies sal baie beter inligting vir die toekoms verseker.

Summary

To optimise profitability by improving internal fruit quality (seedlessness, fibre toughness, juice quality); improving productivity and fruit size distribution (count 48, 56 and 72); improving rind colour, external appearance and peelability; extending the picking period by developing early (June / mid July) and late maturing selections (for hot areas).

The selections were evaluated for the first time this season to determine the fruit size, yield and internal quality of the fruit on the young trees. Evaluations will continue in the future and will provide valuable information to assist producers with the establishment of new orchards.

Objective

To find suitable Valencia selections for the hot inland citrus production areas with superior qualities.

Materials and methods

Field evaluations and laboratory analyses were conducted on Benny 1&2, Bend 8A 1&2, Jassie, Lavelle, Limpopo SL and Ruby at Group 91, Letsitele.

Table 6.2.3.1. List of Valencia selections evaluated at Group 91(Letsitele) during 2008.

Selection	Rootstock	Tree Age	No. of trees
Benny 1	CC/SC	2005	10/10
Benny 2	CC/SC	2005	10/10
Bend 8A 1	SC	2005	10/10
Bend 8a 2	CC/SC	2005	10/10
Jassie	CC	2005	10
Lavelle		2005	50
Limpopo SL		2005	
Ruby	CC	2005	10

Results and discussion

Benny 1

Benny 1 produced average to good yields on both CC and SC with medium to large fruit size (count 88-56). The internal quality will improve when evaluating more mature fruit. The acid levels in the fruit was lower in the case of CC in comparison to SC. SC delays the external colour as well as producing a higher acid in the fruit in comparison to CC by approximately two weeks. The external colour varied between T4 and T6 when fruit were sampled for testing purposes. There was only enough fruit produced on the trees to perform one evaluation.

Benny 2

Benny 2 performed very similarly to Benny 1 at this stage of the evaluations except for Brix levels where Benny 2 was higher than Benny 1. Future evaluations may show additional differences.

Bend 8A1

Bend 8A1 produced slightly oblong fruit with average to good yields on these young trees. Coarse external skin texture is one of the characteristics of young trees and will improve as the trees grow older. The internal quality looks promising with high juice content and sustainable acid levels; Brix already complies with the minimum export requirements.

Bend 8A2

The fruit produced on the trees seems more round in shape in comparison to the fruit from Bend 8A1. Bend 8A2 appears to mature about 2 weeks later than Bend 8A1; future evaluations will have to prove this conclusion.

Jassie

Jassie produced average yields with the fruit having a high fibre content internally. The fruit size varied from medium to large (count 88-48). Internally the sugar content with the second evaluation was 11.0 Brix; very promising being the highest figure for this trial. The acid levels seem to remain fairly high, giving the selection the possible advantage of late hanging. Evaluations will continue.

Lavelle

The production on the trees varied from poor to good, which can be attributed to the age of these young trees. This selection produced from small to large fruit with promising internal quality.

Limpopo SL

The bud union between the scion and rootstock appears to be incompatible with numerous rootstock shoots growing out below the union (CC rootstock). Future evaluations and comparison with the trees on the other trial sites might answer the questions. Limpopo SL at Weipe, Noord Grens boerdery seems fine and most of the trees were planted on Rough lemon rootstock. The juice and acid content in the fruit was fairly low when the evaluation was completed, similar to the trial site at Noord Grens boerdery.

Ruby

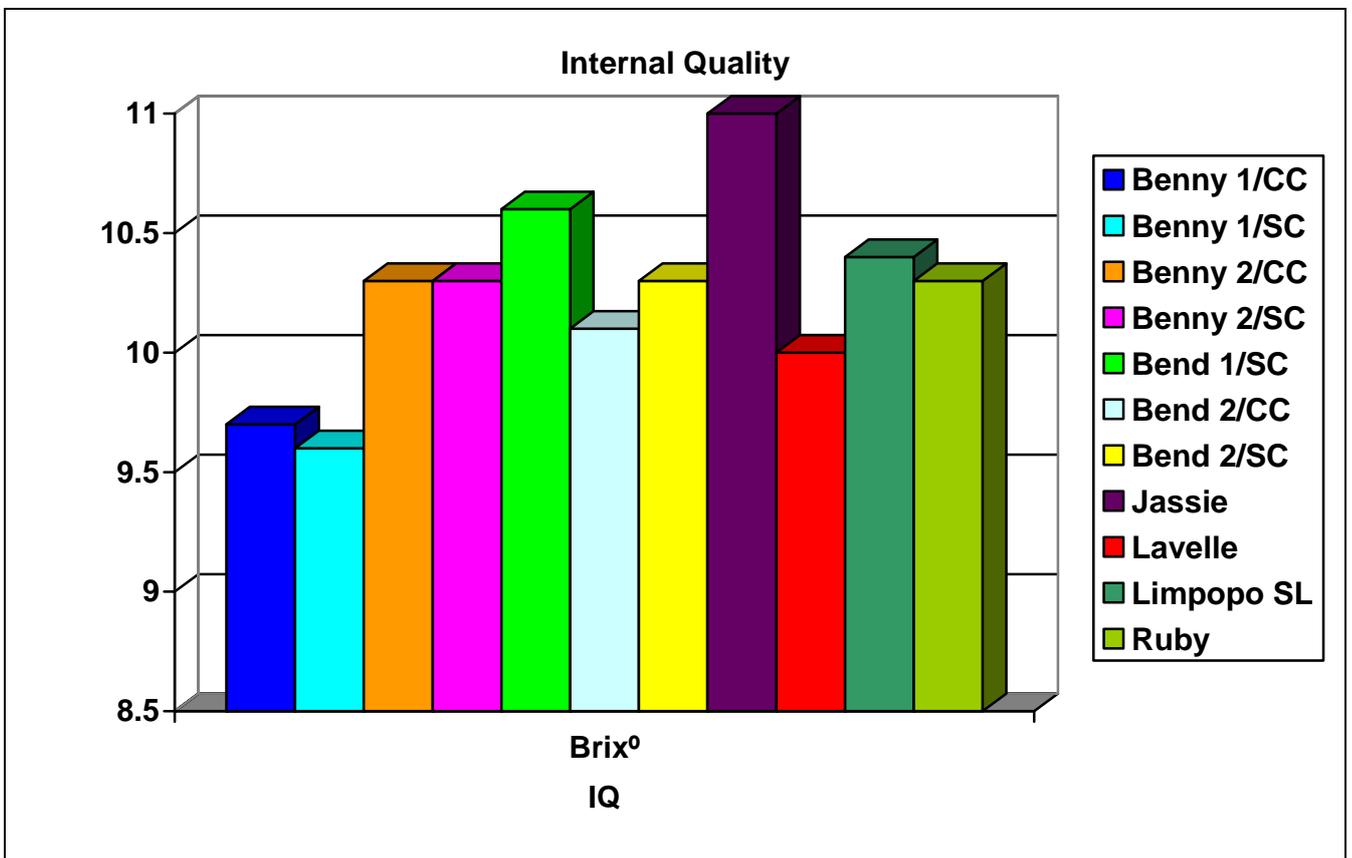
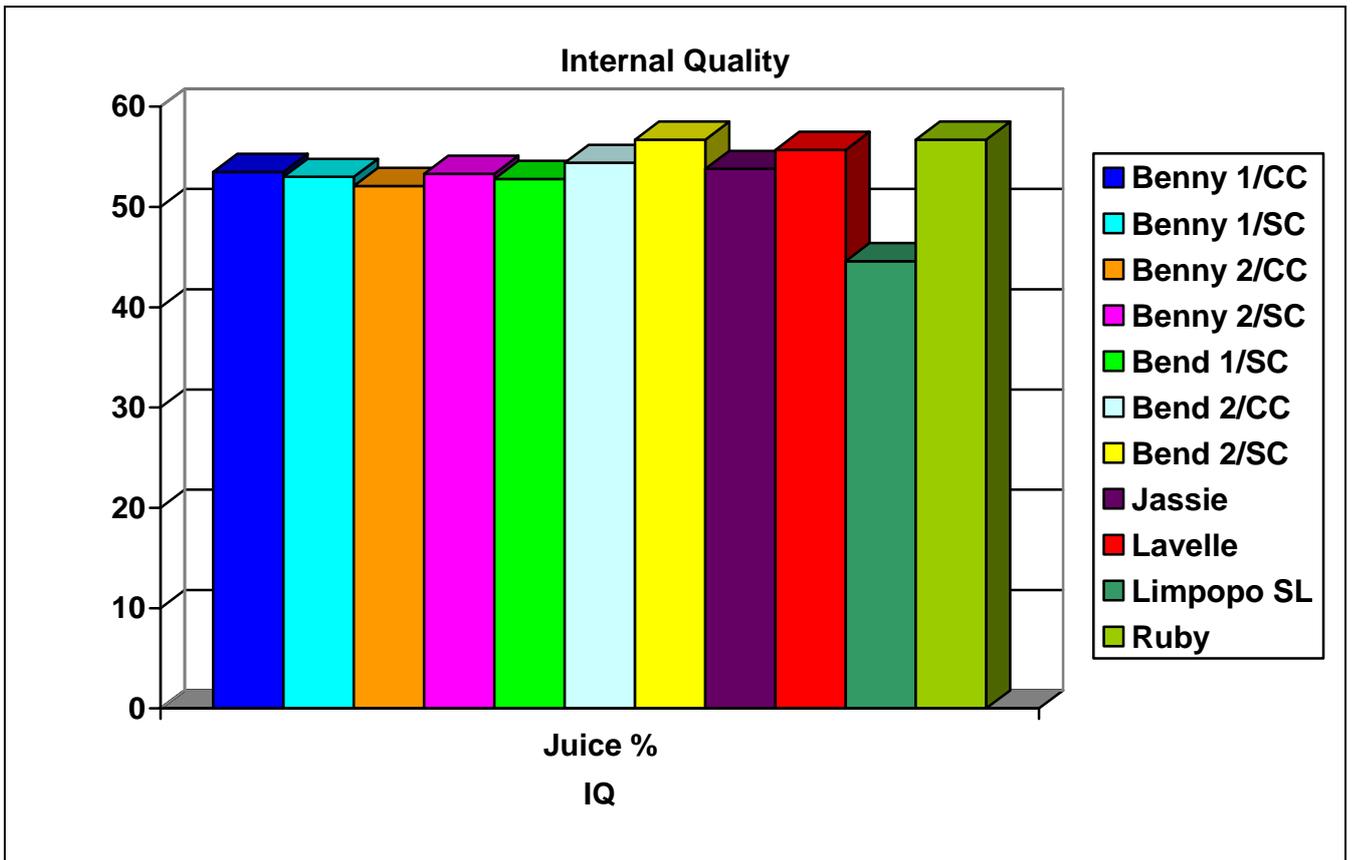
The yield produced on the trees was poor with small to medium fruit size; bear fruit similar to grapefruit in bunches. With the second evaluation the internal quality improved and complied with the export standards. The seed count was 4.3 seeds per fruit on average. Evaluations will continue.

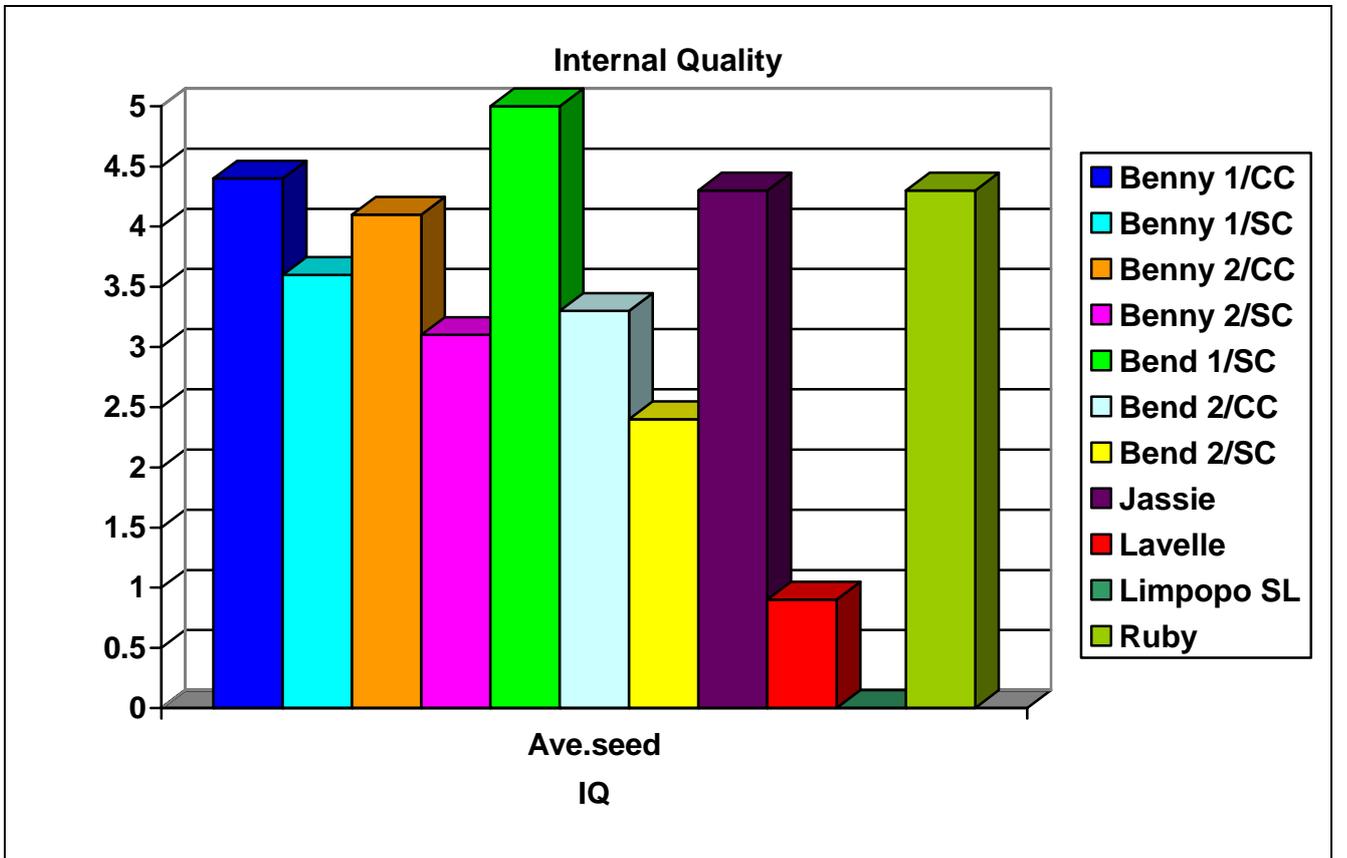
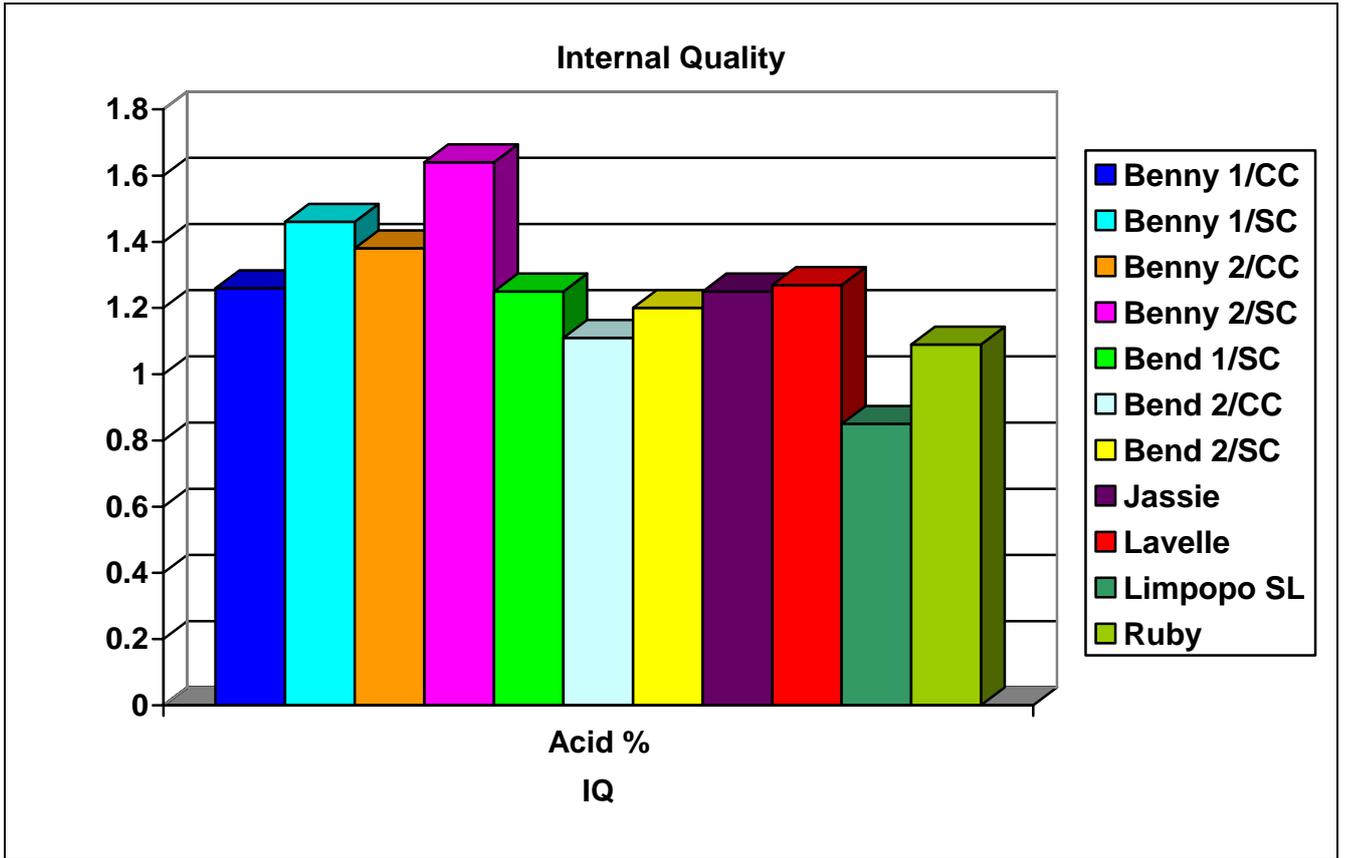
Conclusions and recommendations

This was the first year this trial site was evaluated and it should be kept in mind the trees were still young and the yield produced fairly light. Next season the production will increase and two to three evaluations will be completed. Jassie performed the best for this season, followed by Lavelle, Bend 8A2 and Benny 1&2. Future evaluations should generate valuable information. Evaluations will continue.

Table 6.2.3.2. Internal fruit quality data for Valencia orange selections at Groep 91 (Letsitele) during the 2008 season.

Selection	Root-stock	Date Harvested	Size mm	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Benny 1	CC	04/06/2008	72-81	64-88	53.5	9.70	1.26	7.7	4.4	T4-5
Benny 1	SC	04/06/2008	74-85	56-72	53.0	9.60	1.46	6.6	3.6	T4-6
Benny 2	CC	04/06/2008	76-81	64-72	52.1	10.30	1.38	7.5	4.1	T4-6
Benny 2	SC	04/06/2008	67-84	56-105	53.3	10.30	1.64	6.3	3.1	T4-5
Bend 8A 1	SC	09/07/2008	71-83	56-88	52.8	10.60	1.25	8.5	5.0	T1-3
Bend 8A 2	CC	09/07/2008	78-88	48-64	54.4	10.10	1.11	9.1	3.3	T1-3
Benb 8A 2	SC	09/07/2008	73-81	64-72	56.7	10.30	1.20	8.6	2.4	T2-4
Jassie	CC	04/06/2008	68-83	56-88	52.8	10.30	1.34	7.7	4.4	T3-5
Jassie	CC	09/07/2008	73-86	48-72	53.8	11.00	1.25	8.8	4.3	T1-3
Lavelle		09/07/2008	75-88	48-72	55.7	10.00	1.27	7.9	0.9	T2-4
Limpopo SL		04/06/2008	71-84	56-88	44.6	10.40	0.85	12.2	0.0	T3-5
Ruby	CC	09/07/2008	74-85	56-72	56.7	10.30	1.09	9.4	4.3	T1-2





6.2.4 Evaluation of Lemon selections in the inland areas Experiment 79 by J. Joubert (CRI)

Opsomming

Kouegeharde, doring- en saadlose suurlemoenseleksies met aanvaarbare vruggrootte moet ontwikkel word. Die oesseisoen moet verleng word om aaneenlopende produksie van Maart tot September te verseker, aangesien dit vroeë en laatrypwordende seleksies insluit. Probleme wat met lang blomtyd ge-assosieer kan word, moet verminder word. Goeie vrugkwaliteit soos kleur, skildikte en sapinhoud, moet behou word. Die boomeienskappe en prestasie van nuwe kultivars moet met die kommersieel gekweekte Eureka vergelyk word om te bepaal of hulle aan bogenoemde doelwitte voldoen.

Alle seleksies wat ge-evalueer word in hierdie proef, het tussen 33 en 37% sap geproduseer, effens laer as die vorige seisoen met Villafranca die hoogste (42% sap). Villafranca produseer steeds die laagste saad telling per vrug (0 sade per vrug), gevolg deur Verna met 8.6 sade per vrug. Die grootste nadeel van Villafranca egter is dat hierdie seleksie die 2de laagste produksie (37.34 kg/boom) vir hierdie proef gelewer het.

Die hoogste produksie per boom was op Eureka saadloos (Israel) geproduseer (123.97 kg/boom), en die laagste produksie op Verna met 10.59 kg/boom.

Summary

To develop cold hardy, thornless, seedless lemon selections with acceptable fruit size; to extend the picking period to ensure continuity of supply from March to September, i.e. early and late maturing selections; to reduce the problems associated with protracted flowering and to maintain high fruit quality (colour, rind thickness, juice content). To meet these objectives tree characteristics and performance of new cultivars were compared with the commercially grown Eureka.

All the selections evaluated in this trial produced between 33 & 37% juice content, slightly lower in comparison with the previous season with Villafranca producing the highest quality with 42% juice. Villafranca also produced the lowest seed count (0.3 seeds/fruit) followed by Lisbon on Sour orange with 10.9 seeds per fruit. The biggest disadvantage of Villafranca is the poor yield produced per tree, the second lowest (37.34 kg/tree) for this trial evaluated.

The best production per tree remained Eureka SL (Israel) (123.97 kg/tree), and the lowest on Verna with 10.59 kg per tree.

Objective

To find suitable Lemon selections for the hot inland citrus production areas.

Materials and methods

Field evaluations were conducted on Eureka SL (ARC) as control, Eureka SL (Israel), Fino 49 & 95, Genoa, Limoneira 8A, Lisbon (control), Verna and Villafranca SL on two rootstocks.

Table 6.2.4.1. List of lemon cultivars evaluated at Tekwane (Karino area) during the 2008 season.

Selection	Rootstock	Tree Age	No. of trees
Eureka SL (ARC) *	RL	2000	1
Eureka SL (Israel)	RL	1998	4
Fino 49	RL	1998	3
Fino 95	RL	1998	4
Genoa	RL	1998	4
Limoneira 8A	RL	1998	2
Lisbon	RL,SO	1998	2;2
Verna	RL	1998	4
Villafranca	RL	1998	2

* Situated at Esselen Nursery, Malelane

Results and discussion

Eureka SL (ARC)

Good production of seedless fruit at Esselen Nursery. As this is an ARC trial it was not possible to harvest fruit for internal quality analyses.

Tekwane Estates

Villafranca produced the highest juice content (42%), followed by Fino 49 (37.6%) and Eureka SL (IR) (37.2%). The lowest juice content was produced by Lisbon on Sour orange rootstock with 33.1%. Villafranca produced the lowest seed count per fruit (0.3) followed by Lisbon on RL (10.9) and Verna (12.8). The fruit size peaked at count 144 for all the selections evaluated, except Villafranca and Genoa which peaked at count 115 and produced larger fruit in comparison to the rest on the selections. Verna produced the lowest yield for this trial evaluated (10.4 kg/tree) and the best yield was produced by Eureka SL (IR) on the trees (123.97 kg/tree).

Conclusions and recommendations

Villafranca will be a good option when lower seed counts are important, but the poor yield per tree produced for this season should be borne in mind. Eureka seedless (IR), Fino 49, Limoneira and Genoa performed very well and produced the best crops for the season, although all of the selections contained seeds. All these characteristics should be taken into consideration when deciding on which selection to plant.

Table 6.2.4.2a. Internal fruit quality data for Lemons from Tekwane Estate (Karino) during the 2008 season.

Selection	Root-stock	Date harvested	Juice %	Ave. seed
Eureka seedless(IR)	RL	07/04	37.0	13.7
Eureka seedless(IR)	RL	07/05	37.9	12.2
Eureka seedless(IR)	RL	02/06	40.1	11.8
Eureka seedless(IR)	RL	24/06	33.9	12.6
Fino 49	RL	07/04	37.2	15.3
Fino 49	RL	07/05	40.7	14.2
Fino 49	RL	02/06	39.3	14.1
Fino 49	RL	24/06	33.3	16.8
Fino 95	RL	07/04	37.9	18.8
Fino 95	RL	07/05	38.6	16.4
Fino 95	RL	02/06	37.7	16.9
Fino 95	RL	24/06	33.3	16.8
Genoa	RL	07/04	38.1	19.4
Genoa	RL	07/05	38.3	22.8
Genoa	RL	02/06	42.1	23.3
Genoa	RL	24/06	28.2	27.5
Lisbon	RL	07/04	36.3	15.3
Lisbon	RL	07/05	35.8	8.6
Lisbon	RL	02/06	36.2	9.3
Lisbon	RL	24/06	32.7	10.2
Lisbon	SO	07/04	13.8	15.6
Lisbon	SO	07/05	39.7	15.3
Lisbon	SO	02/06	43.4	15.6
Lisbon	SO	24/06	35.3	16.5
Limoneira	RL	07/04	33.0	18.1
Limoneira	RL	07/05	37.2	17.5
Limoneira	RL	02/06	37.6	18.3
Limoneira	RL	24/06	34.8	17.9
Verna	RL	07/04	37.5	3.4
Verna	RL	07/05	41.7	15.7
Verna	RL	02/06	35.7	15.8

Selection	Root-stock	Date harvested	Juice %	Ave. seed
Verna	RL	24/06	33.3	16.1
Villafranca	RL	07/04	44.4	0.1
Villafranca	RL	07/05	44.9	0.0
Villafranca	RL	02/06	43.1	0.1
Villafranca	RL	24/06	35.7	0.8

Table 6.2.4.2b. Average internal fruit quality data for Lemons from Tekwane Estate (Karino) during the 2008 season.

Selection	Root-stock	Juice %	Ave. seed
Eureka seedless(IR)	RL	37.2	12.6
Fino 49	RL	37.6	15.1
Fino 95	RL	36.9	17.2
Genoa	RL	36.7	23.3
Lisbon	RL	35.3	10.9
Lisbon	SO	33.1	15.8
Limoneira	RL	35.7	18.0
Verna	RL	37.1	12.8
Villafranca	RL	42.0	0.3

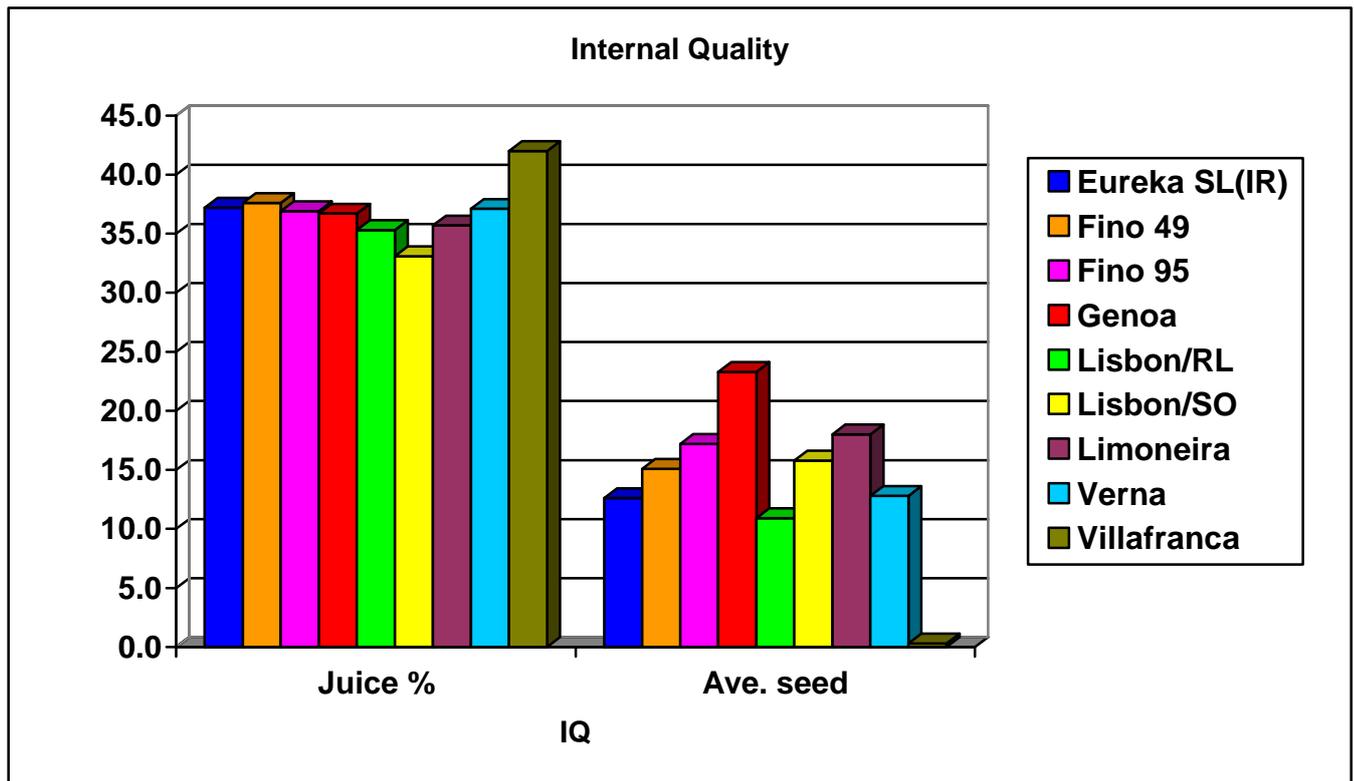


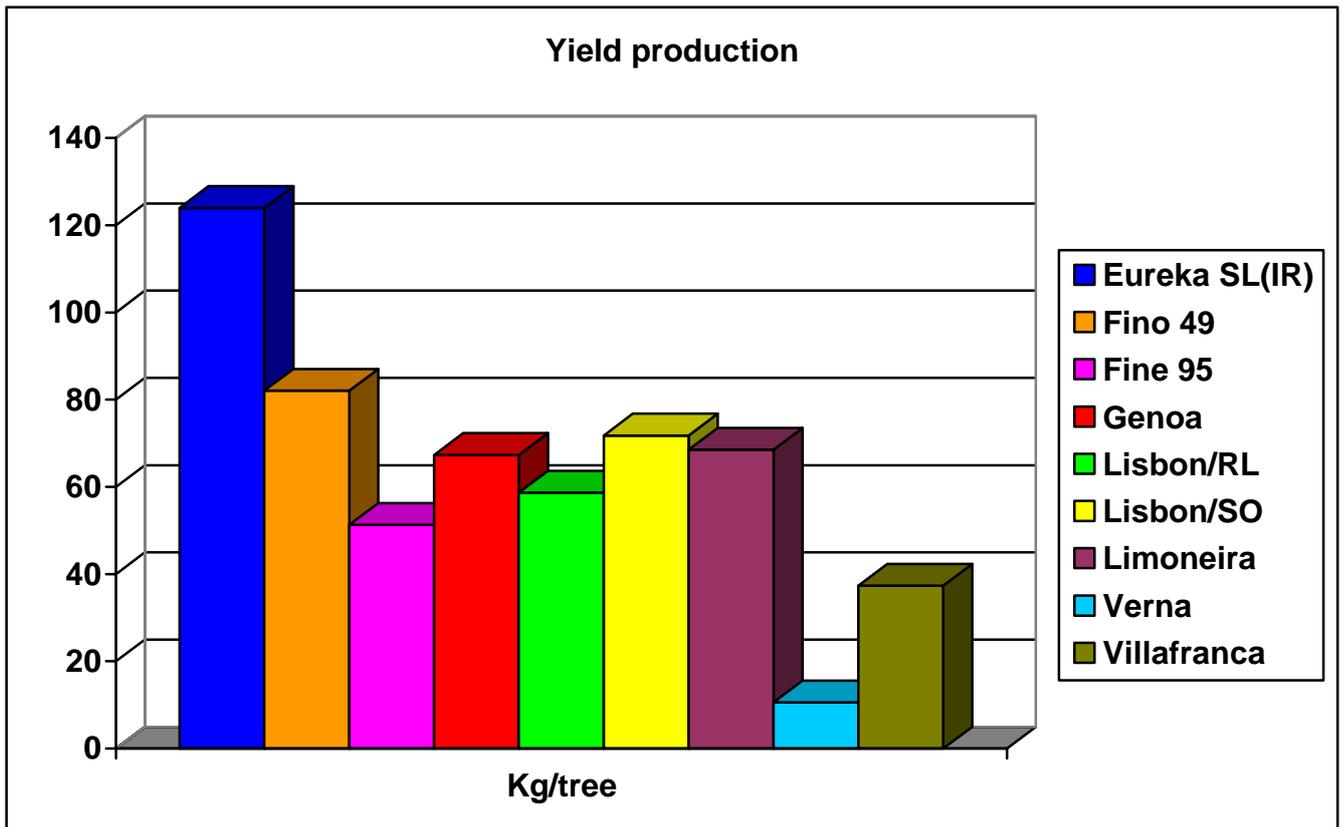
Table 6.2.4.3. Fruit size distribution of Lemons on two rootstocks at Tekwane Estate (Karino) during the 2008 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Eureka seedless(IR)	RL	48	0.30	Lisbon	SO	48	0.00
Eureka seedless(IR)	RL	56	0.56	Lisbon	SO	56	0.00
Eureka seedless(IR)	RL	72	2.77	Lisbon	SO	72	1.02
Eureka seedless(IR)	RL	88	7.82	Lisbon	SO	88	2.21

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Eureka seedless(IR)	RL	115	39.85	Lisbon	SO	115	30.24
Eureka seedless(IR)	RL	144	48.98	Lisbon	SO	144	66.52
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Fino 49	RL	48	0.00	Limoneira	RL	48	0.06
Fino 49	RL	56	0.04	Limoneira	RL	56	0.12
Fino 49	RL	72	0.26	Limoneira	RL	72	0.80
Fino 49	RL	88	2.16	Limoneira	RL	88	3.77
Fino 49	RL	115	33.45	Limoneira	RL	115	42.41
Fino 49	RL	144	64.09	Limoneira	RL	144	52.84
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Fino 95	RL	48	0.00	Verna	RL	48	0.00
Fino 95	RL	56	0.35	Verna	RL	56	0.61
Fino 95	RL	72	3.04	Verna	RL	72	1.22
Fino 95	RL	88	8.76	Verna	RL	88	8.23
Fino 95	RL	115	43.37	Verna	RL	115	35.06
Fino 95	RL	144	44.49	Verna	RL	144	54.88
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	RL	48	0.05	Villafranca	RL	48	0.26
Genoa	RL	56	0.62	Villafranca	RL	56	2.89
Genoa	RL	72	4.11	Villafranca	RL	72	7.87
Genoa	RL	88	11.66	Villafranca	RL	88	13.52
Genoa	RL	115	53.77	Villafranca	RL	115	42.65
Genoa	RL	144	29.78	Villafranca	RL	144	32.81
Cultivar	Rootstock	Size	% Fruit				
Lisbon	RL	48	0.00				
Lisbon	RL	56	0.00				
Lisbon	RL	72	1.97				
Lisbon	RL	88	5.48				
Lisbon	RL	115	41.12				
Lisbon	RL	144	51.43				

Table 6.2.4.4. Production of Lemons on different rootstocks at Tekwane Estate (Karino) during the 2008 season.

Cultivar	Rootstock	Kg/tree(07)	Kg/tree(08)
Eureka seedless(IR)	RL	127.11	123.97
Fino 49	RL	98.09	82.05
Fino 95	RL	66.30	51.28
Genoa	RL	74.89	67.33
Lisbon	RL	65.03	58.69
Lisbon	SO	67.03	71.78
Limoneira	RL	84.72	68.53
Verna	RL	24.65	10.59
Villafranca	RL	36.33	37.34



6.2.5 Evaluation of Valencia selections in the hot inland areas (Swaziland)

Experiment 740A by J. Joubert (CRI)

Opsomming

Die nuwe aanplantings met Jassie en Turkey (nuwe seleksie) beide op Carrizo citrange en Citrange 35 het uitstekend gevaar met goeie opbrengs en interne kwaliteit. Albei seleksies het aan die uitvoer standaarde voldoen. Delta, McClean SL, Portsgate en Rietspruit toon baie potensiaal en het weereens goeie resultate gelewer. Die Tambuti Early seleksie is baie onstabiel, vorm baie kimeras en word glad nie vroeg ryp soos een van sy eienskappe behoort te wees nie. Mouton Early is verwyder a.g.v. Appelstam groef virus, want verdere besmetting van die res van die boorde kan meganies plaasvind. Wanneer skoon materiaal beskikbaar word sal die seleksie weer gevestig word vir evaluasies.

Summary

The new plantings with Jassie and Turkey (new selection) on both Carrizo citrange and Citrange 35 produced excellent yields and internal quality. Both selections complied with the minimum export standards. Delta, McClean SL, Portsgate and Rietspruit also performed well and produced good results during the evaluations. Tambuti Early appears to be unstable, producing high numbers of chimeras. The maturity time of the selection is late in the season in comparison with the other earlier Valencias. Mouton Early was removed from the trial site, because of Apple stemgroof virus. The risk of infecting to the rest of the orchards by mechanical farming methods was too high.

Objective

To optimise profitability by selecting Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), as well as to extend the current harvest period (both earlier and later maturing). To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a hot production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on Delta, Jassie, McClean SL, Portsgate Rietspruit, Tambuti Early and Turkey at Tambuti Esatate, Swaziland.

Table 6.2.5.1. Internal fruit quality data were compared with the minimum export requirements for Valencia types.

Variety	% Juice	Brix ^o	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.75	0.68	1.6%	8.5:1	Colour plate 3 of set no. 34
Delta SL	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

Table 6.2.5.2. List of Valencia selections evaluated at Tambuti Estate (Swaziland) during 2008.

Selection	Rootstock	Tree Age	No. of trees
Delta	CC	2002	20
Jassie	C35/CC	2005	10/10
McClean SL	CC	2002	20
Portsgate	CC	2002	20
Rietspruit	CC	2002	19
Tambuti Early	CC	2002	4
Turkey	C35/CC	2005	10/10

Results and discussion

Delta

Delta produces a good yield on the trees with medium fruit size (count 88-64). The fruit reached optimal internal quality to harvest with a high juice (65.2%) and Brix (11°) content. The Brix:acid ratio of 12.0 indicates that the fruit is mature and complies with the minimum export requirements. Maturity end of June.

Jassie

On both CC and C35 the juice (55.4% and 56.2%) and Brix (12.7° and 12.5°) content of the fruit was excellent, but the acid (1.36% and 1.27%) remained at a fairly high level close to harvest. The average seed count per fruit was 7.02. Jassie on both rootstocks produced a small to medium fruit size on the trees (count 125-64). It should be kept in mind that the trees were evaluated for the first time this season and the trees are still young. Jassie maturity peaks towards the end of July. Evaluations will continue.

McClean SL

The acid levels for this selection were fairly low and decreased considerably towards the harvesting time, while the external colour was delayed. Internally the juice and sugar content was high and acceptable, and the low acid levels increased the Brix:acid ratio above 14.0. The seed count per fruit was very low with only 0.1 seeds per fruit counted during the evaluations. Production on the trees were good with a small to medium fruit size (count 125-64). From the internal quality results peak maturity appears to be mid to end May.

Portsgate

Portsgate produces a good yield on the trees with small to medium fruit size (count 88). The fruit have a slightly elongated shape and a fairly course rind. This selection was completely seedless. Internally the juice, Brix:acid levels comply with the export standards and the ratio was 11.25 on 17/07. Maturity middle of July.

Rietspruit

The fruit size on the trial trees varied from small to medium/large (count 125-56). Production was poor to average on the trees and the fruit produced a fairly thick skin. There was a severe red scale problem on this selection in comparison to the rest of the selections evaluated in the same block with similar treatments. Rietspruit matures mid to end July.

Tambuti Early

This selection seems to be unstable and produced a high number of chimeras. The fruit size on the trees was fairly small to medium (count 125-72) with an excellent crop. The heavy crop load might be the reason for the smaller fruit size produced. The juice content was acceptable, but the Brix and acid levels were fairly low with delayed external colour. This selection does not appear to be early when compared with the other early selections such as Limpopo SL, Turkey and Benny. Maturity early July.

Turkey

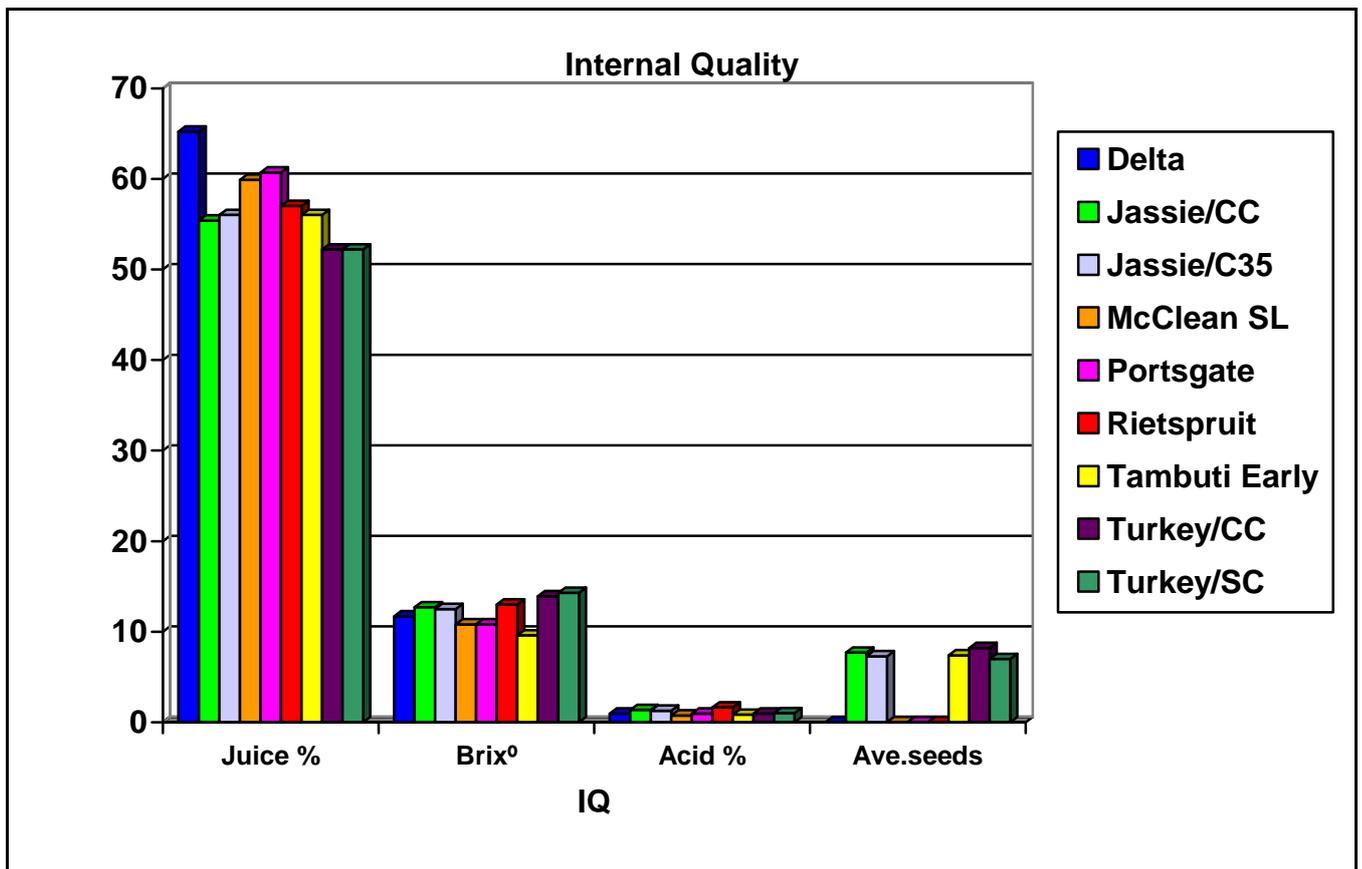
Turkey produced an excellent yield on these young trees with medium to large fruit size (count 88-64), normally associated with young trees. Internally fruit quality was excellent with good juice (52%) and Brix (14°) levels, as well as acceptable acid (1.0%). Maturity mid to end May.

Conclusions and recommendations

Delta, Jassie, McClean SL, Portsgate, Rietspruit and Turkey performed well this season, with good internal quality and optimal fruit size for export. The Tambuti early selection in this trial appears to be unstable producing numerous chimeras and has low Brix levels in comparison with the other Valencia selections Delta as control performed well and will always be a good option for new plantings. Jassie and Turkey on both CC and C35 performed exceptionally well for young trees. Evaluations will continue.

Table 6.2.5.3. Internal fruit quality data for Valencia orange selections at Tambuti Estate (Swaziland) during the 2008 season.

Selection	Root-Stock	Date harvested	Size mm	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Delta	CC	17/07	70-79	64-88	65.2	11.7	0.96	12.19	0.0	T3-4
Jassie	CC	29/04	64-75	72-125	48.5	11.0	1.77	6.21	7.6	T7
Jassie	CC	20/05	66-78	64-105	50.7	11.1	1.50	7.40	5.3	T5-6
Jassie	CC	17/07	69-77	72-88	55.4	12.7	1.36	9.34	7.7	T3-4
Jassie	C35	29/04	64-73	72-125	52.3	9.9	1.68	5.89	7.6	T7
Jassie	C35	20/05	63-78	64-125	52.2	10.5	1.35	7.78	6.6	T5-6
Jassie	C35	17/07	65-78	64-105	56.0	12.5	1.27	9.84	7.3	T3-4
McClean SL	CC	20/05	64-73	72-125	56.2	9.9	0.94	10.53	0.1	T6
McClean SL	CC	17/07	70-80	64-88	59.9	10.8	0.75	14.40	0.0	T3-4
Portsgate	CC	17/07	71-86	48-88	60.7	10.8	0.96	11.25	0.0	T4-6
Rietspruit	CC	20/05	63-74	72-125	53.1	12.4	1.87	6.63	0.3	T5-6
Rietspruit	CC	17/07	70-84	56-88	57.0	13.0	1.64	7.93	0.0	T1-2
Tambuti Early	CC	20/05	60-67	105-125	51.9	8.5	0.97	8.76	2.7	T4-6
Tambuti Early	CC	17/07	66-76	72-105	56.0	9.6	0.83	11.57	7.4	T4-5
Turkey	CC	29/04	71-76	72-88	48.5	11.7	1.17	10.00	6.3	T5-6
Turkey	CC	20/05	70-81	64-88	51.0	12.0	1.09	11.01	8.5	T3-4
Turkey	CC	17/07	67-79	64-105	52.2	13.9	0.95	14.63	8.2	T1
Turkey	C35	29/04	68-79	64-88	51.5	11.8	1.31	9.01	7.3	T5-6
Turkey	C35	20/05	69-77	72-88	50.7	12.6	1.1	11.45	7.1	T3-4
Turkey	C35	17/07	70-78	64-88	52.2	14.3	1.01	14.16	7.0	T1



6.3 PROJECT: ROOTSTOCK EVALUATIONS

6.3.1 Project summary

Commercial rootstock choice is relatively limited, and the best available rootstock option is seldom ideal in addressing all the site limitations as well as production and marketing requirements. The development of a new rootstock is a long and involved process, and it is unlikely that any new rootstock will have all the desirable attributes.

One of the prime objectives of rootstock evaluation is to find reliable size-controlling rootstocks coupled with attributes such as good yield of marketable fruit size and internal fruit quality, pest and disease tolerance or resistance, and adaptability to a wide range of scion cultivars and soil types.

The rootstock research efforts of the 1980s and 1990s led to considerable changes in rootstock use from almost exclusively being rough lemon to Carrizo and Troyer citranges and Swingle citrumelo rootstocks. Yet, there still remains an acute need to seek out, evaluate and commercialise new generation rootstocks.

Malelane

Midnight and Delta on the different rootstock selections produced very good to excellent quality fruit, complying with the minimum export standards for Valencias. Fruit set on all the combinations decreased by up to 50% in comparison with 2007 and fruit size on average increased, probably due to the lighter crop.

Swaziland

This trial was evaluated for the third time this season; production increased substantially in comparison with the 2007 season. Internal quality on Marsh, Star Ruby and Nelruby was very promising although only Nelruby complied with all the export requirements. Marsh and Star Ruby were below the minimum Brix acid ratio of 7:1 in some instances, probably due to the trial being harvested too early. The average fruit size for this season peaked at count 48, followed by count 64 and 40. All the rootstock combinations increased in yield production with some up by 50%.

Hectorspruit

C35 in combination with all the selections performed well and future plantings on this rootstock should be considered. C35 is a semi dwarfing rootstock with trees 25 to 30% smaller after 5 to 6 years being smaller than trees on SC, CC, MxT and X639. Terrabella also has a semi dwarfing effect, but not quite as much as C35 which can be 25 to 30% smaller than CC after 10 to 12 years. Koethen citrange also has a dwarfing effect, but to what extent is not known at present.

Weipe

Limpopo SL in combination with CC, SC and X639 produced fruit complying with the minimum export standards, but fruit from trees on RL was below standard. Fruit size counts were promising with all rootstocks producing fruit in counts 56 to 88. RL being produced the best yields (225 kg/tree) and had the largest tree volume. SC, CC and X639 performed well at slightly smaller tree volumes.

Citrusdal

Genoa in combination with Rangpur Lime produced a good crop and the fruit size ranged from count 216 and 162. Trifoliolate x Sour orange cross produced the second highest yields, but peaked with a high % of fruit in count 216. J. Citroen, RL-C and Volk produced fruit only slightly smaller than Benton citrange (BC), but yields were low. While fruit size was largest on BC yield was low only being higher than trifoliolate. All the scion-rootstock combinations produced fairly small fruit from 48 to 54 mm in diameter. Based on these results Rangpur Lime is the only rootstock that gave a good overall performance. Volk and J. Citroen also performed fairly well while the remaining rootstocks were unacceptable overall.

Projekopsomming

Kommersiele onderstam keuses is relatief beperk, en die beste beskikbare onderstam keuse is selde in staat om al die beperkings van die perseel uit te skakel, asook aan produksie en bemarkings vereistes te voldoen. Die ontwikkeling van nuwe onderstamme is 'n lang en uiteenlopende proses, en dis onwaarskynlik dat 'n nuwe onderstam aan al die vereistes sal voldoen.

Een van die primêre doelwitte van onderstam evaluasies is om geskikte onderstamme te vind wat boomgrootte beheer, gekoppel is aan goeie produksie met bemarkbare vruggrootte en interne vrug kwaliteit, bestand is of weerstand bied teen insekte en siektes, en die aanpasbaarheid met 'n wye reeks bostam kultivars en grond tipes.

Die onderstam navorsings pogings van die 1980's en 1990's het gelei tot aansienlike veranderings in onderstam gebruik van feitlik alleenlik growwe skil in aanplantings na Carrizo en Troyer citrange en Swingle citrumelo onderstamme. Daar bestaan steeds 'n behoefte vir ontwikkeling, evaluasie en kommersialisasie van nuwe generasie onderstamme.

Malelane

Midnight en Delta op verskillende onderstam seleksies het goeie tot baie goeie kwaliteit vrugte geproduseer, en het aan die minimum uitvoerstandaarde vir Valencias voldoen. Vrugset op al die kombinasies het met tot 50% gedaal in vergelyking met die 2007 seisoen, alhoewel vruggrootte toegeneem het moontlik a.g.v. die ligter oes op die bome.

Swaziland

Hierdie proef is vir die derde keer hier seisoen ge-evalueer; produksie het indrukwekkend toegeneem in vergelyking met die 2007 seisoen. Interne kwaliteit op Marsh, Star Ruby en Nelruby was baie belowend gewees, alhoewel slegs Nelruby aan die uitvoerstandaarde voldoen het. Marsh en Star Ruby was onder die minimum Brix:Suur verhouding gewees in sekere gevalle, moontlik omdat die proef redelik vroeg gepluk was. Die gemiddelde vruggrootte vir hierdie seisoen het by telling 48 gepiek, gevolg deur telling 64 en 40. Al die onderstam kombinasies het toegeneem in produksie, sommige selfs met tot 50%.

Hectorspruit

C35 in kombinasie met al die seleksies het goed presteer en nuwe aanplantings op hierdie onderstam moet verseker oorweeg word. C35 is 'n semi verdwergende onderstam met bome tussen 25 tot 30% kleiner na 5 tot 6 jaar, lewer kleiner bome as op SC, CC MxT en X639. Terrabella het ook 'n semi-dwergende effek, maar nie so baie as C35 wat 25 tot 30% kleiner kan wees as CC na 10 tot 12 jaar.. Koethen citrange het ook 'n verdwergende effek, maar tot watter mate is nie huidiglik bekend nie.

Weipe

Limpopo SL in kombinasie met CC, TC en X639 het vrugte geproduseer wat aan die minimum uitvoer standaard voldoen, maar RL was onder die minimum gewees. Vruggrootte tellings was belowend gewees,

alle onderstamme het tussen telling 56 en 88 geproduseer. RL het die beste oes geproduseer (225kg/boom) met die grootste boom volume. TC,CC en X639 het goed presteer met effens kleiner boom volume.

Citrusdal

Genoa in kombinasie met Rangpur lemmetjie het 'n goeie oes geproduseer met vruggroote wat wissel tussen telling 216 en 162. Trifoliaat en Sour orange kruisings het die tweede hoogste oes geproduseer, maar het gepiek met groot hoeveelhede vrugte in telling 216. J. Citroen, RL-C en Volk het effens kleiner vrugte as Benton citrange (BC) geproduseer, maar produksie was laag. Die vruggroote was die beste op BC, maar die oes was ook laag, slags hoer as trifoliaat. Al die onderstam-bostam kombinasies het redelike klein vrugte geproduseer tussen 48 en 54 mm in deursnit. Gebaseer op hierdie resultate was Rangpur lemmetjie die enigste onderstam wat 'n goeie prestasie gelever het. Volk en J.Citron het ook redelik goed gevaar, terwyl die ander ondestamme onaanvaarbaar presteer het oor die algemeen.

6.3.2 Evaluation of Valencias on new imported rootstocks in the Malelane area Experiment 416 A by J.Joubert (CRI)

Opsomming

Die prestasie van Midnight en Delta Valencias op nuwe, ingevoerde onderstamme op herplant grondtipes moet ondersoek word. Die produksie, interne gehalte en skilkleur moet verbeter word, terwyl vruggroote moet toeneem.

Die interne kwaliteit hierdie seisoen het baie goed vergelyk met die vorige seisoen, met die suur wat selfs hoër was, maar het aan die uitvoer standaard voldoen. Ongelukkig het die opbrengste tot en met 50 % afgeneem in vergelyking met die vorige seisoen. Midnight op Sunki 812 het 'n baie belowende vruggroote geproduseer, tussen telling 72 en 56. Die beste Delta kombinasie wat interne kwaliteit van die vrugte aanbetref was Sunki 802 gewees, met 'n Brix:Suur verhouding van 10.2., asook die hoogste oes produksie van 102.3 kg/boom. Vruggroote by al 3 kombinasies het goed vergelyk en selfs toegeneem, met pieke by telling 105/125, gevolg deur telling 72 (vruggroote van telling 88 na 72 opgeskuif).

Summary

The internal quality compared well with the previous season, although acid levels were slightly higher, but still complying with the minimum export standards. Unfortunately there was a decrease in yield production of up to 50% on some combinations in comparison to the previous season. Midnight on Sunki 812 produced the most promising fruit size peaking between count 72 and 56. The best Delta combination for internal quality was Sunki 802, with a Brix:acid ratio of 10.4, as well as the highest yield production of 102.3 kg/tree. Fruit size on all three combinations was promising and increased with peaks at count 105/125, followed by count 72; fruit size increased from count 88 to 72.

Objective

The performance of Midnight and Delta Valencias on new, imported rootstocks on replant soils must be investigated. The production, internal quality and rind colour must be improved and at the same time fruit size must be increased.

Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 were imported and propagated in 1996 by Esselen Nursery, a CIS accredited nursery in Malelane.

Delta Valencia was budded onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: Sunki x Beneke HRS 802, Sunki x Beneke HRS 812 and Sunki x MTO trifoliolate orange (FF6). Midnight Valencia was budded onto Sunki x Beneke HRS 812. The trees were planted at Esselen Nursery in March 1999.

Table 6.3.2.1. Number of trees per rootstock in the Delta and Midnight Valencia trial at Malelane.

Selection	Rootstock	No.of trees
Midnight	Sunki 812	4
Delta	Sunki 812	4
Delta	Sunki 802	4
Delta	FF-6	4

Results and discussion

Midnight Valencia

Internally (Table 6.3.2.2) the fruit complied with the minimum export standards and the Brix (12.3) content decreased slightly in comparison with the previous season. The acid content of 1.42% remained high with the maximum for export being 1.50% on Midnights, but was still acceptable and increased the late hanging ability. The fruit size peaked at count 72, followed by count 56 and another peak at 105/125, producing the optimum fruit size for Midnight (Table 6.3.2.3). Midnight on Sunki 812 produced a 50% lighter yield in comparison with the previous season, from 98.7 kg/tree to 50.4 kg/tree (Table 6.3.2.4).

Delta Valencia

Delta on all three rootstocks produced fruit with good internal quality and complied with the export standards. Sunki 812 tested 13.0 Brix and 1.49 acid by the time of harvest, the highest internal quality in this trial for this season. The acid level on all three combinations increased from the previous season, although still below the maximum export standard (Table 6.3.2.2). All three rootstocks peaked at count 105/125, followed by count 72, indicating a bigger fruit size on average for this season (Table 6.3.2.3). Delta on Sunki 802 set the best crop on the trees with 102.3 kg/tree, followed by FF-6 with 97.2 kg/tree and Sunki 812 with 50.4 kg/tree. Production on all three selections decreased this season with Sunki 812 approximately 50% lower in comparison with the previous season (Table 6.3.2.4).

Conclusions and recommendations

Midnight and Delta on the different rootstock selections produced very good to excellent quality fruit, complying with the minimum export standards for Valencias. The fruit set on all the combinations decreased by up to 50%. The fruit size on average increased because of the lighter crop on the trees, although the loss in production probably had a substantial impact on the potential income for this season.

Table 6.3.2.2. Internal fruit quality of Midnight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 29 July 2008.

Selection	Root-stock	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Midnight	Sunki 812	56.0	12.30	1.42	8.66	0.5	T1
Delta	Sunki 812	55.1	13.00	1.49	8.72	0.0	T1-2
Delta	Sunki 802	59.2	11.70	1.10	10.64	0.0	T1
Delta	FF-6	58.1	12.40	1.41	8.79	0.0	T1-3

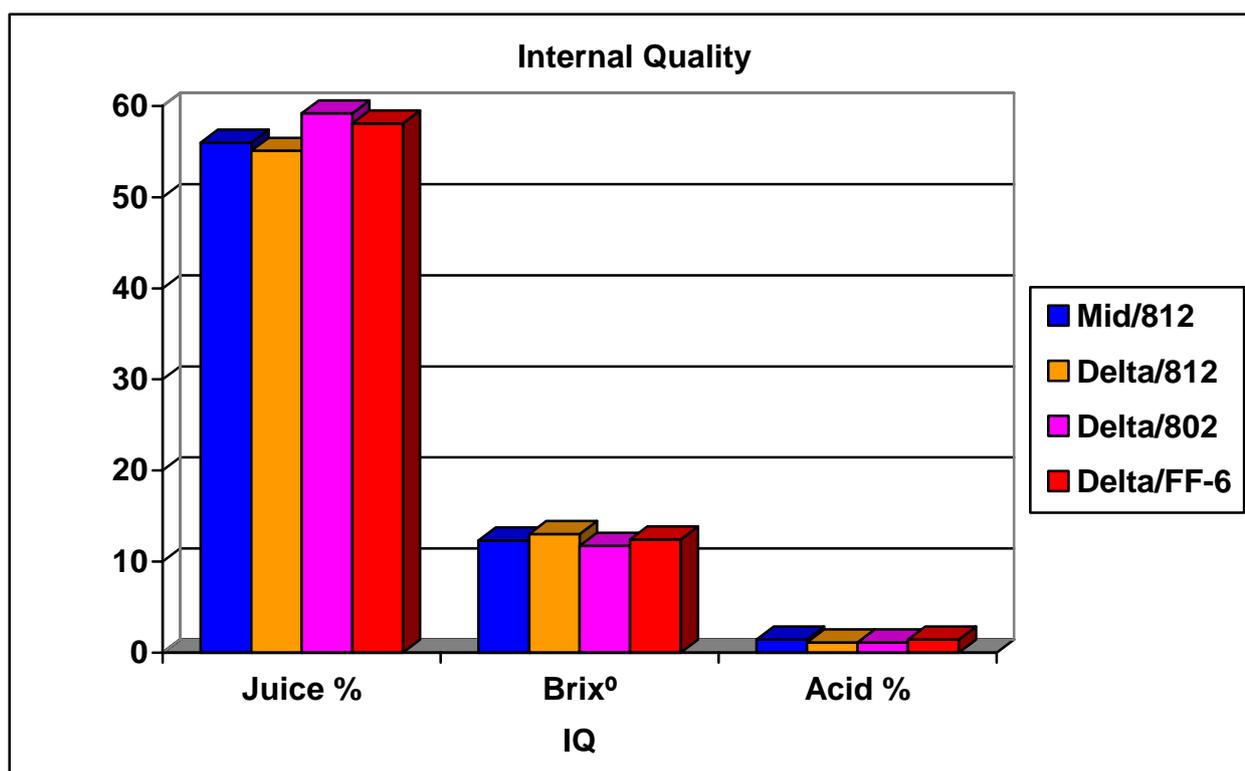
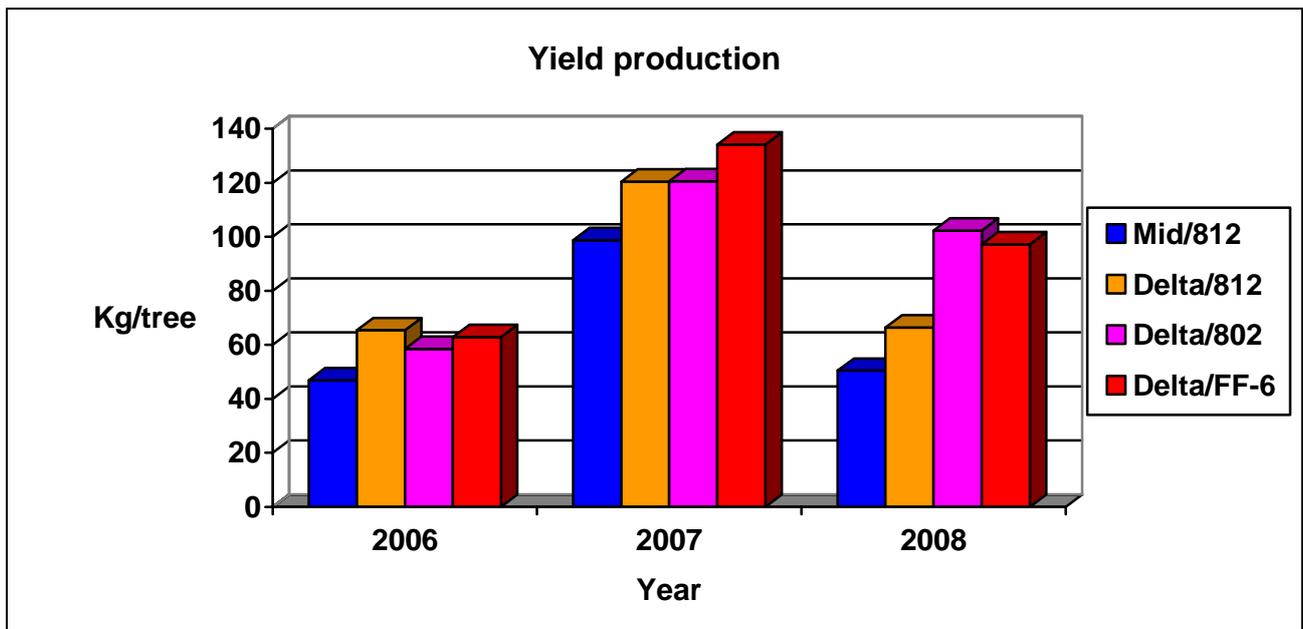


Table 6.3.2.3. Fruit size distribution at Esselen nursery during the 2008 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	Sunki 812	48	8.57	Delta	Sunki 802	48	0.44
Midnight	Sunki 812	56	23.23	Delta	Sunki 802	56	9.87
Midnight	Sunki 812	72	29.76	Delta	Sunki 802	72	25.81
Midnight	Sunki 812	88	12.63	Delta	Sunki 802	88	14.70
Midnight	Sunki 812	105/125	21.63	Delta	Sunki 802	105/125	38.82
Midnight	Sunki 812	144	4.18	Delta	Sunki 802	144	10.36
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 812	48	4.23	Delta	FF-6	48	0.45
Delta	Sunki 812	56	1.82	Delta	FF-6	56	6.01
Delta	Sunki 812	72	21.04	Delta	FF-6	72	17.67
Delta	Sunki 812	88	14.01	Delta	FF-6	88	14.44
Delta	Sunki 812	105/125	47.30	Delta	FF-6	105/125	47.81
Delta	Sunki 812	144	11.60	Delta	FF-6	144	13.61

Table 6.3.2.4. Production per tree of Midnight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2008 season.

Cultivar	Rootstock	Kg/tree (2006)	Kg/tree (2007)	Kg/tree (2008)
Midnight	Sunki 812	46.8	98.7	50.4
Delta	Sunki 812	65.4	120.5	66.4
Delta	Sunki 802	58.5	120.6	102.3
Delta	FF-6	62.9	134.1	97.2



6.3.3 Evaluation of Grapefruit varieties on new imported rootstocks in the Swaziland area Experiment 416 B by J.Joubert (CRI)

Opsomming

Die nuwe aanplantings is nou vir die derde seisoen ge-evalueer. Marsh op SC, Star Ruby op MxT, SC en X639 het nie aan die minimum Brix:suur verhouding van 7:1 vir uitvoere voldoen nie a.g.v. te hoë suur vlakke. Die oes produksie het goed toegeneem (tot en met 50% groei) en veral C35 toon baie potensiaal en het intern baie goed gevaar.

Summary

The new plantings were evaluated for the third time this season. Marsh on SC, Star Ruby on MxT, SC and X639 did not comply with the minimum Brix:acid ratio of 7:1 for export standards due to high acid levels. Yield production increased in some cases with up to 50% and C35 performed very well producing fruit with excellent internal quality.

Objective

The performance of grapefruit cultivars on new rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour need to be improved.

Materials and methods

Trees were planted in 2003, 10 trees Marsh, NelRuby and Star Ruby all on C35, MxT, SC, and X639.

Table 6.3.3.1. Number of trees per rootstock in the grapefruit trial at Tambuti, Swaziland.

Planted 2003		
Selection	Rootstock	No.of trees
Marsh	C35	10
Marsh	MxT	10
Marsh	SC	10
Marsh	X639	10
NelRuby	C35	10
NelRuby	MxT	10
NelRuby	SC	10
NelRuby	X639	10
Star Ruby	C35	10
Star Ruby	MxT	10
Star Ruby	SC	10

Star Ruby	X639	10
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Results and discussion

Marsh

All the combinations produced a Brix° above 9.0, complying with the export standards. SC outperformed the rest of the rootstocks with 11.4 Brix°, although the Brix:acid ratio of 6.55 on SC was below the 7:1 mark for export. C35 produced the best juice content (54.0%) followed by X639 (52.1%) and MxT (50.6%) (Table 6.3.3.5). Marsh peaked at count 48, followed by count 64 and count 40 (Table 6.3.3.6). Marsh on SC outperformed the rest of the combinations and produced 79.3 kg/tree in comparison to 2007 with 67.9 kg/tree. The other rootstocks varied between 57.4 and 66.7 kg/tree (Table 6.3.3.7). C35, MxT and X639 increased fruit production by at least 50% in comparison to 2007.

NelRuby

Internally NelRuby was very promising this season and complied with all the minimum export standards. The highest juice content was produced on C35 with 56.1% and highest Brix° also on C35 at 10.8° (Table 6.3.3.5). Fruit size peaked at count 48 followed by count 64 and count 40 (Table 6.3.3.6). NelRuby on SC produced 95.2 kg/tree (2006-37.5 kg/tree) at time of harvest, followed by X639 (71.4 kg/tree) and MxT (69 kg/tree) (Table 6.3.3.7).

Star Ruby

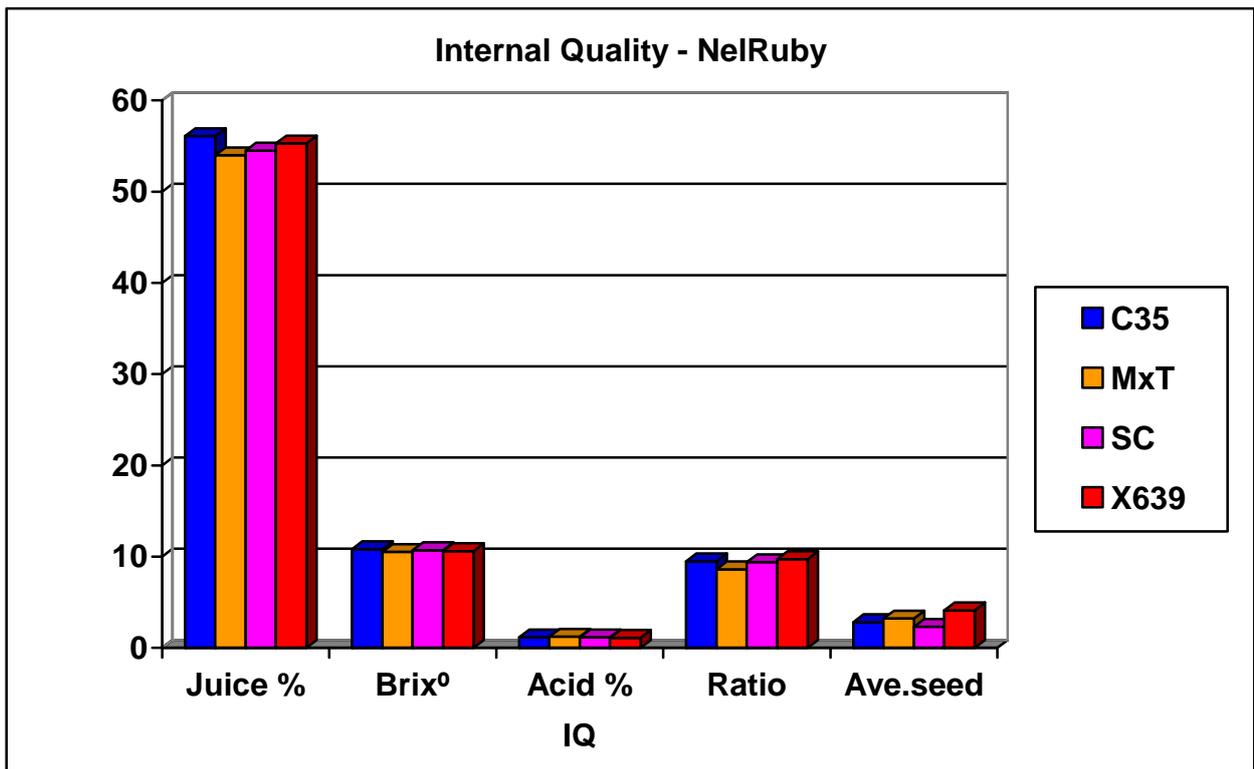
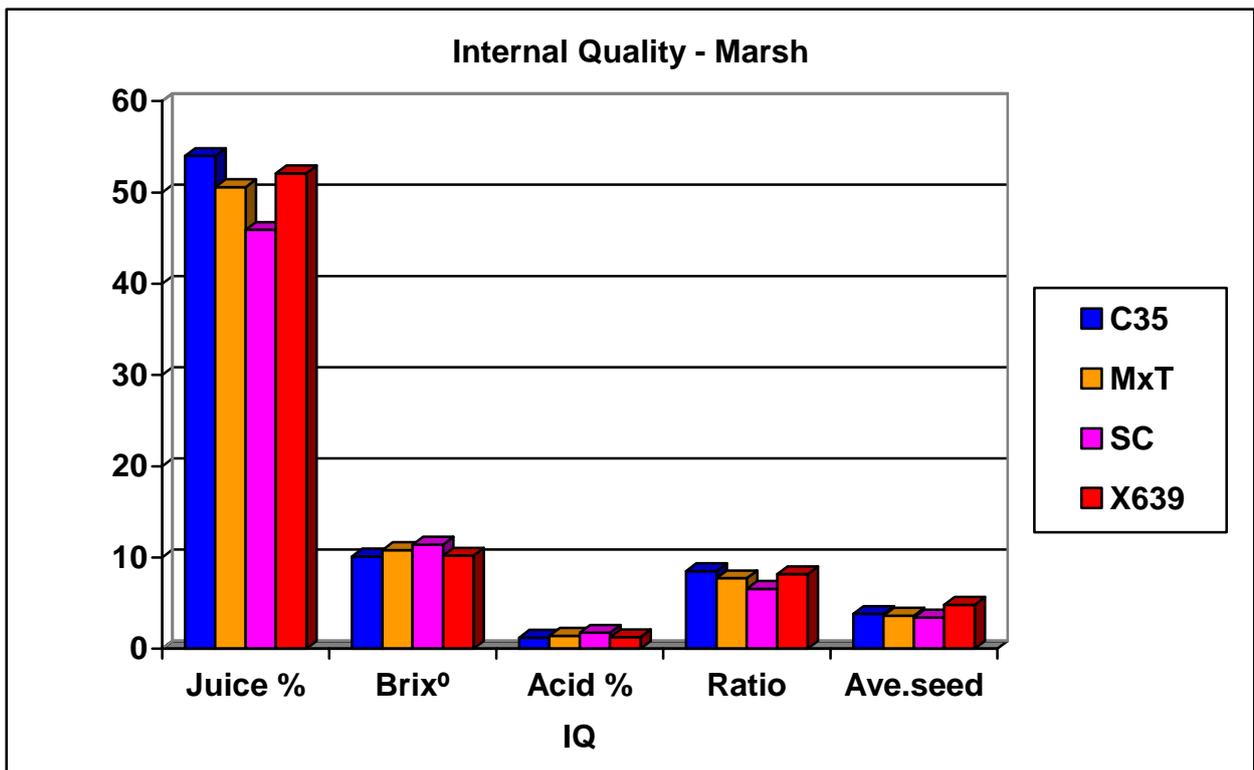
Star Ruby on all the rootstocks complied with the export standards in regards to juice and Brix levels, but MxT, SC and X639 were below the 7:1 ratio for Brix:Acid requirements. The fruit produced on C35 resulted in the best juice content (57.5%) and SC the highest Brix levels (11°) for this trial (Table 6.3.3.5). All the fruit size counts peaked at count 48 except for MxT peaking at count 64 (Table 6.3.3.6). Production peaked at 74.2 kg/tree on MxT (49.2 kg/tree for 2007), followed by C35 (63.7 kg/tree) and SC (60.2 kg/tree) (Table 6.3.3.7).

Conclusion and recommendation

These combinations were evaluated for the third time this season, and the production increased substantially in comparison with the 2007 season. The internal quality on all three selections was very promising and NelRuby complied with all the export requirements. Marsh and Star Ruby were below the minimum Brix:acid ratio of 7:1 in some instances, but the juice and Brix levels were adequate for export. Later harvesting may be the answer to this problem. The average fruit size for this season peaked at count 48, followed by count 64 and 40. All the rootstock combinations increased in yield production with some up by 50%.

Table 6.3.3.5. Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 20 May 2008.

Selection	Root-stock	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Marsh	C35	54.0	10.1	1.19	8.49	3.8	T3-4
Marsh	MxT	50.6	10.8	1.40	7.71	3.6	T3-4
Marsh	SC	45.9	11.4	1.74	6.55	3.4	T5-6
Marsh	X639	52.1	10.2	1.25	8.16	4.8	T3-4
NelRuby	C35	56.1	10.8	1.14	9.47	2.8	T2
NelRuby	MxT	54.0	10.5	1.22	8.61	3.2	T2
NelRuby	SC	54.5	10.7	1.14	9.39	2.3	T2
NelRuby	X639	55.3	10.6	1.09	9.72	4.1	T1-2
TSR	C35	57.5	10.3	1.31	7.86	0.3	T1-2
TSR	MxT	52.4	10.7	1.81	5.91	0.9	T1-3
TSR	SC	53.2	11.0	1.60	6.88	0.2	T1-2
TSR	X639	53.6	10.8	1.67	6.47	0.9	T1-2



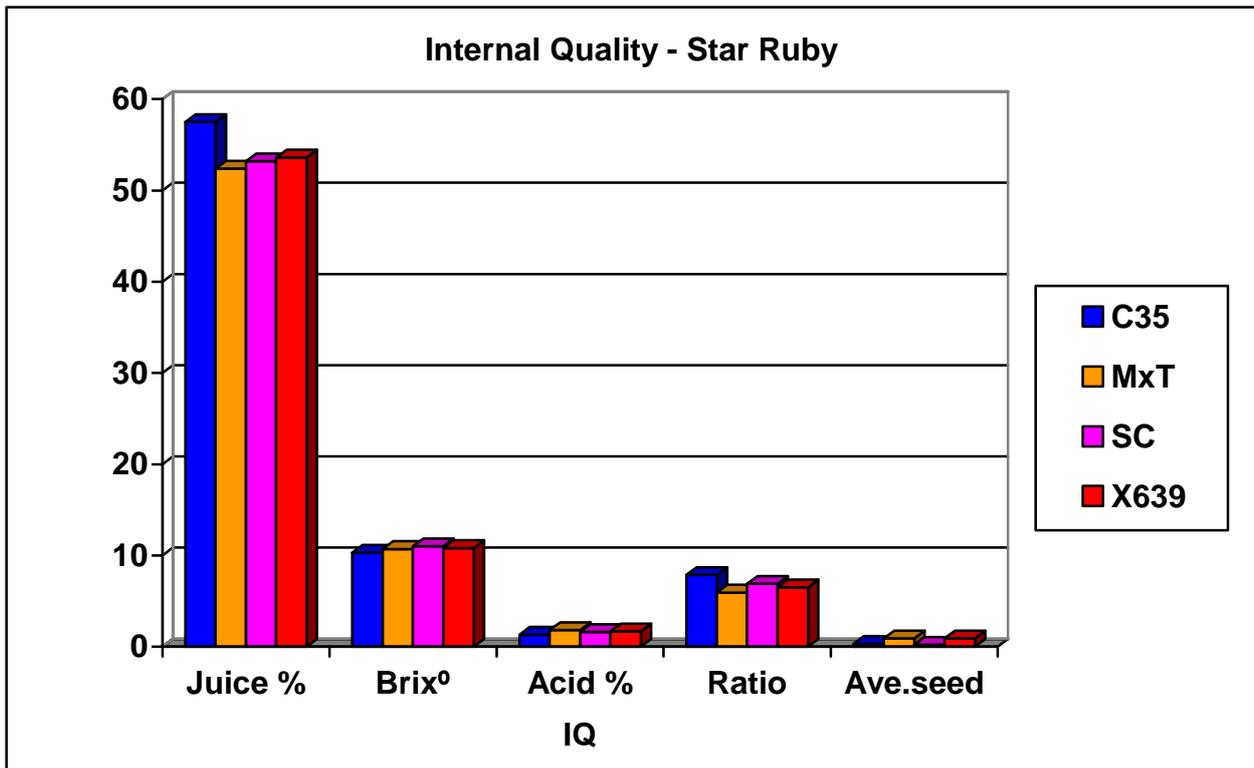
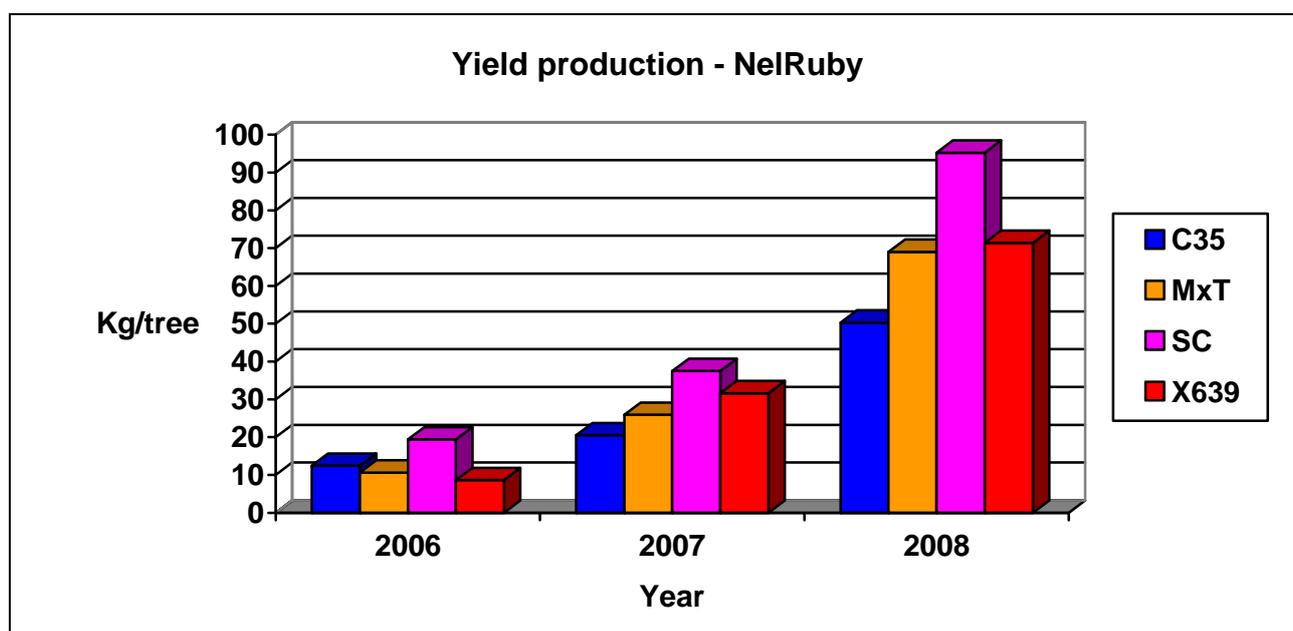
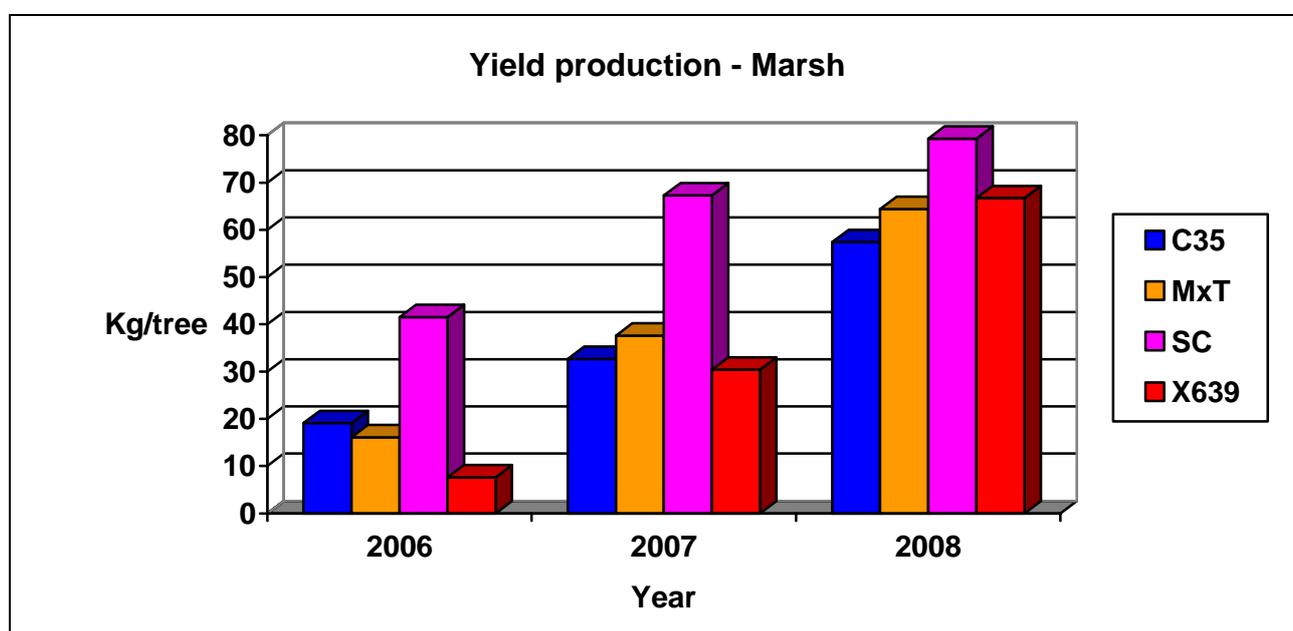


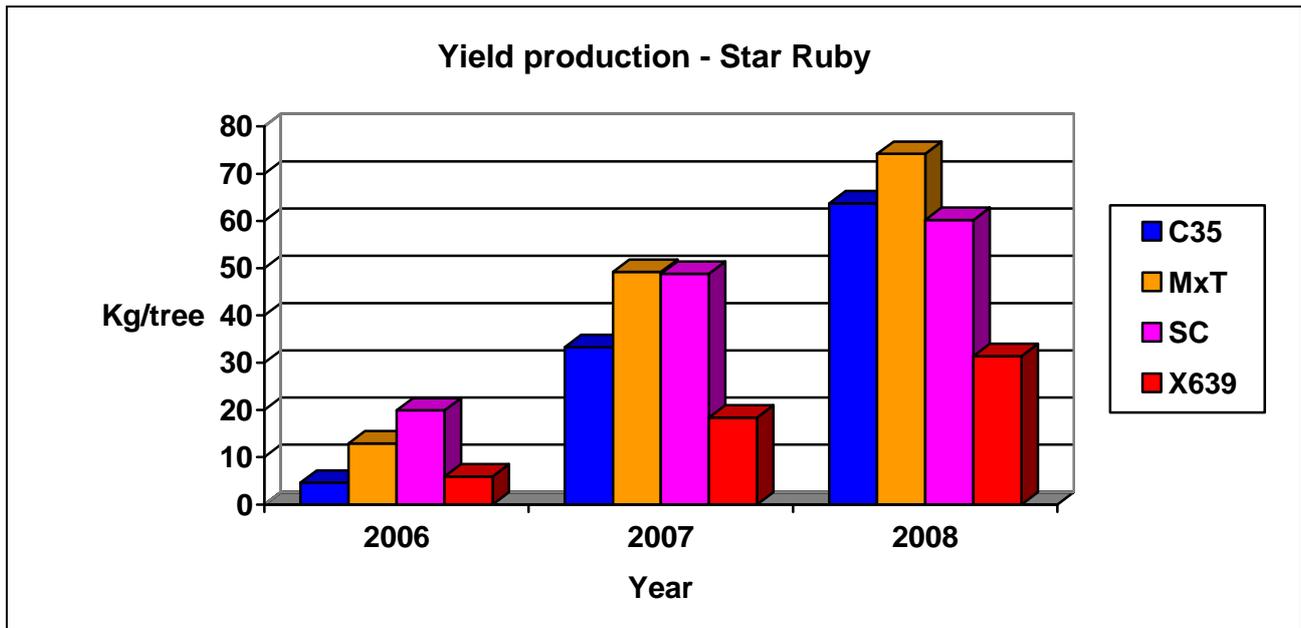
Table 6.3.3.6. Fruit size distribution per rootstock at Tambuti Estate during the 2008 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	C 35	27	2.23	Nelruby	SC	27	0.19
Marsh	C 35	32	1.69	Nelruby	SC	32	0.63
Marsh	C 35	36	6.92	Nelruby	SC	36	4.37
Marsh	C 35	40	18.19	Nelruby	SC	40	16.40
Marsh	C 35	48	48.37	Nelruby	SC	48	49.67
Marsh	C 35	64	22.60	Nelruby	SC	64	28.75
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	MxT	27	4.08	Nelruby	X639	27	0.12
Marsh	MxT	32	2.96	Nelruby	X639	32	0.20
Marsh	MxT	36	12.34	Nelruby	X639	36	1.63
Marsh	MxT	40	19.37	Nelruby	X639	40	11.08
Marsh	MxT	48	39.53	Nelruby	X639	48	54.05
Marsh	MxT	64	21.72	Nelruby	X639	64	32.91
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	SC	27	1.75	Star Ruby	C 35	27	2.49
Marsh	SC	32	1.48	Star Ruby	C 35	32	3.92
Marsh	SC	36	6.61	Star Ruby	C 35	36	14.00
Marsh	SC	40	14.09	Star Ruby	C 35	40	21.89
Marsh	SC	48	42.76	Star Ruby	C 35	48	36.15
Marsh	SC	64	33.31	Star Ruby	C 35	64	21.54
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	X639	27	0.76	Star Ruby	MxT	27	5.51
Marsh	X639	32	1.43	Star Ruby	MxT	32	3.30
Marsh	X639	36	8.74	Star Ruby	MxT	36	8.09
Marsh	X639	40	22.46	Star Ruby	MxT	40	12.26
Marsh	X639	48	52.46	Star Ruby	MxT	48	29.31
Marsh	X639	64	14.14	Star Ruby	MxT	64	41.52
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Nelruby	C 35	27	0.00	Star Ruby	SC	27	12.97
Nelruby	C 35	32	0.30	Star Ruby	SC	32	7.28
Nelruby	C 35	36	2.48	Star Ruby	SC	36	15.57
Nelruby	C 35	40	13.18	Star Ruby	SC	40	19.89
Nelruby	C 35	48	57.68	Star Ruby	SC	48	29.31
Nelruby	C 35	64	26.36	Star Ruby	SC	64	14.98
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Nelruby	MxT	27	0.44	Star Ruby	X639	27	3.24
Nelruby	MxT	32	0.75	Star Ruby	X639	32	2.94
Nelruby	MxT	36	6.53	Star Ruby	X639	36	9.11
Nelruby	MxT	40	19.01	Star Ruby	X639	40	17.81
Nelruby	MxT	48	49.16	Star Ruby	X639	48	44.84
Nelruby	MxT	64	24.11	Star Ruby	X639	64	22.06

Table 6.3.3.7. Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2008.

