



**CRI GROUP  
ANNUAL RESEARCH  
REPORT  
2007-8**



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Citrus Research International, Nelspruit

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## 1 INTRODUCTION

CEO (CRI): Vaughan Hattingh

A change was made to the annual report period so that in future it will coincide with the financial year and consequently, to make the transition, this report spans the 15 month period from January 2007 to end March 2008. The CRI Group approach to coordination of industry research was maintained, including the use of specialist advisory committees and the structuring of CRI's operations in the following divisions: Research, Extension, Citrus Improvement Scheme and Cultivar Development, all with an overarching Market Access priority.

CRI continued to pursue a diversified income stream to augment the core funding provided by the levy on export citrus. CRI's direct income from the commercialization of Intellectual Property continued to provide a valuable funding stream. The SA-EU Pesticide Initiative Programme (PIP) came to an end, but funding proposals were submitted to a new Post Harvest Innovation Fund, provided by the Department of Science and Technology through the initiative of the Fresh Produce Exporters Forum. The citrus industry's technology implementation company, River Bioscience, continued to perform strongly with the prospect of providing additional research funding in the future. The False Codling Moth Sterile Insect Technique was implemented over 1500ha in the Western Cape by Xsit, a subsidiary of River Bioscience. CRI continued to operate the Citrus Foundation Block with a financial self-sufficiency objective and there was a partial recovery in sales. Strong rootstock seed sales during this period indicated the prospect of a stronger recovery in budwood sales in the near future.

CRI expenditure was below budget, primarily due to the existence of a number of vacant posts during the report period. Efforts to fill some of the positions were frustrated by the shortage of available and suitable skills. However, by the end of the report period, only a citrus nutritionist position remained unfilled. A new post was included in CRI's Extension brief, namely an Industry Transformation Extensionist. This post is funded directly by CGA, but is operated by CRI and housed within the CRI Extension Division. The objective of the position is to facilitate integration of new entrant farmers into the industry's technical support structures, including the regional Technology Transfer Groups.

Gaining, retaining and optimising access to markets remained a strategic CRI priority. Progress was made in many Market Access endeavours, including: Europe, Japan, USA, South Korea, Thailand, Australia and other markets. More than 10 years of sustained effort culminated in opening of the Japanese market for SA Clementines in 2007.

A strong focus of attention and resources in the Integrated Pest Management Programme continued to be directed towards improved management of FCM. FCM is recognized as being a research area of top strategic significance to the industry because of its potential implications for access to export markets. Research on a wide range of issues pertaining to fruit flies was also undertaken. This included pre-emptive development of management strategies for exotic fruit flies that do not as yet occur in SA, but may establish in SA in the future. This is strategically important work and aims to minimize the potential detrimental impact of such future developments on the industry's access to export markets.

Within the Disease Management Programme, CBS continued to be a high priority, but here the focus shifted from basic research to Market Access Technical Coordination inputs required to address the phytosanitary regulatory issues. Post Harvest Pathology continued to be a strong focus area in light of the ongoing losses that the industry experiences due to post-harvest decay. Strong research and technical inputs were made in the Graft Transmissible Disease area in recognition of the critical role that this branch of pathology plays in Biosecurity of the industry.

Rind condition research within the Crop and Fruit Quality Management Programme, continued to progress with the prospect of useful practical applications in the future. Within the Production and Quality project, research focused on aspects of improving colour, fruit size and crop load optimization, irrigation optimization and potential manipulation of the size of navel end openings. Unfortunately 2007 research results indicated that sensitivity of citrus fruit rind to a range of irradiation dosages presents a serious constraint to the potential commercial application of this technology as a disinfestation treatment.

CRI Extension made excellent progress in coordinating the establishment and commencement of activities of the Citrus Cold Chain Forum (CCCF). The following structures were put in place to establish the forum: the Packhouse & Handling Panel, the Packaging Working Group, the Exporters Technical Panel, and CRI's Cold Chain Research Project. The formation of the Packhouse & Handling Panel was a particularly noteworthy development. Five regional Packhouse Study Groups were formed to constitute this Panel and the first

round of annual pre-season meetings was held in 2007, with great success. However, sustainability of the CCCF initiative is dependant on adequate resourcing of its structures.

Potential conversion of the Citrus Improvement Scheme to a statutory scheme was frustrated by resignation of the relevant responsible official in the DoA. However, progress was made in developing amendments to the National Variety Listing procedures, such that these would represent less of an obstacle to potential future implementation of the scheme on a statutory basis.

CRI's approach to Cultivar Development was reviewed, culminating in the CRI Board of Directors approving a revised policy guideline that was endorsed by the CGA. The objective of the revised policy is to strengthen focus on the development of cooperation among all industry role players in the cultivar development arena.

The 2007 export season was marked by record volumes, with approximately 90 million 15 kg equivalent cartons being exported. Prices remained strong except for the tail end of the Valencia season. Conditions for production in 2007 were generally good, creating good prospects for the 2008 export season.

## **INLEIDING**

Die jaar se verslagdoeningstydperk is gewysig om in die toekoms saam te val met die finansiële jaar en om gevolglik vir hierdie omskakeling voorsiening te maak, strek hierdie verslag oor 'n tydperk van 15 maande vanaf Januarie 2007 tot Maart 2008. Die CRI Groep se benadering tot die koördenering van die bedryf se navorsing is gehandhaaf deur gebruik te maak van spesialis adviserende komitees en die strukturering van CRI se werksaamhede in die volgende afdelings: Navorsing, Voorligting, Sitrusverbeteringskema en Kultivarontwikkeling, met 'n oorkoepelende marktoegansprioriteit.

CRI het volgehou om 'n diverse bron van inkomste te bekom om die kernfondse wat van die heffing op uitvoersitrus afkomstig is, aan te vul. CRI se direkte inkomste vanaf die kommersialisering van Intellektuele Eiendom het weereens voorsien in 'n waardevolle inkomstebron. Die "SA-EU Pesticide Initiative Programme (PIP)" het tot 'n einde gekom, maar voorleggings vir befondsing is aan 'n nuwe "Post Harvest Innovation Fund" voorgelê. Hierdie fondse is deur die Departement van Wetenskap en Tegnologie beskikbaar gestel deur die inisiatief van die Fresh Produce Exporters Forum. Die maatskappy van die sitrusbedryf wat verantwoordelik is vir die kommersialisering van tegnologie, River Bioscience, presteer steeds goed en die vooruitsigte is daar om addisionele befondsing vir navorsing in die toekoms te verskaf. Die Valskodlingmot Steriele Insek Tegniek is oor 1500ha in die Wes-Kaap deur Xsit, 'n filiaalmaatskappy van River Bioscience, geïmplementeer. CRI bedryf nog steeds die Sitrus Grondvesblok met 'n doelwit van finansiële selfvoorsiening en daar was ook 'n gedeeltelike herstel in verkope. Goeie verkope van onderstamsaad gedurende hierdie periode dui op vooruitsigte van 'n sterker herstel in enthout-verkope in die nabye toekoms.

CRI se uitgawes was onder begroting en kan hoofsaaklik toegeskryf word aan die aantal vakante poste in hierdie verslagtydperk. Die tekort aan beskikbare en geskikte vaardighede het pogings om van die posisies te vul, gekortwiek. Teen die einde van hierdie verslagtydperk was egter slegs die pos van 'n sitrusvoedingskundige nog vakant. 'n Nuwe pos, naamlik Transformasie Voorligter, is in CRI se Voorligtingsopdrag ingesluit. Hierdie pos word deur CGA befonds, maar deur CRI bedryf en is binne die CRI Voorligtingsafdeling gesetel. Die doel van die posisie is om die integrasie van nuwe inkomende produsente in die tegniese ondersteuningstrukture, insluitende die Tegnologie Oordraggroepe in die streke, te fasiliteer.

Om markte te verkry, te behou en te verbeter bly 'n strategiese prioriteit van CRI. Vordering is met baie marktoegangsaksies insluitende Europa, Japan, VSA, Suid-Korea, Thailand, Australië en ander markte gemaak. Meer as 10 jaar se volgehoue pogings het daartoe gelei dat die Japanese mark vir SA Clementines in 2007 geopen is.

Die fokus en hulpbronne van die Geïntegreerde Plaagbestuur Program is steeds daarop gerig om die bestuur van VKM te verbeter. VKM word gereken as 'n strategiese belangrike navorsingsarea van die bedryf weens die potensiële implikasies vir toegang na uitvoermarkte. Navorsing oor 'n wye reeks van vrugtevlieg aangeleenthede is ook onderneem. Dit het voorkomende ontwikkeling van bestuursstrategieë vir eksotiese vrugtevlieë wat nog nie in SA voorkom nie, maar wat in die toekoms in SA kan vestig, ingesluit. Dit is strategiese belangrike werk en is daarop gemik om die potensiële nadelige impak van sulke toekomstige gebeurtenisse op die bedryf se toegang na markte te verminder.

In die Siektebestuur Program geniet SSV ("CBS") nog steeds 'n hoë prioriteit maar die fokus het van basiese navorsing na Tegniese Marktoegang Koördeneringsinsette wat benodig word om die fitosanitêre regulatoriese sake aan te spreek, verskuif. Gesien in die lig van die voortgesette verliese wat die bedryf ondervind weens bederf, word daar nog sterk op Na-oes Patologie gefokus. Goeie navorsing en tegniese

insette is deur die Afdeling - Entoordraagbare Siektes gemaak vanweë die kritiese rol wat hierdie vertakking van Plantpatologie in die Biosekuriteit van die bedryf speel.

Navorsing op skilkondisies binne die Oes en Vrugkwaliteitsbestuur Program het met die vooruitsigte van praktiese toepassings in die toekoms, voortgegaan. In die Produksie en Kwaliteitsprojek is navorsing op die verbetering van kleur, vruggrootte en verbetering van drag en verbetering van besproeiing asok potensiele manipulerings van nawelend groottes gerig. Ongelukkig het 2007 se navorsingsresultate daarop gedui dat die sensitiwiteit van die skil van sitrusvrugte vir 'n reeks van bestralingsdosisse, 'n ernstige stremming vir die moontlike kommersiële toepassing van hierdie tegnologie as 'n disinfestasiebehandeling kan wees.

CRI Voorligting het uitstekende vordering gemaak deur die aktiwiteite vir die stigting en inwerkingtreëding van die Sitrus Koueketting Forum (SKKF) te koördineer. Die volgende strukture is in plek geplaas: Die Paneel vir Pakhuise en Hantering, die Verpakkingswerkgroep, die Uitvoerders Tegnieke Paneel en CRI se Koueketting Navorsingsprojek. Die vorming van die Paneel vir Pakhuise en Hantering was veral noemenswaardig. Vyf Pakhuis-Studiegroepe is in die streke gevorm om hierdie Paneel te konstitueer. Die eerste rondte van die jaarlikse voorseisoen vergaderings het met groot sukses, in 2007 plaasgevind. Voortbestaan van die SKKF-inisiatief is egter afhanklik van genoegsame befondsing van sy strukture.

Moontlike omskakeling van die Sitrusverbeteringskema in 'n statutêre skema is gekniehalter deur die bedanking van die verantwoordelike beampte by die Department van Landbou. Vordering is nogtans met die wysigings aan die prosedures vir Nasionale Variëteitslysing gemaak tot so 'n mate dat dit minder van 'n struikelblok sal wees indien die skema moontlik in die toekoms op 'n statutêre grondslag geïmplementeer gaan word.

CRI se benadering tot Kultivarontwikkeling is hersien en het daartoe gelei dat CRI se Raad van Direkteure 'n hersiene beleidsprosedure goedgekeur het wat deur die CGA onderskryf is. Die doelwit hiervan is om die fokus op die ontwikkeling van samewerking tussen al die rolspelers in die bedryf se kultivarontwikkelingsarena te versterk.

Die 2007 uitvoerseisoen is deur rekord volumes gekenmerk met die uitvoer van byna 90 miljoen 15 kg ekwivalente kartonne. Die pryse was hoog behalwe teen die einde van die Valencia seisoen. Toestande vir produksie in 2007 was oor die algemeen goed wat dus goeie vooruitsigte vir die 2008 uitvoerseisoen skep.

## **2 PROGRAMME: MARKET ACCESS TECHNICAL COORDINATION**

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

### **2.1 PROGRAMME SUMMARY**

Progress was made in improving access to China through authorisation to include additional farms, packing facilities and ports of arrival in the programme. Engagement between SA and the EU on the EU's CBS phytosanitary regulations progressed to SA submitting a final response to EU queries, and a call to finally resolve the issue or refer it to third party mediation. Surveys were initiated to include all of the Western Cape Province as an official CBS pest free area. Quality control inspection procedures were amended to better manage the risk of FCM infested fruit entering the EU market. SA challenged the technical validity of an EU interception report for non-Mediterranean fruit flies in SA citrus. Surveillance trapping was initiated in anticipation of the potential incursion of the fruit fly *Bactrocera invadens* from SA's northern neighbouring countries. The Japanese market was opened for export of SA Clementines in culmination of more than 10 year's research and bilateral negotiation. Progress was made towards amending the time temperature protocols for the export of all citrus types to Japan. Technical input was provided in support of SA's proposals to broaden the scope of citrus cultivars (both navels and soft citrus) permitted for export to Japan. There was extensive engagement with USA to address the many outstanding issues curtailing improved access to this market. These issues included the duration of cold treatment, inclusion of additional pest free areas and inclusion of pest-free-places-of-production. Protocols for the registration of CBS-secure citrus nurseries were developed and relevant amendments made to Control Measures pertaining to restrictions on the movement of citrus propagation material. An application was lodged with South Korea to commence exporting soft citrus to this market. The validity of South Korea's cold treatment requirements was challenged. Technical justification was provided in support of the SA proposals to include lemons and Grapefruit in the South Korean export programme and to apply a systems approach to mitigation measures for FCM (excluding extensive cold treatment). CRI formulated a technical response, with supportive data, to the draft protocol for export of SA citrus to Thailand. A renewed request to pursue access to the Israeli market was assessed. A data pack was provided to Australia in response to its queries relating to SA's request to open this market for SA citrus exports. The processes of gaining official access to the following markets received attention: Vietnam, Lebanon, Jordan, Syria and Malaysia.

## PROGRAMOPSOMMING

Vordering is met die verbetering van marktoegang na China gemaak met die goedkeuring om addisionele plase, pakhuse en hawens in die program in te sluit. Betrekkinge tussen SA en die EU oor die EU se fitosanitêre regulasies vir SSV het gevorder tot 'n finale voorlegging aan die EU. Hierin is die bekommernisse van die EU aangespreek met 'n beroep op 'n finale oplossing in die saak of 'n verwysing na 'n derde party bemiddeling. Opnames om die totale Wes-Kaap Provinsie as 'n amptelike SSV siektevrye area in te sluit, is onderneem. Prosedures vir gehalte-inspeksies om die risiko van VKM besmette vrugte na EU markte beter te kan bestuur, is aangepas. SA het die tegniese geldigheid van EU verslae oor die voorkoms van nie-Mediterreense vrugtevlieë in SA sitrus bevestig. Waarskuwingslokvalle vir die moontlikheid van die potensiële binnekoms van die vrugtevlieë, *Bactrocera invadens* vanaf SA se noordelike buurlande, is uitgeplaas. Die Japanese mark is vir die uitvoer van SA Clementines, na meer as 10 jaar se volgehoue navorsing en bilaterale onderhandelinge, geopen. Vordering met die wysiging aan die tyd temperatuurprotokolle vir die uitvoer van alle sitrustipes na Japan is gemaak. Tegniese insette ter ondersteuning van SA se aansoeke om die reeks van sitruskultivars na Japan uit te brei (beide nawels en sagte sitrus), is gelewer. Daar is intensiewe samesprekings met die VSA gehou om die vele uitstaande sake wat beter toegang tot die mark belemmer, aan te spreek. Hierdie sake sluit in die tydsduur van die koue behandeling, die insluiting van addisionele pesvrye areas en die insluiting van pes-vrye-plekke-van-produksie. Protokolle vir die registrasie van SSV-veilige sitruskwekerie is ontwikkel en relevante wysigings is aan die Beheermaatreëls, soos van toepassing op die beperking van beweging van sitrus voortplantingsmateriaal, aangebring. 'n Aansoek om sagte sitrus na Suid-Korea uit te voer, is ingedien. Die geldigheid van die vereistes van die Suid-Koreaanse koue behandeling is bevestig. Tegniese regverdiging ter ondersteuning van SA se voorleggings om suurlemoene en pomelos in die Suid-Koreaanse program in te sluit is verskaf, asook 'n sisteembenadering vir die bestuur van VKM (uitsluitende intensiewe koue behandeling). CRI het tegniese terugvoering, met die ondersteunende inligting, in antwoord op die konsep protokol vir uitvoer van SA sitrus na Thailand saamgestel. 'n Hernude aansoek om toegang na Israel is ondersoek. 'n Stel van inligting is aan Australië, in reaksie op navrae rakende SA se versoek om marktoegang te verkry, voorsien. Die proses om amptelik toegang tot markte in Viëtnam, Lebanon, Jordanië, Sirië en Maleisië te verkry, het ook aandag geniet.

### 2.2 CHINA

The first citrus was exported to China in 2005 and in 2006 the first Chinese inspectors arrived in South Africa to conduct audit inspections on farms and packing facilities. Only a restricted number of farms and packing facilities could be inspected and therefore at the end of the previous reporting period China was requested by Industry to consider the approval of additional farms and packing facilities in 2007. The invitation from SA was accepted and early in 2007 the inspectors arrived to have additional farms and packing facilities registered for export in 2007. A sample of pack houses and farms in the Eastern Cape and Mpumalanga were inspected by the Chinese officials.

Although the inspections went well, the Chinese were concerned about the levels of FCM in the Eastern Cape and the cultural safeguarding measures for FCM in the orchards. The GAP for FCM was accordingly amended to address this concern. Approval and acceptance of additional farms and packing facilities was granted by China as well as two extra entry ports for citrus from SA. The two ports, Guangzhou and Shenzhen, are in the southern part of China. The acceptance of these two ports will facilitate the shipment of increased volumes.

Outstanding issues to deal with in 2008 are the reversion of the cold treatment protocol from 24d to 22d and the acceptance of bulk shipments. Currently only container shipments are allowed.

### 2.3 EUROPE

The first objection from SA to the phytosanitary measures imposed by the EU with regard to CBS on SA citrus fruit was in 1992. The last set of scientific information to support a call for relaxation of these measures was submitted to the EU by SA in 2004. Response from the EU on these data was only received in November 2006. In June 2007 a South African CBS and PRA Expert Working Group was convened to formulate a response to this report. In this Working Group's report, submitted to the EU in August 2007, SA asked for a final ruling since ample scientific evidence had been provided over the past 7 years. The WG also indicated that if a resolution is not forthcoming, SA will request third party mediation, which is in accordance with the rules and procedures of the International Plant Protection Convention. A synopsis of the interaction between SA and the EU was also supplied to DoA for discussion with the Minister of Agriculture and Land Affairs to highlight the urgency of this matter. In order to facilitate the creation of a political and

trade environment that will be more conducive to a favourable resolution to this issue, a professional EU lobbyist in Brussels was also contracted (by CGA).

Vaughan Hattingh and Justin Chadwick undertook a trip to Europe in September 2007 to expedite consideration of the matter by the European Commission's Standing Committee on Plant Health. Discussions were held with a range of interested parties, including the SA Embassies in Brussels (Ambassador Sooklal, Ian Basson and Winston Makabanyane) and Spain (Ambassador Koloane, Johan Engelbrecht and Alicia Segura), EC DG SANCO officials and the Spanish citrus industry. Several meetings also took place between the Lobbyist and personnel from the SA Embassy, EC officials and representative of EU member states. The SA CBS Expert Working Group's response to the EU was put on the agenda of the SCPH's meeting of 10 December 2007. The meeting could not reach consensus and therefore agreed that the EC will refer the case to the European Food Safety Authority (EFSA) for a scientific opinion based on all the documentation provided to date by SA. The timeframe for this Body to conclude an opinion is expected to be 6 months from the time of submission to EFSA. During a trip to Europe in February 2008 undertaken by Justin Chadwick it was established in meetings with DG SANCO and DG Agri that the issue was not yet referred to EFSA. At the meeting the assurance was again given that the issue will be treated with high priority. The South African documentation was subsequently delivered to EFSA in March 2008.

Official CBS surveys, to gain market access for SA citrus to the USA, were conducted in the Western Cape Province during 1995. Only the magisterial districts in the main citrus producing areas were selected. Whereas the entire Western Cape Province is generally regarded as being free of CBS, the omission of some areas in the earlier surveys, resulted in their exclusion from protection under the SA Control Measures pertaining to the movement of citrus propagation material. In June 2007 preparatory meetings were held between CRI and DoA to conduct surveys to provide the data required to potentially provide protection to all of the Western Cape. It was agreed that the surveys will be done with assistance from CRI, according to the protocol as developed by CRI and DoA, during July (fruit samples) and November/December/January (leaf samples) in the magisterial districts of Knysna, George, Mossel Bay, Vredendal and Van Rhynsdorp. The laboratories of the DoA at the Plant Quarantine Station in Stellenbosch were identified as the responsible laboratory for conducting the analysis of the samples. A total of 450 fruit samples were collected in July in the magisterial districts of Knysna, George, Mossel Bay, Vredendal and Van Rhynsdorp. Leaf samples were collected during November 2007 in the magisterial districts of Vredendal and Van Rhynsdorp and during January and February 2008 in the magisterial districts of Mossel Bay, Knysna and George. At the end of the reporting period the laboratory analyses had not yet been completed by DoA.

A number of notifications of non-compliance due to CBS interceptions were received from EU member countries. Spain also reported finding FCM in SA citrus in Spanish stores. To address this serious matter PPECB was instructed by DoA to intensify its inspection of export consignments destined for sensitive markets by paying specific attention to FCM infestation. It is of the utmost importance that the SA citrus industry ensures that good management programmes are applied for both CBS and FCM.

SA had previously (2006) forwarded an official request to the EU to provide SA with data on how it had conducted the diagnostic identification for its report of an interception of SA citrus infested with *Ceratitis cosyra*. SA specifically requested evidence of technical justification for the reliability of the identification process, as this record was contradictory to SA's recognition of citrus as having a non-host status for this fruit fly. No response was received from the EU. In 2007, SA sent an official request to the EU to accordingly remove this record of interception in the absence of appropriate technical justification.

The exotic fruit fly, *Bactrocera invadens* was reported to have expanded its distribution further south to Zambia. It is widely considered to be only a matter of time before it will expand its distribution into South Africa. Early warning systems for this pest must be implemented as a high priority. Inter-Industry workshops will be arranged in 2008 to prepare for such an event.

## 2.4 JAPAN

Four outstanding phytosanitary issues remained unresolved by the end of the previous reporting period, namely access for South African Clementines, adoption of a revised cold treatment condition for the export of all citrus types, clarification of sweet orange cultivars that may be exported and broadening of the access for soft citrus cultivars.

The initial application to export South African Mandarins to Japan was submitted in 1996. A great deal of supportive data was generated through research in the ensuing years and was the subject of many exchanges between SA and Japan. The final treatment protocol entailed a 14d cold treatment at  $T^{\circ} < 0^{\circ}\text{C}$ , with the treatment commencing once the fruit attains a temperature of  $-0.4^{\circ}\text{C}$ . On 7 June 2007 the good

news was received that Clementines from South Africa and Swaziland are permitted for export from SA to Japan. After communication between DoA and MAFF to sort out the operational details, the first container of fruit was shipped from Durban at the end of June 2007. The sustained efforts of CRI entomologists involved in this work over the past 11 years are to be commended. Likewise, the persistent efforts of SA-DoA, the SA Embassy officials in Japan and the CGA, in particular Justin Chadwick as CEO CGA must be recognised. Of course, this would not have been possible without the patient funding support supplied by the citrus growers.

There had previously been a stand off between SA and Japan regarding the nature of experimentation required to support proposed changes (higher temperatures and longer exposure periods) to the cold treatment conditions for all citrus types (oranges, grapefruit, lemons and soft citrus). Japan notified SA in the first quarter of 2007, that Japan accepts SA's approach to the experimental validation of amendments to the cold treatment conditions. In July 2007 a draft response on the trial procedure was forwarded to DoA for communication with Japan. Japan was advised that those comparative Phase III trials had already been conducted by SA on the different citrus types (in accordance with the test procedures) and that SA will proceed with the Phase IV evaluation of the proposed new treatment condition. The repeat of the Phase IV evaluation on oranges was conducted by CRI in 2007 at a lower temperature than the initial trial that was conducted in 2006 (since inadequate control was achieved in the 2006 trials). In 2007, the target temperature of the fruit was maintained at 1°C for 16 consecutive days in all three replicates, without any survival among the 71756 treated larvae. The results of the experiment will be documented in 2008 and submitted to DoA for submission to Japan, in support of the proposed amendment to the treatment conditions.

Consignments of Cara Cara navels were rejected at the phytosanitary inspection points by the Japanese inspectors in 2007, on the basis that this cultivar is not approved for export to Japan. In response, DoA was requested to forward Table 5 of the Export Standards and Requirements for Oranges and Seville Oranges to Japan, together with a request to broaden access to all cultivars as listed in Table 5. Additional supportive data were included. By year end this matter had not been resolved despite bilateral meetings during a ministerial visit to Japan in October 2007. A decision was taken that the issue will be raised at side bar discussions of the IPPC meeting in April 2008.

A request was put forward by the FPEF for broadening the access of the soft citrus cultivars to Japan. DoA was requested to forward Table 6 of the Export Standards and Requirements for Soft Citrus to Japan, together with the request that access be broadened to include all cultivars listed in this table. During the ministerial visit in October 2007, the broadening of access for soft citrus cultivars was discussed, but DoA did not specifically request the inclusion of all the cultivars as listed in Table 6. A meeting between the FPEF, CGA and CRI was convened to make a recommendation to DoA about the way forward. To avoid the potential for confusion in further communication between SA and Japan, it was recommended that the definition of soft citrus in the Export Standards and Requirements for Soft Citrus be amended. The amendment was approved by the Chairman of the Soft Citrus Variety Group and a letter was sent to DoA by the Chairman with the request that the amendment be officially adopted. By the end of the report period DoA had not as yet amended the Standard and no report of feedback from Japan had been received.

## 2.5 USA

By the end of the previous report period, despite a technical visit by SA scientists to the USA, side bar discussions with the USA at the Geneva WTO-SPS talks, a ministerial visit in December 2006 and the implementation of additional FCM risk mitigation measures, the USA continued to resist reversion from a 24d back to a 22d cold treatment.

A technical visit from USDA-APHIS took place during April 2007. At this meeting all the outstanding issues (reversion of the cold treatment from 24d to 22d, the potential recognition of the N. Cape-, western Free State- and southern North West Provinces as a CBS-free area, verification of the irradiation facilities in Cape Town and the adoption of a system for recognising CBS-free-places-of-production) were discussed. It was agreed that reversion to 22d could take place once there is agreement on and implementation of a Standard Operating Procedure (SOP) for packing facilities to better manage the "FCM inspection approach rate" (level of FCM infestation in fruit presented for pre-shipment inspection). The USDA delegation also visited various orchards in the Western Cape to verify the implementation of the mandatory GAP for FCM.

Subsequent to the technical visit and prior to adoption of the conditions required for reversion to the 22d treatment, the USA market was temporarily closed when the rolling average rejection rate for pests of quarantine concern exceeded 20% over 21 days, in Western Cape navels. The market was reopened with the implementation of new, stricter phytosanitary measures. These new measures are only applicable to citrus that are produced in the Western Cape. By the end of the report period, no agreement had been

reached between SA and USA on the packhouse SOP and consequently reversion to a 22d cold treatment had not taken place.

On the request of growers to support market access for areas other than the specified magisterial districts in the Western Cape, extensive preliminary surveys were conducted by CRI in the N. Cape, western Free State- and southern North West Provinces in 2002 and 2004. An official survey, with assistance from CRI, was conducted in these areas in 2005. No evidence of CBS was found in any of the three surveys. The survey reports together with an application to USDA to include these areas, as areas from which citrus can be exported from SA, were submitted to USDA-APHIS in March 2007. Feedback was received from USDA-APHIS in August 2007. A draft response to the concerns raised by USDA-APHIS was forwarded to DoA in September 2007. The official response regarding the concerns raised was submitted to USDA-APHIS by DoA in October 2007.

Information about a system proposed by CRI for recognising CBS-free-places-of-production in areas of low-pest-prevalence for CBS, was formally submitted to USDA-APHIS in 2004. After various follow-ups by DoA in December 2006 and again in April 2007, a response was received from USDA-APHIS in May 2007. A draft reply to USDA-APHIS was supplied to DoA by CRI in September 2007. The official response regarding the concerns raised was submitted to USDA-APHIS by DoA in October 2007.

A meeting (31 May 2007) was held in the far northern region of the Limpopo Province (Tshipise and Weipe) to discuss a way forward. At this meeting the results of an intensive official survey done in 2005, under the auspices of the DoA with assistance from CRI, were provided. All the role players (growers, industry, nurseries, researchers, DoA-officials) at the meeting agreed that the system must be implemented as soon as possible, using this area as a model for testing the feasibility of the system. Specific procedures were set for the registration of farms, the conducting of the final official surveys in November/December 2007 (leaf samples) and July 2008 (fruit samples), the obtaining of new propagating material, the maintenance of orchards, the amendment of legislation pertaining to the movement of propagating material and the establishing of CBS-secure nurseries. Forms to apply for registration for evaluation as potential CBS-free-places-of-production in the model area were sent to the producers in July 2007, with a cut-off date of 31 August 2007. The official CBS surveys (leaf samples) were conducted during November and December 2007 on the farms that applied for registration. By the end of the report period, the laboratory analyses of the samples (by DoA) had not yet been concluded.

Relevant Control Measures under the Agricultural Pest Act, 1983 (Act No. 36 of 1983) were amended to prohibit the movement of citrus propagation material from areas where the disease occurs to this area of low pest prevalence. The development of a Standard Operating Procedure (SOP) for nurseries in areas where CBS occurs to potentially operate as a CBS-secure source of propagation material for production units within Areas-of-low-pest-prevalence was identified as a high priority at the N-Limpopo meeting. CRI engaged the inputs of relevant expertise and compiled a SOP in cooperation with SACNA and DoA. The draft was reviewed at the CIP Advisory Committee meeting in August 2007 and was adopted by DoA in December 2007 for implementation.

Feedback on the data that was submitted in October and December 2007 pertaining to the outstanding issues (access for Northern Cape, access through pest-free-places-of-production, SOP for packing facilities relating to the duration of cold treatment and revised sampling procedures) was received from USDA-APHIS in February 2008. USDA-APHIS raised some concerns and proposed a visit (in April 2008) to the areas that requested to be recognised as CBS-free areas. CRI forwarded information to address the issues of concern (raised by USDA-APHIS) in March 2008. CRI provided the deciduous fruit industry with technical support in formulating a response to the USA request to obtain access to the SA market for the export of apples from USA.

## 2.6 SOUTH KOREA

Attention was given in 2007 to pursuit of market access for lemons, Grapefruit and soft citrus to this market and to effect a reversion from a 24d to a 22d cold treatment for FCM. An official application for the export of soft citrus was submitted in June 2007 together with a request that the same cold disinfestation protocol that applies for oranges be applied to soft citrus. In 2006 SA provided data to South Korea on non-cold alternative FCM risk mitigation measures for lemons and Grapefruit. During the visit of the Ministerial delegation to South Korea in July 2007, bilateral meetings were held to discuss the outstanding phytosanitary issues. From these meetings it transpired that South Korea required more substantive evidence of the efficacy of the alternative risk mitigation measures proposed in 2006. In particular they required scientific evidence of the FCM host status of lemons. It was also made clear that the primary interest in this market lies with Grapefruit.

Laboratory and field experiments were conducted by CRI to investigate the potential host status of lemons for FCM from 2005 until 2007. From the study it was evident that under artificial forced association situations, there is very low host suitability. Complete non host status was not demonstrated, even in field trials. However, it must be noted that even the field trials included some artificial conditions which could have over-emphasized the potential for host association.

An additional technical justification was drafted by CRI and DoA, to support SA's proposed non-cold FCM risk mitigation measures as proposed by SA for application to lemons and Grapefruit. This proposal comprised a systems-approach for FCM risk mitigation, that included the implementation of mandatory Good Agricultural Practices in the orchards before harvest, stricter quality inspection by PPECB, pre cooling and post-harvest cold treatment as required for Fruit Flies. It was indicated that these measures should be viewed in combination with consideration of the South Korean climate, especially the cold winters, as a barrier to potential establishment of the organism in South Korea. Likewise, the unsuitability of lemons as a host for FCM, would further strengthen the level of security provided. The susceptibility of lemons and grapefruit to chilling injury, and the WTO principle that it is unjustifiable to implement measures that are more restrictive than those that are necessary to reduce the risk to an appropriate level, were used as justification for this approach. It was also requested that DoA combine the lemons, Grapefruit and soft citrus proposals to avoid soft citrus being relegated to the bottom of the priority list for SA - South Korea access submissions. By the end of the report period this document had not been officially submitted to South Korea by DoA.

## 2.7 THAILAND

The revised draft export protocol received from Thailand by DoA in September 2006, was forwarded to Industry in January 2007. The information was evaluated and problems were identified with regard to the following: the proposed phytosanitary treatment (-0.55°C or below for 24 days); pests (fruit fly species) that are not associated with citrus but were specified as requiring disinfestation treatment, the pre- and post-disinfestation inspection procedures and the list of pests that were identified as regulated/actionable. CRI compiled a dossier of data and drafted a technical response to Thailand addressing these concerns and forwarded this to DoA for communication with Thailand.

*Ceratitis quinaria* and *Ceratitis corysa*, both known not to be pests of citrus in SA, were listed as actionable organisms. CRI provided scientific data and information justifying removal of these species from the list.

Thailand proposed an FCM disinfestation cold treatment at -0.55°C or below for 24 days as well as a zero tolerance for FCM interception in pre-treatment inspections. Two large scale validation trials were previously conducted by CRI to prove that a 22 day cold treatment at temperatures below 0°C provides the requisite probit 9 level FCM control efficacy. These data were submitted in support of the proposal that the maximum cold treatment duration should not exceed 22 days. CRI further proposed that the current SA quality standard with regard to FCM infestation (1.5%) be applied as a tolerance during pre-shipment phytosanitary inspections. CRI also proposed a 2% sampling intensity as a substitute for the 600 fruit inspection sample proposed by Thailand.

Thailand proposed that if any actionable organisms are found in post-shipping inspection, additional actions will be required. CRI argued that this should apply only to the interception of live actionable organisms. The Thailand proposed protocol included a list of more than 200 actionable pests. Many of these pests are not associated with citrus, or not with citrus in SA and therefore do not qualify as quarantine pests in association with SA citrus exports. CRI explained this in its response and suggested that the title of the list be changed so that it does not imply that these pests are associated with citrus fruit from SA.

By the end of the report period no response to SA's proposals had been received from Thailand, despite several requests made by DoA. In January 2008 all imports of fresh fruit and vegetables from South Africa to Thailand were suspended. The reason given was that Thailand has embarked upon a process of updating their import conditions.

## 2.8 ISRAEL

In 2006 after evaluating the outcome of the PRA conducted by Israel in 2005, SA decided not to proceed with any further actions until Israel reviewed their PRA according to the rules and regulations of the International Plant Protection Convention.

In 2007 another request was received from Israel's NPPO for the importation of lemons. No indication was given that Israel was prepared to review the PRA and therefore the decision was taken that SA's position

would remain unchanged.

## 2.9 AUSTRALIA

An information data package was prepared by CRI and submitted to Australia by DoA in 2005, in support of the South African application for access of citrus to this market. After numerous enquiries by SA, a response was received from Australia in February 2007. Additional scientific data to support the cold disinfestation protocol for two fruit fly species namely Natal fruit fly (*Ceratitis rosa*) and the five spotted fruit fly (*Ceratitis quinaria*) were requested by Australia.

The scientific data generated by CRI to support the cold disinfestation protocol for the Natal fruit fly were submitted to DoA in September 2007. As part of this response, information was again submitted to confirm that the five spotted fruit fly is not a known pest of citrus in SA and therefore no mitigation measures can be legitimately required.

The priorities of the market access submissions from SA were discussed during a visit of a South African delegation to Australia in 2007 and were finalised as follows: Citrus, Table grapes, Pome fruit and Stone fruit. However, despite this meeting, none of the South African plant products that had applied for access were listed by AQIS in their Biosecurity Australia Policy Memorandum that was published in September 2007. In this document the import applications that were received by AQIS are listed. SA queried this anomaly, but a response from Australia was still outstanding at the end of this reporting period.

## 2.10 OTHER MARKETS

**VIETNAM.** Vietnam responded to SA's long outstanding request for clarification of Vietnam's import requirements for plant products. Fresh citrus fruit was identified as one of the categories for which a PRA must be conducted to determine the import conditions from the specific export country. This will receive further attention in 2008.

**LEBANON, JORDAN AND SYRIA.** An official request was submitted to Lebanon to clarify its official regulations for the export of fresh citrus fruit from SA. The Lebanese Authorities responded with a questionnaire - A Pest Risk Analysis Questionnaire (For export of Agricultural Commodities to Lebanon). On receipt of this document it will be evaluated by their PRA team to determine the import conditions. The questionnaire was completed by CRI and submitted to Lebanon by DoA in September 2007. Feedback from Lebanon was still outstanding at the end of this reporting period, despite several requests from DoA for a response.

Specifications of the import conditions for the export of fresh citrus fruit to **Jordan** were obtained in June 2007. The conditions stipulated among other measures, cold treatment at 1.7°C for 14 days for fruit flies. SA sent correspondence to **Syria**, requesting official authorisation to export citrus to Syria. No response had been received from Syria by the end of the report period. SA requested clarification of the import conditions pertaining to **Malaysia**, but no response had been received by the end of the report period.

## 3 PROGRAMME: INTEGRATED PEST MANAGEMENT

### 3.1 PROGRAMME SUMMARY

By Tim G Grout (Manager: Research & Technical, CRI)

Good progress was achieved in all areas of IPM research in striving to meet both current and future requirements relating to citrus exports. International driving forces have generally resulted in two approaches to the research. One has been to find alternative control methods or products because maximum residue limits (MRLs) have changed and certain plant protection products can no longer be applied to the fruit. The other approach has required an increase in the degree of control achieved for certain pests because their pest status has been elevated in our international markets. Where the required assurance of pest control requires post-harvest treatments that may be damaging to fruit, strategies to reduce or avoid this risk are also under investigation.

Most research funding was once again spent on false codling moth (FCM) and covered a broad spectrum of approaches to the management of this pest without the use of chemicals. The commercialisation of Sterile Insect Release with FCM required the development of various unique pieces of equipment to cope with the mass production and release of FCM. Attempts to increase the residual control of the granulovirus Cryptogran and improve the biocontrol of FCM through the use of larval parasitoids were also made. Through trapping surveys our understanding of this pest has increased and it seems that host plants are

more important in determining its distribution than the climate. Fruit fly research required the second most funding in this programme and included a successful cold disinfestation trial at 1°C for 16 days which, if accepted by the Japanese, may reduce some of the rind condition problems experienced with the shorter and colder treatment. Attempts to avoid fruit residues from fruit fly bait applications by applying the bait to the tree trunks were not successful but M3 bait stations were successful in preventing fruit damage, even when numbers of fruit flies in Capilure traps showed no decline in the population. The lures used for monitoring *Ceratitis* species in citrus in South Africa were compared with one another and with international lures. The identification of fruit fly larvae intercepted by PPECB in packhouses around the country revealed that Medfly was responsible for most rejections and Natal fruit fly was being controlled adequately.

With increased limitations on the number of plant protection products that can be used for the control of mealybugs after petal fall the further research that was conducted on parasitoids of oleander mealybug will prove valuable and the progress in developing an ant bait to reduce the disruption of biocontrol is encouraging. Other research on alternatives to plant protection products included further trials on Helicovir, a nucleopolyhedrovirus for the control of bollworm and the discovery of an excellent alternative to Acarol for the control of citrus bud mite. Further research was also conducted to generate new pre-harvest intervals for various plant protection products for which MRLs have changed in Europe but are not being fully supported by large chemical companies.

As this report is being written, I have just signed a Memorandum of Understanding with an entomological research institute in Kenya, *icip*, where we will shortly be conducting joint research on the post-harvest control of *Bactrocera invadens*, a new fruit fly that is devastating the fruit industry in west, central and east Africa and is moving towards South Africa. It is this type of proactive approach to the threats facing our industry that will ensure that we remain the second largest citrus exporting nation in the world.

## PROGRAMOPSOMMING

Goeie vordering is in al die areas van IPM navorsing gemaak, in die strewe om aan beide die huidige en toekomstige vereistes, met betrekking tot sitrusuitvoere, te voldoen. Internasionale dryfkragte is oor die algemeen vir die twee benaderings wat met hierdie navorsing gevolg word, verantwoordelik. Die een benadering is om as gevolg van veranderde maksimum residuvlakke, en sekere plantbeskermingsprodukte wat nie langer op vrugte aangewend kan word nie, alternatiewe metodes van beheer te vind. Vir die ander benadering is 'n toename in die graad van beheer vir sekere plaë nodig, vanweë hul verhoogde pesstatus in die internasionale markte. Waar die nodige versekering van plaagbeheer van na-oes behandelings, wat skadelik vir die vrugte kan wees, afhanklik is, is strategieë om die risiko te verminder of te verhoed, ook ondersoek.

Meeste van die navorsingsfondse is weereens op valskodlingmot (VKM) spandeer en 'n wye spektrum van benaderings vir die bestuur van hierdie plaag, sonder die gebruik van chemikalieë, is gedek. Die kommersialisering van die Steriele-insek Loslating van VKM het die ontwikkeling van verskeie unieke apparate vir die hantering van massateling en loslating van VKM benodig. Pogings om die beheer van die residue van die granulovirus, Cryptogran, te verhoog, en om die biologiese beheer van VKM deur die gebruik van larwa-parasitoëde te verbeter, is ook gedoen. Inligting van opnames wat uitgevoer is het tot 'n beter begrip van die plaag gelei en dit blyk dat gasheerplante meer belangrik in die bepaling van die verspreiding van die plaag is, as die klimaat. Die tweede meeste fondse in hierdie program is vir vrugtevliegnavorsing aangewend. Dit het 'n suksesvolle koue disinfestasië proef by 1°C vir 16 dae ingesluit, wat, indien dit deur Japan aanvaar sal word, tot 'n vermindering in sommige van die skilprobleme wat met die korter en kouer behandeling ondervind word, kan lei. Pogings om residue op vrugte deur die aanwending van vrugtevlieg-lokaas, deur die aanwending daarvan op die stamme van die bome, te verhoed, was nie suksesvol nie. Die M3-lokaas stasies was egter suksesvol om skade aan die vrugte te verhoed, al het die aantal vrugtevlieë in die Capilure lokvalle getoon dat daar geen afname in die populasie was nie. Die lokmiddels wat vir monitoring van *Ceratitis* spesies in sitrus in Suid-Afrika gebruik is, is met mekaar en met internasionale lokmiddels vergelyk. Die identifikasie van vrugtevlieg larwes wat deur PPECB in pakhuisse regoor die land onderskep is, het getoon dat Medvlieg vir die meeste afkeurings verantwoordelik was en dat die beheer van Natalse vrugtevlieg voldoende is.

Met die toenemende beperkings op die aantal plantbeskermingsprodukte wat vir die beheer van witluis na blomblaarval gebruik kan word, blyk die verdere navorsing wat op parasitoëde van oleander witluis uitgevoer is waardevol te wees, en die proses om 'n mierlokaas te ontwikkel om die ontwinging van biologiese beheer te verminder, blyk belowend te wees. Ander navorsing op alternatiewe plantbeskermingsprodukte sluit verdere proewe op Helicovir, 'n "nucleopolyhedrovirus" vir die beheer van bolwurm in, en die ontdekking van 'n uitstekende alternatief vir Acarol vir die beheer van sitrus knopmyt. Verdere navorsing om nuwe voor-oes intervale vir verskeie plantbeskermingsprodukte, waarvan die MRLs in

Europa verander het, te ontwikkel, maar wat nie deur groot chemiese maatskappye ondersteun word nie, is ook uitgevoer.

Tydens die skrywe van die verslag het ek ook 'n ooreenkoms met 'n entomologiese navorsingsinstituut in Kenia, *icipe*, geteken. Ons gaan binnekort daar gesamentlike navorsing op die na-oes beheer van *Bactrocera invadens*, 'n nuwe vrugtevlug wat vir die vrugtebedryf in Wes-, Sentraal- en Oos-Afrika vernietigend is en wat na Suid-Afrika beweeg, uitvoer. Dit is hierdie tipe van pro-aktiewe benadering tot hierdie bedreigings wat sal verseker dat ons die tweede grootste sitrus-uitvoerder in die wêreld sal bly.

### 3.2 PROJECT: FALSE CODLING MOTH

Project coordinator: Hendrik Hofmeyr (CRI)

#### 3.2.1 Project summary

During the past report year (2007-2008) a diverse range of issues with regard to false codling moth control received the attention of researchers from CRI, the universities of Stellenbosch, Rhodes, Nelson Mandela Metropole, as well as the private sector. Most of the research had commenced in previous years and some of the subjects have been investigated since 2002. The focus ranged from above to below ground, pre- and post harvest control, as well as laboratory and field research. Approximately half of the studies were concluded with this report. The remaining studies will continue for one to two years.

The extensive study concerning chemicals which may individually or in combination with other products, be applied as attractants or deterrents, has not provided a product that could be practically applied for FCM control in the foreseeable future (3.2.2). This study is no longer funded by CRI.

The basic research on radiation biology and F1 sterility of FCM that commenced in 2002, has resulted in the first operational mass rearing unit for a Lepidoptera pest in South Africa (3.2.3). New equipment, as well as the building, had to be designed from scratch and developed to the production stage. Releases of sterile FCM commenced in November 2007 in orchards in certain parts of the Citrusdal region. The effectiveness of these releases have subsequently being monitored regularly using different methods (3.2.12).

Five experimental series addressed the biological control of FCM larvae. Progress was made with the development of a Hymenopterous larval parasitoid (3.2.4), although mass rearing them remains a problem. The orchard persistence (3.2.5) and relative activity 3.2.13) of the granulovirus, Crle-GV, occurring in the spray products Cryptogran and Cryptex, were investigated in attempts to improve management of these biopesticides. Entomopathogenic nematodes were collected country-wide and identified (3.2.6). Selected species will be formulated and produced for semi-orchard trials. A standard was developed to compare the efficacy of the granulovirus, Crle-GV to various FCM populations country-wide (3.2.10).

A survey to determine the distribution of FCM populations country-wide, was continued (3.2.7). This survey aims to develop statistical models that can be used to determine the probability of introduced FCM distributing and establishing in a foreign country importing southern African citrus.

Due to the mandatory cold disinfestation protocol aimed at killing larvae in southern African export fruit, it is not possible to export lemons to certain countries. A study was conducted to investigate the suitability of lemons as hosts for FCM (3.2.8).

The distribution of FCM and the host status of indigenous and cultivated flora in the Citrusdal region has been investigated since 2006 (3.2.9). Various interesting trends have been found which will be useful in future FCM control strategies.

The difficulty of preventing infestation of citrus fruit in the orchard is well-known. A last resort would be to kill the larvae in the pack house. Two potentially appropriate products were evaluated (3.2.11).

#### Projekopsomming

'n Uiteenlopende reeks kwessies ten opsigte van valskodlingmotbestryding, het gedurende die afgelope verslagjaar, 2007-2008, aandag van navorsers afkomstig van CRI, Universiteit van Stellenbosch, Rhodes Universiteit, Nelson Mandela Universiteit en die private sektor, ontvang. Die meeste van die ondersoekes het alreeds in vorige jare begin en sommige onderwerpe geniet alreeds die aandag sedert 2002. Aspekte is oor 'n breë front nagevors en behels faktore wat bo- en ondergronds belangrik is, voor- en na-oesbestryding, asook laboratorium- en boordnavorsing. Ongeveer helfte van die studies wat uitgevoer is, het met die huidige verslag ten einde geloop. Die res word vir nog 'n jaar of twee voortgesit.

Die omvattende studie oor chemikalieë wat moontlik opsigself of in kombinasie met ander produkte as lok- of afstootmiddels aangewend kan word, het nog geen produk opgelewer wat binnekort vir praktiese VKM-bestryding beskikbaar sal word nie (3.2.2). Dié studie word nie langer deur CRI befonds nie.

Basiese navorsing oor die stralingsbiologie en F1-steriliteit van VKM wat in 2002 begin het, het tot die indiensstelling van die eerste massateelinsektarium vir 'n Lepidopteraplaag in Suid-Afrika gelei (3.2.3). Nuwe toerusting en die gebou self, is van meet af ontwerp en tot die produksiefase deurgevoer. Steriele VKM word alreeds sedert November 2007 in sekere dele van Citrusdal losgelaat. Die doeltreffendheid van dié loslatings word sedertdien gereeld op verskillende maniere geëvalueer (3.2.12).

Vyf proefreekse het die kwessie van die biologiese beheer van VKM-larwes aangespreek. Vordering is gemaak met die ontwikkeling van 'n hymenoptera-parasitoïed wat larwes parasiteer (3.2.4). Probleme word egter nog met die massateel van die wesp ondervind. Die boordnawerking (3.2.5) en relatiewe aktiwiteit (3.2.13) van die granulosevirus, Crle-GV, wat in die spuitprodukte Cryptogran en Cryptex voorkom, is ondersoek in pogings om die bestuur van die biologiese beheerprodukte te verbeter. Entomogeniese aalwurms is landswyd versamel en geïdentifiseer (3.2.6). Daar word tans gesoek na 'n maatskappy wat geskikte aalwurmspesies tot 'n bruikbare produk kan formuleer én produseer sodat hulle in semi-boordproewe geëvalueer kan word. 'n Standaard is opgestel wat gebruik kan word om die doeltreffendheid van die granulosevirus, Crle-GV, op VKM-bevolkings in verskillende landstreke te toets (3.2.10).

'n Opname waarmee die verspreiding van VKM landswyd vasgestel word, is voortgesit (3.2.7). Die opname het 'n maatstaf ten doel wat gebruik kan word om die potensiële bedreiging te voorspel wat die plaag inhou in terme van verspreiding en vestiging in lande waarheen suider-Afrikaanse sitrus uitvoer word.

Weens die koue-ontsmettingprotokol wat toegepas moet word om VKM in suider-Afrikaanse uitvoersitrus te dood, kan suurlemoene nie na sommige lande uitgevoer word nie. 'n Ondersoek is geloods om die moontlikheid dat suurlemoene nie VKM sal kan huisves nie en die kouebehandeling derhalwe onnodig sal wees, te ondersoek (3.2.8).

Die verspreiding van VKM en die gasheerstatus van inheemse en aangeplante flora in die Citrusdalgebied is sedert 2006 nagevors (3.2.9). Etlke interessante neigings is opgemerk wat by die opstel van toekomstige strategieë vir VKM-bestryding gebruik sal kan word.

Dit is oorbekend hoe moeilik dit is om die besmetting van sitrusvrugte in die boord te keer. As 'n laaste poging om die uitvoer van lewendige larwes in vrugte te verhoed, kan 'n pakhuisbehandeling wat die larwes dood, baie handig te pas kom. Twee potensiële-bruikbare produkte is geëvalueer (3.2.11).

### 3.2.2 FINAL REPORT: Development of semiochemical odorants for the attraction and repellence of false codling moth in citrus

Experiment 648 (2002-2008): Christo Smit (Desense Pest Control, Citrusdal)

#### Summary

A large collection of odorants were investigated for FCM attraction in laboratory delta trap experiments. Some of the better choices from these experiments were thereafter evaluated in paired trap orchard experiments. The terpene group was investigated extensively and to a lesser extent, the amine/amide group, the O-heterocyclic group and some Lepidopteran pheromones. Highest trap catches, at least double that of the blank controls, were obtained in laboratory experiments with the following combinations with young navel orange fruit, which on its own increased trap catches by an average of 75% in more than 14 experiments:

*Plant extracts and odorants:* Apart from young navel orange fruit, lemon ginger sesquiterpenes, Red palm oil, Chamomile oil and Litsea cubeba oil; also odors from pomegranate fruits, hot pepper sauce (containing capsaicin) and Wistaria flowers.

*Hemiterpenes:* Acids: 2-Methyl butenoic acid (=tiglic acid), 2-methyl pentenoic acid and 2-methyl butyric acid. Alcohols and thiols: 3-Methyl 1-butanol and 3-Methyl 1-butane thiol.

*Monoterpenes:* Geranyl acetone, alpha terpinene and allo-ocimene.

*Sesquiterpenes:* alpha-bisabolol, farnesol and farnesyl acetate.

*Triterpenes:* Squalene.

*Amines and amides:* 8-methyl E6-nonenamide (=sidechain of capsaicin), hippuric acid, acetyl choline chloride.

*Heterocyclics*: 2-Acetyl benzoic acid benzofuran lactone, N-acetyl thiazolidine carboxylic acid and alpha-lipoamide.

*Alkyl (=straightchain) substances*: C30: Triacontanol and C3: 1-Thioglycerol and pyruvaldehyde.

*Lepidopteran pheromones other than FCM sex pheromone*: E2,E13 octadecadienyl acetate (=E2,E13-18Ac).

## Opsomming

’n Groot groep reukstowwe is vir VKM-aantrekking in deltalokval-laboratoriumproewe ondersoek. Sommige van die beter keuses daaruit is daarna in pare deltalokvalle in ’n sitrusboord geëvalueer. Die terpeengroep is die intensiefste ondersoek en, tot ’n mindere mate, die amiene/amiede-, heterosikliese- en Lepidoptera-feromoon-groepe. Die beste lokvalvangste is in hierdie laboratoriumproewe met die volgende verkry:

Jong, onvolwasse nawelvrugte (JNV) het in 14 herhalings gemiddeld 75% meer VKM gevang as die leë kontrole-lokvalle. Die volgende reukstowwe het vangste met JNV alleen, met minstens nog 50% verhoog:

*Plantekstrakte en reukstowwe*: Suurlemoengemmer-seskwiterpene, rooipalmolie, kamille-olie (blou), Litsea cubeba-olie, geurstowwe van granaatvrugte, brandrissiesous (bevat capsaicin) en *Wistaria*-blomme.

*Hemiterpene*: - *Sure*: 2-Metielbutenielsuur (=tigliensuur), 2-metielpentenielsuur en 2-metielbottersuur.

- *Alkohole en tiols*: 3-Metiel 1-butanol en 3-Metiel 1-butaantiol.

*Monoterpene*: Geraniel asetoon, alfaterpineen en allo-osimeen.

*Seskwiterpene*: alfa-bisabolol, farnesol and farnesielasetaat.

*Triterpene*: Skwaleen.

*Amiene and amiede*: 8-metiel E6-nonenamied (=sytak van capsaicin), hippuursuur en asetielcholiënchloried.

*Heterosikliese verbindings*: 2-Asetielbensoësuur benzofuranlaktoon, N-asetiel thiasolidien karboksiesuur en alfalipoamied.

*Alkiel (= reguitkettingverbindings)*: C30 (Triakontanol) en C3 (1-Tioglisierol en piruvaldehyd).

*Lepidoptera-feromone, behalwe VKM-geslagsferomoon*: E2,E13 oktadekadienylasetaat (=E2,E13-18Ac).

## Introduction

The primary aim of this study (CRI experiment 648) was to identify attractant or repellent semiochemical odorants or light stimuli which were effective enough to justify development for FCM control on an orchard scale.

## Materials and methods

**Sources of odorants and odorant groups**: Odorant samples were obtained from the companies Bedoukian and Aldrich (USA), Fluka (Switzerland), RC Treatt (England) and Pherobank (Netherlands).

**Assesment of FCM attraction with Delta traps in laboratory**: For the 2007/8 season, as an interim between the calibrated glass olfactometer tubes and the delta traps in orchards, delta traps were placed in rows on the laboratory floor under ventilated and temperature controlled conditions. Temperature control of the air conditioner was set at 30°C. Approximately 300 g FCM (approximately 6 000 moths) were distributed evenly between the 3 rows of odorant loaded delta traps and blank trap controls. Floor space located per delta trap was about 0.5 square metres. Total floor area was 2.5 m x 6 m which meant an FCM population density of about 400 moths per square metre and 200 moths per trap. For ventilation, 2 heater fans were placed on the floor which carried the odorant loaded air upwards and one larger fan, placed in an aperture in the wall, blew it outwards. Laboratory trap experiments were run for 2 days and nights.

**Orchard experiments**: Initially, i.e. up to the 2005/6 test season, use had been made of natural FCM populations in commercial orchards. However, for the 2006/7 and 2007/8 seasons artificial FCM populations were implemented where each test tree was supplied with a closed paperbag with a tablespoon (15 ml) full of FCM, i.e. approximately 300 FCM. When all traps were placed, the paperbags were cut open to release the moths, which was done in the evening after sunset. A normal placement of traps was every 3<sup>rd</sup>-4<sup>th</sup> tree in a row in every 2<sup>nd</sup>-3<sup>rd</sup> row, depending upon tree spacing. Yellow plastic delta traps from Chempack (Paarl) were suspended individually or in pairs at about eye level (1.8 m) on the south-eastern sides of navel orange trees. Paired traps were placed on the same tree at the same height and approximately 1 m apart. The number of replicates used is mentioned in the results.

## Results and discussion

All results were based on the number of moths trapped.

## 1 Laboratory experiments

### 1.1 Young navel fruit (YNF) and combinations thereof

Over 5 experiments with 2 replicates each, mean trap catches for YNF was 31.3 FCM (with maximum of 36.3) as compared with the blank controls (a mean of 17.0; maximum of 22.8). Because all attractants used in citrus orchards will be subject to interactions with odorants originating from citrus trees and fruits, it was decided to test odorants from all chemical groupings in combinations with YNF, starting with the terpene group.

#### 1.1.1 YNF combinations with the terpene group

To bring order to the investigation, it was decided to investigate the wide range of available odorants according to the chemical groupings of semiochemicals by Howse *et al.* (1998). The first group which was investigated fairly extensively was the terpene group which included (a) the C5 hemiterpenes (= isoprene = methyl butenyl/butyl group) and related chemicals which are the primary building blocks of the higher terpenes, the monoterpenes (C10), the sesquiterpenes (C15), the diterpenes (C20) and the triterpenes (C30) (Table 3.2.2.1).

**Table 3.2.2.1.** FCM trap catches with young navel orange fruit alone and in combination with various hemiterpenes (C5) and related substances (2 reps).

Treatments	Mean/Max
YNF +2-Methyl 2-butenic acid [=Tiglic acid $\text{CH}_3\text{-CH}=\text{CH}(\text{CH}_3)\text{-COOH}$ ]	53/57
YNF + 2-Methyl butyric acid ( $\text{CH}_3\text{-CH}_2\text{-CH}(\text{CH}_3)\text{-COOH}$ )	50/67
YNF + 2-Methyl 2-pentenoic acid [ $\text{CH}_3\text{-CH}_2\text{-CH}_2=\text{CH}(\text{CH}_3)\text{-COOH}$ ]	48/60
YNF + 3-Methyl 1-butane thiol [ $\text{CH}_3\text{-CH}(\text{CH}_3)\text{-CH}_2\text{-CH}_2\text{-SH}$ ]	50/52
YNF + Tiglic aldehyde [ $\text{CH}_3\text{-CH}=\text{CH}(\text{CH}_3)\text{-CHO}$ ]	44/47
YNF +3-Methyl 1-butanol [=Iso amyl alcohol $\text{CH}_3\text{-CH}(\text{CH}_3)\text{-CH}_2\text{-CH}_2\text{-OH}$ ]	43/57
YNF + 3-Methyl 2-buten 1-ol [ $\text{CH}_3\text{-CH}(\text{CH}_3)=\text{CH-CH}_2\text{-OH}$ ]	42.5/43
YNF + 2-Methyl 2-pentenal [ $\text{CH}_3\text{-CH}_2\text{-CH}_2=\text{CH}(\text{CH}_3)\text{-CHO}$ ]	39/57
YNF + Isobutyl tiglate [( $\text{CH}_3$ ) <sub>2</sub> -CH- O-C(=O)-CH( $\text{CH}_3$ )=CH- $\text{CH}_3$ ]	37/38
YNF + 3-Methyl 2-butenal [ $\text{CH}_3\text{-CH}(\text{CH}_3)=\text{CH-CHO}$ ]	33/35
YNF + 2-Methyl 3-buten 2-ol [ $\text{CH}_3=\text{CH-C}(\text{OH})(\text{CH}_3)\text{-CH}_3$ ]	30/31
YNF + 2-Methyl butyraldehyde[ $\text{CH}_3\text{-CH}_2\text{-CH}(\text{CH}_3)\text{-CHO}$ ]	27.5/37
YNF + 2-Methyl 1-butane thiol [ $\text{CH}_3\text{-CH}_2\text{-CH}(\text{CH}_3)\text{-CH}_2\text{-SH}$ ]	22.5/29
YNF + 3-Methyl 2-butanethiol [ $\text{CH}_3\text{-CH}(\text{CH}_3)\text{-CH}(\text{SH})\text{-CH}_3$ ]	19.5/27
<i>Blank control</i>	<b>18.5/25</b>

2-Methyl butenoic acid (= tiglic acid) or 2-Methyl butyric acids seemed to be best choices in combination with YNF with the corresponding 2-methyl pentenoic acid as second choice. The corresponding aldehydes, thiols and isobutyl ester were much less attractive. In the alcohol and thiol group, 3-Methyl 1-butanol, 3-Methyl 2-buten 1-ol and 3-Methyl 1-butane thiol were good attractants but less so for other variations with this molecular structure.

**Table 3.2.2.2.** FCM trap catches with YNF alone and in combination with monoterpenes (C10) (number of replicates indicated in first column).

Treatments and replicates(*)	Mean/Max
YNF + Geranyl acetone (8)	51/54
YNF + alpha-Terpinene (6)	49.2/66
YNF + Allo-ocimene (6)	48.7/54
YNF + Linalyl acetate (8)	40/56
YNF + beta-Pinene (4)	39/50
YNF + Verbenol (6)	38.7/50
YNF + Bergyl acetate (6)	38/61
YNF + Limonene (8)	37/54
YNF + Carveol (6)	37/44

YNF + Myrtenal (4)	35.5/44
YNF + Myrcenyl acetate (6)	35/44
YNF + Geranyl acetate (6)	34.5/43
YNF + Neryl acetate (6)	31.3/38
YNF + Citronellyl acetate(4)	30/40
YNF + Citral (6)	28.3/33
YNF + Neomenthol (2)	28/28
YNF + Linalool (6)	27.5/28
YNF + Verbenone (2)	27/27
YNF + Geraniol (6)	24/29
YNF + Nerol (2)	24/24
YNF + Myrcene (2)	24/24
YNF + alpha-Pinene (2)	22/22
<i>Blank control (8)</i>	<i>21.8/32</i>
YNF + Carvomenthenol (2)	19/19
YNF + Ocimene (2)	18/18
YNF + Sabinene (2)	17/17
YNF + Terpinyl acetate (2)	17/17
YNF + Carvone (2)	14/14

(\*)- Total replicates of up to 4 experiments at 2 replicates per experiment

Geranyl acetone, alpha terpinene and allo-ocimene were the best choices in combination with YNF. Less so, and more comparable with limonene, were linalyl acetate, beta-pinene, verbenol, bergyl acetate and carveol. The reactivity or sensitivity of the different batches of FCM obtained from the Xsit FCM breeding facility in Citrusdal, varied fairly strongly between 4 experiments/dates, e.g. in the screening of monoterpene attractivity; means of blank control trap catches varied from 14 to 18.5, 22 and 32.5 while means of the comparable limonene trap catches in the 4 experiments varied from 28 to 31.5, 34 and 54. Similar variations in reactivity between various experiments and batches of FCM used were found in the tests with FCM sex pheromone (FCM-SP) and its combinations with E2,E13-18Ac mentioned below. Here trap catches in the blank control varied from 12 to 16, 16,5, 17,5, 25 and 39. Comparable mean trap catches for FCM-SP were 21, 23.5, 28, 30.3, 37.5 and 63.3.

There were signs of toxification of experimental moths when testing the monoterpene group; many were dead one day after starting the experiment. In toxicity experiments earlier (2000), odours from the more toxic members of the monoterpene group such as carvone, thujone, citronellal and myrtenal at 0.005 ml/100 ml test vial killed 100% of the exposed FCM in less than 2 minutes. This may indicate that toxicity of attractants such as these monoterpenes should also be taken into consideration when formulating experimental synergistic combinations of odorants, e.g. by using minimal attractive concentrations.

**Table 3.2.2.3.** FCM trap catches with young navel fruit alone and in combination with higher terpenes and related longchain compounds (2 reps.)

Treatments	Mean/max
YNF + Squalene (C30)	70.5/85
YNF + Triacontanol (C30)	67/86
YNF + Bisabolol (C15)	55/60
YNF + Farnesol (C15)	54/73
YNF + Farnesyl acetate(C15)	52/70
YNF + Guaiazulene (C15)	45.5/51
YNF + Nerolidol (C15)	42/54
YNF + Cedrene (C15)	39/41
YNF + Methoprene (C15)	36.5/37
YNF + Farnesene (C15)	27/30
YNF + Farnesene (C15)	27/30
YNF + Farnesyl acetone (C15)	27/28
<i>YNF alone</i>	<i>25/30</i>
YNF + Isomethyl ionone (C15)	16/21

The best choices of FCM attractants in combination with YNF in the group of higher terpenes (C15 to C30) were squalene, alpha-bisabolol, farnesol and farnesyl acetate. Triacontanol, a wax like C30 saturated

alkane alcohol and a natural plant growth regulator, also exhibited strong attraction but should rather be included in the alkane/alkene (straight chain) group which was investigated separately.

**Table 3.2.2.4.** FCM trap catches with young navel fruit alone and in combination with higher terpenes (C15 to C30) (2 reps.)

Treatments	Mean/Max
YNF + alpha-Bisabolol (C15)	83/94
YNF + Squalene (C30)	57.5/63
YNF + Valencene	41/49
YNF + Nerolidol	40/50
YNF + Sinensal	40/45
YNF + Farnesene	39/47
YNF + Elemol	39/48
YNF + Aurantiol	38.5/44
YNF + Retinyl acetate	38.5/45
YNF + Bisabolene	38/40
YNF alone	36/41
YNF + Dimyrcetol	26.5/30
YNF + Caryophyllene	16.5/19
<i>Blank control</i>	21/24

In support of earlier results, alpha-bisabolol and squalene again seemed to be better choices in the test series of higher terpenes used above when combined with YNF.

**Table 3.2.2.5.** FCM trap catches with young navel fruit alone and in combination with plant oils/extracts (2 reps.)

Treatments	Mean/Max
YNF + Red palm oil	34/48
YNF + Lemon ginger sesquiterpenes	31.5/51
YNF + Litsea cubeba oil	26/32
YNF + Chamomile oil	26/28
YNF + Opopanax oil	23/28
YNF + Cedarwood oil	23/28
YNF + Orange oil (cold pressed)	21/24
YNF + Neroli oil	20.5/24
YNF + Cardamom oil	19/20
YNF + Patchouli oil	18.5/22
YNF + Ginger oil	16/18
YNF + Cananga oil	16/18
YNF + Bergamot oil	14/15
<i>Blank control</i>	11/18

The better combinations with YNF includes Red palm oil and lemon ginger sesquiterpenes with Chamomile oil and Litsea cubeba oil as second choices; the latter 2 containing the sesquiterpenes bisabolol and cubebene respectively.

**Table 3.2.2.6.** FCM trap catches with young navel fruit alone and in combination with various higher terpenes (C15 – C30) and plant odours (2 reps.)

Treatments	Mean/Max
YNF + Squalene	32.5/35
YNF + Lemon ginger sesquiterpenes	30.5/33
YNF + Half a pome.g.ranate	30/40
YNF + Farnesol	30/35
YNF + Nerolidol	27/29
<i>YNF alone</i>	24/26
<i>Blank control</i>	15.5/18

In support of earlier findings, squalene and farnesol again seemed to be the better choices of higher terpenes in combination with YNF, as is also the sesquiterpene fraction of Lemon ginger extract. Odorants from pomegranate fruits, which are very attractive to FCM, also attracted FCM strongly in this experiment.

### 1.1.2 YNF combinations with test odorants/attractants other than terpenes

Before deciding which semichemical grouping would be the next to focus on in the investigation, exploratory investigations were first conducted into representatives of various groupings including the amine/amide (AM) group, heterocyclic substances (HC), which often acts as anti-oxidants, Lepidoptera pheromones (LPh) and propionyl substances (PR).

**Table 3.2.2.7.** FCM trap catches with young navel fruit alone and in combination with various odorants and plant odours.

Treatments	Mean/Max
YNF + FCM-SP (LPh)	34/38
YNF + Acetyl choline chloride (AM)	33/38
YNF + 2-Acetyl benzoic acid (Benzofuran form) (HC)	32/34
YNF + Hot pepper sauce (AM)	31/34
YNF + E2,E13-Octadecadienyl acetate (E2,E13-18Ac-1% sol.)(LPh)	28/37
YNF + Wistaria flowers	25/34
<i>Young Navel fruits (YNF) alone</i>	17/18
<i>Blank control</i>	12/16

The FCM sex pheromone exhibited the strongest attraction in combination with YNF, followed by Acetyl choline chloride and 2-Acetyl benzoic acid including its internal benzofuran ester form and another moth sex pheromone E2,E13-18Ac. Hot pepper sauce and *Wistaria* flowers also attracted FCM in combination with YNF.

**Table 3.2.2.8.** FCM trap catches with young navel fruit alone and with various odorants (3 reps.)

Treatments	Mean/Max
YNF + 1-Thioglycerol (PR)	55.7/67
YNF + 8-Methyl E6-nonenamide (AM)	48.3/58
YNF + 2-Acetyl benzoic acid-benzofuran form (HC)	47/62
YNF + Pyruvaldehyde (PR)	45.3/55
YNF + Acetyl choline chloride (AM)	40.3/54
YNF + Wistaria flowers	37.3/51
<i>YNF alone</i>	37/46
YNF + Bisabolene (C15 terpene)	36.5/37
YNF + Acetyl acetone (PR)	34.7/43
YNF + E2-Hexenol	32.3/38
<i>Blank control</i>	16.3/22

In the propionyl group of straight chain substances, 1-thioglycerol and pyruvaldehyde attracted FCM in combinations with YNF. In the AM group, better choices in YNF combinations were 8-methyl E6-nonenamide and acetyl choline chloride, while in the HC group +YNF, 2-acetyl benzoic acid was attractive.

**Table 3.2.2.9.** FCM trap catches with young navel fruit alone and with various odorants (2 reps.)

Treatments	Mean/Max
YNF + N-Acetyl thiazolidine carboxylic acid (HC)	56.5/63
YNF + Hippuric acid (AM)	53/54
YNF + 2-Acetyl benzoic acid –Benzofuran form (HC)	46/55
YNF + 1-Thio-glycerol (PR)	46/47
YNF + 8-Methyl E6-nonenamide (AM)	43.5/50
YNF + Pyruvaldehyde (PR)	41/45
YNF + E2,E13-18Ac – 1% sol. (LPh)	37/50
YNF + Acetyl choline chloride (AM)	31/34
YNF + alpha-Lipoamide (HC/AM)	29/42
<i>Young Navel fruits (YNF) alone</i>	25/30

Blank control	16/18
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More attractive YNF combinations with the various chemical groupings, were the following:

HC: N-Acetyl thiazolidine carboxylic acid, 2-Acetyl benzoic acid and probably alpha-lipoamide.

AM: Hippuric acid, 8-methyl E6-nonenamide (sidechain of capsaicin), acetyl choline chloride and alpha-lipoamide.

PR: 1-Thioglycerol and pyruvaldehyde.

**Table 3.2.2.10.** FCM trap catches with young navel fruit alone and in combination with various carbamides (3 reps.)

Treatments	Mean/max
YNF + 8-Methyl 6-nonenamide* [NH <sub>2</sub> -C(=O)-(CH <sub>2</sub> ) <sub>4</sub> -CH=CH-C(CH <sub>3</sub> ) <sub>2</sub> ]	77/80
YNF + Strong Chilli pepper sauce (Tabasco sauce – with capsaicin **)	71.6/80
YNF + Hippuric acid [C <sub>6</sub> H <sub>5</sub> -C(=O)-NH-CH <sub>2</sub> -COOH]	58.7/62
YNF + Ethyl anthranilate [C <sub>6</sub> H <sub>4</sub> -2NH <sub>2</sub> -C(=O)-O-C <sub>2</sub> H <sub>5</sub> ]	54/65
Young Navel fruit (YNF) only	53.7/61
YNF + 3-Methoxy benzamide [3-CH <sub>3</sub> O-C <sub>6</sub> H <sub>4</sub> -C(=O) -NH <sub>2</sub> ]	51.3/57
YNF + N-(3-Acetyl-4-hydroxy phenyl)-butanamide	47/62
YNF + N-(3-Methoxy phenyl)-acetamide	47/51
YNF + 4-Methoxy benzamide	43.6/57
YNF + 3,5-Dimethoxy benzamide	29.3/35
Blank control	25.3/40

\* Sidechain of capsaicin

\*\*Capsaicin = Vanillyl 8-methyl 6-nonenamide

More attractive YNF combinations with members of the amine/amide group, were the following:

AM: Hippuric acid, 8-methyl E6-nonenamide (sidechain of capsaicin); hot pepper sauce, containing capsaicin, acetyl choline chloride and lipoamide.

*Attraction by Paprika:* With respect to the strong FCM attraction to Paprika fields, it is suspected that the strong FCM attractant transportable odorant signal is partly the detached 8-Methyl 6-nonenamide side chain and that it is less likely to be capsaicin itself because of its fairly large molecule. It consists of a methoxy-hydroxybenzene (=vanillyl-) nucleus the aldehyde group to which is attached the NH<sub>2</sub>/amine group of the 8-Methyl E6-nonenamide side chain. However there is also a large variety of other components in the odorant bouquet of Capsicums which will also have to be investigated.

Considering the above results, the next chemical groupings to be focused on in combinations with YNF, will be the amines/amides and the heterocyclics.

## 1.2 FCM sex pheromone (2% soln.) in Sunspray 7E citrus oil, alone and in combinations with other Lepidoptera pheromones and odors from plant materials

**Table 3.2.2.11.** Trap catches with interactions between FCM sex pheromone and E2,E13-Octadecadienyl acetate (E2,E13-18Ac)

Treatments	Mean/max by dates - trap catches						Mean
	8-9/11	20-21/11	22-23/11	4-5/12	11-12/12	11-12/01	
Blank control	16/18	25/27	12/16	39.3/51	16.5/23	17.5/18	21.1/25.5
FCM-sex pheromone (FCM-SP) 2% soln.	28/29	37.5/43	21/24	63.3/78	30.3/42	23.5/27	33.9/40.5
FCM-SP (2% soln) + E2,E13-18Ac 1% sol	33/36	45/52	30/32	84.3/92	45.3/56	27/30	44.1/49.7

Over 6 experiments with 12 replicates each, the addition of another moth sex pheromone, E2, E13-18Ac, increased FCM trap catches by 30%. Similar fairly large variations in the sensitivity/reactivity of the various FCM batches from different dates used above were experienced, as was the case in the screening of the YNF + monoterpene combinations mentioned earlier.

**Table 3.2.2.12.** Trap catches with FCM sex pheromone alone and in combinations with various oxo-propionyl compounds (2 reps.)

Treatments	Mean/max 08-10/01	Mean/max 11-12/01
FCM-SP + Pyruvaldehyde	-	26.5/31
FCM-SP + Pyruvic acid	28.5/35	25/28
FCM-SP + Hydroxy-acetone	28.5/35	25/34
FCM-SP + Acetyl acetone	-	24/25
FCM-SP + ethyl pyruvate	-	17/18
<i>FCM-sex pheromone (FCM-SP) 2% soln only</i>	26/30	22/27
<i>Blank control</i>	16/18	17/20

The indications of slight improvement of FCM attraction with pyruvic acid, pyruvic aldehyde and hydroxy acetone will be investigated in further experiments.

**Table 3.2.2.13.** Trap catches with FCM sex pheromone alone and in combinations with various amines, heterocyclics and Lepidopteran pheromones (2 reps.)

Treatments	Mean/max 8-9/11/07	Mean/max 2-3/10/07	Mean/max 15-17/9/07
FCM-SP + Hippuric acid (a carbamide)	-	108.5/128	32/40
FCM-SP + Acetyl choline chloride	32/36	84.5/105	50.5/73
FCM-SP + alpha-Lipoamide	32/32	95.5/110	40/52
FCM-SP + E2,E13-18Ac	33/36	-	-
FCM-SP + N-ATC*	-	85.5/92	35/37
FCM-SP + 2-Acetyl benzoic acid (Benzofuran form)	28/45	75/88	29/34
<i>FCM-SP (2% soln only)</i>	28/29	73/101	25/27
FCM-SP + Disparlure	23/27	-	-
FCM-SP + Choline solution	16/21	-	-
Blank control	16/18	-	-
FCM-SP + Octopamine	-	63/68	-
FCM-SP + Cardamom oil + Molasses	-	58/72	-
FCM-SP + Tryptamine	-	47/60	27/33
FCM-SP + Benzofuran carboxaldehyde	8/12	-	-
FCM-SP + Ethyl anthranilate	-	-	26.5/27
FCM-SP + alpha-tocopherol	-	-	21/26
FCM-SP + Trolox**	-	-	20.5/29
FCM-SP + 2-Acetyl thiophene	-	-	20/32
FCM-SP + Gamma aminobutyric acid (GABA)	-	-	18.5/21
FCM-SP + N- Dodecyl trimethyl amine	-	-	17.5/23

\* N-Acetyl thiazolidine carboxylic acid

\*\*Trolox = 6-Hydroxy 2,3,5,7-tetramethyl chromene carboxylic acid, related to alpha-tocopherol

Previous indications of improvement of FCM attraction with hippuric acid, acetyl choline chloride, alpha lipoamide and E2,E13-18Ac, were supported in the above 3 experiments, with N-ATC and 2-acetyl benzoic acid as possibilities. Hippuric acid is a benzamide, as is capsaicin.

All of these odorants which enhances FCM trap catches when applied in combination with FCM-SP, also featured strongly among the better of the YNF attraction enhancing odorants mentioned earlier.

**Table 3.2.2.14.** Trap catches with FCM sex pheromone alone and in combinations (2 reps.)

Treatments	Mean/max
FCM-SP + Bisabolene	28/33
FCM-SP + Retinyl acetate	27.5/30
<i>FCM-SP (2% soln only)</i>	26/29
FCM-SP + Neryl acetate	25/27
FCM-SP + Dodecyl acetate	22/23
FCM-SP + E2,Z6-Nonadienyl acetate	19.5/22
<i>Blank control</i>	16/18

The batch of FCM used in this experiment was fairly insensitive/unreactive. No clear improvement on FCM alone was found.

### 1.3 Combinations with other odorants

- **Acetyl choline chloride** - alone and in combinations

**Discussion:** No clear improvement in FCM trap catches on that of AchCl alone could be found with additives to AchCl including pyruvic aldehyde, octopamine, E2,E13-18Ac-1%, 3-methyl 2-buten 1-ol, bisabolene, 2-acetyl benzoic acid, Opopanax oil, tiglic aldehyde, cinnamyl isobutyrate, hippuric acid, neryl acetate and cardamom oil.

**Table 3.2.2.15.** Acetyl choline chloride alone and in combinations (2 reps.)

Treatments	Mean/max
Acetyl choline chloride (Ach Cl) alone	29/36
AchCl + 1-Thioglycerol	35/38
AchCl + 2-Methyl 1-butanethiol	31/38
AchCl + Pyruvaldehyde	27/30
Blank control	20/30

No strong improvement in FCM trap catches on that of AchCl alone could be found with any additives mentioned in this experiment.

**Table 3.2.2.16.** Trap catches with Pyruvaldehyde alone and in combinations (2 reps.)

Treatments	Mean/max
Pyruvaldehyde + FCM-SP 2% soln	30/33
Pyruvaldehyde alone	26/33
Pyruvaldehyde + 1-thioglycerol	26/27
Pyruvaldehyde + 2-Methyl 1-butanethiol	24/27
Blank control	20/30
Pyruvaldehyde + Octopamine	15.5/21
Pyruvaldehyde + Hippuric acid	14/15
Pyruvaldehyde + Bisabolene	11.5/12

No strong improvement in FCM trap catches on that of pyruvaldehyde alone, could be found with any additives mentioned in this experiment.

**Table 3.2.2.17.** Trap catches with E2,E13 Octadecadienyl acetate (E2,E13-18Ac) (1% solution in Sunspray 7E oil) alone and in combinations; compared to FCM sex pheromone (2% soln.) (2 reps.)

Treatments	Mean/max
FCM-SP 2% alone	37.5/43
E2,E13-18Ac + VKM-SF 2%	45/52
E2,E13-18Ac + gamma dodecalactone	26/34
E2,E13-18Ac – 1% alone	25/27
Blank control	25/26
E2,E13-18Ac + alpha lipoamide	23/28
E2,E13-18Ac + Cinnamyl isobutyrate	22/24
E2,E13-18Ac + N-acetyl thiazolidine carboxylic acid	19/20
E2,E13-18Ac + Ethyl anthranilate	16.5/22
E2,E13-18Ac + Chamomile oil (Blue)	16.5/22
E2,E13-18Ac + Acetyl choline chloride	16/18

E2,E13-18Ac (1%) improved FCM attraction best on addition to FCM-SP 2% and not to any other odorant partner used, although it seemed not to be attractive alone as compared with the blank control.

**Table 3.2.2.18.** Trap catches with 2-Acetyl benzoic acid alone and in combinations (2 reps.)

Treatments	Mean/max
2-Acetyl benzoic acid (ABA) alone	27/36
ABA + gamma Dodecalactone	32/33
ABA + Cinnamyl isobutyrate	24/33
ABA + alpha Lipoamide	22/26
ABA + E2,E13-18Ac	20/22
ABA + Methyl jasmonate	20/32
ABA + cis-Jasmone	20/28
ABA + Acetyl choline chloride	17/18
ABA + Hippuric acid	17/21
ABA + Angelica lactone	8.5/12
FCM-SP 2% alone	54/70
Blank control	11/12

2-Acetyl benzoic acid (ABA) alone attracted FCM against the blank control but no strong improvement on this was obtained on addition to ABA of any other odorant partner used in this experiment.

- **Octopamine** – alone and in combinations (2 reps.)

No clear improvement on the blank control in FCM trap catches could be obtained from octopamine apart and in combinations with pyruvaldehyde, 1-thioglycerol or 2-methyl 1-butanethiol.

- **Orange oil** – alone and in combinations (2 reps.)

No clear improvement on blank control in FCM trap catches could be obtained from orange oil alone and combinations thereof with acetyl choline chloride, alpha lipoamide and choline solution.

## 2 Orchard experiments

**Table 3.2.2.19.** Trap catches with the interaction between FCM sex pheromone with E2,E13-18Ac in paired trap placings in navel orchards (3 reps./treatment/date)

Treatments	14-18/01/08 Mean/max	22-26/01/08 Mean/max	14-18/03/08 Mean/max	Mean/max (9 reps.)
FCM-SP (2% soln) only	1.3/2	2.3/3	3 /4	2.2/4
FCM-SP (2%) + E2,E13-18Ac (1%)	3.7/4	4.7/6	5/10	4.5/6.7

Over 9 replicates on 3 dates FCM trap catches were doubled by the combination of FCM-SP (2%) + E2,E13-18Ac, in comparison with FCM-SP (2%) alone. Apart from E2,E13-18Ac, which gave the most consistent improvement in FCM-SP trap catches, a wide range of other odorants which were selected from the better choices from laboratory experiments, were also tested in similar paired trap orchard experiments with 3 replicates. Less consistent improvements were obtained from FCM-SP combinations with acetyl choline chloride. The orchard experiments were much less sensitive and much more variable than the laboratory experiments. Test moths disperse fairly quickly from the point of application in the paper bags near the traps so that presumably not many of them remain to be caught. It was therefore decided to first screen all odorants from all chemical groups and to test combinations from them before going to orchard experiments with the best choices. Also in future to increase the artificially created FCM populations per test tree and to try to implement measures to prevent their dispersal away from the test trees.

### Conclusion

For application in practical citriculture, the only addition to FCM-SP which was proved experimentally to enhance FCM-SP attraction was another Lepidoptera sex pheromone, i.e. E2,E13-octadecadienyl acetate. For this season, orchard testing of other laboratory identified synergistically acting odorants with FCM-SP or young navel fruits lagged behind in anticipation of anticipated improved orchard testing procedures. Present orchard test procedures were experienced to be too insensitive and variable. Thus, it was decided to first screen all available odorants from all groups in combination with young navel fruits, or to a lesser extent with FCM-SP, and continue with the best of these in orchard experiments.

### Technology transfer

Only annual reports to CRI.

## Reference cited

Howse, P., Stevens, I. and Jones, O., 1998. Insect pheromones and their use in pest management. Chapman & Hall, London, New York. Chapter 5: Chemical structures and diversity of pheromones; pp 135-174.

### 3.2.3 VORDERINGSVERSLAG: Bestryding van VKM met Steriele Insekloslatings (SIL)

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

## Opsomming

Daar is min formele navorsing gedurende die 2007-2008 verslagtydperk uitgevoer. Die Sitrusbedryf het gedurende 2006 besluit dat die Steriele-Insek Tegniek (SIT) so gou moontlik vir valskoddingmotbestryding vir kommersiële toepassing in sitrusboorde ontwikkel moes word. Dit het beteken dat 'n insektarium vir die massateel van genoeg insekte om in 6 000 ha sitrus in die Olifantsriviergebied, Wes-Kaap, los te laat, opgerig moes word. Daarmee saam moes die hele infrastruktuur om die insektarium te bedryf, geskep word. Die taak is aan die skrywers van dié verslag opgedra. Geen toerusting wat voorheen op klein skaal vir VKM-teling gebruik was, kon vir massateling aangewend word nie. Toerusting soos eierlêpanne, teelflesse, pupeersubstraat, larwale versperrings en ontpoppingskabinette, is ontwerp. Die insektarium, wat uiteindelik nagenoeg 2 000 m<sup>2</sup> beslaan, is terselfdertyd ontwerp. Alle konsepvoorstelle is uiteindelik aan die projekingenieurs vir die oprigting van die gebou en vervaardiging van die toerusting, oorgedra.

Daar is vordering gemaak met die toets van alternatiewe voedselbestanddele soos 'n goedkoper graad mieliemeel en melkpoeier, wat in die saamgestelde dieet van die larwes gebruik word. Die veiligheid van 'n interne kleurstof, Calco, vir insluiting in die larwale dieet, is ondersoek. Proewe met osoongas, wat uitgevoer is om 'n plaasvervanger vir die ontsmettingmiddel, formalien, te vind, dui daarop dat die produk groot belofte vir die ontsmetting van eiervelle en produksiekamers inhou.

## Summary

Relatively little formal research was conducted during the 2007-2008 report period. The Citrus Industry decided during 2006 that the Sterile Insect Technique for false codling moth control should be developed as quickly as possible for commercial application in citrus orchards. This meant that an insectary big enough to produce enough insects for release in 6 000 ha of citrus in the Olifants River area, Western Cape, had to be developed and built. The decision included the parallel development of the total infrastructure to operate the facility. The task was assigned to the authors of this report. There was no equipment available from existing FCM insectaries suitable for mass rearing purposes. Equipment such as egg laying containers, diet jars, pupation substrate, larval barriers, and eclosion cabinets therefore had to be designed. The insectary, covering approximately 2 000 m<sup>2</sup>, was designed simultaneously. All concept proposals were presented to the project engineers for erection of the building and manufacture of the equipment.

Progress was made with the evaluation of alternative food ingredients such as a cheaper grade of maize flour and milk powder, for use in the larval diet. The safety of an internal dye, Calco, for inclusion in the larval diet, was investigated. Experiments with ozone gas, aimed at the replacement of the disinfectant, formaldehyde, showed promise for the disinfection of egg sheets and rearing rooms.

## Inleiding

'n Loodsprojek om die doeltreffendheid van die Steriele-insek Tegniek (SIT) met behulp van Steriele-insek Loslatings (SIL) onder boordtoestande te ondersoek, is gedurende die 2006-2007 verslagjaar uitgevoer. Die resultaat was só belowend (raadpleeg die CRI-Jaarverslag vir daardie tydperk) dat die sitrusbedryf besluit het om die projek sonder verwyf te ontplooi sodat dit op groot skaal deur sitrusprodusente vir die kommersiële bestryding van VKM gebruik kan word. Sitrus uit die Wes-Kaap was op daardie tydstip die enigste in suidelike Afrika wat vir uitvoer na die goed-betalende VSA-markte goedgekeur was. Van al die sitrusgebiede rig VKM ook tradisioneel die meeste skade in die betrokke gebied aan. Daar is derhalwe besluit om die projek in die Citrusdal-gebied in te lei en daarna na ander gebiede uit te brei. Die taak is aan die skrywers (Hofmeyr & Hofmeyr) opgedra om 'n nuwe insektarium te ontwerp waarmee genoeg VKM geproduseer kan word om SI-loslatings in alle sitrusboorde in die Olifantsriviervallei, Wes-Kaap, nagenoeg 6 000 ha, moontlik te maak. Daarmee saam moes die nodige toerusting ontwerp word waarmee meer as 14 miljoen insekte per week geteel sou kon word.

VKM word alreeds baie dekades lank in Suid-Afrika geteel. Alle prosesse en toerusting wat deur Ripley *et al* (1939) vasgestel is en deur Theron (1948) en Schwartz (1972) aangepas was, is tot relatief onlangs min of

meer onveranderd deur bestaande VKM-insektaria gebruik. VKM is egter nog nooit op enige skaal vergelykbaar met wat vir SIL benodig word, geteel nie en alle bestaande toerusting was sonder uitsondering om verskeie redes heeltemal ontoereikend vir massateeloeleindes. Daar is derhalwe begin om nuwe toerusting te ontwerp. Teeltegnieke vir verskillende organismes in die algemeen en in besonder vir Lepidoptera-spesies, verskil heeltemal van mekaar. Daar was dus geen bekende internasionale SIT-massateelfasiliteite waarvan die toerusting onveranderd, of selfs met min veranderinge, vir VKM-massateling gebruik kon word nie. Die enigste aspek wat nie aandag gekry het nie, was die larwes se dieet, wat alreeds vantevore deur Moore en Richards (2001) ondersoek en met sukses verander was.

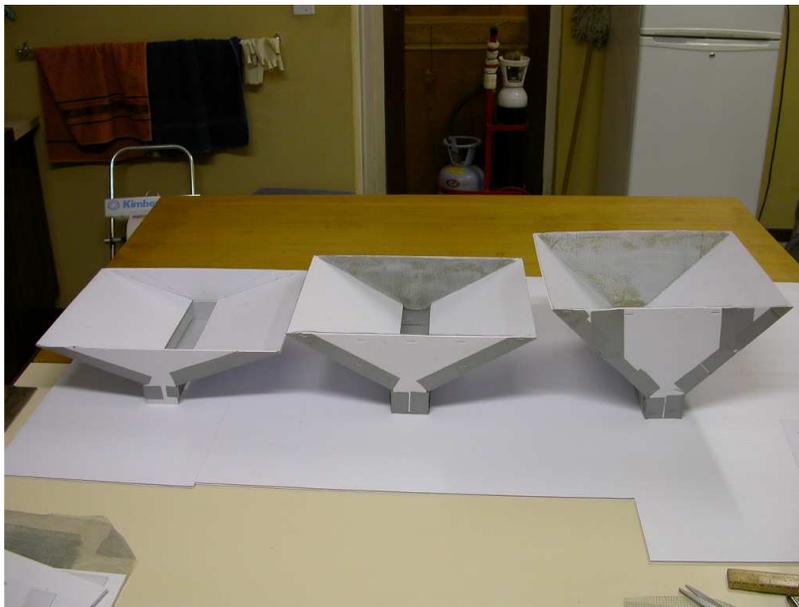
Die verskillende stukke toerusting wat ontwerp is, word vervolgens bespreek. Proewe word ook bespreek waarin alternatiewe metodes om die teelproses te verbeter, ondersoek is.

### 3.2.3.1 Eierlê-apparaat

Die meelsiwwe wat as standaardmetode in VKM-insektaria gebruik was, was om verskeie redes ontoereikend:

- Die siwwe het baie spasie in beslag geneem.
- Dit was arbeidsintensief om die eivelle onder die siwwe in posisie te plaas en weer te verwyder wanneer eiers daarop gelê was.
- VKM is bekend vir die massa skubbe wat hulle verloor. Dié skubbe dryf lank in die lug rond en is uiters gevaarlik omdat dit deur werkers ingeasem word en lugwegprobleme kan veroorsaak. Dit was onmoontlik om dié skubbe met die ou stelsel te verwyder.

Beter eierlêstelsels as die meelsiwwe word internasionaal vir die versameling van Kodlingmot- (Osoyoos, Kanada) en Pienkbolwurmeiers (Phoenix, VSA) gebruik. Eersgenoemde is egter as onnodig ingewikkeld beskou en het iedergeval nie vir die veilige verwydering van skubbe voorsiening gemaak nie. 'n Prototipe wat op die Pienkbolwurmsstelsel gebaseer is, is gebou en getoets. Dit was egter doeltreffend, aangesien die doeltreffendheid daarvan op sekere kenmerke eie aan PBW staatgemaak het. Verskeie prototipes "eierpanne" is vervolgens gebou en getoets (Fig. 3.2.3.1). Die basiese ontwerp was spasievriendelik en kon per eenheidsoppervlakte net soveel as 3,6 meelsiwwe produseer. Dit was ook ontwerp om aan lugsuigapparaat gekoppel te word om skubbe weg te voer. Daarbenewens kon 2 rolle waspapier daaraan gekoppel word wat die versameling van eiers baie vereenvoudig het.



**Fig. 3.2.3.1.** Drie prototipe eierlêpanne (sonder deksels).

Die prototipe eierlêpanne is verfyn en 'n horisontale ontwerp, in plaas van die trogvormige panne is uiteindelik ná toetsing gefinaliseer en gebou (Fig. 3.2.3.2).



**Fig. 3.2.3.2.** Eierlêpanne in die nuwe valskodlingmotinsektarium. Die middelste ry eierpanne is nie in posisie nie. Los skubbe word met behulp van lugsuigpype (blou pype) na 'n sentraalgeleë skubfiltrerder elders in die gebou wegvoer.

### 3.2.3.2 Teelflesse en dekselmembrane

#### Inleiding

Gewone glasheuningflesse, met 'n inhoudsmaat van 375 ml elk, is uitsluitlik in die verlede as teelflesse gebruik. Die flesse het 'n gehad. Eenhonderd en veertig gram dieet is in elke fles geplaas en met 'n stywe watteprop toegemaak. Om die vereiste 15 miljoen motte per week vir die SIT-program in Citrusdal te teel, sou meer as 14 000 heuningflesse per dag ingeënt moes word. 'n Paar verskillende alternatiewe soorte glasflesse is derhalwe gedurende die vorige verslagtydperk (CRI-Jaarverslag vir 2006-2007) getoets om vas te stel of 'n geskikter fles gevind kan word. Daar is uiteindelik besluit om na 'n sogenaamde Consul Glass blatjangfles (500 ml inhoudsmaat) oor te skakel. In vergelyking met die standaard heuningflesse kon dié flesse met dubbel die hoeveelheid dieet (280 g) gevul word, waarmee 2 keer meer larwes per fles geproduseer kon word. Dit het die volgende voordele ingehou:

- Die aantal flesse wat hanteer moes word vir dieettoediening, hitte-ontsmetting, eierinenting, inkubasiespasie en skoonmaak, kon tot nagenoeg 7 000 verminder word.
- Een blatjangfles is goedkoper as 2 heuningflesse.
- Gebruik van die metaal flesdeksel in kombinasie met 'n papiermembraan, was goedkoper en gebruikersvriendeliker as standaard watteproppe.

Verskeie grade Sappi-papier tipes is vervolgens getoets om 'n graad te vind wat deurlaatbaar genoeg was om vogverlies toe te laat teen 'n tempo wat 'n geskikte vogbalans vir die ontwikkelende larwes in die teelflesse sou handhaaf.

#### Materiale en metodes

Drie verskillende grade papier is met mekaar vergelyk. Vyf herhalings, wat elk uit 'n enkele blatjangfles bestaan het, is per behandeling gebruik. Tweehonderd en tagtig gram aangemaakte dieet (140 g dieet plus 140 g water) is in elke fles geplaas en hitte-ontsmet. Elke fles is daarna met formalien-ontsmette VKM-eiers ingeënt en met flesdeksels toegemaak. 'n Ronde gat, 40-50 mm in deursnee is in elke deksel gemaak, die plastiek dekselverseëling is verwyder en met 'n papierskyf (82 mm in deursnee) vervang. Die flesse is by 26°C gehou totdat die larwes hul volwasse grootte bereik het. Die deksels is toe met riffelkartonproppe vervang. Die openinge in die proppe was 2 dae later met kokonne gevul en is met nuwes vervang. Die orige larwes het die daaropvolgende 2 dae daarin puppeer en die flesse is verwyder. Die kartonproppe is 5 dae lank by 26°C gehou waarna alle kokonne oopgemaak en die papies verwyder en getel is (Tabel 3.2.3.1). Tien vroulike en 10 manlike papies is ewekansig per herhaling versamel en individueel op 'n analitiese weegskaal geweeg.

## Resultaat en bespreking

Nagenoeg dieselfde aantal larwes is per fles in 3 van die behandelings geproduseer (Tabel 3.2.3.1). Minder larwes is in die flesse met die High Yield Flute (HYF) 112 g/m<sup>2</sup> dekselmembraan met 'n 50 mm gat in die deksel, geproduseer. Die rede vir dié oënskynde swak prestasie is onduidelik, aangesien dié behandeling vantevore net so goed soos die deksels met 40 mm gate presteer het. Dit kan wees dat die 50 mm gate in dié proef vinniger uitdroging van die diët as die 40 mm gate veroorsaak het wat die ontwikkelende larwes benadeel het. Daar is egter min rede waarom hierdie verskynsel verder ondersoek moet word, aangesien deksels met die kleiner 40 mm gate iedergeval onder praktiese toestande beter beskerming aan die papiermembrane as deksels met groter gate sal bied.

**Tabel 3.2.3.1.** Produksie van valskodlingmotlarwes in flesse met verskillende grade dekselmembrane.

Papier		Grootte van gat in deksel	Gemiddelde aantal papies per fles	Gemiddelde massa (g) per papie	
tipe	graad (g/m <sup>2</sup> )			wyfie	mannetjie
Stratoseal	80	40 mm	608	0.0376	0.0275
High Yield Flute	112	40 mm	622	0.0354	0.0277
High Yield Flute	112	50 mm	571	0.0371	0.0285
High Yield Flute	125	40 mm	608	0.0377	0.0278

Daar is 'n sterk verband tussen die aantal larwes wat per fles geteel word en die grootte (massa) van die papies (derhalwe ook die motte) wat daaruit ontwikkel. Gebaseer op inligting van vorige soortgelyke proewe is die klein verskille in papiemassa tussen die onderskeie behandelings onbelangrik (Tabel 3.2.3.1). Verskille raak eers opsigtelik wanneer die gemiddelde massa van die papies met nagenoeg 0,005 g of meer, van mekaar verskil.

### Gevolgtrekking

Gebaseer op bogenoemde inligting is besluit dat die Consul Glass 500 ml blatjangflesse, toegerus met metaaldeksels met 40 mm gate en Sappi Kraft High Yield Fluting 112 g/m<sup>2</sup> papiermembrane, in die vervolg as standaard teelfles gebruik sal word.

#### 3.2.3.3 Teelflesmandjies

Meer as 140 000 teelflesse sou gedurende 'n 20-dag tydperk in die teelproses benodig word. Dié groot getal flesse moes só gepak word dat lugvloei nie verhinder word nie. Dit moes ook toelaat dat die flesse nie een-een hanteer hoef te word nie. Omdat die flesdeksels verwyder moes word wanneer die volwasse larwes wou puppeer, moes die verpakkingstelsel manipulasie van die flesse toelaat. Daar is aanvanklik beplan om die flesse in vlekvrystaalkiste (25/kis) te plaas. Die ontwerp was egter onder andere veels te duur en daar is alternatiewelik op 'n vlekvrystaalmandjie besluit wat dieselfde aantal flesse kon bevat. Die mandjies is só ontwerp dat die flesse regop in posisie gehou word, maar ook op hul sye gedraai kon word nadat die flesdeksels verwyder is. Die mandjies kon ook in 'n stewige stapel opmekaar gepak word (Fig. 3.2.3.3). Die mandjies het verder voorsiening gemaak dat heuningkoekvelle onder die flesse ingeskuif kon word sodat die larwes daarin kon puppeer.



**Fig. 3.2.3.3.** Vlekvryestaalmandjies, elk met 25 teelflesse. 'n Swart, polikarbonaatplastiek heuningkoekvel is onder die boonste laag flesse ingedruk. Die larwes is nog nie gereed om te puepeer nie en die flesse staan nog regop met hul deksels vasgedraai.

Die konsepvoorstel vir die mandjies is aan Puresteel Products, Kaapstad, opgedra, wat die verskillende eienskappe waaraan hulle moes voldoen, in die mandjie-ontwerp ingebou het. Dié maatskappy sal op die ou end 'n totaal van 5 600 van dié mandjies vir die nuwe insektarium vervaardig (Fig. 3.2.3.4). Die eerste 2 000 mandjies is in September 2007 in gebruik geneem en werk soos beplan.



**Fig. 3.2.3.4.** Stapels van 8 teelflesmandjies elk met 'n heuningkoekvel in posisie onder elke laag teelflesse. Die flesdeksels is verwyder en die flesse lê op hul sye sodat die larwes maklik kan uitklim om te puepeer. Die mandjies is op 'n lae ysterraam met houtbasis geplaas om (i) hantering van die mandjies te vergemaklik en (ii) as 'n platform vir larweversperrings te dien.

### 3.2.3.4 Ontwikkeling van 'n larweversperring

#### Inleiding

Volwasse VKM-larwes verlaat hul voedsel, soos lemoene, waarin hulle ontwikkel het, om elders kokonne te spin en te puepeer. Hulle het 'n instinktiewe drang om voor verpopping so ver en so vinnig as moontlik te versprei om van hulle makkers af weg te kom. Dié reaksie dien as 'n beskermingsmeganisme teen

rooforganismes wat wydverspreide individue moeiliker opspoor as wanneer hulle almal bymekaar bly. In die natuur sorg dié gedrag vir oorlewing van die spesie, maar onder kunsmatige omstandighede in 'n insektarium, waar die larwes om praktiese redes bymekaar gehou moet word, is dit 'n besliste nadeel. Alhoewel die plastiekheuningkoeke 'n goeie pupeersubstraat vir die larwes is, het 'n groot aantal van hulle nog steeds 'n verspreidingsdrang. Dit bring mee dat hulle die heuningkoeke verlaat en met sydraadjies verby die stapel teelflesse en heuningkoeke tot op die grond afsak. Daar pupeer hulle gewoonlik in die hoeke van die teelkamer, waar die papies nie versamel kan word nie (Fig. 3.2.3.5). Dit was derhalwe nodig om 'n larweversperring te ontwerp waarmee die larwes tot die teelflesplatform beperk kon word.

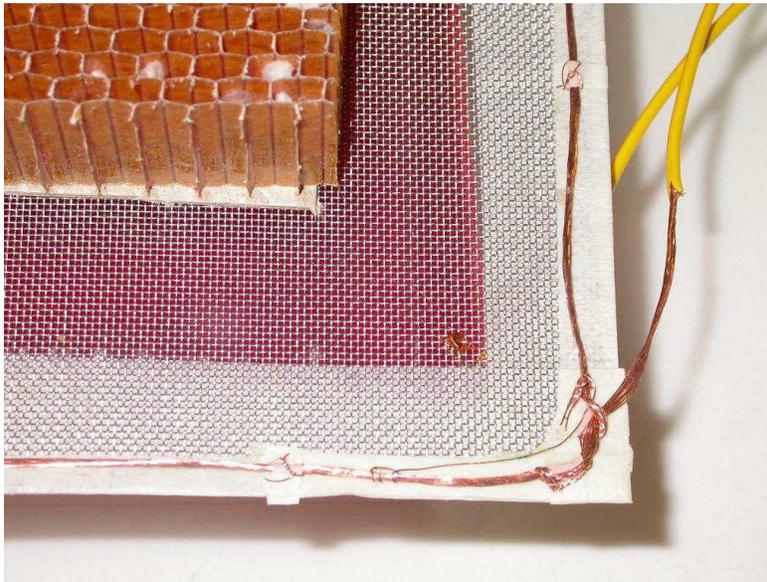


**Fig. 3.2.3.5.** Larwes wat die teelflesstapels verlaat het en in die hoeke van die teelkamer pupeer.

### **Metodes en resultate**

Aanvanklik is gehoop dat 'n tipe materiaal of meganiese versperring gevind sal kan word waaroor die larwes nie sou kon loop of uitklim nie. Nadat 'n groot reeks materiale en verskeie tipes versperrings geëvalueer is, is daar tot die slotsom gekom dat so 'n versperring nie bestaan nie. Die doeltreffendheid van elektriese versperrings is vervolgens as 'n laaste uitweg ondersoek. Verskeie prototipes wat óf van elektriese skokke, óf van hitte, gebruik gemaak het om die larwes te keer, is gebou.

**Eerste prototipe versperring:** 'n Stuk vlekvrystaalgaasdraad, 200 mm x 200 mm, wat die een elektrode gevorm het, is op 'n polistireenbasis vasgemaak. 'n Stuk koperdraad, die tweede elektrode, is met 'n gaping van 2-3 mm rondom die gaasdraad gespan (Fig. 3.2.3.6). Die elektrodes is aan 'n verstelbare voltreguleerder verbind en 'n aantal volwasse larwes is op die gaasdraad geplaas. 'n Voltsterkte van 10V is aanvanklik gebruik en dit was onmiddelik duidelik dat die larwes nie van die swak elektriese skokke gehou het nie. Fyn vonkies was duidelik sigbaar wanneer 'n larwe, wat op die gaasdraad geloop het, met die tweede elektrode kontak gemaak het. So 'n larwe het onmiddelik teruggedeeins en weggeloop om weer op 'n ander plek die versperring te probeer oorbrug. Dié prototipe het gewys dat die stelsel meriete het.



**Fig. 3.2.3.6.** Eerste prototipe elektriese larwe-skokversperring. Die 2 gaas- en koperdraadelektrodes is sigbaar.

**Tweede prototipe versperring:** 'n Tweede prototipe elektriese skokversperring is ontwerp met die praktiese toepaslikheid daarvan in gedagte gehou. 'n Houtbasis, 700 mm x 700 mm, met 'n hoë rand, 20 mm hoog x 20 mm breed, rondom, is gebruik. Die gaas- en draadelektrodes van die eerste prototipe is vervang met vlekvrystaalplate, elk nagenoeg 700 mm x 20 mm breed. Die eerste elektrode het uit 4 plate bestaan wat kontak met mekaar gemaak het en vertikaal teen die binnerand van die houtbasis vasgeheg is. Die tweede elektrode het uit 4 verdere plate bestaan wat horisontaal op die houtbasis vasgeheg is (Fig. 3.2.3.7). Hulle het ook kontak met mekaar gemaak, maar is met 'n (beplande) 1 mm breë gaping van die vertikale plate geskei. Die 2 elektrodes is aan die verstelbare voltreguleerder gekoppel en aangeskakel. Twee flesmandjies gevul met teelflesse en heuningkoekvelle, is as 'n bron van volwasse, migrerende larwes gebruik. Verskeie voltsterktes is getoets om 'n geskikte versperring te vind.



**Fig. 3.2.3.7.** Tweede prototipe elektriese larwe-skokversperring. Die 2 plaaielektrodes is in die regterkantse hoek van die houtbasis sigbaar.

Die skokversperring het goed gewerk teen 15V-18V, alhoewel 2 probleme opgemerk is:

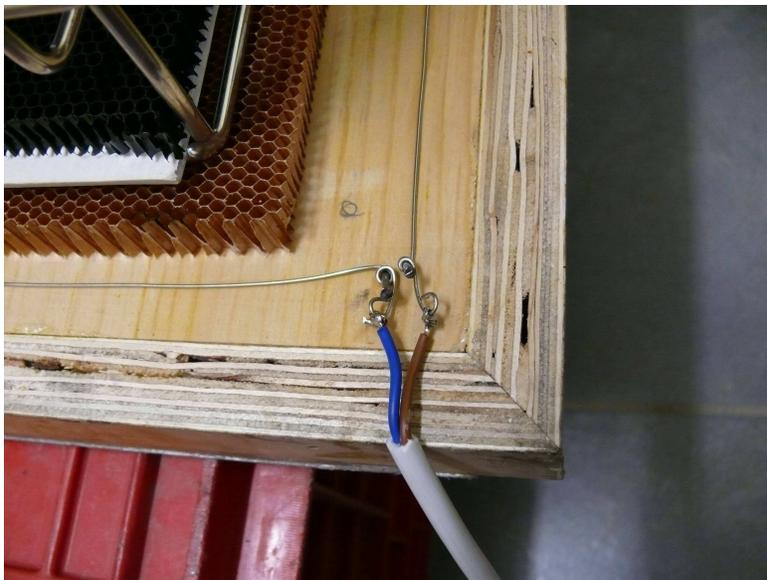
(i) Dit was moeilik om die regte gaping tussen die 2 plaaielektrodes te handhaaf. Dit het veroorsaak dat sommige larwes tussen die 2 plate beland het en sodoende die versperring kon oorbrug sonder om geskok te word. In sommige gevalle het enkele larwes selfs daarin geslaag om tussen die elektrodes te pupeer!

Dié probleem het gewys dat dit in die praktyk uiters moeilik sou wees om versperrings van tot 6 m lank te vervaardig met behoud van die regte elektrodegaping.

(ii) Dit was nie moontlik om 'n voltsterkte kies wat in alle opsigte geskik was nie. Daar was onderlinge verskille in die vatbaarheid van die larwes vir elektriese skokke. Teen 'n laer voltsterkte het sommige larwes oor die elektrodes geklim, terwyl ander deur dieselfde skok geïmmobiliseer is. Teen 'n hoër voltsterkte is sommige larwes doodgeskok. Sulke geïmmobiliseerde en dooie larwes het in kontak met beide elektrodes bly lê en sodoende 'n brug gevorm waarvoor hul makkers die versperring met gerief kon oorsteek sonder om geskok te word.

By gebrek aan 'n beter ontwerp, is vervolgens besluit om hitteversperrings te toets.