Cultivar	Rootstock	Kg/tree (2006)	Kg/tree (2007)	Kg/tree (2008)
Marsh	C35	19.1	32.7	57.4
Marsh	MxT	16.1	37.6	64.4
Marsh	SC	41.5	67.3	79.3
Marsh	X639	7.6	30.4	66.7
Nelruby	C35	12.4	20.5	50.3
Nelruby	MxT	10.6	25.9	69
Nelruby	SC	19.4	37.5	95.2
Nelruby	X639	8.6	31.6	71.4
Star Ruby	C35	4.6	33.3	63.7
Star Ruby	MxT	12.9	49.2	74.2
Star Ruby	SC	19.9	48.8	60.2
Star Ruby	X639	5.9	18.4	31.4





6.3.4 Evaluation of various Valencia selections on different rootstocks in the Komatipoort area Experiment 590 B by J. Joubert (CRI)

Opsomming

Evalueer en bepaal die tuinboukundige potensiaal en vermoë van verskillende Valencia variëteite op verskillende onderstamme. Bepaal die beste onderstam kombinasie vir hierdie nuwe variëteite. Maak betekenisvolle kommersieel aanbevelings vir die produsente. Hierdie onderstam proef is vir die derde keer ge-oes en die bome is nog jonk. Die verskille in oes produksie het nou grootter geword en waardevolle inligting word beskikbaar. Die kapitaal wat uitgelê word vir vestiging kan gouer in winste omgesit word met vroeër produksie op die bome.

Delta valencia in kombinasie met Carrizzo citrange het die hoogste sapinhoud geproduseer (52%), met X639 die hoogste vastestof inhoud (11.9). Terrabella, MxT, Swingle en X639 se vrugsgrootte het by telling 72 gepiek, baie gunstig vir uitvoer spesifikasies. Delta op Swingle het die grootste oes van 73.9 kg per boom vir hierdie proef geproduseer.

McClean saadloos in kombinasie met MxT het die hoogste sap persentasie (59.7%) vir hierdie proef geproduseer, gevolg deur C35 en Terrabella met die beste vastestofinhoud (11.9). C35 het ook die beste oes van 81 kg per boom geproduseer, met vrugsgrootte wat varieer tussen telling 56 en 72.

Midnight op Koethen citrange het 59.6% sap intern geproduseer, met Swingle wat 11.6 Brix vir hierdie proef opgelewer het. Carrizzo citrange, MxT en Terrabella het die grootste vrugsgrootte geproduseer met 'n piek by telling 56. C35 het weereens die beste oes per boom geproduseer.

Portsgate in kombinasie met Swingle het die beste sap persentasie 60.2 geproduseer, met Koethen citrange die hoogste vastestof inhoud van 11.5. Swingle het die beste opbrengs per boom gelever met 55.2 kg.

Summary

This rootstock trial was harvested for the third time this season. The trial was planted in 2002. The difference in yield production escalated and valuable information is available. The capital investment to establish the orchard will bring in returns via early production.

Delta Valencia in combination with Carrizzo citrange produced the highest juice content (52%), with X639 producing the highest solids (11.9). Terrabella, MxT Swingle and X639 peaked at count 72 with their fruit size, a very good size for exporting Valentias. Delta on Swingle produced a very good crop for this season with 73.9 kg per tree.

McClelland seedless in combination with MxT produced the best juice content (59.7%) with C35 and Terrabella outperforming the rest with the highest solids (11.9). C35 also produced the highest yield with 81 kg per tree for this season. Fruit size varied from count 56 to 72 on all rootstocks.

Midnight on Koethen citrange produced a juice content of 59.6% internally, and Swingle produced the best solids with 11.6 Brix. Carrizo citrange, MxT and Terrabella peaked at count 56, producing rather large fruit for Valencias. C35 once again produced the best yield per tree.

Portsgate in combination with Swingle produced the best juice percentage of 60.2, with Koethen citrange producing the best Brix for this trial (11.5). Swingle set the best crop by producing 55.2 kg per tree.

Objective

Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks. Determine the superior rootstock combinations for these new selections. Be able to make credible commercial recommendations.

Materials and methods

Five trees of each cultivar x rootstock combination were planted in 2002.

They were evaluated visually to determine production per tree, trueness to type and compatibility with scion and each tree was harvested with the sizer to determine production per tree as well as fruit size distribution per tree. Samples were taken and internal quality tested and analysed. Fruit colour was evaluated and analysed.

Table 6.3.4.1. List of cultivar and rootstock combinations in the Valencia trial at Golden Frontier Citrus Hectorspruit in the Komatipoort area.

Selection	Rootstock
Delta (Control)	C35
Delta (Control)	CC
Delta (Control)	KC
Delta (Control)	MxT
Delta (Control)	SC
Delta (Control)	Terrabella
Delta (Control)	X639
McClelland SL	C35
McClelland SL	CC
McClelland SL	KC
McClelland SL	MxT
McClelland SL	SC
McClelland SL	Terrabella
McClelland SL	X639
Midnight	C35
Midnight	CC
Midnight	KC
Midnight	MxT
Midnight	SC
Midnight	Terrabella
Midnight	X639
Portsgate	C35
Portsgate	CC
Portsgate	KC
Portsgate	MxT
Portsgate	SC
Portsgate	Terrabella
Portsgate	X639

Results and discussion

Delta Valencia

Internal fruit quality analysis (Table 6.3.4.2)

- Juice %: All the selections complied with the export standards above 52% juice content. CC produced the highest level (61%) followed by SC (59.9%) and C35 (59.6%). The lowest juice content was produced on TB with 56.4%.
- Brix^o: X639 produced the highest Brix^o (11.9) followed by TB and KC (11.5) and CC (11.1). This season all the other combinations were above the minimum levels for packing, except for SC with 10.4°, producing the lowest sugar content.
- Acid: All the rootstock combinations produced low acid level below 0.85 and did not comply with export standards, except MxT with 0.87%.

Fruit size distribution (Table 6.3.4.3)

- The fruit size evaluation shows the largest peak at counts 105/125 for C35, CC, KC and TB. MxT, SC and X639 peaked at count 72, the optimum size for Valencia production.

Production per tree (Table 6.3.4.4)

- SC produced the highest yield per tree (73.9 kg), followed by C35 with 60.1 kg/tree and MxT with 39.4 kg/tree.

McClellan SL

Internal fruit quality analysis (Table 6.3.4.2)

- Juice %: MxT produced the highest juice content (59.7%) followed by KC (58.4%) and C35 (57.4%). All the selections comply with the export standards above 48% juice content.
- Brix^o: C35 and TB produced the highest Brix content (11.9), followed by CC with 11.4 and KC with 11.2 Brix. The lowest Brix content was produced on SC with 10.4°.
- Acid: All the selections comply with the minimum standards of above 0.66%. X639 produced the lowest Brix:acid ratio for this trial of 10.5:1, but still acceptable.

Fruit size distribution (Table 6.3.4.3)

- The fruit size evaluation shows the largest peak at count 56 and then 72 on all the rootstock combinations, except for TB peaking at count 72 and then count 105. The average fruit size for this selection increased to large in comparison with the previous season.

Production per tree (Table 6.3.4.4)

C35 produced the highest yield per tree (81 kg), followed by TB with 46.9 kg/tree and SC with 35.7 kg/tree.

Midnight

Internal fruit quality analysis (Table 6.3.4.2)

- Juice %: All the rootstock selections comply with the export standards above 52% juice content. KC produced the highest level (59.6%) followed by TB (58.7%) and C35 (57.7%). The lowest juice content was produced on SC with 55.8%.
- Brix^o: SC produced the highest Brix^o (11.6) followed by C35, CC, KC (11.3) and TB (11.1). This season all the other combinations were above the minimum levels for packing, except for X639 with 10.3°, producing the lowest sugar content. This is the third season for X639 not complying with the minimum Brix requirements for export.

- Acid: All the rootstock combinations produced an acid content above 0.85 and comply with the export standards, except for CC and TB.

Fruit size distribution (Table 6.3.4.3)

- The fruit size evaluation shows the largest peak at counts 56 by CC, MxT and TB. MxT, C35 and SC peaked at count 48 and KC at count 105/125.

Production per tree (Table 6.3.4.4)

- C35 produced the highest yield per tree (33.9 kg), followed by CC with 20.9 kg/tree and TB with 19.6 kg/tree. The production on Midnight decreased because of over maturity caused by the picking schedule of the producer. Future evaluation timing will be crucial for harvesting at the optimal fruit maturity.

Portsgate

Internal fruit quality analysis (Table 6.3.4.2)

- Juice %: SC produced the highest juice content (60.2%) followed by KC (59.9%) and TB (59%). All the selections complied with the export standards above 48% juice content. CC produced the lowest juice content of 54.7%.
- Brix°: KC produced the highest Brix content (11.5), followed by X639 with 11.2 and C35 with 11.1 Brix. The lowest Brix content was produced on CC with 10.4°.
- Acid: All the selections comply with the minimum standards above 0.66%, except for CC, KC and SC. Harvesting time is crucial for optimal internal quality.

Fruit size distribution (Table 6.3.4.3)

- The fruit size evaluation shows the largest peak at count 56 (C35, CC, SC) and count 72 (KC, MxT, X639) except for TB peaking at count 105/125.

Production per tree (Table 6.3.4.4)

SC produced the highest yield per tree (55.2 kg), followed by X639 with 35.6 kg/tree and C35 with 33.6 kg/tree.

Conclusions and recommendations

C35 in combination with all the selections performed well and future plantings on this rootstock should be considered. C35 is a semi dwarfing rootstock and after 5 to 6 years is smaller than trees on SC, CC, MxT and X639. TB also appears to have a semi dwarfing effect, but not quite as much as C35 which can be 25 to 30% smaller than CC after 10 to 12 years. KC also has a dwarfing effect, but to what extent is not known at present.

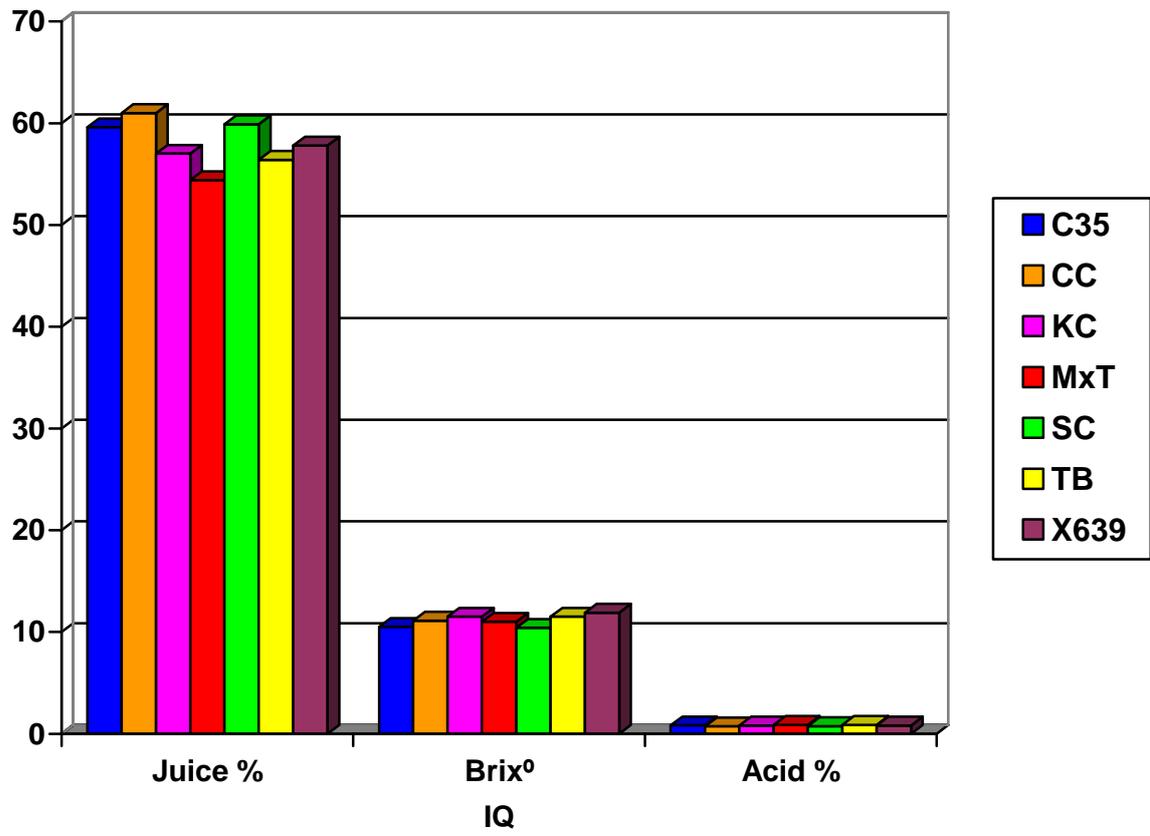
The production on all four selections decreased this season due to fruit drop as a result of late harvesting. Next season special arrangements will ensure optimal harvest time to evaluate the best fruit quality and yield production.

The trial looks promising at this stage. It will be very valuable to evaluate the production increase on the young trees.

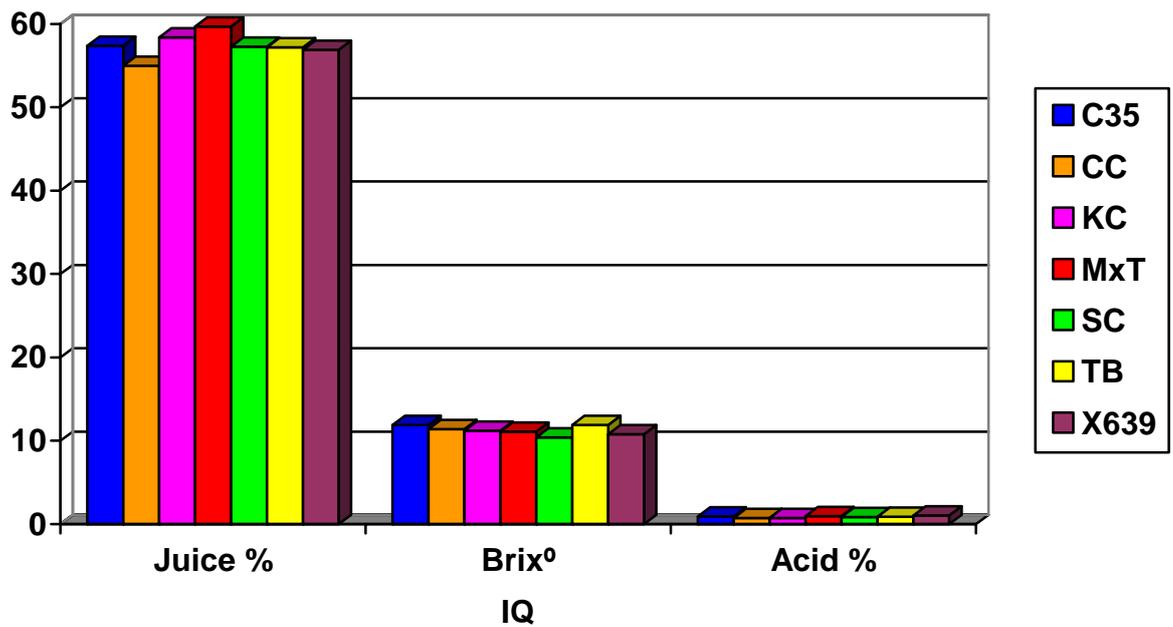
Table 6.3.4.2. Internal fruit quality data for Valencias on different rootstocks at Golden Frontier Citrus Hectorspruit on 18 August 2008.

Selection	Root-stock	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Delta	C35	59.6	10.50	0.82	12.80	0.0	T1
Delta	CC	61.0	11.10	0.72	15.42	0.0	T1
Delta	KC	57.0	11.50	0.80	14.38	0.0	T1
Delta	MxT	54.4	11.00	0.87	12.64	0.0	T1
Delta	SC	59.9	10.40	0.75	13.87	0.0	T1
Delta	TB	56.4	11.50	0.86	13.37	0.0	T1
Delta	X639	57.8	11.90	0.80	14.88	0.0	T1
McCleane SL	C35	57.4	11.90	0.92	12.93	0.0	T1
McCleane SL	CC	55.0	11.40	0.73	15.62	0.0	T1
McCleane SL	KC	58.4	11.20	0.72	15.56	0.0	T1
McCleane SL	MxT	59.7	11.10	0.97	11.44	0.0	T1
McCleane SL	SC	57.3	10.40	0.83	12.53	0.0	T1
McCleane SL	TB	57.2	11.90	0.89	13.37	0.3	T1
McCleane SL	X639	56.9	10.80	1.03	10.49	0.0	T1
Midnight	C35	57.7	11.30	0.85	13.29	0	T1
Midnight	CC	57.0	11.30	0.83	13.61	0.3	T1
Midnight	KC	59.6	11.30	0.93	12.15	0	T1
Midnight	MxT	57.1	11.00	1.05	10.48	0	T1
Midnight	SC	55.8	11.60	0.85	13.65	0	T1
Midnight	TB	58.7	11.10	0.84	13.21	0	T1
Midnight	X639	56.9	10.30	0.91	11.32	0.4	T1
Portsgate	C35	56.2	11.10	0.77	14.42	0	T1
Portsgate	CC	54.7	10.40	0.56	18.57	0	T1
Portsgate	KC	59.9	11.50	0.65	17.69	0	T1
Portsgate	MxT	58.9	10.50	0.77	13.64	0	T1
Portsgate	SC	60.2	10.50	0.65	16.15	0	T1
Portsgate	TB	59.0	10.90	0.75	14.53	0	T1
Portsgate	X639	56.9	11.20	0.73	15.34	0	T1

Internal Quality - Delta



Internal Quality - McClean SL



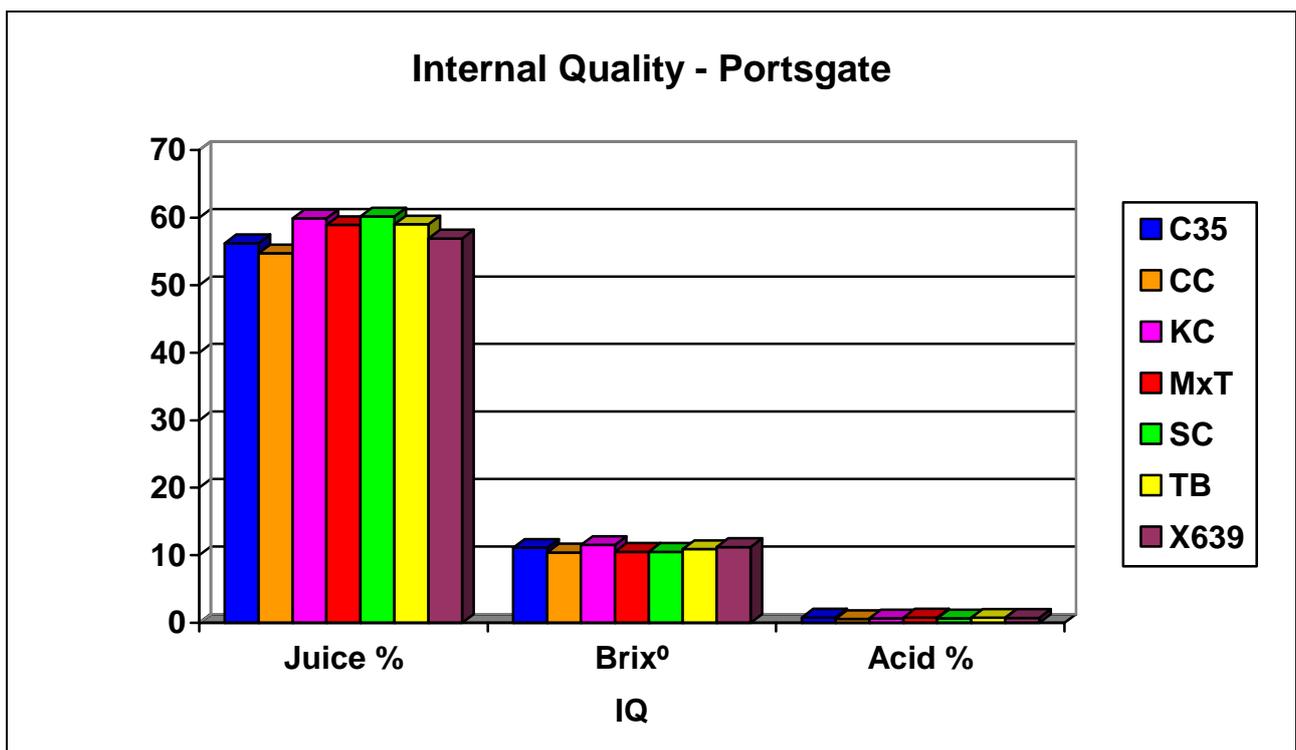
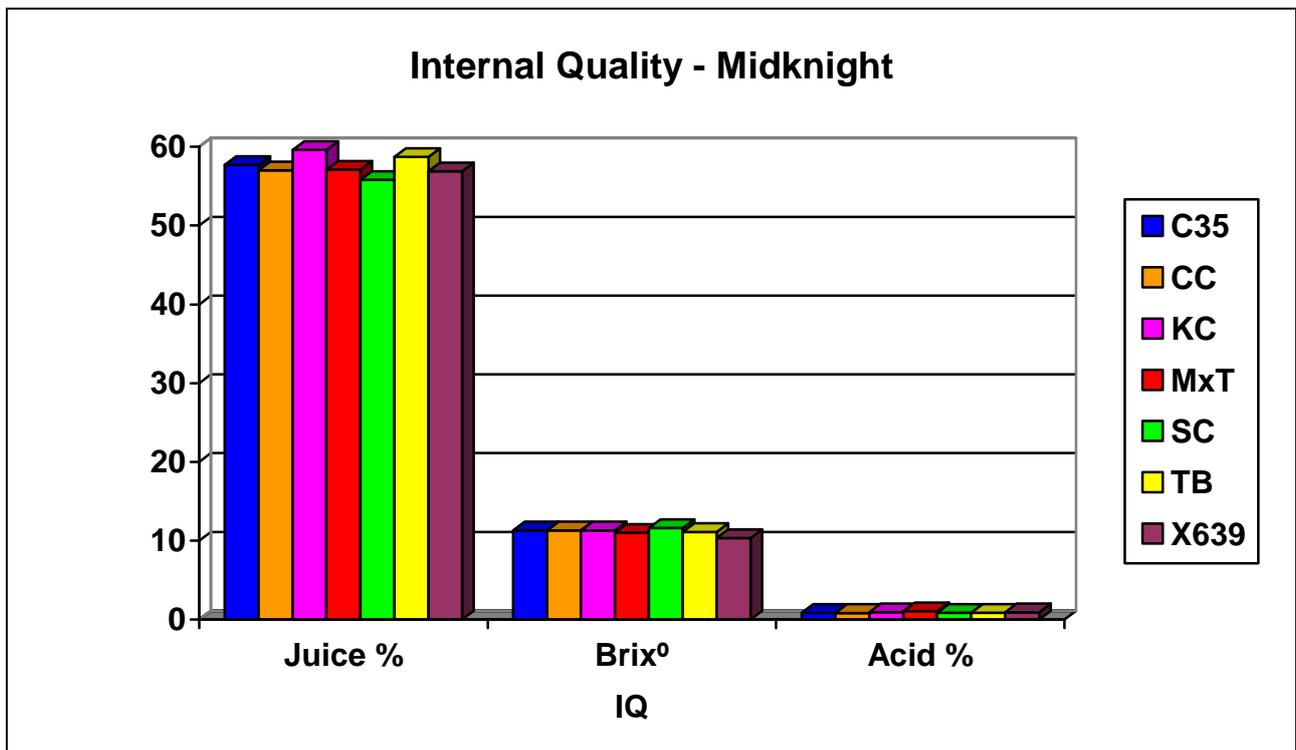


Table 6.3.4.3. Fruit size distribution per rootstock at Golden Frontier Citrus, Hectorspruit during the 2008 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	C35	48	49.00	Delta	C35	48	0.55
Midnight	C35	56	32.70	Delta	C35	56	6.22
Midnight	C35	72	12.46	Delta	C35	72	16.09
Midnight	C35	88	3.49	Delta	C35	88	15.87
Midnight	C35	105/125	1.50	Delta	C35	105/125	41.23
Midnight	C35	144	0.83	Delta	C35	144	20.03

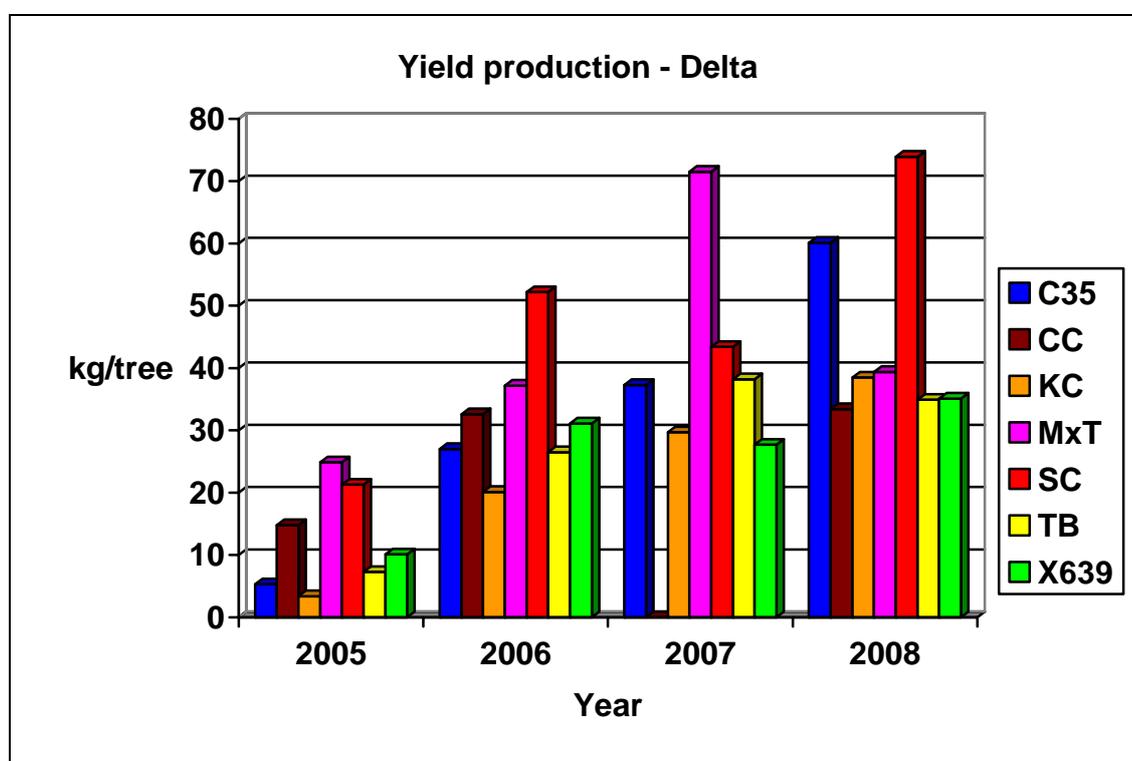
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	CC	48	21.43	Delta	CC	48	0.30
Midknight	CC	56	43.84	Delta	CC	56	6.63
Midknight	CC	72	22.41	Delta	CC	72	23.02
Midknight	CC	88	8.37	Delta	CC	88	2.31
Midknight	CC	105/125	3.45	Delta	CC	105/125	50.75
Midknight	CC	144	0.49	Delta	CC	144	16.98
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	KC	48	6.44	Delta	KC	48	0.37
Midknight	KC	56	19.66	Delta	KC	56	8.42
Midknight	KC	72	11.86	Delta	KC	72	21.61
Midknight	KC	88	11.53	Delta	KC	88	15.84
Midknight	KC	105/125	39.66	Delta	KC	105/125	43.50
Midknight	KC	144	10.85	Delta	KC	144	10.26
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	MxT	48	21.47	Delta	MxT	48	5.06
Midknight	MxT	56	37.42	Delta	MxT	56	24.42
Midknight	MxT	72	24.54	Delta	MxT	72	35.53
Midknight	MxT	88	8.59	Delta	MxT	88	14.52
Midknight	MxT	105/125	5.52	Delta	MxT	105/125	15.07
Midknight	MxT	144	2.45	Delta	MxT	144	5.39
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	SC	48	48.00	Delta	SC	48	1.12
Midknight	SC	56	38.02	Delta	SC	56	13.49
Midknight	SC	72	9.92	Delta	SC	72	36.19
Midknight	SC	88	2.48	Delta	SC	88	15.99
Midknight	SC	105/125	0.83	Delta	SC	105/125	28.25
Midknight	SC	144	0.00	Delta	SC	144	4.96
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	TB	48	42.53	Delta	TB	48	0.71
Midknight	TB	56	43.39	Delta	TB	56	8.79
Midknight	TB	72	10.34	Delta	TB	72	20.51
Midknight	TB	88	1.72	Delta	TB	88	17.88
Midknight	TB	105/125	1.15	Delta	TB	105/125	38.59
Midknight	TB	144	0.86	Delta	TB	144	13.54
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	X639	48	12.73	Delta	X639	48	1.35
Midknight	X639	56	35.45	Delta	X639	56	16.27
Midknight	X639	72	27.27	Delta	X639	72	27.83
Midknight	X639	88	11.82	Delta	X639	88	22.11
Midknight	X639	105/125	12.73	Delta	X639	105/125	29.41
Midknight	X639	144	0.00	Delta	X639	144	3.03
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	C35	48	20.03	McClellan SL	C35	48	1.87
Portsgate	C35	56	46.33	McClellan SL	C35	56	47.37
Portsgate	C35	72	20.80	McClellan SL	C35	72	31.45
Portsgate	C35	88	8.26	McClellan SL	C35	88	10.44
Portsgate	C35	105/125	3.52	McClellan SL	C35	105/125	7.41
Portsgate	C35	144	1.07	McClellan SL	C35	144	1.46
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	CC	48	12.16	McClellan SL	CC	48	14.55
Portsgate	CC	56	38.66	McClellan SL	CC	56	35.87
Portsgate	CC	72	28.31	McClellan SL	CC	72	26.45
Portsgate	CC	88	12.89	McClellan SL	CC	88	13.88

Portsgate	CC	105/125	6.17	McClellan SL	CC	105/125	8.60
Portsgate	CC	144	1.81	McClellan SL	CC	144	0.66
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	KC	48	4.34	McClellan SL	KC	48	15.50
Portsgate	KC	56	33.98	McClellan SL	KC	56	48.97
Portsgate	KC	72	35.66	McClellan SL	KC	72	23.76
Portsgate	KC	88	15.18	McClellan SL	KC	88	8.06
Portsgate	KC	105/125	9.88	McClellan SL	KC	105/125	2.27
Portsgate	KC	144	0.96	McClellan SL	KC	144	1.45
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	MxT	48	3.81	McClellan SL	MxT	48	14.10
Portsgate	MxT	56	27.23	McClellan SL	MxT	56	41.79
Portsgate	MxT	72	39.24	McClellan SL	MxT	72	28.97
Portsgate	MxT	88	16.98	McClellan SL	MxT	88	10.51
Portsgate	MxT	105/125	12.45	McClellan SL	MxT	105/125	4.10
Portsgate	MxT	144	0.29	McClellan SL	MxT	144	0.51
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	SC	48	11.15	McClellan SL	SC	48	15.93
Portsgate	SC	56	37.28	McClellan SL	SC	56	47.37
Portsgate	SC	72	32.84	McClellan SL	SC	72	24.89
Portsgate	SC	88	9.67	McClellan SL	SC	88	6.83
Portsgate	SC	105/125	8.28	McClellan SL	SC	105/125	3.27
Portsgate	SC	144	0.78	McClellan SL	SC	144	1.71
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	TB	48	2.55	McClellan SL	TB	48	3.08
Portsgate	TB	56	16.60	McClellan SL	TB	56	15.81
Portsgate	TB	72	25.64	McClellan SL	TB	72	30.17
Portsgate	TB	88	20.95	McClellan SL	TB	88	19.91
Portsgate	TB	105/125	29.66	McClellan SL	TB	105/125	26.75
Portsgate	TB	144	4.60	McClellan SL	TB	144	4.27
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	X639	48	2.82	McClellan SL	X639	48	10.31
Portsgate	X639	56	28.22	McClellan SL	X639	56	35.84
Portsgate	X639	72	35.09	McClellan SL	X639	72	33.72
Portsgate	X639	88	14.11	McClellan SL	X639	88	13.26
Portsgate	X639	105/125	18.90	McClellan SL	X639	105/125	6.87
Portsgate	X639	144	0.86	McClellan SL	X639	144	0.00

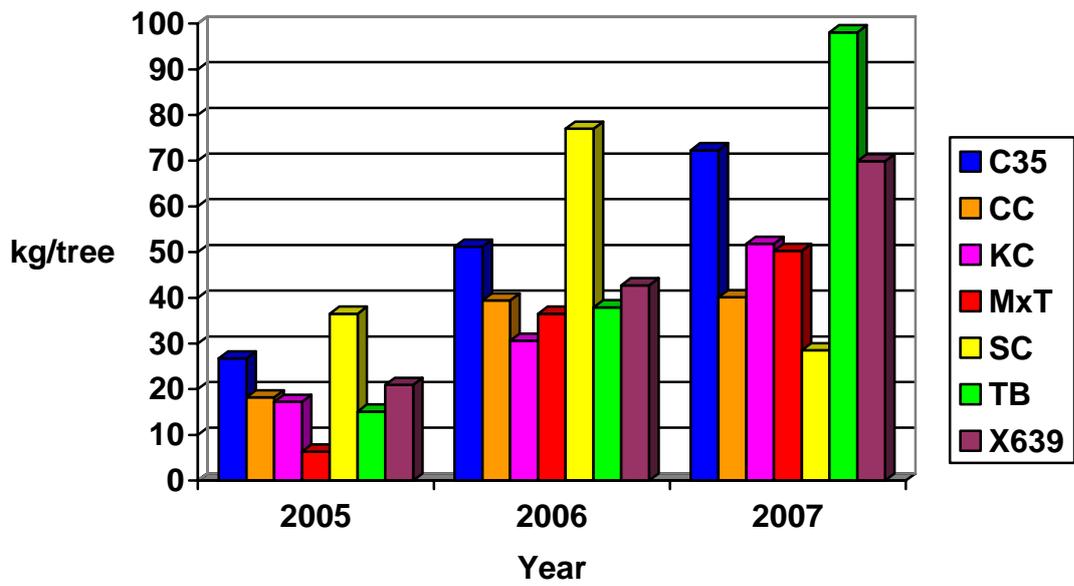
Table 6.3.4.4. Production per tree of Valencia selections on different rootstocks at Golden Frontier Citrus, Hectorspruit during the 2008 season.

Cultivar	Rootstock	Kg/tree(05)	Kg/tree(06)	Kg/tree(07)	Kg/tree(08)
Delta Valencia	C35	5.3	27.0	37.3	60.1
Delta Valencia	CC	14.8	32.6	0	33.4
Delta Valencia	KC	3.4	20.1	29.7	38.5
Delta Valencia	MxT	24.9	37.2	71.5	39.4
Delta Valencia	SC	21.3	52.2	43.4	73.9
Delta Valencia	TB	7.3	26.5	38.2	34.9
Delta Valencia	X639	10.1	31.1	27.7	35.1
McClellan Seedless	C35	26.7	51.2	72.3	81
McClellan Seedless	CC	18.2	39.4	40.1	29.2
McClellan Seedless	KC	17.2	30.6	51.8	24.7
McClellan Seedless	MxT	6.3	36.5	50.3	19.4
McClellan Seedless	SC	36.5	77.0	28.5	35.7
McClellan Seedless	TB	15.1	37.9	98.1	46.9

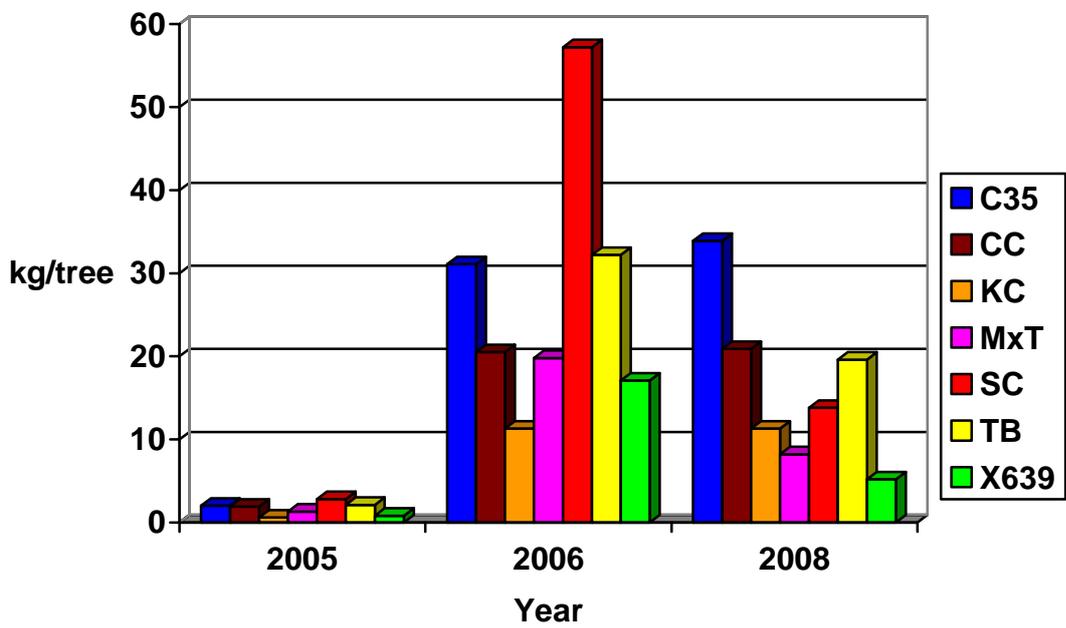
McClellan Seedless	X639	20.9	42.7	69.9	29.3
Midnight Valencia	C35	2.0	31.1	0	33.9
Midnight Valencia	CC	1.9	20.5	0	20.9
Midnight Valencia	KC	0.6	11.3	0	11.3
Midnight Valencia	MxT	1.3	19.8	0	8.2
Midnight Valencia	SC	2.8	57.2	0	13.8
Midnight Valencia	TB	2.1	32.2	0	19.6
Midnight Valencia	X639	0.8	17.1	0	5.2
Portsgate	C35	13.4	43.7	0	33.6
Portsgate	CC	5.4	21.5	0	26.6
Portsgate	KC	14.0	24.5	0	19
Portsgate	MxT	14.2	21.8	0	30.5
Portsgate	SC	32.7	64.4	0	55.2
Portsgate	TB	18.7	44.5	0	48
Portsgate	X639	17.9	22.5	0	35.6

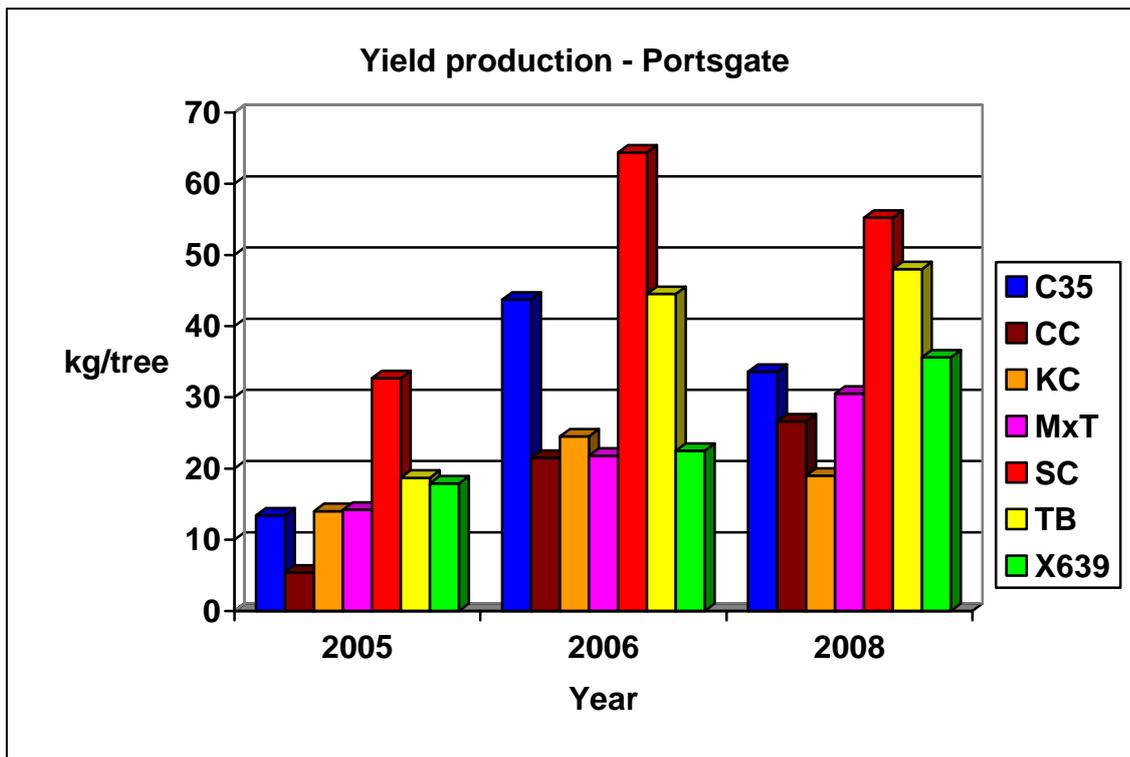


Yield production - McClean SL



Yield production - Midnight





6.3.5 Evaluation of Limpopo Seedless Valencia on four different rootstocks in the Weipe area Experiment 900 by J. Joubert (CRI)

Opsomming

Evalueer en bepaal die tuinboukundige potensiaal en vermoë van Limpopo saadloos op verskillende onderstamme. Bepaal die beste onderstam kombinasie vir hierdie nuwe variëteit. Maak betekenisvolle kommersiële aanbevelings vir die produsente. Hierdie onderstam proef is vir die eerste keer ge-oes; bome ge-topwerk in 2004. Die kapitaal wat uitgelê word vir vestiging kan gouer in winste omgesit word met vroeër produksie op die bome.

Limpopo saadloos het 'n goeie oes op al 4 onderstam kombinasie geproduseer, met die hoogste produksie op Growweskil wat ook die grootste boomvolume beslaan. Die 2de hoogste produksie was op SC gewees, wat ook die 2de grootste boomvolume sal beslaan, gevolg deur CC en X639. Growweskil het die swakste interne kwaliteit kombinasie geproduseer, met die ander drie onderstamme beter Brix:Suur verhoudings vir uitvoere. Die suurvlakke by al vier onderstamme was aan die lae kant gewees, maar het steeds aan die uitvoer standaard voldoen. Die optimale vruggrootte vir Valencias wissel tussen telling 56 en 88. Growweskil en X639 het die meeste vrugte tussen telling 88 en 72 geproduseer, met SC en CC meer tussen telling 105/125, 88 en 72.

Summary

This rootstock trial was harvested for the first time this season; the trial was top-worked in 2004. The capital investment to establish the orchard will bring in returns via early production.

Limpopo seedless produced a good yield on all 4 rootstock combinations, with the best production on RL, the rootstock with the largest tree volume. The second highest yield was on SC, being the second largest tree volume, followed by CC and X639. Rough lemon produced the lowest internal quality with the other three rootstock combinations producing better Brix:Acid ratio's meeting export standards. The acid on all four rootstock was fairly low by the time of harvest, but still acceptable for export purposes. The optimal fruit size count for export varies between count 56 and 88. Rough lemon and X639 produced the highest number of fruit between count 88 and 56, followed by SC and CC with more fruit between count 105/125, 88 and 72.

Objective

Evaluate and assess the horticultural performance and characteristics of Limpopo SL on different rootstocks. Determine the superior rootstock combinations for this new selection to be able to make credible commercial recommendations.

Materials and methods

The cultivar rootstock combinations were top worked in 2004 onto trees planted in 2000. Visual evaluations were conducted to determine production per tree, trueness to type and scion/rootstock compatibility. Each tree was harvested to determine production as well as fruit size distribution per tree. Samples were taken and internal quality tested and analysed. Fruit colour was evaluated and analysed.

Table 6.3.5.1. List of cultivar and rootstock combinations in the Valencia trial at Noord Grens Boerdery in the Weipe (Musina) area.

Selection	Rootstock	No.of Trees
Limpopo SL	RL	14
Limpopo SL	CC	14
Limpopo SL	TC	14
Limpopo SL	X639	14

Results and discussion

Limpopo SL on CC, SC and X639 produced fruit with good internal quality and complied with the export standards. RL produced an average internal quality with acceptable juice (49.45%) and acid (0.81%) levels, unfortunately the Brix level (9.65°) was below the minimum requirement. The Brix:acid ratio for all four rootstocks was above 8.75:1 and complied with the export standards (Table 6.3.5.2).

RL and X639 peaked at count 72; CC and SC peaked at count 105/125. The second highest count figures for the rootstocks was for RL at count 56, for X639 at count 88, for CC at count 72 and for SC at count 88 (Table 6.3.5.3). These are all acceptable counts for export.

Limpopo SL on RL set the best crop on the trees with 225.0 kg/tree, followed by SC with 163.2 kg/tree, CC with 134.8 kg/tree and X639 with 122.6 kg/tree. This trial was harvested for the first time this season and it is expected that production will increase in future (Table 6.3.5.4).

Conclusion and recommendation

Limpopo SL in combination with CC, SC and X639 produced fruit complying with the minimum export standards; RL did not comply with the Brix standards and the 8.5:1 ratio. Internally the acid levels were fairly low early in the season, but still acceptable. The fruit size count appear promising with all four rootstocks producing counts between the 56 and 88, the optimum size for export valencias. RL being the most vigorous rootstock of the four produced the best yield (225 kg/tree) on the trees and also produce the largest tree volume. SC, CC and X639 performed well at slightly smaller tree volumes. Evaluations will continue and more data will be collected to compare for future recommendations.

Table 6.3.5.2. Internal fruit quality data for Limpopo SL on four rootstocks at Noord Grens Boerdery, Weipe on 11 June 2008.

Selection	Root-stock	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Limpopo SL	CC(4)	49.20	10.70	0.89	12.02	0.0	T1-2
Limpopo SL	SC(5)	47.60	10.40	0.79	13.16	0.0	T1-3
Limpopo SL	RL(6)	49.45	9.65	0.81	11.91	0.0	T1-2
Limpopo SL	X639(3)	48.40	10.10	0.76	13.29	0.0	T1-2

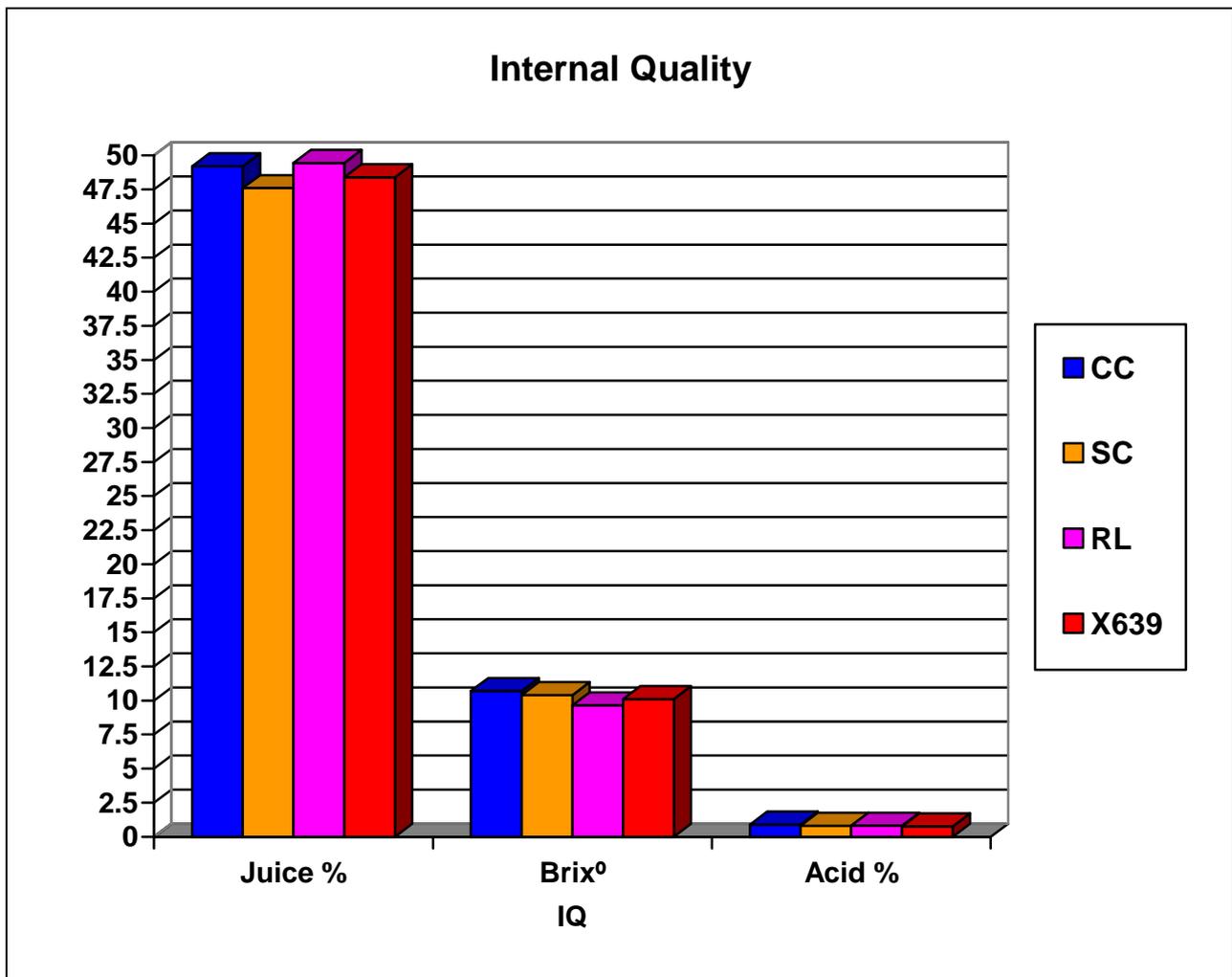
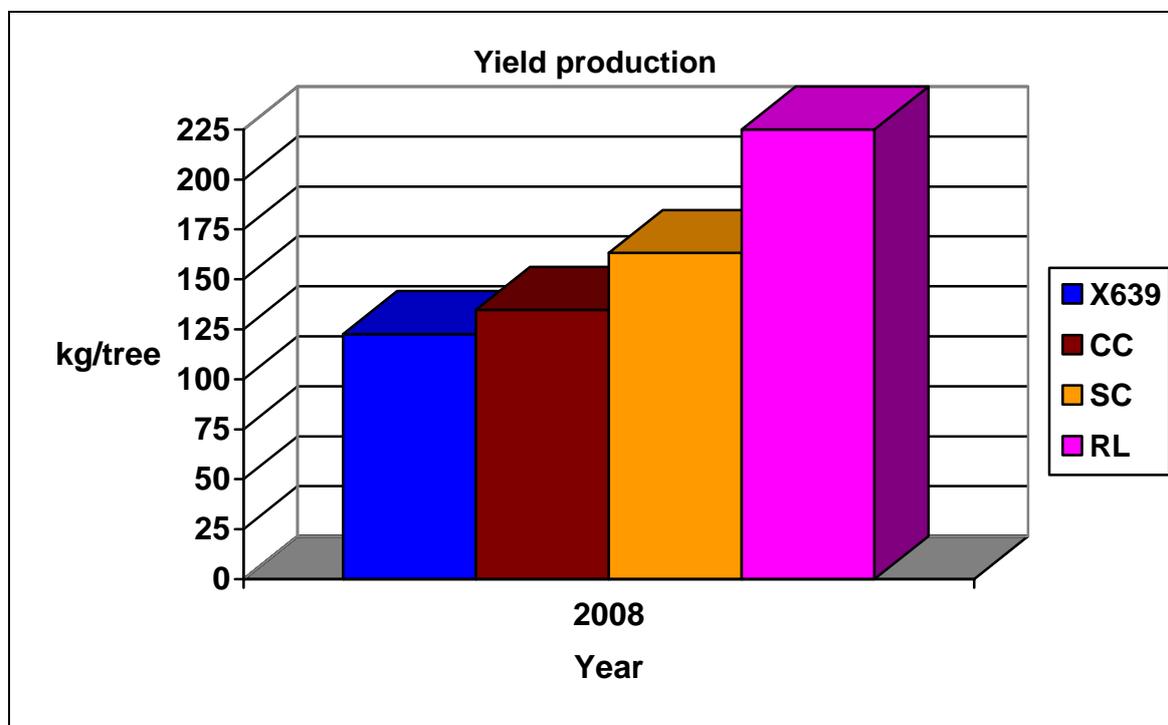


Table 6.3.5.3. Fruit size distribution of Limpopo SL on different rootstocks at Noord Grens Boerdery, Weipe during the 2008 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Limpopo SL	CC	48	1.82	Limpopo SL	RL	48	3.81
Limpopo SL	CC	56	15.55	Limpopo SL	RL	56	24.68
Limpopo SL	CC	72	26.14	Limpopo SL	RL	72	27.76
Limpopo SL	CC	88	25.78	Limpopo SL	RL	88	21.30
Limpopo SL	CC	105/125	26.37	Limpopo SL	RL	105/125	17.52
Limpopo SL	CC	144	5.35	Limpopo SL	RL	144	4.94
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Limpopo SL	SC	48	0.75	Limpopo SL	X639	48	2.09
Limpopo SL	SC	56	9.02	Limpopo SL	X639	56	19.03
Limpopo SL	SC	72	18.92	Limpopo SL	X639	72	28.70
Limpopo SL	SC	88	22.43	Limpopo SL	X639	88	23.79
Limpopo SL	SC	105/125	33.52	Limpopo SL	X639	105/125	22.01
Limpopo SL	SC	144	15.37	Limpopo SL	X639	144	4.37

Table 6.3.5.4. Production per tree of Limpopo SL on different rootstocks at Limpopo SL, Weipe during the 2008 season.

Cultivar	Rootstock	Kg/tree
Limpopo SL	X639	122.6
Limpopo SL	CC	134.8
Limpopo SL	SC	163.2
Limpopo SL	RL	225.0



6.3.6 Evaluation of Genoa lemon on various rootstocks in Citrusdal, Western Cape
Experiment 588 by J. Joubert (CRI)

Opsomming

Probleme wat met lang blomtyd ge-assosieer kan word, moet verminder word. Goeie vrugkwaliteit soos kleur, skildikte en sapinhoud, moet behou word. Die boomeienskappe en prestasie van nuwe onderstam kombinasies moet met die kommersiële varieteite vergelyk word om te bepaal of hulle aan bogenoemde doelwitte voldoen.

Al die bo-onderstam kombinasies het tussen telling 216 en telling 162 gepiek en in meeste gevalle was die vruggrootte eweredig tussen hierdie drie tellings versprei. BC het die grootste vruggrootte vir hierdie proef geproduseer met die meeste vrugte by telling 189. Genoa op Rangpur lime het die hoogste oes geproduseer van 90kg vrugte per boom, gevolg deur Trifoliata x Sweet Orange met 71kg vrugte per boom.

Summary

Reduce the problems associated with protracted flowering and maintain high fruit quality such as rind colour, rind thickness and high juice content. To meet these objectives tree characteristics and performance of new rootstock combinations were compared with the commercially grown selections. All the scion-rootstock combinations produced fruit between count 216 and count 162, with a fairly even distribution in size between the three counts. BC produced the largest fruit size and peaked at count 189 for this trial. Genoa on Rangpur lime produced the highest yield on the trees (90 kg/tree), followed by Trifoliata x Sweet Orange with 71 kg per tree.

Objective

To find suitable rootstock scion combinations between Genoa lemon and various rootstocks for the cold inland citrus production areas.

Materials and methods

Trees were planted at Hexriver Citrus in 1999 on ten rootstock combinations. Assess the horticultural performance and characteristics of Genoa lemon on different rootstocks. Determine the superior rootstock combinations for this lemon selection to be able to make credible commercial recommendations.

Table 6.3.6.1. List of rootstock selections evaluated at Hexriver citrus (Citrusdal) during the 2008 season.

Rootstock	No. of trees
CC	6
Rangpur	6
SC	6
MxT	6
J. Citroen	6
Tri x SO	6
BC	6
Trifoliolate	6
Volk	6
RL-C	6

Results and discussion

Genoa in combination with SC produced the highest number of small fruit and peaked at count 216. MxT, Tri x SO and CC were slightly larger, but also peaked at count 216. The other combinations peaked at count 216, but has a higher percentage of fruit in count 189 and 162. BC produced the largest fruit size by peaking at count 189 and having more fruit in count 162 than count 216, while J. Citroen, RL-C and Volk produced fruit size slightly smaller than BC.

Genoa on Rangpur Lime produced the best yield on the trees with 90kg/tree, followed by Trifoliolate x Sweet orange (71 kg/tree) and Volk with 68 kg/tree. The lowest yield was on BC with RL-C and CC only marginally higher.

Conclusion

Genoa in combination with Rangpur Lime produced a good crop on the trees and the fruit size ranged fairly uniformly from count 216 and 162. Trifoliolate x Sour orange cross produced the second highest yield, but peaked with a high percentage of fruit in count 216. J. Citroen, RL-C and Volk produced fruit only slightly smaller than BC, but yields were low. While fruit size was largest on BC yield was low only being higher than Trifoliolate. All the scion-rootstock combinations produced fairly small fruit from 48 to 54 mm in diameter. Based on these results Rangpur Lime is the only rootstock that gave a good overall performance. Volk and J. Citroen also performed fairly well while the remaining rootstocks were unacceptable overall.

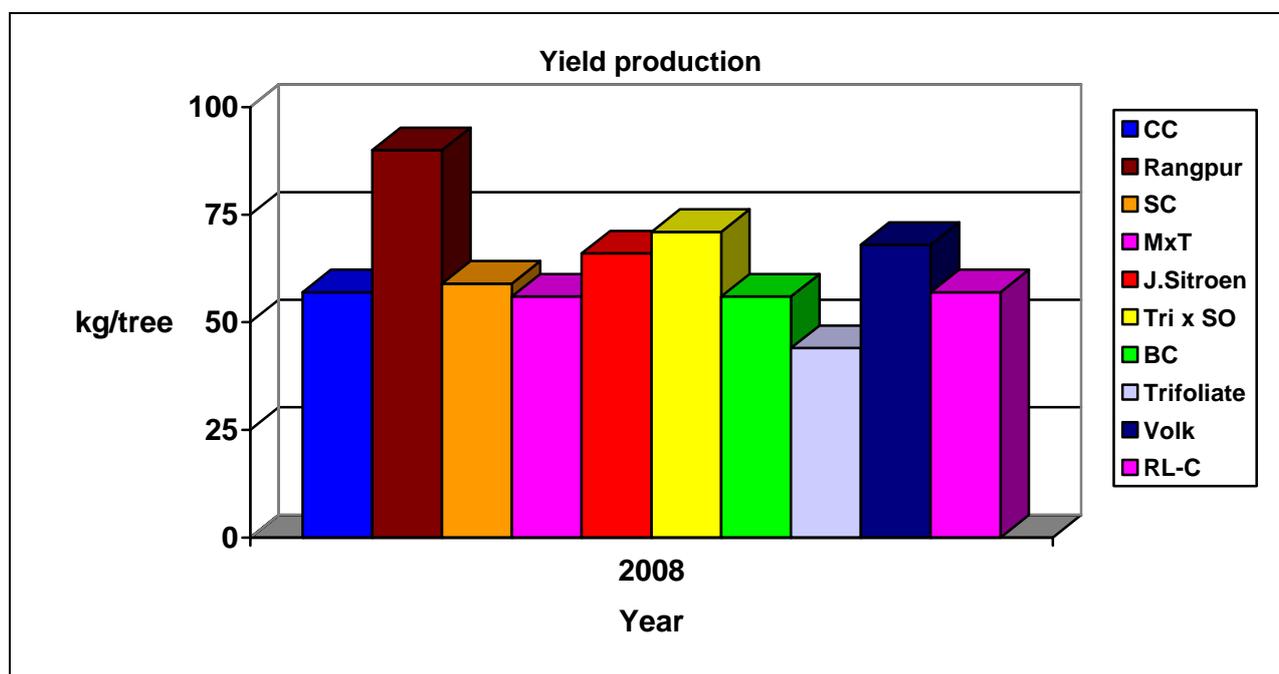
Table 6.3.6.2. Fruit size distribution per rootstock at Hexriver Citrus, Citrusdal during the 2008 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	CC	216	42.40	Genoa	Rangpur	216	25.21
Genoa	CC	189	29.16	Genoa	Rangpur	189	22.47
Genoa	CC	162	17.44	Genoa	Rangpur	162	25.40
Genoa	CC	138	6.91	Genoa	Rangpur	138	14.71
Genoa	CC	113	1.52	Genoa	Rangpur	113	7.19
Genoa	CC	100	2.41	Genoa	Rangpur	100	2.60
Genoa	CC	88	0.14	Genoa	Rangpur	88	2.03
Genoa	CC	75	0.02	Genoa	Rangpur	75	0.33

Genoa	CC	64	0.00	Genoa	Rangpur	64	0.05
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	SC	216	57.53	Genoa	MxT	216	54.78
Genoa	SC	189	24.72	Genoa	MxT	189	27.68
Genoa	SC	162	12.57	Genoa	MxT	162	12.85
Genoa	SC	138	4.14	Genoa	MxT	138	3.72
Genoa	SC	113	0.85	Genoa	MxT	113	0.74
Genoa	SC	100	0.14	Genoa	MxT	100	0.12
Genoa	SC	88	0.00	Genoa	MxT	88	0.09
Genoa	SC	75	0.00	Genoa	MxT	75	0.02
Genoa	SC	64	0.05	Genoa	MxT	64	0.00
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	J. Citroen	216	29.02	Genoa	Tri X SO	216	44.10
Genoa	J. Citroen	189	25.02	Genoa	Tri X SO	189	28.47
Genoa	J. Citroen	162	24.88	Genoa	Tri X SO	162	18.97
Genoa	J. Citroen	138	14.15	Genoa	Tri X SO	138	7.03
Genoa	J. Citroen	113	4.76	Genoa	Tri X SO	113	1.04
Genoa	J. Citroen	100	1.48	Genoa	Tri X SO	100	0.28
Genoa	J. Citroen	88	0.55	Genoa	Tri X SO	88	0.09
Genoa	J. Citroen	75	0.14	Genoa	Tri X SO	75	0.00
Genoa	J. Citroen	64	0.00	Genoa	Tri X SO	64	0.00
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	BC	216	23.43	Genoa	Trifoliolate	216	28.89
Genoa	BC	189	29.15	Genoa	Trifoliolate	189	13.92
Genoa	BC	162	27.03	Genoa	Trifoliolate	162	12.33
Genoa	BC	138	14.79	Genoa	Trifoliolate	138	7.13
Genoa	BC	113	3.91	Genoa	Trifoliolate	113	2.22
Genoa	BC	100	1.42	Genoa	Trifoliolate	100	0.80
Genoa	BC	88	0.20	Genoa	Trifoliolate	88	0.49
Genoa	BC	75	0.08	Genoa	Trifoliolate	75	0.15
Genoa	BC	64	0.00	Genoa	Trifoliolate	64	0.02
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	Volk	216	32.75	Genoa	RL-C	216	28.87
Genoa	Volk	189	24.17	Genoa	RL-C	189	22.75
Genoa	Volk	162	22.04	Genoa	RL-C	162	24.27
Genoa	Volk	138	13.04	Genoa	RL-C	138	14.41
Genoa	Volk	113	5.14	Genoa	RL-C	113	5.34
Genoa	Volk	100	1.78	Genoa	RL-C	100	2.45
Genoa	Volk	88	0.79	Genoa	RL-C	88	1.61
Genoa	Volk	75	0.20	Genoa	RL-C	75	0.30
Genoa	Volk	64	0.11	Genoa	RL-C	64	0.00

Table 6.3.6.3. Production per tree on different rootstocks at Hexriver Citrus, Citrusdal during the 2008 season.

Cultivar	Rootstock	Kg/tree
Genoa	CC	57
Genoa	Rangpur	90
Genoa	SC	59
Genoa	MxT	56
Genoa	J. Citroen	66
Genoa	Tri X SO	71
Genoa	BC	56
Genoa	Trifoliolate	44
Genoa	Volk	68
Genoa	RL-C	57



6.3.7 Cultivar characteristics and climatic suitability of Navel oranges in the cold production regions.

Experiment 74 C (Changed from 74A to 74C as 74A is Burgersfort navel trial) by Z. Zondi (Evaluator) and R. Fenwick (CRI)

Opsomming

Al die nawel seleksies het aan die minimum vereistes vir uitvoer standarde voldoen met Autumn Gold en Powell Summer wat die hoogste Brix gelewer het. Vir die nuwe eksperimentele seleksies het DanSweet en Suitangi die hoogste Brix vlakke geproduseer. Relatiewe rypwordings tye vir al die nawel wat ge-evalueer is word in die Nawel Rypwordings Tye table aan die einde van hierdie gedeelte ingesluit. In die Sondags Rivier Vallei het Lane Late en Cambria die hoogste oes geproduseer; met Palmer, Witkrans en Powel die grootste vrug grootte. By die Sitrus Grondves Blok het Lane Late en Powell Summer die hoogste oes en grootste vrug grootte geproduseer. In die Oos Kaap se middelende het die bome wat ge-evalueer was se ouderdomme verskil. Al die bome het wel 'n goeie oes geproduseer as boom ouderdom en grootte in ag geneem word. Die vrug grootte was die grootste op Washington en Tule Gold gewees, met Autumn Gold wat die kleinste vrug grootte geproduseer. In die Gamtoos Rivier Vallei het Cambria en Palmer die beste opbrengs geproduseer. Van die kommersiele seleksies het Fisher die grootste vrug grootte geproduseer, met die oorblywende seleksies effens kleiner. Die eksperimentele seleksies is gesnoei om enthout te bekom en die vrug grootte en oes was indirek hierdeur geaffekteer.

Summary

All the navel selections evaluated met the minimum quality export standards with Autumn Gold and Powell Summer giving the highest Brix. For the new experimental selections DanSweet and Suitangi produced fruit with the highest Brix levels. Relative maturity dates for all the navels evaluated appear in the Navel Maturity Periods chart at the end of this section. In the Sundays River Valley, Lane Late and Cambria produced the highest yields; Palmer, Witkrans and Powell gave the largest fruit size. At the Citrus Foundation Block, Lane Late and Powell Summer produced the highest yields and the largest fruit size. In the East Cape Midlands evaluations tree ages were variable. All trees, however, produced good yields for tree age and size. Fruit size was largest on Washington and Tulegold with Autumn Gold producing the smallest fruit. In the Gamtoos River Valley, Cambria and Palmer produced the highest yield. Of the commercial selections Fisher produced the largest fruit size with the remaining selections only slightly smaller. As the experimental selections had been pruned for budwood, yield and fruit size was affected accordingly.

Objective

To select Navel cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality which will also extend existing harvest periods, both earlier and later in the season. To describe the cultivar characteristics of these new Navel cultivars and to determine the climatic suitability of these cultivars in various cold production regions. Characteristics for newly discovered navel selections are included in this section.

Materials and methods

Field evaluations and laboratory analyses were conducted on navel selections at the Citrus Foundation Block, Dunbrody Estates in the Sundays River Valley, Riverside and Baddaford in the East Cape Midlands and in the Patensie area of the Gamtoos River Valley.

Results

Table 6.3.7.1. Internal fruit quality data for Navel selections at Dunbrody in the Sundays River Valley (SRV) during the 2008 season (trees planted 2004).

Palmer Navel on CC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
15/4/2008	51.0	8.5	1.11	7.7	0	4-5	Peak
20/5/2008	52.7	9.3	0.81	11.5	0	3-4	
27/5/2008	54.3	10.7	0.97	11.0	0	2-3	

Autumn Gold Navel on CC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
26/6/2008	51.2	9.6	1.05	9.1	0	3-4	
8/7/2008	51.9	10.1	0.94	10.7	0	1-2	Peak
22/7/2008	51.7	12.2	0.92	13.3	0	1	

Cambria Navel on CC - Count 64/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
26/6/2008	53.9	9.6	0.96	10.0	0	5	
8/7/2008	53.6	9.1	1.03	9.8	0	2-3	Peak
15/7/2008	52.9	10.9	0.99	11.0	0	2-3	
22/7/2008	52.9	11.9	0.87	14.1	0	1-3	

Lane Late Navel (CA) on CC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
26/6/2008	55.8	10.5	1.09	9.7	0	3-4	Peak
8/7/2008	54.2	11.6	1.08	10.7	0	2-3	

22/7/2008	55.2	13.3	0.93	14.6	0	1-2	
7/8/2008	55.1	12.7	0.70	18.1	0	1	

Summer Gold Navel on CC - Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
8/7/2008	52.0	10.8	1.00	10.8	0	2-3	Peak
15/7/2008	53.8	11.3	0.87	13.0	0	1-3	
22/7/2008	54.1	11.7	0.89	13.1	0	1-2	

Witkrans Navel on CC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
8/7/2008	53.4	11.1	1.08	10.3	0	2-3	Peak
15/7/2008	54.3	11.3	0.87	13.0	0	1-2	
22/7/2008	52.1	10.5	0.70	15.0	0	1-3	

Powell Summer Navel on CC - Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
26/6/2008	51.5	10.0	1.05	9.5	0	3-4	
8/7/2008	50.4	10.9	1.10	9.9	0	2-3	Peak
22/7/2008	52.8	13.2	1.13	11.7	0	1-3	

Table 6.3.7.1a. Yield and fruit size for Navel selections at Dunbrody in the Sundays River Valley (SRV) during the 2008 season (estimates).

Selection	Rootstock	Yield (kg/tree)	Mean Fruit Size	Year	Estimated Maturity
Palmer	Carrizo citrange	G (30 to 40)	Count 56/64	2004	24/5
Cambria	Carrizo citrange	G to VG (35 to 45)	Count 64	2004	11/7
Lane Late (CA)	Carrizo citrange	G to VG (35 to 45)	Count 64	2004	1/7
Autumn Gold	Carrizo citrange	G (30 to 40)	Count 64	2004	8/7
Summer Gold	Carrizo citrange	M (25 to 35)	Count 64	2004	8/7
Witkrans	Carrizo citrange	G (30 to 40)	Count 56/64	2004	8/7
Powell Summer	Carrizo citrange	M (25 to 35)	Count 56	2004	8/7

Discussion

Tables 6.3.7.1 and 6.3.7.1a show these later maturing navel selections to be from 4 to 6 weeks later than Palmer. There are slight differences between these selections with Lane Late being a week earlier and Cambria being a week later than Autumn Gold, Summer Gold, Witkrans and Powell Summer. When included in a maturity chart of a wide range of navels these later maturing selections all fall in the mid to mid/late part of the navel season. There are only slight differences in internal quality between the late selections, which are marginally superior to Palmer - all selections meet the export standards. Cambria and Lane Late (California selection) gave the highest yields followed by Autumn Gold, Witkrans and Palmer (control). Summer Gold and Powell Summer bore moderate crops. There were only marginal differences in fruit size with Palmer, Witkrans and Powell Summer producing slightly larger fruit on average. These trees are still young so differences at this stage could be misleading.

Table 6.3.7.2. Internal fruit quality data for Navel selections at the Citrus Foundation Block (CFB) during the 2008 season.

Fukumoto Navel on TC - Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
5/4/2008	54.2	10.2	1.03	9.9	0	5-6	Peak
15/4/2008	51.3	10.4	0.96	10.8	0	3-4	
7/5/2008	52.0	10.8	0.74	14.6	0	1-3	

Dream Navel on TC - Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
15/4/2008	54.2	10.2	1.03	9.9	0	4-5	Peak
20/5/2008	51.9	10.6	0.79	13.4	0	2-3	
5/6/2008	52.0	10.8	0.74	14.6	0	1-3	

Autumn Gold Navel on TC - Count 64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
19/6/2008	50.9	12.9	1.48	8.7	0	4-5	
9/7/2008	52.7	12.8	1.40	9.1	0	1-2	
21/7/2008	55.9	15.3	1.44	10.6	0	1	Peak
28/7/2008	56.5	15.5	1.36	11.4	0	1	
18/8/2008	56.5	14.5	1.24	11.7	0	1	

Autumn Gold on SC - Count 72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
19/6/2008	50.3	12.9	1.75	7.4	0	4-5	
5/7/2008	53.6	16.3	1.67	9.8	0	1	
21/7/2008	53.4	15.4	1.38	11.3	0.2	1	Peak
28/7/2008	52.5	15.4	1.47	10.3	0.15	1	
5/8/2008	53.4	15.8	1.29	12.2	0	1	

Barnfield Navel on TC - Count 72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
9/7/2008	56.7	12.9	1.79	7.2	0	1-3	
21/7/2008	57.2	15.0	1.58	9.5	0	1	
28/7/2008	56.9	16.5	1.69	9.8	0	1	Peak

Lane Late Navel (CA) on SC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/7/2008	54.3	14.8	1.46	10.1	0	1	
28/7/2008	52.3	15.2	1.41	10.8	0	1	
5/8/2008	55.4	15.3	1.52	10.1	0	1	Peak

Chislett Navel on TC - Count 72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
19/6/2008	50.2	12.9	1.65	1.7	0	5	
9/7/2008	50.5	14.9	1.58	9.4	0.3	1-3	
21/7/2008	51.6	14.9	1.54	9.8	0.4	1	Peak
28/7/2008	52.7	15.1	1.46	10.3	0	1	

Powell Navel on TC - Count 56/64 (CFB 2008)							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
9/7/2008	53.8	15.4	1.87	8.2	0.3	1	
28/7/2008	57.0	15.1	1.65	9.2	0.3	1	Peak
11/8/2008	54.0	15.0	1.45	10.4	0	1	

Table 6.3.7.2a. Yield and fruit size for Navel selections at the Citrus Foundation Block (CFB) during the 2008 season (estimates).

Selection	Rootstock	Yield (kg/tree)	Mean Fruit Size	Planted	Estimated Maturity
Fukumoto	Carrizo citrange	Screen house	Not of significance		9/4
Dream	Troyer citrange	Screen house			15/4 - CA IQ same
Autumn Gold	Troyer citrange	M (40 to 50)	Count 72/88	1997	21/7
Autumn Gold	Swingle citrumelo	M (40 to 50)	Count 72/88	1994	24/7
Lane Late (CA)	Swingle citrumelo	G (50 to 70)	Count 56/64	2001	21/7
Chislett	Troyer citrange	M (40 to 50)	Count 72/88	1997	24/7
Powell	Troyer citrange	G (50 to 70)	Count 56/64	1995	11/8

In general navel maturity at the Citrus Foundation Block is 2 to 3 weeks later than the Sundays River Valley, Gamtoos River Valley and East Cape Midlands production areas. This is confirmed by the internal quality results in Table 6.3.7.2 and comments in Table 6.3.7.2a. It was not possible to conduct full evaluations on the Fukumoto and Dream navel selections as these selections were not included in the open ground blocks - internal quality tests however were conducted on fruit from the screen house trees. The Fukumoto is reported to have problems with trifoliate hybrid rootstocks in California. Plantings of Fukumotos in South Africa are currently being surveyed and a preliminary report on findings to date appears in this report. The Dream navel is showing promise and has already been included in a new navel trial. Time of maturity has been confirmed using results obtained from the California Citrus Clonal Protection Programme (CCPP). The existing trial which included this selection has been abandoned due to poor tree condition. This selection will be included in new navel trials. The later maturing navels, which include the Barnfield and Chislett selections, have performed in similar manner to the plantings in the Sundays River Valley with the exception that Powell Summer, Lane Late (CA) and Barnfield gave the highest yields. Autumn Gold and Chislett produced moderate yields. Fruit size was variable with Barnfield, Lane late (CA) and Powell Summer producing the largest fruit. Maturity of the later maturing selections was fairly similar to the Sundays River Valley results with only Powell Summer being approximately 2 weeks later than the other selections. Lane Late (CA), Chislett and Autumn Gold were close with Barnfield slightly later maturing. These maturity dates, with adjustment for the Citrus Foundation Block's later maturity, have also been used in the compilation of the navel maturity chart that appears at the end of the navel section.

Table 6.3.7.3. Internal fruit quality data for Navel selections at Riverside and Baddaford in the East Cape Midlands (ECM) during the 2008 season.

Painter Early Navel on TC - Count 64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
14/4/2008	51.1	10.5	1.00	10.5	0	4-5	
26/4/2008	52.0	10.5	1.03	10.2	0	2-3	Peak
10/5/2008	52.1	10.6	1.14	9.3	0	1-2	
21/5/2008	52.5	10.80	1.16	9.3	0	1	

Navelina on TC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
25/4/2008	51.1	11.1	1.23	9.0	0	2-4	
4/5/2008	52.6	11.1	1.15	9.7	0	1-2	Peak
14/5/2008	50.3	13.5	1.13	11.9	0	2	

Tulegold Navel on TC - Count 64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
7/5/2008	51.6	9.6	1.02	9.4	0	2	
14/5/2008	53.5	10.2	0.93	11.0	0	1	Peak
21/5/2008	50.6	9.8	0.90	10.9	0	1	
28/5/2008	56.0	10.1	0.84	12.2	0	1	
4/6/2008	54.8	10.4	0.87	12.0	0	1	
18/6/2008	56.1	9.8	0.85	11.5	0	1	

Washington Navel on TC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/5/2008	52.2	10.8	1.20	9.0	0	1-2	
28/5/2008	52.1	11.7	1.24	9.4	0	1-2	
4/6/2008	53.6	11.6	1.10	10.5	0	2-3	
11/6/2008	51.9	10.7	1.02	10.5	0	1	Peak
18/6/2008	52.8	11.1	1.19	9.3	0	1	
3/7/2008	52.6	11.1	1.06	10.5	0	1	

Autumn Gold Navel on TC - Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
3/7/2008	51.2	10.4	0.96	11.1	0	2-3	Peak
17/7/2008	53.4	13.3	0.91	14.7	0	2	
20/8/2008	52.4	15.1	1.00	15.1	0.3	1	

Powell Navel on TC - Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
15/6/2008	50.2	10.9	1.12	9.7	0	1-2	Peak
3/7/2008	51.3	11.3	0.98	11.5	0	1-2	
17/7/2008	51.5	12.4	0.72	17.2	0	1	
29/7/2008	50.2	12.8	0.76	16.8	0	1	

Table 6.3.7.3a. Yield and fruit size for Navel selections at Riverside and Baddaford in the East Cape Midlands (ECM) during the 2008 season (estimates).

Selection	Rootstock	Yield (kg/tree)	Mean Fruit Size	Planted	Estimated Maturity
Painter Early	Troyer citrange	G (30 to 40)	Count 64	2003	26/4
Tulegold	Troyer citrange	VG(45 to 55)	Count 56/64	2003	18/5
Navelina	Troyer citrange	VG(45 to 55)	Count 64	2004	9/5
Washington	Troyer citrange	VG(70+)	Count 56/64	1995	11/6
Autumn Gold	Troyer citrange	G(50 to 70)	Count 72	1995	3/7
Powell Summer	Troyer citrange	G(50 to 70)	Count 64	1996	24/6

The Painter Early is an early maturing selection made at Riverside farm, Fort Beaufort some time ago and evaluations have been carried out again to establish its characteristics as well as to investigate whether it will help fill a gap in the navel season. As it matures during mid to late April, but holds its quality into May, it would fill a small gap before or overlap with the Navelina and Newhall selections mature. As both these selections tend to bear elongated fruit, have small, but open navel ends and do not develop early rind colour, a slightly earlier maturing selection would be of value. The Painter Early is a round fruit that produces good crops of medium to large fruit of good internal quality.

The Tulegold is a relatively unknown selection with little information available from other sources. In the East Cape Midlands it matures in mid May and produces very good crops of round fruit of good quality and very good flavour. It matures just after Navelina and well before Washington. Washingtons were included in these evaluations as controls for the new selections. This selection produced normal crops of good sized fruit. Autumn Gold produced good crops of medium sized fruit. Maturity was 3/7 which is 2 weeks earlier than Sundays River Valley. This could be due to the heavy soils and differences in the irrigation systems. Powell Summer was even earlier maturing on 24/6, which is definite evidence of a substantial difference in the reaction these later maturing selections in different areas. In addition these trees are in a single variety block in the East Cape Midlands while the trees in the Sundays River Valley are in separate semi commercial blocks.

Table 6.3.7.4. Internal fruit quality data for Navel selections at various sites in the Patensie area of the Gamtoos River Valley (GRV) during the 2008 season.

Patensie Early Navel on RL - C Malan Count 72 - limbsport so fruit for testing limited							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
10/3/2008	Low	9.0	High		0	4-6	
17/3/2008	Medium	9.3	Medium		0	2-4	
24/7/2008	Good	9.6	Good		0	1-3	
31/3/2008	Good	10.0	Good	+ - 10.0	0	1-2	Peak

Fisher Navel on RL - C Rautenbach Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
6/5/2008	51.1	10.6	0.98	10.8	0	2	Peak
15/5/2008	51.8	10.2	1.05	9.7	0	3	Holds IQ and flavour on tree
22/5/2008	50.1	9.9	1.04	9.5	0	2-3	
29/5/2008	50.0	9.9	0.96	10.3	0	2-3	

Palmer Navel on RL - C Rautenbach Count 48/56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
6/5/2008	50.1	9.3	1.07	8.7	0	5	
15/5/2008	50.2	10.7	1.31	8.2	0	3-4	
22/5/2008	52.5	9.8	1.13	8.7	0	2-4	
29/5/2008	51.2	9.8	1.01	9.7	0	3	Peak
5/6/2008	52.2	9.8	0.93	10.5	0	1-3	

EDPN 1 on RL - E Du Preez Count 40/56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
16/7/2008	52.1	12.0	0.65	18.5	0	1	Past
30/7/2008	50.2	12.0	0.65	20.0	0	1	

EDPN 2 on RL - E Du Preez Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
16/7/2008	51.7	12.6	0.77	16.4	0	1	Past
30/7/2008	51.4	12.9	0.80	16.1	0	1	

Cambria Navel on RL - H Malan Count 64/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
25/6/2008	56.0	9.8	1.08	9.1	0	2-4	Peak
9/7/2008	54.3	10.2	0.92	11.1	0	1-3	
23/7/2008	54.3	12.4	0.86	14.4	0	1-2	
8/8/2008	54.4	12.9	0.73	17.7	0	1	

KSCam on RL - K Scheepers Count 56/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
23/7/2008	50.9	11.5	0.78	14.8	0	1	Past*
16/7/2008	51.5	11.8	0.87	13.7	0	1	
8/8/2008	50.1	12.8	0.79	16.3	0	1	

Cambria selected for high % round fruit – Internal Quality similar to standard Cambria

Cambria Navel on SC - Paksaam Farm Count 64/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
25/6/2008	55.8	11.5	1.37	8.4	0	3-4	
9/7/2008	54.3	12.4	1.24	10.2	0	2-3	Peak
23/7/2008	55.1	13.6	1.09	12.6	0	1	
8/8/2008	57.7	14.4	0.93	15.6	0	1	

Robyn Navel on SC - D Rautenbach Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
7/23/2008	51.9	11.9	1.00	11.9	0	1	Past
8/8/2008	53.7	13.1	1.14	11.5	0	1	

Lazy Boy Navel on RL - P Streso Count 56/64							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
16/7/2008	50.6	13.7	1.04	13.2	0	3-5	Past
30/7/2008	51.7	13.4	1.08	12.4	0	1-3	
21/8/2008	50.6	14.9	1.00	14.9	0	1-2	

Suitangi on RL - I Ferreira Count 64/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
23/7/2008	54.7	14.0	1.03	13.6	0	1-2	Past
8/8/2008	59.2	15.0	0.94	16.0	0	1	Flavour holds on tree
21/8/2008	57.6	14.4	0.95	15.2	0	1	
4/9/2008	52.7	13.9	0.91	15.3	0	1	

DanSweet on SC – D Rautenbach Count 56							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
23/07/2008	50.2	14.0	1.53	9.2	0	1-2	
08/08/2008	55.9	14.0	1.62	8.6	0	1	
21/08/2008	52.3	14.7	1.48	9.9	0	1	
04/09/2008	54.2	14.9	1.48	10.1	0	1	Peak
17/09/2008	54.7	14.9	1.34	11.1	0	1	Flavour holds on tree

Note: These data have been used in the development of the navel maturity chart at the end of this section.

Table 6.3.7.4a. Yield and fruit size for Navel selections at various sites in the Patensie area of the Gamtoos River Valley during the 2008 season (estimates).

Selection	Rootstock	Yield (kg/tree)	Mean Fruit Size	Planted	Estimated Maturity
Patensie Early ^{^^}	Rough lemon	Limbsport*	Count 72	1991	31/3
Fisher	Rough lemon	VG (70 to 100)	56/64	2000	6/5
Palmer	Rough lemon	VG (70 to 100)	56/72	2000	5/6
EDPN 1 ^{^^}	Rough lemon	P (25 to 40)**	40/56	1995	16/6 +-
EDPN 2 ^{^^}	Rough lemon	P (25 to 40)**	56	1995	23/6 +-
Cambria	Rough lemon	VG (70 to 100)	Count 64/72	1999	1/7
KSCam ^{^^}	Rough lemon	G (50 to 70)	Count 56/72	2005	1/7
Cambria	Swingle citrumelo	G (50 to 70)	Count 64/72	1987	9/7
Robyn	Swingle citrumelo	G (50 to 70)	Count 56/72	1990	4/7 +- & previous data
Lazy Boy ^{^^}	Rough lemon	G (50 to 70)	Count 56/64	1999	4/7 +-
Suitangi ^{^^}	Rough lemon	G (50 to 70)	Count 64/72	1994	4/7 +-
DanSweet ^{^^}	Swingle citrumelo	M (40 to 50)**	Count 48/56	1990	4/9

- * On Palmer navel tree
- ** Buds cut and/or fruit stolen
- ^^ Experimental selections

Patensie Early

Of these navel selections 8 are still at the experimental stage, but show substantial promise and have therefore been included in this report. The Patensie early is only a limb sport and fruit for evaluation purposes is therefore limited. It has maintained its early characteristics for 3 years now and is similar to the parent Palmer navel tree, except for its earlier colour and internal maturity. It is at colour 1 to 2 by early/mid April and is internally mature by end March/early April. This means it is 6 to 8 weeks earlier than the Palmer navel and at present the earliest maturing navel of South African origin. Young trees have been planted and it will take 2 to 3 years to determine whether it is horticulturally stable. In the meantime material is being put through virus eradication so that clean material is available for further evaluations during the second stage of testing.

Fisher

The Fisher navel has made an impact on the growers in the Patensie area of the Gamtoos River Valley as it colours uniformly before the Palmer navel and appears to be less prone to creasing. Fisher export percentages are higher than the Palmer and it slightly larger fruit size. Yields do not appear to be as high as Palmer, but with 10% higher export fruit plus the extremely uniform fruit colour the Fisher is destined to make its mark in the navel areas in the future. A further positive attribute is that the fruit holds well on the tree with little deterioration of internal quality for at least 4 to 5 weeks after peak maturity.

EDPN 1 & 2

These two promising selections were discovered in a Delta orchard and as a result were only tested after peak maturity. Both selections have large round fruit with very small to no navel and internal quality is very good. A young orchard has already been established with material from EDPN 1 and it should be possible this season to establish whether the selection is horticulturally stable. The maturity of both selections is approximately mid to late June with #2 being slightly later than #1.

Cambria

The Cambria despite its tendency to bear a fairly high proportion of elongated fruit has already made a name for itself as a high quality fruit that is in demand in the more discerning and higher paying markets. It produces very good crops of good size fruit (counts 64 to 72) and has very good internal quality even on rough lemon with Brix levels reaching 12 to 14 on Swingle and Carrizo rootstocks. Peak maturity is during the second week of July and as this selection appears to have two sets, fruit of varying colour, it is picked till the end of July and even into August in some instances. The high yields appear to slow tree growth as Cambria trees are not as vigorous as many other navel selections. This is a further advantage as picking and spraying are simplified for many years.

KSCam

This is a selection of the Cambria that has a much lower proportion of elongated fruit. The trees are still young, but the KSCam is similar in internal quality and other aspects, except fruit shape, to the Cambria.

Robyn

The Robyn was nearly sunk by its reaction on Volckameriana rootstock in the 1980's due to CTV, but it is now making a come back on other rootstocks due to its timing, production, internal quality and fruit size. It matures in early July and appears to be relatively creasing free. Fruit colour is good and fruit size is medium to large (56 to 72), navel ends are small and the fruit is firm.

Lazy Boy

Named for its slowness to mature this selection from the foothills of the Baineskloof has many attributes that recommend its further evaluation. It matures in early July and produces good crops of medium large fruit (Count 56 to 64). It has excellent internal quality which holds well into late July without the fruit losing its firmness. A few trees have been established using material from this tree and it will soon be possible to establish whether it is horticulturally stable.

Suitangi

This late maturing selection was discovered in a Midnight orchard and it has remained stable now for 3 seasons. As it is on rough lemon rootstock it is reasonable to expect that it will be 2 to 3 weeks later on Swingle or Carrizo, which will give it a maturity date of approximately end July early August. Even on rough lemon fruit internal quality is very good and the fruit holds its flavour into August. The fruit has small to no navels and fruit size is medium large (Count 64 to 72). Fruit shape is round with only the occasional oval

fruit. Production is good particularly as it is situated in a Midnight orchard where conditions are not ideal for a navel.

DanSweet

This ultra late navel selection is the latest maturing navel selection in South Africa, and anywhere in the world to the best of my knowledge, and has all the characteristics required of a navel. It has excellent internal quality, is round in shape, has little to no navel ends, produces well when not cut extensively for propagation material, has medium to large fruit size (Count 48 to 56), remains firm and holds its internal quality well after peak maturity which is early September when Brix levels range from 13 to 14 according to year and the ratio is 10.

Navel Maturity Chart

Based on historic data as well as the results included in these tables the following maturity chart has been drafted to highlight the wide range of navel selections now available or soon to be available to the industry. In order to move away from the early (e.g. oleocellosis) and late (e.g. creasing, waste) problems that can occur with individual navel selections picking periods have been reduced to 3 weeks in these charts. While this is not practical at present with the imbalances of navel plantings, it is an example of what can occur in the future with careful planning, i.e. provided that all or most of the new and experimental selections are a commercial success. What is of particular interest in this chart is the season wide range of selections now being produced or evaluated in the cool inland and cold navel areas. There is even interest in producing some of the late and ultra late selections in warmer citrus areas.

6.3.8 **Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region**

Experiment 57A by J. Joubert and R. Fenwick (CRI)

Objective

To select Satsuma cultivars with improved and consistent productivity, fruit size, rind colour, and internal fruit quality (Brix and acidity, ratio), and extended harvest period (both earlier and later maturity). To describe the cultivar characteristics of new Satsuma cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Evaluation Plan

Duration: Planted and topworked 2006; End 2012.

Sites: Whitebridge, Wolseley; Lustigaan, Paarl; Slaley, Stellenbosch.

Cultivars: Dobashi Beni, Ohtsu, Ueno, Aoshima, Imamura, Owari (control), Primosole.

Data collection: Evaluate each selection in production two to three times during the season using suitable control cultivars. Evaluate according to yield, fruit size, rootstock compatibility, seedlessness, trueness to type, colour, maturity and internal quality (juice content, TSS or Brix and titratable acidity) of similar size fruit from one canopy position.

Note: During 2008 these trees were still too small and bore insufficient fruit to begin with evaluations.

6.3.9 **Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region**

Experiment 72A by Z. Zondi (Evaluator) and R. Fenwick (CRI)

Objective

To select Clementine mandarin cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity). To describe the cultivar characteristics of new Clementine mandarin cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Evaluation Plan

This trial is under consideration until new Clementine selections are available for evaluation.

6.3.10 Cultivar characteristics and climatic suitability of Oranges in a cold production region Experiment 972 by J. Joubert and R. Fenwick (CRI)

Objective

To select Orange cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period. To describe the cultivar characteristics of these cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Note: This is the first cooperative trial between CRI and Citrogold, a Private Cultivar Company.

Evaluation Plan

Duration: Planted 2009; End 2017.

Site: Patryberg, Citrusdal

Layout: Randomised block design

Cultivars:

Navels: Glen Ora Late, Krajewski Early. Santa Catharina-1 and 3, Witkrans, Lina and Coetzee Late:

Valencias: Letaba Early, Midnight-1, Benny-2, Limpopo SL: Common: Raratonga

Rootstock: CC (110 trees)

Data collection: Evaluate each selection two to three times during the season. Evaluate according to yield, fruit size, rootstock compatibility, seedlessness, trueness to type, colour, maturity and internal quality (juice content, Brix and titratable acidity) of similar size fruit from one canopy position.

This trial was planted in October 2008

6.3.11 Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region

Experiment 73A by Z. Zondi (Evaluator) and R. Fenwick (CRI)

Opsomming

Die interne kwaliteit en eksterne kleur van Nadorcott op Carrizzo, Swingle en X639 was soortgelyk, met die bome op X639 wat ongeveer een week later ryp geword het. Interne kwaliteit en kleur was uitstekend. Mor het baie hoë Brix vlakke gelewer met voldoende suur om uitstekende smaak te produseer, behalwe by die CFB waar die Brix:Suur verhouding steeds effens laag was met oestyd. Valley Gold het vrugte ger produseer met uitstekende kwaliteit en smaak. African Sunset se kwaliteit en smaak was nie so goed nie, maar steeds aanvaarbaar. Murcott x Clementine (Clemcott) se vrugte het goeie smaak opgelewer, maar het saad geproduseer en dan slegs geskik vir sekere oosterse marke. Kiyomi se interne kwaliteit en skil kleur was baie goed gewees. Nectar en Orri het vrugte geproduseer met hoë Brix vlakke en uitstekende smaak. Winola was slegs beskikbaar gewees by die CFB waar suur vlakke te hoog gebly het om aanvaarbare smaak te ontwikkel. Hierdie seleksie verlang 'n warmer klimaat as die CFB. Dis nog te vroeg om enige afleidings te maak oor die nuwe mandaryn hibriede. Nadorcott en Morr het uitstekende produksie gelewer met medium vruggrootte (telling 2). Valley Gold het ook baie goeie produksie gelewer met medium tot medium-groot vrugte terwyl African Sunset 'n gemiddelde oes met groot tot baie groot vrugte geproduseer het. By die CFB het Murcott x Clementine, Hadass, Nectar, Orri en Winola 'n gemiddelde oes gelewer. Orri, Nectar en Winola het medium tot medium-klein vrugte (telling 2 tot 4) geproduseer. Hadass het effens groter vrugte (telling 1 tot 2) geproduseer, met Murcott x Clementine die grootste vrugte (telling 2 tot 4). Kiyomi het 'n gemiddelde oes met groot vruggrootte (telling 1XX) geproduseer.

Summary

The internal quality and external colour of the Nadorcotts on Carrizo, Swingle and X639 were similar, with trees on X639 maturing approximately one week earlier. Internal quality and colour were excellent. Mors had very high Brix levels with sufficient acid to give excellent flavour, except at the Citrus Foundation Block where ratios were still a bit low at harvest. Valley Gold produced fruit with excellent quality and flavour. African Sunset quality and flavour were not as good, but acceptable. Murcott x Clementine (Clemcott) fruit had very good flavour, but was seedy so is only suitable for certain eastern markets. Kiyomi internal quality and rind colour were very good. Nectar and Orri produced fruit with very high Brix and excellent flavour. Winola was only available at the Citrus Foundation Block where acid levels remained too high for acceptable flavour to develop. This selection requires a warmer climate than the Citrus Foundation Block. It is too early to make any conclusions on the new mandarin hybrids. Nadorcotts and Mors produced excellent yields of

medium sized fruit (count 2). Valley Gold Produced very good yields of medium to medium-large fruit while African Sunset produced moderate yields of large to very large fruit. At the Citrus Foundation Block Murcott x Clementine, Hadas, Nectar, Orri and Winola produced moderate yields. Orri, Nectar and Winola produced medium to medium small fruit (counts 2 to 4). Hadas had slightly larger fruit (counts 1 to 2) and Murcott x Clementine had the largest fruit (count 1X). Kiyomi produced a moderate yield of large fruit (count 1XX).

Objective

To select Mandarin hybrid cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity). To describe the cultivar characteristics of new Mandarin hybrid cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections at the Citrus Foundation Block, Dunbrody Estates in the Sundays River Valley, J&B Citrus in the ECM, W Young in the Hankey region and in the Patensie area of the Gamtoos River Valley.

Table 6.3.11.1 Internal fruit quality data for Mandarin hybrid selections at various sites in the E Cape area during the 2008 season.

Nadorcott on CC - J&B Citrus, Cookhouse ECM - Count 2							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
3/7/2008	58.9	12.2	1.41	8.7	0	1	
10/7/2008	59.2	12.6	1.46	8.8	0.1	1	
17/7/2008	57.8	14.7	1.39	10.6	0.8	1	Peak
29/7/2008	59.2	15.4	1.34	11.5	0.3	1	
20/8/2008	56.3	15.8	1.13	14.0	0.3	1	
3/9/2008	52.0	16.6	1.91	8.7	0.4	1	
17/9/2008	58.6	15.3	1.34	11.4	1	1	

Nadorcott on SC - J&B Citrus, Cookhouse ECM - Count 2							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
3/7/2008	59.6	12.4	1.63	7.6	0	1	
10/7/2008	56.9	11.0	1.12	9.8	0	1	
17/7/2008	56.3	13.7	1.13	12.1	0	1	Peak
29/7/2008	57.8	13.9	1.10	12.6	0	1	
20/8/2008	57.7	16.1	1.33	12.1	0.4	1	
9/3/2008	53.0	16.1	1.33	12.1	0	1	
9/17/2008	60.3	15.6	1.20	13.0	0.3	1	

Nadorcott on X639 - J&B Citrus, Cookhouse ECM - Count 2							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
3/7/2008	59.5	12.5	1.29	9.7	0	1	
10/7/2008	58.2	12.5	1.23	10.2	0	1	Peak
17/7/2008	61.2	15.0	1.24	12.1	0	1	
29/7/2008	59.8	15.9	1.35	11.8	0.3	1	
20/8/2008	56.9	15.3	1.20	12.8	0	1	

Mor on CC - L Ferreira, GRV - Count 1 to 3							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
30/7/2008	61.3	17.5	1.53	11.4	2.8	1-2	
8/8/2008	59.5	17.5	1.63	10.7	3	1	Peak

21/8/2008	58.6	18.6	1.70	11.0	3.1	1	
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Mor on CC - K Scheepers, GRV - Count 1 to 2

Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
23/7/2008	60.1	13.0	1.46	8.9	3.1	2-5	
8/8/2008	61.0	16.2	1.71	9.5	3.1	2-3	
4/9/2008	54.0	15.8	1.48	10.7	2.6	1	Peak
11/9/2008	60.4	18.9	1.60	11.8	2.6	1	

Mor on TC - Citrus Foundation Block - Count 2 to 4

Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/7/2008	61.0	15.4	1.60	9.6	2.8	1-2	
28/7/2008	59.5	15.7	1.60	9.8	3.8	1-3	
5/8/2008	56.3	14.8	1.55	9.6	2.7	1-3	
28/8/2008	61.6	13.7	1.58	8.7	3.4	1-3	
2/9/2008	50.9	17.9	1.66	10.8	0	1	Peak
11/9/2008	60.4	18.9	1.60	11.8	2.6	1	

Valley Gold (B17) on CC - Dunbrody, SRV - Count 1 to 2

Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
26/6/2008	62.0	10.7	1.29	8.3	1.5	1	
2/7/2008	65.6	11.7	1.35	8.6	1.5	1	
8/7/2008	63.2	12.4	1.20	10.3	1.8	1	Peak
15/7/2008	63.3	14.2	1.35	10.5	1.5	1-2	
22/7/2008	61.9	14.8	1.26	11.7	1.3	1	
12/7/2008	60.1	11.4	1.10	10.4	0.2	1	

African Sunset (B24) on CC - Dunbrody, SRV - Count 1XX

Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
26/6/2008	59.3	10.9	1.10	9.9	0	1	
2/7/2008	63.6	10.6	1.07	9.9	0	1	Peak
12/7/2008	60.1	11.4	1.10	10.4	0.2	1	

Murcott X Clementine on TC - Citrus Foundation Block - Count 1X

Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
19/6/2008	58.9	10.0	1.34	7.5	12.3	4-5	
2/7/2008	60.1	9.6	1.16	8.3	10.3	1	Immature

Estimated peak 30/7 - Insufficient fruit for further tests.

Note - small commercial orchards picked 10 July in SRV - CFB 2 to 3 weeks later in maturity.

Hadas Ellendale on TC - Citrus Foundation Block - Count 1 to 2

Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/7/2008	59.9	13.2	1.62	8.1	9	1-3	
28/7/2008	60.6	14.1	1.85	7.6	8.9	1	
5/8/2008	61.3	13.9	1.70	8.2	7.4	1-3	
28/8/2008	59.6	14.4	1.65	8.7	7.1	1	
2/9/2008	51.7	14.6	1.54	9.5	8.6	1	
11/9/2008	63.3	15.1	1.48	10.2	6.8	1	Peak

Estimate for East Cape Region - 22/8.

Kiyomi on TC - Baddaford, ECM - Count 1XX							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
3/7/2008	53.0	8.9	1.13	7.9	0.2	1	
17/7/2008	52.2	11.1	0.95	11.7	0.1	1	Peak
29/7/2008	59.4	12.0	0.93	12.9	1.1	1	
20/8/2008	52.3	11.6	0.98	11.8	0.3	1	
3/9/2008	50.2	11.5	1.02	11.3	0.8	1	
17/9/2008	45.6	12.3	0.81	15.2	0.7	1	

Estimate for East Cape Region - 17/7

Nectar on TC - Citrus Foundation Block - Count 3 to 4							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
19/6/2008	56.3	13.6	1.44	9.4	0.25	2-3	
2/7/2008	53.9	14.2	1.55	9.2	0	1-3	
21/7/2008	58.9	16.4	1.69	9.7	0.3	1	Peak
28/7/2008	53.9	18.1	1.39	13.0	0.1	1	
5/8/2008	53.6	18.1	1.35	13.4	0.1	1	

Estimate for East Cape Region - 5/7

Orr on TC- Citrus Foundation Block - Count 2 to 3							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
28/7/2008	61.6	17.2	1.72	10.0	2.3	1	
5/8/2008	62.5	17.7	1.78	9.9	2.3	1	
28/8/2008	62.0	18.4	1.72	10.7	2.4	1	Peak
2/9/2008	56.0	16.4	1.48	11.1	2.3	1	

Orr on CC- J&B Citrus, Cookhouse ECM - Count 2 to 3							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
17/7/2008	64.5	16.2	1.54	10.5	1.1	1	Peak
29/7/2008	60.5	15.9	1.39	11.4	1.7	1	Peak
20/8/2008	58.2	18.5	1.51	12.3	1.3	1	
3/9/2008	56.1	17.7	1.32	13.4	1.1	1	
17/9/2008	59.8	17.6	1.13	15.7	0.95	1	

Orr on X639 - J&B Citrus, Cookhouse ECM - Count 2 to 4							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
17/7/2008	62.5	15.6	1.52	10.3	1.1	1	Peak
29/7/2008	60.7	15.2	1.35	11.3	1.3	1	
20/8/2008	59.5	16.2	1.39	11.7	1.3	1	
3/9/2008	50.1	17.8	1.44	12.4	0.7	1	
17/9/2008	61.1	17.7	1.21	14.6	0.4	1	

Winola on TC - Citrus Foundation Block - Count 2							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/7/2008	60.1	15.0	2.30	6.5	0.3	1	
28/7/2008	59.1	15.3	2.23	6.9	0.5	1	Immature*

Insufficient fruit - maturity probably mid to late September in ECR - more suited to climate with higher heat units- estimate based on overseas experience

New Mandarin Hybrids

New Mandarin Hybrid (Seedling) - W Young - Count 1							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/8/2008	51.8	15.9	1.47	10.8	5.8	1	Peak

Tasty Mandarin Hybrid (Seedling) - W Young, Hankey - Count 1XXX							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/8/2008	40.1	15.8	1.06	14.9	16	1	Past

Estimated maturity 15/6 to 30/6

Table 6.3.11a. Yield and fruit size for Mandarin Hybrid selections at the CFB, SRV, GRV and the ECM during the 2008 season (estimates).

Selection	Rootstock	Yield (kg/tree)	Mean Fruit Size	Planted	Estimated Maturity
Nadorcott	Carrizo citrange	E(50+)	Count 2	2002	21/7
Nadorcott	Swingle citrumelo	E(50+)	Count 2	2002	21/7
Nadorcott	X639	E(50+)	Count 2	2002	14/7
Mor	Carrizo citrange	E(70+)	Count 1 to 3	2000	15/8
Mor	Troyer citrange	E(70+)	Count 1 to 2	2000	21/8
Mor	Troyer citrange	E(70+)	Count 2 to 4	1999	7/9
Valley Gold	Carrizo citrange	VG(70)	Count 1 to 2	1997	8/7
African Sunset	Carrizo citrange	M(40 to 50)	Count 1XX	1997	8/7
Murcott x Clem	Troyer citrange	M(40 to 50)	Count 1X	1999	30/7 (Est. 10/7 ECR)*
Hadas Ellendale	Troyer citrange	M(40 to 50)	Count 1 to 2	1997	11/9 (Est. 22/8 ECR)*
Kiyomi	Troyer citrange	M(40 to 50)	Count 1XX	1997	17/7
Nectar	Troyer citrange	M(40 to 50)	Count 3 to 4	1997	25/7 (Est. 5/7 ECR)*
Orri (CFB)	Troyer citrange	M(40 to 50)	Count 2 to 3	1999	30/8
Orri	Carrizo citrange	VG(35 to 45kg)	Count 2 to 3	2002	17/7
Orri	X639	E(50+ kg)	Count 2 to 4	2002	17/7
Winola	Troyer citrange	M(40 to 50)	Count 2	1997	15 to 30/9 (Est. ECR)
Mandarin Hybrid	Seedling	Limited information			21/8
Tasty Mandarin	Seedling				15 to 30/6 (Est. ECR)

*ECR - East Cape Citrus Region

Results and discussion

Nadorcott

The Nadorcotts in the ECM produced excellent crops of medium to medium small sized fruit. Internal quality and external colour were excellent and the fruit hung well being harvested over a period of 4 to 5 weeks. Seed counts were always below 1, the highest count being 0.8 seeds per fruit. Peak maturity was slightly earlier on X639 when compared with Carrizo and Swingle.

Mor

The Mors in the GRV and at the CFB bore very good crops of medium to medium small sized fruit. Internal quality and external were excellent and seed counts ranged from 0 to 3.8 seeds per fruit. Peak maturity in the GRV was mid to late August and early to mid September at the CFB, i.e. 3 weeks later.

Valley Gold (B17)

The B17 marketed as Valley Gold is an excellent addition to the range of mandarin now available as it matures approximately 2 to 3 weeks before the Nadorcott. Valley Gold set an excellent crop, too much initially, but a proportion of the fruit drops, finally resulting in a very good crop of medium to medium large fruit. Internal quality was very good and external colour excellent.

African Sunset (B24)

The B24 marketed as African Sunset has not produced the same crops as the Valley Gold and as a result fruit size has been medium large to large. Excessive fruit drop appears to be the reason for this problem. This cultivar is also fairly susceptible to Diplodia dieback and cannot be girdled as this can spread the disease throughout the tree causing more excessive dieback and even tree mortality. It is probable that this

very good quality selection needs more attention or at least different production practices than other mandarin hybrids to produce acceptable yields and fruit size. Peak maturity of the African Sunset is approximately 8/7 similar to the Valley Gold.

Murcott x Clementine

This promising new selection has received attention due to numerous characteristics such as good yields of medium sized, extremely well coloured, firm fruit of very good internal quality. It has seed counts of 10 to 12 seeds per fruit in solid plantings, but this has not been a problem for Far eastern markets as the preference is for large, firm well coloured fruit of very good flavour. It has been planted in numerous other countries and research is now underway to produce a seedless selection for EU and other markets that prefer seedless fruit. It matures in early July in the SRV and as such is a valuable addition to the mandarin range.

Hadas Ellendale

This Israeli selection of Ellendale has still to be tested more fully. In mixed blocks it bears well, but is seedy. Early indications are that its internal quality and time of maturity are typical of Ellendale, but it will be some time before its production characteristics have been evaluated. Fruit size appears to be slightly smaller than standard Ellendales.

Kiyomi

This Satsuma x Trovita hybrid has been available for some time, but few commercial plantings have been established. Reasons for this are probably its Satsuma parentage which can cause unacceptable quality fruit if production practices are not adjusted accordingly. It has many positive characteristics such as good yields, medium fruit size, good external colour, seedlessness and maturity is early to mid July, which could help to fill a small gap in the present mandarin range.

Nectar

This seedless diploid Wilking hybrid is a promising selection that has been available for some time, but has not been planted on a commercial scale to date. The Nectar, which bears very good crops of medium to medium small fruit, has excellent internal quality and should mature in late June to early July in the ECR as maturity at the CFB is approximately 25/7. Its time of maturity falls into the same time slot as Kiyomi. Nectars are upright trees with a more open canopy than average Clementines.

Orri

The Orri in the ECM produced excellent crops of medium sized fruit. Internal quality was excellent and external colour was good. The fruit reached peak maturity in the ECM during mid to late July and hung well into August. At the CFB maturity was late August to early September. Seed counts were always below 2 in solid plantings and below 3 where pollinators were present. Orri on Carrizo and X639 matured at the same time.

Winola

This Minneola x Wilking hybrid is similar in many ways to the Page, except that it has larger fruit size, is a triploid so is seedless in mixed plantings and is extremely late maturing. Like the page it has excellent internal quality and flavour and a deep orange to red external colour. Only limited commercial plantings have been established to date. The Winola could play a very important part in the mandarin season as it matures mid to late September and has outstanding flavour. It does require additional attention however due to the trees grapefruit like downward growth and internal bearing pattern, which makes it an excellent candidate for trellising. Trellised orchards under pulse drip fertigation have reached 2 metres in 2 years and set excellent crops (100 to 150 fruit per tree) on the outside of the erect trees.

New Mandarin Hybrids

The Kleinrivier area of Hankey in the East Cape is a well known 'naartjie' area that has traditionally planted seedling trees due to the high polyembryony of the many selections planted. In every batch of seedlings there are bound to be some hybrids or zygotics and a number of 'off type naartjies' have recently been discovered by an observant grower in this area. The first two selections, Tasty and no name to date, were tested for the first time during 2008. The grower has budded some trees for evaluation purposes so further testing needs to be done before these selections can be rated for horticultural integrity.

Mandarin Hybrid Maturity Chart

Based on historic data as well as the results included in these tables the following maturity chart has been drafted to highlight the wide range of mandarin hybrid selections now available or soon to be available to the industry. In order to move away from the early (e.g. high ratios and poor colour) and late (e.g. soft/puffy fruit

and waste) problems that can occur with individual Mandarin hybrid selections picking periods have been reduced to 3 weeks in these charts. While this is not practical at present with the imbalances of Mandarin hybrid plantings, it is an example of what can occur in the future with careful planning, i.e. provided that these new and semi commercial selections are successful. What is of particular interest in this chart is the almost season-wide range of selections now being produced or evaluated.

6.3.12 **Cultivar characteristics and climatic suitability of Midseason oranges in a cold production region**

Experiment 77A by Z. Zondi (Evaluator) and R. Fenwick (CRI)

Objective

To select Midseason cultivars with improved and consistent productivity, fruit size, rind colour, pigmentation, flavour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity). To describe the cultivar characteristics of new Midseason cultivars and to determine the climatic suitability of these cultivars in a cold production region.

This trial has been concluded and the results will be published in due course. New trials are being planned.

Evaluation Plan

Duration: Plant 2009; End 2018 to allow for 5 years evaluations of bearing trees.

Site: Saxfold Park, Baddaford, Riverside, East Cape Midlands.

Cultivars: Range of available Tarocco selections. As blood oranges only develop internal pigmentation under very cold conditions Taroccos will only be tested in the ECM region.

Sites: Suitable trial sites have been found and new trials will be established during spring 2009.

6.3.13 **Cultivar characteristics and climatic suitability of Valencia oranges in a cold production region**

Experiment 75 A by Z. Zondi (Evaluator) and R. Fenwick (CRI)

Opsomming

By die CFB het McClean SL die hoogste Brix gelewer, gevolg deur Turkey en Midnight. Die oorblywende seleksies het ook Brix vlakke bo 12 gelewer, behalwe vir Delta en Rietspruit met die laagste net oor 10, maar steeds aanvaarbaar. Slegs McClean SL en Portsgate was totaal saadloos, maar in soliede blokke sal meeste van hierdie seleksies saadloos tot feitlik saadloos wees. Alle seleksies het goeie oeste geproduseer met slegs Turkey en Rietspruit effens laer. Vruggrootte het gewissel van medium tot groot (telling 64 tot 88) behalwe vir Valencia Late met vrugte in telling 105 tot 88.

Summary

At the Citrus Foundation Block, McClean Seedless gave the highest Brix followed by Turkey and Midnight. The remaining selections also had Brix levels above 12 except Delta and Rietspruit, which was the lowest at just over 10, but still acceptable. Only McClean Seedless and Portsgate were totally seedless, but it is probable that in solid plantings most of these selections would have very few if any seeds. All selections produced very good yields with only Turkey and Rietspruit being slightly lower. Fruit size ranged from medium to medium large (counts 64 to 88) except for Valencia Late which had fruit in counts 105 to 88.

Objective

To select Valencia cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity). To describe the cultivar characteristics of new Valencia cultivars and to determine the climatic suitability of these cultivars in a cold production region.

Materials and methods

Field evaluations and laboratory analyses were conducted on mandarin hybrid selections at the Citrus Foundation Block, Dunbrody Estates in the Sundays River Valley, Baddaford and Riverside in the ECM and in the Patensie area of the Gamtoos River Valley.

Table 6.3.13.1 Internal fruit quality data for Valencia selections at various sites in the East Cape area during the 2008 season.

Turkey on SC - Citrus Foundation Block - Count 72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Size
21/7/2008	51.7	15.1	1.48	10.2	3.1	1	Peak
5/8/2008	52.3	14.0	1.15	12.1	4.5	1	
28/8/2008	50.5	14.4	1.01	14.2	5.1	1	

Delta on TC - Citrus Foundation Block - Count 72/88							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
7/8/2008	56.3	11.2	1.25	9.0	0	1	
28/8/2008	52.9	11.0	1.21	9.1	0	1	
2/9/2008	52.0	11.6	1.15	10.1	1.2	1	Peak
11/9/2008	56.8	11.1	1.02	10.9	0.1	1	

Rietspruit on SC - Citrus Foundation Block - Count 64/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
7/8/2008	51.9	9.8	1.15	8.5	0.2	1	
28/8/2008	51.5	10.0	1.35	7.4	0.3	1	
2/9/2008	50.2	10.4	1.35	7.7	0.2	1	
11/9/2008	52.4	10.4	1.26	8.3	0.3	1	Immature*

Delicia on TC - Citrus Foundation Block - Count 56/72							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/7/2008	60.4	12.6	1.68	7.5	1.3	1-3	
5/8/2008	61.1	12.6	1.51	8.3	0.8	1-3	
28/8/2008	60.1	13.2	1.36	9.7	0.9	1-3	
2/9/2008	61.6	12.6	1.24	10.2	0.5	1	Peak
11/9/2008	61.0	12.7	1.15	11.0	0.6	1	

Valencia Late on TC - Citrus Foundation Block - Count 88/105							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
7/8/2008	54.1	11.7	1.42	8.2	2.3	1	
28/8/2008	56.0	12.3	1.37	9.0	1.3	1	
2/9/2008	49.2	14.2	1.34	10.6	1.6	1	Peak
11/9/2008	56.0	12.9	1.23	10.5	2.7	1-2	

Midnight on CC - Citrus Foundation Block - Count 64/88							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
21/7/2008	57.1	13.6	1.87	7.3	1.4	1-2	
5/8/2008	58.2	13.7	1.70	8.1	2.2	1	
7/8/2008	56.5	11.7	1.62	7.2	1.5	1	
28/8/2008	57.4	14.0	1.54	9.0	2.6	1	
2/9/2008	57.0	13.5	1.43	9.4	1.2	1	
11/9/2008	56.9	14.1	1.40	10.1	2.3	1	Peak

McClellan Seedless on SC - Citrus Foundation Block - Count 88							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
28/8/2008	56.7	14.1	1.88	7.5	0	1	
2/9/2008	51.2	16.0	1.75	9.1	0	1	
11/9/2008	57.0	14.8	1.56	9.4	0	1	Immature*

Moss Seedless on RL - Mosslands, ECM - Count 88 (late cultivar entry)							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
22/10/2008	62.0	13.7	1.34	10.2	0.6	1	Peak

Portsgate on TC - Citrus Foundation Block - Count 72/88							
Date	Juice (%)	Brix	Acid	Ratio	Seed	Colour	Maturity
5/8/2008	55.5	12.0	1.48	8.1	0	1-2	
28/8/2008	58.7	12.9	1.28	10.0	0	1	Peak
2/9/2008	54.8	13.3	1.28	10.4	0	1	

*Immature - insufficient fruit to finalise testing

Table 6.3.13.2. Yield and fruit size for Valencia selections at the CFB and ECM (Mosslands) during the 2008 season.

Selection	Rootstock	Yield (kg/tree)	Mean Fruit Size	Planted	Estimated Maturity
Turkey	Swingle citrumelo	G(50 to 70kg)	Count 64/72	2001	21/7
Delta	Troyer citrange	VG(70+ kg)	Count 72/88	2001	2/9
Rietspruit	Swingle citrumelo	G(50 to 70kg)	Count 64/72	2001	25/9 Estimate**
Delicia	Troyer citrange	VG(70+ kg)	Count 56/72	1994	2/9
Valencia Late	Troyer citrange	VG(70+ kg)	Count 88/105	1991	7/9
Midnight	Carrizo citrange	VG(70+ kg)	Count 64/88	1991	11/9
McClellan Seedless	Swingle citrumelo	VG(70+ kg)	Count 88	1999	18/9 Estimate**
Moss Seedless	Rough lemon	ECM Mosslands - Limited information			22/10
Portsgate	Troyer citrange	VG(70+ kg)	Count 72/88	1999	28/8

East Cape Citrus Region - Maturity would be 2 to 3 weeks earlier than the CFB

**Insufficient fruit to complete evaluation

Results and discussion

These Valencia selections at the Citrus Foundation Block were evaluated and internal quality was analysed to establish whether they follow similar, but later maturity patterns of the same selections in the northern areas and to check horticultural integrity. From the Valencia maturity chart in the Valencia section of this report it is evident that these selections have followed similar, but substantially later patterns.

First quarter of 2009

During this period a visit was made to Florida and California (A Lee for two weeks) to evaluate late maturing cultivars and discuss arrangements for sourcing, testing and commercializing new cultivars from Florida and California. A full report has been submitted to CEO, CRI.

In addition new and existing cultivar fact sheets were updated to bring total number of drafts 68; these are now ready for inclusion of photos prior to publication. A copy of the first 'completed' fact sheet is attached.

Attended CRI management meetings in Nelspruit during January and planned the year's evaluation programme and new trials for the UC cultivars. Started evaluations and testing of very early maturing cultivars. This data is still being processed.

The survey of the Fukumoto navel on trifoliolate hybrid rootstocks was initiated and a preliminary report is included in this report.

A number of trials have now been completed and will be published during 2009. The Delta rootstock trial at Letaba Estates has been published in the Fruit Journal and the Midnight trial will be finalised for the forthcoming edition of the journal. The publication of these results included soil sampling of these trials for root pathogens as well as the measurement of the trees to establish production per tree volume and per hectare, using tree diameters to calculate suitable planting distances for the different rootstocks.

The first stage of the triploid breeding programme is underway with the selection of 15 trees from the 3 different seed sources extracted from CFB fruit where open pollination took place. To date the results showed that three of the seedlings are triploids.

The blemish standards book has been updated with the inclusion of the Star Ruby colour and Sheepnose charts, as well as the Over Maturity charts for Oranges.

Addressed a meeting at Ryton Estates (J Joubert) with H le Roux to present data on the following:

- The newest selections available for the cool inland areas.
- The different cultivar selections suitable for the Ryton area and possible options to choose for future development.

The second talk described the different cultivar selections suitable for the Ryton area and possible options to choose for future development.

Weipe trial site (Noord Grens Boerdery) and Groep 91 trial sites were evaluated for the first time this year and a visit was made with R Fenwick to the Western Cape trial sites.

Visited the Western Cape to check existing trial sites and obtain co-ordinates and maps where necessary and view proposed trial sites for the establishment and evaluation of new (UC) cultivars. Trials have been arranged at 3 sites to date.

In the Citrusdal region an incompatibility between Thoro Temple and Swingle citrumelo was reported to CRI by one of the local technical advisors. Bark was removed from both stunted/dying trees and apparently healthy trees. Once bark was removed all Thoro Temple showed a dark ring at the bud-union.

Note: Bill Castle of Florida has not observed this problem, but his comments included the fact that there are very few Temples left in Florida.

A number of growers were contacted in the different citrus growing regions of the Eastern Cape to arrange the establishment of trial sites for the new experimental cultivars. Trials have been arranged at 4 sites to date.

Fukumoto Survey

Gamtoos Valley

Area that was surveyed is a farm known as Mistkraal, towards the upper end of the Gamtoos River Valley. The trees in this orchard are 3 to 4 years of age with a good crop. It appears that the rootstocks on which the trees have been planted are mixed. The orchard is documented as being on rough lemon, but some trees are on a trifoliolate type rootstock. All trees are uniform in size, do not show any bud union incompatibility and appear healthy. A few trees are showing some stunting which is most probably due to root health problems. Of the 400 trees examined in the survey (approximately 50% on Rough Lemon & 50% on trifoliolate types), 4 of the rough lemon trees had a few suckers extending just below the bud-union; 15 of the Trifoliolate trees displayed several suckers at ground level. There were no typical bud union suckers or any other bud union deformities. Further evaluations will include bark removal at the bud-union to see if any incompatibility exists between rootstock and scion for both rootstock varieties.

Sundays River Valley

This survey was done in the Fukumoto Navel orchards of Sun Orange Farms in the lower Sundays River Valley. The first orchards are now 6 years old. Trees displayed good uniform crops; there was however some lack of uniformity in tree size and shape. This was not attributed to any bud union problems however and in severer cases iron deficiency was evident with leaf chlorosis and even twig dieback. Bark removal of these trees at the bud-union showed no incompatibility of any kind. Of the 400 trees examined, 30 trees had suckers on the rootstock at ground level, but no incompatibility was observed. Two trees were severely stunted with only minor scion growth protruding from the rootstock. It is probable that these 2 trees were budded on zygotic or off type seedlings in the nursery. All trees had been budded onto Carrizo citrange.

These orchards, which constitute part of a very large Fukumoto planting on Carrizo, will be surveyed again during the course of the year.

Evaluations/Sampling

The start of the citrus season has prompted the sampling and evaluations of various Satsuma types as well as some of the earlier navels. Satsumas have been sampled in the Sundays River Valley, Gamtoos River Valley and in the Eastern Cape Midlands; the sampling is ongoing. Evaluation of some of the early navel varieties has begun. Two new navel varieties have been discovered this year, the 99 Navel, which is thought to be less susceptible to FCM and another navel the Riverside Red, a Cara Cara navel that has earlier external colour and a pink blush on the rind.

Cultivar Fact Sheets

This project has been initiated to supply growers with comprehensive descriptions of all cultivars being produced commercially, semi commercially and on a trial basis in southern Africa. A fact sheet describing the Miho Wase Satsuma is attached as an example layout that will be used for all cultivar selections.

Maturity Charts

Maturity charts for the cultivars being evaluated in this report appear below. These charts are based on the numerous fruit samples (1000 plus) tested during the 2008 season.

Table 6.3.13.2. Navel Maturity Periods in the Cape Regions During the 2008 Season.

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
PEN - New Exp.																												
Fukumoto																												
Newhall/Navelina																												
Fisher																												
Dream - Exp.																												
Tulegold - Exp.																												
PAN - New Exp.																												
Bahianinha																												
Palmer																												
Washington																												
Cara Cara																												
EDPN 1 - New Exp.																												
Chislett																												
EDPN 2 - New Exp.																												
Autumn Gold																												
Barnfield Summer																												
Summer Gold																												
Powell Summer																												
Witkrans																												
Lane Late																												
Cambria																												
LBN - New Exp.																												
SUN - New Exp.																												
DSN - New Exp.																												

Maturity was based on a 3 week period peaking at a ratio of 10:1.

Table 6.3.13.3. Mandarin & Mandarin Hybrid Maturity Periods in the Cold regions during the 2008 season.

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Satsuma (4 Comm.)																												
Clementine (4 Comm.)																												
Fairchild																												
Nova																												
Minneola																												
African Sunset																												
Valley Gold																												
Kiyomi																												
Nectar																												
Nadorcott																												
Mor																												
Hadas Ellendale																												
Winola																												

Note:

Maturity per selection was based on a 3 week period peaking at a ratio of 11:1.

Table 6.3.13.4. Grapefruit Maturity Periods in the Northern regions.

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Marsh																												
Nartia																												
Star Ruby																												
Ray Ruby																												
Henderson																												
Jackson																												
Nelruby																												
Flame																												
Rosé																												

Maturity was based on the minimum export ratio.

Table 6.3.13.5. Valencia Maturity Periods in the Northern region during the 2008 season.

	March				April				May				June				July				Aug				Sept			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Limpopo SL																												
Tambuti Early																												
Turkey																												
Mouton Early																												
Portsgate																												
Alpha																												
Midnight																												
Bennie 1																												
Bennie 2																												
Bend 8A1																												
Bend 8A2																												
Jassie																												
Delta																												
Ruby																												
McClellan SL																												
Lavalle																												

Note:

Maturity was based on a 3 week period peaking at a ratio of 10:1.

7 CITRUS IMPROVEMENT SCHEME (CIS)

By Thys du Toit, Richard Fenwick and Michelle Koegelenberg (CRI)

7.1 Programme summary

Citrus Foundation Block: A total of 1 878 083 buds were supplied during 2008, 131 152 buds less than in 2007. Star Ruby and Midnight have been the two most popular cultivars for five consecutive years. An increase in the demand of seed, from 1 991 litres supplied during 2007 to 2 802 litres supplied during 2008, indicates an increase in the demand for nursery trees. The structure of Shade House One was replaced with the third insect-controlled greenhouse, which was erected over the existing 10 600 increase trees. The area of insect-controlled greenhouses is currently 13 028 m². We have made provision in our budget for the erection of a fourth greenhouse of 2 016 m² during 2009. 4 530 open-ground increase- and evaluation trees were removed.

Tree Certification: During 2008 the amount of 1 611 335 trees were certified as opposed to the 1 238 727 which were certified during 2007.

Nursery Certification: 21 Nurseries were audited in May and November. 20 of these nurseries are certified, including two new nurseries. Another nursery, producing trees for their own use only, has been re-certified as a Farm Nursery.

Statutory Improvement Scheme: Discussions continued with the Department of Agriculture to establish whether the Citrus Improvement Scheme as it currently functions within the citrus industry, can be accommodated as a statutory scheme in terms of the Plant Improvement Act. This act currently requires a variety list, which will not allow for the immediate commercialization of cultivars. The Registrar of Plant Improvement has been promoted to another department and no replacement has been appointed as yet.

Protected Zone around the Citrus Foundation Block: The notification of the intention to declare the area within a 5 km radius from the Citrus Foundation Block as a citrus-free zone, has been completed and the Department of Agriculture is now responsible for ensuring approval by the minister of Agriculture and the implementation thereof. The Department of Agriculture has conducted a survey to determine the quantity of trees within this area and if the property owners are dependent on the income generated from those trees.

Shoot-Tip-Grafting and the Nucleus Block: ITSC and CRI have submitted 23 and 15 new cultivars, respectively, for establishment, evaluation and multiplication at the CFB. During 2008, material was submitted for Shoot-Tip-Grafting: 5 new cultivars at ITSC and 10 new cultivars at CRI-facilities. The ITSC nucleus block currently consists of 418 cultivars and the CRI nucleus block of 235 cultivars.

Phytosanitary status of the CIS material

To ensure that the phytosanitary status of budwood supplied to the industry from the Citrus Foundation Block remains free from graft transmissible diseases, but pre-immunised with a mild strain of the Citrus tristeza virus, budwood needs to be re-indexed every two years. In the last year it was discovered that three navel cultivars were infected with viroids. Nurseries and growers have all been fully informed of the implications in writing. Clean propagation material will be made available shortly. The CIS procedures were amended to reduce the risk of recurrence.

Programopsomming

Sitrus Grondvesblok: 'n Totaal van 1 878 083 okuleerhout is deur die Sitrus Grondvesblok verskaf in 2008 wat 131 152 minder is as in 2007. Star Ruby en Midnight is die twee mees populêre kultivars al die afgelope vyf jaar. 'n Verhoging in die verkope van saad van 1 991 liter in 2007 na 2 802 liter in 2008 voorspel 'n groot toename in die aanvraag van kwekery bome. Skaduhok 1 se struktuur is vervang met die derde insekbeheerde kweekhuis wat bo-oor die bestaande 10 600 vermeerderingsbome op gerig is. Die huidige oppervlak onder insekbeheerde kweekhuise is tans 13 028 m². Ons het begroot vir 'n vierde kweekhuis van 2 016 m² vir oprigting in 2009. Daar is reeds 4 530 oop grond vermeerderings bome en evaluasie bome verwyder.

Boomsertifisering: In 2008 is 1 611 335 bome gesertifiseer in vergelyking met 1 238 727 in 2007.

Kwekery Sertifisering: 21 Kwekerye is in Mei- en November maand ge-oudit en 20 is gesertifiseer waarvan 2 nuwe kwekerye is. Een kwekery wat slegs bome vir eie gebruik produseer is her-geregistreer as 'n plaaskwekery.

Statutêre Verbeteringskema: Voortdurende korrespondensie is nog steeds met die Departement van Landbou gehou om te bepaal of die Sitrus Verbeteringskema soos dit huidiglik in die sitrusbedryf funksioneer ge-akkommodeer kan word as 'n statutêre skema onder die Plantverbeteringswet. Die Plantverbeteringswet in sy huidige vorm vereis 'n Nasionale Variëteitslys wat sal verhoed dat nuwe variëteite/kultivars nie dadelik gekommersialiseer kan word nie. Die registrateur van Plantverbetering is bevorder na 'n ander Departement en daar is nog nie 'n opvolger aangestel nie.

Beskermdede sone rondom die Sitrus Grondvesblok: Die kennisgewing van die voorneme om 'n gebied geleë binne 'n 5 km radius buite die Sitrus Grondvesblok as 'n sitrus vrye sone te verklaar is voltooi en dit is nou die Departement van Landbou se verantwoordelikheid om dit deur die minister te laat goedkeur vir implementering. Onlangs is 'n opname deur die Departement gemaak van die hoeveelheid bome in die gebied, om te bepaal of enige grondeienaar afhanklik is van die inkomste van die bome op sy grond.

Groeipuntenting en Genebron: Vanaf die Groeipuntenting fasiliteite by die ITSG is 23 en vanaf die CRI is 15 nuwe kultivars by die SGB ontvang vir vestiging, evaluering en vermeerdering. In 2008 is daar 5 kultivars by die ITSG en 10 by die CRI ingedien vir groeipuntenting. Die ITSG se genebron bestaan tans uit 418 kultivars en die van die CRI se bystand bron uit 235 kultivars.

Fitosanitiêre status van die SVS materiaal

Om te verseker dat die fitosanitiêre status van die okuleerhout wat deur die Sitrus Grondvesblok aan die bedryf verskaf word, vry van skadelike ent-oordraagbare patogene is, maar tog 'n milde kruisbeskerminsras van die Citrus tristeza virus bevat, moet die okuleerhout op 'n twee-jaarlikse basis her-indekseer word. Die afgelope jaar is gevind dat drie nawel kultivars met 'n viroïde besmet is. Nuwe bron okuleerhout sal so gou as moontlik beskikbaar gestel word. Veranderinge is aangebring aan die SVS prosedures om the risiko van herhaling te verminder.

CITRUS FOUNDATION BLOCK

Budwood Supplied in SA & to Neighbouring countries

Area	2002	2003	2004	2005	2006	2007	2008	2002 - 2008
South Africa	2,193,932	2,341,685	2,286,730	2,571,353	2,154,257	2,005,235	1,878,083	15,431,275
Neighbouring Countries	90,050	37,700	28,000	56,175	5,500	4,000	0	221,425
	2,283,982	2,379,385	2,314,730	2,627,528	2,159,757	2,009,235	1,878,083	15,652,700

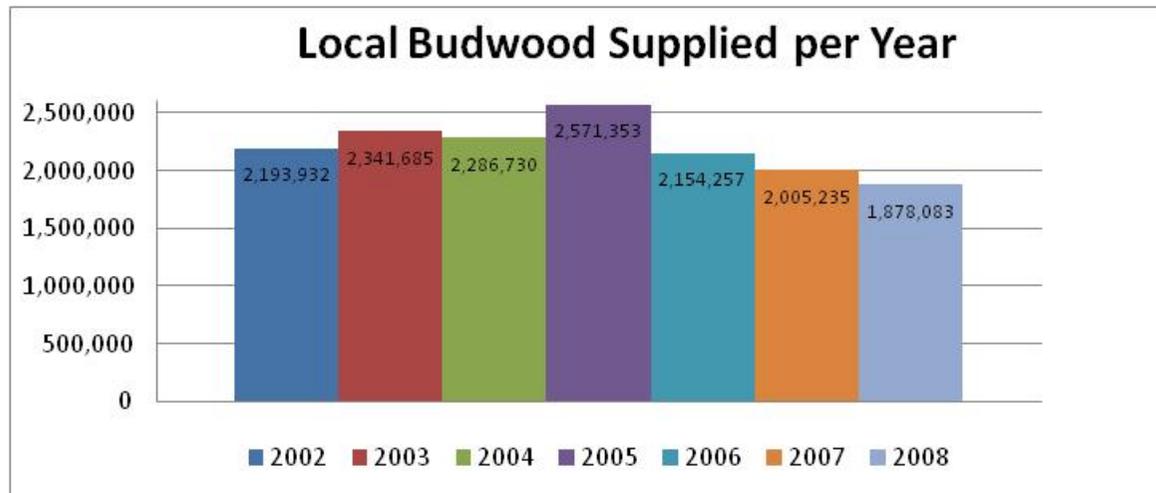
Budwood supplied in South Africa

Area	2002	2003	2004	2005	2006	2007	2008	2002 - 2008
Eastern Cape	441,902	546,660	427,080	478,079	496,900	364,310	293,067	3,047,998
KwaZulu-Natal	20,600	36,030	16,500	15,500	38,750	46,800	22,500	196,680
Limpopo	*	*	1,090,435	1,252,503	806,845	821,460	766,276	4,737,519
Mpumalanga	1,150,325	1,159,240	228,750	312,552	289,788	159,180	243,200	3,543,035
North-West	121,350	101,100	86,990	82,780	130,440	98,800	109,950	731,410
Northern Cape	156,905	116,990	68,720	46,850	39,940	49,000	46,300	524,705
Northern Province	30,000	16,000						46,000
Western Cape	272,850	365,665	368,255	383,089	351,594	465,685	396,790	2,603,928
	2,193,932	2,341,685	2,286,730	2,571,353	2,154,257	2,005,235	1,878,083	15,431,275

* Limpopo's production for 2002 & 2003 is included with that of Mpumalanga.

Budwood supplied to neighbouring countries

Area	2002	2003	2004	2005	2006	2007	2008	2002 - 2008
Mozambique		5,400		12,250	3,000			20,650
Other African States	1,400					4,000		5,400
Swaziland	49,400	10,600		42,225	2,000			104,225
Zimbabwe	39,250	21,700	28,000	1,700	500			91,150
	90,050	37,700	28,000	56,175	5,500	4,000	0	221,425



The ten most popular cultivars supplied during 2008 in comparison with the two preceding years

2008				2007				2006			
Variety	Cultivar	Qty	%	Variety	Cultivar	Qty	%	Variety	Cultivar	Qty	%
Grapefruit	Star Ruby	299500	15.95%	Grapefruit	Star Ruby	317045	15.78%	Grapefruit	Star Ruby	369740	17.12%
Valencia	Midknight	176335	9.39%	Valencia	Midknight	259195	12.90%	Valencia	Midknight	283223	13.11%
Mandarin Hybrid	Nova	126200	6.72%	Mandarin Hybrid	Mor 26	97325	4.84%	Navel	Palmer	117443	5.44%
Mandarin Hybrid	Nadorcott	115960	6.17%	Valencia	Delta	89900	4.47%	Navel	Bahianinha	109520	5.07%
Lemon	Eureka	113840	6.06%	Valencia	Du Roi	89875	4.47%	Valencia	Late	100550	4.66%
Navel	Bahianinha	88100	4.69%	Mandarin Hybrid	Nova	82900	4.13%	Mandarin Hybrid	Nadorcott	88000	4.07%
Navel	Washington	80585	4.29%	Navel	Bahianinha	77320	3.85%	Valencia	Du Roi	76890	3.56%
Valencia	Lavelle	65358	3.48%	Navel	Cambria	76880	3.83%	Navel	Washington	76692	3.55%
Navel	Palmer	53630	2.86%	Valencia	Benny	70775	3.52%	Valencia	Turkey	66700	3.09%
Grapefruit	Nelruby	49900	2.66%	Lemon	Eureka	66750	3.32%	Valencia	Delta	60230	2.79%
Top 10		1169408	62.27%	Top 10		1227965	61.12%	Top 10		1348988	62.46%
2008		1878083		2007		2009235		2006		2159757	

Budwood supplied per Variety and area for the period 2006 - 2008

Area	Year	Clementine	Ellendale	Grapefruit	Grapefruit Hybrid	Kumquat	Lemon	Lime	Mandarin Hybrid	Mid-Season	Navel	Satsuma	Seville	Valencia	Total per Area
Eastern Cape	2008	3,145		7,550			29,480	100	82,720		106,532	14,950		48,590	293,067
	2007	3,300		34,300			30,100		99,050		104,160	11,100		82,300	364,310
	2006	1,700		24,112	200	400	14,912	200	97,336	36	160,674	42,550		154,780	496,900
KwaZulu-Natal	2008			3,500			3,000		2,000		9,000	1,500		3,500	22,500
	2007					2,000	2,500	2,500	5,500		22,300	6,000		6,000	46,800
	2006			2,000		1,000	4,000	6,500	2,000		12,250	5,000		6,000	38,750
Limpopo	2008	700		196,800	6,800	500	36,000		154,650		135,510	3,000		232,316	766,276
	2007	1,500		155,845		5,800	16,100	15,850	51,600		120,320			454,445	821,460
	2006	500		174,720	500	3,000	3,900	6,800	21,060		218,730	2,000		375,635	806,845
Mozambique	2006	100	100	200	100		200	100	400		300	300		1,200	3,000
Mpumalanga	2008	2,000		80,700	300		15,900	400	5,600		55,350	2,200	500	80,250	243,200
	2007	1,150		53,250	150	2,300	3,870	2,000	6,020		13,200	2,700		74,540	159,180
	2006	1,800		132,500	3,900	3,200	15,900	7,300	8,148		46,400	5,100		65,540	289,788
North-West Province	2008	3,150		2,100		700	27,400	11,150	20,700		15,000	1,300		28,450	109,950
	2007	2,200		550	100	3,500	17,600	2,500	24,750	750	38,000	2,850		6,000	98,800
	2006	1,400	200	2,030		3,380	15,330	6,760	23,900	2,500	43,560	1,000		30,380	130,440
Northern Cape	2008	1,000		28,000			2,200		1,000		6,900			7,200	46,300
	2007			30,000			1,000	1,000			8,000	3,000		6,000	49,000
	2006	1,700		22,500			1,000		200		6,540			8,000	39,940
Other African States	2007										2,000			2,000	4,000
Swaziland	2006													2,000	2,000
Western Cape	2008	8,450	750	31,770	24,400	250	34,320	5,600	85,470	60	93,480	13,290	700	98,250	396,790
	2007	21,390	500	52,950	65	6,500	22,600	7,300	172,160	6,100	128,445	5,500		42,175	465,685
	2006	26,750	200	15,680		3,100	17,150	2,910	65,981	9,970	123,695	3,585		78,873	351,594
Zimbabwe	2006							500							500

Summary of Budwood Supplied during 2008

A higher demand was again anticipated after a successful fruit season, but this was not realized. The budwood supplied during 2008 has declined by 131 152 from that of 2007. This is similar to the decrease in budwood supply that was seen in 2007 where 281 674 less buds were supplied than in 2006. A considerable increase in budwood sales is still expected when the emergent rootstock seed sales are taken into account.

2008	1,878,083
2007	2,009,235
2006	<u>2,159,757</u>
Total	<u>6,047,075</u>

Seed supplied in South Africa and abroad during 2008 in comparison with the five preceding years

Seed Supplied in South African and Abroad (litres)

Area	2002	2003	2004	2005	2006	2007	2008	2002 - 2008
South Africa	1,447	1,237	1,917	1,592	951	1,991	2,802	11,937
Countries Abroad	1,690	1,370	1,977	762	586	1,348	1,456	9,189
	3,137	2,606	3,894	2,354	1,537	3,339	4,258	21,126

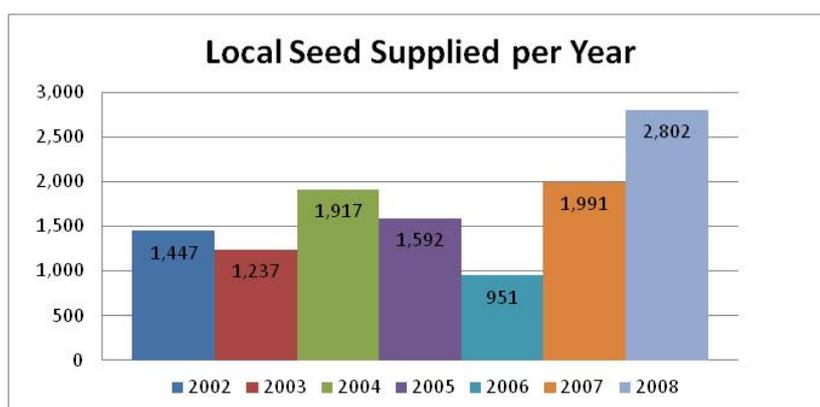
Seed Supplied in South Africa per Area (litres)

Area	2002	2003	2004	2005	2006	2007	2008	2002 - 2008
Eastern Cape	213	164	205	246	190	191	416	1,625
KwaZulu-Natal	9	12	5	18	13	32	10	99
Limpopo	*	776	1,217	886	458	1,181	1,479	5,997
Mpumalanga	808	37	72	58	11	36	76	1,098
North-West Province	46	31	75	38	32	38	51	311
Northern Cape	34	5	22	16	4		154	235
Northern Prov.	29	17						46
Western Cape	308	195	321	330	243	513	616	2,526
	1,447	1,237	1,917	1,592	951	1,991	2,802	11,937

* Limpopo's seed supplied during 2002 has been included with those of Mpumalanga.

Seed Exports per Country (litres)

Area	2002	2003	2004	2005	2006	2007	2008	2002 - 2008
Australia/NZ	405		196	38	64	32	24	759
Caribbean	47	12		7	40	75		181
China	1,040	500	1,500	325	257	1,037	1355	6,014
Europe	12	739		100	30			881
Far East	115	34						149
Mozambique			11		8	6	68	93
Other African States	29	2	157	62	27	14	7	298
South America	28	4				10		42
Swaziland	15	4						19
Thailand				30		24		54
USA		70	110	200	160	150		690
Zimbabwe		5	3				2	10
	1,690	1,370	1,977	762	586	1,348	1,456	9,189



Seed Supplied per Rootstock Cultivar per year: 2006 to 2008 (litres)

Selection	Abbreviation	2008	%	2007	%	2006	%
Carrizo Citrange	CC	1, 701.0	44.40%	1, 261.0	37.77%	579.0	37.67%
C35 Citrange	C35	588.0	17.60%	364.0	10.90%	313.0	20.36%
Swingle Citrumelo	SC	756.0	13.65%	645.0	19.32%	234.0	15.22%
Rough Lemon	RL	343.0	10.27%	158.0	4.73%	121.0	7.87%
X639	X639	228.0	6.83%	129.0	3.86%	37.0	2.41%
Troyer Citrange	TC	391.0	2.72%	500.0	14.98%	127.0	8.26%
Australian Trifoliolate	AT	50.0	1.50%	50.0	1.50%	2.0	0.13%
Minneola X Trifoliolate	MXT	146.0	1.38%	73.0	2.19%	23.0	1.50%
Volckameriana	VA	26.0	0.78%	90.5	2.71%	56.0	3.64%
Flying Dragon	FD	25.0	0.75%	50.0	1.50%	12.0	0.78%
Rough Lemon (Schaub)	RLS	2.0	0.06%	12.0	0.36%	33.0	2.15%
Yuma Citrange	YC	2.0	0.06%	6.0	0.18%	0.0	0.00%
Total Per Year		4258.0	100.00%	3338.5	100.00%	1537.0	100.00%

Summary of Seed Supplied during 2008

During 2008 a substantial increase in the local seed demand was experienced. 811 more litres were supplied than in 2007 and 1 040 litres more than in 2006. This trend substantiates our optimism that the demand for budwood will most certainly increase. Carrizo Citrange is still the most popular rootstock cultivar. The increase in seed exports is due to high demand experienced in China. As the seed export market is quite volatile, it could unexpectedly decline.

Tree Certification

Tree Certification per variety in comparison with the preceding eight years

Varieties Certified: RSA & Neighbouring Countries										2000 - 2008			
Variety	South Africa									Neighbouring Countries			
	2008	2007	2006	2005	2004	2003	2002	2001	2000	2008	2007	2006	2005
Clementine	20,849	8,310	73,892	63,719	70,839	46,442	14,512	81,680	138,902				
Ellendale	1,839		500	250									
Grapefruit	301,374	258,684	295,401	319,151	78,674	146,287	22,778	20,700	200	32,157	11,700	18,020	27,469
Grapefruit Hybrid	1,465	2,600	1,669	1,260	3,139			3,000					
Kumquat	7,517	2,260	100	450		3,000		170	1,512				
Lemon	30,194	20,319	70,012	198,728	113,119	126,905	77,002	93,681	79,619	1,500			
Lime	11,685	3,415	1,260		50	14	2,485	50	6,338				
Mandaryn Hybrid	144,667	50,870	109,714	76,267	108,768	121,421	36,980	31,664	23,042	1,700	350	300	
Midseason			981	1,484		6,630	2,405	2,032	3,927				
Navel	426,134	388,476	572,994	552,377	502,382	468,500	171,645	201,064	160,117	3,260	58,630	2,825	470
Satsuma	8,619	17,202	57,002	49,756	70,049	26,254	17,105	18,061	34,773		250	35	
Valencia	656,992	486,591	355,111	543,535	259,508	509,433	269,933	328,786	256,682	680	31,655	54,098	31,260
	1,611,335	1,238,727	1,538,636	1,806,977	1,206,528	1,454,886	614,845	780,888	705,112	39,297	102,585	75,278	59,199

Trees Certified per area and Variety during 2008 in comparison with the two preceding years

Variety	Year	Botswana	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	Namibia	North-West Province	Northern Cape	Swaziland	Western Cape	Zimbabwe	Other African States	Free State	Total
Clementine	2008		4,234							150		16,465				20,849
	2007											8,310				8,310
	2006									10,000		59,243				69,243
Ellendale	2008				1,654							185				1,839
	2006									500						500
Grapefruit	2008		27,189		34,840	128,822	80,783		4,000	16,450	32,157	9,290				333,531
	2007		15,478		40,257	110,349	89,960		340	2,300	11,700					270,384
	2006		15,474		33,700	89,789	123,831	20	260	12,052	18,000					293,126
Grapefruit Hybrid	2008						1,465									1,465
	2007				1,150	1,450										2,600
	2006				49	1,610										1,659
Kumquat	2008				7,517											7,517
	2007				1,800	460										2,260
	2006											100				100
Lemon	2008		18,031		200	4,530	843					6,590		1,500		31,694
	2007		14,911	1,800	2,725	374	379					130				20,319
	2006		17,585	1,600		1,440	33,429					2,745				56,799
Lime	2008		600			1,000	10,000					85				11,685
	2007					650	2,765									3,415
	2006		250			450						560				1,260
Mandarin Hybrid	2008		86,727		1,400	2,440	10,450		2,300			39,750		1,700	1,600	146,367
	2007	350	8,460	1,000	1,725	700	6,950		12,630			19,405				51,220
	2006		11,039		512	7,786	14,954	300	500	500		42,334				77,925
Midseason	2006										81				81	
Navel	2008	1,400	199,352	1,540	1,300	25,888	98,891	360	12,883	100		86,180		1,500		429,394
	2007	11,050	91,004	9,610	12,246	34,031	95,362		79,019	3,142		64,062	41,080	6,500		447,106

Variety	Year	Botswana	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	Namibia	North-West Province	Northern Cape	Swaziland	Western Cape	Zimbabwe	Other African States	Free State	Total
	2006	2,400	119,869	8,000	3,850	15,065	155,424	425	12,332	39,263		100,446				457,074
Satsuma	2008		4,099			3,500	820					200				8,619
	2007	250	4,551			3,222	449		8,980							17,452
	2006		21,601			5	23,165	35	700			3,287				48,793
Valencia	2008		155,434		5,400	216,950	154,445	180	57,049	4,419		63,295		500		657,672
	2007	2,105	80,322	10,300	7,767	269,542	60,058	1,950	26,123	1,582	9,100	30,897	12,500	6,000		518,246
	2006		48,262	9,400	9,320	100,401	75,192	2,165	2,525	6,777	14,120	21,622	33,863			323,647

Total Trees Certified Per Year:

2008	1,650,632
2007	1,341,312
2006	1,330,207

Summary of Tree Certification during 2008

In 2008 the amount of 372 608 more trees were certified than in 2007 and 72 699 more than in 2006. Awareness of the necessity of the tree certificates as minor-must criteria for GLOBAL-GAP accreditation has been raised amongst the citrus growers. The nurseries have been requested to apply for certificates for all trees that get sold, irrespective if the growers have requested a certificate or not.

The following nurseries were certified during 2008 as conforming to the minimum standard of the Citrus Improvement Scheme

NURSERY	ADDRESS	TOWN	CODE	TEL	FAX	CELL
APAPANZI	Posbus 147	KIRKWOOD	6120	042 230 1483	042 230 0923	082 550 6210
CASMAR	Posbus 3	MOOINOOI	0325	014 574 3152	014 574 3798	082 881 4189
CEDARBERG TREE NURSERY	Posbus 69	SIMONDIUM	7670	021 8741033	021 874 2110	083 255 8980
DU ROI	Posbus 66	LETSITELE	0885	015 3451650	015 345 1650	082 802 5559
ESSELEN	Posbus 100	MALELANE	1320	013 790 0160	013 790 0492	083 325 0565
BF JOUBERT	Posbus 193	KIRKWOOD	6120	042 230 0309	042 230 0280	084 951 1922
H J JOUBERT	Posbus 207	MONTAGU	6720	023 614 2237	023 614 2237	082 578 5747
LETSITELE	Posbus 114	LETSITELE	0885	015 345 1600	015 345 1601	083 259 5590
MISTKRAAL	Posbus 106	KIRKWOOD	6120	042 230 1461	042 230 1461	082 789 5150
NGWENYA	Posbus 36	MALELANE	1320	013 790 3004	013 790 3003	082 443 1231
ORANJERIVIER #	Posbus 369	KAKAMAS	8870	054 441 0183	086 544 9691	082 797 3833
PAKSAAM	Posbus 16	PATENSIE	6335	042 283 0201	042 283 0884	082 897 5438
STARGROW	Posbus 189	CITRUSDAL	7340	022 921 2232	022 921 2747	082 563 0795
SONDAGSRIVIER	Posbus 304	KIRKWOOD	6120	042 230 0349	042 230 0510	083 227 6655
TWEELING	Posbus 190	KIRKWOOD	6120	042 230 1408	042 230 1408	082 560 2179
VAALHARTS	Posbus 317	HARTSWATER	8570	053 474 0565	053 474 1926	082 948 2552
WATERFALL	Posbus 339	ADELAIDE	5760	046 684 0738	046 684 1451	082 695 3433
WESTFALIA	Posbus 14	MODJADJISKLOOF	0835	015 309 0050	015 309 0045	083 652 2138
WITKRANS	Posbus 17	BOSHOK	0301	014 573 3036	014 573 3036	082 922 1579

New Nurseries

Production at the Citrus Foundation Block

The second insect-controlled greenhouse is now in full production with an amount of 45 700 increase trees. The increase trees in the first insect-controlled greenhouse have expired and need to be replaced. The structure of Shade House One has been replaced with the third insect-controlled greenhouse, which was erected over the existing 10 600 increase trees. Provision was made for the erection of the fourth insect-controlled greenhouse in 2009. This structure is similar to Greenhouse 3 and has an area of 2 016m².

The phasing out of open-ground increase and evaluation trees is nearly completed. 4 530 trees were removed, with only 117 Nova and 250 Miho Wase trees remaining. These will be removed once sufficient increase trees have been multiplied and established under insect-controlled structures. We aim to have all increase trees under insect-controlled structures and only seed source trees will remain in the open-ground orchards.

Nursery Certification

During May and November 2008, 21 Nurseries were audited and certified as conforming to the minimum Citrus Improvement Scheme guidelines. Two new nurseries were visited and one nursery was re-certified as a farm nursery, as the trees that they propagate are exclusively for their own use. The general standard is good. However, the growers are urged to visit the different nurseries before they select a nursery, as differences do occur. All tree orders must be placed in writing, stipulating specific requirements. The growers must not accept nor plant substandard trees. In an article to be published in the October/November edition of the SA Fruit Journal the purpose and meaning of tree certification will be explained.

Statutory Improvement Scheme

Discussions continued with the Department of Agriculture to establish whether the Citrus Improvement Scheme as it currently functions within the citrus industry, can be accommodated as a statutory scheme in terms of the Plant Improvement Act. This act currently requires a variety list, which will require evaluations before a cultivar can be added to the variety list. This can cause delays of up to 5 years before such cultivars can be commercialized. No conclusion regarding the proposed amendments of the Plant Improvement Act, also to be considered by the soft fruit and wine industries, has been received. Negotiations will be continued as it is still our aim to have the Citrus Improvement Scheme registered as a statutory scheme. This was further delayed due to the fact that the Registrar of Plant Improvement has been promoted to another department and a suitable replacement has yet to be appointed.

Protected Zone around the Citrus Foundation Block

The notification of the intention to declare the area within a 5 km radius from the Citrus Foundation Block as citrus-free has been completed and the Department of Agriculture is now responsible for ensuring approval by the minister of Agriculture and the implementation thereof. On 31 July 2007 all residents affected by this legislation were invited by the Department of Agriculture to a meeting held at the CFB. This notification was explained by a representative of the Department of Agriculture and a two-week grace period was given for the submission of objections. No objections were received. The Department of Agriculture has recently conducted a survey to determine the quantity of trees within this area and whether the property owners are dependent on the income generated from these trees.

Shoot-Tip-Grafting and the Nucleus Block:

Two institutions are currently responsible for Shoot-Tip-Grafting. The Institute for Tropical and Subtropical Crops in Nelspruit is responsible for cultivars imported under quarantine, as well as local cultivars. The virology department of Citrus Research International (CRI) in Nelspruit deals with local cultivars only. Both of these institutions will be individually providing a full report.

For the period of 01/04/2008 to 31/03/2009

Services Provided	ARC - ITSG	CRI-Virology
Cultivars received for STG	5	10
Cultivars supplied to the CFB for establishment	23	14
Cultivars in the Nucleus Block of each institution	418	235

Phytosanitary status of the CIS material

To ensure that the phytosanitary status of budwood supplied to the industry from the Citrus Foundation Block remains free from graft transmissible diseases, but pre-immunised with a mild strain of the *Citrus tristeza* virus, budwood needs to be re-indexed every two years. In the last year it was discovered that three navel cultivars were infected with viroids.

The Cambria – C is infected by a group IIa viroid which is associated with the induction of dwarfing but with no other harmful effects on the tree. The Advisory Committee advises that material can still be supplied until clean material becomes available; however, very strict control measures need to be in place to prevent the contamination of other trees. A new source is currently in the process of multiplication and should be available by the end of 2009. Nurseries and growers have all been fully informed of the implications in writing.

The Cambria-R (Royal Late) and the Witkrans Navel have been infected with a group III viroid, which along with dwarfism, also induces gum pocketing in trees. The Advisory Committee recommended that the supply of this material temporarily be suspended until more information regarding this virus is known and a risk assessment has been done. A clean gene source of Cambria - R (Royal Late) was available, has been pre-immunised and should be available at the CFB shortly, where it will be multiplied as soon as possible, and should be available to the industry by January 2010. Unfortunately the Witkrans gene source was infected and has to go through shoot-tip-grafting again. Budwood from the new source should be available in January 2011. Nurseries and growers have all been fully informed of the implications in writing.

The following changes were made to the CIS procedures to reduce the risk of recurrence:

1. Combining the re-indexing for CTV and CVd every two years by budding the Etrog indicator on the “Mexican” lime indicator after the CTV indexing was done. This was implemented in 2006. The only problem experienced so far is that Etrog is also sensitive to CTV and when severe CTV is present in the mother tree being indexed, growth of the Etrog is retarded which interferes with the CVd symptom expression.
2. All existing nucleus block plants to be budded with the Etrog indicator for CVd. This was implemented during the beginning of 2009. The tunnel where the plants are housed does not have heating facilities and results may be retarded by the cold winter months.
3. PCR diagnostic protocols are being optimised for implementation in the CIS procedures. This will not replace indexing as the primary diagnostic technique, but will be implemented as a validation phase to confirm all negative results following indexing.
4. All nucleus block material will in future be re-indexed at time of supply to the CFB or for export purposes and no material will be released until indexing results are known.

These changes were implemented during 2008.

New Personnel

Louise Jackson was the CIS administrator for the past 22 years. She resigned at the end of July 2008. Michelle Koegelenberg has been appointed in this position. Richard Fenwick was contracted as the CIS Technician, in July 2008. His duties have been divided between the CFB, cultivar evaluations and horticultural experiments in the Eastern Cape.

8 INTERNATIONAL VISITS

8.1 Combined Report on visit to Wuhan, China, to attend the 11th International Society of Citriculture Congress - 26-30 October 2008

8.1.1 Introduction (Tim Grout)

The history of citriculture in China can be traced back 4 000 years ago to mandarins and pummelos being listed as tributes to the Emperor Da Yu in the book Yu Gong. Commercial citrus production began at least 2 500 years ago. During the last 30 years, citrus production expanded rapidly. In the 1980s the annual growth rate for the world citrus industry was 2.2% but in China it was 16.1%. In 1990 the area planted to citrus in China was already the highest of any country in the world but due to low production they only passed Brazil in having the greatest citrus production in the world in 2007-8. Most citrus is consumed locally as fresh fruit with only 5% being processed but exports have been increasing rapidly and China is now ranked fifth in the world for citrus exports. Approximately 70% of the citrus grown is either mandarins or tangerines with about 15% being oranges. Most citrus is grown in the tropical and subtropical regions with each of the provinces Hunan, Fujian, Guangdong, Sichuan and Guangxi producing more than 2 million tons.

The congress was well attended by international delegates, especially those from Australasia, which was relatively nearby, and Americans who were interested in Huanglongbing. It served as a good opportunity to get an update on citriculture and market access challenges faced by different parts of the world. In Vaughan Hattingh's talk (V. Hattingh & TG Grout. Conflict between some market forces and scientific developments that drive the development of future citrus production and fruit handling practices) he encouraged the development of an international cooperative front on food safety in order to resist the multiplication of standards and phytosanitary regulations that serve as trade barriers.

8.1.2 Integrated Pest Management (Tim Grout)

Visits to citrus farms and institutes

Guangdong province, South China

From around 1974 in China, large farms were broken up into smaller farms, although all land is still owned by the government. This resulted in poorer management of citrus and probably caused an increase in the spread of Huanglongbing (HLB). Around this time, longan and litchi got better prices than citrus which also resulted in neglected citrus orchards. Very recently the tax laws changed so that farmers no longer pay tax. This is helping to boost agricultural production. Two mandarin farms were visited north-east of Guangzhou near the north tributary of the Pearl River on 23 October 2008. Both farms were closely planted without interrow space for tractors and dry grass mulch covered much of the orchard floor. Both farms had Asian citrus psyllid *Diaphorina citri* and trees showed symptoms of HLB. One farm had cement tanks containing an OP mixture in water that could be pumped into knapsack sprayers (Fig. 8.1.2.1). There were various other pests such as mealybug and aphids present with their natural enemies so presumably the trees were not sprayed very frequently.



Figure 8.1.2.1. Pesticide mixture waiting to be used on citrus in Guangdong.

On 24 October I visited the South China Agricultural University in Guangzhou where Prof. Yijing Cen (cenyj@scau.edu.cn) showed me HLB symptoms in citrus, *Murraya paniculata* and *Clausena lansium* (known as wampee) (Figs. 8.1.2.2 & 8.1.2.3). They had a *Murraya* plant grafted onto citrus with HLB symptoms more evident in the citrus than the *Murraya* (Fig. 8.1.1.4). This university has a brand new Entomology and Plant Pathology building with research being conducted on a broad range of topics including fire ants and taxonomic studies on carabid beetles important in agriculture (by Dr Tian Mingyi). The Deputy Director of the university is a nematologist Prof. Xinrong Wang (xinrongw@scau.edu.cn). Dr Xiaoling Deng (xldeng@scau.edu.cn) was very hospitable and is conducting molecular work on HLB with some parallels to Gerhard Pietersen's research.



Figure 8.1.2.2. Blotchy mottle in *Murraya paniculata*. **Figure 8.1.2.3.** Blotchy mottle in *Clausena lansium*.



Figure 8.1.2.4. Citrus showing blotchy mottle on left that grafted onto *Murraya* plant (right) without obvious blotchy mottle symptoms.

Later on 24 October I visited the Guangdong Entomological Institute a government institute working with invasive species and citrus. Approximately 10 years ago their funds for salaries were cut severely so they had to generate their own funds to supplement their salaries. They are therefore breeding animals for medical research and produce a large amount of entomopathogenic nematodes for sale. The Deputy Director Prof. Richou Han (richou-han@163.net) was knowledgeable of the EPN rearing process and the institute was also growing fungi for sale for medicinal purposes. There was no shortage of money for equipment and there were well equipped molecular labs with few researchers to make use of them. Emeritus Prof. Liying Li (lily@gdei.qd.cn) known for biological control is still busy at the institute. Prof. Gecheng Ouyang (vgctzz@sohu.com) was conducting push-pull research with *Diaphorina citri* in citrus where oil sprays were being used to repel and reduce oviposition while *Murraya* plants were being used as trap crops.

The mid-congress tour on 29 October included citrus orchards and glasshouses at a Wuhan university but due to continuous rain nothing could be learnt about the citrus. A solar-powered light trap over two bowls of water (Fig. 8.1.2.5) was being advertised by Shenzhen Fuwaysun Technology who sell various types of traps with and without solar power (www.solarfws.com).



Figure 8.1.2.5. Solar powered light trap.

On 1 November the post-congress citrus tour visited Yichang Xiaoxihong Mandarin farm NW of Wuhan which is a cooperative with 467 ha farmed by 10 groups. In 2007 they sold 7000 tons of fresh fruit, some of which was exported. Most orchards were on steep slopes so a railway system was used to winch picked fruit from the orchard. Both overhead plastic covers and under-tree plastic or foil sheets were used to prevent rain from affecting internal quality shortly before harvest. This farm claimed to use IPM and had many yellow sticky traps on bamboo poles at canopy height that were catching a diverse range of insects, both beneficial and pestiferous (Fig. 8.1.2.6). These traps were also seen at a second farm the same day. Indiscriminate electric exterminators were also used on some electricity poles above the orchards (Fig. 8.1.2.7). Releases of *Neoseiulus barkeri* in small sachets attached to every fifth tree trunk had also been made some time in the past but little information could be obtained about this (Fig. 8.1.2.8).



Figure 8.1.2.6. Yellow traps used in citrus orchards



Figure 8.1.2.7. “Bug zappers” above citrus orchard



Figure 8.1.2.8. Sachets attached to every fifth tree containing predatory mites

Information from talks, posters and discussions at the ISC Congress in Wuhan

Role of Chemical Control in the Integrated Management of *Diaphorina citri* and Huanglongbing Disease in Florida Citrus (81)

Qureshi JA, and Stansly PA (jawwadq@ufl.edu)

Determine shoot density in 36"x36" quadrat and count how many coccinellids seen in 1 minute. Coccinellids taken out by summer sprays for 18 d but ACP only 3 d. Spraying high populations on the flush is ineffective. Foliar treatments in the dormant season are most effective and protect the spring flush. The limit for imidacloprid in the USA is 0.56 kg/ha/yr. The EZ-dose sprayer is used to apply drenches of 8 fl. oz. liquid per tree.

Biologically Based Management of the Citrus Psylla *Diaphorina citri* and Huanglongbing in Florida (85)

Stansly PA, and Qureshi JA (jawwadq@ufl.edu)

Coccinellids are the most important natural enemies of ACP; the impact of *Tamarixia radiata* is low. *Amblyseius swirskii* does prey on ACP in the lab but impact in field unknown. Fogging with oil being investigated for ACP control. Use London fogger not thermal fogger and better if mixed with azadirachtin. Only use 1 gal oil per acre and can spray 500 acres per night. Spray at night so that psyllid not disturbed. Target adults as they are the vectors. One or 2 sprays or a Temik treatment in dormant season most effective. Selective sprays at petal fall and summer OP if necessary.

Olfactory and Electroantennogram Responses of Asiatic Citrus Psyllid Adults to Host and Non-host Plant Volatiles (96)

Cen YJ, Xu D, Qi XX, Beattie GAC, and Liang GW (gwliang@scau.edu.cn)

Used a four arm olfactometer and ground guava leaves or oil extract caused Asian Citrus Psyllid females to stay a shorter time in the test zone than water only. This result was not found with psyllid males. These results differed from other research conducted with a Y-shaped olfactometer.