**Derde prototipe versperring:** Elektriese verhitte draad met 'n weerstand van  $1,7 \Omega/m$ , is gebruik. Die draad is eenvoudig rondom die bodem van die houtbasis vasgekram en aan die voltreguleerder gekoppel (Fig. 3.2.3.8) en aangeskakel. 'n Soortgelyke opstelling as by Prototipe 2 hierbo, naamlik 2 flesmandjies gevul met teelflesse en heuningkoekvelle, is as 'n bron van volwasse, migrerende larwes gebruik. Verskeie voltsterktes is getoets om 'n temperatuur te vind wat as 'n geskikte versperring vir die larwes sou dien. Die draadtemperatuur is met behulp van 'n digitale termometer, toegerus met 'n oppervlakte termokoppel, gemeet.



**Fig. 3.2.3.8.** Derde prototipe elektriese larwe-hitteversperring.

'n Temperatuur van nagenoeg  $70^{\circ}\text{C}$  was warm genoeg om die larwes só af te skrik dat hulle nie die versperring sou oorbrug nie.

Die stelsel is vervolgens op groter skaal op 'n teelflesplatform van  $6,3 \text{ m} \times 0,72 \text{ m}$ , in die nuwe insektarium opgerig. Met die eerste proeflopie het 'n probleem opgeduik wat nie voorsien is nie en ook nie gedurende toetsing in die vorige prototipe waargeneem is nie. Die styf-gespanne weerstanddraad het soveel van die hitte uitgesit dat dit kortsluitings met die omringende staalraam van die platform gemaak het. Groot gapings van tot 20 mm hoog, is ook tussen die draad en die houtbasis gevorm waaronder die larwes gerieflik deurgeloopt het. Die versperring moes dus herontwerp word.

**Vierde prototipe versperring:** In konsultasie met Robbie Rabe, van Hotflo (Edms) Bpg, Kaapstad, is 2 veranderings getoets: Die weerstanddraad is deur 'n vlekvrystaalpyp (8 mm deursnee) gesteek wat uitsetting van die draad sou keer. Alhoewel die pyp ook sou uitsit, sou dit baie minder en hopelik meer beheerbaar, wees. Om kortsluiting met die pyp te verhoed, is 2 tipes insulasie getoets, naamlik porseleinkrale (Fig. 3.2.3.9) en hittebestande silikoonrubberbuis (4 mm deursnee) (Fig. 3.2.3.10). Twee volgrootte versperrings is vervaardig en getoets. Beide versperrings het goed gewerk, maar die porseleinkrale was omslagtig om mee te werk en het maklik gebreek. Daar is dus besluit om die silikoonbuis te gebruik. Laasgenoemde buis is sterk, maklik om te gebruik en behoort lank te hou. Die versperring lyk dus soos volg: Die weerstanddraad word eers deur die silikoonbuis gesteek, waarna die kombinasie deur die vlekvrystaalpyp gesteek word. Die pyp word dan op die korrekte afstand gebuig en met draadkramme

op die houtbasis vasgesit. Uitsettingslaste word op sekere afstande ingesit om uitsetting van die pyp toe te laat.



**Fig. 3.2.3.9.** Die vlekvrystaalpyp met weerstanddraad deur porseleinkrale geïnsuleer.



**Fig. 3.2.3.10.** Die vlekvrystaalpyp met weerstanddraad deur silikoonrubberbuis geïnsuleer.

Die volledige stelsel is getoets en het uitstekend gewerk. Die vlekvrystaalpyp het uiteraard 'n baie groter deursnee as die weerstanddraad, en word oor sy volle oppervlakte warm. Dit bied dus 'n baie groter, hoër, warm versperring as die draad opsigself. Larwes wat daaraan geraak het, het onmiddelik teruggedeeins en vervolgens langs die versperring, maar minstens 15 mm daarvandaan, geloop sonder om verdere pogings om oor te klim, aan te wend.

### **Gevolgtrekking**

Die hitteversperring soos dit hierbo beskryf is, is die enigste tegniek wat gevind kon word wat larwemigrasie doeltreffend verhoed het. Dit kon dus op kommersiële skaal in die nuwe insektarium gebruik word.

### 3.2.3.5 Polikarbonaatplastiek-heuningkoeke vir pupering

Nagenoeg 5 600 heuningkoekvelle word in 'n 20-dag siklus gebruik. Om die groot aantal velle, volgepak met papies, te hanteer, is 'n raamwerk ontwerp waarin hulle vir enkele dae geplaas kan word sodat die puperingsproses voltooi kan word (Fig. 3.2.3.11). Die raamwerk is só ontwerp dat dit net so in die motontpoppingskabinet ingeskuif kon word om hantering te vergemaklik.



**Fig. 3.2.3.11.** Heuningkoeke met wit kokonne in 'n vlekvrystaalraamwerk gestapel. Die hele raamwerk met heuningkoeke word net so in die motkabinet geplaas sodat ontpopping kan plaasvind.

### 3.2.3.6 Motversamelingskabinette

#### Inleiding

Wanneer larwes volwassenheid bereik, spin hulle elk 'n kokon en pupeer daarin. Gedurende die vorige verslagtydperk is melding gemaak van die polikarbonaatplastiek-heuningkoekmateriaal wat uiters geskik was as 'n pupeersubstraat vir die larwes. Die heuningkoekvelle was net dik genoeg om toe te laat dat een larwe per sel kon verpop. Elke heuningkoekvel is op 'n soliede, 3 mm dik basis geplaas sodat pupering slegs van een kant af kon plaasvind en die motte na ontpopping hul selle dus slegs na een kant toe kon verlaat. Toerusting moes vervolgens ontwerp word waarin die velle heuningkoekmateriaal met papies geplaas kon word sodat die motte versamel kon word wanneer ontpopping plaasvind.

#### Materiale en metodes

Dit was nodig dat groot getalle motte na ontpopping outomaties versamel kon word. Die motte sou derhalwe na ontpopping uit die kabinet (hierna vermeld as "motkabinet") waarin die heuningkoekvelle met papies geplaas was, gelok moes word. UV-lig word in Phoenix, Arizona, gebruik om Pienkbolwurm uit hul kabinette te lok. Voorbereidings is getref om 'n soortgelyke stelsel te toets, maar voordat dié studie ingelei kon word, het Marsheille Hofmeyr 'n prototipe motkabinet ontwikkel wat slegs omgewingslig benodig het om die motte aan te lok. Dié ontwikkeling was nie uniek nie, aangesien 'n soortgelyke stelsel in die Ceder Biocontrol Insektarium gebruik was. Dié stelsel het egter swak gewerk aangesien nie alle motte uit die motkabinette gelok was nie. Tot 40% van die wyfies het ook alreeds gepaar teen die tyd dat hulle in plastiekflesse versamel kon word. Twee nuwe prototipe-ontwerpe is vervolgens ontwerp en het soos volg daar uitgesien:

**Eerste prototipe:** Elke heuningkoek het bestaan uit 'n vel heuningkoekmateriaal van aramidpapier, nagenoeg 600 mm x 600 mm x 14 mm groot. Die selle, waarvan daar ongeveer 22 500 per vel was, was regdeur oop en het nie blind geëindig nie. Elke heuningkoekvel is van 'n soliede, los basis van Correx-plastiek van dieselfde grootte voorsien om te voorkom dat die larwes regdeur die heuningkoek kruip en nie in die selle pupeer nie.

Twee heuningkoeke met basisse, waarvan meer as die helfte van die selle met papies gevul was, is deur 'n "agterdeur" vertikaal in 'n kabinet geplaas met hul "oop" kante namekaar en nagenoeg 20 mm spasie tussen-

in. Daar is 'n smal spleet in die voorste wand gemaak wat so geplaas was dat lig van buite deur die spleet tussen die 2 heuningkoeke van binne-in die motkabinet sigbaar sou wees. Die spleet was slegs breed genoeg om toe te laat dat motte daardeur kon klim, maar nie so groot dat die binnekant van die motkabinet noemenswaardig belig sou word nie. Motte wat deur die spleet klim, het in 'n voorste kompartement met 'n glaswand beland (Fig. 3.2.3.12). Dié kompartement is van 'n trogvormige bodem met oorlangse spleet voorsien. Motte wat in dié kompartement rondgefladder het, het na onder deur die trogspleet geval en is in 'n plastiekbak versamel.



**Fig. 3.2.3.12.** Eerste prototipe motkabinet vir motversameling. Die agterste heuningkoek-bevattende kompartement is net-net links agter sigbaar. Die spleet aan die agterkant, die glaswand en trogvormige bodem kan in die voorste kompartement gesien word.

**Tweede prototipe:** Dieselfde basiese ontwerp is gebruik, behalwe dat die kabinet horisontaal gedraai is (Fig. 3.2.3.13).



**Fig. 3.2.3.13.** Tweede prototipe motkabinet vir motversameling. Die splete waardeur die motte uitkruip is agter die glasvenster te sien.

Die toetsinsekte wat deur die trogspleet in die bodem van die kabinet geval het, het geen verdere nut gehad nie en is in 'n plastiekbak met 'n water/seepmengsel opgevang waarin hulle verdrink het. Die idee was dat die waterbak in die produksiemodel met 'n pyp vervang sou word wat aan die trogspleet vasgeheg word. Lug sou teen 'n sekere snelheid deur die pyp gepomp word en motte wat deur die spleet in die pyp beland het, sou met behulp van die lugstroom na 'n aangrensende koelkamer weggevoer het.

### **Resultate en bespreking**

Die eerste prototipe kabinet het goed gewerk en groot getalle motte het van die agterste kompartement deur die spleet na die voorste kompartement deurgeklim. Hulle het in die voorste kompartement afgeval en kon deur middel van die trogvormige bodem buite die motkabinet versamel word. Amper 30 000 motte is suksesvol versamel. 'n Groot probleem is egter met dié motkabinet geïdentifiseer. Voor ontpopping wriemel die papies kop eerste *gedeeltelik* uit hul kokonne. Die papiedop bars dan oop en die mot kom tevoorskyn. Die leë papiedop bly net waar dit is. In die kabinet het die papies in sulke groot getalle ontpop dat die motte baie papiedoppe afgestamp het in hul pogings om die voorste kompartement te bereik. Baie van die motte, veral dié waarvan die vlerke nog nie volledig ontplooi was nie, het saam met die papiedoppe afgeval en tussen die heuningkoeke op die bodem van die kompartement beland. Daar het hulle tussen die papiedoppe verstrengel geraak en gevrek. Papies wat alreeds uit die kokonne gewriemel het, maar nog nie ontpop het nie, is ook deur motte in die kabinet afgestamp en het onderin die kabinet ontpop. Dié motte het ook geen kans op oorlewing gehad nie (Fig. 3.2.3.14). Nagenoeg 1 100 motte het só verlore gegaan.



**Fig. 3.2.3.14.** Agterdeur van motkabinet oopgemaak om digte massa afgevalde papiedoppe en dooie motte wat daarin verstrengeel geraak het, te wys.

Die motkabinet is vervolgens aangepas deur dit horisontaal te draai (tweede prototipe). Dit het meegebring dat die papiedoppe op die heuningkoekvelle bly lê het en nie na onder kon val nie (Fig. 3.2.3.15).



**Fig. 3.2.3.15.** Die horisontale motkabinet se agteraansig. Die bruin papiedoppe kan bo-op die heuningkoekvelle gesien word.

Dié gewysigde motkabinet het uitstekend gewerk en slegs een mot uit 25 000 het in die motkabinet agtergebly. Geen dooie motte is in die kabinet opgemerk nie. 'n Tweede proef is met dieselfde resultate uitgevoer.

'n Steekproef wat later met 'n produksiemodel uitgevoer is, het gewys dat minder as 10% van die versamelde wyfies kans gekry het om met die mannetjies te paar voordat hulle in die koelkamer beland het. Dit is dus 'n heelwat beter resultaat as wat met die motversamelstelsel in die ou insektarium verkry kon word.

## **Gevolgtrekking**

Die horisontaal-ge-oriënteerde prototipe motkabinet het uitstekend gewerk en is as voorbeeld gebruik vir die motversamelingstoerusting wat vervolgens vir die nuwe insektarium vervaardig is. Die voltooide produk, wat sedert Augustus 2007 in die nuwe insektarium gebruik word, is deur Veritech Manufacturing, Somerset-Wes, ontwikkel (Fig. 3.2.3.16).



**Fig. 3.2.3.16.** Motversamelingskabinette in die nuwe insektarium. Die oorspronklike splete waardeur die motte van die agterste na die voorste kompartement beweeg het, het die volle breedte van die kabinet beslaan. Dit is in die produksiemodel met kort, lasergesnyde splete vervang wat eenvoudiger was om te vervaardig en net so goed gewerk het. 'n Lugstroom word deur die blou pype gepomp wat die motte na 'n aangrensende koelkamer vervoer.

### **3.2.3.7 Skoonmaak van heuningkoekmateriaal**

Die velle sintetiese heuningkoek (ca. 600 mm x 600 mm x 11 mm) wat vir gebruik in die nuwe insektarium beoog was, is van polikarbonaatplastiek gemaak. Nadat die papies ontpop het, moet die heuningkoekvelle vir hergebruik skoongemaak word. Die enigste bekende manier is om die heuningkoeke in bleikmiddel te week wat die sykokonne oplos. Verskeie proewe is uitgevoer om die beste konsentrasie bleikmiddel vas te stel.

#### **Materiale en metodes**

Heuningkoekvelle is aan volwasse larwes vir pupering blootgestel. Nadat die papies ontpop het, is die heuningkoekvelle vir verskillende tye in verskillende konsentrasies bleikmiddel geweek.

#### **Resultate en bespreking**

Dit was duidelik dat 3 eienskappe, naamlik die konsentrasie bleikmiddel, die watertemperatuur en die tyd van behandeling, belangrik was om die sykokonne op te los (Tabel 3.2.3.2). Dit was byvoorbeeld moontlik om 'n relatiewe lae konsentrasie bleikmiddel in koue water te gebruik, maar die heuningkoeke vir 'n langer tydperk te week. Dieselfde reaksie kon verkry word deur warm water saam met die bleikmiddel te gebruik en die heuningkoekvelle vir 'n korter tyd daarin te week.

**Tabel 3.2.3.2.** Die doeltreffendheid van natriumhipochloriet om sykokonne van valskodlingmot in polikarbonaatheuningkoek op te los.

Produk	Konsentrasie bleikmiddel	Water-temperatuur	Duur van behandeling	Resultaat
Snowbrite (10% m/m)	0.3% a.b.	17°C	30 min.	Slegs 60% van kokonne opgelos
Snowbrite (10% m/m)	1.0% a.b.	17°C	40 min.	Alle kokonne opgelos
Snowbrite (10% m/m)	1.0% a.b.	15°C	40 min.	Geen kokonne opgelos nie. Bleikmiddel waarskynlik verslaan
Snowbrite (3.5% m/m)	0.35% a.b.	15°C	20 min.	Geen reaksie
	0.35% a.b.	55°C	20 min.	80% opgelos
	1.0% a.b.	45°C	20 min.	87% opgelos
	2.0% a.b.	35°C	20 min. plus 20 min.	20 min = 99% opgelos; 40 min = 100% opgelos
	1.0% a.b.	15°C	60 min.	95% opgelos
Protea (12,5% m/m)	2.0% a.b.	15°C	60 min.	80% opgelos
Protea (12,5% m/m)	1.0% a.b.	53°C	30 min.	90% opgelos
		42°C	plus 10 min.	100% opgelos
Protea (12,5% m/m)	0,4% a.b.	55-60°C	60 min.	Oorblyfsels word met hoëdruk-waterspuit skoongespuut

Daar is uiteindelik aanbeveel dat gebruikte heuningkoeke vir ongeveer 40 minute lank in 1% bleikmiddel in warm water (40-50°C) geweek moet word. 'n Bleikmiddeltenk is in die nuwe insektarium gebou om nagenoeg 60 heuningkoeke per keer skoon te maak. Die aanbevole resep is aangepas om by sekere ontwerpeienskappe van die tenk in te pas en die heuningkoeke word tans 60 minute lank in 0,4% bleikmiddel in warm water (55-60°C) geweek. Enige kokonoorblyfsels word daarna met 'n hoëdrukwaterspuit skoongespuut.

### 3.2.3.8 Produksiepotensiaal van “Spesiale” en “Gesifte” mieliemeel

#### Inleiding

VKM-insektaria gebruik al jare lank sogenaamde “Spesiale” (“Special”) mieliemeel. Niemand weet meer waarom hierdie graad meel in besonder aanvanklik in gebruik geraak het nie. Alhoewel dit 'n fyner graad mieliemeel as die sogenaamde “Gesifte” (“Sifted”) mieliemeel is, is die verskil so gering dat die graadsverskil nie opsigtelik is nie. Die Gesifte meel is egter tans nagenoeg R10 per 50 kg goedkoper as die Spesiale meel. Ongeveer 800 kg meel sal per dag gebruik word wanneer die beoogde insektarium in volle motproduksie is. Indien die growwer meel vir teeldoelindes gebruik kan word sal die skynbaar geringe prysverskil dus mettertyd 'n groot besparing bewerkstellig. Proewe is uitgevoer om die produksiepotensiaal van die 2 grade meel te vergelyk.

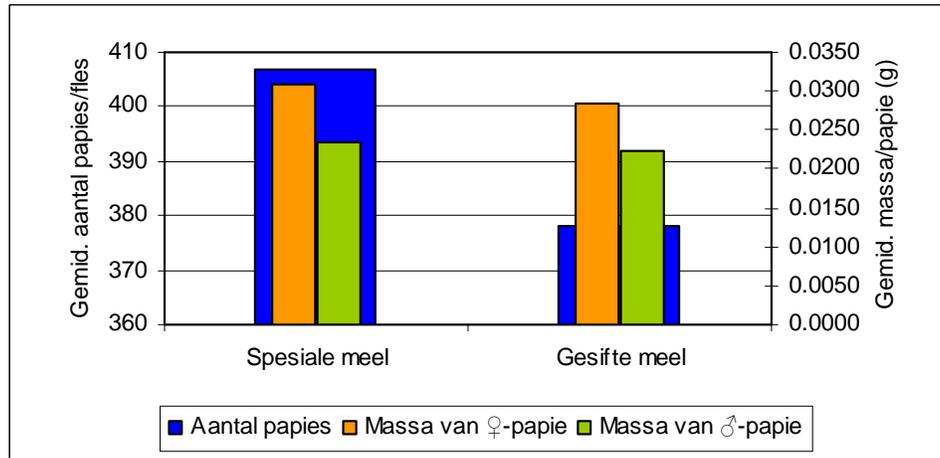
#### Materiale en metodes

Twee proewe is op dieselfde wyse uitgevoer. Tien herhalings, wat elk uit 'n enkele heuningbottel-teelfles bestaan het, is per behandeling gebruik. Eenhonderd en veertig gram aangemaakte dieet (70 g dieet plus 70 g water) is in elke fles geplaas en hitte-ontsmet. Elke fles is daarna met formalien-ontsmette VKM-eiers ingeënt en met watterproppe toegemaak. Die flesse is by 26°C gehou totdat die larwes hul volwasse grootte bereik het. Die watterproppe is toe met riffelkartonproppe vervang. Laasgenoemde is daaglik met nuwes vervang. Die kartonproppe met papies is 5 dae by 26°C gehou, waarna die papies verwyder en getel is. Tien vroulike en 10 manlike papies is ewekansig per herhaling versamel en individueel op 'n analitiese weegskaal geweeg.

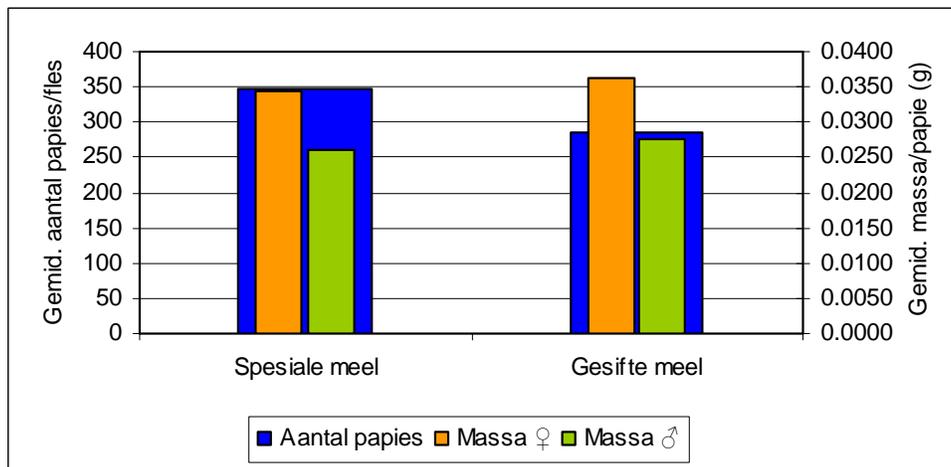
#### Resultaat en bespreking

Meer papies per teelfles is gemiddeld in beide proewe met Spesiale as met Gesifte mieliemeel geproduseer (Fig. 3.2.3.17: 407 teenoor 378 en Fig. 3.2.3.18: 348 teenoor 284). Die gemiddelde massa per papie tussen

die 2 grade meel het baie min van mekaar verskil (Fig'e. 3.2.3.17 en 3.2.3.18). Die rede vir die produksieverskil is onbekend aangesien die 2 grade meel slegs effens in hul relatiewe grofheid verskil, maar nie in voedsaamheid nie. Indien die produksieverskil standhoudend is, kan dit moontlik help om motte 'n paar generasies lank slegs in Gesifte meel te teel sodat hulle daaraan gewoon kan raak. Soortgelyke proewe sal uitgevoer word om die kwessie verder te ondersoek.

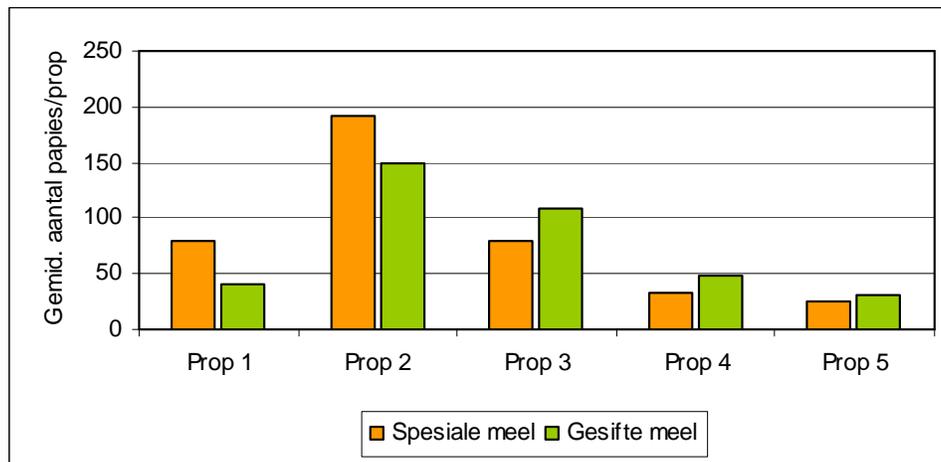


**Fig. 3.2.3.17.** Spesiale teenoor Gesifte meliemeel: Invloed op produksievermoë en massa van papies (Proef 1).

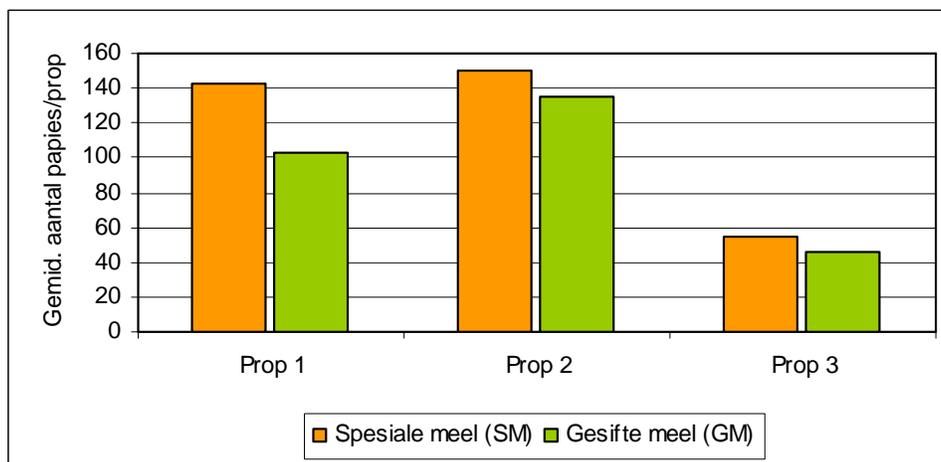


**Fig. 3.2.3.18.** Spesiale teenoor Gesifte meliemeel: Invloed op produksievermoë en massa van papies (Proef 2).

Die ontwikkelings tyd van die insekte, gemeet aan die tydsverloop tussen eierinenting en verpopping, het nie noemenswaardig tussen die 2 grade meel verskil nie (Fig'e. 3.2.3.19 en 3.2.3.20). Die grootste aantal larwes was in die tweede reeks proppe van beide proewe gereed vir ontpopping.



**Fig. 3.2.3.19.** Spesiale teenoor Gesifte mieliemeel: Ontwikkelingstyd van VKM-larwes gemeet aan tydsverloop van eierinenting tot verpopping (Proef 1).



**Fig. 3.2.3.20.** Spesiale teenoor Gesifte mieliemeel: Ontwikkelingstyd van VKM-larwes gemeet aan tydsverloop van eierinenting tot verpopping (Proef 2).

### 3.2.3.9 Produksiepotensiaal van Volroom- en Kremel-melkpoeier

#### Inleiding

Een van die bestanddele in die sintetiese dieet waarmee VKM in die insektarium geteel word, is volroommelkpoeier. Die verkryging daarvan is egter wisselvallig aangesien dit afhanklik is van die beskikbaarheid van vars melk. Dit is derhalwe nodig om 'n alternatiewe produk te vind wat gebruik kan word wanneer volroommelkpoeier onverkrygbaar is. 'n Saamgestelde melkpoeier, Kremel<sup>®</sup>, waarin die diervet van gewone volroom melkpoeier met plantvet vervang is, benewens die byvoeging van sekere vitamien, is met volroommelkpoeier vergelyk. Spesiale en Gesifte mieliemeel is terselfdertyd in dieselfde proef weer met mekaar vergelyk.

#### Materiale en metodes

Sestien herhalings, wat elk uit 'n enkele heuningbottel-teelfles bestaan het, is per behandeling gebruik. 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 ingeënt en met watterproppe toegemaak. Die flesse is by 26°C gehou totdat die larwes hul volwasse grootte bereik het. Die watterproppe is toe met riffelkartonproppe vervang. Laasgenoemde is met nuwes vervang sodra die voriges met kokonne gevul was. Die kartonproppe met papies is 5 dae by 26°C gehou, waarna die papies verwyder en getel is. Tien vroulike en 10 manlike papies is ewekansig per herhaling versamel en individueel op 'n analitiese weegskaal geweeg.

## Resultate en bespreking

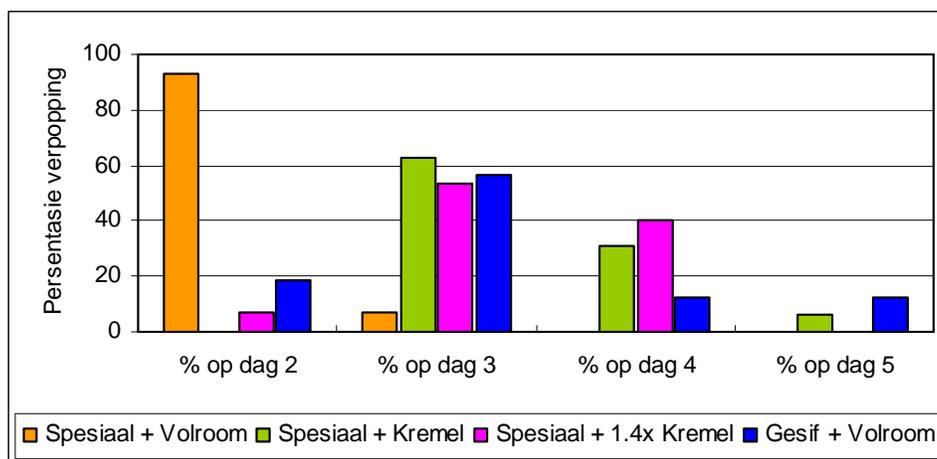
In die vorige 2 proewe (Afd. 3.2.3.8) is meer insekte met Spesiale meel plus volroom melkpoeier (die standaard dieët) as met Gesifte meel (plus volroommelkpoeier) geproduseer. In dié proef het die 2 diëte gemiddeld net soveel papies met Gesifte meel as met Spesiale meel (beide in kombinasie met volroommelkpoeier) geproduseer (Tabel 3.2.3.3). Die vervanging van volroom- met dieselfde hoeveelheid Kremelmelkpoeier in die standaard dieet het oënskynlik 'n swakker produksie veroorsaak. Produksie met Kremel teen 1,4x meer as die volroommelkpoeier, was dieselfde as met laasgenoemde. Dit kom derhalwe voor asof enige voedingstekort wat daar moontlik in die kleiner hoeveelheid Kremel was, deur die verhoging aangevul is. Alhoewel die massa van die papies wat met 1,4x Kremel geproduseer was, gemiddeld effens ligter as in die ander behandelings was, is die verskil te klein om belangrik te wees.

**Tabel 3.2.3.3.** Produksie van valskodlingmotlarwes met verskillende grade mieliemeel en melkpoeier.

Behandeling		Gemiddelde aantal papies per fles	Gemiddelde massa (g) per papie	
Mieliemeel	Melkpoeier		Wyfie	mannetjie
Spesiaal	Volroom (1x)	386	0.0348	0.0263
Spesiaal	Kremel (1x)	342	0.0342	0.0258
Spesiaal	Kremel (1,4x)*	387	0.0308	0.0238
Gesif	Volroom (1x)	379	0.0381	0.0294

\*Die Kremel se dosis is met 37% verhoog sodat dit dieselfde as volroommelkpoeier kos.

Dit is wenslik om in 'n massateelsituasie soveel insekte moontlik in die kortste tyd te teel. Die tempo waarteen die kartonproppe met kokonne gevul raak, is 'n aanwyser van die ontwikkelingsnelheid van die larwes. Meer as 90% van die larwes wat in die standaarddieet (Spesiale meel plus volroommelkpoeier) ontwikkel het, het alreeds grotendeels op die tweede dag puppeer. Die verpoppingstempo van larwes wat in die diëte met die aangepaste bestanddele, Kremelmelkpoeier en Gesifte mieliemeel, ontwikkel het, het eers op die derde dag toegeneem (Fig. 3.2.3.21). Dit is egter vreemd dat dieselfde nie in die vorige 2 proewe gebeur het nie. Dit wil derhalwe voorkom asof die verskynsel wisselvallig is en sal in verdere proewe dopgehou word.



**Fig. 3.2.3.21.** Invloed van alternatiewe mieliemeel en melkpoeier op die ontwikkelingsduur van VKM-larwes

### 3.2.3.10 Invloed van Calco Oil Red kleurstof op die teel van valskodlingmot

#### Inleiding

Dit is vanselfsprekend dat (a) die kwaliteit van geteelde insekte en (b) die sukses van insekloslatings in 'n kommersiële SIL-program, bepaal moet kan word. Daar is verskillende maniere om dit te doen, naamlik met behulp van onder andere vrugvalopnames, loslaat-en-hervangingsmonitering, paringstafelstudies, paringsproewe en mikroskooptechnieke. Alhoewel dit nie opsigself alle inligting kan verskaf nie, bied lokvalle egter die vinnigste en maklikste manier om so 'n moniteringsfunksie uit te voer. VKM wat gesteriliseer en losgelaat word, verskil ongelukkig uiterlik in geen opsig van wilde motte in die boord nie. Die 2 groepe motte kan derhalwe op geen manier met die oog van mekaar onderskei word nie, tensy die losgelate motte vooraf

op een of ander manier gemerk word nie. Daar is alreeds voorheen in proewe van gekleurde, fluoriserende poeiers gebruik gemaak om die motte uitwendig te merk. Dié metode pas egter moeilik in by 'n massateelprogram en kan ook veroorsaak dat die gemerkte motte gedeaktiveer word. 'n Metode wat internasionaal oor die algemeen as veilig beskou word, is om die betrokke insekte inwendig te kleur.

Calco Oil Red is 'n nie-giftige kleurstof wat by die dieet van Kodlingmot en Pienkbolwurm in in-sektaria 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 afgebreek nie. Sodanig gemerkte motte kan ná loslating en hervangs van wilde motte in lokvalle onderskei word deur hul plat te druk en die liggaamsinhoud vir tekens van rooi kleurstof te ondersoek. Die sukses van die loslatings kan onder andere sodoende bepaal word.

Proewe met Calco, waartydens die hervangs van Calco-gemerkte motte ondersoek was, het as gevolg van swak vangste in selfs die onbehandelde kontrolebehandeling, misluk (CRI-Jaarverslag vir 2004-2005, Afd. 3.4.5.8). Resultate was egter wisselvallig en daar kon nie tot 'n gevolgtrekking oor die geskiktheid van die kleurstof gekom word nie. 'n Verdere proef is dus uitgevoer.

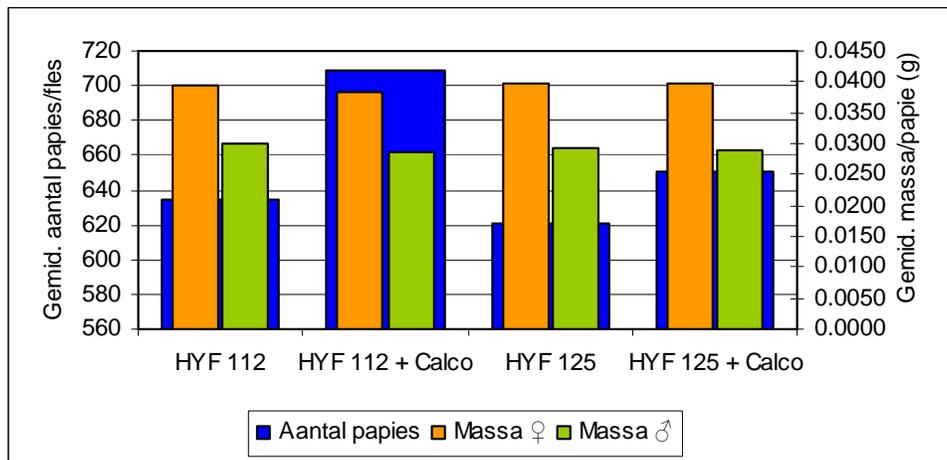
### **Materiale en metodes**

Die maontlike nadelige uitwerking op die produksie van VKM met 'n Calco-bevattende dieet is in dié proef ondersoek. Die geskiktheid van 2 verskillende grade papiermembrane vir die flesdeksels is terselfdertyd getoets. Vyf herhalings, wat elk uit 'n enkele blatjangteelfles bestaan het, is per behandeling gebruik. Tweehonderd en tagtig gram aangemaakte dieet (140 g dieët plus 140 g water) is in elke fles geplaas en hitte-ontsmet. Waar van toepassing, is 0,29 g Calco Oil Red 2144-kleurstof plus 8 ml Canola-olie per 2 000 g dieet bygevoeg. Elke fles is daarna met formalien-ontsmette VKM-eiers ingeënt en met flesdeksels toegemaak. Die flesse is by 26°C gehou totdat die larwes hul volwasse grootte bereik het. Die flesdeksels is toe met riffelkartonproppe vervang. Laasgenoemde is met nuwes vervang sodra die voriges met kokonne gevul was. Die kartonproppe met papies is 5 dae by 26°C gehou, waarna die papies verwyder en getel is. Tien vroulike en 10 manlike papies is ewekansig per herhaling versamel en individueel op 'n analitiese weegskaal geweeg.

### **Resultate en bespreking**

Die Calco-bevattende dieet het gemiddeld meer papies per fles geproduseer wanneer dieselfde graad papiermembraan gebruik was (HYF 112: 709 teenoor 635 en HYF 125: 651 teenoor 621) (Fig. 3.2.3.22). Dié resultaat was ietwat onverwags, aangesien daar verwag was dat Calco eerder 'n na- as voordelige invloed op produksie sou hê. In beide gevalle het die groter aantal papies nie 'n vermindering in liggaamsmassa tot gevolg gehad nie, aangesien so 'n afname eers verwag word wanneer meer as 800 papies per teelfles geproduseer word.

Die flesse wat met HYF 112-grad papiermembrane in hul deksels toegerus was, het beter presteer as die HYF 125-membrane (Sonder Calco: 635 teenoor 621 en met Calco: 709 teenoor 651). Dié verskille is relatief klein, maar kan op daarop dui dat die dikker HYF 125-papiermembraan swakker as die HYF 112 presteer. Daar is vantevore opgemerk dat dieet in flesse wat met die dikker papier HYF 125) toegemaak was, vogtiger gebly het gedurende larwe-ontwikkeling. Larwes vermy dié nat kolle in die dieet en die produksie is gevolglik swakker as gevolg van groter mededinging in die oorblywende droeër dieet.



**Fig. 3.2.3.22.** Produksievermoë van dieet waarby Calco Oil Red kleurstof gevoeg is (HYF = Sappi Kraft High Yield Fluting; 112/125 = papiergradering).

Nog proewe sal met Calco-bevattende dieet uitgevoer moet word om die invloed van Calco op larwe-ontwikkeling verder te ondersoek.

### 3.2.3.11 Die gebruik van osoon vir ontsmettingdoeleindes

#### Inleiding

Afgesien van omgewingstoestande wat insekontwikkeling kan benadeel, speel swamme en virusse in VKM-insektaria 'n bepalende rol in die doeltreffendheid van massateling en sodoende in die sukses waarmee 'n kommersiële SIL-program bedryf kan word. In die CRI-Jaarverslag vir 2004 is daar oor heelwat proewe verslag gelewer wat die onderdrukking van swamme en virusse met behulp van chemiese dospelbehandelings ten doel gehad het. In daardie proewe kon slegs formalien die eiervelle sodanig ontsmet dat virusbesmetting in teelfesse onderdruk kon word. Dié produk is egter onaangenaam om mee te werk en ongesond vir werkers om sonder spesiale beskerming te gebruik. Dit is dus ook nie moontlik om dit vir roetine kamerontsmetting in 'n insektarium te gebruik nie. Osoongas word somtyds in die praktyk vir algemene ontsmetting van instrumente, ens., gebruik en word ook in pakhuse vir vrugbehandeling aangewend om swamme te onderdruk. Alhoewel die gas irriterend vir die mens is en teen hoë konsentrasie selfs gevaarlik kan wees, breek dit vinnig af en vorm weer suurstof, waaruit dit oorspronklik vervaardig word. Dit laat dus geen residu's na nie. Etlke proewe is uitgevoer om die doeltreffendheid van osoon onder verskillende omstandighede te ondersoek. Waar nodig, word tegnieke, resultate, ens., van die proewe wat gemeenskaplik was, saam bespreek.

'n Aeroqual-osoonproduseerder, wat 'n maksimum van 5 000 mg osoon per uur kon produseer, is in alle osoongasproewe gebruik. Dit is in sommige proewe aan 'n instrument gekoppel wat die maksimum produksie van 0,35-0,5 d.p.m. osoongas in 'n vertrek kon beheer.

#### a) Doeltreffendheid teen swamme

##### Materiale en metodes

Drie proewe is uitgevoer om vas te stel of die swamspoorlading in 'n vertrek deur osoonbehandeling verminder kan word. Indien wel, sal osoongas moontlik vir die algemene ontsmetting van vertreke in 'n skoonkamerstelsel gebruik kan word.

*Proef 1:* Die proef is in 'n leë, ongebruikte kamer van die ou VKM-insektarium te Citrusdal uitgevoer. Geen poging is aangewend om die kamer voor, gedurende of na die proef op enige manier skoon te maak of te ontsmet nie. Vyf kontrole Petribakkies met appeldekstrose-agar (ADA) is oop op 'n tafel in die middel van die kamer geplaas. Die lug/stof in die kamer is doelbewus versteur deur 'n plastiekbakdeksel 2 tot 3 keer in die lug te waai om besmetting van die ADA met organismes wat moontlik in die kamer kon wees, te bevorder. Die bakkies is 20 minute later met hul deksels toegemaak en uit die vertrek verwyder. Die osoonproduseerder met beheerder is op 'n aangrensende tafel in dieselfde kamer geplaas. Dit is 6 uur lank aangeskakel om 'n vlak van 0,35-0,5 d.p.m. osoon in die kamer te handhaaf. Vyf ADA-bakkies is 20 minute lank, soos die kontrole-behandeling, na onderskeidelik 2 uur, 4 uur en 6 uur behandeling, in die kamer

blootgestel. Die lug/stof in die kamer is elke keer soos in die geval van die kontrole versteur. Die ADA-bakkies is 40 uur lank by 26°C gehou, waarna alle swamkolonies op die agar getel is.

*Proef 2:* Dieselfde kamer as hierbo in Proef 1 is gebruik. Die kamerdeur is 3 dae lank ná die eerste proef oopgelaat en die kamer is aan normale werkerverkeer in die gang verby die oop deur blootgestel. Daar is weer geen poging aangewend om die kamer voor aanvang van die proef skoon te maak nie. Dieselfde tegniek as in Proef 1 is toegepas. ADA-bakkies is onderskeidelik na 0 uur (kontrole), 1-uur-, 2-uur-, 4-uur- en 6-uurlange osoonbehandeling (0,35-0,5 d.p.m.), vir 20 minute lank blootgestel. Die blootgestelde bakkies is 40 uur lank by 26°C gehou en daarna ondersoek.

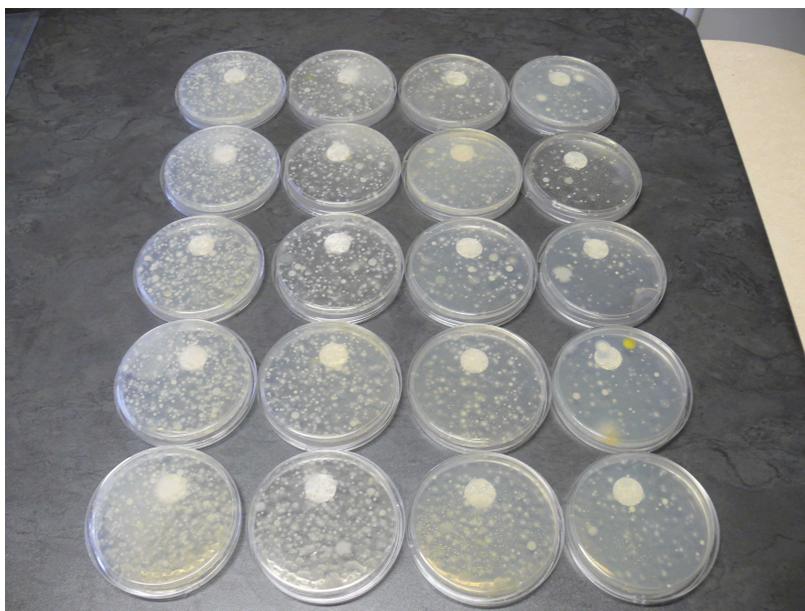
*Proef 3:* Dieselfde kamer as hierbo in Proewe 1 en 2 is gebruik. Die kamerdeur het 6 dae lank na die tweede oopgestaan en die kamer was aan normale werkerverkeer verby die oop deur blootgestel. Daar is weer geen poging aangewend om die kamer voor aanvang van die proef skoon te maak nie. Twee ADA-kontrolebakkies is om 17:00 op die eerste dag voor enige osoonbehandeling blootgestel om vas te stel wat die spoorlading in die vertrek is. Osoon is daarna teen 0,35-0,5 d.p.m. 3 dae lank agtereenvolgens ononderbroke 14 uur lank elke nag vanaf 17:00 tot 07:00 toegedien. Die kamerdeur is vir die verloop van die proef gedurende en tussen behandelings toegehou en is elke dag slegs lank genoeg om 08:00 oopgemaak om ADA-bakkies bloot te stel en 20 minute later weer te verwyder. Die lug/stof is elke keer soos voorheen versteur terwyl die ADA-bakkies blootgestel was. Die blootgestelde bakkies is 40 uur lank by 26°C gehou en daarna ondersoek.

### Resultate en bespreking

Dit was nie gedurende die proewe moontlik om die swamme wat op die ADA gegroei het, te identifiseer nie. Volgens Fourie (pers. kom.) was dit waarskynlik *Penicillium spp.* en ander soortgelyke luggedraagde swamme. Te oordeel aan die aantal swamkolonies wat op die ADA gegroei het, is die spoorlading in die osoonbehandelde vertrek in die eerste 2 proewe sterk verminder soos die osoonbehandeling van 0 tot 6 uur lank verleng is. Dit lyk asof die swambesmetting in die kamer nie so erg herbesmet geraak terwyl dit oopgestaan het tydens die 3 dae wat tussen die 2 proewe verloop het nie – gemiddeld 447 en 95 swamkolonies per ADA-bakkie is in die 2 proewe se kontroles aangeteken (Tabelle 3.2.3.4 en 3.2.3.5; Fig'e. 3.2.3.23 en 3.2.3.24)

**Tabel 3.2.3.4.** Proef 1: Vermoë van osoongas om die swamspoorlading in 'n vertrek te verminder.

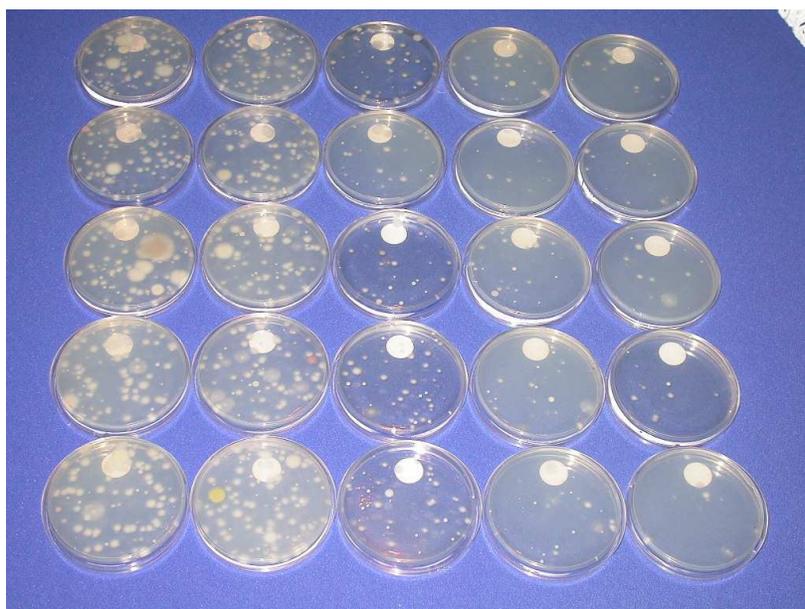
Behandeling	Gemiddelde aantal swamkolonies per ADA-bakkie	Persentasie afname in swamkolonies
Kontrole	446.4	-
2 uur osoon	384.6	13.8
4 uur osoon	237.0	46.9
6 uur osoon	130.2	70.8



**Fig. 3.2.3.23.** Proef 1: Afname in swamkolonies op appeldekstrose-agar na (van links na regs) 0-uur-, 2-uur-, 4-uur- en 6-uurlange behandeling met osoon.

**Tabel 3.2.3.5.** Proef 2: Vermoë van osoongas om die swamspoorlading in 'n vertrek te verminder.

Behandeling	Gemiddelde aantal swamkolonies per ADA-bakkie	Persentasie afname in swamkolonies
0 uur (kontrolle)	94.6	-
1 uur osoon	88.8	6.1
2 uur osoon	45.8	51.6
4 uur osoon	28.6	69.8
6 uur osoon	13.4	85.8

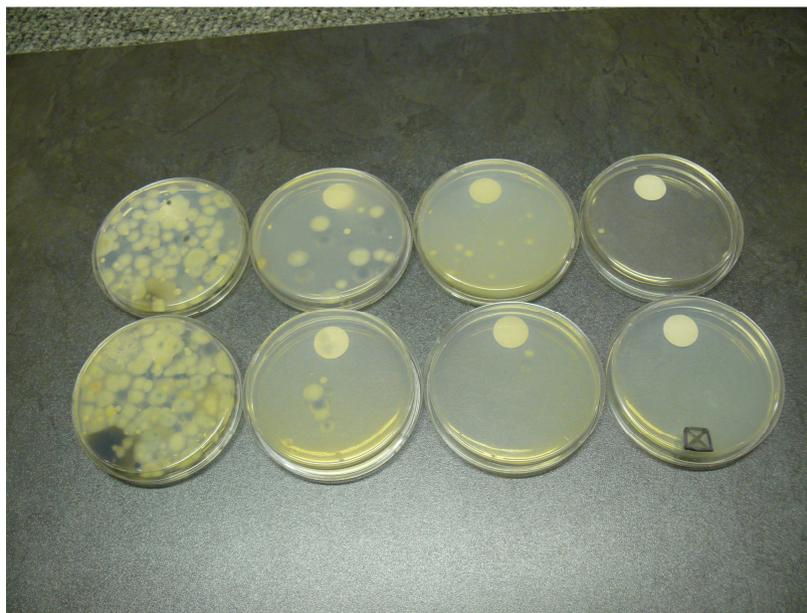


**Fig. 3.2.3.24.** Proef 2: Afname in swamkolonies op appeldekstrose-agar na (van links na regs) 0-uur-, 1-uur-, 2-uur-, 4-uur- en 6-uurlange behandeling met osoon.

Die spoorlading in die derde proef het tot amper nul verminder nadat osoon 14 uur lank op elk van 3 agtereenvolgende dae toegedien is (Tabel 3.2.3.6 en Fig. 3.2.3.25).

**Tabel 3.2.3.6.** Proef 3: Vermoë van osoongas om die swamspoorlading in 'n vertrek te verminder.

Behandeling	Gemiddelde aantal swamkolonies per ADA-bakkie (aantal kolonies per ADA-bakkie)	Persentasie afname in swamkolonies
Dag 0 (kontrole)	97.0 (107, 87)	-
Dag 1	10.0 (13, 7)	89.7
Dag 2	9.5 (17, 2)	90.2
Dag 3	0.5 (1, 0)	99.5



**Fig. 3.2.3.25.** Proef 3: Afname in swamkolonies op appeldeksrose-agar na 14-uurlange osoonbehandeling drie dae namekaar (van links na regs) Dag 0 (kontrole) Dag 1, Dag 2 en Dag 3.

### **Gevolgtrekking**

Die geskiktheid van osoongas om kamers op groter skaal in 'n nuwe insektarium te ontsmet, sal van 2 faktore afhang, naamlik (a) of dit granulosevirus wat ook in die vertreke kan voorkom, sal onderdruk en (b) of dit veilig sal wees vir VKM-eiers en -larwes wat in die kamers kan wees. Bovermelde resultate is belowend en sal op groter skaal op die proef gestel word.

#### **b) Doeltreffendheid van osoongas teen granulosevirus op VKM-eiervelle**

Die proef is uitgevoer om vas te stel of osoongas die viruslading op VKM-eiervelle kan verminder en sodoende besmetting in teelflesse verhoed. Indien wel, sal osoongas die standaard formalienbehandeling kan vervang.

*Proef 1:* 'n Standaardgrootte velletjie waspapier (nagenoeg 40 mm x 40 mm) met nagenoeg 800 eiers, wat met verskillende kombinasies van virus, formalien en osoon behandel was, is in elk van 8 teelflesse per behandeling ingeënt. Die flesse is by 26°C gehou totdat die lewensvatbare larwes tot die 5'de stadium ontwikkel het. Die flesse is daarna vir tekens van virusbesmette larwes ondersoek. 'n Fles met een of meer larwes wat tekens van virusbesmetting getoon het, is as besmet beskou. Die verskillende behandelings en resultate was soos volg (Tabel 3.2.3.7):

**Tabel 3.2.3.7.** Doeltreffendheid van osoongas om die oordraging van granulosevirus van eiervelletjies na teelflesse te verhoed.

Behandeling	Eierbehandeling met			% Flesse met een of meer virusbesmette larwes/fles
	Virus <sup>1</sup>	Formalinen <sup>2</sup>	Osoongas <sup>3</sup>	
Kontrole 1	-	-	-	25
Kontrole 2	-	-	40 minute	25
Kontrole 3	Ja	-	-	100
Osoongas	Ja	-	20 minute	100
Osoongas	Ja	-	40 minute	100
Osoongas	Ja	-	60 minute	100
Standaard	-	Ja	-	0

<sup>1</sup>**Virusbehandeling:** 'n Konsentrasie (LD90) van 1.185E5 (118500 OB's/ml) granulosevirus in water is voorberei. Eiervelletjies is 2 sekondes lank daarin gedompel, toegelaat om droog te word en daarna óf onbehandel óf met formalien of osoon behandel en in teelflesse ingeënt.

<sup>2</sup>**Formalienenbehandeling:** Onbehandelde eiervelletjies is 2 sekondes lank in 20% formalien (20 ml formalien 35% m/m plus 80 ml water) gedompel en daarna in teelflesse ingeënt.

<sup>3</sup>**Osoonbehandeling:** Onbehandelde of virusbehandelde eiervelletjies is 20 tot 60 minute lank met 0,35-0,5 d.p.m. osoongas behandel en daarna in teelflesse ingeënt.

Osoongas kon nie eiervelletjies wat met 'n "natuurlike" of kunsmatige hoë vlak van virus besmet was, teen die getoetste konsentrasies voldoende ontsmet om virusbesmetting in teelflesse te verhoed nie. In teenstelling was die teelflesse waarvan die eiervelletjies met die standaard formalienbehandeling behandel was, vry van virusbesmetting.

*Proef 2:* Dieselfde tegniek as in Proef 2 is gebruik. Die verskillende behandelings en resultate was soos volg (Tabel 3.2.3.8):

**Tabel 3.2.3.8.** Doeltreffendheid van osoongas om die oordraging van granulosevirus van eiervelletjies na teelflesse te verhoed.

Behandeling	Eierbehandeling met				% Flesse met een of meer virusbesmette larwes/fles
	Virus <sup>1</sup>	Formalinen <sup>2</sup>	Chloordioksied <sup>3</sup>	Osoongas <sup>4</sup>	
Kontrole 1	-	-	-	-	75.0
Kontrole 2	-	-	-	6 uur	50.0
Kontrole 3	Ja	-	-	-	100.0
Osoongas	Ja	-	-	3 uur	87.5
Osoongas	Ja	-	-	6 uur	25.0
Chloordioksied	Ja	-	Ja	-	75.0
Standaard	-	Ja	-	-	0.0

<sup>1</sup>**Virusbehandeling:** 'n Konsentrasie (LD90) van 1.185E5 (118500 OB's/ml) granulosevirus in water is voorberei. Eiervelletjies is 2 sekondes lank daarin gedompel, toegelaat om droog te word en daarna óf onbehandel óf met formalien, chloordioksied of osoon behandel en in teelflesse ingeënt.

<sup>2</sup>**Formalienenbehandeling:** Onbehandelde eiervelletjies is 2 sekondes lank in 20% formalien (20 ml formalien 35% m/m plus 80 ml water) afgespoel en daarna in teelflesse ingeënt.

<sup>3</sup>**Chloordioksied:** Virusbesmette eiervelletjies is 2 sekondes lank in 1% chloordioksied (1 ml chloordioksied + 99 ml water) afgespoel en daarna in teelflesse ingeënt.

<sup>4</sup>**Osoonbehandeling:** Onbehandelde of virusbehandelde eiervelletjies is 3 tot 6 uur lank met 5000 mg/uur osoongas behandel en daarna in teelflesse ingeënt.

In die vorige proef was die eiervelletjies relatief kort (tot een uur lank) met osoongas behandel en die kunsmatige hoë vlak van virus kon nie onderdruk word nie. In dié proef het langer osoonblootstelling (3 tot 6 uur lank) wel 'n uitwerking gehad en van die flesse teen besmetting beskerm. Alhoewel die beskermingsvlak nog nie vir massateeltoestande voldoende is nie, lyk dit asof 'n langer behandelingsduur moontlik beter sal werk. Die enigste voorbehoud is uiteraard dat so 'n lang behandeling veilig vir die eiers moet wees.

Chloordioksied het eweneens swak gevaar, terwyl die standaard formaliendompelbehandeling goed presteer het.

c) **Veiligheid van osoongas vir VKM-eiers en larwes**

Osoongas moet heeltemal veilig wees indien VKM-eiers, of teelkamers waarin die larwes gehou word, daarmee behandel word. Die volgende proewe is voorlopers van 'n reeks wat uitgevoer moet word om die veiligheid van osoongas vir die insekte te bepaal.

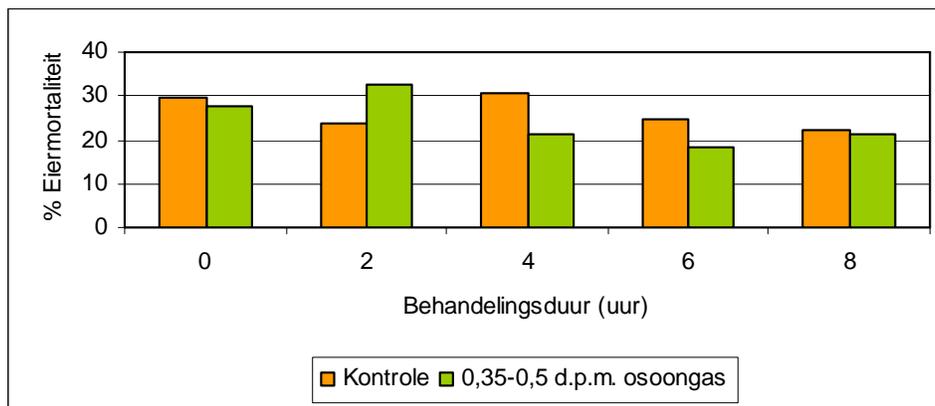
• **VKM-eiers**

*Proewe 1 en 2:* Twee herhalings, wat elk uit 'n enkele vel met minstens 10 000 eiers per vel bestaan het, is per behandeling gebruik. Die velle met eiers is ná versameling 24 uur lank in 'n yskas gehou en die volgende oggend gebruik. Die kontrole-eiers is by 26°C gehou, terwyl die ander eiervelle van 17:00 tot 08:00 met 0,35-0,5 d.p.m osoongas behandel is. Die velle eiers is daarna 5 dae by 26°C gehou sodat ontwikkeling en uitbroeiing kon plaasvind. Alle eiers in onderskeidelik 5 tot 10 mikroskoopvelde per herhaling in die 2 proewe, is daarna per eiervel ondersoek om die persentasie uitbroeiing te bepaal.

Die gemiddelde eiermortaliteit in die onbehandelde kontroles van die 2 proewe was onderskeidelik 98,9% en 98,6%, terwyl eiermortaliteit in die osoonbehandelings van 80,9% tot 91,8% gewissel het. By nadere ondersoek is vasgestel dat daar onregmatig met die yskastermostaat gepeuter was en die temperatuur in die yskas tot -8°C gedaal het. Die eiervelle was derhalwe voor aanvang van die proef 24 uur lank aan dié lae temperatuur blootgestel, wat die abnormale hoë eiermortaliteit in die kontroles verklaar. Dit is tans onverklaarbaar waarom die eiers op die eiervelle in beide proewe, wat vervolgens met osoongas behandel was, nie ook tot dieselfde mate aangetas was nie.

*Proef 3:* Die proeftegniek was basies dieselfde as in die eerste 2 proewe. Die eiers was 24 uur lank voor gebruik in 'n yskas by 5°C gehou. Die eiervelle is onderskeidelik vir tydperke van 2 tot 8 uur lank met 0,35-0,5 d.p.m. (Fig. 3.2.3.26) óf 5 000 mg/uur (Fig. 3.2.3.27) osoongas behandel.

Dit lyk nie asof enige een van die 2 osoongasbehandelings groter eiermortaliteit in vergelyking met die onbehandelde kontrole-eiers veroorsaak het nie. Die "natuurlike" eiermortaliteit in die kontrolebehandelings was egter aan die hoë kant en verdere soortgelyke proewe sal uitgevoer word voordat daar tot enige gevolgtrekking oor die geskiktheid van osoongas as 'n veilige ontsmettingmiddel vir eiers gekom kan word.



**Fig. 3.2.3.26.** Giftigheid van 0,35-0,5 d.p.m. osoongas vir VKM-eiers

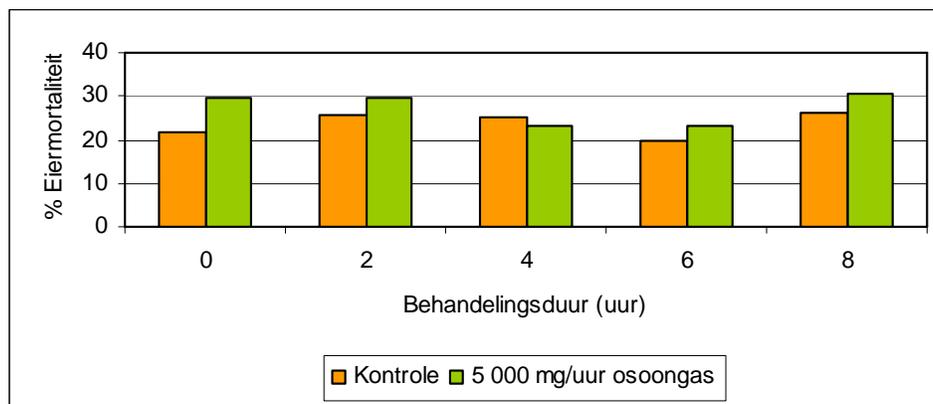


Fig. 3.2.3.27. Giftigheid van 5 000 mg/uur osoongas vir VKM-eiers

- **VKM-larwes**

’n Ongebruikte teelkamer in die nuwe Xsit-insektarium is gebruik. Agt teelflesse met volwasse, 5’de instar larwes wat op die punt van verpopping was, is gebruik. Die flesse was almal met flesdeksels en papiermembrane toegemaak. Vier flesse is as onbehandelde kontroles gebruik en die orige 4 is 14 uur lank met die maksimum hoeveelheid osoon wat die osoonproduseerder kon vervaardig, naamlik 5000 mg/uur, behandel. Die flesdeksels is daarna met riefelkartonproppe vervang om die papies te versamel. Die papies is 5 dae na verpopping verwyder en getel. Die teelflesse se dieët is 5 dae nadat verpopping begin het, versigtig uitgekrap en deursoek vir alle dooie en lewendige larwes wat oorgebly het.

Ongeveer 2,5% van die larwes wat in die kontrole- en osoonbehandelde teelflesse geproduseer is, is dood voordat hulle gepupeer het. Al die onbehandelde, dooie larwes wat in die kontroleflesse agtergebly het, is waarskynlik as gevolg van virusbesmetting dood. Die dooie larwes wat in die flesse in die osoonbehandelde kamer gevind is, het dieselfde simptome van virusbesmetting getoon. Geen ander dooie larwes met verskillende simptome, wat moontlik deur die osoongas veroorsaak is, is opgemerk nie. Dié inligting dui daarop dat osoon moeilik, indien hoegenaamd, deur die flesdeksels se papiermembrane dring. Dit lyk dus tans asof osoon wel vir die ontsmetting van teelkamers geskik sal wees – selfs wanneer daar flesse met ontwikkelende larwes aanwesig is. Dit sal derhalwe gebruik kan word om teelkamers te ontsmet voordat die flesse se deksels verwyder word sodat die larwes in die heuningkoeke kan puppeer. Die proef sal op groter skaal herhaal word om te verseker dat dit veilig is voordat dit as ’n standaardbehandeling in die nuwe insektarium toegepas kan word.

### 3.2.3.12 Die gebruik van ’n girokopter vir die loslaat van gesteriliseerde valskodlingmotte in sitrusboorde

Dit is uit ’n biologiese sowel as finansiële oogpunt nodig om bestraalde motte so vinnig as moontlik onder kommersiële SIL-toestande los te laat. Daar is derhalwe besluit om die potensiële nut van ’n girokopter vir motloslatings te ondersoek. Die doeltreffendheid van motloslatings met ’n girokopter is in September 2007 met loslatings met die hand- én outomatiese toerusting vanaf ’n vierwielmotorfiets vergelyk. Die girokopter is met ’n voorlopige loslaatstelsel, wat uit ’n spesiaal-ontwerpte venturi en pypstelsel bestaan het, toegerus. Motte is vanaf 2 hoogtes, naamlik 12 m en 27 m bo grondvlak, uit die girokopter losgelaat (Fig. 3.2.3.28). Die motte het loslating met behulp van die venturi-stelsel oënskynlik goed deurstaan, maar geen motte is in die week na loslating in sitrusboorde in lokvalle gevang nie. Die aktiwiteitsdrempel vir VKM is nagenoeg 16°C, terwyl metings daarop gedui het dat die temperatuur elke nag gereeld tot ver onder 10°C gedaal het.



**Fig. 3.2.3.28.** Die girokopter in aksie. Indien die foto vergroot word, kan die stroom motte wat losgelaat word, onder die swart pyp wat by die pens van die girokopter uitsteek, gesien word (foto deur Sampie Groenewald).

Drie proewe is vervolgens in Oktober 2007 uitgevoer. Die eerste 2 proewe, soortgelyk aan die vorige ondersoek hierbo, is deur koue nagte in die wiele gery en motvangste was uiters swak. In die derde proef is meer mannetjies gevang, maar weens swak motkwaliteit weer eens nie genoeg om tot 'n gevolgtrekking ten opsigte van die girokopter se geskiktheid te kom nie. Kommersiële loslatings met die girokopter het hierna begin en die geleentheid vir verdere proewe het weggeval. Soortgelyke ondersoeke sal egter in die toekoms uitgevoer moet word.

### 3.2.3.13 Die nuwe insektarium

Die toerusting wat gebruik word het 'n direkte invloed op die grootte en rangskikking van die vertrekke waarin dit gehuisves moet word. Daar kon dus min van bestaande insektariumontwerpe gebruik gemaak word om die nuwe SIT-insektariumgebou te ontwerp. Die ontwerp van die gebou en toerusting moes uiteraard interafhanklik wees, aangesien die gebou groot genoeg moes wees om alle benodigde toerusting te huisves. Daarbenewens moes die gebou só ontwerp word dat die vertrekke waarin die verskillende teelprosesse sou plaasvind, gerangskik is om 'n logiese proses- en arbeidsvloei moontlik te maak.

Die ontwerp van die gebou het min of meer met die ontwerp van verskeie stukke teeltoerusting saamgeval sodat die optimum kamer- en, uiteindelik, gebou-grootte bepaal kon word. Die finale ontwerp is aan die projekingenieurs oorhandig wat die boutekeninge saamgestel het (Fig. 3.2.3.29). Die enigste groot verandering wat aangebring was, was om die gebou se front van Wes na Oos te verander. Dié verandering is genoodsaak omdat die oorspronklike terrein waarvoor die gebou beplan was, ontoereikend geblyk te wees het; die veranderde aansig het beter op die nuwe terrein ingepas.



## Toekomstige doelwitte en werkplan

In dié verslag word die grootste deel van die navorsing wat tot die bou van die nuwe valskodlingmotinsektarium gelei het, bespreek. Die gebou is in Augustus 2007 in bedryf gestel. Daar is besluit dat die teel van motte vir SIL in die Olifantsriviergebied, Wes-Kaap, oor 'n driejaartydperk ingefaseer sal word. Gedurende die eerste jaar sal genoeg motte om SIL in 1 500 ha sitrus toe te pas, geteel word. Die gebied waarin VKM SIL toegepas word, sal gedurende die tweede en derde jare tot 4 500 ha en 6 000 ha uitgebrei word. Dit word ook in die vooruitsig gestel dat, afhange van vordering met die projek, VKM SIT na ander gebiede soos die Oos-Kaap en een of meer van die noordelike provinsies, uitgebrei kan word.

Met die skryf van dié verslag is die insektarium goed aan die gang en meer motte word geproduseer as waarvoor aanvanklik beplan was. As gevolg van die feit dat die gebou en toerusting heeltemal van nuuts af ontwerp moes word, is etlike stelsels wat die toerusting ten volle moet ondersteun, egter nog in 'n ontwikkelingsfase. Daar word egter gedurende vordering gemaak en die gebou sal binne afsienbare tyd in volle bedryf wees.

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- 3.2.4 **FINAL REPORT: Understanding and improving biological control of false codling moth larvae**  
Experiment 690 (April 2002 – March 2008) by Kierryn Keeton (nee Gendall) (RU), Sean D. Moore and Wayne Kirkman (CRI)

## Summary

The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is one of the major pests of citrus in South Africa. Due to problems such as the expense of pesticides, insects developing pesticide resistance, chemical residues on the rind of export fruit and the negative impact of pesticides on the environment, it has become necessary to find alternative methods for pest control. *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae), a larval parasitoid of FCM, recently found only in the Sundays River Valley area, offers a means of control for the pest.

A total of 11 389 navel oranges were collected from various orchards in the Addo/Kirkwood area, and FCM larvae infested 36.09% of the fruit. A single parasitoid species, *A. bishopi*, was reared from these larvae. In 2006 the highest parasitism of larvae, 11.43%, was recorded in May and in 2007, the highest parasitism, 38.9%, was in April. Females of *A. bishopi* live for 18.5 days (n = 20; S.E. = 3.1) and males for 8.25 days (n = 20; SE = 1.23). Females produced an average of 23 offspring in their lifetime, while female FCM produce about 800 eggs each. Captive rearing of *A. bishopi* proved difficult due to viral and fungal contamination. A high number of parasitoids will be required per hectare to reduce the population of FCM. Parasitoids were released into netted FCM infested navel orange trees in the Western Cape. To date no parasitism of larvae has been recorded. However, the trial is still ongoing. *Agathis bishopi* has potential for use in an integrated pest management programme, once the hurdle of mass-rearing has been overcome.

## Opsomming

Die valskodlingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is een van die belangrikste plae wat op sitrus in Suid-Afrika voorkom. Probleme soos die koste van insekdoders, die ontwikkeling van chemiese bestandheid, chemiese residu's op uitvoervrugte en die negatiewe impak van insekdoders aan die omgewing, het ondersoek vir alternatiewe metodes van plaagbeheer nodig gemaak. *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae), 'n parasitoïed van VKM-larwes, wat onlangs net in die Sondagsriviervalleigebied gekry is, het potensiaal as 'n biologiese beheeragent vir VKM.

Altesaam 11 389 nawellemoene is in verskeie boorde in die Addo/Kirkwood-omgewing versamel. VKM-larwes is in 36.09% van die vrugte gekry. 'n Enkele parasitoïedspesie, *A. bishopi*, is uit hierdie larwes verkry. In 2006 is die hoogste vlak van parasitisme van larwes in Mei gekry, d.w.s. 11,43%. In 2007 het parasitisme gedurende April 38,9% bereik. *A. Bishopi*-wyfies leef vir 18,5 dae ( $n = 20$ ;  $SE = 3.1$ ) en mannetjies leef vir 8,25 dae ( $n = 20$ ;  $SE = 1.23$ ). 'n Parasitoïedwyfie het 'n gemiddelde nageslag van 23 gehad, terwyl 'n VKM-wyfie omtrent 800 eiers kan lê. Massateel van *A. bishopi* was moeilik as gevolg van kontaminasie met virus en swamme. 'n Groot aantal parasitoïede sal per hektaar benodig word om die VKM-bevolking te verminder. Parasitoïede is in twee VKM-besmette bome wat in nette toegemaak is, op 'n plaas in die Wes-Kaap vrygelaat. Tot op datum is nog geen parasitisme van VKM-larwes hier gekry nie, maar die proef is nog nie afgesluit nie. Wanneer die struikelblok van massateling oorbrug word, sal daar geleentheid geskep word om *A. bishopi* in 'n geïntegreerde plaagbeheerprogram te gebruik.

## Introduction

Every citrus-producing region has its suite of important pests (Smith & Pena 2002). The false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is regarded as one of the major pests of citrus in South Africa, the others being citrus mealybug (*Planococcus citri*), Mediterranean fruit fly (*Ceratitis capitata*), bollworm (*Helicoverpa armigera*) and the California red scale, (*Aonidiella aurantii*). It is an important pest of citrus throughout southern Africa and has been considered so for many years (Annecke & Moran 1982; Myburgh 1987).

The importance of the pest in citrus is based on its being abundant throughout the year and on the part of the tree which it attacks (Smith & Pena 2002). The larvae infest the fruit, causing the fruit to drop, resulting in a reduction in yield. Secondly, infestation often occurs shortly before the fruit is harvested and can result in post-harvest decay of fruit. Finally, it is considered to be a phytosanitary pest. Detection of the moth outside African markets can result in a great loss of export income (Moore 2002).

Chemical control has proven difficult due to inaccessibility of the burrowing life stage of FCM, development of resistance, incompatibility with integrated pest management (IPM) and integrated crop management, and its expense (Newton 1998). Many studies have been conducted on egg parasitoids, but for advanced control of FCM it is necessary to study parasitoids which attack the larval stage. From preliminary studies conducted in the Eastern Cape from December 2001 to May 2002 (Sishuba 2003), two parasitoid species were reared, *Agathis bishopi* (Nixon) and *Apophua leucotreta* (Wilkinson). Due to the abundance of *A. bishopi* it appeared to be the more valuable parasitoid of FCM on citrus. From a study in the 2003/2004 season it was confirmed that *A. bishopi* was the dominant parasitoid in the Eastern Cape. Due to this the parasitoid was collected and attempts were made to mass-rear the parasitoid. The 2006 season showed that larval infestation was recorded in 32.91% of oranges collected. The highest numbers of parasitoids were obtained from third and fourth instar larvae. The highest parasitism rates were recorded late in the season, from late April to June. An average of 9.99% of the larvae were parasitized over the full survey period.

## Materials and methods

### Parasitoid collection

To investigate parasitism of *Agathis bishopi* in the environment, fruits were collected from various orchards on farms in the Sundays River Valley area on a weekly basis from January to June. Fruit were collected initially from the floor of the orchard and later in the season from the trees. Only fruits which appeared to be infested with FCM were collected. Fruits were collected if they had frass-filled penetration holes or if there was some discolouration around the penetration hole or a mark which was suspected to be a penetration hole.

Fruit were brought back to the laboratory and dissected to find FCM larvae by cutting thin layers of the orange rind away around the penetration hole. The larvae were placed individually in glass vials containing artificial diet (Table 3.2.4.1).

**Table 3.2.4.1.** The ingredients used in the artificial diet for FCM (Moore 2002).

Ingredients	Amount
Maize meal	2 000 g
Wheat germ	200 g
Brewer's yeast	100 g

Milk powder	36.5 g
Nipagin	15 g
Sorbic acid	6.5 g
<b>Total</b>	<b>2 358 g</b>

### Mass-rearing *A. bishopi*

A stock of sterilised honey jars (122 mm x 65 mm) were prepared for the mass-rearing of parasitoids. A hole was cut in the lid of the jar and a cotton-wool plug placed in the hole. The lid was covered with tin foil and autoclaved for 20 min at 127°C, and thereafter allowed to cool under a laminar flow hood. Each jar was prepared with 25 g of dry artificial diet and 25 ml of ddH<sub>2</sub>O mixed together. FCM eggs were obtained weekly from an established culture at Citrus Research International (CRI) in Port Elizabeth, Eastern Cape Province. The moths lay eggs on wax paper provided in their cages. A sheet was removed daily from each cage and cut into squares containing approximately 100 eggs. The egg sheets are then sterilised by placing them in 15% Sporekill (ICA International Chemicals) for 15 min and then dipping them in 25% formalin for 3 seconds to remove or kill fungal spores and any virus particles that may be present. One egg sheet square was placed in each honey jar. The honey jars were left in a constant environment room at 27°C and approximately 60% relative humidity.

Adult parasitoids (2 females: 1 male) were placed in the jars when the first instar larvae were observed. When the adult parasitoids were removed from the honey jar, a large cotton-wool plug was placed in the neck of the honey jar to serve as a pupation substrate for the larvae. When it was observed that the majority of the larvae had pupated, the cotton-wool plug was removed and placed in an emergence jar. A lid was placed on the honey jar in case any larvae had pupated in the diet. The jars were observed daily for the emergence of adult parasitoids, which were then placed in mating jars for a day, to ensure that the female parasitoids mated before being allowed to parasitise the next generation of larvae. The emerged parasitoids were checked for deformations, to ensure the parasitoids were fit for mating. The parasitoids were placed *en mass* in the mating jars, with approximately two males per female to ensure that the females mated. Males were removed from the mating jar when their antennae started to curl, which indicated that the male was going to die and was therefore unfit for mating. The honey and water mixture in the mating jars was replaced daily.

A study was conducted to determine the number of days which were sufficient for the female parasitoids to remain in a single jar in order to parasitise the larvae before being placed into a new jar. This would aid in the mass-rearing process by maximising the number of FCM larvae available for the female parasitoid to parasitise. The data were analysed using a one-way ANOVA.

It was hypothesised that an *Aspergillus* sp. was growing from the bodies of the dead larvae. To test this, honey jars were kept where the larvae were dying due to a virus infection. Dead larvae were removed from 30 bottles and retained in 30 other bottles. Vials were kept with larvae that had died due to virus from the field-collected fruit. These were used to determine whether fungus can start in the oranges from the dead larvae inside them. The fungus was identified by the Rhodes University Department of Microbiology.

### Biological studies

#### 1. *Pre-oviposition, oviposition periods and developmental rates*

Six stages of false codling moth development were used to determine the stage most preferable to *A. bishopi*: eggs only; neonate larvae; and four stages when the surface of the diet was 25%, 50%, 75% or 100% covered by frass, respectively. Each stage contained approximately 100 FCM eggs or larvae. An estimate of instar of the FCM larvae can be made by the percentage frass covering on the surface of the diet. The 25% frass correlates with the second instar, 50% frass with the third instar, 75% frass with the fourth instar and 100% with fifth instar. The parasitoids (2♀♀:1♂) were placed in the jar at the various stages and they were removed from the jar once the next stage began ( $\pm 2$  days). Once the parasitoids had been removed, the lid of the jar was replaced with a cotton-wool plug. It was determined whether there was any parasitoid emergence. Trials with each stage were replicated 10 times. To determine the developmental rate, the time from parasitism to the emergence of adult parasitoids was recorded.

#### 2. *Life span*

Shortly after the emergence of the adult parasitoids, the males and females were placed individually in jars under four different conditions: no food or water (N); water only (W); food and water (FW); for the female, food, water and a male (FW♂) and for the male, food, water and a female (FW♀). The number of days each parasitoid survived was recorded. For each condition 20 female and 20 male parasitoids were used. To

determine if there was a significant difference between the longevity of the females and males, the data were subjected to a t-test.

#### Field releases of *A. bishopi*

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 3.2.4.2). 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. Weekly from 25 March, fruit which had fallen from each tree was collected and inspected for FCM infestation. FCM larvae were extracted from the fruit and placed individually on artificial diet in glass vials, stoppered with cotton wool. Parasitism of these larvae was monitored.

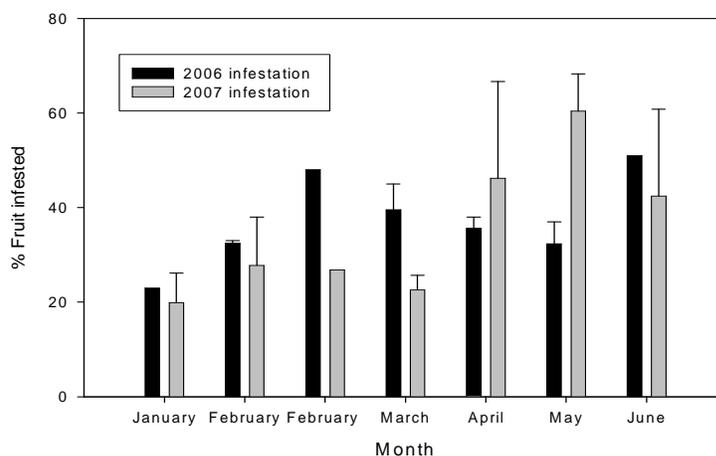
**Table 3.2.4.2.** *A. bishopi* parasitoids released into netted navel orange trees on a farm in Citrusdal.

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
<b>Total</b>			<b>40</b>	<b>20</b>
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
<b>Total</b>			<b>36</b>	<b>17</b>

## Results and discussion

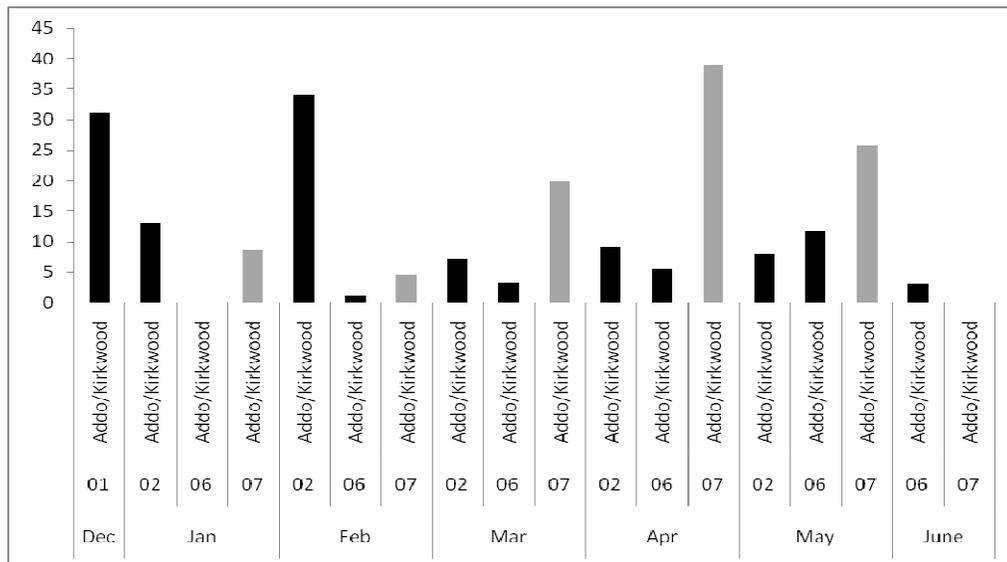
### Parasitoid collection

A total of 11 389 navel oranges were collected from various orchards on farms in the Addo/Kirkwood area for the 2006/2007 season. FCM larvae infested 36.09% of the fruit collected (Fig 3.2.4.1). The infestation rate could be under-estimated as only fruit that larvae were found in were considered infested. Many fruits could be seen to have been infested but no larvae were found, and therefore the fruit was not considered infested. The larvae may have left the fruit to pupate or they were too small to be found in the fruit. In 2006 the percentage of infested fruit ranged from 22% infested fruit per month to 51% and in 2007 from 19% infested fruit per month to 65%.



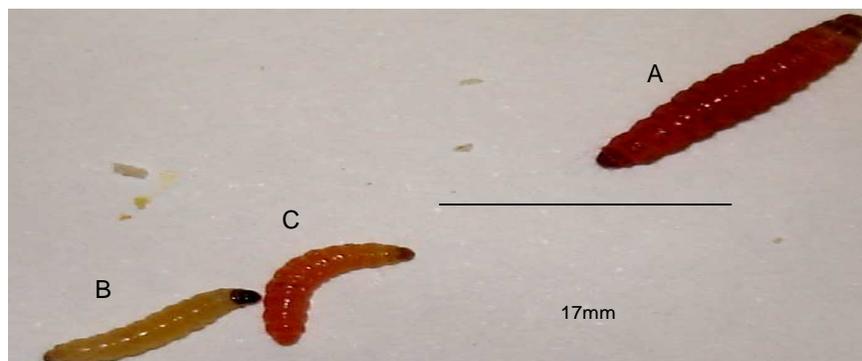
**Figure 3.2.4.1.** The combined average percentage fruit infestation (+ SD) during 2006 and 2007; for the months in which fruit was collected.

A single parasitoid (identified by the Biosystematics division of the PPRI) species, *A. bishopi*, was reared from the FCM larvae collected from the fruit. In 2007, the highest level of parasitism was recorded in April, with 38.9% (parasitism per week was 12.9%) of the FCM larvae yielding parasitoids (Fig 3.2.4.2). In the 2007 season the parasitism rate was low in January with a steady increase in February and March. The changes in parasitism rates may be a result of the monthly fluctuations in the FCM population and factors such as predation, environmental conditions and farming practices such as orchard sanitation. With orchard sanitation, the removal of fruit from the orchard floor, one could be removing parasitoids too. FCM larvae pupate in the soil (Daiber 1979), so by removing the fruit from the orchard floor, the larvae are removed before they can leave the fruit to pupate. This could be detrimental to the success of a biological control programme involving the use of *A. bishopi*. *Agathis bishopi* is a koinobiont, meaning the parasitoid develops inside the host while it is still alive (Hassell *et al.* 1992; Harvey *et al.* 1999; Jervis *et al.* 2001). By removing the fruit early, one is removing the parasitoid along with the fruit, even if not every infested fruit drops. A reason for the low parasitism rates in the early part of the season may be due to the use of chemical sprays in the orchards. These sprays may kill both the host in its first instar and the parasitoid (Moore pers. comm. 2007). Another reason for this may be the decline of the pest and parasitoid population during the winter months (Moore pers. comm. 2007), and therefore both populations will take a few months to build their numbers up again.



**Figure 3.2.4.2.** Monthly rates of parasitism, for seasons 2001, 2002, 2006 and 2007, of FCM by *Agathis bishopi* in the Addo/Kirkwood area. The bars in grey represent the 2007 season.

The second instar yielded the highest number of parasitoids, and there was no emergence of parasitoids from the fifth instar. Sishuba (2003) also found that there was no emergence of parasitoids from fifth instar larvae. This is an indication that the parasitoid parasitizes only early instars of the host. Another possible explanation is that as the parasitoid may retard host development, the larvae may appear to be younger (Fig 3.2.4.3) than they really are.



**Figure 3.2.4.3.** (A) A non-parasitised red fifth instar larva of FCM. (B) A non-parasitised third instar larva of false codling moth and (C) a parasitised larva of false codling moth appearing to be in the third instar but has the colouring (red) of a fifth instar larva.

### Mass-rearing

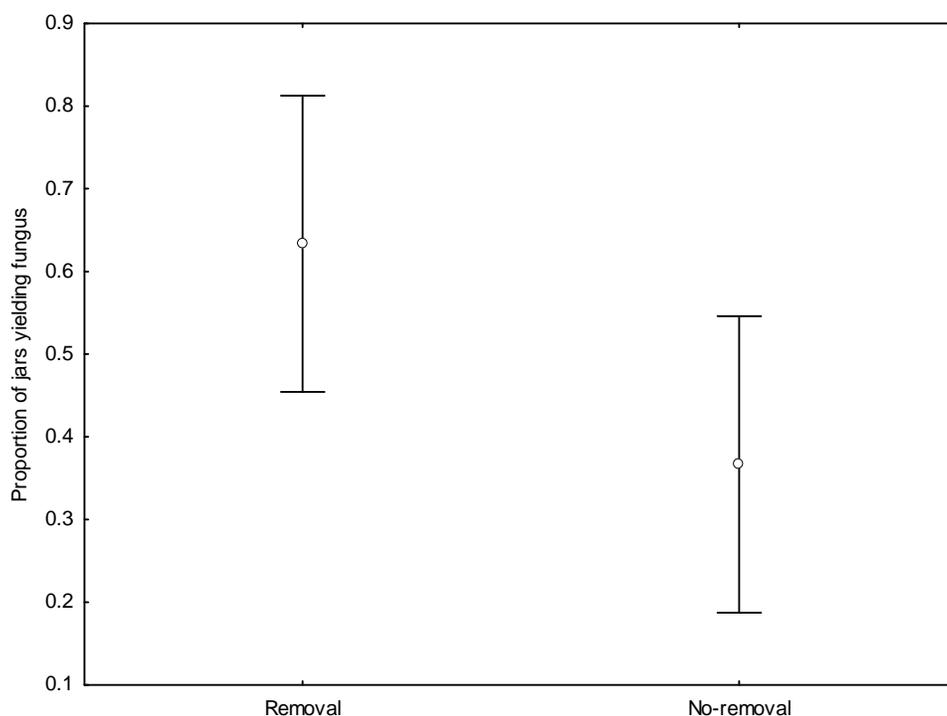
In 2007, an average of 4.06 (std. dev. = 9.29) parasitoids emerged per jar (n = 69). A reason for the low number of parasitoid emergences in 2007 could be the increase in the number of virus, *Cryptophlebia leucotreta* granulovirus (CrleGV), infected larvae in the jars. No parasitoid emerges from a larva which has been killed by virus. Studies are being conducted on the possibility of formulating an artificial rearing medium for mass-rearing parasitoids. This will allow for more parasitoids to be mass-reared and therefore an increase in parasitoid releases into the field (Greany 1986; Thompson 1999).

By 14 days the majority of the larvae had reached their fifth instar and were ready to pupate. There was high variation between the number of progeny produced and the time the parent parasitoids were left in the jar (Table 3.2.4.3). Day 13 (n = 4) showed a high number of parasitoid emergence. It was determined that 3 days was sufficient for the female parasitoid to parasitize larvae, as then an average of 37.8 parasitoids emerged per jar and an average of 9.5 parasitoids emerged per day. There was no statistical difference between the offspring over the days (Kruskal-Wallis; H = 14; p = 0.36). Days 8 and 11 were omitted from the table as there was no progeny emergence. When mass-rearing, the aim is to maximise the use of the parents to produce progeny. The more larvae that can be exposed to parasitoids, the higher the chance of more larvae being parasitised by a female parasitoid. To maximise parasitism for mass-rearing of *A. bishopi*, it would be viable to leave the parasitoids in the honey jar for 4-5 days (approximate recovery rate of 30% parasitoid progeny) and then place them in a new jar until the female parasitoid dies.

**Table 3.2.4.3.** The average number of parasitoid progeny emerging from jars where the parent parasitoids were left in for a varying number of days. Days 8 and 11 were not included as there was no emergence of parasitoids.

Days parent parasitoids left in honey jars	N	Cumulative avg. number of parasitoid progeny emerging	Average offspring / day
2	3	11	3.6
3	4	37.8	9.5
4	6	43.5	7.3
5	5	28.8	5.8
6	5	37.6	7.5
7	4	36.0	9
9	3	26.0	8.7
10	3	32.0	10.6
12	4	19.3	4.8
13	4	47.3	11.8
14	2	14.0	7.0

The presence of a fungus, *Aspergillus* sp., in the culture had a disastrous effect on the emergence of the parasitoids from the diet. The fungus became visible in the late stages of the mass-rearing process, just before the cotton-wool plug was to be removed from the jar. It took approximately 3 days from the first day of seeing the fungus for the entire diet to be affected and the jar had to be discarded. As the majority of the female parasitoids emerged from the diet, this ultimately affected the mass-rearing process, as it is the females that ultimately lead to the next generation. It was suspected that the fungus was starting from the bodies of larvae dying due to virus infection. There was a significant difference (one-way ANOVA; F = 4.44; df = 1; p = 0.039) between fungus growth in the jars where dead larvae were not removed and those where they were (Fig 3.2.4.4). In jars where there was no removal, 63% yielded fungus and from jars where dead larvae were removed, 36% yielded fungus. One problem was that not all dead larvae could be removed as some of the larvae died within the diet, which made them difficult to find. From the vials, 43% with dead larvae yielded fungus.



**Figure 3.2.4.4.** The mean proportion of jars ( $\pm$  SD) yielding fungal growth on the surface of the diet by the removal and non-removal of dead larvae.

#### Biological studies

##### 1. *Pre-oviposition, oviposition periods and developmental rates*

It was observed that the *A. bishopi* female searched for the host larvae by probing around in the diet with her ovipositor and feeling around on the surface of the diet with her antennae. A number of different methods are used by parasitoids to locate their host (Thompson 1986), including chemical cues, sound, heat, sight and vibrations (Hassell *et al.* 1992; Van Baaren *et al.* 2005; Fellows *et al.* 2005). The same type of behaviour was observed occurring in an *Agathis* sp. that parasitises *Greya subalba* (Lepidoptera: Incurvariidae) (Thompson 1986).

There was no probing during, or adult parasitoid emergence from, the egg stage, which was expected, as *A. bishopi* is not an egg parasitoid. In the neonate larval stage there was probing by the female parasitoid but there was no emergence of adult parasitoids. The female parasitoid probed around in the diet for approximately 35 seconds at varying intervals. Probing by the female started once the neonate larvae started to burrow into the diet. This stage lasted only 1 day. In the 25% and 50% frass stages there was vigorous probing and 9.7 adult parasitoids emerged per jar from these stages. These two stages coincide with the second and third instar of the FCM larvae. It is suggested that female parasitoids prefer second and third instar larvae of FCM in which to lay eggs. In the 75% frass stage, the female parasitoid probed around in the diet for approximately 13 seconds at various intervals for about the first 4 minutes when placed in the jar. Only 3.1 adult parasitoids emerged per jar. In the 100% frass stage there was no probing and no emergence of adult parasitoids (Table 3.2.4.4).

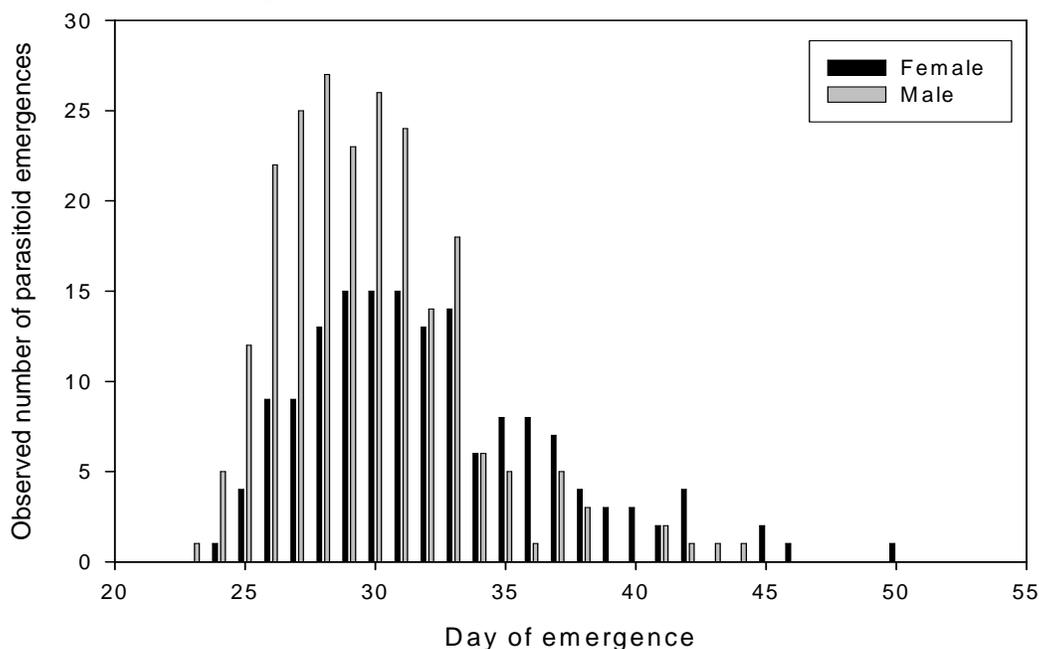
**Table 3.2.4.4.** Probing behaviour of females of *Agathis bishopi* and subsequent emergence of adult parasitoids from six stages of development of *T. leucotreta*.

Stage	N	Probing	% Parasitised larvae per jar ( $\pm$ SD)
Eggs only	10	None	0
Neonate larvae	10	Yes, some interest by the female was shown	0
1-25 % frass cover	10	Probe for 35 seconds at varying intervals	10.1 ( $\pm$ 2.86)
26-50 % frass cover	10	Yes, probing occurred for about 18 seconds at variable intervals	9.3 ( $\pm$ 2.39)
51-75 % frass cover	10	Probe for about 13 seconds at variable intervals for	3.1 ( $\pm$ 2.04)

		about 4 minutes after being placed in the jar, and then seemed to lose interest	
76-100 % frass cover	10	None	0

In the last two stages the majority of the larvae were in the fourth or fifth instars. It is apparent that female parasitoids do not oviposit in these instars. Although Table 3.2.4.4 shows that the parasitoids do oviposit in fourth instar larvae, the number of emerged parasitoids was low. A reason could be that these larvae are too far into their development cycle and there will not be enough time for the parasitoid to develop inside the host. The length of the females' ovipositor is approximately 5mm. Some of the larvae had burrowed too far into the diet and could not be reached by the parasitoid's ovipositor, but others could be reached as they were just below the surface of the diet. From this study it is estimated that the pre-oviposition stage is approximately 1 - 2 days and the oviposition period is 3 - 7 days.

The developmental rate of *A. bishopi* varied between sexes. The males had a faster developmental rate than females; this is based on the males emerging before the females (Fig 3.2.4.5). More studies are needed in order to confirm this assumption. The developmental rate of the parasitoid was in synchrony with the development of FCM. The larvae that were not parasitised emerged 1 to 2 days earlier than the parasitoids, as would be expected, as the parasitoids would need the moths to lay eggs and produce larvae for the parasitoids to parasitise. There were 23 days from the time the parent parasitoids were placed in the honey jar to when the first parasitoid progeny emerged. From Day 23 to approximately Day 34 the emergence of parasitoids was dominated by males. From Day 35 to approximately Day 45 the emergence of parasitoids was dominated by females (Fig 3.2.4.5).



**Figure 3.2.4.5.** The observed number of times parasitoid progeny emergence occurred on the various days after parent parasitoids were placed into the honey jars (temperature 27°C).

The majority of the parasitoids emerged from Day 26 to Day 33. Even though these days were dominated by the emergence of male parasitoids, a high number of females also emerged. If the female can mate shortly after emerging it will be more beneficial as it will allow her to produce both male and female progeny from the time she starts parasitizing larvae, instead of producing only male progeny if the female has not mated. It is speculated that the females take longer to develop as they are slightly larger in size and because of egg maturation; therefore they emerge later than the males. Males emerged for up to 44 days and females emerged up to 50 days after the parent parasitoids were placed in the honey jar (Fig 3.2.4.5). For the rearing of *A. bishopi*, from the time the parent parasitoids are placed in the jar it takes 23 days for the first parasitoid to emerge and up to 50 days for the last parasitoid to emerge.

## 2. Life span

Females of *A. bishopi* lived for up to 18.5 days and males for up to 8.25 days under environmental conditions of 27°C, 60% relative humidity and a photoperiod of 12D:12L. When males and females were under conditions of no food or water (N) and water only (W), males lived longer ( $p < 0.05$ , t-test for independent

samples) than females in both cases. When they were under conditions of food and water (FW) or food and water plus the opposite sex (FW♂/♀), females lived longer ( $p < 0.05$ , t-test for independent samples) than males in both cases (Table 3.2.4.5). For males there was a significant difference (Kruskal-Wallis test;  $p < 0.05$ ) in life span under all of the conditions except between FW and FW♀ (Kruskal-Wallis test;  $p > 0.05$ ) (Table 3.2.4.6). For females there was a significant difference under all of the conditions except between N and W and between FW and FW♂ (Table 3.2.4.7).

**Table 3.2.4.5.** Life spans of male and female *Agathis bishopi* without the host under four different conditions.

Condition	Sex	Sample size	Mean Longevity (days ± S.E.)
<b>no food or water</b>	Males	20	2.45 ± 0.89
	Females	20	1.4 ± 0.59
<b>water only</b>	Males	20	4.2 ± 0.69
	Females	20	3.15 ± 0.58
<b>food and water</b>	Males	20	8.25 ± 1.23
	Females	20	18.45 ± 1.79
<b>food, water and a mate</b>	Males	20	6.55 ± 2.09
	Females	20	18.5 ± 3.1

**Table 3.2.4.6.** Kruskal-Wallis (probability) tests of the life span for male parasitoids under different conditions. The numbers in bold show significant differences.

	<b>No food or water</b>	<b>Water only</b>	<b>Food &amp; water</b>
<b>Water only</b>	0.048265		
<b>Food &amp; water</b>	0.000000	0.000046	
<b>Food, water &amp; a female</b>	0.000000	0.027883	0.602050

**Table 3.2.4.7.** Kruskal-Wallis (probability) tests of the life span for female parasitoids under different conditions. The numbers in bold show the significant differences.

	<b>No food or water</b>	<b>Water only</b>	<b>Food &amp; water</b>
<b>Water only</b>	0.069550		
<b>Food &amp; water</b>	0.000000	0.000145	
<b>Food, water &amp; a female</b>	0.000000	0.000208	1.000000

For both male and female parasitoids, food and water were important for survival. *Xanthopimpla stemmator* (Thunberg) parasitoids lived significantly longer when provided with food and water (Moore & Kfir 1996). There is no effect of males on females' survival or vice versa. For females, the presence of food and water is possibly more important than for males, as they live longer when both are provided. For females, feeding directly affects reproduction as it determines the amount of eggs matured and the quality of the eggs (Quicke 1997; Rivero & Cass 1999). The female is also slightly bigger than the male, therefore needing more nutrients for survival.

#### Field releases of *A. bishopi*

At the time of compiling this report, no parasitoids had yet been recovered from FCM larvae collected from the netted navel oranges. However, monitoring will continue until the fruit is harvested in May.

#### **Conclusion**

Due to the expense of pesticides, problems such as insects developing pesticide resistance (Hogsette 1999), chemical residues on the skin of the fruit and the negative impact on the environment, it has become necessary to find alternative methods for pest control. Integrated pest management offers a suitable solution. The example of the control of red scale is proof that an integrated pest management program can be successful (du Toit 1996). The use of parasitoids in an integrated pest management system is important, especially in citrus, when the economic damage potential of the pest is high. Judicious biological and cultural practices serve to enhance the activity of parasitoids in the citrus orchards (Viggiani 2000).

The life cycle of *A. bishopi* is in synchrony with that of FCM. Therefore it is potentially a good agent for biological control of FCM. A single female moth can live for 2 - 3 weeks and lay up to 800 eggs in her life time. Generally only a few survive, as if there are many females laying eggs the larvae will die due to cannibalism and lack of food (Stibick 2006) and importantly due to a range of adverse environmental conditions. A female *A. bishopi* parasitoid can live for 2 - 3 weeks and under mass-rearing conditions, can lay 4 – 23 (Ave. 13.43) eggs per 100 larvae. Using the figure of 13.43 eggs per 100 larvae, it can be estimated that 87 FCM larvae per 100 are developing into adults (this is based on ignoring other possible mortality factors). Therefore per 100 FCM larvae, approximately 4 fecund female parasitoids are required to parasitise all the larvae. Over a single female moth's life time, approximately 32 wasps would be required to parasitise all of its progeny larvae. For a mass-release programme it would be necessary to find out the number of female moths per hectare in-order to calculate the number of female wasps to release into the field.

*A. bishopi* has potential to be used successfully in an integrated pest management program once the hurdle of mass-rearing has been overcome as many parasitoids will be required per hectare in-order to reduce the population of FCM. A programme incorporating the release of parasitoids at the start of the season to reduce the number of FCM and then periodic releases to maintain the high number of parasitoids in the field, with orchard sanitation and the use of the *Cryptophlebia leucotreta* granulovirus (CrleGV), could be used for the control of FCM.

### Future research

As no further funding is available, no further work is planned. However, other experiments that need to be done include rate and duration of development at various temperatures and host suitability. The rate and duration of development at various temperatures will give an indication of the minimum, optimum and maximum temperatures at which the parasitoid can survive. The host suitability test will give an indication of which other major lepidopteran pests such as *Thaumatotibia batrachopa* (Tortricidae), *Cryptophlebia peltastica* (Tortricidae) and *Cydia pomonella* (Tortricidae), can be parasitised by *A. bishopi*. Most parasitoids are host specific, therefore it is best to test other species from the same family or genus. Another experiment is to test whether the female parasitoid can determine whether or not a larva is infected with a virus, therefore being able to determine the suitability of a larva for development of the parasitoid's offspring. These results will allow one to determine when in an IPM programme the virus should be used and when to release the parasitoid or whether they can be used at the same time.

Evaluations of parasitoid releases in Citrusdal will be completed.

### Technology transfer

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

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### 3.2.5 PROGRESS REPORT: Investigating and improving field persistence of Cryptogran

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

#### Summary

This experiment aimed to identify, quantify and resolve persistence problems with Cryptogran and to improve the field persistence through formulation and management practices. Three bioassays were conducted which indicated that Cryptogran appeared to be rain fast, although results were not totally conclusive. Laboratory bioassays and a field/detached fruit bioassay indicated that lignin offered UV protection to the virus. A field trial also indicated that lignin improved the efficacy Cryptogran, especially when applied at midday. However, the lignin product is expensive and did not provide much additional benefit above evening applications of Cryptogran. Morning and midday applications were less effective than evening applications. Laboratory bioassays and field trials indicated that Cryptogran is compatible with most black spot treatments, except copper. Field trials indicated that an additional early application in October did not improve FCM control, and Cryptogran performed significantly better than Cryptex. A field trial showed that reducing the molasses dosage by half resulted in only slightly lower efficacy of Cryptogran. Substituting brown sugar and mannitol for molasses had a similar effect. These products could be used in the event of a molasses shortage. Break-Thru was as effective as Agral 90 when used with Cryptogran and molasses.

#### Opsomming

Die doel van hierdie eksperiment was om probleme met die nawerking van Cryptogran te identifiseer, te kwantifiseer en die produk hopelik deur beter formulاسie en bestuurspraktyke te verbeter. Drie biotoetse het aangedui dat Cryptogran reënvas is, alhoewel die resultate nie heeltemal afdoende is nie. Biotoetse en

boordproewe het getoon dat lignien UV-beskerming aan Cryptogran verleen. Ongelukkig is die lignienprodukt duur en dit het nie die uitklopaksie en nawerking van Cryptogran veel verbeter in vergelyking met bespuitings wat in die aand toegedien was nie. Bespuitings wat soggens en vroegmiddag toegedien is, was minder doeltreffend as dié wat saans gespuit is. Biotoetse en boordproewe het gewys dat Cryptogran verenigbaar is met die meeste swartvlekbehandelings, koper uitgesluit. Boordproewe het gewys dat 'n addisionele vroeë Cryptogran-bespuiting tydens Oktober nie VKM-beheer verbeter het nie, terwyl Cryptogran beduidend meer doeltreffend as Cryptex was. Waar die dosis van melasse met 50% verlaag was, is die werking van Cryptogran baie min benadeel. Bruinsuiker en Manitol het soortgelyke resultate gelewer en kan dalk gebruik word in die geval van 'n melasse tekort. Break-Thru was net so doeltreffend soos Agral 90 wanneer dit met Cryptogran en melasse gebruik was.

## Introduction

Field trials have been conducted with Cryptogran since the year 2000. Cryptogran is also in its fourth 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). Many of the trials were aimed at improving practical management practices and usage of Cryptogran. Timing of application was investigated, as well as the compatibility of Cryptogran with various products, mainly black spot treatments. The efficacy of lignin and Wetcit as UV protectants was further investigated. Substitutes for molasses and Agral 90 were investigated.

## Materials and methods

### Rainfastness bioassay 1

A previous trial showed Cryptogran to be rainfast (Moore *et al.*, 2004b). This concurred with experiences in field trials, where Cryptogran remained effective in controlling FCM after rainfall had occurred. Previously, treated fruit were dipped into water, so as to simulate a worst case scenario of rain, and then exposed to neonate FCM larvae. However, in this trial, an attempt was made to simulate rainfall more accurately by using a rain simulation machine (Hattingh, 1998). On 11 June 2007, 75 Cara Cara navel oranges were harvested from the Citrus Foundation Block. Fifty of these fruit were treated with Cryptogran, by dipping the fruit in the registered concentration of Cryptogran, molasses and Agral 90 (Moore *et al.*, 2004a) (Table 3.2.5.1). Fruit were then allowed to dry for 24 h. Half the number of fruit were then exposed to simulated rainfall, while the other half were not. Twenty-five fruit were retained untreated, as a control. The rainfall was applied as 36 mm of rain in 5 minutes, which would be classified as a cloudburst (Aaron *et al.*, 1986). The amount of rainfall was determined by placing a rain gauge under the simulated rain shower. The fruit were then left to dry, after which they were inoculated with four neonate FCM larvae each. If the product proved rainfast under these extreme conditions, no further testing would be considered necessary.

**Table 3.2.5.1.** Treatments on Autumn Gold navel oranges to test the rainfastness of Cryptogran

	Treatment	Dose	Rainfall
1	Untreated control		None
2	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	None
3	Cryptogran + molasses + Agral 90	10 ml + 250 ml + 18 ml	36 mm in 5 minutes

The fruit were then left for two weeks, after which they were inspected for penetration marks and the presence of FCM larvae. Treatments were compared using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 1996).

### Rainfastness bioassay 2

On 10 July 2007, 90 Autumn Gold navel oranges were harvested from Orchard 39 on Carden Farm in the Sundays River Valley. They were used in a rainfastness bioassay, which was conducted as described in the previous trial. In this trial each treatment was replicated 30 times. A higher concentration of Cryptogran (20 ml/100 l water) was used in an attempt to obtain clearer results.

### Rainfastness bioassay 3

On 10 July 2007, 90 Valencia oranges were harvested from the Citrus Foundation Block. They were used in a rainfastness bioassay, which was conducted exactly as described in the previous trial.

### Compatibility bioassay 1

Compatibility of Cryptogran with other products is important as the timing of application of these products and Cryptogran could coincide. If they could be applied together, mechanical application costs could be reduced. A detached fruit bioassay was conducted to test the compatibility of certain black spot treatments with Cryptogran. One hundred and fifty Cara Cara navel oranges were harvested from the Citrus Foundation Block on 1 June 2007. Glass beakers of the various suspensions were prepared (Table 3.2.5.2.), and 30 fruit were dipped in each. 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. Treatments were compared using ANOVA and the Bonferroni multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 1996).

**Table 3.2.5.2.** Treatments applied to Cara Cara navel oranges to test the compatibility of certain black spot treatments with Cryptogran, evaluated on 18 June 2007.

<b>Treatment (Doses in ml or g per 100 ℓ water)</b>	
1	Distilled water
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>Flint (10 g)</b>
4	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>Ortiva (20 ml)</b>
5	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>

### Compatibility bioassay 2

A detached fruit bioassay was conducted to test the compatibility of copper oxychloride treatments with Cryptogran. Copper oxychloride (200 g/100 ℓ water) was added to a Cryptogran suspension (10 ml/100 ℓ water). On 8 August 2007, 150 Lane Late navel oranges were harvested from the Citrus Foundation Block. Thirty fruit were dipped into the solution at various intervals after preparation, i.e. immediately after mixing, 1 hour and 2 hours thereafter (Table 3.2.5.3). The fruit were allowed to dry on a mesh rack, and were then inoculated with 3 neonate FCM larvae each. The trial was then evaluated as described for the previous trial.

**Table 3.2.5.3.** Treatments applied to Lane Late navel oranges to test the compatibility of copper oxychloride with Cryptogran, evaluated on 23 August 2007.

<b>Treatment (Doses in ml or g per 100 ℓ water)</b>		<b>Time from preparation of solution to fruit dip (hours)</b>
1	Distilled water	0
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	0
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>	0
4	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>	1
5	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>	2

### UV bioassay 1

A bioassay was conducted to determine the effectiveness of a lignin sulphate carrier and Wetcit as UV protectants. A suspension of Cryptogran was again prepared at  $1.34 \times 10^5$  OBs/ml and 15 ml was inoculated into each of twelve Petri-dishes. The lignin carrier (200 ml/100 ℓ water; source and formulation proprietary) was added to four of the twelve Petri-dishes. Wetcit (200 ml/100 ℓ water) was added to four other Petri-dishes. These Petri-dishes were then exposed to a germicidal UV lamp for periods ranging from 60 minutes to 360 minutes. After exposure the suspensions were inoculated onto artificial diet and bioassayed against neonate FCM larvae. Two distilled water treated controls were used and one control of each of Cryptogran and Cryptogran with lignin, which were not exposed to UV. Where possible, a probit analysis was conducted to establish a relationship between the different exposure times to UV and survival of neonate FCM larvae.  $SD_{50}$  values were calculated where probit analyses were conducted.

### UV bioassay 2

A second bioassay was conducted in exactly the same manner as the previous one.

### UV bioassay 3

A third bioassay was conducted in the same manner as previously described, but the Wetcit treatment was excluded.

### Field trial 1: Dunbrody Farm

A field trial was conducted to test the efficacy of Cryptogran when applied at different times of the year, both as single and multiple applications (Table 3.2.5.4). This included an application corresponding with the minor peak in FCM activity in October, indicated by pheromone trap catches. An orchard of Lane Late navel oranges was selected on Dunbrody Farm in the Sundays River Valley. The orchard was divided into 12 blocks of approximately 150 trees each. Each treatment was applied to 2 randomly selected blocks. An average of 15.0 – 15.5 l of spray mix was applied per tree for all applications. The orchard, in which trees were spaced at 6 m x 2 m (rows x trees), was planted in 1997. After application, evaluation commenced three weeks later on 30 November 2006, in the following manner. Fruit drop from 14 data trees per treatment was evaluated from 3 weeks after application, until there was a substantial decline in efficacy. 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 1996).

**Table 3.2.5.4.** Timing of Cryptogran applications (Cryptogran 10 ml/100 l + molasses 250 ml/100 l + Agral 90 18 ml/100 l) in an orchard of Lane Late navel oranges on Dunbrody Farm.

Treatment	Timing of Cryptogran applications		
1	-	-	-
2	26 Oct 2006	5 Dec 2006	-
3	26 Oct 2006	-	5 Feb 2007
4	26 Oct 2006	5 Dec 2006	5 Feb 2007
5	-	5 Dec 2006	-
6	-	5 Dec 2006	5 Feb 2007

### Field trial 2: Dunbrody Farm

A second similar field trial was conducted to test the efficacy of Cryptogran when applied at different times of the year, both as single and multiple applications (Table 3.2.5.5). 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) was 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, and evaluation was conducted as in the previous trial

**Table 3.2.5.5.** Timing of Cryptogran and Cryptex applications in an orchard of Lane Late navel oranges on Dunbrody Farm.

Treatment		Concentration (ml / 100 l water)	Timing of virus applications				
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
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#### Field trial 3: Riverbend Farm.

An exact replicate of the previous trial was conducted in two adjacent Lane Late navel orange orchards on Riverbend 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 1999. The idea was to spray 2 trials, to begin evaluating both, but only to continue evaluations on the site with the higher FCM levels. The first treatments, as described in the previous trial, were applied on 25 October 2007, followed by the second applications on 5 December 2007. Approximately 8750 l per ha was applied for all treatments. The trial was evaluated as described for the previous field trials.

#### Field trial 4: Dunbrody Farm.

A third field trial was conducted to compare a standard Cryptogran programme (i.e. 2 – 3 applications per season at 10 ml/100 l water) with a programme in which Cryptogran was included with all sprays applied in the orchard at half the registered rate (5 ml/100 l water) (Table 3.2.5.6). Two neighbouring orchards of the same cultivar, variety (Lane Late navel oranges), age and FCM history were selected. The trial was applied by the grower, according to the instructions of CRI.

**Table 3.2.5.6.** Cryptogran treatments applied in orchards N1 and N2 on Dunbrody Farm in the 2006/07 season, and the products applied as a tank mix with Cryptogran.

Orchard	Cryptogran application date	Cryptogran concentration (per 100 l water)	Pesticides applied with Cryptogran	Adjuvants applied with Cryptogran
N1	26 Oct 2006	5 ml	Nemesis	Oil
N1	31 Oct 2006	5 ml	Agrimec	Oil
N1	9 Nov 2006	5 ml	Agrimec	Oil
N1	24 Nov 2006	5 ml	Tartox	Sugar
N1	27 Nov 2006	5 ml	Ultracide	Leafcote, Commodabuff
N2	27 Nov 2006	10 ml	-	Molasses, Leafcote
N1	18 Jan 2007	5 ml	Agrimec	Oil
N1	31 Jan 2007	5 ml	Giberellic acid, 2-4 D	Commodabuff
N2	14 Feb 2007	10 ml	-	Molasses, Leafcote

In orchard N1, Cryptogran was applied seven times at 5 ml/100 l water. In orchard N2, Cryptogran was applied twice at the registered concentration (10 ml/100 l water). Twenty data trees were selected per orchard, and the trial evaluation was conducted as described for the previous trial.

#### Field trial 5: Compatibility

A field trial was conducted to test the compatibility of Cryptogran with various fungicides. Simultaneously, a reduced concentration of a registered wetter (i.e. alkylated phenol-ethylene oxide wetters, e.g. Agral 90) was tested, as was Break-Thru, (a trisiloxane surfactant) in the place of the registered wetter. Cryptex was also included (Table 3.2.5.7). Treatments were applied on 6 December 2006, as full cover sprays, in an orchard of Lane Late navel oranges. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees) and was planted in 1996, was on the farm of Willy Killian in the Kirkwood district of the Sundays River Valley. The trial was laid out in a single-tree randomised block format, replicated 10 times. Treatments were applied as full cover sprays, using hand-held spray guns. An average of 23.5 l/tree of spray mix was applied for all treatments, and an untreated control was retained. Weekly evaluations were conducted as in the previous trials, commencing 3 weeks after application, on 27 December 2006.

**Table 3.2.5.7.** Treatments applied to test the compatibility of various fungicides with Cryptogran, on Willy Killian's farm on 6 December 2006, in the Sundays River Valley.

Treatment no	Treatment (dosages per 100 ℓ water)
1	Untreated control
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Flint (10 g)
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Cabrio (10 ml)
4	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Flint (10 g)
5	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Copper Oxychloride (200 g)
6	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Sporekill (100 ml) + Mancozeb (150 g)
7	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)
8	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (6 ml)
9	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (3 ml)
10	Cryptex (5 ml) + molasses (250 ml) + Agral 90 (18 ml)

Field Trial 6: Time of day + lignin

In a sixth field trial, Cryptogran was applied at different times of the day: morning (08H15 – 08H35), midday (12H30 – 12H50) and evening (17H30 – 20H30) of 31 January 2007. The trial was applied in an orchard of Lane Late navel oranges on Atmar farm near Kirkwood in the Sundays River Valley. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 1999. An average of 22.9 ℓ spray mix was applied to single trees in a randomised block format, replicated 10 times. The midday and evening sprays were applied with and without lignin. Lannate was sprayed in order to check its compatibility with Cryptogran. Surround (Kaolin) was applied on its own (Table 3.2.5.8.)

**Table 3.2.5.8.** Treatments applied to Lane Late navel orange trees on Atmar Farm in the Sundays River Valley on 31 Jan 2007, to test the effect of the time of day and lignin on efficacy of Cryptogran against FCM.

Treatment no.	Treatment (dosages per 100 ℓ water)
1	Untreated control
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) applied <b>Morning</b>
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) applied <b>Midday</b>
4	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) applied <b>Evening</b>
5	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>lignin</b> (200 ml) applied <b>morning</b>
6	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>lignin</b> (200 ml) applied <b>evening</b>
7	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Lannate (20 g)
8	Cryptex (5 ml) + molasses (250 ml) + Agral 90 (18 ml)
9	Surround (6 kg)

Field trial 7: Lignin (by Stephan Honiball)

The experiment was conducted at The Baths, a farm with a long history of FCM damage, located in the Bo-Rivier area 20 km north of Citrusdal. The trial plot formed part of Block 8 (called Kraal), consisting of Washington navel oranges (year of planting: 1954) planted on rough lemon root stock, spaced 4 m x 7 m (~360 trees / ha). The trees did not touch each other.

The experimental lay-out was a randomized complete block design consisting of five treatments replicated in each of 10 blocks. Each replicate was a single tree and no guard trees were used in between. An untreated control was retained.

Cryptogran (L 7598) was applied according to the instructions on the label (10 ml Cryptogran + 250 ml molasses + 18 ml Leafcote) as a full cover film spray (20 Bar pressure) with adjustable handguns. A UV stabilizer, lignin, was added to two of the treatments (Table 3.2.5.22), at a concentration of 200 ml per 100 ℓ water. Approximately 20 ℓ of spray mixture was applied per tree.

The noon sprays commenced at 12h05 and the evening sprays at 17h25 on 13 February 2007. Each spraying session lasted for just over two hours.

The temperature reached a high of 41°C on the day of spraying. No rain fell during the first week after spraying. A Hobo data logger was used to measure the temperature at half hourly intervals. Three weeks after the treatment, all infested / rotting fruit were removed from under the data trees as well as hanging on the trees, as these fruit were probably infested before the treatment date. Weekly fruit evaluations started from four weeks after treatment, and lasted for five more weeks. Fruit infested with FCM larvae that were Split or *Alternaria*-infested were discarded. Dropped fruit were carefully dissected in the field to determine the cause of drop. Fruit containing FCM larvae (dead or alive) or the characteristic granular frass, were counted as infested.

Cumulative counts were transformed to  $\log_{10}(x+1)$  before being subjected to analysis of variance (ANOVA) using Statistica (2006). Residual deviations were tested for non-normality, and Bartlett's Chi-Square test was performed at a 5% level of significance to compare means of significant effects.

#### Field trial 8: Lignin

A trial was conducted on Valencia orange trees at the Citrus Foundation Block, to see if the addition of lignin would increase the persistence of the virus. Cryptogran, with and without lignin, was applied to the northern side of trees with a handgun applicator, at a rate of 9 l/tree. Thirty fruit were then picked from trees sprayed with the two treatments, as well as from unsprayed trees, at the following intervals after application: 0 days (immediately after spray had dried), 1 day, 3 days, 7 days, 14 days and 21 days. The fruit were taken to the laboratory, where 3 neonate FCM larvae were placed onto each fruit. The fruit were kept at 24°C for two weeks after inoculation with larvae, and were then dissected and inspected for penetration marks and the presence of FCM larvae. Mean numbers of FCM per fruit per treatment were compared using ANOVA and Duncan's multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 1996).

#### Field trial 9: Molasses

Due to possible shortages of molasses, a field trial was conducted in an orchard of Lane Late navel oranges on Lone Tree Farm in the Sundays River Valley, to test the effect on the efficacy of Cryptogran of reduced rates of molasses, as well as other additives which could possibly be used if molasses was unavailable (Table 3.2.5.9). 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. The trial was evaluated as described in the previous field trials.

**Table 3.2.5.9.** Cryptogran treatments, with various additives, applied to an orchard of Palmer navel orange trees on Lone Tree Farm in the Sundays River Valley on 10, 11 & 12 December 2007.

Treatment	Additives (per 100 l) to Cryptogran (10 ml / 100 l)
1	Untreated control
2	-
3	Agral 90 (18 ml)
4	Voermol molasses (250 ml) + Agral 90 (18 ml)
5	Voermol molasses (250 ml) + Break-thru (3 ml)
6	Voermol molasses (250 ml) + Break-thru (5 ml)
7	Voermol molasses (125 ml) + Agral 90 (18 ml)
8	White sugar (200 g) + Agral 90 (18 ml)
9	White sugar (400 g) + Agral 90 (18 ml)
10	Brown sugar (200 g) + Agral 90 (18 ml)
11	Brown sugar (400 g) + Agral 90 (18 ml)
12	Imported molasses (250 ml) + Agral 90 (18 ml)
13	Imported molasses (125 ml) + Agral 90 (18 ml)
14	Voermol molasses (250 ml) + oil (300 ml)
15	Voermol molasses (125 ml) + oil (300 ml)
16	Oil (300 ml)
17	Mannitol (1 kg) + Agral 90 (18 ml)

#### Field trial 10: Patensie

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 11: Letsitele

Two semi-commercial demonstration type trials were conducted in the Letsitele area. In the first of these trials, two adjacent mature Star Ruby grapefruit orchards were used on Laeveld Sitrus Farm. One of these was sprayed with Cryptogran (at the registered rate) on 30 November 2006. From 22 December until 21 February, weekly evaluations of fruit drop from five data trees in each of the sprayed and unsprayed orchards were conducted. Evaluations were conducted by Du Roi IPM staff, namely, Felix Hacker, Hannah Otto and Christo Breytenbach.

#### Field trial 12: Letsitele

In the second trial, two adjacent Turkey Valencia orchards on Bosveld Sitrus Farm (four and a half years old), were used. Again, one of the orchards was sprayed with the registered concentration of Cryptogran – on 29 November 2006. This orchard received a second spray of Cryptogran on 13 May 2007. From 20 December until 12 June, weekly evaluations of fruit drop from five data trees in the sprayed and unsprayed orchards were conducted. Evaluations were again conducted by Du Roi IPM staff. As the recorded level of infestation was low, a revised method of evaluation was incorporated from 10 May and used until 12 June. Twenty trees in each block were marked. Twice a week, each tree was inspected for approximately 1-2 mins for any damaged fruit. Damaged fruit was picked and all fallen fruit was collected. Fruit were then dissected to determine the level of FCM infestation.

### **Results and discussion**

#### Rainfastness bioassay 1

Unfortunately neither of the Cryptogran treatments worked very well, although the Cryptogran plus rainfall treatment did result in a statistically significant reduction in mean number of larvae infesting fruit (Table 3.2.5.10). It was not clear why Cryptogran was not more effective in this trial, as it had been in the initial rainfastness trial – reducing infestation by more than 60% (Moore *et al*, 2004b). Nevertheless, for this reason, it was necessary to repeat this trial.

**Table 3.2.5.10.** Damage to and infestation of Cara Cara navel oranges treated with distilled water, Cryptogran or Cryptogran with simulated rainfall. Four neonate larvae were placed per fruit; 25 fruit per treatment, evaluated on 28 June 2007.

Treatment	Fruit infested (%)	Mean no of larvae per fruit
Control	52	0.76a*
Cryptogran	45	0.45a
Cryptogran + rain	60	0.72a

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

#### Rainfastness bioassay 2

This bioassay showed very little difference in infestation between the treatment which was exposed to 'rain', and the Cryptogran treatment which was not (Table 3.2.5.11). This would indicate that the product is rainfast. Unfortunately, the differences between the two Cryptogran treatments and the untreated control were not significant, so the bioassay was repeated.

**Table 3.2.5.11.** Damage to and infestation of Autumn Gold navel oranges treated with distilled water, Cryptogran or Cryptogran with simulated rainfall. Four neonate larvae were placed per fruit; 30 fruit per treatment, evaluated on 24 July 2007.

Treatment	Fruit infested (%)	Mean no of larvae per fruit
Control	70	1.20a*
Cryptogran	70	0.93a
Cryptogran + rain	70	0.90a

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

#### Rainfastness bioassay 3

This bioassay delivered similar results to the previous one. Where 'rain' was applied, infestation was very similar to the standard treatment (Table 3.2.5.12), which would indicate that the product is rainfast. Once again, unfortunately these treatments were not significantly different from the untreated control, so the bioassay needs to be repeated.

**Table 3.2.5.12.** Damage to and infestation of Valencia oranges treated with distilled water, Cryptogran or Cryptogran with simulated rainfall. Four neonate larvae were placed per fruit; 30 fruit per treatment, evaluated on 31 August 2007.

Treatment	Fruit infested (%)	Mean no of larvae per fruit
Control	66.7	0.93a*
Cryptogran	46.7	0.60a
Cryptogran + rain	56.7	0.67a

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

#### Compatibility bioassay 1

There was no significant difference in infestation (mean no of larvae per fruit) between the treatments. However, they differed significantly from the untreated control (Table 3.2.5.13). This would indicate that they had little or no detrimental effect on the efficacy of Cryptogran. Surprisingly, the copper oxychloride did not have the expected effect on the virus. The duration of exposure in suspension was probably too brief.

**Table 3.2.5.13.** Mean penetration marks and FCM infestation of fruit in a detached fruit bioassay to test compatibility of products with Cryptogran, evaluated on 18 June 2007.

Treatments (concentrations per 100 ℓ water)		Mean penetration marks per fruit	Fruit penetrated (%)	Mean no of larvae per fruit	Fruit infested (%)
1	Distilled water	1.17a	80	0.9a	67
2	Cryptogran (10ml) + molasses (250 ml) + Agral 90 (18ml)	0.73b	57	0.53b	47
3	Cryptogran (10ml) + molasses (250 ml) + Agral 90 (18ml) + <b>Flint (10g)</b>	0.60b	50	0.47b	40
4	Cryptogran (10ml) + molasses (250 ml) + Agral 90 (18ml) + <b>Ortiva (20ml)</b>	0.8ab	57	0.53b	43
5	Cryptogran (10ml) + molasses (250 ml) + Agral 90 (18ml) + <b>copper oxychloride (200g)</b>	0.73b	47	0.56b	37

\* Different letters in the same column denote significant differences ( $P < 0.05$ , LSD multiple range test)

#### Compatibility bioassay 2

The detrimental effect of copper oxychloride was clear in all treatments. Surprisingly, where Cryptogran was kept in suspension for longer periods, the efficacy was higher. However, the differences were not significant, and might therefore have been meaningless (Table 3.2.5.14). This is contrary to expectation, which was that the longer Cryptogran was in suspension with copper oxychloride, the more harm would be done to the virus, resulting in lower efficacy. The trial needs to be repeated.

**Table 3.2.5.14.** Mean penetration marks and FCM infestation of fruit in a detached fruit bioassay to test compatibility of copper oxychloride with Cryptogran, evaluated on 23 August 2007.

	Treatments (Concentrations per 100 ℓ water)	Time from preparation of solution to fruit dip (hours)	Mean penetration marks per fruit	Fruit penetrated (%)	Mean no of larvae per fruit	Fruit infested (%)	
1	Distilled water	0		1.33a	90.0	1.30a	90.0
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	0		0.63c	53.3	0.60c	53.3
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>	0		1.23ab	90.0	1.13ab	80.0
4	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>	1		1.13ab	86.7	0.93bc	73.3
5	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + <b>copper oxychloride (200 g)</b>	2		0.97bc	70.0	0.80bc	63.3

\* Different letters in the same column denote significant differences (P<0.5, LSD multiple range test)

#### UV Bioassays

Two bioassays were conducted to determine if lignin and Wetcit gave UV protection to Cryptogran. These were repeats of previous bioassays.

In the first bioassay, mortality was very high for the lignin and Wetcit treatments (Table 3.2.5.15). Both these additives appeared to give some UV protection, as indicated by the higher mortality after 6 hours of exposure to UV. One concern was the high mortality in the lignin control treatment (52%), as this could indicate that lignin itself had some effect on the larvae, and the higher mortality where lignin was added may not be due to the fact that it added UV protection to the virus. This trial was repeated.

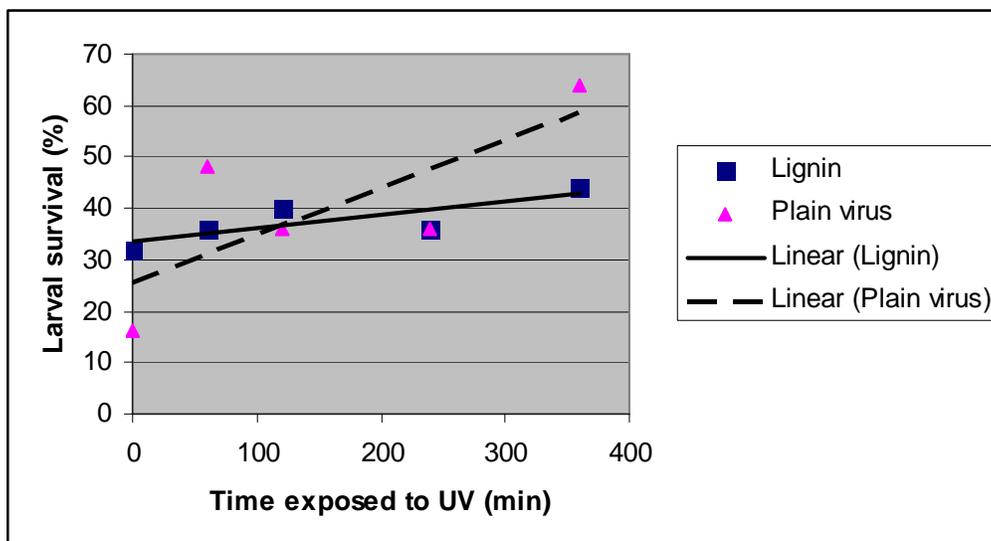
**Table 3.2.5.15.** Impact of UV-irradiation (sunlight) on Cryptogran ( $1.34 \times 10^5$  OBs/ml), with and without lignin, measured by mortality of neonate FCM larvae in a dose-response bioassay.

Treatment no	Treatment (All Cryptogran treatments at a concentration of $1.34 \times 10^5$ OBs/ml)	Larval mortality (%)		
		Cryptogran	Cryptogran + Wetcit	Cryptogran + Lignin
1	Distilled water	20	16	52
2	Cryptogran – no exposure to sunlight	100	100	100
3	Cryptogran – exposed for 60 minutes	100	100	100
4	Cryptogran – exposed for 120 minutes	96	100	100
5	Cryptogran – exposed for 240 minutes	92	100	100
6	Cryptogran – exposed for 360 minutes	56	100	100

In the second bioassay, lignin alone did not influence mortality of neonate FCM larvae. Once again, Wetcit and lignin appeared to protect the virus from UV irradiation. This is reflected in the higher mortality recorded after the longer exposures to UV, where the protectants were added (Table 3.2.5.16). When plotted (Fig 3.2.5.1), the trend line for the treatment where lignin was added is almost horizontal, which would indicate that exposure of the virus to UV had little effect on the survival of neonate FCM larvae exposed to Cryptogran. Lignin appeared to give more UV protection than Wetcit, This was reflected in the higher larval mortality after 6 hours of exposure to UV, where lignin was added, as compared to Wetcit.

**Table 3.2.5.16.** Impact of UV-irradiation (sunlight) on Cryptogran ( $1.34 \times 10^5$  OBs/ml), with and without lignin, measured by mortality of neonate FCM larvae in a dose-response bioassay.

	Treatment (All Cryptogran treatments at a concentration of $1.34 \times 10^5$ OBs/ml)	Larval mortality (%)		
		Cryptogran	Cryptogran + Wetcitt	Cryptogran + Lignin
1	Distilled water	16	28	16
2	Cryptogran – no exposure to sunlight	84	76	68
3	Cryptogran – exposed for 60 minutes	52	60	64
4	Cryptogran – exposed for 120minutes	64	52	60
5	Cryptogran – exposed for 240 minutes	64	68	64
6	Cryptogran – exposed for 360 minutes	36	48	56



**Fig 3.2.5.1.** Survival of neonate FCM larvae exposed to Cryptogran, with and without lignin, exposed to a germicidal UV lamp for periods from 0 to 360 minutes, in laboratory bioassays.

In the third bioassay, mortality in the distilled water control was too high (44%), which rendered the results meaningless. The bioassay therefore needs to be repeated.

#### Field trial 1: Dunbrody

Unfortunately FCM levels were very low over the period of evaluation (Table 3.2.5.17). However, results indicated that an already low level of FCM can be suppressed to an almost negligible level. There were unfortunately no detectable trends to indicate the most effective timing of spray applications or the most effective programmes. A higher level of FCM infestation would be necessary to determine this.

**Table 3.2.5.17.** FCM infestation for different Cryptogran programmes in an orchard of Lane Late navel orange trees on Dunbrody Farm, evaluated from 30 November 2006 to 27 March 2007

Treatment	Timing of Cryptogran applications			Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	-	-	-	0.122a*	
2	26 Oct 2006	5 Dec 2006	-	0.077ab	21.05
3	26 Oct 2006	-	5 Feb 2007	0.020b	84.21
4	26 Oct 2006	5 Dec 2006	5 Feb 2007	0.051ab	63.16
5	-	5 Dec 2006	-	0.056ab	57.89
6	-	5 Dec 2006	5 Feb 2007	0.026b	78.95

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

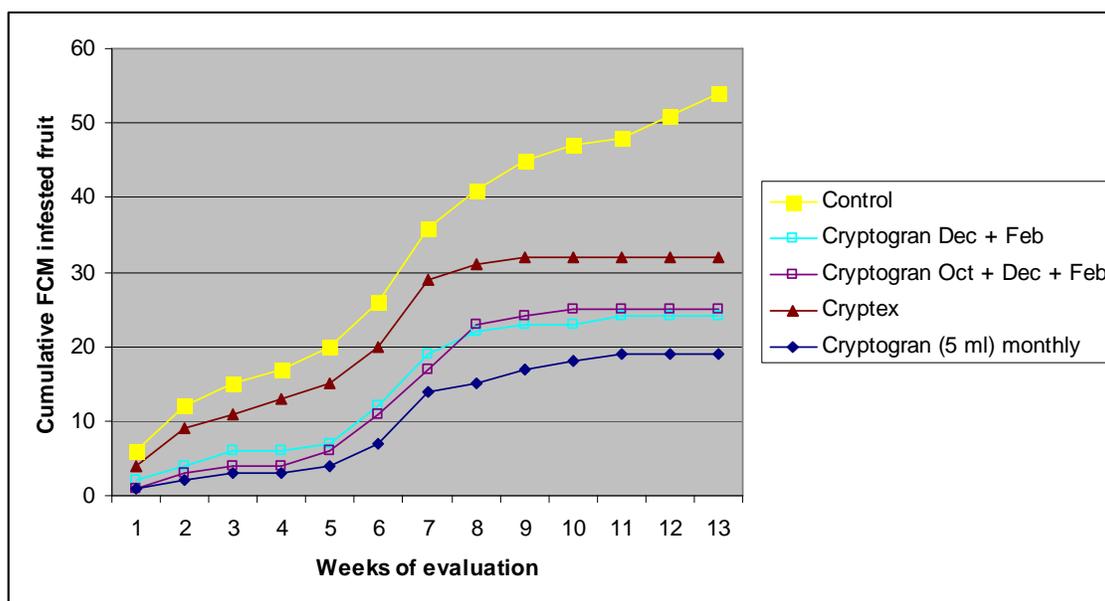
### Field trial 2: Dunbrody

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 3.2.5.18) (Figure 3.2.5.2). The greatest reduction in infestation occurred where Cryptogran was applied more frequently (monthly) at half the registered rate (5 ml/100L water). The Cryptex sprays resulted in the smallest reduction in infestation, and was the only treatment that did not result in a significant reduction of FCM infestation (Table 3.2.5.18) (Figure 3.2.5.2). Evaluations shall continue until May, and the final results will be recorded in the next annual report.

**Table 3.2.5.18.** FCM infestation for different Cryptogran programmes in an orchard of Lane Late navel oranges on Dunbrody Farm, evaluated from 2 January 2008 to 31 March 2008

Treatment		Concentration (ml/100 ℓ water)	Timing of Cryptogran applications					Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control				-	-	-	0.33a*	
2	Cryptogran	10		04 Dec 2007	-	14 Feb 2008	-	0.15b	55.6
3	Cryptogran	10	24 Oct 2007	04 Dec 2007	-	14 Feb 2008	-	0.16b	53.7
4	Cryptex	3.3		04 Dec 2007	09 Jan 2008	14 Feb 2008	-	0.21ab	40.7
5	Cryptogran	5	24 Oct 2007	04 Dec 2007	09 Jan 2008	14 Feb 2008	13 Mar 2008	0.12b	64.8

\* Different letters in the same column denote significant differences between values (P<0.5, Bonferroni multiple range test).



**Fig 3.2.5.2.** Cumulative number of infested fruit for various treatments and untreated control in an orchard of Lane Late navel oranges on Dunbrody Farm in the Sundays River Valley evaluated from 2 January 2008 to 31 March 2008.

#### Field trial 3: Riverbend Farm.

One evaluation was done on 2 January 2008. Only one FCM larva was discovered in the untreated control, and none in any of the treatments. It was decided to terminate the trial, as FCM levels were higher in the trial at Dunbrody, which was an exact replica of this trial.

#### Field trial 4: Dunbrody Farm.

In total only 14 and 16 infested fruit were discovered respectively for the two treatments (Table 3.2.5.19). It was interesting to note, however, that in orchard N1, where Cryptogran applications began on 26 October 2006, there were no infested fruit found until 28/12/2006, which could indicate some benefit of early applications (beginning in October). However, infestation was too low for conclusive results, and it would have been advantageous to keep and evaluate an untreated control.

**Table 3.2.5.19.** FCM infested fruit for various treatments applied to 2 similar Lane Late navel orange orchards on Dunbrody farm in the Sundays River Valley, evaluated from 30 November 2006 to 11 April 2007.

Date	FCM infested fruit / 20 trees/week	
	Orchard N1	Orchard N2
	7 Cryptogran sprays @ 5 ml / 100 ℓ water from 26 October 2006 to 31 January 2007	2 Cryptogran sprays @ 10 ml / 100 ℓ water on 27 November 2006 and 14 February 2007
30/10/2006	0	1
07/12/2006	0	1
14/12/2006	0	1
20/12/2006	0	1
28/12/2006	1	1
04/01/2007	0	0
11/01/2007	0	0
17/01/2007	0	0
25/01/2007	0	0
01/02/2007	2	1
07/02/2007	4	2
15/02/2007	0	1
22/02/2007	2	0
01/03/2007	0	2
08/03/2007	0	0
15/03/2007	2	0
21/03/2007	1	0
28/03/2007	1	1
03/04/2007	0	0
11/04/2007	0	1
18/04/2007	1	1
24/04/2007	0	2
<b>TOTAL</b>	<b>14</b>	<b>16</b>

#### Field trial 5: Compatibility

The addition of Flint and Ortiva to Cryptogran resulted in slightly lower efficacy of the virus, but the differences were not significant. Copper oxychloride is known to be harmful to viruses, so the lower reduction in infestation where this product was added to the virus was expected (Table 3.2.5.20). Cryptogran results with Agral 90 at 18 ml/100L were marginally better than at 6 ml/100 ℓ. It was surprising that the results with Break-Thru were poorer, and more work should be done with this product at different concentrations. Cryptex performed better than it had done in previous trials.

**Table 3.2.5.20.** FCM infestation for various treatments in an orchard of Lane Late navel orange trees on Willy Killians Farm, evaluated from 27 December 2006 to 31 January 2007.

Treatment no	Treatment (dosages per 100 ℓ water)	FCM infestation (infested fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	1.61a	
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Flint (10 g)	1.05b	37.6
3	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Cabrio (10 ml)	0.77b	55.4
4	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Flint (10 g)	0.98b	41.6
5	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Copper Oxychloride (200 g)	0.95b	44.6
6	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml) + Sporekill (100 ml) + Mancozeb (150 g)	0.80b	52.5
7	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	0.72b	57.4
8	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (6 ml)	0.82b	51.5
9	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (3 ml)	0.11b	36.6
10	Cryptex (5 ml) + molasses (250 ml) + Agral 90 (18 ml)	0.73b	56.4

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

#### Field Trial 6: Time of day + lignin

Cryptogran applied in the morning and at midday performed poorly (Table 3.2.5.21). Both these applications are against registration, as it is known that Cryptogran is UV-sensitive, particularly when still wet. It is speculated that this is due to faster UV breakdown while the virus is still in suspension. UV rays could be refracted within the water droplet, thus encountering more virus particles and causing greater inactivation of the virus. Cryptogran is therefore registered to be applied in the evening. The addition of lignin to both the midday and evening sprays improved their efficacy. Evening applications of Cryptogran, with and without lignin, resulted in significant reductions of FCM infestation, as did the midday application where lignin was added. This was not only due to improved persistence (as a result of UV-protection, but also to improved knock-down. It is not clear whether there was incompatibility with Lannate, as results were fairly poor. However, this is unlikely and the mixture of Cryptogran and Lannate should therefore be retested. Results with Cryptex were poor. Contrary to earlier reports (Graham Barry, unpublished data), Surround resulted in an increase in FCM infestation. This may have been due to its impact on egg parasitoids, which are known to be very effective against FCM in the Eastern Cape.

**Table 3.2.5.21.** FCM infestation for various treatments in an orchard of Lane Late navel orange trees on Atmar Farm, evaluated from 21 February 2007 to 3 April 2007.

Treatment	Infested fruit per tree per week							Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT		
Untreated control	1.2	0.9	1.0	0.7	0.5	0.4	0.8	0.79a*	
Cryptogran Morning	0.8	0.5	1.1	0.4	0.2	0.1	0.7	0.54ab	31
Cryptogran Midday	1.0	0.9	0.8	0.3	0.2	0.1	0.6	0.56ab	29
Cryptogran Evening	0.8	0.6	0.4	0.2	0.1	0.2	0.4	0.39b	51
Cryptogran + lignin midday	0.5	0.8	0.2	0.1	0.3	0.1	0.4	0.34b	56
Cryptogran + lignin evening	0.4	0.5	0.6	0.3	0.1	0.1	0.3	0.33b	58
Cryptogran + Lannate	0.6	0.5	0.5	0.2	0.7	0.1	1.0	0.51ab	35
Cryptex (5 ml)	0.7	1.1	0.6	0.2	0.2	0.2	0.7	0.53ab	33
Surround	1.9	1.0	1.0	0.5	0.4	0.3	0.6	0.81a	-4

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

#### Field trial 7: Lignin (by Stephan Honiball)

The results can be considered as reliable because there was not enough evidence against normality ( $p > 0.236$ ) (Table 3.2.5.23). Even though some interesting trends can be observed when scrutinizing these results, statistically there was no significant difference (significant increase in efficacy) between any of the different treatments on any of the dates, nor between the treatments and the control ( $p > 0.05$ ) (Table 3.2.5.23). Hence, the following inferences may be provisionally made, but they need to be confirmed in future trials:

The FCM infestation began as a light infestation, and almost no FCM infested fruit dropped in the control during the first two weeks of the trial (Table 3.2.5.22). The infestation increased steadily from week three (at six weeks after treatment (WAT)) when the economic threshold of one infested fruit dropped per tree per week was exceeded. Spraying this orchard was thus economically justifiable. At eight WAT, FCM infestation reached a peak, and none of the Cryptogran treatments could effectively suppress FCM to below the economic threshold of one infested fruit per tree. However, when considering the entire data collection period (12 March – 16 April), all the Cryptogran treatments (even the least effective one) succeeded in suppressing FCM to below the economic threshold (a mean of less than one infested fruit per tree per week for the entire period).

During the first four weeks of data collection (at seven WAT), both Cryptogran evening sprays (with and without lignin) reduced fruit drop by 45% compared to the control. At nine WAT the lignin evening spray provided a six percent improvement on the straight evening spray (without lignin) (40% reduction in fruit infestation (RFI)) vs. 34%), thus it seems adding lignin can improve Cryptogran's field persistence slightly.

The Cryptogran noon spray applications were only marginally less effective at seven WAT than the evening sprays (38% RFI vs. 45%), thus it appears as though the time of application has a limited effect on efficacy (provided climatic conditions are conducive to spraying).

The least effective treatment consisted of Cryptogran and lignin applied at noon (28% RFI up to 2 April 2007). The fact that no statistically significant difference was found between any of the treatments and the control, are surprising in view of previous similar trials that were conducted (e.g. Hofmeyr & Hofmeyr, 2003, and Kirkman *et. al*, 2005). According to Dr. K Pringle (pers. comm), no deductions can be drawn from such

experiments' data, since the difference in treatments could be a coincidence, or due to some other external factor.

Though rare, instances have been recorded with Cryptogran (unformulated, known as CrleGV), where seemingly good results were obtained that were not statistically significant. An example of this would be a trial at Vergenoeg Boerdery (Moore *et. al*, 2003), where CrleGV was applied at rates of  $9.694 \times 10^{13}$  OBs and  $9.694 \times 10^{15}$  OBs/ha, which resulted in fruit drop reduction / FDR of 52% and 61% respectively, none of which were significantly different from the control.

Hence, even though there were no significant differences in treatment means, this experiment can probably act as a guide to the different treatments' relative field efficacy.

The efficacy of the different treatments at seven WAT (RFI ranging between 38% and 45%) are in the same range as a previous trial, conducted at Carden Farm where the RFI ranged from 36% to 41% (Moore *et. al*, 2004b). Incidentally during the same Carden trial, Cryptogran was also applied to a continuous block of trees, where it afforded a reasonable 74% RFI. Thus though it may seem as if Cryptogran did not perform satisfactorily during this trial, it must be borne in mind that had Cryptogran been applied to a block of trees, there may have been a significant decrease in the percentage FCM infested fruit.

The most plausible reason for the poor performance of the lignin noon treatment is probably due to the unfavourable climatic conditions at the time of application. When the first Cryptogran spray (without lignin) was applied between 12h05 and 13:15, the mean temperature measured by the data logger was 24°C (Table 3). The mean temperature (between 13h35 and 14h40) of the subsequent Cryptogran / lignin treatment was 36°C. Though relative humidity readings were not taken during the course of the trial, it is known that an increase in air temperature and relative humidity, lead to an increase in the speed of evaporation and decrease the lifetime of a droplet (Basson, 1996). These factors likely impeded the Cryptogran / lignin noon spray's ability to suppress FCM attack.

At nine WAT, the Cryptogran / lignin evening spray was the best performer (at 40% RFI). Based on these results however, it is doubtful whether the addition of lignin to Cryptogran will have any real (significant) beneficial effect.

**Table 3.2.5.22.** Mean cumulative count of FCM infested fruit / tree and reduction in fruit infestation (% RFI) for each treatment

Treatment	Time of application	12 Mar (4 WAT)		19 Mar (5 WAT)		26 Mar (6 WAT)		2 Apr (7 WAT)		% RFI (12 Mar - 2 Apr)
		log (x + 1)	Mean cumulative count	log (x + 1)	Mean cumulative count	log (x + 1)	Mean cumulative count	log (x + 1)	Mean cumulative count	
Control	–	0	0	0.090309	0.3	0.4334454	2	0.5355643	2.9	
10ml Cryptogran + molasses + Agral	Noon	0.060206	0.2	0.120412	0.5	0.3362482	1.4	0.4089481	1.8	37.9
10ml Cryptogran + molasses + Agral + lignin	Noon	0	0	0.1380211	0.5	0.3209515	1.4	0.4179552	2.1	27.6
10ml Cryptogran + molasses + Agral	Evening	0	0	0.0477121	0.2	0.180618	0.7	0.3686636	1.6	44.8
10ml Cryptogran + molasses + Agral + lignin	Evening	0.0477121	0.2	0.0477121	0.2	0.2885361	1.1	0.3788451	1.6	44.8
Std. error		0.066341		0.111993		0.254677		0.373571		

No treatments were significantly different at P=0.05 (Bartlett's Chi Square)

(Table 1 continues on next page)

**Table 3.2.5.22.** (continued) Mean cumulative count of FCM infested fruit / tree and reduction in fruit infestation (% RFI) for each treatment.

Treatment	Time of application	9 Apr (8 WAT)		16 Apr (9 WAT)		% RFI (12 Mar - 16 Apr)
		log (x + 1)	Mean cumulative count	log (x + 1)	Mean cumulative count	
Control	—	0.7843208	6	0.8361722	6.8	
10ml Cryptogran + molasses + Agral	Noon	0.5918785	3.3	0.6957703	4.3	36.8
10ml Cryptogran + molasses + Agral + lignin	Noon	0.6670037	4.3	0.7673706	5.5	19.1
10ml Cryptogran + molasses + Agral	Evening	0.6066043	4	0.6568718	4.5	33.8
10ml Cryptogran + molasses + Agral + lignin	Evening	0.5635484	3	0.641903	4.1	39.7
Std. error		0.684586		0.817927		

**Table 3.2.5.23.** Analysis of variance performed on the log transformed cumulative counts over time

Source	df	12-Mar		19-Mar		26-Mar		02-Apr		09-Apr		16-Apr	
		MS	p										
Treatment	4	0.024333	0.830699	0.102046	0.467588	0.129004	0.731158	0.091534	0.910844	0.111474	0.955828	0.098906	0.974144
Block	9	0.029559	0.900840	0.029365	0.980937	0.063217	0.984208	0.052796	0.998025	0.095113	0.998152	0.110702	0.998331
Error	37	0.066341		0.111993		0.254677		0.373571		0.684586		0.817927	
Total	50												
Bartlett			0.877603		0.53832		0.236054		0.618847		0.426771		0.431588

### Field trial 8: Lignin

In this detached fruit bioassay, the addition of lignin did not appear to improve the efficacy of Cryptogran from days 0 to 7 (Table 3.2.5.24). However, the addition of lignin appeared to improve the residual efficacy of Cryptogran thereafter, as seen in the lower levels of infestation where lignin was added, measured 14 and 21 days after application. These differences were, however, not significant. Continued monitoring of this trial for a further week or more may have revealed whether a trend was developing or not.

**Table 3.2.5.24.** FCM infestation in a detached fruit bioassay where fruit was harvested at various intervals after application with Cryptogran to the northern side of the trees, with and without lignin.

Treatment	Penetration marks	No of fruit penetrated	Fruit infested	Mean no of larvae per fruit	Reduction in infestation (%)
<b>Day 0</b>					
Control	20	20	19	0.80a*	
Cryptogran	16	13	10	0.43b	47.4
Cryptogran + lignin	15	14	13	0.43b	31.6
<b>Day 1</b>					
Control	20	18	15	0.53a	
Cryptogran	9	9	7	0.23b	53.3
Cryptogran + lignin	11	8	5	0.20b	66.7
<b>Day 3</b>					
Control	21	16	16	0.67a	
Cryptogran	19	16	12	0.47a	25.0
Cryptogran + lignin	18	16	12	0.40a	25.0
<b>Day 7</b>					
Control	32	23	22	0.97a	
Cryptogran	24	17	15	0.67a	31.8
Cryptogran + lignin	30	23	20	0.83a	9.1
<b>Day 14</b>					
Control	27	24	23	0.83a	
Cryptogran	23	20	19	0.73a	17.4
Cryptogran + lignin	29	20	17	0.7a	26.1
<b>Day 21</b>					
Control	57	30	21	1.0a	
Cryptogran	21	15	13	0.5b	38.1
Cryptogran + lignin	21	16	11	0.43b	47.6

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

### Field trial 9: Molasses

Very good control of FCM was achieved with Cryptogran during this trial (Table 3.2.5.25). The standard registered application of Cryptogran 10 ml/100 l water) with Voermol molasses (250 ml/100 l water) and Agral 90 (18 ml / 100 l water), gave the best control. Similar control was achieved where Agral 90 was substituted with Break-Thru at 5 ml per 100 l water. Satisfactory control was obtained where white sugar was added instead of molasses, but results with brown sugar were poor. Where the dosage of Voermol molasses was reduced by half to 125 ml per 100 l water, control was satisfactory, but slightly (not significantly) poorer than where the full molasses rate was applied. In the case of a thicker molasses (apparently a product imported by Tate & Lyall), control with the reduced rate of molasses was not significantly different from control with the full rate. In fact, reduction in infestation was higher where the reduced rate of molasses was used. Control was poorer where oil, instead of Agral 90, was added to the reduced rate of Voermol molasses. Mannitol gave similar results to white sugar where they were used instead of molasses.

**Table 3.2.5.25.** FCM infestation for various treatments in an orchard of Palmer navel oranges on Lone Tree Farm, evaluated from 2 January 2008 to 12 February 2008.

Additives (per 100 ℓ) to Cryptogran (10 ml / 100 ℓ)	Infested fruit per tree per week							Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
	3 WAT	4 WAT	5 WAT	6 WAT	7 WAT	8 WAT	9 WAT		
Untreated control	2.1	1.5	0.6	1.9	1.2	0.6	1.1	1.27a*	
-	0.8	1.1	1.0	1.0	0.7	0.6	1.1	0.90ab	30
Agral 90 (18 ml)	0.2	0.7	0.2	0.4	0.4	0.4	0.5	0.40bc	68.9
Voermol molasses (250 ml) + Agral 90 (18 ml)	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.17c	86.7
Voermol molasses (250 ml) + Break-thru (3 ml)	0.1	0.3	0.2	0.9	0.3	0.1	0.4	0.33bc	74.4
Voermol molasses (250 ml) + Break-thru (5 ml)	0.2	0.3	0.1	0.4	0.4	0.1	0.1	0.23c	82.2
Voermol molasses (125 ml) + Agral 90 (18 ml)	0.1	0.5	0.1	0.4	0.3	0.3	0.2	0.27bc	78.9
White sugar (200 g) + Agral 90 (18 ml)	0.0	0.5	0.0	0.3	0.5	0.5	0.3	0.30bc	76.7
White sugar (400 g) + Agral 90 (18 ml)	0.3	0.6	0.1	0.4	0.3	0.5	0.3	0.36bc	72.2
Brown sugar (200 g) + Agral 90 (18 ml)	0.6	0.3	0.5	0.7	0.6	0.3	0.4	0.49bc	62.2
Brown sugar (400 g) + Agral 90 (18 ml)	0.1	0.3	0.2	0.7	0.5	0.3	1.0	0.44bc	35.6
Imported molasses (250 ml) + Agral 90 (18 ml)	0.5	0.2	0.1	0.4	0.4	0.3	0.5	0.34bc	73.3
Imported molasses (125 ml) + Agral 90 (18 ml)	0.2	0.5	0.1	0.1	0.2	0.4	0.3	0.26c	80.0
Voermol molasses (250 ml) + oil (300 ml)	0.3	0.1	0.4	0.1	0.5	0.4	0.3	0.30bc	76.7
Voermol molasses (125 ml) + oil (300 ml)	0.5	0.2	0.2	0.4	0.7	0.7	0.7	0.49bc	62.2
Oil (300 ml)	0.4	0.5	0.1	0.6	0.2	0.4	0.5	0.38bc	70.0
Mannitol (1 kg) + Agral 90 (18 ml)	0.3	0.1	0.2	0.5	0.8	0.5	0.2	0.37bc	71.1

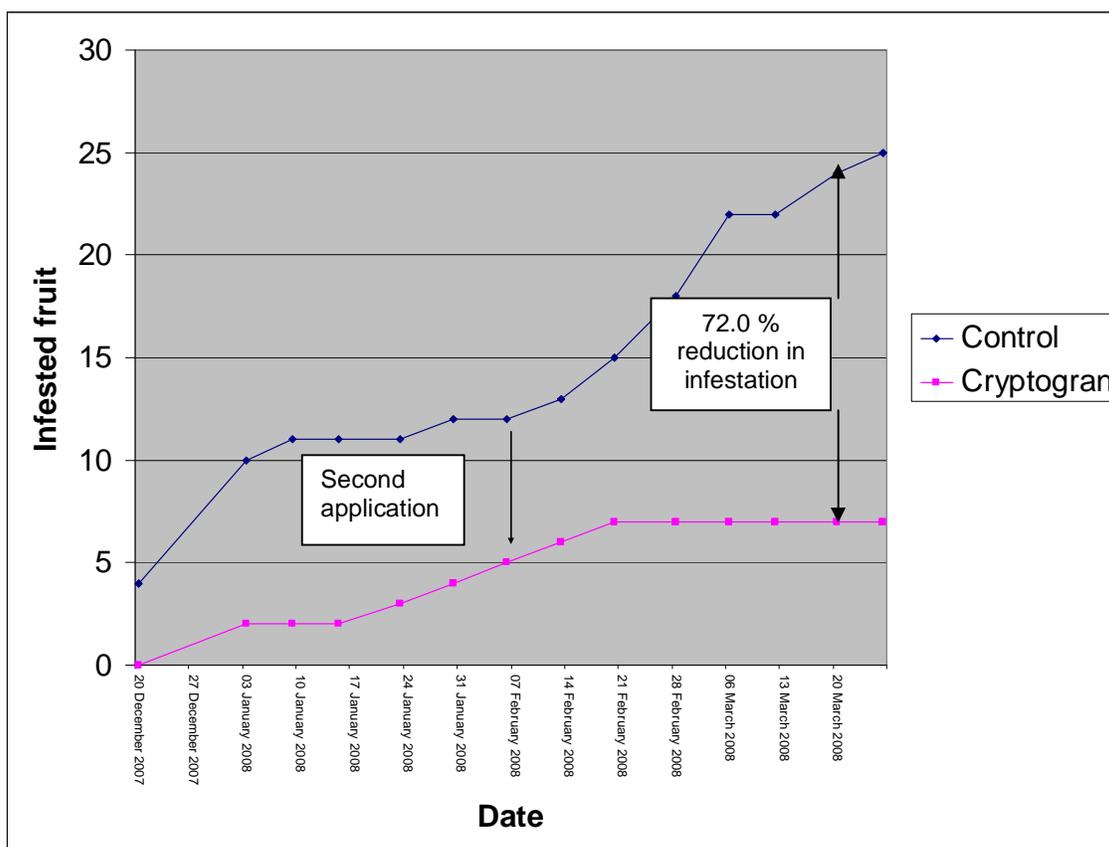
\* Different letters in the same column denote significant differences between values ( $P < 0.05$ , Bonferroni multiple range test).

Field trial 10: Patensie

FCM infestation in the trial was low, but FCM control by Cryptogran was very good, with the two Cryptogran applications resulting in a 72.0% reduction in infestation over a 14 week period of evaluation (Table 3.2.5.26), (Fig 3.2.5.3).

**Table 3.2.5.26.** 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 26 March 2008.

Treatment no	Treatment (dosages per 100 ℓ water)	Mean no of FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	0.15a	
2	Cryptogran (10 ml) + molasses (250 ml) + Agral 90 (18 ml)	0.04b	72.0



**Fig 3.2.5.3.** Cumulative number of infested fruit for Cryptogran treatment and untreated control in an orchard of Palmer navel oranges on Paksaam Farm in the Gamtoos River Valley evaluated from 20 December 2007 to 26 March 2008.

Field trial 11: Letsitele

Unfortunately, no FCM was found in any of the fruit. However, an average of 1.87 fruit dropped per tree per week in the treated orchard, compared to an average of 2.60 in the untreated orchard. There was no evidence that this difference could be attributed to FCM.

Field trial 12: Letsitele

From 20 December until 12 June, weekly evaluations of fruit drop from five data trees in the sprayed and unsprayed orchards were conducted. Over this period, only one infested fruit was found in the sprayed orchard and two in the unsprayed orchard. Therefore, a revised method of evaluation was incorporated from 10 May and used until 12 June. Over this time an average of 0.20 infested fruit were recorded per tree per

week in the untreated block. An average of 0.14 infested fruit were recorded per tree per week in the treated block – 30% less than in the untreated block. A total of 23.4% of the fruit collected from the untreated block were infested, whereas only 8.8% of fruit collected from the treated block were infested.

## Conclusion

Bioassays and field trials proved that lignin can protect Cryptogran against UV inactivation, thus improving the efficacy and extending the residual activity of the virus. However, the lignin product is expensive. Cryptogran appears to be rainfast. Additional early applications of Cryptogran, coinciding with a minor FCM peak in October, resulted in no better FCM control than the standard two-spray programme (December and February). Reduced rates of Cryptogran applied more frequently gave good control. Cryptex was shown to be less effective than Cryptogran in Eastern Cape field trials. Cryptogran was shown to be compatible with Lannate and most black spot treatments, except copper. Surround offers no FCM control. Evening applications of Cryptogran are far more effective than morning and midday applications. Reduced rates of molasses, and substitution of molasses with brown sugar and Mannitol resulted in satisfactory FCM control. Break-Thru was shown to be an adequate substitute for Agral 90. Cryptogran gave very good FCM control in the trials at Dunbrody (79% reduction in infestation), Paksaam (72% reduction in infestation) and Lone Tree farm (87% reduction in infestation). Wayne Kirkman completed his MSc thesis on the work completed in this project and graduated in March 2008.

## Acknowledgments

Felix Hacker, Christo Breytenbach and Hannah Otto of Du Roi IPM are thanked for their assistance with the evaluation of trials in the Letsitele area. All growers on whose farms trials were conducted are also thanked.

## Future research

Research will be continued to further test potential UV-protectants, including sugar-alcohols, both in laboratory bioassays and in field trials, in an effort to improve the formulation of Cryptogran. 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.

## Technology transfer

Wayne Kirkman and Sean Moore made presentations on related topics at a total of 17 grower study group meetings throughout the country. See Section 9 on Technology Transfer for details.

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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 (SU) and S D Moore (CRI)

#### Summary

A total of 202 soil samples from citrus orchards throughout South Africa were analyzed for the presence of entomopathogenic nematodes (EPN) of which 32 (16%) were positive. The following species have been identified by means of molecular techniques: *Heterorhabditis bacteriophora*, *H. zealandica*, *Heterorhabditis* spp., *Steinernema khoisanae*, *S. yirmagelensis* (new record for South Africa), *Steinernema* spp. The steinernematids are probably new species or not reported for South Africa and are close to *S. feltiae*. From all the available EPN isolates four final selections were made for the control of false codling moth (*Thaumatotibia leucotreta* Meyerick) (FCM) larvae. These four EPN isolates were used to inoculate cocooned FCM larvae in sterilized sand. Two nematode selections from the sand bioassay with high mortality (78-94%) were inoculated onto cocooned FCM larvae in three unsterilized citrus soil samples from three orchards. *Heterorhabditis bacteriophora* (SF351) gave 100% control in all three soil types, followed by *H. zealandica* (98%). To determine the minimum concentration for effective control, six concentrations of *H. bacteriophora* (SF351) were used to infect soil with cocooned FCM larvae. Concentrations of 200, 100, 50 and 25 IJ/FCM larva gave >90% control while 25 and 6 IJ/FCM larva gave mortality of 70% and 68% respectively. Sterilized soil from citrus orchards was also used to determine the persistence of the final EPN selection (SF351) in soil. Up to day 35, infection of FCM larvae added to the infested soil was obtained with 24 (60%), 50 (80%) and 200 (100%) IJ/FCM larva.

#### Opsomming

’n Totaal van 202 grondmonsters vanuit sitrusboorde regoor Suid-Afrika is vir die voorkoms van entomopatogeniese nematodes (EPN) ontleed. Die volgende spesies is identifiseer deur gebruik te maak van molekulêre tegnieke: *Heterorhabditis bacteriophora*, *H. zealandica*, *Heterorhabditis* spp., *Steinernema khoisanae*, *S. yirmagelensis* (nuwe aanmelding vir Suid-Afrika) en *Steinernema* spp. Die *Steinernema* spp. is moontlik ’n nuwe spesie of nog nie aangemeld in SA nie en is baie naby aan *S. feltiae*. Van al die beskikbare EPN isolate, is vier geselekteer vir die beheer van valskodlingmot (*Thaumatotibia leucotreta* Meyrick) (VKM) en gebruik om toegespinde VKM-larwes in sand te behandel. Die twee seleksies met die hoogste mortaliteit (78-94%) in die sandbiotoets is geselekteer om toegespinde larwes in drie ongesteiriliseerde sitrusgrondtipes te behandel. *Heterorhabditis bacteriophora* (SF351) het 100% beheer in al drie grondtipes gegee, gevolg deur *H. zealandica* (SF41) (98%). Om die minimum konsentrasie van nematodes vir suksesvolle beheer van VKM te bepaal, is ses konsentrasies van *H. bacteriophora* (SF351) gebruik om grond met toegespinde VKM-larwes in ongesteiriliseerde grond te behandel. Konsentrasies van 25, 50, 100 en 200 IJ/VKM-larwe gee beheer van >90%, terwyl konsentrasies van 6 en 25 IJ/VKM-larwe besmetting van 68% en 70% respektiewelik tot gevolg het. Vir die bepaling van die nawerking van die nematodes is daar 35 dae nadat grond besmet is met SF351 steeds 100% kontrole van VKM-larwes verkry. Tot op 35 dae word VKM-larwes wat by die grond gevoeg is nog steeds besmet. Met konsentrasies van 25 is 60%, met 50 is 80% en 200 is 100% besmetting verkry.

#### Introduction

Current control practices employed against false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick), include chemical, mating disruption and biological control using a *Cryptophlebia leucotreta* granulovirus (Moore et al., 2004). With the onset of the use of the Sterile Insect Technique for the control of FCM, the use of additional biological control agents especially against the soil stages of FCM became imperative. Soil

is the natural habitat for EPN. During the period when the larvae leave the fruit and fall onto the soil to burrow and pupate, the pupae as well as the emerging moth offer a window period for EPN to be used as a biological control agent against FCM. The ability of different EPN strains to control a specific pest can vary greatly (Shapiro-Ilan *et al.*, 2002). The key to success of EPN as a biological control agent against a specific pest is dependent on obtaining a good nematode match for the target insect.

The aim of this study was first to obtain EPNs, locally adapted to citrus orchards, by doing a survey in the different citrus producing areas. Obtained EPN isolates were identified to species level using molecular techniques. Selected isolates were subjected to more natural conditions such as cocooned larvae in sand and unsterilized soil from different orchards to determine their potential as biological control agents. Thirdly, soil from an orchard was used to determine the minimum concentration and to test for persistence of nematode activity for the selected isolate.

## Materials and methods

To identify new isolates obtained from the citrus survey, the rDNA internal transcribed spacer region from rDNA extracted from the first generation of females or hermaphrodites was amplified, using the polymerase chain reaction. The amplification DNA product was visualized on an agarose gel, cleaned up and sequenced directly. The identity of the species was confirmed by alignment with sequences from Genbank. Moist sand in plastic containers was used to determine the potential of four previously selected EPNs to penetrate cocooned FCM larvae. Ten last instar FCM larvae were added to 100 ml moist sand in each of five 500 ml plastic containers and closed with a lid. The containers were left at 25°C for 24 hours to give the larvae time to burrow into the soil and spin their cocoons. Nematodes (200 IJ/FCM larva) were entered into the middle of the sand and after four days infection was determined by washing the cocooned larvae from the soil.

Soil from three different citrus orchards in the Nelspruit area and the Eastern Cape was used to determine the infectivity of two selected EPN isolates, as well as *S. khoisanae*, on cocooned larvae of FCM. The containers were inoculated by spraying IJ of *H. zealandica* (SF41), *H. bacteriophora* (SF351) and *S. khoisanae* (SF 106-C) onto the surface of the soil. The nematodes were sprayed by adding 2000 IJ (58 IJ/cm<sup>2</sup>) to 5 ml of filtered tap water to a 20 ml cylinder, using the nozzle of a spray bottle to spray it evenly onto the soil surface. The containers were closed with a lid and returned to a growth chamber at 25°C. After 5 days the larvae were removed by sieving from the soil and dissected to confirm infection with nematodes.

To determine the minimum number of IJ to apply, six concentrations (6, 12, 25, 50, 100 and 200 IJ/FCM larva) of the selected isolate were inoculated by spraying them onto unsterilized orchard soil, to which 10 FCM larvae were added 24 hours earlier. After 5 days at 25°C the number of infected larvae was determined by looking at the colour change to red and if unsure the larvae were dissected to confirm infection.

To test for persistence soil from citrus orchards were sterilized by freezing. Soil (100ml) was placed in 500 ml plastic containers and inoculated with 25, 50 and 200 IJ/FCM larva by spraying them onto the soil. The containers were kept at room temperature and after 5, 15, 25 and 35 days 10 FCM larvae were added to the soil and the infectivity determined after 5 days, and then every 10 days up to 35 days after inoculation.

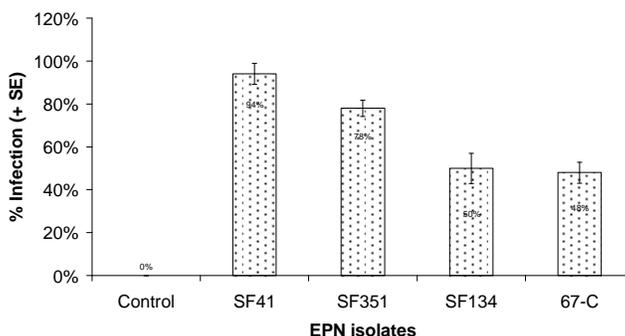
## Results and discussion

A total of 202 soil samples from citrus orchards throughout South Africa were analyzed for the presence of entomopathogenic nematodes (EPN) of which 31 (15%) were positive. The following EPN species were identified: *Heterorhabditis bacteriophora*, *H. zealandica*, *Heterorhabditis* sp., *Steinernema khoisanae*, *S. yirgalemense* and two *Steinernema* spp. (Table 3.2.6.1). The unidentified *Steinernema* spp. are probably new species or unreported for South Africa, but close to *S. feltiae*. *Heterorhabditis bacteriophora* was found to be the most common species, followed by *H. zealandica* and *S. khoisanae*. *Steinernema yirgalemense* is a first report for South Africa. It has been previously reported from Ethiopia (Nguyen *et al.*, 2004) and Kenya (Nguyen *et al.*, 2007). These results showed a similar species distribution as for a general survey for EPNs in SA (Malan *et al.*, 2006).

**Table 3.2.6.1.** Species and isolates of entomopathogenic nematodes identified from a survey of citrus producing areas throughout South Africa.

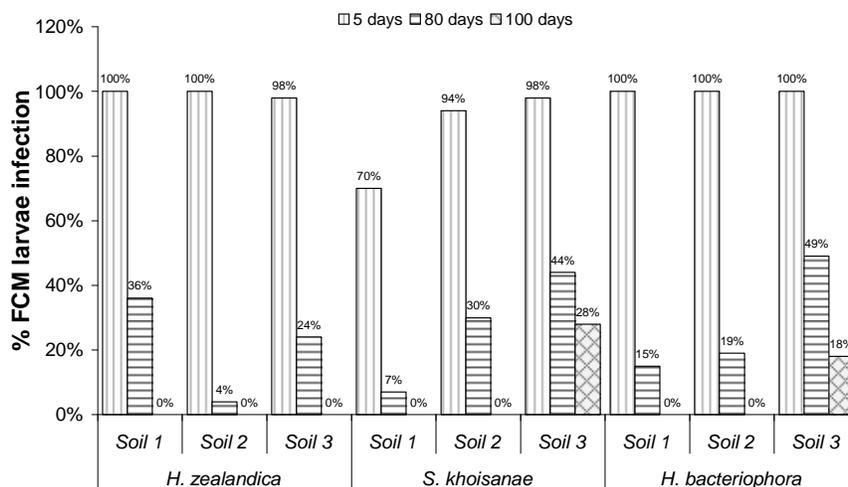
Province	Area	Species	Isolate	Total
Western Cape	Citrusdal	<i>H. bacteriophora</i>	42-C; 111-C; 113-C; 153-C; 154-C	5
	Montagu	<i>H. bacteriophora</i>	117-C	2
		<i>H. zealandica</i>	118-C	
	Mooreesburg	<i>H. zealandica</i>	130-C	1
	Piketberg	<i>H. bacteriophora</i>	149-C	1
		<i>Steinernema</i> sp.	143-C	1
	Porterville	<i>H. bacteriophora</i>	104-C; 142-C; 147-C	4
		<i>S. khoisanae</i>	06-C	1
		<i>Steinernema</i> sp. (close to <i>S. feltiae</i> )	41-C	1
Stellenbosch	<i>H. bacteriophora</i>	65-C	1	
Wellington	<i>H. bacteriophora</i>	26-C	1	
Mpumalanga	Nelspruit	<i>H. bacteriophora</i>	63-C; 65-C; 66-C; 67-C	9
		<i>S. yirgalemense</i>	57-C	1
		Unknown	58-C	1
		<i>H. zealandica</i>	59-C	1
		<i>H. zealandica</i>	60-C	1
Eastern Cape	Addo	<i>H. bacteriophora</i>	29-C	1
	Kirkwood	<i>H. bacteriophora</i>	51-C; 20-C	2
	Knysna	<i>H. bacteriophora</i>	56-C	1
	Patensie	<i>H. bacteriophora</i>	89-C; 136-C	2
	Sundays River Valley	<i>H. bacteriophora</i>	17-C	1
<b>Total number of positive samples:</b>				<b>31</b>

The FCM larvae in sand were found to be in tightly woven cocoons with sand particles clinging to the outside. The highest percentage infection was obtained for *H. zealandica* and *H. bacteriophora* (Fig. 3.2.6.1). The other two isolates were discarded for future testing against FCM. These results indicate that the cocoon spun by the larvae was no barrier for nematode infection.



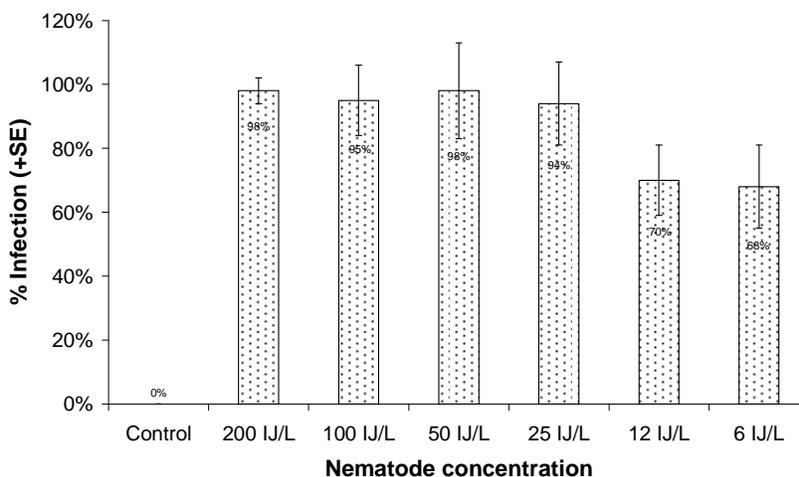
**Fig. 3.2.6.1.** Infectivity of four entomopathogenic nematode isolates to cocooned false codling moth larvae in sterilized sand.

All three of the selected EPN species performed well in all three soil types and infectivity was high (Fig. 3.2.6.2). *Heterorhabditis bacteriophora* (SF351) gave 100% control in all three soil types followed by *H. zealandica*. *Steinernema khoisanae* (only available steinernematid) was included in the study to give an indication of persistence. The nematode did not perform well in soil type 1, but in the other soil types infectivity was comparable in the other two isolates. The nematodes performed even better in unsterilized soil than in the sand. After a period of 100 days some infection was still obtained in the soil for *S. khoisanae* and *H. bacteriophora*. Heterorhabditids are not known to persist for long periods in the soil in comparison with steinernematids.



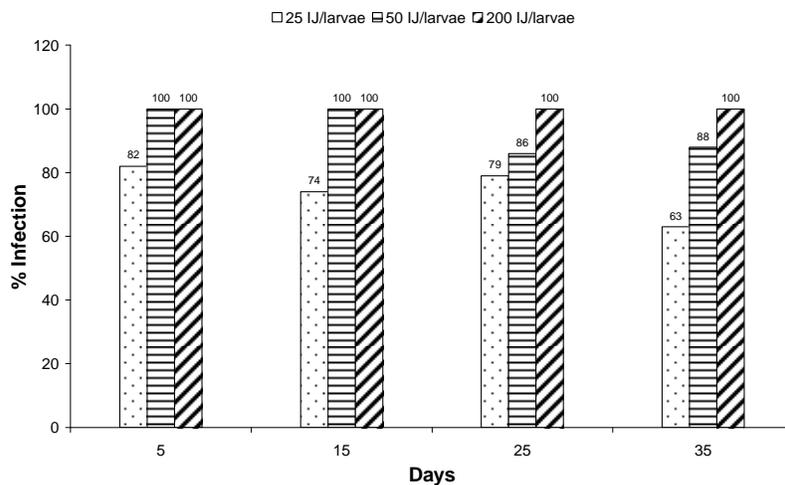
**Fig. 3.2.6.2.** Tree types of unsterilized citrus orchard soil were used to test the infectivity of three EPN species after five days. The ability of the soil to infect FCM larvae after 80 and 100 days were also tested.

Concentrations of 25-200 IJ/FCM give no statistically significant differences between the mean percentages of infectivity. Between 6 and 12 /FCM larva there was also no statistical difference. However, infectivity at these concentrations differed significantly from the four higher concentrations (Fig. 3.2.6.3).



**Fig. 3.2.6.3.** Six different concentrations of the selected EPN isolate (SF351) were used to test for infectivity of cocooned FCM in unsterilized soil.

For the selected isolate (SF351) a 100% infection was found up to day 25 with a concentration of 50 and 200 IJ/FCM larva. With half the concentration the infection was still 100% up to 15 days after which it started to decline. With a concentration of 25 IJ/FCM larval infection stayed the same up to 35 days (Fig. 3.2.6.4).



**Fig. 3.2.6.4.** Three concentrations (25, 50 and 200 IJ/FCM larva) were used in sterilized citrus orchard soil to determine the infectivity after a period of 5, 15, 25 and 35 days.

### Conclusion

During the survey, three *Steinernema* isolates were found, which were not *S. khoisanae*, the only steinernematid currently described for SA. This is a first report for the occurrence of *S. yirgalemense* in South Africa. The two *Steinernema* spp. need to be identified and tested, for example against infectivity of FCM pupae, since steinernematids have shown in other studies to be very effective against Lepidoptera. Two EPN species from previous selections were chosen in the sand bioassay. All three of the selected EPN species performed well in all three soil types and infectivity was high. *Heterorhabditis bacteriophora* (SF351) was selected in the soil type bioassay for the control of FCM. It was shown that 25-50 IJ/FCM larva will be an effective concentration to be used in semi-field trials. Persistence in the soil of the selected isolate seems to still be effective after 35 days at a concentration of 50 IJ/FCM larva.

### Future research

The selected EPN isolate will be presented to a biological company for formulation and production. The formulated product will be applied in semi-field trials in a citrus orchard at the Welgevallen experimental orchard in Stellenbosch. The semi-field trials will be conducted in different environmental conditions of which soil temperature, moisture and ultraviolet light will be the most important. Optimum concentration and persistence of the formulated EPNs will also be determined in orchard conditions.

### Technology transfer

- Malan, A.P. & Moore, S.D., 2007. Potential of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae for the control of false codling moth. 16th Symposium of the Nematological Society of Southern Africa (oral presentation).
- De Waal, J.Y., Malan, A.P. & Ferreira, T., 2007. Influence of temperature on the infectivity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) to false codling moth *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). Symposium of the Nematological Society of Southern Africa (poster).
- Malan, A. P. & Moore, S.D., 2008. Entomopathogenic nematodes for the control of false codling moth. Deciduous Fruit Producers Truist IPM meeting (oral presentation)

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### 3.2.7 **PROGRESS REPORT: Determine the potential global distribution for false codling moth** Experiment 805 (March 2006 – March 2010): M de Villiers (SU)

#### **Summary**

False codling moth, *Thaumatotibia leucotreta* (Meyrick), is a pest of various South African fruit. Due to phytosanitary concerns, this pest hinders international fruit trade. Knowing its current distribution, its potential global distribution can be modelled and the relevance of associated phytosanitary regulations evaluated. Delta traps baited with pheromone, were placed in different South African climatic regions to trap false codling moth. Three traps, serviced monthly, were used per monitoring area. 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.

#### **Opsomming**

Valskodlingmot, *Thaumatotibia leucotreta* (Meyrick), is 'n plaag van 'n verskeidenheid van Suid-Afrikaanse vrugte. Weens fitosanitêre belang, verhinder hierdie plaag internasionale vrugtehandel. Kennis van sy huidige verspreiding maak dit moontlik om sy potensieële globale verspreiding te modelleer om sodoende die toepaslikheid van geassosieerde fitosanitêre regulasies te evalueer. Deltavalle met feromoon as lokmiddel is in verskillende klimaatstreke van Suid-Afrika geplaas om valskodlingmot te monitor. Drie valle, wat maandeliks nagegaan is, is per monitorarea gebruik. Die volopheidsverspreiding van valskodlingmot het nie ooreengestem met klimaatsverskille nie, wat voorstel dat relatiewe volopheid waarskynlik meer sensitief is teenoor faktore soos gasheerbeskikbaarheid as beperkings wat deur die klimaatsreeks van die studiearea gestel word.

#### **Introduction**

False codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is a pest of international phytosanitary concern (EPPO 2007). In South Africa, this pest infests a wide range of fruits. By determining its current distribution it becomes possible to model its potential to invade other parts of the world. Such information provides a scientific basis to evaluate the relevance of current and future phytosanitary restrictions imposed on international citrus trade to minimize the risk of introducing this pest into importing countries. The availability of reliable, science-based, technical information of such a nature is the only legitimate basis for contesting phytosanitary restrictions placed on trade. Therefore, such information is considered critical in both maintaining and gaining market access.

To be able to model the potential future global distributions, detailed distribution data need to be obtained. This should be achieved by gaining relevant information from the literature to determine historical distribution. The power of the modelling exercise can be greatly enhanced by including more detailed distribution and abundance data. Such information is not currently available and will require surveys. The surveys will be focussed on generating more detailed information on the pests' occurrence within the southern African zone of distribution. The objective of such surveys will be two-fold. Firstly, the geographical limits of distribution ranges within southern Africa should be determined and secondly, some measure of comparative abundance, across the distribution range, within southern Africa.

#### **Materials and methods**

To determine relative abundance of false codling moth (FCM) across the country, 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, Tom Burke, Tzaneen, Rustenburg and Harare. These areas are representative of the different climatic regions in South Africa (Earle *et al.* 1996). In each of these areas, three yellow delta traps, baited with FCM pheromone, were used to monitor FCM. These traps were placed mostly in home gardens and in host plants. All traps were rebaited and trap catches collected on 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

The abundance data of FCM is given in Table 3.2.7.1. FCM was absent from Swellendam, Hondeklipbaai, Onseepkans and Britstown. The maximum monthly average over the period January 2007 to March 2008, was low (<10 moths/trap/month) in Knysna, Addo and Tshipise, moderate (10-19.9 moths/trap/month) in Komatipoort, Tom Burke and Tzaneen, high (20-49.9 moths/trap/month) in Citrusdal, Jan Kempdorp, Nkwalini, Groblersdal/Marble Hall and Nelspruit, and very high ( $\geq 50$  moths/trap/month) in Stellenbosch, Keimoes, King William's Town, Bloemfontein, Pietermaritzburg and Rustenburg. 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 appeared to be absent from Onseepkans and 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. Data of various months still need to be obtained from Tom Burke.

## Conclusion

FCM occurred across all climatic regions, with localised occurrence of both high numbers and apparent absence within the same climatic regions. Therefore, FCM did not show an abundance distribution pattern corresponding with climatic regions, suggesting that relative abundance is probably more sensitive to factors like host availability than to restrictions imposed by the climatic range covered by the study area.

## Technology transfer

No technology transfer occurred during January 2007 to March 2008. However, a poster will be presented at the International Congress of Entomology during July 2008 and a presentation at the Citrus Research Symposium during August 2008.

## Future research

Trapping in each area will continue until two year's data is obtained. A CLIMEX course will be attended in Australia. This will ensure reliability of the model that will be created for potential global distribution, using CLIMEX.

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**Table 3.2.7.1.** Abundance data of false codling moth, *Thaumatotibia leucotreta*, across southern Africa.

Area	2007												2008		
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Stellenbosch	36.7	88.3	147.7	182.3	212.7	134.7	27.3	8.7	10.7	14.7	29.7	1.3	5.7	7.3	46.3
Citrusdal	2.3	2.7	12.0	48.3	21.7	16.7	13.7	5.3	2.0	10.7	14.3	4.0		26.0*	36.0
Swellendam	0	0	0	0	0	0	0	0	0	0	0		0*	0	0
Knysna	0	0	2.0	0.3	0	0	0	0	0.3	0.3	0.3	0.7	0.3	0.7	0.7
Hondeklipbaai	-	-	-	-	-	-	-	0	0	0	0	0	0	0	-
Onseepkans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Keimoes		121.0*		198.3*	90.3		22.0*	11.3	37.0	45.3		117.7*			168.7*
Britstown	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan Kempdorp	7.7	13.7	9.0	25.0	4.3	1.0	0.3	0	1.3	2.7	2.0	2.7	14.7	11.7	13.0
Addo	0	0.3	4.3	0.7	4.0	5.3	2.3	2.3	1.3	0.7	1.3	1.0	2.3	1.3	4.7
King William's Town	5.0	38.3	82.7			136.0*			28.3*	1.7	9.7	16.0	0.7	9.0	29.3
Bloemfontein	54.0	90.3	58.7	163.0	37.0	2.3	0.3	0.3	3.7	6.7	8.3	28.7	6.7	18.7	74.3
Pietermaritzburg	-	-	-	18.3	25.7	52.0	32.3	11.0	11.0	9.0	23.0	6.0	0.3	7.7	10.7
Nkwalini	14.7	21.0	19.7	6.7	3.3	1.0	2.7	1.3	11.0	-	-	34.3	9.7	6.7	0.7
Groblersdal/Marble	27.7	12.0	-	-	-	-	-	-	-	9.3	13.3	31.7	3.3	-	-
Nelspruit	6.7	9.7	11.3	6.0	10.0	36.0	24.3	23.7	14.3	24.7	28.7	3.0	0	6.0	11.0
Komatipoort	14.7	8.3	6.7	3.0	1.7	2.7	2.7	3.7	7.0	3.3	6.7		10.0*	2.0	6.0
Tshipise	2.3	3.7	0.7	0.7	0.3	0	0.7	0.3	0	0	0	1.0	2.3	0.3	0
Tom Burke	-	-	11.3	0	0.7	5.0	0.7	2.0	-	-	-	-	-	-	-
Tzaneen	6.0	13.0	8.7	7.0	1.0	3.3	2.0	2.3				13.0*		12.7*	7.7
Rustenburg	100.0		43.7*			56.5*	5.0		21.0*		43.0*		77.3*		83.3*
Harare	27.7*				38.3*		24.7*			20.3*			55.7*		36.0*

\* Collective samples, taken over more than one month – monthly averages not available

- Data not obtained

### 3.2.8 FINAL PROGRESS REPORT: The host status of lemons for FCM

Experiment 828 (April 2005 – July 2007): Sean D. Moore and Wayne Kirkman (CRI)

#### Summary

The Chinese market has recently opened for exports of South African citrus fruits. The protocol for export stipulates that fruit must be cold disinfested in transit to China. Cold disinfestation of lemons is not feasible. This experiment proposes to determine the suitability of lemons, at various stages of development, as hosts for FCM. The objective is to provide the Chinese authorities with sufficient data to demonstrate that lemons – possibly of particular color standards – do not host FCM. Attached lemons of a range of colors were netted and pairs of moths introduced into the nets. Evaluations conducted three weeks later revealed that 2.61% of the fruit were infested with FCM larvae. This was in comparison with 53.97% of navel oranges, used as a control. In a laboratory trial, 57.9% of small marble-sized fruit were infested with FCM. This may explain the high numbers of FCM adults trapped in lemon orchards. Although both of these trials are not entirely representative of a natural situation, it is unlikely that it will be possible to demonstrate a non-host status of lemons for FCM.

#### Opsomming

Die Chinese mark is onlangs vir uitvoere van Suid-Afrikaanse sitrusvrugte oopgestel. Die uitvoerprotokol vereis dat vrugte op pad na China aan koue-ontsmetting blootgestel moet word. Koue-ontsmetting van suurlemoene is nie moontlik nie. Die doel van hierdie eksperiment is om die geskiktheid van suurlemoene as gasheer vir VKM teen verskeie stadiums van kleurontwikkeling te bepaal. Die mikpunt is om die Chinese beamptes te voorsien met data wat voldoende sal wees om te bewys dat sekere kleurstandaarde van suurlemoene nie gasheer vir VKM kan wees nie. Suurlemoene op bome met 'n reeks kleure is in nete saam met pare motte toegemaak. Evaluasies wat drie weke later gedoen is, het getoon dat 2.61% van die vrugte met VKM-larwes besmet was. Dit is in vergelyking met ryp nawellemoene, wat as 'n kontrole gebruik is, waar 53.97% van die vrugte besmet was. In 'n laboratoriumproef is 57.9% van klein, albastergroottes vrugte met VKM besmet. Hierdie inligting verduidelik heel waarskynlik die hoë lokvalvangste van VKM in suurlemoenboorde. Alhoewel beide proewe nie heeltemal verteenwoordigend van 'n natuurlike situasie is nie, is dit onwaarskynlik dat 'n nie-gasheerstatus van suurlemoene vir VKM bewys sal kan word.

#### Introduction

The Chinese market has recently opened for exports of South African citrus fruits. Originally, the relevant protocol stated that only fruit from FCM-free orchards is admissible. The Chinese market has now accepted an alternative protocol of cold sterilisation of fruit in transit. However, the cold sterilisation treatment for lemons is not a feasible option as lemons damage easily at such low temperatures. The first protocol, in effect means that if any FCM adults are caught in pheromone traps in an orchard, even if the fruit is not attacked, then the fruit from that orchard cannot be packed for China. However, it is known that lemons are an unsuitable host for FCM (Newton, 1998). Consequently, trials to examine the exact host status of lemons for FCM were initiated two years ago (Moore et al., 2005). Season-long monitoring of pheromone traps confirmed that lemon orchards are not free of FCM. Laboratory trials revealed notable levels of infestation of detached lemons of all degrees of colour. However, this was associated with a high level of fruit decay. Field trials in which FCM eggs were pasted onto lemons at different stages of maturity, conducted in order to avoid high levels of fruit decay, revealed only one infested fruit (Moore et al., 2005). During the following season it was shown that releasing moths into nets covering fruit clusters was a viable technique (Moore & Kirkman, 2006). This experiment proposed to use this methodology to determine the suitability of lemons, at various stages of development, as hosts for FCM. A laboratory trial was also conducted to test the susceptibility of very small immature lemons to FCM.

#### Materials and methods

##### Trial 1

On 29 March 2007, a mature Eureka lemon orchard on Luthando Farm in Sundays River Valley, with fruit ranging from T1-T8 in colour was used for a trial. Branches holding between 15-30 lemons were bagged with mosquito netting. Twenty such nets were enclosed over fruit on various adjacent trees. Four pairs of mating FCM adults were released into each bag. Approximately 130 Palmer navel oranges (T2 colour) in an orchard on the Citrus Foundation Block near Uitenhage, were subjected to the same protocol, as a positive control. A week later, bags of fruit were opened and the colour of each fruit was categorised and marked on the fruit with an indelible pen. Observations were conducted to confirm that FCM eggs had been laid. All

live FCM observed were killed. Bags were again sealed. After another two weeks, fruit were collected and inspected in the laboratory for eggs, penetration marks and larval infestation.

### Trial 2

A total of 70 small lemons were harvested from the psylla-house at the Citrus Foundation Block, Uitenhage. Fruit were separated into three categories: marble sized, fruit averaging a circumference of 115 mm and fruit averaging a circumference of 140 mm. Three neonate FCM larvae were placed onto each fruit. Fruit were kept at 24°C. After 17 days fruit were inspected for penetration and infestation.

## Results and discussion

### Trial 1

Only 9 out of a total of 347 bagged lemons were infested with FCM – 2.61% of all fruit. In comparison, 53.97% of the navel oranges were infested (Table 3.2.8.1). It was hoped that infestation of lemons would be exclusive to very ripe fruit (colour plates 1 and 2). Unfortunately this was not so, with even green fruit being infested (colour plates 6, 7 and 8).

**Table 3.2.8.1.** FCM egg laying, penetration and infestation of lemons on Luthando Farm and oranges on the Citrus Foundation Block, three weeks after being netted with four pairs of FCM adults per net.

Cultivar	Colour plate per colour plate	Total fruit	Mean eggs/fruit	Fruit with penetration marks		Fruit infested with larvae	
				No.	%	No.	%
Lemons	1	1	0.0	0	0	0	0
	2	4	2.5	4	100	1	25.0
	3	11	4.8	9	81.8	0	0
	4	26	3.9	24	92.3	0	0
	5	63	6.0	55	87.3	0	0
	6	104	5.4	87	83.6	4	3.85
	7	87	10.2	68	78.2	1	1.15
	8	49	7.5	37	75.5	3	6.12
Palmer navel oranges	2	126	3.3	76	60.3	68	53.97

### Trial 2

FCM pheromone traps placed in lemon orchards in the Sundays River Valley over the last three seasons have indicated high levels of moth presence in these orchards (Moore *et al.*, 2005). If lemons have been shown to be an unsuitable host for FCM, it is necessary to discover the reason for the high trap catches. It was hypothesised that very small fruit, which did not yet have unfavourably high acid juice levels, might be facilitating FCM. Of the two categories of larger fruit, only 4.3% and 14.3% of fruit were infested (Table 3.2.8.2). Even though this was higher than infestation levels of mature fruit in some previous trials (Moore *et al.*, 2005; Moore & Kirkman, 2006), it was still relatively low. However, 57.9% of marble-sized fruit were infested, indicating that this might be the reservoir for FCM in lemon orchards.

**Table 3.2.8.2.** FCM infestation of small lemons in a laboratory trial.

Fruit size category	Total fruit exposed	Fruit with penetration marks (%)	Fruit infested (%)
Marble size	19	73.7a	57.9a
115 mm	23	39.1b	4.3c
140 mm	28	39.3b	14.3b

Values in the same column followed by the same letter are not significantly different (Bonferroni LSD Multiple Range Test; P<0.05).

## Conclusion

Entry of lemons into markets which require cold-sterilisation of fruit cannot be achieved through current protocols. Lemons cannot be cold sterilised in transit and it is unlikely that many lemon orchards could be declared FCM free on the basis of pheromone baited trap catches. In a field trial in which fruit were bagged with pairs of moths, nine infested lemon fruit (2.6% of all fruit) were recorded, compared to 54% of oranges (used as a susceptible control).

Although, it is clear that FCM can infest a small percentage of lemons in a high pressure artificial test, FCM infestation of lemons under commercial practices is an unrecorded phenomenon. It is still possible that further trials, closer to a natural-type situation, could be conducted to test the host status of lemons for FCM. However, given the results of these exploratory trials, it seems likely that the outcome of further such trials may demonstrate non-preferred or marginal host status of lemons for FCM, but conclusive demonstration of non-host status (even related to green fruit) seems unlikely.

A high level of infestation of very small immature lemons was recorded in a laboratory experiment. This might explain the high FCM adult trap catches experienced in many lemon orchards. However, this is irrelevant for market access purposes.

## Future research

No further research is planned on this experiment.

## Technology Transfer

A report was compiled on all work conducted since the inception of this experiment, for the purpose of submission to relevant phytosanitary markets. No presentations have been made on this work at grower meetings, since a presentation was made at the Citrus Research Symposium in 2006.

## Acknowledgements

The shareholders and manager of Luthando Farm in Sundays River Valley are thanked for the use of their farm.

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- 3.2.9 **FINAL REPORT: Spatial and Temporal Distribution of False Codling Moth across landscapes in the Citrusdal area (Western Cape Province, South Africa)**  
Experiment 859 (April 2006 – October 2007). R. Stotter (SU)

## Summary

The False Codling Moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is an indigenous pest of citrus fruit in southern Africa, and is a pest of high phytosanitary concern, impacting negatively on the export of fresh citrus fruit from South Africa to some international markets. FCM is a particularly serious pest in the Citrusdal area in the Western Cape Province of South Africa. FCM is known to infest most types of citrus, with navel oranges being particularly prone to attack, whereas lemons are not considered to be a suitable host. Conventional control strategies that rely on the use of insecticides are of limited use due to high levels of insecticide resistance in FCM populations. Mating disruption, the Sterile Insect Technique (SIT) and the integration of different control techniques are options that are currently being adopted.

Little is known about FCM host preferences in this important geographical area, or about its dispersal capacity. The ability of FCM to migrate between various host patches, including citrus orchards and

indigenous fynbos vegetation, and its ability to maintain a viable population in alternative host plants while there is no fruit available for infestation in citrus orchards has not been well studied. Knowledge of these largely behavioral facets is important in planning an effective control strategy for FCM.

Towards addressing this dearth of knowledge, FCM pheromone traps were set out in transects in the Citrusdal area. These transects included citrus orchards, and extended beyond citrus orchards, to include a range of habitat types and elevational gradients. This provided a mechanism to monitor the spatial and temporal distribution of male FCM in the area. In addition, intensive sampling and inspection of plant material was undertaken in the area in an attempt to identify any alternative host plants.

Results showed that male FCM were mostly confined to citrus orchards, while those occurring outside orchards were close to those orchards, or close to identified alternative host plants. However, some male FCM were caught up to 1,5 kilometers from the nearest orchards, but only in small numbers. Guava fruit (*Psidium guajava*) and acorns (*Quercus robur*) were the only alternative fruits found to be naturally infested by FCM in this geographical area. It would therefore seem that indigenous fynbos plant species in the area are not significant hosts for FCM, and that male FCM are concentrated within or very near to citrus orchards. This suggests that mass migrations of FCM between citrus orchards and surrounding vegetation are not a general occurrence. This means that control practices need only be applied to citrus orchards, or, in exceptional cases, to areas with a high density of alternative host plants, such as oak trees or guavas. These findings will have important applications in the future development of control practices, especially the use of the Sterile Insect Release programme that is currently being implemented in the area.

### Opsomming

Die Valskodingmot (VKM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is 'n inheemse plaag in suidelike Afrika en van groot fitosanitêre belang, veral ten oopsigte van vars sitrusuitvoermarkte. VKM is veral 'n belangrike plaag in die Citrusdalomgewing, Wes-Kaap. VKM is bekend dat dit die meeste sitrustipesbesmet, waarvan nawelmoene die grootste vatbaarheid toon, terwyl suurlemoene as 'n ongeskikte gasheer beskou word. Konvensionele bestuurspraktyke wat hoofsaaklik op insektedoders steun, se effektiwiteit is beperk weens hoë immuniteit deur VKM-bevolking. Paringsontwrigting, Steriele-Insek Tegniek (SIT) en integrasie van verskillende beheerstrategieë word tans toegepas.

Weinig is oor VKM se gasheervoorkeure of verspreidingspatrone in hierdie belangrike geografiese area bekend. VKM se vermoë om tussen verskillende gasheerbondels soos sitrusboorde en inheemse fynbos te migreer, asook hul vermoë om volhoubare bevolkings in habitatte sonder vrugte te onderhou, was nog nie doeltreffend bestudeer nie. Kennis van hierdie gedragpatrone is belangrik vir die beplanning van effektiewe bestuurstrategieë van VKM.

Ten einde die tekort van kennis aan te spreek is VKM-feromoonlokvalle in "deursnitte" in die Citrusdalgebied uitgeplaas. Hierdie deursnitte het sitrusboorde, 'n verskeidenheid natuurlike habitatte en topografieë ingesluit ten einde ruimtelike en tydgebonde verspreiding van manlike VKM in die area te bestudeer. Intensiewe monitoring en inspeksie van plantmateriaal is in die area onderneem om potensiële alternatiewe gasheer te identifiseer.

Resultate het gewys dat manlike VKM hoofsaaklik tot sitrusboorde beperk was, terwyl dié wat wel buite boorde voorgekom het, in die onmiddellike omgewing daarvan of by geïdentifiseerde gasheerplante teenwoordig was. Manlike motte, alhoewel in klein getalle, is egter tot 1,5 kilometer van die naaste boord gevang. Koejawelvrugte (*Psidium guajava*) en akkers (*Quercus robur*) is as die enigste alternatiewe gasheer vrugte wat natuurlik deur VKM in hierdie area besmet word, geïdentifiseer. Dit blyk dus dat die inheemse fynbos in hierdie omgewing geen noemenswaardige gasheer vir VKM is nie en dat manlike VKM slegs in, of in die direkte nabyheid van, sitrusboorde gekonsentreer is. Dit impliseer dus verder dat massamigrasie van VKM tussen sitrusboorde en die omliggende omgewing nie 'n algemene verskynsel is nie. Beheermaatreëls moet dus slegs binne sitrusboorde of in uiterste gevalle, in areas waar hoë digtheid van natuurlike gasheer soos koejawels en akkerbome voorkom, toegepas te word. Hierdie bevindings sal belangrike implikasies vir die toekomstige ontwikkeling van VKM-bestuurmaatreëls hê, veral vir Steriele-Insek loslatings soos dit tans in die area toegepas word.

### General Introduction

Primary reliance on the use of insecticides to effect long term management of insect pest populations on agricultural crops has generally been a failure, because the target pests often become resistant to the insecticides (Hofmeyr and Pringle, 1998; Dent, 1995; Norris et al., 2003), as well as regulations on chemical residues on crop products becoming stricter. Insect pests are often difficult to target within fruits with

insecticides or biological control. As a result, a multidisciplinary approach with integrated methodologies is needed when planning control strategies for such pests.

This multidisciplinary approach must be based on a sound understanding of the pests' biology and habits, including knowledge of their host plant preferences, population dynamics of both the pests and their natural enemies, and the pests' migration and dispersal capabilities. Within an agroecosystem, comprising various crops interspersed among patches of indigenous vegetation, it is particularly important to understand the movement of insect pest species between patches of varying land usage and their host preferences and the potential for crop infestation to be associated with the proximity of alternative hosts. It is particularly important to understand patterns of pest population fluctuation throughout the year, both within cropping systems and outside them. The estimate of pest abundance or change in numbers provides the essential measure by which control decisions for that pest are often made (Dent 2000).

Whilst population dynamics of FCM and of its egg parasitoids have been studied within citrus orchards (Fuller, 1901; Gunn, 1921; Schwartz, 1981; Newton, 1988a, 1988b, 1998), very little attention has been given to population studies outside of citrus orchards. FCM is known to feed on a variety of alternative host plants besides citrus, allowing it to be active and pose a threat throughout the year. This problem may be compounded by the cultivation of numerous cultivars of citrus within an area, which bear fruit at varying times of the year, extending the duration of host resource availability in orchards, especially if there is shuttling between orchards of different cultivars.

A study of FCM population levels within an agroecosystem and its immediate surroundings would therefore provide an invaluable contribution to the understanding of FCM behaviour within such a system, and be of considerable benefit to the planning of future strategies for the effective management of the pests' population levels.

The aims of this study were to:

1. Evaluate the spatial and temporal distribution of FCM within and across an agricultural landscape mosaic, to answer questions regarding: 1) the population fluctuations of the moth over time within an area, 2) population levels of FCM outside citrus orchards, and, 3) the capabilities of FCM to migrate into or out of orchards at certain times of the year.
2. Identify possible alternative host plants within the area of study to provide insight into: 1) the likelihood of FCM infesting citrus orchards from sources outside orchards, and 2) their ability to maintain populations outside orchards on certain alternative hosts, that may lead to infestation of citrus orchards. Particular attention was paid to plants within the fynbos biome, as well as known alternative hosts.
3. Identify any parasitoids of FCM found within collected host plant material to provide insight into the effects of natural biological control, including that which occurs outside orchards.

### **3.2.9.1 Evaluating the spatial and temporal distribution of FCM within and across an agricultural landscape mosaic**

#### **Summary**

Over a trapping period of 18 months, it was noticed that male FCM catches were highest within citrus orchards, or just outside orchards, decreasing with increasing distance from citrus orchards. However, hotspots do occur outside citrus orchards, in areas with populations of alternative host plants such as Oak trees, which support large populations of FCM.

There was no observed mass migration of FCM between citrus orchards and surrounding vegetation at any time in the year.

#### **Introduction**

This experiment was conducted in the Citrusdal area over 18 months, from March 2006 to October 2007, to encompass two full harvests of the various citrus cultivars in this geographical area, as well as the period in-between successive harvests, to gain an understanding of the distribution of FCM throughout the year in an agricultural landscape, and to gain insight into any possible movements of FCM between the various components of an agricultural landscape, including citrus orchards, alternative host plants and surrounding indigenous vegetation (Fig. 3.2.9.1).

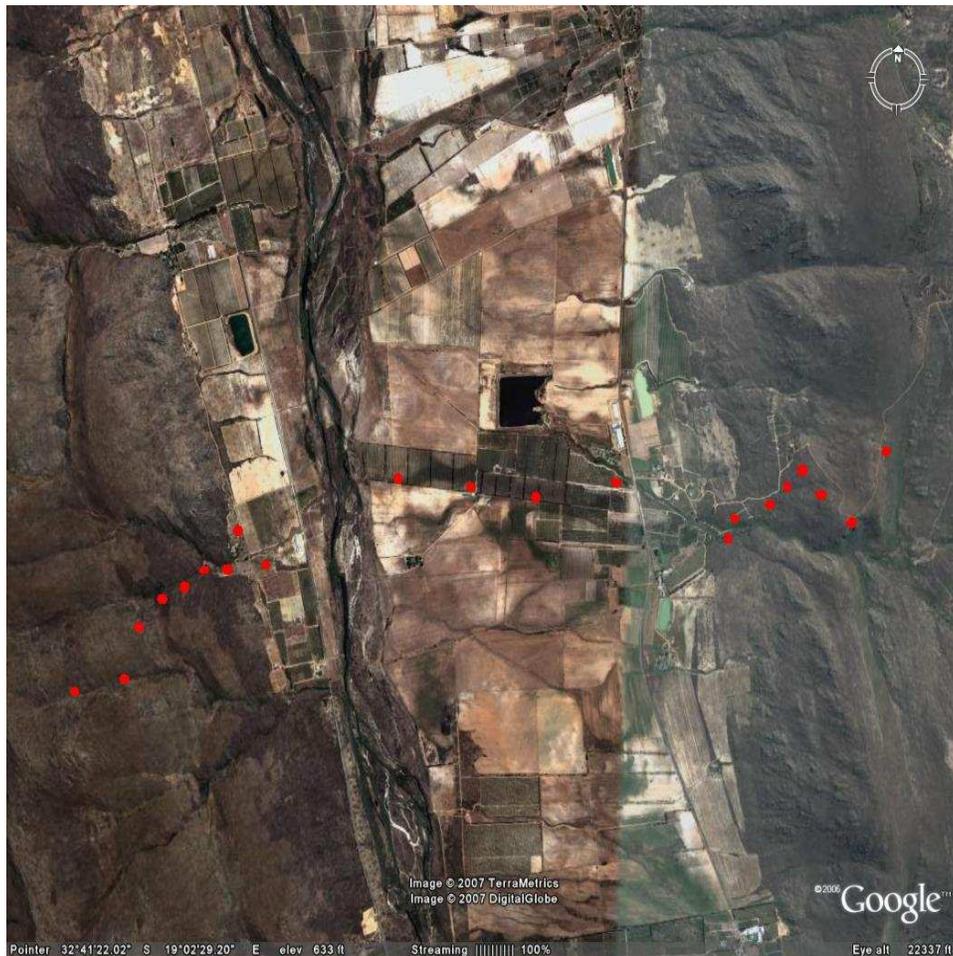


**Fig. 3.2.9.1.** An agricultural land mosaic in the Citrusdal area.

### **Materials and methods**

Pheromone traps were laid out in transects across the Olifants River valley in the Citrusdal area, to ascertain male FCM population levels over both space and time. The observation site was a river valley bordered on either side by mountains. The valley floor was mostly citrus, with some wheat, rooibos tea and patches of grapevines and apricots. Mountain slopes were vegetated with mostly indigenous fynbos vegetation. This study site was chosen for its enclosed situation, with citrus in the middle and natural vegetation on either side. FCM did not occur in this area prior to 1974 (Honiball, S. J. 2004), but is now a serious pest of citrus in this enclosed area.

Six transects were laid out, with the aid of 1:50 000 topographical maps of the area, a valley which runs roughly in a north-south direction. Each transect comprised 21 traps: seven traps in citrus orchards, spanning the valley floor from west to east, seven traps outside orchards on the western side of the valley, and seven traps outside orchards on the eastern side (Fig. 3.2.9.2). A total of 16 farms were used for trapping.



**Fig. 3.2.9.2.** Example of a transect (Transect 3) showing trap placements in citrus orchards and up mountains in indigenous vegetation.

Each Transect was spaced between five and ten km apart, spanning a total distance of approximately 55 km along the valley. Each transect was approximately 6 km long. This gives a fair representation of FCM numbers in as much of the valley as possible, particularly as climate conditions differ slightly from the more elevated transects in the south, to the lower transects in the north.

Traps were placed in citrus orchards where no other FCM pheromone traps were present, or, where no such orchards were available, traps were placed within an orchard at a distance no less than 200 m from the nearest farmers' pheromone trap, to avoid trap interaction.

Orchards were mainly Valencia and navel orange varieties, as well as a few mixed orchards, including the mandarin varieties clementines and satsumas. This range of varieties was employed to determine FCM presence in the various cultivars at different times of year. In this area, navels mature between April and July, and Valencias between June and the end of September. Both early and late cultivars of citrus were used.

Traps outside orchards were spaced out according to distance elevation units (Distance from orchard x Elevation above orchard). This was due to the fact that the slope on either side of the valley is not even, so spacing of traps at varying distances from citrus orchards alone would not provide a fair indication of FCM presence at different elevations.

Traps closest to citrus orchards were placed no further than 200 m from the orchard and no higher than 20 m above the orchard. Traps farthest away from orchards were placed at a distance greater than 1500 m from the orchard, and at an elevation of about 200 m above the nearest orchard, with other traps placed at intervals between these two values.

Chempac yellow delta traps were used, as they are light for carrying up the mountains, and are highly visible from a distance (Fig. 3.2.9.3). Within the delta traps, the Lorelei® pheromone dispenser was used. I chose this as opposed to other pheromone dispensers as there is a threshold value for FCM catches using these

pheromones, of ten male FCM individuals/trap/week, on which the farmers base their decisions to apply control practices, and the pheromone within the Lorelei dispenser tends to last longer than other dispensers (up to seven months) and is emitted at a relatively constant rate over this time. It should be noted that this threshold value is for the Lorelei pheromone dispenser used in a PVC pipe trap, and values might differ when used in other trap types such as the delta trap (CRI Integrated Production Guidelines for Export Citrus), but for the purpose of this study, threshold values were not important. Pheromone dispensers were replaced when the liquid pheromone began to run out. The advantage of these dispensers is that one can visibly see how much pheromone is left within each dispenser. Within the delta trap, I used Chempac sticky pads with which to catch the male FCM. These were replaced as needed, particularly when many insects and debris occurred on the sticky pads, which would produce decomposition odours that might have inhibited moth catches. Traps were hung according to the CRI guidelines set out for farmers (Hofmeyr in CRI Integrated Production Guidelines for Export Citrus). In orchards, traps were hung as high as possible in trees, at approximately 2 m from the ground, in the outer canopy, so as to be visible from both sides, and have a free flow of air through the trap. To assist this, traps were hung on the windward, southern side of the trees, and, orientated in a north-south direction. The traps were also on the windward side of the orchard, as male moths fly upwind to find a mate (Carde and Minks, 1995). Traps were generally each hung in the fifth tree in the fifth row from the perimeter of the orchard and at least 20 m from windbreaks. The traps were hung with the provided wire, wrapped around small branches to prevent the trap from being blown away. Branches were constantly pruned around traps to prevent new growth from obscuring the traps, which would otherwise inhibit moth catches.

Outside orchards in the natural vegetation, traps were hung according to the same principles, although hanging traps at a 2 m height was not always possible, as few plants had grown to this height. Generally, traps were hung in either wild olive (*Olea europaea* subsp. *africanum*) trees, or in protea species, or any other sizeable plants, including oak trees in some instances.

Traps were monitored every two weeks for a total of 18 months, from the beginning of April 2006 until the end of September 2007, to cover two full harvests and one period in-between harvests, to assess male FCM individuals both over space and time.

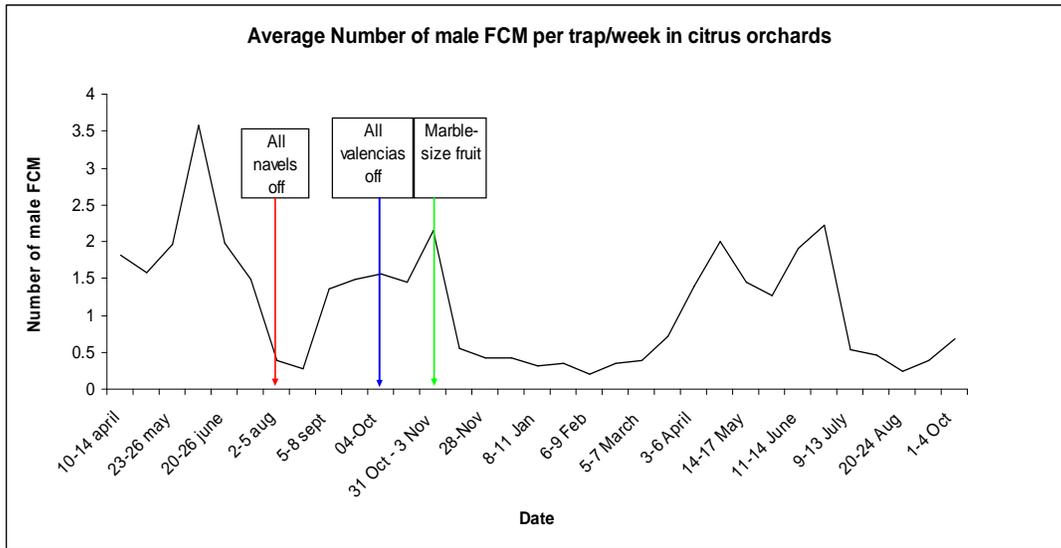


Fig. 3.2.9.3. Delta trap with Lorelei pheromone dispenser in a guava tree up a mountain.

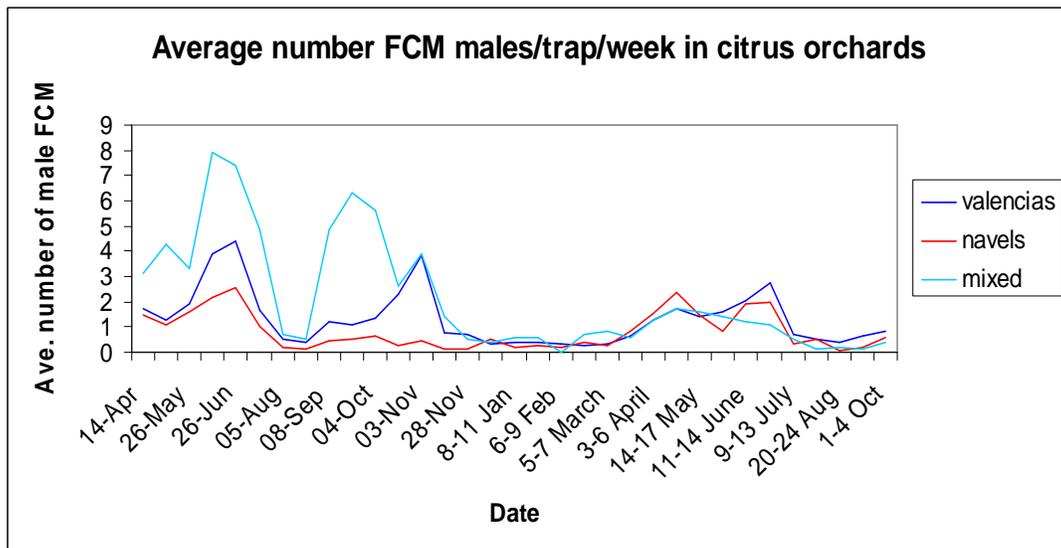
### Results and discussion

Results of pheromone trapping clearly indicated that FCM individuals were concentrated within citrus orchards, or very close to them. Citrus orchards varied considerably in the number of male FCM caught, largely due to differences in citrus cultivars. In addition, the application of various control techniques within orchards, including mating disruption, seriously reduced trap catches. Trap catches in citrus orchards were highest in the first week of June in both seasons (Fig. 3.2.9.4), with very little being caught in mid-winter (July-August) and from late November until early March, when fruit starts to ripen.

It is important to note that trap catches within orchards were often higher within Valencia orange cultivars than in Navel orange cultivars (Fig. 3.2.9.5), even though Valencia cultivars are deemed to be poor hosts compared with navel cultivars. This can largely be attributed to more intensive control practices being applied against FCM within Navel orchards, but should not be discounted. Valencia orchards are often overlooked in terms of monitoring for FCM and application of control mechanisms.

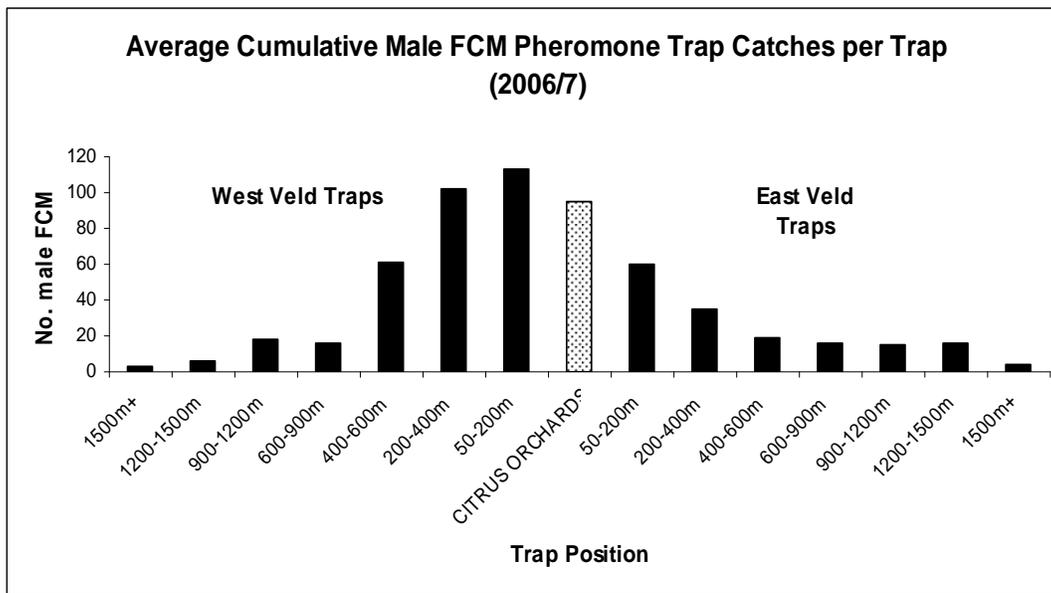


**Fig. 3.2.9.4.** Average weekly catches of male FCM in pheromone traps in citrus orchards.



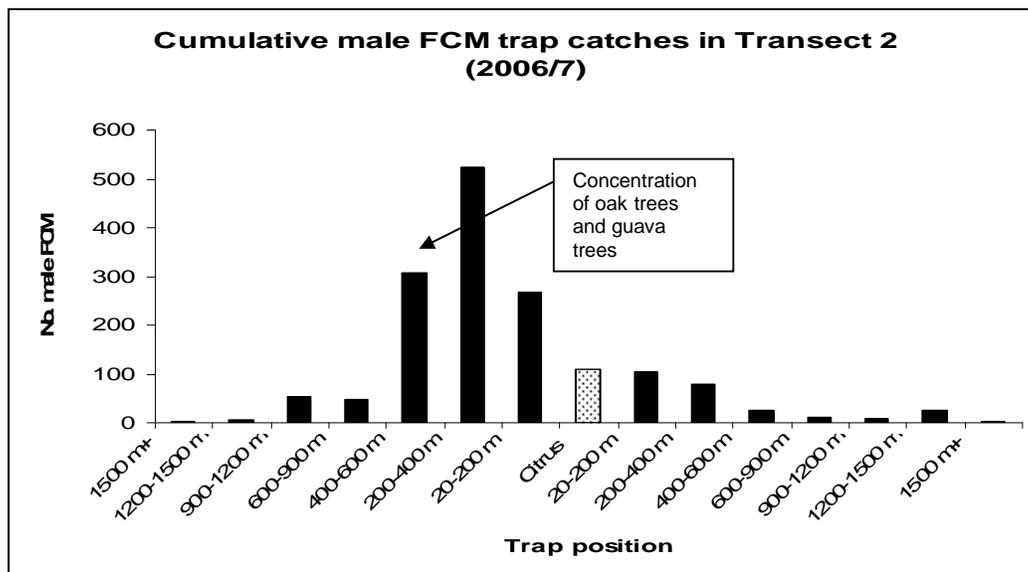
**Fig. 3.2.9.5.** Average weekly catches of male FCM in navel cultivars versus Valencia cultivars and mixed orchards.