Study on Potential Distributions of *Bactrocera dorsalis* (Diptera: Tephritidae) in Middle Reaches of Yangtze River by CLIMEX (246)

Li XX, Li JF, Qiu GQ, Cai WL, and Zhang HY (hongyu.zhang@mail.hzau.edu.cn)

Bactrocera dorsalis is not present in China. Cold stress and dry stress is minimal in SE China. Cold stress is probably the most important with respect to global warming but many other factors play a role. One scenario showed *B. dorsalis* moving inland as far as the Yellow River by 2020.

Biological Control of Citrus Key Pests in Spain (247)

Urbaneja A1, Jacas JA (aurbaneja@ivia.es)

More than 100 pests occur in Spanish citrus but most are under biocontrol. *Euseius stipulates* is effective against citrus red mite and *Anagyrus pseudococci* is more effective against citrus mealybug than *Leptomastix*. The parasitoid *Diachasmimorph tryoni* is to be released soon against Medfly and releases of *Fopius arisanus* and *D. longicaudata* are planned in the future. Carabid beetles were found to prey on twice as many Medfly pupae in the lab as wolf spiders. Releases of pupae in the field showed some impact from these predators. California red scale is becoming increasingly problematic and releases of *Aphytis melinus* are being made

because the biocontrol by other natural enemies is inadequate. Cover crop management is important for *Tetranychus urticae*. A cover of *Festuca arundinacea* grass resulted in less mites and less aphids in the citrus because natural enemies could increase on the ground cover. The grass can be sown in Autumn without extra irrigation. Aphids can reduce the flowering of Clementines.

The Integrated Management of Diseases and Pests in Sicily Citrus (248)

Tumminelli R, Calcaterra S, Pasciuta G, Pasotti L, Raciti E, Riolo G, Sapienza E, Sciacca V, Tamburino V, Pedrotti C, and Vinci A

Aspidiotus nerii is still the key pest of lemons in Sicily. Medfly and *Pezothrips kellyanus* are problematic on navels and California red scale is the most important pest of Tarocco. Chlorpyrifos is used for thrips control. Degree-day timing is being used to release parasitoids and apply treatments for red scale. Have €1 million for a seven year programme of rearing and releasing *Aphytis*.

Insecticide Resistance Management of California Red Scale and Citricola Scale Populations in the San Joaquin Valley of California (249)

Ouyang YL, and Grafton-Cardwell EE (yuling@uckac.edu)

OP and carbamates resistance test can be done in one day using enzyme test and alpha-navarone. Pyriproxyfen used since 1998 but no field failures yet. Resistance test can't be done with enzymes so a ring is drawn around newly settled crawlers on a fruit, dipped for 10 s in pyriproxyfen 10 ppm and evaluated after 14 d. No cross resistance with OPs. OP resistance test for citricola scale: ring first instar on leaf, dip in 178 ppm chlorpyrifos solution and place leaf on wet sponge. Evaluate after 5 d.

Current Status of Citrus IPM in the San Joaquin Valley of California (250)

Grafton-Cardwell EE (bethgc@uckac.edu)

Many orchards only receive two pesticide treatments a year in the SJV. Spinosad has been the dominant pesticide since 1998. Imidacloprid is not very effective against red scale because the dose used is one third of the drench dosage registered in South Africa. They get control of scale on the fruit but not on the leaves or wood where the dosage is enough to kill *Aphytis* and therefore results in a scale repercussion the next season. Pyriproxyfen is therefore preferred but is only used every second year. An OP is used in the alternate years for citricola scale and katydids. Imidacloprid as a systemic is toxic to *Rodolia* for 6 months and pyriproxyfen sprays for 7 months. Pyriproxyfen sprays are delayed until *Rodolia* has done its job. Mealybug is not a problem in SJV, only southern California. Spinetoram is now more popular for thrips control than spinosad. Sometimes a very low dose of pyrethroid is used for katydid control instead of an OP and this is usually combined with spinosad for thrips. Some intervention thresholds used are as follows: red mite 8 females per leaf, look at 100 leaves per 10 acres. Thrips look at 100 fruit twice a week while 4-30 mm diameter. Katydid spray on sight. Red scale 1000 males per pheromone trap in previous autumn; spray in spring. *Aphytis melinus* released at 5000/acre every 2 weeks from 1 Mar to 31 Oct = 100 000 per acre which same cost as spray. Also use pressure washers in packhouse. Spirotetramat being used experimentally. Phil Stansly said it wasn't effective against Asian Citrus Psyllid. Only had citrus leafminer since 2005 and doesn't occur on spring flush. Same parasitoids as peel miner but latter getting worse and attacking more cultivars. Glassy winged sharpshooters only sprayed once every three years with imidacloprid or acetamiprid. ARC IMS being used by growers to enter scouting data on a map that is available on the internet. Rearing and releasing *Euseius tularensis* does not work because it is a generalist feeder but pruning trees generates flush that results in higher numbers of *Euseius*. Branches tapped over sticky sheet to monitor ACP. Fenpyroximate and acequinocyl are being used for mite control. Spinosad is often used with oil.

Evidences to support that Citrus leprosis virus and its mite vector interact in a circulative – but not propagative – manner (P349)

Freitas-Astua J, Bastianel M, Nicolini F, Schons J, Kitajima EW, and Machado MA (jfastua@centrodecitricultura.br)

Citrus leprosis virus does not reproduce within the *Brevipalpus phoenicis* vector.

Molecular Fingerprinting and Population Dynamics of the False Spider Mite *Brevipalpus* spp (P380)

Mata J, Sétamou M, and Louzada ES (elouzada@ag.tamu.edu)

Where *B. californicus* and *B. phoenicis* were present in the same orchard in the Lower Rio Grande valley they could be readily distinguished using molecular techniques.

Biological control in citrus in Spain: from classical to conservation biological control (P366)

Jacas JA, and Urbaneja A (jacas@camn.uji.es)

Abamectin sprays are considered harmful to *Rodolia cardinalis* and *Cales noacki*. Staphylinids and spiders are the most important predators on the ground. Oleander is being used as a windbreak because the oleander aphid *Aphis nerii* is specific to oleander but its natural enemies are beneficial against citrus pests. *Oxalis pes-caprae* from South Africa also provides for alternative prey that natural enemies can feed on.

Ground-Dwelling Predators in Citrus Orchards in Spain and Its Predatory Ability on the Mediterranean Fruit Fly (P387)

Monzó C, Sabater B, Urbaneja A, and Castañera P (castan@cib.csic.es)

In Spain, most abundant ground-dwelling predators are staphylinids 38.6%, spiders 28.9%, Dermaptera 18% and carabids 12.7%. A PCR was successfully used to detect Medfly DNA in predators that had fed on flies using ITS2. This was effective 50% of the time for up to 87 h after feeding.

Field Experiments towards the Development of Strategy for the Control of the MedFly (*Ceratitis capitata*) using ADDRESS (Syngenta) in Citrus Orchards (P381)

Mazih A, Eltazi S, Srairi I, Sahil S, Bouguiri H, Miloudi M, Moubaraki Y, Bourachidi Y, Elmourhir T, and Lamqadem K (mazih@iavcha.ac.ma)

A plastic yellow bait station containing attractants Trimedlure, trimethylamine and ammonium acetate held a gel with lufenuron as a bait. Twenty to 25 bait stations per ha gave similar control to baiting when started 6 to 8 weeks before fruit susceptible.

Manipulation of Soil-Dwelling Predatory Mite Populations for Citrus thrips IPM (P370)

Baker G, and Crisp P (baker.greg@saugov.sa.gov.au)

Municipal green waste at 200 m³ per ha provided better control of *Pezothrips kellyanus*. Cost of application was offset within one year by improved crop (better water retention?) and reduced input costs. Soil predatory mites such as *Pergamasus* sp. increased significantly. Measured impact of sprays on soil fauna by spraying at 2000 L/ha with run-off and collecting samples of soil for Berlese funnels before and 2 d after spraying. Run-off from thiamethoxam, buprofezin, spinetoram and spinosad had little effect on soil predatory mites that were harmed by run-off from chlorpyrifos or methidathion. The success of this technique is largely because 100% of this thrips pupate in the soil unlike our citrus thrips that also pupates in the tree.

Recommendations – IPM

- Investigate possibility of using spirotetramat and spinetoram for citrus psyllid control.
- Guangdong Entomological Institute may be prepared to share techniques used for commercial rearing of entomopathogenic nematodes.
- Preblossom thrips treatments that will control citrus psyllid may be effective in preventing psyllid numbers from building up later in the season. Treatments that would cause a residue problem later in the season could be used at this time.
- Research on Attract and Kill fruit fly solutions should continue.

Presentations by CRI staff

Grout TG, Moore SD, Hofmeyr JH, and Hattingh V **The Evolution of Citrus IPM in Southern Africa.**

8.1.3 Crop & Fruit Quality Management (Stephan Verreyne & Paul Cronjé)

Stephan Verreyne

In this report I am not going to address all the papers presented in this area, but rather focus on some specific papers or parts of papers that we can apply in our research efforts. The highlight for me was the session on Citrus regulation using hormones. In this section possible alternatives for fruit size improvement, such as thinning with NAA and using 2,4-D (without thinning), was identified. NAA also gave good results in reducing fruit splitting and creasing incidence. The 2,4-D research on the reduction of the navel end size of navel oranges presented by me (Stephan Verreyne) (sv@cri.co.za) [Abstr. 163] got a good response. I had some good discussions with some Chilean researchers on improving the method of application of 2,4-D.

Carol Lovatt (carol.lovatt@ucr.edu) [Abstr. 157] presented some work on the use of the cytokinin, CPPU and 3,5,6-TPA (Maxim) on 'Washington' Navel Orange for fruit size and yield improvement. CPPU reduced total yield and had some phytotoxic effects and 3,5,6-TPA gave similar results on fruit size to what we have seen in South Africa..

Joseph Greenberg (yogreen@shaham.moag.gov.il) [Abstr. 158] reported that naphthaleneacetic acid (NAA, 300 mg·L⁻¹) applied on 'Nova' mandarin at 20mm fruit diameter thinned 34% of the fruit resulting in larger fruit that resulted only in a 9% yield reduction. Application of NAA at 28 mm diameter did not thin and had no effect on fruit size. 5% Bonus –NPK was applied in combination with all the NAA applications. Early and later NAA applications reduced fruit splitting and creasing incidence (from 38% to 7%). GA₃ reduced creasing incidence (from 38% to 5-11%), but had no effect on fruit splitting.

The work on 2,4-D for fruit size improvement by Thomas Chao (cthomaschao@gmail.com) [Abstr. 159] concluded that 2,4-D at 24 ppm applied at 30 days after 75% petal drop gave the best fruit size improvement on Nadorcott and Minneola Tangelo. 2,4-D is already registered on navel, valencia and grapefruit in California. It would be interesting to see what fruit size response NAA and 2,4-D would give on grapefruit under South African conditions.

Graham Barry (ghbarry@gmail.com) [Abstr. 162, 164] presented his work on colour improvement and vegetative growth retardation using growth retardants, similar to what was presented at the 2008 CRI Symposium.

In the session on abiotic stress physiology, Jim Syvertsen (jmsn@ufl.edu) [Abstr. 227] reported that 50 % shade cloth increase net CO₂ assimilation and stomatal conductance but had no effect on leaf transpiration of two-year old 'Valencia' orange trees. It is interesting since shade net was put up on 2 ha of Mihowase Satsuma this season in Citrusdal. In a separate presentation by A Sadka (vhasadka@volcani.agri.gov.il), [Abstr. 71] anti-hail nets on Or Mandarin increased minimum and reduced maximum temperatures, increased relative humidity and reduced wind. The white and transparent nets used reduced water consumption, reduced the number of leafless flowers (white blossom) by more than 5-fold and increased the number of vegetative shoots 3-fold and increased yield.

In a study by TTM Pham (T.Pham@exchange.curtin.edu.au) [Abstr. 222] different surfactants were tested on 'Washington' navel orange to reduce albedo breakdown (creasing). Five 2% Ca(NO₃)₂ applications at 10-day intervals starting from 81 days after full bloom in combination with the surfactants Tween 20, Tween 80, Triton X-100 or Tergitol at 0.05% were compared. Tween 20 was the most effective surfactant with Ca(NO₃)₂ in reducing albedo breakdown.

Ahmed Ait-Oubahou (aoubahou@iavcha.ac.ma) [Abstr. 223] reported that foliar application of Ca or K in the summer period reduced the number of 'Nova' mandarin fruit, and the severity of splitting and creasing at harvest.

One aspect of concern was the low number of nutrition papers presented. Japie Kruger (ohsss@bigpond.com) [Abstr. 202] presented a review on open hydroponics systems (OHS) and its uses and advantages in citrus. Apart from that one, there were a few papers on spraying different nutrients to control or reduce disorders such as creasing, splitting, rind staining and rind breakdown. This lack of nutrition research presents a great opportunity for the future CRI nutritionist to make his mark in this area.

Post-Congress Tour

On the post-congress tour we visited some commercial citrus orchards. Although they are producing citrus mainly for the local market we saw two interesting horticultural practices implemented in some orchards. Covering citrus trees completely with plastic, basically growing it in greenhouses ensures longer hanging of fruit and keeps rain away from the rootzone to increase total soluble solids in fruit just before harvest, something similar to what we do when we water stress Satsumas before harvest to increase the solids. Reflective plastic or foil ground cover in some of these greenhouses as well as in the open fields (non-covered trees) is also implemented in some orchards to ensure a drier rootzone to increase solids (sugars) in the fruit before harvest. Harvest time normally coincides with the rainy season and excess water at this time normally results in the decrease of the total soluble solids in fruit.

Recommendations – Crop & Fruit Quality Management

A lot of work still needs to be done or work already done are not at a stage yet where a commercial recommendation can be made from the papers presented. However, from a research point of view a few new research ideas came out (2,4-D and NAA for fruit size improvement), some of which were probably not even considered a few years back in South Africa.

Papers and posters presented by CRI staff

Paper

Verreyne, J.S. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges - A preliminary study. 11th International Citrus Congress. Wuhan, China, 26-30 October 2008.

Poster

Verreyne, J.S., van Kerwel. W. The benefits of hand thinning Nules Clementine mandarins (Poster). 11th International Citrus Congress. Wuhan, China, 26-30 October 2008.

Paul Cronjé

Presentation

The effect of potassium, calcium and magnesium foliar applications on postharvest rind breakdown of 'Nules Clementine' mandarin

Paul J.R. Cronjé¹, Graham H Barry², Marius Huysamer³

Rind breakdown (RB) is a postharvest physiological disorder of 'Nules Clementine' mandarin developing 5-10 weeks into storage, which has a negative economic impact on exports. The first visible symptom of rind breakdown is a darkening of an oil gland followed by the adjacent tissue, leading to tissue discoloration. This physiological collapse of the oil gland structure occurs randomly over the fruit surface resulting in the oil leaking into the subcutaneous flavedo tissue. During 2007 an experiment studying the effect of foliar nutrient applications on this postharvest condition was conducted. Foliar applications commenced after fruit set in October and were repeated at monthly intervals until April. The nutrient applications were chosen as high and low concentrations according to industry guidelines: potassium 2 and 6%, magnesium 2 and 6% and calcium 1 and 4%. Fruit were harvested from inner shaded and outer exposed bearing positions during 19-21 May, degreened, packed (including fungicides and wax) before being cold stored at 7.5°C. After 4, 6 and 8 weeks of storage RB incidence was scored. Thereafter the flavedo was removed to determine the total mineral content of this fruit tissue. The incidence of RB followed the familiar pattern of inner shaded fruit having significantly higher occurrence than outer exposed fruit. The 6% Mg treatment resulted in a significant reduction of RB on the inner shaded fruit. The same trend was evident on outer exposed fruit, but due to lower RB incidence the effect was not significant. These results indicate that the condition of the rind with respect to the mineral content, and especially magnesium, seems to play a vital role in determining the sensitivity of the rind towards RB. The exact role of magnesium, or other minerals, in limiting RB incidence is unclear and requires further research.

The following presentations were of relevance to my field of study - problems associated with postharvest handling of citrus fruit and the physiological disorders

Regulation of carotenoid biosynthesis in citrus fruit: progress and biotechnological application [9] Alquezar, B, Rodrigo MJ, Pons E, Pena L and Zacarias L. (lzacarias@jata.csic.es)

Carotenoids are the molecules in the rind and pulp that not only make up their attractive appearance but also greatly contribute to the antioxidant capacity. The identification of their metabolic pathways and the regulatory genes involved will give a better understanding of their synthesis and also offer opportunities for manipulation. This research has added to the current fundamental knowledge on colour development of citrus fruit and should be taken note of in future research on colour research.

Postharvest research programs on citrus at the USDA/ARS citrus and Subtropical products laboratory. [42] Baldwin EA. (liz.baldwin@ars.usda.gov)

This laboratory does their majority of research on citrus (70%) and they have two primary projects. The first project involves the quality of citrus juice, which includes a flavour and sensory panel which aims to discern the influence of greening disease on the juice quality. The second project aims to develop and evaluate edible coatings to reduce water loss, decay and improve fruit appearance. In a further spin-off they try to develop products from citrus processing waste.

Effects of postharvest application of hot water and molybdenum dips on the production of total antioxidants in lemon rind during cold storage and their ability to scavenge reactive oxygen species. [45] Mathaba N, Bower JP, and Bertling I. (2031300865@uknz.ac.za)

The focus of this research, financed by CRI, is to increase the antioxidant content in the flavedo in order to decrease the susceptibility to chilling damage. They showed that hot water and Mo dips do increase the amount of antioxidants in the rind but the original amount at harvest could be the most influential factor in determining chilling susceptibility of lemon fruit.

Changes in sensitivity to mechanical damage in relation to cultivar, maturity, degreening process and storage [49] Larrigaudiere C, Schotsmans W, and Recasens I. (irecasens@hbj.udl.cat)

This group showed that respiration could be used as a marker to indicate mechanical injury of citrus fruit. They showed that the difference between cultivars susceptibility to mechanical damage is due to rind thickness. Immature (more green) fruit were also reported to have higher sensitivity to mechanical stress. Degreening did not increase the sensitivity to mechanical damage and sensitivity to mechanical damage decreased significantly during storage.

Washing methods influence colour development during degreening of grapefruit, oranges and tangelo fruit. [52]. Ritenour MA. (ritenour@ufl.edu)

Mark studied the impact of different washing techniques in the pack line on colour development. He found that colour development of red grapefruit washed on brushes or high-pressure washer systems after degreening was decreased. The treatments that inhibit fruit colour development also resulted in the greatest rate of water loss during degreening and storage. This study indicates that there is a certain amount of damage being done to the epidermal cell layer during washing and brushing which is negative for fruit colour development.

Ethanol fermentation metabolism and accumulation of off flavours in citrus fruits [54] Shi JX, Goren R, Goldschmidt EE and Porat R. (rporat@volcani.agri.gov.il)

The results presented by the Israeli postharvest research group explained why mandarin fruit and not grapefruit developed off tastes during postharvest storage. The difference in ethanol and acetaldehyde content in the juice are due to the higher respiration rates of mandarins as well as their less permeable rind resulting in a build up of gasses and anaerobic conditions in the internal fruit atmosphere. This research highlighted the need for different handling practices for specific citrus cultivars.

Grappling with granulation in imperial mandarins [149]. Hofman H, Smith M and Walsh K. (walsh@cqu.edu.au)

Granulation or internal dryness is a significant problem affecting Imperial mandarins in Australia. However, after 60 years of research the underlying causes still remained unclear. In this study they pursued the hypothesis that the cell wall thickening and gelation associated with granulated vesicles could be related to high water potential in the juice cells. This could be due to too low sugar content of high turgor pressure. Various field experiments will be done to test this hypothesis.

Difference of cell wall metabolism in pericarp of citrus fruit and its relation to creasing fruit ratio [150]. Li J, Zhang PP, Chen JZ and Yao Q. (cjzlx@scau.edu.cn)

In this study looking at creasing of 'Honglian' and 'An liu' sweet orange during ripping it was shown that the activities of polygalacturonase, cellulose and pectin esterase in the pericarp increased more in the 'Hongjiang' than the 'An liu'. This breakdown of the cell walls could be responsible for the higher creasing found in the 'Hongjiang'. This study added some much needed basic information to the understanding of this complex physiological disorder.

Factors affecting peel browning of Afourer 'Nadorcot' mandarin fruit in Morocco [151] Ait-Oubahou a, El Krouchin T, Talhi M, Goumari M, and Laamim M. (aoubahou@iavcha.ac.ma)

Skin browning found in Afourers after harvest is a serious problem in Morocco. Fruit with thin rind have been found to be more susceptible to browning. Foliar application of Ca and K as well as postharvest dipping in ascorbic acid has reduced the incidence of the disorder. The use of low pH water (5.5-6) in postharvest handling however increases the incidence of browning. No real conclusions were made as yet.

The regulation of aconitase a central enzyme of citrus acid metabolism in citrus fruit [152] Degu A, Prakash S, Schlizerman L, Zur N, Hatew B, Blumwald E, and Sadaka A. (vhasadka@volcani.agric.gov.il)

Accumulation of citric acid in the juice sacs is the major quality determinant in citrus fruit. Manipulation of aconitase in the TCA would therefore impact on °Brix/TA. This basic scientific research will give a better

understanding of citric acid metabolism and is expected to result in the development of a practical tool to control the levels. This is especially important to manipulate internal quality without the use of calcium arsenate.

Fruit shading changed photosynthate partitioning, sugar metabolism and accumulation in developing satsuma mandarin (C unshiu) fruit [156]. Chen JW, Zhang SL, Xie M, Xu HX and Xu JG. (chenjunwe@tom.com)

By using individual fruit shading experiments this group showed that the rind can become a stronger sink for carbohydrate during fruit development at the detriment of the pulp. Under normal conditions the rind only imports carbohydrates from the leaves after colour break but when the fruit are shaded during stages II or III it started much sooner, resulting in a reduced Brix in the pulp. This research showed that the citrus rind is a photosynthetically active organ, which can produce its own carbohydrates until colour development.

General impression

It becomes clear to me that we as an industry will have to be responsible for conducting most of our own research on these specific postharvest disorders and the factors contributing to their occurrence, e.g. peteca spot, chilling injury and rind breakdown. Other citrus industries are not under the same export constraints (temperature and time) as we are and therefore do not experience the same sort of rind quality problems. However, we can obtain a lot of basic information on the physiological processes that occur in the rind from the Spanish, Israeli and USA research groups as they have a much more fundamental focus in their research.

Post congress tour

During the visits to the soft citrus production areas near the Three Gorges Dam it became clear that the Chinese industry have a very different mindset when it comes to citrus production compared to us. In the vast orchards visited mechanisation was not well employed but individual trees were very well managed. We did not see a packhouse in operation as all the fruit had been picked and packed on site before transported to the next door town with 3 million plus people for consumption. Plastic ground covers are widely used to reduce the impact of late rain on the internal quality of 'Ponkan' mandarins. This technique could be useful for some of our growers in the Western Cape that have serious oleo problems. Applying plastic ground covers prior to rain could result in a drier soil which will lead to less turgid oil glands and possibly reduce the incidence of oleocellosis.

8.1.4 Disease Management (Paul Fourie & Hennie le Roux)

Citrus pathology

Graft transmissible diseases [Compiled by Hennie le Roux and Paul Fourie]

An IOCV workshop was held, in which Bove presented an overview of the IOCV and of all graft transmissible diseases in citrus. Nuria Duran-Vila presented information on the IOCV website on which descriptions and photos of most of these diseases are freely available: <http://www.ivia.es/iocv/>

Huanglongbing (HLB)

Huanglongbing, better known as citrus greening in South Africa, has rocked the citrus world. As a result of this two full sessions at the congress were spend on this disease. HLB can be caused by one of three different pathogens. Asian HLB is caused by *Liberibacter asiaticus* (*Las*), American HLB by *Liberibacter americanus* (*Lam*) and African greening by *Liberibacter africanus* (*Laf*). The first two candidates spp is spread by the psyllid vector, *Diaphorina citri* and the third by *Trioza erytrae*. A Chinese presentation (Paper 92) also made note about a phytoplasma related to *Ca. Phytoplasma asteri* (Group 9) that con-infected citrus with *Las* in 48% of the HLB symptomatic trees, 29% was infected by the phytoplasma alone and 14% by *Las* alone. However, the role of this phytoplasma in HLB is still being disputed, most fervently by Bove who does not believe it to be an HLB pathogen.

Studies done to determine the genetic diversity in *Las* in China (paper 94) have concluded from the homology between Chinese isolates that no new introduction of the pathogen occurred in China.

The most important citrus producing countries that have become infected with HLB during the last five to six years are Brazil, Florida, Cuba and Iran. In all cases Asian HLB is the cause with the exception of Brazil where American HLB can also be found. Interesting the incidence of American HLB in Brazil shows a decline whereas Asian HLB is becoming more and more dominant.

According to Richard Lee the disease in Brazil and Florida are too widespread to attempt eradication. Efforts to remove symptomatic plants have not been successful so far. Their field tests show that psyllids may be able to transmit the disease well before plants become symptomatic. According to the researchers at the Centro APTA

Citros Sylvio Moreiro in Sao Paulo, more than 500 000 trees has been mandatory diagnosed for HLB and removed whereas another 2,5 million trees were voluntarily removed from infected blocks in Sao Paulo province since 2005. A law requiring eradication of diseased trees was promulgated in March 2005. Unfortunately this law was only enforced for the first two years leaving it without teeth since. In areas where the overall strategy of the removal of inoculum and vector control was followed growers coped fairly well with the disease. However, where this was not done the disease is on the increase.

In China, the amount of resources made available to study and control HLB is staggering (paper 93). Resources are invested into regulation (remove trees, control psylla), extension (8563 extension classes; 1.5 million technical guides) and financial support for extension, eradication, nurseries and research.

Several countries have drawn up action plans for HLB and its vectors. According to Luque-Williams of California the State of California Department of Food and Agriculture in cooperation of the citrus industry in California have developed an action plan to detect and to prevent the spread of the vector. This action plan has already been activated successfully after the detection of the vector in San Diego county. Argentina has a similar action plan in place. It will, however, be much more difficult to execute as the vector is already present in Argentina and the disease is only 200 km from the Argentinean border. The South African Citrus Industry needs to draw up a more formal action plan for Asian/American HLB and *Diaphorina citri*.

Though the Florideans, according to Qureshi, realise that the reduction in psyllid populations is a key to slow the spread of HLB through the use of soil applied systemic insecticides (eg. Imidacloprid, thiamethoxam, aldicarb) or foliar sprays (eg. Imidacloprid, fenprothrin) it was my impression that the average Floridean citrus producer was still trying to control the vector with contact insecticides applied as foliar sprays. This had an impact on non-target organisms resulting in repercussion pests becoming a problem. The foliar sprays will also ensure that neither predation nor the exotic parasitoid, *Tamarixia radiate* will be successful. Ironically the registration of methamidaphos as a stem-applied systemic will never be allowed in the USA as it is an organophosphate. They would rather apply much harsher products as foliar sprays.

One of the major differences between the Greening situation in South Africa and HLB in the Americas is the presence of alternative hosts. In South Africa citrus is by far the most important host for *Trioza*. In the Americas the ornamental plant *Murraya paniculata* (L.) Jack also known as jasmine orange, is as good a host for *Diaphorina* as citrus. If the vector is not controlled on the *Murraya* plants together with the citrus, vector control will according to Sara from INTA in Bella Vista not be successful. The recent importation of *Murraya* spp by the ornamental plant industry into South Africa and its distribution throughout South Africa pose a definite threat to our citrus industry and should be followed up. The seasonal abundance of *Diaphorina* in Argentina is similar to that of *Trioza* in South Africa where there are adult peaks in spring and again in late summer. The low incidence in *Trioza* numbers in South Africa during the last number of years is difficult to explain. In Argentina it seems as if the parasitoid *Tamarixia radiate* could play a role in reducing the *Diaphorina* numbers.

One should take notice of the spread of Asian HLB through Cuba. The disease symptoms were first noticed in 2007 according to Cueto from the Research Institute for Tropical Fruit Crops in Havana. It might, however, be that the Cuban industry only became aware of the symptoms at that stage because of what happened in Florida. The fact is that when Cuba was visited by Bove in March 2008, the disease has spread throughout the whole length of the island. It can be expected that a large number of grapefruit trees will be eliminated as a result of HLB in Florida and Cuba during the next five years and that this could result in shortages on the world markets. It may be a good idea to react to this by planting red grapefruit to fill this anticipated gap that is expected to occur in the near future.

HLB symptoms are characteristic, not specific and can often be confused with zink or other deficiency symptoms or girdling effects weather caused mechanically or by root rot pathogens. The use of PCR and real time PCR to confirm the presence of *Liberibacter*, and thus HLB is used worldwide with good success. Once the presence of HLB in an area has been confirmed the presence of blotchy mottle can be used as a quick diagnostic tool. In China, Deng found that there was a 93% correlation between blotchy mottle and positive PCR results. This is similar to Bove's findings. Spann from CREC in Florida only found a 51-71% correlation. In lemons there could be blotchy mottle symptoms, similar to those that represent HLB in oranges, which are not HLB-induced. Take notice that the blotchy mottle on lemons is often much larger mottles than on oranges and mandarins.

Spann found that there were lower P, Ca, S, Zn, Mn and Cu levels in HLB infected leaves whereas the K levels were higher. He maintained that nutrient analyses may be good early indicators of HLB infection since these tests were able to detect significant differences apparently before the bacterial titer was high enough for detection by PCR. Personally I could not support this as there are large differences in the nutritional status of orchards even in HLB free areas.

The repellent effect of guava interplantings on psylla was studied by Chinese researchers (paper 96). The results showed that guava leaf oil, fresh guava leaf, and Chinese giant hyssop oil repelled psyllid females. Volatile oil from guava leaf was the most repellent of the oils tested. Odours from fresh jasmine orange and

sweet orange leaves, and oils extracted from lemon, grapefruit and sweet orange leaves, attracted psyllid females. The odour of fresh jasmine orange leaves was the most attractive. In contrast to the responses of females, oils from both host and non-host plant species did not repel or attract psyllid males.

Citrus Tristeza virus (CTV)

Zhou (paper 167) summarised the research on CTV being conducted in China. Efforts were mostly aimed at differentiating between isolates and therewith determining the genetic variability in isolates, which could not be spatially correlated based on origin of isolates. Based on the p25/Hinf I RFLP groupings, they have mostly groups 1, 3 and 6 in China. They found that isolates in grapefruit were less complex than in sweet orange and it was questioned whether the aggressive isolates dominated in the more susceptible varieties. In studies on transmission, they used single aphid transmission to separate strains, but found that Group 3 was better transmitted by *Toxoptera*. Studies on cross-protection failure are also ongoing. Hu (paper 168) presented similar work on CTV diversity and management in Hawaii where CTV has a 74% incidence and high diversity. They find mostly Group T3, T30, T36 and VT groups, but recently found a 'new' group similar to NS25 from New Zealand, which seemed to be able to break Trifoliolate resistance. A high degree of sequence homology of the coat protein allowed them to construct a synthetic coat protein construct with 94.6% homology to all known strains of CTV. This construct was transformed into Mexican lime and transgenic lines are presently being evaluated. In Japan (poster 510), marker assisted breeding techniques are used to expedite resistance breeding against CTV.

Citrus Viroids (CVd)

George Vidalakis (California, USA) reported on the use of non-pathogenic CVd IIIb and IIa strains as dwarfing agents (paper 173). Longterm evaluation has demonstrated a 50% reduction in canopy volume with no concomitant yield reduction in high density plantings. A single CVd IIb infection on Carizzo did not yield the desired results and they demonstrated that a mixture of strains would be needed (paper 203). They believe their unique CVd IIIb strain to be genetically stable.

Molecular detection

Cambra, Bove and other co-workers (paper 171) described and compared several direct methods of sample preparation for quantitative real-time PCR of viruses and bacteria from citrus plant material and vectors. The most exciting development is direct tissue blots (even insects) onto a membrane from where ELISA (most effective for CTV) or Real-Time PCR could be conducted. False negatives remain a constraint.

Wang and co-workers (paper 176) studied the species and diversity of CVd in China. In the process, they developed and published a rapid one-step RT-PCR assay for CVd (Eur.J.P.Path) and also placed a lot of sequence data on GenBank. Interestingly, they observed only 5 nucleotide difference between CVd IIa (non-pathogenic) and CVd IIb and IIc (cachexia-causing).

Interesting posters (photos available)

- Multi-locus sequence typing system for *Las* (poster 186)
- HLB pathogen genomic DNA subtraction enrichment approaches (poster 320)
- Role of *Murraya* species on HLB spread (poster 324)
- Citrus leprosis virus are transmitted by mites in a persistent, circulative manner, but not propagative (poster 349)
- Gene expression of *Citrus aurantifolia* following CTV infection (poster 350)
- Coat protein analysis of severe and mild CTV strains (poster 355)
- Performance of citranges following infection by Exocortis viroid (poster 360)
- Citrus improvement in Albania (poster 361)
- Nested RT-PCR detection of tatter leaf virus (poster 362)
- Influence of 34 sweet orange cultivars on the polymorphism of CTV isolates CT14 and CT36 (poster 363)
- Mechanical inoculation of citrus with *Spiroplasma citri*, cause of Stubborn Disease (poster 519)
- CVC Vectors and Population Dynamics of *Dilobopterus costalimai* in Valencia Orange in Argentina (poster 384)
- First report of citrus measles in California (poster 40)

Lessons/actions for South African citrus industry

- Asian and American HLB is much more devastating than the Citrus Greening that occurs in South Africa today and will destroy the southern African citrus industry if it is neglected in the same way Greening is currently neglected.
- Ensure that an action plan is in place to cope with *Liberibacter asiaticus* and *L. americanus* as well as *Diaphorina citri* if it is introduced.
- Discourage the planting of *Murraya* plants as ornamentals in South Africa. Determine if the newly established *Murraya* sp introduced into South Africa went through plant quarantine. If not ensure that the Department of Agriculture takes on its responsibilities and eradicate all those plants that were introduced.
- Determine the impact which HLB will have on the production of orange juice concentrate in Florida and Brazil and determine if it would not be viable for BEE growers in South Africa to produce citrus for processing.
- Determine what opportunities could be created for the South African grapefruit industry if the Floridean and Cuban industries collapse as a result of HLB.
- Determine the impact that HLB will have on the Argentinean and Uruguay citrus industries once it reaches these industries. It is 200 km from the Argentinean border and it is not a case of "if" but "when" it will affect these industries.
- A lot of research is being conducted on molecular detection of graft transmissible diseases worldwide and this aspect should be accommodated in the Diagnostic Centre at CRI-Nelspruit.

Fruit and Foliar diseases [Compiled by Paul Fourie]

Citrus canker

This important disease received a lot of attention at the congress and deservedly so, as Jim Graham (Florida, USA) described how they struggle to control bacterial canker with Copper sprays at 21-day intervals (paper 178). Frequent storms, wind-blown rain and wounds (especially leafminer damage) favour disease spread and severity and Graham (paper 189) described how the use of wind breaks and leaf miner control alone could reduce disease. Very susceptible grapefruit needs to be protected at 21-day intervals (up to 14 sprays per season) in order to produce clean fruit, which is allowed to be exported from Florida state. However, control is not guaranteed and a preharvest canker incidence of 5% 'disqualifies' an orchard for fresh fruit market.

Deng and co-workers (China) are developing transgenic plants for resistance against citrus canker (Paper 180) through the use of various techniques. Results seem promising. In China, work is also ongoing in the attempt to eradicate this disease and up to 930,000 trees were already removed (Paper 186). In Sao Paulo state in Brasil, Fundecitrus manages a huge monitoring effort of by surveying all trees in 10% of all blocks (Paper 187). The level of eradication (though burning trees) is based on the disease incidence: all trees within a 30 m radius from infected trees are removed in blocks with a canker incidence of $\leq 0.5\%$, while complete blocks are eradicated when disease incidence exceeds 0.5%. In suspected areas, all blocks will be surveyed. Re-inspections are done at a 30-30-60-60-90-day schedule and clean orchards are allowed to replant after 2 years. Through this thorough programme, as well as expansions into urban and non-commercial plantings and nurseries, the overall incidence of citrus canker is declining from 0.7% to 0.17% infected blocks. A cost benefit analysis has indicated that this approach (\$16 million for Fundecitrus alone) is cheaper than the alternative, which might lead to up to 3 extra copper sprays per season (\$40 million). Growers do not receive any government compensation for eradication.

In Argentina, chemical control of citrus canker involves 4-5 sprays of up to 25 L/tree with copper formulations alone, or in combination with mancozeb or QAC sterilants, as well as a strong focus on leaf miner control. Top hedging and pruning also improved control as the highest incidence of canker was observed in the top half of trees.

Work from Argentina by Roberto (Paper 182) demonstrated that postharvest treatments with chlorine dioxide (5 ppm for 2 min at 15-20 C) reduced citrus canker incidence on fruit to undetectable levels. Apparently ClO_2 is not adversely influenced by organic matter in aqueous solutions (i.e. dirty baths) in the same manner as chlorine and has a 2.5 times higher oxidative power than sodium hypochlorite.

Lopez and co-workers (Spain) developed a molecular detection protocol for viable bacterial cells of citrus canker on fruit. As conventional PCR and primers detected dead cells, they focused their real-time RT-PCR primers on mRNA, which has a 30 min half-life.

Citrus Black Spot (CBS)

Only one oral paper and two posters were presented on CBS. Andrew Miles (Queensland, Australia) presented preliminary results from trials aiming to control CBS through mulching, fungicide application and pruning (Paper

243). Very low CBS levels made conclusions difficult, although the lowest disease incidence involved a combination of all three practices. De Goes and co-workers (paper 418) evaluated several contact fungicides, benlate, carbendazim and pyraclostrobin for CBS control in Brazil. All fungicides improved CBS control statistically, with the latter fungicide being superior to the benzimidazoles.

Alternaria Brown Spot (ABS)

Two oral papers were presented on ABS. Paul Fourie (CRI, Stellenbosch) used ABS as model pathogen to demonstrate the detrimental effect spray run-off had on spray deposition and biological efficacy (paper 238). Megan Dewdney (Florida, USA) demonstrated a leaf wetness and temperature interaction for infection of *Alternaria*, and also showed that cultivar susceptibility influenced this interaction with leaf wetness periods as short as 4-6 h were sufficient to support infection on the most susceptible cultivars at optimum temperatures of 24-28 C. Mineolla, then Murcott and then Nova appeared to be most susceptible to ABS.

Interesting posters (photos available)

- Copper application for Citrus canker control in Uruguay (poster 335 and 341))
- Effect of wind breaks on canker incidence in Argentina (poster 347)
- Verification of eradication of canker in Australia (poster 346)
- Image recognition of citrus canker symptoms (poster 344)
- Effect of antibiotic gentamicin for canker control in Argentina was shown to be insufficient (poster 342)
- Mixture of silicon and copper improved canker control (poster 343)
- *In vitro* and *in vivo* Inoculation of the *Alternaria alternata* in different tangerine varieties (poster 413)
- Resistance research of tangerine hybrids and cultivars to *Alternaria alternata* (poster 416)
- Surveying rumple disorder in a 7-year--old lemon orchard in Turkey (poster 417)
- Chemical control of *Guignardia citricarpa* in fruit of sweet orange infected by conidia (poster 418)
- Assessment of the sanitary conditions of certified citrus nurseries production in Souss Massa Valley (Morocco) (poster 419)

Lessons/ actions for South African citrus industry

- Promote awareness to ensure early-detection of citrus canker in the event of an incursion.
- As part of a 'readiness plan', continue collaboration with South American researchers in order to keep abreast of effective control strategies of citrus canker.

Soilborne diseases [Compiled by Paul Fourie]

Soilborne diseases received relatively little attention compared with graft transmissible diseases and citrus canker. Most discussion revolved around *Phytophthora citrophthora* branch canker, with reports from Syria (paper 237) and Spain (paper 241). In the latter paper, Antonio Vicent investigated data relative to soils, cultivars, rootstocks, irrigation, pruning, techniques to improve fruit set, fungicide treatments, presence of brown rot of fruit and frost damage were recorded from affected as well as from non affected orchards in 110 orchards. Factors such as flood irrigation, girdling and clay-type soils promoted disease. Interestingly, the Spanish have disregarded genetic drift or host specificity in *P. citrophthora* populations as a reason for the recent outbreak of this disease worldwide. Several papers addressed molecular (and other) detection techniques (papers 236-237), which should be considered for implementation in the CRI Diagnostic Centre. Similar techniques were also discussed for diagnosis of 'Mal secco' (paper 240), which does not occur in SA.

Catara and co-workers described how dry-root rot, caused predominantly by *Fusarium solani*, was emerging as an important disease in Italy (paper 235). This was largely due to the industry changing from Sour Orange rootstock to CTV-tolerant Troyer and Carizzo. The disease was also mostly associated with stress, in particular compacted soils.

Interesting posters (photos available)

- Root rot tolerance of various citrus rootstocks and hybrids against *Phytophthora* (poster 26).
- Intercropping with hosts of micorrhizal fungi (poster 233)
- Effects of micorrhizal fungi on seedlings (poster 232)
- Variability of *Fusarium* spp. in Citrus rhizosphere (poster 401)
- Real-Time PCR to assess the host colonisation and soil survival of *Phoma tracheiphila* (poster 402)
- Epidemiological and etiological aspects of dry root rot in nurseries and orchards in Tunisia (poster 406)
- Characterization of *Phytophthora* spp. strains affecting Citrus rootstocks in Cuba (poster 408)

- Chemical and biological control of Phytophthora root rot of container-grown Citrus plants (poster 410)
- Potential role of snails as vectors of *P. citrophthora* causing branch cankers on Clementine trees in Spain (poster 428)

Lessons/actions for South African citrus industry:

- Continue collaboration with Spanish researchers on *Phytophthora citrophthora* research.
- Compare methodology on rootstock tolerance testing.

Postharvest pathology [Compiled by Paul Fourie]

Host-pathogen interaction

[Paper 31] Samir Droby and co-authors - *Penicillium digitatum* Actively Suppress Defense-related Hydrogen Peroxide Burst During Infection of Citrus Fruit. Wound healing and active host response to infection in citrus rind involves an oxidative burst, which was accentuated following wound-infection with non-pathogens. Enzymatic removal of H₂O₂ from wound sites inoculated with the non-pathogen, *Penicillium expansum*, allowed it to cause lesions. *P. digitatum* is able to suppress this oxidative burst, probably through production of citric acid and catalase.

[Paper 34] Samir Droby presented another talk on the role of volatile compounds in recognition and germination of *P. digitatum* and *P. italicum* on citrus fruit. *Penicillium* spores needed wounds to penetrate, although the spores needed be in the immediate vicinity of the wound site. Through a process called chemotaxis, volatile compounds from the citrus peel promote germination and directional growth toward the wound site.

Alternative approaches to decay control (Biocontrol + GRAS chemicals)

[Paper 32] Torres Leal and co-authors evaluated the biofungicidal effects of lactic acid bacteria against *Penicillium digitatum*. Several lactic acid producing bacterial strains effectively controlled against green mould, but a mixture of organic acids (Lactic, acetic, propionic and phenyllactic acids) was as effective as 100 ppm imazalil and guazatine.

[Paper 35] Ippolito (Italy) presented a talk on the use of various salts for control of green mould. The most effective treatments involved a combination of pre- and postharvest applications with sodium bicarbonate, sodium carbonate, potassium carbonate and/or sodium silicate. They even reported that preharvest application with sodium bicarbonate was as effective as imazalil.

[Paper 38] Chinese researchers found a specific strain of the yeast *Kloeckera apiculata* (34-9) to be as effective in controlling green mould as 200 ppm carbendazim in packhouse trials and had no detrimental effect on quality parameters. However, the yeast needs time to colonise the wound site in order to be effective against the pathogen, most likely through competition and/or direct antagonism. In follow-up research (an unscheduled talk), Lui demonstrated that crude heat and protease tolerant extracts from the yeast was effective against all postharvest pathogens. They identified farnesol and the primary component, which led to hyphal and nuclear membrane disintegration and nuclear condensation. Several posters also address further research on farnesol. Other decay control options that were discussed, included X-ray irradiation (poster 192), which increased the scoparone levels in mandarin flavedo.

Chemical control

[Paper 33] Hongye Li and co-workers (China) are studying the mechanism of imazalil resistance in *P. digitatum* and have three biotypes: R1, R2 and R3. R1 and R2 have genetic insertions before the DMI-target gene, while they cloned a gene of a major facilitator superfamily transporter from R3. Transporters function in the secretion of endogenous fungal pathogenicity factors (e.g. toxins) and in protection against exogenous toxic compounds, such as plant defense compounds (e.g. phytoalexins) and fungicides and might be involved in multi-drug resistance.

[Paper 36] Lanza gave a broad overview of the status and new strategies for postharvest decay control in Italy. They use similar fungicides as in SA, with the notable exception of metalaxyl during preharvest stages for brown rot control. They found fludioxonil effective against green mould although not as curative as imazalil. Synergism was observed between this product and 800 ppm peroxy acetic acid. A curing period of 24-72 h at 32-36°C is recommended to enhance host defence. An alternative would be hot water dipping in 52°C for 3 min or hot water brushing at 62°C.

[Paper 36] Nancy Cunningham presented the Australian experience with fludioxonil, a reduced risk phenylpyrrole fungicide. They observed no phytotoxicity, even at rates as high as 1500 ppm, and good control of green mould, notably also of thiabendazole resistant strains of *Penicillium*. Interestingly, Fludioxonil gave better control of *P. italicum* and *P. digitatum* on lemons than when same fungi occurred on oranges or

mandarins. They concluded that fludioxonil may require enhancement to achieve commercially acceptable levels of control in some citrus cultivars.

[Paper 39] Joe Smilanick (California, USA) presented a collaborative paper between Danny Bylemans (Janssen Pharmaceutica), Torres Leal (INTA, Argentina) and Keith Lesar (CRI, South Africa) on a new postharvest fungicide, pyrimethanil (PYR). Its mode of action is different than the other postharvest fungicides and targets enzyme secretion. It can be used at rates of 500-1000 ppm in a bath application (with better residue loading at 40-50 C) while 2000 ppm in a light wax is also recommended. A heavy wax negatively affected its efficacy. Effective treatments were associated with fruit residues of 1 to 2 mg/kg, below the MRL of 10 mg/kg. Thiabendazole and imazalil-resistant *P. digitatum* isolates were controlled by PYR. The addition of sodium bicarbonate or potassium sorbate improved PYR performance. PYR was not compatible with chlorine. According to Smilanick, PYR has good eradicant activity, although not as good as imazalil, and did not provide sporulation inhibition. A combination product of PYR and imazalil (Philabuster) combines the benefits of both fungicides and is also beneficial in terms of resistance management. No resistance to PYR has been observed in US packhouses.

[Paper 41] Lanza and co-workers evaluated preharvest Chitosan, beeswax and gibberelic acid treatments to control water spot of Clementine in Italy. All treatments yielded firmer better quality fruit, although no statistical differences were observed with the control.

Interesting posters (photos available)

- Postharvest treatments for the preservation of Tangor Murcott (poster 450)
- Effect of tea polyphenol on inhibition of *Diplodia natalensis* infections (poster 454)
- Improved postharvest disease control of *Penicillium digitatum* on citrus fruits by combined application of compatible biocontrol agents (poster 455)
- Inhibition of citrus *Penicillium digitatum* with propolis extracts (poster 457)
- A real-time PCR assay for the detection the frequency of imazalil-resistant *Penicillium digitatum* (poster 459)
- Combining biocontrol agents to optimise the biological control of *Penicillium digitatum* (poster 461)
- Control of *Penicillium digitatum* (green mold) by sodium bicarbonate in lemon fruit in Tucumán (Argentina) (poster 462)
- Amplification and partial sequencing of the intergenic regions (IGS) of ribosomal DNA from *P. digitatum*, *P. ulaiense* and *P. italicum* (poster 463)
- Farnesol induces apoptosis in the citrus fungal pathogen *Penicillium expansum* (poster 464)
- Farnesol influence *Penicillium expansum* morphological change reveals a possible mechanism for antagonistic yeasts (poster 465)
- Antagonistic substances produced by antifungal strain 34-9 to *Penicillium digitatum* (poster 466)
- Postharvest application of auxins to control calyx senescence in clementines submitted to degreening treatment (poster 469)
- Controlling blue and green moulds of Citrus by UV-C light (poster 470)
- Control of green mould of stored oranges by phenolic compounds (poster 471)

Lessons/ actions for South African citrus industry:

- Investigate possible implementation of the use of salts in South African packhouses.
- Screen pyrimethanil and fludioxonil as reduced-risk postharvest fungicides against green mould and latent pathogens and also to broaden our postharvest fungicide arsenal.
- Intensify fungicide resistance screening in *Penicillium*.
- Integrated application of alternative control options should be investigated, as opposed to the 'silver bullet' approach.

Presentation: P. Fourie, M. du Preez, J.C. Brink and T. Schutte. The effect of run-off on spray deposition on citrus leaves and fruit and control of Alternaria brown spot of mandarins. 11th Int. Soc. Citriculture Congress, 26-30 Oct 2008, Wuhan, China.

8.1.5 Cultivar Development and Improvement (Andrew Lee, Thys du Toit & Hannes Bester)

Introduction

While many researchers are still following traditional as well as new methods of cultivar breeding and improvement there has been a swing in the emphasis and priorities with the presence and spread of HLB over

the past 4 years in numerous citrus producing countries. While traditional methods are still being used to combat CTV and other diseases HLB is a disease that needs an entirely new approach to control. Once seldom mentioned now transgenic plant development makes up a large % of current work.

In addition the present demands plus diversity of markets worldwide has led to a substantial increase in breeding to meet changing market demands. Improved flavour and freshness, better eating quality, firmer fruit and even some seed is now acceptable to certain markets provided that the other criteria are met.

Oral presentations

Citrus Genomics and Breeding

M L Roose – UCR (mikeal.roose@ucr.edu)

While there has been rapid progress in genomic research in recent years the restrictive results tend to reduce the efficacy and therefore funding of this work. This work is aimed at assisting conventional breeding methods select parent material more efficiently by identifying traits that will identify suitable and/or non suitable genes for the development of new hybrids.

Unfortunately this knowledge will not reduce the juvenility of such new hybrids and evaluation periods will still have to run the normal course.

Somatic Hybrids

J W Grosser – CREC (jgrosser@ufl.edu)

Numerous somatic hybrids have been developed using protoplast fusion to be used as Germplasm for breeding conventional crosses. The generation of seedless triploids, using somatic hybrids as the tetraploid parents, and the creation of tetraploid rootstocks for tree size control are two major benefits of this process. This technique allows the identification and use of particular traits such as disease tolerance to be incorporated with other sought after attributes into a single, new scion or rootstock selection.

Transgenic Plants

M Dutt – CREC (manjul@ufl.edu)

The use of antimicrobial peptides (AMP's), which occur in all classes of life and are noted for their broad antibiotic properties, in developing transgenic plants that would be resistant to Huanglongbing (HLB) is currently receiving considerable attention in Florida. Using standard *in vitro* transformation techniques several genes containing AMP's have been incorporated in the citrus genome. These plants are now being tested for HLB resistance.

More recently systemic acquired resistance (SAR) is also being investigated in an attempt to induce immunity against HLB in previously infected citrus trees. Transformed plants are now being prepared for HLB resistance trials.

Mandarin Hybrids

T Kahn – UCR (tracy.kahn@ucr.edu)

The previously conservative approach to releasing new hybrids from the collection at UCR has changed in recent times and a number of mandarin hybrids have been released to the Californian industry as well as to other countries over the past 10 years. These selections are the Tahoe Gold, Yosemite Gold and Shasta Gold, formerly known as the TDE's, the Gold Nugget, Pixie and Tango. Of these selections Tango as the irradiated seedless mutation of W. Murcott or Nadorcott is probably the most promising and is currently being planted on a large scale in California. The TDE's are triploid hybrids of Temple x (Dancy x Encore) and are mid to late maturing. The Pixie has not shown much promise, but the other selections have been imported for testing in SA.

USDA Rootstocks

K Bowman – USDA (kim.bowman@ars.usda.gov)

US - 812 (Sunki x Trifoliolate), US - 802 (Pummelo x Trifoliolate) and US - 897 (Cleopatra x Trifoliolate) are recent releases of the USDA in Fort Pierce, Florida. US - 812 gives a moderate size tree, US - 802 a large tree and US - 897 a dwarfed tree with standard scions. All are tolerant to CTV, while 812 and 802 also have good resistance to citrus blight. 897's blight resistance is not mentioned, but if it is succeeding in Florida it is probably also blight tolerant.

Breeding for a Subtropical Climate

M W Smith - DPI Queensland (smithmw@dpi.qld.gov.au)

Rising heat units in Australia's main mandarin growing area have caused Queensland DPI to establish a breeding programme in a warmer area than the current commercial orchards. In this way should temperatures continue to rise at the present rate the new hybrids will be suited to the future expected climate. Using traditional breeding techniques 52 000 hybrids have been included in the programme of which 340 selections have already undergone a second evaluation. To date five of these second stage selections have been released as semi commercial and they are now dominating new commercial plantings.

This novel approach and the scope of this programme make it a worthwhile site to investigate further and visit if considered necessary.

USDA Breeding

E W Stover - Fort Pierce (Ed.Stover@ars.usda.gov)

The USDA in Florida has a long history of citrus breeding and still continues to work on hybrids that can meet the demands and challenges facing the Florida industry. Included in their programme are numerous seedless mandarin hybrids bred for the discerning markets as well as rootstocks already mentioned.

More recently transgenic plants transformed using AMP's for HLB resistance have been developed and laboratory testing is underway to establish whether any HLB resistant plants have been produced. Once this stage is complete field trials, which are not in favour with food safety and environmental groups, will need to be established.

UCR Breeding Programme

T E Williams – UCR (timwill@ucr.edu)

UCR also has a long history of citrus breeding, but for many years releases were few and far between. Recently with the inclusion of an irradiation programme UCR has been far more active in the production and release of new seedless mandarin selections developed from formerly seeded cultivars.

To date 68 low seeded selections have been made of which Tango, an irradiated W Murcott, was the first to be patented and released. Further selections due for release during 2008 are Daisy SL, Kinnow SL and Fairchild SL. Selections from irradiated Fremont, Encore and Nova are due for release in 2009/10.

UC reserves new releases for CA growers for three years before releasing their new cultivars to other citrus producing countries.

Tango is a very low seeded irradiated W Murcott or Nadorcott with a mean seed count of 0.4 seeds per fruit - most fruit are therefore completely seedless. The low pollen fertility implies that this selection will not cause seediness in Clementines or similar cultivars. This has been confirmed with hand pollination experiments - large field plantings have been planted and should come into bearing next season. Fruit and tree characteristics are similar to its parent and maturity is in late January, but fruit holds well on the tree till April.

Daisy SL, Fairchild SL and Kinnow SL are low seeded irradiated selections that have a mean of 2 to 3 seeds per fruit under high pollination pressure. These selections mature in late December in Riverside and hang well into February. All produce very good to excellent crops and eating quality is outstanding.

Morocco Breeding Programme

N Handaji - INRA Kenitra Maroc (citrusinra@yahoo.fr)

This breeding programme consists of 35 clones of Afouer as well as 1000 hybrids of Clementine with other mandarins. Based on initial evaluations numerous diploids as well as three triploids were identified as being promising and are to be tested in larger trials for further information.

H Benyahia- INRA Kenitra Maroc (hamidbenyahia2002@yahoo.fr)

Initial trial work to replace sour orange using citrange rootstocks was not successful due to calcareous soils and CEV sensitivity. As a result the programme decided to make use of somatic hybrids bred from selected callus material using protoplast fusion. In this way they are developing a series of somatic hybrids bred from material selected for the traits they require to face their particular soil, climatic and other issues.

Tarocco Selections

G R Recupero - Acireale Sicily (giuseppe.reforgiato@entecra.it)

Blood oranges represent over 50% of Italy's orange crop which is approximately 2 million tonnes per annum. The majority of Italy's citrus is produced in Sicily and research on a wide range of cultivars is centred in this region. Of the blood oranges available Tarocco was singled out as being superior to the others. In 2001 a trial planting including 17 Tarocco selections was established to decide which selection or selections would be the best for the Italian industry.

Consumer Surveys USA

F G Gmitter Jr – CREC (fgg@crec.ifas.ufl.edu)

CREC has had extensive marketing and consumer surveys carried out by professional marketing companies. The results have shown that freshness and flavour were the most important factors influencing the purchase of fresh fruit, followed in order by appearance, juiciness, price, seed count, size and ease of peeling. Fruit used for these surveys were Spanish Clementines, Dancy's, 2 new hybrids and Californian Cuties (Clementines). Three US markets were surveyed with over 150 consumers, including children, being questioned in each survey. The Florida hybrids even though lightly seeded and not as easy to peel were preferred to the Spanish Clementines and Cuties and topped the popularity charts in the surveys.

Turkish Seedless Lemons

A Uzun - Alata Mersin (uzun38s@yahoo.com)

Kutdiken lemons make up approximately 40% of the Turkish lemon industry, which is planted mainly in the Adana/Mersin region. Its susceptibility to mal secco is overlooked evidently due to its very good storage ability. Three seedless irradiated selections have now been developed and are being evaluated by the Alata Research Institute in Mersin. All selections are seedless while the original Kutdiken contains from 7 to 13 seeds.

Tami Mandarin Hybrid

J Kanonich - Extension Bet Dagan (shukan@shaham.moag.gov.il)

Tami is an early maturing seedless mandarin hybrid of Temple x Michal that is harvested in mid October in the south of Israel near Gaza. Trees are slow growing and thorn less, yields are good and fruit is medium to large in size. Rind colour is a deep orange/red and easy to peel. Internal quality is very good and the Tami is not susceptible to 'Alternaria brown spot'. Fruit set treatments are not necessary in this region so this selection could be a suitable replacement for certain Clementine selections.

Poster presentations

Somatic Hybrid Rootstocks

D Dambier - CIRAD Montpellier (patrick.ollitrault@cirad.fr)

The dominance of sour orange as a rootstock in the Mediterranean region combined with the spread of CTV and the BCA in the region has led to the search for alternative rootstocks that are CTV resistant and that can also withstand the other problems present such as drought, salinity, high pH levels, calcareous soils, *Phytophthora* and nematodes.

In association with Research Institutes in Spain, Morocco, Turkey, Tunisia the French Organisation CIRAD has established a rootstock breeding programme to replace sour orange and to meet the problems facing the region.

The main approach to date has been to develop tetraploid somatic hybrids (4) and diploid cybrids (3) for testing in the various citrus areas of these countries. A wide range of citrus species has been used in order to test as many combinations as possible.

S Blumer - Piracicaba Brazil (blumer@esalq.usp.br)

Citrandarin rootstocks developed from Sunki and Cleopatra with a range of trifoliate selections showed tolerance to CTV and citrus blight over a 13 year period. Carrizo and Troyer citrange were included in the trial as well as a Rangpur x Carrizo selection. The Rangpur hybrid showed bud union incompatibility with all scion selections. All trees showed CTV and blight tolerance and seedlings of Cleopatra crossed with Swingle, Rubidoux and Trifoliate (1574) were more *Phytophthora* resistant than the other rootstocks.

C35 Citrange Rootstock

J Bouffin - San Giuliano Corsica (jean.bouffin@cirad.fr)

C35 has become a popular rootstock and it is extremely important to ensure accurate selection of nucellar seedlings. In the trial 86 plants were selected based on visual uniformity the rest being discarded as zygotic. In order to test the molecular uniformity of these 86 plants 5 SSR markers were used on DNA extracts from these plants. The results showed that 28% of the plants were zygotic and that the visual selection had not been adequate. Based on these findings there is cause for concern for the commercial use C35 as normal visual selection that is carried out in commercial nurseries does not appear adequate in removing all zygotic. This could lead to losses in field situations due to lack of uniformity as well as the loss of disease tolerance.

UCR Citrus Variety Collection (CVC)

T Siebert - UC Riverside (tsiebert@ucr.edu)

The CVC contains more than 1000 accessions of citrus and citrus relatives and serves as of information and propagation material on a worldwide basis. The collection's website is being expanded in association with the California Citrus Nursery Society (CCNS) and will include a comprehensive description of each accession. The current website is www.citrusvariety.ucr.edu

Australian Scion Programme

S Sykes - Merbein Victoria (steve.sykes@csiro.au)

The development of new scion cultivars is seen as an extremely important priority for the Australian citrus industry. Since 1991 a breeding programme has been developed which includes conventional diploid hybrids, triploid production for seedlessness and mutation breeding. The objectives are to produce new scion cultivars suited to Australia's diverse citrus regions, to produce superior quality easy to peel seedless selections with excellent internal quality and of the correct fruit size. Production aspects such as ease of picking and producing cultivars that fill early and late season as well as any other gaps are also included as criteria for new cultivars. A further emphasis of this programme is to meet the market demands identified by the industry for fruit for the export markets.

Australian Rootstock Programme

S Sykes - Merbein Victoria (steve.sykes@csiro.au)

Priorities for rootstock research and improvement were determined at a national workshop in 1895. CTV tolerance, *Phytophthora* resistance and salinity tolerance for certain regions were seen as important due to the majority of regions now being in a replant phase. Local selections, imports from China, Vietnam, Brazil, Florida, California and South Africa as well as new hybrids from their own breeding programmes form part of their evaluations. Initial glasshouse disease screening precedes field trials which are then evaluated in short term trials from which the more promising selections are included in longer term trials.

UCR Rootstocks for Iron Deficiency

M Roose - UC Riverside (mikeal.roose@ucr.edu)

Greenhouse screening on rootstock seedlings has been conducted on standard versus new hybrid selections for 4 years. Sour orange, C35 and trifoliolate were compared with the new hybrids in soil with high calcium carbonate content. Several promising hybrids have been identified.

Zygotic Embryo Rescue

M Ghasemi - Ramsar Iran (malek_ghasemi@yahoo.com)

GA concentration and timing of embryo rescue were investigated at different stages based on days after pollination (DAP). Results showed that up to 50 DAP no germination was obtained and that the best time for germination in most cultivars was between 65 to 80 DAP. GA at 5 mg/l was better than 1 mg/l in the Murashige and Tucker medium used.

Recommendations – Citrus Genomics and Breeding

- Somatic hybrids - we need to consider the establishment of a facility with a suitable breeder to take advantage of this technique.
- Transgenic plants (TP's) - an offer has already been made to test the US TP's in view of the problems they are experiencing in obtaining approval to carry out field tests in the US. We should also consider following their example and producing our own HLB and CBS resistant TP's.
- UCR Breeding Programme - we have already obtained a number of their new releases and need to keep in regular contact to ensure a lasting relationship.

- USDA Florida Breeding Programme - we are currently negotiating with USDA Florida to obtain the rights to their new seedless cultivars and need to consider acquiring their rootstocks as well if available.
- CREC Florida Breeding Programme - CREC has a wide range of new hybrids which are to be released in the near future - we need to make every effort to obtain the rights to these cultivars. In addition the results of their consumer surveys are extremely interesting - we need to consider looking into doing surveys in our markets on a regular (?annual) basis.
- I saw Tami and Kedem in the trial in southern Israel (near Gaza) and was impressed with both of these cultivars. We need to urge Citrogold to put more emphasis on these cultivars as they may be replacements for some of the 'problematic' Clementine selections.
- The somatic hybrid breeding programme of Mediterranean group - France, Spain, Morocco, Turkey and Tunisia - could prove to be a good source of rootstock material if they are willing to share it with us. Once again we could offer climatic testing conditions that they do not have in their countries.
- The Citrandarin rootstocks being evaluated in Brazil appear promising and it may be worthwhile including their programme during the next visit to Brazil.
- The low polyembryony of C35 is well known, but the results from Corsica are disturbing as a substantial % of zygotics could be slipping through the normal culling procedures of commercial nurseries.
- UCR's citrus variety collection and library is a valuable source of information and knowledge.
- The Australian scion and rootstock breeding programmes are actively focused at Australia's problems, future markets, expected climatic changes and replanting situation. We need to become more actively involved with them on these issues.
- UCR has a number of standard rootstocks as well as hybrids bred for reducing iron chlorosis. We need to look into acquiring these rootstocks for evaluation in South Africa.
- Iran's work on embryo rescue techniques appears to be of considerable interest.

No presentations by CRI staff.

8.2 M.M.N. DU TOIT

Visit to China to attend the 8th International Citrus Nursery Congress in Chongqing – 22-25 October 2008

Summary

The congress was attended by 53 delegates from 14 countries, excluding China. China was represented by 151 delegates. Nine nurseries, able to produce 10 million trees, were established with the financial support of the Chinese government. 120,000 hectares were established in the Chongqing province. Various presentations regarding many of the different aspects of the citrus nursery industry were delivered.

- Graft transmissible diseases
- Nursery industries and certification scheme of the following countries:
 - China
 - Brazil
 - Argentina
 - Peru
 - South Africa
- New promising rootstock- & scion cultivars from various breeding programmes
- Development of DNA fingerprint technology to differentiate between various cultivars
- Processing of rootstock seed
- Propagation of organic nursery trees
- Open ground nursery trees under plastic coverage
- Fertilization and growing media
- Shoot Tip Grafting; and
- Control of greening in China, California and Guangxi.

Introduction

The continuity of the organisation was again in the balance. In June 2007, Mr. Hassan Marei of Egypt, President of the ISCN, had the ball rolling by selecting a suitable country to host the congress. The necessary arrangements have been made and slowly but surely everything fell into place. It was suggested that the ISCN be held together with the International Citrus Congress in Wuhan, China, but the members decided that it is important that the organisation maintain its own identity. The congress was therefore held before the ICC, but in

Chongqing, the biggest city in China. Chongqing has a population of 30 million. In China, 1.8 million hectares is dedicated to the production of citrus (only exceeded by the production of apples) and ± 18 million tons are produced each year. The congress was attended by 53 delegates from 14 countries, excluding China. China was represented by 151 delegates. Summary of presentations and posters:

Chongqing Citrus Industry: Achievements and Strategies

Xia Zuxiang, Chongqing Agricultural Committee

With the construction of the new Three Gorges dam, the citrus industry of Chongqing plays an important role with the relocation of their citizens. Nine nurseries, with financial aid of the government, were founded. These nurseries have the capability of producing 10 million trees and 120,000 hectares are already established.

Indexing for Graft-transmissible Diseases of Citrus. It's History, Importance, Current Status and use in Certification Programs

Roistacher, C.N. (chetroist@charter.net)

Before 1945 the indexing for graft transmissible pathogens were virtually not practiced. Growers selected the budwood from the best and most productive trees. Later it was established that these trees, not clearly indicating symptoms, are the bearers of harmful graft transmissible pathogens. Top working, a generally practiced technique, is responsible for distribution of these harmful pathogens. During 1945 in California, Wallace has discovered the indexing of seedlings to identify Psorosis. This was ground-breaking and the beginning of biological indexing. In 1947, Fawcett and Wallace have described Tristeza as a virus and with a second break-through have discovered that the Mexican Lime can be used as an indicator. During 1957, the researchers were united due to the destructive nature of the Tristeza virus and so the International Organisation of Citrus Virologist was born. New technology was rapidly developed to identify graft transmissible pathogens with the use of different indicators. Initially it was suggested that the indexing of the following viruses be done for a certification programme: tristeza, sporosis, exocortis, cachexia and stubborn. Shoot Tip Grafting has developed in South Africa during 1977. With the development of technology, indexing is now done by means of ELISA, sPAGE and PCR. The devastating effect of greening experienced world-wide, has necessitate the early detection. RT-PCR, new technology, can detect the presence of bacteria within the insect vector two years prior to the symptoms being visible in the field and detection by PCR.

The Citrus Nursery Industry in Brazil

Dibbern Graf C.C. (cesar@citrograf.com.br)

In the state of Sao Paulo, the production of citrus nursery trees free of CVC and other pathogens have gone through various phases. In 1997 the main actions started with an incentive program for the production of pathogens-free trees. The official standards have been updated during 2005, where all trees must be propagated within an protected environment. Regular testing for the detection of greening, CVC, Phytophthora, nemathodes, and Citrus Canker must be done. The nurseries of Sao Paulo have converted to companies and are professionally managed, with improvement of in all levels of the citrus production chain. Their aim is to be a world leader in the production of citrus and to be an example to other countries and Brazilian states.

General Introduction on Citrus Varieties and Certification Program in Argentina and other South American Countries

Anderson, C. (canderson@correo.inta.gov.ar)

The American Continent is responsible for 45% of citrus production; and 84% of the trade in fresh- and processed citrus, worldwide. The popular cultivars in Argentina are Lisboa-, Eureka Lemon, Pera-, Midnight-, Delta Valencia, Salustiana and other midseasons, as well as, Navelina-, Lane Late-, and other Navels. Satsumas, Nova, Murcott and Clementine mandarins and limited pomelo's are of interest too. Growers requests new patented cultivars. The majority of South American countries have a certified citrus nursery programme to ensure that only propagation material of superior quality be supplied to their industry. The success of the implementation of the various programmes fluctuates. Greening has been encountered and is rapidly spreading in South America. This pose a great treat to the citrus industries of many South American countries and places great pressure on the nurseries to safely grown citrus nursery trees.

Some native citrus germplasm resources in China

Zhong Guangyan, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China

China is rich with nucleus. Certain wild mandarins, primitive and variant in its morphology, have been discovered. In Yunnan, Ichang papeda oranges naturally occur in nature. This is also the origin of the citron and its variants. To date, neither wild sweet oranges nor pummelos have been found. Trifoliate oranges like the

unique always-green trifoliate orange are widely spread. Wild Kumquat trees can also be found. It is estimated that many more germplasm materials that are not yet discovered.

Three new citrus rootstocks: “Bitters”, “Carpenter” and “Furr” Trifoliate hybrids

Roose Mikeal roose@ucr.edu

These three new rootstocks will possibly be released in June 2009 to the University of California. High quality fruit can be achieved with these new rootstocks like small trees, tolerant calcareous soils (“Bitters”) and medium-large trees (“Carpenter”). All three rootstocks seem tolerant to CTV, but vary in their tolerance towards *Phytophthora* and nematodes. They produce uniform seedlings due to their high levels of nucellar embryony. They are graft-compatible with sweet oranges, lemons, grapefruit and Mandarins.

Development of Transgenic Dwarfing Rootstocks by Introducing *phyB* and *ro/ABC* Genes

Yuan Feirong, National Centre for Citrus Improvement, Changsha, Hunan Agriculture University

Poncirus trifoliate is the most important rootstock in China and it is dependent on light for growth. Citrange rootstocks are used worldwide and grow into large trees after grafting. This research is aimed on the development of a trans-genetic trifoliate orange and Troyer citrange by transferring the *phyB* and *ro/ABC* genes into two genomes to obtain dwarfing rootstocks and improve their photosynthetic rate. Stable dwarfing characteristics, with between 10% to 25%, and higher photosynthetic efficiency were obtained.

Irradiated Mandarin Selections from the University of California Riverside

Williams, T. E. timwill@ucr.edu

Daisy SL, Fairchild SL and Kinnow SL is developed by the University of California by means of radiation and can be deemed as low seed cultivars with 2-3 seeds per fruit (with various cross pollinating situations) in comparison with their parent cultivars which has 20-30 seeds per fruit (with various cross pollinating situations). All three cultivars ripen in early winter (late December in California).

Daisy SL has large fruit (67mm in diameter and 59mm in height) with a deep orange colour, smooth rind, very sweet and excellent flavour (15.5° Brix). Daisy SL peels more easily than the regular Daisy. Alternative bearing does occur, but can be manipulated.

Fairchild SL has reasonably large fruit (65mm in diameter and 61mm in height), slightly elongated shape, orange and medium-textured rind and has a sweet rich taste at maturity (14.0° Brix). The compacted trees have a good production from the third year. Alternative bearing occurs and can be moderated with cultural practises.

The Kinnow SL fruit are flat and medium sized (63mm in diameter and 55mm in height), with a pale orange, exceptionally smooth rind. The fruit are sweet, rich and has an exceptional flavour (15.20° Brix). The growth habit is upright, excellent production on the three year, but severely bears alternatively. This can be moderated with cultural practices.

All three cultivars are estimated to be distributed outside of California, in 2011, by the master licensees.

The Irradiation Mutation Breeding Program at the University of California Riverside

Williams, T.E. timwill@ucr.edu

During 1995 a mutation breeding component were initiated within the existing breeding programme, utilizing irradiation to induce mutations of selection previously released by the University, with the intended purpose of significantly reducing the seed contents. Using variable doses of gamma irradiation (30 – 80Gy), to date 37 separate varieties and selections were irradiated. From these 68 low-seeded selections were derived from 25 varieties. Some of their successes include the “Tango”, a very low seed selection of “W. Murcott” mandarin, as well as the three selections as mentioned in the section above.

The general time-frame for the evaluation and release of a new cultivar ranges from 11 to 13 years. This is substantially faster than the development of new hybrid varieties, which ranges from between 18 to 20 years. New cultivars are patented and for the first three years, released only to the licensed Californian growers, before it is released outside of the state.

Native Australian Citrus Species as Rootstocks for Tangerine and Lemon

Smith, M.W. smith@dpi.qld.gov.au

In Australia and Papua New Guinea some of the world’s most unusual citrus species can be found, on which very little rootstock evaluation have been done. Graft-compatibility tests were done on numerous wild rootstock varieties, with Imperial mandarin and Eureka Lemon as the scion cultivar. Seven of which were found to graft-compatible. Unfortunately, these performed unsatisfactory in the field. This could possibly be attributed to their susceptibility to CTV.

Wuhe Xuegan – A Seedless Orange Variety Bred by Irradiation

Hong Qibin, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China

Wuhe Xuegan is a new seedless sweet orange which was developed through irradiation (60 Gy). On average, 0.7 seeds per fruit were found in seven year old trees under open pollination conditions and slightly more under artificial pollination, depending on the pollen sources. This cultivar produces large bright orange fruit and is suitable as fresh fruit or it can produce deep-orange coloured juice of good quality.

Scion and Rootstocks of Screen House Citrus Nursery Production in Sao Paulo State, Brazil

Zanetti Marcelo Marcelo.zanetti@citrofrut.com.mx

A recent survey showed that 556 nurseries with 36 million trees, the scion cultivars were represented as follows: Valencia 32%, Pera 26%, Hamlin 15%, Natal 10%, Folha Murcha 4% and Westin 2%. The rootstocks that were used are Rangpur lime 67%, Swingle 22%, Sunki 5% and Cleopatra 4%.

(Poster) A brief of Citrus Rootstocks used in the Mainland of China

Zhao Xueyuan, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China

Traditional rootstocks: Trifoliate orange, Suanju (Citrus sunki), Hongju (Citrus tangerine), Guotuocheng (sour orange hybrid), Honglimeng (Citrus limonia), Pummelo and citron, Sanhuhongju (Citrus erythrosa), Ziyang Xiangcheng (Citrus junos) and Carrizo citrange.

(Poster) Tango – A new, very low-seeded, Late-season Irradiated selection of W. Murcott Mandarin from the University of California Riverside.

Williams, T.E. timwill@ucr.edu

Tango is a mandarin selection developed by the University of California, by the irradiation of the W. Murcott. The Tango is distinct in being low-seeded (less than 1 seed per fruit) in all pollination situations. In California this cultivar ripens during late January and retains its fruit quality into April. The fruit is 59mm in diameter and 48mm in height, with a smooth, deep orange coloured rind. It has a deep orange flesh, juicy with a sweet flavour at optimum maturity (14.8° Brix) and is an easy-peeler. The growth habit is upright and has a good production. Overbearing can be problematic, but can be managed with the thinning of fruit. Alternative bearing with the application of appropriate cultural practices does not appear to have a significant problem. Tango has low fertility pollen and is very unlikely to cause seediness in adjacent Clementine plantings. This cultivar has been patented and will be available outside of California in 2009.

(Poster) Plant Tissue Culture, a potential tool in Citrus Propagation and Crop Improvement.

Usman, M. musman74@gmail.com

In Pakistan the lack of plant tissue culture is being investigated to improve the citrus production and resistance to the development against biotic and abiotic stresses, which cannot be achieved by means of regular breeding. This research is still in a developing phase.

(Poster) Construction of DNA Fingerprinting database for Citrus Cultivars

Lei Tiangang scchen2004@vip.sina.com

Construction of a DNA fingerprint database of major citrus cultivars is useful for citrus variety and purity testing. In this study, 102 cultivars were analysed using simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers. The results have shown that this SSR and ISSR markers are suitable for the construction of a DNA database.

(Poster) New Citrus Rootstocks released in Spain

Forner-Giner, M.A. forner_margin@ivia.gva.es

In 1974 a project for the breeding, with the use of traditional breeding techniques, of new rootstocks commenced. The purpose is to obtain suitable cultivars tolerant to generally calcareous and salinity of the soils in Spain. The main parental used is Trifoliate orange, Troyer citrange, Cleopatra mandarin, common mandarin, King Mandarin and Citrus volkameriana. To escalate the evaluation, rooted cutting were used in a fog system. This enabled testing before the mother trees were able to produce seed. The following eight new rootstocks were registered for protection in the European Union: Five selections of Cleopatra x Poncirus trifoliate (two of which is resistant towards CTV and tolerant towards salinity and flooding and it is semi-dwarfed); one is tolerant towards calcareous soils and the other is susceptible. One of these hybrids is resistant to water stress and all of them are very productive with good quality fruit. King mandarin x Poncirus Trifoliate is dwarfed, with good production. It is resistance to citrus nematode, CTV and is tolerant towards soils of a calcareous nature. Other

dwarfed rootstocks are Troyer x common mandarin (tolerant towards CTV and is highly productive) and Troyer x Cleopatra mandarin (also productive and induces late maturation in fruits).

(Poster) Construction of High Effective Regeneration System and Agrobacterium-mediated Transformation of Citrus Mature Explants

Chen Shanchunscchen2004@vip.sina.com

The influence of the incubation period on the in vitro rootstocks, and maturity and sterilization time of Shinanui branches on survival of grafted mature auxiliary buds were studied in order to establish a high frequency regeneration system for mature explants of Shinanu

(Poster) A Seedless Mutation Produced by Irradiating Seeds of Kinnow Mandarin.

Cao Li, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China

Kinnow mandarin seed were irradiated and the selection CR030321 was grafted onto a trifoliolate rootstock. After five years the trees were 2 meter high with half of the fruit being seedless and the remainder had less than 4 seeds per fruit. Kinnow normally has more than 25 seeds per fruit. The fruit ripens in December and weighs between 110 en 130g with 13.9 % Brix with a good flavour.

(Poster) A promising citrus rootstock 'Ziyang Xiangcheng'

Liu Jianjun, Citrus Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu, China

Ziyang xiangcheng (*Citrus junos* Sieb ex Tanaka) is a natural hybrid of Swingle *Citrus reticulata* Blanco, which is local to China, and has been identified as diploid. Good compatibility status has been achieved with Oranges, Mandarins, Lemons and Kumquat yielding high production and fruit size.

Processing Citrus Seed in a Modern Enviroment

Tolley Oam, I.S.ist@riverland.net.au

Ian Tolley has produced a new set of equipment made from stainless steel that can be used in the processing of seeded fruit. The separation of peel and seed is achieved through a vibrating band rather than using water as is the case with older machines. A new warm water treatment method has also been produced that simplifies the entire process. No running costs have yet been given but more information is available from him.

Development of Citrus Container Tree Production Technology in China

Wu Houjiu, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Beibei, Chongqing, China

The production for trees in containers was first implemented in 2000. Technologies and consultations from around the world were used and now the number of trees grown in this method have reached 15 million of which 15% forms the total number of trees planted yearly. Trees are grown in greenhouses, shade houses and mostly outdoors as they are dependant on sufficient sunlight. A peet mos mixture is the dominant medium that is housed in a firm, but flexible, plastic square container. The container tapers towards the bottom where a large opening is present to provide adequate drainage. These containers can be reused. Budding height varies from 10–20 cm, which previously was as low as 3 – 5 cm, with "chip budding" being the technique mostly used.

Propagation techniques for efficient sustainable citrus nursery tree production

Bederski, Klaus, Topara Fruit Nursery, PO Box 0472, Lima 18, PERU

The Topara Nursery is 400m above sea level amongst the Andean Mountains 15 km from the Pacific Ocean in Peru. They produce certified organic citrus trees according to EU regulations since 2002 which is renewed annually. The change towards organic involved a lot of replanning in order to remain economical and competitive. Production costs and the time involved in producing a tree has been lowered due to new management techniques.

Using High Tunnels for the rapid development of citrus trees

Smith, R.W.smith@citrustreesource.com

In California, growers prefer to use large open ground nursery trees as precautionary measures against possible cold damage. With the increased risk of disease carrying vectors, open ground nurseries will soon become a thing of the past. Using High Tunnels over open ground nurseries, the time it takes for tree production has decreased from 28 months to 18 months. The capital output of these non-permanent tunnels is very high, but with the associated advantages it becomes economical.

(Poster) Improving the performance of growing media in a citrus research nursery

Smith, M.W. smith@dpi.gld.gov.au

A reliable and cost effective growth medium is essential for research projects and specifically for breeding programmes. For plants in a breeding programme, the risks involved with use of a poor quality medium such as poor growth or possible plant dieback must be eliminated. The importance of using medium components that are consistent and of high quality is declared. Problems relating to coarse sand with fine particles, toxicity of poorly composted bark and the importance of batch testing of pH and steam pasteurisation was discussed and also how a quality management system can correct these problems and maintain the standard.

(Poster) Technique for propagating virus free citrus container trees in Chongqing

Xia Pingyou, *Chongqing Cash Crop Technique Extending Station, Chongqing, China*

The major techniques to propagate virus free container trees is described. Plants are sprayed 15-15-15 with a 0.2% NPK solution every week from germination until seedlings reach a height of 6 cm. The medium is sprayed with a Potassium Nitrate NPK type mixtre from a height of 6 cm until seedlings get transplanted. Three days after transplanting, a 0.3% Urea solution should be sprayed every 10 days. The first 2 Urea treatments should be accompanied by a 0.3% NPK solution which must be increased to a 0.4% NPK solution after that. During warm days the NPK mixture concentration must drop to 0.15%–0.2 %. Once seedlings reach 55 cm, the shoot tip is removed. The stems are then budded using the T-method (height not mentioned). Three days after opening, the seedling is bent above the graft. The grafted trees are fertilized the same way for the next three months using a 0.3% solution after which it gets increased to 0.4%. At the 8–10 leaf stage, trees are pinched. This causes the second shoot to develop better during the next growth spurt. Only then does the bent portion of the rootstock removed and the remaining plant supported by a bamboo pole. Trees are grouped according to size and should reach 60 cm and have a good root system after 18 months, ready for transplantation.

(Poster) Study on drip irrigation for citrus container tree production in China

Cui Guangzu, *Chongqing Jincheng Techonlogy Co. Yubwei Chongqing, China*

Drip irrigation was compared to hand irrigation. Under drip irrigation, only 13% of water and fertilizer is used and 2.5% labour. That is a saving of Rmb 0.5 yuan (R 0.77) per tree for water, fertilizer and labour and trees grow 10% faster.

(Poster) Relationship between Magnesium content and Photosynthesis in Magnesium-deficient leaves of Beibei 447 Jingchen Sweet Orange – China

Ling Lili, *Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China*

The correlation between Magnesium content and chlorophyll was investigated and explained.

(Poster) Effects of exess B on growth, gas exchange, B Status in four scion-rootstock combinations of Navel orange

Ou Sheng, *College of Horticulture and Forestry Sciences, Huazhong Agriculture University, Wuhan, China*

Information is available in English on request.

(Poster) Effect of soil with different ph on growth of citrus rootstocks and graftd budlings

Deng Lie liedeng@163.com

High pH soils of 7.5 and 8.5 are found in the Sichuan Basin. This creates an iron deficiency on trifoliolate rootstocks. Four rootstocks; Hongju, *Poncirus trifoliata*, Xiangcheng and Carizzo citrange; were used to determine the uptake of iron in soils with a pH of 7.3, 8.0 and 8.5. All seedlings showed a decrease in size as the pH rose. The Xianhcheng rootstock performed the best and did not display any chlorosis even at a pH of 8.5.

(Poster) The Physiological effects of Arbuscular Mycorrhizal Fungi (AMF) on the growth of citrus nursery trees

Zeng Ming zengming@swu.edu.cn

The experiment showed a meaningful stimulated effect when AMF is present in growth medium. Phosphate uptake improves and a strong rootsystem develops giving a good rootstock/scion relationship and enhances photosynthesis.

(Poster) In vitro culture of Pommelo and Sweet Orange budwood under Various high temperatures.

Liu Ying changyoung@hotmail.com

Through shoot-tip-grafting it was found that sucrose in fluid growth medium results in contamination and is therefore left out.

(Poster) Introduction of technique for propagating virus-free citrus seedbed trees

L.I. Taisheng, Citrus Research Institute, Chongqing, China

An informative discussion on how a nursery should be laid out including information that is already well known regarding the fundamental processes is discussed here.

Huanglongbing (HLB) and its control in the mainland of China

Zhao Xueyaun, Citrus Research Institute, Chongqing, China

HLB, namely greening has been present in China for a long time. The “mottling yellow leaf” is a typical identifiable symptom to the presence of greening. Since 1990 PCR has been used to identify the organism as *Candidatus liberibacter asiaticus*. Recently another pathogen *Candidatus phytoplasma asteri* has been discovered in Guangdong where its ability to be shoot-transmissible is varied. The transmission of HLB through psylla was proven in an experiment in 1970. All citrus types and Fortunella can be infected by HLB as well as *Murraya paniculata* and *Clausena lansium*. HLB can be successfully controlled through strict control of the psylla and disease free trees.