Traps outside orchards resulted in more male FCM being caught in traps closest to citrus orchards, with decreasing numbers caught with increasing distance and elevation from citrus orchards (Fig. 3.2.9.6). Generally, fewer male FCM were caught outside citrus orchards than within citrus orchards.



**Fig. 3.2.9.6.** Average cumulative trap catches of male FCM in all transects, showing the average catch per trap within citrus orchards compared with the average catch in traps at various distances from the nearest citrus orchard.

At first glance, it would appear from the figure above that more FCM occur on the Western side of the valley than on the Eastern side. However, this is skewed largely due to one particular transect (Fig. 3.2.9.7) that had a high concentration of oak trees (*Quercus robur*) and guava trees (*Psidium guajava*) outside the citrus orchards on the Western side of the valley.



**Fig. 3.2.9.7.** Cumulative trap catches in transect 2, showing high trap catches outside citrus orchards on the western side of the transect due to concentration of oak trees and guava trees.

The vital importance of this experiment is that it indicates that FCM in this area predominantly stems from citrus orchards. However, areas outside orchards with concentrations of alternative host plants do support FCM populations, which can then migrate into citrus orchards. Timm (2005) found no evidence to suggest that populations of FCM sampled from different host plants within a specific geographical area were genetically differentiated. This suggests that FCM could move from host plant to host plant. FCM are highly polyphagous, and as such can survive on a variety of host plants. Newton (1998) describes FCM as a poorly dispersing species. However, in this monitoring and sampling exercise, FCM males were caught up to 1.5 km from the nearest known host plant, albeit in very small numbers.

### 3.2.9.2 The search for alternative host plants for FCM in the Citrusdal area

#### Summary

Over a sampling period of 18 months, FCM was only found to infest Oak trees (*Quercus robur*) and guavas (*Psidium guajava*) aside from citrus varieties, in this geographical area. It was noticed that Oak trees close to citrus orchards were much more highly infested by FCM than oak trees far from citrus orchards.

#### Introduction

FCM is a highly polyphagous insect with over 50 species of plant being known host plants (Stibick et al. 2007). These include many well-known cultivated crops such as citrus, maize, apples, pears, grapes, peaches, plums, guavas, macadamia nuts, cotton and avocados to name just a few.

Little is known about the status of fynbos species and other indigenous plant species as host plants for FCM in the Citrusdal area. Honiball, (2004) successfully reared FCM in a laboratory on the indigenous wild plum (*Harpephyllum caffrum*) and wild almond (*Brabejum stellatifolium*) and recorded it naturally infesting exotic Port Jackson willow (*Acacia longifolia*) galls in the Citrusdal area.

This project aimed to identify possible host plants for FCM in this geographical area, and coincided with the above-mentioned experiment (3.2.9.1). The sampling period therefore covered 18 months from March 2006 until October 2007.

#### Materials and methods

While pheromone traps were being monitored in the above-mentioned experiment (3.2.9.1), a variety of fruits and plants were sampled over an 18 month period and checked for infestation by FCM. Samples were bagged and placed in emergence boxes for up to 4 months to await possible emergence of FCM. Plant species sampled included known cultivated host plants, fynbos species and windbreak plants cultivated around orchards.

<b>Plant species sampled</b>	
Apricot ( <i>Prunophora armeniaca</i> )	Prickly pear ( <i>Opuntia sp.</i> )
Chinese Poplar ( <i>Populus simonii</i> )	<i>Protasparagus</i> spp.
Common Oak ( <i>Quercus robur</i> )	Real Yellow wood ( <i>Podocarpus latifolius</i> )
<i>Diaspyros glabrata</i>	<i>Solanum</i> spp.
Eucalyptus ( <i>Eucalyptus</i> spp)	Sugar bush ( <i>Protea repens</i> )
Guava ( <i>Psidium guajava</i> )	Syringa ( <i>Syringae</i> spp.)
<i>Mytenus oliodes</i>	Wild Almond ( <i>Brabejum Stellatifolium</i> )
Pecan nut ( <i>Carya illinoensis</i> )	Wild melon
<i>Phyllica</i> spp.	Wild olive ( <i>Olea europea</i> subsp. <i>africana</i> )
Pomegranate ( <i>Punica granatum</i> )	Wild Peach ( <i>Kigellaria Africana</i> )
Port Jackson willow ( <i>Acacia saligna</i> )	Wild Plum ( <i>Harpephyllum caffrum</i> )

#### Results and discussion

After 18 months of sampling, FCM larvae were only found in acorns, *Quercus robur* (Fig. 3.2.9.8), and guavas, *Psidium guajava*, in the Citrusdal area. I do not discard the possibility of other known host plants such as yellow wood, apricots, pomegranates and wild olives, being able to sustain FCM, but none were found in any other known host species that I sampled. I have found FCM in both wild olives, *Olea europea* subsp. *africana*, and commercially cultivated olives, *Oleae europea*, in the Stellenbosch and Paarl areas from 2005-2007, but not in the Citrusdal area, even though hundreds of thousands of olives were collected.



**Fig. 3.2.9.8.** Acorns infested by FCM close to citrus orchards.

Guavas in the area are either grown in home gardens, or, are found growing wildly. The wildly growing guavas were particularly highly infested with FCM, whilst those growing in home gardens were not particularly infested. The wildly growing guavas could only be harvested for a very limited period of the year in February and March, as baboons eat them before they are mature.

FCM is also known to occur in Port Jackson willow (*Acacia saligna*) galls. These trees are common throughout the Western Cape and they are widespread in the Citrusdal area. I sampled galls from numerous stands on various farms, but found no live larvae in any galls. However, many galls contained signs of feeding by larvae, and I have found numerous pupal casings of emerged moths, which look very similar to those of FCM. However, I cannot conclude that these were definitely FCM and that Port Jackson's willow is an alternative host plant for FCM in this area, as the litchi moth, *Cryptophlebia peltastica* is also known to infest these galls and looks relatively similar to FCM.

No indigenous plant species were found to contain FCM larvae or eggs. Wild almond (*Brabejum stellatifolium*) and wild plum (*Harpephyllum caffrum*) were sampled intensively with no detection of FCM infestation. Whilst many species were sampled, and numerous samples of each taken, I cannot discard the possibility that some of these species might be host plants for FCM, but can confidently conclude that they are not highly sought after by FCM, which obviously prefers citrus, guavas and acorns.

Infested acorns may play a significant role in reinfestation of nearby citrus orchards from year to year, as the lifecycle duration of FCM is dramatically slowed within acorns, probably due to limited nutrition. I found the lifecycle could take well over 5 months when developing in acorns. Kelly (1914) found the duration of the lifecycle within acorns in the Pietermaritzburg area to average 121 days. This is opposed to the lifecycle in citrus which takes between 37-60 days to be completed in summer and 68-100 days in winter (Stofberg, 1954). Acorns can remain viable for infestation on the ground for many months, meaning FCM could develop under these trees on acorns at a slow rate over winter (June – September), and emerge as adults in time to infest immature citrus fruit in nearby orchards by the beginning of November.

### 3.2.9.3 Identification of any parasitoid species found in the Citrusdal area

#### Summary

Sadly, no larval parasitoids were found in any infested material found during sampling for the preceding experiment (3.2.9.2). Egg sheets containing codling moth eggs were not parasitized by egg parasitoids. However, at the time of going to press, I have discovered egg parasitoids naturally parasitizing FCM eggs in a house garden in the area. It remains to be seen which species of egg parasitoid this is.

## Introduction

An investigation was initiated in April 2006 to investigate the extent of natural larval parasitism of FCM outside citrus orchards in the Citrusdal area.

## Materials and methods

Samples of alternative host plants were collected from the Citrusdal area over an 18 month period and monitored for infestation by FCM as well as the possibility of any larval parasitoids being found, which would give indications of the level of natural control of FCM outside citrus orchards in the Citrusdal area. In addition, 12 sheets of wax paper covered with Codling moth (*Cydia pomonella*) eggs were hung up within trees in an organic navel orchard in March 2007. These were replaced weekly for 6 weeks to see whether egg parasitism by *Trichogrammatoidea cryptophlebiae* occurred

## Results and discussion

No larval parasitoids were found from infested alternative host plant material, including acorns from oak trees and guavas. Additionally, no codling moth eggs were parasitized in an organic navel orchard over a six week period. These results hint that very poor natural control of FCM by parasitoids occurs in the Citrusdal area.

## Conclusion

The aim of this study was primarily to gain insight into the distribution of the false codling moth, *Thaumatotibia leucotreta*, across an agricultural landscape, and in response to seasonal fluctuations, in the Citrusdal area of South Africa, where it is a major pest of citrus. In particular, the project aimed to gain insight into the size of FCM populations maintained outside of citrus orchards, and the ability of FCM to move between citrus orchards and surrounding vegetation.

A better understanding of the dispersal capabilities and seasonal movements of FCM within such an agricultural system is invaluable in planning future control strategies against FCM in this area as well as others.

Additionally, it was aimed to identify any possible unknown or alternative host plants for FCM, and identify any larval parasitoids that might be found in infested plant material. The accomplishment of these aims is outlined below.

### 1. Spatial and temporal distribution of FCM across an agricultural landscape

Pheromone trapping in transects across the agricultural landscape has shown that FCM males are concentrated within citrus orchards, or very close to them. However, some FCM individuals are caught at distances of up to 1.5 km from the nearest citrus orchard or known host plant. This raises questions about FCM dispersal ability. Perhaps certain male individuals are able to disperse over greater distances than previously thought. Most male FCM appear to prefer to remain close to citrus orchards or other host plants.

With regard to seasonal distribution, there is no evidence to suggest that mass migrations of FCM occur between citrus orchards and surrounding vegetation at any particular time during the year. Rather, it seems that when FCM populations are high within citrus orchards, populations are also higher than usual outside orchards, suggesting a relationship between the two.

It is clear that FCM populations are higher both within citrus orchards and in surrounding vegetation when there is citrus fruit available for infestation, while population sizes, or movement of males, are significantly lower during winter, and while there is no citrus fruit on trees.

Areas of high FCM presence do occur outside of citrus orchards, but only where alternative host plant patches are present.

### 2. Alternative host plants

While the area concerned was intensively sampled for possible alternative host plants for FCM, this study only proved that guavas, *Psidium guajava*, and oak trees, *Quercus robur*, are host plants of FCM in this area. It is unlikely that other plant species in this area play a significant role in supporting FCM populations, with the exception perhaps of other cultivated crop plants, such as fruit trees. It is highly evident that local

fynbos plant species and trees such as the wild olive, *Olea europea* subsp. *africanum*, are probably not hosts of FCM in this area.

The implications of this to farmers in the area, is that they should either remove oak trees and guava trees from their farms, or destroy fruit from these trees or implement a control practice within these trees, targeted at FCM, even though infestation probably stems from nearby citrus orchards. Many farmers in the area are already removing both oak trees and guava trees from their farms

### 3. Identification of larval parasitoids

No larval parasitoids of FCM were found during this study. This may either be due to larval parasitoids not playing a prominent role in suppressing FCM numbers in this area, where FCM was not recorded previous to 1974, or maybe due to not enough fruit being sampled during the survey. Either way, it seems that larval parasitoids of FCM, if present in this area probably play only a very minor role, if at all, in suppressing FCM.

In conclusion, it is evident that control practices for FCM in the Citrusdal area can be concentrated on citrus orchards themselves, and in special cases, certain identified hotspot areas outside citrus orchards.

In an area that has viable citrus fruit in orchards for 10-11 months of the year, it is always going to be more difficult to control a pest such as FCM, especially if control practices are only applied for a small portion of the year. It is apparent that Valencia orange cultivars can support significant populations of male FCM within the orchards, particularly in late June when the fruit is ripening. Many farmers apply little or no control practices against FCM in these cultivars. In addition, very little monitoring takes place in these orchards, as it is assumed that they are not significant hosts for FCM. Perhaps this is something to be re-investigated in the near future.

### Future research

Due to the seemingly limited dispersal capabilities of FCM and its apparent concentration within and close to citrus orchards, it makes FCM a very suitable candidate for control practices such as mating disruption and Sterile Insect Technique.

With the initiation of SIT against FCM in the Citrusdal area in November 2007 arises the opportunity for future studies into the effects of SIT on FCM populations in the area, and the possible application of it in other citrus producing areas. It will be important to monitor the dispersal capabilities of released sterile FCM within the agricultural landscape and to see the effect of this control practice on FCM population sizes both within citrus orchards and outside orchards. It is not yet certain whether SIT will be effective if applied as a stand-alone control practice or whether it should be used in conjunction with another control practice such as mating disruption. This is something that will be investigated in the very near future.

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### 3.2.10 PROGRESS REPORT: Geographic variation in the susceptibility of FCM populations to a granulovirus, CrleGV-SA (CRYPTOGRAN®)

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

#### Summary

Differences in the susceptibility of false codling moth (FCM) populations, in some key citrus growing areas in South Africa, to CRYPTOGRAN® (CrleGV, a virus used for the biological control of FCM) was investigated. An initial benchmark for pathogenicity was established with laboratory reared FCM. Surface dose-response bioassays were conducted with the five larval stages, using CrleGV-SA (unformulated virus product). For the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> instars, the LC<sub>50</sub> values for the assays were calculated to be 4.095 x 10<sup>3</sup>, 4.516 x 10<sup>4</sup>, 1.662 x 10<sup>5</sup>, 2.205 x 10<sup>6</sup> and 2.678 x 10<sup>7</sup> OBs/ml, respectively. The LC<sub>90</sub> for these instars was calculated to be 1.185 x 10<sup>5</sup>, 4.287 x 10<sup>6</sup>, 9.992 x 10<sup>6</sup>, 1.661 x 10<sup>8</sup> and 9.118 x 10<sup>9</sup> OBs/ml, respectively. A standard protocol for determining the LC<sub>50</sub> and LC<sub>90</sub> values for the 1<sup>st</sup> to 5<sup>th</sup> FCM instars with CrleGV-SA has been established. This protocol is being used in carrying out bioassays with field collected FCM larvae. Susceptibility to CrleGV-SA has been found to decline with larval stage and time of exposure. Conducting bioassays with field collected FCM larvae, on a non-agar based diet is more suitable than an agar-based diet.

#### Opsomming

Verskille in die gevoeligheid van valskodlingmot(VKM)-bevolkings vir CRYPTOGRAN (CrleGV, 'n virus wat vir die biologiese beheer van VKM gebruik word), word in sekere uiters belangrike sitrusproduksiestreke in Suid-Afrika ondersoek. Eerstens is 'n standaard gestel vir die patogeniese werking van die virus met laboratorium-geteelde VKM. Oppervlakte-dosis biotoetse is met die vyf larwale stadia en met ongeformuleerde CrleGV uitgevoer. Vir die 1ste, 2de, 3de, 4de en 5de stadia is die LC<sub>50</sub>-waardes bereken op onderskeidelik 4.095 x 10<sup>3</sup>, 4.516 x 10<sup>4</sup>, 1.662 x 10<sup>5</sup>, 2.205 x 10<sup>6</sup> en 2.678 x 10<sup>7</sup> OBs/ml. Die LC<sub>90</sub> vir hierdie stadia is bereken op onderskeidelik 1.185 x 10<sup>5</sup>, 4.287 x 10<sup>6</sup>, 9.992 x 10<sup>6</sup>, 1.661 x 10<sup>8</sup> en 9.118 x 10<sup>9</sup> OBs/ml. 'n Standaard protokol om die LC<sub>50</sub>- en LC<sub>90</sub>-waardes vir die 1ste tot 5de larfstadia met CrleGV te bepaal, is uitgewerk. Hierdie protokol word tans gebruik om biotoetse met boordversamelde VKM-larwes uit te voer. Dit is gevind dat gevoeligheid vir CrleGV verminder met larfstadium en met duur van blootstelling aan die virus. Dit is ook gevind dat 'n nie-agar dieet geskikter vir hierdie biotoetse is as 'n dieet wat agar bevat.

#### 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 fruits 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; Fritsch *et al.* 2006). 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. It is possible that differences in the susceptibility of FCM to CRYPTOGRAN® (CrleGV-SA) could also exist.

In view of the above, the main objective outlined in this study was to investigate whether; there were any differences in the susceptibility of FCM populations to CrleGV-SA, between geographically distinct key citrus growing areas in South Africa. Some interesting observations have been made which have been captured in this report.

#### Materials and methods

Firstly, a benchmark for pathogenicity was established. Surface dose-response bioassays were conducted with laboratory reared FCM larvae with CrleGV-SA (unformulated virus product). These were conducted to establish a dose-response relationship for each FCM larval instar with CrleGV-SA, which were used as our benchmark. The LC<sub>50</sub> and LC<sub>90</sub> values for the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar FCM larvae were determined in these

studies. However, the LC<sub>50</sub> and LC<sub>90</sub> values for the 1<sup>st</sup> and 5<sup>th</sup> larval instars were previously established by Moore (2002). Comparative bioassays were also conducted with field collected FCM larvae from infested citrus fruits, across a range of geographic regions.

In order to obtain a constant supply of larvae for the surface dose-response bioassay tests, a standard dry diet preparation consisting of maize meal, wheat germ, brewer's yeast, milk powder, sorbic acid and nipagin was used in the rearing of the FCM larvae (Moore 2002).

FCM were reared in 370 ml jam bottles. A mixture containing 50 g standard dry diet and 50 ml distilled water (dH<sub>2</sub>O) was weighed and then dispensed into each jam bottle. Afterwards, individual bottles were stoppered with cotton wool and autoclaved at 121°C for 20 minutes and then placed within a laminar flow cabinet to cool (Moore, 2002). FCM eggs were collected from wax paper sheets attached to a custom-built moth emergence box. The moth emergence box consisted of a ten compartment facility specially designed for the moths (Moore, 2002).

Once the jam bottles had adequately cooled down in the laminar flow cabinet, the cotton wool stoppers were removed. FCM eggs laid on the wax paper sheets fitted inside the wire mesh of the emergence box were collected for incubation on the same day. Several pieces of egg sheets (containing approximately 100-150 eggs) were cut into approximately 10mm X 10mm size with a scissors. Thereafter, they were sterilized in 25% formaldehyde solution using a pair of forceps, sterilized with alcohol and a flame (Moore, 2002). The individually cut egg sheets, were then transferred into each jam bottle using the forceps. Once done, all the jam bottles now containing FCM eggs, were fitted with cotton wool stoppers. The bottles were finally sent to the incubation chamber with a temperature of 27°C ± 1°C to hatch and grow into the required instars (Moore 2002).

In establishing a dose-response relationship for the laboratory reared FCM larvae, an agar-based diet (described below), was used. The 5<sup>th</sup> instars were however, assayed on a non-agar based diet (as described in the diets used in conducting bioassays with field collected larvae, outlined below) (Moore 2002).

It was however established beforehand by Moore (2002) that, it was necessary to use polypots (30 ml capacity - Evron, South Africa) with holes (± 15mm diameter) cut in the centre of each lid. Cotton wool was plugged into each hole, to ensure good ventilation in the polypots and prevent larvae from escaping (Moore, 2002). Therefore, for each trial a total of 150 individual polypots were used (Moore, 2002).

The following diet formulation was used in conducting bioassays with laboratory-reared FCM larvae. A diet preparation consisting of 150 g standard dry diet, 501 ml distilled water and 18 g agar was autoclaved at 121°C for 20 min. The resulting mixer was then poured into each polypot to a depth of 5-7 mm. Once the diets had adequately cooled and set, 275 µl of the virus suspension was pipetted onto the surface of the diet in each pot. Individual batches (25 larvae per batch) of larvae were tested at five concentrations of the virus sample (CrleGV-SA), using a five-fold serial dilution technique (Moore, 2002).

The same diet formulation, as described in the assays with laboratory-reared FCM instars, was initially used in dose-response bioassays with field collected FCM. The diet was however modified to exclude agar due to the extremely high levels of mortality observed in the control (Table 3.2.13.2). The protocol adopted was a diet consisting of 200 g standard diet plus 200ml 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 hot pie-dishes (containing diet) were allowed to cool in a laminar flow cabinet. Once cool, individual diet plugs were cut using a polypot which had the bottom removed (Moore 2002). Individually cut diet plugs, were transferred into each polypot using sterilized (using 2% sodium hypochlorite) forceps (Moore 2002).

For the field-collected FCM larvae, all infested fruits were individually cut open with the aid of sharp knives to locate the larvae. Consequently, with laboratory-reared FCM larvae, a total of 150 larvae were used per trial, (with 25 larvae per treatment, including the control). Afterwards the inoculated diet containing larvae were transferred to the incubation chamber until evaluation. The 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> FCM larvae were evaluated after 8, 8 and 10 days respectively. The larvae or pupae were recorded as dead or alive. 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 LC<sub>50</sub> (concentration required to elicit 50% mortality in the test insects) and LC<sub>90</sub> (concentration required to elicit 90% mortality in the test insects) were calculated. PROBAN transformed the doses to log<sub>10</sub> 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).

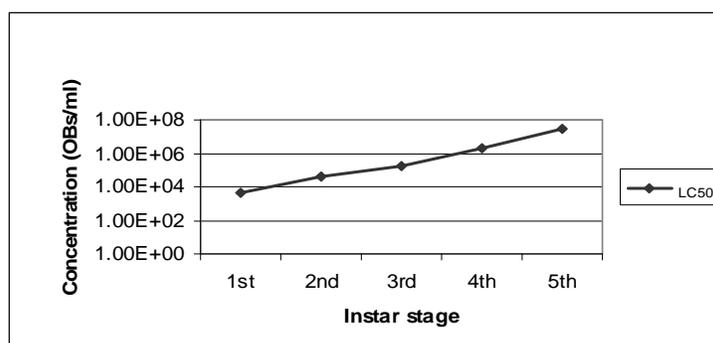
A lot of trial and error was involved in arriving at the appropriate series of concentrations to give a good dose-response curve. This was therefore quite a lengthy process.

Bioassays were replicated three times for the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars. Only good bioassays, with control mortality not exceeding 20%, were used for data analysis.

## Results and discussion

### Surface dose-response bioassays with laboratory reared FCM larvae

In surface dose-response bioassays with laboratory reared 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars with CrleGV-SA, the mean LC<sub>50</sub> values were calculated to be;  $4.516 \times 10^4$ ,  $1.662 \times 10^5$  and  $2.205 \times 10^6$  OBs/ml respectively. The LC<sub>90</sub> values were also calculated to be,  $4.287 \times 10^6$ ,  $1.113 \times 10^7$  and  $1.661 \times 10^8$  OBs/ml respectively (Table 3.2.10.1). Fig. 3.2.10.1 shows that there was a general increasing trend in LC<sub>50</sub> values with larval stage. This increasing resistance (lower susceptibility) with larval stage has been reported by several authors (Hugh & Shapiro 1997; Hunter-Fujita *et al.* 1998; Escribano *et al.* 1999; Jones 2000; Moore 2002).

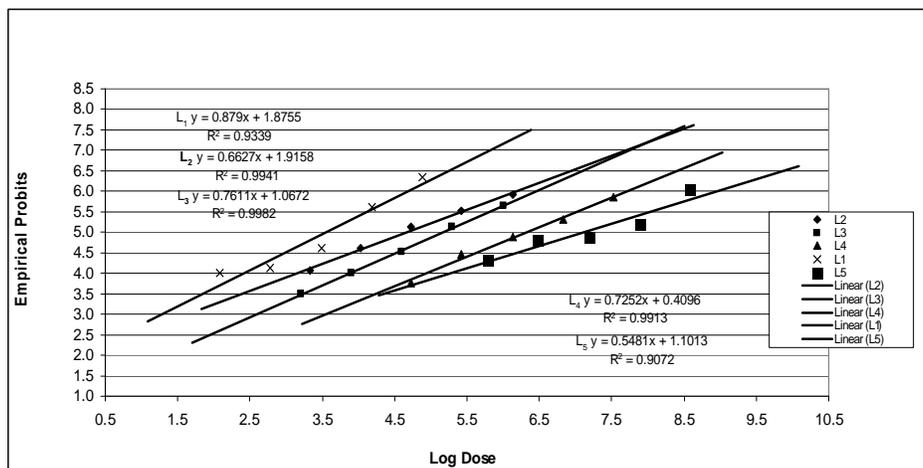


**Fig. 3.2.10.1** Graphical representation of the mean LC<sub>50</sub> values for all instars.

Similar observations were made by Escribano (*et al.* 1999), on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In droplet feeding bioassays with *S. frugiperda* against its NPV (Nicaragua isolate), Escribano *et al.* (1999), observed a significant increase in the LC<sub>50</sub> values for all instars. The LC<sub>50</sub> values increased with larval stage from  $2.03 \times 10^5$  OBs/ml for the 2<sup>nd</sup> instars to  $1.84 \times 10^8$  OBs/ml for the 5<sup>th</sup> instars.

In this study, the LC<sub>50</sub> values established for the FCM instars, increased by 11, 3.6, 14 and 12 fold in-between larval stages (1<sup>st</sup> to 5<sup>th</sup> instars) respectively. However, there was a relatively small increase in the LC<sub>50</sub> values, from the 2<sup>nd</sup> to 3<sup>rd</sup> instars, showing only 3.6 fold increase. Escribano *et al.* (1999) also observed a similar 3.9 fold increase in the LC<sub>50</sub> value from the 2<sup>nd</sup> to 3<sup>rd</sup> *S. frugiperda* instars. The LC<sub>50</sub> values increased with larval stage from  $2.04 \times 10^5$  OBs/ml for the 2<sup>nd</sup> instars to  $8.05 \times 10^5$  OBs/ml for the 3<sup>rd</sup> instars (Escribano *et al.* 1999). This observation probably indicates that reduced susceptibility to CrleGV-SA from the 2<sup>nd</sup> to the 3<sup>rd</sup> larval stage is relatively marginal.

In addition, the virus exposure time exhibited a somewhat increasing trend with larval stage (Table 3.2.10.1). The exposure time for FCM larvae on virus inoculated diet increased from 7, 8, 8, 10 and 14 days for the 1<sup>st</sup> to 5<sup>th</sup> FCM instars (Table 3.2.10.1). Again, some discrepancy was observed with the 3<sup>rd</sup> instar. Preliminary surface dose-response bioassays conducted (data not shown) with the 3<sup>rd</sup> instars at a CrleGV-SA concentration of  $1.34 \times 10^6$  OBs/ml showed an almost total response (100% mortality) in all batches, after an exposure time of 9 and 10 days. As a result, virulence of CrleGV-SA appeared to increase dramatically in the 3<sup>rd</sup> instar after 9 and 10 days due to the instars marked increased susceptibility to the virus at longer exposure times. The LC<sub>50</sub> value for the 4<sup>th</sup> instar was established after 10 days exposure. This phenomenon has been reported by Federici (1997) with type 2 GVs (such as CrleGV-SA), typically lasting between 5-10 days in larvae infected during the 4<sup>th</sup> instar stage. The LC<sub>50</sub> value for the 5<sup>th</sup> instar ( $2.678 \times 10^7$  OBs/ml), on the other hand was established after 14 days (longest exposure time).



**Fig. 3.2.10.2.** Comparison of dose-response probit lines for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> FCM instars (\* L<sub>1</sub> (first FCM instar), L<sub>2</sub> (second FCM instar), \*L<sub>3</sub> (third FCM instar), \*L<sub>4</sub> (fourth FCM instar) and L<sub>5</sub> (fifth FCM 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 1<sup>st</sup> – 5<sup>th</sup> FCM instars respectively. The  $R^2$  values for all instars (1<sup>st</sup> to 5<sup>th</sup>) were all higher than 91%.

Using Bartlett's test for homogeneity of residual variances (Van Ark, 1995), the residual variances of the three lines (Table 3.2.10.1.) for all instars concerned, were determined to be homogenous making the chi-squared test more applicable than would have been the case if deviations were heterogeneous (Van Ark, 1995). The chi-squared test showed the lines to be parallel and their slopes to be comparable (Fig. 3.2.10.1.). The elevations of all the lines did not differ significantly from one another (for all instars). The mean LC<sub>50</sub> and LC<sub>90</sub> values calculated, for all instars, were within the 95% lower and upper fiducial limits (Table 3.2.10.1.).

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 (Sun 2005; Jehle *et al.* 2006). However, unlike the early instars, the late instars (such as the 5<sup>th</sup> FCM instars) on the other hand tend to have a much thicker peritrophic membrane, which makes it difficult for the virus to penetrate and access the midgut epithelium in order to initiate infection (Sun, 2005). This phenomenon, probably explains why the 5<sup>th</sup> instar is more resistant to CrleGV-SA than the lower instars. For instance the LC<sub>50</sub> for the 5<sup>th</sup> instars was  $2.678 \times 10^7$  OBs/ml, much higher than the LC<sub>90</sub> values established for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instars. Again, the LC<sub>90</sub> values for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars, increased by 36, 2.3 and 16.7 fold respectively from one larval stage to the next. However, the LC<sub>90</sub> values from the 4<sup>th</sup> to the 5<sup>th</sup> instar increased by an appreciable 54.89 fold.

**Table 3.2.10.1.** PROBAN (Van Ark, 1995) output of probit analysis of dose-response data with CrleGV-SA, against laboratory reared 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> FCM larvae in surface dose bioassays.

Instar Stage	Five-fold serial dilutions of CrleGV-SA dosages (OBs/ml) tested per batch	Mean LC <sub>50</sub> (OBs/ml)	Mean LC <sub>90</sub> (OBs/ml)	$\chi^2$ Reps 1, 2 & 3	Regression lines For Reps 1, 2 & 3	SE slopes	G - for Fiducial limits	LC <sub>50</sub> , 95%	LC <sub>50</sub> , 95%	LC <sub>90</sub> , 95%	LC <sub>90</sub> , 95%	Probabilities For individual lines (P<0.05) (Reps; 1, 2 & 3)								
								(Fiducial limits) Lower (mean)	(Fiducial limits) Upper (mean)	(Fiducial limits) Lower (mean)	(Fiducial limits) Upper (mean)									
1 <sup>st</sup>	1.221 x 10 <sup>2</sup>	4.095 x 10 <sup>3</sup>	1.185 x 10 <sup>5</sup>	6.919	y = 1.931 + 0.841x	0.1318	0.0943	2.29 x 10 <sup>3</sup>	7.05 x 10 <sup>3</sup>	4.92 x 10 <sup>4</sup>	5.36 x 10 <sup>5</sup>	0.0730								
	6.104 x 10 <sup>2</sup>			13.462	y = 2.176 + 0.789x	0.1119	0.0773					0.001								
	3.052 x 10 <sup>3</sup>											3.574	y = 0.673 + 1.200x	0.1667	0.0741	0.311				
	1.526 x 10 <sup>4</sup>															0.194	y = 2.493 + 0.556x	0.1309	0.2131	0.973
	7.630 x 10 <sup>4</sup>																			2.528
2.14 x 10 <sup>3</sup>	1.339	y = 1.176 + 0.784x	0.1482	0.1372	0.724															
1.07 x 10 <sup>4</sup>					0.283	y = 1.523 + 0.650x	0.1351	0.1659	0.958											
5.36 x 10 <sup>4</sup>									0.599	y = 0.650 + 0.839x	0.1476	0.1189	0.895							
2.68 x 10 <sup>5</sup>	0.328	y = 0.769 + 0.839x	0.1444	0.1137									0.950							
1.34 x 10 <sup>5</sup>													0.256	y = 0.873 + 0.636x	0.1295	0.1591	0.963			
1.60 x 10 <sup>3</sup>	0.988	y = 0.124 + 0.777x	0.1364	0.1185	0.806															
8.00 x 10 <sup>3</sup>					0.443	y = 0.493 + 0.725x	0.1331	0.1295	0.928											
4.00 x 10 <sup>4</sup>									1.681	y = 1.072 + 0.537x	0.0925	0.1142					0.645			
2.00 x 10 <sup>5</sup>																	5.036	y = 1.133 + 0.498x	0.0940	0.1367
1.00 x 10 <sup>5</sup>	1.355	y = 1.889 + 0.490x	0.0925	0.1372	0.513															
5.34 x 10 <sup>4</sup>					1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>					0.645							
2.67 x 10 <sup>5</sup>	0.928	y = 1.889 + 0.490x	0.0925	0.1372					0.167											
1.34 x 10 <sup>6</sup>									1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>	0.513							
6.68 x 10 <sup>6</sup>													1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>	0.645			
3.34 x 10 <sup>7</sup>																	0.167			
4 <sup>th</sup>	6.145 x 10 <sup>5</sup>	2.678 x 10 <sup>7</sup>	9.118 x 10 <sup>9</sup>	1.681	y = 1.072 + 0.537x	0.0925	0.1142	1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>	0.645								
	3.072 x 10 <sup>6</sup>			5.036	y = 1.133 + 0.498x	0.0940	0.1367					0.167								
	1.536 x 10 <sup>7</sup>											1.355	y = 1.889 + 0.490x	0.0925	0.1372	0.513				
	7.681 x 10 <sup>7</sup>															1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>	0.645
	3.841 x 10 <sup>8</sup>																			0.167
5 <sup>th</sup>	6.145 x 10 <sup>5</sup>	2.678 x 10 <sup>7</sup>	9.118 x 10 <sup>9</sup>	1.681	y = 1.072 + 0.537x	0.0925	0.1142	1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>	0.645								
	3.072 x 10 <sup>6</sup>			5.036	y = 1.133 + 0.498x	0.0940	0.1367					0.167								
	1.536 x 10 <sup>7</sup>											1.355	y = 1.889 + 0.490x	0.0925	0.1372	0.513				
	7.681 x 10 <sup>7</sup>															1.20 x 10 <sup>7</sup>	7.56 x 10 <sup>7</sup>	1.41 x 10 <sup>9</sup>	5.02 x 10 <sup>11</sup>	0.645
	3.841 x 10 <sup>8</sup>																			0.167

\*Bioassays for 1<sup>st</sup> and 5<sup>th</sup> instars were carried out by Moore (2002).

### Surface dose-response bioassays with field collected FCM larvae (Eastern Cape)

In surface dose-response bioassays with field collected larvae, only the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> larval instars were used for preliminary bioassays. The number of 1<sup>st</sup> instar larvae, isolated from infested fruits collected from the field (during the months of December 2007 to February 2008) was inadequately small to enable the satisfactory execution of bioassays (Gendall *et al.* 2006; Sishuba, 2003; Moore & Kirkman 2004). In addition these first instar larvae were extremely delicate and exhibited very high levels of mortality (Stofberg, 1939; Catling & Ascheborn, 1978). Moreover, the methodology used in isolating these larvae (by dissecting infested fruits with the aid of sharp knives, to isolate larvae), also proved to be fatal to many of the first instars (field collected).

The 5<sup>th</sup> instar larvae were also excluded from these bioassays, as adequate feeding could not take place before the initiation of pupation.

One virus concentration was used for all bioassays with field collected larvae. As it was not possible to predict the instars obtained from a particular sample, and their numbers, a concentration which was useful for all instars had to be selected. A concentration that would not elicit a total response in all instars had to be selected. The LC<sub>90</sub> for the 4<sup>th</sup> instar larvae ( $1.661 \times 10^8$  OBs/ml) was selected.

**Table 3.2.10.2.** Control mortality results on bioassays with field collected larvae, from Lane Late navel oranges from Addo (Sundays River Valley, Eastern Cape) on an agar-based diet.

Instar	Rep 1 %Mortality	Rep 2 %Mortality	Rep 3 %Mortality	Rep 4 %Mortality	Mean (Av) %Mortality
2 <sup>nd</sup>	80.0	80.0	100.0	66.7	81.68
3 <sup>rd</sup>	55.6	80.0	100.0	93.3	82.23
4 <sup>th</sup>	50.0	100.0	100.0	86.7	84.18

**Table 3.2.10.3.** Treatment mortality results on bioassays with field collected larvae (using CrleGV-SA at  $1.661 \times 10^8$  OBs/ml) from Lane Late navel oranges from Addo on an agar-based diet.

Instar	Rep 1 (% Mortality)	Rep 2 (% Mortality)	Rep 3 (% Mortality)	Rep 4 (% Mortality)	Mean (% Mortality)
2 <sup>nd</sup>	100.00	100.00	100.00	76.91	94.23
3 <sup>rd</sup>	100.00	91	100.00	100.00	97.75
4 <sup>th</sup>	100.00	100.00	100.00	100.00	100.00

Control mortality was very high (Table 3.2.10.2). Consequently, treatment mortality was even higher (Table 3.2.10.3). This prevented any meaningful comparison with benchmark mortalities, as given in Table 3.2.10.1. The high mortality levels in the control were in part attributed to the type of diet being used. It was decided that subsequent diets would be modified to exclude agar. Indications were that control mortality would decline. Results from these bioassays will appear in a later report.

Bioassay results with field collected FCM larvae against CrleGV-SA, from some selected orchards in the country are still under way. A detailed report in relation to this phenomenon will be compiled in due course.

### **Conclusion**

A standard protocol for determining the LC<sub>50</sub> and LC<sub>90</sub> values for the 1<sup>st</sup> to 5<sup>th</sup> FCM instars with CrleGV-SA (CRYPTOGRAN®) has been established. These values were established for all five of the instars, obtained from a laboratory culture. Susceptibility to CrleGV-SA declines from early instars to late FCM larval instars, as well as time of exposure. Conducting of bioassays with field collected FCM larvae on a non agar-diet is more suitable than an agar-based diet. Bioassays with field collected FCM larvae, from a range of geographic citrus growing areas are underway.

### **Future research**

1. Bioassays will be conducted with field collected FCM larvae from the Eastern Cape, Western Cape and Mpumalanga.

2. Laboratory colonies will be established from FCM populations found in the Eastern Cape, Western Cape and Mpumalanga. These will be used to conduct dose-response bioassays against neonate larvae, using Cryptex® and Cryptogran®.

### Technology Transfer

No technology transfer conducted yet. Once meaningful results are produced, these will be conveyed.

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3.2.11 **FINAL REPORT: Investigating post-harvest control of FCM using Xterminator and Break-Thru**  
Experiment 906 (July - August 2007): Wayne Kirkman and Sean Moore (CRI)

**Summary**

Goedehoop Citrus approached CRI to test the efficacy of Xterminator and Break-Thru for the post-harvest control of FCM. Two trials were conducted. The first trial was a very basic trial designed to investigate if Break-Thru at 25 ml/100 L had any effect on neonate FCM larvae. The second was a detached fruit bioassay conducted to test the efficacy of Xterminator, with and without Break-Thru, against FCM larvae once they have penetrated the fruit. Break-Thru alone had no effect on neonate FCM larvae. Xterminator did induce some mortality of FCM larvae in the fruit. The addition of Break-Thru increased the mortality at the earlier stages of penetration and development. However, the increase in mortality was not significant. There could be some benefit in adding these products to dump tanks, possibly at higher concentrations, dependent on cost.

**Opsomming**

CRI is deur Goedehoop Sitrus genader om Xterminator en Break-Thru te evalueer vir die na-oesbeheer van VKM. Twee proewe is gedoen. Die eerste was 'n baie eenvoudige proef om te bepaal of Break-Thru alleen teen 25 ml/100 ℓ water enige effek op VKM het. Die tweede was 'n biotoets op afgeplukte vrugte om die invloed van Xterminator, met en sonder Break-thru, te toets op VKM-larwes wat reeds vrugte ingedring het. Break-Thru alleen het geen invloed op pas uitgebreide VKM larwes getoon nie. Xterminator het wel mortaliteit van VKM-larwes binne die vrugte veroorsaak. Die mortaliteit was hoër waar Break-Thru bygevoeg was, veral met jong larwes kort nadat hulle die vrugte ingedring het, hoewel die verhoging in mortaliteit nie betekenisvol was nie. Dit kan voordeel inhou om die produkte in die dompelbaddens by te voeg, moontlik teen hoër konsentrasies, afhangende van die koste daarvan.

**Introduction**

Goedehoop Citrus approached CRI to test the efficacy of Xterminator and Break-thru for the post-harvest control of FCM. They had performed some preliminary trials, which indicated that the addition of Xterminator (50 ml/100 ℓ water) and Break-Thru (25 ml/100 ℓ water) to their dump tanks resulted in high mortality of young FCM larvae which had just penetrated the fruit. The addition of Xterminator without Break-Thru resulted in much lower mortality. Many of the rejections of fruit by the USDA inspectors were due to these young FCM larvae.

**Materials and methods**

Two trials were conducted. The first trial was a very basic trial designed to investigate if Break-Thru at 25 ml/100 ℓ water had any effect on neonate FCM larvae. As a control, 30 neonate larvae were placed in a coffee filter bag, and immersed in distilled water for 1 minute. Another 30 larvae in a new filter bag were dipped into a Break Thru (25 ml/100 ℓ water) solution for 30 seconds, after which the bag was placed in distilled water (agitated gently to rinse). The bags were then torn open and placed on tissue paper to dry. After 5 minutes any larvae moving were counted as alive and removed.

Thereafter a detached fruit bioassay was conducted to compare the efficacy of Xterminator, with and without Break-Thru, against FCM larvae once they have penetrated the fruit. On 3 July 2007, 240 Lane Late navel oranges from the Citrus Foundation Block were each inoculated with 4 neonate FCM larvae. In an effort to simulate a packhouse situation, 20 fruit were dipped into buckets containing the trial solutions (Table 3.2.11.1) for 2 minutes (dump tank simulation), and then dipped in water for 30 seconds to simulate the effect of the fungicide bath. The untreated control was dipped in water. This process was conducted at various intervals after inoculation with FCM larvae (Table 3.2.11.1).

**Table 3.2.11.1.** Treatments applied to Lane Late navel oranges at various intervals in a detached fruit bioassay, to evaluate the post-harvest effect of Xterminator, with and without Break-Thru, on FCM larvae.

Treatment		Dosage (per 100 ℓ water)	Time dipped (and then rinsed in water for 30 seconds)	Time between inoculation with larvae and application of treatments (days)
1	Water		2 min	1, 3, 6 and 9
2	Xterminator	50 ml	2 min	
3	Xterminator	50 ml	2 min	

	Break-thru	25 ml		
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Approximately 10 oranges were inoculated with 8 neonate FCM larvae and put aside to evaluate the depth of penetration and the stage of development of the larvae at the different times that the treatments took place.

The fruit were kept at 24°C, and then dissected and inspected for the presence of live FCM larvae on 16 July 2007. The efficacies of the treatments were then compared using ANOVA and the LSD multiple range test.

### Results and discussion

In the first trial, when the larvae were dipped into distilled water, 27 of the 30 larvae survived. When the larvae were dipped in break-Thru at a concentration of 25 ml/100 l, 28 out of 30 survived. It therefore appeared that the Break-Thru alone had no effect on neonate FCM larvae.

In the second trial, it appeared that Xterminator did induce some mortality of FCM larvae in the fruit. (Tables 3.2.11.2; 3.2.11.4 & 3.2.11.5). The addition of Break-Thru increased the mortality at the earlier stages of penetration and development (Tables 3.2.11.2; 3.2.11.4 & 3.2.11.5). However, the increase in mortality was not significant.

**Table 3.2.11.2.** FCM larval infestation of fruit, subjected to various treatments **1 day** after inoculation with neonate FCM larvae.

Treatment	Dipping time	Mean no of larvae per fruit*	Reduction in infestation (%)	Penetration depth and larval development at time of treatment
Water	2 min 30 seconds	0.80a	-	Larvae penetrated through rind, just into the albedo, in the outer 1 mm of the fruit
Xterminator (50 ml/100 l) Water	2 min 30 seconds	0.75a	6.25	
Xterminator (50 ml/100 l) + Break-Thru (25 ml/100 l) Water	2 min 30 seconds	0.63a	21.25	

\*Different letters in the same column denote significant differences (P<0.5, LSD multiple range test)

**Table 3.2.11.3.** FCM larval infestation of fruit, subjected to various treatments **3 days** after inoculation with neonate FCM larvae.

Treatment	Dipping time	Mean no of larvae per fruit*	Reduction in infestation (%)	Penetration depth and larval development at time of treatment
Water	2 min 30 seconds	0.60a	-	Some larvae in albedo; most already penetrated the flavedo.
Xterminator (50 ml/100 l) Water	2 min 30 seconds	0.65a	-8.33	
Xterminator (50 ml/100 l) + Break-Thru (25 ml/100 l) Water	2 min 30 seconds	0.50a	16.67	

\*Different letters in the same column denote significant differences (P<0.5, LSD multiple range test)

**Table 3.2.11.4.** FCM larval infestation of fruit, subjected to various treatments **6 days** after inoculation with neonate FCM larvae.

Treatment	Dipping time	Mean no of larvae per fruit*	Reduction in infestation (%)	Penetration depth and larval development at time of treatment
Water	2 min 30 seconds	0.95a	-	All larvae penetrated the flavedo; small 2 <sup>nd</sup> instar
Xterminator (50 ml/100 l) Water	2 min 30 seconds	0.65a	31.58	

Xterminator (50 ml/100 ℓ) + Break-Thru (25 ml/100 ℓ) Water	2 min 30 seconds	0.50a	47.37	
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\*Different letters in the same column denote significant differences (P<0.5, LSD multiple range test)

**Table 3.2.11.5.** FCM larval infestation of fruit, subjected to various treatments **9 days** after inoculation with neonate FCM larvae.

Treatment	Dipping time	Mean no of larvae per fruit*	Reduction in infestation (%)	Penetration depth and larval development at time of treatment
Water	2 min 30 seconds	0.80a	-	Larvae deeper into the flesh of the fruit, mostly larger 2 <sup>nd</sup> instar.
Xterminator (50 ml/100 ℓ) Water	2 min 30 seconds	0.65a	18.75	
Xterminator (50 ml/100 ℓ) + Break-Thru (25 ml/100 ℓ) Water	2 min 30 seconds	0.75a	6.25	

\*Different letters in the same column denote significant differences (P<0.5, LSD multiple range test)

### Conclusion

Break-Thru itself has no detrimental effect on FCM. It appears that the addition of Break-Thru to Xterminator does enhance control of FCM larvae once they have entered the fruit. Reduction in infestation was, however, not significant. There could be some benefit in adding these products to dump tanks, dependent on cost. Xterminator is registered as a post-harvest treatment for the control of grain chinch bug at a 10 times higher rate of 5 ml/ℓ water, added to the dump tanks. It could have the added benefit of inducing some mortality of young FCM larvae which have just penetrated the fruit.

### Future research

The project is completed. Further trials could be conducted using this higher concentration of Xterminator, if prioritized by the growers.

### Technology transfer

These results have not been presented in any meetings, but have been informally transferred to Goedehoop Sitrus due to their initiative in this work.

### 3.2.12 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): R. Stotter (SU)

### Summary

Commercial releases of sterile FCM in the Citrusdal area have been underway for nearly 7 months. Monitoring of field ratios of sterile: wild FCM has shown that a ratio of 10: 1 is difficult to achieve. It is expected that this ratio will be more easily attainable in the following years.

Experiments carried out to investigate the optimal trapping method have shown that delta traps with the Lorelei pheromone lure catch the most male FCM. Height is also an important factor, particularly in orchards where rows are orientated against the prevailing wind direction.

Weekly flight tests with irradiated facility-reared FCM are showing that flight, dispersal and recapture of released moths is largely dependent on temperature at night. This could have an influence on the effectiveness of SIT, particularly during winter.

### Opsomming

Kommersiële loslatings van steriele VKM in die Citrusdalgebied het aan die begin van November 2007 begin. Monitoring van die verhouding van steriele:wilde VKM in die boorde het gewys dat 'n verhouding van

10:1 moeilik bereik word. Daar word verwag dat hierdie verhouding in die volgende jare makliker bereik sal word.

Proewe is uitgevoer om die beste lokvalstelsel te vind waarmee bestraalde VKM bestudeer kan word. Resultate wys dat die Delta-lokval saam met die Lorelei-feromoonvrysteller die meeste VKM-mannetjies vang. Lokvalhoogte is 'n belangrike faktor, vernameklik in boorde met rye wat teen die heersende wind georiënteer is.

Weeklikse vliegtoetse met bestraalde VKM wys dat vlieg, verspreiding en hervangs afhanklik is van nagtemperatuur. Dit kan 'n invloed op die doeltreffendheid van SIT hê, veral in die winter.

## **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, and virus 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 false codling moth 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<sup>st</sup> November 2007. The initial aim was to achieve a ratio of 10 sterile FCM males to every one feral FCM male (Hofmeyr et al. 2005). Releases take place bi-weekly over a total of just over 1 500 ha of citrus orchards. Approximately 3000-4000 sterile FCM are released per ha of citrus orchards per week.

Irradiated moths were initially dyed internally with Calco Red dye, to be able to distinguish between released and wild FCM. However, the use of this dye was halted due to fears that it was affecting moth quality and competitiveness. As a result, trapped moths are now distinguished based solely on appearance, which has proved to be reliable, although not 100% reliable. Stephens et al. (2008) showed that the use of Calco Red dye did not negatively affect the competitiveness or rearing of painted apple moth (PAM) (*Teia anartoides*), but that external fluorescent dyes affected the ability of males to detect a component of the female PAM pheromone.

This experiment 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. (2007). Whilst *T. cryptophlebiae* is no longer commercially reared in South Africa, combinations of the two practices may be investigated during this project.

Furthermore, monitoring and evaluation of sterile moth performance has been undertaken in order to gain an indication of moth performance with regard to temperature and various other limiting factors, including the use of different dyeing techniques to monitor SIT.

### **3.2.12.1 Optimizing trapping techniques to monitor ratios of sterile: feral FCM males in orchards**

#### **Summary**

Trapping of FCM males has historically relied on the use of Lorelei pipe traps and the Lorelei pheromone lure. Traps are hung on the windward side of citrus trees, approximately 2m above ground, with minimal

obstructions, to optimize flow of air through the trap, and hence dispersal of pheromone. Hofmeyr and Hofmeyr (2004) found that yellow delta traps with the Lorelei pheromone lure trapped approximately 1,4 to 1,6 times more male FCM than the pipe traps. Farmers in the Citrusdal area currently use pipe traps predominantly, with delta traps rapidly gaining favour, as they are less cumbersome to use. Experiments were set up to investigate the relationships between trap type, pheromone lure type, and height of traps with regard to optimizing trap catches of male FCM for monitoring purposes.

Results from these experiments indicate that delta traps are more effective at catching male FCM, and that the Lorelei pheromone lure is the most effective commercially available lure for trapping male FCM. Additionally, it seems that trap height may be a limiting factor with regards to FCM catches in orchards with rows orientated against the prevailing wind, where tree canopies overlap and wind cannot pass directly through the orchard, thereby limiting pheromone dispersal. In such cases, placement of traps on poles at the top of the tree canopy may be of more use as a monitoring tool for FCM.

### 3.2.12.1.1 Investigation into the relationship between trap type, trap height and male FCM catches

#### Introduction

This experiment aimed to investigate the differences between catches of male FCM from delta traps and pipe traps, as well as investigate the relationship between trap height and catches with regard to row orientation of orchards.

#### Materials and methods

This experiment has been conducted since 28 February 2008, and is ongoing. A 12 year-old, 22 hectare block of Robyn navels was selected on the farm Swartvlei. The block is naturally split into 4 equal-size sections by windbreaks. In the centre of each section, 4 traps were placed 2 trees apart from each other. A total of 16 traps were hung out. Two pipe traps were used in each of 4 repetitions, and two delta traps. One of each trap type was hung from a 3.5m steel pole, to reach the top of the tree canopy. Poles were placed between two trees (Fig 3.2.12.2). The other two traps of each repetition were hung in the conventional manner on the windward side of trees, which in this area is the Southern side. The Lorelei pheromone lure was used in all traps. Traps are rotated every two weeks. The trees in this orchard are orientated North-South. Traps are monitored every week.

#### Results and discussion

Delta traps consistently trap more male FCM than pipe traps. After 11 weeks, a total of 478 male FCM had been caught in delta traps, and 175 in pipe traps. Delta traps therefore catch approximately 2.7 times more male FCM than pipe traps in this instance (Fig 3.2.12.1), which differs from Hofmeyr and Hofmeyr's value of 1.3 to 1.4 in 2003 (See Fig 3.4.5.5. *CRI Group Annual Research Report 2004*).

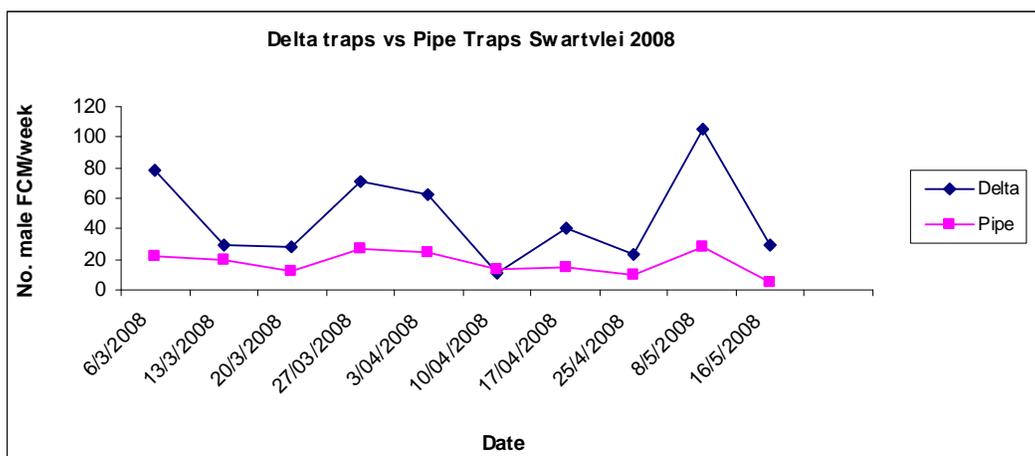
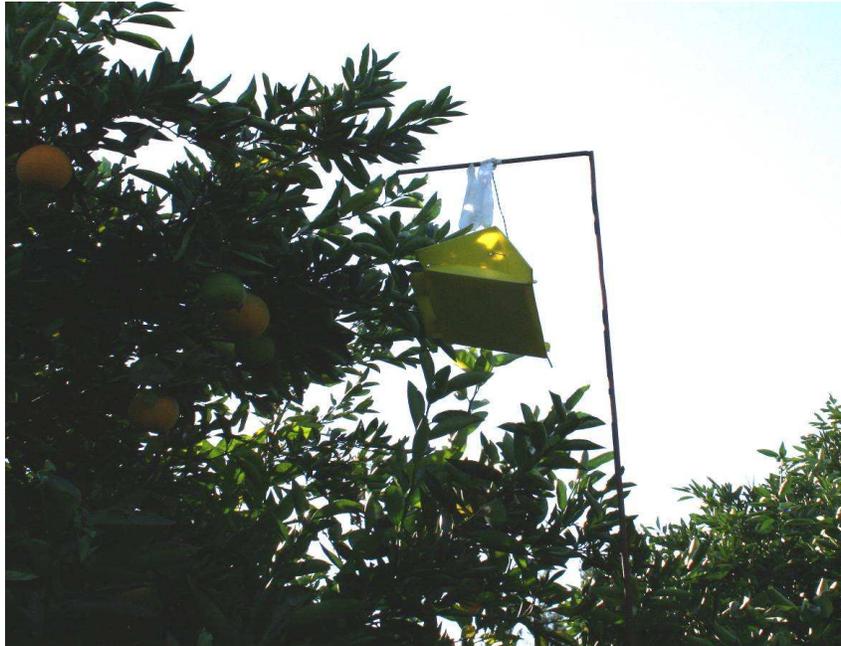
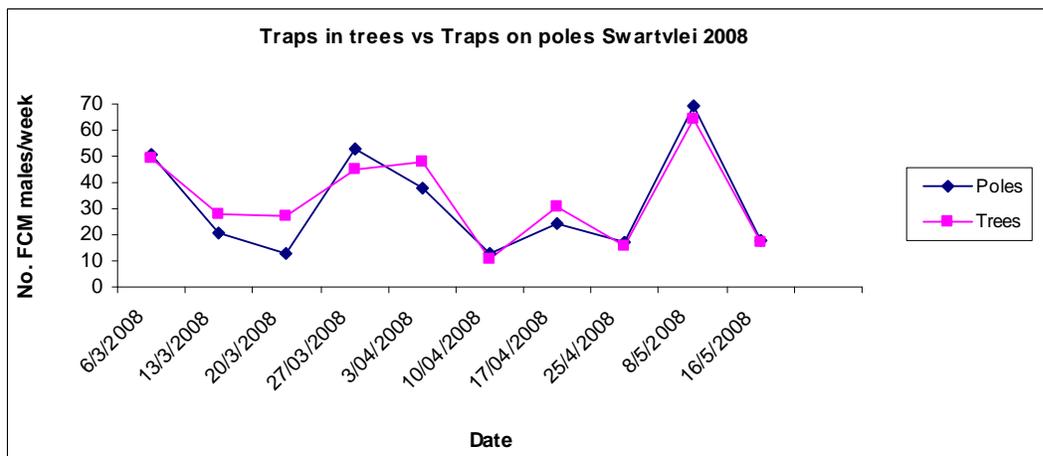


Fig. 3.2.12.1. Catches of male FCM in Delta traps versus Lorelei pipe traps on the farm Swartvlei.

There appears to be no difference with regard to trap height in this orchard, with trap catches from traps placed in the conventional manner, and traps hung from 3.5 m poles, showing no difference (Fig. 3.2.12.3).



**Fig. 3.2.12.2.** Delta trap on a pole at the top of the tree canopy to trap male FCM.



**Fig. 3.2.12.3.** FCM male trap catches with regard to trap placement in an orchard with rows orientated North-South.

The results from the above figure differ considerably from a similar experiment in which trap height was compared with regard to trap catches in an orchard with East-West row orientation (See Fig 3.2.12.4), where air cannot flow directly through the orchard. This experiment would suggest that delta traps should be used to monitor FCM males in citrus orchards, particularly where SIT is being implemented, to effectively monitor ratios of sterile: feral FCM.

### 3.2.12.1.2 Investigation into the relationship between pheromone lure type, trap height and male FCM catches

#### Introduction

Traditionally, the Lorelei pheromone lure has been used for monitoring FCM in citrus orchards. However, three different pheromone lures for FCM are now commercially available, and it was deemed necessary to investigate the differences between these lures with regard to optimizing the monitoring system for FCM.

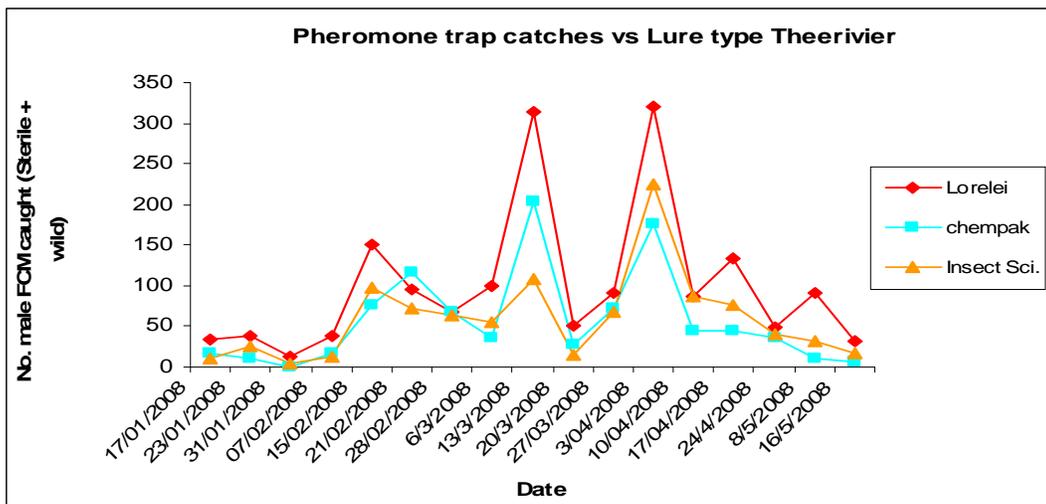
Additionally, this experiment was also used to further investigate the effects of trap placement with regards to height.

**Materials and methods**

This experiment was set out on the 10<sup>th</sup> of January 2008, and is ongoing. A 55 hectare block of 14 year-old Washington navels was selected on the farm Theerivier. 18 delta traps were set out approximately 200m apart. Nine of these were placed on steel poles 3.5 m high at the top of the tree canopy. The other 9 were hung from trees, in the conventional manner, on the windward (Southern) side of trees. This orchard has rows orientated East-West. Tree canopies overlap, limiting air flow through the orchard. Three different commercially-available pheromone lures were used, to give a total of 6 traps for each lure type. Three of each of these lures were placed in traps on poles and three in traps in trees respectively. This gave a total of 6 treatments, with 3 repetitions of each. Traps were then placed in a random block design. Traps are monitored weekly, and all pheromone lures are replaced every ten weeks, as one of the lures is registered to be replaced every 3 months.

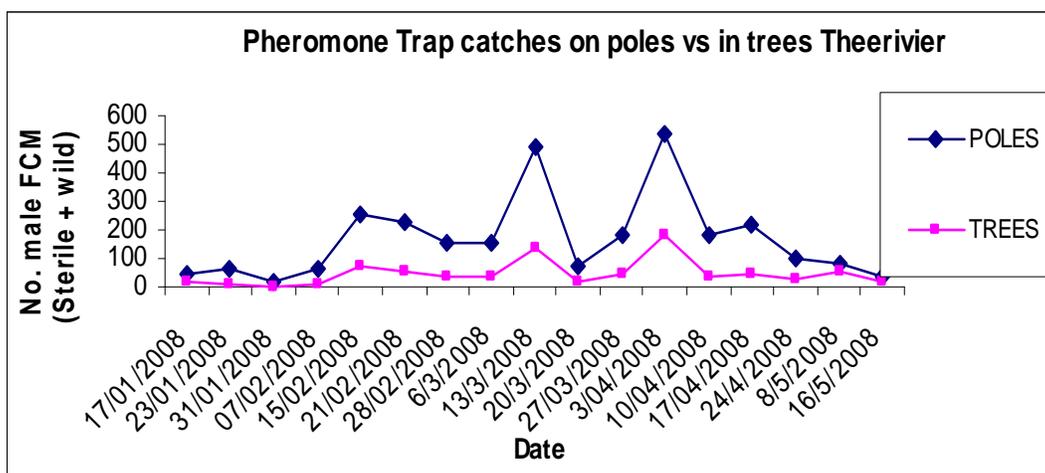
**Results and discussion**

With regards to pheromone lure type, the Lorelei pheromone has caught considerably more FCM males than either the Insect Science FCM lure or the Chempak FCM lure (Fig. 3.2.12.4), with 1707, 1006 and 963 male FCM caught to date respectively.



**Fig. 3.2.12.4.** FCM male trap catches with regard to pheromone lure type.

In this orchard, considerably more FCM have been caught in traps placed on poles at the top of the tree canopy than in traps placed in the conventional manner (Fig. 3.2.12.5), with a total of 2880 and 796 caught respectively. This indicates that in this orchard, traps placed on poles catch approximately 3.6 times more FCM males than the conventional trapping method. This differs considerably to the preceding experiment (3.2.12.1.1), where catches did not differ between traps on poles and traps in trees (Fig. 3.2.12.3). This can probably be attributed to row orientation, and a reduction in air flow and pheromone dispersal in orchards with rows orientated against the prevailing wind direction.



**Fig. 3.2.12.4.** FCM male trap catches with regard to trap placement in an orchard with rows orientated East-West.

This experiment indicates that the Lorelei pheromone lure is the most optimum lure to use for monitoring FCM in citrus orchards, due to higher catches, and also due to the dispenser lasting considerably longer (up to 7 months) than the other two commercially available dispensers.

Correct trap placement could provide an interesting debate, but this experiment hints that increasing the trapping height in orchards orientated against the prevailing wind may greatly improve trap catches of FCM, particularly in older plantings where tree canopies overlap.

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

#### Summary

Monitoring the ratio of sterile: wild FCM in the SIT area has been underway since early November 2007, with the use of delta pheromone traps. Results to date show that it has been difficult to achieve the proposed ratio of 10 sterile FCM: 1 wild FCM in field conditions. Only in one week, has this ratio been surpassed. This may be due to a larger-than-expected wild FCM population in the area, particularly due to poor orchard sanitation, a failure to remove out-of-season fruit from orchards and the lack of implementation of additional control practices.

However, wild FCM catches and fruit infestation in monitored orchards have been kept below economic threshold values for most of the season

#### Introduction

This experiment was initiated to monitor releases of sterile FCM from the XSIT facility over a period of approximately three years, to record the effects of SIT against FCM in this area. In particular, the ratio of sterile: wild FCM males is being monitored using delta pheromone traps to gain insight into the performance of sterile FCM under field conditions. In addition, fruit infestation surveys serve as a measure of control achieved by the programme.

#### Materials and methods

Seven farms were selected 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 traps were set in each orchard of a bearing size, excepting lemon orchards. Yellow Delta traps are used, with sticky pads and the Lorelei pheromone lure. A total of 77 traps were set out at the end of October to coincide with the first releases of sterile FCM on 2<sup>nd</sup> November 2007. Traps are hung generally in the middle of the orchard, 10 trees in from the edge, on the Southern side of the orchard, which is the windward side. 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. Therefore, sterile FCM within traps are identified purely by sight, which is relatively

easy, as the facility-reared moths lose most of their scales, and are easily distinguished from wild FCM (Fig 3.2.12.5). Identification is also very accurate.



**Fig. 3.2.12.5.** Sticky pad from a delta trap showing the difference in visual appearance of a facility-reared sterile FCM male (left) and a wild 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. Fallen fruit is collected from beneath these trees every week and inspected for FCM damage, starting on the 1<sup>st</sup> of December, until fruit is harvested 5-6 months later

### **Results and discussion**

To date, it has proven difficult to achieve the recommended ratio of 10 sterile FCM/ wild FCM in orchards (Fig. 3.2.12.6). This may be due to a rather high population of wild FCM in the area this year, as well as the fact that all farms being monitored are considered to be “hot spots”. Only during one week in early February was this ratio surpassed, with the ratio generally hovering around 4:1. This ratio seems to be decreasing with the onset of winter, as was recorded by Hofmeyr and Hofmeyr during the pilot project in 2007 (Pers. Comms). Obviously, the more wild FCM present on a particular farm, the lower the ratio of sterile: wild will be.

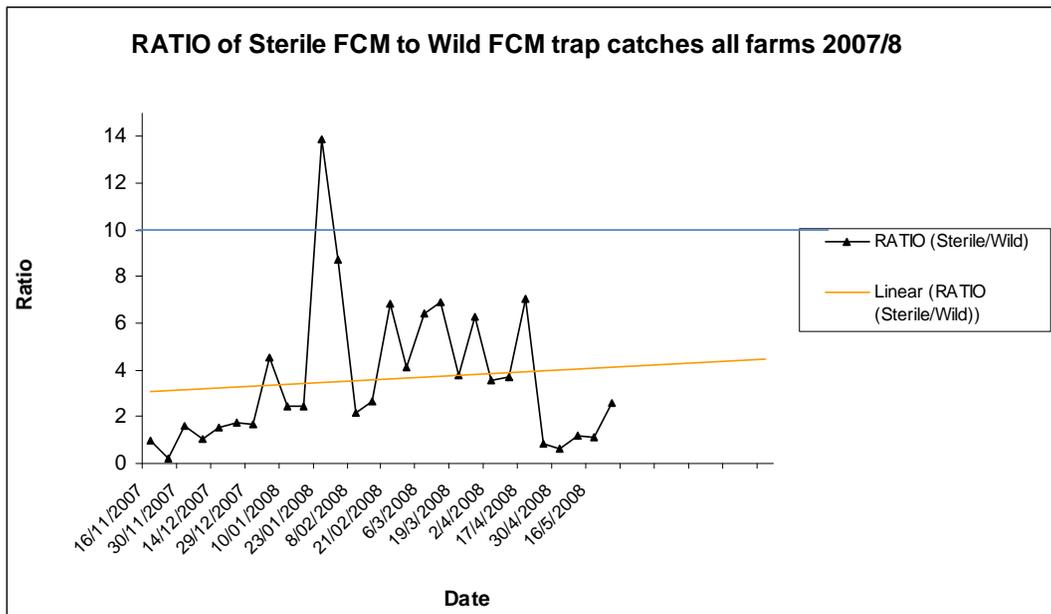


Fig. 3.2.12.6. Graph indicating the ratio of sterile: wild FCM trapped in 77 orchards in the SIT area.

Trapping data from 77 delta traps shows the weekly trap catches of wild FCM males to be well below the threshold for Lorelei pipe traps of 10 males/trap/week. This ratio has often been surpassed in certain orchards, but the average is well below this value, with the highest average trap catch per week being 4 wild males/week (Fig.3.2.12.7).

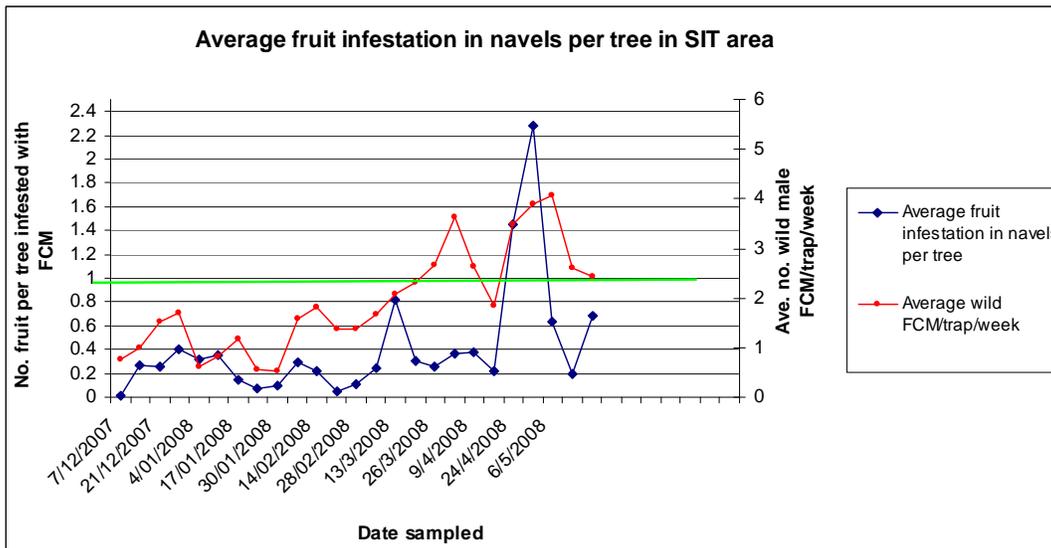


Fig. 3.2.12.7. Graph showing the average fruit infestation/tree/week in 13 navel orchards in the SIT area, compared with the trapping data of wild male FCM/trap/week from 77 delta traps in the same area.

The average fruit infestation in 13 navel orchards has also remained below the recommended economic threshold of 1 infested fruit/tree/week for most of the season, excepting a rather high peak of fruit infestation at the end of April (Fig 3.2.12.7). This can largely be attributed to two specific orchards on a farm where orchard sanitation has not taken place since Christmas, and where no other control practices have been applied against FCM.

### 3.2.12.3 Monitoring flight ability and longevity of facility-reared sterile FCM

#### Summary

Mark-recapture performance tests for facility-reared sterile FCM have been underway since 12<sup>th</sup> April 2008,

and will continue for at least another 11 months. Results to date show disappointingly poor recaptures, with no batch of moths achieving more than 3.5 % recapture of male FCM. Many released batches have 0 % recapture. This can possibly be attributed to the low temperatures experienced at this time of the year. However, wild FCM are still caught, and catches seem to peak towards the end of June (Personal experience). This may suggest that facility-reared sterile FCM males are less cold-tolerant than their wild counterparts.

**Introduction**

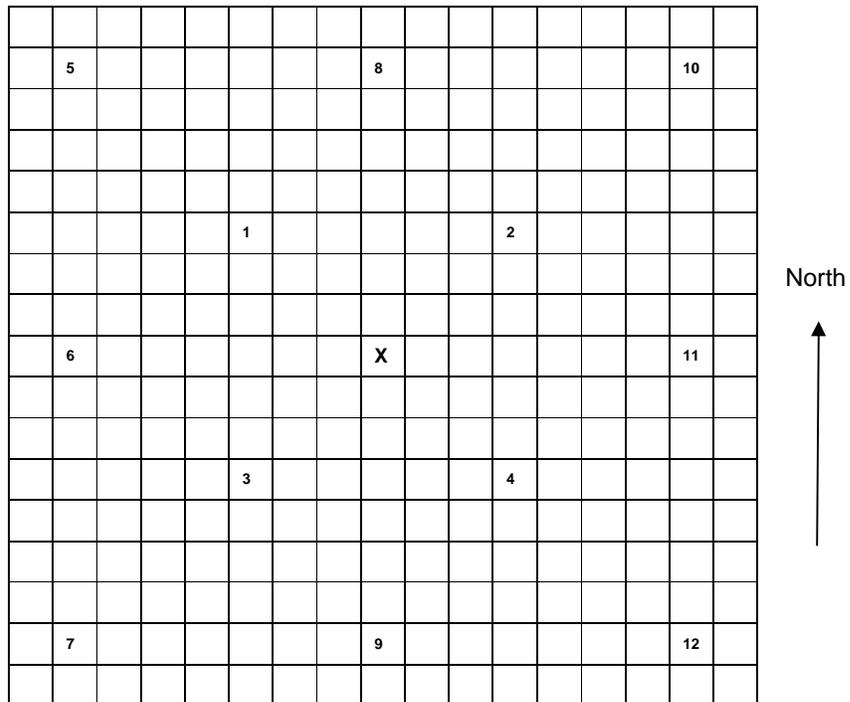
This experiment is a remake of some of Hofmeyr and Hofmeyr’s very similar experiments performed in 2004 (See 3.4.5.4 -3.4.5.7 *CRI Group Annual Research Report 2004*). It is not meant to disprove anything and was set up simply to monitor released sterile moth quality from the XSIT facility, over a long period. Sadly, it was only set up in mid-April, and with winter upon us, recapture rates of released moths has been very poor. This experiment will continue, with releases bi-weekly for another 11 months to provide a year’s data.

Results from this experiment will provide valuable information regarding sterile FCM performance at certain times of the year, such as winter, when temperatures are low, and will provide a means of monitoring moth quality from the facility. Data indicating dispersal distance and longevity in the field will be collected.

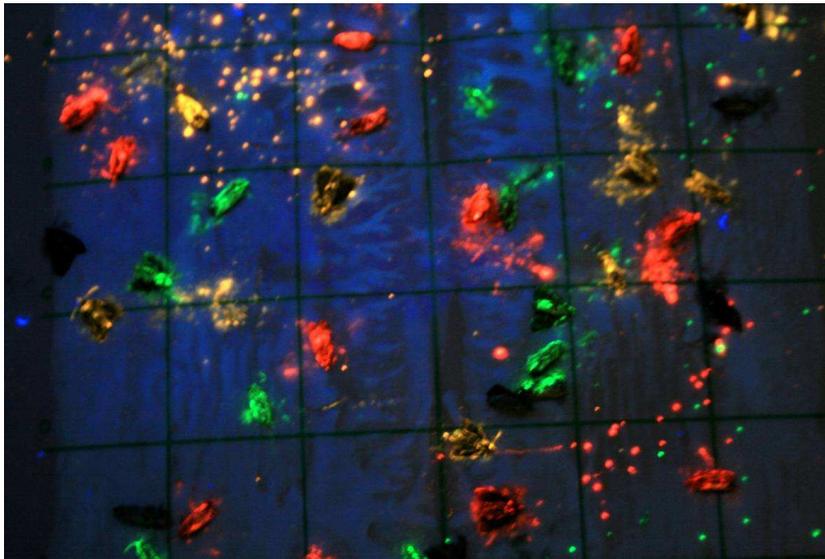
**Materials and methods**

This experiment is being conducted on the farm Robyn, just outside Citrusdal in a 4 Ha block of Washington navels. 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 3.2.12.8). Trees in this block are spaced 4x6. Traps are no further than 60m from the release point. Lorelei pheromone lures are used within the delta traps.

Approximately 3000 mixed FCM irradiated with 150 Gy are released every Tuesday and Friday. Each batch of moths is dyed with a fluorescent powder dye (Fig. 3.2.12.9) 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 collected. An Escort® data logger is kept permanently within a delta trap in the centre of the orchard to constantly monitor temperature and humidity.



**Fig. 3.2.12.8.** Trap layout for orchard flight tests with central release point marked “X”. Each block represents a tree.



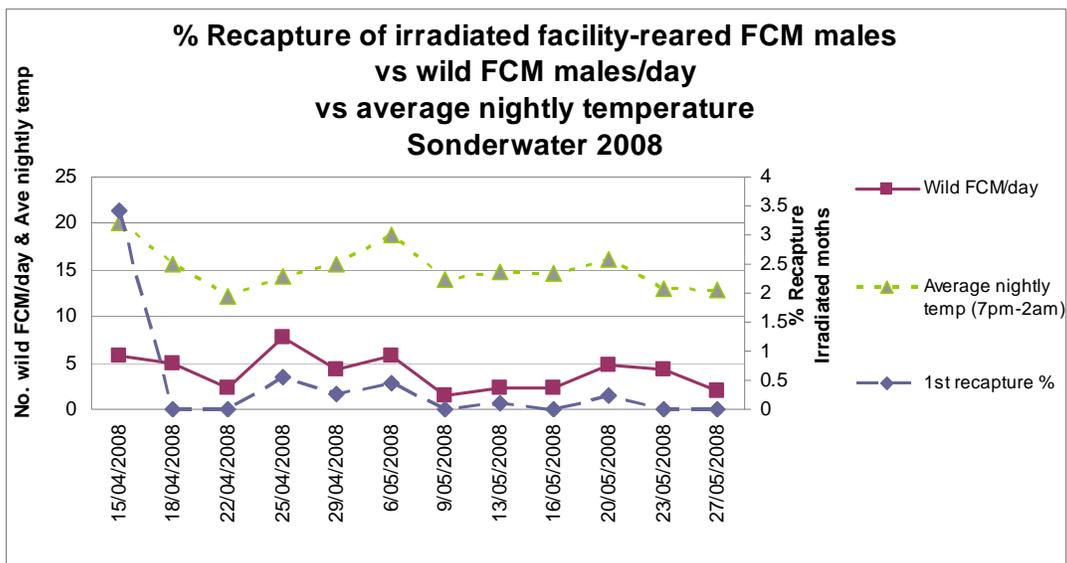
**Fig. 3.2.12.9.** Sticky pad under a UV lamp, showing dyed FCM, as well as a few wild FCM (black in colour)

### Results and discussion

Recapture of irradiated moths has been very poor to date (Fig. 3.2.12.10). Often, no released moths are caught at all. This may be attributed to low nightly temperatures at this time of the year. It seems that when the average nightly temperature (between 7 pm and 2 am) falls below 15°C, no released moths are caught, and very few wild moths are caught either.

It remains to be seen what the effect of winter will be on irradiated released FCM, and whether they are less cold-tolerant than their wild counterparts. As they lose most of their scales during the rearing process, this may play a role in affecting their cold tolerance and competitiveness, particularly during winter.

As so few released moths have been recaptured, one cannot draw any conclusions with regard to longevity or dispersal distance, although some males have been recaptured up to 60 m from the release point, and one male survived for a total of 10 days before being trapped. 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 (3.4.5.4 *CRI Group Annual Research Report 2004*).



**Fig. 3.2.12.10.** Graph showing 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.

### 3.2.12.4 The effects of Calco red on quality of facility-reared FCM

#### Summary

Initial investigations into the effects of the presence of Calco red dye in rearing diet on FCM quality show that presence of Calco in the diet does not affect the number of larvae that develop to pupation, or the incidence of granulovirus in diet jars.

#### Introduction

It was initially planned that Calco red dye would be implemented into the larval rearing diet at the XSIT facility to provide a means of distinguishing between released sterile FCM and wild FCM trapped in Delta traps. However, the use of this dye was suspended indefinitely pending an investigation into the effects of the dye on moth quality and on production. Stephens et al. (2008) found that painted apple moths (*Teia anartoides*) were not adversely affected by the implementation of Calco red dye in their diet. Hofmeyr and Hofmeyr (2004) could make no conclusions about any detrimental effects of Calco red dye on flight ability of released FCM when compared with externally dyed moths.

#### Materials and methods

An investigation was initiated to gain insight into the possible effects of Calco on both production of mass-reared FCM at the XSIT facility and on moth quality.

To date, two experiments have been performed to investigate any effects of Calco on production.

On two separate occasions, a week apart, 7 diet jars were prepared, incorporating Calco red into the diet, and 7 jars were prepared with the standard diet, without Calco. Each jar was inoculated with approximately 2000 FCM eggs, dipped in a solution of 20% Formalin (30% Conc.) and 80% distilled water, and rinsed immediately in distilled water. Jars were left at 26°C for 13 days and then turned on their sides with PVC lids with SFK cardboard stoppers placed in the mouth of the jar for larvae to pupate within. Stoppers were removed after 7 days and opened 5 days later and inspected for pupae and larvae affected by virus.

#### Results and discussion

There seemed to be very little difference between the treatments in terms of either production (number of pupae per jar) or number of larvae affected by virus Fig. 3.2.12.11). Jars with Calco red dye produced slightly fewer pupae by average, on both occasions. It was noticeable however, that larvae in jars with Calco red dye developed slightly slower than larvae in the conventional diet.

It would therefore seem that in terms of production, Calco red dye does not adversely affect the production. It remains to be seen whether the dye has an effect on the fecundity or competitiveness of FCM.

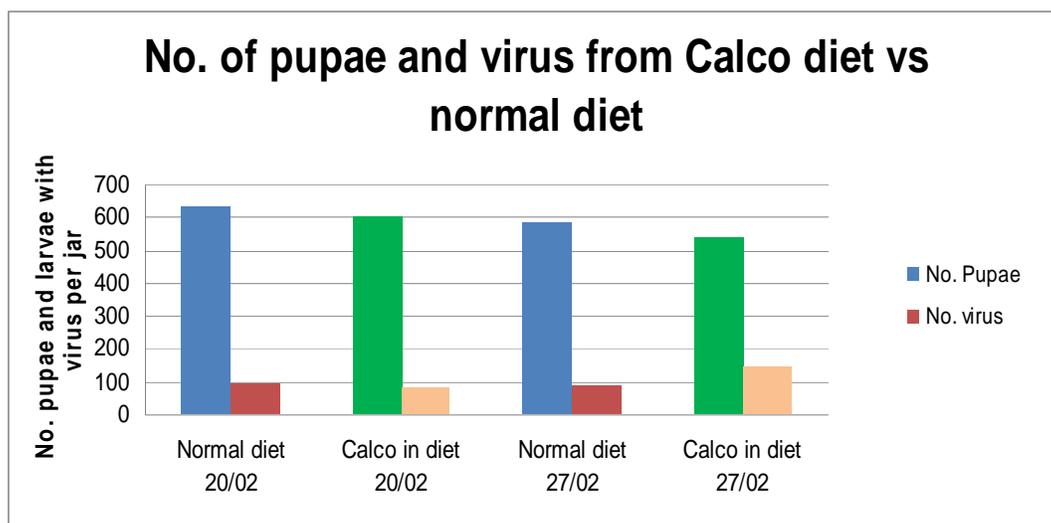


Fig. 3.2.12.11. Comparison of average pupal counts and average number of larvae affected by virus in diet jars with and without Calco red dye.

## Further Objectives and work plan

Monitoring of sterile/wild FCM ratios and fruit infestation by FCM on the 7 farms will be monitored until November 2010, to provide 3 years' data. In addition, more farms will be selected by November of the current year to commence monitoring in the area where the second phase of releases will commence. These farms will be monitored for 2 years. Each monitored orchard will have a treatment history, and different treatments will be compared based on their effectiveness combined with SIT.

Flight performances of released sterile moths will continue to be monitored bi-weekly for another 11 months, to provide a years' data. This will provide useful information regarding flight performance of sterile FCM with regard to season and temperature, as well as longevity in the field and dispersal distance of released moths. Further research is already underway on the effects of different dyeing techniques for monitoring of sterile FCM. Currently, no dye is used due to fears of negative effects of using either Calco red internal dye or fluorescent external powder dyes. The effects of Calco red on fecundity of FCM will be investigated, as well as the effects of Calco red and external fluorescent powder dyes on flight ability and competitiveness of released moths.

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### 3.2.13 FINAL REPORT: The genetic characterisation and biological activity of the South African *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) in two biopesticides, Cryptogran® and Cryptex®

Experiment (February-October 2007): by Tarryn Goble (RU)

## Summary

Cryptogran® (River Bioscience, South Africa) and Cryptex® (Andermatt, Switzerland) are two registered biological control products that have been formulated using the South African isolate of CrleGV-SA for augmentative control of false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick on citrus in South Africa. Genetic characterisation of these viral isolates was undertaken using restriction fragment length polymorphism analysis (RFLP) with the restriction enzymes *Bam*HI, *Xho*I, *Eco*RI, *Hind*III and *Pst*I. It was revealed that the granulovirus formulated as the active ingredient of these biopesticide products, is in fact two geographically distinct genotypes of the CrleGV-SA variant. To further characterise these viral isolates, partial amplifications of both the *granulin* and *egt* genes, as amplicons of 690 base pairs (bp) and 1290 bp was carried out revealing 99% and 98% nucleotide identity respectively using a BLAST search on the NCBI database. *Egt* gene alignments also showed that there was some divergence between the SA variant and the Cape Verde (CV3) variant; BLAST searches revealed a 97% nucleotide identity between these 'sub-species' of virus. The differences between the Cryptogran® and Cryptex® viral genotypes, based on restriction analysis, was further supported by significant differences (F-value= 5.59; p = 0.003) in their biological activity determined by the elevations of the probit lines during surface dose-response bioassays with neonate FCM larvae (lethal concentration LC<sub>50</sub> and LC<sub>90</sub>). LC<sub>50</sub> and LC<sub>90</sub> for Cryptogran® were

estimated to be  $4.054 \times 10^3$  and  $7.372 \times 10^4$  OBs/ml (occlusion bodies/ml) while these values for Cryptex® were estimated to be  $8.460 \times 10^3$  and  $1.950 \times 10^5$  OB/ml. This study highlighted the differences in the genetic and biological activities of the CrleGV-SA isolates formulated in both Cryptogran® and Cryptex®. In this preliminary study, Cryptogran® was shown to be more pathogenic than Cryptex® in dose-response bioassays.

## Opsomming

Cryptogran® (River Bioscience, Suid-Afrika) en Cryptex® (Andermatt, Switzerland) is twee geregistreerde biologiese beheer produkte wat geformuleer is met 'n Suid-Afrikaanse isolaat van CrleGV-SA vir die bykomende beheer van valskodlingmot, *Thaumatotibia leucotreta* Meyrick, op sitrus in Suid-Afrika. Genetiese karakterisering van hierdie virusisolate is onderneem met die gebruik van restriksie ensiem lengte polimerase analiese (RFLP) met die ensieme *Bam*HI, *Xho*I, *Eco*RI, *Hind*III en *Pst*I. Daar is gevind dat die granulovirus wat die aktiewe bestanddeel van hierdie twee produkte is, in werklikheid twee duidelik verskillende genotipes van die CrleGV virus is. Om verder hierdie virusisolate te karakteriseer, is gedeeltelike vermenigvuldiging van albei die *granulin* en *egt* genes, as ampikone van 690 basispare (bp) en 1290 bp, uitgevoer. Met die gebruik van 'n BLAST-ondersoek op die NCBI databasis, is 'n 99% en 98% nukleotied identiteit onderskeidelik onthul. *Egt* gene-opstellings het ook gewys dat daar ietwat afwyking tussen die SA variant en die Kaap Verde (CV3) variant is. BLAST-onderoeke het 'n 97% nukleotied identiteit tussen hierdie 'subspesies' van virus onthul. Die verskille tussen die Cryptogran® en die Cryptex® virusgenotipes, gebaseer op restriksie-analiese, is verder ondersteun deur betekenisvolle verskille (F-waarde = 5.59; p = 0.003) tussen hulle biologiese aktiwiteit. Dit is bepaal deur die elevasies van die probit lyne gedurende dosis-respons biotoetse met pas-uitgebroeide VKM-larwes (dodelike konsentrasie LC<sub>50</sub> en LC<sub>90</sub>). LC<sub>50</sub> en LC<sub>90</sub> vir Cryptogran® is geskat op  $4.054 \times 10^3$  en  $7.372 \times 10^4$  OPs/ml (okklusie partikels/ml), terwyl hierdie waardes vir Cryptex® geskat is op  $8.460 \times 10^3$  en  $1.950 \times 10^5$  OPs/ml. Hierdie studie het die genetiese en biologiese aktiwiteite van die CrleGV-SA isolaat wat in Cryptogran® en Cryptex® geformuleer is, na vore gebring. In hierdie voorlopige studie is dit in dosis-respons biotoetse gewys dat Cryptogran® meer patogenies as Cryptex® is.

## Introduction

*Cryptophlebia leucotreta* granulovirus (CrleGV) is a tortracid-specific baculovirus which is highly pathogenic to false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick. This highly virulent pathogen has proven to be a highly effective biological control agent against FCM and has been used as part of an augmentative IPM strategy in citrus orchards in South Africa (Moore, 2002). The South African CrleGV isolate has a circular, covalently closed, double stranded DNA genome about 112 kilobase pairs in size and potentially encodes 129 open reading frames (ORF) with an AT content of 67.6% (the highest found thus far in sequenced baculoviruses). Genetic engineering of baculoviruses can result in the improvement of biopesticides by either deleting or adding foreign genes. In order to manipulate granuloviruses, essential genes need to be isolated and identified within the genome. Within the CrleGV genome, the *granulin* and *egt* genes have been considered as important genes.

The *granulin* gene locus for example, has received much attention because it can be replaced by foreign genes, which are expressed at high levels due to this gene possessing a strong promoter (Singh, 2001). Although expressed in the late stages of infection, the *granulin* gene is encoded in the first open reading frame (ORF 1) of the CrleGV genome and is 747 base pairs in size (Lange & Jehle, 2003). *Granulin* is an important structural protein, which forms part of the capsid protein matrix, which is characteristic to all granuloviruses. It is also a highly conserved gene among the granuloviruses with an identity of 98% to *Cydia pomonella* granulovirus (GV); 94% to *Phthorimea operculella* GV; 85% to *Plutella xylostella* GV and 88% to *Xestia c-nigrum* GV, respectively (Lange & Jehle, 2003).

Ecdysteroid UDP-Glucosyltransferase (*egt*) is a non-structural protein enzyme, which catalyzes the transfer of glucose from UDPglucose to ecdysteroids. Ecdysteroids are a family of steroid hormones essential for the induction of both larval and pupal molts in insects (O'Reilly & Miller, 1990). In CrleGV, the gene encodes a protein of 506 amino acids, which is subsequently secreted from infected cells. CrleGV infection is thought to block this molting process through the expression of the *egt* gene. The *egt* gene is encoded in open reading frame 128 (ORF 128) and is 1391 base pairs in size. The *egt* function is therefore to increase the feeding time after infection, which is beneficial to the pathogen as it allows further virus propagation but causes prolonged damage to crops (Singh, 2001). Genetic engineering of viruses which lack the *egt* function have enormous potential as biopesticides because insects infected with these recombinant viruses stop feeding and succumb to viral infection sooner, thus increasing crop yield (Singh, 2001).

To date, three different geographic, naturally occurring virus isolates have been identified and described from infected larvae, namely from the Cape Verde Islands (CV), the Ivory Coast (IC) and South Africa (SA)

(Lange & Jehle, 2003). The wild-type isolates consist of a mixture of genotypes, which can be distinguished using restriction endonuclease analysis of the viral DNA (Jehle *et al*, 2003). Studies have been conducted which show that recombination occurs between two genotypes of the Cape Verde isolate of the CrleGV through mixed infection experiments with FCM larvae, further suggesting that novel genotypes are common among geographically distinct viral isolates and may contribute to the genetic diversity in this virus group (Jehle *et al*, 2003). It is this genetic variation which renders the pathogen persistent and able to adapt to changes which may occur within the host, FCM.

Timm *et al* (2006) showed using amplified fragment length polymorphism (AFLP) analysis that a high level of genetic variation exists in FCM populations in South Africa, with 98.3% of the AFLP fragments being polymorphic; this value is amongst the highest recorded for insects. In populations with high genetic variation, novel genotype evolution is common and it is assumed that the species is more persistent, this is true of FCM. With this in mind it seems prudent to assume that CrleGV-SA may also have various viral biotypes, having evolved with a particular FCM population in isolated geographic regions within South Africa.

Two commercially produced products have been formulated using this entomopathogenic virus and are available on the South African pesticide market for augmentative use on citrus and other important economic crops. Both Cryptogran<sup>®</sup> and Cryptex<sup>®</sup> have apparently been formulated using naturally occurring South African isolates of the CrleGV and are registered for use on citrus in South Africa. The products are available in suspension concentrates which contain virus concentrations of at least  $5 \times 10^{10}$  virus OBs/ml (occlusion bodies/ml) (Internet source 1) and  $2 \times 10^{13}$  OB/litre (Internet source 2) for Cryptogran<sup>®</sup> and Cryptex<sup>®</sup> respectively. Virus characterisation is required from the manufacturer for all formulated biological control products before application for registration is approved (Moore, 2002).

Characterisation of granulovirus isolates is important because it permits monitoring of insect resistance to particular viral stains, enables mutants to be detected during biopesticide production (which may cause changes in pathogenicity or host range) and allows development of assays for detecting viral residues (Singh, 2001). The determination of viral pathogenicity is an important consideration; virulent viral isolates have a faster speed of kill, causing death to the pest and limiting crop damage (Singh, 2001). The relative success and integrity of the biopesticide market relies heavily on products that are reliable and formulated from the most virulent microbial strains, so that adequate control of these pest populations may be achieved.

We hypothesize that the biopesticide products, Cryptogran<sup>®</sup> and Cryptex<sup>®</sup>, have been formulated with distinct isolates of the CrleGV-SA variant. To test this we genetically characterized the viral isolates obtained from the products using restriction fragment length polymorphism (RFLP) with five restriction enzymes. A second aim was to amplify the *egt* and *granulin* genes from the viral isolates by polymerase chain reaction (PCR) to determine if there were any differences in these important genes which may aid in further genetic analysis and delineation of the viral isolates. A final aim was to test the pathogenicity of the viral isolates using surface dose-response bioassays with neonate FCM larvae using lethal concentration ( $LC_{50}$  and  $LC_{90}$ ) which may support the heterogeneity of the viral isolates obtained from the products.

## Materials and methods

South African isolates of the *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) were obtained from two registered biological control products, Cryptogran<sup>®</sup>, manufactured by the South African company, River Bioscience, and Cryptex<sup>®</sup>, manufactured by the Swiss company Andermatt. Products were supplied as suspension concentrates in five hundred ml bottles by Dr S. Moore (Citrus Research International). The Cryptex (Batch 2) was supplied to him by Andermatt's South African counterpart, BCP (Biological Control Products). Products were stored at  $-10^{\circ}\text{C}$ , as endorsed by the manufacturers, prior to experimentation and while experimentation was in progress. The maintenance of the original pathogenicity of both products was therefore ensured.

### Isolation of CrleGV-SA occlusion bodies (OBs)

Viral OBs were purified according to the protocol by Ludewig (2003); 12 ml of product was added to 18 ml of 0.1% SDS. Total volume was transferred to two sterile tubes and centrifuged for 30 minutes at 10 000 rpm in a Beckman JA-20 rotor (Beckman, Johannesburg). Re-suspended OB pellets in 3 ml of 0.1% SDS. Two 25-90 % (v/v) glycerol gradients in 0.1% SDS were prepared in Beckman SW-28 swing out rotor centrifuge tubes and rotating them on a BIOCAMP<sup>™</sup> 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). Viral OB band was extracted into two Beckman JA-20 centrifuge tubes. Tubes were filled with distilled water and centrifuged for 14

minutes at 10 000 rpm. Pellets were re-suspended in distilled water and the spin repeated. Final pellet re-suspension in 1.5 ml of distilled water and stored at -20°C.

#### Estimation of the OB concentration

OB concentration was determined using a spectrophotometer (Shimadzu, model 1240, Johannesburg). A 1/100 dilution was made of each viral isolate; absorbance was read at 350 nm and 260 nm. Number of OBs was calculated according to the following standard formulae obtained from Dr S. Wormleaton (Horticulture Research International, U.K).  $OD_{350nm}/13x \text{ dilution factor} = \text{mg/ml}$  plus  $OD_{260nm}/31x \text{ dilution factor} = \text{mg/ml}$  obtained the averages of both equations and multiplied by the standard formulae:  $1 \text{ mg/ml} = 3.83 \times 10^{10}$  OBs/ml.

#### Isolation of CrleGV-SA genomic DNA

Modification of the CTAB extraction protocol by Aspinall *et al* (2002) was used to extract total genomic DNA from purified OBs. Two hundred  $\mu\text{l}$  OBs from both products were aliquoted into Eppendorf tubes and 80  $\mu\text{l}$  of sodium carbonate (1M) was added to each tube. Solutions were incubated at 37°C for 30 minutes and then neutralised with 120  $\mu\text{l}$  of 1M Tris-HCl (pH 6.8). After the addition of 90  $\mu\text{l}$  of 10% SDS and 60  $\mu\text{l}$  of proteinase K (25 mg/ml), solutions were further incubated at 37°C for 60 minutes. Samples were microcentrifuged for 3 minutes at 15 000 rpm. Supernatants were transferred to 1.5 ml microcentrifuge tubes and 500 $\mu\text{l}$  of CTAB buffer added. Tubes were mixed by inversion and incubated at 70°C for 60 minutes. Five hundred  $\mu\text{l}$  of chloroform was added to each tube. Solutions were mixed and microcentrifuged for 10 minutes at 10 000 rpm. The upper aqueous layers were transferred to 1.5ml tubes and 400  $\mu\text{l}$  of 100% isopropanol added to each tube. Solutions were incubated at -20°C overnight. The following day, after a 20 minute centrifugation at 13 000 rpm, supernatants were discarded and pellets resuspended in 1 ml of 70% ethanol. Solutions were microcentrifuged at 13 000 for 5 minutes and ethanol discarded after; tubes were inverted to air dry. Pellets were resuspended in RNase-free water and stored at -20°C.

#### Quantification of genomic DNA concentration

Concentration of the DNA was determined using a spectrophotometer (Genequant, Pharamicia Biotech, Johannesburg). 1/100 dilution was made using triple distilled water. Absorbance was read at 260 nm and the concentration of the double stranded DNA was calculated according to the standard equation that 1 absorbance unit at  $OD_{260nm} = 50 \mu\text{l/ml}$

#### Characterisation of viral isolates using RFLP and agarose gel electrophoresis

Single restriction enzyme (RE) digestion was carried out using five RE's and the appropriate buffers (Promega Corp, Cape Town), according to Singh (2001). Twenty  $\mu\text{l}$  of genomic DNA was digested in a total volume of 30  $\mu\text{l}$  with three units each of the restriction enzymes *Bam*HI, *Xho*I, *Eco*RI, *Hind*III and *Pst*I. Digests were then incubated at 37°C for 180 minutes. Samples were loaded on 1% agarose gels in TAE buffer run at 30 V for 16 hours. A  $\lambda$ *Pst* molecular weight marker was used to determine the size of restriction fragments. Gels were stained with ethidium bromide for 60 minutes after electrophoresis. Stained gels were visualised using an UV transilluminator and gel images were captured using UVIprochemi (Version 12.4 for windows) manufactured by UVIttec in Pretoria.

DNA fragment sizes were estimated by comparison with the  $\lambda$ *Pst* molecular weight marker. This was done by plotting the distance migrated by the  $\lambda$ *Pst* marker fragments against the log of its known size in kilobase pairs (kbp). The standard curve obtained was used to estimate restriction fragment sizes determined by the standard curve equation. The size of the  $\lambda$ *Pst* marker was inadequate to determine the sizes of some of the larger bands because they fell outside the range of the marker. These bands were allocated a not applicable (NA) symbol because no estimated sizes were known (Table 3.2.13.3 in Appendix).

#### Amplification of granulin and egt genes using PCR

The Expand High Fidelity PCR system (Roche, Johannesburg) was used to amplify CrleGV-SA genes through the polymerase chain reaction (PCR). PCR was performed using 15.86  $\mu\text{g/ml}$  DNA isolated from viral OBs, 5  $\mu\text{l}$  buffer with  $\text{MgCl}_2$  (25 mM), 2  $\mu\text{l}$  dNTP mix (10 mM of each nucleotide), 0.3  $\mu\text{l}$  of high fidelity DNA polymerase, 33.7  $\mu\text{l}$  distilled water and 1  $\mu\text{l}$  (100  $\mu\text{M}$  stock) of each of the respective primers listed in Table 3.2.13.1 to a total volume of 50  $\mu\text{l}$ . Primers used to amplify the *granulin* and *egt* genes from the CrleGV-SA genome were designed based on the gene sequences from Singh *et al* (2003) and Lange & Jehle (2003) (Table 3.2.13.1).

**Table 3.2.13.1.** Primers designed to amplify the *granulin* and *egt* from CrleGV-SA genomic DNA. Note that sequences are in a 5' to 3' direction.

Oligonucleotide name	Sequence
Granulin Forward	ATG GGA TAT AAC AAA TCT TTG AGG
Granulin Reverse	TTA ATA GGC TGG ACC GGT GAA TAG G
Egt Forward	TTA TTT ATT TTC GTT AAA CAT AAA CAT TAC
Egt Reverse	TGT ATA GTA TCT TTG TTG TGC TGT TG

After amplification (95°C for 1 min 30 secs, 30x (95°C for 30 secs, 54°C /51°C for 40 secs, 72°C for 4-5 secs), 1x (72°C for 5 mins), 5 µl of PCR products were visualised by agarose gel electrophoresis. Ethidium bromide stained gels were visualized using a UV trans-illuminator. PCR products were purified using the Wizard® SV quick purification kit (Promega Corp, Cape Town).

#### DNA sequencing

Sequencing of the CrleGV-SA genes was performed using the Big Dye® Sequencing Kit v.3.1, (Applied Biosystems, Johannesburg) with 2 µl of purified PCR product, 3 µl of 5x reaction buffer, 2 µl Sequencing Big Dye Terminator, 12.8 µl of distilled water and 0.2 µl of either the forward or reverse respective primer (100 µM stock) listed in Table 1; making a total volume of 20 µl and placed in a thermocycling machine set on a 30 cycle revolution/regime. All samples were sequenced using an ABI 3100 genetic analyzer at Rhodes University. The resulting sequence trace files were edited using GeneStudio Professional Edition v.1.03.72 (GeneStudio Inc). Sequence data was imported into MEGA version 3.1 (Kumar *et al*, 2004) aligned automatically using the CLUSTAL W algorithm (Chenna *et al*, 2003) and the alignment checked manually. Control product gene sequences were then BLAST (Altschul, 2004) checked and aligned with Genbank sequences from the NCBI database (Internet source 3) and compared.

#### Determination of isolate biological activity using dose-response bioassays

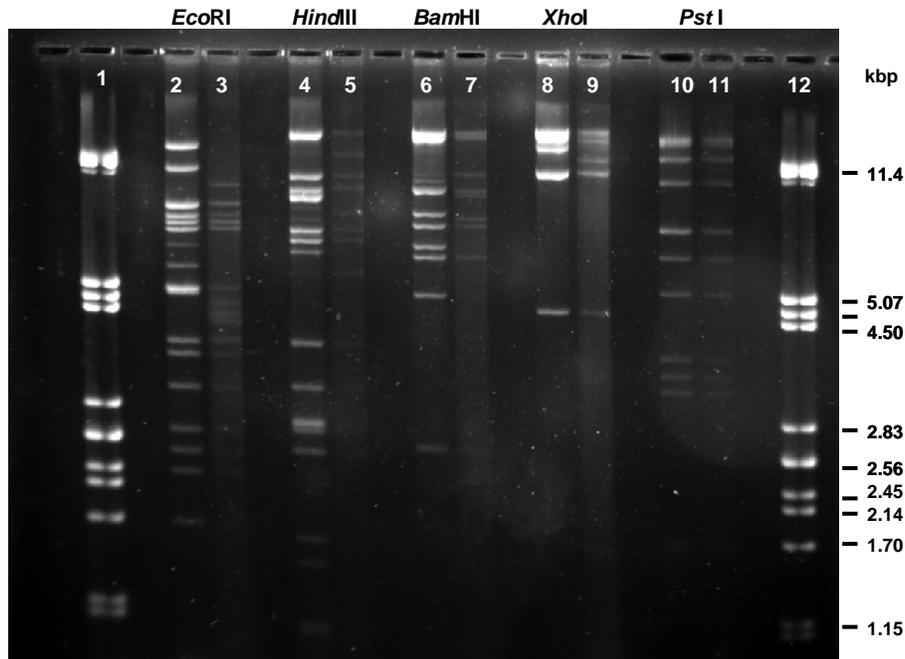
Surface dose-response bioassays with 24-h-old *FCM* larvae, supplied by Dr S. Moore (Citrus Research International), were conducted in 25 cell bioassay trays (Sterilin, South Wales UK) and carried out according to Moore (2002). Each cell was filled with a layer of 10mm of artificial diet comprising: 12.7% (wt: vol) maize meal, 1.8% (wt: vol) agar, 1.2% (wt: vol) wheat germ, 0.6% (wt: vol) brewer's yeast, 0.2% (wt: vol) milk powder, 0.1% (wt: vol) Nipagen, 0.05% (wt: vol) sorbic acid. Five-fold serial dilutions of CrleGV-SA isolates were carried out in sterile distilled water, with a sterile distilled water control. The concentrations of the virus dilutions were adjusted according to the specification of the virus content of each specific product (Cryptogran 5x10<sup>10</sup>GV/ml and Cryptex 2x10<sup>10</sup> GV/ml). Fifty micro litres (µl) of each virus dilution and of the control was pipetted onto the centre of the diet surface of each cell and spread evenly over the diet surface. One neonate larva was placed in each cell. Trays were sealed and kept at a constant temperature of 27°C for 7 days after which, trays were opened and evaluated. Larvae were recorded as either dead or alive. The prevalence of *Aspergillus* spp. became more severe the longer the bioassay trays were left to incubate, thus it became necessary to check the trays after several days of incubation rather than the recommended two week incubation period. Four replications were carried out per viral isolate with 25 larvae per treatment (dose); replications were combined so the total number of responses was corrected to 100 larvae.

The dose-response curve was calculated using PROBAN (Van Ark, 1995), a computer software package for calculating probit analysis (Finney, 1971). PROBAN took into consideration the mortality of the control insects and corrected the mortality of treated larvae according to Abbott's formula (Abbott, 1925). From this the lethal concentrations of each product, LC<sub>50</sub> and LC<sub>90</sub> (concentrations required to kill 50% and 90% of larvae in a sample) were calculated. When larvae react to a stimulus (in this case a viral dose), the larger the dose, the more individuals will react and consequently a cumulative curve of the successive percentage of larvae reacting is obtained. Further, a frequency distribution can be fitted to these percentage reactions to each dose and this normally takes the form of a flat normal distribution (Van Ark, 1995). Probit analysis or probit function is simply an equivalent of an accumulative normal distribution. The fit of the probit lines used the sum of least squares to determine the application of the probit line to the data set; minimising the possibility of outliers was achieved by combining replications. When the dose was transformed to log-dose, a characteristic sigmoid curve (S-curve) was obtained and could be fitted to the cumulative frequency distribution. When the percentage reactions were transformed to empirical probits, this curve became a straight line (probit line). A chi-squared ( $\chi^2$ ) test was used to determine if the fit of the probit lines were acceptable and parallel, and the quantity G was used in the calculation of the fiducial limits. The elevations of the probit lines were also calculated and compared using Bartlett's test (F-test) for homogeneity of the residual variances.

## Results and discussion

### Characterisation of viral isolates using RFLP and agarose gel electrophoresis

Cryptogran® yielded an average of  $5.766 \times 10^{10}$  OB/ml per glycerol purification while on average Cryptex® was found to yield  $5.603 \times 10^{10}$  OB/ml per purification. CrleGV-SA genomic DNA was extracted from viral OBs and the concentration of the DNA was calculated to be 15.86 µg/ml and 30.56 µg/ml for Cryptogran® and Cryptex® respectively. Single enzyme digestion was carried out; in this event, only restriction fragments which could be reliably viewed on a particular gel were included, estimated and compared, were fragments could not be estimated they were allocated a NA symbol (Table 3.2.13.3 in Appendix). The resolution of faint Cryptogran® fragments (Fig. 3.2.13.1) was increased by running more gels (Fig. 3.2.13.5 and 3.2.13.6 in Appendix).



**Fig. 3.2.13.1.** DNA profiles of two CrleGV-SA isolates from Cryptex® (lanes 2, 4, 6, 8 and 10) and Cryptogran® (lanes 3, 5, 7, 9 and 11) digested with five restriction enzymes. A  $\lambda$ Pst molecular marker was loaded in lanes 1 and 12 on a 1% agarose gel run at 30V for 16 hours.

### The comparison of the CrleGV-SA isolates from Cryptogran® and Cryptex®

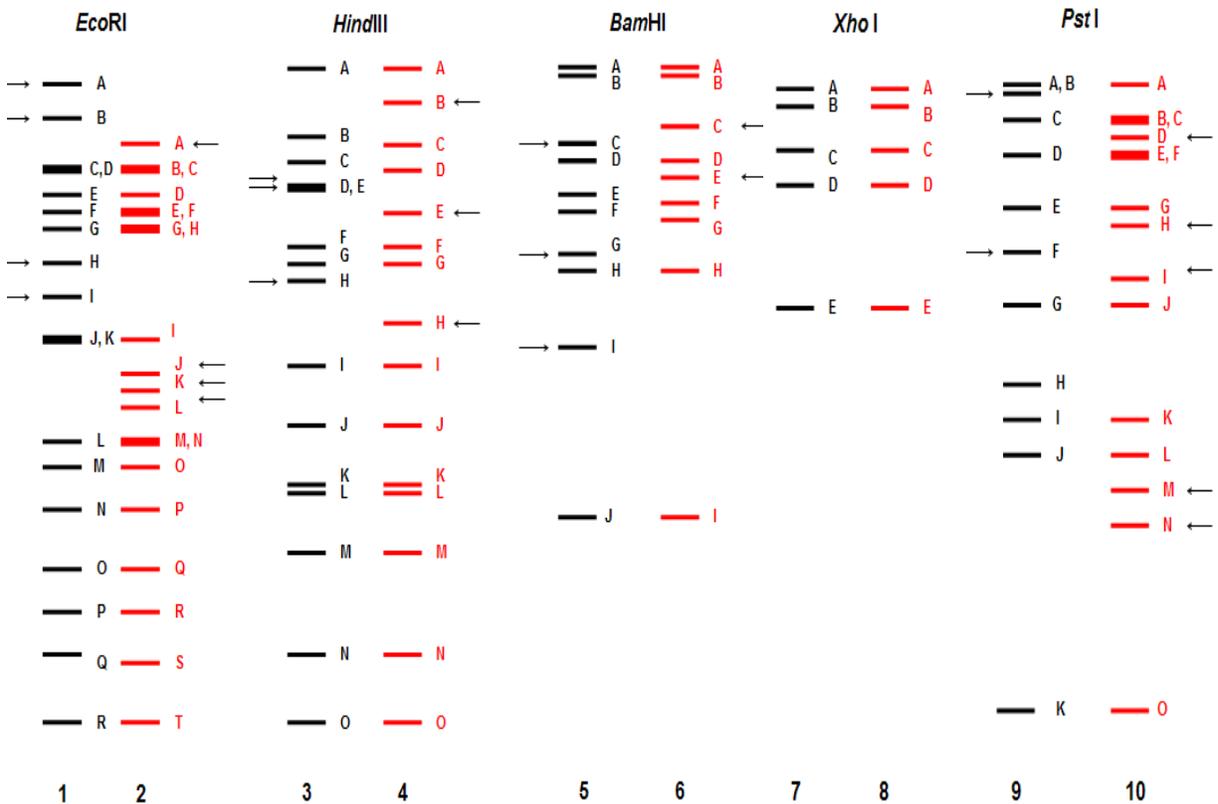
Both South African (SA) isolates of the CrleGV show their own distinctive restriction enzyme profiles (Fig. 3.2.13.2). The differences in the profiles are due to the loss or addition of enzyme specific restriction sites or by the insertion or deletion of DNA, which are common among granulovirus variants (Singh, 2001). An arrow indicates a fragment which does not appear in the opposite viral DNA profile indicating the loss or addition of a particular restriction site. Fragments are labelled in order of decreasing size with A being the largest fragment of any particular digest.

Using the RE *EcoRI*, 18 fragments were identified with the CrleGV-SA DNA isolated from Cryptex®. 20 restriction fragments were generated from the Cryptogran® viral isolate. Fragments, A (size could not be determined), B (9.77 kbp), H (6.85 kbp) and I (6.20 kbp) of the Cryptex® isolate do not occur in the *EcoRI* digested Cryptogran® isolate. Similarly, the Cryptogran® isolate contains fragments A (8.85 kbp), J (5.09 kbp), K (4.87 kbp) and L (4.61 kbp) which did not occur in the Cryptex® isolate (Fig. 3.2.13.2).

Using *HindIII*, 15 fragments were identified from both the Cryptex® and Cryptogran® isolates. Fragments D (8.70 kbp), E (8.55 kbp) and H (6.56 kbp) appeared in the Cryptex® isolate but not in the Cryptogran® isolate. The Cryptogran® profile differed with the inclusion of fragments B (10.2 kbp), E (7.69 kbp) and H (5.87 kbp) (Fig. 3.2.13.2).

*Bam*HI generated 10 fragments which were identified from the Cryptex® isolate while the Cryptogran® isolate contained 9 restriction fragments. Cryptex® contained fragments C (9.50 kbp), G (6.68 kbp) and I (5.27 kbp) which were not present in the Cryptogran® isolate. Similarly, the Cryptogran® profile differed by the fragments, C (10.97 kbp) and E (9.55 kbp) (Fig. 3.2.13.2).

Using *Xho*I, no differences were observed in the DNA profiles of either isolate. Both were identified as having 5 fragments (Fig. 3.2.13.2).



**Fig. 3.2.13.2.** Comparative sketch of the restriction enzyme DNA profiles of CrleGV-SA isolated from Cryptex® (lanes 1, 3, 5, 7 and 9) and Cryptogran® (2, 4, 6, 8 and 10). Letters correspond to restriction fragments which are labelled in order of decreasing size, A being the largest fragment. Arrows indicate DNA restriction fragments which are not present in the opposite CrleGV-SA isolate. Distances are not to scale.

*Pst*I generated 11 and 15 fragments for the Cryptex® and Cryptogran® isolates respectively. Fragments B (size could not be determined) and F (6.57 kbp) were present in the DNA profile of Cryptex® but absent from the oppositely compared DNA profile, while fragments D (size could not be determined), H (7.16 kbp), I (5.95 kbp), M (3.41 kbp) and N (3.09 kbp) were recorded in Cryptogran's® profile but absent in the Cryptex® isolate (Fig. 3.2.13.2).

The viral isolates characterised in this study were compared to the characterisation of the SA CrleGV in studies done by Moore (2002), Singh (2001) and Fritsch (1989), based on these studies, it was apparent that the viral isolates in the present study were indeed the CrleGV-SA. Not only are there sufficient similarities in the DNA profiles of Cryptex® and Cryptogran® isolates to state that they are closely related but there were distinctive differences observed in four of the five restriction digests, suggesting that they are in fact distinctive geographic isolates of the South African variant of the CrleGV.

The DNA profile of Cryptex® and Cryptogran® were compared to the SA isolate in the study done by Fritsch (1989); only the Cryptex® isolate appeared to be very similar to Frisch's SA isolate. The only differences noted between these viral isolates was the appearance of a fragment at around 6.85 kbp, produced by the *Eco*RI digest and the absence of a fragment at around 10 kbp, produced by the *Bam*HI digest in the Cryptex® SA isolate. When Moore (2002) compared his obtained SA isolates to Fritsch's study he concluded that there were sufficient differences to consider these SA isolates genetically distinct from one another. In the comparison of the Cryptogran® and Cryptex® SA isolates with Moore's (2002) study of the SA isolate, there were similarities in the *Eco*RI and *Xho*I digests of Cryptogran® but no similarity in Cryptex®. There was however some differences observed in the *Bam*HI digests of Cryptogran® and Moore's SA isolate,

particularly in the absence of a fragment in Cryptogran® at around 10.97 kbp, which was present in the SA isolate obtained by Moore (2002). Further, Singh (2001) in her characterisation of the SA isolate also noted the absence of this fragment. The Cryptogran® isolate appears to be identical in the DNA profiles obtained using *EcoRI*, *BamHI* and *XhoI*, to the SA isolates characterised by both Moore (2002) and Singh (2001).

Based on the evidence of studies done by Moore (2002), Singh (2001) and Fritsch (1989) and the differences noted between the isolates formulated in Cryptex® and Cryptogran® from the RFLP analysis, it appears that there may be many more isolates of the SA variant which occur in various geographically isolated areas within South Africa. It would appear that this virus 'species' has a high degree of genetic variation which allows for the evolution of novel genotypes. This is further supported by the high degree of genetic variation in the lepidopteran host, FCM. Further confirmation by Jehle *et al*, (2003) was obtained when they showed that many wild type GV isolates consist of a mixture of different genotypes; for example the Cape Verde CrleGV is known to have two distinct genotypes, namely the CV3 and CV4 isolates, which can intra-specifically recombine during co-infection of the host species, FCM.

These results may be explained by geographic isolation which has produced a high level of genetic variation between FCM populations in South Africa (Timm *et al*, 2006). Using the multilocus technique, AFLP, these authors found a total of 98.3% of the AFLP fragments polymorphic; among the highest recorded thus far for any insect species. Inbreeding caused by geographic isolation often leads to a decrease in genetic variation within populations but FCM populations obtained from various provinces within South Africa, indicated some mechanism for maintaining increased levels of genetic variation within populations which has not yet been identified (Timm *et al*, 2006). In populations where genetic variation levels are high, novel genotype evolution is common and often allows for local environmental adaptation and persistence of the species (Timm *et al*, 2006). It seems prudent to assume that where high levels of genetic variation occur in these pest populations, the same may be true of the pathogens which infect them. The evolutionary arms race between insect and pathogen thus relies on genetic modification of genotypes so as to invoke resistance against a particular pathogen and subsequently the genetic change in the pathogen, rendering the insect susceptible once again.

Moore (2002) investigated the possibility of different isolates of the SA variant of the CrleGV obtained from infected larvae in various citrus growing regions within South Africa using restriction fragment length polymorphism (RFLP). He briefly concluded that there were some observed differences in the DNA profiles of isolates obtained from Zebediela, Citrusdal and a confirmed CrleGV-SA laboratory culture but that future work would better reflect these observed differences and confirm the distinction of these geographically isolated South Africa viral isolates.

The CrleGV-SA DNA in this study was digested singly and therefore smaller fragments were not resolved, this may indicate that there may be more differences in the DNA profiles of these two isolates, which can only be determined using double digestion or a wider range of restriction enzymes in single digestion, for a more extensive characterisation.

#### Amplification of the granulin and egt genes from CrleGV-SA isolates using PCR

The *granulin* genes of both viral isolates obtained from the products, produced amplicons of 690 bp each while the *egt* genes produced amplicons of 1290 bp each. The CrleGV-SA *egt* and *granulin* gene sequences obtained from the viral isolates were then aligned with Genbank sequences, using the BLAST option on the NCBI database (Internet source 3), to ascertain whether the obtained sequences were in fact *egt* and *granulin* genes.

When the amplified *granulin* genes obtained from Cryptex® and Cryptogran® were aligned with one another on the database, no nucleotide changes were observed between these product isolate sequences (Fig. 3.2.13.5 Appendix). There was a maximum nucleotide identity of 100% shared between Cryptex® and Cryptogran® *granulin* gene sequences. However there were three nucleotide changes which occurred between the Genbank CrleGV-SA *granulin* gene sequence (accession number: AY293731.1) and the product isolates (Fig. 3.2.13.5 Appendix). Both Cryptogran® and Cryptex® isolates showed a 99% maximum nucleotide identity when compared to the Genbank CrleGV-SA *granulin* gene sequence.

When the amplified *egt* genes obtained from Cryptex® and Cryptogran® were aligned with one another on the database, only four nucleotide changes were noted between these isolates. The maximum nucleotide identity shared between these product isolates was 99%. However, when Cryptogran® was aligned with the CrleGV-CV3 *egt* sequence (accession number: AY229987.1), there were twenty nucleotide changes which occurred between this product isolate and the Genbank CrleGV-CV3 isolate (Fig. 3.2.13.7 Appendix) and the nucleotide identity shared between these isolates was 97%. When Cryptex® was aligned with this Genbank

sequence, twenty four nucleotide changes occurred and the nucleotide identity shared between these alignments was also 97%. The amino acid sequences reveal no non-functional protein changes or stop codons.

The Genbank *granulin* gene which was obtained from the database was the SA variant of the CrleGV; our results indicate that there appears to be very little difference between this isolate and the isolates obtained from the products. When comparing the *egt* gene sequences of both products and the Genbank CrleGV-CV3 isolate, there were many observed nucleotide changes, indicating considerable differences and possible divergences between the SA variant and the CV variant of the CrleGV. The amplification of the conserved *granulin* and *egt* genes revealed some nucleotide changes which indicate evidence of mutation but do not provide data for isolate delineation.

These results do not seem to support the restriction enzyme analyses; according to the DNA profiles, Cryptex® closely resembles the SA isolate obtained by Fritsch while Cryptogran® resembles that obtained by Moore (2002), but Singh *et al* (2003) published a *granulin* gene sequence of the SA isolate which resembles that of the Cryptogran® isolate. It would seem that if any changes were likely, it would render the Cryptex® isolate different from Cryptogran®; however these isolates were identical in nucleotide and amino acid sequences. Replication of the PCR amplifications and sequencing of the *granulin* gene is considered important and would possibly narrow any error of misreading a particular nucleotide base.

Jehle & Backhaus (1994) reported that the *granulin* gene is highly conservative within the granuloviruses and forms the major component of the large protein capsule occluding these viruses. Any changes in the protein translation of this gene may render the occlusion body, which is vital for the environmental survival of these viruses, dysfunctional. This would have enormous evolutionary consequences for virus infectivity and host specificity. Results here indicated that both Cryptogran® and Cryptex® isolates showed a 99% nucleotide identity when compared to the Genbank CrleGV-SA *granulin* sequence. There were however many more nucleotide changes observed in the *egt* gene alignments, particularly when the SA isolates were compared against the CV3 isolate, indicating considerable divergence of these geographically remote viral isolates, despite them being obtained from the same host species.

No amino acid changes occurred between the alignments of the *granulin* protein sequences of Cryptex® and Cryptogran® viral isolates (Fig. 3.2.13.6 Appendix) and there was 100% amino acid identity obtained from a BLAST query on the database. When either Cryptex® or Cryptogran® translated *granulin* protein sequences were aligned with the CrleGV-SA *granulin* Genbank protein sequence there were no observed differences between these amino acid sequences and the results of the BLAST query revealed 100% amino acid identity.

In the determination of the *egt* protein sequences however, there were two amino acid changes which occurred between the Cryptex® and Cryptogran® isolates (Fig. 3.2.13.8 Appendix). Further, there were seven amino acid changes observed when the Cryptex® isolate was compared to *egt* protein sequence of the CrleGV-CV3 isolate (Fig. 3.2.13.8 Appendix). Cryptogran® on the other hand showed six amino acid changes when compared to the CrleGV-CV3 isolate. A BLAST query indicated a 98% amino acid identity between both product isolates and the Genbank *egt* protein sequence of the CrleGV-CV3 isolate.

Protein translation revealed several amino acid changes which indicated that the *egt* gene is less conservative than *granulin*. *Egt* encodes an ecdysteroid UDPglucosyltransferase which catalyses the transfer of glucose from UDPglucose to ecdysteroid insect molting hormones (O'Reilly & Miller, 1990), expression of this gene product allows the virus to block molting and pupation of infected larvae. However, the importance of this gene is questionable as many biopesticides are now genetically engineering baculoviruses without a functional *egt* gene; causing larvae to molt and cease feeding more rapidly, thus increasing crop yield and biopesticide control. There appears to be a greater variation among the lepidopteran *egt* genes compared with *granulin* and this is observed in the number of amino acid changes obtained in this study. Despite these amino acid changes, the translated protein sequences of Cryptex® and Cryptogran® are identical and do not indicate any data which may support isolate delineation.

#### Determination of product isolate pathogenicity using dose-response bioassays

The computer software program, PROBAN (Van Ark, 1995) transformed the viral doses to log<sub>10</sub> and the percentage response to empirical probits thus the sigmoid curves obtained from these reactions to the biopesticides became straight lines (probits) which were then compared.

The regression or probit lines were fitted to the corrected data in Table 3.2.13.2 and had the equations,  $y = 1.306 + 0.940x$  (standard error (SE) of slope = 0.118) for Cryptex® and  $y = 1.329 + 1.017x$  (SE of slope =

0.113) for Cryptogran®. The  $\chi^2$  test used to determine if the fit of the probit lines were acceptable used the test level set at  $\alpha = 0.05$  and deviations from these two probit lines were considered homogenous. This implied that the deviations of the observed mortalities from the expected larval mortalities were no larger than can be expected from normal sampling variation (expected mortalities were not very large or very small).

The five calculated viral doses (OBs/ml) used per product, as well as the percentage larval mortality per dose, is shown in Table 3.2.13.2. The table illustrates combined data obtained from four replicated experiments; larval percentage mortality and the standard errors (SE) are shown. Approximately 20% of the control insects died per control per product treatment per experiment and are shown in the table. The empirical probits per dose (treatment) are also recorded but no standard errors were shown for these or the corrected control mortality because these values were obtained from the PROBAN analysis.

**Table 3.2.13.2.** Mean number ( $n \pm SE$ ) of neonate FCM larval percentage mortality in dose-response bioassays with five concentrations of CrleGV-SA. PROBAN corrects for percentage control mortality and empirical probits, thus no SE were determined for these values. The data presented is the combination of four bioassay replicates using a total of 100 larvae tested.

Combined replicates	Cryptogran			Cryptex		
	Larval mortality (%)	Corrected for control mortality (%)	Empirical probit (%)	Larval mortality (%)	Corrected for control mortality (%)	Empirical probit (%)
Control	20.00 $\pm$ 0.00	NA	NA	20.00 $\pm$ 0.00	NA	NA
1.221 x 10 <sup>2</sup>	27.00 $\pm$ 0.40	8.75	3.644	25.00 $\pm$ 0.25	6.25	3.466
6.104 x 10 <sup>2</sup>	37.00 $\pm$ 0.70	21.25	4.202	28.00 $\pm$ 0.63	10.00	3.718
3.050 x 10 <sup>3</sup>	52.00 $\pm$ 0.40	40.00	4.747	50.00 $\pm$ 0.00	37.50	4.681
1.520 x 10 <sup>4</sup>	80.00 $\pm$ 0.85	75.00	5.674	67.00 $\pm$ 0.40	58.75	5.221
7.630 x 10 <sup>4</sup>	92.00 $\pm$ 0.25	90.00	6.282	85.00 $\pm$ 0.47	81.25	5.887

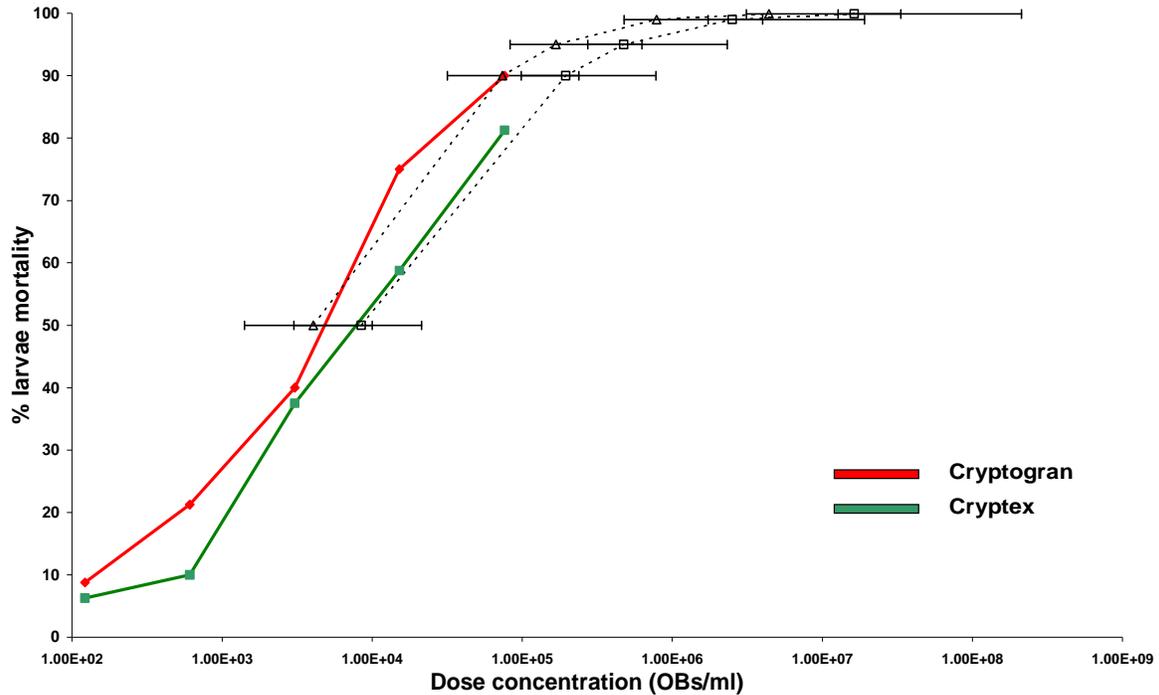
G is used in the calculation of the fiducial limits; the deviations from the probit lines were considered homogenous and G was determined to be 0.0477 and 0.0610 for Cryptogran® and Cryptex® respectively. Moore (2002) states that if these values are larger than approximately 0.025 it means that the variation of the mortalities is rather large, as is seen with these results, however he also states that experimental procedures or the value of the probit lines need only come into question when G exceeds 0.25, which is not the case here.

The variances of the two lines were considered homogenous (F-value= 47.47; p= 0.8920) and the slope of the probit lines were subsequently comparable. The  $\chi^2$  test showed the lines to be parallel and their elevations to be comparable ( $\chi^2= 3.841$ ; p= 0.6440). The elevations of these probit lines were shown to be significantly different from one another (F-value= 5.59; p = 0.003); the greater elevation of the Cryptogran® isolate probit line infers that this isolate may be more pathogenic than the Cryptex® isolate because it requires a slight increase in viral concentration to elicit a increased response from larvae, whereas a slight increase in Cryptex concentration elicits a slower response from larvae.

The LC<sub>50</sub> and LC<sub>90</sub> for Cryptogran® were estimated to give 4.054 x 10<sup>3</sup> OBs/ml and 7.372 x 10<sup>4</sup> while the LC<sub>50</sub> and LC<sub>90</sub> for Cryptex® were estimated to be 8.460 x 10<sup>3</sup> OBs/ml and 1.950 x 10<sup>5</sup> OBs/ml (Fig. 3.2.13.3). The 95% fiducial limits of the LC<sub>50</sub> and LC<sub>90</sub> of Cryptogran® were estimated to range from 2.645 x 10<sup>3</sup> - 5.933 x 10<sup>3</sup> and 4.210 x 10<sup>4</sup> - 1.648 x 10<sup>5</sup> OBs/ml respectively. Furthermore, the 95% fiducial limits of the LC<sub>50</sub> and LC<sub>90</sub> of Cryptex® were estimated to range from 5.443x 10<sup>3</sup>- 1.291 x 10<sup>4</sup> and 9.646 x 10<sup>4</sup> - 5.822x 10<sup>5</sup> OBs/ml respectively. Larval mortality never reached 100% and the control mortality was never zero, this implies that the LC<sub>90</sub> values from both Cryptogran® and Cryptex® is an estimation of the actual values and thus explains the large variation in the fiducial limits (confidence intervals) nearer this value.

Figure 3.2.13.3 indicates that Cryptogran® (red) lies further to the left and has a greater elevation ( $y = 1.329 + 1.017x$ ). This implies that it requires less product concentration to kill the same amount of neonate larvae than Cryptex® (green) which shows a flatter elevation ( $y = 1.306 + 0.940x$ ). This flatter elevation value may indicate slower absorption or a slower mode of action by the Cryptex® viral isolate which elicits a significantly (F-value= 5.59; p = 0.003) delayed response in neonate FCM larvae than the Cryptogran® viral isolate. Although the difference in LC<sub>50</sub> values, which are considered the standard determination of response frequency, appears insignificant due to the overlap of the fiducial limits, the significant difference between the

probit line elevations suggests that the viral virulence's are different and supports the heterogeneity of these distinct viral isolates.

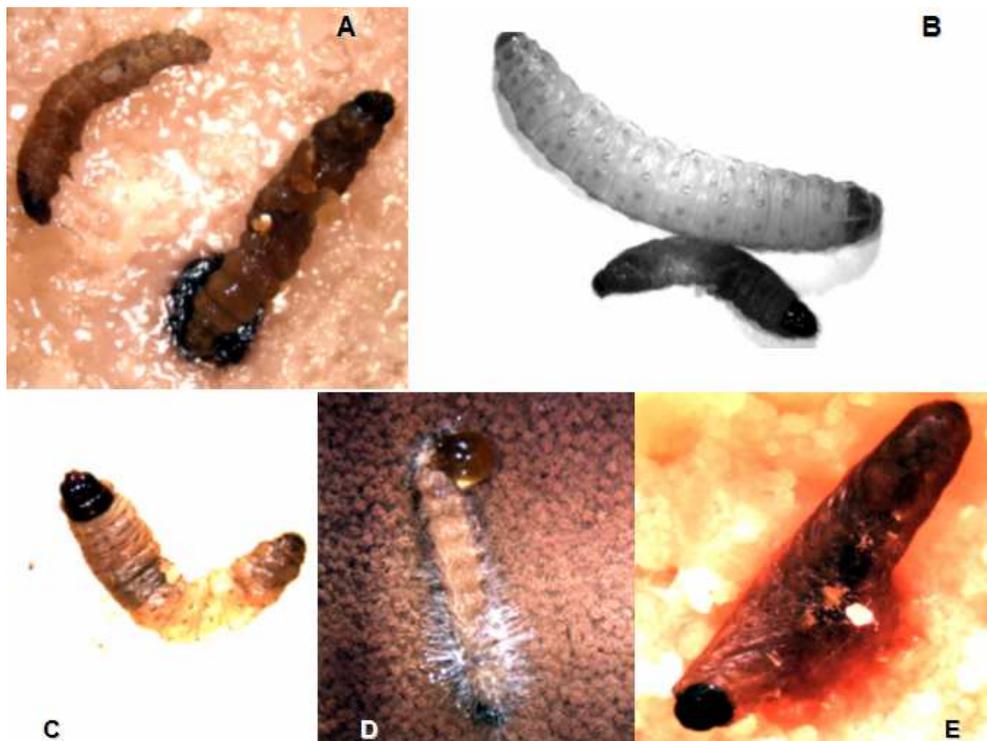


**Figure 3.2.13.3.** Dose-response of one hundred *FCM* neonate larvae to five concentrations of CrleGV-SA in the biological control products Cryptogran® (red) and Cryptex® (green). The LC<sub>50</sub>- LC<sub>99.9</sub> have been plotted with the upper and lower fiducial limits of the dose shown as horizontal bars on the graph.

The LC<sub>50</sub> and LC<sub>90</sub> values revealed for Cryptogran® in this preliminary study were similar to those reported by Moore (2002) ( $4.095 \times 10^3$  and  $1.185 \times 10^5$  OBs/ml respectively), when he investigated the pathogenicity of CrleGV-SA, highlighting the similarity of Cryptogran® to that of Moore's SA isolate. In Fritsch's study (1989), other dose-response tests recorded with CrleGV against neonate larvae revealed LC<sub>50</sub> values to range from  $3.93 \times 10^3$  to  $5.35 \times 10^3$  OBs/ml and LC<sub>90</sub> values to range from  $6.83 \times 10^4$  to  $1.86 \times 10^5$  OBs/ml respectively between the SA, IC and CV variants. The Cryptex® isolate showed similar biological activity to that recorded in Fritsch's study (LC<sub>50</sub> and LC<sub>90</sub> values of  $8.460 \times 10^3$  OBs/ml and  $1.950 \times 10^5$  OBs/ml respectively); once again reiterating the probability of this isolate being the same SA isolate that Fritsch reported in her study.

### Symptomatology

*FCM* larvae showed morphological infection which is characteristic of CrleGV. Neonate larvae were most often noticed dead at higher virus concentrations, presumably because they came into contact with OBs sooner, due to the prevalence of the virus. Second and third instar cadavers were also noted with infection but no observation was made of fourth and fifth instar larval infection. This could be because bioassay trays were checked after several days rather than after two weeks, not allowing sufficient larval growth to observe these later instar infections, which would take longer to manifest than would be the case with the younger instars. Larval bodies appeared flaccid and glossy, often turning black upon infection (Fig. 3.2.13.4 A, C and E). In the latest stages of infection, larval cadavers were often noticed disseminating fluid, which was presumably, loaded with viral occlusion bodies (Fig. 3.2.13.4. A and E). Most infected larvae either moved to the surface of the diet upon which they were feeding or were observed hanging upside down on the paper towelling or the sides of the bioassay trays. There was no observed difference in the symptomatology of the two viral isolates obtained from Cryptogran® and Cryptex® when *FCM* larvae were infected.



**Fig. 3.2.13.4.** (A) Symptomatically CrleGV-SA infected third instar FCM larvae disseminating viral OBs, (B) Comparison of a healthy fourth instar larva (above) against an infected third instar larva (below), (C) Third instar larva showing flaccid and darkening body, (D) Infected second instar larva on a mat of *Aspergillus niger* spores, riddled with fungal hyphae, (E) Characteristic disseminating third instar larval cadaver. (Images captured using a Leica®, Johannesburg, dissecting microscope).

### Conclusions

The biopesticide products, Cryptogran® and Cryptex® were registered to have been formulated with the SA variant of CrleGV, however there is sufficient genetic and biological evidence to support the distinction of these viral isolates and their delineation in the form of isolate numbers should be considered for future formulations. Cryptogran® has been formulated with a genotypically distinct isolate of the SA variant and the results from the surface-dose bioassays highlighted that this genotype is significantly more pathogenic against a laboratory culture of FCM, based on the elevations of the probit lines. Cryptogran® was shown to be more pathogenic in surface-dose bioassays as it required a slight increase in viral concentration to elicit an increased response from FCM larvae, whereas a slight increase in Cryptex® concentration elicited a slower response from larvae. The differences in the pathogenicity observed in the laboratory may not result in significant differences in pathogenicity between these products in the field. Laboratory work is often considered more conservative because external factors such as host population (origin, fitness, resistance) and environmental factors (temperature, humidity, diet) are not considered, however further testing is needed to resolve this. Based on these initial results it appears that the Cryptogran® isolate has a faster speed of kill, which has been a perceived disadvantage of baculovirus biopesticides in general. However this needs to be assessed using time-response bioassays. Empirical testing of the speed of kill between Cryptex® and Cryptogran® using time-response bioassays is currently underway at Rhodes University. The improvement of biopesticide formulation by selecting geographically virulent isolates should have considerable implications for this market as it would increase the effectiveness of the product and reduce the amount of product required per hectare to attain this level of control.

### Future research

Time-response bioassays, which would aid in determining the speed of kill of Cryptex® and Cryptogran® isolates is currently being investigated. Also, additional surface-dose response bioassays using Cryptogran® and Cryptex® are currently underway. Future studies should try to identify precisely how many isolates of the SA variant of CrleGV exist geographically by surveying orchards and characterising obtained viral isolates; empirical testing of these isolates should be undertaken so that the biopesticide formulation may be improved. Future evolutionary considerations however should include resistance studies. It may be prudent to study the genetic mechanisms of insect resistance and also to investigate the possibilities of formulating

control products with multiple isolates of the virus, so that resistance to any one particular strain may be slowed.

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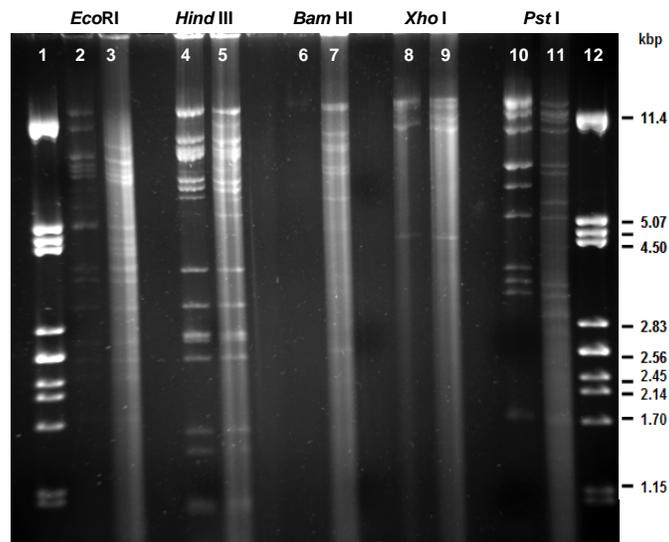
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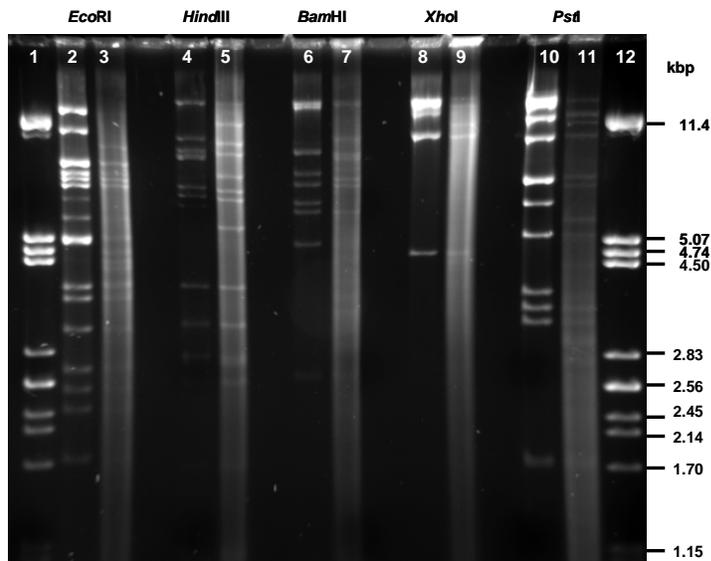
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**Appendix**

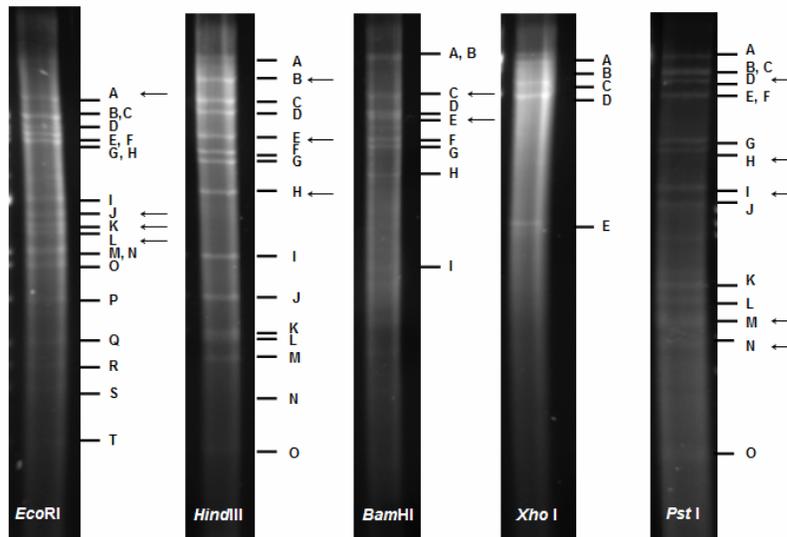
**Characterisation of viral isolates using RFLP and agarose gel electrophoresis**



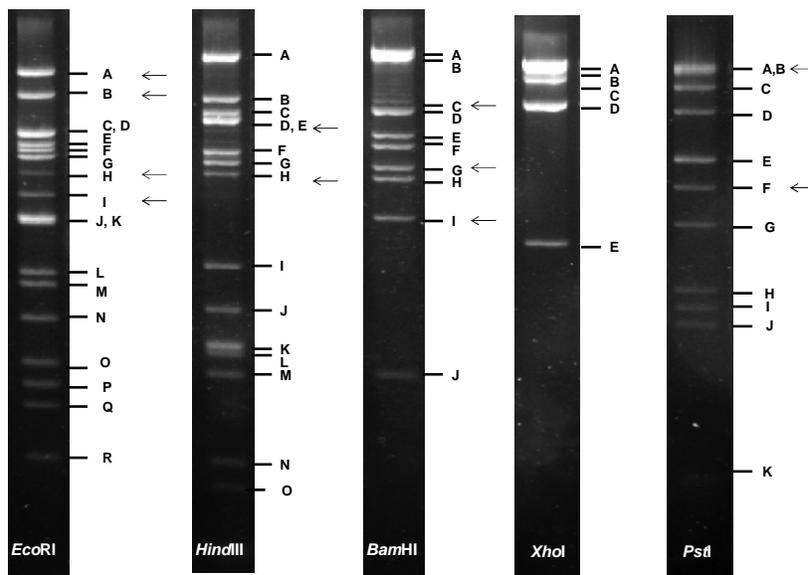
**Fig. 3.2.13.5.** Restriction digest DNA profiles of two CrleGV-SA isolates from Cryptex (lanes 2, 4, 6, 8 and 10) and Cryptogran (lanes 3, 5, 7, 9 and 11). A  $\lambda$ Pst molecular marker was loaded in lanes 1 and 12 on a 1% agarose gel run at 30V for 16 hours.



**Fig. 3.2.13.6.** Restriction digest DNA profiles of two CrleGV-SA isolates from Cryptex (lanes 2, 4, 6, 8 and 10) and Cryptogran (lanes 3, 5, 7, 9 and 11). A  $\lambda$ Pst molecular marker was loaded in lanes 1 and 12 on a 1% agarose gel run at 30V for 16 hours.



**Figure 3.2.13.7.** Restriction enzyme DNA profiles of CrleGV-SA isolated from Cryptogran®. Letters correspond to restriction fragments generated and arrows indicate fragments which are not present in the Cryptex® CrleGV-SA isolate.



**Figure 3.2.13.8.** Restriction enzyme DNA profiles of CrleGV-SA isolated from Cryptex®. Letters correspond to restriction fragments generated and arrows indicate bands which are not present in the Cryptogran® CrleGV-SA isolate.

**Table 3.2.13.3.** Estimated sizes in kbp of restriction fragments of singly digested CrleGV-SA DNA isolated from Cryptogran®, designated (\*) and Cryptex®. Fragments are labelled in order of decreasing size, **A** being the largest fragment. NA refers to bands which could not be estimated due to  $\lambda$ Pst. Values highlighted in bold correspond with arrows in Figures 3.2.13.2.

Fragment	<i>EcoR1</i> *	<i>Cryptex EcoR1</i>	<i>HindIII</i> *	<i>Cryptex HindIII</i>	<i>BamH1</i> *	<i>Cryptex BamH1</i>	<i>Xho1</i> *	<i>Cryptex Xho1</i>	<i>Pst</i> *	<i>Cryptex</i>
A	8.85	NA	NA	NA	NA	NA	NA	NA	NA	NA
B	8.24	<b>9.77</b>	<b>10.2</b>	9.68	NA	NA	NA	NA	NA	NA
C	8.12	8.24	9.24	9.01	<b>10.97</b>	<b>9.50</b>	NA	NA	NA	NA
D	7.79	8.12	8.60	<b>8.70</b>	9.65	8.93	NA	NA	<b>NA</b>	9.37

E	7.56	7.79	<b>7.69</b>	<b>8.55</b>	<b>9.55</b>	7.96	4.95	4.86	9.37	7.46
F	7.46	7.56	7.16	7.29	7.86	7.42			9.24	<b>6.57</b>
G	7.26	7.26	6.76	6.92	7.45	<b>6.68</b>			7.36	5.62
H	7.16	<b>6.85</b>	<b>5.87</b>	<b>6.56</b>	6.12	6.33			<b>7.16</b>	4.17
I	5.47	<b>6.20</b>	4.29	4.08	2.61	<b>5.27</b>			<b>5.95</b>	3.83
J	<b>5.09</b>	4.27	3.52	3.30		2.42			5.47	3.57
K	<b>4.87</b>	5.39	3.01	2.76					3.94	1.71
L	<b>4.61</b>	4.29	2.97	2.67					3.62	
M	4.29	3.99	2.61	2.36					<b>3.41</b>	
N	4.23	3.41	1.61	1.52					<b>3.09</b>	
O	3.99	2.76	1.48	1.29					1.63	
P	3.41	2.47								
Q	2.72	2.23								
R	2.50	1.71								
S	2.20									
T	1.68									
U										
V										
W										
X										

### 3.3 PROJECT: FRUIT FLY

Project coordinator: Tim G Grout (CRI)

#### 3.3.1 Project summary

The status of fruit fly as a phytosanitary pest continues to increase so the large amount of money being spent on this pest is readily justified. All three economically-important species of *Ceratitis* in southern Africa continue to be reared at CRI in Nelspruit and some small experiments to modify the diet for Natal fruit fly were conducted (3.3.2). The phase 4 of cold disinfestation research involving temperatures above 0°C for the post-harvest control of Medfly in fruit destined for Japan was repeated at a mean temperature of 1°C for 16 days. The treatment was successful and there were no survivors. The report has been submitted for consideration by the Japanese (3.3.3). Attempts to find alternatives to organophosphates and carbamates as toxicants in fruit fly baits have suggested that imidacloprid and fipronil are the most promising. However, high control mortalities after 48 hours required changes to the technique and attempts to develop a means of evaluating slow-acting toxicants in the field (3.3.4). No further progress has been achieved by CSL in the UK in developing a rapid diagnostic test for Medfly larvae so this agreement will be terminated (3.3.5). Attempts to avoid residues from fruit fly bait applications by applying the bait to the tree trunk rather than the foliage and fruit were not successful and a small investigation of artificial substrates onto which bait could be sprayed indicated that non-absorbent surfaces were best (3.3.6). Various comparisons of lures showed that Capilure and Ceratitislure were more effective than 3-component lure for male Medfly and male marula fruit fly, respectively. However, Capilure was at least three times less effective for Natal fruit fly males than for Medfly males. The 3-component lure was more effective than Questlure in catching females of all three fly species (3.3.7). The results from monitoring Natal fruit fly at different locations throughout southern Africa are showing a good correlation with climatic differences which suggest that it is a good candidate for modelling its potential global distribution based on climate (3.3.8). For a second time, fruit flies intercepted by PPECB at nine packhouses around the country were sent to CRI for identification. Only Medfly was recovered and most of these were from mandarins. This confirms that the control methods used for Natal fruit fly are adequate (3.3.9). With the possibility that some of our markets may request proof in the future that marula fruit fly is not a phytosanitary threat in our exports, further laboratory trials were conducted on the likelihood of oviposition and survival in citrus. Both likelihoods were extremely slight but research on an improved bait for all three *Ceratitis* species is being conducted because it is known that protein hydrolysate baits are not very effective against marula fruit fly (3.3.10). In a further attempt to learn more about the control of fruit fly without the risk of residues on fruit, the level of fruit fly infestation in blocks treated with M3 bait stations was compared with untreated blocks near Uitenhage. Although the numbers of fruit fly recovered in Capilure traps in the treated blocks did not decline, fruit fly damage and infestation was completely eliminated (3.3.11). Future research in this project will concentrate on improving control efficacy

for all *Ceratitis* species and conducting both pre- and post-harvest research in other countries on *Bactrocera invadens*, a fruit fly that may arrive in South Africa within the next two years.

## Projekopsomming

Die status van vrugtevlieg as 'n fitosanitiere plaag neem steeds toe en daarom kan die groot bedrae wat op hierdie plaag spandeer word maklik geregverdig word. Die teling van al drie die ekonomies belangrike spesies van *Ceratitis* in suider-Afrika word in Nelspruit voortgesit en verskeie klein eksperimente om die dieet van die Natalse vrugtevlieg te wysig, is uitgevoer (3.3.2). Die fase 4 van die koue disinfesteringsnavorsing met temperature bo 0°C vir die na-oes beheer van Medvlieg in vrugte wat vir Japan bestem is, is by 'n gemiddelde temperatuur van 1°C vir 16 dae herhaal. Die behandeling was suksesvol en daar was geen oorlewende nie. Die verslag is aan die Japanese vir oorweging voorgelê (3.3.3). Pogings om alternatiewe vir organofosfate en karbamate as gifstowwe in vrugtevlieg lokaas te vind het daarop gedui dat imidacloprid en fipronil die belowendste is. Wysigings aan die tegniek weens die hoë mortaliteit van die kontrole na 48 ure was egter nodig asook pogings om 'n manier vir die evaluering van stadig-werkende gifstowwe in die veld te ontwikkel (3.3.4). Geen verdere vordering is deur CSL in die VK gemaak met die ontwikkeling van 'n vinnige diagnostiese toets vir Medvlieg larwes nie en hierdie ooreenkoms gaan beëindig word (3.3.5). Pogings om residue van vrugtevlieg lokaas toedienings te verhoed deur die lokaas op die stam van die boom toe te dien in plaas van op die blare en vrugte was nie suksesvol nie en 'n klein ondersoek na kunsmatige substrate waarop lokaas gespuit kan word het getoon dat die nie-absorberende oppervlaktes die beste was (3.3.6). Verskeie vergelykings van lokaas het getoon dat Capilure en Ceratitislure meer effektief is as die 3-komponent lokmiddel vir Medvlieg mannetjies en Maroela vlieg mannetjies, onderskeidelik. Capilure was egter ten minste drie maal minder effektief vir Natalse vrugtevlieg mannetjies as vir Medvlieg mannetjies. Die 3-komponent lokmiddels was meer effektief as Questlure om wyfies van al drie die vrugtevlieg spesies te vang (3.3.7). Die Natalse vrugtevlieg moniteringsresultate vanaf verskillende plekke regdeur suider-Afrika het goeie korrelasie met die klimaatverskille getoon wat daarop dui dat dit 'n goeie kandidaat is vir modellering van globale verspreiding wat op klimaat gebaseer is (3.3.8). Vir 'n tweede keer is vrugtevlieë wat deur PPECB by 9 pakhuis landwyd onderskep is, vir identifikasie na CRI gestuur. Slegs Medvlieë is gevind en die meeste hiervan was in mandaryne. Dit bevestig dat die metodes vir beheer van Natalse vrugtevlieg voldoende is (3.3.9). Met die moontlikheid wat bestaan dat sommige van ons markte in die toekoms bewyse sal verlang dat maroela vlieg nie 'n fitosanitiere bedreiging is in ons uitvoere nie, is verdere laboratoriumtoetse op die moontlikheid van eierlegging en oorlewing in sitrus uitgevoer. Beide moontlikhede is uiters skraal maar navorsing op 'n verbeterde lokaas vir al drie *Ceratitis* spesies is uitgevoer omdat dit bekend is dat proteïen-hidrolisaat nie baie effektief teen maroela vlieg is nie (3.3.10). In 'n verdere poging om meer uit te vind aangaande die beheer van vrugtevlieg sonder die risiko van residue op vrugte is die vlak van vrugtevlieg besmetting in blokke naby Uitenhage wat met M3 lokvalle behandel is vergelyk met onbehandelde blokke. Alhoewel die getalle van die vrugtevlieë in Capilure lokvalle in die behandelde blokke nie afgeneem het nie, was daar geen vrugtevlieg skade en besmetting nie (3.3.11). Verdere navorsing in hierdie projek sal konsentreer daarop om die effektiwiteit van beheer vir alle *Ceratitis* spesies te verbeter en om beide voor- en na-oes navorsing in ander lande op *Bactrocera invadens*, 'n vrugtevlieg wat Suid-Afrika binne die volgende twee jaar kan binnekom, uit te voer.

### 3.3.2 PROGRESS REPORT: Rearing of fruit fly

Experiment 407 (1999 onwards) by John-Henry Daneel, Rooikie Beck and Tim Grout (CRI)

#### Summary

Cultures of *Ceratitis capitata*, *C. rosa* and *C. cosyra* are being maintained in Nelspruit to provide flies for research on baits and post-harvest treatments. Some fruit flies were supplied to other institutions during 2007/8 and more wild marula fruit fly were added to the culture of that species to improve the gene pool. Diet investigations showed that Natal fruit fly should not be reared at more than 1000/160 g larval medium and perhaps lower numbers should be investigated. A 2:1 ratio of carrot powder: Torula yeast plus 10% sugar resulted in better adult eclosion than a 5:1 ratio of carrot: yeast with 10% sugar. Rearing of all three species will continue.

#### Opsomming

Kulture van *Ceratitis capitata*, *C. rosa* en *C. cosyra* word in Nelspruit onderhou om in vlieë vir navorsing op lokaas en na-oes behandelings te voorsien. 'n Aantal vrugtevlieë is in 2007/8 aan ander instansies voorsien en meer wilde maroela vrugtevlieë is by die kulture gevoeg om die genepoel te verbeter. Ondersoeke na die dieet het getoon dat Natalse vrugtevlieg nie geteel moet word teen meer as 1000/160 g larwale medium nie en laer getalle moet dalk ondersoek word. 'n 2:1 verhouding van wortelpoeier: Torula gis plus 10% suiker het

in beter ontpopping van volwassenes tot gevolg gehad as in 5:1 verhouding van wortel:gis met 10% suiker. Die teling van al drie spesies sal voortgesit word.

### Collaboration with other researchers

The three cultures were successfully maintained during the year. Natal fruit fly pupae were sent to Dr. Carlos Caceres at the Agriculture and Biotechnology laboratory in Seibersdorf, Austria. Dr. Caceres is trying to crossbreed Natal fruit fly with Medfly in an attempt to rear sterile offspring. Natal fruit fly pupae were also sent to the Department of Conservation, Ecology and Entomology, Stellenbosch University, to assist Dr. Juanita Heunis with her studies. Natal fruit fly eggs (+/- 1 ml) placed in vials filled with water were sent to Dr. Brian Barnes, Coordinator: Sterile Insect Release Programme, ARC Infruitec-Nietvoorbij to establish an adult culture. Seven samples were sent in total.

### Natal fruit fly diet development

In order to improve the Natal fruit fly rearing technique a trial was conducted to establish the ideal number of eggs to use in a container to maximize the ultimate number and health of adults. Egg numbers of 1000, 2000, 3000, 4000 and 5000 were placed on 160 g medium (2:1 dry carrot powder: Torula yeast).

After applying the correct number of eggs to each container, the eggs were allowed to hatch, form larvae and develop into pupae. The results indicated that there was a slight but non-significant ( $P > 0.05$ ) change in the male: female ratio with increasing egg numbers (Table 3.3.2.1). In containers with 1000 eggs, the male: female ratio was on average 1.09:1. This gradually changed to more females per container as the number of eggs in the containers increased. In the containers with 5000 eggs the ratio was 0.95:1. It was also noticed that with the higher numbers of eggs per container the percentage of pupae eclosing to adults increased, even though the percentage of larvae forming pupae, dropped. This could be due to the larvae benefiting from the extra protein derived from other dead, decomposing larvae.

As expected, increasing egg numbers resulted in smaller pupae being produced (Table 3.3.2.1). A volume (or weight) loss was already significant ( $P < 0.05$ ) between 1000 and 2000 suggesting that less than 1000 should be evaluated and that more work is needed in creating a correctly balanced medium. This was also confirmed by the poor eclosion rate, which was on average 48.8% per container.

**Table 3.3.2.1.** Pupal volumes after rearing larvae at different densities in 160 g medium (2:1 carrot: yeast)

Larvae per 160 g medium	Sex ratio (♂/♀)	Pupal volume (mm <sup>3</sup> )
1000	1.09 a	16.14 d
2000	1.00 a	15.11 c
3000	0.99 a	12.73 b
4000	0.98 a	10.87 a
5000	0.95 a	10.88 a

Means in the same column followed by a different letter are significantly different at  $P = 0.05$  (SNK test)

It was thought that the failure of many Natal fruit fly adults to emerge successfully from the pupal cases may be due to a lack of energy. A preliminary laboratory trial was therefore conducted where 18% sugar was added to the dry larval diet (2:1 carrot: yeast). The percentage eclosion in four replicates of two hundred pupae each was determined after the larvae had been on medium with either no sugar (normal diet) or with 18% sugar. In the sugar-enriched diet, eclosion varied between 47% and 68% with an average of 57%. In the normal diet the average eclosion rate was 54% and varied between 40% and 67%. There was therefore no obvious improvement with the addition of this amount of sugar.

A third trial was conducted to see if an increase in the ratio of the carrot powder to yeast in the larval medium would improve adult eclosion. Six containers each of two ratios (2:1 and 5:1) were compared. In another six containers of each ratio, 10% sugar was added and in another six of each ratio, 20% sugar was included. The increase in the amount of carrot powder decreased the emergence rate, although this was only significant when comparing mixtures with 10% sugar (Table 3.3.2.2). With the 2:1 ratio the emergence rate improved slightly when 10% sugar was added but this improvement was not significant ( $P > 0.05$ ).

**Table 3.3.2.2.** Differences in adult Natal fruit fly eclosion from larval diets with variations in carrot powder and sugar content.

Treatments	Eclosion (%)
Carrot: yeast 2:1, no sugar	80.2 ab
Carrot: yeast 2:1, 10% sugar	85.0 b
Carrot: yeast 2:1, 20% sugar	78.5 ab
Carrot: yeast 5:1, no sugar	69.7 a
Carrot: yeast 5:1, 10% sugar	69.6 a
Carrot: yeast 5:1, 20% sugar	66.0 a

Means followed by a different letter are significantly different at P=0.05 (SNK test)

### Culture maintenance

Marula fruit infested with fruit fly (*Ceratitis cosyra*) were collected in the Nelspruit-area. The fruit was placed in crates lined with plastic bags. The base of the crate was then covered with coarse sand and wire mesh baskets were placed upside-down on top of the sand. Fruit was then evenly distributed on top of the baskets to avoid the sand becoming saturated with moisture. The sand was sieved once a week to ease the collection of the pupae from the sand. A total of 7429 pupae were collected over a month and placed in a new adult cage. New emerging adults from the insectary were added to the cage to help with egg laying. Eggs collected from this cage will be used to maintain the colony.

### Conclusion

Fruit flies were supplied to some other institutions and more wild marula fruit fly were added to the culture of that species. Diet investigations showed that Natal fruit fly should not be reared at more than 1000/160 g larval medium and a 2:1 ratio of carrot powder: Torula yeast plus 10% sugar resulted in better adult eclosion than a 5:1 ratio with 10% sugar.

### Future research

All cultures will continue to be maintained for various pre- and post-harvest research purposes.

### Technology transfer

A poster depicting the rearing technique used for Natal fruit fly was displayed at the 2006 Citrus Research Symposium but generally this work is not of interest to growers because it just provides flies for other research.

### 3.3.3 FINAL PROGRESS REPORT: Cold disinfestation of Medfly-infested lemons, grapefruit, oranges and Clementines using temperatures above 0°C

Experiment 772 (2004-2007) by Peter Stephen, John-Henry Daneel, Rooikie Beck and Tim Grout (CRI)

### Summary

In order to increase the temperature used for in-transit cold disinfestation of *Ceratitis capitata* for all citrus going to Japan, research is being conducted with temperatures above 0°C. Earlier research had shown that there were no significant differences in susceptibility of young Medfly larvae when using different types of citrus so the following Phase 4 disinfestation trial was conducted with Valencia oranges using a 16-day period at a mean temperature of 1°C. Three replicates were conducted, requiring a total of 1548 fruit in the control and 6206 fruit in the treatment. The mean control mortality was 8% of 21 801 larvae and the cold treatment mortality was 100% of 71 756 larvae. This treatment therefore exceeds the Probit 9 level of assurance and steps will be taken to develop this into a new export protocol for Japan.

### Opsomming

Om die temperatuur wat gebruik word vir die in-transit koue disinfestasië van *Ceratitis capitata* vir alle sitrus na Japan te verhoog, is navorsing met temperature bo 0°C gedoen. Vroeëre navorsing het getoon dat daar geen noemenswaardige verskille is in die vatbaarheid van jong Medvlieg larwes met verskillende sitrus tipes is nie. Die volgende fase 4 disinfestasiëproef is met Valencia lemoene vir 'n 16-dag periode, by 'n gemiddelde temperatuur van 1°C uitgevoer. Drie herhalings wat 'n totaal van 1 548 vrugte vir die kontrole en 6 206 vrugte vir die behandeling benodig het, is uitgevoer. Die gemiddelde mortaliteit van die kontrole was 8% van 21 801 larwes en die mortaliteit van die koue behandeling was 100% van 71 756 larwes. Die

behandeling oorskry dus die Probit 9 sekerheidsvlak en stappe om hierdie as 'n nuwe uitvoerprotokol vir Japan te ontwikkel, sal geneem word.

## **Introduction**

Due to chilling injury problems when using the current cold disinfestation protocol for Japan research is being conducted in order to apply for a longer treatment period at a higher temperature as used by some other countries that export citrus to Japan. Earlier phases of this research have been conducted with various citrus cultivars and indicated that younger larvae (6-d-old) were most tolerant to cold treatments in oranges (Ware et al. 2006) and Clementine mandarins (Ware et al. 2000) but that there was no significant difference between susceptibilities of different ages of larvae when cold treated in lemons or grapefruit (Ware et al. 2006). Due to the availability of Valencia oranges over a long period and the fact that they are less susceptible to post-harvest decay than Clementine mandarins, Valencia oranges were chosen for the phase four evaluation. This research therefore covers the phase 4 evaluation conducted with Valencia oranges at a mean core temperature of 1°C.

## **Materials and methods**

*Fruit type:* Small Valencia oranges 70-80 mm in diameter were used. Fruit preparation entailed the removal of the calyces and dipping in a combination of Sporekill (didecyl, dimethyl, ammonium chloride) (100 ml/100 l water) and guazatine (480 ml/100 l water) for one minute to surface sterilize and control fungal growth in inoculated fruit. At least two thousand five hundred fruit were treated in each of three replicates.

*The work area:* The entire work space of 60 m<sup>2</sup> (Fig. 3.3.3.1) and the surrounding area of ± 160 m<sup>2</sup> were sprayed with a high pressure sprayer using 100 ml/100 l Sporekill to surface-sterilize all working surfaces. A solution of guazatine was used for sterilizing all apparatus in contact with the fruit, and workers dipped their gloved hands in this solution before handling fruit.

*Test insects:* Mediterranean fruit fly (*Ceratitidis capitata* [Weidemann]) reared at the CRI facility in Nelspruit, were used to produce the eggs for inoculating test fruit. A minimum of 30 000 larvae exposed to the cold treatment is required to determine whether there are any survivors.

*Inoculation:* Eggs were collected within 24 h of oviposition and placed in deionized water. The egg/water ratio was adjusted until the number of eggs per 0.025 ml aliquot removed using an automatic pipette, was ± 40 which were then placed in a 5 mm-diameter hole drilled ± 30 mm deep into the fruit from the calyx end. Before the eggs were inserted, a small quantity of yeast was placed in the fruit using a syringe to act as a protein source for the developing larvae. The hole was plugged with cotton wool before it was sealed using molten wax. At least two thousand five hundred Valencia oranges were inoculated with ± 40 eggs each on 15 August 2007. The second replicate was inoculated on 14 September and the third replicate was inoculated on 4 October 2007.

*Incubation:* The fruit was individually placed into brown paper bags and packed into plastic crates (lug boxes) at 50 per crate. All fruits were systematically packed into these crates by loading the total number of fruit (2 500) into 50 crates two at a time per crate until the total (50 fruit) was reached. This ensured that fruit were shared equally among the crates as they completed the inoculation process. A few extra fruit were added to each crate to ensure that correct numbers of fruit were obtained. These crates were then placed in a temperature-controlled room at 26°C to boost larval development (Fig. 3.3.3.2). This primary incubation period lasted for 6 d (or 144 h) after which the fruit were removed and 2 000 fruit (40 crates) were transferred to the cold disinfestation room and the remaining 500 were retained as controls and dissected in the laboratory to determine the number of live larvae per fruit (ideally an average of 5+ larvae per fruit). Approximately 20 000 larvae were used in each of three replicates.



**Figure 3.3.3.1.** Laboratory used for inoculations that was sprayed with Sporekill before hand.



**Figure 3.3.3.2.** Fruit after inoculation wrapped in individual paper bags and stored in crates for primary incubation. Note datalogger at top left.

*Cold disinfestation:* Thermoprobes were calibrated before each disinfestation session using the freezing point method where the probes were immersed in melting ice and the temperature recorded when they reach equilibrium. A thermometer immersed in the melting ice was used to confirm the temperature. At least three calibration runs were conducted and the mean result for each probe was used for correction purposes. Calibration was done immediately prior to any of the tests being conducted. Temperatures were recorded at the inlet and outlet of the cooling coil in the room and from 14 probes placed 30 mm inside fruit that were

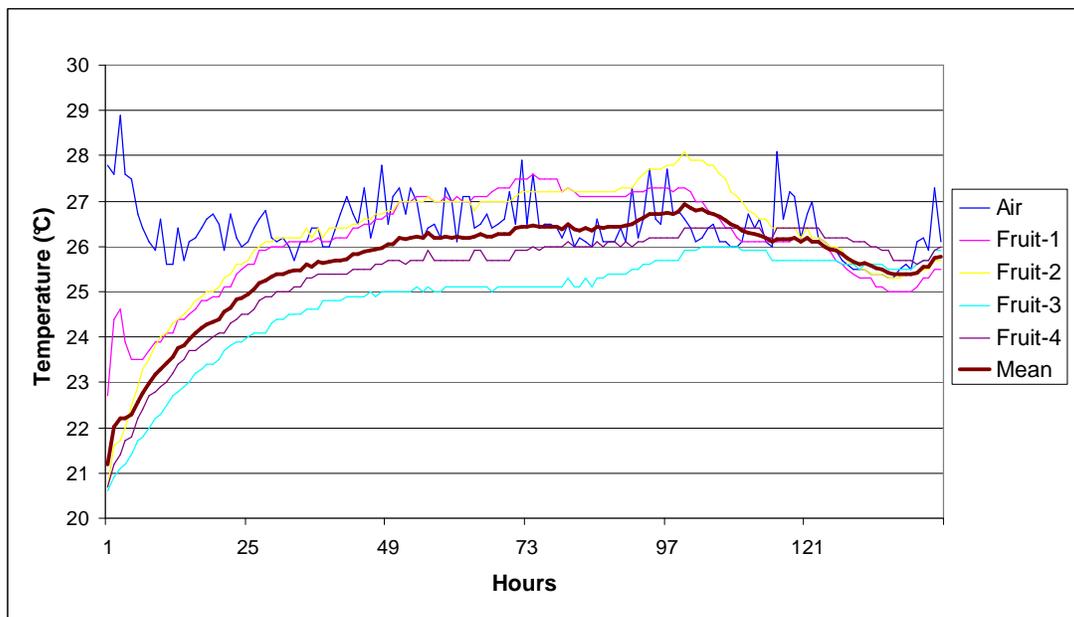
arbitrarily scattered amongst the fruit in the crates. The positions of the latter probes were recorded. Readings were recorded hourly on a Grant Squirrel data logger. The 16 d cold period was deemed to have started when at least 7 of the 14 fruit-core probes reached a temperature of 1.0°C. This was approximately 50 hours after the fruit was moved into the cold room. The fruit were removed 15 d and 23 h later and transferred to another room held at 26°C. The disinfestation period was intentionally terminated 1 h early to prevent any variance in interpretation of temperature records from extending the period beyond 16 d.

**Evaluation:** After a secondary incubation period of at least 48 h at 26°C the fruit were dissected and the numbers of live and dead larvae recorded. The actual numbers of insects were used rather than an estimate based on the numbers found in the control fruit.

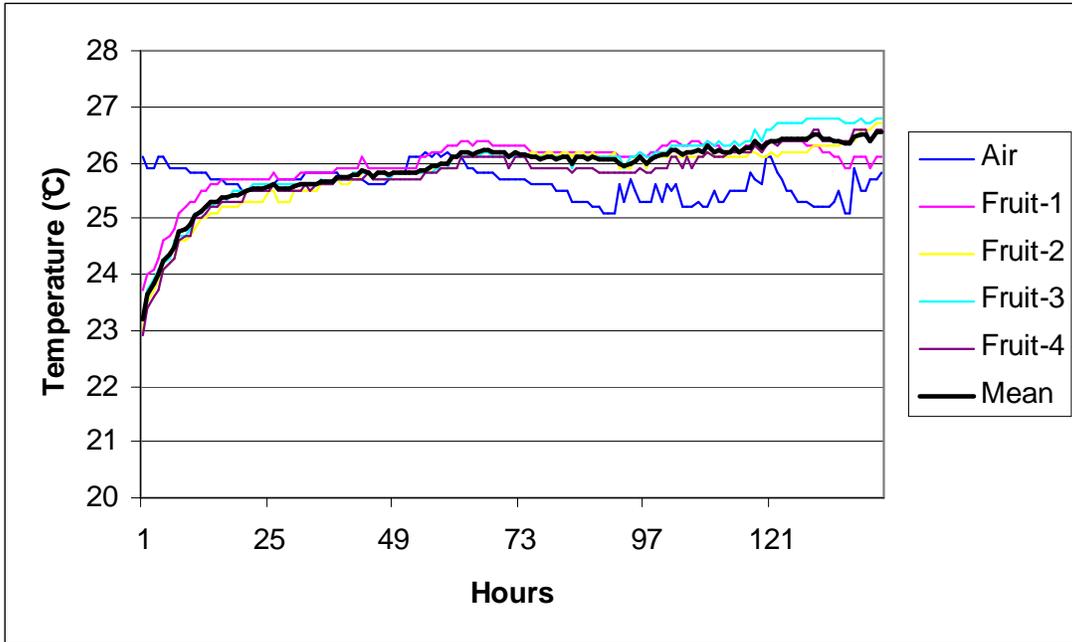
**Temperature data:** Temperatures were recorded in 4 fruit in all replicates during the primary incubation periods at 26°C to confirm that the fruit was being held at the correct temperature for larval development. During the cold disinfestation treatments, temperatures were recorded within 14 fruit as described above. The mean temperature of these 14 probes and the highest and lowest probe readings were plotted for each replicate. Temperatures were also recorded in the first replicate of the secondary incubation period to demonstrate that the fruit are being removed for dissection well after they have reached a temperature of 26°C.

## Results and discussion

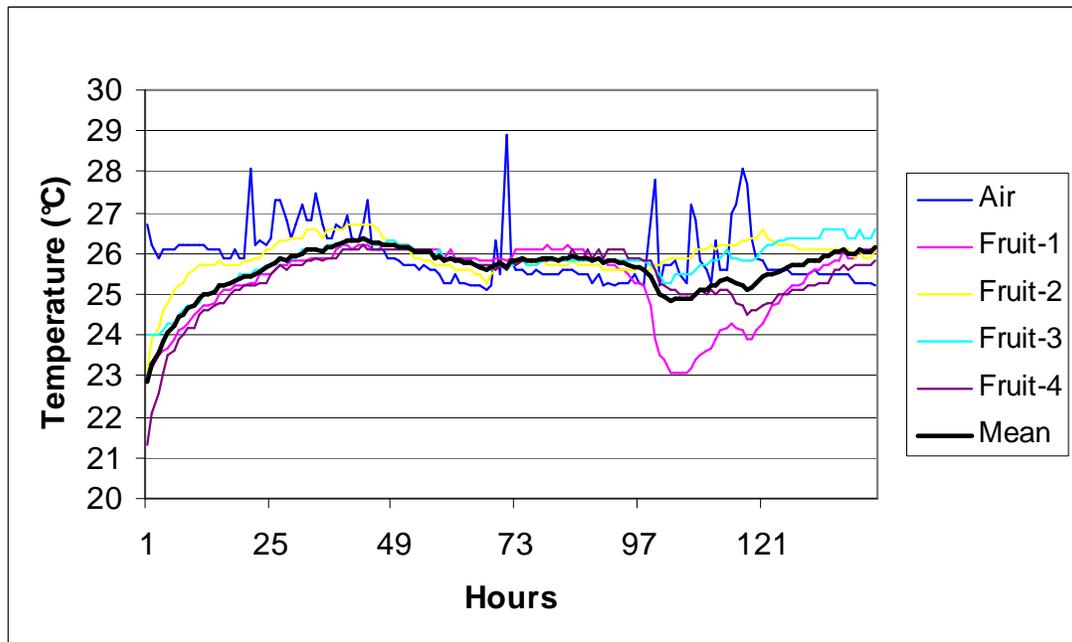
In the primary incubation period of the first replicate, a mean fruit temperature of 26°C was reached after 46 h (Fig. 3.3.3.3). In the primary incubation period of the second and third replicates, it took 58 h and 30 h, respectively, to reach 26°C (Figs. 3.3.3.4 and 5). In the first replicate of the cold treatment, the requirement for 7 or more probes to be at 1°C or below was reached after 53 h (Fig. 3.3.3.6). In the second replicate this took 51 h (Fig. 3.3.3.7) and in the third replicate, 51 h (Fig. 3.3.3.8). Thereafter the temperatures were very stable at a mean of 1.048°C in replicate 1, 1.040°C in replicate 2 and 1.042°C in replicate 3. When fruit was removed from the cold treatment, a mean temperature of 26°C was achieved after 36 h in the secondary incubation period for the first replicate (Fig. 3.3.3.9). This showed that the fruit would always be at 26°C when dissected because fruit were removed only after at least 48 h had elapsed. Control mortality in the first replicate was found to be higher than in the other two replicates due to some sour rot (*Galactomyces citri-aurantii*) infections in some fruit (Table 3.3.3.1). However, after switching to a new batch of guazatine the control mortality was lower in the second and third replicates with the overall control mortality for all three replicates amounting to 8.1%. No survivors were found out of a total of 71 756 larvae in all three replicates. The 16 d cold treatment at a mean temperature of 1°C therefore complies with the Probit-9 efficacy standard of 99.9968% mortality.



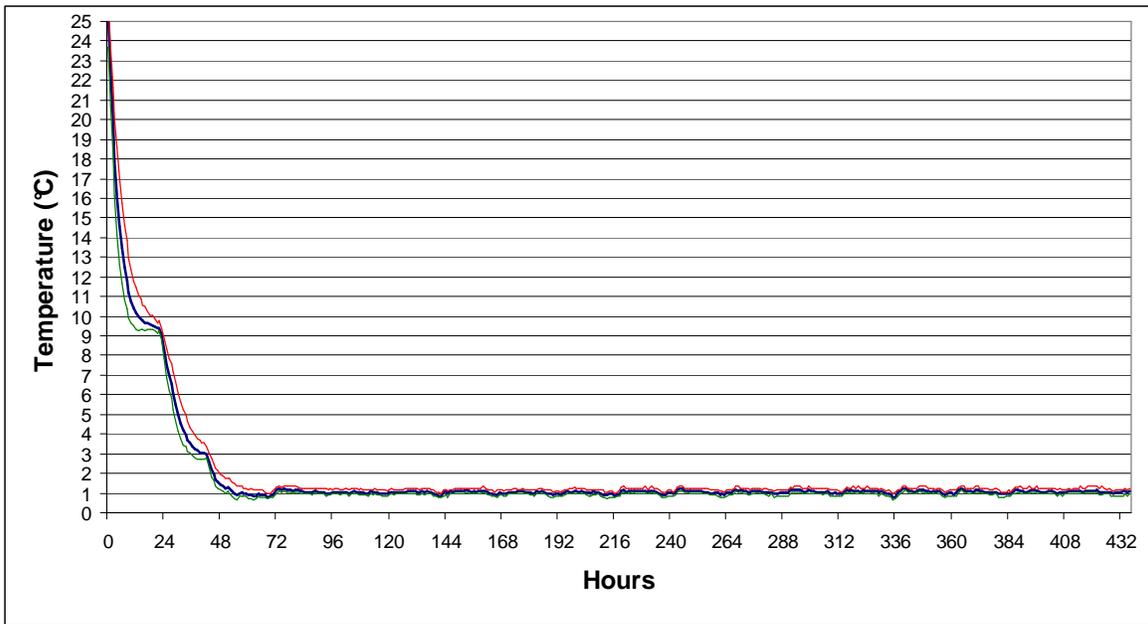
**Figure 3.3.3.3.** Temperatures in the air, four fruit and the mean fruit temperature, during the primary incubation period of the first replicate at 26°C when larvae were developing.



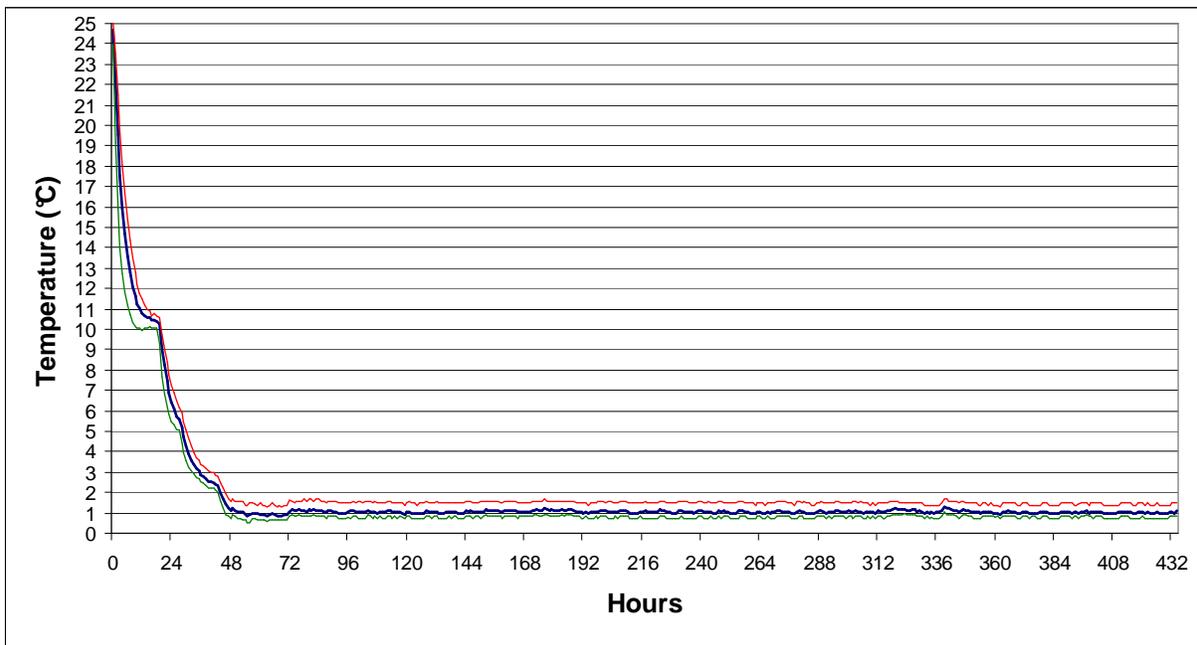
**Figure 3.3.3.4.** Temperatures in the air, four fruit and the mean fruit temperature, during the primary incubation period of the second replicate at 26°C when larvae were developing.



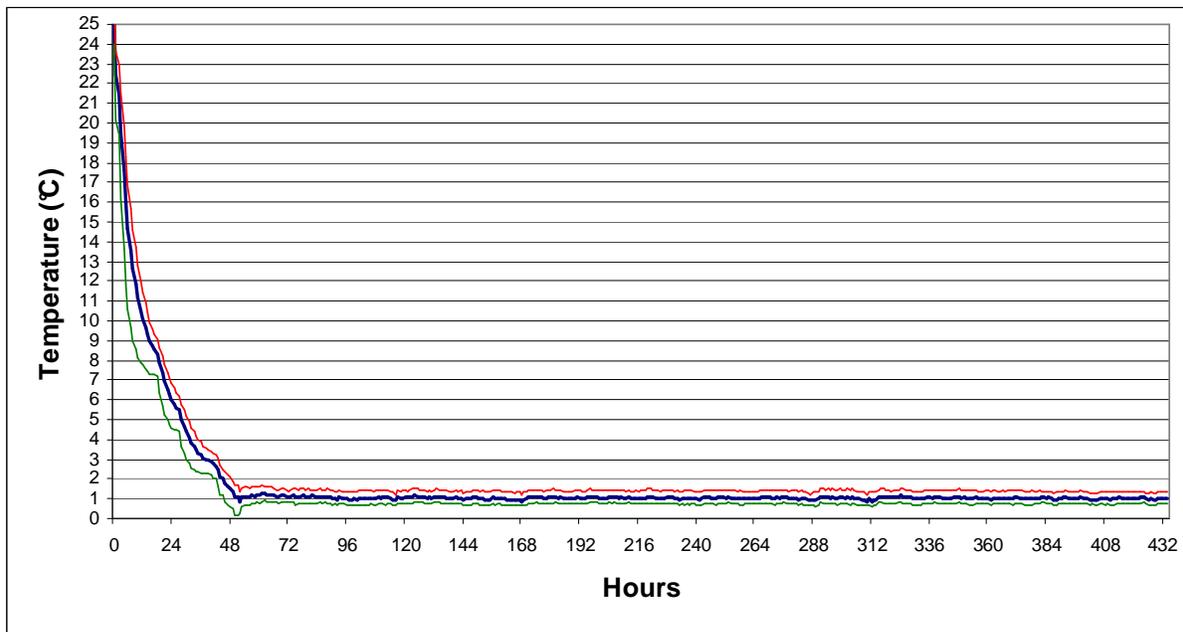
**Figure 3.3.3.5.** Temperatures in the air, four fruit and the mean fruit temperature, during the primary incubation period of the third replicate at 26°C when larvae were developing.



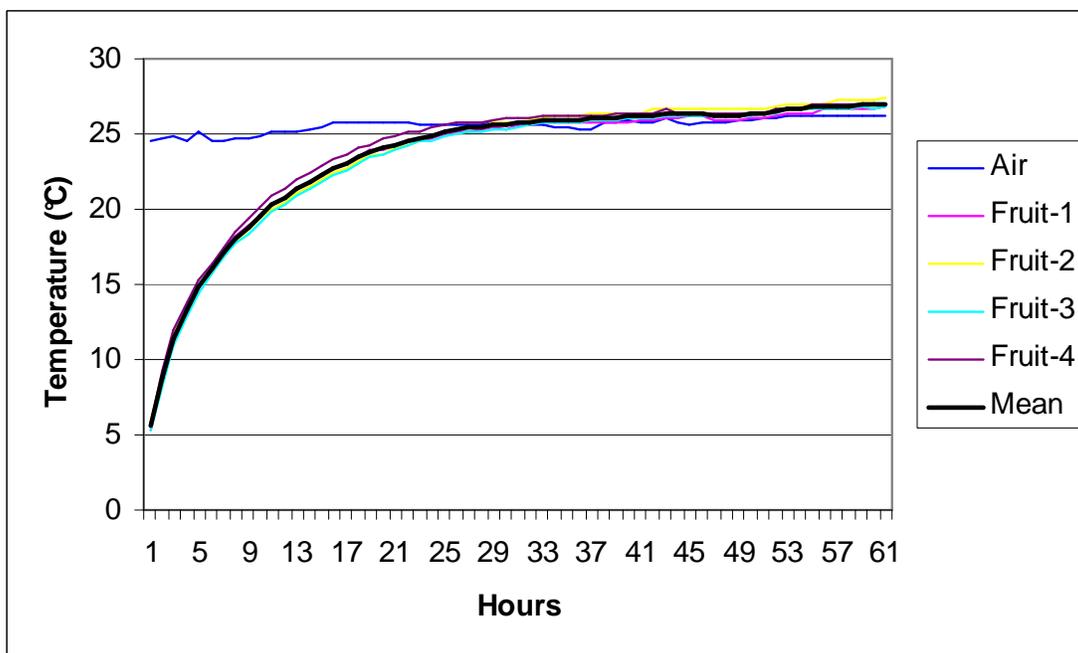
**Figure 3.3.3.6.** Highest (red line), lowest (green line) and mean (blue line) temperatures of fruit in replicate 1 during cold disinfestation. **Long-term mean temperatures: mean 1.048, low 0.928, high 1.221°C.**



**Figure 3.3.3.7.** Highest (red line), lowest (green line) and mean (blue line) temperatures of fruit in replicate 2. **Long-term mean temperatures: mean 1.040, low 0.797, high 1.493°C.**



**Figure 3.3.3.8.** Highest (red line), lowest (green line) and mean (blue line) temperatures of fruit in replicate 3. Long-term mean temperatures: mean 1.042, low 0.747, high 1.398°C.



**Figure 3.3.3.9.** Temperatures in the air and four fruit, and the mean fruit temperature, during the second incubation period of the first replicate at 26°C after ter fruit has been removed from the cold treatment.

**Table 3.3.3.1.** *Ceratitis capitata* mortality in controls after 6 d at 26°C and in the treatment after 15 d 23 h at 1.0°C.

Rep	Control treatment at 26°C for 6 days				Cold treatment at 1.0°C for 16 days			
	Fruit used	Larvae found	Larvae dead	Mortality %	Fruit used	Larvae found	Larvae dead	Mortality %
1	500	7512	1022	13.6	1998	20697	20697	100
2	520	6977	490	7.0	2089	22415	22415	100
3	528	7312	260	3.6	2119	28644	28644	100
Overall	1548	21801	1772	8.1	6206	71756	71756	100*

\*Probit-9 requirement is 99.9968% mortality

## Conclusion

After precooling for approximately 50 h, a cold treatment of 15 d and 23 h at a mean temperature of 1.04°C killed 100% of 71 756 *Ceratitis capitata* young larvae. This treatment should therefore be developed into a protocol for post-harvest treatment of export citrus.

## Future research

A response from the Japanese is now awaited. If they request that another phase 4 replicate be conducted in the presence of a Japanese researcher then this research will be conducted. No other cold disinfestation work with *C. capitata* is planned but research in Kenya on *Bactrocera invadens* will be conducted.

## Technology transfer

A poster on this research will be presented at the Citrus Research Symposium in August 2008.

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- Ware, A.B., Tate, B., Daneel, J-H., Stephen, P. and Beck, R. 2006. Sensitivity of Mediterranean fruit fly eggs and larvae in lemons, grapefruit and oranges to cold treatment of 1°C. pp. 100-106. In: CRI Group Annual Research Report for 2005. Citrus Research International, Nelspruit.

### 3.3.4 FINAL REPORT: Fruit fly baits – Organophosphate alternatives

Experiment 773 (2005-2008) by B. Tate, T. Grout, A. Ware and P. Stephen (CRI)

## Summary

With the trend to move away from organophosphates it was considered important to evaluate alternative toxicants for fruit fly baits. Bioassays were therefore conducted with *Ceratitis capitata* in large cages to evaluate various prospective toxicants. Based on the LD levels, mevinphos, tartar emetic and chlorfenapyr did not show potential as cost-effective alternatives and no further testing will be done on these products. Methomyl and formetanate did show potential but being carbamates they may also be phased out soon so no further testing will be conducted. The two most promising toxicants tested were imidacloprid and fipronil. The results indicated that mortality did not increase above a Confidor SC dosage of 12 ml/hl bait mixture. However, fipronil is slow-acting and results after 48 h were not reliable because the control mortality level increased dramatically after this time. An uncorrected mortality of around 95% after 48 h was obtained with fipronil at 30 ml/hl bait mixture. The reason for the high control mortality was eventually traced to inadequate water supply from water dispensers used in the cages. These were different to those used in the mother cultures. Attempts to develop a means of testing imidacloprid and fipronil further with wild fruit flies in an orchard were not successful. This will receive further attention in the future.

## Opsomming

Met die neiging om weg te doen met organofosfate, is dit belangrik geag om alternatiewe gifstowwe as vrugtevlug lokaas te evalueer. Biotoetse is dus met *Ceratitis capitata* in groot hokke uitgevoer om die verskillende gifstowwe te evalueer. Gebaseer op die LD waardes, het mevinpos, tartar emetic en chlorfenapyr nie moontlikhede getoon as koste effektiewe alternatiewe nie en geen verdere toetse sal op die produkte uitgevoer word nie. Methomyl en formetanate het wel moontlikhede getoon, maar is egter carbamates wat dalk binnekort uitgefaseer gaan word, en geen verdere toetse sal dus uitgevoer word nie. Die twee belowendste gifstowwe wat getoets is, was imidacloprid and fipronil. Volgens die resultate het mortaliteit nie bokant 'n Confidor SC dosis van 12ml/hl lokaas-mengsel toegeneem nie. Fipronil is egter stadig werkend en die resultate na 48 uur was nie betroubaar nie omdat die kontrole se mortaliteitsvlak dramaties na die periode toegeneem het. 'n Foutiewe mortaliteit van rondom 95% is na 48 ure verkry met fipronil teen 30 ml/hl lokaas-mengsel. Die rede vir die hoë mortaliteit van die kontrole is uiteindelik teruggespeur na 'n onvoldoende water toevoer vanuit die waterhouers wat in die hokke gebruik is. Hierdie houers het verskil van dié wat in die moeder kulture gebruik is. Pogings om 'n manier te ontwikkel vir die verdere toetsing van imidacloprid en fipronil met wilde vrugtevlieë in 'n boord was nie suksesvol nie en verdere aandag sal in die toekoms hieraan gegee word.

## Introduction

When this research was proposed it was considered that the continued use of organophosphates in fruit fly baits would not be permitted and alternatives were required. The retailers in Europe have targeted Dipterox (trichlorfon) and several companies will not accept fruit with residues of this product even though this may not be the official EU position on this chemical. Malathion (mercaptotion) residues remain officially acceptable to the EU and will for several years to come but in the long term, movement away from organophosphates is advisable. The first research on alternative toxicants to Malathion was conducted by Ware, Stephen and Tate in 2005. This was then taken further by Tate and Grout in 2006. However, fipronil is a slow-acting toxicant and should be evaluated after 48 h, but with the cage methods previously used, control mortality after 48 h was unacceptably high. Attempts were therefore made to design a cage that could be used in the field to evaluate slow-acting toxicants against wild fruit flies, but the fruit flies did not readily enter the cages to feed on the bait. Further research was therefore conducted in 2007 by Tate, Grout and Stephen with the previously-used cages to determine the reason for the high control mortality and to further evaluate imidacloprid and fipronil after 48 h exposure. The evaluation of Hymlure as a possible source of mortality showed that Hymlure was not playing any role.

## Materials and methods

### Cage tests 2005

One citrus seedling of approximately 1.2 m in height was treated with exactly 1 ml of bait mixture of any one of the concentrations tested. One 10 µl drop was placed on each of 100 leaves using a micropipette. The bait was allowed to dry for 3 hours after which the trees (one per cage) were suspended from the roof of gauze cages (1.1 X 0.6 X 1.85 m (l X b X h)). The product rates used are shown in Table 3.3.4.1. In general the highest rate tested was twice that registered for use on citrus (Nel et al. 2002). Mevinphos (150 EC) is registered at 100 ml/100 l water for bollworm (*Helicoverpa amigera* (Hübner)), methomyl (200 g/l SL) at 450 ml/100 l for red scale (*Aonidiella aurantii* (Maskell)) and for thrips (*Scirtothrips aurantii* Faure), formetanate (500 g/kg SP) at 25 g/100 l, tartar emetic (995 g/kg SP) at 400 g/100 l, chlorfenapyr (360 g/l SC) at 30 ml/100 l and fipronil (200 g/l SC) at 10 ml/100 l. The traditional bait mercaptotion (500 g/l EC) was used as a standard at 175 ml/100 l.

For six days after their emergence, adult laboratory-reared Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]) were fed granulated sugar and water *ad lib.* (i.e. were protein starved). Approximately 100 flies ( $\pm$  equal numbers of each sex) were released into each cage shortly after the trees were positioned. Exactly 24 hours later all the dead flies were removed and counted. The total numbers of flies in the cages were assessed and the percentage mortality determined. POLO-PC (LeOra Software) was used to determine LD<sub>50</sub> and LD<sub>90</sub> data.

### Cage tests 2006

The same method was used as described above for 2005. In general, the highest rate tested was 2-3 times that registered for citrus. All products were tested in combination with a 2% hydrolysed protein bait (Hymlure) which was also used as a control spray (Table 3.3.4.3). The floor of each cage was covered with plastic to facilitate the counting of dead flies. For six days after their emergence, adult laboratory-reared Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]) were fed granulated sugar and water *ad lib.* but no protein. Approximately 100 flies ( $\pm$  equal numbers of each sex) were released into each cage shortly after the trees were positioned. Exactly 24 hours later all the dead flies were removed and counted. The total numbers of flies in the cages were assessed and the percentage mortality determined. A further evaluation was conducted after 48 hours.

### Field test to compare imidacloprid with mercaptotion in Hymlure bait 2006

A comparison of imidacloprid (Confidor) and the standard toxicant mercaptotion in protein hydrolysate bait mixtures was made in a citrus orchard near Nelspruit. Confidor (350 g/l SC) at 4 ml was mixed with Hymlure protein hydrolysate at 400 ml/hl water and compared with mercaptotion (500 g/l EC) at 175 ml and 400 ml Hymlure/hl water. The bait mixtures were applied at 10 ml per branch using a hand operated spray bottle of 500 ml capacity that delivered 1 ml spray mixture with each squirt. Twelve oval plastic basins (650 x 500 x 250 mm (LxBxH)) of approximately 40 l capacity with the bottoms removed and replaced with plastic gauze to prevent any liquid accumulation, were mounted on steel spikes at each end to hold them in close proximity to the treated branches which were manipulated to fit into the basins. Six branches were treated per bait in an alternating pattern where no treatments were closer than 10 m apart. Protection against ant predation of dead flies was done using Ant-Bar (polybutene) applied around the base of the treated branches and on the

metal spikes holding the basins. Preparation for the trial was done the day before the bait was applied so that the bait application could be made early the following day. This comparison was conducted four times.

#### Reducing control mortality in cage evaluations of alternative toxicants 2007

The same cages were used as before (Ware et al. 2006; Tate and Grout 2007) with dimensions 1.1 x 0.6 x 1.85 m (LxBxH) and these were usually placed outside, in natural light but under a tin roof. In all cases, Hym lure protein hydrolysate liquid was used as an attractant at a concentration of 2% and this was applied to leaves on a grapefruit seedling suspended in the middle of the cage. The attractant and bait mixtures were applied with the use of a micropipette so that each cage had exactly the same amount of product (100 drops of 10 µl each). Five-day-old *Ceratitis capitata* that had only been fed on sugar since eclosing were used in the experiments. All cages had sugar in a petri dish and water in an inverted specimen jar that could be accessed through holes in the lid. Approximately 100 flies were used per cage and a single dosage rate or treatment used per cage. Mortality was determined by removing dead flies after 24 and 48 hours, then counting the number of remaining flies. A sex ratio of approximately 50:50 was used. Six trials were conducted between 13 February and 9 May 2007. Three of these involved a dosage series of fipronil (Regent 200 SC) as a toxicant, two involved imidacloprid (Confidor 350 SC) and one compared two collection techniques without any toxicants. For all trials the technique was similar.

A trial was also conducted (between 30 January and 1 February 2008) to determine whether Hym lure itself was responsible for high mortality. Medflies were collected by scooping flies from rearing cages and released into trial cages as used above. Three cages received a treatment of 2% sugar (m/v) and another three cages were treated with Hym lure at 2% formulated product. The treatments were applied as 100 drops of 10 µl each to a suspended citrus tree in the cage. Each cage had some granular sugar for food and two specimen-jar water dispensers as used in the previous trials. Fly mortality was determined after 24 and 48 hours. Two further trials were conducted to evaluate the water dispensers used in the cages as it was thought that poor water availability may lead to high control mortality. In these trials, two cages contained the specimen-jar water dispensers as used in previous tests and another two cages contained honey-jar dispensers as used in the fruit fly cultures. Approximately 100 Medflies were scooped from the culture cages and released in the trial cages on 8 February 2008. The flies were approximately 10 d old. Apart from two water containers in each cage, the flies were also supplied with granulated sugar and yeast. During the evaluation, temperatures ranged between 21 and 30°C. Mortalities were determined every 24 hours for five days. This experiment was repeated from 14 February 2008 using Natal fruit fly *C. rosa*.

### Results and discussion

#### Cage tests 2005

The mortality of the flies exposed to the various compounds is shown in Table 3.3.4.1. Based on the LD levels, mevinphos, tartar emetic and chlorfenapyr did not show potential as cost-effective alternatives to the currently used organophosphates (Table 3.3.4.2) and no further testing will be done on these products. Methomyl and formetanate showed potential but, as they belong to the carbamate group which may also be phased out, no further testing will be carried out. Results with fipronil were poor but as this product is known to be slow-acting, it may appear more promising after a longer evaluation period.

**Table 3.3.4.1.** The percentage mortality of Mediterranean fruit fly after 24 hours exposure to various concentrations of formulated products in Hym lure fruit fly bait

Mercaptothion	ml/100 l	0	0.175	0.875	3.5	17.5	52.5	175
	Mortality (%)	12.4	12.4	24.2	72	84.5	92	97
Mevinphos	ml/100 l	0	0.5	1	10	25	100	250
	Mortality (%)	3.0	9.4	10.4	14.4	17.9	39	31
Methomyl	ml/100 l	0	2	10	30	100	225	450
	Mortality (%)	17.9	30	67	86	97	97	100
Formetanate	g/100 l	0	2	10	25	75	200	-
	Mortality (%)	9.6	11.8	52	80	95	94	-
Tartar emetic	g/100 l	0	10	30	100	200	400	800
	Mortality (%)	3.9	4.5	14	12	15	18	37
Chlorfenapyr	ml/100 l	0	0.3	1	3	10	30	60
	Mortality (%)	10.3	2.3	1.1	17.9	29	32	37
Fipronil	ml/100 l	0	0.1	0.3	1	3	10	30
	Mortality (%)	8.6	9.4	8.5	40	34	59	50

**Table 3.3.4.2.** The LD<sub>50</sub> and LD<sub>90</sub> levels (% active ingredient) after 24 hours exposure of Mediterranean fruit flies to various toxicants in Hymlure fruit fly bait

Product	LD <sub>50</sub>		LD <sub>90</sub>	
	mℓ/100 ℓ	% ai	mℓ/100 ℓ	% ai
Mercaptothion	3.12	0.002	34.4	0.017
Mevinphos	1644	0.247	682666	102.4
Methomyl	7.6	0.002	46	0.0092
Formetanate	11.25	0.006	47	0.024
Tartar emetic	42950	42.38	164090	163.27
Chlorfenapyr	115	0.041	5698	2.05
Fipronil	15.5	0.003	1146	0.229

#### Cage tests 2006

Imidacloprid showed the most promise as it resulted in more than 50% kill after 24 hours at 4 ml product per 100 litres bait mixture (Table 3.3.4.3). The related product thiacloprid had very little effect with the highest mortality obtained being 11%. Acetamiprid, another chloronicotinyl, caused mortality levels between the two former products with no effect below 25 ml product/hl. Chlorfenapyr and fipronil both showed a slow increase in mortality with dosage when evaluated after 24 hours (Table 3.3.4.3). Although mortality improved with the 48-hour evaluation (Table 3.3.4.2), the control mortality for both these series of bioassays was unacceptably high so these results are not reliable. Proteus was similar to thiacloprid in having a negligible effect in both evaluations. The experimental product 0316423 caused 59% mortality after 24 hours at the highest dosage used, but this would probably not be cost effective. Fluvalinate caused some mortality at the lowest dosage tested, then some at the higher dosages with no effect in between (Table 3.3.4.3). This may be due to a repellent effect at the intermediate dosages and contact mortality at the high dosages. Based on the 24-hour bioassay results, field tests of bait mixtures containing imidacloprid were conducted.

**Table 3.3.4.3.** Percentage mortality of Mediterranean fruit fly after 24 hours for products (and their dilution rates) in protein hydrolysate baits screened in cages. 2% Hymlure (protein hydrolysate) was used as the control treatment.

Product	Formulation	Dilution (ml/100 ℓ water and 2% Hymlure) and mortality (%) below						Control mortality (%)
		0.3	1.0	3.0	10	30	60	
Chlorfenapyr	360 g/ℓ SC	0.3	1.0	3.0	10	30	60	-
	Mortality:	2.3	1.1	17.9	29.0	32.0	37.0	10.3
Fipronil	200 g/ℓ SC	0.1	0.3	1.0	3	10	30	-
	Mortality:	9.4	8.5	40.0	34.0	59.0	50.0	8.6
Imidacloprid	350 g/ℓ SC	0.5	1.5	4.0	12	35	105	-
	Mortality:	1.0	42.0	58.0	60.0	71.0	85.0	1.0
Thiacloprid	480 g/ℓ SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	6.0	2.6	0.0	11.4	10.0	5.7	4.4
Acetamiprid	222 g/ℓ SL	3.125	6.25	12.5	25	50	150	-
	Mortality:	2.0	0	0	21.0	35.6	58.5	0
Fluvalinate	240 g/ℓ EW	1.875	3.75	7.5	15	30	90	-
	Mortality:	26.0	2.0	6.0	2.0	23.0	23.0	12.0
Proteus	170 g/ℓ OD	6.25	12.5	25	50	100	300	-
	Mortality:	12.0	5.4	14.0	8.5	8.1	14.3	2.0
0316423*	SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	1.3	0.0	1.0	10.3	16.3	59.6	0

\*Experimental Bayer CropScience product – Ketanol group

**Table 3.3.4.4.** Percentage mortality of Mediterranean fruit fly after 48 hours for products (and their dilution rates) in protein hydrolysate baits screened in cages. 2% Hym lure (protein hydrolysate) was used as the control treatment.

Product	Formulation	Dilution (ml/100 ℓ water and 2% Hym lure) and mortality (%) below						Control mortality (%)
		0.3	1.0	3.0	10	30	60	
Chlorfenapyr	360 g/ℓ SC	0.3	1.0	3.0	10	30	60	-
	Mortality:	51.1	26.6	74.5	83.14	92.8	87.2	80.5
Fipronil	200 g/ℓ SC	0.1	0.3	1.0	3	10	30	-
	Mortality:	69.4	79.8	84.1	84.5	89.1	96.6	44.4
Imidacloprid	350 g/ℓ SC	0.5	1.5	4.0	12	35	105	-
	Mortality:	11.62	72.44	91	92.2	92.5	97.8	2
Thiacloprid	480 g/ℓ SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	12.1	5.2	0	13.9	16.2	17.2	14.3
Acetamiprid	222 g/ℓ SL	3.125	6.25	12.5	25	50	150	-
	Mortality:	5	1	7.4	46	64.3	84.1	2.2
Fluvalinate	240 g/ℓ EW	1.875	3.75	7.5	15	30	90	-
	Mortality:	39.4	26.7	29	0	49.5	75.6	26.4
Proteus	170 g/ℓ OD	6.25	12.5	25	50	100	300	-
	Mortality:	42.6	17.4	29	41.5	12.6	48	3.2
0316423*	SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	2.5	17	38.3	44.3	51	84.4	1.3

\*Experimental Bayer CropScience product – Ketanol group

#### Field tests 2006

The first two tests yielded no flies in the basins despite moving the basins to a different site. The third test yielded only 3 *Ceratitis* spp. flies caught in 6 basins for the imidacloprid baits, and 4 flies for the mercaptothion baits. The fourth test was delayed and moved to an orchard known to have large numbers of flies, and the application volume was increased to 20 ml per branch. The yield here was an improvement over previous runs but still poor with 6 flies killed in total for imidacloprid and 22 for mercaptothion (Table 3.3.4.5).

**Table 3.3.4.5.** Dead fruit flies collected in basins beneath branches sprayed with bait containing either imidacloprid (0.0014% a.i.) or mercaptothion (0.0875% a.i.) as a toxicant.

Test no.	Product	Volume/branch	Fruit flies collected
3	Imidacloprid	10 ml	3
3	Mercaptothion	10 ml	4
4	Imidacloprid	20 ml	6
4	Mercaptothion	10 ml	22

#### Reducing control mortality in cage evaluations of alternative toxicants 2007

Control mortalities after 24 hours were acceptable in the Regent series but once again increased above 10% after 48 hours, even with a lot of attention being given to changing positions of cages, avoiding direct sun and making sure that water was available (Table 3.3.4.6). The mean results shown in Table 3.3.4.6 indicate that a surprisingly high dosage of Regent is required to kill 90% of the flies so ideally the bioassay should be conducted for an even longer period, but this was not possible due to the high control mortality.

**Table 3.3.4.6.** Corrected mean percentage mortalities of *Ceratitis capitata* from three bioassay series conducted in large cages when flies were released at temperatures of 30, 28 and 25°C

Bait treatments	Corrected mean mortality (%) after 24 h*	Corrected mean mortality (%) after 48 h*
Regent 0.1 ml/hl water + Hym lure 2%	6.2	15.8
Regent 0.3 ml/hl water + Hym lure 2%	11.5	38.8
Regent 1 ml/hl water + Hym lure 2%	30.8	37.6
Regent 3 ml/hl water + Hym lure 2%	33.2	67.5
Regent 10 ml/hl water + Hym lure 2%	36.5	76.1
Regent 30 ml/hl water + Hym lure 2%	65.0	90.6

\* Corrected with Abbott's (1925) correction relative to mortality in a control with Hym lure 2% only.

The two bioassay series with Confidor had extremely high control mortalities but this may well have been exacerbated by releasing the flies in both bioassays when the temperature was 35°C, which according to Grout and Stoltz (2007), is above their lethal temperature of 33°C. However, this was not the only factor responsible for mortality because a cage held in an air conditioned room at 25°C still had extremely high control mortality after 24 h (Table 3.3.4.7). The results in Table 3.3.4.7 were not corrected for control mortality due to the unacceptable level but they did suggest that mortality did not increase above a Confidor dosage of 12 ml/hl water.

**Table 3.3.4.7.** Uncorrected mean percentage mortalities of *Ceratitis capitata* based on two bioassays, showing unacceptably high control mortalities when flies were released at temperatures of 35°C

Bait treatments	Uncorrected mean mortality (%) after 24 h	Uncorrected mean mortality (%) after 48 h
Hym lure 2% only	53.8	73.6
Hym lure 2% but in an A/C room at 25°C	41.0	55.4
Confidor 350 SC 0.5 ml/hl water + Hym lure 2%	49.0	82.0
Confidor 350 SC 1.5 ml/hl water + Hym lure 2%	74.6	91.9
Confidor 350 SC 4 ml/hl water + Hym lure 2%	70.5	90.2
Confidor 350 SC 12 ml/hl water + Hym lure 2%	91.2	97.4
Confidor 350 SC 35 ml/hl water + Hym lure 2%	82.2	96.3
Confidor 350 SC 105 ml/hl water + Hym lure 2%	81.9	96.8

The positions of the cages on the veranda did not show any consistent pattern with regard to high mortality in controls (Table 3.3.4.8). There were also no significant differences between two different methods used to collect the flies from the mother culture and transfer them to the cages (Table 3.3.4.9).

**Table 3.3.4.8.** Percentage mortalities of *C. capitata* in all trials when exposed to Hym lure 2% without toxicant and when cages were in different positions under a galvanised roof.

Trial number	Cage position	Mortality (%) no toxicant 24 h	Mortality (%) no toxicant 48 h
1	7	0	21.1
2	5	1.9	27.2
3	5	50.6	70.1
3	Rotunda	41	55.4
4	2	56.9	77.1
5	2	3.4	13.5
5	4	8	20.5
6	3	14.3	26.7
7	3	5.1	46.8
7	5	15.6	38.9
7	2	6.5	55.1
7	1	12.1	42.2
7	4	6.9	28.2
7	6	10.2	49.0
<b>Mean control mortality per trial:</b>		<b>19.1</b>	<b>39.3</b>

**Table 3.3.4.9.** Mortalities of *C. capitata* from two different collecting methods in large cages using Hym lure 2% without toxicant and flies released at 28°C

Collection method	Mortality (%) 24 h	Mortality (%) 48 h	Mortality (%) 72 h
Consol jar & funnel	7.9 a	48.1 a	79.8 a
Bottle & vacuum	10.9 a	38.7 a	63.0 a

There were no significant differences between treatments at P=0.05

The evaluation of Hym lure as a possible source of mortality showed that Hym lure was not playing any role. After 24 hours, the mean mortality per cage was 4.5% for sugar only and 4.1% for Hym lure. After 48 hours, the mean mortalities had risen to 39.4% for sugar only and 39.8% for Hym lure.

The evaluation of water dispensers showed that mortality of Medfly was higher in cages with the specimen-jar water dispensers than in those with honey jars (Fig. 3.3.4.1). It also showed that the males were more susceptible to a lack of water than the females. The evaluation of Natal fruit fly showed very similar results (Fig. 3.3.4.2) and the mortality remained below 10% for female Natal fruit fly for as long as 72 hours when honey-jar water dispensers were used. These will therefore be used in future bioassays.

A means of evaluating slow-acting toxicants against wild flies must still be devised in order to confirm field rates of bait. Further research on other possible toxicants will be incorporated in research on a new bait that is effective against all *Ceratitidis* spp.

### Conclusion

Problems with techniques led to unreliable results after 48 h exposure to toxicants in cage trials but indications were that imidacloprid and fipronil were the most promising toxicants for further testing in baits.

### Future research

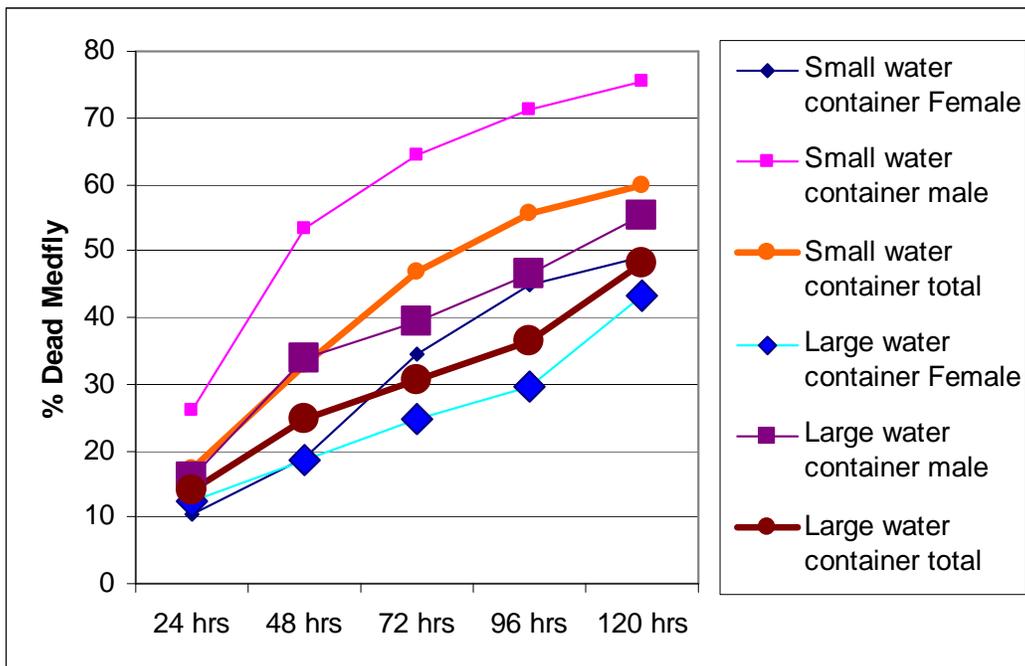
Further research will be conducted under the experiment number 915.

### Technology transfer

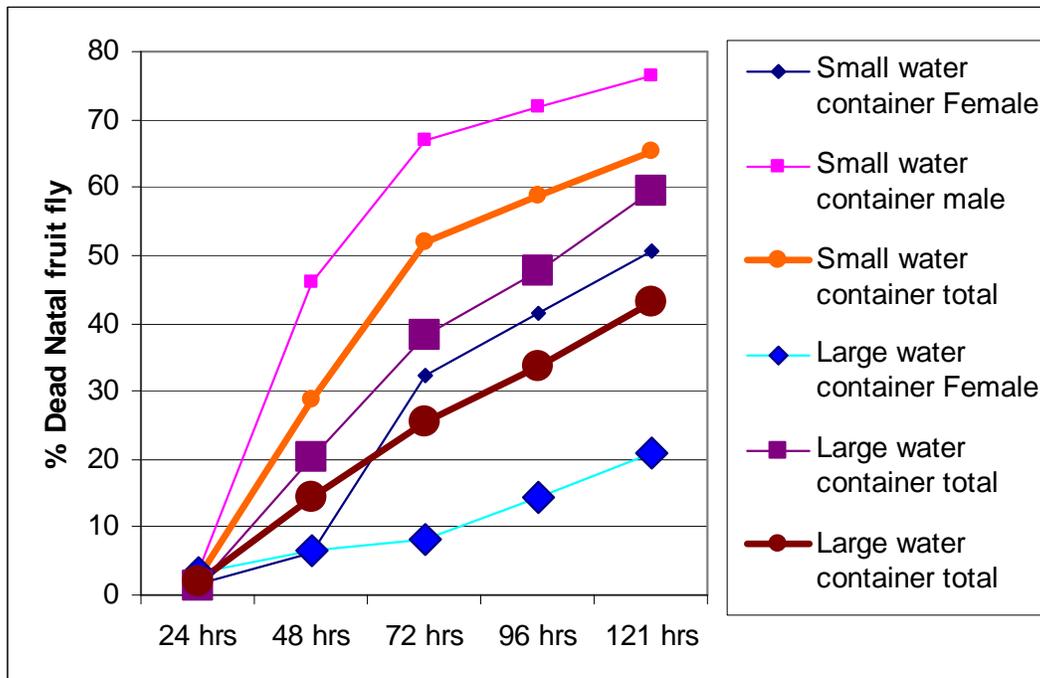
The results of this research are not yet at the point where technology transfer other than internal reporting is possible.

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**Figure 3.3.4.1.** Medfly mortalities in cages with small water dispensers compared with cages with large water containers.



**Figure 3.3.4.2.** Natal fruit fly mortalities in cages with small water dispensers compared with cages with large water containers.

**3.3.5 FINAL PROGRESS REPORT: Development of a rapid diagnostic test to distinguish Medfly larvae from other larvae**

Experiment 774 (2004-2008) by T. Grout and J-H. Daneel (CRI)

**Summary**

Central Science Laboratories in the United Kingdom had been contracted to investigate the possibility of developing a lateral flow, ELISA-type system to identify squashed larvae of *Ceratitis capitata* within minutes.

However, after producing two ineffective prototypes this work was handed over to a spin-off company Forsite Diagnostics who despite promising to continue the investigation have got no further. The contract will now be formally terminated.

### **Opsomming**

Central Science Laboratories (Verenigde Koningkryke) is gekontrakteer om die moontlikheid van 'n laterale vloei, ELISA-tipe sisteem te ondersoek om fyngedrukte larwes van *Ceratitis capitata* binne minute te identifiseer. Die werk is egter aan 'n filiaalmaatskappy, Forsite Diagnostics, oorhandig nadat twee oneffektiewe prototipes geproduseer is. Ten spyte van beloftes is daar egter ook nie deur hierdie maatskappy enige vordering gemaak nie. Die kontrak sal nou amptelik beëindig word.

**3.3.6 FINAL PROGRESS REPORT: Will fruit fly bait applications be effective if applied to the tree-trunk to avoid fruit residues? In: Fruit fly - Field control other than OP substitutes**  
Experiment 801 (2005-2008) by J-H. Daneel, T. Grout, and P. Stephen (CRI)

### **Summary**

In order to investigate further means of avoiding residues from organophosphates when applying fruit fly baits to the foliage, the application of protein hydrolysate bait to tree trunks was compared to conventional applications to foliage. All numbers of fruit fly species were low in three different trials but foliar sprays were significantly more effective ( $P < 0.05$ ) than trunk sprays in one case and the foliar sprays often had the lowest numbers of fruit flies trapped per day. Trunk sprays therefore appear to be inferior. If artificial substrates are to be used for the application of baits between trees, non-absorbent surfaces or sponge allow for more up-take of bait by the flies. However, a suitable technique to evaluate bait on artificial substrates in the orchard could not be developed. Further research on baits will be conducted in experiment 915.