State of California action plan for Huanglongbing (HLB) and its vectors

Luque-Williams, M.J. mlwilliams@cdfa.ca.gov

The devastating effects of HLB has been observed in all major citrus producing lands (Brazil, China, Florida) where severe losses of commercial plantings and money have been experienced which number in the millions. The State of California does not have the African, Asian or American strains of HLB or its vectors. Because of the devastation accompanying HLB, an action plan has been set up to identify the disease and its vectors and to prevent it from entering the state. The action plan involves communication, potential entryways, economical impacts, protocols and survey procedures, control methods, special treatments, research and education. The law and control programmes and personnel are in place to prevent and hopefully eliminate this threat. The research will focus on an improved understanding of the disease and counteracting it. The educating of lawmakers, regulatory personnel and those working in the citrus industry as well as the general public is necessary to inform them of the potential devastation that would follow HLB.

Citrus Huanglongbing control in Guangxi

Bai Xianjin, Guangxi Department of Agriculture

Citrus is one of the most important economical fruit industries in Guangxi with a production of 2,345 million tons in 2007. In the last 50 years, 66,700 hectares of citrus trees have been infected with HLB have been removed. The following regulation methods have been implemented in 2005: The removal of infected trees, strict psylla control, planting of disease free trees and the implementation of mass prevention and mass treatment systems. More than 15 million HLB infected trees have been removed by the end of 2007. A virus free citrus production centre has since been established. The production of virus-free trees has risen from 1.5 million in 2005 to 15 million. HLB has decreased from 3.9% to 2.16%.

Phytosanitary status of California citrus

Krueger, R.R., USDA-ARS-NCGRCD, Riverside California

The Californian citrus industry maintains a high phytosanitary status. Propagation material is supplied through the Citrus Clonal Protection Program of the University of California. Two vector transmitted pathogens CTV and *Spiroplasma citri* are endemic to the production areas. Trees showing positive signs of CTV are identified and removed and that keeps the infection rate as low as 0.1%. Other pathogens such as citrus psorosis virus and other viroids are of low incidence. The threat to a healthy industry includes diseases like HLB and the changes in the transmissibility of the endemic CTV strains.

Chinese Citrus Virus-free Propagation Scheme and establishment of Citrus Quarantine Pest-free Zone in Chongqing, P.R. China

Zhou Changyong zhoucy@swu.edu.cn

To ensure high production rates, high quality nursery trees are essential. This is the motive behind the National Citrus Virus-free Propagation Scheme which was implemented in 2000 and the National Centre for Citrus Virus Exclusion which is operated by the Citrus Research Institute of CAAS and financed by the State. They provide virus free mother trees and budwood for both national and international exchange. Attention is also given to the development 40 new modern nurseries. Indexing for pathogens and molecular biological techniques have been applied on a large scale to screen virus free mother trees in orchards and select these in the first three years of the scheme. Trifoliolate is the dominant rootstock used, however over the last few years Carrizo and Troyer seed that has been imported is now being used. The Minister of Agriculture is paying a lot of attention on production

safety within the three top production regions and an early warning system for quarantining of citrus pests. Another proposal will be the implementation of a pest-free zone in low epidemic areas.

(Poster) Application of Molecular Biological means to monitor major citrus diseases in China

Li Zhongan zhongan369@yahoo.com.cn

Samevatting is op aanvraag in Engels beschikbaar.

(Poster) Detection of Citrus Leaf Blotch Virus using two-step RT-PCR (Briefing)

Xie Rangjin, Citrus Research Institute of Southwest University, China

Information is available in English on request.

(Poster) Preliminary studies on symptoms caused by various Citrus Tatter-leaf Virus isolates

Yang Fangyun, Citrus Research Institute, Chongqing, China

Information is available in English on request.

(Poster) Susceptibility status of Panonchus citri field populations to different acaricides.

Ran Chun, College of Plant Protection Southwest University, Chongqing, China

Information is available in English on request.

(Poster) Effects of soil fungicides on disease control and quality of Citrange seedlings

Lu Zhihong, Citrus Research Institute of Southwest University, Chongqing, China

Steam sterilisation is generally used to sterilize growth medium. In this study, various fungicides were tested for the control of harmful fungi during the seedling stage. None of the fungicides had a negative influence on seedling growth. Formalin, Aetna Health, Prochloraz amines, Azimsulf-uron and Mycophenolate Ling delivered better results than Mancozeb M45, Triophanatemethyl and Ferrous sulphate. The fungicides can also improve germination.

(Poster) Occurrence and control of major citrus insect and mite pest in China.

Dou Wei, Key Laboratory of Entomology and Pest Control Engineering, Southwest University, Chongqing, China

Various insects and mites occur in China of which the following three are the most important, *Panonychus citri* (Red Mite), *Phyllocopthruta oleivora* Ashmead (Rust Mite) and *Phyllocnistis citrella* (Leaf Miner). For many years these insects have been kept under control through acaricides, however resistance develops and an alternative needs to be found. Secondary pests include Citrus White Fly, Mealy Bug, Scale (cottony cushion and arrowhead), swallowtails and fruitfly. Integrated Pest Management is used.

Occurrence and control of Citrus Leaf Miner on Citrus Seedlings

Liu Yonghua wangjinjun@swu.edu.cn

Leaf Miner occur in all the citrus producing areas of China. The larvae bore through the leaf epidermis, this develops a site of infection from fungi and bacteria as well as stunting the growth of the plant since young flush is most often affected. Chemical control is expensive and frequent use often results in the development of resistance. Biological control looks promising and natural enemies like aphids lions, ants and some parasitoids are used.

(Poster) Infestation and controle of Citrus Red Mite Panonychus citri in China.

Wang Jinjun wangjinjun@swu.edu.cn

Red mite is an important pest in China. By rotating between various effective acaricides, resistance development is successfully prevented. The main biological control agent is *Amblyserius cucumeris* and *A. nicholsi*. Commercial production of *A. cucumeris* is already in place.

(Poster) Selection of Low-toxicity Insecticide for controlling Unaspis Yanonsis Kuwan

He Lin helinok@tom.com

Arrowhead scale is an important pest because its small waxy body makes it difficult to control chemically. Highly toxic substances such as Omethate has been banned from frequent use in citrus orchards and a safer alternative is investigated. Through careful evaluation of 21 insecticides, the chemicals emamectin benzoate, abamectin, buprofezin, imidacloprid, acephate and chloropyrifos all showed favourable effectiveness and toxicity.

(Poster) Research and implication of the Preventive Control Technology of Citrus Huanglongbing

Chen Yuefei, Fujian Yongchun Agricultural Bureau

The future spreading trend is predicted and control measures, successes and problems discussed.

Citrus Nursery Trees Traceability Program in Argentina

Anderson, C.M. canderson@correo.inta.gov.ar

In order to remain commercially competitive, citrus growers require high production rates and high fruit quality. Argentina has a Certification programme since 1998, but will only be fully implemented in 2010. In 2007, the Citrus Nursery Traceability Program (CTNP) was developed to determine the genetic origins and sanitary status of nursery trees. The CTNP is a database that is available online to import or export data for reporting nursery information. The program makes provision for the following information: A) Documentation: Scion and rootstock list, sanitary status, nursery and type of material (seed, seedlings, budwood, nursery trees). B) Registration and transactions: Includes production of propagation material and stock, delivery and barcode tags for material identification. C) Data query: Results can be supplied in various formats.

Southern African Citrus Nursery Certification Scheme

Du Toit, M. tdt@cri.co.za

For long term profitability and growth of the South African citrus industry it is important that nursery trees of the highest standard be provided through nurseries. The South African Citrus Growers Association (SACGA) is responsible for the Citrus Improvement Scheme and mandated Citrus Research International for scheme management and operation. A Certified citrus nursery must be managed through a quality management system (QMS) and is audited twice yearly and must conform to the standards set by the Scheme. The QMS focuses on water treatment and testing every three months, media treatment and testing with each new batch used, root pathogen testing every three months, pest control and monitoring, and only certified propagation material can be used. Only certified nurseries may apply for tree certificates provided the trees conform to the minimum standard.

(Poster) Risk management of citrus nursery tree production

Huang Sen, Citrus Research Institute of Southwest University, Chongqing, China

With the rapid development of China's citrus industry, the demand for citrus nursery trees increased dramatically. Many citrus nurseries were constructed by the central government. A virus free Citrus Scheme with three levels has initially been established, however the various levels have not been organised properly. Citrus nursery tree production faces various risks, namely market, costs, pests and diseases, natural disasters policy and social risks. The article identifies and evaluates the risks and proposes strategies in order to minimise economical losses.

(Poster) The present situation of Propagation Technique and Quality Standards of citrus nursery trees in China

Wang Chengqiu, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, China

In China a shift from open ground nursery trees to virus-free container trees has occurred. A quality standard has now been set and developed towards standardising, normalising and centralising. Similar to the more developed citrus countries, the normal management and quality of citrus has to be improved. The improvement of quality standards, the implementation of a national production licence for virus-free nursery trees and effective management and administration of product quality and safety is what the industry is striving for.

Current status of application of DNA barcoding technique in plants

Yan Huaxue, College of Horticulture and Landscape Architecture, Southwest University, Chongqing, China

Certain key questions need to be resolved in the application of DNA barcoding in the identification of plants.

Du Roi Nursery

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Du Roi Nursery was established in 1989 near Letsitele in the Limpopo Province of South Africa. The nursery has been certified by the SA Citrus Improvement Scheme and is under the mandate of the Citrus Nurserymen's Association and produce high quality citrus, mangos, pomegranates and guava trees. All commercially available cultivars and rootstocks are grown from certified material supplied from the Citrus Foundation Block in the Eastern Cape. Du Roi is the largest citrus nursery in South Africa. Client services are available before and after planting regarding rootstocks, cultivars, ground preparation, irrigation, fertilization and pest management. The nursery has special greenhouses where blackspot free trees are grown for certain areas. Another service

provided is Du Roi IPM, the largest commercial agricultural insectory in South Africa and the Du Roi Laboratories which specialise in the tissue culture of bananas.

8.3 PAUL FOURIE

Visit to Chile, 22-29 June 2008

Bayer Cropscience in Chile (BCS-Chile) invited me to present a lecture on “Fruit + Foliar Disease Management in South African Table Grapes”, with special emphasis on powdery mildew, Botrytis and fungicide spray application, at the Chilean launch of a new fungicide formulation called Twist Duo (mixture of pyrimethanil and trifloxystrobin). BCS-Chile agreed to cover all expenses, and also to escort me on a 2-day tour of citrus orchards and packhouses, which warranted CRI’s approval for my acceptance of this invitation.

Twist Duo should also be effective against Alteraria Brown Spot (ABS) and Citrus Black Spot (CBS), and given its double-action and mobility, should be investigated for potential use in South African citrus orchards in an anti-resistance strategy aimed at protection of the strobilurin fungicide group. The mix-partner for trifloxystrobin, pyrimethanil is regarded by FRAC as a medium-risk compound. Longterm monitoring of spray trials of pyrimethanil alone in French vineyards has not indicated any resistance development, according to Dr Helene Lachaise from Bayer in France (also chairperson of the FRAC Dicarboximide and Anilinopyrimidine work groups) who was also invited to Chile by BCS-Chile.

Meetings were held with two of the major citrus producing companies in Chile, Agricom and Propal. Lemon fruit production and export is the major citrus activity of these companies, with major markets being Japan and the USA. The citrus industry in Chile is small, relative to table grapes and deciduous fruit. Nonetheless, in excess of 20,000 hectares are produced, of which 7,856 ha are lemons, 9,231 ha under oranges, 355 under grapefruit and 3,391 ha under Clementines and Mandarins.

Orchards

Orchards were visited at Agricom only, but disease management at both organisations were discussed. Given the prevailing climate and clay soil type, lemons (Eureka and Fino49) are grown on Macrophilla rootstock and on ridges in order to improve fruit size and limit *Phytophthora* root rot, respectively. Irrigation is carefully managed through anti-leach pulse-systems with 4 to 12 pulses per day. In the case of avocados, which are grown on the slopes (to prevent frost damage), up to 80 pulses per day can be given to provide the water and nutrient demands of trees. Systemic insecticides are also frequently applied through the irrigation system. As far as I could ascertain, they do not have any experience with application of phosphonates through the irrigation system. *Phytophthora* root rot or *Fusarium* dry root rot is only a problem in cases of over-irrigation or non-ridged orchards. However, *Phytophthora* brown rot of fruit is the major preharvest disease. *Phytophthora* root and brown rot are both caused by *P. citrophthora*. However, trunk and branch cankers (gummosis) are not frequently observed, especially since the industry started to bud higher and to plant on ridges.

Control of brown rot is done mainly by ridging, skirting and up to 3 foliar applications of phosphonates and/or soil (and foliar) application of copper fungicides (at 2,000 L/ha). Phytotoxicity following phosphonate application is sometimes observed, which is overcome by recommending the phosphonate application prior to copper. The packhouse also pre-selects orchards in terms of brown rot risk, and will also take remedial action should more than 0.5 infected fruit per bin be monitored. Branches of lemon trees in visited orchards hung very low, and given the predominance of favourable weather conditions (rainy, wet and cool) one might expect high levels of brown rot infection.



Given the large number of lemon tree plantings on *Macrophilla* rootstock, citrus nematode (*Tylenchulus semipenetrans*) is an important problem. In affected orchards (with a threshold count of 2,000 larvae per 250 g soil), the pest is controlled by means of nematicide (Rugby, NemaCur, Mocap) application once a year in December. At Agricom, this application is followed by a biological product 'QL Agri', which is an extract from a native Chilean tree. QL Agri stimulates root growth and also acts as a nematicide, although not as toxic as the synthetic products [this product is being evaluated by MC Pretorius under South African conditions]. Chemical control of nematodes is very expensive (250 US\$/ha) and growers opt for more resistant cultivars such as Swingle, Citrange and C35 when replanting citrus. At Propal, soil analyses are frequently done, but they struggle to interpret the variable results due to the lack of reliable economic threshold levels.

Botrytis infection on lemons during flowering period is sometimes a problem, which leads to ridges on lemon fruit and in some cases to postharvest grey mould. It is controlled with 2 applications of Strobry (kresoxymethyl). These applications are made by 5-10% of Agricom growers, with the decision to spray based largely on historical data. High levels of Botrytis infection on flowers and fallen fruit was observed in a lemon orchard.



Green mould is the major postharvest disease. Green mould incidence differs between growers and seasons. Preharvest control measures include orchard sanitation and in some cases sprays with chlorine dioxide, which according to anecdotal evidence showed promising results. However, disease incidence in sprayed plots was not compared with unsprayed controls, and merely with historical incidence. Sour rot (*Geotrichum candidum*) is not controlled as it is not regarded as an important problem.

From what I could find out, *Alternaria* brown spot is not an important disease, although it occurs on Lane Late according to discussions with Propal. For control, they reduce the inoculum source by pruning out dead wood. At Propal, they were complaining about a new disease causing tree dieback: symptoms include brown lesions in the xylem tissue, with apparently healthy roots and no fruit or foliar symptoms.

Packhouses

The two packhouses visited, Agricom (1.4 million cartons; 25% of Chilean lemon exports) and Propal, handle most of the Chilean citrus exports. My general impression at both these facilities was the very high hygiene and sanitation standards. The packhouse flow was fairly similar, and is largely based on Californian recommendations.

Applications in sequence

1. An on-farm Thiabendazole+Chlorine drench treatment is done by Agricom only, as Propal does not believe this treatment is effective. In the past, they used a chlorine + sodium bicarbonate mixture in the on-farm drench systems.
2. At Agricom, a wet-dump system into a chlorine bath (200 ppm) followed by high-pressure wash is used. Propal uses a wet-dump system into a cold chlorine (200 ppm) + sodium bicarbonate (3%) bath with 1 min exposure time (pH of 8-9). They replace this bath every day.



3. Separated sanitation section where all visibly decayed fruit are removed before entry into the packhouse. At Propal, they used an inline UV-sorter (manual), but this was stopped due to 'over-selection'.



4. Warm (40°C) sodium bicarbonate (3%) treatment for 2-5 min wash with a 2-5 min exposure time. This bath is replaced every 2 weeks (Agricom). Instead of this treatment, Propal treats fruit in a 45-47°C soda ash (sodium carbonate) bath at 3% for 3 min (pH of 10-11). In these long baths, fruit is kept submerged by 'belt-paddles' to ensure adequate exposure. This bath is replaced every week.



5. Rinse by low pressure sprays or water shower.



6. Agricom: Warm IMZ (750 WP formulation) bath treatment for 5-10 second exposure at 150 – 250 ppm. Titrations are done every hour to ensure the correct IMZ concentration is maintained. Propal uses 3× hot (50°C in reservoir; 42-48°C on fruit) IMZ showers at 150-250 ppm, depending on disease pressure and time in season.



7. Fruit are dried and waxed with a storage wax + 500 ppm IMZ (500 EC formulation). Propal uses 1500 ppm IMZ in the storage wax. TBZ is sometimes used in the wax at 3000 ppm, although its use has declined due to resistance in *Penicillium* populations.



8. Following these treatments, the IMZ residue on fruit is 1.5 to 2.5 ppm resulting in very good green mould control and sporulation inhibition.



9. Automatic colour sorting, manual sorting for blemishes and re-binning.





10. Each bin's fruit gets a peteca and quality rating, from which the maximum storage time is estimated.
11. Degreening storage at 16°C for up to 21 days, with or without ethylene at 3 ppm. Storage period depends on colour and peteca+quality rating, as well as marketing considerations.



12. Following degreening, fruit is treated through a whole line (similar to what was described above), but waxed with export wax containing 500 ppm IMZ and exported. At Propal, the second line does not use a hot IMZ application, but only 2000 ppm in a Chellac or Carnauba+Chellac wax.
13. Retention samples are kept to monitor postharvest quality and waste. From green mould lesions, *Penicillium* isolates are screened for IMZ (at 2 ppm) and TBZ (at 10 ppm) resistance. Should resistance frequencies exceed certain thresholds, the whole packline is sanitised thoroughly.
14. Degreening rooms are sanitised with QAC formulations, the packhouse with QAC misters overnight, and the surfaces with a foamy chlorine mixture at Agricom or alcohol-mixture at Propal (used since they are uncertain about QAC residues on fruit, which really should not be a problem). All rotten fruit are discarded in closed containers to prevent aerial spread of inoculum.



15. At Agricom, Clementines and Navels are packed over a dry line, and IMZ is applied at 3000 ppm in the wax. At Propal, oranges are packed using one packline only with a hot IMZ treatment and 500 ppm IMZ in Carnauba wax. Clems were packed on a dry line with 3000 ppm IMZ in the wax, but they are changing to a wet-line. Peteca used to be a big problem at Propal, but they changed to a system where they keep the lemons in degreening chambers for 1 to 10 days before export packing. This allows them to monitor fruit for peteca symptom development, if any. Lemons are also kept on farms for 1-2 days after picking, followed by 3-5 days at ambient temperatures under a roof. From pick to packhouse intake, it normally takes 3 to max 7 days. One application that I wanted to experience was postharvest fungicide application with the Bayer-patented electrostatic system Typhoon. According to my discussions, Typhoon is only used in 3 table grape packhouses and not in citrus. Only two of these systems are in use in Chile.

General recommendations

- Study and implementation of salts (sodium carbonate or sodium bicarbonate).
- Pre-packline sorting of decayed fruit to prevent contamination of packhouse.
- UV-sorting of quiescent postharvest decay lesions.
- Pre-degreening colour sorting of fruit to prevent super-optimal degreening times.
- Use of risk indicators to manage postharvest handling of different fruit lots.
- Split application of imazalil in bath/shower and wax to ensure adequate residue loading.
- Promote the use of belt-paddles to ensure adequate exposure in fungicide baths.
- Institute fungicide resistance monitoring as an industry service.
- Automated sanitation systems in packhouses (i.e. QAC misters).

Contacts established

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8.4 G.C. SCHUTTE

8.4.1 Tour to Torino, Italy to attend the 9th International Congress of Plant Pathology

Summary

New contacts with researchers from other countries working on various diseases were made and old ties strengthened with known ones. Researchers from France working on citrus canker were of great importance, since they have detected canker in Africa. The new head of the Diagnostics division of the Plant Protection Service in Wageningen, Hans de Gruyter, was also of importance since he receives and identifies the CBS fruit that is intercepted in the harbours. He was also part the team of researchers with Pedro Crous that identified a new strain of *Guignardia* on citrus. New cooperative research projects with researchers from Spain, Brazil, Holland, Taiwan and Uruguay also resulted from this congress.

Itinerary

Saturday 23 August	Travel: Johannesburg – Zurich- Torino
Sunday 24 August	Register for conference
Monday 25 August	Conference started
Friday 29 August	Conference ended
Saturday 30 September	Travel: Torino-Zurich-Johannesburg

I presented two posters entitled:

- Reduction of contact fungicide tank rates by addition of a patented DDAC (Sporekill®) for the control of *Guignardia citricarpa* and *Alternaria alternata* on citrus in South Africa
- Identification and control of *Phytophthora citrophthora*, the cause of a new trunk disease of Clementines in South Africa

Keynote address:

PLANT HEALTH RESEARCH IN EUROPEAN PROGRAMMES
T.J. Hall. DG Research, European Commission.

Agriculture and forestry have received increased political and public attention in the context of today's global challenges, including climate change, bio-fuels, food security and the potential competition between food and biomass production, globalization as well as food safety issues and biosecurity – many have implications for plant health research. The European Commission has been supporting trans-European and wider international scientific cooperation, both on pre-defined topics as well as on investigator-driven research activities through its

multi-annual Framework Programmes (FPs). Since plant diseases and their control are important for farmers, consumers and the environment, research on plant health, pesticide usage, low-input farming, food chain issues and associated genomics and biotechnology have been important components of recent FPs and will continue to be in the 7th FP. The latter provides increased opportunities for plant health research and crop-related research in general, under the Theme Food, Agriculture and Fisheries, and Biotechnology, but also in other parts. Since European Commission research funding represents only a small fraction of the overall European effort, it is vital that a more coherent approach is developed across Europe to maximize research output. In recent years there has been progress towards realizing a more complete European Research Area in the plant sciences but further advances are still needed. Several Technology Platforms are playing an important role in this respect, together with a number of ERANET coordination actions which bring together managers of similar national programmes from different countries with a view to developing complementary priorities, joint calls and possibly, ultimately, joint programming.

Six sessions ran concurrently and it was impossible to attend all the interesting sessions. Here are abstracts of some of the interesting lectures and posters:

a) **Market access**

BIOSECURITY IN THE MOVEMENT OF COMMODITIES AS A COMPONENT OF GLOBAL FOOD SECURITY.
N.A. van der Graaff and W. Khoury. Former Chief, Plant Protection Service, Food and Agriculture Organization of the United Nations
(FAO). Email: vdgraaff@tiscali.it

Demand for agricultural produce will closely follow the continued growth of the world population and the change in consumption patterns in major developing countries. International agricultural trade, which more than doubled between 1987 and 2003, is vital for access to food. Biosecurity (regulatory regimes for food safety, animal and plant health, and the introduction of genetically modified organisms) results in technical barriers to trade. The WTO Sanitary and Phytosanitary Agreement (SPS Agreement, 1995) seeks to avoid arbitrary or unjustifiable discrimination among countries. Phytosanitary biosecurity addresses the introduction and spread of plant pests. Phytosanitary policies and measures have a dominant effect on market access and many countries have started a regulatory review to ensure a scientific base for phytosanitary measures. The SPS agreement recognizes the international standards for food safety set by the FAO/WHO Codex Alimentarius as well as International Standards for Phytosanitary Measures (ISPMs) concluded within the framework of the International Plant Protection Convention (IPPC), an intergovernmental FAO Treaty. The latter has led to major changes in the international plant health framework: setting of international phytosanitary standards has been taken up and the number of IPPC Contracting State Parties has increased to 164. Future developments might include the international recognition of pest free areas. Many countries are seeking synergies among their national regulatory systems for Biosecurity allowing for consistency of decision-making and best use of limited resources. At international level, there is a need for further alignment between the above-mentioned agreements, and the Convention on Biological Diversity and its Cartagena Protocol. While private standards gain importance in food safety, phytosanitary biosecurity remains primarily a government concern.

EFSA SPECIAL SESSION

RISK ASSESSMENT AT THE EUROPEAN FOOD SAFETY AUTHORITY.
R.L. Majjala. European Food Safety Authority, Risk Assessment Directorate, Largo N. Palli 5/A, 4300 Parma, Italy. Email: Riitta.Majjala@efsa.europa.eu

The European Food Safety Authority is the keystone of EU risk assessment regarding food and feed safety. In close collaboration with scientists, national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and communication on existing and emerging risks. Since EFSA's advice serves to inform scientific knowledge for risk managers, a large part of EFSA's work is undertaken in response to specific requests for scientific advice. Requests for scientific assessments are received from the European Commission, the European Parliament and EU Member States. EFSA also undertakes scientific work on its own initiative, so-called self-tasking. The EFSA risk assessment is based on available scientific evidence and is undertaken in independent, objective and transparent manner. The EFSA Scientific Committee and the Scientific Panels provide opinions on issues related to specific biological risk factors for human health, animal and plant health and regulated substances and products. Aiming at strengthening the European food safety system EFSA

further develops risk assessment approaches and methodologies. Databases, monitoring and reporting systems are built jointly with the EU Member States to ensure that the risk assessments are supported by comprehensive data. Scientific excellence of EFSA experts in risk assessment matters matched with high scientific standards, independence, transparency and efficiency supports the EU risk managers in their decision making processes.

THE EFSA PANEL ON PLANT HEALTH: ACCOMPLISHMENTS AND CHALLENGES FOR THE EU PEST RISK ASSESSMENT.

Jan Schans, E. Ceglarska, S. Cheek and G. Stancanelli.
European Food Safety Authority, Panel on Plant Health, Largo
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Directive 2000/29/EC of the European Community enforces protective measures against the introduction into and spread within the Community of organisms harmful to plants or plant products. The right to take such measures has been established (IPPC, 1997; WTO-SPS, 1995), provided that these are based on scientific principles and are not maintained without sufficient scientific evidence. Internationally endorsed guidelines for 'Pest Risk Analysis' have been developed (ISPM No. 2, No. 5, No. 11, No. 21). The EFSA Plant Health (PLH) Panel provides scientific advice on the risks of plant pests to the safety and security of the food production chain and its environment in the EU, underpinning risk management decisions. The PLH Panel started its activity in June 2006. It is composed of 21 Members with expertise in various fields of risk analysis and plant health. It operates with the support of the EFSA PLH secretariat through four permanent working groups. Since its start, it has published six scientific opinions on the risk posed by invasive plants, weeds, a citrus bacterial disease and a citrus insect pest. The PLH Panel is currently reviewing pest risk assessments of banana and citrus pests for the French overseas departments. The target of the PLH Panel is to contribute to development of a harmonized approach for pest risk assessment in the EU, in cooperation with EU Member States and international organizations. Noteworthy challenges are the analysis of uncertainties relating to pathways and impacts and evaluation of their effects on conclusions of pest risk assessment.

b) Post harvest diseases

MECHANISMS MODULATING FUNGAL ATTACK IN POSTHARVEST PATHOGEN INTERACTIONS.

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As biotrophs, insidious fungal infections of postharvest pathogens remain quiescent during fruit growth while at a particular phase during ripening and senescence the pathogens transform to necrotrophs causing typical decay symptoms. Exposure of unripe hosts to pathogens (hemi-biotrophs or necrotrophs), initiates defensive signal-transduction cascades that limit fungal growth and development. Exposure to the same pathogens during ripening and storage activates a substantially different signaling cascade which facilitates fungal colonization. His presentation focused on modulation of postharvest host-pathogen interactions by pH and the consequences of these changes. Host pH can be raised or lowered in response to host signals, including alkalization by ammonification of the host tissue as observed in *Colletotrichum* and *Alternaria*, or acidification by secretion of organic acids as observed in *Penicillium* and *Botrytis*. These changes sensitize the host and activate transcription and secretion of fungal hydrolases that promote maceration of the host tissue. This sensitization is further enhanced at various stages by accumulation of fungal ROS that can further weaken host tissue and amplifies fungal development. Several particular examples of coordinated responses which follow this scheme are described, followed by discussion of the means to exploit these mechanisms for establishment of new approaches for postharvest disease control.

c) Soil borne diseases

TECHNIQUES FOR THE DETECTION OF AGENTS OF DECAY AND ROT IN STANDING TREES.

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Detection of wood decay in standing trees and identification of the causal agents are crucial for assessment of hazardous trees and prediction of severity and development of decay. The VTA instrumental approach represents an advance compared with the use of the increment borer in the past, but penetrometers rarely allow precise evaluation of the extent of rot. Recently there has been an improvement in diagnostic techniques like electrical conductivity meters, ultrasonic detectors, X-rays and γ -rays, densimeters and nuclear magnetic resonance, but these instruments only give local information concerning the material surrounding the sensors. Some of these techniques have recently been developed for tomographic investigation allowing reconstruction of the section investigated, measuring the energy passing through the trunk. Different types of energy give information about the different physical properties of wood: ultrasonic waves describe elasticity, electric fields and radar electromagnetic waves reveal conductivity, γ -rays and X-rays measure density. Although modern technologies are improving the detection of wood decay, identification of the fungal agents is not always feasible without the presence of fruit bodies. Their identification is important to predict the pattern of spread within the tree and the risk of spread to neighbouring trees. Fungal DNA extraction directly from wood is a promising method for specific and sensitive diagnoses. **PCR methods, based on taxon-specific nuclear or mitochondrial primers, have proved effective for identification, at different taxonomic levels**, of the most important and widespread decay fungi responsible for tree failures.

MANAGING NEMATODES IN THE POST-METHYL BROMIDE ERA.

N. Greco and L.W. Duncan. CNR - Istituto per la Protezione delle Piante, Sezione di Bari, Via Amendola 122D, 70126 Bari, Italy. Email: n.greco@ba.ipp.cnr.it

The phase-out of methyl bromide has caused problems in the management of some economically important plant parasitic nematodes. Scientists are trying to implement new strategies that are effective, convenient, safe, sustainable and profitable. Future management programmes will rely more on the integration of resistant cultivars with crop rotation and other control tactics. Increased reliance on nematode identification, spatial monitoring, and the adoption of precision agricultural methods are also likely. Greater emphasis will be placed on some existing management tactics and new tactics will be adopted. Increased reliance on quarantine measures will help to reduce the spread and establishment of exotic nematodes. Plant nurseries can play a key role by releasing only material that is free of nematodes and other pests and diseases. Managing the water and nutrient holding capacity of soil can help plants tolerate nematode infestations and some industrial and municipal wastes used as soil amendments can also suppress nematode populations. **Many plant extracts are being studied for their nematicidal activity and some are available as commercial formulations.** Micro-organisms that are antagonistic to nematodes or that induce resistance in plants are being commercially formulated. Physical methods of controlling nematodes are being re-evaluated. Solarization and biofumigation are increasingly adopted and new machinery is being developed to produce hot water or steam at an acceptable cost for use in protected cultivation and in the field. Although conventional nematicides (primarily organophosphate, carbamate, halogenated hydrocarbons) have decreased in availability, some new nematicides and **nematicide mixtures** being studied appear promising.

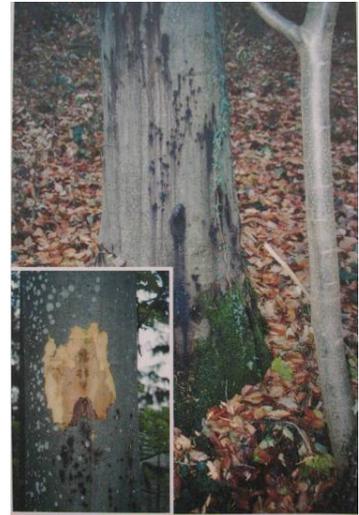
INVOLVEMENT OF PHYTOPHTHORA ROOT AND BARK INFECTIONS IN THE WIDESPREAD DECLINE OF EUROPEAN BEECH IN BAVARIA.

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Fig. 5: Active (red) and inactive (yellow) aerial bark necrosis caused by *P. citricola* on mature beech.

During the past decade, and in particular after the wet summer of 2002, an increasing number of trees and stands of Euro- Journal of Plant Pathology (2008), **90** (2, Supplement), S2.81-S2.465 S2.105 pean beech (*Fagus sylvatica* L.) in Bavaria were showing symptoms typical for *Phytophthora* diseases: thinning and dieback of the crown, small and often yellowish leaves, root and collar rot and aerial bleeding cankers up to stem heights of >20 m. Between 2003 and 2007, 100 mature beech stands on a broad range of soils were examined, and typical *Phytophthora* symptoms were found in 94 stands. In most stands the majority of beech trees were declining, and scattered or clustered mortality occurred. Eight different *Phytophthora* species were recovered from symptomatic tissues or rhizosphere soil of 243 of 300 trees investigated. The most frequent species were *P. citricola* and *P. cambivora* followed by *P. cactorum*, *P. gonapodyides*, *P. pseudosyringae* and *P. syringae*. Primary *Phytophthora* lesions were soon infected by a series of secondary bark pathogens and wood-rotting fungi; **the predisposed trees were usually attacked by several bark- and wood-boring insects leading to rapid mortality.** A small-scale survey in nine Bavarian nurseries demonstrated regular infestations of all 12



Figs. 2-3: Severe collar rot caused by *P. cambivora* on a mature beech

beech fields with the same range of *Phytophthora* species. The results indicate that (1) *Phytophthora* species are regularly associated with beech decline, and (2) widespread *Phytophthora* infestations of nursery stock might endanger current and future silvicultural projects aiming to replace pure conifer stands by beech-dominated mixed stands.

EFFICACY OF FUNGICIDES APPLIED TO THE SOIL FOR MANAGEMENT OF PHYTOPHTHORA ROOT AND CROWN ROT ON CHILE PEPPERS.

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The oomycete pathogen *Phytophthora capsici* can cause extensive losses in pepper plantings. Studies were initiated to evaluate and compare several new and existing fungicides for their ability to protect pepper plants from *Phytophthora* crown and root rot when applied to the soil. In 2005 and 2006, soil was collected from within the root zone of chile pepper field plants infected with *P. capsici*. Five parts of this soil was thoroughly mixed with 2 parts sand in a large container, then dispensed into a series of 0.5-liter plastic pots. A bell pepper seedling (approximately 8 cm tall) was transplanted into each pot, and the soil in each container was then drenched with 200 ml of a solution containing one of the chemical treatments. Plants were maintained in a greenhouse for approximately 2 months. Additional applications of materials to the soil were made after 1-month in 2005 and after 3- and 6-

weeks in 2006. Average survival time for pepper plants grown in infested soil not treated with a fungicide was 5 and 29 days in 2005 and 2006, respectively. On the other hand, in both years the survival of plants in soil treated with **cyazofamid, fluazinam, fluopicolide, dimethomorph, mandipropamid, and fenamidone + propamocarb** did not differ significantly from plants grown in soil not containing *P. capsici*. These ingredients were all effective for management of root and crown rot on pepper plants when applied as a soil drench in these trials.

COORDINATED DEFENCE RESPONSES ELICITED AGAINST PHYTOPHTHORA. R. Daniel and D.I. Guest. Faculty of Agriculture, Food & Natural Resources, The University of Sydney, NSW 2006, Australia. Email: d.guest@usyd.edu.au

Disease resistance in plants results from the timely signaling and expression of complex defences; when these signals fail or are suppressed, plants succumb to disease. *Phytophthora* species have globally-significant

cultural, ecological and economic impacts, and can be effectively managed using phosphonate. Phosphonate targets the plant–pathogen interaction but we have yet to discover the particular components of the signalling network on which it acts. We have shown that phosphonate restricts pathogen development by enhancing defence responses in *Arabidopsis thaliana* following challenge by *Phytophthora palmivora*. Within 3 hours of inoculation, *P. palmivora* is arrested within the first three cell layers in phosphonate-treated seedlings. Extracellular release of superoxides occurs 6-7 hours after inoculation, immediately preceding hypersensitive cell death. **Phosphonate treatment also enhances the accumulation of soluble phenolics including the phytoalexin camalexin and the deposition of lignin.** Our studies indicate that application of phosphonate to *A.thaliana* induces a similar host defence response to that observed in other incompatible host-pathogen interactions.

INFLUENCE OF ENVIRONMENTAL FACTORS AND RELATIVE WATER CONTENT ON THE SUSCEPTIBILITY OF CITRUS CULTIVARS TO PHYTOPHTHORA CITROPHTHORA AND P. NICOTIANAE.
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Variability in the susceptibility of three *Citrus* cultivars: mandarin Clementine cv. 'Hernandina', Fortune hybrid and sweet orange cv. 'Lane-Late' to monthly inoculations of *P. citrophthora* and *P. nicotianae* (syn. *P. parasitica*) was evaluated from October 2004 to December 2006. Inoculations were made on branches in the field and excised branches *in vitro* with both pathogens. The areas of canker obtained were found to be correlated with environmental parameters and relative water content. In field assays, cultivars inoculated with *P. citrophthora* **developed largest lesions during March-June (spring)** and with *P. nicotianae* from June to August. However, canker areas on excised citrus branches inoculated with both pathogens were largest during March–May. **There were no correlations between environmental parameters during the 27-month period and the extent of colonization by *P. citrophthora*.** Nevertheless, a significant relationship was observed for mean relative humidity and mean ambient temperature with the length of lesions during October to May of each year. There was a strong positive correlation between mean maximum relative humidity and mean maximum temperatures with the size of cankers caused by *P. nicotianae*. Canker areas were significantly related to relative water content of Fortune and Lane-late cultivars in orchard assays. In excised branches, the lesions caused by both pathogens were not significantly related to any seasonal change in water content. Seasonal changes in susceptibility of citrus cultivars to *P. citrophthora* and *P. nicotianae* may facilitate timing of disease control measures to coincide with periods when disease development is greatest.

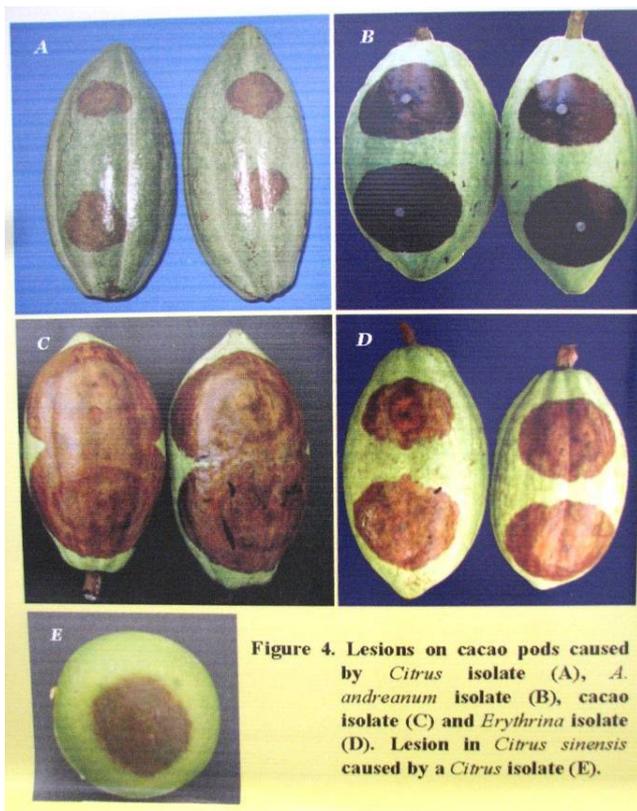
ROOT PATHOGENS IN CITRUS NURSERIES OF FARS PROVINCE (IRAN).

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In different seasons during 2002-2003 most of the *Citrus* nurseries of Fars province were visited and samples showing symptoms of wilt, dieback, and decline were removed to the laboratory with care. After washing the roots, 5 mm lengths of root were treated in 10% chlorax for 1-3 minutes and put on the culture media WA and PDA. Fungi isolated and purified by hyphal tip or single spore culture were inoculated on lemon seedlings and pathogenic isolates were studied taxonomically. 52 pathogenic isolates were found, belonging to the five following fungi: *Rhizoctonia solani*, *Fusarium solani*, *Pythium aphanidermatum*, *Phytophthora citrophthora*, and *Phytophthora parasitica*. *F. solani* is described for the first time as a cause of seedling damping-off from Iran. 105 Samples of soil and roots from different part of commercial citrus nurseries were collected, screened and centrifuged to separate out the nematodes, and preparations were made. **The following species were identified: *Tylenchulus semipenetrans*, *Helicotylenchus pseudorobustus*, *Paratylenchus hamatus*, *Xiphinema pachtaicum*.** Of which *Helicotylenchus pseudorobustus* and *Xiphinema pachtaicum* are reported for the first time in Fars province.

TAXONOMIC STUDIES ON TROPICAL ISOLATES CLASSIFIED AS PHYTOPHTHORA CITROPHTHORA.

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Phytophthora citrophthora is pathogenic to *Citrus* and other economically important crops. In Brazil, a species putatively classified as *P. citrophthora* is the most aggressive among the ones causing black pod disease on *Theobroma cacao*. Taxonomic studies were conducted to compare Brazilian isolates from cacao and *Citrus*. Random amplified polymorphic DNA (RAPD) and sequence analyses were performed on 83 isolates (69 from cacao, 12 from *Citrus* spp., one from *Erythrina glauca* and one from *Anthurium andraeanum*). Fragments of three genes (ITS region, translation and elongation factor 1a and b-tubulin) were sequenced and used for phylogenetic analyses. RAPD and sequence analyses of each or the three gene fragments combined showed the existence of two distinct groups: one formed by isolates from cacao, *Erythrina glauca* and *Anthurium andraeanum* and the other composed of isolates from *Citrus* spp. Isolates from cacao were more closely related to *P. capsici* than to isolates obtained from *Citrus*. **Sporangia were bigger (63.9 × 30.6 µm in average) in cacao isolates than in *Citrus* isolates (42.6 × 29.0 µm).** The shape of the sporangia and the ability to form chlamydospores also differed between the two groups. Cacao isolates were unable to infect *Citrus* fruits while the latter group was capable of infecting cacao pods.

NEMATICIDAL EFFECT OF FURFURAL AND SOME BIOCONTROL AGENTS ON CITRUS NEMATODES INFESTING CITRUS TREES.

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Studies of the effect of furfural and nematode-trapping fungi on yields of citrus infested with citrus nematode (*T. semipentans*) were carried out under field conditions. Both furfural and nematode-trapping fungi increased the yield of Valencia orange (*Citrus sinensis* L.) when they were applied **at the rate of 3000 ppm** for furfural, 1L and 1kg per 10L water in the drip irrigation system. The furfural treatment was superior to the nematode-trapping fungi treatments. Citrus nematode populations in soil and citrus roots were decreased by furfural and nematode-trapping fungi treatments compared with untreated trees (controls). Citrus nematode populations were decreased more by furfural than by the nematode-trapping fungi during the first three months after application.

COMPARISON OF KUMQUAT ISOLATES OF PHYTOPHTHORA CITROPHTHORA WITH CITRUS ISOLATES IN TAIWAN.

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In 1995, a severe disease caused by *Phytophthora citrophthora* broke out in Kumquat (*Fortunella margarita*) orchards in northeastern Taiwan. In order to determine if the pathogen is a recent introduction, a survey of *P. citrophthora* on members of the Rutaceae in Taiwan was conducted from 1995 to 2006. Among 200 orchards in 14 counties surveyed, *P. citrophthora* was detected in 23 orchards, and 83 isolates (all A1 mating type) were obtained including 35 isolates from kumquat and 48 isolates from citrus species. Kumquat isolates showed colony patterns on PDA plates different from other citrus isolates and produced more sporangia, and were more pathogenic to kumquat than citrus isolates. The similarity values of rDNA sequences in ITS regions ranged from 97 to 100% among all isolates tested, the length of ITS regions was 779 bp for kumquat isolates and 782-784 bp for citrus isolates. In comparison with citrus isolates, all kumquat isolates have 4 base pair deletions and 2 base substitutions in ITS1 regions, and 6 base substitutions in ITS2 regions. **Our results suggest that the pathogen causing severe kumquat decline in Taiwan in recent years is a new invasive strain of *P. citrophthora*.**

CONTRIBUTION OF GENOMICS IN REVEALING THE MODE OF ACTION OF FUNGICIDES.

R. Beffa, F. Schmitt, D. Nennstiel, J.L. Zundel, P. Perret, A.L. Mauprivez, A. Lappartient, V. Toquin, F. Villalba, T. Knobloch, V. Lempereur and M.-H. Lebrun. BCS Biochemistry department and UMR5240 CNRS-UCB-INSA-BCS, Functional Genomics of Plant Pathogenic Fungi, Bayer Cropscience, La Dargoire, Research center, 14-20 rue P Baizet, 69263 Lyon Cedex 09, France. Email: roland.beffa@bayercropscience.com

The expanding field of fungal genomics fuels the development of genome wide functional tools and comparative analyses in plant pathogenic fungi. As a consequence, transcriptomics, proteomics and metabolomics studies coupled with high throughput forward and reverse genetics are now available in a significant number of fungal plant pathogens (e.g. *Ustilago maydis*, *Magnaporthe grisea*, *Fusarium graminearum*, *Botrytis cinerea*). These functional and their associated bioinformatics tools are essential in accelerating the discovery of biochemical targets required to develop novel fungicides. These tools also offer the possibility to accelerate the identification of the biochemical mode of action of novel fungicides. This knowledge is required to characterize and follow efficiently the emergence of resistance. We will review the available genomic tools in plant pathogenic fungi. Several case studies will be discussed by comparing the information obtained using classical biochemical approaches and adequate genomic and bioinformatics tools. These studies have shown that the efficient discovery of modes of action requires a multi-disciplinary approach involving cellular biology, biochemistry, molecular genetics and genomics.

Fluopicolide – a new fungicide for *Phytophthora*

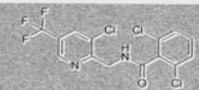
Fluopicolide

Chemical class: Acylpicolides

Chemical name: Fluopicolide

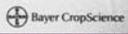
Molecular formula: $C_{14}H_8Cl_2F_3N_2O$

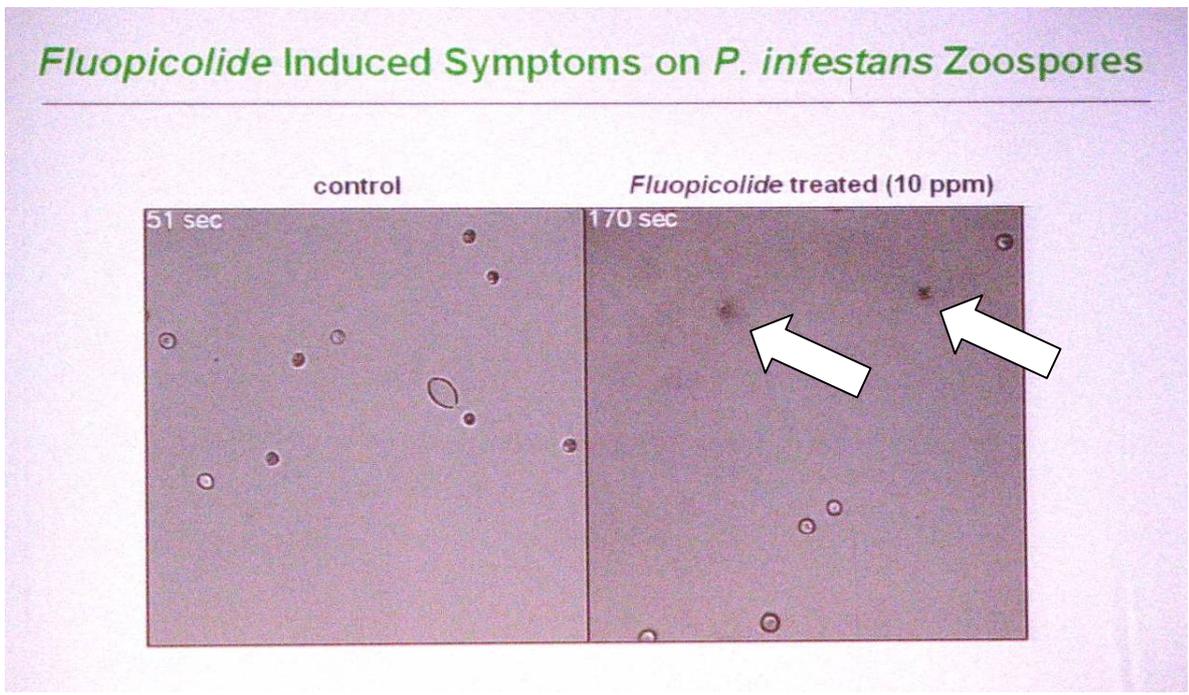
Structural formula:



Chemical name (IUPAC): 2,6-dichloro-N-[(3-chloro-5-trifluoromethyl)-2-pyridinyl]methylbenzamide

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d) Greening

HUANGLONGBING DISEASE OF CITRUS AND THE GENUS CANDIDATUS LIBERIBACTER.

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Huanglongbing (HLB), an insect-vector-transmitted disease, is destructive and endangers the very existence of commercial citrus. Leaves with blotchy mottle and lopsided fruits with aborted seeds are characteristic of HLB. The causal agents are endogenous bacteria, restricted to the phloem sieve tubes, and members of the genus *Candidatus Liberibacter*, a new genus within the alpha-proteobacteria. The liberibacters have never been obtained in culture. Their phylogenetic and taxonomic characterizations are based on 16SrDNA sequence comparisons. Until 2004, two species of liberibacters causing HLB were known: *Candidatus Liberibacter africanus* and *Ca. L. asiaticus*, responsible for the disease, respectively in Africa and Asia. In 2004, characteristic symptoms of HLB were observed in São Paulo State, Brazil. Some trees were infected with *Ca. L. asiaticus*, but most trees were PCR-negative with the primers specific for the African or the Asian liberibacter. PCR amplification with universal primers for prokaryotic 16SrDNA led to the discovery of a third species of liberibacter, *Ca. L. americanus*, and the development of specific primers for its detection. HLB was noticed in Florida in 2005, and only *Ca. L. asiaticus* was found to be involved. For further characterization, the *nusG-rplKJL-rpoBC* gene cluster was sequenced for the three liberibacter species, and used to determine the percentage sequence identity. An estimation of speciation dating has been attempted. It appears that *Ca. L. asiaticus* and *Ca. L. africanus* diverged some 150 million years ago, while speciation of *Ca. L. americanus* might have started some 300 million years ago.

CONTROL OF CITRUS YELLOW SHOOT DISEASE (HUANGLONGBING) IN SOUTH AND NORTH AMERICA.

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Huanglongbing (HLB) is a destructive disease affecting all citrus species and *Murraya paniculata*. In Brazil, it is caused by *Candidatus Liberibacter americanus* and *Ca. Liberibacter asiaticus*, disseminated by *Diaphorina citri*, and first reported in July 2004 in Araraquara municipality of São Paulo State. In October 2007 HLB had been detected in 149 municipalities with 80% of the estimated 2 million affected trees found in Araraquara and the vicinity. To suppress HLB, Fundecitrus and governmental institutions have implemented a strong campaign to advise growers of the need to frequently inspect and immediately eliminate symptomatic trees (this is required by law), to control the vector, and to use healthy trees for planting (mandatory production of nursery trees under insect-proof conditions since 2003). Today, a team from Fundecitrus trains growers on symptom recognition and disease control and helps the government in farm inspection. Most farmers who rigorously adopted the management practices have succeeded in suppressing the disease. Low disease incidence and absence of other affected farms in the vicinity are factors that decisively contribute to the success of control. In Florida, *Ca. Liberibacter asiaticus* was first discovered in August 2005 in a commercial nursery in Florida City and in the residential communities of Pinecrest and Coral City. By October 2007, HLB had been reported in 28 counties, predominately in the southern half of the Florida peninsula. Florida is moving to certified greenhouse nurseries, but there is no law for HLB eradication. **Psyllids are controlled and symptomatic trees eradicated spontaneously by conscientious growers.**

XYLELLA FASTIDIOSA IN GRAPEVINE AND CITRUS.

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Various strains of the xylem-limited bacterium *Xylella fastidiosa* cause diseases in numerous crops and forest trees in the subtropical and tropical Americas, but grapevine and sweet orange are the most seriously affected crops. Significant new epidemics of citrus variegated chlorosis disease in sweet orange in Brazil and Pierce's disease of grapevine in California have accelerated research on this bacterium over the past 10-15 years. Disease prevention is the only available disease management strategy. In California control of leafhopper vectors is the main method of management of Pierce's disease. **In Brazil, a rigorous sanitation of planting stock and disease removal combined with vector control is used. A citrus strain of *X. fastidiosa* was the first plant pathogenic bacterium to be completely sequenced, and promising new ideas for control of *X.***

fastidiosa have emerged from molecular based studies employing various knockout bacterial mutants deficient for fimbriae, pili, hemagglutinins, cell-cell communication, Type I and Type V secretion systems. For example, over expression of *X. fastidiosa*'s cell-cell signalling molecule within plants suppresses bacterial movement and reduces symptom severity. A dominant gene (*PdR1*) for resistance to Pierce's disease has been identified and mapped from hybrids of *Vitis vinifera* × *Vitis arizonica*, and backcrosses to *V. vinifera* are underway to produce resistant wine and table grape cultivars with commercial quality.

e) **Fruit and foliar diseases**

THE CHALLENGE OF CHEMICAL CONTROL OF PLANT DISEASES

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Since the first fungicide, sulphur, was used to control powdery mildew on grapes, production of most crops has become dependent on the use of fungicides to avoid disease losses. In the late 1840s the Irish potato famine proved the necessity for chemical intervention to prevent human and economic disaster. Recently it has become increasingly difficult for growers to control crop diseases. Genetic resistance of crops towards diseases has been in many cases short-lived (for example cereal rusts), GMOs have only limited success for disease control and acceptability. With more intensive cropping, new diseases have arisen which are devastating if not controlled, such as Asian Rust of soybean. In addition, new more aggressive pathotypes of diseases have arisen. All these changes require the rapid development of chemical control measures to prevent economic disaster, since reliance on genetic resistance and cultural techniques have been insufficient. Intensive use of chemical control measures has in turn led to its own challenges, including fungicide resistance. The sustainable use of fungicides to prolong their effectiveness and usefulness to growers is key, and the implementation of resistance management strategies an essential part of this. Only if the long-term effectiveness of fungicides can be ensured will industry invest the money and resources required for their discovery and development, especially considering the high standards of today's registration requirements. The Fungicide Resistance Action Committee (FRAC) and its network play a vital role in the design and support of these strategies.

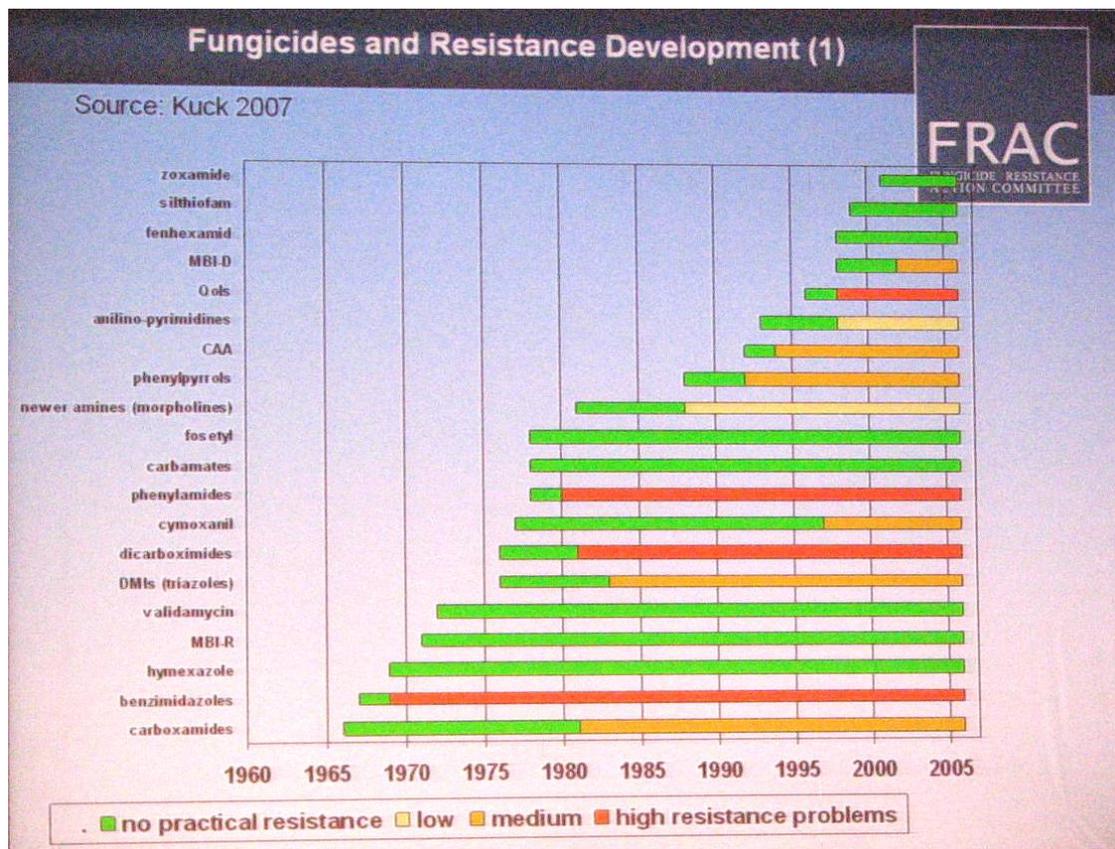
History of Fungicides up to 1940

Year	Fungicide	Primary use
BC	Natural products	Cankers, mildews
c. 60-1760	Wine, brine, arsenic, Copper sulphate	Cereal seed treatment
1824	Sulphur (dust)	Powdery mildews and other foliar pathogens
1833	Lime sulphur	Broad spectrum, fruit, vines
1885	Bordeaux mixture	Broad spectrum
1891	Mercuric chloride	Turf fungicide
1900	CuOCl ₂	Broad spectrum, especially <i>Phytophthora infestans</i>
1932	Cu ₂ O	Seed and foliar broad spectrum
1934	Dithiocarbamates	Broad spectrum protectants
1940	Chloranil, Dichlone	Broad spectrum, generally seed treatments

Key Fungicide Introductions

Year	Fungicide	
1940-1960	thiram, zineb, nabam, biphenyl, oxine copper, tecnazene, captan, folpet, fentin acetate, fentin hydroxide, anilazine, blastidicin S, maneb, dodine, dicloran	13
1960-1970	mancozeb, captafol, dithianon, propineb, thiabendazole, chlorothalonil, dichlofluanid, dodemorph, kasugamycin, polyoxins, pyrazophos, ditalimfos, carboxin, oxycarboxin, drazoxolon, tolyfluanide, difenphos, benomyl, fuberidazole, guazatine, dimethirimol, ethirimol, triforine, tridemorph	24

1970-1980	iprobenfos, thiophanate, thiophanate-methyl, validamycin, benodanil, triadimefon, imazalil, iprodione, bupirimate, fenarimol, nuarimol, buthiobate, vinclozolin, carbendazim, procymidone, cymoxanil, fosetyl-AI, metalaxyl, furalaxyl, triadimenol, prochloraz, ofurace, propamocarb, bitertanol diclobutrazol, etaconazole, propiconazole tolclofos-methyl, fenpropimorph	29
1980-2000	benalaxyl, flutolanil, mepronil, pencycuron, cyprofuram, triflumizole, flutriafol, penconazole, flusilazole, diniconazole, oxadixyl, fenpropidin, hexaconazole, cyproconazole, myclobutanil, tebuconazole, pyrifenoxy, difenoconazole, tetraconazole, fenbuconazole, dimethomorph, fenpiclonil, fludioxonil, epoxyconazole, bromuconazole, pyrimethanil, metconazole, fluquinconazole, triticonazole, fluazinam, azoxystrobin, kresoxim-methyl, metaminostrobin, cyprodinil, mepanipyrim, famoxadone, mefenoxam, quinoxyfen, fenhexamid, fenamidone, trifloxystrobin, cyazofamid (acibenzolar s methyl)	42
2000 - present	picoxystrobin, pyraclostrobin, prothioconazole, ethaboxam, zoxamide, fluopicolide, flumorph, benthiavalicarb, iprovalicarb, mandipropamid, boscalid, silthiofam, meptyldinocap, amisulbrom, orysastrobin, metrafenone, ipconazole, proquinazid	ca. 18
Future	Many known pipeline products	



- Industry continues to invest heavily into providing new innovative solutions.
- Despite increased costs and legislation.
- Sustainability is essential to maintain this investment.

Conclusions

- The challenges of feeding a growing world population mean that agricultural intensification will increase. Crop protection chemicals are a key part of this.

- Despite great technological advances over the past century, the “theoretical yields” of crops are not realized and much more potential exists for optimization of agricultural production.
- Despite the increasing costs of new product R&D, and increasing legislative requirements, industry continues to invest and is successful in providing new innovative solutions.
- Fungicide diversity is essential to maintain to ensure sustainable crop production, control new threats arising and to manage fungicide resistance.
- The maintenance of diversity and resistance management should be considered as one of the key factors in regulatory legislation.

DETECTION AND MONITORING OF AIRBORNE PATHOGENS BY OPTICAL REFLECTANCE AND AIR SAMPLING.

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To detect and monitor progress of airborne pathogens it is possible to assess disease symptoms or to detect airborne inoculums before infection occurs. Numerous optical techniques have been used to detect early disease symptoms. Chlorophyll fluorescence often changes before visible symptom expression but is difficult to measure in crops and provides only a warning of imminent disease without identifying the causal agent. Changes in the canopy spectral signature vary with host-pathosystem but generally a reduction in the ratio of green to far-red light reflected from the canopy indicates chlorophyll reduction associated with disease development. Imaging rather than spectrographic techniques are usually needed to distinguish chlorophyll reductions caused by discrete leaf lesions from overall reductions caused by nutrient deficiency. In practice, it is often necessary to apply fungicides to entire fields by the time visible foci of diseases such as stripe rust are observed. Monitoring inoculum in air can provide, an earlier warning of potential disease. **Immunological or molecular diagnostic techniques applied to air samples have improved accuracy and quality of information gained, compared to identifying and counting spores by microscopy.** Spore numbers can be estimated directly from the amount of pathogen DNA detected by quantitative PCR and additional genotypic information, such as presence of genes conferring fungicide resistance, can be obtained. Recent research suggests that integration of spore trapping with quantitative PCR has great potential for forecasting diseases of arable crops and for monitoring changes in populations such as development of fungicide resistance.

DEVELOPMENT AND IMPLEMENTATION OF SAMPLING-DRIVEN PROGRAMS FOR MANAGEMENT OF GRAPEVINE POWDERY MILDEW IN EASTERN WASHINGTON.

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Powdery mildew of viniferous grape (*Vitis vinifera*), caused by the ascomycete *Erysiphe necator*, is one of the most severe diseases of grapevine worldwide. Disease management can be expensive because in many instances control requires the intensive use of QoI, DMI, sulfur, and oil fungicides. Widespread resistance of *E. necator* to QoI and DMI fungicides has been documented. Therefore, lowering selection pressure is a key component of fungicide resistance management in grapevine IPM programs. A PCR assay using species-specific primers was developed to identify propagules of *E. necator* in vineyard air samples collected by Rotorod sampling devices. Molecular techniques provide a rapid and accurate way to identify *E. necator* in air samples, compared to the more conventional tools. Our research indicates that microscopic signs of powdery mildew occur about 1 week days after PCR detection, and 7 days after *E. necator* conidia were detected by the Rotorod sampler/PCR assay. Volumetric spore traps confirmed the presence of *E. necator* ascospores in the vineyard during the period when PCR detected the fungus in air samples. Fungicide programs initiated upon first detection of *E. necator* in the vineyard reduced fungicide usage without compromising control. Four years of field research indicate that **PCR used in combination with Rotorod samplers can be used to signal the initial presence of *E. necator* in the vineyard air**, and represents the first step in incorporating an inoculum component in the powdery mildew risk assessment model in widespread use in Eastern Washington.

EFFECT OF COPPER-BASED FUNGICIDES ON FRUIT QUALITY OF TART (SOOR) CHERRY.

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Blumeriella jaapii causes cherry leaf spot (CLS), a serious disease of tart (sour) cherry in North America and Europe. Copper based fungicides control *B. jaapii* but have been associated with phytotoxic symptoms on the

leaves of sprayed trees. We compared the phytotoxic effects of copper fungicides to the damage caused by CLS in terms of fruit quality. Fresh cherry fruit mass and brix (g of sucrose per 100 g of H₂O) were compared for trees that were either sprayed with copper fungicides, sprayed with synthetic fungicides, or not sprayed, during the final stage of mesocarp maturation (14 June – 20 July) in 2007. On 14 June, there were no significant differences between fungicide treatments for fruit mass ($P = 0.68$) or for brix ($P = 0.17$) measurements. However, on the last sample date, 20 July, copper-sprayed trees had significantly lower fruit mass compared to the nonsprayed controls ($P = 0.002$) and synthetic-fungicide-sprayed trees ($P = 0.005$). Also, on 20 July, copper-sprayed trees had significantly lower ($P = 0.02$) brix measurements than did synthetic fungicide-sprayed trees. However, there was no difference in brix measurements between copper-sprayed and non-sprayed trees ($P = 0.20$). In summary, **this 1-year study demonstrated that copper fungicides, despite their sufficient control of *B. jaapii* infection, could be having a detrimental effect on fruit mass and fruit sucrose content.** Mechanisms explaining copper's negative effect on fruit mass and fruit sucrose content, such as amount of visible leaf damage or photosynthetic processes, are being determined.

METHOD OF INOCULATION OF *GUIGNARDIA CITRICARPA* (*PHYLLOSTICTA CITRICARPA*) ON 'PÊRA RIO' SWEET ORANGE FRUIT.

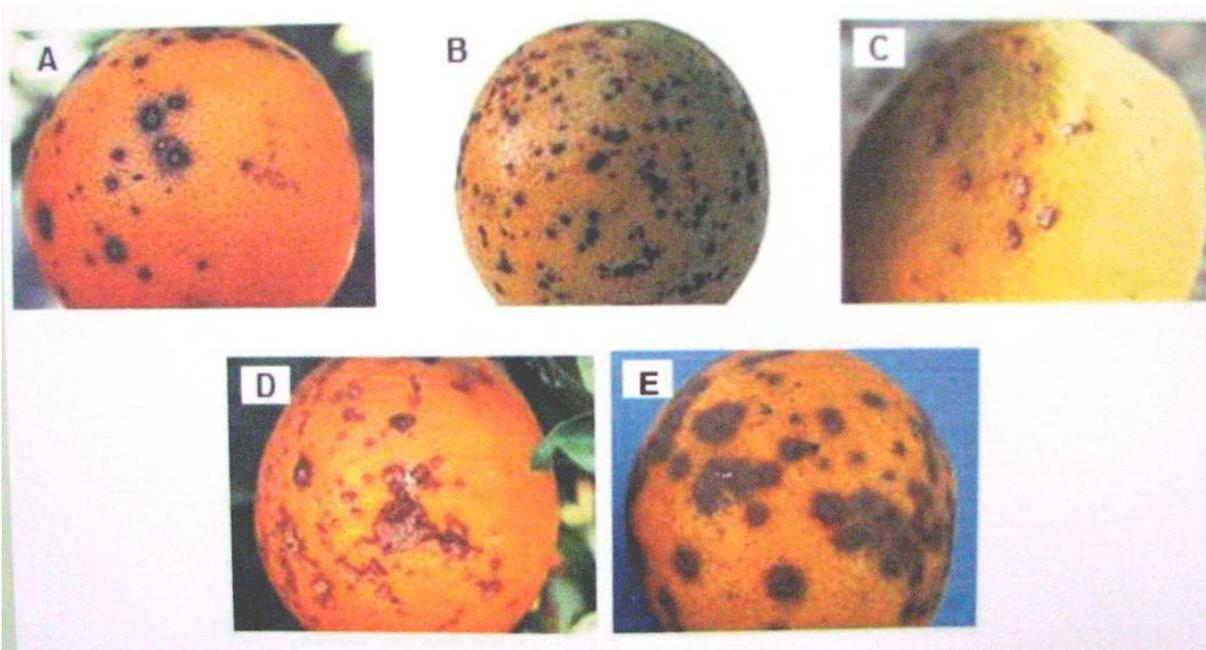
T.F. de Almeida, R.F. dos Reis and A. de Goes. São Paulo State University, Campus of Jaboticabal, Jaboticabal, SP, Brazil. Email: agoes@fcav.unesp.br



The objective this work was to evaluate the method of inoculation using conidia of *Guignardia citricarpa* on 'Pêra-Rio' sweet orange fruit and to verify the symptom types of citrus black spot expressed. Colonies of *G. citricarpa* were produced on OPDA (orange peel-dextrose-agar) medium, **prepared with 200 g of orange peel + 20 g of dextrose + 16 g of agar.** Mature sweet orange peel was triturated using a blender, and 1l of water was added and was filtered through two layers of cheesecloth. The plates were maintained at 25°C for 12/12 h photoperiod for 30 days. The conidia were removed from colonies by adding **10 ml of sterile water, and scraping with a hard brush, then filtering the suspension through two layers of cheesecloth.** **Ten g/l of sucrose and orange juice (1%v/v) was added to the suspension and the number of conidia adjusted to 10⁴ and 10⁸ conidia/ml.** Fruit measuring 2-2.5 cm of diameter was inoculated using a hand sprayer and enclosed in plastic bags. The plants were maintained in a mist bed for 48 h at 25-27°C, and transferred to the greenhouse. All fruits inoculated showed symptoms typical of CBS approximately 40 days after inoculation. Fruits inoculated with 10⁴ conidia/ml showed symptoms of hard spot, whereas 80% of fruit inoculated at 10⁸ showed symptoms of false melanose, and 20% of freckle spot.

GENETIC DIVERSITY OF *GUIGNARDIA CITRICARPA* ISOLATED FROM DIFFERENT CITRUS GENOTYPES. A.C.O. Silva-Pinhati, A. Goes, R.L. Boscariol-Camargo and M.A. Machado. Centro APTA Citrus Sylvio Moreira-IAC, C.P. 04 CEP. 13490-970 Cordeirópolis, SP, Brazil. Email: raquel@centrodecitricultura.br

Citrus black spot, caused by the fungus *Guignardia citricarpa* Kiely, causes an economically important disease in Brazil and other regions of the world, affecting various citrus species. The disease is characterized by the occurrence on the fruits of necrotic lesions that can vary in colour, shape and size. The disease also causes early maturation and premature drop, with significant production losses. The main **objective of this work was to evaluate the genetic diversity of this fungus, particularly isolates associated with different types of symptoms and different citrus hosts.** *G. citricarpa* mycelia were isolated from different citrus genotypes presenting various black spot symptoms. Genetic diversity was assessed using molecular markers based on the fungal ITS region. A total of 37 isolates from 29 genotypes from the Citrus germplasm active bank of the Centro APTA Citrus Sylvio Moreira-IAC, Cordeirópolis, SP, Brazil, were analyzed. Preliminary phylogenetic analyses, based on ITS 1 and ITS 2 of the fungus, suggest high similarity among the isolates and very small intraspecific genetic diversity. However, we detected three distinct groups based on different host species, but not on different symptoms. **Our data suggest that there may be some specificity between *G. citricarpa* isolates and citrus genotypes. This is an ongoing project and new molecular markers will be used in order to confirm our results.**



f) Citrus Improvement

THE FRENCH CITRUS CERTIFICATION SCHEME.

J.P. Thermoz. INRA-GEQA, 20 230 San Giuliano, France. Email: thermoz@corse.inra.fr



Many major threats around the world can cause important citrus yield losses, due to viruses, phytoplasmas, bacteria and fungi. When accessing new varieties or propagating trees we have to take care not to spread the pathogens. Within the French certification scheme for fruit trees, partners from research, development, official services and nurseries cooperate to propagate citrus trees free of diseases. French citrus production is located in Corsica and in overseas departments. Corsica is free of airborne and vector-transmitted diseases. For 50 years INRA-GEQA has managed a large germplasm collection of about 5000 trees grown outdoors and is able to provide seeds of rootstocks, and budwood of most citrus varieties. INRA-GEQA is moving the scheme of sanitary control to a quality insurance system in order to secure budwood quality.

Conclusions and recommendations

- Various new contacts with new researchers were established.
- Three cooperative research programmes were established with other countries.
- Direct communications with the Dutch authorities that identify CBS on exported citrus to the EU were established.
- A better understanding was obtained of how the FRAC group operates.
- Various new fungicides were mentioned and should be investigated.

8.4.2 Tour to Argentina and Uruguay

Summary

I was invited by SA San Miguel for an official visit. The aim was to inspect their orchards for the status of citrus black spot, canker, *Alternaria* brown spot, *Phytophthora* branch canker and melanose. Furthermore, the aim was also to strengthen ties with the CBS researchers in both countries and to look for opportunities for cooperative research projects. Spray machines in operation were also investigated to determine how effective their spray applications are carried out.

Itinerary

Wednesday 18 March	Travel: Johannesburg – Buenos Aires (BA)
Thursday 19 March	Travel: BA – Concordia; visit citrus orchards and a packing house in the Chajari region.
Friday 20 March	Presented a talk at the INTA experimental station. Afterwards a meeting was held with Sergio Garran at INTA and Dr Guillermo Miguel Marco regarding CBS and market access to the EU. Also met Prof Victor Rodriques of the University of UNNE and discussed joint research projects on <i>Alternaria</i> brown spot.
Saturday 21 March	Visited orchards of Ayui in the Concordia region. CBS was detected in their orchards.
Sunday 22 March	Travel to Uruguay.
Monday 23 March	Visit a packing house of Caputto in Salto. Visited INIA (Salto Grande) in Salto and presented a talk on CBS. Also had a meeting with Drr. Hector Mara, Roberto Bernal and Elena Perez regarding market access and joint research projects on <i>Alternaria</i> brown spot.
Tuesday 24 March	Traveled to Montevideo and to BA.
Wednesday 25 March	Traveled to Tucuman. Meetings with SA San Miguel researchers at their offices. Presented a talk on CBS and <i>Phytophthora</i> trunk and branch canker afterwards.
Thursday 26 March	Visited Citromax and presented a talk on <i>Phytophthora</i> trunk and branch canker. Went to orchards to collect <i>Phytophthora citrophthora</i> isolates. Then visited Argentinini lemon to have a look at their CBS problems. A spray demonstration was held in a lemon orchard to see the coverage obtained with their spray machine from the USA.
Friday 27 March	Visited SA San Miguel packing house where they just started to pack with Jacquelin Ramallo an ex-INTA plant pathologist. Visited Gabriella Fogliata at EEOC to organize fruit, leaf and soil isolations from collected samples and to collect CBS isolates for Pedro Crous.
Saturday 28 March	Traveled to BA.
Sunday 29 March	Return to SA.

ARGENTINA

Visit to Entre Rios

Concordia

The researchers in INTA Concordia are doing nothing new with regards to CBS and concentrate mainly on *Alternaria* brown spot and post harvest disease control. They only mentioned that the EU does not allow the use of dithiocarbamates on exported citrus fruit, which is not true.

A field trial was visited about 40 km north of Concordia where they were spraying and evaluating an *Alternaria* brown spot trial. They were trying to position a single pyraclostrobin application with oil only (without mancozeb) with 5 – 6 copper oxychloride applications to save the growers some spray rounds. They were also evaluating the Alter-rater prediction model. Although it was dry, they have had some infection on their untreated control fruit (Fig. 8.4.2.1).



Fig. 8.4.2.11. Alternaria brown spot on 'Nova' north of Concordia

Joint research projects were discussed and agreed upon between CRI and INTA with regards to Alternaria brown spot research. I will draft the protocol and write the manuscript depending on the outcome of the trial results.

Chajari

The visit to the packing house at Chajari showed an interesting concept with a moveable drenching machine. In this case more than 20 containers could be simultaneously treated (in comparison with the 4+ in South Africa) saving a lot of time before the fruit was sent for degreening (Fig. 8.4.2.2.)



Fig. 8.4.2.2. Moveable drenching machine for the bulk treatment of containers

Visit to Tucuman

SA San Miguel

They had not started picking lemons at the time of my visit. Everything was ready to start packing and some lemons were de-greened earlier. They expect a normal season with a normal crop size.

Jacqueline Romallo from the local experimental station was employed by them as pre- and postharvest pathologist. I helped her to set up some techniques to isolate *Phytophthora* pathogens from soil. She will also coordinate some field trials and it seems that I will have to help her in this regard in future. I presented a talk on CBS and *Phytophthora* trunk and branch canker. Especially the latter drew their attention due to the sudden increase in the amount of trees that died the past season.

Citromax

They also have not started to pack yet, but they were ready to start on Thursday 26 of March. I also presented a talk on CBS and *Phytophthora* trunk and branch canker. Again the latter disease drew their attention due to the sudden increase in the amount of trees that died the past season. They took me to one of their affected lemon orchards south of Tucuman along the mountain where the temperatures are cool and humid. Upon arrival, it was obvious that the problem is widespread and that the symptoms are similar to that experienced on Clementines (Fig. 8.4.2.3). Apart from the gumming, the lesions also expand upwards on the trunks eventually girdling the trunk, which results in tree death. Upon investigation, an ant trail was seen that ran over the infected part that expanded upwards beyond 4 metres! This confirms the findings of Luis Alvarez that ants can also serve as vectors for the disease (Fig. 8.4.2.4).



Fig. 8.4.2.3. Die-back of a 'Lisbon' lemon at Citromax, Tucuman, Argentina. The outside circumference of the trunk is more than 60% infected and shows that it is not economical to treat the tree anymore.



Fig. 8.4.2.4. *Phytophthora citrophthora* branch canker expanding along an ant trail \pm 3 m into a lemon tree showing excretion of gumming.

URUGUAY

Visit to Salto

A visit was made to INIA research station north-east of Salto. Here I met with Drs. Hector Mara, Roberto Bernal and Elena Perez regarding market access and joint research projects on *Alternaria* brown spot. When I asked them if they are interested in a joint CBS project, they immediately denied that they have black spot. A meeting was also organized the same evening where I gave a lecture on CBS. Just before the meeting, I was introduced to a representative from the Uruguayan Ministry of Agriculture whom assured me that they do not have CBS and

also asked why I am talking on CBS. My answer was that I wanted to share my experience on CBS and was looking for partners in our joint effort to ensure market access to the EU. (EU inspectors intercepted CBS on fruit from Uruguay back in 2001 and researchers across the river in Concordia are of the opinion that research is being conducted in Uruguay on CBS.) I also presented a short talk on *Alternaria* brown spot and *Phytophthora* trunk and branch canker. The latter was very interesting to them as they have seen the same lesions on citrus trees in the south-western parts of Uruguay.

A joint research project was established between SA, Argentina and Uruguay with regards to *Alternaria* control. I will head the project and either Sergio Garran or Elena Perez will present the results at future conferences.

Thrips

During a visit to Caputto's packing house I came across ready to be exported Satsuma fruit with apparent thrips or leafroller damage (Fig. 5). According to the pack house manager, it was "wind" damage and not thrips. Clearly they do not recognize thrips as part of their pest complex.

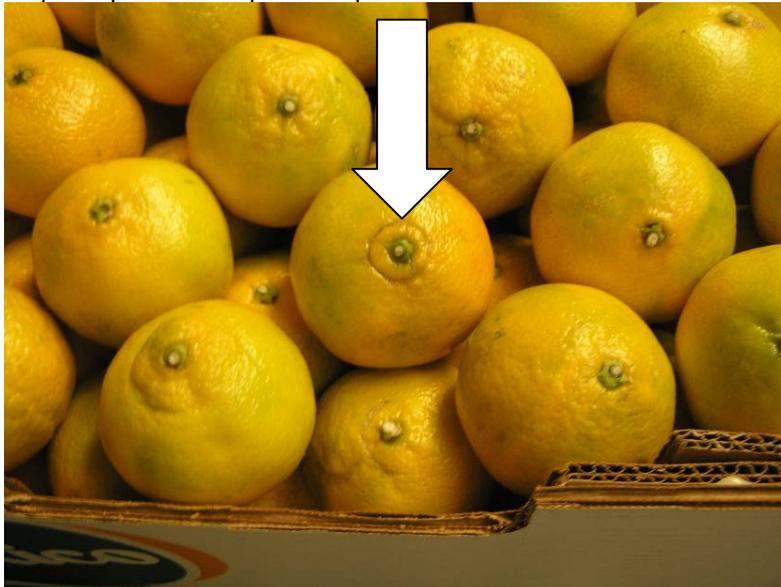


Fig. 8.4.2.5. Uruguayan fruit with probable thrips damage

General recommendations and conclusions

- Citrus canker is a widespread problem in both countries and copper is included in all their spray programmes. The consequence is that copper stippling is a huge problem which has an influence on pack out.
- Alternating copper with QAC (which is registered there for canker control) will solve the problem of stippling.
- *Phytophthora citrophthora* (Pc) is becoming a serious problem in Tucuman. All lemon cultivars are susceptible. We should look into this and screen all the lemon cultivars in SA for their susceptibility.
- Ants were seen to carry Pc higher up in lemon trees. Ant bands will solve this problem but according to them, it is too labour intensive.
- Lemon trees are not skirted in Tucuman, because the bottom parts according to them, bear a lot of fruit. However, if their trees are not skirted, they will not be able to control *P. citrophthora* effectively with fungicides that must be applied with hand guns. Skirting of trees will also prevent additional pathways for ants into the trees.
- Joint research projects were proposed between CRI, INTA Concordia, Argentina (Sergio Garran) and INIA, Uruguay (Roberto Bernal) with regards to *Alternaria* brown spot.
- CBS isolates were also imported to enlarge our culture collections for future research projects.

8.5 G. PIETERSEN

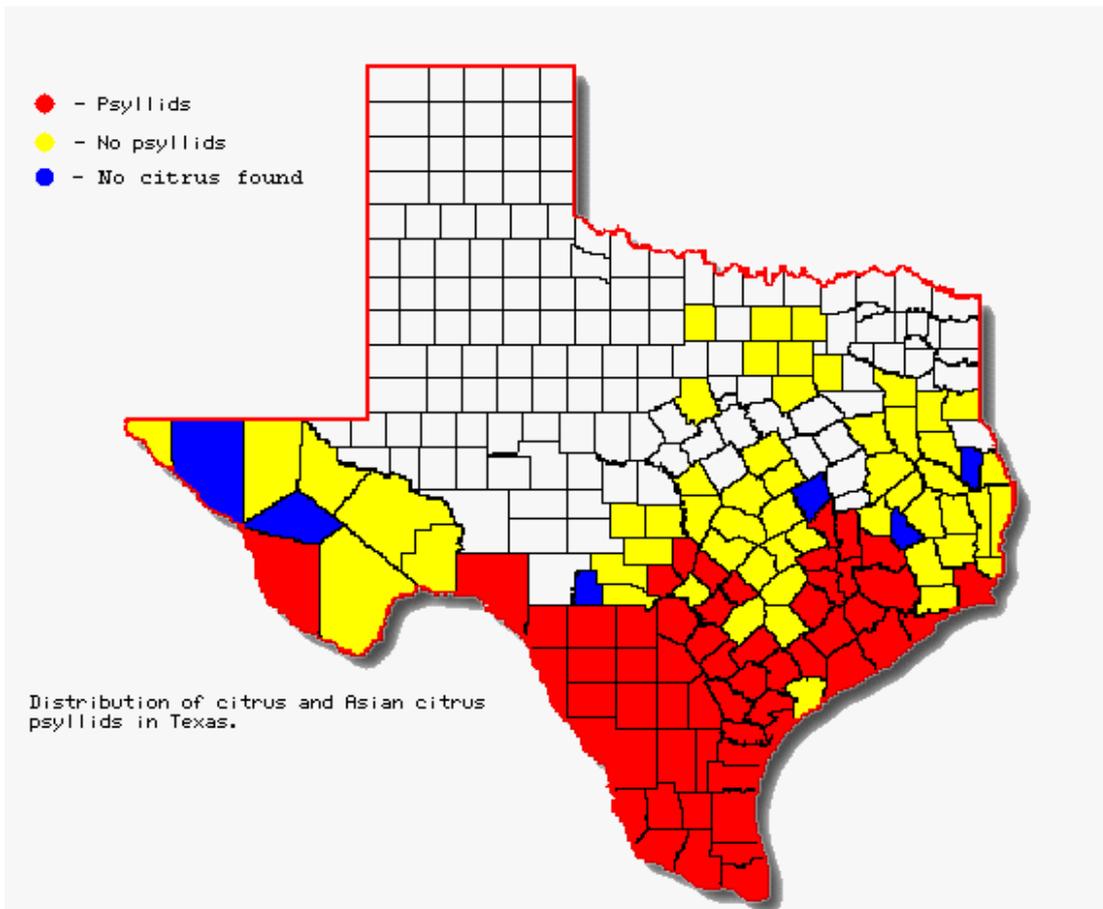
8.5.1 Attendance of the International Huanglongbing Conference, 1-5 December 2008 in Orlando, Florida

This report deals with the attendance of the above meeting. The meeting was attended by 425 delegates from 26 countries. The vast majority of delegates were American (researchers, inspectors, consultants, producers and regulators) followed by a large contingent of Brazilians. The conference was housed at the Caribe Royale Hotel and was clearly very well sponsored, with superb facilities and meals. The conference was very well organized and very informative. The abstracts of the conference were made available to all delegates in the form of a pdf file, and any person interested in it is welcome to get a copy from me at gerhard.pietersen@up.ac.za. The most salient points, from the South African perspective regarding this conference are highlighted below under various themes.