### **Opsomming**

Om verdere maniere te ondersoek om residu van organofosfate te verhoed wanneer vrugtevlieg lokaas op loof toegedien word, is proteïen hidrolisaat lokaas wat op die stamme van bome toegedien is vergelyk met konvensionele blaartoedienings. Alle vrugtevlieg spesies se getalle in die drie verskillende proewe was laag, maar in een geval was die blaarbespuitings betekenisvol meer effektief ( $P < 0.05$ ) as die stambespuitings en die blaarbespuitings het dikwels die laagste aantal vrugtevlieë per dag gevang. Stambespuitings blyk dus om nie geskik te wees nie. As kunsmatige substrate vir die aanwending van lokaas tussen die bome gebruik moet word, laat sponse of nie-absorberende oppervlaktes toe dat meer van die lokaas deur die vlieë opgeneem kan word. 'n Geskikte tegniek vir die evaluering van lokaas op kunsmatige substrate in die boord kon egter nie ontwikkel word nie. Verdere navorsing op lokaas sal in eksperiment 915 uitgevoer word.

### **Introduction**

Earlier attempts were made to determine whether fruit fly bait sprays would be effective when sprayed on the ground or ground-cover rather than tree foliage and fruit (Daneel et al. 2007). These showed that the ground treatments were ineffective. A further attempt to avoid fruit residues by applying baits to the tree trunks was then initiated as well as research to determine whether spraying bait on an artificial substrate hung between the trees would be effective.

### **Materials and methods**

#### Orchard trials with trunk sprays

A mango orchard on Oewersig farm near Alkmaar, 20 km west of Nelspruit, Mpumalanga was used to evaluate trunk applications of fruit fly bait as an alternative to foliar sprays. Once the presence of fruit flies was established, the orchard was divided into three zones and each of these further divided into three treatment blocks, each from 5 to 7 rows wide and approximately 40 trees long. The three treatments were: an untreated control, a bait spray on the trunk from the crotch down and a foliar bait spray. One Sensus trap containing Ceratitislure and another Sensus trap containing Capilure were each hung inside a tree in each treatment block, approximately 1.5 m above the ground. Bait applications started on 10 August 2007 and finished on 8 September. The trees were approximately 3.5 m high. The bait mixture comprised Hym lure protein hydrolysate (800 ml/hl water) and Malathion EC (175 ml/hl water) and approximately 100 ml bait mixture was applied to each tree in each row in the block using a knapsack sprayer. The foliar sprays were applied to only one side of the row as a 1 m-wide band approximately 1.5 m above ground. When reapplied, the other side of the row was sprayed. Treatments were reapplied after rain. Sprays were applied on 10, 21, 28, 30 August and 4, 8 September and traps were emptied on the same dates and 12 September. Trap

counts were recorded as numbers of flies caught per day as the intervals were not regular. Where graphs of trap counts showed noticeable differences between treatments on certain dates, numbers of flies caught per day were compared with a 2-way ANOVA after a square root plus 0.5 transformation. If the F-test was significant at  $P=0.05$ , means were further compared using Student-Newman-Keuls test.

In November and December 2007 a second trial was conducted in the same mango orchard at Oewersig due to a lack of other suitable sites. By this time the weather had become hotter and wet and Natal fruit fly had become dominant. The trial was laid out as before with three treatments in three zones. However, a high pressure (25 bar) spray machine was used to apply the bait through a 1 mm diameter orifice without a whirler plate. The spraygun was moved up and down while moving past the tree. The amount of bait applied per tree was more than before with 380 ml bait mixture being applied to the foliage of each tree and 340 ml per trunk. Both sides of each tree were sprayed and both sides of the trunk, so the volumes per side were 190 ml and 170 ml, respectively. Three sprays were applied on the 21, 27 November and 5 December. Flies were monitored as before but Questlure in Sensus traps was also used (one per treatment replicate).

A third trial was conducted in the same mango orchard at Oewersig between the 4 March and 4 April 2008. Due to poor results in the previous trial it was decided to use two larger blocks of trees per treatment rather than three but the treatments were applied in the same manner as the second trial on 6, 20 and 25 March 2008. However, a larger nozzle orifice of 1.5 mm diameter was used and approximately 410 ml Hymlure 2x mixture plus Malathion was applied to the foliage of every tree (205 ml per side). A 4x concentration of Hymlure was used with Malathion in the trunk sprays and 380 ml (190 ml per side) was applied to every tree trunk. Six Sensus traps with Capilure and six Sensus traps with Questlure were used in each treatment replicate and emptied approximately every five days. Trap catches were reflected as numbers caught per day and the percentage reductions relative to the trap catches before the first sprays were applied, were determined.

#### Applying bait to other substrates between trees

In an attempt to reduce toxicants applied to fruit in the orchard, alternative artificial substrates hung between trees in an orchard might be suitable for bait application. A trial was conducted in the laboratory to determine how available protein bait would be to adult flies when applied to different materials and whether there was any preference between substrates. The results from this trial were then expanded on in the orchard.

Yeast Hydrolysate Enzymatic (0.5 g) and an Apple Green Food Colorant (Robertson) (0.04 ml or 0.2 ml) were dissolved in 2 ml water. The mixture was applied to different materials (30 mm x 30 mm) placed in 10 petri dishes, 90 mm in diameter. Adult flies were placed in each petri dish and inspected later for the presence of any food colorant in their abdomen, a clear sign that colorant had been ingested.

For orchard evaluations, nine substrates (300 x 200 mm) of different materials were placed between trees in an orchard at Brackenhill in the Nelspruit environs. During the same period, one Sensus trap with Questlure and another Sensus trap with Capilure were hung in the orchard to monitor fruit fly presence. Substrates comprised corrugated cardboard, yellow Correx and mutton cloth and were hung with crocodile clips from a wire fastened between the trees in the orchard. A 60 l oval basin was placed underneath each trap to collect dead flies. The basin was kept in place on two metal stands treated with Ant Bar (polybutene) to prevent ants from removing dead ants. Traps were sprayed with a standard registered fruit fly treatment, Malathion (175 ml/100 l water) and Hymlure 2x (800 ml/100 l water).

The substrates were sprayed on two separate days in dry, sunny weather. On 18 May, as separate droplets in a typical bait application, and on the 21 May, as a cover spray to runoff. Basins were inspected 24 h later and traps were also emptied after 3 d exposure.

Following this experiment, three new materials were hung in the same orchard consisting of a 5 mm-thick sponge, yellow dust cloth and a paraffin wick. Another oval basin was also placed underneath a branch to act as a standard. The new substrate dimensions were similar to the previously used materials. The substrates were sprayed as before on 24 May and basins were inspected 24 h later.

Further sprays were applied on 28 May and evaluated 24 h later. On 29 May, the substrates received another spray but were turned horizontal this time after the spray, changing the angle of availability of the bait.

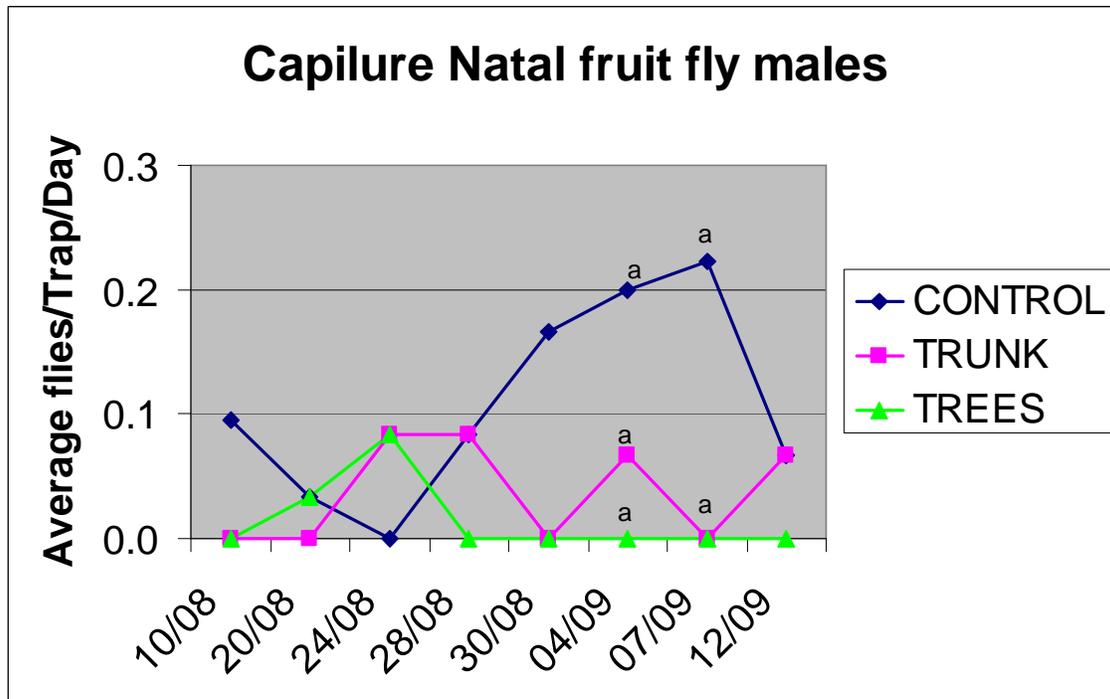
Traps placed at Crocodile Valley and the Lowveld Agricultural College indicated fruit fly presence but at very low numbers. Twelve 60 l oval basins were placed in trees at Crocodile Valley. The aim of this trial was to

see how effectively flies could be sampled using the basins as a collection method to test the effectiveness of an application. Each basin was placed underneath a branch and held in position with strings. Six branches were sprayed with the registered Malathion and Hymlure (2x) application and six branches with Kombat Fruitfly (cypermethrin EC 20 g/l) at 1ml/l and Hymlure (2x). The basins were inspected 8 and 24 h after the application.

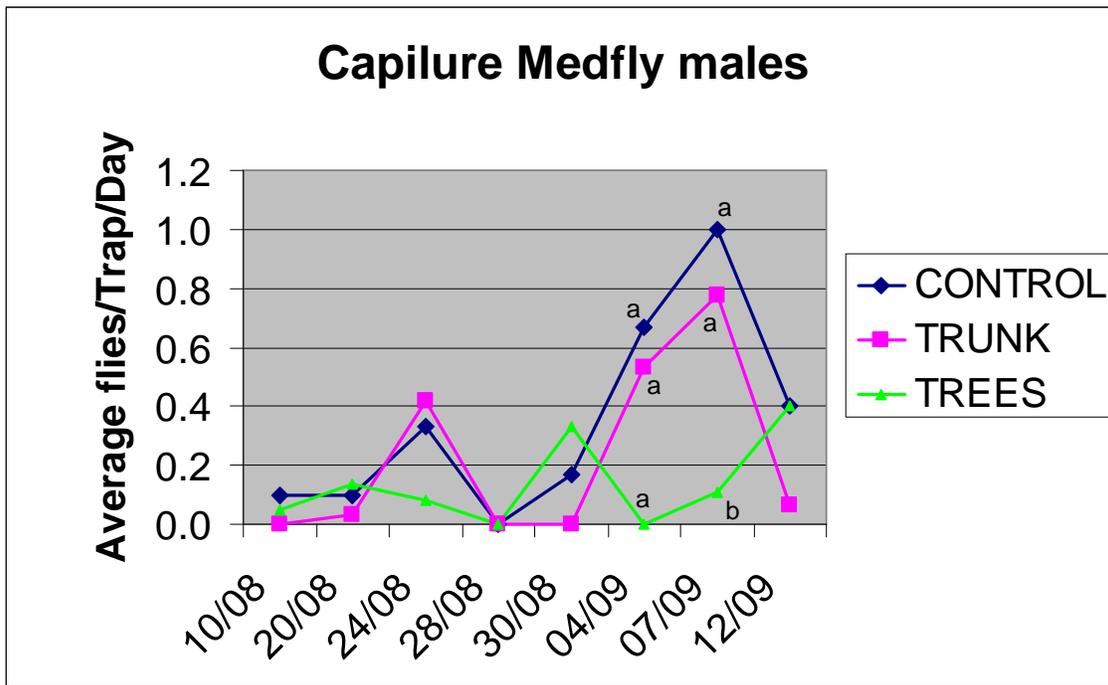
## Results and discussion

### Orchard trials with trunk sprays

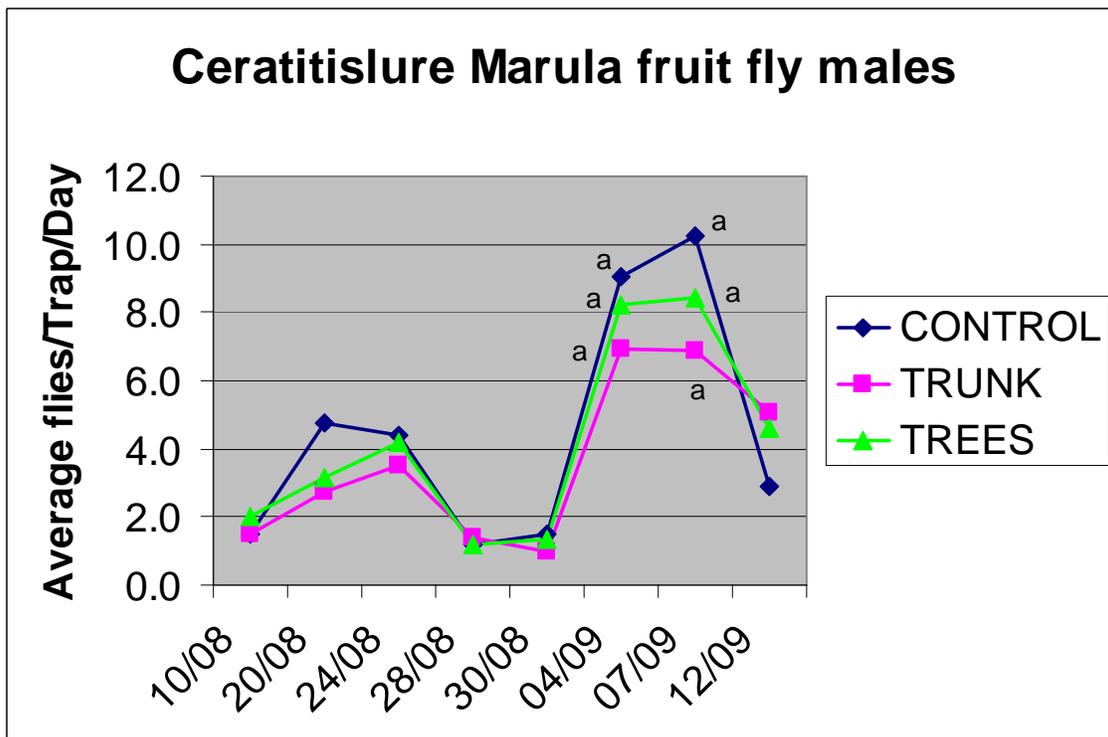
Numbers of fruit flies during the trial at Oewersig in August and September 2007 remained low throughout the period and seldom exceeded the treatment threshold of 4 flies per trap per week for *Capilure* when used in citrus orchards. This was particularly the case for Natal fruit fly which did not provide any useable data (Figure 3.3.6.1). However, Medfly numbers did show a trend (Figure 3.3.6.2) and fly catches on 7 September in blocks treated with the foliar sprays were significantly lower than catches in the other two treatments. This indicates that the trunk treatment is not as effective as the traditional foliar applications. Numbers of Marula fruit fly were relatively high in this orchard at this time but the trap catches showed very little difference between the treatments and nothing significant (Figure 3.3.6.3).



**Figure 3.3.6.1.** Mean numbers of *Ceratitis rosa* caught per trap per day in treatments at Oewersig using Capilure in Sensus traps. There were no significant differences at  $P=0.05$  on the dates with letters.

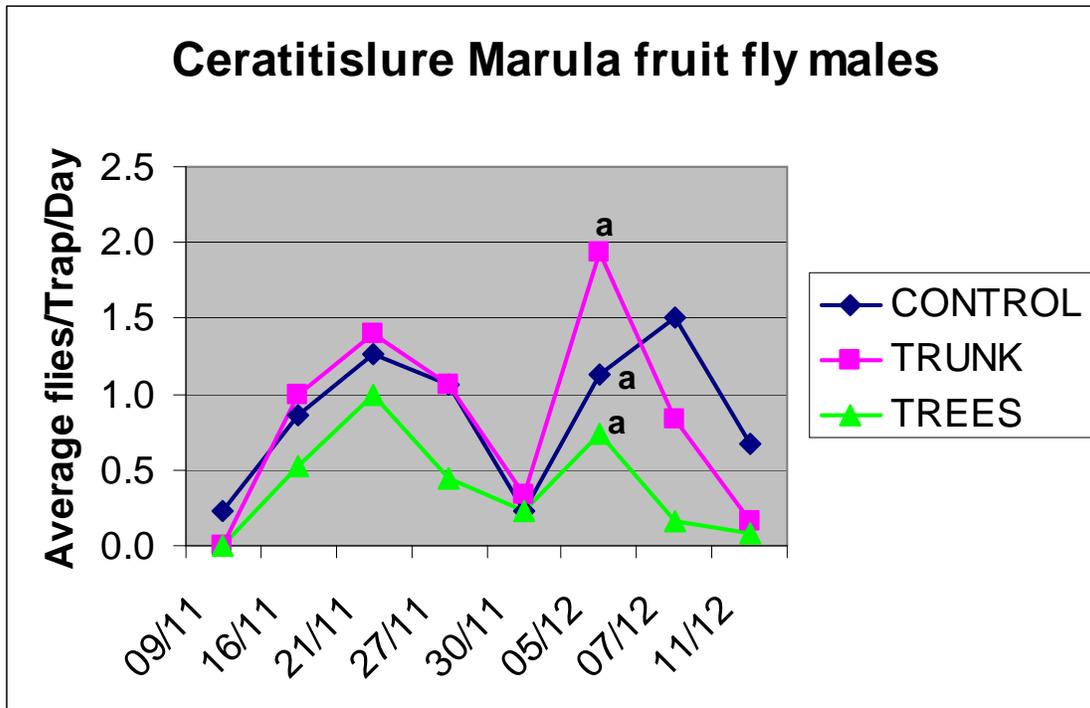


**Figure 3.3.6.2.** Mean numbers of *Ceratitis capitata* caught per trap per day in treatments at Oewersig using Capilure in Sensus traps. Different letters denote significant differences at P=0.05 on a particular date

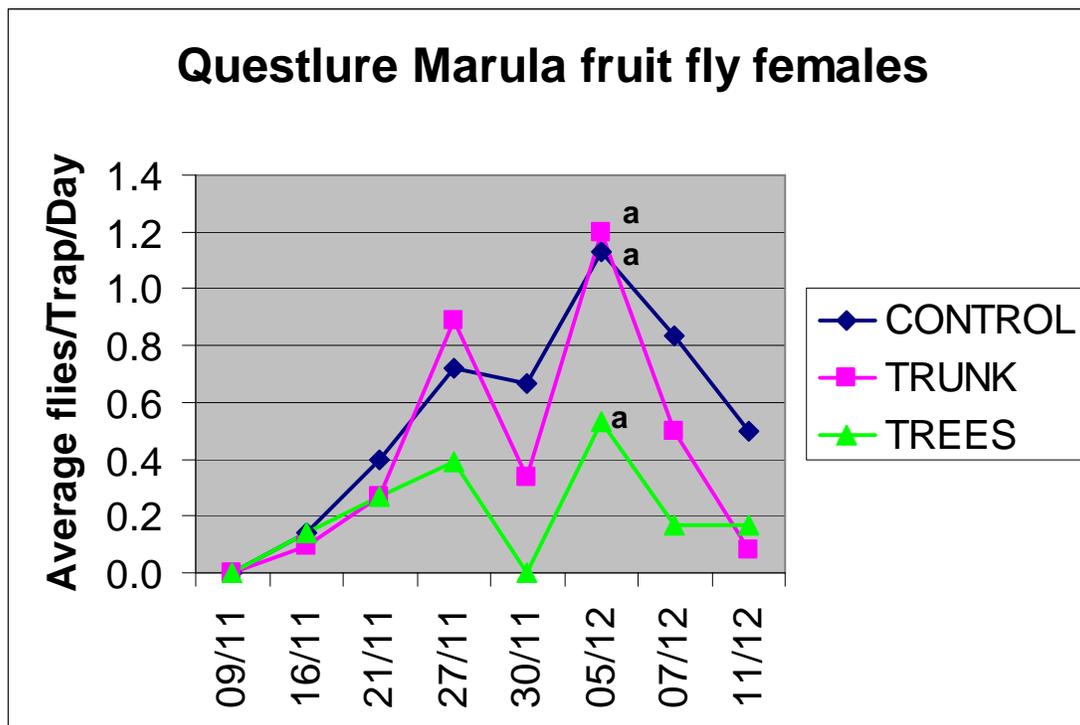


**Figure 3.3.6.3.** Mean numbers of *Ceratitis cosyra* caught per trap per day in treatments at Oewersig using Ceratitislure in Sensus traps. There were no significant differences at P=0.05 on the dates with letters.

The second trial at Oewersig with higher bait volumes was disappointing because the fly numbers remained even lower than in the first trial and did not exceed the Capilure treatment threshold at any time. Numbers of Marula fruit flies were also lower than before in traps with Ceratitislure and although there were no significant differences between treatments, the lowest counts were always in the blocks with the foliar sprays so perhaps there is a slight treatment effect (Figures 3.3.6.4 and 3.3.6.5).

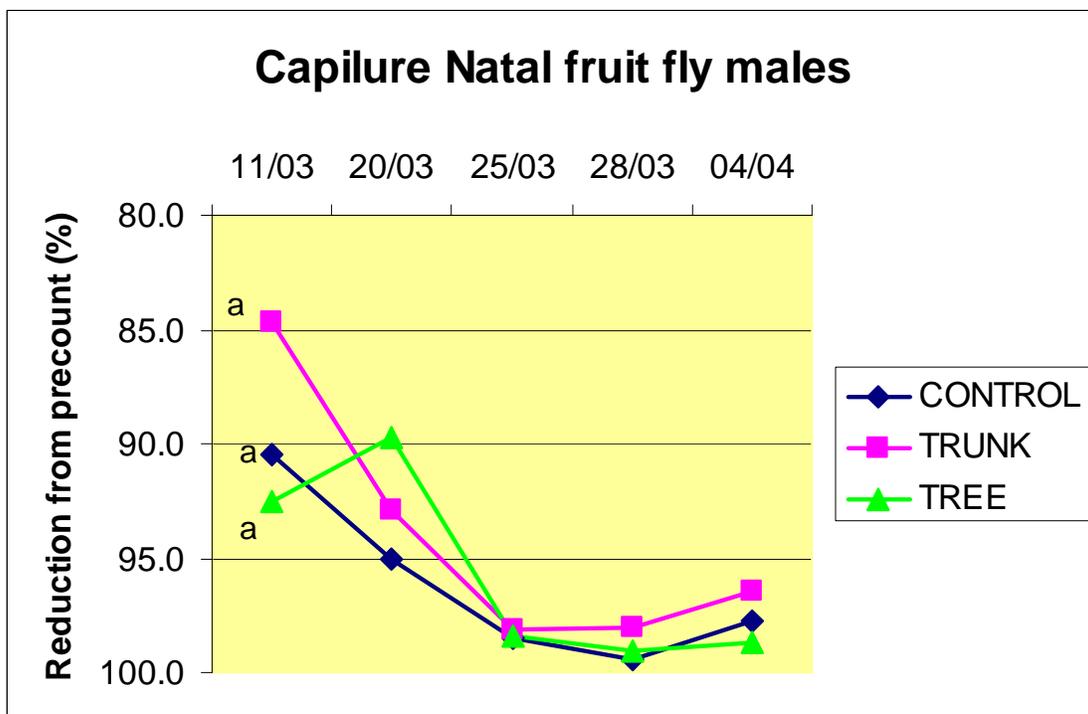


**Figure 3.3.6.4.** Mean numbers of *Ceratitis cosyra* caught per trap per day in treatments at Oewersig using Ceratitislure in Sensus traps. There were no significant differences at  $P=0.05$  on the date with letters.



**Figure 3.3.6.5.** Mean numbers of *Ceratitis cosyra* caught per trap per day in treatments at Oewersig using Questlure in Sensus traps. There were no significant differences at  $P=0.05$  on the date with letters.

In the third trial the numbers of fruit flies did not start from a low base and build up, but were dropping naturally after the mangoes had been harvested. Unfortunately, this general decline in numbers seemed to be a stronger influence than the bait treatments as numbers in all treatments declined. The highest trap catches were Natal fruit fly in traps with Capilure but even here there were no significant differences between treatments (Figure 3.3.6.6).



**Figure 3.3.6.6.** Percentage reduction in the mean numbers of *Ceratitis rosa* caught per trap per day in treatments at Oewersig in March 2008 using Capilure in Sensus traps. There were no significant differences at  $P=0.05$  on the date with letters.

#### Applying bait to other substrates between trees

Flies could not absorb bait from the corrugated cardboard and with the exception of the soaked sponge, droplets applied to a hard surface were taken up more readily (Table 3.3.6.1).

**Table 3.3.6.1.** Comparison between different substrates to establish the availability of the bait to adult flies.

No	Material used as a substrate	Food colorant used (ml)	Total flies exposed	Flies that have fed ♂	Flies that have fed ♀	Percentage successful ingestion
1	Corrugated carton (soaked with bait)	0.04 ml	6	0	0	0
2	Mutton cloth (soaked with bait)	0.04 ml	6	0	1	15
3	Yellow Correx with droplets (3 to 5 mm)	0.04 ml	6	1	1	33
4	Sponge (3 ml) soaked	0.04 ml	6	1	2	50
5	Corrugated carton with droplets (3 to 5 mm)	0.2 ml	4	0	0	0
6	Mutton cloth with droplets (3 to 5 mm)	0.2 ml	6	1	0	15
7	Citrus leaf with droplets (3 to 5 mm)	0.2 ml	4	0	2	50
8	Droplets (3 to 5 mm) applied to the petri dish base	0.2 ml	4	1	2	75
9	Yeast Hydrolysate Enzymatic with sugar (1:3) dampened with green food colourant applied to the petri dish base	No water	4	2	2	100

No flies were caught in the basins below the substrates in the orchard. During the same three-day period, the Questlure trap caught 5 Medfly females and the Capilure trap caught 26 Medfly males and 3 Natal fruit fly males. Both traps therefore indicated the presence of fruit fly in the orchard.

On 25 May 2007, no flies were collected in the basins or traps.

On 28 May, in the morning before spraying, 1 Natal female was collected in the Questlure trap and 10 Medfly males and 1 Natal male were caught in the Capilure trap. In the basin underneath the treated branch, 1 Medfly female was caught. At 16:00 the Capilure trap caught 6 Medfly males and 1 Natal male. The treated branch caught 1 Medfly female and 1 Natal male and female. On 29 May the Capilure trap caught 2 Medfly male and 1 Natal male, but all the basins were empty. On the 30 May, only the basin underneath the treated branch caught 4 Medfly females and 1 Natal male.

No flies were collected in the basins below branches sprayed with Malathion plus Hymlure or cypermethrin plus Hymlure. This might have been due to the low numbers of flies in the orchard.

The basin technique did catch flies below treated branches when they were present but did not appear to be effective below alternative substrates. Perhaps the fly behaviour is different on these surfaces and they do not spend as long on these surfaces as on foliage. This technique or something similar will need to be evaluated further in order to determine whether alternative substrates for baits can be effective.

## Conclusions

Numbers of fruit flies were generally low in the orchard evaluations of bait sprays on trunks but there was one significant difference in the efficacy of baits applied to the foliage and applied to the trunks and a few trends that indicated that the foliar sprays were the most effective. Bait applied to alternative substrates could not be readily taken up by fruit flies when materials were very absorbent.

## Future research

Research focus will shift to the development of a gelatinous bait that could be applied to main branches and improved female attractants for bait station or attract and kill devices.

## Technology transfer

The results of this research and the earlier work that showed that bait applied to the ground cover was ineffective, will be presented at the Citrus Research Symposium in August 2008.

## Reference cited

Daneel, J-H., T.G. Grout and R. Beck. 2007. Fruit fly - Field control other than OP substitutes. pp. 117-129. In: CRI annual research report 2006. Citrus Research International, Nelspruit.

### 3.3.7 FINAL REPORT: Differences between lures used for fruit fly monitoring. In: Fruit fly - Field control other than OP substitutes

Experiment 801 (2005-2008): John-Henry Daneel, Tim Grout, Tony Ware, Rooikie Beck (CRI)

## Summary

The citrus industry in southern Africa has been using Sensus traps with Capilure for 14 years and with Questlure for 8 years but there is still much to learn about these lures and how they compare to internationally-accepted lures. To determine the relative efficiencies of our present trapping systems, experiments were conducted where laboratory-reared *Ceratitis capitata*, *C. rosa* and *C. cosyra* were released in a mango orchard within a few metres of Sensus traps with either Capilure, Ceratitislure or Questlure. The experiment was run twice using flies of 3 and 12 days old. When using 12 d old flies, Capilure caught 3 times more Medfly males than Natal fruit fly males and this difference was more extreme when 3 d old flies were considered. Ceratitislure caught significantly more 12-d-old marula male fruit flies than 12-d-old Medfly males but the difference was reversed when 3-d-old flies were compared. Both these differences are due to Medfly being sexually mature before the other two species. Questlure showed the least differences between species and age but recovered the lowest proportion of released species. Further comparisons were conducted with wild flies in a citrus orchard using other known attractants in large yellow Probodelt traps. Capilure caught more male Medflies than 3-component lure but 2-component lure was more effective than Questlure for Medfly females. 3-component lure was also more effective than both

Capilure and Questlure for male and female Natal fruit flies, respectively. Ceratitislure was the most effective lure for male Marula fruit flies and 3-component lure was more effective than Ceratitislure and Questlure for female Marula fruit flies. It is recommended that the intervention threshold of 4 flies/trap/week previously used with Capilure for Natal fruit fly, be lowered to 2 Natal fruit flies/trap/week.

## Opsomming

Die sitrusbedryf in suider-Afrika gebruik Sensus lokvalle met Capilure en Questlure al vir 14 en 8 jaar onderskeidelik. Daar is egter nog baie om te leer omtrent hierdie lokmiddels en hoe hul met internasionaal aanvaarde lokmiddels vergelyk. Om die relatiewe doeltreffendheid van ons huidige lokvalsisteme te bepaal, is eksperimente met *Ceratitis capitata*, *C. rosa* en *C. cosyra* wat in die laboratorium geteel en vrygelaat is in 'n mangoboord, binne 'n paar meter vanaf Sensus lokvalle met Capilure, Ceratitislure of Questlure, uitgevoer. Die eksperiment is twee maal herhaal met 3- en 12 dag oue vlieë. Capilure het 3 maal meer Medvlieg mannetjies as Natalse vrugtevlug mannetjies gevang as 12d oue vlieë gebruik is. Hierdie verskil was meer drasties met die gebruik 3d oue vlieë. Ceratitislure het betekenisvol meer 12d oue maroela vrugtevlug mannetjies as 12d oue Medvlieg mannetjies gevang. Hierdie verskil was egter omgekeerd in vergelyking met 3d oue vlieë. Beide van hierdie verskille kan toegeskryf word daaraan dat Medvlieg vroeër seksueel volwasse is as die ander twee spesies. Questlure het die minste verskille tussen die spesies en ouderdom getoon maar het ook die laagste proporsie van die vrygelate spesies gevang. Verdere vergelykings is met wilde vlieë in 'n sitrusboord uitgevoer deur ander bekende lokmiddels in groot geel Probodelt lokvalle te gebruik. Capilure het meer Medvlieg mannetjies gevang as die 3-komponent lokmiddel. Die 2-komponent lokmiddel was meer effektief as Questlure vir Medvlieg wyfies. Die 3-komponent lokmiddel was ook meer effektief as beide Capilure en Questlure vir Natalse vrugtevlug mannetjies en wyfies onderskeidelik. Ceratitislure was die mees effektiewe lokmiddel vir Maroela vrugtevlug mannetjies en die 3-komponent lokmiddel was meer effektief as Ceratitislure en Questlure vir Maroela vrugtevlug wyfies. Dit word aanbeveel dat die aksie drempelwaarde van 4 vlieë/lokval/week soos voorheen met Capilure vir Natalse vrugtevlug gebruik, na 2 Natalse vrugtevlug/lokval/week verlaag word.

## Introduction

One of the assurances that we give export markets for our citrus is that our fruit flies are monitored in orchards and control methods are stepped up when thresholds are exceeded. However, the lures used in our monitoring system have not been compared with internationally recognised lures such as the 3-component lure or Biolure, in the same containers. Relative attraction to the South African lures by the three economically important *Ceratitis* spp. in South Africa is also unknown because the size of wild populations in orchards are never known when traps and lures are compared. It was therefore decided to compare the South African lures in Sensus traps, as used by citrus farmers, by releasing large numbers of laboratory-reared fruit flies. This was conducted twice using flies of different ages.

## Materials and methods

### The competitive efficiency of Questlure, Ceratitislure and Capilure for a known number of released flies

In 2005, laboratory-reared Mediterranean fruit flies were marked with Dayglo fluorescent dye and packaged in lots of 500 (equal numbers of males and females). Protein was withheld, although the adults were allowed water and granulated white sugar *ad lib*. The flies were released 2-3 days after eclosion. Natal fruit fly and Marula fruit fly were treated in the same way.

The flies were transported to the release site, a harvested mango orchard at Neos Estates in the Onderberg area of Mpumalanga Province. A single lot of Mediterranean fruit flies was placed under a tree in a selected row (designated the release row). The lid of the holding container was removed and the flies were allowed to disperse naturally. Marula fruit flies were released under the adjacent tree and Natal fruit fly under the following tree. The sequence of releases was repeated 18 times and a total of 9 000 individuals of each species were released. Releases were made between 10h00 and 11h00 during fine weather. The trial was repeated three times on 27 May, 9 June and 15 July 2005.

Traps were emptied before each release of the flies. Sensus traps were placed in trees that were two rows east of the release row. A Sensus trap containing Questlure was placed in the first tree. Three trees further down the row a Sensus trap with Ceratitislure was hung and a further three trees down the row a Sensus-Capilure trap was used. The sequence was repeated until six traps of each Sensus-lure combination were positioned. The arrangement of Sensus traps with lures was repeated two rows west of the release row. A Probodelt yellow plastic bucket trap (McPhail-type but with 3 additional holes near the lid - Figure 3.3.7.1) containing Biolure was placed 12 trees in from the first tree in the fourth row from the release row on the east

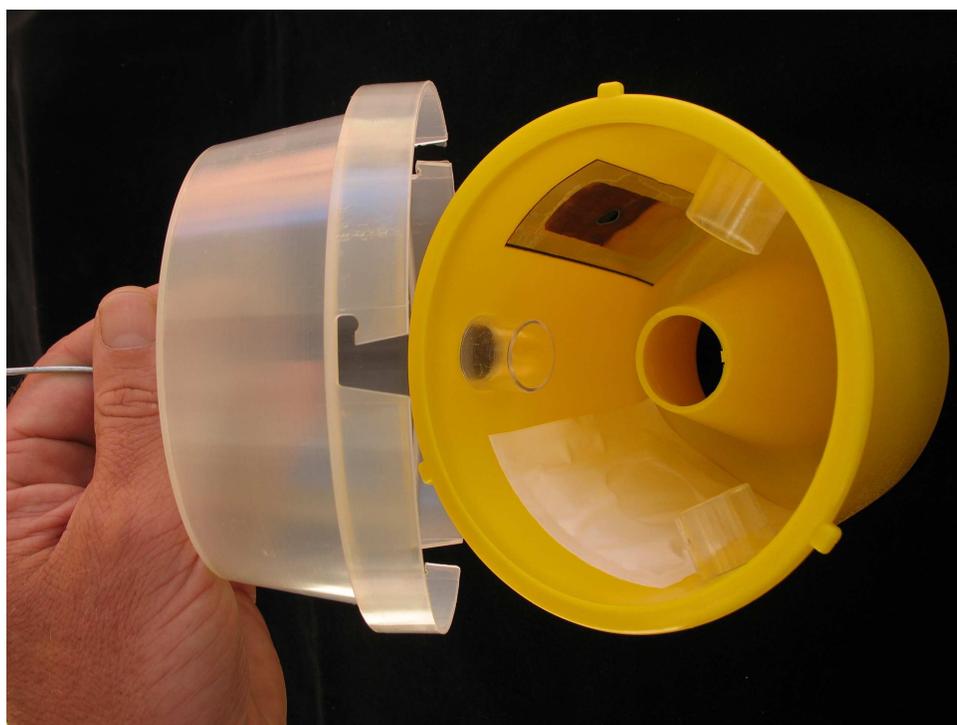
side. A second Probodelt trap with Biolure was placed in a similar position to the first but in the twelfth tree from the last tree in the fourth row from the release row on the west side. All lures were replaced every six weeks.

The traps were monitored and emptied daily for the first six days after a release of flies, and weekly thereafter. All flies caught were taken to the laboratory where they were checked for the presence of dye and identified to species and sexed. The recovery rate for the flies and trap types could then be determined.

The work conducted in 2005 with 3-day-old flies above was repeated in 2006 at the same mango orchard (Neos Estates in the Onderberg area of Mpumalanga Province) but using 12-day-old flies. All techniques were the same as before apart from the age of the flies. Protein was withheld, although the adults were allowed water and granulated white sugar *ad lib*. The flies were released (9000 per species) on 29 May, 21 June and 23 September 2006.

Traps were emptied before each release of the flies. Sensus traps were placed in trees that were two rows east of the release row. A Sensus trap containing Questlure was placed in the first tree. Three trees further down the row a Sensus trap with Capilure was hung and a further three trees down the row a Sensus-Ceratitislure trap was used. The sequence was repeated until six traps of each Sensus-lure combination were positioned. The arrangement of Sensus traps with lures was repeated two rows west of the release row. All lures were replaced every six weeks. Two larger, yellow, Probodelt traps with Biolure (3-component) were placed on either side of the rows of Sensus traps, two rows away and diagonally opposite one another. They were each 4 rows away from the release row and flies had to first pass the Sensus traps before reaching the Biolures.

The traps were monitored and emptied daily for the first six days after a release of flies and weekly thereafter. However, by the fifth day after release, numbers of released flies being caught were almost negligible so the first four days after release were used for comparison purposes. Flies with traces of dye were identified to species and sexed in the laboratory.



**Figure 3.3.7.1.** Probodelt trap with 3-component sachets attached to inner walls

A comparison of Questlure, Ceratitislure and Capilure with 3-component and 2-component lures using Probodelt yellow traps

A comparison of Questlure, Ceratitislure and Capilure with Biolure 3-component lures consisting of sachets of ammonium acetate, trimethylamine and putrescine was conducted in March and April 2008. A mature Protea mid-season orange orchard on rough lemon rootstock at Oewersig farm near Nelspruit was used where all three *Ceratitis* species had been shown to be present and the orchard was close to other tree crops such as mangoes and avocados. The trees were 27 years old and 3.5 m high. The large Probodelt

traps were used in order to include the commercially available Biolure sachets that are too large to use in Sensus traps without partially blocking the entry holes. Each South African lure was placed on a sponge in the capsule normally used in Sensus traps. A special holder was made from wire to hold the capsule in a horizontal position at the same height as the hole in the Biolure sachets. The capsules had the sponge facing upwards and a coarse mesh grid was placed over the top to prevent flies making direct contact with the lure. In all cases except one where capsules were used, the substrate for absorbing the lure was polyethylene sponge. In the exception, two dental rolls (4 cm long) were forced into the capsule so that a different chemical could be placed on each roll in case they reacted when mixed together. The lures used were as described in Table 3.3.7.1. In the case of the capsules, 2.5 g lure was added to the sponge in each capsule. The capsules were carefully weighed before being placed in the field and weighed again when removed to determine how much lure had evaporated. The traps were hung just inside the tree on the south-eastern side at a height of between 1.6 and 1.9 m above ground. The traps were hung in every tenth tree in the row and every fifth row. This meant that a distance of at least 35 m separated any two traps. Four replicates were used per lure and the trap positions in the orchard were initially randomised within each of four zones in the orchard. Thereafter, when the traps were emptied, each trap was moved onwards two positions so that by the end of the trapping period the traps were back in the first position again. The traps were first hung out on 27 February 2008 and emptied and moved every 7 d until 2 April 2008. All *Ceratitis* flies in the traps were identified and an average number of flies per lure per week determined for each sex of each species. Numbers of trapped flies were compared using analysis of variance after a square root transformation of the number of flies plus 0.5.

**Table 3.3.7.1.** Details on lures compared in the trial at Oewersig

Abbreviation used	Description of lure
3-COMP	3-component Biolure comprising sachets with trimethylamine, ammonium acetate and putrescine
2-COMP	2-component Biolure comprising sachets with trimethylamine and ammonium acetate
DEN-1:2	2-components of trimethylamine (0.75 g) and ammonium acetate (1.5 g dissolved in 0.75 g distilled water) in a 1:2 ratio absorbed into separate dental rolls placed in the capsule from a Sensus trap
SEN-1:2	2-components (as for DEN-1:2) of trimethylamine and ammonium acetate in a 1:2 ratio absorbed into the standard sponge in the capsule from a Sensus trap
SEN-1:10	2-components of trimethylamine (0.15 g) and ammonium acetate (1.5 g dissolved in 0.5 g distilled water) in a 1:10 ratio absorbed into the standard sponge in the capsule from a Sensus trap
CAP	Capilure absorbed into the standard sponge in the capsule from a Sensus trap
CER	Ceratitislure absorbed into the standard sponge in the capsule from a Sensus trap
QUEST	Questlure absorbed into the standard sponge in the capsule from a Sensus trap

## Results and discussion

### The competitive efficiency of Questlure, Ceratitislure and Capilure for a known number of released flies

The numbers of flies trapped during the first four days after releases in 2005 were determined from the raw data of that year and are expressed in Table 3.3.7.2. These results were then used in various comparisons with the data from 2006 (Table 3.3.7.3). Unfortunately, there was a lot of variability in the data between releases of the same year so very few significant differences were found. After the work was conducted in 2005, concern was expressed that Natal fruit flies were immature when released and this may have affected their interest in certain lures. This concern appears to have been justified because the recovery of Natal fruit fly in traps with Capilure and Ceratitislure was higher with 12-day-old flies than with 3-day-old flies (Figure 3.3.7.2). Capilure is a parapheromone or type of sex attractant and Ceratitislure contains caryophyllene which is a sesquiterpene found in citrus rind and presumably is more attractive when the flies are looking for oviposition sites. From the work conducted by Grout and Stoltz (2007) it is known that Medfly reared at 26°C is ready to oviposit 3.4 d after eclosion, whereas Marula fruit fly requires 5.1 d and Natal fruit fly 6.6 d. With the releases of young flies in 2005, many of the Natal fruit flies would have died before seeking oviposition sites or mates and to a lesser extent this would have applied to some of the Marula fruit flies. The highest total number of flies trapped with 3-day-old flies was for Medfly males (Table 3.3.7.2) but the highest number trapped of the 12-day-old flies was Marula fruit fly males (Table 3.3.7.3). Questlure showed the fewest differences between the ages of the flies because it is primarily a food-type of lure and its attractiveness is not affected by sexual maturity. However, the recovery percentages for Questlure were the lowest of all the lures (Figure 3.3.7.2).

**Table 3.3.7.2.** Flies trapped in 12 traps per lure type during the 4 days following each release of 9000 flies (3 days old) per species in 2005.

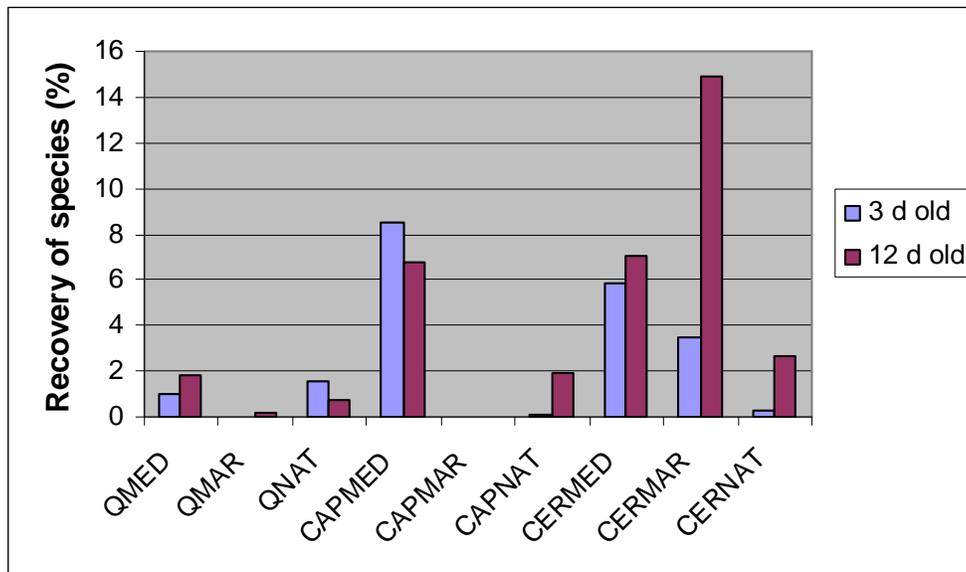
Lure	Species	Sex	Release 1	Release 2	Release 3	Total trapped
Questlure	Medfly	M	1	24	5	30 ab
		F	20	120	106	246 ab
	Marula	M	2	0	2	4 ab
		F	2	5	1	8 ab
	Natal	M	121	67	24	212 ab
F		106	79	19	204 ab	
Capilure	Medfly	M	550	440	1033	2023 d
		F	71	39	170	280 ab
	Marula	M	0	0	0	0 a
		F	0	0	0	0 a
	Natal	M	16	4	12	32 ab
		F	1	1	3	5 ab
Ceratitislure	Medfly	M	262	278	727	1267 c
		F	28	72	208	308 b
	Marula	M	192	446	291	929 c
		F	1	3	2	6 ab
	Natal	M	26	20	10	56 ab
		F	13	9	8	30 ab

Means in the last column followed by the same letter are not significantly different at P=0.05 (SNK)

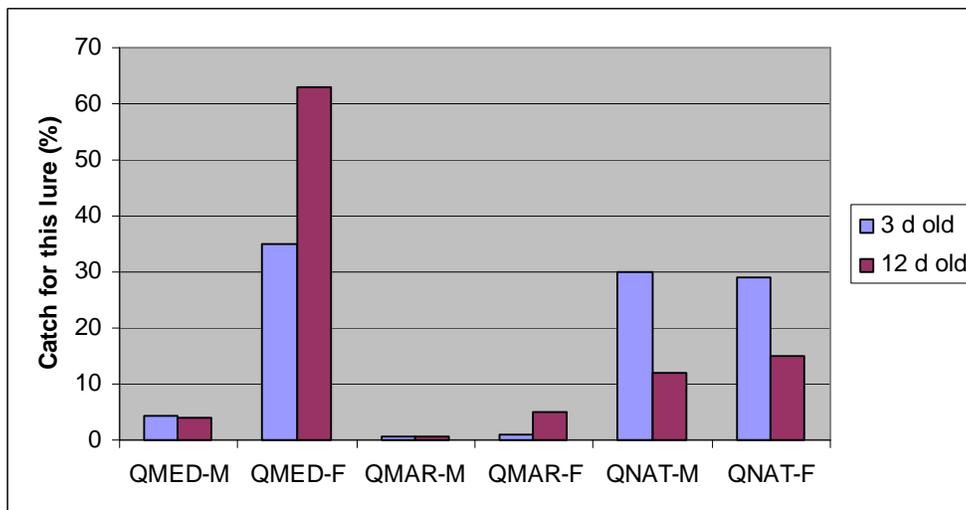
**Table 3.3.7.3.** Flies trapped in 12 traps per lure type during the 4 days following each release of 9000 flies (12 days old) per species in 2006.

Lure	Species	Sex	Release 1	Release 2	Release 3	Total trapped
Questlure	Medfly	M	6	16	7	29 ab
		F	213	194	55	462 bc
	Marula	M	3	1	2	6 a
		F	28	5	4	37 ab
	Natal	M	26	39	24	89 abc
F		34	46	29	109 abc	
Capilure	Medfly	M	440	557	794	1791 d
		F	5	9	30	44 ab
	Marula	M	0	0	0	0 a
		F	0	0	0	0 a
	Natal	M	13	102	406	521 abc
		F	0	1	5	6 a
Ceratitislure	Medfly	M	776	609	330	1715 d
		F	72	51	69	192 abc
	Marula	M	2036	861	1096	3993 e
		F	7	23	5	35 ab
	Natal	M	72	282	328	682 c
		F	8	9	12	29 ab

Means in the last column followed by the same letter are not significantly different at P=0.05 (SNK)

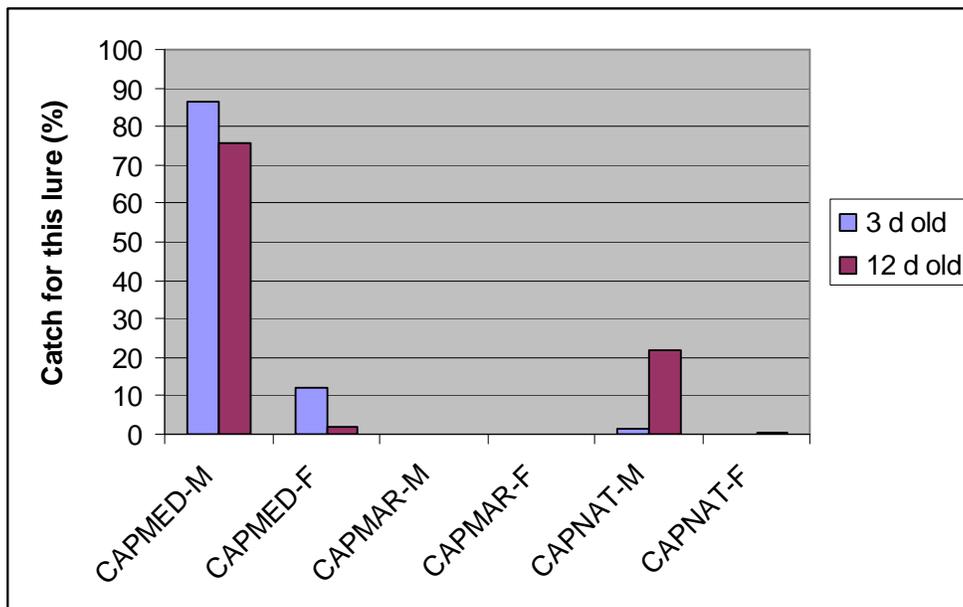


**Figure 3.3.7.2.** Percentage recovered of each species released at different ages where Q, Cap and Cer refer to Questlure, Capilure and Ceratitislure, and Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively. None of the differences due to age at any particular lure and species combination were significant at  $P=0.05$ .

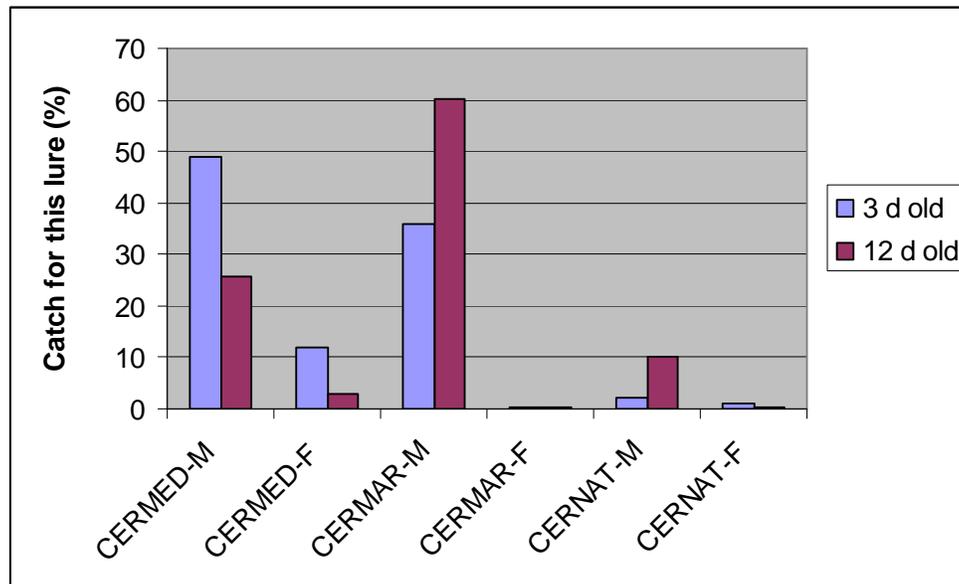


**Figure 3.3.7.3.** Percentage catch for Questlure for different sexes and species released at two different ages, where Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively, and M and F refer to the sex. None of the differences due to age at any particular sex and species combination were significant at  $P=0.05$ .

Considering the catches per sex and species as a percentage of the total catches per lure, provided a slightly different perspective. For 3-day-old fruit flies, catches with Questlure were fairly evenly distributed between female Medflies, and both male and female Natal fruit flies (Figure 3.3.7.3). Capilure is known to be ineffective for Marula fruit fly (Hancock 1987) and this was confirmed (Figure 3.3.7.4). This lure mostly caught male Medfly with an increase in male Natal fruit fly when 12-day-old flies were used.



**Figure 3.3.7.4.** Percentage catch for Capilure for different sexes and species released at two different ages, where Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively, and M and F refer to the sex (Capilure is known to have no attraction for Marula fruit fly). The difference due to age for Medfly females attracted to this lure was significant ( $P < 0.05$ ) but there were no other significant differences.



**Figure 3.3.7.5.** Percentage catch for Ceratislure for different sexes and species released at two different ages, where Med, Mar and Nat refer to Medfly, Marula fruit fly and Natal fruit fly, respectively, and M and F refer to the sex. None of the differences due to age at any particular sex and species combination were significant at  $P = 0.05$ .

Ceratislure was most effective for Medfly males and Marula fruit fly males, the latter showing an increased attraction with fly age (Figure 3.3.7.5). Although not part of the main comparison, the two McPhail traps with Biolure showed that the fly age had no apparent effect on this trap-lure combination (Table 3.3.7.4). Biolure was not very effective for either sex of Marula fruit fly and caught primarily female Medfly. Numbers of caught female Natal fruit flies only amounted to approximately one-third of the numbers of female Medfly.

In crops such as mangoes where Marula fruit fly is an important pest, Biolure would not be a good choice for monitoring purposes and Ceratislure would be the best option. Ceratislure provided the best recovery of all three *Ceratitis* species but is more effective for mature flies. It must be remembered that these results were obtained where the flies had the option to go to the different lures. Where one particular type of lure is

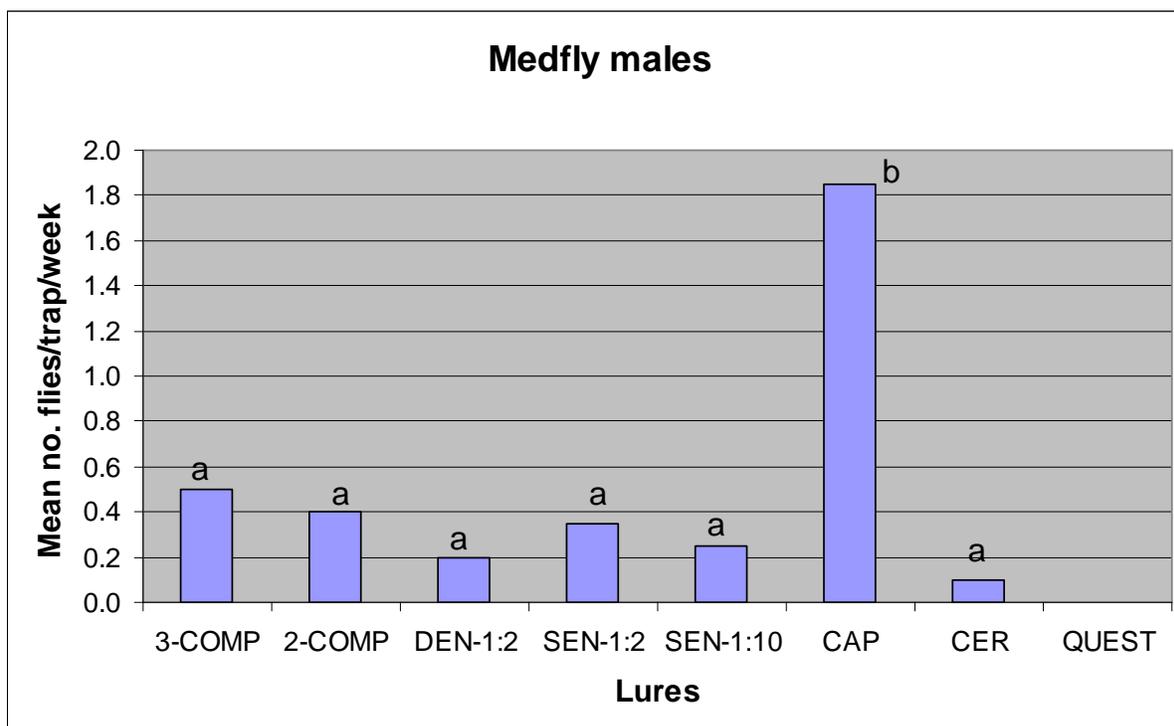
used in an orchard as a monitoring system without competition with other lures, the recovery level may be higher.

**Table 3.3.7.4.** Mean totals of flies caught in two yellow plastic Probodelt traps with Biolure over the 4 days after each release.

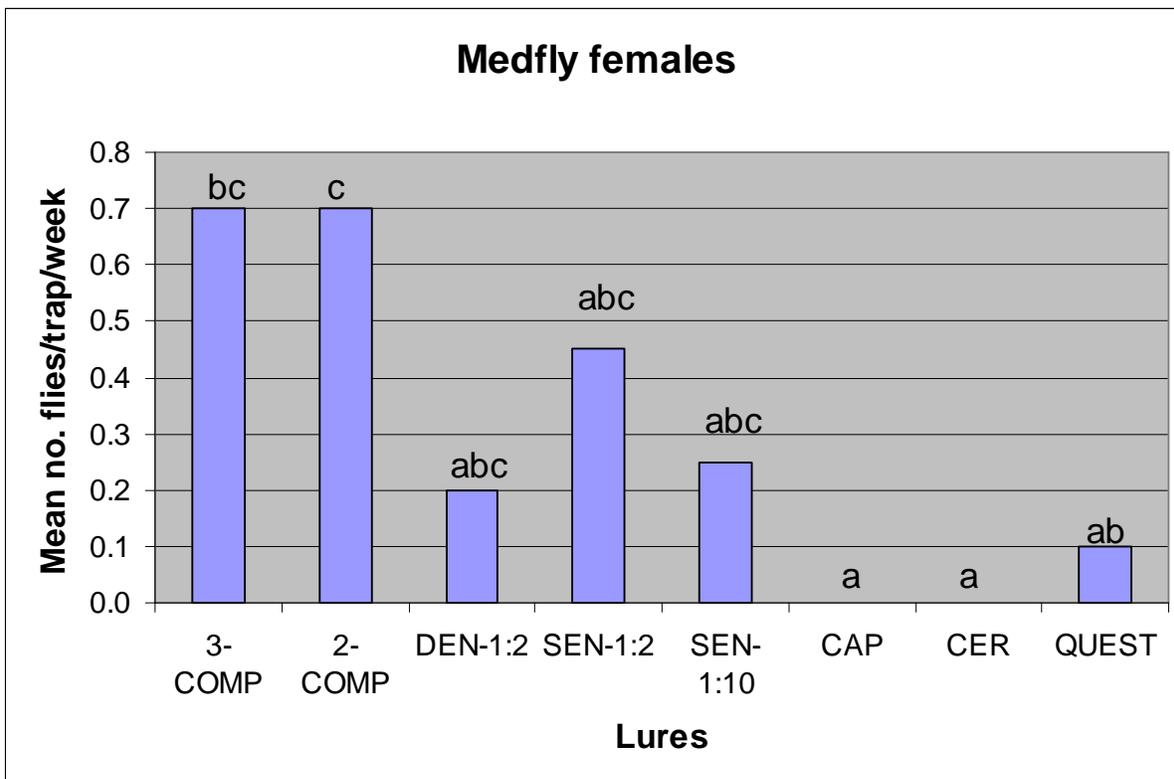
Species	Sex	3-day-old flies	Catch (%) 3-d	12-day-old flies	Catch (%) 12-d
Medfly	M	35.7	8.7	8.5	2.2
	F	215.0	52.2	255.0	65.1
Marula	M	3.3	0.8	3.0	0.8
	F	6.0	1.4	6.0	1.5
Natal	M	70.7	17.2	47.0	12.0
	F	81.0	19.7	72.0	18.4

A comparison of Questlure, Ceratitislure and Capilure with 3-component and 2-component lures using Probodelt yellow traps

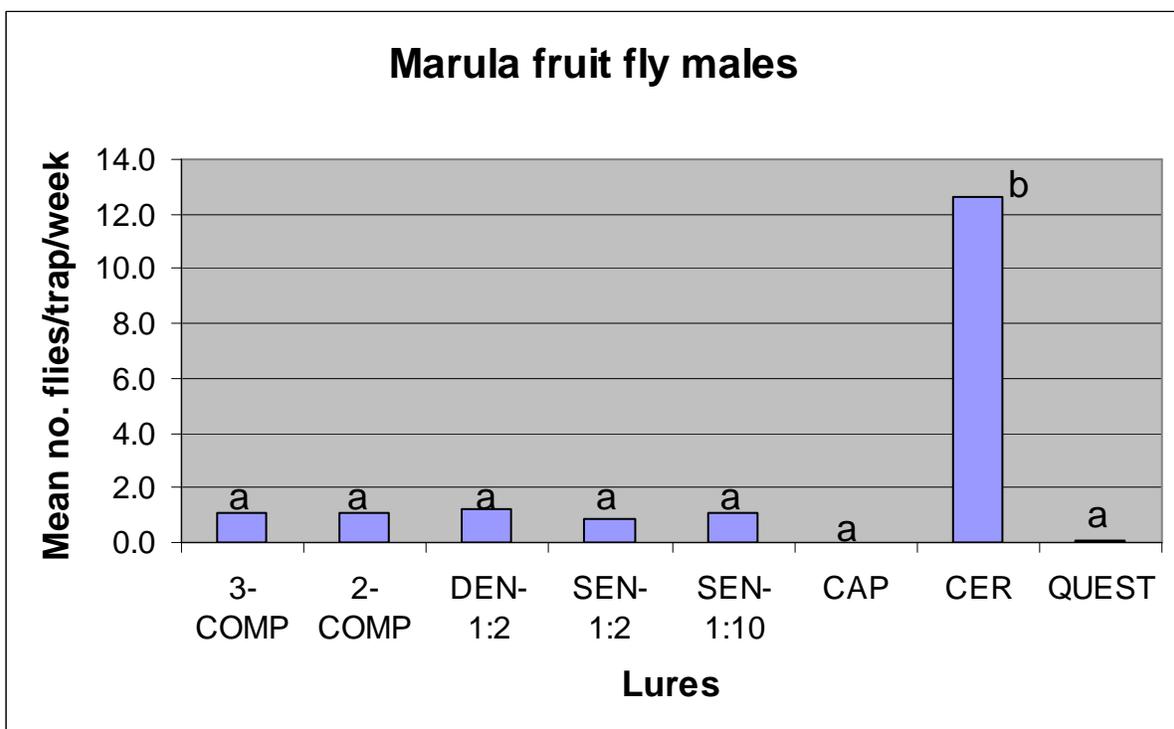
The problem with this comparison is that it involves wild fly populations of unknown densities. Although all three species were present it appears that numbers of Natal fruit fly may have been the most abundant. This means that lures can only be compared within the same species in the graphs below. Capilure was significantly ( $P < 0.05$ ) more effective than any other lure for Medfly males (Fig. 3.3.7.6) and there were no significant differences between the 3-components and the 2-components or other lures. Questlure did not catch any male Medfly but this may have been due to the relatively low numbers of this species. Female Medfly were most attracted to the 2- and 3-component lures with the Biolure 2-component sachets catching significantly more than Questlure and of course Capilure and Ceratitislure, which are primarily male lures (Fig. 3.3.7.7). Marula fruit fly males were significantly more attracted ( $P < 0.05$ ) to Ceratitislure than any other lure and the other lures were not significantly different from one another.



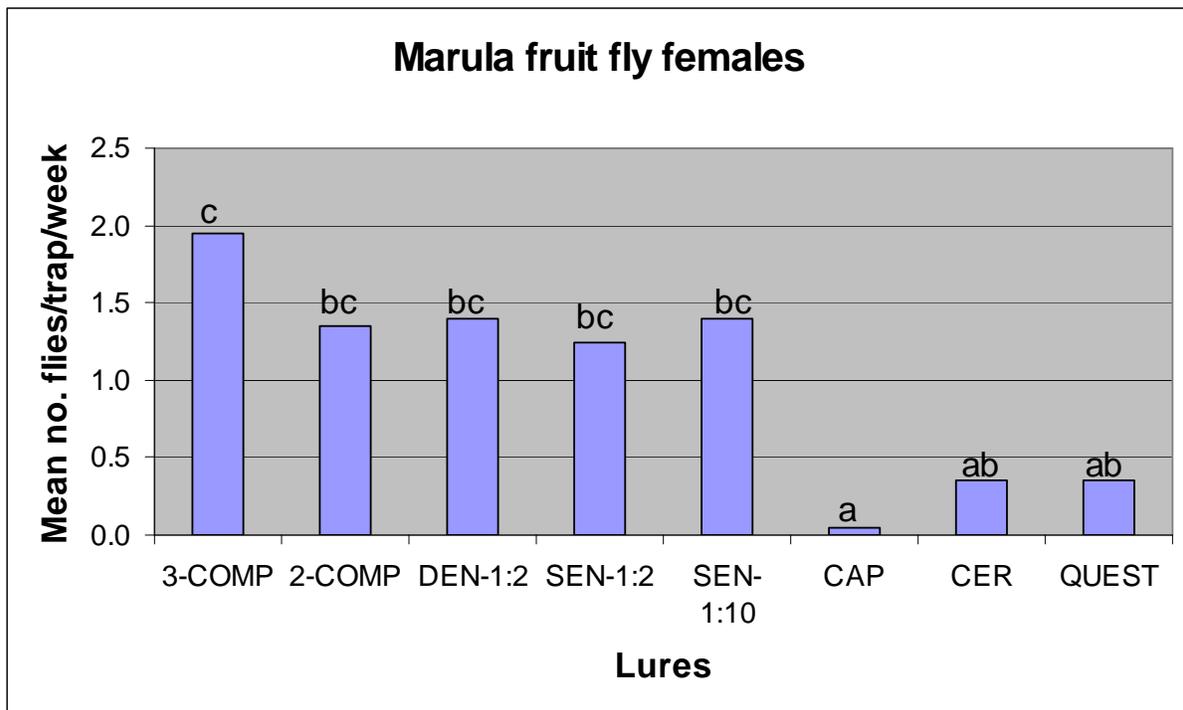
**Figure 3.3.7.6.** Numbers of male *Ceratitis capitata* caught with different lures (see Table 3.3.7.1 for an explanation of the lure abbreviations).



**Figure 3.3.7.7.** Numbers of female *Ceratitis capitata* caught with different lures (see Table 3.3.7.1 for an explanation of the lure abbreviations)

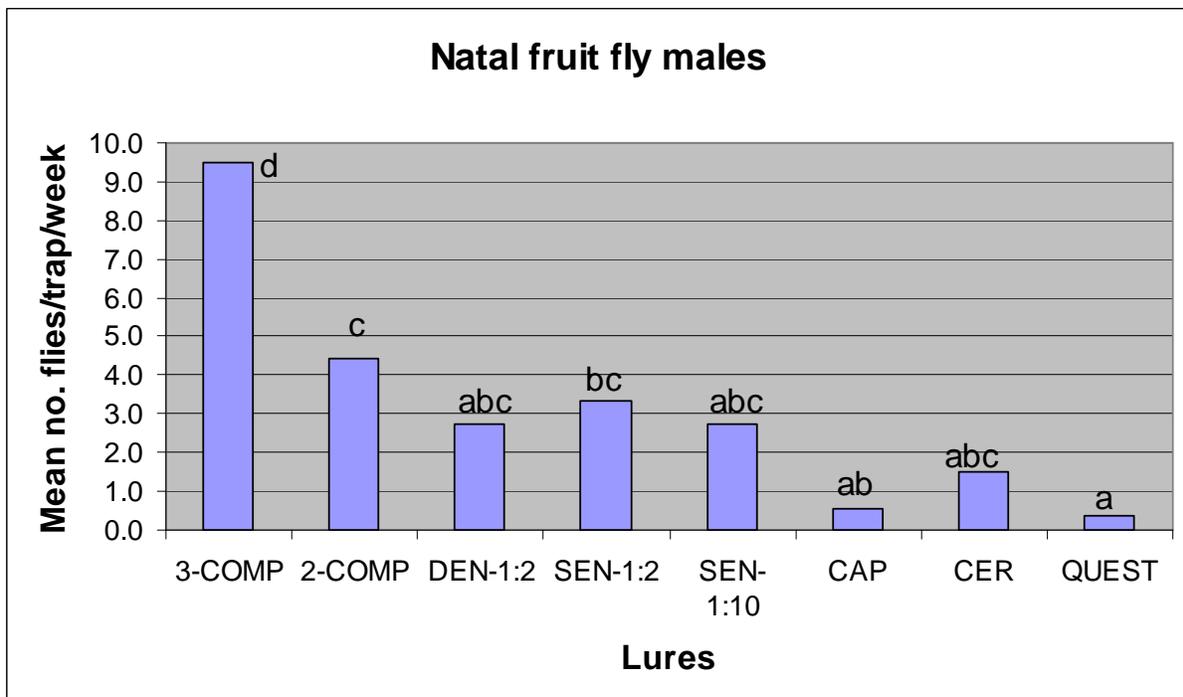


**Figure 3.3.7.8.** Numbers of male *Ceratitis cosyra* caught with different lures (see Table 3.3.7.1 for an explanation of the lure abbreviations)

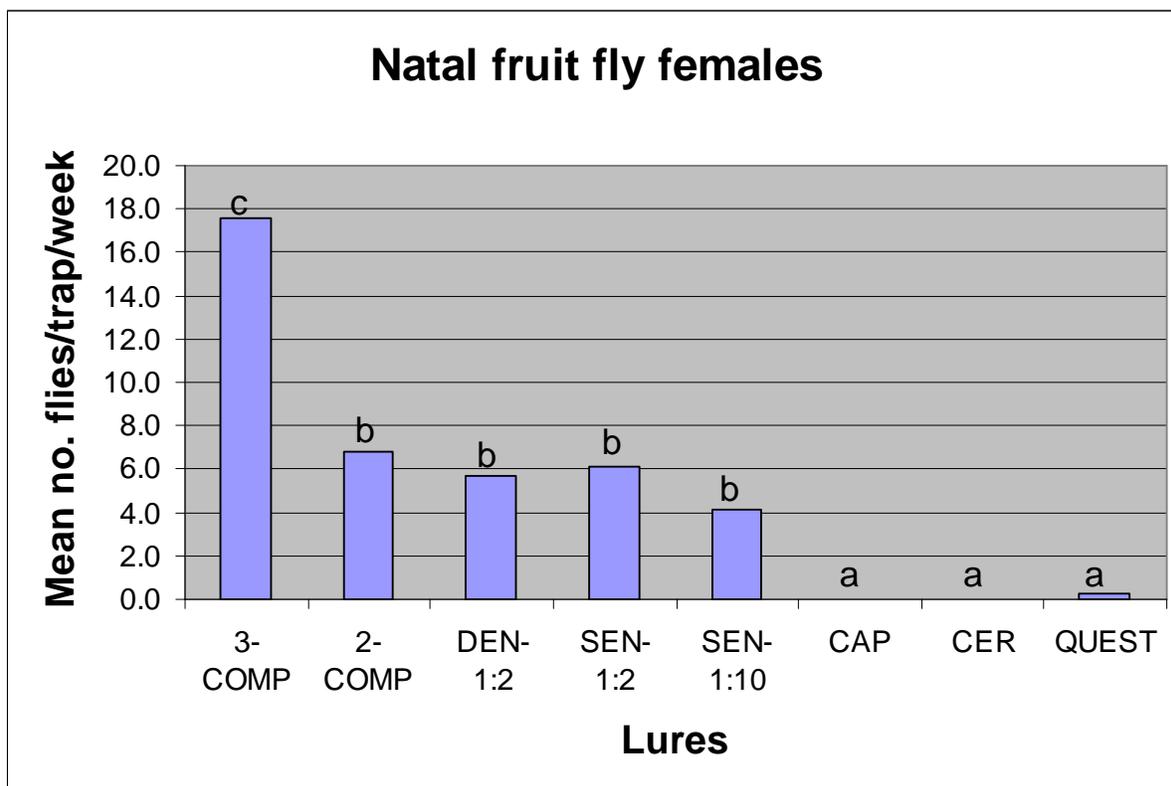


**Figure 3.3.7.9.** Numbers of female *Ceratitis cosyra* caught with different lures (see Table 3.3.7.1 for an explanation of the lure abbreviations)

Marula fruit fly females were significantly more attracted to 3-component lure than to Ceratitislure or Questlure but there were no significant differences between the various types of 2-component lure and 3-component lure (Fig.3.3.7.9). Numbers of male Natal fruit fly were significantly more attracted to 3-component lure than the various 2-component lures (Fig. 3.3.7.10). The 2-component sachets attracted significantly more male Natal fruit flies than Capilure. This was the opposite of the trend with Medfly males. The 3-component lure was significantly more effective than any of the 2-component lures for the female Natal fruit fly (Fig. 3.3.7.11). The 2-component lures were in turn significantly more effective ( $P < 0.05$ ) than Questlure or the two male lures.



**Figure 3.3.7.10.** Numbers of male *Ceratitis rosa* caught with different lures (see Table 3.3.7.1 for an explanation of the lure abbreviations)



**Figure 3.3.7.11.** Numbers of female *Ceratitis rosa* caught with different lures (see Table 3.3.7.1 for an explanation of the lure abbreviations)

Previous research (Table 3.3.7.4) had shown that when equal numbers of species were released, 3-component lure caught more female Medfly than female Natal fruit fly. With wild fruit flies, this trend seemed to be reversed which emphasised the relatively low numbers of Medfly in the orchard. The use of the 2-components in a 1:2 ratio in the Sensus capsule attracted at least three times as many females of each of the species then Questlure did. This mixture of the 2-components in the Sensus capsule evaporated faster than the same two chemicals in dental rolls (Table 3.3.7.5). The same two chemicals in the 1:10 ratio in the Sensus capsule lost weight more slowly than the 1:2 ratio so it must be the trimethylamine that is the most volatile.

**Table 3.3.7.5.** Loss of mass of lures in Sensus capsules while exposed for 5 weeks

Abbreviation used	Mean loss of mass (g)	Mean loss of mass (%)
DEN-1:2	0.72	9.8
SEN-1:2	1.10	16.0
SEN-1:10	0.35	5.6
CAP	0.06	0.8
CER	0.03	0.5
QUEST	0.00	0.0

One of the more striking observations in both types of comparisons was that Capilure was more effective for Medfly males than for Natal fruit fly males. With the wild flies this is only obvious from relative differences between male catches with Capilure and male catches with all the other lures (Figs. 3.3.7.6 and 3.3.7.10) but with the released flies direct comparisons can be made (Tables 3.3.7.2 and 3.3.7.3). More than three times as many released male Medflies were caught than released male Natal fruit flies. This difference was more extreme when young flies were compared because the immature adult Natal fruit fly males were even less attracted to Capilure. This difference between species regarding Capilure would suggest that there should be more rejections for citrus infestation by Natal fruit fly but the detections made by PPECB (section 3.3.9) show that all rejections are due to Medfly. The longer time between adult eclosion and oviposition in Natal

fruit fly (Grout and Stoltz 2007) may be the reason for this as Natal fruit fly females will have twice the chance of encountering bait in an orchard before ovipositing than Medfly females. However, with there being increased concern about infestations of Natal fruit fly in export fruit the intervention threshold of 4 flies per Capilure trap per week previously used for both Medfly and Natal fruit fly, should be lowered to 2 flies in the case of Natal fruit fly. This will mean that counting will take longer because the species of fly will have to be identified.

## Conclusions

Capilure is the most effective lure for Medfly males and Ceratitislure is the most effective lure for Marula fruit fly males. Three-component lure is the most effective lure for both sexes of Natal fruit fly. The use of 2-component lure in a 1:2 ratio in the Sensus capsule caught at least three times more females of all species than Questlure. Capilure catches at least three times more male Medflies than male Natal fruit flies. The lowering of the intervention threshold of 4 flies per trap per week for Natal fruit fly when using Capilure should therefore be lowered to 2 Natal fruit flies per trap per week to prevent fruit infestation by this fruit fly.

## Future research

Some of the results from these comparisons will be used in developing an improved bait for all three species of *Ceratitis* (experiment 915).

## Technology transfer

A decision on whether to change the Capilure threshold will first be made before communicating these results further.

## References cited

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### 3.3.8 **PROGRESS REPORT: Determine the potential global distributions for Natal fruit fly** Experiment 805 (March 2006 – March 2010): M de Villiers (SU)

## Summary

Natal fruit fly, *Ceratitis rosa* Karsch, is a pest of various South African fruit. Due to phytosanitary concern, this pest hinders international fruit trade. Knowing its current distribution, its potential global distribution can be modelled and the relevance of associated phytosanitary regulations evaluated. Bucket traps baited with BioLure® were placed in different South African climatic regions to trap Natal fruit fly. Three traps, serviced monthly, were used per monitoring area. The abundance distribution of Natal fruit fly seemed to correspond with climatic differences, with lower numbers or absence observed in areas with hot, dry summers, suggesting it is a good candidate to model its potential global distribution based on climate.

## Opsomming

Natalse vrugtevlieg, *Ceratitis rosa* Karsch is 'n plaag van 'n verskeidenheid van Suid-Afrikaanse vrugte. Weens fitosanitêre belang, verhinder hierdie plaag internasionale vrugtehandel. Kennis van sy huidige verspreiding maak dit moontlik om sy potensiële globale verspreiding te modelleer om sodoende die toepaslikheid van geassosieerde fitosanitêre regulasies te evalueer. "Emmer"-valle met BioLure® as lokmiddel is in verskillende klimaatstreke van Suid-Afrika geplaas om Natalvlieg te monitor. Drie valle, wat maandeliks nagegaan is, is per monitorarea gebruik. Die volopheidsverspreiding van Natalvlieg het geblyk om ooreen te stem met klimaatsverskille, met laer getalle of afwesigheid in areas met warm, droë somers. Dit stel voor dat Natalvlieg 'n goeie kandidaat is om sy potensiële globale verspreiding, gebaseer op klimaat, te modelleer.

## Introduction

Natal fruit fly, *Ceratitis rosa* Karsch (Diptera: Tephritidae), is a pest of international phytosanitary concern (EPPO 2007). In South Africa, this pest infests a wide range of fruits. By determining its current distributions

it becomes possible to model its potential to invade other parts of the world. Such information provides a scientific basis to evaluate the relevance of current and future phytosanitary restrictions imposed on international citrus trade to minimize the risk of introducing this pest into importing countries. The availability of reliable, science-based, technical information of such a nature is the only legitimate basis for contesting phytosanitary restrictions placed on trade. Therefore, such information is considered critical in both maintaining and gaining market access.

To be able to model the potential future global distributions, detailed distribution data need to be obtained. This should be achieved by gaining relevant information from the literature to determine historical distribution. The power of the modelling exercise can be greatly enhanced by including more detailed distribution and abundance data. Such information is not currently available and will require surveys. The surveys will be focussed on generating more detailed information on the pest's occurrence within the southern African zone of distribution. The objective of such surveys will be two-fold. Firstly, the geographical limits of distribution ranges within southern Africa should be determined and secondly, some measure of comparative abundance, across the distribution range, within southern Africa.

## Materials and methods

To determine relative abundance of Natal fly across the country, 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, Tom Burke, Tzaneen, Rustenburg and Harare. These areas are representative of the different climatic regions in 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. These traps were placed mostly in home gardens and in host plants. 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

The abundance data of Natal fly is given in Table 3.3.8.1. Natal fly was absent from Porterville, Vanrhynsdorp, Swellendam, Beaufort West, Hondeklipbaai, Garies, Springbok, Onseepkans, Keimoes and Tom Burke. The maximum monthly average over the period January 2007 to March 2008, was very low (<10 flies/trap/month) in Citrusdal, Clanwilliam (only one specimen found), Britstown, Olifantshoek, Gariëpdam, Tshipise and Vryburg, low (10-29.9 flies/trap/month) in Jan Kempdorp, moderate (30-99.9 flies/trap/month) in Piketberg, Nkwalini and Groblersdal/Marble Hall, high (100-499.9 flies/trap/month) in Onrus/Vermont, Riebeeck Kasteel, Knysna, King William's Town, Bloemfontein, Komatipoort, Tzaneen, Rustenburg and Harare, and very high ( $\geq 500$  flies/trap/month) in Somerset West, Stellenbosch, Paarl, Addo, Pietermaritzburg and Nelspruit. Data of various months still need to be obtained from Gariëpdam, Tom Burke and Vryburg.

Although the study focuses on Natal fruit fly, other fruit flies were also recorded. With the exception of Hondeklipbaai, Mediterranean fruit fly, *C. capitata* (Wiedemann), was present in all these areas. Marula fruit fly, *C. cosyra* (Walker), was present in Groblersdal/Marble Hall, Harare, Jan Kempdorp (only one specimen found), Komatipoort, Nelspruit, Pietermaritzburg, Rustenburg, Tom Burke, Tshipise, Tzaneen and Vryburg (only one specimen found).

## Conclusion

The abundance distribution of Natal fruit fly seemingly corresponded with climatic differences, being generally absent or present in lower numbers in areas with dry, hot summers. This suggests that Natal fly is a good candidate for modelling its potential global distribution based on climate.

## **Technology transfer**

No technology transfer occurred during January 2007 to March 2008. However, a poster will be presented at the International Congress of Entomology during July 2008 and a presentation at the Citrus Research Symposium during August 2008.

## **Further objectives (milestones) and work plan**

Trapping in each area will continue until two years' data are obtained. A CLIMEX course will be attended in Australia. This will ensure reliability of the model that will be created for potential global distribution, using CLIMEX.

## **References cited**

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**Table 3.3.8.1.** Abundance data of Natal fruit fly, *Ceratitis rosa*, across southern Africa

Area	2007												2008		
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Onrus/Vermont	107.3	52.3	24.0	43.3	20.3	5.3	1.0	2.0	2.0	8.7	10.0	28.7	132.3	55.7	52.3
Somerset West	515.3	96.3	143.0	69.0	36.3	54.7	21.3	7.3	6.7	18.0	79.0	180.3	196.7	129.7	-
Stellenbosch	609.3	206.7	507.3	204.7	177.0	21.3	23.0	7.3	5.3	52.7	436.0	247.3	478.0	276.0	366.7
Paarl		1288.7*	55.7	151.3	32.0	15.0	12.0	7.7	4.3	9.7	27.7	128.7	420.0	682.0	60.5
Riebeeck Kasteel	12.7	3.3	32.3	84.7	118.7	65.3	26.0	6.3	0	4.3	1.0	4.7	10.7	16.7	25.0
Piketberg	22.3	1.0	2.3	2.3	14.3	11.0	6.0	2.7	3.0	5.0	55.0	-	-	-	-
Porterville	0	0	0	0		0*	0	0	0	0	0	0	0	0	0
Citrusdal	0.3	0	1.0	1.0	1.7	0	0	1.0	0.7	0	3.7	0.7		0*	0
Clanwilliam	0	0	0	0.3	0	0	0	0	0	0	0	0		0*	0
Vanrhynsdorp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Swellendam	0	0	0	0	0	0	0	0	0	0	0		0*	0	0
Knysna	136.3	18.7	8.0	0.7		13.0*	5.7	0.7	3.7	1.0	89.7	23.0	15.0	27.3	-
Beaufort West	0	0	0	0	0	0	0	0	0	0	0	0		0*	0
Hondeklipbaai	-	-	-	-	-	-	-	0	0	0	0	0	0	0	-
Garies	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Springbok	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Onseepkans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Keimoes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Britstown	0	0.7	0.7	0	0	0	0	0	0	0	0	0	0	4.0	8.3
Olifantshoek	0.3	0.3	0	0	0	0	0	0	0	0	0	0.3	0	1.0	4.3
Jan Kempdorp	0	1.0	0.3	0	4.3	0	0	0	0	0	0	6.3	23.0	4.3	1.3
Addo	82.7	33.3	59.3	158.7	1069.0	483.0	317.7	48.3	117.7	49.3	62.7	77.0	124.0	256.7	190.0
King William's Town	469.3	136.0	209.0			355.3*			27.0*	11.7	24.3	97.7	135.0	220.0	24.7
Bloemfontein	175.0	118.7	47.0	47.7	34.7	0.3	0.7	0	0	0	1.0	14.0	66.0	208.0	350.3
Gariepdam	0.3		1.3*	0.3	2.3	-	-	-	-	-	-	-	-	-	-
Pietermaritzburg	-	-	-	324.0	392.7	237.7	211.7	102.3	22.7	34.3	144.7	391.7	437.0	637.0	316.3
Nkwalini	-	-	-	-	-	61.7	48.3	36.7	94.0	16.0	8.3	6.7		35.7	19.3
Groblersdal/Marble Hall	33.0	37.3	-	-	-	-	-	-	-	0.7	50.3	72.3	25.0	-	-
Nelspruit	492.7	183.3	101.7	49.7	58.3	28.7	44.7	33.3	16.3	54.7	76.3	268.3	535.3	497.3	184.3
Komatipoort	110.0	78.3	17.0	5.7	8.7	8.0	8.7	7.3	5.7	3.0	5.0		174.3*	54.7	4.7
Tshipise	1.0	3.3	1.3	0	0	0	0.7	0	0	0	0	0	3.0	0	0
Tom Burke	-	-	0	0	0	0	0	0	-	-	-	-	-	-	-
Tzaneen	23.0	36.7	65.7	129.0	83.0	47.7	58.0	64.7				114.3*		95.7*	201.3
Vryburg	7.7	6.0	0.7	3.0	2.0	0.3	0	0	-	-	-	-	-	-	-
Rustenburg	118.3		124.3*			158.7*	24.3		22.7*		34.0*		175.3*		249.0*
Harare	1109.0*				385.7*		210.7*		213.0*	335.7			868.3*		287.0*

\* Collective samples, taken over more than one month – monthly averages not available

- Data not obtained

3.3.9 **FINAL REPORT: Fruit fly recovery from fruit inspected by PPECB**  
Experiment 874 (2007/8) by John-Henry Daneel and Tim Grout (CRI)

**Summary**

Sampling by PPECB inspectors at nine packhouses around the country only resulted in larvae of *Ceratitis capitata* being recovered and no *C. rosa*. Most of the fruit flies were also recovered from Satsuma mandarins. These results suggest that the control measures being used are adequate for Natal fruit fly.

**Opsomming**

Monsterneming deur PPECB inspekteurs by nege pakhuise regoor die land het slegs larwes van *Ceratitis capitata* opgelewer maar geen van *C. rosa* nie. Meeste van die vrugtevlieë is in Satsuma mandaryne gevind. Hierdie resultate dui daarop dat die beheermaatreëls vir Natalse vrugtevlieg voldoende is.

**Introduction**

Comparisons of trapping systems (section 3.3.7) indicated that some lures caught few lab-reared Natal fruit flies. However, the only true indication that the use of a single trap-lure combination in an orchard without competition with other traps is not working for Natal fruit fly, is to see whether there are a high proportion of fruit fly rejections due to Natal fruit fly. In the same way, the most reliable means of determining whether citrus is a host for Marula fruit fly is to determine whether any fruit destined for export is found to contain larvae of this species. A previous, unpublished survey had been conducted by Ware in 2005 in collaboration with the Perishable Products Export Control Board (PPECB) to rear out and identify fruit fly larvae from nine different citrus packhouses sampled by PPECB inspectors. Only a few Medfly larvae were recovered from this exercise. This type of study was repeated in 2007 and again covered nine different packhouses representing different production regions across the country. The report on this research follows.

**Materials and methods**

Firstly a laboratory trial was conducted to evaluate the suitability of the medium placed in vials distributed to the different inspection points, for different numbers of larvae. Larvae (second and third instar) from all three fruit fly species reared at CRI were placed into six vials. Two vials contained +/-20 larvae, two contained +/- 10 larvae and two contained 5 larvae. The medium consisted of 5 g dry medium mixed with 11 ml boiled water. Each vial was then placed in a gauze-covered container with the bottom covered with sand. The larvae were allowed to pupate and emerge as adults.

**Table 3.3.9.1.** Suitability of the medium distributed to the different inspection points

<b>Medfly</b>	<b>Number of pupae</b>	<b>Adult males emerging</b>	<b>Adult females emerging</b>	<b>Total number of adults</b>	<b>Percentage emergence</b>
+/- 20 larvae	24	13	10	23	96
+/- 20 larvae	21	14	7	21	100
+/- 10 larvae	12	9	3	12	100
+/- 10 larvae	10	7	3	10	100
5 larvae	5	3	1	4	80
5 larvae	5	1	4	5	100
<b>Marula fruit fly</b>	<b>Number of pupae</b>	<b>Adult males emerging</b>	<b>Adult females emerging</b>	<b>Total number of adults</b>	<b>Percentage emergence</b>
+/- 20 larvae	21	11	10	21	100
+/- 20 larvae	22	13	8	21	95
+/- 10 larvae	17	9	8	17	100
+/- 10 larvae	10	7	3	10	100
5 larvae	5	4	1	5	100
5 larvae	5	1	4	5	100
<b>Natal fruit fly</b>	<b>Number of pupae</b>	<b>Adult males emerging</b>	<b>Adult females emerging</b>	<b>Total number of adults</b>	<b>Percentage emergence</b>
+/- 20 larvae	25	8	7	15	60
+/- 20 larvae	21	8	9	17	81
+/- 10 larvae	10	4	6	10	100
+/- 10 larvae	4	1	3	4	100
5 larvae	3	3	0	3	100

5 larvae	3	3	0	3	100
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From Table 3.3.9.1 it is clear that the Natal fruit fly emergence rate dropped to an average of 70%, when +/- 20 larvae were placed into the vial. Marula fruit fly also had a 5% loss in one of its containers with higher larval numbers. From this we concluded that it would be safe to recommend that not more than 10 larvae should be placed in a vial at any one time.

The following instructions were sent to each collection point:

**Sampling of larvae for verification purposes**

1. Add 11 ml of **boiling** water to the sampling bottle containing the dry mixture. Mix thoroughly with a small spoon or spatula to form a paste. Press the paste down into the bottom of the container to form a solid layer. **Allow to cool down.**
2. When the paste reaches room temperature transfer larvae from the infested fruit to the paste ensuring that the collected larvae are not damaged. Avoid transferring any unwanted material with the larvae.
3. Complete the label on the sampling bottle with the following information: Packhouse name, date, cultivar and the collector's name.
4. Sample bottles containing the live pupae must be dispatched within 24 hours of sampling for the attention of Mr. John-Henry Daneel at:  
Citrus Research International  
P.O. Box 28  
Nelspruit  
1200  
Tel: (013) 759-8046  
Fax: (013) 744-0578  
Cell: 083 235 9467  
E-mail: [john-henry@cri.co.za](mailto:john-henry@cri.co.za)
5. Storage and dispatch must take place at ambient temperatures.
6. Immediately after dispatch the tracking number must be confirmed with the above person to ensure that there is no delay in receiving the package.

**Results and discussion**

Apart from the drosophilids, only Medfly larvae were received (Table 3.3.9.2). It is also significant to note that most of the hosts were Satsuma mandarins which ripen in late summer when numbers of Natal fruit fly are relatively high compared with Medfly. The mandarins have thinner peels than other citrus types so the fruit flies are more often able to get their eggs below the toxic chemicals and oils in the flavedo. The fact that none of these mandarins were infested with Natal fruit fly is encouraging because it indicates that our control measures for that species are probably adequate. It may be worthwhile to compare the length of the aculeus (ovipositing device) in Medfly and Natal fruit fly because if it is shorter in Natal fruit fly, this will also limit its ability to oviposit eggs below the flavedo and therefore restrict egg survival. Alternatively, the longer time between eclosion and oviposition in Natal fruit fly allows for more time to control the flies with baits before they oviposit.

**Table 3.3.9.2.** Samples received from PPECB during 2007

Sample	Date	Cultivar	Total Pupae	Emerged Adults
1	18/04/07	SATSUMA	2	DROSOPHILID X 1
2	19/04/07	SATSUMA	2	MEDFLY: 2xmale
3	24/04/07	SATSUMA	2	MEDFLY: 1xmale 1xfemale
4	01/05/07	SATSUMA	2	MEDFLY: 1xmale 1xfemale
5	11/07/07	MARSH	1	DID NOT EMERGE

**Conclusion**

Only Medflies were recovered from PPECB fruit inspections and these were mostly found in mandarins.

## Future research

This research will not be repeated unless reports are received of other fly species being found in export fruit.

## Technology transfer

This will be included in a talk at the Citrus Research Symposium in August 2008.

### 3.3.10 FINAL REPORT: Marula fruit fly survival in citrus fruit and bait efficacy

Experiment 890 (2007/8) by Tim Grout, Bruce Tate, John-Henry Daneel and Kim Stoltz (CRI)

## Summary

In a natural orchard situation it is highly unlikely that marula fruit fly would oviposit in citrus shortly before harvest. No-choice oviposition studies showed that no adults emerged from Clementine mandarins or Valencia oranges exposed to 200 mature adult females for three days, although one adult emerged from Star Ruby grapefruit under the same conditions. The survival of inoculated eggs was also poor in Clementines (0.8%) and Star Rubies (2.5%), showing that if marula fruit fly did ever oviposit in citrus in a natural situation, the chances of survival to adulthood are extremely small. Marula fruit fly did feed on Solgel bait but the mixture was not very attractive from a distance. Further research will be conducted on a bait that will be suitable for all *Ceratitis* species.

## Opsomming

In 'n natuurlike boord situasie is dit hoogs onwaarskynlik dat maroela vrugtevlieg kort voor oes eiers in sitrus sal lê. Nie-keuse eierlegging studies het getoon dat geen volwassenes vanuit Clementine mandaryne of Valencia lemoene, na blootstelling aan 200 volwasse wyfies vir drie dae, te voorskyn gekom het nie. Een volwassene het egter vanuit Star Ruby pomelos, onder dieselfde kondisies, te voorskyn gekom. Die oorlewing van geïnokuleerde eiers in Clementines (0.8%) en Star Rubies (2.5%) was ook swak, wat daarop dui dat al sou maroela vrugtevlieg onder natuurlike omstandighede in sitrus eiers lê, die kans op oorlewing tot 'n volwassene baie skraal is. Maroela vrugtevlieg voed wel op Solgel lokaas maar die mengsel was op 'n afstand nie baie aantreklik nie. Verdere navorsing sal op 'n lokaas wat geskik is vir alle *Ceratitis* spesies uitgevoer word.

## Introduction

In 2007, unsubstantiated claims were made that marula fruit fly larvae had been found in export citrus. This claim provided support for further investigations of the susceptibility of different citrus cultivars to *Ceratitis cosyra*. The following research was conducted to verify earlier no-choice oviposition tests (Grout 2000) and to determine the survival of *C. cosyra* eggs inoculated into citrus artificially. The efficacy of protein hydrolysate baits for marula fruit fly had been shown to have no significant impact on marula fruit fly populations in the experiments where baits were applied to the ground cover or the foliage (Daneel et al. 2007) and similar results were obtained in the experiments in 2007 where protein baits were applied to tree trunks and foliage (section 3.3.6). Some preliminary research was therefore conducted on the suitability of Mangan and Moreno's (2007) Solgel bait for *C. cosyra*.

## Materials and methods

In the no-choice oviposition tests, the objective was to expose unwaxed citrus fruit in a cage to high numbers of Marula fruit fly that had no substrates for oviposition besides the citrus, then determine how many second-generation flies would emerge. Clementine mandarins, Star Ruby grapefruit and Valencia oranges were used with four cages for each fruit type. The fruit were placed in Netlon bags and suspended within each cage on 17 May to 20 May. With the Clementines, 75 fruit were used per cage and with the Star Rubies, 50 fruit were used per cage. The Valencia trial was conducted later between 7 and 10 August using 75 fruit per cage. Fifty male and fifty female approximately 9-day-old adult marula fruit flies were released into each cage. The cages were all supplied with granulated sugar and yeast for food and water. After three days of exposure in a natural day-night cycle, all the fruit were removed and inspected for stinging marks. The fruit was then stored at a constant temperature of 26°C on a thin layer of sand in crates lined with garbage bags for 14 days, by which time any larvae should have left the fruit and pupated. The sand was then sieved to collect any pupae which were then left in a screened box to see whether they would develop to adults.

The second approach to this research was to inoculate eggs into the fruit and see how many resultant adults emerged. Two hundred each of Star Ruby grapefruit and Clementine mandarins were inoculated with  $\pm 5$

eggs per fruit using the same technique used for cold disinfestation inoculations (section 3.3.3). The inoculated fruit were placed on a thin layer of sand in crates lined with garbage bags and stored at a constant temperature of 26°C. The sand from all the above crates was sieved after 14 days and all pupae were collected. The pupae were kept in containers until adults emerged from each treatment.

Solgel bait was made up according to the recipe in Mangan and Moreno (2007) with the use of both gum Arabic and corn starch to thicken it into a paste consistency. Hym lure was used in the place of Solulys in the recipe. Small-scale experiments were conducted to determine whether marula fruit fly would feed on this gel by mixing a drop of blue food colorant into some gel and placing this in a large plastic cake box with some marula fruit flies. Approximately 2 ml of gel was also placed in the lid of a Sensus trap with a dichlorvos block and hung in a home garden at the same time as a Sensus trap containing ammonium acetate and trimethylamine in sachets (Biolure) to see how attractive the gel was to *Ceratitidis* species. The sachets had to be trimmed around the edges in order to fit in the trap without obstructing the entrance holes. The traps were 5 m apart and were exposed from 6 November to 14 December 2007.

## Results and discussion

In the no-choice oviposition tests, only one Clementine mandarin had a noticeable sting when fruit were placed on the sand. However, no pupae and therefore no adults were collected from the sand under the mandarins. With the Star Ruby grapefruit, one pupa was collected from which an adult did successfully eclose. No stings and no pupae were recorded from the Valencias.

With inoculation, 17 pupae were collected from Clementines that resulted in eight adult flies emerging (0.8% survival). Forty-three pupae were collected from the inoculated Star Rubies, resulting in 25 adult flies (2.5% survival). The definition of host status is extremely subjective (Aluja and Mangan 2008) and probably depends on the requirements of those defining it. The no-choice tests conducted here and the inoculation comparison show that an extremely low percentage of marula fruit flies may complete a generation if an adult female oviposits in a fruit. However, in a natural situation where preferred hosts are present, this is probably very unusual and would explain why infestations in mature fruit have not been confirmed in South Africa.

The Solgel no-choice feeding experiment showed that marula fruit fly did feed on sufficient quantities of Solgel for their abdomens to change colour from the food colorant. However, the lure did not attract any Natal fruit fly or marula fruit fly in the home garden when the 2-component trap was 5 m away. Solgel therefore needs a change to the mixture in order to be more attractive.

**Table 3.3.10.1.** A comparison of Solgel with 2-component lure in Sensus traps in a home garden in Nelspruit.

Lure	<i>C. capitata</i> male	<i>C. capitata</i> female	<i>C. rosa</i> male	<i>C. rosa</i> female	<i>C. cosyra</i> male	<i>C. cosyra</i> female
2-component	0	0	1	2	2	0
Solgel	0	0	0	0	0	0

## Conclusions

The no-choice oviposition study and the inoculation experiment showed that a very low percentage of eggs may reach adulthood if laid in citrus fruit shortly before harvest. Marula fruit fly did feed on Solgel but the mixture was not very attractive from a distance.

## Future research

A bait will be developed that is effective against all *Ceratitidis* species and possibly other attract and kill devices.

## Technology transfer

This information is not yet complete enough to be distributed further.

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### 3.3.11 **FINAL REPORT: Fruit fly damage reduction and management of male fruit fly numbers**

Experiment 929 (April 2007 – September 2008) by Sean D. Moore, Wayne Kirkman and Peter R. Stephen (CRI)

#### **Summary**

Due to the seriousness of the fruit fly pest problem, it has previously not been possible to conduct a fruit fly control trial including an untreated control. This trial was able to do so, and proposed to measure not only the impact of the treatments on adult fruit flies, measured by traps, but also on damage and infestation. A pilot trial comparing M3 fruit fly bait stations with untreated controls was conducted from September to November 2007. Although M3s appeared unable to reduce numbers of adult fruit flies, they completely eliminated fruit fly damage and infestation, whilst damage and infestation remained conspicuous in the untreated blocks. A new trial will be initiated in March 2008, expanding on this investigation.

#### **Opsomming**

As gevolg van die ernstigheid van die vrugtevlieg probleem is dit voorheen nie moontlik gewees om 'n vrugtevlieg beheer proef met die insluiting van 'n onbehandelde kontrole uit te voer nie. Dit is wel in hierdie proef bereik. In hierdie proef is dit beplan om nie net die impak van behandelings op volwasse vrugtevlieë te meet nie, maar ook op vrugskade en besmetting. 'n Loodsproef wat M3 vrugtevlieg lokstasies met onbehandelde kontroles vergelyk het is van September tot November 2007 uitgevoer. Alhoewel dit voorgekom het dat M3s nie die getalle van volwasse vrugtevlieë kon verminder nie, het hulle vrugtevlieg skade en besmetting heeltemaal uitgewis, terwyl skade en besmetting in onbehandelde blokke opletbaar gebly het. 'n Nuwe proef, wat op hierdie proef uitbrei, sal in Maart 2008 begin word.

#### **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 (Ware et al., 2003). All of these methods are considered to work adequately well. However, due to the seriousness of the fruit fly threat, no trial has ever been designed to measure damage reduction relative to an untreated control. This trial proposes to attempt to measure exactly this. Although this experiment was only approved for the 2008/09 financial year, a pilot trial (comparing only M3 fruit fly bait stations with untreated controls) was conducted from September to November 2007.

#### **Materials and methods**

Four blocks of citrus trees, of approximately half a hectare in size each, were selected at the Citrus Foundation Block near Uitenhage. Blocks consisted of mixed cultivars and varieties. A common denominator between all four blocks was the occurrence of Autumn Gold navel orange trees in each block. Although the trial was initiated in August, fruit was still hanging on the trees. No harvesting had taken place.

On 21 August 2007, two Sensus traps were hung in each of the four blocks, in order to determine the level of fruit fly activity and hence the suitability of the site for the trial. One of the traps was loaded with Capilure and the other with Questlure (Ware et al., 2002). These traps were monitored for two weeks before the treatments were applied.

On 4 September 2007, M3 bait stations were hung in two of the four blocks (GVB4 and GVB7), at a density of 350 bait stations per hectare. The other two blocks were used as untreated controls (GVB5 and GVB8). In order to monitor fruit fly numbers, one Questlure-loaded and four Capilure-loaded Sensus traps were spaced throughout each block on the same day. Traps were positioned on the northern, southern, eastern and western aspects of each block and one trap in the middle. The Questlure-loaded traps were hung on the eastern aspect of all blocks except GVB5, where they were hung on the northern side of the orchard. Traps were monitored weekly. At the same time, fruit drop under 10 data trees in the middle of each block, was collected and inspected for fruit fly infestation and any signs of damage which could possibly be related

to fruit flies. Monitoring was continued for a nine-week period.

## Results and discussion

Trap catches confirmed that there were sufficient numbers of fruit fly at the site in order to conduct the trial (Table 3.3.11.1). On a couple of occasions, fruit fly adults were actually seen resting on fruit. This is not a usual sight in an orchard where fruit fly is under any measure of commercial control and was therefore indicative that there was a fruit fly problem in the trial orchards.

**Table 3.3.11.1.** Fruit fly trap catches per trap per week before the application of treatments.

Date traps removed	Treatment to be applied	Block	Capilure				Questlure			
			Medfly		Natal fly		Medfly		Natal fly	
			M	F	M	F	M	F	M	F
28/08	M3	GVB4	6	0	0	0	2	10	1	1
		GVB7	55	0	0	0	0	0	0	0
	Control	GVB5	33	0	0	0	0	0	0	0
		GVB8	68	0	0	0	0	0	0	0
04/09	M3	GVB4	5	0	0	0	0	17	0	0
		GVB7	102	0	0	0	0	0	0	0
	Control	GVB5	51	0	0	0	1	48	0	0
		GVB8	65	0	0	0	0	0	0	0

Fruit fly numbers in traps remained relatively high in all blocks throughout the trial, with little difference between treated and control blocks (Table 3.3.11.2). The M3s did not appear to reduce the numbers of male Medflies at all (Table 3.3.11.2). This may have been due to the relatively small size of the blocks i.e. no larger than a quarter of a hectare each, and the persistent fruit fly pressure from all around the trial orchards. No fruit fly control measures were employed anywhere on the farm.

**Table 3.3.11.2.** Mean male Medfly numbers caught per Capilure-loaded Sensus trap per approximate week.

Date	Fruit flies (male Medfly) per trap per period			
	M3		Control	
	GVB4	GVB7	GVB5	GVB8
13/09	11.5	37.0	40.5	24.7
19/09	9.5	34.0	34.7	28.0
26/09	3.2	44.5	46.0	30.7
03/10	3.7	75.5	55.5	51.5
10/10	3.7	43.7	33.0	22.5
16/10	21.7	122.7	45.2	51.0
23/10	14.7	88.7	42.7	51.7
29/10	14.0	116.2	96.2	82.5
05/11	28.0	156.0	150.7	100.2
<b>Average</b>	<b>12.2</b>	<b>79.8</b>	<b>60.5</b>	<b>49.2</b>
	<b>46.0</b>		<b>54.8</b>	

However, there were indications that the M3 bait stations might have reduced the numbers of fruit fly females. A total of 6 adult female fruit flies (both Medfly and Natal fruit fly) were caught in the Questlure-loaded traps in the two M3-treated blocks over the full 9 week trial period (Table 3.3.11.3). During this same period, 53 adult female fruit flies were caught in the two control blocks. It might be argued that this is not a fair comparison, as the attractant used in the M3s and in Questlure is very similar (Ware et al., 2002; 2003). It is therefore possible that the M3s could lead to a type of “shut-down” of a Questlure-loaded trap.

**Table 3.3.11.3.** Fruit flies caught per Questlure-loaded Sensus trap per approximate week.

Date	Fruit flies per trap per week															
	M3								Control							
	GVB4				GVB7				GVB5				GVB8			
	Medfly		Natal fly		Medfly		Natal fly		Medfly		Natal fly		Medfly		Natal fly	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
13/09	0	1	0	0	0	0	0	0	0	12	0	0	0	0	0	0
19/09	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0
26/09	0	0	0	0	0	0	0	0	1	8	0	2	0	0	0	0
03/10	0	1	0	0	0	0	0	0	0	10	0	1	0	0	0	0
10/10	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
16/10	0	0	0	0	0	0	0	0	2	1	1	1	0	0	0	0
23/10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
29/10	0	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0
05/11	0	2	0	0	1	0	0	0	1	9	0	1	0	2	0	0
<b>Total</b>	<b>0</b>	<b>4</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>46</b>	<b>2</b>	<b>5</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>

Although fruit fly numbers in traps remained high (i.e. well above the thresholds of 4 flies per Capilure-loaded trap per week and > 1 female fly per Questlure-loaded trap per week), M3s seemed to work very well in completely eliminating fruit fly damage and infestation. For the first four weeks of evaluation there was little difference in fruit damage and infestation between the treated and control blocks (Table 3.3.11.4). However, during the last five weeks of evaluation, no fruit fly damaged or infested fruit were recorded from the data trees in the M3 blocks (Table 3.3.11.4). It may be that the M3 bait stations were effective in controlling fruit fly right from the first week. However, fruit damaged by or infested with fruit fly would probably take a few weeks to drop after occurrence of the damage.

**Table 3.3.11.4.** Fruit fly damage and infestation from fallen fruit per 20 data trees per treatment per week.

Date	Fruit drop				Fruit with penetration marks				Fruit infested			
	M3		Control		M3		Control		M3		Control	
	GVB4	GVB7	GVB5	GVB8	GVB4	GVB7	GVB5	GVB8	GVB4	GVB7	GVB5	GVB8
13/09	64	17	5	22	2	3	3	2	0	2	0	1
19/09	69	7	4	27	2	1	0	8	1	1	0	2
26/09	72	24	7	69	5	4	3	3	4	2	0	3
03/10	48	43	20	144	3	9	1	8	3	5	0	6
10/10	23	2	25	25	0	0	2	0	0	0	2	0
16/10	4	1	9	7	0	0	1	0	0	0	0	0
23/10	18	5	10	4	0	0	2	1	0	0	1	1
29/10	15	1	22	9	0	0	5	0	0	0	3	0
05/11	6	1	20	3	0	0	3	0	0	0	2	0
<b>Total during last 5 weeks</b>	<b>66</b>	<b>10</b>	<b>86</b>	<b>48</b>	<b>0</b>	<b>0</b>	<b>13</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>8</b>	<b>1</b>

### Conclusion

M3 fruit fly 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.

### Future research

During March 2008 another trial will be initiated at the same site. In this trial M3 bait stations will be compared with conventional baiting. A third treatment of M3 bait stations interspersed with "male" M3s will also be included. The use of an untreated control will enable an assessment of the effectiveness of the treatments in reducing numbers of adult flies and fruit fly damage to fruit.

## Technology Transfer

The information generated from this trial has already been presented to three grower study group meetings in Zimbabwe during January 2008.

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### 3.4 PROJECT: COSMETIC PESTS

Project coordinator: Tim G Grout (CRI)

#### 3.4.1 Project summary

With the focus being on phytosanitary research requirements it may be easy to overlook the fact that export quality fruit must have excellent external appearance in order to compete in the market. Over the last 10 years, the usage of many plant protection products required for the control of cosmetic pests has been curtailed or stopped completely due to changes to the maximum residue limits in the markets. Research focus has therefore shifted to the search for acceptable alternative means of controlling these pests. In the case of bollworm, a nucleopolyhedrovirus has been found to be effective which is also less disruptive to natural enemies than some of the other chemicals used (3.4.2). Hopefully this product will be registered shortly. An attempt to develop a rearing technique for the only known parasitoid of citrus thrips succumbed to the extreme difficulty of rearing citrus thrips. A culture that had been maintained for a year at very low levels suddenly collapsed and could not be restarted. This work was therefore terminated (3.4.3). The possibility of using entomopathogenic fungi to control citrus thrips pupae in the soil beneath citrus trees is under investigation but progress has been slow due to low numbers of citrus thrips and an inability to find isolates of these fungi in soil samples from 41 different locations (3.4.4). Research on citrus grey mite has proved almost as frustrating because although several regions list it as their most important pest, it is under control on most farms and efforts to monitor the movement of the mite in the air from natural bush to citrus have proved fruitless (3.4.5). Fortunately, research on finding a chemical alternative to Acarol for the control of citrus bud mite has been much more promising and a product has been found that is as effective as Acarol and should have a short enough pre-harvest interval for it to be used at the most effective time in autumn (3.4.6).

### Projekopsomming

Met die fokus op fitosanitiere navorsingsbehoefte, kan die feit dat kwaliteit uitvoervrugte 'n uitstekende eksterne voorkoms moet hê om te kan kompeteer in die mark, maklik vergeet word. Oor die laaste tien jaar is die gebruik van baie plantbeskerende produkte vir die beheer van kosmetiese plae beperk of verbied weens die veranderinge aan die maksimum residu vlakke in die markte. Die navorsing se fokus het dus verskuif na 'n soektog vir aanvaarbare alternatiewe maniere vir die beheer van hierdie plae. In die geval van bolwurm is 'n "nucleopolyhedrovirus" wat effektief is, maar ook minder ontwrigtend teenoor natuurlike vyande is as sommige van die ander chemikalieë wat gebruik word, gevind (3.4.2). Hopelik sal hierdie produk binnekort geregistreer kan word. 'n Poging om 'n tegniek te ontwikkel om die enigste bekende parasitoïed van sitrus blaaspootjie te teel, het gefaal weens die moeilikheidsgraad verbonde aan die teling van sitrus blaaspootjies. 'n Kultuur wat vir 'n jaar teen baie lae vlakke instand gehou is, het skielik tot niet gegaan en kon nie weer aan die gang gekry word nie. Die werk is dus beëindig (3.4.3). Die moontlikheid om entomopatogeniese swamme te gebruik om sitrus blaaspootjie papies in grond onder sitrusbome te beheer, is ondersoek maar vordering was stadig omdat die getalle van sitrus blaaspootjie laag was en isolate van hierdie swamme nie in grondmonsters vanaf 41 verskillende plekke gevind kon word nie (3.4.4). Navorsing op die sitrus grysmyt was feitlik net so frustrerend. Alhoewel verskeie streke dit as hul belangrikste plaag gelys het, is dit op die meeste plase onder beheer en pogings om die beweging van die myt deur die lug vanaf natuurlike plantegroei na sitrus te monitor, was vrugteloos (3.4.5). Gelukkig was die navorsing om 'n alternatiewe chemikalie vir Acarol te vind om sitrus knopmyt te beheer, meer belowend. 'n Produk, met dieselfde effektiwiteit as Acarol wat ook 'n kort genoeg voor-oes interval sal hê vir gebruik in die mees effektiewe periode tydens herfs, is gevind (3.4.6).

### 3.4.2 FINAL PROGRESS REPORT: Evaluation of the *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) for control of bollworm on citrus

Experiment 782 (April 2005 – March 2008) by Sean D. Moore, Wayne Kirkman and Peter Stephen (CRI)

#### Summary

From 1996-1998 a South African isolate of the *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) was tested against bollworm (*Helicoverpa armigera*) on citrus, with very promising results. Despite this, further work was not conducted on this experiment, as the virus was not commercially available. Recently, an Australian company began producing a HearNPV product. The product was originally known as Vivus and Vivus Gold. However, the product is currently being registered and commercialised by River Bioscience, as Helicovir. During the 2006/07 season, three trials were conducted in navel orange orchards and one in a Midnight Valencia orchard. Helicovir was compared with mevinphos or Lannate, Dipel, Beta-Bak and a combination of virus and Bt. Beta-Bak is a locally produced Bt isolate. Although bollworm infestation in one of the trials had reached the highest level yet recorded in any of the trials conducted by the authors (77.5%), fruit cull and yield loss due to bollworm were significantly reduced by Helicovir, relative to the untreated control. In this case, none of the other treatments managed to significantly reduce fruit cull. Despite this, it was apparent that one Helicovir application was probably not adequate to satisfactorily reduce bollworm damage. Indications were also that Helicovir was at least somewhat rainfast. A further two trials were conducted during the 2007/08 season. Infestation remained reasonably low. However, it was observed that a mixture of Helicovir and mevinphos gave better control of bollworm than mevinphos alone. No further work is planned on this experiment.

#### Opsomming

Gedurende 1996-1998 is 'n Suid-Afrikaanse isolaat van *Helicoverpa armigera* nukliêre polihedrovirus (HearNPV) teen bolwurm (*Helicoverpa armigera*) met uitstekende resultate op sitrus getoets. Desondanks is navorsing daarop nie voortgesit nie omdat die virus nie kommersieel beskikbaar was nie. 'n Australiese maatskappy het onlangs 'n HearNPV-produk begin vervaardig. Die produk is oorspronklik as Vivus en Vivus Gold bekend maar word nou deur River Bioscience as Helicovir geregistreer. Gedurende die 2006/07 seisoen is Helicovir in twee boordproewe met Dursban, Dipel en Beta-Bak vergelyk. Beta-Bak is 'n plaaslik vervaardigde Bt isolaat. In al twee proewe het die Helicovir behandelings bolwurm beskadigde vrugte betekenisvol verminder. In een proef is vrugverlies ook betekenisvol verminder tot dieselfde mate as wat met Dursban bereik is. Beta-Bak het swak gewerk. In een van die proewe was daar 'n positiewe tendens tussen bolwurm skade en uitstop nawels. Gedurende die 2006/07 seisoen, is drie proewe in navel lemoen boorde uitgevoer en een proef in Midnight Valencias. Helicovir is vergelyk met mevinphos of Lannate, Dipel, Beta-Bak en 'n mengsel van virus en Bt. Alhoewel bolwurm besmetting in een van die proewe 'n baie hoë vlak bereik het (77.5%), is vruguitskot en oesvermindering betekenisvol verminder deur die gebruik van Helicovir. In hierdie geval kon nie een van die ander produkte uitskot betekenisvol verminder nie. Nietemin het dit geblyk dat een Helicovir toediening heel waarskynlik nie voldoende was om bolwurm besmetting genoegsaam te verminder nie. Indikasies is gekry dat Helicovir minstens 'm mate van reenvastheid het. 'n Verdere twee proewe is gedurende die 2007/08 seisoen gespuit. Besmetting in redelik laag gebly, maar dit is opgetel dat 'n mengsel van Helicovir en mevinphos beter as mevinphos op sy eie gewerk het. Geen verdere werk word op hierdie eksperiment beplan nie.

#### Introduction

From 1996-1998 a South African isolate of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) was tested against bollworm (*Helicoverpa armigera*) on citrus, with excellent results (Moore et al., 2004c). Despite this, work on this experiment was terminated, as the virus was not commercially available. Recently, an Australian company, Ag-Biotech Australia, began commercially producing HearNPV. The product was previously known as Vivus and Vivus-Gold, but will be commercialised in South Africa by River Bioscience as Helicovir. In the trials recounted in this report, a suspension concentration formulation of  $2 \times 10^9$  viral occlusion bodies (OBs) per ml, was used. However, the formulation which will be registered will have a concentration of  $5 \times 10^9$  OBs/ml. During the 2006/07 season, four trials were conducted with Helicovir. During the 2007/08 season, a further two trials were conducted. Good results with this commercial product, as were achieved during the 2005/06 season, will strongly support an application for its registration for the control of bollworm on citrus in South Africa. If Helicovir could replace the organophosphate, carbamate or pyrethroid usually used for bollworm control in spring, growers will be greatly assisted towards a reduction in the use of chemicals and the implementation of a bio-intensive IPM programme.

## Materials and methods

### General

Four field trials were conducted during spring of 2006, to test Helicovir – and other products – for control of bollworm on citrus. During the spring of 2007 a further two trials were conducted. Treatments were applied as medium cover film sprays, using high pressure hand-held spray guns. All trials, except the 2007 trial in Citrusdal, were laid out in randomised single-tree plot designs, replicated 10 times. Efficacy of sprays was initially assessed by inspecting blossom or fruitlet clusters for infestation with bollworm larvae, approximately one week after spraying and weekly thereafter for between one and four weeks. Clusters were categorised as clean or infested. For the 2006 trials, this information is given in last year's report. The 2007 trial in Citrusdal was not laid out and evaluated as described for the other trials. This trial was conducted by the grower, according to the researchers' instructions. Details of this trial are given in the relevant sections below.

During April 2007 and March 2008, fruit damage caused by bollworm was evaluated. Twenty randomly-selected fruit from each tree were inspected and categorised as clean, blemished, or culled (non-exportable) according to stipulated standards (Anonymus, 1995). The crop load was then estimated – at two of the trial sites (2006 trials) – by placing a 0.125 m<sup>3</sup> frame at uniform height (1 m above the ground) into both the northern and southern sides of each tree, and counting the number of fruit within the frame. Fruit counts from both aspects of the tree were added together and the mean counts calculated for each treatment.

In all trials (except the 2007 Citrusdal trial), data were analysed using ANOVA and means compared using the Bonferroni LSD multiple range test at the 95% significance level. Proportions (percentages/100) were subjected to an arc sine transformation, where necessary, in order to normalise the data. A zero percentage was counted as 1/(4n) and 100% as (n - 0.25)/n before transformation (Bartlett, 1947; Snedecor & Cochran, 1980). Data from the two treatments in the 2007 Citrusdal trial were compared by using the Student's t-test.

### Hoedspruit, Mpumalanga Province (2006/07)

A spray trial was conducted in an orchard of 8-year old Midnight Valencia orange trees at Blydevallei Canyon Pakkers in Mpumalanga. Treatments were applied on 18 September 2006. Average temperature during time of application was 24°C; relative humidity (RH) was 42.5%; there was 60% cloud cover; and a very gentle northerly wind. An average of 10.4 L of spray mixture was applied per tree for each treatment. The following treatments were applied (at concentrations per 100 L water): Helicovir (20 ml), Helicovir (25 ml), Lannate (20 g), Dipel (12.5 g) plus Kynobuff (100 ml), Beta-Bak (40 g) plus Kynobuff (100 ml), Beta-Bak (80 g) plus Kynobuff (100 ml), and Beta-Bak (40 g) plus Helicovir (20 ml) plus Kynobuff (100 ml). Fruit damage was evaluated on 8 May 2007.

### Sundays River Valley, Eastern Cape Province (2006/07)

A spray trial was conducted in a navel orange orchard, on Scheepersvlakte Farm in the Sundays River Valley. Treatments were applied as soon as hatching of bollworm eggs was observed – on 28 and 29 September. On 28 September, mean temperature was 18°C, RH was 89% and there was a very gentle south-easterly breeze. Conditions on 29 September were similar: temperature was 17.3°C, RH was 82% and wind was as for the previous day. No precipitation fell during the course of the two days. At this time, 57.5% of all blossom clusters inspected, were infested with one or more life-stages of bollworm. The following treatments were applied (at concentrations per 100 L water): Helicovir (25 ml), Helicovir (30 ml), Mevinphos (100 ml), Dipel (12.5 g) plus Comodobuff (50 ml), Beta-Bak (40 g) plus Comodobuff (50 ml), Beta-Bak (80 g) plus Comodobuff (50 ml), Beta-Bak (40 g) plus Helicovir (20 ml) plus Comodobuff (50 ml), Dipel (12.5 g) plus Comodobuff (50 ml) plus Helicovir (20 ml), an experimental HearNPV (5 g). The 5 g per 100 L water concentration of the experimental HearNPV had an equivalent number of viral occlusion bodies to the 30 ml per 100 L water concentration of Helicovir. The grower had applied Profenofos to the remainder of the orchard on 12 October 2006 as a full cover spray at 100 ml/hl. This was in order to control bollworm, as well as mealybug and thrips. Twelve Profenofos-treated trees alongside the trial rows were also evaluated to compare the bollworm inflicted damage and yield with that of the trial treatments. In addition, the 12 control trees in the trial (20 fruit per tree) were evaluated to see whether the majority of bollworm damage occurred on the sides of the fruit or at the navel end.

### Swellendam, Western Cape Province (2006/07)

The trial at Swellendam was applied to an orchard of six year old Washington navel oranges on Thornlands farm. The trees were spaced at 6 m x 3 m. The following treatments were applied (at concentrations per

100 L water): Helicovir (25 ml) plus Comodobuff (50 ml), Helicovir (25 ml) plus Comodobuff plus Agral 90 (18 ml), Helicovir (25 ml) plus Comodobuff plus Agral 90 (18 ml) plus molasses (250 ml). Shortly after treatments were applied (on 9 October 2006), light rain (1 mm in total) was experienced. Consequently, treatments were reapplied the following day (10 October) on different trees adjacent to the original trial block. Other climatic conditions during the two days of application were that the mean temperature was 17°C and no wind was evident. On 16 & 17 April, fruit damage and yield were evaluated.

Citrusdal, Western Cape Province (2006/07)

A trial was conducted in October on Uitvlug Farm in Citrusdal. An orchard of Washington navel orange trees was used. Exactly the same treatments were applied as in the Scheepersvlakte trial described above, on 10 October 2006.

Sundays River Valley, Eastern Cape Province (2007/08)

Bollworm infestation levels in citrus orchards in the Eastern Cape were very low in spring 2007. From mid-September countless farms and orchards were visited and inspected in order to find a suitable site. A site was eventually found where approximately 25% of blossom clusters were infested, mainly with bollworm eggs. The trial was marked out and sprayed with Helicovir and a standard treatment on 1 October.

Citrusdal, Western Cape Province (2007/08)

A Washington navel orange orchard on Silwerspruit Farm in the Citrusdal Valley was split in two. Half of the orchard was sprayed with Phosdrin (30 ml/100 L water) and the other half was sprayed with a mixture of Phosdrin and Helicovir (12 ml/100 L water). Bollworm infestation was not evaluated thereafter. However, on 25 February bollworm damage on fruit was evaluated.

## Results and discussion

Hoedspruit, Mpumalanga Province (2006/07)

At the time of applying the treatments at Blydevallei Canyon Pakkers, 20.0% of blossom or fruitlet clusters were infested with bollworm. At this time, only 22.7% of larvae were smaller than 12 mm in length; 45.4% of larvae were between 12 mm and 19 mm long; and 31.8% of larvae were larger than 19 mm long. It is generally accepted that both Bt and baculoviruses are far less effective against larger larvae. Despite this, it was surprising that only nine days after application, five of the treatments were free of bollworm larvae (Moore *et al.*, 2006). Although the post-treatment infestation analysis showed that all treatments were effective, there was little difference in fruit damage between treatments (Table 3.4.2.1). This was because treatments were applied too late. Oddly, significantly more Dipel-treated fruit than untreated fruit were culled.

**Table 3.4.2.1.** Fruit (navel orange) damage and yield for various bollworm treatments at Blydevallei Canyon Pakkers Farm in the Hoedspruit region. Treatments were evaluated on 8 May 2007.

Treatment	Fruit damage (%)		Yield index
	Scar	Cull	
Untreated control	6.5a	3.5a	4.3a
Lannate 20 g	5.9a	3.2a	6.8a
Dipel (12.5g) + Kynobuff (100ml)	15.0a	9.1b	5.2a
Helicovir (20ml)	8.2a	3.6a	5.5a
Helicovir (25ml)	11.8a	4.5ab	5.8a
Beta-bak (40g) + Kynobuff (100ml)	8.0a	3.0a	4.4a
Beta-bak (80g) + Kynobuff (100ml)	8.0a	4.5ab	3.7a
Beta-bak (80g) + Helicovir (20 ml) + Kynobuff (100ml)	5.5a	2.3a	5.5a

Sundays River Valley, Eastern Cape Province (2006/07)

At the Scheepersvlakte trial site used during the 2006/07 season, a higher level of bollworm infestation was recorded than had ever previously been recorded during the course of these virus trials, dating back to 1996 (Moore *et al.*, 2004b). Consequently, treatments seemed to be less effective than in other trials. Also, the higher concentration of Helicovir (30 ml/100 L water), which was the most effective treatment at three weeks after application, was notably (but not significantly) more effective than the lower concentration (25 ml/100 L

water). It was this result, which swayed River Bioscience to register the product at a rate of no less than 30 ml per 100 L water. Despite HearNPV having worked well in all trials to date, its slow knock-down will not make it easily acceptable to growers. However, if Helicovir is able to significantly and acceptably reduce bollworm damage, growers should be convinced of its efficacy through an intensive education programme.

The higher concentration of Helicovir was the most effective treatment in reducing fruit cull (Table 3.4.2.2), being the only treatment which differed significantly from the control. Yield results were dramatic (Table 3.4.2.2). Yield for Helicovir and mevinphos treatments was significantly higher than for the untreated control. None of the Bt treatments (Dipel and Betabak) worked very well, even when in combination with virus. The experimental virus was also not very effective.

**Table 3.4.2.2.** Fruit (navel orange) damage and yield for various bollworm treatments at Scheepersvlakte Farm in the Sundays River Valley, evaluated on 2 & 3 May 2007.

Treatment	Concentration per 100 L water	Fruit damage (%)		Yield index
		Scar	Cull	
Untreated control		29.92abc	28.75c	2.33a
Mevinphos	100 ml	25.00abc	21.25abc	9.42cd
Dipel Commodobuff	12.5g 50 ml	27.92abc	23.75abc	7.17abcd
Helicovir	25 ml	22.50ab	20.00ab	10.33cd
Helicovir	30 ml	20.42a	15.83a	11.75abcd
Beta-bak Commodobuff	40 g 50 ml	30.42c	28.75c	3.58ab
Betabak Commodobuff	80 g 50 ml	27.08abc	25.00bc	4.58abc
Beta-bak Helicovir Commodobuff	40 g 20 ml 50 ml	24.17abc	25.00bc	8.92bcd
Helicovir Dipel Commodobuff	20 ml 12.5 g 50 ml	27.08abc	24.17abc	7.17abcd
Experimental virus	5 g	28.33bc	22.50abc	5.75abcd

Results with the grower-applied Profenofos were very good (Table 3.4.2.3).

**Table 3.4.2.3.** Fruit (navel orange) damage and yield for Profenofos, applied by the grower, at Scheepersvlakte Farm in the Sundays River Valley, evaluated on 3 May 2007.

Treatment	Concentration per 100 L water	Fruit damage (%)		Yield index
		Scar	Cull	
Profenofos	100 ml	19.17	10.83	12.00

As has previously been observed, the majority of the bollworm damage was inflicted on the navel ends of fruit (Table 3.4.2.4).

**Table 3.4.2.4.** Bollworm damage on sides of fruit (navel oranges) and navel ends at Scheepersvlakte Farm in the Sundays River Valley, evaluated on 3 May 2007.

	Fruit scarred		Fruit culled	
	Number	Relative %	Number	Relative %
Navel end	48	65.8	56	78.9
Side of fruit	25	34.2	15	21.1

Swellendam, Western Cape Province (2006/07)

The rainfall experienced at the Swellendam trial site did not reduce the efficacy of Helicovir, measured by post-treatment bollworm infestation (Moore et al., 2006). The rainfall could have been too light, or the virus might be rainfast in a similar manner to that recorded for Cryptogran (*Cryptophlebia leucotreta* granulovirus) (Moore et al., 2004a). The addition of molasses also did not improve the efficacy of the virus. Most of the larvae found during the evaluations were first or second instar larvae, which would indicate that a "second" infestation took place after the treatments were applied (Moore et al., 2006).

The light rain did not appear to affect the efficacy of the treatments, as in both trials, all treatments significantly reduced scarring and fruit cull, relative to the control (Tables 3.4.2.5 & 3.4.2.6). Bollworm infestation was too low to have an effect on yield.

**Table 3.4.2.5.** Fruit (navel orange) damage and yield for various bollworm treatments at Thornlands Farm in the Swellendam region. Treatments evaluated on 16 April 2007 (Trial 1 – with rainfall).

Treatment	Concentration per 100 L water	Fruit damage (%)		Yield index
		Scar	Cull	
Untreated control		10.56b	3.33b	349a
Helicovir	25 ml	4.17a	1.67ab	348a
Commodobuff	50 ml			
Helicovir	25 ml	6.11a	1.11a	311a
Commodobuff	50 ml			
Agral 90	18 ml			
Helicovir	25 ml	6.67ab	0.83a	316a
Commodobuff	50 ml			
Agral 90	18 ml			
Molasses	250 ml			

**Table 3.4.2.6.** Fruit (navel orange) damage and yield for various bollworm treatments at Thornlands Farm in the Swellendam region. Treatments were evaluated on 17 April 2007 (Trial 2 – no rainfall).

Treatment	Concentration per 100 L water	Fruit damage (%)		Yield index
		Scar	Cull	
Untreated control		18.33b	6.67b	364a
Helicovir	25 ml	10.00a	1.11a	416a
Commodobuff	50 ml			
Helicovir	25 ml	9.44a	1.94a	373a
Commodobuff	50 ml			
Agral 90	18 ml			
Helicovir	25 ml	10.28a	2.50a	409a
Commodobuff	50 ml			
Agral 90	18 ml			
Molasses	250 ml			

Citrusdal, Western Cape Province (2006/07)

Before spraying, it was determined that 51.7% of blossom and fruitlet clusters were infested with bollworm. Unfortunately, the trial was inadvertently sprayed out by the grower, approximately 10 days after treatments were applied. This was before any evaluations could be conducted.

Sundays River Valley, Eastern Cape Province (2007/08)

Unfortunately the trial was inadvertently sprayed out by the grower before evaluations could be conducted. Various other sites were subsequently visited but none were suitable, as bollworm infestation was either too low or life stages were too advanced.

Citrusdal, Western Cape Province (2007/08)

Where Phosdrin alone had been sprayed, 2.5% of fruit were damaged and 1.7% of fruit were culled. Where Phosdrin and Helicovir were applied together, 1.25% of fruit were scarred and 1.25% of fruit were culled. Therefore a total of 4.2% of fruit were damaged for the Phosdrin treatment and 2.5% of fruit were damaged for the Phosdrin and virus treatment.

Where bollworm infestation is high, it is often necessary to spray an orchard more than once during spring, particularly if a short residual product such as Phosdrin is used. The purpose of the trial was to determine whether applying Phosdrin with virus would be sufficiently efficacious to avert the application of a second treatment. Unfortunately bollworm levels were too low to determine this. It was nevertheless possible to ascertain that control of bollworm was enhanced by the addition of Helicovir.

## Conclusion

Helicovir adequately succeeded in reducing bollworm damage. Even in the trial in which bollworm infestation was the highest, Helicovir managed to significantly reduce fruit damage and crop loss. In this case, none of the other treatments managed to significantly reduce fruit cull. Despite this, it was apparent that one Helicovir application was probably not adequate to satisfactorily reduce bollworm damage. Indications were also that Helicovir was at least somewhat rainfast. A mixture of Helicovir and mevinphos gave better control of bollworm than mevinphos alone. An application for the registration (according to Act 36 of 1947) of Helicovir for control of bollworm on citrus, has been submitted by River Bioscience.

## Future research

Funding for this experiment has come to an end. Therefore no further research is planned with HearNPV in the immediate or short term. However, further research on the following aspects would be valuable: 1. The benefit of application with various adjuvants; 2. The efficacy of an organophosphate-virus combination as an alternative to a double organophosphate treatment, in the case of a heavy and protracted bollworm infestation. (This is a repeat of what was attempted in the 2007 Citrusdal trial).

## Technology Transfer

Grower talks on general bollworm control were presented by Sean Moore at the following study group meetings: Southern KZN, Nkwalini, Kat River Valley, Sundays River Valley, Gamtoos River Valley, Swellendam, Breederivier, Paarl, Swartland, Citrusdal, Benede Oranjerivier, Vaalharts (all September 2007).

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- 3.4.3 **FINAL PROGRESS REPORT: Develop a rearing technique for the citrus thrips parasitoid *Goetheana incerta***  
Experiment 809 (2005-2007) by Tim G Grout and Kim C Stoltz (CRI)

## Summary

After many delays due to changes required to apparatus and techniques a citrus thrips culture was successfully started on *Bryophyllum pinnatum* after collecting adults that had discovered these plants in the open outside the laboratory building. The culture was maintained for approximately one year but never increased in numbers sufficiently to consider using some thrips for parasitoid rearing. Then the culture died out inexplicably and could not be restarted. At this stage the decision was taken to terminate the research as it was too labour intensive and did not appear to have much chance of succeeding.

## Opsomming

Na vele vertraging weens die nodige veranderinge aan apparate en tegnieke, is 'n kultuur van sitrus blaaspootjies suksesvol op *Bryophyllum pinnatum* aan die gang gekry nadat volwassenes wat op hierdie plante aan die buitekant van die laboratoriumgebou ontdek is, versamel is. Die kulture is vir ongeveer een jaar instandgehou, maar die getalle het nooit sodanig vermeerder dat dit oorweeg kon word om van die blaaspootjies te gebruik om die parasitoïede te teel nie. Die kultuur het skielik onverklaarbaar uitgesterf en

kon nie weer aan die gang gekry word nie. In hierdie stadium is daar besluit om die navorsing te beëindig omdat dit te arbeidsintensief was en dit wou voorkom of die kanse op sukses skraal was.

#### 3.4.4 **PROGRESS REPORT: Investigation of entomopathogenic fungi for control of citrus thrips** Experiment 879 (2007/8-2009/10) by Tim G Grout, Kim C Stoltz and Sean D Moore (CRI)

##### **Summary**

Progress in this experiment has been slow due to the lack of citrus thrips for bioassays with purchased isolates of entomopathogenic fungi and not being able to find natural EPF isolates in 41 soil samples. Preliminary bioassays using citrus thrips larvae on citrus leaves that had been sprayed with PPRI EPF isolates showed that the *Metarhizium anisopliae* isolate caused more mortality than *Beauveria bassiana* or *Verticillium lecanii*. Two more *M. anisopliae* isolates have been purchased for evaluation and further samples of soil will be tested for the presence of EPFs.

##### **Opsomming**

Die stadige vordering met hierdie eksperiment kan toegeskryf word aan die tekort aan sitrus blaaspootjies vir biotoetse met isolate van entomopatogeniese swamme wat aangekoop is asook die onvermoë om natuurlike EPF in 41 grondmonsters te vind. Voorlopige biotoetse waarin sitrus blaaspootjie larwes op sitrusblare wat met PPRI EPF isolate bespuit is gebruik is, het getoon dat die *Metarhizium anisopliae* isolate meer mortaliteit as *Beauveria bassiana* of *Verticillium lecanii* veroorsaak het. Nog twee *M. anisopliae* isolate is vir evaluasie aangekoop en verdere grondmonsters sal vir die teenwoordigheid van EPFs getoets word.

##### **Introduction**

Citrus thrips, *Scirtothrips aurantii*, is the most important cosmetic pest of citrus crops in southern Africa, with the capacity to cause severe external damage to fruit, rendering them unmarketable. Control of citrus thrips is difficult, due to their ability to quickly build up large populations, rapid development of resistance to insecticides, and the shortage of affordable/efficacious IPM options. In addition, non-target impacts of pesticides and human-and environmental-health risks associated with their use are of increasing concern.

Many species of phytophagous thrips drop from host plants to pupate in the soil or leaf litter on the ground and pupation rates are affected by abiotic (e.g., moisture) and biotic (e.g., natural enemies) factors (Lewis, 1973). Work by Schweizer & Morse (1997) in Californian citrus orchards indicated that organisms living in leaf material under trees significantly reduce survival of pupating *Scirtothrips citri*, and suggested that manipulation of these organisms could significantly benefit thrips management programmes. Recently, work conducted by AgResearch Limited (New Zealand) and the University of Wales-Swansea, revealed impressive results in controlling Western Flower thrips, *Frankliniella occidentalis*, pupae by applying entomopathogenic fungi (EPF) to soil, in potting mixes or compost. Previous researchers have had similar success applying fungi to compost and other substrates for thrips control (Helyer et al. 1995, Butt & Brownbridge 1997). It is proposed that a similar approach for controlling pupae of citrus thrips be investigated. Local isolates of EPFs should be identified and tested for their potential utility as a component of a bio-based thrips IPM strategy (Brownbridge 2006).

##### **Materials and methods**

The main objective in the first year of research was to assess the virulence of South African isolates of EPFs against citrus thrips and establish cultures of five promising pathogens. Two approaches were used to acquire EPFs, one was to test some isolates from the PPRI collection in Pretoria and the other was to try to collect our own isolates from soil samples. A total of 41 soil samples were collected from citrus orchards such as Crocodile Valley, the Lowveld Agricultural College, Bufland, Groblersdal, Karino, Rustenburg and Malelane, organic Macadamia orchards in Hectorspruit, in addition to home gardens in Nelspruit, Hazyview, White River and Lumphisa, ant nests and compost heaps. Each week three-soil samples were tested on half-strength Sabouraud Dextrose Agar. Ten grams of soil was added to 90 g sterile water then blended for a few minutes before taking 0.2 ml and spreading it across each plate. Three sub-samples and five plates per sub-sample were used, giving a total of fifteen replicates per original soil sample. Seven days later each petri dish was carefully examined to determine what type of fungi growth was present. Anything slightly different or possibly unknown was placed on full strength SDA for further evaluation seven days later.

Initially, one isolate each of *Beauveria bassiana* (7910), *Metarhizium anisopliae* (6717) and *Verticillium lecanii* (6752) were obtained from the Biosystematics Division of the PPRI in Pretoria. These were cultured on quarter strength SDA for 12 days, then a sterilised scalpel was used to harvest spores and mix them in

100 ml sterile water. Sufficient spores were added until the suspension contained  $10^5$ - $10^7$  spores per gram. The spores were counted with a spectrophotometer (Bausch & Lomb Spectronic 20). Initially, a technique similar to that used for non-target effect bioassays of *Trichogrammatoidea cryptophlebiae* was tried where a grapefruit leaf was sprayed with the spore suspension using a Sigma Spray Kit, then once dried, this served as the substrate on a piece of glass with citrus thrips larvae being placed on the leaf inside an inverted petri dish. However, the environment was too dry for the thrips larvae and 64% of the untreated larvae had died by 48 hours so this technique was abandoned. These suspensions were then sprayed onto the abaxial sides of grapefruit leaves which after drying were used to cut leaf disks from that were placed abaxial-side up on a wet sponge covered with cotton-wool. Twenty disks were used for each of the three isolates and the untreated control. Five second-instar citrus thrips larvae collected from *Caesalpinia pulcherrima* were then placed on each leaf disk and provided with *Typha* pollen as supplemental food. Mortality of the citrus thrips was determined after 48 hours on 2 March 2008.

An attempt was made to develop a technique to be used in the next step of the research where sterile soil is used as a substrate for pupation after late second instar thrips larvae have walked across leaf disks that have been treated with fungi. Petri dishes (9 cm diameter) were used for this with a 3 mm depth of soil in the bottom. Sterile water was added to the soil to obtain 10-25% moisture. The petri dish lids were sprayed with Tanglefoot (polybutene) to catch any adult citrus thrips that emerged from the soil. The intention was to compare the numbers of adults successfully eclosing and getting caught in the Tanglefoot with the different fungal treatments. The technique was tested with 25 citrus thrips larvae being placed on untreated leaf disks and left for 14 days. However, no adult thrips were recovered and problems were experienced with condensation on the inside of the lids. It is possible that the soil was too shallow for the larvae and they wandered out of the dishes in the air gap between the petri dish wall and the lid. This technique will have to be developed further, perhaps with the petri dish lids being inverted so that they seal properly, but less moisture will have to be added to the soil

## Results and discussion

Although various recognisable fungi such as *Trichoderma sp.*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Penicillium sp.* were commonly found and at least 45 unidentified fungi, no cultures of EPFs could be established on half-strength Sabouraud Dextrose Agar from the soil samples.

Citrus thrips were not available on *C. pulcherrima* for several months so this delayed the efficacy bioassays. It had been hoped that citrus thrips from a laboratory culture would be readily available but as mentioned in section 3.4.3 the culture collapsed and could not be revived so this delayed this part of the research considerably. The results of the small scale bioassays on leaf disks indicated that all the PPRI isolates caused some mortality but that the *Metarhizium anisopliae* isolate was the most promising (Table 3.4.4.1). More citrus thrips were recovered in the control treatments than in any of the other treatments so perhaps the fungi were irritating the thrips and causing them to run into the water barrier and drown. These were not counted as dead in the evaluation. On the strength of these results, two further *M. anisopliae* isolates collected from grass in Pretoria and KwaZulu-Natal have been purchased and will be compared with the former isolate and other pathogens in further tests.

Due to the slow progress in this research the intended visit by Michael Brownbridge from New Zealand was cancelled.

**Table 3.4.4.1.** Mortality of citrus thrips larvae after 48 h exposure to three different fungi from PPRI

Fungus on leaf	PPRI isolate no.	Live thrips	Dead thrips	Mortality (%)
Untreated control	-	47	0	0.00
<i>Beauveria bassiana</i>	7910	22	4	15.4
<i>Metarhizium anisopliae</i>	6717	18	9	33.3
<i>Verticillium lecanii</i>	6752	23	1	4.2

## Conclusion

Progress in this research has been slow and disappointing with no new EPF isolates being found. Isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* from the PPRI all caused some mortality in citrus thrips placed on treated leaves in a moist environment, but *M. anisopliae* was the most promising.

## Future research

Two more isolates of *M. anisopliae* have been purchased for evaluation and further attempts to collect fungi from the soil will be made. Promising isolates from research being conducted by Tarryn Goble at Rhodes University may also be evaluated against citrus thrips.

## Technology transfer

Progress is not yet sufficient for any technology transfer.

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### 3.4.5 **PROGRESS REPORT: Improving management of citrus grey mite, *Calacarus citrifolii*** Experiment 856 (2006-2008/9) by Tim Grout (CRI)

#### Summary

Citrus grey mite is a severe pest in certain production regions and due mostly to its microscopic size is extremely difficult to manage. Some growers claim that orchards adjacent to natural vegetation are more likely to have the pest than other orchards. Vertical, sticky, pipe traps were therefore placed around and through such an orange orchard at Bufland farm near Mookgopong that had severe concentric ring blotch symptoms from grey mite. The traps were monitored from November 2006 until March 2008 but no further infestations of grey mite occurred. Citrus farms near Ohrigstad in the past had problems with grey mite but an annual spray in late summer or autumn has successfully controlled the pest and no sites were available for research. An infested site was eventually found near Brits in April 2008 and some chemical trials were initiated that will be reported on in the next annual report.

#### Opsomming

Sitrus grysmyt is 'n ernstige plaag in sekere produksieareas en is hoofsaaklik weens sy mikroskopiese grootte uiters moeilik om te beheer. Sommige produsente beweer dat boorde, wat aangrensend aan natuurlike plantegroei is, meer geneig is om las van hierdie plaag as ander boorde te ondervind. Vertikale, taai pyplokvalle is om hierdie rede rondom en deur so 'n lemoenboord op Bufland plaas naby Mookgopong, met ernstige konsentriese ringvlek simptome van grysmyt, geplaas. Die lokvalle is vanaf November 2006 tot Maart 2008 gemonitor, maar geen verdere infestaties van grysmyt het voorgekom nie. Sitrusplase naby Ohrigstad het in die verlede probleme met grysmyt gehad, maar die plaag is suksesvol beheer met 'n jaarlikse bespuiting in die laat somer of herfs en geen persele was vir navorsing beskikbaar nie. 'n Geïnfesteerde perseel is uiteindelik naby Brits in April 2008 gevind en daar is begin met 'n paar chemiese proewe, waarvoor daar in die volgende jaarverslag gerapporteer sal word.

### 3.4.6 **PROGRESS REPORT: Alternative chemicals for citrus bud mite control** Experiment 916 (2007-2008/9) by Tim G Grout and Peter R Stephen (CRI)

#### Summary

The most effective time of the year to control citrus bud mite *Aceria sheldoni* is between February and May and for several years the acaricide of choice has been Acarol (bromopropylate). However, residues of this product are not accepted on citrus destined for the USA and from December 2008 residues will no longer be accepted in the EU either. There is therefore an urgent need for an effective replacement with a pre-harvest interval that will allow for an application between February and May. An investigation of products used for other eriophyoid mites in other countries led to the inclusion of RJU37PY in four evaluations. Other products that are registered on citrus in South Africa for other pests were also evaluated in addition to a new acaricide. The results of two trials on lemons in Mpumalanga and one trial on lemons and another on Valencias in the North-West Province showed that RJU37PY (EC) at 150 ml/hl water was as effective as

Acarol 30-50 ml/hl in three out of four trials. All other products tested were significantly less effective than Acarol. These included abamectin at 20, 40 and 60 ml/hl, Torque (15 ml/hl), Torque plus Tedion (55 + 200 ml/hl), Dithane 200 g/hl, Envidor 15 ml/hl, Acramite 50 ml/hl and Smito 30 ml/hl. Hopefully these results will assist in a prompt registration of RJU37PY on citrus for bud mite control.

## Opsomming

Die beste tyd van die jaar om sitrus kopmyt, *Aceria sheldoni* te beheer is tussen Februarie en Mei, en vir baie jare was Acarol (bromopropylate) die voorkeur mytdoder. Residue van hierdie produk is egter nie toelaatbaar op sitrus wat vir die VSA bestem is nie en vanaf Desember 2008 sal residue ook nie meer in die EU aanvaar word nie. 'n Dringendheid bestaan dus om 'n plaasvervanger, met 'n voor-oes interval wat toediening tussen Februarie en Mei toelaat, te vind. 'n Ondersoek na produkte wat teen eriophyidae myte in ander lande gebruik word, het tot die insluiting van RJU37PY in vier evaluasies gelei. Ander produkte wat in Suid-Afrika op sitrus teen ander plae geregistreer is, is ook tesame met 'n nuwe mytdoder geëvalueer. Die resultate van twee suurlemoenproewe in Mpumalanga en een suurlemoenproef plus een Valenciaproef in die Noordwes Provinsie het getoon dat RJU37PY (EC) teen 150 ml/hl water net so effektief soos Acarol 30-50 ml/hl in drie van die vier proewe was. Al die ander produkte wat getoets is was betekenisvol minder effektief as Acarol. Dit het abamectin teen 20, 40 en 60 ml/hl, Torque (15 ml/hl), Torque plus Tedion (55 + 200 ml/hl), Dithane 200 g/hl, Envidor 15 ml/hl, Acramite 50 ml/hl en Smito 30 ml/hl ingesluit. Hopelik sal hierdie resultate ondersteuning kan bied in die vinnige registrasie van RJU37PY op sitrus vir die beheer van knopmyt.

## Introduction

Although tydeid mites are clearly associated with citrus bud mite (CBM) (*Aceria 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, some acaricides such as propargite and amitraz that were registered against CBM can no longer be used after petal fall. Furthermore, the usage of bromopropylate after petal fall will be stopped in December 2008 when the maximum residue limit is reduced to the limit of detection. Growers who export to the USA currently cannot use bromopropylate and are having to resort to lime sulphur. 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. Due to a lack of citrus grey mite infestations, the first two trials of CBM research were conducted with citrus grey mite funding from experiment 856 but are reported here under 916 for convenience.

## Materials and methods

Three different lemon orchards and one Valencia orchard were used for the trials. Two lemon orchards were situated in Mpumalanga, one at the Lowveld College of Agriculture and the other at Bakgat Farm in the Schoemanskloof valley. Another lemon orchard and a Valencia orchard were situated on Mooinooi Farm near Brits in the North-West Province. In all orchards a randomised block design was used with single-tree plots. Four to ten trees were used as replicates per treatment, depending on their size. If the trees were touching in the row, untreated buffer trees were left between each treated tree. The trees were sprayed by hand as a medium-cover film wet spray using a pressure of 2000 kPa. Treatment details and application dates are provided in the result tables. In the first two lemon trials in Mpumalanga, two evaluations were conducted, 2 and 4 wk after treatment. However, it was found that some mites were still dying after 2 wk and the single evaluation after 4 wk gave a more realistic result. For the trials in North-West Province, only one evaluation after 4 wk was conducted. In each evaluation, six twigs (when four trees) or four twigs were cut from each tree, placed in a small brown paper bag and this placed in a plastic bag to retain relative humidity. The plastic bag was placed in a cold box and returned to the laboratory. When cutting the twigs, care was taken to ensure that there were at least five axil buds on each twig, excluding the youngest one at the distal end. In the laboratory a stereo-microscope was used to view the area at the base of each leaf and the axil bud while it was prised open. Each bud was then rated as being infested or not with live CBM. It was sometimes necessary to prod the bud mites to determine whether they were still alive, this was particularly the case with the evaluations after 2 wk. After an arc sine-square root transformation, infestation levels were compared by two-way ANOVA using treatments and replicates as main effects. If the F-test for treatments showed a significant value ( $P < 0.05$ ), means were compared further using Student-Newman-Keul's test.

## Results and discussion

The results of the initial trial at Bakgat farm (Table 3.4.6.1) showed more differences between treatments with time as the population of CBM increased. After two weeks, Torque appeared to be as effective as

Acarol but after a further two weeks it was significantly ( $P < 0.05$ ) inferior to Acarol. After four weeks the 100 ml rate of RJU37PY was slightly, but not quite significantly, inferior to the higher rates. The higher rates of RJU37PY were as effective as Acarol. The lowest rate of 100 ml was therefore dropped in further trials. In the second trial (Table 3.4.6.2), the combination of Torque and Tedion did not appear to be any better than Torque alone was in the previous trial. The levels of infestation in all treatments except the control decreased between two and four weeks after treatment. For this reason the next two trials were only evaluated after four weeks. As with the previous trial there were no significant differences between RJU37PY at 150 ml/hl water and at 200 ml/hl. The single rate of 150 ml/hl was therefore used in the next two trials. The high rate of abamectin plus oil was surprisingly effective so the lower registered rate of 20 ml/hl plus oil for citrus thrips must cause some suppression of CBM populations.

**Table 3.4.6.1.** Efficacy of various acaricides on lemons against citrus bud mite at Bakgat farm, Schoemanskloof

Treatments per hl water (Sprayed 14/06/2007)	Buds infested with bud mite (%)	
	27/06/07	11/07/07
Water control	65.8 a	83.3 a
Torque (fenbutatin oxide 55% SC) 55 ml	6.7 c	18.3 b
Acarol (bromopropylate 50% EC) 50 ml	0.8 c	5.0 c
RJU37PY 100 ml	3.3 c	10.0 bc
RJU37PY 150 ml	4.2 c	4.2 c
RJU37PY 200 ml	2.5 c	3.3 c
Unsprayed control	45.0 b	80.0 a

Means in the same column followed by a different letter are significantly different at  $P=0.05$  (SNK test)

**Table 3.4.6.2.** Efficacy of various acaricides on lemons against citrus bud mite at Lowveld College of Agriculture, Nelspruit

Treatments per hl water (Sprayed 4/09/2007)	Buds infested with bud mite (%)	
	18/09/07	2/10/07
Unsprayed control	88.6 a	95.0 a
Acarol (bromopropylate 50% EC) 50 ml	10.0 c	1.4 d
Abamectin (1.8% EC) 60 ml plus medium grade narrow range oil 300 ml	22.1 c	12.1 c
Torque (fenbutatin oxide 55% SC) 55 ml plus Tedion (tetradifon 8.1% EC) 200 ml	39.3 b	37.9 b
RJU37PY 150 ml	21.4 c	10.0 c
RJU37PY 200 ml	15.0 c	10.0 c

Means in the same column followed by a different letter are significantly different at  $P=0.05$  (SNK test)

In the trial on lemons near Brits in the North-West Province (Table 3.4.6.3), RJU37PY at 150 ml/hl was again as effective as Acarol at 30 ml/hl and all other treatments were significantly inferior to these. This included the registered Smite treatment which was not significantly different from the unsprayed control. In this trial, abamectin was used without oil and was not very effective with the 20 ml rate not causing a significant impact ( $P > 0.05$ ). Perhaps the oil is important to assist penetration into the bud. Torque at 15 ml/hl, Acramite, Envidor and Dithane all caused a significant degree of suppression but not control. In the Valencia trees near Brits the population densities of CBM were not as high as in the lemons but the trends in treatment efficacy were similar (Table 3.4.6.4). Acarol and RJU37PY were again significantly better than any other treatment and not significantly different from one another. Dithane, Smite and Torque were the next best treatments while the remaining treatments were not significantly better than the control. The addition of BreakThru to Smite made the treatment completely ineffective on the Valencias and reduced the efficacy slightly on the lemons. Perhaps the wetter is causing the product to run off in the areas where it is needed.

**Table 3.4.6.3.** Efficacy of various acaricides on lemons against citrus bud mite at Mooinooi Farm near Brits

Treatments per hl water (Sprayed 10/4/2008)	Buds infested with bud mite (%) 9/5/2008
Acarol (bromopropylate 50% EC) 30 ml	26.5 a
Torque (fenbutatin oxide 55% SC) 15 ml	63.0 b
RJU37PY 150 ml	30.5 a
Acramite (bifenazate 48% SC) 50 ml	62.5 b
Envidor (spirodiclofen 24% SC) 15 ml	66.5 bc
Abamectin (1.8% EC) 20 ml	78.5 cd
Abamectin (1.8% EC) 40 ml	69.5 bc
Abamectin (1.8% EC) 60 ml	62.0 b
Dithane (mancozeb 80% WP) 200 g	63.5 b
Smite (etoxazole 10% SC) 30 ml	77.0 bcd
Smite (etoxazole 10% SC) 30 ml + Break-thru 3 ml	80.0 cd
Unsprayed control	84.5 d

Means in the same column followed by a different letter are significantly different at P=0.05 (SNK test)

**Table 3.4.6.4.** Efficacy of various acaricides on Valencias against citrus bud mite at Mooinooi Farm near Brits

Treatments per hl water (Sprayed 10/4/2008)	Buds infested with bud mite (%) 9/5/2008
Acarol (bromopropylate 50% EC) 30 ml	5.5 a
Torque (fenbutatin oxide 55% SC) 15 ml	25.0 bc
RJU37PY 150 ml	6.5 a
Acramite (bifenazate 48% SC) 50 ml	36.5 bcd
Envidor (spirodiclofen 24% SC) 15 ml	39.5 cd
Abamectin (1.8% EC) 20 ml	32.5 bcd
Abamectin (1.8% EC) 40 ml	33.5 bcd
Abamectin (1.8% EC) 60 ml	32.5 bcd
Dithane (mancozeb 80% WP) 200 g	19.5 b
Smite (etoxazole 10% SC) 30 ml	24.0 bc
Smite (etoxazole 10% SC) 30 ml + Break-thru 3 ml	44.5 d
Unsprayed control	44.0 d

Means in the same column followed by a different letter are significantly different at P=0.05 (SNK test)

### Conclusions

RJU37PY appears to be a good alternative to Acarol and the only acaricide tested that comes close to having similar efficacy. Several other treatments such as abamectin plus oil and Dithane provided some suppression which may be adequate on cultivars other than lemons if sprayed more than once.

### Future research

Research will be conducted on the residue breakdown of RJU37PY and non-target effects on natural enemies in order to accelerate the registration process.

### Technology transfer

These results will be communicated at the Citrus Research Symposium in August 2008 and articles will be published in the SA Fruit Journal and Cutting Edge once the product becomes available.

### 3.5 PROJECT: MEALYBUG AND OTHER PHYTOSANITARY PESTS

Project coordinator: Sean Moore (CRI)

#### 3.5.1 Project summary

Five experiments were conducted within this project from January 2007 to March 2008. In the first, *Leptomastix* spp. parasitoids were captured from the field and reared on oleander mealybug (3.5.2). This experiment has come to an end. However, it has led into a new experiment which aims to develop and exploit *Leptomastix* spp. as biocontrol agents for oleander mealybug. In the second experiment, citrus mealybug pheromone trap catches were related to mealybug scouting results in 4 orchards (3.5.3). No meaningful or significant correlations could be established between trap catches and mealybug infestation. Also, no threshold value could be attached to trap catches to indicate whether intervention of some sort is necessary. In the third experiment, Break-Thru was tested as an adjuvant for corrective control of mealybug, with 3 different insecticides (3.5.4). Results were sufficiently convincing to be used towards the registration and recommendation of Break-Thru for this role. The fourth experiment, aimed to investigate the biology and biocontrol of oleander mealybug (3.5.5). Initiation of this experiment was delayed, due to difficulty in acquiring the involvement of a research student. In the fifth and final experiment, a laboratory colony of the grain chinch bug was established with limited success (3.5.6). This retarded the ability to examine the efficacy of gamma irradiation as a post-harvest sterilisation treatment for the bugs.

#### Projekopsomming

Vyf eksperimente is onder hierdie projek van Januarie 2007 tot Maart 2008 uitgevoer. In die eerste is *Leptomastix* spp. parasiete in die veld lewendig gevang en op oleander witluis geteel (3.5.2). Hierdie eksperiment het tot einde gekom maar het tot 'n nuwe eksperiment gelei. Die doel hiervan is om *Leptomastix* spp. as biologiese beheer agente vir oleander witluis te ontwikkel. In die tweede eksperiment is sitrus witluis feromoon lokval vangstes en resultate van witluis verkenning in 4 boorde vergelyk (3.5.3). Geen betekenisvolle korrelasie of verhouding tussen lokval vangstes en witluis besmetting kon gewys word nie. Dit was ook nie moontlik om 'n drempelwaarde aan lokval vangstes te heg nie. In die derde eksperiment is Break-Thru as 'n byvoegmiddel vir korrektiewe beheer van witluis, met 3 verskillende plaagdoders, getoets (3.5.4). Resultate was goed genoeg om Break-Thru in hierdie rol aan te beveel en te laat registreer. Die doel van die vierde eksperiment was om die biologie en biologiese beheer van oleander witluis te ondersoek (3.5.5). Omdat daar 'n probleem was met die beskikbaarheid van 'n navorsings student, is hierdie eksperiment vertraag. In die vyfde en finale eksperiment is laboratorium kultuur van die graanstinkluis met beperkte sukses gestig (3.5.6). Dit het die vermoë om die effek van gammabestraling as 'n na-oes sterilisasie behandeling vir die besies belemmer.

#### 3.5.2 FINAL REPORT: Investigating biocontrol agents of mealybug species other than citrus mealybug

Experiment 692 (April 2002 – March 2008) by Sean D. Moore and Wayne Kirkman (CRI)

#### Summary

The pest status and importance of oleander mealybug, *Paracoccus burnerae*, on citrus has increased in South Africa, especially in the Eastern Cape. Important and effective parasitoids have been identified for certain of the mealybug species which occur on citrus. Such information is still required for *P. burnerae*. This experiment was designed to collect such information.

A laboratory culture of *P. burnerae* was established on potato seedlings. In addition, another culture of *P. burnerae* was established on citrus seedlings, maintained in an outdoor tunnel house. By placing *P. burnerae* infested citrus and potato seedlings into mealybug infested citrus orchards for two weeks at a time, parasitoids were attracted to attack the mealybug on the seedlings. In emergence chambers in the laboratory, *Leptomastix* sp. and *Coccidoxenoides perminutus* were obtained. *Leptomastix* sp. parasitoids, which emerged from mealybug in the emergence boxes, were caught and exposed to more *P. burnerae* on potato seedlings. By so doing, a laboratory culture of *Leptomastix* sp. parasitoids was established. To date, this culture has completed several generations.

This experiment has been completed. However, in its place a new experiment – “*Leptomastix* spp. as biocontrol agents for control of mealybug, with particular reference to oleander mealybug, *Paracoccus burnerae*” – has been initiated. Rearing techniques for the *Leptomastix* sp. will be developed with the objective of conducting augmentative releases for oleander mealybug control.

## Opsomming

Die plaagstatus en derhalwe die belangrikheid van *Paracoccus burnerae* op sitrus, het in Suid-Afrika verhoog, veral in die Oos-Kaap. Belangrike en doeltreffende parasitoïede van 'n paar wiluisspesies wat op sitrus voorkom, is geïdentifiseer. Soortgelyke inligting word egter nog vir *P. burnerae* benodig. 'n Proef is uitgevoer om sulke inligting te versamel.

'n Laboratorium kultuur van *P. burnerae* is op aardappel saailinge gevestig. Deur om *P. burnerae* besmette sitrus en aardappel saailinge in wiluis besmette boorde te plaas vir twee weke op 'n slag is parasiete gelok om die wiluis op die saailinge aan te val. In uitbroeiingskaste in die laboratorium is *Leptomastix* sp. en *Coccidoxenoides perminutus* gekry. *Leptomastix* sp. parasiete wat van wiluis op die saailinge uitgebroei het is gevang en aan nog oleander wiluis op aardappel saailinge blootgestel. Sodoende is 'n laboratorium kultuur van *Leptomastix* sp. gevestig. Tot op datum het hierdie kultuur 'n hele paar generasies voltoei.

Hierdie eksperiment het tot einde gekom. Alhoewel, in sy plek is daar met 'n nuwe eksperiment begin – “*Leptomastix* spp. as biocontrol agents for control of mealybug, with particular reference to oleander mealybug, *Paracoccus burnerae*”. Teel tegnieke vir *Leptomastix* spp. sal ontwikkel word met die doel om aanvullende loslatings vir oleander wiluis beheer uit te voer.

## Introduction

Citrus mealybug, *Planococcus citri*, is known to be effectively controlled by natural enemies. This control has been substantially enhanced with the development of the augmentation technique for the parasitoid *Coccidoxenoides perminutus* (*peregrinus*). However, it has been shown that the oleander mealybug, *Paracoccus burnerae*, is approximately 100 times less suitable a host for *C. perminutus* than is *P. citri* (Hattingh & Tate, 1997). What makes this a serious situation justifying further investigation is that *P. burnerae* is regarded by certain important markets e.g. USA, Korea and China (and potentially many others) as being a phytosanitary pest; and that *P. burnerae* recently increased in dominance in citrus orchards, at least in the Eastern Cape. Of late, Wakgari & Gilliomee (2002) qualified and quantified the species of parasitoids in the natural enemy complexes attacking citrophilous mealybug, *Pseudococcus calceolariae*, longtailed mealybug, *Pseudococcus longispinus*, and *P. citri*. This experiment set about identifying the important and effective natural enemies of *P. burnerae*, something which had not been done until now. Moore & Kirkman (2006) identified 5 species of parasitoids as natural enemies of oleander mealybug: *Leptomastix* sp., *Coccidoxenoides perminutus*, *Leptomastix thukumiensis*, *Anagyrus* sp. and *Allotropa* sp. The last 3 species had not previously been recorded as parasitoids of oleander mealybug. The *Leptomastix* spp. appeared to be dominant. Ultimately, the objective of this study should be to establish an augmentation technique with natural enemies effective against *P. burnerae* (and possibly other mealybug species too).

## Materials and methods

A culture of *P. burnerae* was reared on potato seedlings. The purpose of this was two-fold: to attract and capture mealybug parasitoids in citrus orchards; and to rear these parasitoids in the laboratory. Starter material for the *P. burnerae* culture was sourced from the National Department of Agriculture (NDA) in Stellenbosch. In addition, a new *P. burnerae* culture was initiated from field-collected material. In order to be able to rear the mealybug on citrus seedlings, a fine gauze-tunnel was erected and citrus seedlings placed inside. Seedlings were infested with *P. burnerae*.

Infested citrus seedlings were placed into mealybug-infested citrus orchards in the Sundays River Valley and Gamtoos River Valley, in order to trap particularly *Leptomastix* spp. parasitoids. These were a Delta Valencia orchard on the farm of Steve Nichols in the Sundays River Valley, and a Palmer navel orange orchard on Tierhok Farm in the Gamtoos River Valley. Infested trees were left in the orchard for a period of two weeks, and then returned to the laboratory and placed in an emergence box. Four such trappings were conducted, on 17 January, 31 January, 13 February and 27 February 2007. Emerging parasitoids were kept in the emergence box and additional *P. burnerae* individuals were added from time to time, in an attempt to start a *Leptomastix* sp. culture. Before placing trees in the emergence boxes, a twig with some infested leaves was removed from each seedling and placed in a smaller emergence box, after recording the mealybug life stages on the twig. Parasitoids emerging from the recorded mealybug were stored in 70% alcohol, counted and identified. *Leptomastix* parasitoids emerging from the trees in the large emergence boxes were caught and exposed to more *P. burnerae* on potato seedlings.

During the 2008/09 season, potato seedlings infested with *P. burnerae* were placed in an orchard of navel orange trees on Paksam Farm in the Gamtoos River Valley. Again, the objective was to trap and collect

parasitoids, particularly *Leptomastix* spp. The infested potatoes were left in the orchard for a period of two weeks, and then returned to the laboratory and placed in an emergence box.

## Results and discussion

Several problems were experienced with the *P. burnerae* culture on potato seedlings. Firstly, the culture became contaminated with *P. citri*, and later with long-tailed mealybug, *Pseudococcus longispinus*. A new *P. burnerae* culture was therefore initiated from field-collected material. As citrus seedlings had previously seemed to be a more favourable substrate and food source on which to rear oleander mealybug, this was again used (in addition to the potato seedlings). However, a problem previously encountered was that the Grow-lux lighting used for the seedlings in the laboratory did not seem to be an adequate source of ultraviolet light and seedlings therefore did not survive for very long. Consequently, a fine gauze-tunnel was erected outdoors used for rearing *P. burnerae* on citrus seedlings, under natural light.

Results of the parasitoids collected from mealybug on the twig samples from field recovered seedlings, during December 2006 and January and February 2007, are recorded in Table 3.5.2.1. Parasitoids were identified by the authors. During the previous year, samples of parasitoids were sent to the Biosystematics Unit of the PPRI in Pretoria for identification. Identifications during this study were therefore based on what was learned the previous year.

**Table 3.5.2.1.** Parasitoids emerging from *P. burnerae* individuals placed in orchards for two weeks at a time from December 2006 to February 2007.

Farm & orchard no. (area)	Dates trap tree was in orchard	Mealybug life stages recorded on twig placed into emergence box				Parasitoids emerged		
		Egg sacs	Crawlers	Pre-adults	Adults	Total	<i>Leptomastix</i> sp.*	<i>Coccidoxenoides perminutus</i>
Steve Nichols #23 (SRV)	13-27/12/06	2	27	56	8	93	8	1
	13-27/02/07	3	65	29	6	103	2	1
Tierhok #8 (GRV)	26/12-09/01/07	1	81	23	2	107	-	-
	23/01-06/02/07	1	14	41	5	61	-	-

\*Exact identification of whether the species was *Leptomastix thukumiensis* or an undescribed *Leptomastix* sp., was not made.

Last season 5 species of parasitoids were identified from *P. burnerae* from the November surveys (Moore & Kirkman, 2006). Three species were identified for the first time, namely *Leptomastix thukumiensis*, *Anagyrus* sp. and *Allotropa* sp. Previous work reported by Prinsloo (1984) and Hattingh *et al.* (1998) lists the occurrence of various species of parasitoids and the relative dominance of *Anagyrus* sp. (probably *pseudococci*) and *Coccidoxenoides perminutus* in the complex. However, it is acknowledged that there is insufficient information on which parasitoid species attack which mealybug species, of which there are 7 occurring on citrus (Hattingh & Moore, 2003). Of the 5 species of parasitoids recorded in the 2006 study, *C. perminutus* and *Anagyrus* sp. have been previously reported (Prinsloo, 1984). *Allotropa* sp. could be *Allotropa kamburovi* (Gerhard Prinsloo, personal communication). Both *Leptomastix* sp. (which is not *Leptomastix dactylopii*) and *L. thukumiensis* appear to be new recordings.

During the 2007/08 study, only *C. perminutus* and *Leptomastix* sp. were obtained (Table 3.5.2.1). The *C. perminutus* individuals (of which there were only 2) were discarded. The *Leptomastix* sp. were used to initiate a laboratory culture of the parasitoid. It was not confirmed whether this species was *Leptomastix thukumiensis* or if it was the unidentified species of *Leptomastix*, recorded last season. Once the culture has been strongly established, individuals will be sent to the PPRI for identification.

From October 2007 until March 2008, potato seedlings infested with *P. burnerae* were periodically placed into a mealybug infested orchard on Paksaam Farm in the Gamtoos River Valley. Frequent catches of *Leptomastix* sp. parasitoids were made. However, these were not enumerated. All *Leptomastix* sp. parasitoids collected were used to bolster the *Leptomastix* sp. laboratory culture. The laboratory culture has now been running for a few months. Therefore, several generations of the parasitoid have been completed.

## Conclusion

A laboratory culture of *P. burnerae* was established on potato seedlings. In addition, another culture of *P. burnerae* was established on citrus seedlings, maintained in an outdoor tunnel house. Parasitoids were trapped by placing infested potato and citrus seedlings into orchards. By so doing, a laboratory culture of *Leptomastix* sp. parasitoids was established. To date, this culture has completed several generations.

## Future research

This experiment has been completed. However, in its place a new experiment has been initiated. This is experiment 934: "*Leptomastix* spp. as biocontrol agents for control of mealybug, with particular reference to oleander mealybug, *Paracoccus burnerae*". Rearing techniques for the *Leptomastix* sp. will be developed with the objective of conducting augmentative releases for oleander mealybug control.

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### 3.5.3 FINAL REPORT: Evaluation of *Planococcus citri* pheromone traps for monitoring infestation levels

Experiment 845 (September 2006 – September 2007): by Sean D. Moore and Wayne Kirkman (CRI)

## Summary

The objective of this study was to determine whether it was possible to correlate citrus mealybug, *Planococcus citri*, pheromone trap catches with mealybug infestation on citrus fruit and thereby enable growers to use mealybug traps instead of scouting, for monitoring mealybug levels. Two navel orange orchards, 1 Delta Valencia orchard and 1 lemon orchard were used for the trial. One sticky yellow delta trap impregnated with PheroLure (female *P. citri* pheromone analogue) was placed into each orchard and monitored weekly. Mealybug infestation of fruit in the same vicinity was monitored simultaneously. Mealybug infestation peaked at a range of 14-46% fruit infested, whereas trap catches ranged between peaks of 22 and 247 males per trap per week. No meaningful or significant correlation could be established between trap catches and mealybug infestation in the four citrus orchards monitored. Also, no threshold value could be attached to trap catches to indicate whether intervention of some sort is necessary. However, this experiment should be repeated in more orchards over more than one season, before a reliable conclusion can be drawn. One possible impediment to the reliability of this trapping system is that it is specific to *P. citri*. Therefore, it is possible that there could be a high level of infestation of other mealybug species, which will not be reflected on the traps. This experiment has come to an end. However, additional research is warranted.

## Opsomming

Die doel van hierdie studie is om te bepaal of dit moontlik sal wees om sitrus witluis, *Planococcus citri*, feromoon lokvalle vangstes met witluis besmetting op sitrus vrugte te korreleer. Dit sal vir produsente die geleentheid gee om witluis lokvalle in die plek van verkenning te gebruik, vir die monitering van witluis

vlakke. Twee nawellemoen boorde, 1 Delta Valencia boord en 1 suurlemoen boord is vir die proef gebruik. Een taai geel delta-lokval met 'n PheroLure (wyfie *P. citri* feromoon analogon) is in elke boord gehand en weekliks gemonitor. Witluis besmetting van vrugte is gelyktydig in dieselfde omgewing gemonitor. Witluis besmetting in die 4 persele het gepiek tussen 14-46% vrugte besmet, terwyl lokval vangstes gepiek het op tussen 22 tot 247 mannetjies per lokval per week. Geen betekenisvolle korrelasie of verhouding tussen lokval vangstes en witluis besmetting in enige van die 4 boorde kon gewys word nie. Dit was ook nie moontlik om 'n drempelwaarde aan lokval vangstes te heg nie, om aan te dui of aksie van een of ander soort teen witluis benodig was nie. Nietemin moet hierdie eksperiment in meer boorde oor meer as een seisoen herhaal word, voordat 'n betroubare gevolgtrekking gemaak kan word. 'n Moontlike struikelblok vir die betroubaarheid van hierdie lokval stelsel is dat hulle spesifiek vir *P. citri* is. Daarom is dit moontlik dat daar 'n hoë vlak van besmetting van ander witluis spesies kan wees, en hulle word nie op die lokval opgetel nie. Hierdie eksperiment het tot einde gekom, maar verdere navorsing word regverdig.

## Introduction

During the 2004/05 citrus growing season, a trial was conducted to compare the sensitivity of five different trap types for monitoring of citrus mealybug, *Planococcus citri*, males with the Insect Science PheroLure product in citrus orchards. PheroLure is a dispenser impregnated with an analogue of the *P. citri* female pheromone. The study determined that of 5 different trap types used, small yellow delta traps caught the highest numbers of mealybug males (Moore & Kirkman, 2005). Surprisingly, these results directly contradicted those from another study (Zada *et al.*, 2004). These traps also tend to be more user friendly than card traps, as their sticky surface is protected within the trap. The follow-up study, described in this report, was thus conducted with this trap. The work entailed the investigation of the usefulness of pheromone impregnated traps for accurate monitoring of citrus mealybug and hence decision making on the need for and timing of intervention (chemical or biological). The objective was to determine whether it was possible to correlate trap catches with infestation and thereby enable growers to use mealybug traps instead of scouting, for monitoring mealybug levels.

## Materials and methods

Four sites were used for this trial. These were two navel orange orchards in the Gamtoos River Valley (GRV) and a Delta Valencia orchard and a lemon orchard in the Sundays River Valley (SRV). The GRV orchards were orchards 1 and 8 on Tierhok Farm. Both the SRV orchards were on the farm of Steve Nichols: orchards 23 (Delta Valentias) and 10 (lemons). One small yellow delta trap, furnished with a sticky floor and a fresh PheroLure (*P. citri*) pheromone dispenser (replaced once every three weeks), was placed in each of the four orchards. These traps were monitored weekly. Simultaneously, mealybug infestation of fruit on 10 data trees in the immediate vicinity of the trap was determined. Percentage of fruit infested was determined and all life stages present were identified and counted individually. Monitoring was initiated in November 2006 and continued until mid-March 2007, at which time there was a general decline in mealybug infestation and further monitoring was considered superfluous.

Data from each site were subjected to a regression analysis. Data were examined for significant correlation between trap numbers and fruit infestation, numbers of each life stage and total mealybug numbers.

## Results and discussion

When the trial was initiated (traps were hung) on 7 November, a low level of mealybug infestation was already detected in each of the four orchards (Tables 3.5.3.1 - 4). Mealybug infestation peaked at 33% and 46% fruit infested in the two navel orange orchards (Tables 3.5.3.1 & 2). This was in early to mid-January. Thereafter, mealybug infestation declined, almost certainly due to biological control (Hattingh & Moore, 2003).

**Table 3.5.3.1.** Trap counts, mealybug infestation (%) and life stages in Orchard 1 (navel oranges), Tierhok farm, Gamtoos River Valley. (Dates with asterisk denote replacement of pheromone dispenser).

Date	Trap count	Fruit infested (%)	Numbers of mealybug per life-stage				
			Egg sacs	Crawlers	Pre-adults	Adults	Total
07/11/06*		9					
15/11	16	11	0	2	10	0	12
22/11*	Trap stolen						
29/11	5	13	1	7	6	2	16
05/12	3	23	2	35	4	4	45

12/12*	4	24	5	55	8	7	75
19/12	0	18	1	29	7	1	38
26/12*	3	28	3	33	9	0	45
02/01/07	4	33	3	106	3	2	114
09/01*	5	33	1	49	5	0	55
16/01	11	27	0	34	5	2	41
23/01	22	17	1	13	11	2	27
30/01*	16	9	1	5	3	0	9
06/02	5	9	0	7	4	0	11
13/02	9	6	0	4	4	0	8
20/02*	11	4	0	4	0	0	4
27/02	10	4	0	3	2	0	5
06/03	12	5	0	6	1	0	7
13/03	10	6	1	4	3	0	8

**Table 3.5.3.2.** Trap counts, mealybug infestation (%) and life stages in Orchard 8 (navel oranges), Tierhok farm, Gamtoos River Valley. (Dates with asterisk denote replacement of pheromone dispenser).

Date	Trap count	Fruit infested (%)	Numbers of mealybug per life-stage				
			Egg sacs	Crawlers	Pre-adults	Adults	Total
07/11/06*		4					
15/11	3	6	0	3	3	0	6
22/11	5	5	1	3	0	1	5
29/11*	6	16	0	7	12	1	20
05/12	2	28	0	65	13	0	78
12/12	4	33	2	43	5	6	56
19/12*	8	33	7	32	12	1	52
26/12	62	41	4	35	31	9	79
02/01/07	39	43	4	72	17	4	97
09/01*	48	41	2	98	24	1	125
16/01	247	46	4	111	11	2	128
23/01	194	39	0	31	47	0	78
30/01*	106	28	0	52	10	10	72
06/02	241	19	1	15	17	0	33
13/02	83	12	3	35	2	0	40
20/02*	87	10	0	6	5	0	11
27/02	57	7	2	24	1	0	27
06/03	79	6	3	2	1	0	6
13/03	15	7	2	3	1	1	7

Mealybug infestation remained low in both of the SRV orchards, without any distinct peak and decline in infestation (Tables 3.5.3.3 & 4). Despite this, trap counts continued to rise, peaking in January in the Delta Valencia orchard and in February in the lemon orchard. Trap catches in both orchards actually peaked at a higher level than in orchard 1 in GRV (Table 3.5.3.1), despite infestation being a lot lower than in orchard 1.

**Table 3.5.3.3.** Trap counts, mealybug infestation (%) and life stages in Orchard 23 (Delta Valencia oranges), Steve Nicholls' farm, Sundays River Valley. (Dates with asterisk denote replacement of pheromone dispenser).

Date	Trap count	Fruit infested (%)	Numbers of mealybug per life-stage				
			Egg sacs	Crawlers	Pre-adults	Adults	Total
08/11/06*		6					
17/11	8	7	0	7	1	0	8
23/11	6	10	0	3	7	0	10
30/11*	6	14	0	15	4	2	21
07/12	4	15	1	15	3	2	21
13/12	0	10	0	8	2	0	10
20/12*	2	11	2	14	7	1	24
27/12	7	10	1	12	3	5	21

04/01/07	9	15	1	28	2	2	33
10/01*	5	8	1	10	1	3	15
17/01	21	15	1	16	0	3	20
24/01	68	15	0	15	4	0	19
31/01*	21	11	0	8	5	1	14
08/02	24	10	0	7	3	1	11
14/02	9	12	0	10	2	0	12
21/02*	49	8	1	4	3	0	8
28/02	79	7	0	6	1	0	7
07/03	45	12	2	6	5	0	13
14/03	11	10	2	6	3	0	11

**Table 3.5.3.4.** Trap counts, mealybug infestation (%) and life stages in Orchard 10 (lemons), Steve Nichols' farm, Sundays River Valley. (Dates with asterisk denote replacement of pheromone dispenser).

Date	Trap count	Fruit infested (%)	Numbers of mealybug per life-stage				
			Egg sacs	Crawlers	Pre-adults	Adults	Total
08/11*		14					
17/11	6	5	1	1	2	1	5
23/11	5	8	2	5	9	1	17
30/11*	3	5	0	10	3	0	13
07/12	4	5	0	4	0	1	5
13/12	2	7	1	16	0	1	18
20/12*	4	8	0	10	1	0	11
27/12	5	5	0	4	4	0	8
03/01/07	14	14	0	13	3	2	18
10/01*	19	7	0	5	3	0	8
17/01	20	8	0	9	1	1	11
24/01	27	5	1	5	1	0	7
31/01*	19	4	0	20	2	0	22
08/02	22	2	0	0	1	1	2
14/02	16	5	0	4	2	0	6
21/02*	10	6	0	4	0	2	6
28/02	34	4	0	2	2	0	4
07/03	27	7	0	4	3	0	7
14/03	5	6	1	4	2	0	7
14/03	5	6	1	4	2	0	7

No statistically significant correlation could be established between trap numbers of mealybug males and any of the scouted parameters at any of the four sites (Tables 3.5.3.5 - 8). The relationship between trap numbers and crawlers at Tierhok 1 was significant at a probability of 99.32%. However, there was only a 41.76% correlation between these two variables (Table 3.5.3.5). This was in contrast with similar studies conducted with the grape vine mealybug, *P. ficus*, on grapes, where strongly significant positive correlations were established between trap catches, mealybug infestation and mealybug damage in California (Millar et al., 2002) and South Africa (Walton et al., 2004).

**Table 3.5.3.5.** Regression analyses to determine the relationship between mealybug pheromone trap catches (adult male mealybug) and mealybug infestation on Palmer navel oranges in orchard 1 on Tierhok Farm (7 November 2006 to 13 March 2007).

Variable pairs	n	Correlation coefficient (%)	Standard error	P-value
Trap and fruit infested (%)	20	28.28	0.55	0.034
Trap and number of egg sacs	20	43.70	0.58	0.052
Trap and number of crawlers	20	41.76	0.50	0.0068
Trap and number of sub-adults	20	0.82	0.67	0.748
Trap and number of adults	20	27.37	0.72	0.287
Trap and total number of mealybugs	20	34.70	0.53	0.016

**Table 3.5.3.6.** Regression analyses to determine the relationship between mealybug pheromone trap catches (adult male mealybug) and mealybug infestation on Palmer navel oranges in orchard 8 on Tierhok Farm (7 November 2006 to 13 March 2007).

Variable pairs	n	Correlation coefficient (%)	Standard error	P-value
Trap and fruit infested (%)	20	5.35	1.59	0.356
Trap and number of egg sacs	20	0.36	1.44	0.852
Trap and number of crawlers	20	7.50	1.57	0.272
Trap and number of sub-adults	20	2.24	1.60	0.566
Trap and number of adults	20	15.02	1.39	0.269
Trap and total number of mealybugs	20	10.24	1.55	0.195

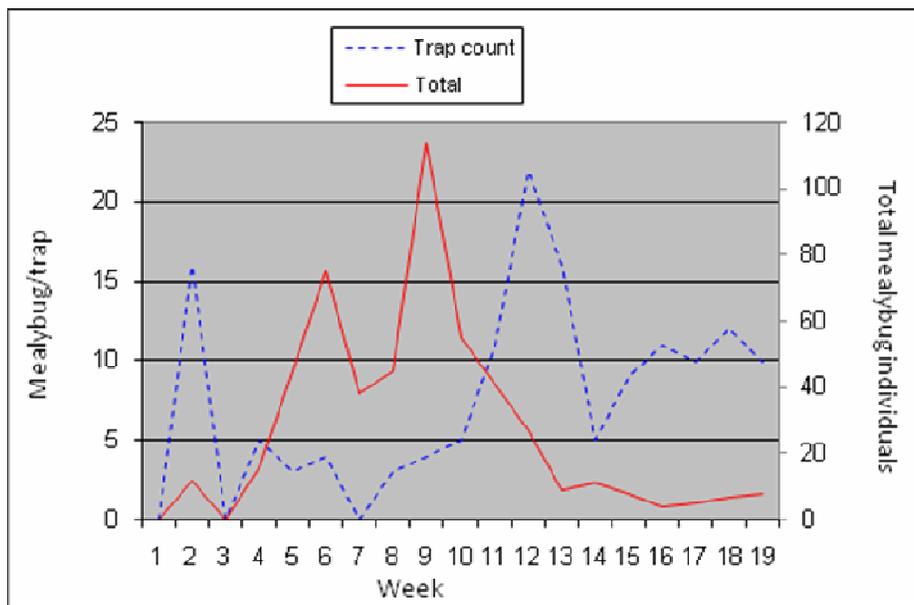
**Table 3.5.3.7.** Regression analyses to determine the relationship between mealybug pheromone trap catches (adult male mealybug) and mealybug infestation on Delta Valencia oranges in orchard 23 on Steve Nichols' Farm (7 November 2006 to 13 March 2007).

Variable pairs	n	Correlation coefficient (%)	Standard error	P-value
Trap and fruit infested (%)	20	1.531	1.09	0.636
Trap and number of egg sacs	20	0.046	1.16	0.956
Trap and number of crawlers	20	9.112	1.05	0.239
Trap and number of sub-adults	20	1.412	1.12	0.661
Trap and number of adults	20	0.980	0.91	0.800
Trap and total number of mealybugs	20	20.222	0.98	0.070

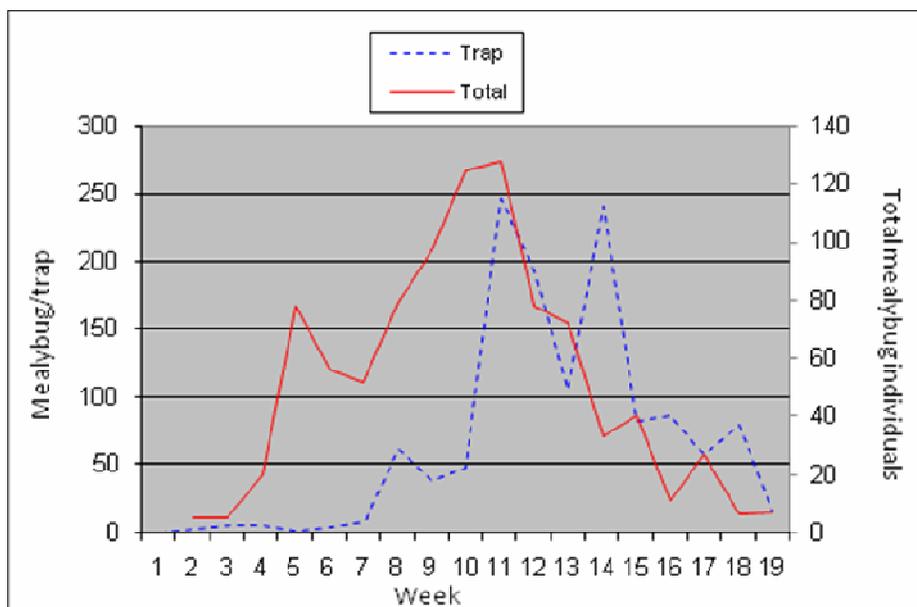
**Table 3.5.3.8.** Regression analyses to determine the relationship between mealybug pheromone trap catches (adult male mealybug) and mealybug infestation on lemon oranges in orchard 10 on Steve Nichols' Farm (7 November 2006 to 13 March 2007).

Variable pairs	n	Correlation coefficient (%)	Standard error	P-value
Trap and fruit infested (%)	20	4.552	0.88	0.395
Trap and number of egg sacs	20	1.283	1.08	0.856
Trap and number of crawlers	20	2.612	0.90	0.535
Trap and number of sub-adults	20	11.99	0.80	0.206
Trap and number of adults	20	8.969	0.86	0.471
Trap and total number of mealybugs	20	11.63	0.85	0.166

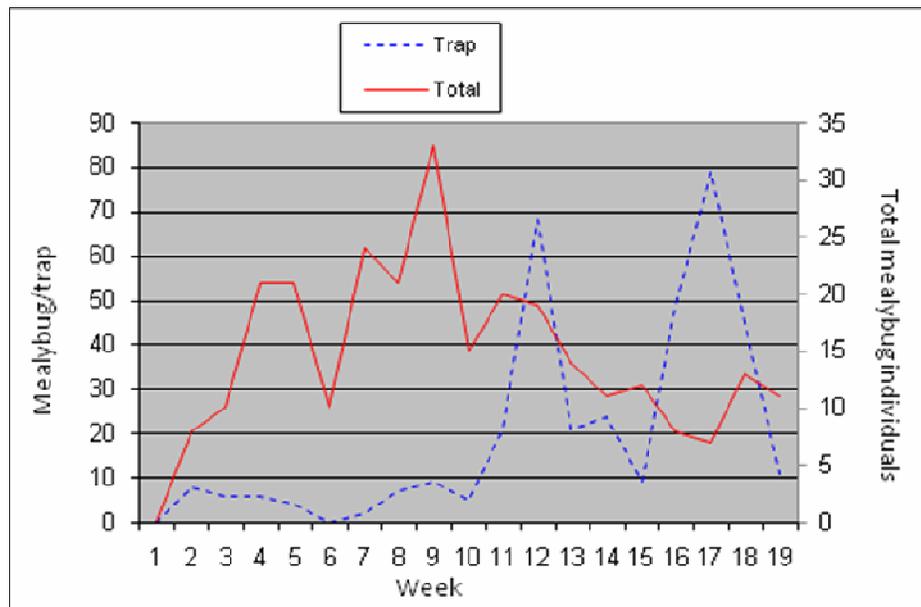
At all four sites, the probability of a correlation between trap numbers and total numbers of mealybug and/or numbers of crawlers was closer to significance than the relationship between trap catches and any of the other factors measured (scouted) (Tables 3.5.3.5 - 8). Graphic displays of trap numbers and total numbers of mealybug on fruit were therefore generated. Inspecting these graphs appeared to indicate that peaks in trap catches followed peaks in infestation (Figs. 3.5.3.1 - 4). This was particularly apparent in Tierhok 8 and the two SRV orchards (Figs. 3.5.3.2 - 3.5.3.4). Although it could not be determined with certainty, there seemed to be about a 3 week gap between peaks in infestation and trap peaks. As the bulk of individuals infesting fruit were crawlers (Tables 3.6.3.1 - 3.6.3.4), this trend makes sense. The life cycle of *P. citri* in mid-summer is around 4 weeks (Hattingh *et al.*, 1998), which means that duration from crawler to adult male may well be in the region of 3 weeks. Nevertheless, the fact that trap peaks lagged behind infestation peaks, means that the predictive value of these traps is compromised.



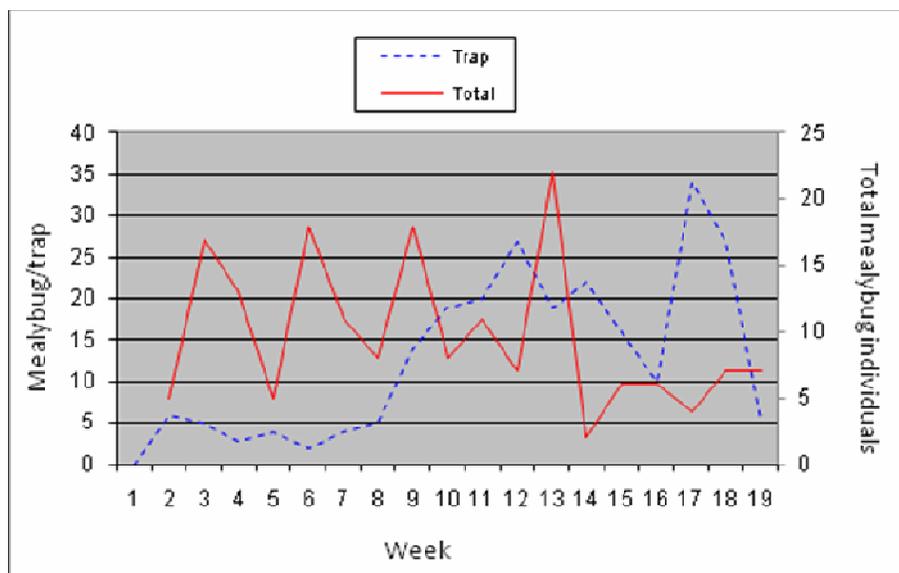
**Fig. 3.5.3.1.** Male *P. citri* trap catches and total number of mealybug individuals on fruit per week in orchard 1 (navel oranges) on Tierhok Farm (November 2005 to March 2006).



**Fig. 3.5.3.2.** Male *P. citri* trap catches and total number of mealybug individuals on fruit per week in orchard 8 (navel oranges) on Tierhok Farm (November 2005 to March 2006).



**Fig. 3.5.3.3.** Male *P. citri* trap catches and total number of mealybug individuals on fruit per week in orchard 23 (Delta Valencia oranges) on Steve Nichols' farm (November 2005 to March 2006).



**Fig. 3.5.3.4.** Male *P. citri* trap catches and total number of mealybug individuals on fruit per week in orchard 10 (lemons) on Steve Nichols' farm (November 2005 to March 2006).

Not only would one want the traps to be able to forewarn one of an impending mealybug infestation (which the traps did not seem to do), but one would also want the level of trap catches to indicate whether a treatment for mealybug might be necessary or not. In other words, can a threshold value be applied to the traps? In orchard 1 at Tierhok, trap catches peaked at 22 individuals per trap per week (on 23 January), whereas infestation peaked at 33% fruit infested (2 January) (Table 3.5.3.1). In orchard 8 at Tierhok, trap catches peaked at 247 individuals per trap per week (on 16 January), whereas infestation peaked at 46% fruit infested (on the same date) (Table 3.5.3.2). In orchard 23 on Nichols' farm, trap catches peaked at 79 (on 28 February), whereas infestation peaked at 15% fruit infested (on 4 January) (Table 3.5.3.3). In orchard 10 on Nichols' farm, trap catches peaked at 34 (on 28 February), whereas infestation peaked at 14% fruit infested (3 January) (Table 3.5.3.4). It therefore did not appear that trap catch numbers were indicative of level of infestation. Although the orchard with the highest trap catch was also the orchard with the highest percentage fruit infested (Tierhok 8), this trend was not followed in the other 3 orchards. When using total numbers of mealybug individuals on fruit instead of percentage fruit infested, again no trend was apparent.

## Conclusion

No meaningful or significant correlation could be established between mealybug (*P. citri*) pheromone trap catches and mealybug infestation in the four citrus orchards monitored in the Eastern Cape. However, this does not mean that these traps do not have a use as a monitoring tool. This experiment should be repeated in more orchards over more than one season, before a reliable conclusion can be drawn. One possible impediment to the reliability of these traps is that they are specific to *P. citri*. Therefore, it is possible that there could be a high level of infestation of other mealybug species, which will not be reflected on the traps. It will therefore be important to verify which mealybug species occur in an orchard, meaning that it will probably never be possible to completely replace visual scouting with trapping.

## Future research

This experiment has come to an end. However, additional research is warranted.

## Technology Transfer

As the outcome of this research is not yet conclusive, further studies should be conducted before there is any technology transfer to grower communities.

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### 3.5.4 FINAL REPORT: Using Break-Thru to improve corrective control of mealybug on citrus Experiment 873 (2005/6-2007/8) by Sean D. Moore and Wayne Kirkman (CRI)

## Summary

Break-Thru S 240 appears to have an uncanny ability to cause spreading and therefore improve penetration of sprayed treatments into nooks and crannies. Consequently, it is possible that the addition of this product to mealybug treatments could improve corrective control of mealybug. An orchard trial was conducted to test this. Three different products were used in this trial to measure the corrective control of mealybug on citrus. These products were applied with Agral 90 or with Break-Thru at one of three different concentrations. All treatments reduced mealybug infestation, Ultracide being the most effective of the products. The addition of Break-Thru was at least as effective as was the addition of Agral 90 and possibly marginally better. Indications are that there should be no need to use Break-Thru at a concentration any higher than 3 ml per 100 L water. Results were sufficiently convincing to be used towards the registration and recommendation of Break-Thru as an additive to corrective treatments for mealybug control on citrus.

## Opsomming

Dit wil voorkom dat Break-Thru S 240 'n ongelooflike vermoë het om verspreiding te veroorsaak en sal daarom penetrasie van bespuitings tot in hoekies en gaatjies verbeter. As gevolg hiervan is dit moontlik dat die byvoeging van hierdie produk tot wtluis behandelings die werking van korrektiewe behandelings vir wtluis kan verbeter. 'n Boordproef is uitgevoer om hierdie te toets. Drie verskillende produkte is in hierdie proef gebruik om die korrektiewe beheer van wtluis op sitrus te meet. Hierdie produkte is saam met of Agral

90 of Break-Thru, teen 3 verskillende konsentrasies, gebruik. Alle behandelings het witluis besmetting verminder. Ultracide was die mees doeltreffend van die produkte. Die byvoeging van Break-Thru was minstens so doeltreffend as die byvoeging van Agral 90 en moontlike effens beter. Die resultate het aangedui dit die heel waarskynlik nie nodig sal wees om Break-Thru teen 'n hoër dosis as 3 ml per 100 L water te gebruik nie. Die resultate is oortuigend genoeg om te lei na die registrasie en aanbeveling van Break-Thru as 'n byvoegmiddel saam met korrektiewe behandelings vir witluis beheer op sitrus.

## Introduction

Good corrective control of mealybug is difficult to achieve. Once mealybug has packed underneath the calyx of the fruit and inside the navel-end (of navel oranges), it is well protected against chemical sprays. Corrective spray trials conducted during the 2003/04 season with a range of insecticides, demonstrated that acceptable control was impossible with all treatments except Applaud (Moore & Kirkman, 2004). However, substantial reduction in infestation was only recorded for Applaud, eight weeks after application. Break-Thru S 240 is a polyether trisiloxane wetter and spreader. It appears to have an uncanny capacity to cause spreading and therefore improve penetration in nooks and crevices. For this reason it is possible that the addition of this product to mealybug treatments could improve their ability to correctively control mealybug. Last season (2006/07), Break-Thru was tested at one concentration with four different products. Results with Break-Thru were as good as those with Agral 90 (Moore & Kirkman, 2006). The trial described in this report is a more detailed extension of the work initiated during the previous year.

## Materials and methods

A Midnight Valencia orange orchard (planted in 1997) with a conspicuous level of mealybug infestation was selected for this trial. This orchard was on Scheepersvlakte Farm in Sundays River Valley, Eastern Cape. Before application of the trial, mealybug infestation on untreated control trees was evaluated by inspecting 10 randomly selected fruit on each of the 10 trees. Inspections were also conducted underneath the calyx of each fruit. The trial was laid out in single tree randomised block format, replicated 10 times. Three different products were used, either with Agral 90 (alkylated phenol-ethylene oxide) or with one of three different concentrations of Break-Thru: 3 ml, 5 ml and 10 ml per 100 L water (Table 3.5.4.1). Sprays for mealybug control are commonly recommended to be applied with a wetter (Hattingh & Moore, 2003). Agral 90 is one of the wetters most commonly used. Treatments, for corrective control of mealybug, were applied on 8 February 2007, using a high pressure spray machine with hand guns, as full cover film sprays. An average of 20.0 L of spray mix was applied to each tree. Trees were spaced at 5.5 m x 2.5 m apart, making a total of 727 trees per hectare. This would extrapolate to an application of 14540 L of spray mix per hectare.

Infestation was evaluated twice: 6 days after application on 14 February and again 41 days later on 21 March. This was done by inspecting 10 fruit on each tree. Fruit was classified as clean or infested. Data were statistically analysed by comparing means for treatments using an ANOVA and the LSD multiple range test. Infestation on Applaud-treated trees was only evaluated on 21 March, as this product does not have the rapid knock-down effect of the other two products, Ultracide and Phosdrin (both organophosphates).

**Table 3.5.4.1.** Various treatments applied on 8 February 2007 for the corrective control of mealybug on Midnight Valencia orange trees on Scheepersvlakte Farm.

	Treatment	Active ingredient & formulation	Concentration in 100 L water	Wetter	Concentration in 100 L water
1	Untreated control	-	-	-	-
2	Phosdrin*	Mevinphos SL 500 g/L	50 ml	Agral 90	18 ml
3	Phosdrin*	Mevinphos SL 500 g/L	50 ml	Break-Thru	3 ml
4	Phosdrin*	Mevinphos SL 500 g/L	50 ml	Break-Thru	5 ml
5	Phosdrin*	Mevinphos SL 500 g/L	50 ml	Break-Thru	10 ml
6	Applaud	Buprofezin WP 500 g/kg	30 g	Agral 90	18 ml
7	Applaud	Buprofezin WP 500 g/kg	30 g	Break-Thru	3 ml
8	Applaud	Buprofezin WP	30 g	Break-Thru	5 ml

9	Applaud	500 g/kg Buprofezin WP 500 g/kg	30 g	Break-Thru	10 ml
10	Ultracide	Methidathion EC 420 g/L	150 ml	Agral 90	18 ml
11	Ultracide	Methidathion EC 420 g/L	150 ml	Break-Thru	3 ml
12	Ultracide	Methidathion EC 420 g/L	150 ml	Break-Thru	5 ml
13	Ultracide	Methidathion EC 420 g/L	150 ml	Break-Thru	10 ml

\* Not a registered option for mealybug control, but known to be moderately effective.

## Results and discussion

On 8 February, the date on which the trial was applied, 29% of fruit on the untreated control trees was infested with mealybug. It was clear that infestation had been a lot higher, earlier in the season and had declined, most probably due to natural biological control. This biocontrol trend was considered likely to increase towards harvest. The first evaluation of the trial was therefore conducted only 6 days after application, in order that infestation was still of such a level that differences between treatments could be detected. At this stage infestation in the untreated control had fortunately not yet begun to decline. Mealybug infestation was significantly reduced for all treatments, except Phosdrin with 3 ml Break-Thru and Ultracide with Agral 90 (Table 3.5.4.2). There was no significant difference in efficacy between any of the 8 treatments evaluated 6 days after application (Table 3.5.4.2).

**Table 3.5.4.2.** Mealybug infestation of Midnight Valencia oranges on Scheepersvlakte Farm 6 days and 41 days after application of treatments.

	Treatment	Fruit infested (%)*	
		14 February	21 March
1	Untreated control	29.0a	17.0a
2	Phosdrin + Agral 90	6.0b	14.0ab
3	Phosdrin + Break-Thru 3 ml	15.0ab	6.0abc
4	Phosdrin + Break-Thru 5 ml	4.0b	11.0abc
5	Phosdrin + Break-Thru 10 ml	7.0b	6.0abc
6	Applaud + Agral 90	-	4.0bc
7	Applaud + Break-Thru 3 ml	-	3.0bc
8	Applaud + Break-Thru 5 ml	-	3.0bc
9	Applaud + Break-Thru 10 ml	-	1.0c
10	Ultracide + Agral 90	14.0ab	1.0c
11	Ultracide + Break-Thru 3 ml	4.0b	0c
12	Ultracide + Break-Thru 5 ml	5.0b	0c
13	Ultracide + Break Thru 10 ml	4.0b	1.0c

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

The second evaluation was conducted about 6 weeks after application of treatments – mainly in order to give Applaud an opportunity to work out its full efficacy. Earlier trial work showed that Applaud required well over three weeks in order to show its maximum efficacy (Moore & Kirkman, 2004). At this time (21 March) mealybug infestation in the untreated control had declined to 17% (Table 3.5.4.2). The inferior efficacy of Phosdrin was shown up at this stage, as none of the Phosdrin treatments were significantly better than the untreated control. Although the knock-down of Phosdrin appeared to be fairly good on the 14 of February, the lack of residual efficacy was evident 5 weeks later. Surprisingly, the control achieved with Ultracide was marginally, but consistently better than with Applaud. However, this difference was not significant. This was in contrast with the results of a corrective treatment trial conducted on navel orange trees in 2004 (Moore & Kirkman, 2004). This may have been an indication that the timing for the application of Applaud was not ideal. Applaud is an insect growth regulator (IGR) and is therefore only effective against younger life-stages of mealybug. Ultracide might also be more effective than Applaud on Valencia oranges, whereas Applaud might be more effective than Ultracide on navel oranges. This may be because Ultracide has a superior knock-down action, whereas Applaud is more effective against emerging crawlers, which can be very effectively hidden from Ultracide within the navel-end of a navel orange fruit.

A total of 20 L of spray mix was applied to each tree, extrapolating to an application of 14540 L of spray mix per hectare. It is utterly imperative that treatments for mealybug control be applied as heavy full cover film sprays with good penetration (Hattingh & Moore, 2003). Break-Thru was mixed at concentrations of 3 ml, 5 ml and 10 ml per 100 L water. This means that Break-Thru was applied at 436 ml per hectare, 727 ml per hectare and 1454 ml per hectare. The product label states that Break-Thru should not be applied at more than 500 ml per hectare but that it should be applied at a concentration of 25-50 ml/100 litres of water. It can therefore be argued that either too much (5 ml and 10 ml doses) or too little Break-Thru was applied. This discrepancy on the label needs to be cleared up.

There was no detectable trend in efficacy for the different Break-Thru concentrations. Break-Thru, at all concentrations, was at least as effective as was Agral 90. In the case of Phosdrin and Applaud, results with Break-Thru were marginally, but not significantly, better than with Agral 90 (Table 3.5.4.2). The results recorded in this trial seem to indicate that it should not be necessary to add more than 3 ml of Break-Thru per 100 L water for use with a corrective control treatment for mealybug on citrus.

## Conclusion

Three different products were used in a trial to measure the corrective control of mealybug on citrus. These products were applied with Agral 90 or with Break-Thru at one of three different concentrations. All treatments reduced mealybug infestation, with Ultracide being the most effective of the products. The addition of Break-Thru was at least as effective as was the addition of Agral 90 and possibly marginally better. Indications are that there should be no need to use Break-Thru at a concentration any higher than 3 ml per 100 L water. Results were sufficiently convincing to be used towards the registration and recommendation of Break-Thru as an additive to corrective treatments for mealybug control on citrus.

## Future research

No further research is planned on this topic.

## Technology Transfer

Numerous grower talks have been made on mealybug control over the past year. At the upcoming 2008 Citrus Research Symposium, the authors will present a paper on the specific work covered in this report.

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### 3.5.5 PROGRESS REPORT: Biology of the oleander mealybug, *Paracoccus burnerae* Experiment SU-E-2006 (April 2006 - December 2009) by JH Giliomee and Todd Johnson (SU)

## Summary

The aim of the project is to study the biology (particularly the rate of development, fecundity and parasitism) of the oleander mealybug, *Paracoccus burnerae*, which is an endemic species of quarantine importance attacking citrus. The species is reared on young citrus trees in pots in a greenhouse. Seedlings in small containers are infested and reared in incubators at various constant temperatures. Older trees in pots were also obtained and are being infested in the greenhouse. Permission was obtained to place them out in an abandoned citrus orchard near Stellenbosch to expose the mealybugs to parasitism. Todd Johnson, a student, will do part of the experiment towards an MSc.

## Opsomming

Die doel van van die projek is om die biologie (veral die tempo van ontwikkeling, vrugbaarheid en die parasitisme) van die oleanderwitluis, *Paracoccus burnerae*, te bestudeer. Hierdie witluis is 'n endemiese spesies wat sitrus aanval en van kwarantainbelang is. Dit word op jong sitrusplante in 'n glashuis aangeteel. Saailinge in klein houers word besmet met die witluis en dan in inkubators by verskillende konstante

temperature dopgehou. Ouer bome in houers is ook verkry en word besmet in die glashuis. Toestemming is verkry om hulle in 'n verwaarloosde sitrusboord naby Stellenbosch uit te plaas om die witluise aan parasitisme bloot te stel. Todd Johnson, 'n student, sal 'n deel van die projek as navorsing vir 'n MSc-graad aanbied.

## **Introduction**

The oleander mealybug is a widespread endemic species that attacks citrus and is therefore of great phytosanitary importance. Major importers of our fruit such as the USA and Korea are well aware of this species and will not accept fruit on which it is found. Also, for reasons not well understood, it has recently become the dominant species on citrus in certain areas of the Eastern and Western Cape. While the biology of the other major citrus mealybug species i.e. *Planococcus citri*, *Pseudococcus calceolariae* and *Ps. longispinus* has been studied by Wakgari & Giliomee (2003a) and their parasitoids determined (Wakgari & Giliomee 2003b) this kind of information is not available for *P. burnerae*. A study of the biology and parasitoids of this species has therefore become imperative for the timing of chemical sprays and the development of suitable biocontrol agents. Attempts by Moore & Kirkman (2003, 2004) to find its parasitoids in the Eastern Cape have not been very successful.

## **Materials and methods**

First a colony of the oleander mealybug had to be established in a greenhouse. The mealybug will then be reared on citrus seedlings at a range of constant temperatures in incubators to determine the temperature for development, the rate of development in degree days and the fecundity. At the same time young citrus trees in pots will be infested in the laboratory and placed out in an abandoned orchard to expose the mealybugs to parasitoids, returned after two weeks and the parasitoids allowed to emerge for determination of the species involved. Trees in the abandoned orchard will also be infested with the mealybug to study its biology under natural conditions.

## **Results and discussion**

The project took some time to get off the ground, but now most of the obstacles have been overcome. A student, Mr Todd Johnson, has been found to do post-graduate studies on the project, a colony of the species was located and is now thriving in a greenhouse on one year old citrus plants; citrus seedlings have been established in small containers, to be infested for development studies at various constant temperatures in incubators; and an abandoned citrus orchard has been found near Stellenbosch where we have permission to conduct field studies of the development rate and parasitism.

## **Conclusion**

Work on this project has been delayed, due to the difficulty in acquiring the involvement of a post-graduate student. This has now been resolved and progress is being made.

## **Further objectives (milestones) and work plan**

By April 2008 the present laboratory culture of *P. burnerae* would have expanded sufficiently to start the experiment. During July to September 2008 the trees will be sterilised from other mealybugs and infested with *P. burnerae*. From October 2008 to March 2009 biweekly observations will be made. Parallel with this laboratory studies on the growth rate, number of instars and generations under controlled and room conditions will be conducted.

## **Technology Transfer**

None yet.

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Wakgari, W.M. & Giliomee, J.H. 2003b Natural enemies of three mealybug species found on citrus in South Africa, *Bulletin of Entomological Research* 93: 243-254.

### 3.5.6 **FINAL REPORT: Development of laboratory culture methods for grain chinch bug, *Macchiademus diplopterus* and initial assessment of irradiation as a post harvest control treatment against this pest**

Project USSJ1 (April 2007 – March 2008): S Johnson (SU)

#### **Summary**

The grain chinch bug, *Macchiademus diplopterus*, is a phytosanitary pest of South African export fruit. Post-harvest control treatments against grain chinch bug are required to mitigate the risks posed to our export markets and to maintain trade. Grain chinch bugs have an active and dormant phase during their life cycle. Field-collections are difficult during their active phase as there is no chemical attractant available. Actively reproducing individuals are required to test treatments such as irradiation. In an effort to provide material for such research, different methods to maintain a laboratory colony of grain chinch bug were investigated. In the field, the insects become dormant during summer and autumn months. Grain chinch bugs were successfully maintained on potted wheat seedlings in the laboratory however, dormancy could not be prevented. Maintaining an active laboratory colony requires further investigation. Field-collected insects were used to assess the use of irradiation to sterilize bugs as a mitigation treatment. The low numbers of active grain chinch bugs obtained during field collections, and the difficulty of maintaining an active colony in the laboratory to assess the effects of irradiation, highlighted the need for basic research on this phytosanitary pest before results of irradiation treatments can be properly confirmed.

#### **Opsomming**

Die graanstinkluis, *Macchiademus diplopterus*, is van fitosanitêre belang vir die uitvoer van Suid-Afrikaanse vrugte. Na-oes beheer is nodig vir die bestuur van die risikos wat dit vir uitvoermarkte inhou om sodoende handelsooreenkomste instand te hou. Graanstinkluise het aktiewe en dormante fases in hul lewenssiklus. Versameling in die veld is moeilik in die aktiewe fases omdat daar geen feromoon lokvalle beskikbaar is nie. Aktiewe insekte wat kan voorplant is nodig vir die toetsing van behandelings soos bestraling. In 'n poging om resultate beskikbaar te stel, is verskeie metodes vir die aanhouding van graanstinkluise in die laboratorium getoets. In natuurlike omstandighede is graanstinkluise gedurende die somer en herfs dormant. Alhoewel graanstinkluise suksesvol op koring saailinge in die laboratorium geteel is, kon die dormante fase nie voorkom word nie. Verdere navorsing word dus benodig vir die instandhouding van 'n aktiewe kolonie van graanstinkluise. Insekte wat in die veld versamel is, is bestraal om steriele insekte as 'n moontlike voorkomende maatreël te evalueer. Lae getalle van versameling in die veld, en die probleme om 'n kolonie in die laboratorium instand te hou, beklemtoon die belang van basiese navorsing alvorens die effek van bestraling doeltreffend bepaal kan word.

#### **Introduction**

The grain chinch bug, *Macchiademus diplopterus* is endemic to the Western Cape and is consequently classified as a quarantine pest. A direct pest of wheat, grain chinch bugs move from wheat fields when wheat is harvested, into orchards and vineyards to find aestivation sites. These sites include the stalk and calyx area of fruit. Although not a direct pest of fruit, the presence of live grain chinch bugs on packed fruit has led to rejections of fruit cartons presented for export. The seasonal cycle of grain chinch bugs, in which they are active and reproducing on wheat from late winter to early summer, and aestivating through summer and autumn, makes obtaining insects from the field for research purposes difficult. One of the objectives of this project is to assess methods of rearing and maintaining a culture of grain chinch bugs that does not go into aestivation. There is currently no suitable pre-harvest control treatment against the grain chinch bug in orchards. A post-harvest treatment currently used for grain chinch bug on citrus fruit involves the use of the broad spectrum pesticide, pyrethrum, in dip tanks. Other possible non-chemical post harvest treatments such as cold treatment, heat treatment, controlled atmosphere, irradiation or combinations of these, require investigation. Cold treatment is not effective against grain chinch bug as the species appears to be extremely cold tolerant (Addison 2005). Heat treatment and controlled atmosphere (low O<sub>2</sub> and high CO<sub>2</sub> levels) are being investigated, but these treatments may not be suitable for citrus as they could have detrimental effects on fruit quality. Irradiation can be used at insecticidal levels or, at considerably lower doses, as a means of sterilizing an insect. Preliminary laboratory trials have shown that irradiation at high doses are ineffective in killing grain chinch bug (P. Addison, pers comm). A dose of at least 1000 Gy is required for grain chinch bug mortality, but this is unacceptable for maintaining fruit quality. According to the International Database on Insect Disinfestation and Sterilization (IDIDAS) the mean dose for sterilization ranges from 10 to 180 Gy in Hemiptera (Bakri et al. 2005). The second objective of this project is to assess the use of irradiation for

sterilization (rather than mortality) as a method of control against grain chinch bug.

### **Materials and methods**

Actively reproducing adults and nymph grain chinch bugs were collected from wheat fields around Riebeeck-Kasteel and Malmesbury in the Western Cape during August, September and October 2007. In the laboratory individuals were used to test three different culture techniques: wheat seedlings potted in soil; sprouted wheat seedlings in petri dishes; and crushed sunflower seeds in petri dishes. Pots and petri dishes were placed inside ventilated clear perspex boxes to contain the bugs on each set-up. In previous work varying temperatures and humidities did not prevent the colony from aestivating. Here we considered the effect of day length as a trigger for aestivation. The perspex boxes were maintained at either 20°C or 25°C at 12:12 (L:D).

Adult grain chinch bugs collected in October 2007 and maintained on wheat in the laboratory were exposed to an irradiation dose of 180 Gy. The treatment was replicated once using 40 adult grain chinch bugs (20 males and 20 females). Treated bugs were returned to wheat seedlings and observed for mating and egg-laying.

### **Results and discussion**

Of the three different culture techniques tested, field-collected grain chinch bugs survived for the longest on wheat seedlings potted in soil. This was also the easiest technique to maintain as the sprouted wheat seedlings in petri dishes required more watering and were prone to fungal contamination, while the diet of sunflower seeds did not support the bugs at all. Lygaeid bugs are considered to be predominantly seed feeders and some species have been reared successfully on hulled sunflower seeds (Sweet, 1960; Burgess & Weegar, 1986). Together with sunflower seeds, sprouted mustard and canola seedlings in petri dishes have been used to rear the false chinch bug, *Nysius ericae* (Burgess & Weegar, 1986). However, although the grain chinch bug survived well on potted wheat seedlings, aestivation was not prevented and the laboratory colony became quiescent in early January 2008.

Irradiated grain chinch bugs survived for up to 6 weeks on wheat seedlings in the laboratory. Mating was observed, and only three nymphs were found on wheat during that time. However, due to the small sample size, and the fact that reproduction in the laboratory control colony was also very low, the low levels of reproduction seen in the treated grain chinch bugs cannot, at this stage, be attributed to irradiation treatment.

Field collections of grain chinch bug from wheat fields during their active phase can be time-consuming and laborious, since there is no attractant available for grain chinch bug. Within wheat fields the distribution of grain chinch bugs was found to be very patchy and unless sites with very large numbers of grain chinch bugs were found, collecting sufficient numbers of actively reproducing bugs for laboratory use is difficult.

### **Conclusions**

Potted wheat seedlings can be used to maintain the grain chinch bug in the laboratory, however, two main obstacles remain an issue and require further research: 1] maintaining a continuously active laboratory colony that does not become dormant and provides sufficient insects for trials; and 2] collecting enough actively reproducing adults from the field to do the irradiation trials, together with successful rearing methods to maintain treated individuals in order to monitor the effects of treatment.

### **Future research**

Considering the requirement for research to be done on the grain chinch bug, a laboratory colony for the continuous supply of insects is essential. Techniques to maintain grain chinch bug colonies need to be developed.

Research into the chemical ecology of grain chinch bug is required in order to identify aggregation or sex pheromones that can be used to formulate lures for pheromone traps and the development of monitoring systems.

Without successful methods to maintain a colony, the effects of irradiation can be assessed by the use of cytological techniques to identify translocations in sex chromosomes caused by irradiation.

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### 3.6 PROJECT: BIOCONTROL DISRUPTION

Project coordinator: Tim G Grout (CRI)

#### 3.6.1 Project summary

No non-target effect bioassays were conducted during the report period so the only research in this project concerned the management of ants. The search for an ant repellent was terminated after a small extension to the work done in 2006 was unsuccessful (3.6.2). Only a summary is provided. Research on baits was more promising with indications that the amount of Sieges required per hectare can be reduced when using the product in a bait station (3.6.3). A suitable attractant for both the brown house ant and the pugnacious ant has now been developed and future research will now focus on finding a suitable toxicant.

## Projekopsomming

Geen nie-teiken biotoetse is gedurende die verslagtydperk uitgevoer nie. Die enigste navorsing in hierdie projek was gemoeid met die beheer van miere. Die soektog na 'n afweermiddel vir miere is beëindig nadat 'n klein uitbreiding aan die werk in 2006 onsuksesvol was (3.6.2). Slegs 'n opsomming word voorsien. Navorsing op lokaas was meer belowend met aanduidings dat die hoeveelheid Sieges benodig per hektaar verminder kan word as die produk in 'n lokval gebruik word (3.6.3). 'n Geskikte lokmiddel vir beide die bruin huismier en die malmier is nou ontwikkel en verdere navorsing sal daarop fokus om 'n geskikte gifstof te vind.

### 3.6.2 FINAL PROGRESS REPORT: To develop an ant repellent that will keep ants out of citrus trees without destroying their nest

Experiment 798 (2005/6-2007/8) by Tim Grout and Kim Stoltz (CRI)

## Summary

Controlling ants with the use of repellents is attractive because the predatory benefits of ants on the orchard floor can be utilised without their disruptive influence on natural enemies in the trees. Although the termination of this experiment was covered in the last CRI annual research report it was subsequently felt that the application of a repellent to a fibrous surface was worth testing. Prospective repellents were therefore sprayed onto the fibrous surface of Ant Cap Protectors and the barriers attached to trees having *Pheidole* activity but the repellents were ineffective and the ants circumvented the barriers. A repellent formulation containing 5% of an extender called Duratech was also sprayed onto the trunks of five trees having *Pheidole* activity but no repellent effect was evident after 3.5 days. This experiment was therefore not revived and no further research was conducted on ant repellents.