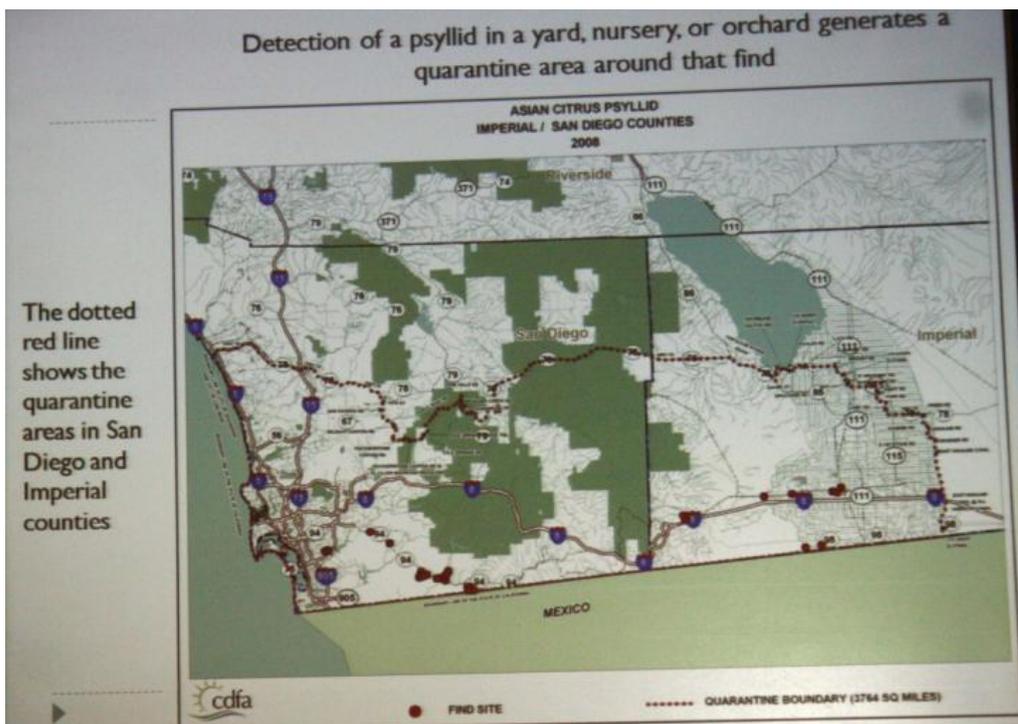
1. Distribution and spread of HLB and *Diaphorina citri*

During the course of the Conference various presentations dealt with the continued spread of Huanglongbing (HLB) globally. The following was garnered:

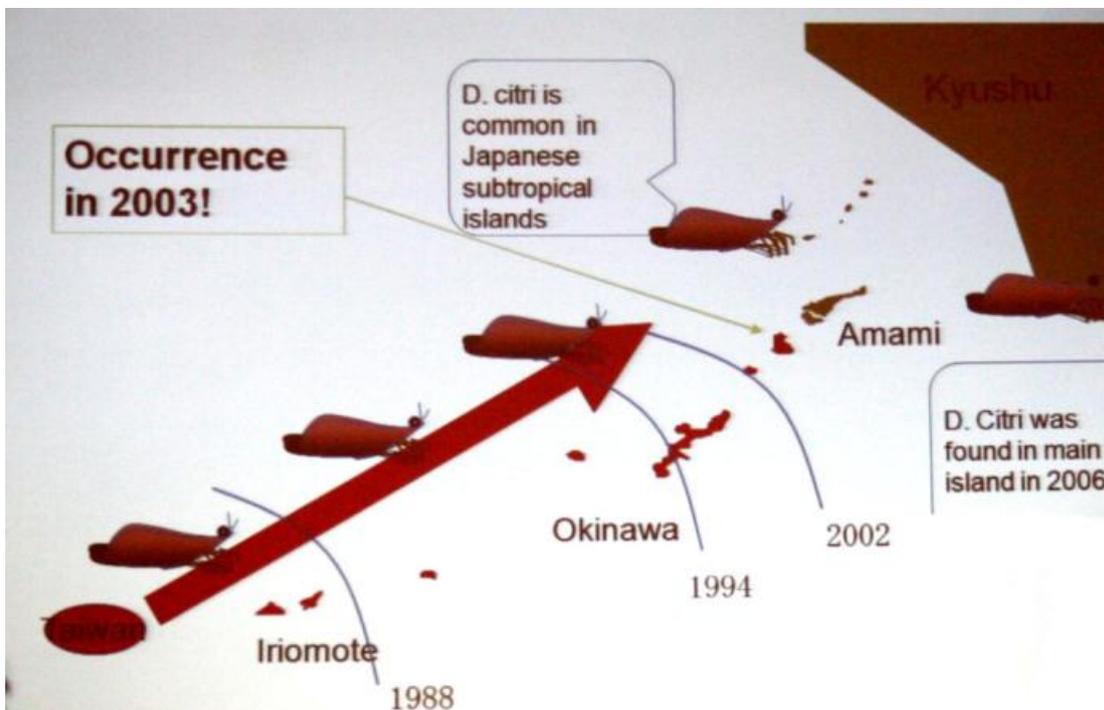
- 1) The spread of HLB in Brazil from Arraquarema, Sao Paulo has continued since 2004 and the disease can now also be found in Parana State, Brazil.
- 2) Whereas *Liberibacter americanus* (Lam) was the predominant species found during the initial phases of the Brazilian epidemic this has now changed to primarily *Liberibacter asiaticus* (Las).
- 3) Las has now spread to the Dominican Republic (this is not public knowledge at this stage, but the information is expected to be published shortly).
- 4) The presence of HLB in Cuba is now widely accepted and apparently widespread (unfortunately the Cuban delegation could not get Visas and were unable to present their results).
- 5) HLB has spread to 32 counties in South and Central Florida,
- 6) *Diaphorina citri* (Dci) has been spread mainly on ornamental plants (*Murraya*) to the Northern-most counties in Florida.
- 7) Dci has been sighted in Jamaica, and unofficial reports suggest that it is on all the Caribbean Islands.
- 8) Dci is found in Louisiana, and HLB is reported at one location.
- 9) Dci is fairly widespread in Texas (see figure below), where an USDA-APHIS certified diagnostic laboratory has been established for HLB PCR testing. After one scare in which a sample tested positive for HLB by PCR, the sample tested negative subsequently and has remained so after repeated tests, plants in the vicinity have also remained negative. A widespread survey for citrus trees, including backyard trees has been conducted over the past two seasons. 822 plants and 815 psyllids have been tested thus far. All were negative for HLB.



- 10) Dci is considered widespread in Mexico, in all states producing citrus, all the way to the US border.
- 11) Dci is present in the Southernmost two counties in California and quarantine protocols have been imposed on citrus below a specified line (Figure Below).



12) Spread of HLB is occurring from Taiwan along a chain of Islands in the direction of Japan. Dci has already been reported on all intermediate Islands as well as on the southernmost tip of the Japanese main island. HLB was reported on about two-thirds of the islands on the way to Japan, but not reported on the Japanese main island yet (Figure Below).



- 13) Dci has been detected for an unspecified period on the Iran/Pakistan border, and HLB symptoms are apparently now also occurring in this area, this report needs to be confirmed and plants tested by PCR for Liberibacters. Prof. Bové will be travelling to this area in mid-January to inspect this report.

2. Diagnostic assays

Most researchers and diagnosticians in Florida and Brazil utilize real-time PCR (mainly with the primers of Li et al. (2007), in spite of the high cost of the test, and the limit to the number of tests that can be done in parallel. To address the problem of low throughput and high cost of purchasing of a real-time PCR, a group in Japan, probably in anticipation of having to do thousands of PCR tests shortly for HLB, have developed a new technique called Cycleave ICAN, in which DNA is amplified using a chimeric (DNA/RNA) primed amplification under isothermal conditions (i.e. no need for an expensive thermocycler; can use an incubator or heating/cooling block), and amplification detected by FRET in a single microtitre tube. The test can be conducted in a single tube within one hour. This assay has the potential to allow thousands of assays in parallel. The test has been shown to be up to 25 times as sensitive as conventional PCR. Another group of researchers has modified the conventional nested PCR protocol, which, while exquisitely sensitive, is severely prone to false positives. This is usually due to cross contamination of samples during the amplicon handling step when the product of the first round is transferred to the second round. The test has been modified to no longer require physical transfer of the first round product to the second round, and the two rounds are conducted in a single closed tube. This was achieved through very careful design of 4 primers with the first and second round pair differing considerably regarding their annealing temperatures. This nested PCR to HLB is claimed to be even more sensitive than the real-time PCR.

A privately owned laboratory for HLB tests has been set up in Florida and conducts PCR tests for free to growers. The cost of the test, a real-time PCR, is calculated to be US\$6.00 per test. This laboratory has conducted an incredible 65000 tests in 2 years. A positive/negative threshold value has been established at a cycling cross over point (Ct value) of 32, based on an observed bimodal distribution of Ct values obtained during the 65000 tests. The laboratory has a strict requirement for data on submission of the test and this has allowed data mining to be conducted on the samples. This has produced some interesting information, including a) highest titres are found between July and February; B) the most susceptible trees are those of 6-9 years old (I am not sure how this inference is made); c) grapefruit and oranges are the most "susceptible" (probably based on titres); d) tangerines and tangelos are the least susceptible; and e) a very marked edge effect of infection is noted.

Numerous posters and presentations report on detection of Liberibacters at real time in excess of Ct's of 32 including those dealing with seed-transmission, which are dealt with below. This is really pushing the limit of reliability of the real-time PCR and such data must be interpreted with care.

Tests on Dci may be a more reliable early warning test of infection than on plants as the titre tends to be higher and less problems are associated with tissue distribution.

3. Seed transmission

A number of groups in the US have conducted experiments to determine whether Las is seed transmitted. Results have been ambiguous and the general consensus is that "they don't know". However, there appears to be a somewhat generally accepted theory that some unknown component of what is now being thought of as the "Las complex" is being seed transmitted. All groups involved had followed roughly the same methodology, by collecting fruit from HLB infected branches of trees, testing these by PCR for Las, and then either removing seed coats and germinating seeds in tissue culture flasks, or by planting the seeds out directly. The results of the individual groups can be summarized as follows.

1. John Hartung's group initially found no evidence of seed transmission with none of their seedlings testing positive for Las using real-time PCR (Li et al., 2007 primers). A number of the seedlings were abnormal in appearance, but this could be ascribed to heterozygotes. After other groups indicated that there is some evidence of seed-transmission John Hartung sent samples of his seedlings to the Shatters group, who had just developed a different real-time PCR method, also based on amplification of the 16S region of Las. Using this method a number of samples yielded very high Ct values, indicative of a low titre of bacteria. These included samples with some abnormalities and others which appeared healthy. The amplicon was sequenced from duplicate reactions and was found to have a 100% match to the 16S gene sequence from several strains of Ca Las deposited in Genbank.

2. The laboratory of Duan contend that using nested PCR (using duplicate tests as “at the detection limit”) they obtained seed transmitted seedlings from Citrus and Periwinkle. In the case of citrus, they claim they have found 57 infected plants out of 170 tested. The periwinkle and citrus progenies from HLB-affected plants did not show blotchy-mottling but did exhibit atypical HLB symptoms, denoted by vein yellowing, leaf curling and yellowing only when they were stressed by nutrient deficiency. Symptoms disappeared after the stress was removed. They interpreted their results as suggesting that although Las was seed transmitted, it was either not the form that caused severe HLB symptoms and death, or a second, undescribed component of an HLB disease complex was not transmitted. The researchers contend that they have retested these plants and that some of them are still positive after 2 years.
3. In a poster, one of the persons helping John Hartung reported that they detected HLB-like symptoms on seedlings of Duncan grapefruit emerging in insect-free glasshouses. Using their newly developed Las 16S rDNA primer based real-time PCR they detected Las sequence in under 10% of the seedlings; however, the detection of Las did not correlate with the symptoms. Later, with Ruby Red Grapefruit and Hamlin, the 16S rDNA sequence was detected in seedlings that were surface sterilized and germinated in sterile Magenta jars. The sequence of the 16S rDNA was 100% identical to Las. Dissection of the sterile-grown seedlings showed that the highest detectable level of Las was in the seedling roots. As plants grew, HLB-symptomatic plants developed more slowly than asymptomatic plants, however, most lost HLB symptoms over time.
4. In a poster by Mike Irey’s group, they report on tests on Pineapple sweet orange seedlings. Seeds and seed coats were separated and tested separately by real-time PCR using primers based on Li et al. (2006). All surviving seedlings from seed with RT-PCR positive seed coats as well as 45 plants with negative seed coats were sampled and tested by RT-PCR. From the 59 plants sampled, 7 plants were either positive or questionable. The Ct value of one plant was 28.22 and a second was 31.38. The remaining suspects were between 32.3 and 33.4. Upon re-assay, 3 of the 7 plants were positive and yielded amplicons using the nested PCR that the Duan group uses. The three plants testing positive were transferred to the USDA-ARS facility in Ft. Pierce to conduct psyllid transmission studies to partially fulfill Koch’s postulates. Of these three plants, only one tested positive in subsequent real-time PCR testing. In addition to the 59 plants tested, 356 plants not tested in the original group of 56 were tested and none found positive or questionable. In November 2007, fruit were collected from 8 symptomatic trees at the same location as in 2006. Seed were planted and the resultant 723 seedlings were assayed by RT-PCR in February 2008. From this group of seedlings, 6 had Ct values less than or equal to 32 after two assays. In 2008, HLB was confirmed for two Carrizo seed source trees in a FL citrus nursery. From each positive tree, fruit were collected for extraction and germination of at least 200 seedlings. In seedlings assayed by RT-PCR for transmission, 142 seedlings from the first source yielded two seedlings with Ct values 32 or less and of 148 seedlings from a second source, 5 seedlings had Ct values 32 or less.

Note: Some of the rates of seed transmission reported above are exceptionally high, even in terms of well known seed-transmitted viruses, occurring throughout mesophyll tissue, and appear to be almost impossible for a phloem transmitted pathogen to achieve. Furthermore the interpretation of being positive is based on extremely high Ct values of 16S rDNA regions and in my opinion could rather indicate contamination and/or cross reaction with the 16S region of another bacterium. Furthermore, it is not evident from any of the groups if a large amount of seedlings derived from healthy fruits were tested in parallel with the infected fruit (this is usually not done when wishing to determine seed-transmission rates). I am not convinced that any evidence exists thus far for seed transmission. Interestingly, this aspect was considered the most important research aspect to be conducted during a survey of research priorities.

5. Exploring for existing resistance in Citrus and citrus-relatives to Las or the psyllid

Boscariol-Camargo *et al.* test progression of Las in citrus-related plants and showed that *Atalantia* sp., *Eromolemon* sp. and *Poncirus* sp. showed a slower replication rate of Las. Only *Atalantia* had a few replicates that were still negative after 6 months post-inoculation.

Subandiyah *et al.*, from Indonesia, were looking for citrus and citrus-related plants that may be resistant to psyllid colonization and Las. They found that *Diaphorina citri* heavily colonized *Berbera*, *Murraya*, *Swinglea*, citron and *C. hystrix* but were lower on a number of the citrus genotypes tested including mandarins and pumellos. A number of plants in their experiments were found to be positive for Las after two years.

Folimonova *et al.* screened 31 citrus and citrus-relatives for disease symptoms in glasshouse and climate controlled cabinet experiments. All plants tested positive for Las by the conclusion of the experiment and all plants showed symptoms ranging from mild through intermediate to severe. No correlation between symptom severity and Las concentration was found. Symptoms could be enhanced by exposing the plants to a 24-hour light regime, to increase starch build-up.

Unique local mandarin cultivars are found in Japan. As HLB is expected to shortly spread to the main island of Japan, where the major citrus production areas occurs, it was decided to evaluate these cultivars for resistance to Las. No immunity was found in spite one cultivar remaining PCR negative in the field in the presence of high Las pressure. It is possible that graft evaluation negates obtaining information on resistance, due to resistance to vector rather than to Las.

Stuchi *et al.* evaluated a number of somaclonal variants for resistance to CVC, and are currently extending these evaluations to HLB.

So far, there appear to be some indications of tolerance in some citrus or citrus relatives to Las but no instances of immunity.

6. Transgenic plants for control of HLB

Progress in the development of a transgenic citrus, resistant to HLB has occurred at a phenomenal pace, given that work just started a maximum of four (Brazil) or two (Florida) years ago, and that researchers are dealing with a woody perennial host (much more time consuming to work with than herbaceous annuals). A number of groups presented data on progress in this field including Allelyx and Centro de Citricultura Sylvio Moreira (Brazil), University of Florida, and USDA-ARS.

All research groups clearly have infrastructure and essential protocols to produce transgenic citrus completely in place, having sorted out the essential requirements in transformation protocols (use of protoplasts, embryonic callus tissue), regeneration, selection and evaluation. Some differences in approach depending on aim of research groups were evident, for example, the use of protoplasts is especially useful a) where transformation of polyembryonic seedless cultivars is required as these would be difficult to transform with *Agrobacterium*; b) transformation of some rootstocks and cultivars of mandarins (eg. Murcott), which are recalcitrant to *Agrobacterium* transformation, is required; c) in cases where it is critical that engineered plants lack antibiotic marker. A fair amount of work is, however, going on to find promoters and other regulatory elements for more controlled resistance gene expression in citrus.

A number of strategies and constructs have been prepared and used for transformation, e.g. antimicrobial peptides (AMP) (CREC, Ed Stover, Machado, Allelyx), enhancing systemically acquired resistance (SAR), virus genes to disrupt pathogen functions. It is accepted that the first prize is cis-transgenic plants (genes from citrus or related genus used; e.g. as used by Allelyx). AMP, which can be systemically spread, would be especially useful if produced in rootstock from where it could spread to scion (obviates the need to replace existing tree, and to transform all cultivars). This is an especially attractive option as rootstocks appear to be easier to transform. Replacement of existing trees can also be avoided with the use of virus-based expression vectors, and this is being addressed by at least one research group.

As very little is known about Liberibacter-Host interactions on molecular level, resistant strategies utilizing AMPs are still very much applied with AMP with a wide range of action against bacterial pathogens.

Thousands of transformants, produced by the various research groups are already in glasshouses, with a number already being challenged and some looking promising. For example, Machado: while most transformants altered using AMP genes have been shown to remain susceptible to Las, they do have a "NRF" transgene transformed plant line where all of the plants currently remain free from HLB after a long period of Las challenge. LIMA (an AMP) -transformed Duncan grapefruit plant, grafted with HLB shoot looks promising as the shoot is still testing positive for Las but the transformed plant has been PCR negative now for 13 months (CREC).

6. Guava for repelling Psyllids

A number of papers at the conference dealt with this aspect. The first involved laboratory tests, using cages, jars and Y-tube olfactometers, which confirmed the repellent effect of guava on Dci. A second paper presented data

on identification of the volatile compound in guava, which potentially may be responsible for the repellent effect. Seven sulfur volatiles were detected: hydrogen sulfide, sulfur dioxide, methanethiol, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methional and dimethyl trisulfide (DMTS). DMDS is an insect toxic, defense volatile produced only by wounded guava and not citrus leaves. The authors therefore speculate that DMDS may be one of the components responsible for the protective effect of guava against DCi.

A third paper reports on observations suggesting that the use of guava interplanting was effective in preventing HLB infection. They report on visiting 17 orchards, where either guava-interplanting, chemical control of pests, biological control by weaver ants or no control was conducted. The ages of the orchards varied from two to four years (i.e. very young). The authors collected five leaves from 10 to 20 trees in each orchard through an unspecified sampling procedure and tested the leaves by PCR to confirm the HLB diagnosis. The mean infection percentage was lowest in guava-interplanting orchards ($12.4 \pm 2.8\%$), followed by chemical control ($20.2 \pm 4.8\%$). Biological control seems to be much less effective than these two managements ($65.7 \pm 11.2\%$) or even no control ($39.5 \pm 6.6\%$). The authors therefore initiated a field experiment in MDR Vietnam to test the efficacy of guava-interplanting (GI). In the first 14 months, no infected trees were detected in the GI block, but 30 % trees were infected in the non-interplanted block (NG). HLB infection reached 20% in the GI in two and half years, while 70% in NG in two years. They therefore conclude that guava-interplanting reduced the invasion of HLB. However, during the presentation (not written in the abstract) the authors present data on a trial in which a block of citrus treated with systemic insecticides is compared to a block with GI. HLB infection arose very rapidly in both blocks, casting doubts on the usefulness of guava interplanting, and in fact the authors themselves appear doubtful of the value of the GI. Private discussion with the Brazilian delegates revealed that they had also searched for guava orchards in close proximity to Citrus orchards in Sao Paulo state. They had not obtained any disease gradients away from the guava orchard, and had in fact noticed the clear edge effect that Las often induces in close proximity to the guava block.

8.6 M.C. PRETORIUS

8.6.1 Report on a visit to Brisbane, Australia on 13-18 July 2008 to attend the 5th International Congress of Nematology

Introduction

Information from talks, posters and discussions at the 5th International Congress of Nematology.

Session Six - Nematodes in Farming Systems

Enhanced Soil Carbon: The Key to Improving Soil Health and Suppressing Nematode Pests

Stirling, G.R. & M.J. Bell

Observations on sugarcane cropping soils in Australia have shown that conserving soil organic matter through practices such as residue retention and minimum tillage increases soil C levels (particularly the labile fraction that is oxidized by 33mM potassium permanganate). This results in an improvement in aggregate stability, rainfall infiltration rates, cation exchange capacity, amounts of potentially mineralisable N and a reduction in bulk density and surface crusting: all positively associated with soil and plant health. Suppressive mechanisms against plant-parasitic nematodes are also enhanced due to increased microbial activity. Experiments in microplots and the field have shown that *Pratylenchus zae* and *Meloidogyne javanica* do not multiply as readily in soils that receive continual C inputs from a mulch layer of plant residue, are not disturbed by tillage, or are amended with high C/N residues. Numerous suppressive mechanisms are probably operating, but predatory fungi that obtain N from nematodes in low N environments appear to be involved. Results of experiments in other cropping systems also confirm the key role of organic matter in biological suppression of nematodes. In minimum till cereal cropping systems, *Pratylenchus thornei* reaches high population densities at depth but does not multiply readily in surface soils, where C levels are highest due to stubble retention and suppressive mechanisms are operating. In capsicum cropping systems, where biological activity is limited by low C inputs, excessive cultivation and reliance on soil fumigation, populations of *M. incognita* and the level of damage caused by the nematode can be reduced by a combination of organic amendments, minimum tillage and mulching with plant residues. Collectively, these results suggest that the key to improving sustainability and reducing losses from nematode pests is to introduce practices that reverse the decline in soil C that currently characterizes most cropping systems.

Session Seven – Current and Future Trends for Insect Control through EPN

Status and Future of Insect Control with Entomopathogenic Nematodes in Asia

Choo, H.Y., D.W. Lee, H.H. Kim, S.M. Lee & S. Yamanaki

EPNs are widely used against insect pests in Korea, Japan and China from the 1980s. Recently, India, Thailand, Vietnam and Turkey began to have an interest in EPNs. In Korea, native EPNs are extensively used against vegetable, ornamental, and turfgrass insect pests. In addition, newly-occurring insects in tea plantations, seedling beds, medicinal plant plantations, forest, propagation houses, greenhouses, and sustainable farms will be targets for developing effective methods. New strains of EPNs will also be continuously isolated from various habitats for effective control of native and introduced insects. In Japan, commercially-produced *Steinernema carpocapsae* and *S. glaseri* are used against turfgrass, orchard, and sweet potato insects. These are or will be selectively used against soil insects and sudden occurrences of insects. Commercialization of EPNs will be active for the time being in Japan. In China, invasive pests such as oriental fruit fly, Asiatic palm weevil and banana moth are good targets for EPNs. Local insects invading fruit and some other crops will be targets of EPNs. A combination of EPNs with insecticides will be attempted to augment efficacy. Insects of vegetable and aromatic plants are targets of *S. carpocapsae* in Thailand. Thai strains will be continuously isolated and used against the above pests with mass production. In Vietnam, black cutworm and armyworm were initially tested against Vietnamese EPN strains. However, turfgrass insects including billbug and white grub will be controlled using Korean EPNs in golf courses belonging to Korean owners. In India, Indian isolates of *S. thermophilum* and *S. riobrave* have received attention against vegetable, maize, and sugar cane insects. *S. thermophilum* and newly isolated Indian strains will be continuously evaluated against locally important insects.

Session Ten – Sustainable and Organic Management through Biofumigation, Amendments and Suppressiveness

Impact of Green Leaf Application on the Management of Plant Parasitic Nematodes and its Effect on the Population of Predatory and Saprophytic Nematodes and Microflora in Soil

Sheela, M.S., K. Ajith & M.S. Nisha

Green leaves of a number of plants are well documented for nematode suppressant properties. Neem (*Azadirachta indica* A Juss.) and eupatorium (*Cromolaena odorata* L.) are two plants with potentially nematicidal properties. However, knowledge on the impact of these green leaves on the soil fauna is meagre. Hence an attempt was made to study the effect on the soil fauna and their consequent effect on the yield of okra and cowpea during rainy and summer seasons. Field experiments were laid out in a randomized block design. Chopped neem and eupatorium green leaves were applied 15 days prior to sowing the seeds at rates of 7.5 and 15 t per ha at a depth of 30cms. Nematodes were extracted by Cobb's sieving and sifting techniques and cleared by modified Baermann funnel method, while fungi, bacteria and actinomycetes were isolated by dilution plate method of Timonin using different media. The result showed that application of neem and eupatorium leaves at both doses reduced reniform nematode, *Rotylenchulus reniformis* in the root zone of okra and cowpea in soil both in summer (214 to 427 per 200g soil sample as against 727/200 g in untreated) and during the rainy period (324 to 646 per 200g as against 933 per 200 g in untreated). The population of predatory (two to three fold) and saprophytic nematodes (three fold) also increased significantly in the root zone of okra and cowpea. The rate of increase was more in eupatorium leaves when compared to neem leaves. The pre-sowing application of eupatorium leaves increased the population of fungi, bacteria and actinomycetes in the root zones of okra and cowpea whereas neem leaf at higher dose (15t/ha) reduced the multiplication of microbial population (except for the fungi) in the root zone of okra and cowpea in both the seasons. The stimulatory effect of eupatorium leaves in the multiplication of microbial flora and predatory and saprophytic nematode in our investigation is being reported for the first time. The pre-sowing application of neem and eupatorium at 15t ha resulted in significant improvement in plant growth characters which contributed the increase in yield of okra and cowpea in both the seasons. Thus application of green leaves suppressed the multiplication of plant parasitic nematodes by the nematicidal principles released during decomposition and it also indirectly managed the plant parasitic nematode population by stimulating the multiplication of predatory nematodes and beneficial microflora.

Session Twenty-One – Nematode Management in Subsistence and Smallholder Agricultural Systems

Addressing Root-knot Nematodes in Horticulture: Diagnostics Resistance and Integrated Management Practices in Turkey

Söğüt, M.A. (1), I.H. Elekcioğlu (2), Z. Devran (3) & A. Özarslandan (4)

Root-knot nematodes are the most important nematode species for the both protected vegetable, horticulture and potato cultivation in Turkey. When root-knot nematodes is not controlled, they cause significant yield losses (between 50% and 80%) every year. Identification of root-knot nematodes on various groups has been achieved by using morphological, host reaction and molecular techniques. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the common species found in Turkey. *Meloidogyne incognita* race 2 and *M. javanica* race 1 are widespread in vegetable growing areas eastern Mediterranean Region of Turkey. Recently a new finding of *Meloidogyne chitwoodi* on potato has been identified in the middle Anatolian region. With the phase out of Methyl Bromide in Turkey by 2008, a large integrated project supported by World Bank and UNIDO in Mediterranean region of Turkey enabled alternative management practices to be investigated in greenhouse horticultural production systems. A number of treatment and treatment combinations were investigated including Solarization + Trichoderma, Solarization + Dazomet, Solarization + Fresh Chicken Manure, Solarization Fresh Cow Manure, Grafting and Resistant varieties to control root-knot nematodes. The most cost effective alternatives to methyl bromide were Solarization + Trichoderma and Solarization + Organic Manures. Also, other treatments were found to be viable and cost effective alternatives to methyl bromide in greenhouses. Another management strategy was backcrossing breeding to incorporate Mi-1 resistant gene into a commercial fresh cultivar of tomatoes. The effective transfer of the gene was validated by using MAS (Marker Assisted Selection). However, one of the challenges with Mi-1 gene is the resistance has not been effective in several locations, and also other resistance cultivars have broken down in the West Mediterranean Region. In order to address this further research on tomatoes is being carried out on the Mi-gene breaking population of virulent root-knot nematode on tomatoes in West Mediterranean Region supported by TÜBİTAK.

Session Twenty-two – Funding Applied Nematology, Extension and Teaching: Government and Commercial

Governmental Influence (by Funding) on Research in The Netherlands and the EU

Den Nijs, L.J.M.F.

Insight is given into how research funding is organised in The Netherlands; a distinction is made between public and private funding. Nematological aspects are covered in general plant health programs and in phytosanitary (statutory plant health) programs specifically related to quarantine matters. Some examples are given of policy supporting research related to the quarantine nematodes *Globodera rostochiensis/pallida* and *Meloidogyne chitwoodi/fallax*. Results of these projects are used for developing new policy and policy adjustments. EU Framework Programmes are a source for financing plant health research. Within the EC 6th Framework Programme ERA-Net (European research area network) EUPHRESKO is funded, meaning European Phytosanitary REsearch Coordination. The aims of this project is to increase the co-operation and co-ordination of national phytosanitary research programmes and to fund at the EU level through networking of research activities and mutual opening of national programmes. The *Globodera* project will be used as an example to explain the process.

Funding for Applied Nematology in the UK

Pickup, J.

A review of the public and commercial funding provided for applied and extension nematology in the UK is presented. In the UK, the public funding of applied nematology is largely limited to the responsibilities of statutory bodies in relation to the implementation of phytosanitary legislation. The most significant example of this is statutory pre-crop testing for potato cyst nematodes. Surveillance in relation to other quarantine species constitutes another important area of work, particularly in relation to the examination of imported plants and plant products. Research in applied nematology may be funded by either the public or commercial sectors and often by both. The extent to which public funding is likely to be involved will depend upon the degree to which the outcome is considered to be to the 'public good'. There is an increasing tendency for government funded research to be of benefit to government policy making.

Support for Nematology in Developing World Agriculture: How is the Future Looking?

Coyne, D., J. Nicol & B. Sibanda

Globally, nematology tends to have a low profile with research organisations, universities and consequently donors, but especially in developing countries and Africa in particular. Within the consultative group for international agricultural research (CGIAR), designed to support and promote developing country agricultural research, nematology expertise has declined with nematologists a rarity in a system dedicated to supporting national programmes. This creates difficulties for nematologists to attract research funding and support for training, often through a limited critical mass and voice to champion the discipline. Fellowships, specifically

designed to cater for needs of developing world scientists provide opportunities, but without the disciplinary advocates, nematology opportunities are often missed. Since the widely acclaimed International *Meloidogyne* Project (IMP) (1975–1983), funded by USAID, which involved some 200 nematologists over 70 countries, there have been few substantial nematology interventions. Some notable examples include the Postgraduate International Nematology Course (PINC) (since 1992), now EUMAINE, funded by the Belgian Government through the Flemish Interuniversity Council (VLIR) and The Nematology Initiative in Eastern and Southern Africa (NIESA) (2005–2010), funded by the Gatsby Charitable Trust, both designed to build capacity in plant nematology. However, the weaknesses and needs of tropical and developing world nematology, such as basic knowledge and a lack of awareness among key actors, remain prominent, while becoming increasingly necessary to confront, as cropping intensification and cropping of more marginal lands exaggerate nematode problems. In particular, there is need to follow on the success of the IMP, especially in respect to meeting the challenge of the most important plant parasitic nematode group, *Meloidogyne* spp. With a bleak future perspective, there is urgent need to address nematology training and support to create greater disciplinary prominence and bring it more into line within agricultural research agendas.

Session Twenty-four – Climate Changes, Soil Health Monitoring and Nematode Bioindicators

Molecular Nematology as a Tool for Soil Monitoring

Neilson, R., S. Donn, S.N. Vink, B.S. Griffiths & T.J. Daniell

Climate change is now recognized as one of the most important challenges for the planet. Whilst the gross geophysical changes such as receding glaciers capture the headlines and are in relative terms simple to measure, the impact upon soil ecosystems is potentially more difficult to detect due to the resilient nature of soil. The combination of climate change and constant perturbation by agronomic practices may accelerate degradation of soils in agricultural zones with soil erosion for example an increasing issue. Intensive land use has been implicated in declining soil health with concomitant concerns over sustainability of agronomic production. Monitoring soil health is problematic although soon to become a reality in the European Union due to forthcoming legislation. Nematodes have been proposed as indicators of soil health due to their ubiquity, short generation times and trophic composition, however identification based on morphology is time consuming and challenging. Previously univariate and multivariate analysis of classical morphological data have been used to interpret nematode assemblage data with limited success. Alternative molecular approaches to profiling soil nematode assemblages have been applied here, based on Terminal Restriction Fragment Length Polymorphism (T-RFLP) of small subunit ribosomal DNA. Two approaches are described, the first entailing digestion of fluorescently labelled PCR product with a single enzyme, combined with multivariate analysis of the resulting fragment profile. Application of this method on agricultural sites under differing management regimes revealed significant differences in assemblage composition by agronomic treatment. The second approach utilises a directed method where, from collected sequence information, a restriction digest has been designed to separate nematode taxa present at the study sites into terminal restriction fragments of known size. We envisage the resulting semi-quantitative profiles may be combined with existing nematode diversity indices and other soil (a)biotic data as a potential tool to monitor soil health.

Session Thirty-six – New Technologies for Plant Nematode Control

Innovations in Nematode Management on Turf in the USA

Crow, W.T.

The state of Florida in the United States has over 2.5 million ha of turfgrass which brings in over \$10 billion US to the state economy annually. Turfgrass is used on lawns, golf courses, athletic fields, sod farms, parks, and other venues. Plant-parasitic nematodes are among the most important pests on turfgrasses in Florida, however effective nematicides are not available for most turf uses. Fenamiphos has been the most widely used turfgrass nematicide in the U.S. for over thirty years, but as of 2006 it is longer being manufactured in the U.S. due to environmental concerns. Over the past eight years a great deal of research effort in Florida has sought to find innovative ways to manage plant-parasitic nematodes on turfgrass in the absence of fenamiphos. These efforts include: a) new uses of older nematicides such as 1,3-dichloropropene, b) development of new nematicides based on chemicals and plantderivatives such as furfural, mustard-bran, and sodium azide, c) new nematicides based on novel chemistries, d) biological control organisms such as *Pasteuria* spp. and *Paecilomyces lilacinus*. These efforts have met with varying degrees of success from complete failure to registration and industry acceptance of commercial products. Experimental results from field trials with numerous management tactics will be presented and future directions will be discussed.

Session Thirty-nine – Ecology and Biogeography of Entomopathogenic Nematodes

Do Natural Enemies Regulate Entomopathogenic Nematode Spatial Patterns?

Duncan, L.W., F.E. Elborai, R.J. Stuart, D.L. Bright & J.H. Graham

Entomopathogenic nematodes (EPN) have been shown to be important natural enemies of *Diaprepes abbreviatus*, a major weevil pest of citrus in Florida and the Caribbean Basin, and recently introduced into Texas and California. In different regions of Florida where endemic EPN species diversity and predation of weevil larvae are high, the insect is a minor pest; whereas, the weevil can cause growers to abandon citriculture in regions with fewer species and little predation by EPN. Accordingly, we are studying biotic and abiotic factors that regulate spatial patterns of EPN across the Florida citrus industry. Nematophagous fungi (NF) have been shown to respond in a density dependent manner when EPN emerge in high numbers from insect cadavers and when EPN are added to soil as an augmentation biocontrol tactic. Predation rates by NF also vary depending on the species combinations of NF and EPN, suggesting the possibility that some EPN species may have a competitive advantage in habitats that favor particular NF. Among the EPN endemic in Florida citrus orchards, the numbers of *Steinernema diaprepesi* and *S. glaseri* were unaffected by three species of *Arthrobotrys* (trapping fungi) in soil bioassays, whereas numbers of *Heterorhabditis indica*, *H. zealandica* and *S. riobrave* were reduced significantly. In contrast, two endoparasitic fungi (*Catenaria* sp. and *Myzocyrtium* sp.) whose zoospores require free water to locate and infect nematodes, had no effect on numbers of *H. indica*, but preyed heavily on the other four EPN species. *H. indica* is frequently the dominant species detected in parts of Florida with poorly drained soils and high water tables. Ongoing research is characterizing the spatial patterns of NF in Florida to better understand their habitat requirements and their potential to affect EPN communities.

Session Forty-six – Cereal and Potato Cyst Nematodes

Progress in the Quantification of Potato Cyst Nematodes

Blok, V.C., A. Paterson, J. Heilbronn, A. Holt, L. Pylypenko, J. Pickup & M.S. Phillips

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* occur in many potato growing regions world-wide and their widespread occurrence is of increasing concern to growers. Monitoring and detecting their presence in seed and ware land is of importance to limit their spread and for deployment of appropriate management strategies. To improve the efficiency of detection and to enable quantification of the two species, a quantitative PCR assay has been developed. The assay has been used with DNA extracted from three types of samples; pure cysts, soil 'floats' and soil to determine sensitivity and specificity parameters. The application of the assay to an experiment to determine how the two species of PCN compete on potato genotypes with differing degrees of resistance is described. The use of the assay for the detection and quantification of the two PCN species in typical statutory samples is demonstrated. In addition the potential to use the assay for soil samples will be discussed.

Topic Six – Entomophilics

Morphometric, Molecular and Biological Characterization of Spanish Native Steinernematid Strains and their Relationship with Bioassay to Assess their Activity and Sex-ratio

Campos-Herrera, R. & C. Gutiérrez

Twenty-two native nematode entomopathogenic (EPN) strains were isolated from La Rioja soils (Northern Spain). The morphobiometric study rendered: 16 *Steinernema feltiae*, 4 *S. carpocapsae* and 2 *S. kraussei* strains. The genotype agrees with those described for *S. carpocapsae* and *S. kraussei*, and with *S. feltiae* A2 RFLP type. Statistical analysis was performed to study their morphometric similarity with respect to 3-5 reference strains, and their biological similarity was assessed through out penetration, migration and one-on-one bioassays using *Galleria mellonella* (Lepidoptera: Pyralidae) as host. Native strains showed morphometric intraspecific variation, with low morphometric similarity percentage. In penetration assay, mean values of larval mortality were 42.3-96.1%, 79.9-85.4% and 86.2% due to *S. feltiae*, *S. carpocapsae* and *S. kraussei*, respectively, with the IJs penetration being 1.9-15.4%, 2.3-5.3% and 7.0%, respectively, in the same order. These values, although slightly reduced in the sand column migration study, were agreed with those observed by other authors in the Mediterranean region whereas show higher differences with those of other geographic regions. The differences observed in both assays might be due to different searching behavior of each species in relationship with the intraspecific variations in IJs size. Female proportion of the three EPNs species from La Rioja developed into *G. mellonella* was significantly higher than the male one. Larval mortality values in the one-to-one assay were lower to those observed by other authors, being 0-21.7% for *S. feltiae*, 8.7-8.9% for *S. carpocapsae* and 6.3% for *S. kraussei*. Some strains very active in the penetration and migration assays showed

0% penetration in the one-to-one assay, suggesting that a part of the IJs population might not be infective. The results of this survey provide new knowledge of three steinernematid species and show the interest of their biological characterization to assess more accurately their activity.

Topic Ten – Host Plant Resistance and Genetic Markers Development

From Virology to Nematology: A New Approach to Plant Resistance to Nematodes

Wang, Z., S. Liu & M.G.K. Jones

Root-knot nematodes are economically important plant parasites with a wide host range. They develop a unique interaction with cells of host plant roots that involves the induction of highly specialised giant cells from which they feed. Giant cells are the sole nutrient source for these nematodes that enable them to complete their life cycle and to reproduce. Strategies to develop synthetic resistance include inhibiting feeding cell function, but the requirement for this approach is strict control of gene expression to nematode feeding sites using a giant cell-specific nematode-responsive promoter. However, this approach has achieved limited success because a completely specific nematode-responsive promoter, which shows no other expression in any other cell type in the plant, has not been identified, although some promoters that drive high expression in giant cells have been reported. An alternative approach is to use two nematode-responsive promoters rather than one to develop the required specificity of gene expression. We are developing such an approach, based on a Tobacco Yellow Dwarf virus (TYDV) replicase gene and a target gene with modified structure which is not expressed in the absence of the virus replicase. Expression of the target gene requires over-lapping activities in giant cells of two different promoters, and this approach may provide the tight control of target gene expression that is needed to restrict it only to giant cells. The current status of this work will be presented that may lead synthetic plant resistance to nematodes by inhibiting development of host feeding cells.

Topic Twelve – Pathogenicity and New Host Records

Efficacy of Fumigant and Non-fumigant Nematicides for the Management of *Meloidogyne chitwoodi* in Idaho Potatoes

Hafez, S.L & P. Sundararaj

Two field experiments were conducted at the University of Idaho, Idaho, USA to study the efficacy of different combinations of Vapam HL, Temik 15G, Mocap 6EC, Vydate C-LV and different application methods of fosthiazate for the control of *Meloidogyne chitwoodi* in potato. The experiments were laid out in a randomized complete block design with seven and six treatments in a field each with five replications for the first and second experiment respectively. Mocap treatments were surface broadcast using a tractor-mounted plot sprayer and Vapam was applied as broadcast by fumigation bar. Temik and Admire were applied in furrow at planting. Vydate was applied at planting and chemigated subsequently after planting. Preplant treatments (PPI) of fosthiazate were broadcast sprayed or shanked at 6 inch deep or shanked followed by rototilling or broadcast surface spray with the nozzles attached to the front of the rototiller. Potato cv. Russet Burbank seed pieces were planted on 25th April in rows three feet apart. Weeding and other normal cultural practices were followed. Five months after planting, the tubers were hand-harvested on 2nd October from 15 feet of the middle two rows of each plot and weighed. The tubers were graded and evaluated for nematode infection. Data from both experiments indicated that there was an increase in total yield in different combinations of treatments compared to control plot. Percent of nematode infected tubers in treated plots ranged from 6 to 27.4 and 5.6 to 35.5 for the first and second experiment respectively. Lowest level of nematode infection was observed in the Mocap 2gal + Temik 20 lb + Vydate 2.2 pt (6.0 %) applied plots and; Fosthiazate 4.5 ai/A + Temik 20lbs at planting (5.6 %) applied plots in the first and second experiment respectively.

Topic Fourteen – Epidemiology and Population Dynamics

Occurrence of Soil-transmitted Helminths in Developing Country Women

Joshi, S.D., P.R. Chaudhary & K. Panday

Objective: To find the occurrence pattern and prevalence of the soil transmitted helminths in women of child-bearing age group in developing country Nepal. **Methods and Materials:** The study was conducted in 7 districts at an altitude of 2100 metres above sea level. Faecal samples of 2478 women of child-bearing age (15 to 45 years) were taken randomly and examined for the ova of soil transmitted helminths during year 2007. The data were analysed and edited by EPI info program. **Results:** The occurrence pattern was 53.0%, 20.0% and 2.7% for Hookworms, *Ascaris lumbricoides* and *Trichuris Trichuria* respectively. Both *Ascaris* and *Hookworm* prevalence rates noticeably increased with increasing age, with the highest infection rate between the ages of 36 - 45 years while *trichuris* infection was highest in women of 15-25 years of age. **Conclusion:** Due to the lack of

medicine and healthcare facilities in remote areas, there is a high prevalence of hookworm and *Ascaris* in women of child-bearing age and intervention is needed according to WHO guidelines. The government should make special policies and programs for health care access in these areas.

Topic Fifteen – Food Webs, Soil Ecology and Biodiversity

Does Long-term Organic Fertilization Simultaneously Enhance the Structural and Functional Stability of Soil Ecosystems?

Chen, X., M. Liu, B. Griffiths, F. Hu, H. Li & B. Zhang

The stability of soil ecosystem function depends on soil community, of which nematodes can be used as model organisms to represent the soil food web. To test whether organic fertilization enhances the stability of soil ecosystem structure and function simultaneously, we compared the resilience and resistance of nematode community and overall soil function (decomposition of plant residues) of two soils (degraded soil and its counterpart with longterm organic fertilization) after applying an experimental stress of copper, heat, chloroform or drying as well as a control treatment, respectively. In soils before applying stress, organic fertilization sharply promoted soil physio-chemical, microbiological properties, nematode abundance, diversity and structural complexity and soil function. After different stress applied, however, neither the stability of nematode community nor soil function was consistently higher in organically fertilized soil, but depended on the type of experimental stress. Soil function with organic fertilization showed significantly lower resistance ($P < 0.05$) after heat or drying but higher ($P < 0.05$) resilience irrespective of stress type. Further, we found different respects of nematode community showed distinctive responses even imposed by a similar stress. Nematode abundance, diversity, trophic structure exhibited significantly higher resilience and/or resistance following the stress of copper, chloroform or drying in organically fertilized compared to degraded soil, but both maturity index (MI) and structure index (SI) showed reverse trends after applying copper, heat or drying stress. Thus, the higher abundance and complexity of nematode community initially established in soil do not mean higher stability of community and function after stress. Our results indicated that, besides organic fertilization, both the historical legacy of stresses soil originally undergone and the type of stress soil subsequently received determined the structural and functional stability of soil ecosystem. The pre-cautionary roles of nematodes' response for indicating soil functional performance under currently increasing natural and anthropogenic stress require further study.

Long-term Changes in Soil Nematode Communities under the Impact of Fertilizers

Gruzdeva, L., E. Matveeva & T. Kovalenko

Changes taking place in the communities of soil nematodes of an artificially sown meadow under the impact of annually applied mineral fertilizers have been studied in a field experiment for nine years. Changes in the species composition, eco-trophic community structure, and the number of nematodes from different genera depend on the fertilizer applied. The application of mineral fertilizers had a more pronounced effect on the nematode community in the plots without background manuring; the soil manuring weakened the mineral fertilizer effect. The most significant changes in the soil nematode numbers were observed for the trophic groups of bacterial feeders and plant feeders during the first 5-6 years of the experiment. The population of bacterial feeders increased in response to the increasing rates (from 60 to 180 kg/ha annually) of the complete mineral fertilizer *NPK* and nitrogen-containing fertilizers. The population of plant feeders increased in response to the potassium fertilizers. The spectra of nematode genera sensitive to *NPK* and to the particular nutrients have been identified with the use of parameters, including the maturity index of nematode communities, the biotope preferences of the particular nematode genera, and the general pattern of nematode habitats. It was found that the nematode community structure stabilized by the seventh--ninth year of the succession; after this, the effect of annual application of mineral fertilizers on the nematode community structure was not very significant. The results obtained can be used to assess the effect of mineral fertilizers on the soil fauna and to suggest optimum application rates of mineral fertilizers ensuring the sustainable development of meadow herbs.

Effect of Cadmium and Lead Salts on Soil Nematodes

Suschuk, A.A., L.I. Gruzdeva & E. Matveeva

Sensitivity of soil nematodes from 15 genera extracted from spruce forest at different concentrations $CdSO_4$ (1.5, 3.0, 6.0 mg/l) and $PbSO_4$ (16, 32, 64 mg/l) was studied under laboratory conditions. Toxicity of *Cd* and *Pb* was estimated on mortality of individuals. Distilled water was used as control. In water 50% of nematodes died in 11 days, the majority of taxa passed away in 16 days. Representatives of *Plectus* and *Eudorylaimus* were the most sensitive to such environmental conditions; their death was registered in 7 and 11 days respectively. Nematodes from genera *Plectus*, *Eudorylaimus* and *Tylenholaimus* were the most sensible to *Cd* (mortality were

observed in 2 days). Representatives of *Aphelenchoides*, *Rhabditis* and *Acrobeloides* showed a high resistance. They survived up to 15 days (dose 1.5 mg/l). With increasing cadmium dose up to 3.0 and 6.0 mg/l death of nematodes was registered in 9 and 8 days respectively. The first ones who responded earlier to Pb (in 3 days) were nematodes from genera *Metateratocephalus*, *Teratocephalus*, *Eudorylaimus*. Representatives of genus *Plectus* lost the vitality on 4-8 days. Nematodes from genera *Eucephalobus*, *Cervidellus*, *Malenchus*, *Ditylenchus*, *Paratylenchus* exhibited higher resistance to lead. The representatives of *Rhabditis*, *Aphelenchoides*, *Cephalobus* and *Acrobeloides* were the most resistant. Mass nematode mortality was fixed in 12-13 days for low Pb concentration and 11 days –for high concentrations (32, 64 mg/l). On the whole, cadmium was more toxic to nematode populations. It was exhibited in early terms of total nematode mortality (in 8-9 days for Cd, 14 - for Pb). Research was supported by the Programme of Fundamental Research of Biology Department, RAS, № 01.2.006 08823.

Topic Sixteen – Organic Amendments and Management

Effect of Application Sequences of Brassica Green Manures, Mustard Seed Meal and Nematicide on Root-knot Nematode Suppression, Starch Reserves, Yield and Juice Characteristics in Grapevine

Rahman, L. & B. Orchard

Inter-row cultivation of brassicas may substantially reduce *Meloidogyne* spp in vineyards but the optimal number of years of cultivation to reduce the population levels below damage threshold is less known. We investigated effects of two brassicas (Indian mustard cv Nemfix, BQMulch™) as green manure, mustard seed meal and Nematicur® on the suppression of *M. javanica* in soil and roots when applied in one to three consecutive years in potted Semillon vines. Each vine was inoculated with 500 *M. javanica* J2 three months after planting. Two control treatments, infected and uninfected, were also maintained. Brassica seeds @ 20 kg/ha were sown in early May; plants grew until early September, and then slashed and incorporated into top soil. Mustard seed meal @ 2t/ha and Nematicur® @ 30 L/ha were also applied on the same day. Data on nematode populations in soil and roots, starch contents in shoots and roots, yield and juice characteristics were recorded from each treatment. Results indicated that *M. javanica* J2 populations were reduced in all treated soils compared to the inoculated control treatment. Populations were increasingly reduced from one to three years of repeated treatment. Root populations were also consistently reduced in the BQMulch™ and Nematicur® treated vines. Yields from treated vines were similar to that of inoculated vines in one and two consecutive years of treatment but increased significantly following three consecutive years of treatment. Similarly vines applied with brassica green manures for three consecutive years produced similar yields to that of uninoculated vines. Starch contents in roots (% dry weight) of brassica and seed meal treated vines were considerably higher (21-24%) than that in roots of untreated and inoculated vines (17%) after three consecutive years of application. The number of treatment years did not affect juice characteristics.

Effect of Neem Based Biopesticides on *Meloidogyne incognita* and *Rotylenchulus reniformis* Attacking Tomato

Siddiqui, M.A.

Pot experiments were conducted in two successive years to evaluate the potential of some neem, *Azadirachta indica* A. Juss, based biopesticides viz., Achook 0.15%; Neem Raj 0.15%, Fortune aza 0.15% and Neem drop on the root-knot development caused by *Meloidogyne incognita* (Kofoid and White) Chitwood and population of reniform nematode *Rotylenchulus reniformis* (Linford and Oliveira), attacking tomato (*Lycopersicon esculentum* Mill. cv 'Pusa Ruby'). These neem based biopesticides were applied @3 ml/pot and all the biopesticides significantly reduced the nematode multiplication. The highest reduction in nematode infection was observed in pots treated with neem drop followed by Achook, Neem Raj and Fortune aza respectively. The reduction in the nematode infection simultaneously enhanced the plant growth characters, the highest being in neem drop treated pots.

Topic Seventeen – Nematode Biological Control Agents

Impact of *Pseudomonas*-based Biocontrol Agents and Solarization on *Mesocriconea xenoplax* Populations and Tree Survival in a Peach Tree Short Life Site

Nyczepir, A.P., D.A. Kluepfel & W.P. Wechter

Soil solarization, alone or in combination with other disease management practices, has been shown to be effective in reducing inoculum density of many soilborne diseases, including nematodes. *Pseudomonas synxantha* (BG33R), isolated from a peach orchard site suppressive to peach tree short life (PTSL), was demonstrated to suppress ring nematode, *Mesocriconea xenoplax*, reproduction in field soil under greenhouse conditions and inhibit egg hatch *in vitro*. In 2005, a field study was initiated to determine the influence of

combining solarization and application of BG33R plus four additional bacterial, nematode antagonists through the irrigation system for management of *M. xenoplax* and prevention of PTSL tree death. Soil treatments include: i) solarized soil alone (S); ii) solarized soil + biocontrol cocktail (SB); iii) nonsolarized soil alone (NS); iv) nonsolarized soil + biocontrol cocktail (NSB); v) solarized soil + wheat (SW); vi) solarized soil + wheat + biocontrol cocktail (SBW); vii) nonsolarized soil + wheat (NSW); viii) nonsolarized soil + wheat + biocontrol cocktail (NSBW); and ix) methyl bromide (MBr) fumigated soil. Controls include, fumigated and nonsolarized nonfumigated soil. Four and 13 months (June 2005 & March 2006) after planting trees, ring nematode populations were greatest in three nonsolarized treatment plots (NS, NSBW, and NSW) than in all four solarized (SW, SB, SBW, S), or MBr fumigated plots. Twenty-one months after MBr application (November 2006), ring nematode population density did not differ among most of the treatment plots. In May 2006 and 2007, more trees in the nonsolarized treatment plots (NSW, NS, & NSB) developed typical PTSL symptoms and died than in the solarized (S, SW, SBW, & SB) or MBr fumigated plots. Soil solarization alone significantly reduced PTSL tree mortality.

Influence of Fungicides on a Nematode-Suppressive Soil

Timper, P. & A.K. Culbreath

We identified a field in Georgia, USA that was moderately suppressive to *Meloidogyne* spp. In the greenhouse, reproduction of both *M. incognita* on cotton and *M. arenaria* on peanut was greater in microwave-heated soil than in natural soil from this field suggesting that nematode suppression was caused by a heat-sensitive organism. Because fungi antagonistic to nematodes are common in soils and are frequently associated with suppressive soils, we hypothesized that fungicides would reduce the activity of these fungi and allow greater nematode reproduction. To test this hypothesis, we collected soil from the suppressive field and placed it in 6 liter pots. Peanut was planted and 2 weeks later inoculated with 3,000 eggs of *M. arenaria*. Starting 5 weeks after planting (WAP), the peanuts were sprayed with one of four fungicide treatments: 1) five applications of chlorothalonil, 2) four applications of tebuconazole, and 3) two applications each of flutolanil, and 4) azoxystrobin. These fungicides are commonly sprayed on peanut at the rates and frequencies used. There were eight replications of each treatment and control (no fungicide). The number of eggs/g root was determined 15 WAP. In both trials of the experiment, azoxystrobin was the only fungicide that led to an increase ($P = 0.0004$) in nematode densities relative to the control; nematode densities in the other fungicide treatments were not different from the control. The number of eggs/g root was 20,391 in control pots and 44,315 in azoxystrobin-treated pots, a two-fold increase in nematode reproduction. Further research is underway to determine whether azoxystrobin has a similar effect on nematode densities in heat-treated soil.

Studies on the Nematicidal Activity of Plant Extracts and their Control of Plant Disease Caused by Nematodes

Wen, Y-H., L-Y. Peng, G-J. Wang & H. Xie

Ethanol extracts of 46 species of plants (belong to 33 families) from China were screened for nematicidal activity (NA) against *Bursaphelenchus xylophilus* and *Meloidogyne incognita* by the fungal-feeding and dipping bioassays method. *Croton tiglium* L., *Ruta graveolens* L., *Cerbera manghas* L., *Axillary Choerospondias* Fruit, *Sapium sebiferum* (Linn.) Roxb, *Tripterygium Wilfordii* Hookf., *Dysosmavens ipelis* (Hance) M.Cheng, *Lantana camara* L. and *Artocarpus heterophyllus* Lam showed very strong NA against *Bursaphelenchus xylophilus* (80%-90%); while *Lagerstroemia speciosa* (L.)Pers, *Tripterygium Wilfordii* Hookf. *Ophiopogon japonicas* (Linn.f.)Ker-Gawl., *Colocasia gigantean*, *Eucalyptus citriodora*, *Croton tiglium* L., *Sapium sebiferum* (Linn.) Roxb., *Aconitum hemsleyanum*, *Dysosmavens ipelis* (Hance) M.Cheng, and *Schima superba* showed strong NA against second stage juvenile of *Meloidogyne incognita* (85%-100%). Pot experiments showed that *Dysosmavens ipelis* (Hance) M.Cheng, *Celastrus angulatus*, *Eucalyptus citriodora*, *Sapium sebiferum* (Linn.) Roxb, *Croton tiglium* L., *Lantana camara* and *Cerbera manghas* L. could effectively control the root-knot disease by powder or root irrigation methods. Soil add the powder of this plants or water with the extracts of this plants could significantly reduce the nematode infection, the number of galls in root and the number of nematode in galls; and inhibit the egg hatching.

Topic Eighteen – Chemical and Integrated Management

Cross-degradation of Novel Non-fumigant Nematicides by Soil Biotic Factors

Cabrera, J.A., A. Schouten & R.A. Sikora

Inconsistent efficacy of non-fumigant nematicides is a world-wide problem, especially in tropical agriculture with intensive cultivation, high temperatures and frequent use of pesticides. Due to the large scale application of nematicides soil-inhabiting microorganisms have in several cases become capable of rapidly degrade the active ingredients. In soil with no nematicide-application history no nematicide metabolization is observed. The

objectives of our study are to further investigate the role of microorganisms in the rapid degradation of non-fumigant nematicides and to determine the biodegradability of novel nematicides in known nematicide-biodegradable soils. Metabolization studies using high-performance liquid chromatography (HPLC) are conducted in this investigation. Results of cross-biodegradation are discussed. Cross-biodegradation may easily occur since some new nematicides belong to the same chemical family as the previously used biodegradable ones. The nematicide degrading microorganisms and the mechanism(s) of metabolization are characterized. The impact and implications of 'cross-degradation' of nematicides by soil microorganisms are discussed.

Observations on the Nematicidal Activity of 1,3,7,-trimethylxanthine (caffeine)

Ciancio, A.

A nematicidal activity was discovered for caffeine (1,3,7-trimethylxanthine) through *in-vitro* and pot tests. In a first assay, *Meloidogyne incognita* eggs were placed in watch glasses with caffeine solutions ranging from 300 to $1.27 \cdot 10^4$ ppm. Hatching was measured after two weeks at 25°C. A juvenile (J2) mortality assay was carried out in watch glasses with caffeine solutions from $0.075 \cdot 10^4$ to $1.0 \cdot 10^4$ ppm. Mortality was measured after two weeks at 25°C, adding 50 µl of lactic acid and counting the J2 reacting to the pH change. Three replications were used for both tests, with distilled water as control. Data showed a progressive dose dependent hatching reduction and a significant J2 mortality increase. No hatching was observed at 7500 ppm or higher concentrations (DL50 = 2500 ppm). Highest J2 mortality (100%) was at $1.27 \cdot 10^4$ ppm (DL50 = 1400 ppm). In pot tests, caffeine was applied to UC82 tomato plants kept at 25±2 °C, transplanted to soil containing 533 J2 · litRE-1 of *M. hapla*. Treatments (six doses) consisted in 20 ml additions of caffeine solutions from 1500 to 104 ppm, in five replicates, with water as control. At 1500-3000 ppm plant growth was higher than control, with a marginal effect decrease at 5000 ppm and higher levels. Height increase was 70% at 1500 and 3000 ppm, and 40% at 5000 ppm. No difference was found between control and highest dose. Similar trends were observed for root and leaves weights, highest at 1500 and 3000 ppm, at which less galls and eggs were found. At more than 5000 ppm, galls and eggs increased and root weight decreased, due to phytotoxicity. However, at 104 ppm, the total number of eggs per plant was 35.7% lower than control. Caffeine was known for its activity against snails, but not for phytoparasitic nematodes.

Agri-Terra: A New Low-rate Nematicide

McGawley, E.C.

2007 was the seventh consecutive year of trials evaluating the efficacy of Agri-Terra against nematode species associated with major crops in Louisiana. Trials with soybean, cotton, rice and carrot were conducted in microplots. Concentrations of 0.5 and 1% and rates of 0, 5, 10, 20, 40 and 80 gallons per acre were applied to microplot soil infested with one or a combination of five nematodes. The 10GPA/1% concentration was the most optimal treatment for soybean, resulting in harvest plant weights, pod numbers and pod weights that were significantly greater than those of controls. This concentration/rate combination of Agri-Terra was also the best treatment for cotton, producing statistically significant increases in plant growth and boll production while providing nematode control. The 0.5% concentration of Agri-Terra produced slight to moderate increases in the overall growth of rice and the 1% concentration produced moderate growth inhibition. All Agri-Terra treatments of soil in microplots of rice resulted in marginal control of ring, spiral and stubbyroot nematodes. Agri-Terra treatments also resulted in significant reductions in populations of root-knot nematode on carrot. Residual nematode populations, however, caused marked root galling and plant damage at harvest. Commercial vegetable production protocols were employed in field trials with tomato, cucumber and bell pepper. With tomato, Agri-Terra successfully managed reniform nematode populations and resulted in significant increases in yields of tomato fruit in the Extra-Large and Large size categories. With cucumber, treatment of soil with Agri-Terra reduced reniform nematode populations significantly and increased yields of fruit in the Super-Select and Select size categories. Results of field trials with cotton conducted in 5 consecutive years showed that the application of Agri-Terra to soil as an at-planting, infurrow (fine mist) spray treatment produced highly significant decreases in nematode populations and highly significant increases in yield.

MCW-2: A 'True' Nematicide Belonging to the Fluoroalkenyle Group

Oka, Y, M. Berson & A. Barazani

Nematicidal efficacy of MCW-2 (Makhteshim Chemical Works, Beer-Sheva, Israel), which belongs to the fluoroalkenyle group, was studied in laboratory, growth chamber and in fields. MCW-2 showed an irreversible nematicidal activity after exposure of *Meloidogyne javanica* second-stage juveniles to 0.5 µg/ml solution for 48 hr and rinse in water for another 24 hr, in contrast with fenamiphos or cadusafos, which had only reversible nematostatic effect at the same or higher concentrations (~ 8 µg/ml). An EC formulation of MCW-2 inhibited the

nematode hatching at a high concentration (8 µg/ml), but the hatching recovered after rinse in water. In pot experiments, the compound at a concentration as low as 0.25 mg/l soil showed the same or better control efficacy against *M. javanica* than those of fenamiphos or cadusafos at the same concentration. The duration of the nematocidal activity of the EC formulation lasted at least two weeks after application into an alkaline sandy soil; however, fenamiphos showed a tendency of longer nematocidal activity in the soil than that of MCW-2. In microplot experiments, soil drench with an EC formulation at 2.0 kg a.i./ha had the same control level of fenamiphos at 4.0 kg/ha or cadusafos at 3.0 kg/ha, based on galling index of tomato roots caused by *M. javanica*. No phytotoxic symptoms were observed on tomato plants at the concentration. The compound has a far lower toxicity to rats (acute oral LD50: >500 mg/kg) and non-target organisms (non-toxic to bees and earthworms) comparing with organophosphate or carbamate nematocides. MCW-2 has also a low leaching potential in the soil. The results indicate that MCW-2 has a great potential as a nematocide, which belongs to a new chemical group, and probably has a novel mode of action. Results from field experiments will be also presented.

8.6.2 Report on a visit to Minneapolis, Minnesota, USA on 26-30 July 2008 to attend the APS 2008 Centennial Meeting

Introduction

Information from talks, posters and discussions at the APS 2008 Centennial meeting.

Abstracts of Plenary Session Presentations

When agriculture fails

Ray D. Martyn, Ph.D. Professor of Plant Pathology and APS President, Purdue University, West Lafayette, IN, USA. Phytopathology 98:S1.

Agriculture is one of the world's great success stories. Our ability to grow food and fiber to feed, clothe and shelter almost 7 billion people is nothing short of remarkable. Agriculture's success is attributed to the many advances in research and production over the last 100 years each adding to the overall production efficiencies. And while the U.S. and other developed countries of the world have benefited, agriculture in other less developed countries continues to struggle. Starvation and malnutrition is still rampant throughout much of the world. In 2003, 38 countries faced a serious food shortage emergency, as defined by FAO. In many of these countries, food shortages are compounded by the impact of pandemics of HIV-AIDS, vector-borne diseases such as malaria and water-borne intestinal diseases. Polluted and contaminated water contributes to the death of 15 million children annually. Paradoxically, the process of growing the world's food, feed and fiber is a major contributing factor to the public health crisis. Agriculture accounts for about 70% of all water withdrawals globally and up to 95% in some developing countries. As a result many people have to choose between water for growing food and water for sanitation and public health. The link between agriculture and human public health is one that cannot be ignored. If the world is to make significant gains in alleviating the global public health crisis we first have to improve the quantity, quality, security and availability of food, and do so, in an environmentally-neutral and sustainable manner. All the medicines in the world cannot cure starvation. Global agriculture and, in fact, humanity itself, faces many new challenges. The increasing rate of decline in the planet's rich biodiversity is looming large. Deforestation, particularly in the Amazon Basin of Brazil, is increasing dramatically and, ironically, much of this loss is a result of habitat destruction in the name of agriculture. Additionally, global climate change resulting from increased anthropogenic greenhouse gases will negatively impact agriculture in many ways. These are global issues and they will require global solutions. But there is reason for optimism. It is time for a new paradigm in world agriculture – one that views agriculture as an instrument for public health and is focused not only on the quantity of food it produces but the nutritional quality, particularly in regard to the micronutrients such as iron and zinc, vitamins, proteins and even plant-derived pharmaceuticals. If the developed world is intent upon improving the public health of hundreds of millions, even billions of people, a revitalization of, and support for, agriculture must be a vital 'first step' in the process. What we do as agricultural scientists is paramount and a big piece of the solution. When agriculture fails, humanity fails. This special Centennial Plenary Session entitled "Agriculture, Food Security, and Public Health: Global Issues – Global Solutions" will address the issues discussed above.

Biodiversity and agriculture

Peter S. Raven, Ph.D. President, Missouri Botanical Garden and Engelmann, Professor of Botany, Washington University in St. Louis, MO, USA, Phytopathology 98:S1

Agriculture is the enemy of biodiversity. Since crop agriculture was developed starting about 10,500 years ago in the eastern Mediterranean region (“the Fertile Crescent”), the global human population has grown from an estimated 3-4 million to 6.6 billion people, and is not expected to level off until at least 2 billion additional people have been added. This explosive growth, which took place over 400 generations after approximately 2 million years of human existence on earth, has brought about 11% of the earth’s land surface into crop cultivation and an additional 22% into use as natural pastures, virtually all of which are unsustainable. Human impacts, exacerbated by rapidly rising demands for meat and higher consumption of all kinds as well as the use of traditional polluting technologies on a much wider scale than earlier, are using all aspects of global productivity more rapidly than they are being replenished naturally. With the progressive loss of topsoil, natural vegetative cover, and agricultural land, pressures on the environment are mounting even though half the people in the world are living in extreme poverty and one-eighth of us are literally starving.

Human impacts are felt everywhere on earth, but with special severity in the third of the land surface where agriculture is practiced. Habitats are being destroyed throughout the world for many reasons, the extraction of natural products such as wood and the conversion of forests and natural pastures to often temporary cattle pastures, as well as rapid, low-density urbanization and road building being among them. As a result of these pressures and the steady increase in global temperatures that accompanies the industrial production of carbon dioxide and other greenhouse gasses it is estimated that as many as two thirds of all kinds of living organisms may be driven to the brink of extinction or actually be extinct by the end of the century we have just entered. Since only about 1.7 million of an estimated 12 million or more species of eukaryotic organisms even have a scientific name, and only a very small fraction of the estimated number of species of bacteria (prokaryotes), it is clear that most of the species that are being lost will be completely undetected at the time they vanish, a disturbing consideration. We have built our agricultural systems and much of the rest of our economy on the basis of the properties of plants, animals, fungi, and microorganisms, and certainly hope to use them extensively in building a sustainable world – one in which we use no more than the world is capable of producing on continuing basis – for the future. In view of those expectations, it is clearly in our interest, particularly in the early years of our understanding of the molecular basis of life, to preserve as much biodiversity as we possibly can.

The global water crisis: Balancing water for agriculture and public health Shiney Varghese

Access to clean drinking water and sanitation is a basic need and a human right. According to the U.N. General Comment on the right to water (2002) “Water is required for a range of different purposes, besides personal and domestic uses, to realize many of the Covenant rights. For instance, water is necessary to produce food (right to adequate food) and ensure environmental hygiene (right to health).” Unfortunately, half of humanity does not have access to sanitation; more than one in six people still lack reliable access to drinking water. This has led to a severe public health crisis. On the other hand it has been predicted that if the current water use patterns are continued there will not be enough water to produce food for growing populations by 2050. Much of this water crisis is closely connected to current practices associated with irrigated agriculture. The amazing productivity of irrigated, chemical intensive agriculture in the United States helped feed post World War II Europe, as they were re-building the agricultural infrastructure in those countries. It also helped meet challenges of securing food for many newly independent nations of the south in the second half of 20th century. Yet it has come with a huge cost. Many of our rivers are either dammed or run dry for much of the year; most of them are polluted; our aquifers are getting depleted and our groundwater is contaminated. This affects the quality and quantity of water available for rural environments, and the people who are directly dependent on rural water sources for meeting their basic water needs. In fact, a majority of the world’s 1.1 billion people affected by the world water crisis are rural inhabitants.

The challenge is to ensure that our development trajectories do not lead to a conflict or choice between food production on the one hand and drinking water and sanitation needs on the other hand. This challenge must be considered in the face of growing populations, extreme fluctuations in rainfall pattern and increasing vulnerability of rain-fed farming systems that contribute to 60 percent of worlds’ food production.

Plant biotechnology and agriculture: Is there a role for public sector scientists? Roger N. Beachy,

The scientific advances in microbial genetics and recombinant DNA sciences in the 1970’s lead directly to applications of technologies that produced new pharmaceutical products, remediation of oil spills, and novel products for a host of applications. By the late 1970’s scientists were probing the genetic bases of diseases, including plant diseases, using the new scientific tools, and soon uncovered the basis of the grown gall disease

caused by *Agrobacterium tumefaciens*. The rest is history, and by the early 1980's the first transgenic cells and whole plants were developed to express a variety of transgenes, including from other plants, bacteria, viruses. When the first field trials were conducted there was great expectation that new crop varieties would have higher yields, require fewer chemical inputs and use less water, non-legumes would fix atmospheric nitrogen, increase nutritional content in foods, among other goals. Now, nearly 25 years since the development of the first transgenic crops, few of the goals have been achieved. Nevertheless, large numbers of farmers in many countries have embraced the use of transgenic crop plants with resistance to certain insects or viruses and tolerance to selected herbicides, and consumers, many of whom are unaware of the differences between the new and old varieties have benefitted from their planting. Others remain indifferent or vocally opposed to the crops, due in large part to vocal groups that seek to retain the status quo, and in part due to governmental agencies in some countries that are unable to make strong policy recommendations for acceptance. The vast majority of transgenic (genetically modified, GM) crops currently grown around the world are products developed and released by successful private sector seed companies, following approval by government agencies. The new varieties have increased efficiency of agriculture and crop yields significantly. However, much of the promise of scientific discovery in agriculture and food biotechnology remains to be realized in future years. The untapped wealth of scientific discoveries made during the past 20 years in research institutions around the globe have yet to be harnessed, and crops with heightened resistance to attack by pathogens and insects, or have increased levels of vitamins and minerals, have yet to be approved and released to farmers and consumers. Reasons for this seem to be due to unfamiliarity of public researchers with the biosafety and product approval process imposed on biotechnology and the attendant high costs for the process. Interestingly, governmental agencies in India and China are encouraging product development by public sector scientists while agencies in the U.S. and EU countries do little to advance applications of biotechnology. Meanwhile governments in less privileged countries, including those in Africa and Asia seem unable to make decisions on acceptance or rejection of biotechnology in their plant breeding programs, and not knowing whether the policies of the EU or the India/China positions should be adopted in their country. As a consequence, public sector scientists who might become engaged in development of new crop varieties using the full repertoire of advanced technologies remain on the sidelines. Relieving some of these constraints would lead to greater applications of biotechnology to agriculture and increase sustainable production and improve nutritional value and safety of foods in the U.S. and around the globe.

Abstracts of Centennial Session Presentations

Biology of Plant Pathogens

100 Years of The American Phytopathological Society Staging a centennial: Milestones in the development of the American Phytopathological Society

P.D. Peterson and K.-B. G. Scholthof

As part of the growing specialization generally in the biological sciences between 1880 and 1920, the creation of the American Phytopathological Society (APS) in 1908 was a response to the developing professionalism within the agricultural sciences in the United States. Inspired and led by senior federal plant pathologist C. L. Shear, the organization and early membership reflected both the growing impact of plant disease losses and the expansion of problem-solving research by the United States Department of Agriculture, agricultural colleges and state agricultural experiment stations. At the time of the first regular meeting in 1909, at Harvard Medical School, Boston, Massachusetts, 130 plant pathologists from the U.S. and Canada had become charter members. Hosted by the American Association for the Advancement of Science (AAAS) in both 1908 and 1909, APS then met jointly with AAAS for its first 33 years before meeting alone or with other specialized scientific societies. As the profession grew in the 20th century, the Society reflected increased specialization within university departments, government, and industry, while membership increased to nearly 5000 scientists worldwide. An examination of pivotal developments and influential scientists in plant pathology during the last century provides a backdrop for understanding the past, current, and future role of the APS.

The Future of Plant Pathology

Educating the next generation of plant pathologists

G. W. Hudler.

Despite our best efforts, plant diseases continue to diminish food supplies, disrupt fragile economies, and precipitate human misery. There is no reason to think that any of that will change soon, and – if anything – the demand for skilled people to teach, learn, and apply concepts of plant diseases and disease management to

contemporary agriculture is destined to grow. Challenges associated with attracting and educating the next generation of plant pathologists are also destined to grow, but they are not insurmountable. First, students with interests in biology or natural history or agricultural science have to become aware that our profession exists. Not so many as before are enrolled in agricultural science curricula and fewer than ever learn about us by taking our courses. We can fix that...if we think out of the box. That's what I try to do and you could, too. Second, the best students of the next generation have to become convinced that the mission of the profession is one that they will find intellectually stimulating and personally rewarding. We can fix that, too...by putting those among us who are successful because of the passion they bring to the job into the classroom to share their enthusiasm for what they do and how it impacts on human affairs. That's what I hope you'll try to do. Third, the education of the next generation must include experiences that teach students to embrace a good mystery, to ask good questions, and to design experiments that will yield data leading to reliable answers. That's for all of us to do, as individuals, as departments, as a society and perhaps most importantly, as a unified team of dedicated professionals working toward a common goal.

Abstracts of Presentations

Phytophthora nicotianae zoospores evade pressure and agitation stress but are completely destroyed by CO₂ injection

M.O. AHONSI, T. J. Banko, S.R. Doane, A.O. Demuren, W.E. Copes, C.X. Hong

Phytophthora nicotianae is a known pathogen of numerous herbaceous and some woody ornamental plants, and is commonly isolated from recycled irrigation ponds. Zoospores are the most important propagules of *Phytophthora* spp. Using simulated recycled irrigation water we investigated Vol. 98, No. 6 (Supplement), 2008 S11 the survival of *P. nicotianae* zoospores as affected by hydrostatic pressure, agitation, and aeration with CO₂ or air. Exposing zoospores to hydrostatic pressure of 840 kPa for 8 min or agitation of mixing intensity G = 6483 1/s for 4 min did not kill any zoospores. However, bubbling CO₂ into zoospore infested water at 110.4 ml (0.2 g)/min for 5 min consistently killed up to 81% of the zoospores. Further extending CO₂ injection up to 30 min did not increase percent zoospores killed although fewer were killed with a shortened injection time. When we exposed zoospores to CO₂ pressure of 630 kPa (16.3 g CO₂) or 70 kPa (3.85 g CO₂) for 30 seconds or longer, percent zoospore kill did not differ from one another and did not differ from bubbling CO₂ at 110.4 ml/min for 5 min. In contrast, when the same treatments were done using pressurized air in place of CO₂, all zoospores survived. In further experiments, when we minimized cyst formation during zoospore-infested water preparation by avoiding vigorous shaking, CO₂ injection consistently resulted in over 98% zoospore kill. We concluded that the percent zoospores not killed by CO₂ injection in previous experiments were zoospores that had encysted before exposure to CO₂. Similarly, hydrostatic pressure and agitation treatments induced cyst formation and consequently allowed 100% survival. Results indicate that CO₂ treatment may be a promising alternative technology for disinfecting recycled irrigation water contaminated with *P. nicotianae*.

Nested PCR is essential for the detection of extremely low titer of Candidatus Liberibacter asiaticus from citrus and its vector psyllid *Diaphorina citri*

L. Benyon, L. Zhou, A. Weathersbee, Y. DUAN

Citrus huanglongbing (HLB), transmitted by the psyllids *Diaphorina citri* and *Trioza erytrae*, is one of the most devastating diseases of citrus worldwide. The disease is associated with three different species of Candidatus *Liberibacter*: *Ca. L. asiaticus* (Las), *Ca. L. americanus* and *Ca. L. africanus*. Currently detection and diagnosis of HLB rely on the typical symptoms of HLB such as leaf blotchy mottle and PCR confirmation using various sets of Las-specific primers. Due to the complex nature of HLB, conventional PCR and real-time PCR were not able to detect the bacterium in certain phenotypes of the disease or from psyllids that carried a low titer of the bacterium. Three sets of primers for nested PCR were developed which targeted three different genetic loci, 16S rDNA, beta-operon and the outer membrane protein gene of the bacterium. With any one or a combination of these nested PCR Las-specific primer sets, the detection rate of Las bacterium in psyllids collected from HLB infected citrus groves was 20–30% higher than the detection rate by real-time PCR. Using nested PCR coupled with improved sampling and bacterial DNA isolation methods, we also increased detection of the Las bacterium from citrus exhibiting atypical HLB symptoms. These results demonstrated that nested PCR is essential in the detection of Las bacterium when the pathogen is present at a very low level, and it is extremely important in screening for HLB-free germplasm in citrus.

Number of insecticide sprays has no effect on the incidence of citrus huanglongbing in a commercial orchard in São Paulo, Brazil

A. BERGAMIN-FILHO, M.G. Gasparoto, L. Amorim, R.B. Bassanezi

A total of 716,476 citrus plants (*Citrus sinensis*), from five to ten years old, distributed in 357 blocks (mean of 2006.9 trees per block) were submitted to different number of insecticide sprays (3 to 12) during three growing seasons (2004/2005, 2005/2006, and 2006/2007) in a farm located in São Paulo state, Brazil. Insecticide sprays were aimed to control citrus huanglongbing (HLB) throughout the control of its vector, *Diaphorina citri*. Eradication of symptomatic trees was carried out in the whole area 4 to 8 times per growing season. Incidences of HLB in all blocks ranged from 0.0 to 8.35% of symptomatic trees. The relationships between the number of eradicated plants and the number of insecticide sprays were investigated considering (i) each one of the three growing season, (ii) same than previous but grouping blocks according to classes of initial disease incidence, and (iii) eradicated plants in the last season (to minimize the influence of a long latent period) and the total number of insecticide sprays in all seasons. We did not find any significant negative relationship between the number of sprays and HLB incidence. We suggest that, in the conditions of the farm, the low incidence of HLB was mainly due to eradication of symptomatic trees than to insecticide sprays.

Characterizing resistance to infection by the root pathogen *Armillaria mellea* in tolerant and susceptible grapevine rootstocks

R. BHAT, K. Baumgartner, P. Fujiyoshi

Grapevine rootstocks that are resistant to the basidiomycete fungus *Armillaria mellea* have not been identified, mainly due to lack of a rapid and reliable inoculation technique. The aim of our research was to develop an in planta assay for inoculating grapevines and tracking root infection. We propagated tolerant (Freedom) and susceptible (3309C) rootstocks from green cuttings rooted in tissue culture medium in a growth chamber at 25C. After 6 weeks, surface of the culture medium of each plant was inoculated with four mycelia agar plugs of a strain virulent on grape (n = 25 per rootstock). Within days, hyphae proliferated the medium, and by week 2, were in direct contact with roots. At 0, 2, 4, 6, and 8 weeks post-inoculation, plants were harvested (n = 5 per rootstock), roots were washed of culture medium, and pathogen growth in root tissue was confirmed by treatment with wheat germ agglutinin conjugated to a fluorophore (AlexaFluor 488) and confocal microscopy. Hyphae and roots were scanned on separate channels; hyphae appeared green, roots appeared red. Percent colonization of root area by hyphae was quantified (ImageJ,v1.37). Analysis of variance indicated significant differences in root colonization between rootstocks; 3309C had significantly higher root colonization than Freedom starting at week 4 and continuing throughout the experiment ($P < 0.0001$). The pathogen was detectable in roots, via microscopy, only two weeks after inoculation. This is a dramatic improvement over the previous inoculation technique, which resulted in earliest pathogen detection months to years after inoculation. With the ability to infect plants by *Armillaria* in a much shorter amount of time and on a reliable basis, we are now able to investigate the mode of root penetration by the pathogen, to identify susceptible portions of the root system, and to determine when foliar symptoms form in response to infection.

Detection of phytoplasma and Candidatus Liberibacter asiaticus in citrus showing Huanglongbing (yellow shoot disease) symptoms in Guangdong, P.R. China

J. CHEN, X. Deng, S. Liu, X. Pu, H. Li, E. Civerolo

Huanglongbing (HLB) or yellow shoot disease (ex. greening disease) is highly destructive to citrus production worldwide. HLB is currently known to be associated with Candidatus Liberibacter asiaticus in China. However, Koch's postulates have not been fulfilled. It also remains unclear if other plant pathogens are involved. In two surveys performed in Guangdong, P. R. China in 2006 and 2007, 141 samples were collected from citrus trees showing typical symptoms of HLB from 11 different cities. PCR using phytoplasma specific primer sets fU5/rU3 nested with primer set P1/P7 identified 110 (78.0%) positive samples. A 1,785 bp amplicon was obtained with primer set P1/P7 and showed a 100% identity to three strains of *Ca. Phytoplasma asteri* (onion yellows (Japan), aster yellows 'watercress' (Hawaii), and valeriana yellows (Lithuania)). Meanwhile, 89 (63.1%) samples were positive for *Ca. Liberibacter asiaticus*. Sixty-nine (48.9%) samples were positive to both phytoplasma and *Ca. L. asiaticus*. Transmission electron microscopy (TEM) showed both walled and wall-less bodies in symptomatic citrus tissue. HLB phytoplasma was further demonstrated using periwinkle (*Catharanthus roseus* (L.) G. Don.) through dodder (*Cuscuta campestris* Yunck) transmission. PCR detected the same phytoplasma in the affected periwinkle, along with *Ca. L. asiaticus*. In addition to yellowing/mottling, the infected periwinkle showed typical symptoms of virescence and phyllody that are commonly associated with phytoplasmal diseases. TEM revealed bacteria-like organisms with pleomorphic morphology. Data from this study showed that in addition to *Ca. L. asiaticus*, a phytoplasma related to *Ca. P. asteri* was also associated with citrus HLB in Guangdong.

Evaluation of alternative fungicides for organic apple production in Vermont

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The objective of this trial was to compare the efficiency of potassium bicarbonate, neem oil, and *Bacillus subtilis* to a standard organic lime sulfur/sulfur fungicide program and a non-sprayed treatment for control of apple scab and other fungal diseases. Treatments were applied to 'Empire' trees arranged in a completely randomized design with five single-tree replications at the University of Vermont Horticultural Research Center in South Burlington, VT. Fungicides were applied with a handgun to drip, using maximum label rates. Applications began on 26 April and continued on approximately a weekly schedule through the end of June and then every two weeks through 23 July. Data obtained, representing the first year of a two year study, were analyzed by analysis of variance and significance between means was determined by Fisher's Protected LSD Test ($P < 0.05$). The alternative fungicides showed some activity against foliar apple scab compared to the non-sprayed treatment, and the potassium bicarbonate and neem oil treatments had significantly less fruit scab than the non-sprayed treatment. However, the lime sulfur/sulfur treatment provided the best overall control of scab. There were significantly more necrotic leaf spots in the neem oil and potassium bicarbonate treatments compared to all other treatments. On fruit, there was a significantly greater incidence of phytotoxic burn and russetting in the lime sulfur/sulfur treatment.

Antiserum development from an outer membrane protein (*omp*) of *Candidatus Liberibacter asiaticus*

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Huanglongbing (HLB) is considered one of the most destructive diseases of citrus in the world because it affects most citrus cultivars and causes rapid decline of infected trees. The causal agent of HLB present throughout Asia and in parts of North and South America is *Candidatus Liberibacter asiaticus*. Two additional bacteria, *L. americanus* and *L. africanus*, are associated with HLB only in South America and Africa, respectively. We used polymerase chain reaction primers to an outer membrane protein (*omp*) to clone a 964 bp product for developing an antiserum for *L. asiaticus*. The product was subcloned using a pQE TriSystem® expression vector for recombinant protein expression. The expressed protein was tagged by 6xHis on the N-terminus. The 39.82 kDa product was further purified using an Ni⁺ NTA agarose column. SDS-PAGE analysis revealed a single band corresponding to its molecular weight. This purified protein will be used for antibodies production and tested for specificity to *L. asiaticus*.

Soil application of imidacloprid and related SAR-inducing compounds produces effective and persistent control of citrus canker

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Soil drenches of the systemic insecticide imidacloprid (Admire®) produce season-long control of citrus canker caused by *Xanthomonas citri* ssp. *Citri* (Xcc). Imidacloprid (IM) is a neonicotinoid that breaks down in planta into 6 chloronicotinic acid, a closely-related compound to the systemic acquired resistance (SAR)-inducer isonicotinic acid (INA). Potted seedlings of Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) were treated with IM, INA and the inducer acibenzolar-s-methyl (ASM) as soil drenches or as a spray of foliage (ASM) one week prior to inoculation of immature leaves with Xcc. Plants were cut back and re-inoculated four times over a 6 month period. SAR induction was confirmed by expression of the PR gene, beta-1,3 glucanase. Soil drenches of IM, INA and ASM induced a high and persistent upregulation of PR gene expression and reduced canker lesions for up to 6 months compared to less than a month for foliar ASM. Soil inducers of SAR reduced canker lesions up to 70% compared with the untreated check (UTC). Lesions were small, necrotic and flat compared to pustular lesions on UTC leaves. Populations of Xcc per leaf were reduced 1-2 log units in soil treated plants.

Taegro: A biofungicide with broad spectrum of activity towards soilborne or foliar fungal and bacterial pathogens

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Taegro is an EPA-registered biofungicide whose active ingredient is 5.0×10^{10} cfu/g of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24. It can be applied as soil drench or foliar spray at the rate of 2.5 to 3.5 oz per 100 gallons of water/A. Laboratory profiling of its bioefficacy in the laboratory has revealed that it has a wide spectrum of biological activity towards both fungal and bacterial pathogens. This includes the fungal pathogens, *Rhizoctonia*, *Fusarium*, *Phytophthora* (including mefenoxam-resistant field strains of *P. erythroseptica*) and *Venturia inaequalis* and strains of bacterial pathogens, *Xanthomonas campestris*, *Pseudomonas syringae* pathovars, *Ralstonia solanacearum*, and *Erwinia amylovora*. In greenhouse trials, we observed more than 75% reduction of bacterial wilt in tomato when Taegro was incorporated into the potting mix at the labeled rate of 1.23 g/gallon mix. Our laboratory and greenhouse observation are supported by crop data from field trials. In earlier field trials conducted during 1995–1998 treatment of tomatoes led to delay in *Fusarium* wilt development and also to 10-to-20% yield increases. In potatoes and ornamentals, FZB24 treatments led to consistent increase of

tuber yield, marketable plants as well as number of flowers. From recent field trials conducted in different CA locations during 2007, significant disease reductions due to Taegro treatments were recorded for the suppression of (i) bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (greater than that was afforded by kocide 2000), (ii) downy mildew of organic head lettuce caused by *Bremia lactucae* when compared to chemical standards, kaligreen and other conventional chemistry combinations, and (iii) bottom rot of lettuce caused by *Rhizoctonia solani* over the chemical standard botran 75-W. These results suggest that soil drench and foliar applications of Taegro show no phytotoxicity and can be reliably used to control fungal and bacterial pathogens.

Zoospore responses to environmental pH of seven *Phytophthora* species commonly isolated from irrigation reservoirs at ornamental plant nurseries

P. KONG, G. Moorman, J. Lea-Cox, D. Ross, S. Umeha, P. Richardson, C. Hong

Phytophthora species are commonly referred to as water molds. However, there is little information about how long and at what rate individual species may survive aquatic environments that often undergo significant daily and seasonal changes chemically, physically and biologically. In the present study, the responses of zoospores to a range of pH from 3 to 11 were tested in seven species that are frequently isolated from nursery irrigation reservoirs including *P. citricola*, *P. citrophthora*, *P. drechsleri* (Dre II), *P. irrigata*, *P. megasperma*, *P. nicotianae* and *P. tropicalis*. Hoagland's solution at 15% strength was the primary test medium. Additional tests using irrigation water as the medium were performed for *P. citrophthora*. Aliquots of a zoospore suspension were added to the test media pre-adjusted to pH levels of 3, 5, 7, 9 and 11 with NaOH or HCl. Infested media were incubated in the dark at room temperature for 0, 1, 3, 5, 7 days. Then, 1 ml was plated in 10-cm Petri dishes with PARP-V8 agar. Emerging colonies were counted after 48–72 h culture and pH responses were assessed by number of days on which zoospores remained recoverable and rate of survival (calculated by dividing colonyforming units in each replicate dish by that in optimal treatment of the same species). The longest survival was 7 days at pH 3 and 11 for *P. citricola* and *P. drechsleri* and at pH 3 only for the remaining species tested. The greatest recovery rates were observed always in dishes of day 0 at a range of pH 5 to 9, depending on species. Comparatively, *P. citrophthora* survived longer and at a higher rate in irrigation water than in Hoagland's solution. The underlying mechanisms and implications of these zoospore responses are discussed.

Quorum sensing operates in *Phytophthora nicotianae*

P. KONG, C. Hong

The term quorum sensing was introduced to describe the control of gene expression in bacteria species in response to cell density. Bacteria produce, detect and respond to hormone-like signal molecules called autoinducers to coordinate communal behaviors. Most autoinducers (e.g. acyl-homoserine lactones, AHL) promote intraspecies communication, but autoinducer 2 (AI-2) allows interspecies communication and regulates gene expression of many important behaviors including virulence. Apart from bacteria, no organism has been shown to have a quorum-sensing system involving AI-2. Here we show operation of quorum sensing involving AI-2 in *Phytophthora nicotianae*. Using two autoinducer reporters, we demonstrated that zoospores produce an AI-2-like signal but not AHL. We also demonstrate chemical communication among zoospores prior to or during plant infection by *P. nicotianae*. Autoaggregation and plant infections that usually require a high concentration of zoospores occurred at a low concentration or single spore level when provided with zoospore free fluid (ZFF) from a highly concentrated suspension. Moreover, zoospores at low concentration did not move toward to plant tissue unless supplied with ZFF. These results indicated *Phytophthora* species may share a similar quorum sensing mechanism with bacteria although their autoinducers may be produced through different pathways. This mechanism may allow *Phytophthora* species to maximize infection potential by use of widespread bacterial autoinducer (AI-2) in nature. It may thus be possible to develop novel methods to control *Phytophthora* diseases through interfering with the pathogen's communication systems.

Compost and biological amendment effects on soilborne disease and soil microbial communities

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Effects of compost and biological amendments on soilborne diseases and microorganisms were assessed in field trials in northern Maine under both conventional and organic potato production practices. Three different biocontrol amendments, hypovirulent *Rhizoctonia solani* Rhs 1A1 (HvRs), *Bacillus subtilis* (Bsub), and *Trichoderma virens* (Tvir), as well as a nontreated control were used in conjunction with plots both amended and not amended with a conifer-based (Hemlock bark) compost (19 Mg/ha). At the conventional site, compost amendment reduced incidence and severity of black scurf by 12–27%, and increased tuber yields by 13–23%. Biocontrol treatments (Tvir and HvRs) reduced incidence and severity of black scurf by 9–31% but had no significant effect on yield. The combined effect of compost and biocontrol amendments reduced black scurf by

30–48% and increased yield. At the organic site, where soil was already rich in organic matter, compost did not significantly reduce scurf or scab, but increased tuber yield (9–30%). Biocontrol treatments (Tvir and Bsub) reduced incidence and severity of black scurf by 10–48%, scab by 5–20%, and the total of all diseases by 15–30%. All treatments significantly affected soil microbial communities, with compost amendments generally resulting in more pronounced changes in community characteristics than biological amendments. Overall disease levels were lower and yields higher at the organic site. Vol. 98, No. 6 (Supplement), 2008 S87
Appropriate compost and biological amendments had significant positive effects on soil quality, disease reduction, and yield, and should play an important role in sustainable soil and disease management programs.

Monitoring management of Huanglongbing disease of citrus in Brazil

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Huanglongbing (HLB), or citrus greening disease, was first reported in Brazil in 2005. The disease is spread in nature by psyllid vector, *Diaphorina citri*. *Candidatus Liberibacter asiaticus* and *Ca. L. americanus* have been found to be associated with the disease in Brazil. It is difficult to detect the presence of Liberibacters in plants early enough for successful management of the disease. A prolonged incubation period of two years or longer in some trees was indicated from a field trial. Selected blocks of citrus trees from three orchards practicing varying levels of disease management practices were selected for the present study. Weekly samples of psyllids were collected from these blocks over a period of one year and analyzed for the presence of both species of Liberibacters by real time PCR analysis. Selected samples were also analyzed by conventional PCR. Development of disease symptoms in these blocks was also monitored. Results show that the presence of Liberibacters can be identified in psyllids long before symptoms become visible in plants and show the usefulness of psyllid analysis in monitoring different management practices.

Effectiveness of the biopesticides Actinovate and Kaligreen within a management program for powdery mildew on cantaloupe

M. E. MATHERON, M. Porchas

Powdery mildew on cantaloupe and other melon crops, caused by the fungus *Podosphaera xanthii*, can result in significant yield losses. A field trial was conducted to compare the efficacy of a new biopesticide Actinovate (*Streptomyces lydicus*), an established biopesticide Kaligreen (potassium bicarbonate) and the conventional fungicide Procure (triflumizole), applied alone or within a rotation program with each other, for control of powdery mildew on cantaloupe. Most treatments were applied five times at weekly intervals to each of the five replicate plots arranged in a randomized complete block design. Powdery mildew was not observed in plots until the third treatment application date; however, a high level of disease developed on nontreated cantaloupe plants by crop maturity. Disease ratings at plant maturity revealed that reduction of powdery mildew due to five applications of the biopesticides Actinovate or Kaligreen alone at weekly intervals was 72 and 59%, respectively, whereas similar application timing for the conventional fungicide Procure resulted in 100% disease control. In treatment programs where the conventional fungicide was alternated with one of the biopesticides, reduction of powdery mildew by Procure alternated with Actinovate or Kaligreen was 82 and 85%, respectively. Alternate application of Actinovate and Kaligreen reduced final disease severity by 79% compared to nontreated plots. These preliminary results suggest that a treatment program alternating application of the conventional fungicide Procure with either of the biopesticides Actinovate or Kaligreen could provide a high level of powdery mildew control on melon crops. Further evaluation of these products is in progress.

Citrus stubborn symptom severity and *Spiroplasma citri* location within the tree canopy

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The severity of symptoms of citrus stubborn disease (CSD) within an orchard can range from mild to severe, but whether factors other than pathogen titer or duration of infection impact severity is not known. We tested the hypothesis that the canopy distribution of the pathogen, *Spiroplasma citri*, is related to symptom severity. When fruits were harvested randomly from the canopies of trees from a commercial orchard and *S. citri* presence assessed by culturing from fruit receptacles, a greater percentage of samples from severely symptomatic trees yielded cultures, and bacteria grew to log phase more quickly, than from mildly symptomatic trees. In a second experiment, both fruits and leaves from two canopy aspects (east and west) and three canopy tiers (top, middle, and base) were subjected to q-PCR, using P58 gene-based primers, on DNA from fruit columellas and leaf petioles. The percentage of samples testing PCR-positive was significantly greater from severely symptomatic than from mildly symptomatic trees, and columellas yielded more q-PCR positives than did petioles. However, neither canopy aspect (east vs west) nor tier (upper, middle or lower) correlated significantly with percentage *S. citri* detection. The data suggest that, within this orchard, CSD severity is unrelated to the overall within-tree pathogen distribution, but is correlated with the percentage of samples containing the pathogen.

Effects of glucosinolates from brassicaceous plants on nematode populations

K. ONG, K. Steddom, J. L. Starr

Plants from the brassica family have been used as biofumigant agents in managing soilborne pathogens. We utilized several varieties of these plants in a sweet potato production system to evaluate their ability to suppress nematode population levels. Brassicas, used as winter cover crops, were destroyed and tilled under as green manures. Samples of the plants were frozen and later analyzed for concentration of two glucosinolates, Vol. 98, No. 6 (Supplement), 2008 S117 glucotropaeolin and sinigrin. Nematode populations were sampled prior to brassica incorporation, 4-weeks post incorporation and at sweet potato harvest. There were no significant differences in nematode population between treatments at 4-week post incorporation of the winter cover crop. However, regression analysis of the data suggested that the amount of sinigrin and glucotropaeolin in the cover crops could predict final nematode population at sweet potato harvest. A 0.22% decrease of ring nematode at the end of the growing season is predicted with 1 mmol sinigrin/m² increase. On the other hand, there is a 1.47% increase in stunt nematode population at the end of the growing season for every 1 mmol/m² increase of glucotropaeolin. Although glucosinolates are not expected to be in the soil for long periods of time, it may be one of several indicators to predict the trend of nematode population during the growing season.

Survey of Huanglongbing (HLB) and citrus canker in the Rio Grande Valley

B. SALAS, P. Parker

Huanglongbing (HLB) and citrus canker are the two most dreaded diseases for citrus production in the Rio Grande Valley (RGV), Texas. The objective of this study was to detect as early as possible these diseases in the RGV and thus to mitigate the tremendous economic losses that can be inflicted on the citrus industry. Within each one mile square quadrat of the RGV, one to four citrus trees have been strategically selected and used routinely to monitor populations of the Mexican fruit fly (*Anastrepha ludens*). Presently, these trees are also being used as sentinel trees to detect HLB and citrus canker. Before the survey, fruit fly trappers from the Texas Department of Agriculture were trained on the recognition of HLB and citrus canker and, thereafter, asked to collect diseased citrus leaves in addition to their normal duties. During years 2006 and 2007, a total 314 and 1685 citrus leaf samples were collected, respectively. The majority of leaf samples were from grapefruit and less from orange. Regardless of host, most samples were from groves and less from dooryards. Samples were examined in the laboratory for symptoms of HLB and citrus canker. Citrus trees showing symptoms of Zn deficiency, yellowing or lop sided fruit were revisited. Other foliar diseases or insect damage were also recorded. None of the citrus leaves or fruits examined showed diagnostic symptoms of HLB or citrus canker. However, a follow up survey is suggested of trees showing symptoms of Zn deficiency (2006 = 15%; 2007 = 8%), mosaic or yellowing (2006 = 3%; 2007 = 7%), or psyllid attack (2007 = 5%). Greasy spot (*Mycosphaerella citri*), Fe deficiency, and Mg deficiency were the most frequent diseases found in this survey. Close monitoring of HLB and citrus canker throughout the Valley must continue.

Influence of carbon source amendments on population density, resource use, and antibiotic phenotypes of soilborne *Streptomyces*

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Disease suppression by soil microbial communities is often a function of antibiotic inhibition and resource competition. These competitive phenotypes are likely to be dependent on available nutrients. This study explores the effects of repeated carbon source inputs on population densities, resource use, and antibiotic inhibitory phenotypes of *Streptomyces* in soil. High and low doses of glucose, cellulose, and lignin were added weekly to mesocosms of native prairie soil for 9 months. Culturable population densities were estimated for each community. Resource utilization was determined for individual *Streptomyces* isolates from each treatment using Biolog SF-P2 plates. Antibiotic inhibition profiles for these isolates were determined against a collection of 5 standards. The greatest densities were produced by cellulose and lignin amendments. Communities with high resource doses had *Streptomyces* with stronger inhibitory phenotypes than low-dose communities. *Streptomyces* communities with low resource inputs used substrates more efficiently than *Streptomyces* from high-input communities. These findings Vol. 98, No. 6 (Supplement), 2008 S141 suggest that dense communities with high resource availability select for good inhibitors while low resource communities select for efficient nutrient use. Thus, resource inputs that promote high *Streptomyces* densities may produce an environment with strong resource competition and better inhibitory phenotypes that would be more likely to suppress pathogens.

Evaluation of resistance to *Phytophthora megasperma* in rootstocks for species of *Prunus*

L. S. SCHMIDT, G. T. Browne

Phytophthora megasperma can cause high incidences of tree death following periods of prolonged soil water saturation in almond and other stone fruit orchards in California. Although many new rootstock selections are available for *Prunus* species, relatively few evaluations of resistance to *Phytophthora* spp. have been completed for them. We examined resistance to *P. megasperma* in three rootstocks used widely among almonds and other stone fruits (Nemaguard, Lovell, and Marianna 2624) and twelve additional rootstocks used either less frequently or experimentally for *Prunus* species. The stocks were rooted as hardwood cuttings, grown in 1-liter pots of UC mix, and transplanted into 2-liter pots of UC mix that was either non-infested or artificially infested with *P. megasperma*. There were 5 randomized complete blocks per treatment. Once every 2 weeks the soil was flooded for 48 h. Between periods of flooding the soil was watered as needed and drained freely. Six months after transplanting, the root systems were washed free from soil and rated for severity of crown and root rot. The six plum hybrids and species (Marianna 2624, AC941 [Microbac], Krymsk#86 [Kuban#86], Ishtara, Empyrean#2, and Hiawatha) were resistant to *P. megasperma* (mean 0 to 5% of crown length rotted, 2 to 7% of roots rotted). In contrast, the peaches and peach hybrids, (Lovell, Nemaguard, Cadaman, and Empyrean#1) were moderately susceptible (21 to 37% of crown length rotted; 21 to 28% of roots rotted) as were the peach × almond hybrids (Cornerstone, Floraguard × Alnem, GxN 15 [Garnem], Hansen 536, and Nickels) (26 to 48% of crown length rotted, 20 to 56% of roots rotted). Apparently, the plum parentages offer resistance to *P. megasperma*.

Limited cultivation of *Candidatus Liberibacter asiaticus*, suspected causal agent of Huanglongbing of citrus

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Candidatus Liberibacter asiaticus (Las) and *L. americanus* (Lam), suspected causal agents of Huanglongbing (HLB) disease of citrus have been categorized as unculturable, phloem-limited bacteria based upon in situ electron micrograph images and 16S rDNA sequence of prokaryote DNA associated with diseased tissues. Using a citrus extract supplemental (CES) agar and a microaerophilic environment, we report the growth of bacteria associated with HLB. Isolations were made by streaking extracts of sterilized leaf petioles and veins onto CES agar. Plates were sealed and incubated for 3-4d at 28°C, until colonies were visible with a binocular microscope. The colonies, positive in a Las-specific and Lam-specific RT-PCR assays, were 0.1 mm or less, irregular, and consisted of a coagulum of cells in a tight matrix after 7d. Cells were 0.3 to 0.4 × 0.5 to 0.8 microns with numerous fimbriae; filaments were occasionally observed. Similar masses of cells were observed in infected tissue by SEM. Serial transfers to new media resulted in very small, single colonies after 3-5d on CES agar. Growth in liquid CES medium resulted in extensive biofilms. Final identification of the causal agent of HLB awaits completion of Koch's postulates.

Control of *Pythium* root rot in a tobacco float system with surfactants

K. W. SEEBOLD, E. Dixon

Pythium root rot is the most commonly encountered disease on tobacco transplants produced in float beds, a hydroponic system utilized by the majority of producers in KY and other areas in the U.S. Control recommendations include sanitation and the preventive use of etridiazole; however, adequate sanitary practices are not employed universally and etridiazole, although effective, is costly and can be phytotoxic. Surfactants have been shown to be effective against Oomycete pathogens in hydroponic vegetable and ornamental systems, and could be of value in transplant production to manage *Pythium* root rot. Five surfactants were applied at 100 ppm to plastic containers containing 1L of water. An untreated control (inoculated and non-inoculated) was included and etridiazole was applied at 50 ppm to serve as the chemical standard. Treatments were arranged in a completely random design with 3 replications. Four-week old 'KY 14' tobacco seedlings in 4 × 6-cell styrofoam trays were then floated in the containers. Trays were inoculated with *Pythium* one day after chemical treatment, and plants were evaluated for wilting, root necrosis, and phytotoxicity 14 days later. All treatments reduced wilting of tobacco seedlings compared to the untreated control. Tergitol and Naiad at 100 ppm were as effective in reducing root symptoms as etridiazole at 50 ppm. Silwet, SM-9, Tergitol, and Tween-20 caused greater levels of plant damage than etridiazole. The use of a surfactant, Naid, to manage *Pythium* root rot shows promise. Additional research is needed to identify other effective surfactants, optimal rates and treatment intervals to achieve adequate control of disease.

Influence of fungicides applied before harvest on postharvest gray mold of table grapes

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Thiophanate methyl (THM), iprodione (IPR), cyprodinil (CYP), pyraclostrobin+boscalid (PS/BO), pyrimethanil (PYR), or fenhexamid (FEN) reduced colony size of 4 fungicide-sensitive *Botrytis cinerea* isolates by 50% at

12.4, 2.5, 0.61, 0.29/0.57, 0.26, or 0.17 microg/ml, respectively. THM, IPR, CYP, PS/BO, PYR, or FEN were applied at equivalent of maximum approved rates to detached Thompson Seedless (TS) berries at 600, 500, 270, 59/116, 370, or 290 microg/ml, respectively, 24 or 48 h before or after *B. cinerea* inoculation. Postharvest gray mold after 2 weeks at 15°C was lowest after FEN, followed by PYR, CYP, IPR, PS/PO, or THM. In a Ruby Seedless vineyard, water, IPR, CYP, PS/BO, PYR, or FEN were applied at bunch closure and 2 weeks before harvest and decay was assessed after 1 month at 1°C. Only FEN reduced it significantly, from 15.0% among water-treated grapes to 5.9%. Fungicide residues were about 1/3 USEPA maximums. The following were applied in a TS vineyard at flowering, bunch closure, onset of veraison, and 2 weeks before harvest: 1) water; 2) FEN, PS/BO, THM, then IPR; 3) THM, IPR, PS/BO, then FEN; or 4) IPR+FEN, PS/BO+IPR, IPR, then FEN+PS/BO. Postharvest decay after 7 weeks at 0°C was 5.5, 1.9, 1.3, or 0.7%, respectively. In San Joaquin Valley vineyards, isolates resistant to these fungicides occurred, although FEN resistance was rare.

Efficacy of various brassica varieties for the suppression of root knot, ring, and stunt nematodes

K. STEDDOM, K. Ong, J. Starr

Plant parasitic nematodes are a serious limit to vegetable production in East Texas. Green manures of “Florida Broadleaf” (FB), “Southern Giant” (SG), and “Bionute White” (BN) mustards, “Garza” radish (GR), “Purple Top” turnip (PT), “Vates” collards (VC), and “Dwarf Blue” kale (DB) were investigated as biofumigants for the suppression of Root Knot (RKN), Sting (SN), and Ring (RN) nematodes in a sweet potato production system. Controls consisted of plots left fallow (FC) or planted to “Elbon” rye (ER). Brassicas were incorporated into the soil 58 days before planting sweet potatoes. Prior to planting sweet potatoes, incorporating GR reduced ($P = 0.0043$) populations of RKN better than all other treatments and also resulted in fewer ($P = 0.0374$) RN at harvest of sweet potatoes than FC. The rate of reproduction of RN in GR plots was lower ($P = 0.0030$) than all other treatments. Total nematode populations, RKN + SN + RN, were lower ($P = 0.0091$) than FC for BN and VC. Some brassicas are known to be hosts of root knot nematode. Plots with GR had higher populations ($P = 0.0034$) of root knot nematodes than other treatments prior to incorporation. Yield was not significantly different between any treatments. Stepwise regression indicated that the most important factors for yield were rate of increase of RKN + RN, initial populations of RKN + RN, and RKN populations prior to planting sweet potatoes. This work demonstrates the effectiveness of brassicas as a winter cover crop for vegetable production in the East Texas Region.

Soil microbial communities among different cropping sequences and their effect on the occurrence of peanut soilborne pathogens

H. SUDINI, R. N. Huettel, C. Arias, K. L. Bowen

Microbial communities associated with the agro-ecosystems play an important role in the crop health and productivity. Crop rotations can alter the diversity and richness of microbial communities which appear to influence levels of soilborne plant pathogens. In this study, we are investigating the effect of soil microbial communities associated with different peanut rotation sequences on the occurrence of important soilborne pathogens, such as *Sclerotium rolfsii* (causal agent of stem rot of peanut) and mycotoxigenic *Aspergillus flavus*. We have used ARISA (Automated Ribosomal Intergenic Spacer Analysis) to fingerprint fungal microbial communities in a peanut rotation system. The results indicated similarities among fungal ARISA profiles of the same cropping sequence even though the replicated plots are widely spaced. In order to reveal the frequency of occurrence of the soilborne pathogens in the total fungal communities, we extracted DNA from pure cultures of *S. rolfsii* and *A. flavus*. The DNA from these two fungi are being used to monitor their occurrence in each peanut rotation system at three sampling times, pre-plant, pegging, and harvest. The banding patterns corresponding to these soilborne pathogens could aid in determining their frequency of occurrence over different sampling dates as compared to total fungal populations.

Fungicide sensitivity in North Carolina populations of *Colletotrichum cereale* and molecular characterization of benzimidazole- and Qolinsensitive strains

L. P. TREDWAY, M. D. Soika, M. L. Bunting

Isolates of *Colletotrichum cereale* were obtained from creeping bentgrass and annual bluegrass putting greens exhibiting symptoms of anthracnose basal rot or foliar blight. Seventy-one isolates were obtained from 6 locations in NC and 1 location each in VA and TN. Sensitivity to thiophanate-methyl, azoxystrobin, and propiconazole was determined *in vitro*. All 71 isolates grew uninhibited on media containing 10 µg/ml thiophanate-methyl. Sequence analysis of the TUB1 and TUB2 genes from selected isolates revealed E198K and E198A amino acid substitutions in TUB2. One isolate was sensitive to azoxystrobin, 9 were moderately sensitive, and the remaining isolates were insensitive to azoxystrobin. Sequence analysis of the CYTB gene in selected isolates revealed an F129L amino acid substitution in moderately sensitive isolates and a G143A amino

acid substitution in insensitive isolates. All F129L isolates were obtained from a single location in Western NC, where application of QoI fungicides provided approximately 50% control of anthracnose in replicated trials. EC50 values for propiconazole ranged from 0.27 to 1.33 µg/ml. Resistance to the benzimidazole and QoI fungicides has become widespread in *C. cereale* populations in NC and surrounding states, and some locations are also likely to observe reduced efficacy from DMI fungicides. Populations dominated by isolates with F129L mutations in CYTB may be partially suppressed by QoI fungicide applications.

Accelerated degradation of metam-sodium in soil: Occurrence and possible mechanism

S. Triky-Dotan, M. Austerweil, D. Mintz, Y. Katan, A. GAMLIEL

Accelerated degradation (AD) of soil fumigants can result in insufficient pest control. We have documented AD and reduced effectiveness of Methylisothiocyanate (MITC), the active ingredient of Metam-sodium in soil, resulting from repeated applications under field conditions. AD of MITC may result from enrichment in the population of microbial degraders in the soils, from increased enzymatic activity of the degraders, from the transfer of extrachromosomal elements from the degraders to the other components of the soil microbial community, or from a combination of these factors. Revealing the microbial mechanism underlying this AD may provide tools for managing it. The objective of the present study was to identify and characterize the microorganisms responsible for the occurrence of accelerated degradation of MITC in soil. Under controlled conditions, we were able to induce accelerated degradation of MITC in six different agricultural soils, by inoculating natural (nonhistory) soil with 10% of MS history soil (three repeated MS applications). Disinfestation of a history soil by steam or solarization eliminated the AD phenomenon. In contrast, fumigation with methyl bromide, iodomethane, or formalin did not reduce AD in history soil. We developed a soil extraction method in order to increase the density of the degrading organisms. Inoculation of nonhistory soil with such extract from a history soil resulted in AD in the soil, indicating the presence of degraders in the extract. Liquid culture of the soil extract from history soil had a high potency for rapidly degrading MITC. Heating the soil extract from a history soil to 60°C for two hours eliminated the accelerated degradation. Initial results indicate that the DNA profiles in history and nonhistory soils after MS application are different. We isolated culturable gram negative bacteria which rapidly degrade MITC in liquid culture as well as in soil.

Methyl Iodide and Sulfuryl Fluoride as quarantine treatments for solid wood packing material

K. M. TUBAJIKA, A. V. Barak

The threat of wood-inhabiting fungi to American hardwood forests, lumber industries, and tourism has enormous economic significance, and the aesthetic and dollar values of properties are potentially disastrous. The efficacy of Methyl Iodide (Mel) and Sulfuryl Fluoride (SF) for eradicating woodinhabiting fungus, *Ceratocystis fagacearum* was assessed in wood blocks of birch, maple, poplar and red pine based on in-vitro experiments. In a series of replicated controlled experiments, wood blocks were inoculated with a 1 g macerated mycelium/spores mixture of *C. fagacearum* and fumigated with 160 and 240 g/m³ of Mel, SF and methyl bromide (Mebr) as control for 24, 48, and 72 hours. Analysis of variance showed that fumigant types, fumigant concentrations, and exposure time as well as their interactions (C × T) had an effect on *C. fagacearum* recovery on tested wood species. Colonization of birch, maple, red pine, and poplar by *C. fagacearum* was significantly greater in non-fumigated samples than fumigated samples. *C. fagacearum* was greatly inhibited by Mel than SF in all wood species tested. Overall, the C × T products of < 4,108 for Mel and < 8,755 for SF were not effective in killing the fungus. These results suggest that longer treatment exposure time might achieve the goal of complete eradication of *C. fagacearum* and imply that Mel performed as well as MeBr in killing the fungus in some wood species by exposure time combination. Overall, Mel was most effective in killing the fungus than SF under the conditions of this study with potential implications for quarantine use.

Aggressiveness of *Phytophthora cactorum* and *Phytophthora citricola* isolates on European beech and lilac

J. E. WEILAND, A. H. Nelson, G. W. Hudler

Inoculation experiments were conducted to compare the aggressiveness of *Phytophthora cactorum* and *P. citricola* isolates on European beech and lilac seedlings grown in a greenhouse. The isolates were obtained from bleeding cankers on European beech from 5 cities (Albany, Ithaca, Oyster Bay, Plainview, and Rochester) in New York. Isolates of *P. citricola* were subdivided into 2 clades (*P. citricola* 1 and 2) based on distinct differences within selected DNA sequences. Stems, roots, and leaf disks of both hosts were inoculated with 3 single-spore isolates of *P. cactorum*, 4 of *P. citricola* 1, and 3 of *P. citricola* 2. Stems were inoculated with colonized agar plugs, roots via infested soil at 3 inoculum levels, and leaf disks with a zoospore suspension. Disease incidence was independent of isolate in all inoculated stems and leaf disks (100%), but was dependent on isolate in the soil infestation assay (0–100%) for both hosts. Severity (canker length, rate of mortality, and affected leaf disk area) was dependent on isolate regardless of inoculation site (stem, root, or leaf, respectively) or host, with *P.*

cactorum isolates usually causing less necrosis than either clade of *P. citricola*. However, the range of disease severity caused by isolates of *P. citricola* 1 was similar to that of *P. citricola* 2. Lilac was less severely affected by inoculation than beech, regardless of isolate. No effect of inoculum level on root infection was observed.

Control of bacterial spot of tomato with a phosphorous acid product

A. WEN, B. Balogh, M. Momol, S. M. Olson, J. B. Jones

K-PHITE, a phosphorous acid-containing product, was evaluated alone or with a combination of other products for management of bacterial spot of tomato incited by *Xanthomonas perforans* in eight greenhouse and seven field experiments in two locations in Florida during a 3-year period. Treatments included (i) a weekly schedule of K-PHITE alone, (ii) K-PHITE combined with full rate or half rate of standard copper-bactericide, (iii) K-PHITE alternated with a standard copper-bactericide, and (iv) K-PHITE plus biweekly Actigard. Both greenhouse and field experiments showed that overall disease control with those treatments was similar to that obtained using the standard copper-bactericide program. Treatment of K-PHITE combined with full rate or half rate of standard copper-bactericide was not superior to the standard copper-bactericide program. Yields in the field experiments were not affected by any of these treatments. Under greenhouse conditions phytotoxicity was observed due to foliar application to tomato seedlings; however, drench application did not cause phytotoxicity. These data suggested that K-PHITE and K-PHITE plus Actigard could be used for managing bacterial spot of tomato in the field in Florida where prevalent natural inoculum is race 3 or 4 of *Xanthomonas perforans*; and that K-PHITE could be used as a new tool in greenhouse transplant production using drench application. A direct effect on bacterial spot of tomato was shown by the greenhouse data with the evidence that K-PHITE acts as antibacterial agent toward *Xanthomonas perforans*, which was confirmed by *in vitro* test. No indirect effect was observed from the greenhouse experiments.

Genome-wide pyrosequencing analysis of a *Citrus tristeza virus* (CTV) complex revealed large-scale recombination throughout the viral genome

Z. XIONG, Z. Weng, Y. Yu, S. Gowda, X. Liu, D. W. Galbraith, R. A. Wing, W. O. Dawson

Recombination is a major force driving virus evolution, especially in viruses that have high genome stability and persistently infect hosts with multiple strains. CTV, an RNA virus with a genome of approximately 20 kb, occurs frequently as a complex of multiple strains in its natural hosts, vegetatively propagated and long-lived citrus plants. To examine the CTV complex at the sequence level and to assess the extent of inter-strain recombination, we performed deep-sequencing analysis of a natural CTV complex, FS2-2, using the high-throughput, 454 pyrosequencing technique. Entire CTV genomes in FS2-2 were amplified with four sets of universal RT-PCR primers and subjected to the 454 sequencing analysis in a 1/16-region sequencing run in the Genome Sequencer FLX. Over 2.2 megabases of high quality sequences were obtained from 8722 sequencing reads with an average read length of 256 nucleotides. Three divergent, high coverage (27X-43X) genome contigs corresponding to the genomes of the three co-infecting CTV strains were subsequently assembled from the sequence reads. Additionally, a large percentage (4.7%) of the sequencing reads represented recombinants between the three strains. A genome-wide recombination map for each of the CTV strains in FS2-2 was constructed using these recombinant sequences, revealing a systematic and unprecedented scale of recombination activity throughout the CTV genome. Recombination was more active in the more highly conserved 3' halves of the CTV genomes. This unprecedented, genome-wide recombination provides a plausible explanation for rapid evolution and extreme diversity of an RNA virus whose genome is remarkably stable for years in hosts infected with a single pure strain.

The efficacy of methyl bromide and alternatives on *Agrobacterium tumefaciens* and *Phytophthora cactorum*

L. E. YAKABE, S. R. Parker, D. A. Kluepfel

The presence of *Phytophthora* species, causal agents of root and crown rot, and *Agrobacterium tumefaciens*, causal agent of crown gall, diminishes the value of nursery walnut stock and reduces the yield and lifespan of production orchards. Current management strategies for these diseases rely on pre-plant fumigation with methyl bromide and 1,3-dichloropropene. While *Phytophthora* root and crown rots are effectively controlled, crown gall control has been inconsistent. In *in vitro* assays, methyl bromide, chloropicrin, iodomethane, metam sodium and dazomet eliminated *A. tumefaciens* and, *Phytophthora cactorum* populations in soil while 1,3-dichloropropene only reduced populations by 89% and 96%, respectively. Five days after treatment, methyl bromide had significantly reduced general aerobic bacterial populations while its alternatives did not suppress microbial populations below levels found in untreated soils. Data will be presented on the ability of *A. tumefaciens* to recolonize: 1) methyl bromide treated, 2) Telone C35 treated, 3) thrice autoclaved, and 4) untreated field soils. These results may be useful in understanding the inconsistent nature of crown gall control often observed in field conditions.

Potential of phosphorous acid-containing products for control of Phytophthora blight on squash

J. YIN, K. L. Jackson, A. S. Csinos, P. Ji

The potential of several phosphorous acid-containing products for control of *Phytophthora* blight, incited by *Phytophthora capsici*, was evaluated by comparing their efficacy in suppressing the pathogen in *in vitro* studies and disease development in greenhouse assays. The phosphorous acid-containing products, including ProPhyt, K-Phite, Lexx-A-Phos, Agri-Fos and NutriPhite, were applied at the same concentrations based on phosphorous acid equivalent. EC50 values of the phosphorous acid-containing products in inhibiting mycelial growth on PDA ranged from 80 to 370 ppm based on phosphorous acid equivalent, and from 80 to 900 ppm in suppressing sporangia production. None of the phosphorous acid-containing products at the concentrations used was effective in reducing germination rates of zoospores, while zoospore germ-tube elongation was affected by higher concentrations of the products. EC50 values of mefenoxam (Ridomil Gold) in suppression of mycelial growth and sporangia production were lower compared to the phosphorous acid-containing products, and zoospore germination was inhibited completely by mefenoxam. In greenhouse studies, the products were used to treat soil artificially infested with *P. capsici* and squash seedlings were transplanted in the treated soil. Mefenoxam and some phosphorous acid-containing products reduced disease on squash compared with the non-treated control with mefenoxam being the most effective. Soil and root drench using the phosphorous acid-containing products or mefenoxam did not provide systemic protection of squash seedlings against *P. capsici* that was inoculated onto the leaves, suggesting that a direct effect of these products on the pathogen probably contributed more than induced host resistance in disease control. These studies indicated that some phosphorous acid-containing products had the potential to suppress *P. capsici* but were less effective than mefenoxam. Development of integrated approaches, rather than use of these products alone, would be beneficial for more efficient management of the disease.

Genetic diversity of *Citrus tristeza virus* isolates spreading in Central California

R. K. YOKOMI, M. Polek, M. Saponari

A rapid increase in trees infected with *Citrus tristeza virus* (CTV) was observed in several locations in Tulare, County, CA in 2007. Leaf and bark tissue were sampled from infected trees and used for molecular characterizations. Real-time RT-PCR using a universal CTV TaqMan probe detected all isolates. No reactions were obtained with a CTV-stem pitting TaqMan probe but several samples reacted to a TaqMan probe for California MCA13 positive isolates which do not induce decline. All isolates had a T30 genotype and SSCP analysis of the CP showed most isolates had a profile identical to the mild P81 isolate. However, three different SSCP patterns were observed. Sequencing of the CP gene showed at least two different isolates were present along with the common P81-like isolate. Pairwise alignments of these isolates shared only 91.5% and 90.7% nucleotide identity with mild isolates which suggests that they are genetically distinct isolates. Phylogenetic relationships indicated that one isolate was closely related to but distinct from the Florida T36 strain (94% nucleotide identity); whereas the other isolate was in a separate clade with a grapefruit stem-pitting isolate from Argentina (C269-6) and a sub-isolate (P108-35) of the Dekopon isolate, a virulent CTV strain intercepted in central California. Host range biocharacterization of these isolates are ongoing. We continue to monitor the spread and diversity of these CTV isolates in an effort to explain a trend of rapid spread even in some areas of the Central Valley where CTV-infected trees are consistently removed.

Silicon: Virus friend or foe?

W. L. ZELLNER, S. M. Leisner

Silicon is a beneficial element that aids plant resistance to certain fungal and bacterial pathogens, such as powdery mildew and bacteria spotted wilt, respectively. However, the effects of silicon on viral infections are poorly understood. To develop a model system for these studies, ten *Arabidopsis thaliana* ecotypes were inoculated with *Tobacco ringspot nepovirus* (TRSV) to determine infectivity. One, three, and six ecotypes were resistant, susceptible, and tolerant, respectively. *A. thaliana* Sf-1, a TRSV susceptible ecotype, was then grown hydroponically, inoculated with TRSV, and supplemented with soluble silicon at 0.1 mM or 1.0 mM. Surprisingly, 1.0 mM silicon treatment resulted in a higher percentage of symptomatic plants compared to those amended with 0.1 mM silicon. *Nicotiana tabacum* is also susceptible to TRSV infection and was used as a second host for our study. TRSV symptoms on hydroponically-grown *N. tabacum* leaves amended with 0.1 mM soluble silica covered more surface area than leaves of plants grown under higher silicon concentrations, similar to previous reports on silicon-aided resistance. Our results suggest that silicon amendments aid virus resistance in a host-specific manner.

Seed transmission of *Candidatus Liberibacter asiaticus* in periwinkle and dodder resulted in low bacterial titer and very mild disease in periwinkle

L. Zhou, Y. DUAN, D. Gabriel, T. R. Gottwald

Canadidatus Liberibacter asiaticus (Las) is the most widely-distributed of three species of *Liberibacter* that are associated with citrus Huanglongbing (HLB), a lethal disease of citrus worldwide. In addition to citrus, periwinkle (*Catharanthus roseus*) and dodder (*Cuscuta pentagona*) are two experimental hosts in which the bacteria can multiply well. Symptoms of HLB in inoculated-periwinkle were characterized by progressive vein and leaf yellowing, resulting in death of most HLB-infected periwinkles within six month after first appearance of symptoms. Dodder plants did not exhibit symptoms, even when they contain high titers of the bacterium. Las was detected in up to 53% of all seeds tested both from HLB-infected periwinkle and dodder without resorting to nested PCR. The PCR amplicons were confirmed by sequence analysis. Germination rates of these Las-positive seeds from both plant species were normal. Over 80% of the periwinkle plants germinated from the infected seeds showed initial HLB symptoms of vein yellowing and leaf yellowing only when they were stressed by nutrient deficiency. Surprisingly, the disease progressed slowly, and did not cause plant death, and all symptomatic plants became asymptomatic after the stress was removed. The Las population remained in very low titer; in most cases, detected only by nested PCR or regular PCR by increasing the concentration of the bacterial DNA. The periwinkles-infected with Las via seed transmission have been maintained for over six months. These results suggest that although Las was seed transmitted, a second, undescribed component of an HLB disease complex was not.

Assembling the Fungal Tree of Life: From Linnaeus to Deep Hypha and Beyond

The Oomycota

C. A. LÉVESQUE, A. W. A. M. de Cock, G. Robideau, N. Desaulniers, and K. Bala

Oomycetes are no longer part of the Eumycota, or true fungi. Although oomycetes are different from true fungi in many ways, the two groups still have many common ecological features. Molecular taxonomy and phylogenies have confirmed for the most part the traditional classification of oomycetes. The two main orders of arogniales and Peronosporales are still well separated by phylogenies. Most important plant pathogen genera such as *Pythium* and *Phytophthora* are still monophyletic and their species morphological taxonomy is generally supported by molecular analyses. There are a few exceptions though. There are some species that are being split (e.g. *Py. irregulare*), genera that are within a genus clade (e.g. *Pythiogeton*), and clades that might require a new genus status (e.g. *Pythium vexans* clade). Most phylogenetic studies of oomycetes have been done with the ribosomal DNA cistron and mitochondrial cytochrome oxidase genes but multigene phylogenies were performed in *Phytophthora*. These were made possible by the large amount of genome sequence information available for different species of this genus. The genome of *Pythium ultimum* was recently sequenced, opening new possibilities of multigene studies in Peronosporales. There are also efforts to sequence the genome of *Saprolegnia parasitica* which would greatly facilitate broader phylogenetic studies in oomycetes.

Phytophthora: A Global Problem with Continued and Historical Importance

A historical review of *Phytophthora* diseases

In many ways *Phytophthora* has defined the science, the practice, and the promise of plant pathology, from the potato famine to the new world of pathological genomics. *Phytophthora infestans* spawned plant pathology and thrust it onto the world stage, and in 2007, more articles in the APS journals *Phytopathology* and *Plant Disease* addressed *Phytophthora* than any other genus of plant pathogens. Our understanding of pathogenesis has been enhanced by work with *Phytophthora* phytoalexins and elicitors, and now with complete genome sequences for 4+ species available, *Phytophthora* will continue to lead the way in genomics research in plant pathology. *Phytophthora* provided early and dramatic examples of the importance of nomenclature, taxonomy, and today phylogenetics to plant pathologists. *Phytophthora* first demonstrated the dangers of invasive pathogens through the globalization of agriculture, and continues to force the issue as newly recognized exotic *Phytophthora* species threaten wild as well as agricultural ecosystems. *Phytophthora* species have been instrumental for epidemiological research and the development of disease forecasting models. It's not all about potatoes and peppers, either. *Phytophthora ramorum*, described only 7 years ago, now leads the annual *Phytophthora* citation index. The history of *Phytophthora* and the diseases it causes is the history of plant pathology, and today's research on *Phytophthora* gives a preview of the directions plant pathology will take in the future.

Phytophthora capsici: A serious threat to vegetable industries in the world

M. Babadoost.

Phytophthora capsici Leonian was first described on peppers in New Mexico in 1922. Subsequently, it was reported on more than 50 plant species in 15 families. Among the affected plants, cucurbits and peppers are the most susceptible hosts. In the past 10 years, *P. capsici* has been reported from many vegetable growing areas throughout the world, causing up to 100% crop losses. *P. capsici* can infect plants at any growth stage, causing a range of symptoms including root rot, stem lesions, leaf spot, and fruit rot. Variation in genetics and virulence among isolates of *P. capsici* has been reported from different parts of the world, which makes management of the pathogen very challenging. *P. capsici* is a soil-borne pathogen and can survive in the soil for more than three years. *P. capsici* also spreads by air movement. There is limited information about biology of *P. capsici* and epidemiology of the diseases caused by this pathogen. There is no single method with adequate control measure against this pathogen. Integrated approaches of cultural practices and chemical application are used to minimize crop losses by *P. capsici*. Regional, national, and international efforts are needed to carry out comprehensive studies on epidemiology of the diseases caused by *P. capsici* and develop effective strategies for management of *P. capsici*.

Itinerary for the period 8 July – 4 August 2008

Day	Date (2008)	Place	Contact person	Contact details	Flight		
					No.	Depart	Arrival
Tu	08/07	JNB - Hong Kong	Travelling	-	CX748	12:45	07:50
Wed	09/07	Hong Kong - Sydney	Travelling	-	QF128	21:10	07:55
Thur	10/07	Sydney	Day at leisure	-			
Fri	11/07	Sydney	Day at leisure	-			
Sat	12/07	Sydney - Brisbane	Congress, Brisbane		QF520	11:05	12:35
	13-18/07	Brisbane	5 th International Nematology Congress	West End Central (07) 3100 8333 (07) 3011 8399(F)			
Sat	19/07	Brisbane - Sydney	Travel	-	QF517	10:55	11:45
Sat	19/07	Sydney – San Francisco	Travel	-	QF73	13:55	10:15
Sun	20/07	San Francisco – Los Angeles – San Diego	Travel	-	AA2450 AA3083	12:05	15:59
Mon	21/07		Lawrence Marais	Desert King			
Mon	21/07	San Diego – Los Angeles - Fresno			AA3048 AA3023	13:45	16:42
Tu	22/07	Bakersfield	Dr. L Marais	Desert King			
Wed	23/07	Fresno- Dallas- Tampa (Florida)			AA1216 AA1498	06:55	17:20
	24/07	Lake Alfred	Prof. L. Duncan	University			

Day	Date (2008)	Place	Contact person	Contact details	Flight		
					No.	Depart	Arrival
Thur				of Florida			
Fri	24/07	Lake Alfred	Prof. L. Duncan	University of Florida			
Sat	26/07	Tampa – Dallas - Minneapolis	Travel	-	AA1053 AA450	07:15	12:25
Sun - Wed	27-30/07		APS Centennial Meeting, Minneapolis				
Thur	31/07	Minneapolis - Washington	Travelling	-	AA580	18:15	23:10
Fri	01/08	Washington - London	Travelling	-	BA292	21:40	10:05
Sat	1&2/08	London	Prof. Roland Perry	Rothhamsted, UK			
Sun	03/08	London - Madrid	Travelling	-	IB3167	18:50	22:10
Mon	04/08	Madrid - JNB	Travelling	-	IB6051	01:30	11:20

8.6.3 Visit to Ghana from 22-28 September 2008

Introduction

Pathologists from Florida, Spain and South Africa were invited to visit Ghana to assist local pathologists to identify and confirm the presence of *Pseudocercospora*, fruit and leaf spot disease, and citrus cancer. The pathologists involved were Prof. P Timmer and Dr. M McDowney, Dr. Antonio Vicent and MC Pretorius.

Itinerary for the period 22-28 September 2008

Days & Date	Action	Place
Monday, 22 Sept 2008	Arrival of researchers	Kota International Airport, Accra
Tuesday, 23 Sept 2008	Welcome by Director & Provost of Institute Travel to Kade, Agricultural Research Centre – 120 km from Accra	University of Ghana, Legon, Accra
Wednesday, 24 Sept 2008	Visit orchards at Agricultural Research Centre, Kade. Inspect orchards, collect samples, ID diseases	Agricultural Research Centre, Kade
Thursday, 25 Sept 2008	Visit Cocoa Research Institute and citrus farms along the road	Return to Accra – visit orchards along the road
Friday, 26 Sept 2008	Seminar at main University campus	University of Ghana, Legon, Accra
Saturday, 27 Sept 2008	Visit Kakum National Park	Day at leisure
Sunday, 28 Sept 2008	Departure	Kota International Airport, Accra to Johannesburg ORT airport

Citrus Production in Ghana

Commercial citrus production in Ghana started with the introduction of the West Indian lime in 1913 when about 5.2 ha of seedling trees were established at Asuansi Agricultural Station. Around 1938, it was apparent that some trees were not productive and many were dying. The decline in trees became widespread and by 1947. The decline was caused by Citrus Tristeza virus (CTV), and was spread by the vector *Toxoptera citricidus*. In 1947, a campaign was started by the Department of Agriculture to rehabilitate the lime industry, using Rough lemon as rootstock.

In an attempt to find alternative rootstocks more resistant or tolerant to the Citrus Tristeza virus disease, the then Department of Agriculture brought in Cleopatra mandarin and Rangpur lime, which had shown tolerance to the disease in Brazil. The University of Ghana's Agricultural Research Station at Okumaning near Kade (ARS-Kade), was set up among other things to start research programmes on citrus and find other citrus relatives whose use as rootstocks would encourage production of better quality fruit and also prove tolerance to virus diseases particularly CTV.

Serious efforts to find solutions to the CTV and other virus diseases led to the collection of budwood of different cultivars from other agricultural stations at Asuansi, Bunso and Aiyinasi, these budwood were used to establish large citrus experimental trials the University of Ghana Agricultural Research Station at Okumaning near Kade (ARS-Kade) in 1960. The budwood comprised of the 'foreign' cultivars, which had been imported into the country earlier, and the 'local', which were named after local towns and villages where the mother seedlings, were collected. Other citrus varieties were introduced and evaluated at ARS-Kade and Crop Research Institute Station at Bunso (now Plant Genetic Resources and Research Institute), through the assistance of the FAO.

Currently Ghana's citrus industry consists of a total of between 26 - 30 000 ha of citrus with an average orchard size of 2-5 ha (small scale farmers – Fig 8.6.3.1) and an average yield production of 40 metric tons per annum. The current rootstock choices are restricted to Rough Lemon (90%), Cleopatra Mandarin (8%) and Rangpur Lime (1%). Volckameriana, Shekwansha, Troyer citrange, Carizzo citrange, Swingle citrumelo and Sunki mandarin are all still experimental.



Fig 8.6.3.1. A typical citrus farm in Ghana.

Citrus trade

The oranges were exported mainly to the neighboring countries such as Burkina Faso, Ivory Coast and Togo. Currently not much processing of citrus is done in the country. About 80-90% of the sweet orange fruits are consumed locally as fresh fruits. The large processing companies such as Athena Foods in Tema process a small quantity locally. A number of cottage industries and home based processing units also process some but these quantities are negligible compared to the fresh fruit consumption.

Introduction of Sweet Orange cultivars

The time of introduction of sweet oranges in the country is not very well documented. The sweet orange (cv. Obuasi) seems to have been introduced into the country much earlier than the lime, through the activities of the Europeans who were prospecting for gold in the 1820s. According to chiefs in the Obuasi area, the Europeans grew the sweet oranges at their bungalows, so the natives used to call the sweet oranges "Bungalow Ankaa", literally meaning Bungalow Sweet orange. Local staff who worked for the Europeans took some seeds from the "Bungalow Ankaa" and planted them in their farms. In terms of land area cultivated, it comes after cocoa and oil palm; however, in terms of income per unit area, it is the highest yielding crop. In fact in most farming communities, farmers are replacing their cocoa farms with citrus orchards.

Types of Citrus cultivated in Ghana

Commercially important citrus varieties in Ghana originate from two species; the sweet orange, *Citrus sinensis* (Linn.) Osbeck; whereas the mandarins originated from *C. reticulata* Blanco. It is however important to note that grapefruit and other citrus hybrids (tangelos; tangors) are also cultivated although not in commercial quantities.

Sweet oranges

Sweet oranges may be separated into three recognizable groups. Within these groups, there are a number of varieties, which cannot be easily distinguished. The groups are (1) Those with the normal fruits such as Late Valencia, Pineapple, Mediterranean Sweet and Obuasi, (2) those with abnormal or navel fruits such as the Washington Navel and (3) those with red or red-streaked pulp commonly called “blood” oranges such as the Ruby red and Tarocco varieties. All these varieties are available and cultivated in the country. The “blood orange” is noted for its slow acid content, compared to other sweet orange varieties, and is popular among consumers in the Ashanti region.

Mandarin

There are two groups of mandarin oranges of commercial importance. (1) The Satsuma group composed of several varieties and (2) the tangerine group, commercially developed in Florida. These include varieties such as Dancy and Clementine. Some varieties were introduced into the country several years ago, and are known by the towns where these were popularly grown such as Aburi and Adeiso varieties. Satsuma has however maintained its name. The recommended rootstocks for the mandarins include the Cleopatra mandarin, Clementine and Rangpur lime. Rough lemon is not recommended as a rootstock for the mandarins. Satsuma mandarin has very good potential for establishment on a larger scale because of its high sweetness. The major constraint to its production in Ghana is the high levels of fruit drop as a result of Mediterranean fruit fly.

Grapefruit

Grapefruit (*C. paradise Macf*) is cultivated on a small scale, mainly in the research institutions. Most of the grapefruits have been budded onto Rough lemon, Cleopatra mandarin and Rangpur lime.

Lime

Some trees can be found in backyards of houses, however lime is cultivated on a commercial basis (not large scale), mainly in the Central region.

Major constraints to citrus production in Ghana

- Lack of certified budwood
- Land tenuresystems and irrigation facilities
- Agricultural Extension Service
- Marketing of citrus fruits
- Fruit fly
- Restricted post-harvest practices

Environmental requirements for citrus production

Climatic requirements – Temperature, rainfall amount and distribution, and relative humidity

Citrus can be grown in most of the country, provided the soil and soil moisture requirements are satisfactory. Although the crop can be grown under irrigation, this is not practiced in Ghana. Currently, citrus is grown under rain-fed conditions, and the cultivation is therefore limited to areas where annual rainfall is not less than 1000 mm per annum and is evenly distributed throughout the year. Producing areas in Ghana have a bimodal rainfall pattern.

The major citrus producing districts include Kwaebibrem, Birim South, West Akim, Birim North, (Eastern region); Assin Fosu, Twifo-Hemang-Lower Denkyira, Asebu-Kwamankese, Jukwa (Central region); Ejisu-Juaben, Mankranso, Atwima (Ashanti region), Hohoe, Jasikan and Kpano (Volta region). Prevailing temperature conditions are not a limiting factor to citrus cultivation. The temperature range in the citrus producing regions range between 24°C and 38°C and relative humidity of 85-90% (morning) - 60-80% (afternoon) is conducive to its cultivation.

Some initial problems associated with the Citrus Industry

The movement of citrus propagation material in the world is associated with diseases and Ghana was not spared. It is believed that indiscriminate imports of citrus budwood from different sources such as Trinidad, California, Brazil and Nigeria might have complicated the virus disease problem in Ghana. No official certification programme or proper nursery practice guidelines are available.

Current research into Citrus

The University of Ghana (ARS-Kade) is conducting research in several aspects of citrus. It has established a 16 ha citrus museum comprising over 35 citrus varieties budded onto four main rootstocks. It has also established a 4 ha rootstock museum, comprising 10 different rootstocks (Figs. 8.6.3.2 & 8.6.3.3). In addition to the provision of quality planting materials, the station also provides technical support to farmers for plantation establishment. Other institutions working on citrus include the Plant Genetic Resources Research Institute at Bunso and the Crops Research Institute at Kumasi.



Figs. 8.6.3.2 & 8.6.3.3. Different varieties and rootstocks in the citrus museum at Kade Research station.

Discussion

No official certification programme is currently in place to monitor and guide nurserymen with regards to providing healthy and true to type planting material to farmers. The Kade Research station is the major supplier of new trees to farmers but privately owned part-time nurseries also exist, some are well managed and others are poorly managed (Figs. 8.6.3.4 & 8.6.3.5). *Alternaria* damage was not visible in the orchards visited but researchers confirmed the existence of the pathogen. However *Alternaria* and Scab symptoms were observed on leaves in nursery trees at Kade research station's nursery. Current control measures are 2 weekly foliar sprays with copper. It was suggested that the nursery be covered with shade cloth to prevent the continual wetness of leaves.



Fig. 8.6.3.4. Ghana's main nursery at Kade Research station.



Fig. 8.6.3.5. A privately owned citrus nursery.

Researchers involved in citrus research are aware of potential catastrophes with regards to illegal importation of certified plant material. The importation of plant materials has been discussed numerous times with the authorities but no definite actions have yet been taken. The importance of a certification programme was highlighted to them and contact details of CRI's manager of the citrus improvement programme were handed to them.

Pseudocercospora angolensis was found on leaves and fruit in citrus trees surrounding the laboratory and offices at the research station in Kade (Figs. 8.6.3.6, 8.6.3.7 & 8.6.3.8).



Figs. 8.6.3.6, 8.6.3.7 & 8.6.3.8. Kade Research station Laboratory with infected trees, Fruit and leaf symptoms of *Pseudocercospora angolensis*.

The disease was also observed in orchards along the road through the central parts of Ghana. Isolation techniques were demonstrated to the local researchers to assist them with further studies and identification of the disease (Figs. 8.6.3.9 & 8.6.3.10).



Figs. 8.6.3.9 & 8.6.3.10. Kade Research station Laboratory.

The orchards visited were riddled with black spot, *Guignardia* (Fig. 8.6.3.11). Benomyl and copper are sprayed to control the disease. Research to evaluate the effectiveness of the strobilurins are currently under investigation.



Fig. 8.6.3.11. Blackspot lesions on fruit.

Fruit losses due to fruit fly are estimated to be in excess of 40%. No sanitation with regards to fruit fly is being done. The Mediterranean fruit fly is their major insect pest, however the new invasive fly, *Bactrocera invadens*, is also present in all the production regions (Figs.8.6.3.12 & 8.6.3.13).



Figs. 8.6.3.12 & 8.6.3.13. Fruit fly trap with Mediterranean Fruit Fly.

Phytophthora root and collar rot damage was visible and shown to the researchers especially in orchards where trees were planted in poorly drained soils (Figs. 8.6.3.14 & 8.6.3.15). Researchers identified the problem/disease

as Diplodia and not a Phytophthora related disease. It was identified by the overseas research group as a Phytophthora related problem due to continuous wet soil conditions.



Figs. 8.6.3.14 & 8.6.3.15: Phytophthora damage to tree trunks.

The tropical rainfall pattern and average rainfall per annum as well as the fact that 90% of the industry's orchards are established on rough lemon rootstock, worsen their situation.

No citrus cancer lesions/damage was observed during the field visits, however on the last day of the seminar one of the farmers, farming in the northern regions of the country, showed fruit and leaf samples with visible cancer-like symptoms. Unfortunately he was only able to provide the team with one leaf and two fruits but we all agreed that it was a typical citrus cancer symptom (Fig. 8.6.3.16).



Fig. 8.6.3.16. Citrus Cancer lesions found on fruit and leaf.

Thereafter, other farmers also reported that they had also seen similar symptoms in their orchards, on both fruit and leaves. A seminar organized by the University of Ghana to report back on our findings was well attended by senior lecturers, lecturers, researchers, Department of Agriculture officials, farmers and students. The following topics were addressed by presenters.

<u>Topic</u>	<u>Presented by</u>
➤ Brief background of the Seminar	Dr. F. Ofori (Director-IAR)
➤ Black Spot of Citrus	Prof. L.W. Timmer (USA)
➤ Pseudocercospora fruit and leaf spot disease on citrus – control measures	M.C. Pretorius (South Africa)
➤ Phytophthora/Alternaria diseases of citrus	Dr. A. Vicent Civera (Spain)
➤ Greening Disease of Citrus	Dr. M.D. Megan (USA)
➤ Overview of the Citrus Industry in Ghana	Dr. K.G. Ofori-Budu (Ghana)

Concluding remarks

1. All the researchers we met were well informed and had the theoretical back ground with regards to their respective disciplines but the **lack of** applied research skills and **implementation of the theory** was very clear. The industry is battling due to very little support from government. Everyone was highly appreciative and thankful towards the inputs by the foreign researchers.
2. The lack of official (Government), support towards the importation (Legally and illegally) of plant material remains the most important challenge that needs to be addressed in Ghana, (no hope for near future implementation/ support).
3. Finally Pseudocercospora and citrus canker are present in Ghana. The country has much potential towards citrus production but the challenges regarding their tropical climatical conditions remain a problem.

Contact details of the citrus research group and the head of PPRI in Ghana

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8.7 J.J. BESTER

Report on a visit to Spain 17 - 24 April 2008

Purpose

The Post Harvest Innovation Fund (DST) made funds available for technology transfer to the Citrus Industry on post-harvest related issues. The trip to Spain was undertaken to investigate the latest technology and best operating practices related to the cold chain for possible implementation and improvements in the Citrus Industry of southern Africa.

Background

Spain is by far the biggest exporter of citrus in the world with a total export volume of more than 3200 tons. The country is also known for its ability to develop and manufacture state of the art packhouse equipment, as well as products for the control of post-harvest diseases.

Therefore, exposure to the Spanish citrus industry is extremely valuable from the point of view that CRI is not only closely involved in the coordination of the activities of the Citrus Cold Chain Forum (CCCF), in the sense

that the CCCF is housed within CRI, but CRI is also primarily responsible for facilitating technology transfer to the southern African citrus industry.

Production in Spain

Orchards were only visited in the Valencia area and surroundings, mainly Cacer, where citrus is mostly produced on small plots of land, even on pieces of land in the city and in smaller towns in the area. The size of orchards varies from less than half a hectare to only a few hectares, with apparently no fulltime farmers. This area accounts for 60% of the total citrus production in Spain, which is more than the total production of South Africa. In the south of Spain the production units are larger and apparently better managed, accounting for the rest of the production.

As a result of the high cost of labour, the orchards in general seem to be neglected, with the exception of some of the larger orchards. Though it is the onset of the blossom period, the appearance of the trees is yellowish due to low nitrogen levels. Tree size per orchard is often very uneven with many interplants or replacements of dead trees. Weed control is poor. Flood irrigation is the general means of watering the trees and very few dripper lines was observed. However, the trees are kept short for ease of picking and are pruned fairly open on the inside.

Harvest is often delayed by spraying gibb in the orchards to enable the fruit to hang later and therefore manage the flow of fruit to the market and optimize marketing opportunities. The internal quality of the fruit that was packed at this late stage was very good, but the colour of the fruit entering the packhouses varied from deep orange to fruit with a few tints of green, due to the application of gibb.

The small production units lend themselves to a system where the vast majority of the fruit gets packed and marketed by co-operative businesses; some of these are buying the fruit from the growers and take full ownership while others market the fruit on consignment on behalf of the growers.

General overview of packhouse practices and equipment

Packhouses that were visited include AMC, Copal, Motella, Alzicoop, Citricos Valencianos, Fontestad, Greenmed and Crisbabe SA. Since practices and equipment used differ from packhouse to packhouse, only a general overview will be discussed.

After harvest the fruit gets delivered to the packhouses in lugboxes, pre-graded, drenched if not packed chem-free or having to be degreened, and then gets sized and either packed for consignment or stored in the cold rooms to be packed at a later stage.

All packhouses visited have cold rooms to store the fruit before packing, as well as cold rooms for the fruit that has been packed, with automated storage and retrieval systems. The temperature and relative humidity of each cold room is controlled from a central point. Some packhouses are temperature controlled from the point of intake throughout the whole packing process up to the loading bays, although temperature varies between different sections of the packhouse. Transport to the markets in Europe is with cooling trucks at a temperature of 5 - 8°C. Relative humidity in the cold rooms is controlled to between 90 – 94%, which is higher than the RSA recommendation of 85 – 90%.



Temperature controlled packhouse

Automated and sophisticated equipment are being widely used to speed up and streamline the whole packing process. Sophisticated electronic sizers and graders (e.g. the Globalscan) can be calibrated to sort the fruit with a very high degree of accuracy on various factors like colour, weight, shape, blemishes and diameter; and can also identify bruised fruit. They can, however, not yet pick up minor damage like insect stings, bruises and decay at a very early stage of development, but will hopefully be able to do so after the next stage of development.

Although most packing takes place by hand, automated weight packers for jumble packs and place packers for place packing do exist. Other automated machinery that is generally used in these packhouses includes bin and box tippers, bin immersion and sanitizing systems, palletizers, strapping machines, carton erection machines, empty and packed carton feeders and bag-filling and net-filling machines.



Place packing machine



Weight packing machine

These automated packing lines and standard systems substantially improved the efficiency, capacity, accuracy, flexibility, quality, as well as gross margin of packing by dramatically cutting the cost of labour. Less handling of

fruit occurs, and therefore less labour is needed for packhouse operations, resulting in reduced costs and greater ease of human resource management.



Palletizing machine



Strapping machine

Hygiene throughout the packhouses is very good, from the pre-sorting to the final packing. Pipes are generally installed at each packing table for removal of culled fruit directly out of the packhouse, where it gets mulched and pumped into a container for removal by trucks once it is filled. Where big bins are used inside the packhouse for culled fruit, they are properly isolated with plastic covers until they are removed. Packhouses and cold rooms are equipped with a number of spraying application systems for automatic disinfection of critical points.

The cold and logistical chain

The fruit is packed in cartons and wooden boxes, or pre-packed in nets which are packed in cartons or plastic crates. The packaging material is all well ventilated and more than strong enough to withstand the short journey to the market. A carton sample was brought back to South Africa and tests done by SAPPI revealed that the carton weighs less per area than those locally manufactured and the quality of paper is slightly inferior to that used in the RSA, resulting in a slightly lower stacking strength. However, taking into account that the longest journey to the market is a maximum of four days, these cartons are quite suitable to stand up to the test.



Strapping and corner pieces

The wooden pallet bases used were very neat, with all the slats exactly the same measurements - the result of automated manufacturing equipment. All wooden pallet bases conform to a high specification standard. Corner pieces are used on all pallets with four or more straps evenly spaced from bottom to top. As a result of automated palletization, the packed pallets all look perfect: secure, stable and very neat.

One outstanding factor in the post-harvest handling of citrus in Spain is the effective management of the cold chain. After harvest, the fruit gets cooled down as soon as possible after arrival at the packhouse, at controlled temperature and RH, slowing down the physiological ageing of the fruit. As mentioned already, in some cases the whole packhouse is temperature controlled. Once fruit is packed, it is put into the cold rooms, loaded under controlled conditions and gets transported in cooling trucks to the market. Thus, very little fluctuation in temperature happens during handling in the cold chain, giving the fruit the edge in terms of extended shelf-life.



Centralised temperature and RH controlled cold rooms



Temperature controlled loading bays

It is quite evident that there is remarkable input in Spanish packhouses to ensure that a superior product, in terms of quality, shelf-life and appearance, lands on the shelves. The management and labour in the

packhouses seems to be well trained, organized and productive. Although equipment is expensive, it is being seen as an investment to ensure long term sustainable marketing of a superior quality product.

Technology

Two companies were visited in Spain to learn about new packhouse and post-harvest technology. Roda Iberica (Maf Roda, Spain) is based in Alzira (Valencia) and specializes in manufacturing of sophisticated and automated packhouse equipment, most of which was seen in packhouses in Spain.



Bagging machine



Humidifier

Technidex is based in Valencia and specializes in manufacturing of post-harvest products like waxes, cleaning products, disinfectants, phytosanitary treatments, degreening chambers and equipment, dosage and application systems, humidifiers and purification systems.

The Euroagro Exhibition was also visited where post-harvest equipment and products, new innovations, packaging material and general agricultural information were displayed. It was interesting that although many types of plastic pallet bases were on display, none of them was seen in the packhouses visited. Many types of plastic bins and crates were also displayed, most of which are used with other fruit and vegetable types. Whether plastic will become a substitute for wood in the manufacturing of pallet bases and cartons, remains to be seen.



Packaging



Plastic pallet base

General

The cost of labour, packaging material and road transport in Spain is significantly higher than in South Africa. However, subsidies of close to 50% from the EU, on packhouse equipment, make it very attractive to install automated packing lines and equipment, whereas this kind of support to the citrus industry will never happen in South Africa.

With the continuous rising of cost of labour in SA, more and more packhouses might consider automating their pack lines and start to invest in sophisticated and automated equipment.

Due to lack of time, it was not possible to investigate all the components of the Spanish citrus cold chain.

Conclusions

Lots of information on handling and BOP's regarding the citrus cold chain in SA is well documented and readily available. The formation of the Citrus Cold Chain Forum (CCCF) during February 2007 and subsequent updating and redistribution of this information to all relevant parties can be considered as one of the most important efforts to upgrade the operational activities and efficiencies of all components in the cold chain. Despite all the input into the citrus cold chain since the formation of the CCCF, ignorance is the one most important reason for not accomplishing the necessary desired results.

The Spanish citrus post-harvest industry is a very good example of how the cold chain should be consistently managed. Attention to detail and the commitment to do it right the first time is the main reason why they are so successful in managing their cold chain. The fruit packed year-to-date in SA has already shown too many problems, of which by far the most originated at packhouse level, namely the use of sub-standard pallet bases and poor palletization practices. Added to this is the wrong configuration and poor stabilisation of pallets during road transport, as well as poor handling at the depots. There is much room for improvement in these components of the cold chain. The use of automated equipment in the packhouses could address many of the problems, but the equipment is expensive and could only be used at the cost of the existing labour force.



Automated pack lines

The extended distribution of the southern African citrus producing areas and the long journey of fruit to world markets makes transport an expensive cost item. Transport of fruit to the local ports under cooling is the ideal, but in most cases uneconomical. Thus, improvement in this component will be costly, but improved packhouse practices and better logistical planning might be sufficient to compensate for this.

There is a definite need to build capacity in the citrus industry to ensure a dedicated focus on all components of the citrus cold chain for long term economic sustainable citrus exports and marketing. This must be considered a very small input into a very lucrative industry, to the benefit of many individuals.

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2. Gerard Meyer (Maf Roda, RSA) for organizing the trip.
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9.1 Tegnologieoordraging (Hennie le Roux & Hannes Bester – CRI)

2008-seisoen

Die aanvanklike skattings vir die 2008-seisoen wat deur die variëteitsfokusgroepe opgestel is, het op 'n afname van 1% teenoor 2007 se volumes gedui. Suurlemoene het met bo-verwachting goeie pryse afgeskop. Dit kon toegeskryf word aan 'n 20% afname in oes in Argentinië na die vorige jaar se koueskade en die feit dat die wêreldproduksie van suurlemoene oor die afgelope paar jaar teen 3% per jaar gedaal het. Vruggrootte op nawels was klein, maar die oes was groot. Kraakskil was vroeg in die seisoen die grootste oorsaak van uitskot op nawels en by tye is minder as 50% in sekere areas uitgepak. Gehalte was wisselvallig omdat te veel marginale vrugte gepak is as gevolg van die sterk markte.

Die 2008 seisoen het nie so gunstig afgesluit as wat dit begin het nie. Daar is aanvanklik buitensporige hoë pryse vir onder andere die suurlemoene, nawels en sagtesitrus behaal. Dit was nie die geval met die laat Valencias nie. Hierdie vrugte het swak pryse behaal en is gevolg met pryse vir sitrus uit die noordelike halfgrond wat so swak was dat produsente in Spanje op 'n stadium nie bereid was om hulle vrugte te pluk nie. In Desember 2008 was die pryse vir prosesserings-suurlemoene en -pomelos in Spanje beter as die vir vars vrugte. Daar is in totaal 89,3 miljoen kartonne sitrus uit Suid-Afrika uitgevoer teenoor 'n oorspronklike skatting van 89,6 miljoen. Pomelos en Valencias was onderskeidelik 1.8 m en 3.4 m laer. Nawels was met 2.8 m op, Valencias met 3.4 m en sagtesitrus met 0.6 m kartonne. Hierdie skatting was uitstekend en die wyse waarop dit bereken is verdien 'n pluimpie.

Die gehalte van die vrugte was aansienlik swakker as wat normaalweg die geval is. Bederf, hoofsaaklik groenskimmel, was baie hoog, gevolg deur skildefekte, veral gepokte skil en skilafbraak by verskeie variëteite. Heelwat koueskade op vrugte onder kouesterilisasie het ook voorgekom. Baie klagtes oor sagte, pofferige vrugte, veral by sagtesitrus en nawels, is ontvang.

Verskeie faktore het bygedra tot die swak gehalte van die vrugte in die mark. As 'n vertrekpunt het Moeder Natuur nie die beste gehalte vrugte gelewer nie; die vrugte was meer sensitief en het beslis 'n korter raklewe as normaalweg gehad. Ongelukkig is te veel van die beheerbare faktore nie na behore bestuur nie en het dramaties bygedra om die situasie te vererger – lang staantyd in sommige pakhuisse, vragmotors wat tot ses dae buite die hawe staan en wag om af te laai, tekort aan koelkamer-kapasiteit, ongereelde skedulering van skepe wat lang staantyd in depots tot gevolg het, swak koelgeriewe op skepe, swak bestuur van die koueketting, swak kartonne wat gebruik is, swak palettisering, ens. Die buitensporige pryse vroeg in die seisoen het die dryfveer geword om volumes te jaag, ten koste van kwaliteit.

Studiegroepe

Feitlik al die onderwerpe is die vorige twee jaar omvattend by die studiegroepe gedek en met die Sitrusnavorsingssimposium wat Augustus aangebied is, is daar ietwat afgeskaal op die aantal vergaderings wat gehou is. Die grootste behoefte was die oordra van die nuutste inligting oor bemesting en besproeiing, en ook kultivars in sekere gevalle. Na afloop van die 5^{de} Sitrusnavorsingssimposium wat vanaf 3-6 Augustus 2008 by die Champagne Sports Resort gehou is, is daar 'n volledige terugvoersessie by elk van die studiegroepe gehou om alle produsente op hoogte te bring van die vorige twee jaar se navorsing. Waar studiegroepe egter 'n behoefte gehad het om spesifieke onderwerpe te bespreek, het die CRI navorsers die nodige insette gelewer (Tabel 9.5 bevat 'n volledige lys van al die onderwerpe wat by elke studiegroep aangespreek is).

Die studiegroepe is oor die algemeen goed bygewoon, veral tydens die terugvoer van die Sitrusnavorsingssimposium. Dit beklemtoon die behoefte en waarde wat produsente daaraan heg. Die CRI is groot lof toegeswaai oor die groot sukses van die simposium. 'n Opname by al die studiegroepe het gewys dat Augustus die mees geskikte tyd van die jaar is om die simposium aan te bied.

Die noodsaaklikheid om die beheer van VKM dramaties op te skerp ten opsigte van veral Europese markte, is onder al die studiegroepe se aandag gebring en verskeie areas het maatreëls in plek gesit om hierdie risiko te bestuur. Die erns van die saak is sterk beklemtoon. Die uitdaging is egter om alle produsente te kry om die erns van die probleem te beseef, sodat almal hul volle samewerking gee. Vir die eerste keer in drie jaar is kultivars by die studiegroepe aangespreek en soveel as moontlik van die rolspelers in kultivar

ontwikkeling was betrokke. Die samewerking wat tussen die onderskeie rolspelers begin is, is bemoedigend en die produsente het groot waardering vir hul werk uitgespreek.

Dit is bykans onmoontlik om vooraf 'n jaarprogram vir die studiegroepe op te stel, aangesien hul behoeftes verander na gelang van die probleme wat gedurende die seisoen ervaar word. Behoeftes verskil ook van area tot area en studiegroep tot studiegroep. Die CRI Produksieriglyne wat in die toekoms as 'n 'lewendige dokument' elektronies beskikbaar sal wees, behoort baie by te dra om in al die behoeftes van al die studiegroepe te voorsien. Sekere navorsers het onmiddellik gereageer daarop dat die Produksie Riglyne voortdurend opgedateer moet word. Andere moet nog eers aan die konsep gewoond raak.

Citrus Cold Chain Forum

Voorseisoen werksinkels is in Februarie 2008 baie suksesvol met elk van die vyf Pakhuisstudiegroepe gehou, waartydens verskeie onderwerpe deeglik gedek is. Die werksinkels is oor twee dae gehou en is uitstekend in al die areas bygewoon, met goeie terugvoer na die tyd. Tussen 65 en 95 persone het elk van die vergaderings bygewoon.

Versoeke deur al die pakhuisstudiegroepe, Uitvoerders Tegnieese Paneel en lede van die Verpakkings-werkgroep dat die rolspelers in die logistieke kettings by die CCCF betrokke begin raak om probleme in die ketting aan te spreek, het gelei tot verskeie vergaderings in die Wes-Kaap en KZN. Kapasiteit binne die regte strukture sal geskep moet word om namens die hele sitrusbedryf die proses aan die gang te kry om al die ingewikkelde logistieke probleme in die bedryf aan te spreek. Dis veral die gebrek aan kommunikasie om akkurate inligting tydig aan betrokke rolspelers te verskaf, groei in die volume vrugte wat met beide 'hi-cubes' en houers verskeep gaan word en gevolglike gebrekkige beplanning wat groot risiko's vir die bedryf inhou. Die CGA het gedurende sy besoek aan die verskillende areas goedkeuring gekry om meer betrokke te raak by sekere van die logistiese aangeleenthede en daar is addisionele tantieme hiervoor goedgekeur. Die CCCF sal dus betrokke wees by die fisiese hanteringsprobleme wat in die koueketting ondervind word, terwyl die CGA meer insette sal lewer oor die oorhoofse logistiese aangeleenthede wat deur die sitrusbedryf vir die toekoms in die gesig gestaar word.

'n Vergadering is op 8 Februarie 2008 in Johannesburg met die mees belangrike kartonvervaardigers gehou, met die doel om 'n poging aan te wend om fondse te ontsluit wat vir tegnieese ondersteuning in die koueketting aangewend kan word. Hoewel die rolspelers die beginsel ondersteun, was die gevoel dat die kartonvervaardigers nie alleen hierdie funksie kan vervul nie, maar dat alle skakels in die logistieke ketting ook behoort by te dra tot sodanige kostes.

Die minimum spesifikasies vir die vervaardiging van kartonne en palette, asook die palettiseringsprotokolle, is deur verskeie kanale aan die bedryf uitgestuur. Prosedures vir die evaluering en toetsprosedure van kartonne is ook opgestel. Dis egter skokkend om te sien hoe baie foute steeds voorgekom het agv verkeerde palettisering. Die hantering van palette in die hawens was by tye chaoties en dit wil voorkom of niemand verantwoordelikheid daarvoor aanvaar nie. Baie spesifikasies, riglyne en protokolle kan opgestel en versprei word, maar dit dien geen doel as dit nie geïmplimenteer word nie. Om dit te bereik is toegewyde, gefokusde, voltydse tegnieese ondersteuning aan die koueketting nodig.

'n Besoek is gedurende April aan Spanje gebring om blootstelling aan hul koueketting te kry waaruit ons voordeel kan trek. Dit is ongelooflik hoe baie moeite die Spanjaarde doen om die gehalte en raklewe van hul vrugte te verhoog. Effektiewe bestuur van die koueketting, geoutomatiseerde pakhuis-toerusting, effektiewe palettisering en goeie gehalte verpakkingsmateriaal dra by tot die goeie gehalte produk wat hulle in die mark plaas. Gekontroleerde temperatuur en humiditeit, vandat die vrugte by die pakhuis gelewer word totdat dit in die mark arriveer, dra baie by tot die goeie toestand en raklewe van hulle produk.

Gedurende Februarie 2009 is weer voorseisoen werksinkels met al die pakhuisstudiegroepe gehou. Die werksinkels is weereens uitstekend bygewoon en goeie terugvoer is verkry. Daar is hoofsaaklik gefokus op die faktore wat verlede seisoen die meeste verliese tot gevolg gehad het, nl bederf, skildefekte en pakverwante probleme. Bestuur van die koueketting, logistiek en uitvoerstandaarde vir 2009 is ook gedek.

5^{de} Sitrus Navorsingsimposium

The 5th Citrus Research Symposium was held at the Champagne Sports Resort in the Drakensberg from Sunday, 3 August 2008 to Wednesday, 6 August 2008. Champagne Sports Resort's unique combination of

convenient location, country club atmosphere, accommodation, good food and friendly staff made it the ideal venue for the symposium.

During the symposium, feedback was given on citrus research conducted for the southern African citrus industry during the previous two years. This research has been conducted by researchers from CRI, the Universities of Pretoria, Stellenbosch, Rhodes, Kwa-Zulu Natal, the ARC (ITSC) and researchers from the private sector. (See Addendum A for the Programme). The symposium was attended by 435 delegates which included citrus producers and their technical staff, the citrus consultants (SASCCON), citrus exporters, the citrus nursery industry (SACNA), Citrus Cold Chain Forum (CCCF) which included packhouse managers, the paper and carton manufacturing companies, PPECB and logistics, the chemical industry and delegates from other related industries.

This symposium hosted several guest speakers from abroad as keynote speakers. These included Prof Etienne Rabe from California who discussed Global citrus trends, Prof Bové from France who updated us on the worldwide increase in Huanglongbing (Citrus greening), Dr Zacharias from Spain who discussed Rind disorders and their prevention, and Dr Smilanic from California on the prevention of Post Harvest Diseases. Dr Wolfgang Thielert from Bayer Germany discussed the future of Agricultural chemicals and Dr Peter Johnston from the UCT updated us on the impact that Climate change could have on citrus production in South Africa.

Bayer Crop Science was the main sponsor. **SAPPI** sponsored the Gala Dinner with their magnificent dancing girls and **Avello** sponsored the Golf Day. River Bioscience hosted the welcoming dinner and Illovo, Oro Agri, Makhteshim Agan, Mondipak and Jansen Pharmaceuticals acted as Golden sponsors. The Silver sponsors consisted of DOW, Villa Crop Protection, Ag-Chem Africa, FMC, Philagro, PPECB, SASCCON, Lowveld Agro-Chem, Madumbi BCP, Nampak, Du Roi IPM & Nursery, Green Trading and Unifrutti. The Bronze sponsors were: Poupart UK, SAFE, Colors, Capespan, APL Cartons, Monsanto, Esselen Nursery, Glenrand MIB, Improcrop, Hygrotec/ICI, Du Pont, Nexus, Plaaskem, Qwemico, RT Chemicals, Subtropiese Agrodienste, Dana Travel, XSIT, Tsunami, Dole and Budget.

Gedurende die Gala-ete was daar 'n geleentheid om persone wat 'n uitsonderlike bydrae tot die sitrusbedryf gelewer het, te vereer. Hierdie eer het vanjaar die volgende persone te beurt geval: Dr Hannes Coetzee vir sy bydrae t.o.v. die bemesting van sitrus, Hendrik Hofmeyr vir die ontwikkeling van die Steriele Insek Tegniek vir Valskodlingmotbeheer, en Dr Carel Buitendag vir sy bydrae t.o.v. die beheer van die vergroeningsvektor, *Trioza erytraeae*.

Tydens die simposium is 'n vraelys uitgehandig om vas te stel wat die opinie is van die simposiumgangers t.o.v. die plek, sy standaard en die tyd van die jaar wat die mees gepaste maand is om die simposium in te hou. Die meerderheid van die vraelyste het getoon dat die simposiumgangers dieselfde standaard wil handhaaf wat betref die verblyf en etes. Die beste tyd van die jaar is steeds Augustus gevolg deur September. Omdat die vraelyste slegs voltooi is deur diegene wat wel die simposium kon bywoon is daar tydens die terugvoer aan die verskillende sitrusstudiegroepe weereens 'n vraelys uitgedeel om vas te stel watter maand van die jaar die mees geskikte maand is om die simposium aan te bied. Die uitslag was as volg:

Maand	% Produsente wat in die Noordelike studiegroepe hierdie betrokke maand verkies	% Produsente wat in die Suidelike studiegroepe hierdie betrokke maand verkies	% van Simposiumgangers wat die betrokke maande verkies
Januarie	3	0	5
Februarie	14	19	0
Maart	11	18	0
April	3	1	0
Mei	0	0	0
Junie	1	1	10
Julie	11	3	15
Augustus	33	27	30
September	13	16	25
Oktober	12	14	15
November	0	1	0
Desember	0	0	0

Dit is duidelik dat Augustus en September die mees geskikte maand vir produsente is om die simposium by te woon. September is egter nie geskik vir die konsultante in die Noorde nie aangesien die lenteploegkompleks dan reeds aktief is en dit die konsultante se besigste tyd van die jaar is. Februarie / Maart pas die meeste navorsers nie omdat dit dan sowat 'n maand of twee is voordat hulle hulle proewe kan monitor vir bespuitings en toedienings wat gedurende die afgelope somer gedoen is. As die simposium in Augustus aangebied word kry produsente hierdie resultate vars uit die perd se bek. As dit in Februarie of Maart aangebied word is hierdie uitslae nog nie gereed nie en sal dit eers twee jaar later by die simposium aangebied kan word. Daar sal dus volstaan word met Augustus as die maand waarin die simposium aangebied word.

Die vraelys by die simposium het verder versoek dat die vordering wat gemaak is t.o.v. die verskillende onderwerpe ge-evalueer moes word. Die onderstaande tabel toon die persepsie van die bedryf hieroor:

Onderwerp	Vordering 1= Onvoldoende 2=Voldoende 3=Uitstekend
Valskodlingmot	2,5
Vrugtevlieg	2,0
Ander plae bv Dopluise, myte	2,2
Skilprobleme	1,6
Vrugproduksie & Kwaliteit	2,0
Entoordraagbare siektes	2,5
Vrug, blaar en taksiektes	2,1
Grondgedraagde siektes	2,2
Na-oes patogene	2,3
Marktoegang	2,0

Uit die resultate is dit duidelik dat die huidige persepsie is dat daar uitstekende vordering gemaak word op die gebied van Valskodlingmotbeheer en Entoordraagbare siektes terwyl Na-oes patologie ook bo gemiddeld presteer het. Die gebied waar die simposiumgangers wel aangetoon het dat hulle nie tevrede is met die huidige vordering nie, is op die gebied van skildefekte. Of dit geregverdig is kan bespiegel word aangesien daar goeie terugvoer deur Paul Cronje gegee is oor Peteca en die bestuur van die probleem. Dit is waarskynlik die swak terugvoer oor 'n baie lang tydperk oor vordering t.o.v. kraakskil wat hierdie persepsie gevestig het.

Simposiumgangers het versoek dat daar in die vervolg 'n Plakkaatsessie aangebied moet word en dat die simposium eerder die volle drie dae moet duur as twee en 'n half. Daar is nie genoeg tyd vir vroeë gelaat nie en die tee-breke moet ook effens langer wees om simposiumgangers meer tyd te gee om onderling te kommunikeer. Die simposiumgangers verkies om by een plek te bly. Wat die borge betref is dit duidelik dat die borge bereid is om te borg indien die plek waar die simposium gehou word op standaard is. Hulle is nie bereid om te borg indien dit 'n afskeep geleentheid is nie. Die Sitrusnavorsingsimposium is op die oomblik die bes bygewoonde landbounavorsingsimposium op die Suid-Afrikaanse kalender.

Transformasie

Die sitrusbedryf se Transformasieproses het 'n verdere hupstoot gekry met die aanstel van Andrew Mbedzi as die Transformasie Voorligtingsbestuurder. Sy aanvanklike taak is om die ooreenkoms tussen die CGA, die CRI en die Limpopo Departement van Landbou gestand te doen en die Voorligters van Limpopo op te lei sodat die sitrusvoorligtingsprogram in die provinsie suksesvol kan wees. As gevolg van die transformasieproses, sal daar oor die volgende paar jaar plase wat tans minstens 10 miljoen uitvoerkartonne lewer, van eienaar verwissel in die Limpopo en Mpumalanga provinsies. Ongelukkig lyk die situasie in die Onderberg waar talle plase die afgelope jaar oorgedra is aan voorheen benadeelde gemeenskape, redelik mismoedig. In plaas van welvaartskepping is talle van hierdie plase tans verwaarloos, die infrastrukture is vernietig en daarmee saam talle werksgeleenthede. Die transformasieproses is effektief wat betref die oordrae van grond maar dit is 'n grootskaalse mislukking wat betref welvaartskepping. Hoewel daar 'n gebrek aan tegniese kundigheid is, is die primêre probleem die onvermoë van die nuwe eienaars om fondse te mobiliseer om hulle kontantvloei aan die gang te hou.

Andrew Mbedzi se werk is besig om momentum te kry. 'n Hoogtepunt was 'n werkwinkel wat saam met die Limpopo se Departement van Landbou se voorligters betrokke met sitrus, gehou is. Agtergrond is gegee oor die sitrusbedryf en daar is duidelik uitgespel wat die sitrusbedryf se verwagting is tov die samewerkingsooreenkoms tussen die Limpopo Regering, die CRI en die CGA. Teikenmipunte is uitgestip en daar heers 'n gees van opgewondenheid onder die staatsvoorligter oor hulle rol in die toekoms.

Transformation will be one of the future pillars of the citrus industry. A full report on the progress made by Andrew Mbezi as Transformation Extension officer is attached to this report. A few aspects should however be highlighted. Andrew is doing excellent work with regard to the previously disadvantaged growers and the Limpopo Department of Agriculture. The one problem that we do experience with regard to the Limpopo DoA is the fact that they do not have the necessary funding available to allow all 23 of their citrus extension officers to attend the meetings/courses which are organized by Andrew. Only 2 or 3 of these courses will be arranged on the different topics during the season. It is thus important that all the Limpopo Extension officers should attend these.

The second problem which has been encountered is the negativity against white people by BEE citrus producers in certain areas. CRI was requested by the Land Claims Commissioner in Nelspruit to give them advice on the citrus farms in the Badplaas area in order to recover these farms. The farms were visited by both Hennie le Roux and Andrew Mbedzi. Three farms were visited which should be producing approximately 4000 tons of lemons this season. The only farm which produced any fruit was that which was taken over from a Davel, but was still run by his daughter, Vivian. This farm produced 1400 t (70t/ha) in 2008. The production on the other two should have been similar but has dropped to almost zero. Andrew's full report will state the reasons for this. What needs to be mentioned is the poor attitude which they had against Hennie as a white person. This was not the case with the Land Claims Commissioner but by the community who owns the property. It is clear that the white man gets the blame for the failures of the new owners. A large portion of the failure can be laid in front of the Land Claims Commissioner and the CGA should bring it to their attention. The rest of the problem can be attributed to the fact that "the communities receive these properties mahala and are not prepared to invest sweat equity into these projects" (Quote from Andrew).

Die eerste jaar waartydens CRI betrokke was by die Limpopo se Departement van Landbou om hulle voorligtingsaksie te probeer ophef, was 'n sukses. Andrew Mbedzi se verslag word hierby aangeheg. 'n Vergadering is op die 17de November in Polokwane met die LDA gehou waartydens die afgelope jaar bespreek is en beide die hoogtepunte en laagtepunte uitgelig is. Die LDA het sowat 20 voorligters getaak om by sitrus betrokke te raak. Onder hulle is 'n groot aantal leergierige persone en die opleidingskursusse wat deur Andrew gereël is het goed afgegaan en is met entoesiasme bygewoon. Die grootste probleem is dat daar onvoldoende fondse is om almal op hierdie kursusse te stuur. Dit het soms tot gevolg dat daar vanuit sekere streke niemand is wat die kursusse bywoon nie. Aangesien hierdie 'n spesiale fase is en daar weens die groot aantal plase wat in die transformasieproses is 'n groot behoefte is vir tegniese kundigheid binne die departement, kan daar nie bekostig word dat hierdie voorligters nie almal al die kursusse bywoon nie.

Daar is talle sitrusprodusente beide in die Letsitele vallei en Tshipise wat hulle gewig agter die transformasieproses ingooi en op verskillende maniere betrokke raak. Hierdie is waarskynlik die enigste manier wat aanleiding sal gee tot suksesverhale. Waar plase eers in die transformasieproses ingegaan het sonder dat die produsent betrokke gebly het as mentor, is die suksesverhale maar yl gesaai. Dit help ook nie om 'n mentor te probeer aanstel nadat die plaas eers geruïneer is nie, aangesien dit te lank vat om so 'n verwaarloosde sitrusplaas weer reg te ruk. Die Boyes-groep, wat alles in hulle vermoë gedoen het om verskeie van die ou landgoedere, soos Lisbon, weer te rehabiliteer, moes die handdoek ingooi en loop gevaar om gelikwideer te word. In die Limpopo en Mpumalanga provinsies is daar minstens 10 sitrusplase waarby niemand kans sien om saam met die eienaars of die regering betrokke te raak en die plase weer op te bou nie.

CRI was genooi om 'n Mpumalanga Agri-Sektor Forum (MASF) in Witbank by te woon op die 20ste November 2009 om die "Land and Agrarian Reform Programme" te bespreek. Dit was 'n vermorsing van tyd en al wat MASF hiermee wou bereik is om te kan sê dat hulle wel die besluite wat die ANC in Polokwane geneem het ten opsigte van die Landbou, met die onderskeie Agri-sektore bespreek het. Niks is bereik nie en die meerderheid van die persone wat die vergadering bygewoon het, het nie na middagete teruggekeer om die verrigtinge verder by te woon nie. Die vergadering het oogklappe aan en skryf byvoorbeeld die swak prestasie van die plase wat oorgeneem is toe aan aardverwarming en die feit dat volgens hulle die blanke boere met al die besproeiingswater sit. Dit ten spyte van die feit dat elk van hierdie plase nog presies

dieselfde waterkwotas het. In die meeste van die gevalle waar die transformasieproses misluk, is die patroon die volgende: Die plaas word oorgeneem deur die voorheen benadeeldes, die elektrisiteitsrekening word nie betaal nie. Na 'n paar maande sny ESKOM die krag af. Hierna word die transformator oopgebreek en geplunder vir koper, gevolg deur die hidrante. Omdat dit in die reënseisoen is, lyk dit of die sitrusbome heel goed presteer onder droë-land toestande. Die gras tussen die bome groei dakhoogte, dit word winter. Die bome kan nie die droogtetoestande hanteer nie en omdat die gras nie gesny word nie, verbrand die besproeiingstelsel. Die volgende stap is vir die grondeisekommisaris om CRI te kontak en te versoek dat ons saam met hulle die betrokke plase moet besoek om vir hulle tegniese hulp te verskaf en vas te stel wat het fout gegaan. Die antwoord is eenvoudig: Dit gaan oor die bestuur van die plase, nie oor tegniese kundigheid nie. Die proses val plat lank voordat dit daarby kom. Andrew Mbedzi vat dit so pragtig saam as hy sê: "There is a lack of sweat equity!"

Die regering se oplossing vir die problem is om 1000 nuwe voorligters aan te stel. Dit gaan niks help nie want die bestaandes het nie die middele om hulle werk te verrig nie. Suid-Afrika stuur af op presies dit wat in China in die laat 1950s, tydens hulle "Great leap", gebeur het, toe sowat 50 miljoen Chinese van honger gesterf het omdat die regering nie sy foute wou besef nie. Zimbabwe bevind homself huidiglik in dieselfde posisie en die denkproses van die staatsamptenare binne die Departement van Landbou is oorwegend dieselfde. Suid Afrika het verander van 'n netto uitvoerder na 'n netto invoerder van voedsel. Die publiek voel dit nog nie omdat daar nog genoeg voedsel beskikbaar is en omdat oorgewig eerder as hongersnood tans die land se grootste probleem is. Transformasie sal sorg dat hierdie oorgewigprobleem uitgesorteer word.

'n Nuwe voorligter vir die Suide is aangestel om ondersteuning te verleen aan die swartbemaatigingsprojekte in veral die Oos-Kaap en KZN. Melton Mulaudzi het vanaf 1 April 2009 diens aanvaar en sal in Fort Beaufort gestasioneer wees. Die SEB-projekte wat saam met Melton en Colin Painter in die Katrivier-area besoek is, lyk verbasend goed, danksy die ondersteuning van Colin en sy vennote Lew en Jonny Roberts. Daar is ongeveer 22 produsente wat deel uitmaak van die SEB-projekte in hierdie area en tesaam het hulle ongeveer 250 000 kartonne gedurende 2008 uitgevoer.

Melton sal die eerste paar weke van sy tyd in die Katrivier spandeer om sy voete te vind en al die rolspelers te ontmoet alvorens hy in die Sondagsrivier en Gamtoosrivier betrokke sal raak. Een van die eerste doelwitte sal wees om 'n studiegroep vir die SEB-produsente op die been te bring.

Die Limpopo Departement van Landbou ondervind ernstige finansiële probleme en vir alle praktiese doeleindes het die voorligtingsdienste wat deur die Departement gelewer word tot stilstand gekom. Die werk sal eers weer hervat kan word wanneer die nuwe finansiële jaar in aanvang neem. Dit is skokkend om die minste te sê.

Zimbabwe, Mosambiek en Angola

Zimbabwe: Dit gaan onder omstandighede redelik met die sitrusprodusente in die suide van Zimbabwe. Hulle het gedurende die 2007 seisoen weer meer as 2,5 miljoen kartonne uitgevoer. Ironies genoeg is hulle grootste probleem plukkers om die oes gepluk te kry. Talle uitvoerkartonne kon gedurende 2007 nie betyds geoes word nie en moes uiteindelik na die sapfabriek gaan. In die sitrusproduserende gebiede verder noord is daar in 2008 sowat 500 000 kartonne uitgevoer wat heelwat meer is as die vorige jaar, maar dit is steeds maar sowat 10% van dit wat die verwagte uitvoere was, sou die blanke boere nie onteien gewees het nie. Hennie le Roux, Thys du Toit en Sean Moore het Zimbabwe gedurende Januarie 2008 besoek en studiegroepvergaderings is gehou in Beitbrug, Chegutu en Mazowe. Bywoning was in al drie gevalle goed en die meeste Zimbabwe sitrusprodusente wat nog sitrus produseer was teenwoordig.

Mosambiek: Produsol Kwekery is in die Chicamba area in Mosambiek besoek deur Hennie le Roux en Thys du Toit. David en Kathie Sole is besig om met Nederlandse hulp 'n sitruskwekery te vestig om in die toekoms sitrusbome aan Mosambiek en Zimbabwe te verskaf. Dit is vir die Suid-Afrikaanse sitrusbedryf van strategiese belang dat hierdie kwekery sy plantmateriaal van die Grondvesblok sal ontvang en nie van ander lande soos Suid-Amerika nie. Net soos in die Angolese geval is sitrusplantmateriaal wel die afgelope jaar vanuit Brasilië ingevoer na Mosambiek. Dit was egter slegs saad. CRI het versoek dat dit desnieteenstaande vernietig sal word om te verseker dat siektes soos sitruskanker en "Citrus Variegated Chlorosis" nie per ongeluk saam met die saad die land binnekom nie.

Argentinië

Hennie le Roux en Andy Lee het Argentinië besoek waar Hennie opgetree het as 'n spreker by 'n konferensie oor Huanglongbing (Vergroening) in Concordia. HLB het in Brasilië beweeg tot sowat 200 km van die Argentynse grens. Daar is geen natuurlike grense wat die verspreiding van HLB na Argentinië sal keer nie en die vektor, *Diaphorina citri* is reeds in Argentinië. Tydens die byeenkoms het Prof Bove van INRA daarop gewys dat nie alleen Florida nie, maar die hele Kuba ook besmet is met vergroening. Dit sal die pomelo mark in die toekoms beïnvloed.

Die Tucuman provinsie is ook besoek en samesprekings is met die navorsingstasie aldaar gevoer oor moontlike toekomstige samewerking. 'n Volledige verslag oor die Argentynse besoek is aan die CRI Direksie voorgelê.

Vergroeningstoer (Prof JM Bove/ Argentyn / Brasilië)

Na afloop van die simposium in Augustus is 'n toer onderneem deur KwaZulu-Natal, Mpumalanga, die Limpopo Provinsie en Noord-Wes saam met Prof Bove, Fernando Carera van Argentinië en vier afgevaardigdes van Brasilië. Gedurende gesprekke met die Brasilië was dit duidelik dat die uitwissingsprogram wat sowat vier jaar gelede met soveel entosiasme in Brasilië aangepak is, nie slaag nie. Die rede daarvoor is omdat die bestaande wetgewing nie toegepas word nie en die kleiner produsente nie besmette bome vernietig soos wat die wet vereis nie.

Die groter landgoedere het 'n geweldige intensiewe opsporingsprogram ("scouting") en boorde word tot agt keer per jaar geïnspekteer. Meer as 'n miljoen bome is reeds vernietig. In teenstelling met Suid-Afrika speel 'n alternatiewe gasheer, die *Murraya* plant, ook 'n groot rol om die siekte te help versprei. Hierdie gasheer is 'n uiters gunstige gasheer vir beide die vektor, *Diaphorina citri*, en die patogene, *Liberibacter asiaticus* en *Liberibacter americanus*. In hulle beheerprogramme konsentreer die Brasilië op kontakdoderbespuitings en daar word dikwels soveel as 14 keer per jaar gespuit. Hierdie behandelings is onsuksesvol en alvorens hulle nie sistemiese insekdoders meer algemeen deel maak van hulle programme nie, sal hulle die siekte nie effektief onder beheer kry nie.

Vergroening het reeds tot sowat 200 km noord van die Argentynse grens versprei en daar is geen geografiese hekkies wat die verspreiding van die siekte na Argentinië sal stuit nie. Die vektor is reeds in Argentinië.

Vergroeningstoer (Universiteit van Florida)

'n Groep afgevaardigdes van die Universiteit van Florida sowel as Floridaanse sitrusprodusente het Suid-Afrika besoek om vas te stel hoe ons daarin slaag om met vergroening saam te leef. Hulle het belang gestel in Afrika-vergroening, die gebruik van sistemiese insekdoders en hoë-digtheidsaanplantings. Dit was vir hulle baie duidelik dat *Liberibacter africanus* nie naastenby so aggressief is soos *L. asiaticus* nie. *Diaphorina citri* het ook 'n meer uitgebreide gasheerreëks as *Trioza erytreae* en is moeiliker om in die boorde te vind omdat dit nie die jong blaartjies aanval nie, maar die jong lote. Dit veroorsaak dus nie die pokmerke wat ons met bladvlooi besmettings assosieer nie.

Wat Amerika aan betref kom die vektor en Asiatiese vergroening nou in feitlik al die "counties" in Florida voor. Dit het versprei na Louisiana en die vektor is nou ook in Kalifornië gevind net noord van die Meksikaanse grens. Die Amerikaners probeer die vektor beheer met kontakinsekdoders en tot twintig bespuitings word per jaar gedoen. Die bespuitings is nie effektief nie en pesreperkussies kom voor. Tensy die Amerikaners meer aandag gee aan die gebruik van sistemiese insekdoders, is hulle kanse om met hierdie siekte saam te leef, skraal. Daar is sekere Amerikaners wat arrogant is oor die siekte en wat voel dat as die Chinese produksie so dramaties kon toeneem hulle ook saam met vergroening sal kan leef. Wat hulle nie besef nie is dat die Chinese in die koeler areas inbeweeg het waar die vektor nie voorkom nie. Florida het nie koel genoeg areas om van die vektor weg te beweeg nie.

Kuba is ook totaal deurtrek met Asiatiese vergroening en ons kan verwag dat daar geleenthede vir die bemarking van pomelos in die tradisionele markte van hierdie twee lande gaan ontstaan.

Wat besproeiing betref het die Amerikaners hier afgeklim met die persepsie dat druppers nie vir hulle sal werk nie. Hulle het nie van hierdie voorafopgestelde idee afgewyk nie en dit was ook nie ons doel om hulle

hiervan te oortuig nie. Elke ou soen sy vrou op sy eie manier. Hulle was wel van mening dat die hoërdigtheidsaanplantings vir hulle belofte kan inhou.

Angola (“Biosecurity”)

Nadat dit onder die aandag van CRI gekom het dat Angola sitrusplantmateriaal van Brasilië af ingevoer het, het CRI alles in hulle vermoë gedoen om 'n afspraak met die Angolese Departement van Landbou te maak om hierdie invoere te staak en vas te stel of die vorige invoere nie dalk reeds een van die gevreesde siektes uit Suid Amerika na Afrika gebring het nie. Dit sluit siektes in soos Sitruskanker, Asiatiese- en Afrika-vergroening, Citrus Variegated Chlorosis, Sudden Death, Leprosis virus en Rubilose virus.

Dit was aanvanklik onmoontlik om met hulle kontak te maak en nadat die kontak nie d.m.v. die amptelike kanale gemaak kon word nie, het CRI self deur Antonio Nascimento van die Angolese ambassade in Pretoria 'n vergadering gereël gekry met die Angolese Ministerie van Landbou. Vaughan Hattingh en Hennie le Roux het op 19 September 'n vergadering gehou met die Angolese Minister van Landbou, die Adjunk Minister en drie van hulle senior direkteure. Hulle het begrip vir ons probleem gehad en onderneem om enige verdere invoere van sitrusmateriaal uit lande anders as Suid-Afrika te staak. Vaughan Hattingh het hulle aangemoedig om van die Suid-Afrikaanse Plantkwarantynkanale gebruik te maak tot tyd en wyl hulle eie kwarantynstelsel weer op die been is. Daar is ook besluit om gesamentlik die aanplantings uit Brasilië te besoek. 'n Datum vir so 'n besoek moet nog bepaal word.

Die besoek was hoogs suksesvol en die bande tussen CRI en die Angolese Ministerie van Landbou moet opgepas word.

11de Internasionale Sitruskongres: Wuhan, China

'n Volledige verslag oor die kongres is gepubliseer. Die kongres is deur Huanglongbing, oftewel Vergroening, oorheers. Die siekte het oor die laaste paar jaar na verskeie prominente sitrusproduserende lande soos Brazilië, Florida, Kuba en Iran versprei en gaan beslis die speelveld verander. In Florida is die dosis wat toelaatbaar is per hektaar om die vektor te beheer, onvoldoende. In Brazilië regverdig die sappryse nie die gebruik van die sistemiese middels nie en in Kuba en Iran het die siekte so versprei voordat dit ontdek is dat dit waarskynlik te laat is om dit te keer.

Tegnologie en Tegnologie-oordrag

Tegnologie is deurentyd aan die verbeter en dit is van kardinale belang dat Voorligting op hoogte moet bly van wat in die res van die wêreld aan die ontwikkel is, om terugvoer aan die navorsers te gee, asook geleenthede vir CRI en River Bioscience te help identifiseer. 'n Groot probleem-area in die bedryf is die besoedeling van die natuur ag.v. afloopwater van die pakhuis. Daar bestaan reeds tegnologie om hierdie afloopwater te neutraliseer en as CRI en River Bioscience nie die geleentheid baie gou gaan benut om dit in die land in te bring en te versprei nie, gaan ander instansies dit doen.

Verskeie ander geleenthede bestaan ook en word nie aangespreek nie: Niemand in die sitrusbedryf is besig om na nuwe innoverende verpakking te soek nie. Nie net is houtpalette 'n probleem nie, maar ook ander materiaal om kartonne of houers vir verpakking te vind. Goedkoper toerusting moet gevind word om mee te oes, ringeleer, palettiseer, boordsanitasie te doen, ens. Alternatiewe boord-, oes- en pakpraktyke moet ondersoek word, asook metodes om die koueketting meer effektief te bestuur.

In samewerking met die Sitrus Akademie word daar gewerk aan 'n video reeks wat verskeie aspekte van sitrusverbouing sal aanspreek.

Algemeen

Die positiewe sentiment wat tydens die CGA “roadshow” teenoor CRI/CGA ervaar is, is verblydend. Die werk wat deur CRI/CGA gedoen word, word hoog aangeslaan en word as 'n belangrike komponent vir die langtermyn voortbestaan van die sitrusbedryf beskou. Die toestaan van 'n addisionele 2c/ karton het CGA in 'n posisie geplaas om die logistieke probleme in die bedryf te begin aanspreek.

Kommer is uit verskeie oorde uitgespreek oor opvolgbeplanning vir sleutelposisies in die sitrusbedryf. Die bedryf is midde in 'n periode van sewe of agt jaar waarin bykans 20 tot 30 van die mees kundige tegniese persone in die proses is om af te tree, terwyl daar weinig jong toetreders tot die bedryf is.

Die voorkoms van swartvlek in die Oos-Kaap het dramaties verhoog, tot so mate dat in sommige weke soveel as 70% vrugte na Iran afgekeur is. Swartvlek het hierdie seisoen vir die eerste keer op nawels in die Oos-Kaap problematies begin raak. Vroeër was dit net 'n probleem op suurlemoene en tot 'n mindere mate op Valencias. Die vraag is tot watter mate die CFB blootgestel is aan CBS-besmetting. Een van die opsies wat in die verlede genoem is, is om die CFB te skuif na 'n geïsoleerde gebied wat naby 'n lughawe is, by Kimberley of Uppington. Daar sal weer oorweging aan hierdie voorstel geskenk moet word.

2009 Seisoen

Die voorseisoen in die noorde was effens droër en omdat Corosil-E van die mark onttrek is, kon produsente nie chemiese uitdunning, veral op die pomelos, doen nie. Produsente was versigtig om Maxim te gebruik. Daar kan dus verwag word dat die vrugtelting op die pomelo-oes een vrugtelting kleiner kan wees in die 2009 seisoen, vergeleke met die vorige oes. In die suide is die vroeë aanduiding ook dat die vrugte kleiner gaan wees as verlede seisoen, veral in die Oos-Kaap waar 'n ernstige droogte met warm winde ondervind word. Blaaspootjie is ook erg en saam met die baie wind word laer uitpakte verwag. Die situasie ten opsigte van VKM lyk beter. Suigmot is op satsumas in die Oos-Kaap gerapporteer, maar hopelik sal die skade nie weer so dramaties wees as in 1999 nie.

Die totale oesskatting vir 2009 is soortgelyk aan 2008. Nawels, pomelos en sagtesitrus is op teenoor verlede jaar, terwyl suurlemoene en Valencias laer is. Die verwagting is dat sommige markte minder vrugte sal koop, maar dat redelike aanvaarbare pryse behaal kan word en groot volumes steeds uitgevoer kan word indien die kwaliteit uitstekend is en pryse onderhandel word op 'n vlak wat sal verseker dat die vrugte steeds teen 'n goeie tempo verkoop en almal in die ketting geld kan maak. Die simposiumprogram is in die volledige weergawe van die Navorsingsjaarverslag beskikbaar.

9.2 Extension Coordinator 2008 (A. Mbedzi – CRI)

The Limpopo Department of Agriculture (LDA), the CGA and CRI signed a Memorandum of Understanding (MoU) in July 2007. To implement the MoU a structure (engine room) was set up and as a first step, agricultural technicians who will serve as citrus commodity coordinators were identified. (See table listing these coordinators in the detailed version of the Research Annual report). These officers are in the process of being capacitated through citrus skills training courses and mentoring programmes.

After the identification of the citrus commodity coordinators an orientation workshop was organized by the Extension coordinator. The orientation workshop was funded by the CRI, CGA and LDA. It was held at Eiland Spa on 23 and 24 April 2008. The purpose of the orientation workshop was to orientate the identified citrus commodity coordinators about the citrus industry. Both clusters and their respective sub-districts were represented as well by senior officers of the Limpopo Department of Agriculture.

The Extension coordinator and two citrus commodity coordinators from Vhembe District (Thulamela sub-district) in Limpopo Province attended a citrus production management course from 11-15 February 2008 in the Western Cape. The training was on how to facilitate using unit standard aligned learning materials. The learning materials (citrus production management) were from National Qualification Framework (NQF) Level 2 to 5. The training workshop was organized by the Citrus Academy. Some of the Citrus Commodity Coordinators and Senior Managers of the LDA attended the Citrus Research Symposium which was held at Champagne Sports Resort in KwaZulu-Natal in August 2008.

On the 17-19 September 2008 a citrus scouting course was held in the Vhembe District, at the Madzivhandila College of Agriculture. A total of seventeen agricultural officers (scientists and technicians) from the Limpopo Department of Agriculture attended the course. These officers are coordinating citrus as a commodity in their districts. The course was facilitated by Kevin Language's Pest Solution company. The scouting course's practical training was done at Lungane and Easy Farms.

Challenges

Communication is still a major challenge. In one of the meetings in Polokwane it was indicated that the CRI should use the project approach when working with the citrus commodity coordinators. This approach is working in some districts but it does not work at all in some of the other districts. There is a need to use a combination of project approach and top down approach. This will avoid a situation where CRI is seen as an organization that wants to control but rather a partner that wants to see things happening on the ground with

regard to the production and marketing of citrus as a commodity. Madzivhandila College was playing a major role and should be applauded for that. However, of late it was clear that there were some districts who were questioning the role that they were playing in making this programme a success.

Another challenge is that some of the citrus commodity coordinators do not have the necessary communication tools such as computers and e-mail. When documents are sent to these officers, through districts coordinators, they do not reach them in time or do not reach them at all which makes communication with these officers difficult.

A lack of funds was also one major challenge in 2008. As a result some of the citrus commodity coordinators missed important training workshops. Sometimes only a few officers were sent to attend the training workshops and some remained behind. This created a conflict as some officers felt that others were given preference because they are liked by their seniors which was not the case. Rotation of officers was also not good because it created a situation where there was no continuity in what the officers were doing. This lack of funds created a division among the citrus commodity coordinators and this is not acceptable as CRI wants them all to grow their skills simultaneously and to have enthusiasm for citrus.

The citrus commodity coordinators indicated to the Extension coordinator that they do not have good relationship with the farmers or beneficiaries that are having strategic partners on their farms or projects. This has made it difficult for them to obtain the farm profiles (statistics). They indicated that they are ignored when they visit these farms and this made them feel uneasy when visiting these farms.

Where the beneficiaries are more than one per farm the potential for a conflict is high due to a clash of interests, a clash of personalities and misunderstandings. In most cases officers find themselves sympathising with one of the groups that are in conflict with the other. This makes it difficult for the officer to work in that environment and ultimately he or she finds himself or herself no longer visiting that farm.

Recommendations

- The Head Office should nominate one of the officers to help with the implementation of the memorandum of understanding and the districts and sub-districts should be informed about this officer. Madzivhandila Agricultural College (Mr K.S. Mudau) is doing a wonderful job in the implementation of the MoU, but he needs the support of the districts and sub-districts. This will help in a situation where project approach alone is not working. The officer will help in implementing a top-down approach where necessary.
- The citrus commodity coordinators should have their own computers and, if not possible, the district coordinators should be informed to pass information in time to the citrus commodity coordinators. Some information (i.e. Cutting Edges) are important and need to filter down to farmers immediately and if delayed becomes useless.
- Communication between the district coordinators, the citrus commodity coordinators and the CRI extension coordinator should be two ways and not one way. This will facilitate in accomplishing our obligations and ensure that everything is in order.
- The citrus activities that need be to carried out during the year must be identified and a LDA budget should be allocated to those identified activities to easy-up financial challenges.
- The beneficiaries who are having strategic partners should allow the citrus commodity coordinators to visit their farms or projects. They must realise that the strategic partners have exit plans and one day they are going to leave the farm. When the strategic partners exit the farm the beneficiaries will need the services of these agricultural officers. So, it is best to start building a good relationship now and, obviously, this will prevent a situation were the farmers would complain about the unavailability of the Extension officers.
- The citrus commodity coordinators must avoid getting involved in farm politics. They must not take sides when the beneficiaries are having conflicts, instead, they should help both the conflicting sides to resolve their conflicts amicably.

The South African Pesticide Initiative Program (SAPIP) European Union (EU) evaluators visited the CRI offices and Tzaneen (Hlanganani and Mariveni Farmers Cooperative). The aim of the visits was to evaluate the SAPIP funded programmes (Mentorship Programme). They interviewed the CGA Transformation Manager, CRI Extension Coordinator and CGA Mentor and also visited Mariveni citrus farm and interviewed the Farm Manager and the Farmers.

There is good relationship developing between the Citrus Research International (CRI) and the Mpumalanga Land Claims Commission. This relationship, if nurtured well, will help a lot especially during the transition period of claimed citrus farms in Mpumalanga.

The Formation and Type of the Growers Voice

Andrew Mbedzi (AM) was tasked by the CGA to interact with the growers with regard to the formation of the growers' representative structure and the formulation of its objectives as well as the type of the structure. There was a lot of mixed feelings from the growers about the formation and the type of the structure to represent them within the CGA. The following were the feelings of the farmers that were contacted physically and telephonically by AM.

- Some growers feel that the formation of the structure is a waste of time as it is like forming a structure within another structure which already exists. These growers indicated that it is better to continue to use CGA as the growers' voice. Forming another structure according to these growers is just a mere duplication.
- Other growers are welcoming the idea of forming a black growers' representative structure within CGA. They see this black representative structure within CGA as something that will bring an opportunity for the black growers to be able to link with the relevant government departments. This representative structure will also help in solving challenges that are faced by black growers.
- Some growers were in favour of the formation of the black growers' representative structure within CGA, but they do not want it to be in a form of a Trust. The growers feeling about a trust is that it can be used to source funds from donors and government to find out that the money is no longer used to achieve the objectives of the trust, but for something else that does not benefit the black growers. These growers strongly feel that the issue of the type of black growers' representative must be left to the growers themselves to decide. They suggested that a Task Team should be formed and this task team will come up with the best type of black representative structure. They indicated that the Task Team structure will be formed by all the growers from the different regions; therefore it will be well represented by members from all the regions. These growers feel that the decision that will be taken by this Task Team about the type of the body to be created to represent the black growers will be legitimate.

The types of structures (bodies) that were suggested by the growers were the following:

- Citrus Growers Transformation Committee (CGTC)
- Citrus Growers Development Trust (CGDT)
- Citrus Growers Development Forum (CGDF)
- CGA Board Representative

9.3 FINAL REPORT OF THE PHIP/CRI CITRUS RIND CONDITION WORKSHOP, 2-6 FEBRUARY 2009, NELSPRUIT

The aim of the workshop was to discuss all aspects relevant to the condition of a citrus fruit rind during pre-harvest development and postharvest handling. In order to thoroughly examine these aspects, international and local researchers were invited to share their experience in a 2-day workshop with local technical personnel, representing all the citrus production areas in South Africa.

In the first session, insight into the problems associated with rind condition and disorders was given from the perspective of the grower/exporter by representatives from the different production areas of South Africa, viz. Hans le Grange, Piet Smit (Western Cape), Christo Theron (Eastern Cape), Frans Olivier (Mpumalanga) and Dr. Fanus Swart (Limpopo). This session was helpful in identifying and distinguishing the various disorders as either specific to one locality or prevalent in all production areas.

In subsequent sessions, the various researchers presented an overview of research results in their fields of expertise. Prof. John Bower from UKZN presented his outlook on the impact of pre-harvest environment on fruit and rind development. He focused on aspects that would influence allocation and assimilation of mineral nutrients and carbohydrate in the fruit rind. He stressed the importance of optimising cultural practises such as pruning, irrigation and fertilisation on eventual rind quality.

The influence of the prevailing climate and postharvest handling practises from the orchard to the packhouse on rind condition was addressed by Dr. Fernando Alférez (IATA, Valencia). He highlighted the importance of

climatic conditions prior to and during harvest on especially the rind water balance, and the influence of fluctuating temperature and humidity, prior to harvest, on predisposing the fruit rind to rind staining. In addition, a sudden change in relative humidity (influencing vapour pressure deficit) in the days after picking and prior to packing have been linked by his research to the mechanism causing rind pitting in various citrus species.

Dr. Lluís Palou, a postharvest pathologist from IVIA in Valencia, shared his experience on alternative methods of control and reduction of pathogens and resultant decay during the packhouse phase of fruit handling. He gave emphasis to the importance of sanitation in the packhouse, as well as using complementary technologies such as traditional fungicides, temperature control and GRAS compounds, to reduce spore load and decay incidence.

Paul Cronje, from CRI, with input from Dr. Lorenzo Zacarias from IATA Valencia, identified the key issues in susceptibility of the citrus fruit rind to chilling injury during export shipment. The interaction between climatic fluctuation (temperature and RH) and genetics accounts for the difference in chilling incidence between cultivars, areas and within a season. New research avenues and technologies, such as hot water treatment, thiabendazole, CO₂ and gibberellic acid were discussed with regard to their potential in strategies to reduce the incidence of chilling injury in citrus fruit.

After these sessions the participants were split into three working groups, each focusing on a specific topic, *viz.* chilling injury, progressive disorders and postharvest pathology, to identify critical research areas and compile an action plan to obtain these results. The feedback from these groups was summarised in an action plan for research (short, medium and long term) as well as technology transfer of available information. This action plan is available in the detailed version of the Annual Research report for 2008.

General impression

The rind condition workshop served as an excellent opportunity for not only local and international researchers to interact, but also in exposing the technical personnel to the new research directions and results. From these discussions, the complexity surrounding rind condition and factors influencing it became very clear. It is evident that no easy solution or “silver bullet” will be available for any of the physiological rind disorders or the pathogen complex affecting rind integrity. However, only by collaboration between local and international researchers will a better understanding of the mechanisms involved in rind disorders and decay evolve, which would lead to implementable strategies/technologies to reduce or prevent economically damaging levels of these problems in the market.

Acknowledgement

The Post Harvest Innovation Programme is thanked for funding this workshop and subsequent presentations to various technical personnel in the citrus industry.

9.4 **THE RELATIVE FUNDING SUPPORT FOR RESEARCH PROGRAMMES AND PROJECTS FOR 2008-9**
 By Tim G Grout (CRI)

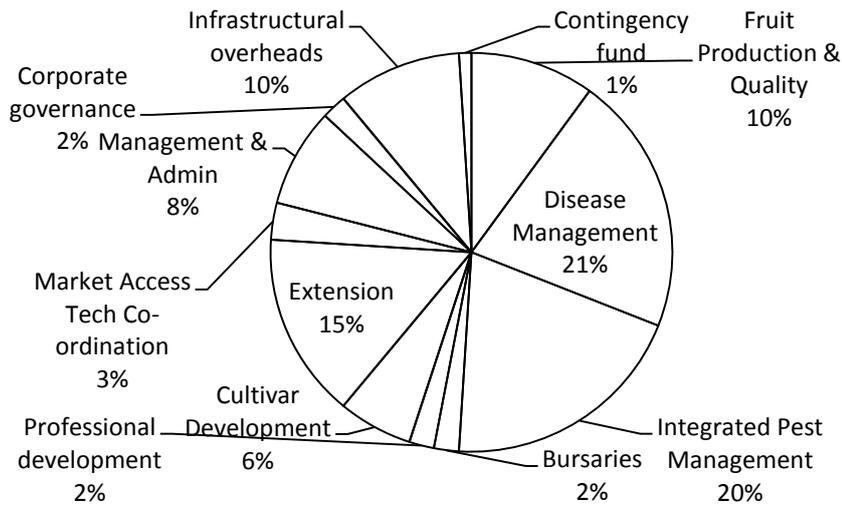


Fig. 9.4.1. Percentage funding in each CRI programme and rest of budget for 2008-9.

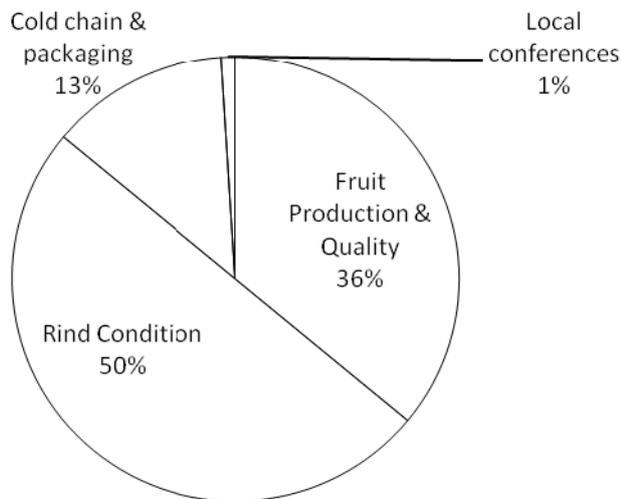


Fig. 9.4.2. Percentage funding to projects in the CRI Research Programme: Fruit Production and Quality Management for 2008-9. Cold Chain and Packaging is also included.

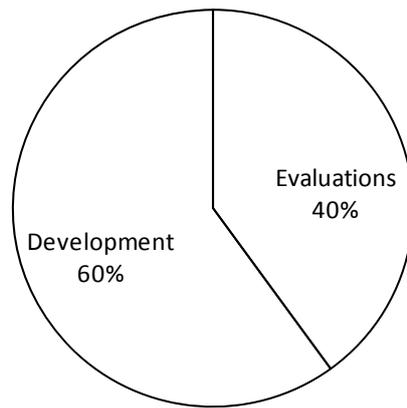


Fig. 9.4.3. Percentage funding to projects in the CRI Research Programme: Cultivar Development and Evaluation for 2008-9.

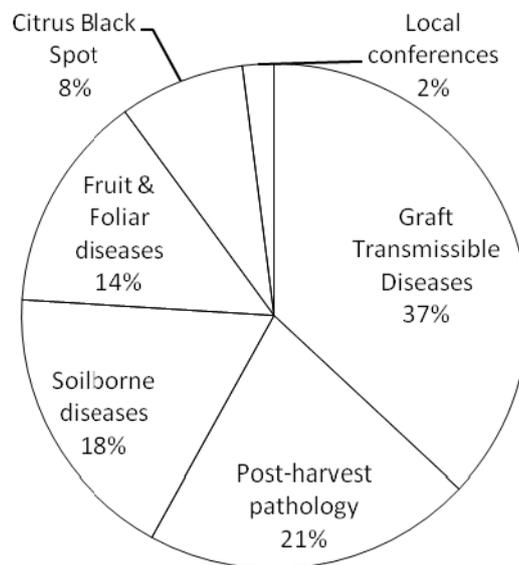


Fig. 9.4.4. Percentage funding to projects in the CRI Research Programme: Disease Management for 2008-9.

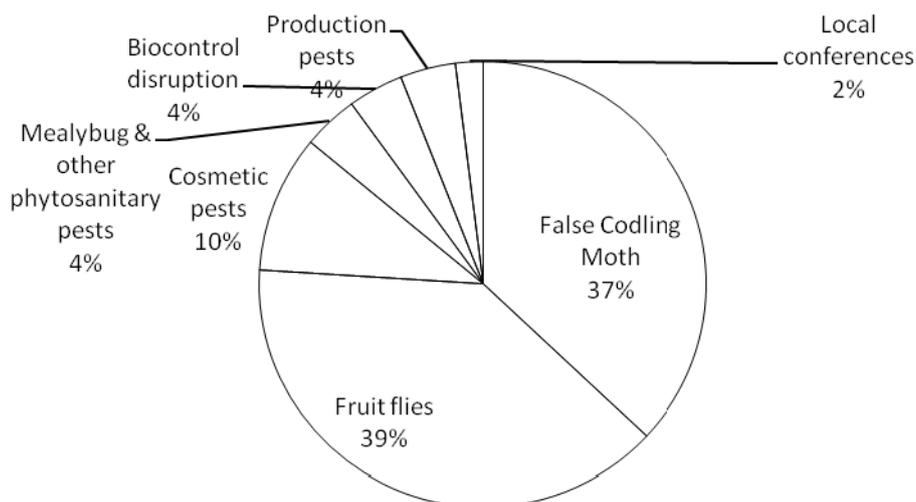


Fig. 9.4.5. Percentage funding to projects in the CRI Research Programme: Integrated Pest Management for 2008-9.

9.5 EXTENSION PRESENTATIONS BY CRI GROUP RESEARCHERS IN 2008-9

Name	Date	Place	Topic
Breytenbach, J.H.J.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	CTV cross-protection of Marsh and Star Ruby grapefruit trees
Cronjé P.J.R.	04-29/02/08	Citrus Cold Chain Forum: Packhouse workshop at Gordons Bay, Western Cape, Letsitele, Limpopo, Loskopdam, Mpumalanga, Nkwaleni Valley, KwaZulu-Natal	Peteca Spot on Lemons
	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	The effect of potassium, calcium and magnesium foliar applications on post-harvest rind breakdown of Nules Clementine mandarin The effect of CO ₂ , ethylene and nitrogen as well as 1-MCP applied as postharvest treatment on the occurrence of peteca
De Villiers, Marelize	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Distribution of Natal fruit fly and FCM, two pests of citrus in SA
Fourie, P.H.		Addo, Hoedspruit	Orchard spray demonstration
		Study Group meeting in Constantia/Letsitele	Orchard spray demonstration
		Western Cape growers	Ad hoc lecture on study tour to Hoedspruit
	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	The effect of run-off spray deposition and control of Alternaria brown spot of mandarins. Poster. Development of selected adjuvants for application in southern African citrus orchards.
Grout, T.G.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Can fruit fly baits be applied without leaving residues on fruit?

			Bud mite control after Acarol
Hattingh, V.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Market Access
Joubert, J.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	The performance of Limpopo Seedless Valencia orange on four rootstocks (RL, SC, CC and X639) on a replant soil
Keeton, Kierryn	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Larval parasitism of FCM on citrus in SA and biocontrol opportunities
Kirkman, W.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Factors influencing the field persistence of Cryptogran
			Break-Thru as a wetter in combination with various pesticides and black spot treatments
	25/08/2008	Swellendam	Symposium feedback, EU FCM
	26/08/2008	Ashton, Riebeek Kasteel & Citrusdal	Symposium feedback, EU FCM
	27/08/2008	Benede-Oranjerivier	Symposium feedback, EU FCM
	28/08/2008	Hartswater	Symposium feedback, EU FCM
Lee, A.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	CRI Cultivar Development
Lesar, K.H.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	The potential role of GRAS chemicals in the control of the major postharvest citrus pathogens
			The evaluation of the new postharvest fungicide Philabuster for the control of the postharvest disease <i>Penicillium digitatum</i> after harvest
Malan, A.P.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Potential of entomopathogenic nematodes for the control of false codling moth, <i>Thaumatotibia leucotreta</i> , (Lepidoptera: Tortricidae) in laboratory bioassays
Manrakhan, A.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Current status of the exotic fruit fly, <i>Bactrocera invadens</i> (Diptera: Tephritidae) in Africa, and future research perspectives
	03/03/2009	Swaziland & Pongola	Fruit fly monitoring and control
	04/03/2009	Malelane & Komatipoort	Fruit fly monitoring and control
	26/03/2009	Burgersfort & Ohrigstad	<i>Bactrocera invadens</i> – a new fruit fly threat
Meitz, J.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Poster: Characterization and detection of citrus <i>Phytophthora</i> species in South Africa.
Moore, S.D.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	A comparison of different methods of controlling fruit fly, measured by fly numbers and fruit damage
			Soft green scale: life cycle and control options
	01/09/2008	Kat River (Grower Study group meetings [GSGM])	Symposium feedback FCM and the EU
	02/09/2008	Sundays River [GSGM]	Symposium feedback FCM and the EU
		Patensie [GSGM]	Symposium feedback FCM and the EU
		Baviaans [GSGM]	Symposium feedback FCM and the EU
	20/10/2008	Nelspruit [GSGM]	FCM management
	21/10/2008	Swaziland [GSGM]	FCM management

		Komatipoort [GSGM]	FCM management
	11/11/2008	Nelspruit (Extension Workshop)	FCM research
			Mealybug research
Opoku-Debrah, John, K.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Geographic variation in the susceptibility of FCM populations to a granulovirus (CrleGV-SA) in SA
Phahladira, M.N.B.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Identification of alternative hosts to citrus of <i>Candidatus Liberibacter africanus</i> amongst indigenous rutaceous plants of SA
Pietersen, G.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Survey for "Candidatus" <i>Liberibacter</i> species on citrus in SA
Pretorius, M.C.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	The invasion of Huanglongbing citrus greening disease in the Western Cape and evaluation of new control strategies
			Evolution of control strategies for the citrus nematode, <i>Tylenchulus semi-penetrans</i> , in SA citrus orchards
		Swellendam	Orchard demonstration.
Read, D.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Detection of Citrus Tristeza Virus using real-time RT-PCR
Schutte, G.C.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	New spray programmes for the control of citrus black spot
			New spray programmes for the control of <i>Alternaria</i> brown spot on Mandarins in the summer and winter rainfall regions in SA
			The status and control of <i>Phytophthora citrophthora</i> , the cause of trunk and branch canker of Clementines in SA
Scott, K.A.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Strain differentiation of CTV isolates by PCR and DNA Microarray in SA
Stotter, R.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Spatial and temporal distribution of FCM across landscapes in the Citrusdal area (W. Cape province, South Africa)
Van der Westhuizen, L.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Sequence characterization of selected genes in four CTV mild sources potentially useful in mild strain protection of soft citrus cultivars
Van Vuuren, S.P.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Searching for resistance in the battle against greening
			Cross-protection to reduce the effect of CTV in citrus production of southern Africa
Verreynne, J.S.	03-06/08/2008	5 th Citrus Research Symposium, Drakensberg	Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges – a preliminary study

VOORLIGTING

Datum	Gebeurtenis	Onderwerp	Betrokkenes/Sprekers
02/01/2008	Winterveldt	Besoek BEE projek en gee raad	HIR
03/01/2008	S. Kamburov	Hulpverlening met Plantpatologie gedeelte van nuwe boek.	HIR
10/01/2008	Limpopo Extension meeting	Verduideliking van samewerkingsooreen-	HIR, AM

		koms tussen CRI/CGA/LDA	
16/01/2008	Verpakkingswerkgroep	Minimum spesifikasies en protokolle	DG/HB
	5 ^{de} Sitrusnavorsing-vergadering	Simposiumbeplanning	Bayer. HIR, HS, JdG
21/01/2008	Angolese ambassade	Besoek aan Angolese Ministerie van Landbou	HIR
23/01/2008	Beitbrug	Studiegroepvergadering: Sitrusverbeteringskema, VKM, Myte, Wolluis	HIR, TdT, SM
24/01/2008	Chegutu	Studiegroepvergadering: VKM ea peste en plae, Eksotiese sitrussiektes	HIR, TdT, SM
	Mazowe	Studiegroepvergadering: VKM ea peste en plae, Eksotiese sitrussiektes	HIR, TdT, SM
25-26/01/2008	Produsol Kwekery	Gee riglyne vir 'n geakkrediteerde sitruskwekery	TdT, HIR
30-31/01/2008	CRI Management Meeting	Agenda	VH/HIR/TG/TdT/AL/HB
04-05/02/2008	Wes-Kaap Pakhuisstudiegroep Werkswinkel	Rypheidsindeksing Pakhuispraktyke Verkoeling en Ventilasio Fisiologiese skildefekte Tyd-en temp protokolle Verpakkingswerkgroep PPECB Logistiek	Hannes Bester Keith Lesar Peter Hoekstra Paul Cronje Frikkie v Wyk Dawid Groenewald Cyril Julius Lynette Grobler
	Studiegroepvergadering: Letsitele	Vrugsetprobleme	SV, HIR
6-7 Feb 2008	Oos-Kaap Pakhuisstudiegroep Werkswinkel	Rypheidsindeksing Pakhuispraktyke Verkoeling en Ventilasio Fisiologiese skildefekte Tyd-en temp protokolle Verpakkingswerkgroep PPECB Logistiek	Hannes Bester Keith Lesar Peter Hoekstra Hannes Bester Frikkie v Wyk Dawid Groenewald Cyril Julius Lynette Grobler
08/02/2008	Verpakkingswerkgroep	Strategiese Sessie	VH/HIR/HB
11-12/02/2008	Limpopo Pakhuisstudiegroep Werkswinkel	Rypheidsindeksing Pakhuispraktyke Verkoeling en Ventilasio Fisiologiese skildefekte Tyd-en temp protokolle Verpakkingswerkgroep PPECB Logistiek	Hannes Bester Keith Lesar Hannes Bester Paul Cronje Frikkie v Wyk Dawid Groenewald Cyril Julius Lynette Grobler
13-14/02/2008	Mpumalanga Pakhuisstudiegroep Werkswinkel	Rypheidsindeksing Pakhuispraktyke Verkoeling en Ventilasio Fisiologiese skildefekte Tyd-en temp protokolle Verpakkingswerkgroep PPECB	Hannes Bester Keith Lesar Hannes Bester Paul Cronje Frikkie v Wyk Dawid Groenewald Cyril Julius

		Logistiek	Lynette Grobler
20/02/2008	Logistics meeting	Logistics	HB/LG
	5 ^{de} Sitrusnavorsings-simposium	Beplanningsvergadering	Champagne Sports Resort, Bayer, HIR, HS, JdG.
21-22/02/2008	KZN Packhouse Study Group Workshop	Maturity Indexing Packhouse Practices Cooling and Ventilation Physiological Disorders Time and temp protocols Packaging Work Group PPECB Logistics	Hannes Bester Keith Lesar Hannes Bester Paul Cronje Hannes Bester Dawid Groenewald Cyril Julius Lynette Grobler
26/02/2008	Patensie CGA meeting	Statutory Levies	PH/VH/HB
	Sundays River CGA meeting	Statutory Levies	PH/VH/HB
	Letsitele CGA Meeting	Statutory Levies	JC/HIR/MH
27/02/2008	Katrivier CGA meeting	Statutory Levies	PH/VH/HB
28/02/2008	Limpopo CGA Meeting	Statutory Levies	JC/HIR/MH
	Exporters Technical Panel	Verpakkingsforum Tyd- en temp protokolle Logistiek	Dawid Groenewald Hannes Bester Lynette Grobler
	Logistieke vergadering	Logistiek	HB/DG/LG
29/02/2008	Magalies	Winterveldt BEE	HIR
04/03/2008	Benede-Oranjerivier CGA vergadering	Statutêre Heffings	JC/HB/MvN
05/03/2008	Vaalharts CGA vergadering	Statutêre Heffings	JC/HB/MvN
06/03/2008	VSA Werkswinkel	Agenda	HB
10/03/2008	Limpopo Extension	MoU discussions & organogram	AM
11/03/2008	Southern Fruit Growers	Fruitnet	HB/PD
12/03/2008	CMF	Agenda	HB
13/03/2008	Studiegroepvergadering Burgersfort & Ohrigstad	VKM. Vrugtevlieg, Myte. Phytophthora bruinvrot, Post harvest	HIR, TG, KL, MCP
18/03/2008	Verpakkingsvergadering	Min specs en protokolle	HB/DG/RK
19/03/2008	Cultivar meeting	Agenda	AL/TdT/HB/JJ
26/03/2008	Angolese ambassade	Bespreek fitosanitêre aangeleenthede	HIR
27/03/2008	Southern Fruit Growers	Fruitnet	HB/PD
	Soetdoring Boerdery Dendron	Yster chlorose/ Swingle probleme	HIR
03/03/2008	Baviaans Studiegroep	Rypheidsindeksering en oespraktyke Ontgroening Fisiologiese skildefekte Pakhuispraktyke	Hannes Bester Hannes Bester Hannes Bester Hannes Bester

17-24/04/2008	Besoek Spanje	Koueketting	Hannes Bester
12/05/2008	Swellendam Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	John Edmonds Andy Lee Andy Lee Peter Turner / Hennie Prins
13/05/2008	Breederivier Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	John Edmonds Andy Lee Andy Lee Peter Turner / Hennie Prins
	Paarl / Stellenbosch en Swartland Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	John Edmonds Andy Lee Andy Lee Peter Turner / Hennie Prins / Waldo Maree
	Citrusdal Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	John Edmonds Andy Lee Andy Lee Peter Turner / Hennie Prins / Waldo Maree / Eben vd Walt
14/05/2008	Benede-Oranjerivier Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	John Edmonds Andy Lee Andy Lee Freek Veldman
15/05/2008	Vaalharts Studiegroep	Gekanselleer agv geen belangstelling	
19/05/2008	Southern Natal Study Group	Industry statistics Cultivar development CIS Cultivar options	John Edmonds Andy Lee Andy Lee Andy Lee
20-31/05/2008	Argentinië	HLB Kongres. Concordia Tucuman besoek	Hennie le Roux Andrew Lee
20/05/2008	Nkwalini Study Group	Cancelled due to land claims	
21/05/2008	Katrivier Study Group	Industry statistics Cultivar development CIS Cultivar options	John Edmonds Andy Lee Andy Lee Freek Veldman
22/05/2008	Sondagsrivier Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	John Edmonds Andy Lee Andy Lee Peter Turner/Freek Veldman
	Patensie Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	Hannes Bester Andy Lee Andy Lee Peter Turner/Freek Veldman
	Baviaans Studiegroep	Bedryfsstatistiek Cultivarontwikkeling SVS Cultivar-opsies	Hannes Bester Hannes Bester Hannes Bester Freek Veldman
23-24/05/2008	Voorligtingswerkswinkel	SA Sitrusbedryf Sitrusvoorligting in die Limpopo Provinsie na Transformasie	Hennie le Roux Andrew Mbedzi Maxwell Hawes
25/05/2008	Henley Nursery	Vernietiging van sowat 1 miljoen saailinge van saad	Hennie le Roux

		afkomstig van Florida	
29/05/2008	SASCCON	Agenda	Hannes Bester
9-10/06/2008	Angolese Ambassade	Onderhandelings rakende 'n besoek aan die Angolese Ministerie van Landbou	Hennie le Roux
20/06/2008	Winterveldt	Boordbesoeke	Hennie le Roux Andrew Mbedzi
23/06/2008	Swellendam Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee Hannes Bester
24/06/2008	Breederivier Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee Hannes Bester
	NDA en PPECB	PPECB monster-nemingsprosedure	Hannes Bester Hanlie Wessels Cyril Julius
	Paarl / Stellenbosch en Swartland Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee Hannes Bester
25/06/2008	Citrusdal Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee Hannes Bester
	SAPPI en APL	Verpakkingswerk-groep	Hannes Bester D Groenewald Roche Kenny
30/06/2008	Vaalharts Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee Hannes Bester
30/06-02/07/08	Transformasie Vergadering	CGA Transformasie proses	Justin Chadwick Maxwell Hawes Hennie le Roux Andrew Mbedzi
01/07/2008	Benede-Oranjerivier Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee
01-02/07/2008	CGA Transformation Workshop	Transformation within the Citrus Industry	Hennie le Roux Andrew Mbedzi
04/07/2008	Tala Valley	Visit BEE project	Hennie le Roux Dr Joe Stevens (UP)
07/07/2008	Southern-Natal Study Group	Fertilisation Irrigation	Hannes Coetzee Hannes Coetzee
08/07/2008	Nkwalini Study Group	Fertilisation Irrigation	Hannes Coetzee Hannes Coetzee
10/07/2008	Ethiopian Delegation	Citrus discussions	Hennie le Roux Andrew Mbedzi
15/07/2008	Patensie Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee
	Baviaans Studiegroep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee
16/07/2008	Katrivier Study Group	Fertilisation Irrigation	Hannes Coetzee Hannes Coetzee
17/07/2008	Sondagsrivier Studie-groep	Bemesting Besproeiing	Hannes Coetzee Hannes Coetzee
	Hoedspruit Studiegroep Phalaborwa	Boordprobleme BEE	Hennie le Roux Andrew Mbedzi Dr J. Stevens
25/07/2008	Logistics and Cold Chain Interactive Workshop	Packhouse Practices Agenda	Hannes Bester Other
01-06/07/2008	Citrus Research Simposium	Program	Program

07-12/08/2008	Huanglongbing Survey	Orchard visits	Hennie le Roux Prof Bove Brazilians Argentinean
13/08/2008	Cultivar Development Cooperation Group	Agenda	HB/AL/TdT/JJ/HIR
14-15/08/2008	Angolan Embassy	Organise meeting with Angolan Ministry of Agriculture	Hennie le Roux
18-20/08/2008	Angola	Biosecurity visit with the Angolan Minister of Agriculture	Vaughan Hattingh Hennie le Roux
21/08/2008	Kamburov	Comments on Pathology part of book	Hennie le Roux
25-31/08/2008	Floridian visitors	Huanglongbing, OHS & High density plantings	Hennie le Roux
25/08/2008	Swellendam Studiegroep	Simposium Terugvoer	Hannes Bester Wayne Kirkman
26/08/2008	Breederivier Studiegroep	Simposium Terugvoer	Hannes Bester Wayne Kirkman
	Paarl/Stellenbosch en Swartland Studiegroep	Simposium Terugvoer	Hannes Bester Wayne Kirkman
	Citrusdal Studiegroep	Simposium Terugvoer	Hannes Bester Wayne Kirkman
27/08/2008	Benede-Oranjerivier Studiegroep	Simposium Terugvoer	Hannes Bester Wayne Kirkman
28/08/2008	Vaalharts Studiegroep	Simposium Terugvoer	Hannes Bester Wayne Kirkman
01/09/2008	Katrivier Study Group	Symposium Feedback	Hannes Bester Sean Moore
02/09/2008	Sondagsrivier Studiegroep	Simposium Terugvoer	Hannes Bester Sean Moore
	Patensie Studiegroep	Simposium Terugvoer	Hannes Bester Sean Moore
	Baviaans Studiegroep	Simposium Terugvoer	Hannes Bester Sean Moore
	Hoedspruit Studiegroep	Simposium Terugvoer	Hennie le Roux Tom vd Meulen
03/09/2008	Nkwalini Study Group	Symposium Feedback	Hannes Bester Sean Moore
	Tshipise & Weipe Studiegroep	Simposium Terugvoer	Hennie le Roux
04/09/2008	Southern Natal Study Group	Symposium Feedback	Hannes Bester Sean Moore
	Waterberg Studiegroep	Simposium Terugvoer	Hennie le Roux
	Marble Hall Studiegroep	Simposium Terugvoer	Hennie le Roux
08/09/2008	Nelspruit Studiegroep	Simposium Terugvoer	Hennie le Roux
09/09/2008	Malelane Studiegroep	Simposium Terugvoer	Hennie le Roux Chris Kellerman
	Komatipoort Studiegroep	Simposium Terugvoer	Hennie le Roux Chris Kellerman
10/09/2008	Swaziland Study Group	Symposium Feedback	Hennie le Roux Chris Kellerman
	Pongola Studiegroep	Simposium Terugvoer	Hennie le Roux Chris Kellerman
11/09/2008	Burgersfordt & Ohrigtsadt	Simposium Terugvoer	Hennie le Roux Clive Pountney
12/09/2008	Sundagsriviervallei FCM Strategy Meeting	FCM-EU Strategy	Hannes Bester Other
15/09/2008	Supply Chain Workshop	SA Supply Chain	Malcolm Dodd

	Stellenbosch	Citrus Packhouses in Spain Horticulture in New Zealand	Hannes Bester Errol Hewett
16/09/2008	Constantia/Letsite Studiegroep	Simposium Terugvoer	Hennie le Roux Andrew Mbedzi
17/09/2008	FCM-meeting SRV	GAP's for FCM	Hannes Bester
19/09/2008	Supply Chain Workshop Nelspruit	SA Supply Chain Citrus Packhouses in Spain Horticulture in New Zealand	Malcolm Dodd Hannes Bester Errol Hewett
25/09/2008	Patensie- en Baviaans Studiegroepe	Pro-Gibb: Vrugset en Kraakskil	Ian Garden
	Visit BEE Projects with Land Claims Commissioner	Badplaas Lemon farms	Hennie le Roux Andrew Mbedzi
30/09/2008	Winterveldt BEE Projek	Advisering en PR	Hennie le Roux Hannes Bester
	Magalies Sitruskoöperasie	Sapaanleg	Hennie le Roux Hannes Bester
01/10/2008	Loskopdam produsente	Kultivaraanbevelings	Hannes Bester Hennie le Roux
02/10/2008	Rustenburg Sitrusstudie-groep	Simposium terugvoer	Hannes Bester Hennie le Roux
07/10/2008	Oesopbrengs & Vrugkwaliteit-Bestuursprogram Stellenbosch	Navorsingsprogram-vergadering	Hennie le Roux Hannes Bester
08/10/2008	Kultivar vergadering PE	Navorsingsprogram-vergadering	Hennie le Roux Hannes Bester
09/10/2008	Siektebestuur program Jhb	Navorsingsprogram-vergadering	Hennie le Roux
14/10/2008	Geïntegreerde bestuursbeheer program	Navorsingsprogram vergadering	Hennie le Roux
	Patensie en Baviaans studiegroep	Swartvlek Alternaria	Tian Schutte Hannes Bester
	Terason	Swartvlek Alternaria	Tian Schutte Hannes Bester
15/10/2008	Transformasie	Samewerking tussen CRI en UP	Hennie le Roux Dr Joe Stevens Andrew Mbedzi
16/10/2008	Midnight Studiegroep Marble Hall	Lente Plaagkompleks Swartvlek Simposium terugvoer	Tim Grout Hennie le Roux
	FCM Task Team Sondagsrivier	FCM GAP's en SOP's	Hannes Bester
17/10/2008	CGA Mentors vergadering	Stand van plase wat gementor word	Andrew Mbedzi Hennie le Roux
20/10/2008	Nelspruit Sitrusstudiegroep	Valskodlingmot	Sean Moore
22/10/2008	Unifrutti-vergadering	Gehalte-werkswinkel	Hannes Bester Paul Cronje
21/10/2008	Swaziland Sitrusstudiegroep	Valskodlingmot	Sean Moore
	Komatipoort Sitrusstudiegroep	Valskodlingmot	Sean Moore
25/10-05/11/08	lide ICC, Wuhan, China	Internasionale Sitruskongres	Hennie le Roux Hannes Bester
06/11/2008	SASCCON	Algemene jaarvergadering	Hennie le Roux
11/11/2008	Extension workshop	SWOT analysis	Hennie le Roux Hannes Bester Paul Fourie

			Tim Grout Sean Moore Stephan Verreyne
	XSIT Stellenbosch	Amptelike openingsgeleentheid	Hennie le Roux Hannes Bester
14/11/2008	Waterberg Sitrusstudiegroep Mokopane	Oorsig van sitrus wereldwyd	Hennie le Roux
19/11/2008	Magalies Brits	Winterveldt BEE projek	Hennie le Roux
20/11/2008	CRI Raadsvergadering Johannesburg	Raadsvergadering	Hennie le Roux
21/11/2008	FCM Task Team Sondagsrivier	GAP's en SOP's	Hannes Bester
25/11/2008	Mpumalanga Agri- Sector Forum Witbank	Grond en Landbou hervormingsprogram	Hennie le Roux
26/11/2008	Patologievergadering Nelspruit	Situsviroiede	Hennie le Roux CRI Patoloee
27/11/2008	Viroied Schoemanskloof	Ondersoek Rustenburg Nawels vir simptome van viroiede	Fanie v vuuren Hennie le Roux
	ETP Meeting	Verpakking Gehalte- terugvoer	Hannes Bester
02/12/2008	Watnavorsings-kommissie Pretoria	Werkswinkel	Hennie le Roux
03/12/2008	Limpopo Departement van Landbou Polokwane	Nabetragting van Voorligtingspoging vir BEE produsente gedurende 2008	Andrew Mbedzi Hennie le Roux
04/12/2008	Lisbon Landgoed Hazyview	Besoek saam met Limpopo Grondeise kommissaris	Hennie le Roux Andrew Mbedzi
08/12/2008	Marble Hall	Vergadering met Maf Roda re 6de Sitrusnavorsingsimp	Hennie le Roux Hannes Bester Henry Skinner
10/12/2008	Verpakkings-werkgroep: Pretoria	Verpakkings spesifikasies	Hannes Bester
11/12/2008	Twycross Landgoed	Kultivaraanbevelings	Johan Joubert Hennie le Roux
	Baviaans studiegroep	Oorsig oor 2008	Hannes Bester
TRANSFORMATION EXTENSION (A. Mbedzi)			
Date	Venue	Reason for the Meeting	
10/01/2008	Limpopo/Madzivhandila College	CGA, CRI and LDA MoU and Identification of Citrus Coordinators	
05/02/2008	Limpopo/Lungane Farm	Skills Assessment with Citrus Academy	
06/02/2008	Limpopo/Lungane Farm	Skills Assessment with Citrus Academy	
07/02/2008	Limpopo/Makwarela	Discussions on the identification of Coordinators and Channels to follow when meeting the Citrus Coordinators.	
19/02/2008	Mpumalanga/Emnotwen Sun/Nelspruit	Discussion between CRI and LDA about the MoU and Identification of the Citrus Coordinators in the Districts and Sub-districts	
29/02/2008	Mpumalanga/CRI Offices/Nelspruit	SAPIP Evaluation by the EU Evaluators.	
04/03/2008	Limpopo/Tzaneen (Hlanganani and Mariveni Farm)	SAPIP Evaluation by the EU Evaluators	
06/03/2008	Limpopo/Giyani	Discussions on the identification of Coordinators and Channels to follow when meeting the Citrus Coordinators	
10/03/2008	Limpopo/Madzivhandila College	CGA, CRI and LDA MoU and Identification of Citrus Coordinators	
09/04/2008	Gauteng/Magaliesburg Processing	Organising Mentorship Programme for Winterveldt	

	Company	Citrus Project.
09/04/2008	Limpopo/Thabazimbi/ Dept. of Agriculture	Meeting the Extension Officer and the farm with regard to citrus production of the area
10/04/2008	Gauteng/O.R. Tambo Airport	CGA Transformation for the Emerging Citrus Farmers
28/05/2008	Limpopo/Phalaborwa/ Mashishimale Farm	To Discuss the Training and Production Improvement of the Citrus Farm
29/05/2008	Limpopo/Thohoyandou/Dept. of Agriculture	To discuss the Citrus Extension Programme
30/05/2008	Limpopo/Makwarela/Dept. of Agriculture	Discussion on the Existing Study Group Involvement and Formation of New study Groups
20/06/2008	Mpumalanga/Nelspruit/ CRI offices	CRI Staff Meeting
25/06/2008	Limpopo/Polokwane/ Dept. of Agriculture	Meeting with LDA and CGA to Discuss Approaches and Communications Channels with Citrus Coordinators
16/07/2008	Limpopo/Vhembe/Lungane Citrus Farm	Helping out the extension officer with the marketing of oranges
17/07/2008	Limpopo/Phalaborwa/Mogotle Citrus	Accompanied by Manger Extension to help solve extension problems.
25/07/2008	Limpopo/Vhembe/ Easy Farm	Help with the transport problems for delivering citrus fruit at the Port in Durban
28/07/2008	Limpopo/Letsetele/ Risavi Citrus Farm	Help Organise the processing market with the Extension Officer
18/08/2008	Mpumalanga/Malelane/Mhlaba Farm & Cairns Lemon Farm	Mentorship Programme Assessment with Dr. Richard Bates, Bruce Andrews & Frank Fakude
19/08/2008	Mpumalanga/Eilandshoek/Sibonelo Farm	Mentorship Programme Assessment with Dr. Richard Bates & Bruce Andrews
20/08/2008	Limpopo/Mopani/Mabunda & Mariveni	Mentorship Programme Assessment with Dr. Richard Bates & Melton Mulaudzi
21/08/2008	Limpopo/Vhembe/Easy Farm/Lungane Farm	Mentorship Programme Assessment with Dr. Richard Bates & Melton Mulaudzi
28/08/2008	Limpopo/Mopani/Homu Irr. Scheme	Investigate Possibility of Establishing Citrus Crop.
03/09/2008	Limpopo/Vhembe/Tshipise	Tshipise Study Group Symposium Report back
16/09/2008	Limpopo/Mopani/Junction	Constantia Study Group Report back
22/09/2008	Mpumalanga/ Nelspruit/CRI Offices	Meeting between CRI and Land Claims Commission over Ndwandwa lemon farms
25/09/2008	Mpumalanga/Gert Sibande/Badplaas	Visit with CRI Manager Extension and Land Commission to establish the state of the Ndwandwa Lemon Farms
06/10/2008	Limpopo/Mopani/ Homu Irrigation Scheme	Visit Giyani Helping out with the possibility of establishing citrus at Homu Irrigation Scheme
16/10/2008	Limpopo/Sekhukhune/ Groblersdal	Meeting between CRI, LDA and Citrus Academy to discuss the training of the Citrus Coordinators (Extension Officers)
17/10/2008	Mpumalanga/Nelspruit/ CRI Offices	Meeting between, CRI, NDA and CGA for the Mentorship Programme Assessment
27/10/2008	Mpumalanga/Nelspruit/Schagen Offices	Visit aim was to help with the Interview of the Sibonelo (Eilandshoek lemon farm) managerial position.
17/11/2008	Limpopo/Mopani/Letsitele/Du Roi IPM and Nursery	Collecting the Fungi and meeting the CGA mentor.
20/11/2008	Mpumalanga/Badplaas/Ndwandwa Citrus Farms	Accompanying the NDA to visit the Ndwandwa Citrus farms
03/12/2008	Limpopo/Carpricorn/ Polokwane	Meeting between CRI and LDA on MoU Progress Report.
10/12/2008	Mpumalanga/Hazyview/Lisbon Estate	Visit the Lisbon Estate with CRI Extension manager and Land Claims Commissioner Project Coordinator to observe the condition of the farm

9.6 OTHER MEANS OF TECHNOLOGY TRANSFER

9.6.1 SA Fruit Journal by Tim G Grout (CRI)

The SA Fruit Journal is distributed to every citrus grower who is paying the levy on export citrus because the subscription is paid out of the levy funds. It therefore is one of the best means of transferring technology on technical issues. Bimonthly Extension Briefs are provided as reminders for growers of practices that need to be implemented at that time. These are edited by Hennie le Roux and Hannes Bester and all researchers contribute to these on a regular basis. In-depth, semi-scientific research articles are also provided that are usually of a practical nature and other topical or news articles are sometimes included. The citrus articles published in the SA Fruit Journal during 2008/9 are listed in Table 9.6.1.1. Due to the lag time of two months between submission of the articles and circulation of the journal, urgent information is circulated to growers as Cutting Edge or Snykant articles via CRInet and emails to the technology transfer groups.

Table 9.6.1.1. S.A. Fruit Journal articles by CRI group members during 2008/9.

Issue	Article	Author
April/May 08	CRI appoints Transformation Extension Officer	H.F. le Roux & J.J. Bester
	Tydeoid mites on citrus in southern Africa	T.G. Grout
June/July 08	Sitrusboomsertifisering	M.N.N. du Toit
	Factors affecting sheeppnose incidence in grapefruit	S. Verreynne
	Voorseisoen Pakhuiswerkwinkels herleef soos in die verlede	J.J. Bester
	Plaaslike Sitrus Mutasies – ‘n Nuwe insentief / Local Citrus Mutations – A New Incentive	A. Lee
	5 th Citrus Research Symposium	H.F. le Roux
	Citrus Research Focus for 2008-9	T.G. Grout & V. Hattingh
Aug/Sept 08	Recent CRI research appointments	
	Sitrus Verbeteringskema Administrateur	M.N.N. du Toit
	Thresholds and guidelines for intervention against citrus pests	S. Moore, T. Grout, V. Hattingh & H. Hofmeyr
Oct/Nov 08	An inexpensive ant bait station	T.G. Grout
	Sitrusverbeteringskema tegnikus	M.N.N. du Toit
	5 th Citrus Research Symposium 2008	T.G. Grout & P. Stephen
Dec 08/Jan 09	Citrus orchard sanitation with emphasis on false codling moth control	S. Moore & W. Kirkman
	Awards presented by Citrus Research International	
	Clementine selections evaluated in the cool inland area of Burgersfort	J. Joubert & A. Lee
Feb/March 09	Combating the African Invader fly <i>Bactrocera invadens</i>	A. Manrakhan, T. Grout & V. Hattingh

9.6.2 CRI website by Tim G Grout (CRI)

The usage of the website remains fairly stable with only slight fluctuations from month to month (Table 9.6.2.1). Apart from requests from unknown IP addresses, dot-com and dot-net domains, South African domains were the highest. Other countries in order of decreasing page requests from our website were Argentina, Spain, Germany, Russian Federation, India and Brazil. Access to technical information such as the Integrated Production Guidelines remains limited to residents of southern Africa. Some updated guidelines were uploaded during the course of the year in addition to various reports and publications.

Table 9.6.2.1. Visits and page requests on www.cri.co.za since April 2008.

Month	Unique visitors	Number of visits	Pages	Hits
Apr 2008	578	827	3580	16698
May 2008	600	894	4819	19340
Jun 2008	633	904	4102	16291
Jul 2008	710	1051	4865	22235
Aug 2008	563	957	4453	17788
Sep 2008	573	846	3851	16511
Oct 2008	757	1117	6196	26017
Nov 2008	632	1030	4794	15442
Dec 2008	518	970	4101	13101
Jan 2009	645	1055	5316	16099
Feb 2009	698	1129	8515	25963
Mar 2009	886	1430	9288	30546
Total	7793	12210	63880	236031
Mean/month	649.4	1017.5	5323.3	19669.3

9.6.3 CRInet by Tim G Grout (CRI)

The number of messages circulated on CRInet during 2008/9 increased slightly from the previous year but is still a fraction of what it was four or five years ago (Table 9.6.3.1). The reduced number of emails is due to fewer emails originating from people outside of CRI which hopefully indicates that other methods of technology transfer are being more effective than in the past. The number of people belonging to CRInet is 280.

Table 9.6.3.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2009	1	7	3										
2008	3	6	1	8	5	2	7	3	3	5	3	4	50
2007	5	2	7	1	1	2	4	2	5	4	3	3	39
2006	18	3	1	2	13	9	9	2	1	2	13	2	75
2005	14	11	3	3	3	14	8	3	23	5	11	5	103
2004	7	26	13	28	27	26	12	9	15	12	12	0	187

9.6.4 Cutting Edge by Tim G Grout (CRI)

During 2008/9, issues 61 to 79 were circulated via email and made available on the CRI website. The titles covered during this period are given in Table 9.6.4.1. Most were involved with residue issues.

Table 9.6.4.1. Cutting Edge issues during 2008/9.

No.	Title	Issue	Author
61	MRL (PHI) March 2008	April 08	P. Hardman
62	Management of Imazalil concentration in fungicide baths	April 08	K. Lesar
63	Responsible use of fungicides in citrus packhouses	May 08	P. Fourie K. Lesar
64	Problems with fruit fly control related to birds	May 08	S. Moore A. Manrakhan
65	Methomyl residue trial results on Southern African citrus fruit	June 08	P. Hardman
66	Restriction on Sodiame Ortho Phenyl Phenol (SOPP)	July 08	P. Hardman
67	Post Harvest decay warning 2008	July 08	K. Lesar

68	MRL matters – SOPP and Malathion	July 08	P. Hardman
69	Philabuster Recommendations	July 08	K. Lesar P. Fourie
70	Update on EU MRL status for some Plant Protection Products	Sept 08	P. Hardman V. Hattingh
71	Precaution on use of Dichlorprop (Corasil E)	Oct 08	P. Hardman V. Hattingh
72	Fruit size Management Strategies on Citrus	Oct 08	S. Verreyne
73	A team effort is needed for the control of CBS in the E. Cape	Oct 08	G.C. Schutte D. Gerber
74	Amendment to the Fenpropathrin (Meothrin) Usage Restriction	Nov 08	P. Hardman V. Hattingh
75	Compatibility of Cryptogran with Agro-chemicals	Dec 08	S. Moore
76	Amendment to the Chlorfenapyr (Hunter) Recommended Usage Restrictions	Dec 08	P. Hardman V. Hattingh
77	Preventing the spread of the invasive Fruit Fly, <i>Bactrocera invadens</i> in southern Africa	Dec 08	A. Manrakhan
78	Detection surveys for the new invasive Fruit Fly, <i>Bactrocera invadens</i>	Jan 09	T. Grout A. Manrakhan
79	Summary of known management practices to reduce common citrus fruit rind disorders	March 09	P.J.R. Cronjé

10 PUBLICATIONS IN 2008/9

10.1 Refereed publications (or ISI ranked journals)

- Doddapaneni, H., Liao, H., Lin, H., Bai, X., Zhao, X., Civerolo, E.L., Irely, M., Coletta-Filho, H., and Pietersen, G., 2008. Comparative phylogenomics and multi-gene cluster analyses of the Citrus Huanglongbing (HLB)-associated bacterium *Candidatus Liberibacter* BMC Research Notes 1(75).
- Fourie, P.H., M. du Preez, J.C. Brink and G.C. Schutte. 2008. The effect of run-off on spray deposition and control of *Alternaria* brown spot of mandarins. *Australasian Plant Pathology* 38: 173-182.
- Pietersen, G., Arrebola, E., Bové, J-M., Breytenbach, J.H.J., Korsten, L., Le Roux, H.F., La Grange, H., Lopes, S.A., Meyer, J.B., Pretorius, M.C., Schwerdtfeger, M., Van Vuuren, S.P., and Yamamoto, P. 2009. Survey for "*Candidatus*" *Liberibacter* species in South Africa confirms the presence of only *Ca. L. africanus* in commercial Citrus. (Accepted by Plant Disease).
- Stotter, R.L., Terblanche J.S., 2009. Low-temperature tolerance of false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa. *Journal of Thermal Biology* (In press).
- Van Vuuren, S.P., J.G.J. Maritz and N. Combrink. 2009. *Citrus tristeza virus* cross-protection of 'Palmer' navel orange in the Eastern Cape Province of South Africa. *S. Afr. J. Plant & Soil* 2009, 26(2): 98-101.
- Verreyne, J.S. and C.J. Lovatt. 2009. The effect of crop load on bud break influences return bloom in alternate bearing *Citrus reticulata* (Blanco). *Amer. J. Hort. Sci.* 134(3): 299-307.

10.2 Semi-scientific publications

- Grout, T.G. and V. Hattingh. 2008. Citrus research focus for 2008-9. *SA Fruit J.* 7(3): 26-29.
- Grout, T. and P. Stephen. 2008. 5th Citrus Research Symposium. *SA Fruit J.* 7(5): 55-57.
- Grout, T.G. and E.A. Ueckermann. 2008. Tydeoid mites on citrus in southern Africa. *SA Fruit J.* 7(2): 47.
- Manrakhan, A., T.G. Grout & V. Hattingh, 2009. Combating the African Invader fly, *Bactrocera invadens*, *SA Fruit J.* 8(1):57-61.
- Moore, S., T. Grout, V. Hattingh, H. Hofmeyr. 2008. Thresholds and guidelines for intervention against citrus pests. *SA Fruit J.* 7(4): 77-78, 80-81.
- Moore, S.D. & W. Kirkman. Citrus orchard sanitation with emphasis on false codling moth control, *SA Fruit J.* 7(6):57-60.
- Stotter, R.L. 2009. Spatial and Temporal Distribution of False Codling Moth across landscapes in the Citrusdal area (Western Cape Province, South Africa). MSc Thesis Dissertation, University of Stellenbosch (Graduated *cum laude* March 2009).
- Verreyne, J.S. 2008. Factors affecting sheepsnose incidence in grapefruit, *SA Fruit J.* 7(3):16-18.

11 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

- Brink, J.C., G.C. Schutte & P.H. Fourie. 2009. Influence of selected adjuvants on fungicide spray retention on Satsuma Mandarin leaves. Oral presentation at 46th Congress of the SASPP, Gordon's Bay (25-28 January 2009).
- De Villiers, M. & Hattingh, V. 2008. Distribution of Natal fruit fly, *Ceratitis rosa* Karsch (Diptera: Tephritidae), and false codling moth (*Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), two pests of citrus, in South Africa. XXIII International Congress of Entomology, 6-12 July 2008, Durban South Africa: cd rom. (Conference poster).
- Fourie, P.H., J.C. Brink, G.C. Schutte & T.G. Grout. 2009. Efficiency and uniformity of fungicide spray deposition in citrus orchards. Oral presentation at 46th Congress of the SASPP, Gordon's Bay (25-28 January 2009).
- Fourie PH, Brink JC, van Zyl S, Schutte T. 2008. Improving fungicide application: reality, options and impact. Invited presentation at Western Cape branch symposium of SASPP, Stellenbosch, 8 May 2008.
- Fourie PH, Brink JC, van Zyl S, Schutte T. 2008. Improving fungicide application: reality, options and impact. Invited presentation at Northern branch symposium of SASPP, Pretoria, 29 May 2008.
- Fourie, P.H., M. du Preez, J.C. Brink and T. Schutte. The effect of run-off on spray deposition on citrus leaves and fruit and control of *Alternaria* brown spot of mandarins. 11th Int. Soc. Citriculture Congress, 26-30 Oct 2008, Wuhan, China.
- Grout, T.G., J.H. Hofmeyr and S.D. Moore. 2008. Changes to Citrus IPM strategy in southern Africa. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa, p 1318.
- Grout, T.G., S.D. Moore, J.H. Hofmeyr and V. Hattingh. The evolution of citrus IPM in southern Africa. 11th International Citrus Congress, 26-30 October 2008, Wuhan, China.
- Hattingh, V. and T.G. Grout. Conflict between some market forces and scientific developments that drive the development of future citrus production and fruit handling practices. 11th International Citrus Congress, 26-30 October 2008, Wuhan, China.
- Keeton, K.L., Sishuba, N., Moore, S.D. & Villet, M.H. 2008. Rates of larval parasitism of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), on citrus in South Africa. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa p 718.
- Kirkman, W., Moore, S. & Malan, A. 2008. Realities and prospects for microbial control of citrus pests in South Africa. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa.
- Kirkman, W. 2008. Understanding and improving the residual efficacy of the *Cryptophlebia leucotreta* granulovirus. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa.
- Malan, A.P. & Moore, 2008. Entomopathogenic nematodes for the control of false codling moth. IPM meeting, Stellenbosch.
- Malan, A.P. & S.D. Moore, 2008. Potential of entomopathogenic nematodes for the control of false codling moth, *Thaumatotibia leucotreta*, (Lepidoptera: Tortricidae) in laboratory bioassays. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban South Africa, p 563.
- Meitz, J., Pretorius, M.C., Z. Buhlungu, Z, Botha, W.J., Huisman, L., Langenhoven, S. and McLeod, A. (2009). (Poster). A survey of *Phytophthora* species on citrus in South Africa, and the development of a real-time PCR method for detection of citrus *Phytophthora* species from soil. January 2009, South African Plant Pathology meeting, Gordon's Bay.
- Moore, S.D. 2008. Analysis of a market-focussed biorational product business. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa, p 1331.
- Moore, S.D., Kirkman, W. & Chambers, C. 2008. Insect rearing for commercial virus production. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa, p 1338.
- Moore, S.D., Malan, A.P., Kirkman, W., Grout, T.G. & Goble, T. 2008. Realities and prospects for microbial control of citrus pests in South Africa. Proceedings of XXIII International Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa, p 1331.
- Mupambi, G., Verreynne, J.S. Studies to reduce the size of the navel end opening. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.
- Opoku-Debrah, John, K. 2008. Geographic variation in the susceptibility of *Thaumatotibia leucotreta* (FCM) populations to a granulovirus (CrleGV-SA) in South Africa. Proceedings of XXIII International

- Congress of Entomology, 6-12 July 2008, International Convention Centre, Durban, South Africa, p 1708.
- Phiri, Z.P., Verreyne, J.S. Studies in citrus creasing/albedo breakdown. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.
- Pietersen, G., Kotze, A., Phahladira, M.N.B. and Schwerdtfeger, M. 2008. Survey for “*Candidatus*” *Liberibacter* species in South Africa. HLB International Conference, Orlando, Florida, USA, 1-5 December, 2008.
- Pretorius, M.C. 2009. *Phytophthora citrophthora*, the cause of trunk and branch canker of Clementine mandarins in South Africa and Spain. 46th Congress of the South African Society for Plant Pathology, 25-28 January 2009, Gordon’s Bay, South Africa.
- Schutte, G.C., C. Kotze & M.C. Pretorius, 2008. (Poster). Identification and control of *Phytophthora citrophthora*, the cause of a new trunk disease of Clementines in South Africa. International Congress for Plant Pathology, Turin, Italy.
- Scott, K.A. & Pietersen, G. 2009. Strain differentiation by PCR and DNA microarray of Citrus Tristeza Virus isolates in South Africa. 46th Congress of the South African Society for Plant Pathology, 25-28 January 2009, Gordon’s Bay, South Africa.
- Verreyne, J.S., van Kerwel, W. The benefits of hand thinning Nules Clementine mandarins (Poster). Proceedings of the 11th International Citrus Congress. Wuhan, China, 26-30 October 2008. (In press).
- Verreyne, J.S. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges - A preliminary study. Proceedings of the 11th International Citrus Congress. Wuhan, China, 26-30 October 2008. (In press).
- Verreyne, J.S., van Kerwel, W. The benefits of hand thinning Nules Clementine mandarins (Poster). 11th International Citrus Congress. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Stellenbosch, 19-22 January 2009.