## Opsomming

Die beheer van miere deur die gebruik van afweermiddels is aanloklik, omdat die predatoriese voordele van miere op die vloer van 'n boord benut kan word, sonder hul ontwrigtende invloed op natuurlike vyande in die bome. Daar is in die vorige CRI jaarverslag oor die beëindiging van die eksperiment gerapporteer, maar agterna is daar gevoel dat die aanwending van 'n afweermiddel op 'n veselagtige oppervlakte die moeite werd is om te toets. Moontlike afweermiddels is dus op veselagtige oppervlakte van Ant Cap Protectors gespuit en die versperrings is aan bome aangebring waar daar *Pheidole* aktiwiteit was, maar die afweermiddels was oneffektief en die miere het die versperrings vermy. 'n Afweermiddel formulاسie wat 5% van 'n aanvuller bekend as Duratech bevat, is ook op die stamme van vyf bome met *Pheidole* aktiwiteit gespuit, maar daar was geen afweringseffek na 3.5 dae sigbaar nie. Die eksperiment is om hierdie rede nie weer aan die gang gesit nie en geen verdere navorsing is op mierafweermiddels uitgevoer nie.

### 3.6.3 PROGRESS REPORT: Development of ant baits and the use of bait stations Experiment 857 (2006/7-2008/9) by Tim Grout, Kim Stoltz and Bruce Tate (CRI)

#### Summary

Ants are important indirect pests because they disrupt the biological control of several pests that are becoming more difficult to control due to fewer chemical options being available, e.g., mealybug and soft scales. Keeping ants alive on the ground but out of the trees is still the best option for IPM but this approach is labour intensive and not always possible with trees that have a low main branches. Research on ants is difficult because their behaviour is influenced by various climatic, edaphic and dietary factors. Research was delayed due to frequent rain showers in the first part of summer but some good progress was achieved. It does seem possible to use less Siege for the control of *Pheidole* than recommended if the product is kept out of water and direct sunlight. Confirmation of this would be ideal but suitable trial sites are difficult to find. An attractant for an ant bait has finally been developed that is effective for both pugnacious ants and *Pheidole* but further research for a suitable toxicant and dosage is required.

#### Opsomming

Miere is belangrike indirekte plaë omdat hulle die biologiese beheer van verskeie plaë ontwrig waarvan die beheer al hoe moeiliker raak weens minder beskikbare chemiese opsies, bv. wilvluis en sagte dopvluis. Om miere op die grond aan die lewe te hou maar uit die bome is nog steeds die beste opsie vir IPM, maar hierdie aanslag is arbeidsintensief en nie altyd moontlik vir bome waarvan die hooftak laag is nie. Navorsing op miere is moeilik omdat hul gedrag deur verskillende klimaat-, grond- en dieet faktore beïnvloed word. Navorsing is vertraag as gevolg van gereelde reënbuie in die eerste gedeelte van die somer, maar goeie vordering is wel gemaak. Dit lyk moontlik om minder Siege as aanbeveel vir die beheer van *Pheidole* te gebruik as die produk uit direkte sonlig en water gehou word. Bevestiging hiervan sal ideaal wees, maar geskikte proefpersele is moeilik om te vind. 'n Lokmiddel vir mierlokaas is uiteindelik ontwikkel wat effektief is vir beide malmiere en *Pheidole*, maar verdere navorsing vir 'n geskikte gifstof en dosis word nog benodig.

#### Introduction

Mealybugs are largely being controlled by the use of organophosphates at the moment but these products may not be available in the future and growers would 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 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 not widely used due to it being expensive and only effective against one of the two major ant pests, *Pheidole*. Recent research in pineapples has shown that by placing Siege in a bait station where it is not exposed to water and UV light the amount of product required per hectare is less than half. This needs to be evaluated in citrus. Grout conducted research on ant baits for the pugnacious ant between 1988 and 1991 (unpublished) but although the attractant was successful, the old toxicants were not. Now there are several new toxicants available such as hydramethylnon, fipronil, imidacloprid and pyriproxyfen that should be evaluated.

#### Materials and methods

##### Bait station for Siege

Siege is an effective product for the control of *Pheidole* and is registered as 10 g per tree, to be scattered around the base of the tree. When applied in this manner the product is exposed to the sun and irrigation water and does not last very long. A trial was conducted in a mature Empress mandarin orchard at Brackenhill Farm, Hilltop outside Nelspruit to compare the registered treatment (only 9 g was used which was the original registered rate) with other methods of application involving the use of a bait station constructed from a plastic 35 mm film canister with a roof made from a 9 cm diameter petri dish lid (Fig. 3.6.3.1). The lid of the canister was glued to the petri dish and a hole was drilled into the side of the canister just below the lid to provide access for the ants. The bait station was attached to the tree by means of a piece of string. The treatments involving the bait station were 9 g Siege per bait station per tree, 4.5 g per bait station per tree and 9 g per bait station on every second tree. An untreated control was also included.

Each treatment was applied to 7 trees in a row with the 5 centre trees being monitored for ant activity and the end two trees as buffers. Three replicates were used per treatment and a randomised block layout was used with the allocation of the 5 rows within the block to the treatments being randomised. The trial was laid out on 9 March 2007 and counts of ants moving up and down the trunk per minute were made on 16, 22, 31 March, 5, 12, 19, 27 April and 3, 10, 18 May. On 28 May, counts of ant activity in untreated trees 4 rows below the lowest treated row in each block were compared with the untreated rows in the randomised blocks.



**Figure 3.6.3.1.** Ant bait station used for trial with Siege at Brackenhill Farm.

No further suitable sites with uniform distributions of *Pheidole* throughout an orchard could be found to compare several dosages of Siege in bait stations versus being scattered at the base of a tree. A simple bait station was developed from irrigation tubing that presumably will work, but its efficacy could not be verified. The bait station (called Ant Bait Tube) was made from a 300 mm length of 15 mm diameter low pressure polypropylene irrigation tubing. One end was folded over and held in place with a ring cut from a 25 mm diameter irrigation tube. At a distance of 180 mm from the fold, a hole of 8 mm diameter was melted into the side of the tube. When the tube was filled with Siege from the open end of the tube above the hole, up to the level of the hole, it held 10 g, the registered dose for one tree. The top of the tube could then be folded over and fastened with another plastic ring. The ends of the tube were folded in opposite directions to one another so that the tube formed the shape of an “S” when placed on the ground. This improved stability. When in this position the entry hole for the ants was in the side of the tube (Fig. 3.6.3.2).



**Figure 3.6.3.2.** Ant Bait Tube made from 15 mm diameter irrigation tubing to protect Siege from water and UV light. Arrow shows 8 mm diameter access hole for ants.

#### Development of ant bait gel

Earlier research on an attractant that would be effective for both *Pheidole* and pugnacious ant indicated that a combination of peanut butter, animal protein and a sugar source was the most effective (Grout and Tate 2007). However, this work did not include combinations of protein and a sugar source alone so this combination was compared with the three-component attractant for both ants.

During the first part of summer, frequent rain showers prevented work with ants. Preliminary investigations were first possible in December 2007. Statistical ant bait evaluations were conducted during January 2008 at Brackenhill Farm, Hilltop for *Pheidole* and Lowveld College for pugnacious ants. The attraction of sugar gel, tinned cat food, gel and tinned cat food as a 50:50 mixture, and peanut butter, gel and tinned cat food in a 33:33:33 mixture were determined. Six grams of each attractant mixture was placed in a 39 mm diameter petri dish lid. Each experiment was repeated three times on separate days with five replicates per attractant. Peanut butter was not tested on pugnacious ants because it is known to be ineffective. With the *Pheidole* evaluations the petri dishes were placed directly on the ground around the base of each infested tree with a 15 cm gap between different attractants. With pugnacious ants, a 65 mm diameter petri dish was attached to the top of a 46 cm dowel rod (diameter 18 mm) using a drawing pin. The smaller petri dish with the attractant was placed inside the larger dish. The rods were separated by about 15 cm and placed around an active nest opening. Attractants were evaluated 30 minutes after setting up with *Pheidole* and after 3 hours for pugnacious ants. For each evaluation the number of ants feeding on the attractant within one minute was determined.

Two further series of trials were conducted in February 2008 at the same sites. The treatments compared were a blended mixture of sugar gel and protein as before in a 50:50 ratio, the same two products kept as separate food sources and a proprietary mixture called Saga. These three treatments were evaluated against both ants at the same two sites as before, using the same technique.

From the above trials it appeared that Saga was the best attractant for both species of ants so after some improvements were made with the addition of a thickener, trials were initiated with fipronil as a toxicant. Regent (fipronil 200 g/l SC) was blended with the Saga mix at 0.03% Regent by weight. This is equivalent to 0.006% a.i. which was between the  $1 \times 10^{-5}$ % a.i. used by Hooper-Bui and Rust (2000) for *Linepithema humile* and the 0.05% used by Ulloa-Chacón and Jaramillo (2003) for *Tapinoma melanocephalum*. The mixture was blended for several minutes to ensure thorough mixing.

Twelve *Pheidole* nests separated by at least 10 m were flagged and peanut butter was used in a small petri dish to get an idea of ant activity at each nest as a "precount" on 25 March 2008 at the CRI research campus

in Nelspruit. Each dish was left for 2 hours before the number of ants feeding within a minute was determined. The peanut butter dishes were then removed and replaced with dishes with Saga and Regent 0.03% (9 g per dish). Counts of ants feeding per minute were taken after 24 hours at 14:30 when the temperature was 28°C. The baits were refreshed, if necessary, then 24 hours later another count was taken and the baits refreshed as needed. This continued until 31 March with the temperature always being between 28 and 29°C when counts were taken. On 1 April it rained so counts could not be taken but a final count was taken on 2 April using peanut butter in small petri dishes.

## Results and discussion

### Bait station for Siege

Evaluations of the commercial bait Siege presented in a simple bait station to protect it from water and UV light initially showed that the 9 g dosages as registered on the ground or in the bait station were significantly more effective than the control ( $P < 0.05$ ), whereas the half dosages were not significantly different from the control (Table 3.6.3.1). However, after 22 days there were no significant differences between any of the treatments and the numbers in the control were the lowest. By 34 days after treatment was initiated, the activity in all treatments had declined to below 1 ant per minute. Numbers remained close to zero until a final count was taken 73 days after the initial treatment when there was no activity at all in any of the control trees. The untreated trees four rows below the treatment blocks still had a mean ant activity of 8.9 ants per minute at this time. This shows that lower amounts per hectare could be used against *Pheidole* than registered but that the treatment effect will be slower. As the ants were clearly moving between treatments, further work will be required to confirm what distance should separate the bait stations. Suitable sites with uniform *Pheidole* infestations that would allow for larger blocks with further separation between treatments could not be found. Farmers should experiment with the Ant Bait Tube and see whether it provides control of *Pheidole* with fewer applications of Siege, perhaps by treating alternate trees or alternate rows.

**Table 3.6.3.1.** Number of *Pheidole* passing a point per minute on trees that had received various treatments involving Siege (hydramethylnon) at various intervals after treatment started.

Treatments	Ants per minute		
	7 DAT	22 DAT	34 DAT
9 g Seige spread around base of every tree	0.3 a	2.4 a	0.8 a
9 g Seige in a bait station on every tree	0.6 a	2.5 a	0.0 a
4.5 g Seige in a bait station on every tree	2.3 ab	2.2 a	0.0 a
9 g Seige in a bait station on every second tree	2.5 ab	3.5 a	0.7 a
Untreated control	5.1 b	0.8 a	0.2 a

Means in the first data column followed by a different letter were significantly different ( $P < 0.05$ )

### Development of ant bait gel

With pugnacious ants, the two-component attractant was always more attractive than the three-component attractant, but not significantly so ( $P > 0.05$ ) (Table 3.6.3.2). Sugar in a gel was variably attractive on different days but not significantly different from the previous attractants. Protein alone varied from the best to the worst attractant in different replicates, but again without significant differences. With *Pheidole*, sugar gel alone was significantly unattractive in two of three replicates but there were no significant differences between the other treatments (Table 3.6.3.3). In the third replicate, protein alone was significantly more attractive than the other options. These results confirm earlier trials in showing that in an orchard situation where ants probably have access to honeydew, sugar alone is not a good attractant for *Pheidole* and is similar to other attractant combinations for the pugnacious ant. Further work focussed on a combination of protein and sugar without peanut butter.

**Table 3.6.3.2.** Mean numbers of pugnacious ants visiting attractants in three trials at Lowveld College of Agriculture in January 2008.

Treatments	Mean ants feeding per min (15 Jan 2008)	Mean ants feeding per min (16 Jan 2008)	Mean ants feeding per min (17 Jan 2008)
PNB-Protein-Sugar	5.6 a	35.6 a	53.6 a
Protein & gel	8.8 a	58.0 a	81.8 a
Protein only	15.0 a	33.8 a	30.8 a
Sugar gel only	4.8 a	43.8 a	82.0 a

Means in the same column followed by the same letter are not significantly different ( $P > 0.05$ ) (SNK)

**Table 3.6.3.3.** Mean numbers of *Pheidole* ants visiting attractants in three trials at Brackenhill Farm, Hilltop in December 2007 and January 2008

Treatments	Mean ants feeding per min (15 Jan 2008)	Mean ants feeding per min (16 Jan 2008)	Mean ants feeding per min (17 Jan 2008)
Peanut butter only	53.0 b	110.0 b	47.2 ab
PNB-Protein-Sugar	44.0 b	99.0 b	37.2 ab
Protein & gel	46.0 b	63.2 b	27.0 a
Protein only	78.0 b	96.0 b	87.0 b
Sugar gel only	2.4 a	2.4 a	20.4 a

Means in the same column followed by the same letter are not significantly different ( $P>0.05$ ) (SNK)

In the next two series of trials, there were no significant differences between any of the three treatments in each of the trials with pugnacious ants (Table 3.6.3.4) and only one significant difference ( $P<0.05$ ) in one of the three trials with *Pheidole* where Saga was significantly better than the other two attractants (Table 3.6.3.5). Once again the differences in ant behaviour each day were marked. Separate gel and protein seemed to be slightly more attractive to pugnacious ant than a mixture, although not significantly so. With *Pheidole* this trend was not apparent.

**Table 3.6.3.4.** Mean numbers of pugnacious ants visiting attractants in three trials Lowveld College of Agriculture in February 2008.

Treatments	Mean ants feeding per min (5 Feb 08)	Mean ants feeding per min (6 Feb 08)	Mean ants feeding per min (7 Feb 08)
Separate gel & protein 50:50	46.0 a	23.8 a	14.4 a
Mixed gel & protein 50:50	16.4 a	3.0 a	8.6 a
Saga	18.8 a	5.4 a	3.0 a

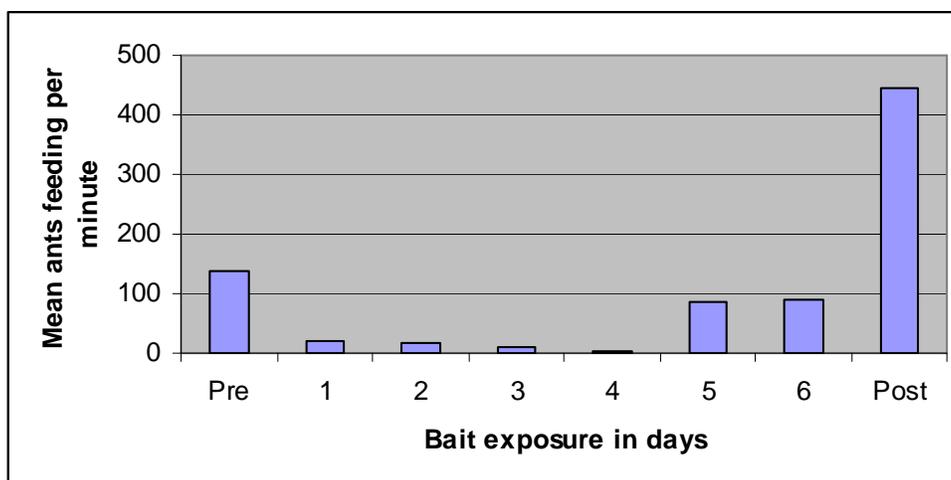
Means in the same column followed by the same letter are not significantly different ( $P>0.05$ ) (SNK)

**Table 3.6.3.5.** Mean numbers of *Pheidole* ants visiting attractants in three trials Brackenhill Farm, Hilltop in February 2008.

Treatments	Mean ants feeding per min (5 Feb 08)	Mean ants feeding per min (6 Feb 08)	Mean ants feeding per min (7 Feb 08)
Separate gel & protein 50:50	73.0 a	53.0 a	88.0 a
Mixed gel & protein 50:50	58.4 a	103.0 a	58.8 a
Saga	152.8 b	104.0 a	114.6 a

Means in the same column followed by the same letter are not significantly different ( $P>0.05$ ) (SNK)

The incorporation of Regent 0.03% with Saga did not have any noticeable affect on feeding by *Pheidole* (Fig. 3.6.3.3). The drop in feeding after a few days may have been due to weather patterns as numbers feeding on days 5 and 6 increased to the highest levels on Saga and if the ants were being repelled by this stage the numbers would have dropped. Obviously this dosage is too low.



**Figure 3.6.3.3.** Mean number of *Pheidole* feeding per minute on peanut butter on pre- and post counts and on Saga plus Regent 0.03% on other days

### Conclusion

The use of Siege in a bait station did kill ants in adjacent rows to those being treated so the amount of product used per hectare can be reduced in this manner. An effective attractant for both *Pheidole* and the pugnacious ant was developed that now requires a suitable toxicant.

### Future research

Further research will be conducted with Saga and fipronil and if possible the Ant Bait Tube will be evaluated.

### Technology transfer

This research is not yet at a point that is suitable for technology transfer apart from farmers that want to try the Ant Bait Tube which will be demonstrated at the Citrus Research Symposium.

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## 3.7 PROJECT: PRODUCTION PESTS

Project coordinator: Tim G Grout (CRI)

### 3.7.1 Project summary

In today's competitive export markets, good production is essential in order to cover costs. For most growers this is now taken for granted and their focus is on quality and phytosanitary issues. For this reason there were no CRI-funded experiments in this project during the 2007/8 financial year. However, some contract research was conducted on the possible benefit of adding Break-Thru to horticultural mineral oil for the control of red scale (3.7.2). Unfortunately, the red scale infestation in the orchard used was too severe to show meaningful results with a single oil spray and the work should be repeated if this concept is to be pursued.

## Projekopsomming

Uitvoermarkte is vandag kompetierend, daarom is goeie produksie noodsaaklik om kostes te dek. Meeste van die produsente aanvaar dit as vanselfsprekend en hul fokus is op kwaliteit en fitosanitêre aangeleenthede. Vir hierdie rede was daar geen CRI-befondste eksperimente in hierdie projek gedurende die 2007/8 finansiële jaar nie. Enkele kontraknavorsing op die moontlikheid van byvoeging van Break-Thru by hortologiese minerale olie vir die beheer van rooi dopluis is egter uitgevoer (3.7.2). Ongelukkig was die rooi dopluis besmetting in die boord wat gebruik is te erg vir enige betekenisvolle resultate met 'n enkele oliebespuiting. Die werk sal dus herhaal moet word indien daar met die konsep voortgegaan moet word.

### 3.7.2 Evaluation of the efficacy of oil with Break-Thru for red scale control

Experiment 897 (July 2006 – June 2007) by Sean D. Moore and Wayne Kirkman (CRI)

#### Summary

The objective of this trial was to determine whether the addition of the adjuvant, Break-Thru OE444 to a medium grade mineral spray oil, could enable a reduction in the oil concentration and still achieve an adequate level of red scale control. An orchard of navel orange trees which was heavily infested with red scale, was sprayed in winter with treatments including a 1% oil and a 0.5% oil, both with and without OE444. Surprisingly poor control was achieved with all of the treatments. The orchard was in a poor condition and the trees were undoubtedly stressed. This may in some way have led to the high level of red scale infestation and the poor control. However, there were indications that the addition of the OE444 might improve control of red scale. The only treatment for which there was a significant reduction in red scale was the 1% oil plus Break-Thru. As the good efficacy of mineral oils for control of red scale is widely documented, it would be advisable to repeat this trial in a less infested orchard. However, the suppliers of the products do not wish to proceed further.

#### Opsomming

Die doel van hierdie proef is om te bepaal of die byvoeging van Break-Thru OE444 tot 'n medium graad minerale spuitolie, na 'n vermindering in die olie konsentrasie kan lei sonder dat daar enige verlies in werking teen rooidopluis is. 'n Boord nawellemoen bome wat met rooidopluis hewig besmet was, is in die winter met verskeie behandelings gespuit. Dit sluit in 'n 1% en 'n 0.5% olie behandeling, albei met en sonder die OE444. Uiteens swak beheer is met al die behandelings behaal. Die boord is in 'n swak toestand en die bome is ongetwyfeld onder stres. Dit kon op 'n manier het na die hoë vlak van besmetting en die swak beheer gelei het. Nietemin was daar aanduidings dat die gebruik van OE444 saam met olie, dopluis beheer kon verbeter. Die enigste behandeling wat enige betekenisvolle vermindering in dopluis veroorsaak het was die 1% olie plus Break-Thru. Omdat dit bekend is dat minerale olies rooidopluis doeltreffend kan beheer, sal dit wys wees om hierdie proef in 'n meer gesonde boord te herhaal. Ongelukkig is dit nie meer in die belang van die verskaffers van die produkte om voort te gaan met die werk nie.

#### Introduction

CRI was contracted by Degussa and H & R to test the use of a specialized formulation of Break-Thru (OE444) as an oil additive for control of red scale on citrus. The objective was to determine whether the addition of OE444 could enable a reduction in oil concentration and still achieve the same level of red scale control.

#### Materials and methods

The trial was conducted in a seven-year-old (planted 2000) orchard (orchard no. 1) of Palmer navel orange trees on Hopefield Farm in Sundays River Valley. The orchard was 0.51 ha in size, with the trees spaced at 6 m (between rows) x 2 m (between trees within rows). Four treatments were applied (Table 3.7.2.1) and an untreated control was retained. Treatments were applied on 17 August 2006 in a single-tree randomized block format, replicated 15 times. An average of 17.3 L of spray mix was applied per tree using high pressure hand-held spray guns. On 19 February 2007, 20 fruit per tree were evaluated for red scale infestation and categorized as clean, blemished (fewer than eight red scale per fruit) or culled (eight or more red scale per fruit).

**Table 3.7.2.1.** Treatments applied to Palmer Navel orange trees on 17 August 2006 on Hopefield Farm in the Sundays River Valley.

Treatments
Untreated control
*H & R Medium Spray Oil (1%)
H & R Medium Spray Oil (1%) + Break-Thru OE444 (0.5%)
H & R Medium Spray Oil (0.5%)
H & R Medium Spray Oil (0.5%) + Break-Thru OE444 (0.5%)

\* H & R Medium Spray Oil was previously known as BP Medium oil.

## Results and discussion

Red scale infestation in the orchard was generally extremely high and would have required at least two oil sprays to achieve commercial control. The orchard was in a poor condition and the trees were undoubtedly stressed. This may in some way have led to not only the high level of red scale infestation but the surprisingly poor control achieved with all of the treatments (Table 3.7.2.2). There were indications that the addition of the OE444 might improve control of red scale and therefore facilitate a reduction in the concentration of oil required. However, the only treatment in which significantly fewer fruit were culled for scale than in the untreated control was the 1% oil plus Break-Thru.

**Table 3.7.2.2.** Red scale infestation of Palmer navel oranges for different oil and Break-Thru treatments applied on Hopefield Farm and evaluated on 19 February 2007.

Treatment	Fruit blemished (%)	Fruit culled (%)*
Untreated control	24.33	74.33 a
*H & R Medium Spray Oil (1%)	38.67	56.67 ab
H & R Medium Spray Oil (1%) + Break-Thru	42.33	51.67 b
H & R Medium Spray Oil (0.5%)	30.00	68.67 ab
H & R Medium Spray Oil (0.5%) + Break-Thru	37.33	61.67 ab

\*\*Values in the same column followed by the same letter are not significantly different ( $P > 0.05$ ; Bonferonni LSD Multiple Range Test).

It is well known that medium grade narrow distillation range mineral spray oils can control red scale on citrus very effectively (Georgala, 1984; Grout, 1993; Grout & Stephen, 1993) but that multiple applications will be required in emergency situations. It may therefore be worthwhile to repeat the trial with more moderate infestations of red scale.

## Conclusion

It could not conclusively be shown that the addition of Break-Thru OE444 significantly improved the control of red scale with a medium grade mineral oil. However, red scale infestation was very high and control with all treatments was poor. It is the opinion of the authors that it would be worth repeating this trial in a less infested orchard.

## Future research

No further work is planned on this experiment. The suppliers of the two products used, H & R Medium Spray Oil and Break-Thru OE444, have expressed that they do not wish to proceed with this work.

## Technology Transfer

The results of this trial have been reported to the companies involved. These results have not been conveyed within the citrus industry.

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### 3.8 PROJECT: RESIDUE TRIALS FOR PHI AND MRL DEVELOPMENT

Project coordinator: Tim G Grout (CRI)

#### 3.8.1 Project summary

Due to the increasing difficulties that fresh produce industries in South Africa are having in meeting the changing Maximum Residue Limits on produce that is to be sold in the European Union, the EU provided some funding in the form of the SA Pesticide Initiative Programme for research to establish pre-harvest intervals for chemicals that were not being supported by chemical companies. The following research on methamidophos (3.8.2), triflumuron (3.8.3), mercaptothion (3.8.4), buprofezin (3.8.5), methiocarb (3.8.6) and tetradifon (3.8.7) benefitted from this funding source. The research was conducted in collaboration with SABS and as some of it required GLP standards it was very labour intensive and required detailed records of everything. However, the results will be very valuable for the industry.

#### Projekopsomming

Die Suid-Afrikaanse bedrywe van vars produkte ondervind toenemend probleme om aan die verandering in maksimum residu vlakke op produkte wat in die Europese Unie verkoop moet word, te voldoen. Die EU het daarom fondse in die vorm van die "SA Pesticide Initiative Programme" vir navorsing om voor-oes intervalle vir chemikalieë wat nie deur chemiese maatskappye ondersteun word nie te ontwikkel, beskikbaar gestel. Die volgende navorsing op methamidophos (3.8.2), triflumuron (3.8.3), mercaptothion (3.8.4), buprofezin (3.8.5), methiocarb (3.8.6) en tetradifon (3.8.7) het by die befondsing gebaat. Die navorsing is in samewerking met die SABS gedoen en omdat vir sommige hiervan GLP standaarde benodig is, was dit baie arbeidsintensief en is volledige rekords van alles benodig. Die resultate sal egter vir die bedryf van baie waarde wees.

#### 3.8.2 FINAL PROGRESS REPORT: Methamidophos residue trials

Experiment 851 (April 2005 – April 2007): Peter Stephen and Wayne Kirkman (CRI) and Vincent Nel (SABS)

#### Introduction

Trials were conducted to determine the decline of Methamidophos residues in citrus fruit when applied for control of citrus psylla. The study was planned to be conducted during two seasons, and started in the 2005/2006 season. This report covers the final trials conducted during the 2006/2007 season.

#### Materials and methods

During the first season, trials were conducted on Grapefruit, Oranges (Mpumalanga) and Mandarins (E. Cape). For the second season, trials were required to be conducted on Mandarins only.

Two sites were chosen on Carden Farm in the Sundays River Valley, for trials to be conducted during the 2006/2007 citrus growing season. These trials were not conducted under GLP conditions, but all the principles of GLP were applied.

The circumference of each tree trunk was measured using a verified tape measure at the intended point of application. The method of application was refined from the instructions on the product label and verified syringes were used to apply the correct dose for each tree. The applications were made to 5 trees in each site (Table 3.8.2.1.). Residue and control samples were taken immediately and then at various intervals (Table 3.8.2.2). All samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI in Port Elizabeth, and sent to the SABS at a later stage.

**Table 3.8.2.1.** Application schedule for Citrimet to citrus.

Season	Location	Trial no	Selection	Application time	Principal investigator
2006/2007	Sundays River Valley	Trial 1	Orr	Application 1 17 November 2006	Wayne Kirkman
				Application 2 18 January 2007	
				Application 3 19 March 2007	
	Swellendam	Trial 2	Affourer	Application 1 17 November 2006	Wayne Kirkman
				Application 2 18 January 2007	
				Application 3 19 March 2007	

**Table 3.8.2.2.** Residue and control samples taken after 3 applications of Citrimet and sent to SABS for analysis.

Sample number	Sample type	Sample (DALA)	Time	Selection	Date
<b>Trial 1 - Orr</b>					
A1213/012	Control sample	T-1		Orr	19/03/07
A1213/002	Residue sample	T-1		Orr	19/03/07
A1213/003	Residue sample	T20		Orr	05/04/07
A1213/004	Residue sample	T40		Orr	26/04/07
A1213/005	Residue sample	T60		Orr	17/05/07
A1213/006	Residue sample	T80		Orr	04/06/07
A1213/013	Control sample	T80		Orr	04/06/07
<b>Trial 2 - Affourer</b>					
A1213/014	Control sample	T-1		Affourer	19/03/07
A1213/007	Residue sample	T-1		Affourer	19/03/07
A1213/008	Residue sample	T20		Affourer	05/04/07
A1213/009	Residue sample	T40		Affourer	26/04/07
A1213/010	Residue sample	T60		Affourer	17/05/07
A1213/011	Residue sample	T80		Affourer	04/06/07
A1213/015	Control sample	T80		Affourer	04/06/07

## Results and discussion

### Description of sample

Ten samples of citrus fruit, type mandarin, taken from a test site at Eastern Cape were submitted for analysis during July 2007.

According to the field trial protocol the trees were treated with Citrimet 500 AL. The product was applied undiluted to the bark of the trees immediately below the fork/first branches to three or four zones around the circumference of the trunk to ensure even uptake and translocation. A first application was made in November 2006 followed by another 60 days later and a final application 60 days after the second.

An untreated control sample was submitted with the samples. The samples were frozen when received. They were immediately placed and stored in a deep-freeze until the analysis commenced on 25 July 2007.

### Test requested

To determine the methamidophos residue content of the peel and the flesh separately and to calculate the content on the whole fruit

### Method of test

The fruit of each sample was peeled, the peel and flesh weighed and treated as separate samples.

Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. Each sample was analysed in duplicate employing the method *Acephate and Methamidophos in apples, beans, carrots etc - Gas-chromatographic determination*, as described in the Manual of Pesticide Residue Analysis Volume 1 p. 81. Matrix matched standards were used for the regression curve and relative low recoveries were obtained. Recovery determinations were done by adding known amounts of methamidophos to portions of the control sample and analysing these concurrently with the samples.

### Notes

- Generic equivalents may have been used for solvents or equipment. However, chemical and instrument verification is regularly performed.
- If changes are made to methods, such changes are described in the official report. However, slight changes to chromatographic conditions are only documented internally.

**Table 3.8.2.3.** Methamidophos residue content of fruit samples – Trial 1

Date sampled	No. of days after last application	Methamidophos residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
2007-03-19	Before last application	ND ; ND	ND ; ND	ND
2007-04-05	20	1,7 ; 1,6	1,2 ; 1,1	1,4
2007-04-26	40	0,32 ; 0,32	0,29 ; 0,30	0,30
2007-05-17	60	0,07 ; 0,07	0,07 ; 0,08	0,07
2007-06-04	80	0,03 ; 0,02	0,04 ; 0,03	0,03

ND = Not detected/determined.

**Table 3.8.2.4.** Methamidophos residue content of fruit samples – Trial 2

Date sampled	No. of days after last application	Methamidophos residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
2007-03-19	Before last application	ND ; ND	ND ; ND	ND
2007-04-05	20	1,8 ; 1,7	0,66 ; 0,64	0,98
2007-04-26	40	0,08 ; 0,08	0,17 ; 0,15	0,14
2007-05-17	60	0,02 ; 0,02	0,03 ; 0,03	0,03
2007-06-04	80	ND ; ND	0,02 ; 0,02	0,01

ND = Not detected/determined.

**Table 3.8.2.5.** Methamidophos recovery at various levels of insertion

Recovery level mg/kg	Recovery (%)	
	Peel	Flesh
0,05	49	-
0,10	-	46
0,20	-	48
0,25	43	-
1,0	45	-

No residues of methamidophos were detected in the control sample used for the recovery determinations. Under the conditions of test employed the lowest limit of determination was 0,01 mg.

### Conclusion

These results will be used for the determination of a revised PHI for methamidophos.

### 3.8.3 FINAL PROGRESS REPORT: Triflumuron residue trials

Experiment 867 (April 2005 – April 2006): Peter Stephen and Wayne Kirkman (CRI) and Pieter van Zyl (SABS)

#### Introduction

These trials were designed to evaluate the residue levels of the insecticide triflumuron after application of the formulated product Alsystin. This report covers trials conducted during the second and final season of this study

#### Materials and methods

A total of 16 trials were planned for two Citrus-growing seasons, the 2005/2006 season and 2006/2007 season. This report covers 8 trials done during the second season, 4 in Mpumalanga on oranges and 4 in the Eastern Cape on soft citrus. All applications were made at the highest permitted dosage rate with the shortest possible withholding period. The trials were conducted in accordance with the European Commission guidelines for residue trials. These require meticulous attention to detail and recording of all operations, with independent auditing at all critical phases. Peter Stephen (Nelspruit) and Wayne Kirkman (Port Elizabeth) were appointed as Principal Investigators for the study and Pieter van Zyl (SABS) was appointed Study Director. Residue data were collected from the treatments.

Treatments consisted of an untreated control and a 1X dosage rate of Alsystin equivalent to its current registration on citrus in South Africa, i.e. 20 ml per 100 litres of water. The compound was applied as a full cover spray simulating the applications for false codling moth control on citrus used by growers. Control treatments consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees. Before spraying, samples of the water used for spraying were checked for suspensibility, which could influence the integrity of the formulation. During spraying at each trial, samples of each tank mix were taken for analysis. Applications were made using calibrated hand-gun spray machines. The sprays were applied as “full cover” applications to wet the entire tree to the point of drip/run-off (Table 3.8.3.1).

In the Eastern Cape, four sites of late mandarins (3 Affourer and one Orr) orchards were selected in the Sundays River Valley, two on Carden Farm and the other two on Sun Orange Farm. In Mpumalanga four Valencia sites were selected at Friedenheim, Crocodile Valley Citrus (2) and Karino.

**Table 3.8.3.1.** Application details for triflumuron residue trials

Cultivar	Trial site area	Date of application	Tank mix sample number	Average volume of spray mix per tree (L/tree)
Orr	EC trial 1	08/03/07	06/23/105	10.18
Affourer	EC trial 2	08/03/07	06/23/106	10.19
Affourer	EC trial 3	08/03/07	06/23/107	10.80
Affourer	EC trial 4	08/03/07	06/23/108	11.20
Valencia	MP trial 1	19/02/07	06/23/205	10.8
Valencia	MP trial 2	19/02/07	06/23/206	17.3
Valencia	MP trial 3	19/02/07	06/23/207	27.3
Valencia	MP trial 4	19/02/07	06/23/208	21.1

Fruit residue samples were taken at various intervals according to the schedules (Table 3.8.3.2). The samples were taken in duplicate, and sent to the SABS for residue analysis. In addition to the normal 2 kg fruit residue samples, at T79 at the 4 Mpumalanga sites, an additional 35 kg sample was collected for investigation of the residues after processing.

**Table 3.8.3.2.** Triflumuron samples taken for trials in the Sundays River Valley and Mpumalanga

Sundays River Valley		Mpumalanga	
Type	Time of sampling (DALA)	Type	Time of sampling (DALA)
Control	T0	Control	T0
Residue	T0	Residue	T0
Residue	T79	Residue	T79
Control	T79	Control	T79
Residue	T90	Residue	T91
Residue	T120	Residue	T120
Control	T120	Control	T149
		Residue	T149

## Results and discussion

### Description of samples

Seventy-two samples of citrus, GLP study No. 06/23 were submitted for analysis during 2005/2006 citrus season. According to the study protocol accompanying the samples the trees were treated with Alsystin 480 SC at a concentration of 20 mL product/hL.

Details of the treatments are shown in Table 3.8.3.3.

**Table 3.8.3.3.** Details of the treatments applied in the Sundays River Valley and Mpumalanga

Area	Trial No.	Locality
Mpumalanga	1	ARC Friedenheim
	2	Cottage 6 Croc valley
	3	Xhosa 3b (Croc Valley)
	4	KDK Packhouse
Eastern Cape	1	Addo
	2	Addo
	3	Kirkwood
	4	Kirkwood

Untreated control samples were submitted with the samples. The samples were in a frozen state when received. They were immediately placed in a deep-freeze and were kept under these conditions until the analysis was commenced on 30 October 2006.

### Test required

To determine the triflumuron residue content of the peel and the flesh separately and to calculate the content on the whole fruit. The fruit of each sample was peeled. The peel and the flesh weighed and treated as separate samples.

### Method of test

Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. The analysis was carried out in duplicate employing *method No. RA179 viz Provisional Method for the determination of SIR 8514 Residues in Plants, Soil and Water by HPLC and GC.*

Recovery determinations were done by adding known amounts of triflumuron to portions of untreated control samples and analysing these concurrently with the samples.

Notes:

- a) Generic equivalents may have been used for solvents or equipment. However, chemical and instrument verification is regularly performed.  
 b) If changes are made to methods, such changes are described in the official report. However, slight changes to chromatographic conditions are only documented internally.

Analyses results can be seen in Tables 3.8.3.4, 3.8.3.5. and 3.8.3.6

**Table 3.8.3.4.** Triflumuron residue content of fruit samples taken in Mpumalanga

Trial no.	No. of days after application	Triflumuron residue content, mg/kg		
		Peel	Flesh	Whole fruit
1	0 Control	0,02 ; 0,02 *	ND ; ND	ND
	0	1,4 ; 1,3	0,11 ; 0,08	0,45
	7	1,3 ; 1,3	0,09 ; 0,07	0,46
	14	1,2 ; 1,3	0,08 ; 0,07	0,41
	28 Control	ND ; ND	ND ; ND	ND
	28	0,86 ; 0,94	0,05 ; 0,05	0,30
	35	0,97 ; 0,99	0,07 ; 0,07	0,33
	79 Control	ND ; ND	ND ; ND	ND
	79	0,82 ; 0,83	0,05 ; 0,06	0,29
	2	0 Control	ND ; ND	ND ; ND
0		1,5 ; 1,5	0,17 ; 0,21	0,61
7		1,5 ; 1,6	0,13 ; 0,12	0,57
14		2,4 ; 2,3	0,22 ; 0,17	0,74
28 Control		ND ; ND	ND ; ND	ND
28		1,6 ; 1,9	0,09 ; 0,08	0,53
35		2,0 ; 2,1	0,11 ; 0,15	0,61
79 Control		ND ; ND	ND ; ND	ND
79		1,5 ; 1,6	0,07 ; 0,07	0,45
3		0 Control	ND ; ND	ND ; ND
	0	1,7 ; 2,2	0,16 ; 0,17	0,70
	7	1,3 ; 1,4	0,09 ; 0,11	0,45
	14	1,4 ; 1,4	0,11 ; 0,11	0,47
	28 Control	ND ; ND	ND ; ND	ND
	28	1,2 ; 1,2	0,06 ; 0,06	0,38
	35	1,2 ; 1,2	0,08 ; 0,07	0,38
	79 Control	ND ; ND	ND ; ND	ND
	79	1,1 ; 1,0	0,06 ; 0,06	0,33
	4	0 Control	ND ; ND	ND ; ND
0		2,3 ; 2,2	0,17 ; 0,17	0,90
7		1,7 ; 1,6	0,14 ; 0,16	0,64
14		1,8 ; 1,8	0,17 ; 0,19	0,73
28 Control		ND ; ND	ND ; ND	ND
28		1,6 ; 1,6	0,11 ; 0,14	0,57
35		1,6 ; 1,6	0,09 ; 0,07	0,54
79 Control		ND ; ND	ND ; ND	ND
79		1,8 ; 1,8	0,11 ; 0,10	0,64

ND = Not detected/determined

Note \* = Duplicate sample will be re-analysed.

**Table 3.8.3.5.** Triflumuron residue content of fruit samples taken in the Sundays River Valley

Trial no.	No. of days after application	Triflumuron residue content, mg/kg		
		Peel	Flesh	Whole fruit
1	1 Control	ND ; ND	ND ; ND	ND
	1	3,0 ; 2,8	0,30 ; 0,33	1,3
	7	2,4 ; 2,4	0,30 ; 0,28	1,1
	14	2,7 ; 2,7	0,26 ; 0,28	1,1
	28 Control	ND ; ND	ND ; ND	ND
	28	2,0 ; 2,0	0,15 ; 0,18	0,68
	35	1,6 ; 1,5	0,07 ; 0,07	0,51
	79 Control	ND ; ND	ND ; ND	ND
	79	1,3 ; 1,2	0,05 ; 0,05	0,34
2	1 Control	ND ; ND	ND ; ND	ND
	1	2,8 ; 2,9	0,39 ; 0,41	1,4
	7	2,4 ; 2,4	0,23 ; 0,30	1,1
	14	2,3 ; 2,4	0,16 ; 0,16	0,94
	28 Control	ND ; ND	ND ; ND	ND
	28	2,0 ; 2,1	0,20 ; 0,21	0,79
	35	1,6 ; 1,7	0,09 ; 0,08	0,53
	79 Control	ND ; ND	ND ; ND	ND ; ND
	79	1,5 ; 1,4	0,07 ; 0,07	0,44
3	1 Control	ND ; ND	0,06 ; 0,06*	0,03
	1	3,0 ; 2,7	0,43 ; 0,45	1,5
	7	2,0 ; 2,0	0,22 ; 0,22	1,0
	14	2,0 ; 2,2	0,23 ; 0,20	0,95
	28 Control	ND ; ND	ND ; ND	ND
	28	1,4 ; 1,4	0,14 ; 0,16	0,58
	35	1,2 ; 1,1	0,01 ; 0,01	0,39
	79 Control	ND ; ND	ND ; ND	ND
	79	1,1 ; 1,1	0,04 ; 0,04	0,35
4	1 Control	ND ; ND	ND ; ND	ND
	1	2,0 ; 1,9	0,27 ; 0,27	0,91
	7	1,8 ; 1,7	0,22 ; 0,21	0,79
	0,79	2,0 ; 2,1	0,17 ; 0,20	0,80
	28 Control	ND ; ND	ND ; ND	ND
	28	2,1 ; 2,0	0,13 ; 0,12	0,67
	35	1,3 ; 1,3	0,08 ; 0,09	0,46
	79 Control	ND ; ND	ND ; ND	ND
	79	1,2 ; 1,2	0,05 ; 0,05	0,34

ND = Not detected/determined

Note \* = Duplicate sample will be re-analysed

**Table 3.8.3.6.** Triflumuron recovery at various levels of insertion

Recovery level	Recovery (%)	
	Peel	Flesh
0,05	102	108
0,10	108	104
0,15	-	97
0,50	107	100
1,0	98	92
2,5	90	-

The results reported were corrected for recovery using the appropriate value. Under the conditions of test employed the lowest limit of determination was 0,01 mg/kg.

### Conclusion

These results will be used for the determination of a revised PHI for triflumuron (Alsystin).

#### 3.8.4 FINAL PROGRESS REPORT: Malathion residue trials

Experiment 872 (April 2005 – April 2006): Peter Stephen and Wayne Kirkman (CRI) and Vincent Nel (SABS)

### Introduction

This study was conducted to determine the decline of mercaptothion residues in citrus fruit when applied as Avi Gard in a bait for the control of fruit fly.

### Materials and methods

For this study 16 trials were to be conducted over two citrus-growing seasons, i.e. 2006 and 2007. This report covers 8 trials done during the second season, 4 in Mpumalanga on oranges and 4 in the Eastern Cape on mandarins. All applications were made at the highest possible dosage rate with the shortest possible withholding period. The trials were conducted in accordance with the European Commission guidelines for residue trials. These require meticulous attention to detail and recording of all operations, with independent auditing at all critical phases. Peter Stephen (Nelspruit) and Wayne Kirkman (Port Elizabeth) were appointed as Principal Investigators for the study and Vincent Nel (SABS) was appointed Study Director.

The applications were made through the use of a baiting method. In this system, fruit fly attractant was made up in water as a mixture with a pesticide. The tank mixture was then applied as coarse droplets to the trees at an application rate of 50 to 150 ml per one side of the tree. A knapsack was used with a 1 bar pressure regulator and a 56 whirler with a D3 disc. Hym-Lure was used as the fruit fly attractant and AviGard (malathion) as the insecticide. Applications were made once a week for 5 weeks.

In the Sundays River Valley the trials were conducted on 4 orchards of late mandarins on Carden Farm and Dunbrody Estates. In Mpumalanga the trials were conducted on 4 orchards of Valencias at Friedenheim Estates (2), Crocodile Valley Citrus and KDK Packhouse.

### Spray mixture and dosage rates

The applications were made according to schedule (Table 3.8.4.1) using the equivalent spray mixture concentration rate of 175 ml AviGard and 400 ml Hym-Lure per 100 l of water.

**Table 3.8.4.1.** Application schedule for 4 sites for Malathion to citrus in the Sundays River Valley.

Citrus growing season	Citrus type	Cultivar	Application no.	Date application	of	Dosage Gard <sup>®</sup> mL/100 L	Avi
2007	Mandarin	Orr and Affourer	1	29/05/2007		17.5 ml	
			2	04/06/2007		17.5 ml	
			3	13/06/2007		17.5 ml	
			4	20/06/2007		17.5 ml	
			5	27/06/2007		17.5 ml	
2007	Oranges	Valencias	1	23/05/2007		17.5 ml	
			2	30/05/2007		17.5 ml	
			3	07/06/2007		17.5 ml	
			4	13/06/2007		17.5 ml	
			5	20/06/2007		17.5 ml	

Tank mix samples were taken for each application at each site, frozen and sent to SABS.

Fruit samples were taken at all four sites according to schedule (Table 3.8.1.4.2). Samples were taken in duplicate and sent to the SABS for residue analysis.

**Table 3.8.4.2.** Samples taken in 2007

Cultivar	Sample time	Sampling date	Sample number			
			Site 1	Site 2	Site 3	Site 4
Orr and Affourer	T0 Control	27/06/07	06/144/096	06/144/105	06/144/113	06/144/121
	T0 Residue	27/06/07	06/144/095	06/144/104	06/144/112	06/144/120
	T7 Residue	04/07/07	06/144/097	06/144/106	06/144/114	06/144/122
Valencia	T0 Control	20/06/07	06/144/129	06/144/137	06/144/145	06/144/153
	T0 Residue	20/06/07	06/144/128	06/144/136	06/144/144	06/144/152
	T7 Residue	27/06/07	06/144/130	06/144/138	06/144/146	06/144/154

## Results and discussion

### Analysis of samples

The following is an extract from the report by SABS compiled by HV Garbers, MANAGER: CHROMATOGRAPHIC SERVICES and PFC van Zyl, SUBJECT SPECIALIST.

48 samples of citrus, GLP study No. 06/144 were submitted for analysis during 2005/2006 citrus season.

According to the study protocol accompanying the samples the trees were treated with Avi Gard 500 EC at a concentration of 175 ml product/hl.

**Table 3.8.4.3.** Application details for malathion residue trials

Area	Trial No.	Locality
Mpumalanga	5	Friedenheim
	6	Friedenheim
	7	Croc Valley

	8	KDK Packhouse
Eastern Cape	1	Carden
	2	Carden
	3	Sun Orange
	4	Sun Orange

Untreated control samples were submitted with the samples.

The samples were in a frozen state when received. They were immediately placed in a deep-freeze and were kept under these conditions until the analysis was commenced on 11 December 2006.

#### Test required

To determine the malathion residue content of the peel and the flesh separately and to calculate the content on the whole fruit. The fruit of each sample was peeled. The peel and the flesh weighed and treated as separate samples.

#### Method of test

Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. The analysis was carried out in duplicate employing *SABS In-house Method No. 019/2000 Multi residue Analysis in Citrus*.

Recovery determinations were done by adding known amounts of malathion to portions of untreated control samples and analysing these concurrently with the samples.

#### Notes

- Generic equivalents may have been used for solvents or equipment. However, chemical and instrument verification is regularly performed.
- If changes are made to methods, such changes are described in the official report. However, slight changes to chromatographic conditions are only documented internally.

The results of analyses can be seen in Tables 3.8.4.4., 3.8.4.5. and 3.8.4.6

**Table 3.8.4.4.** Malathion residue content of fruit samples taken in Mpumalanga

Trial no.	No. of days after application	Malathion residue content, mg/kg		
		Peel	Flesh	Whole fruit
5	T-1	0,02 ; 0,02	ND ; ND	0,006
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,17 ; 0,18	ND ; ND	0,05
	3	0,07 ; 0,07	ND ; ND	0,02
	7	0,04 ; 0,04	ND ; ND	0,01
	14	0,03 ; 0,03	ND ; ND	0,008
6	T-1	0,03 ; 0,03	ND ; ND	0,008
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,18 ; 0,18	ND ; ND	0,05
	3	0,11 ; 0,11	ND ; ND	0,03
	7	0,09 ; 0,08	ND ; ND	0,02
	14	0,04 ; 0,03	ND ; ND	0,009
7	T-1	ND ; ND	ND ; ND	ND
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,13 ; 0,11	ND ; ND	0,03
	3	0,06 ; 0,06	ND ; ND	0,02

Trial no.	No. of days after application	Malathion residue content, mg/kg		
		Peel	Flesh	Whole fruit
	7	0,02 ; 0,02	ND ; ND	0,005
	14	0,01 ; 0,01	ND ; ND	0,003
8	T-1	0,03 ; 0,03	ND ; ND	0,008
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,18 ; 0,18	ND ; ND	0,05
	3	0,06 ; 0,07	ND ; ND	0,02
	7	0,05 ; 0,05	ND ; ND	0,01
	14	0,02 ; 0,02	ND ; ND	0,005

ND = Not detected/determined

**Table 3.8.4.5.** Malathion residue content of fruit samples taken in the Sundays River Valley

Trial no.	No. of days after application	Malathion residue content, mg/kg		
		Peel	Flesh	Whole fruit
1	T-1	0,15 ; 0,15	ND ; ND	0,04
	T-1 Control	ND ; ND	ND ; ND	ND
	0	0,66 ; 0,67	ND ; ND	0,17
	3	0,15 ; 0,15	ND ; ND	0,04
	7	0,11 ; 0,10	ND ; ND	0,03
	14	0,07 ; 0,07	ND ; ND	0,02
2	T-1	0,83 ; 0,83	ND ; ND	0,23
	T-1 Control	ND ; ND	ND ; ND	ND
	0	1,4 ; 1,3	ND ; ND	0,36
	3	0,89 ; 0,92	ND ; ND	0,24
	7	0,94 ; 0,85	ND ; ND	0,24
	14	0,61 ; 0,60	ND ; ND	0,16
3	T-1	1,2 ; 1,1	ND ; ND	0,34
	T-1 Control	0,78* ; 1,0*	ND ; ND	-
	0	2,6 ; 2,7	ND ; ND	0,76
	3	2,0 ; 2,0	ND ; ND	0,57
	7	0,95 ; 0,96	ND ; ND	0,27
	14	1,6 ; 1,4	ND ; ND	0,42
4	T-1	1,8 ; 1,8	ND ; ND	0,48
	T-1 Control	0,47* ; 0,34*	ND ; ND	-
	0	2,8 ; 2,1	ND ; ND	0,66
	3	2,0 ; 1,8	ND ; ND	0,51
	7	0,95 ; 1,0	ND ; ND	0,30
	14	1,0 ; 1,1	ND ; ND	0,27

ND = Not detected/determined

Note \* = Duplicate sample will be re-analysed

**Table 3.8.4.6.** Malathion recovery at various levels of insertion

Recovery level	Recovery (%)	
	Peel	Flesh
0,01	92	76
0,05	91	84
0,10	73	80
0,50	84	83
1,0	92	78

The results reported were corrected for recovery using the value of 86% (mean). Under the conditions of test employed the lowest limit of determination was 0,01 mg/kg.

### **Conclusion**

These results will be used for the determination of a revised PHI for malathion.

### **3.8.5 FINAL PROGRESS REPORT: Buprofezin residue trials**

Experiment 892 (April 2006 – April 2007): Peter Stephen and Wayne Kirkman (CRI) and Vincent Nel (SABS)

### **Introduction**

The trials were designed to evaluate the residue levels of the insecticide buprofezin in citrus after application of the formulated product Applaud.

### **Materials and methods**

The trials were conducted over the 2006/2007 season only. A total of 2 trials were conducted on oranges, one in the Eastern Cape and one in Mpumalanga.

An orchard of Palmer navel oranges was selected on Carden Farm in the Sundays River Valley and in Mpumalanga, an orchard, also Palmer navels, was selected at Crocodile Valley Citrus Co. Treatments consisted of an untreated control and single and double applications of Applaud. A 1X dosage rate of Applaud equivalent to its current registration on citrus in South Africa was used. The compound was applied to 5 to 9 trees per application as a full cover spray simulating the applications for red scale and mealybug control as used by growers. Control treatments for residue trials consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees. The applications were made using calibrated hand-gun type applicators. The spray machines had suitable agitation and calibration was achieved by selection of the appropriate pressure and nozzles. The sprayer was adjusted to deliver coarse droplets at 20 - 30 bar pump pressure to enable application of a full cover spray. The Applaud was applied in a mixture with spray oil, a wetter and water as registered. The equivalent of 30 g Applaud, 500 ml medium distillation range oil and 10 ml Agral 90 per 100 L water was used as the highest registered dosage rate. Applications were "full cover" so as to wet all above ground parts of the tree.

The spray mixtures were prepared on the day of application. The required amount of Applaud was premixed in a bucket of water to an homogenous slurry and then added to about half the required water in the spray tank. The tank was agitated while the wetter and oil were added and the tank filled to the required volume. The applications were made to the point of drip.

Applications (Table 3.8.5.1; 3.8.5.2) and sampling (Table 3.8.5.3) were done according to scheduling.

**Table 3.8.5.1.** Schedule of applications for trial 1 (Mpumalanga)

Treatment number	Treatment description	Date of Application	Average L per tree of spray mix applied
1	Single application in <b>October</b>	12/10/2006	24.6
2	Application in <b>October</b> + <b>November</b>	12/10/2006 & 09/11/2006	24.6 & 22.6
3	Single application in <b>November</b>	09/11/2006	22.6
4	Single application in <b>February</b>	13/02/2007	30.0

**Table 3.8.5.2.** Schedule of applications for trial 2 (Eastern Cape)

Treatment number	Treatment description	Date of Application	Average L per tree of spray mix applied
1	Single application in <b>December</b>	12/12/06	14.5
2	Application in <b>December</b> and <b>January</b>	17/12/06 + 18/01/07	14.5 + 14.0
3	Single application in <b>January</b>	18/01/07	14.0
4	Application in <b>January</b> and <b>February</b>	18/01/07 + 21/02/07	14.0 + 16.0
5	Single application in <b>February</b>	21/02/07	16.0

**Table 3.8.5.3.** Schedule of sampling for all trials

Sample Time (DALA)	Sample type	Date sampled Eastern Cape	Date sampled Mpumalanga
T0	Control sample	21/02/07	13/02/07
T0	Residue sample	21/02/07	13/02/07
T28	Residue sample	-	13/03/07
T30	Residue sample	22/03/07	-
T60	Residue sample	23/04/07	-
T62	Residue sample	-	16/04/07
T82	Residue sample	14/05/07	-
T82	Control sample	14/05/07	-
T90	Residue sample	-	14/05/07
T90	Control sample	-	14/05/07

Control samples were taken as recorded above. Residue samples were taken from all treatments at each sampling time. All samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at the respective CRI laboratory and sent to the SABS at a later stage.

### Results and discussion

Twenty fruit samples from the Eastern Cape and 16 samples from Mpumalanga were submitted for analysis. The samples were frozen when received and were immediately placed in a deep-freeze until the analysis commenced on 12 July 2007.

The fruit from each sample were peeled and peel and flesh treated as separate samples. Each sample was analysed in duplicate using SABS inhouse-method No.019/2000. This is a multi-residue method for pre-

harvest pesticides in citrus. Recovery determinations were done by adding known amounts of buprofezin to portions of control samples and analyzing these concurrently with the samples.

Eastern Cape

**Table 3.8.5.4.** Buprofezin residue content of fruit samples taken in the Sundays River Valley - Single application in December

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
21/02/07	0	0,10 ; 0,12	ND ; ND	0,03
22/03/07	30	0,10 ; 0,07	ND ; ND	0,03
23/04/07	60	0,11 ; 0,12	ND ; ND	0,03
14/05/07	82	0,08 ; 0,09	ND ; ND	0,02

**Table 3.8.5.5.** Buprofezin residue content of fruit samples taken in the Sundays River Valley - Application in December and January

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
21/02/07	0	0,41 ; 0,42	0,01 ; 0,01	0,13
22/03/07	30	0,40 ; 0,41	ND ; ND	0,11
23/04/07	60	0,22 ; 0,22	ND ; ND	0,06
14/05/07	82	0,21 ; 0,22	ND ; ND	0,05

**Table 3.8.5.6.** Buprofezin residue content of fruit samples taken in the Sundays River Valley - Single application in January

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
21/02/07	0	0,28 ; 0,27	ND ; ND	0,08
22/03/07	30	0,13 ; 0,13	ND ; ND	0,03
23/04/07	60	0,17 ; 0,17	ND ; ND	0,05
14/05/07	82	0,14 ; 0,14	ND ; ND	0,03

**Table 3.8.5.7.** Buprofezin residue content of fruit samples taken in the Sundays River Valley - Application in January and February

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
21/02/07	0	4,2 ; 4,2	0,07 ; 0,07	1,3
22/03/07	30	0,80 ; 1,0	0,02 ; 0,02	0,25
23/04/07	60	0,58 ; 0,56	0,01 ; 0,01	0,15
14/05/07	82	0,60 ; 0,61	0,02 ; 0,02	0,16

**Table 3.8.5.8.** Buprofezin residue content of fruit samples taken in the Sundays River Valley - Single application in February

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
21/02/07	0	0,41 ; 0,42	0,01 ; 0,01	0,13
22/03/07	30	0,40 ; 0,41	ND ; ND	0,11
23/04/07	60	0,22 ; 0,22	ND ; ND	0,06
14/05/07	82	0,21 ; 0,22	ND ; ND	0,05

**Table 3.8.5.9.** Buprofezin residue content of fruit samples taken in the Sundays River Valley - Application in December and January

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
21/02/07	0	3,2 ; 3,5	0,07 ; 0,07	1,1
22/03/07	30	0,54 ; 0,54	0,01; 0,01	0,15
23/04/07	60	0,38 ; 0,38	0,01; 0,01	0,11
14/05/07	82	0,51 ; 0,50	0,01; 0,01	0,14

Mpumalanga

**Table 3.8.5.10.** Buprofezin residue content of fruit samples taken in Mpumalanga - Single application in October

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
13/02/07	0	0,03 ; 0,03	ND ; ND	<0,01
13/03/07	28	0,02 ; 0,02	ND ; ND	<0,01
16/04/07	62	0,02 ; 0,02	ND ; ND	<0,01
14/05/07	90	0,01 ; 0,02	ND ; ND	<0,01

**Table 3.8.5.11.** Buprofezin residue content of fruit samples taken in Mpumalanga - Application in October and November

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
13/02/07	0	0,07 ; 0,07	ND ; ND	0,02
13/03/07	28	0,06 ; 0,06	ND ; ND	0,02
16/04/07	62	0,05 ; 0,04	ND ; ND	0,01
14/05/07	90	0,05 ; 0,05	ND ; ND	0,01

**Table 3.8.5.12.** Buprofezin residue content of fruit samples taken in Mpumalanga - Single application in November

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
13/02/07	0	0,07 ; 0,07	0,08; 0,08	0,02
13/03/07	28	0,03 ; 0,03	ND ; ND	0,01
16/04/07	62	0,03 ; 0,03	ND ; ND	0,01
14/05/07	90	0,02 ; 0,01	ND ; ND	<0,01

**Table 3.8.5.13.** Buprofezin residue content of fruit samples taken in Mpumalanga - Single application in February

Date sampled	No of days after application	Buprofezin residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
13/02/07	0	2,7 ; 2,3	0,02; 0,01	0,73
13/03/07	28	0,10 ; 0,10	ND ; ND	0,03
16/04/07	62	0,10 ; 0,09	ND ; ND	0,03
14/05/07	90	0,04 ; 0,05	ND ; ND	0,01

ND = Not detectable/determined

**Table 3.8.5.14.** Buprofezin recovery at various levels of insertion

Recovery level mg/kg	Recovery (%)	
	Peel	Flesh
0.01	-	95
0.02	97	101
0.04	69	85
0.06	87	-
0.10	85	92
0.20	80	104
0.40	78	83

The results reported were corrected for recovery using the appropriate value. No residues of buprofezin were detected in the control sample used for the recovery determinations. Under the conditions of test employed the lowest limit of determination was 0.01 mg.

### Conclusion

These results will be used for the determination of a revised PHI for buprofezin (Applaud).

### 3.8.6 FINAL REPORT: Methiocarb residue trials

Experiment 902 (February 2007 – July 2007): Peter Stephen (CRI) and Vincent Nel (SABS)

#### Introduction

The trials were designed to evaluate the residue levels of the insecticide methiocarb after application of the formulated product Mesurol.

#### Materials and methods

The trials were conducted over the 2006/2007 season only. A total of 2 trials were conducted on oranges, both in Mpumalanga.

Two orchards of Valencias were selected at Crocodile Valley Citrus Co. in Mpumalanga. Treatments consisted of an untreated control and single and double applications of Mesurol as per Table 3.8.6.1. The Mesurol was applied in a mixture with white sugar and water as registered. The equivalent of 10 g Mesurol, 200 g sugar per 100 L water was used as the highest registered dosage rate.

The applications were made to 4 to 6 trees per treatment using a calibrated hand-gun type applicator. The sprayer had suitable agitation and enabled accurate measuring and calibration. The sprayer was adjusted to deliver 20 - 25 bar (2000 – 2500 kPa) pump pressure and a 1.5 mm nozzle was used to enable application of 3 to 10 litres of spray mixture per tree, depending on tree size. Application was as a “diffuse bait spray” so as to cover the foliage canopy with fine droplets without merging of droplets. This is the type of application used by growers to achieve citrus thrips control with this type of material.

Control treatments for residue trials consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees.

Applications (Table 3.8.6.1) and sampling (Table 3.8.6.2) were done according to scheduling.

**Table 3.8.6.1.** Schedule of applications for trials 1 & 2

Treatment number	Treatment description	Application date
1	Single application in <b>February</b>	15/02/2007
2	Application in <b>February and March</b>	15/02/2007 & 15/03/2007
3	Single application in <b>March</b>	15/03/2007

**Table 3.8.6.2.** Schedule of sampling for all trials

Sample Time (DALA)	Sample type	Sampling Date
T0	Control sample	15/03/2007
T0	Residue sample	21/02/2007
T32	Residue sample	16/04/2007
T60	Residue sample	14/05/2007
T90	Residue sample	12/06/2007
T119	Control sample	12/07/2007
T119	Residue sample	12/07/2007

Control samples were taken as recorded above. Residue samples were taken from all treatments at each sampling time. All samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI Nelspruit and sent to the SABS at a later stage.

## Results and discussion

### Description of samples

Twenty samples of citrus fruit, variety Valencia, taken from test sites at Nelspruit were submitted for analysis during 2006/2007 season. According to the field trial protocol the trees were treated with Mesuro at a concentration of 10 g product + 200 g sugar/100 L water. Untreated control samples were submitted with the samples.

The samples were frozen when received. They were immediately placed and stored in a deep-freeze until the analysis commenced on 28 August 2007.

### Test requested

To determine the methiocarb residue content of the peel and the flesh separately and to calculate the content on the whole fruit

### **Method of test**

The fruit of each sample was peeled, the peel and flesh weighed and treated as separate samples. Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. Each sample was analysed in duplicate employing method No. RA-917 as provided by Bayer viz *Method for Gas Chromatographic Determination of Mercaptodimethur Residues in Rape*. Final analysis was performed by post column derivitization (HPLC).

### **Note:**

The peel samples, as well as the highest flesh sample was re-analysed by HPLC-MS-MS for confirmation.

Recovery determinations were done by adding known amounts of methiocarb to portions of control samples and analysing these concurrently with the samples.

### **Notes:**

- Generic equivalents may have been used for solvents or equipment. However, chemical and instrument verification is regularly performed.
- If changes are made to methods, such changes are described in the official report. However, slight changes to chromatographic conditions are only documented internally.

**Table 3.8.6.3.** Methiocarb residue content of fruit samples – Trial 1, Treatment 1.

Date sampled	No. of days after last application	Methiocarb residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
2007-03-15	0	0,02 ; 0,02	ND ; ND	ND
2007-04-16	32	0,02 ; 0,02	ND ; ND	ND
2007-05-14	60	0,02 ; 0,02	ND ; ND	ND
2007-06-12	89	0,01 ; 0,01	ND ; ND	ND

2007-07-12	119	0,01 ; 0,1	ND ; ND	ND
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ND = Not detected/determined.

**Table 3.8.6.4.** Methiocarb residue content of fruit samples – Trial 1, Treatment 2.

Date sampled	No. of days after last application	Methiocarb residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
2007-03-15	0	0,15 ; 0,13	ND ; ND	0,03
2007-04-16	32	0,03 ; 0,03	ND ; ND	ND
2007-05-14	60	0,02 ; 0,02	ND ; ND	ND
2007-06-12	89	0,03 ; 0,03	ND ; ND	ND
2007-07-12	119	0,02 ; 0,02	ND ; ND	ND

ND = Not detected/determined.

**Table 3.8.6.5.** Methiocarb residue content of fruit samples – Trial 2, Treatment 1.

Date sampled	No. of days after last application	Methiocarb residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
2007-03-15	0	0,03 ; 0,03	ND ; ND	ND
2007-04-15	32	0,02 ; 0,02	ND ; ND	ND
2007-05-14	60	0,02 ; 0,02	ND ; ND	ND
2007-06-12	89	0,03 ; 0,02	ND ; ND	ND
2007-07-12	119	ND ; ND	ND ; ND	ND

ND = Not detected/determined. See 4.5

**Table 3.8.6.6.** Methiocarb residue content of fruit samples – Trial2, Treatment 2.

Date sampled	No. of days after last application	Methiocarb residue content, mg/kg		
		Peel	Flesh	Whole fruit (mean)
2007-03-15	0	0,29 ; 0,29	0,02 ; 0,02	0,08
2007-04-15	32	0,02 ; 0,03	ND ; ND	ND
2007-05-14	60	0,02 ; 0,02	ND ; ND	ND
2007-06-12	89	0,01 ; 0,01	ND ; ND	ND
2007-07-12	119	ND ; ND	ND ; ND	ND

ND = Not detected/determined. See 4.5

**Table 3.8.6.7.** Methiocarb recovery at various levels of insertion

Recovery level mg/kg	Recovery (%)	
	Peel	Flesh
0,008	88	-
0,012	73	-
0,02	72	106
0,024	-	106
0,032	80	-
0,04	-	73
0,40	89	74

The results reported were not corrected for recovery.

No residues of methiocarb were detected in the control sample used for the recovery determinations.

Under the conditions of test employed the lowest limit of determination was 0,01 mg, but lower levels could be reached by HPLC-MS-MS.

## Conclusion

These results will be used for the determination of a revised PHI for methiocarb (Mesuro).

### 3.8.7 FINAL REPORT: Tetradifon residue trials

Experiment 904 (March 2007 – July 2007): Peter Stephen (CRI) and Heinz Loots (SABS)

## Introduction

The trials were designed to evaluate the residue levels of the acaricide tetradifon after application of the formulated product Tedion.

## Materials and methods

The trials were conducted over the 2006/2007 season only. A total of 4 trials were conducted on citrus in Mpumalanga.

Two sites of Valencias were selected in the Nelspruit area, Mpumalanga, one at Friedenhiem Estates and one at Crocodile Valley Citrus Co. Sites with lemons were selected at Bakgat Farm, Schoemanskloof, and KDK Packhouse, Karino. Treatments consisted of an untreated control and a single application of Tedion as per schedule of application. The Tedion was applied only once at the registered rate of 200 ml per 100 litres of water. The spray mixture was prepared on the day of application. The required amount of Tedion was added once about half the required water in the spray tank had been filled. The tank was then filled to the required volume.

The compound was applied to 6 to 10 trees per application as a full cover spray simulating the applications for spider mite control as used by growers.

Control treatments for residue trials consisted of untreated trees of the same cultivar, but located at a suitable distance from the test trees.

The applications were made using a calibrated hand-gun type applicator. The spray machine had suitable agitation and calibration was achieved by selection of the appropriate pressure and nozzles. The sprayer was adjusted to deliver coarse droplets at 20-30 bar pump pressure to enable application of a full cover spray.

Applications (Table 3.8.7.1; 3.8.7.2) and sampling (Table 3.8.7.3) were done according to scheduling.

**Table 3.8.7.1.** Schedule of applications for tetradifon (Tedion)

<b>Trial number</b>	<b>Site</b>	<b>Date of Application</b>	<b>Average L per tree of spray mix applied</b>
1	Friedenhiem Estates	27/03/2007	14.0
2	Crocodile Valley Citrus	27/03/2007	15.3
3	Bakgat Farm	24/04/2007	12.3
4	KDK Packhouse	24/04/2007	11.0

**Table 3.8.7.2.** Schedule of sampling for all trials

Sample Time (DALA)	Sample type	Date	
		Trials 1 & 2 (Valencias)	Trials 2 & 3 (Lemons)
T0	Control sample	27/03/2007	24/04/2007
T0	Residue sample	27/03/2007	24/04/2007
T15	Residue sample	11/04/2007	10/05/2007
T30	Residue sample	26/04/2007	24/05/2007
T60	Residue sample	25/05/2007	25/06/2007
T90	Control sample	25/06/2007	23/07/2007
T90	Residue sample	25/06/2007	23/07/2007

Control samples were taken as recorded above. All samples were taken in duplicate. One sample was sent to SABS, and the duplicates kept in freezers at CRI Nelspruit and sent to the SABS at a later stage.

## Results and discussion

### Analysis of samples

The following is an extract from the report by SABS compiled by WS Louw, MANAGER: CHROMATOGRAPHIC SERVICES and PFC van Zyl, SUBJECT SPECIALIST.

Twenty samples of citrus, varieties Valencia and Eureka lemon, taken from test sites at Nelspruit were submitted for analysis during 2006/2007 citrus season.

According to the study protocol the trees were treated with Tedion 81 g/l EC at a concentration of 200 ml product/100 l water and applied at a rate of 12-15 L spray mix per tree.

Untreated control samples were submitted with the samples.

The samples were in a frozen state when received. They were immediately placed in a deep-freeze and were kept under these conditions until the analysis was commenced on 20 August 2007.

### Method of test

To determine the tetradifon residue content of the peel and the flesh separately, and to calculate the residue content on the whole fruit. The fruit of each sample was peeled. The peel and the flesh weighed and treated as separate samples.

Each sample was shredded in a Stephan food cutter and was mixed thoroughly to render it homogeneous. The analysis was carried out in duplicate employing *SABS In-house Method No. 019/2000*. This is a multi residue method for pre-harvest pesticides in citrus.

Recovery determinations were done by adding known amounts of tetradifon to portions of untreated control samples and analysing these concurrently with the samples.

### Notes

a) Generic equivalents may have been used for solvents or equipment. However, chemical and instrument verification is regularly performed.

b) If changes are made to methods, such changes are described. However, slight changes to chromatographic conditions are only documented internally.

Results of analyses can be seen in **Tables 3.8.7.3 to 3.8.7.7**

**Table 3.8.7.3.** Trial 1 – Valencias

Date applied	Date sampled	No. of days after application	Tetradifon residue content, mg/kg		
			Peel	Flesh	Whole fruit (mean)
27/03/2007	27/03/2007	0	3.1 ; 3.2	0.03 ; 0.05	0.81
	11/04/2007	15	3.4 ; 3.1	0,03 ; 0,05	0,76
	26/04/2007	30	3.0 ; 2.6	0,04 ; 0,03	0,61
	25/05/2007	59	2.6 ; 2.6	ND ; ND	0,55
	25/06/2007	90	2.0 ; 2.2	0.01 ; ND	0,41

**Table 3.8.7.4.** Trial 2 - Valencias

Date applied	Date sampled	No. of days after application	Tetradifon residue content, mg/kg		
			Peel	Flesh	Whole fruit (mean)
27/03/2007	27/03/2007	0	3.9 ; 3.7	0.05 ; 0.07	1.1
	11/04/2007	15	1.9 ; 2.0	0,07 ; 0,04	0,57
	26/04/2007	30	2.8 ; 2.9	0,03 ; 0,02	0,59
	25/05/2007	59	2.3 ; 2.2	0.01 ; 0.01	0,51
	25/06/2007	90	1.1 ; 1.1	0.01 ; 0.01	0,24

**Table 3.8.7.5.** Trial 3 – Lemons

Date applied	Date sampled	No. of days after application	Tetradifon residue content, mg/kg		
			Peel	Flesh	Whole fruit (mean)
24/04/2007	24/04/2007	0	1.5 ; 1.3	0.09 ; 0.05	0.66
	10/05/2007	17	2.0 ; 2.0	0,01 ; 0,02	0,66
	24/05/2007	30	1.5 ; 1.7	ND ; 0,01	0,55
	25/06/2007	62	0.82 ; 0.79	0.01 ; ND	0,26
	23/07/2007	90	0.55 ; 0.57	ND ; ND	0,22

**Table 3.8.7.6.** Trial 4 – Lemons

Date applied	Date sampled	No. of days after application	Tetradifon residue content, mg/kg		
			Peel	Flesh	Whole fruit (mean)
24/04/2007	24/04/2007	0	3.4 ; 3.8	0.05 ; 0.04	1.1
	10/05/2007	17	3.1 ; 2.8	0,03 ; 0,04	0,92
	24/05/2007	30	2.5 ; 2.5	ND ; ND	0,72
	25/06/2007	62	0.89 ; 0.90	ND ; ND	0,30
	23/07/2007	90	0.75 ; 0.79	ND ; ND	0,24

**Table 3.8.7.7.** Recovery determinations gave the following mean values:

Recovery level mg/kg	Recovery (%)	
	Peel	Flesh
0.01	93	73
0.05	-	79
0.10	83	-
0.50	78	-

The results reported were corrected for recovery using the appropriate value  
No residues of tetradifon were detected in the control sample used for the recovery.

**Conclusion**

These results will be used for the determination of a revised PHI for tetradifon.

## 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 2007/8 reporting period is briefly summarised below.

Apart from research, the Graft Transmissible Diseases project provided essential services for the Citrus Improvement Programme 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. Several experiments are under way to select and evaluate new mild CTV strains for pre-immunisation of grapefruit, soft citrus and Valencia oranges. Molecular detection techniques for the pathogens of citrus Tristeza and greening were improved through the acquisition and use of a real-time PCR system in the laboratory of Prof Gerhard Pietersen (CRI at UP). This technology is an essential tool in experiments aimed at determining the variability in the citrus greening pathogen and failure of cross-protection against CTV. A country-wide survey confirmed that only "*Candidatus Liberibacter africanus*" is the cause of greening in South Africa, although its subspecies *capensis* was again confirmed in Cape Chestnut trees. Good progress has been made in the investigation of host resistance to citrus greening and two promising clones have been planted in the field and will be evaluated shortly. Investigation into methods of eradicating citrus greening in existing orchards, such as chemical or heat treatment, proved unsuccessful as the bacterial titre could be reduced, but not eliminated.

In the Citrus Black Spot project, several experiments were again completed during this report year. A review article of research from University of Pretoria is currently in its final stages completion. This article will prove invaluable during market access negotiations. The developed technologies, such as leaf-wilting, PCR detection and nursery monitoring, were successfully integrated in a protocol for determination of the CBS status of nursery plants or orchards. Previous studies on important epidemiological aspects will be included in new experiments aimed at disease forecasting. Strategies that are being studied to improve CBS control include inoculum management and improved spray programmes. For the latter, new fungicides and new formulations of copper and mancozeb, as well as selected spray adjuvants, were studied and the relevant recommendations made for registration or further development. It was also shown that combinations of certain of these compounds with Sporekill resulted in good control and reduced rates.

New spray programmes were evaluated in the Fruit and Foliar Diseases Project for control of *Alternaria* brown spot (ABS) in summer and winter rainfall areas. Unfortunately, the trials in the winter region could not be completed as a result of financial problems at the estate. However, trials in the summer rainfall region showed very good results with three strobilurins + mancozeb tank mix applications with either mineral spray oil or Sporekill, thereby saving growers 5 spray rounds. Effective control was obtained and stippling was reduced with new WG copper formulations. From the spray application project, it was clear that biological efficacy declined with increased run-off. Future experimentation should thus focus on optimising application to ensure adequate deposition of the active ingredient with minimal run-off.

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. Promising results were obtained in the experiment aiming to stimulate nematode egg hatching, which would improve the efficacy of nematicides. Formulated products will be evaluated in ongoing experiments. Several alternative products were evaluated for nematode control and showed some potential, but further research is required to confirm these results and a better understanding of the products' mode of action and residual activity. Trunk and branch cankers of Clementines, caused by *Phytophthora 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. Snails were also observed during the evaluations and their role as potential vectors, should be investigated. Clementine cultivars such as 'Marisol', 'Clemlate', 'Oroval', 'Tardino' and 'Oroblanco' were found to be susceptible to *Phytophthora citrophthora*. Residue analyses of potassium phosphonate levels in the roots following application as foliar sprays or through the irrigation system showed similar levels. No effects from phosphonates applied through irrigation systems were recorded on fruit size or production during the 2006 / 2007 growing season.

Post Harvest Diseases remain a very high priority and several experiments were directly aimed at improving post harvest disease management in packhouses. Preliminary screenings for imazalil and guazatine resistance in *Penicillium* spp. indicate elevated levels of resistance development. Further *in vitro* testing is under way to characterise resistance levels. *In vivo* trials with imazalil-sensitive and resistant strains

indicated that resistant strains were not preventatively controlled by dip-treatments in up to 4x the recommended dosage, nor was sporulation inhibited. The aims of a new study are to investigate imazalil application in commercial citrus packhouses, to determine the residue levels required for control of green mould infection and sporulation as caused by imazalil-sensitive and resistant strains, and to investigate and optimise current and novel application methods. In order to improve residue loading of fruit in packhouses, a split application of imazalil, i.e. in fungicide bath and in wax, was evaluated. Findings show that favourable residue levels are retained (ca. 3 ppm), without exceeding the MRL of 5 ppm. Registration trials with Philabuster, which is a mixture between imazalil and pyrimethanil, have been completed and the product was registered early in 2008. This product will prove invaluable in anti-resistance management strategies. Various trials were also conducted with a biological control agent (identified as *Bacillus subtilis*), sanitisers, GRAS chemicals and fungicides. Synergistic activity was observed in mixtures of Sporekill and imazalil or guazatine, and guazatine sodium carbonate, while the latter product, lime sulphur and sodium bicarbonate also demonstrated a fair degree of green mould and sour rot control. Certain plant growth regulators demonstrated some potential as replacements for 2,4-D sodium salts for calyx retention. Preliminary studies by the University of Pretoria determined that postharvest contamination often occurs further down the export chain. This creates an optimal environment for the onset of postharvest decay and in particular by various *Penicillium* spp.

While research progress is made, so too do we encounter new challenges, most notably the constant threat against biosecurity of citrus production in southern Africa, the dramatic rise of crop protection costs and therewith the need for sustainable production utilising integrated means of disease management, markets demanding reduced chemical use, fungicide resistance, and maintenance of market access to sensitive but lucrative markets. Most of these aspects are currently being addressed in the Disease Management Research portfolio as part of short- medium- and long-term strategies that are based on grower priorities. These strategies are discussed at various forums on an ongoing basis to ensure the relevance of research conducted. Moreover, through research alliances with reputable researchers at universities (Pretoria and Stellenbosch) and private companies (QMS Agriscience), we strive to ensure and build capacity to adequately address current and new plant pathological challenges.

### **Programopsomming**

Alle projekte in die Siektebestuurprogram toon goeie vordering en die meeste navorsingsprioriteite wat deur die bedryf geïdentifiseer is, word aangespreek in eksperimente gemik daarop om sekere kort-, medium- en langtermyn strategiese doelwitte aan te spreek. Die vordering gemaak in die 2007/8 verslagperiode word kortliks hieronder opgesom.

Buiten navorsing, het die Projek Entoordraagbare Siektes onmisbare dienste aan die Sitrusverbeteringskema gelewer, hoofsaaklik deur herindeksing van moederbome in die grondvesblok, groeipunt-enting en pre-immunisering van nuwe seleksies. Virus-reiniging is suksesvol op verskeie seleksies gedoen wat gevolglik gepre-immuniseer is en aan die Grondvesblok vir vermeerdering voorsien is. Verskeie eksperimente poog om nuwe matige Tristeza virusrasse vir die pre-immunisasie van sagte-sitrus, pomelo's en Valencia lemoene te selekteer en evalueer. Molekulêre opsporingstegnieke van die patogene van sitrus Tristeza en vergroening is verbeter deur die aankoop van 'n 'real-time PCR' sisteem in die laboratorium van Prof Gerhard Pietersen (CRI te UP). Hierdie tegnologie is 'n onmisbaar in eksperimente wat die variasie in die vergroeningspatogeen en verlies aan kruisbeskerming teen CTV bestudeer. 'n Landswyde opname het bevestig dat slegs "*Candidatus Liberibacter africanus*" die oorsaak van vergroening in Suid-Afrika is, terwyl die subspesie *capensis* in Kaapse kastaiingbome bevestig is. Goeie vordering is in die soektog na weerstand teen vergroening gemaak, en twee belowende klone is uitgeplant vir verdere evaluasie. Metodes, soos chemiese en hitte-behandelings, om vergroening in bestaande boorde uit te wis, is ondersoek, maar was onsuksesvol siende dat die bakterie-voorkoms slegs verlaag is, en nie uitgewis is nie.

In die Sitrus Swartvlek Projek is verskeie projekte in hierdie jaar voltooi. 'n Oorsigartikel van navorsing spruitend uit Universiteit Pretoria is tans in finale fases van afhandeling. Hierdie artikel sal baie waardevol vir marktoegangsonderhandelinge wees. Die ontwikkelde tegnologie, soos PKR opsporing, blaarverwelking om sporulasie van die swam te induseer en kwekery-monitering, is suksesvol in 'n protokol vir CBS statusbepaling in kwekerye en boorde geïntegreer. Vorige studies op belangrike epidemiologiese aspekte sal in nuwe eksperimente wat op siektevoorspelling fokus, ingesluit word. Verskeie strategieë om CBS beheer te verbeter word bestudeer, insluitend inokulumbestuur en verbeterde spuitprogramme. Vir laasgenoemde, is nuwe swamdoders en formulasies van koper en mancozeb, asook benatters bestudeer. Toepaslike aanbevelings is vir registrasie-doeleindes of opvolgnavorsing gemaak. Sekere kombinasies van kontakdoders en Sporekill was effektief teen verlaagde dosisse.

Nuwe spuitprogramme is in die Vrug- en Blaarsiekte Projek teen *Alternaria* bruinvlek in somer en winterreëngediede ge-evalueer. Ongelukkig kon proewe in die winterreëngediede weens geldelike tekorte by die betrokke landgoed nie voltooi word nie. Baie goeie resultate is egter uit die somerreëngediede verkry: drie bespuitings met strobilurine + mancozeb mengsels (+ olie of Sporekill) het effektiewe beheer van bruinvlek gegee, en sal daarmee kwekers 5 spuit rondtes kan spaar. Effektiewe beheer is met nuwe BG koperformulasies gekry. Aanvanklike resultate uit die spuittoedieningsprojek dui daarop dat biologiese effektiwiteit van spuite met toenemende afloop afneem. Navorsing in hierdie projek sal dus op optimisering van spuittoediening fokus om voldoende bedekking met minimale afloop te verseker.

Verskeie kontrakproewe is in die Grondgedraagde Siektes projek gedoen. Uit hierdie proewe is waardevolle inligting oor beheer van aalwurms met alternatiewe produkte versamel. Belowende resultate is uit 'n eksperiment gekry wat poog om uitbroei van aalwurmeiers te stimuleer en om sodoende beheer met aalwurmdoders te verbeter. Geformuleerde produkte is ge-evalueer in veldproewe. Verskeie alternatiewe produkte is vir aalwurmbespreking ge-evalueer. Party hiervan het potensiaal getoon, maar opvolgwerk is nodig om die meganisme en residuele werking daarvan te bepaal. Kraagvrot van Clementines in die suid-Kaap is veroorsaak deur *Phytophthora citrophthora* en is effektief gestuit met 'n laatwinter blaarbespuiting met 'n fosfonaat, gevolg deur 3 stambespuitings (elke 2 maande) met 'n Sporekill+kaptan mengsel gedurende die winter. Slakke is tydens evaluasies versamel en hul moontlike rol as vektore word tans ondersoek. Clementine kultivars soos 'Marisol', 'Clemlate', 'Oroval', 'Tardino' en 'Oroblanco' was vatbaar vir *Phytophthora citrophthora*. Residu-analises van kalium-fosfonaatvlakke in wortels na blaar- en besproeiingstoediening het soortgelyke vlakke gewys. Geen effek van die fosfonaat is egter op vrug grootte of produksie gedurende die 2006 / 2007 seisoen waargeneem nie.

Na-oessiektes is steeds 'n belangrike prioriteit en verskeie eksperimente word op verbeterde beheer in pakhuis gemik. Voorlopige resultate het gewys dat imazalil en/of guazatine weerstand in *Penicillium* spp. voorkom. Navorsing om weerstandsvlakke te karakteriseer en te bepaal watter van hierdie vlakke tot praktiese weerstand (verlies aan beheer) sal lei, duur voort. *Penicillium* isolate wat weerstand biedend teen imazalil is, kon nie voorkomend deur dip-behandelings van tot 4x die voorgeskrewe dosis beheer word nie, en sporulasie is boonop nie geïnhibeer nie. Die doel van 'n nuwe studie is om imazalil toediening in kommersiële sitruspakhuis te ondersoek, om die residuvlakke vir beheer van infeksie en sporulasie van imazalil sensitiewe en weerstand biedende isolate te bepaal, en huidige en nuwe toedieningsmetodes te ondersoek en te optimaliseer. 'n Dubbele aanwending van imazalil in pakhuis, i.e. in dompelbad asook in waks, is ondersoek. Voldoende residu-lading is opvrugte verkry (ca. 3 ppm), sonder dat MRL vlakke oorskry is. Registrasie-proewe met 'n nuwe swamdoder in die sitrus na-oes arena, Philabuster, 'n mengsels van imazalil and pyrimethanil, is afgehandel en die produk is vroeg in 2008 geregistreer. Hierdie sal 'n belangrike produk in teen-weerstand strategieë in pakhuis wees. Verskeie proewe is ook met 'n biologiese beheeragent (geïdentifiseer as *Bacillus subtilis*), GRAS chemikalieë, saniteerders en swamdoders is uitgevoer. Sinergistiese werking is tussen Sporekill en imazalil of guazatine, en tussen natrium karbonaat en guazatine teen groenskimmel en suurvrot waargeneem. Boonop het natrium karbonaat, natrium bikarbonaat en kalkswael redelike beheer van hierdie siektes getoon. Alternatiewe tot 2-4 D is ondersoek, en sekere plantgroeireguleerders lyk belowend. Voorlopige studies deur die Universiteit van Pretoria het bepaal dat besmetting gereeld verder af in die uitvoerketting plaasvind. Dit skep ideale omstandighede vir na-oes bederf, veral deur verskeie *Penicillium* spp.

Terwyl goeie vordering met navorsing gemaak word, kry ons terselfdertyd met nuwe uitdagings, soos veral die kontante druk op die bio-sekuriteit van sitrusproduksie in suider Afrika, die dramatiese verhogings in koste van gewasbeskerming en daarmee saam die behoefte na volhoubare produksie met gebruik van geïntegreerde metodes van siektebestuur, markte wat op verminderde chemiese gebruik aandring, bestandheid teen swamdoders, en ontsluiting en instandhouding van marktoegang na sensitiewe dog winsgewende markte. Meeste van hierdie aspekte word tans in die Siektebestuur navorsingsportefolio as deel van kort-, medium- en langtermyn strategieë aangespreek. Hierdie strategieë word gereeld op verskeie vlakke bespreek om die relevansie van navorsing te verseker. Boonop, poog ons om met alliansies met navorsers by universiteite (Pretoria en Stellenbosch) en privaatinstansies (QMS Agriscience) kapasiteit te verseker en uit te bou om huidige en nuwe plantpatologiese uitdagings aan te spreek.

## 4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project coordinator: S.P. van Vuuren (CRI)

### 4.2.1 Projekopsomming

Daar is meer as 40 entoordraagbare siektes in die wêreld bekend waarvan ongeveer 16 in Suid Afrika voorkom. Van die uitheemse siektes is uiters gevaarlik vir die bedryf en dit is noodsaaklik dat hulle uit die land gehou word. Die mees belangrikste siektes in Suid Afrika is *Citrus tristeza virus* (CTV), Sitrus viroïde (CVd) (eksokortis, cachexia, gomsaksiekte), Psorose virus (CPV), Appelstam groefvirus (voorheen "Citrus tatter leaf" virus) (ASGV), Impietratura (CID) en Sitruskroei ("Citrus Blight") (CB). Indeksering van die siektes word hoofsaaklik biologies gedoen deur indikatorplante. Indikatorplante vir elk van hierdie siektes vereis spesiale temperature wat dit genoodsaak om indeksering in temperatuurbeheerde glashuiskamers te doen. Die indekseringstyd varieer van 6 tot 12 maande, afhangende van die siekte.

Biologiese indeksering is gebruik om die CTV strafheid in die moederbome van die Sitrus Grondvesblok te bepaal. Strawwe rasse is in 8 moederbome geïdentifiseer. Die bome word getermineer as enthoutbronne. Sewe-en-sestig moederbome (hoofsaaklik losskil tipes) het negatief getoets. Die rede is onbekend maar dit kan wees dat die gashere die spesifieke CTV bron onderdruk en sodoende vermeerdering en beweging beperk. Ondersoek word ingestel na 'n alternatiewe kruisbeskermingsbron. Vir die eerste keer is herindeksering van CVd in moederbome gedoen. Ses-en-sewentig bome is getoets en was almal negatief. Dertig groeipunt entingsplante word tans geïndekseer (afdeling 4.2.2). Sewe nuwe cultivars is gedurende die jaar by die 235 cultivars van die genebron gevoeg (afdeling 4.2.3). 'n Uitgebreide studie word gedoen om die afbreek van kruisbeskerming met die GFMS 12 CTV bron, te ondersoek. Meer as 500 enkel-plantluis oordragings is vanaf die bron gedoen om die verskillende CTV rasse in die bron te skei (afdeling 4.2.4). Nege-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red op Swingle citrumelo onderstam, het baie eenvormig gereageer met vier ligte CTV bronne (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Bome met die vier CTV bronne verskil nie betekenisvol van mekaar ten opsigte van boom grootte, produksie (kg/boom), produksie doeltreffendheid (kg/m<sup>3</sup> kroon volume), kumulatiewe produksie oor 'n tydperk van drie jaar, oeswaarde (die produksie van klein vrugte) en die voorkoms van stamgleuf nie (afdeling 4.2.5). Die Marsh en Star Ruby boompies wat in Swaziland en die Nkweleni Vallei geplant is, is nou 4 jaar oud en daar is aanduidings dat van die sub-isolate groei strem en bome met ander soortgelyk as bome met die huidige kruisbeskermingsbronne presteer. Dit wil voorkom of daar interaksies tussen die sub-isolate en cultivars is, maar dit kan moonlik aan klimaatverskille tussen die twee persele toegeskryf word (afdeling 4.2.6). Star Ruby boompies wat met dieselfde bronne en sub-isolate as in die proewe in afdeling 4.2.6 geïnkuleer is, is in die Kakamas omgewing geplant. Na 3 jaar toon die bome geen effek van die verskillende CTV inokulasies nie (afdeling 4.2.7). Nuwe belowende ligte isolate wat in verskillende pomelo produksiegebiede versamel is, word gebruik om Marsh en Star Ruby boompies te preïmmuniseer. Die boompies is gedurende 2007 by Bosveld Sitrus in Letsitele en Riverside te Malélane geplant (afdeling 4.2.8).

Vruggrootte is 'n groot probleem by clementines in die Oos- en Weskaap en daar was 'n versoek om die invloed van kruisbeskerming op clementines te bepaal. 'n Proef is by die Addo Navorsingstasie gevestig waar gepreïmmuniseerde en virusvrye bome van sewe clementine seleksies en 'n Satsuma seleksie vergelyk word. Boomgrootte is bepaal en alhoewel die proef nog te jonk is, wil dit voorkom of al die clementine seleksies nie dieselfde op CTV besmetting reageer nie. Van die bome wat virusvry geplant is, het binne 4 jaar met CTV besmet geraak. Die vergelyking tussen gepreïmmuniseerde en virusvrye bome kan dus nie gedoen word nie (afdeling 4.2.9). 'n Proef om geskikte CTV bronne vir Turkey Valencia te identifiseer is gedurende 2007 te Riverside, Malélane gevestig (afdeling 4.2.10). Boomgrootte van McClean Saadlose Valencia is betekenisvol kleiner as die van McClean en Delta Valencia maar hul produksie was 50% hoër. Bome met twee CTV bronne, SM 41 en SM 49, se produksie was hoogs betekenisvol beter as die van bome wat virusvry geplant was (afdeling 4.2.11).

In die proef waar 17 onderstamme vir CB toleransie ge-evalueer word, toon bome op Swingle citrumelo, Sun Chu Sha, X639 en Orlando tangelo onderstamme die meeste toleransie en kwekers moet hierdie onderstamme oorweeg in sitruskroei gebiede. Bome op C35 citrange, Zhu luan en Sunki mandaryn onderstamme is die meeste ge-afgete deur die siekte (afdeling 4.2.12).

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. Twee klone, GTC-E2 en GTC-T2, is in 2006 geïdentifiseer as simptoombloos na blootstelling aan die vektor. Die klone is op onderstamme vermeerder en afsonderlik met twee CTV bronne gepreïmmuniseer en is 'n boord geplant vir verdere evaluasies. Sitrus bladvlooi populasies was te laag om die vier nuwe klone van 2007 te evalueer (afdeling 4.2.13). Twee potproewe en 'n boordproef is begin om chemiese en/of hittebehandeling in

vergroeningsbesmette bome te evalueer. Resultate tot dusver was teleurstellend. Die finale evaluasies sal gedurende die komende winter en lente gedoen word en die resultate sal bepaal wat die opvolgnavorsing sal behels (afdeling 4.2.14). Die bevestiging van die voorkoms van vergroening in alternatiewe gashere is tot dusver negatief. Die sub-spesie *capensis* is egter in verskeie bronne van *Calodendron capensis* (Kaapse kastaiing) opgespoor (afdeling 4.2.15). Slegs Afrika vergroening is gevind in monsters wat uit die hoof produksie gebied van Suid Afrika versamel is. Die Asiatiese vergroening word dus nog as eksoties beskou (afdeling 4.2.15).

### Project summary

There are more than 40 graft transmissible diseases known in the world of which approximately 16 occur in South Africa. Some of the foreign diseases are very dangerous for the citrus industry and it is important that they should be kept out of the country. The most important diseases in South Africa are *Citrus tristeza virus* (CTV), Citrus viroids (CVd) (Exocortis, Cachexia, Gum pocket), Psorosis virus (CPV), Apple stem grooving virus (formerly Citrus tatter leaf virus; ASGV), Impietratura (CID) and Citrus Blight (CB). Indexing of the diseases is mainly done biologically through the use of indicator plants. Indicator plants for each of the diseases require specific temperatures, which necessitate the use of temperature-controlled glasshouse rooms. The indexing time varies from 6 to 12 months.

Biological indexing was used to determine the CTV severity in mother trees at the Citrus Foundation Block. Severe strains were identified in 8 mother trees. These trees will be terminated as budwood sources. Seventy-six of mother trees (the majority soft citrus types) tested negative. The reason is unknown but it is possible that the hosts restrict the multiplication and movement of the specific CTV source. An investigation to find an alternative cross-protecting source is in progress. For the first time re-indexing for CVd was done on the mother trees. Seventy-six trees were indexed and were all negative. Thirty shoot tip grafted plants are currently being indexed (section 4.2.2). Seven new cultivars were added this year to the 235 cultivars in the nucleus block (section 4.2.3). An extensive study has been initiated to investigate the cross-protection breakdown that occurred when the GFMS 12 CTV source was used. More than 500 single-aphid transmissions were done to separate the different strains in this source (section 4.2.4). Nine-year-old trees of seven red grapefruit selections, viz. Star Ruby, Rio Red, Hendersen, Nel Ruby, Flame, Ruben and Oran Red on Swingle citrumelo rootstock, reacted very similar to four CTV sources (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as cross-protecting agents. Trees with these four sources did not differ significantly from each other regarding growth, yield (kg/tree), yield efficiency (kg/m<sup>3</sup> canopy volume), cumulative production over a period of 3 years, crop value (production of small fruit), and the occurrence of stem pitting (section 4.2.5). The Marsh and Star Ruby trees that were planted in Swaziland and the Nkwaleni Valley are now 4 years old and there are indications that some sub-isolates suppressed growth and with others, the trees are similar to those with the standard CTV sources. It appears that there are interactions between the sub-isolates and scions but it could possibly be ascribed to the climatic differences of the two sites (section 4.2.6). Star Ruby trees that were pre-immunised with the same sources and sub-isolates as the trials in section 4.2.6 were planted in the Kakamas region. After 3 years the trees are not showing any affect of the CTV inoculations (section 4.2.7). New promising mild isolates that were collected in different grapefruit production areas, are being used to pre-immunise Marsh and Star Ruby trees. The trees were planted during 2007 at Bosveld Citrus in Letsitele and Riverside at Malelane (section 4.2.8).

Fruit size is a great problem with Clementines in the eastern and western Cape and there was a request to evaluate the effect of pre-immunisation on fruit size. A trial was established at Addo Research Station where pre-immunised trees of seven Clementine selections and a Satsuma selection are compared to trees planted virus-free. Tree sizes and yield were determined and although the trees are still young, it appears that all the Clementine selections do not react similarly to CTV infection. Some of the trees that were planted virus-free became CTV infected within 4 years after plant. A comparison of pre-immunised and virus-free trees can no longer be done (section 4.2.9). A trial to assess CTV sources to pre-immunise Turkey Valencia has been established at Riverside Malelane during 2007 (section 4.2.10). Tree size of McClean Seedless Valencia was significantly smaller than those of McClean and Delta but their production was 50% better. The production of trees with 2 CTV sources, SM 41 and SM 49, was highly significantly better than trees that were planted virus-free (section 4.2.11).

In the trial where 17 rootstocks were evaluated for CB tolerance, trees on Swingle citrumelo, Sun Chu Sha, X639 and Orlando tangelo rootstocks appeared to exhibit the most tolerance and growers should consider these rootstocks in CB areas. C35 citrange, Zhu luan and Sunki mandarin rootstocks were affected the most by the disease (section 4.2.12).

Attempts are made to generate greening-resistant plants against greening by the rescuing of immature embryos out of healthy chimera sectors in fruits infected with greening and growing them on artificial

medium. Two clones, GTC-E2 and GTC-T2 were identified in 2006 as being symptomless after challenging them with the vector. These clones were multiplied on rootstocks and pre-immunised separately with two CTV sources for orchard evaluation. Psylla populations were too low to challenge the four new clones that were generated in 2007 (section 4.2.13). Two pot trials and a field trial to assess chemical and/or heat treatments of greening infected trees were initiated. Results so far are discouraging. Final evaluations will be made during the coming winter and spring and the results will determine if any future research is necessary (section 4.2.14). The search for the occurrence of greening bacteria in alternate hosts has thus far not identified any potential alternate hosts. However, the sub-species *capensis* was found in several sources of *Calodendron capensis* (Cape chestnut) trees (section 4.2.15). Only African greening was found in citrus samples that were collected in the major citrus producing areas of South Africa. The Asiatic form of greening is still considered an exotic disease (section 4.2.15).

#### 4.2.2 **PROGRESS REPORT: Diagnostic services for graft transmissible diseases** Experiment 796 (2005 - 2025) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

### **Opsomming**

Die sukses van die sitrusverbeteringskema (SVS) berus op 'n fitosanitêre program wat op 'n diagnostiese bepaling van die teenwoordigheid van skadelike patogene, die eliminerings daarvan en die onderhou en verspreiding van gesonde voortplantingsmateriaal gebaseer is. Indeksering, of die bepaling of entoordraagbare siektes in plantmateriaal teenwoordig is, word tans hoofsaaklik deur biologiese indikatorplante gedoen. Serologiese tegnieke soos ELISA en Kol Klard word hoofsaaklik gedoen om pre-immunisering met *Citrus tristeza virus* (CTV) te bevestig, asook om die teenwoordigheid van Sitruskroei te bevestig. Molekulêre tegnieke vir indeksering (bv. PCR) van verskeie sitrussiektes word tans ge-optimeer en sal baie tyd bespaar. Behalwe vir indeksering wat op SVS materiaal gedoen word, word daar ook indeksering op materiaal wat vanaf kwekers ontvang of versamel word, gedoen. Dit is verder nodig om die oorsaak van siektetoestande te bevestig om sodoende sinvolle aanbevelings vir beheer te maak. Spesifieke virusvrye indikatore is vir elk van die entoordraagbare siektes waarvoor ge-indekseer word in die glashuis gekweek. Die moederbome by die Sitrus Grondvesblok is ge-indekseer om te bepaal of enige strawwe CTV rasse in die moedermateriaal voorkom. 'n Totaal van 151 moederbome is getoets waarvan 8 bome met strawwe CTV geïdentifiseer is en 67 sonder CTV. Die rede vir laasgenoemde scenario is huidige onbekend. 'n Totaal van 76 moederbome is ook getoets vir die teenwoordigheid van Sitrus Viroïede. Almal het negatief getoets. Virusvrye bome in die genebron is geïndekeer vir appelstamgroef-virus (ASGV), sitrus psorosis virus (CPV) en sitrus impietratura siekte (CID). Enthout wat vanaf kwekers ontvang of versamel is, is op verskeie indikatorplante geïnkuleer en by optimale temperatuur in die glashuis gehou sodat siektesimptome kan ontwikkel indien dit teenwoordig is. Twee monsters is onderskeidelik vir sitrus viroïede en psorose virus getoets waarvan die resultate nog onbekend is.

### **Summary**

The framework of disease-free planting material in the Citrus Improvement Scheme is a diagnostic service for detection of disease causing agents, the elimination thereof, and the maintenance and distribution of healthy propagation material. Indexing, or the determination whether graft transmissible disease agents are present in plant material, is done mainly by means of biological indicator plants. Serological methods such as ELISA and Dot Blot are used to confirm pre-immunisation of CTV, while the latter technique is also used to determine the presence of Citrus Blight. Laboratory techniques (i.e. PCR) for indexing a range of citrus diseases are currently being optimised and will save a lot of time. Indexing for graft transmissible diseases is not only done on Citrus Improvement Scheme material, but also on material received from or collected at growers. It is necessary to determine the cause of the disease in order to do a meaningful recommendation. Specific virus-free plants are propagated in the glasshouse for each of the graft transmissible diseases to be indexed for. The mother trees at the Citrus Foundation Block were indexed to determine if severe CTV strains are present. A Total of 151 mother trees were indexed of which 8 were identified with a severe strain and 67 without CTV. A total of 76 mother trees were indexed for the presence of CTV's. All of them tested negative. Virus free trees in the gene source indexed for ASGV, CPV and CID. Budwood sent in by growers or collected during visits were budded to indicator plants and kept in the glasshouse at optimum temperatures according to the requirements for disease detection. Two samples were sent in by growers of which the results are still awaiting.

### **Introduction**

As with any commercial tree crop, citrus species are subjected to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria and in some cases, unknown organisms. The GTD affect the vigour, longevity of the trees, as well as the yield and quality of fruit. The framework of disease-free planting material

is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and is used in South Africa since 1977 (de Lange *et al.*, 1981). Mainly biological indexing is used for the detection of GTD in STG material while ELISA is used to confirm pre-immunisation (Roistacher, 1991). Since *Citrus tristeza virus* (CTV) and its vector, *Toxoptera citricida*, is endemic in South Africa, virus-free material should be protected by pre-immunisation with a suitable virus source (Müller & Costa, 1987). Currently three CTV sources are used in the southern African Citrus Improvement Scheme (CIS) depending on the scion material to be protected (von Broembsen & Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000). The STG and pre-immunisation procedures have been improved to suite South African conditions (Fourie & van Vuuren, 1993). Re-indexing to establish that mother material at the Citrus Foundation Block (CFB) remains free of graft transmissible diseases and that the pre-immunising agent remains mild is done on an annual basis for CTV and Huanglongbing (greening), bi-annually for Citrus viroids (CVd) and every 10 years for the other GTD. Indexing for GTD where growers have problems of disease infections is necessary to recommend proper control measures. Indexing includes biological as well as serological techniques (ELISA, Dot Blot, PCR) (Roistacher, 1991).

## Materials and methods

Virus-free indicator plants for the different graft transmissible diseases (GTD) are propagated from seed or clonally from virus-free material in an insect-free glasshouse and kept in stock until needed (except for Citrus leaf blotch virus and Citrus Variegated Chlorosis, which do not occur in South Africa, GTD are not seed transmissible). When budwood from the source to be indexed is received, two buds are budded on each of three indicator seedlings for each disease. Hereafter the plants are cut back to force new growth and kept in the glasshouse at a temperature required for symptom expression of the specific disease. Known positive and negative samples are included as controls. A minimum indexing time of 6 months is required for CTV, CVd's, Apple stem grooving virus (tatter leaf) (ASGV) and greening, while 12 months are required for Citrus psorosis virus (CPV) and Citrus impietratura disease (CID).

Field material is usually not suitable for the serological or molecular techniques (ELISA, PCR, s-PAGE, etc.), since the organisms are usually present in low concentrations or are poorly distributed, and therefore false results may be obtained. Such material is multiplied on suitable plants at optimal temperatures in the glasshouse and can be collected 3 months after inoculation for a specific test. Results can be obtained quicker and is a confirmation of the biological result.

When indexing is completed the client is informed of the result.

## Results and discussion

**A. Shoot tip grafted (STG) material:** After Citrus cultivars underwent STG they are initially indexed for CTV, ASGV and CVds (Table 4.2.2.1). Indexing for CPV and CID continues for an additional 6 months (Table 4.2.2.2). However, when a cultivar indexed negative for CTV, ASGV and CVds, which takes 6 months, pre-immunised budwood is supplied to the CFB to establish mother trees and the cultivar is introduced into the Nucleus Block source (Table 4.2.2.3). The presence of greening is continuously monitored since all the cultivars and selections, except the trifoliolate types, are self-indexed.

**Table 4.2.2.1.** Current status of STG'd plants indexed biologically for CTV, ASGV and CVds<sup>1</sup>.

Cultivar	Number of plants	CTV			ASGV			CVDs		
		+	-	±	+	-	±	+	-	±
Navel	11	0	6	5	0	0	11	0	4	7
Midseason	4	0	2	2	0	0	4	0	2	2
Mandarin	1	0	1	0	0	0	1	0	1	0
Clementine	3	1	1	1	0	0	3	0	0	3
Valencia	1	0	1	0	0	0	1	0	0	1
Grapefruit	1	0	0	1	0	0	1	0	0	1
Lemon	2	0	0	2	0	0	2	0	0	2
Ornamental citrus	6	2	3	1	0	0	6	0	0	6

<sup>1</sup> ± = awaiting results.

**Table 4.2.2.2.** Current status of STG'd plants indexed biologically for CPV and CID.

Cultivar	Number of plants	Results
Navel	3	Awaiting results
Midseason	2	Awaiting results
Valencia	1	Awaiting results
Reticulata	1	Awaiting results

**Table 4.2.2.3.** The number of new additions to the gene source that were pre-immunised with an approved CTV source.

Cultivars	Additions to nucleus Block	Pre-immunisation to be confirmed by ELISA	Pre-immunisation confirmed by ELISA	Budwood supplied to the CFB
Navel	8	5	3	3
Midseason	4	2	2	2
Valencia	1	0	1	1
Reticulata	1	0	1	1
Grapefruit	1	1	0	0
Clementine	2	2	0	0
Total	17	10	7	7

**B. Mother trees at the Citrus Foundation Block (CFB):** It is important that the CTV status of mother trees at the CFB remains stable. Therefore all mother trees should be indexed annually to establish the CTV severity in each mother tree. Because of limited glasshouse space, the mother trees are indexed bi-annually. The CTV status of 151 mother trees indexed during 2007 is presented in Table 4.4.2.4. The Mexican lime indicators showed the presence of severe CTV in 8 of the mother trees. It is suggested that these trees should be terminated as budwood sources. There is also evidence that 67 mother trees are CTV-free. These trees were all pre-immunised with the LMS 6 CTV source. The reason for this is unknown. At first, it was speculated that the source is sensitive to high temperatures, but glasshouse tests as well as the erratic occurrence of the virus in newly pre-immunised soft citrus, showed that the problem is not due to high temperature. It is possible that multiplication and movement of the LMS 6 source is restricted in some cultivars.

Similarly the mother trees should be re-indexed to assure that they are still CVd-free. The CVd status of 76 mother trees of 32 cultivars that were indexed during 2007 is presented in Table 4.2.2.5. The Etrog citron indicators showed no symptoms. Six months later another 120 mother trees of 27 cultivars as indicated in Table 4.2.2.6 were indexed. The final results of these are not available yet.

**Table 4.2.2.4.** CTV status of pre-immunised mother trees of different citrus cultivars at the CFB.

Cultivar	Number of mother trees	Number of trees with severe SP <sup>1</sup>	Number of trees with mild CTV	Number of trees negative for CTV
Clara	3	0	0	3
Gold Nugget	3	0	0	3
Tacle	3	0	0	3
<b>Total Mandarins</b>	<b>9</b>	<b>0</b>	<b>0</b>	<b>9</b>
Tarocco Scire (nuc)	3	0	2	1
Tarocco Tapi	4	0	1	3
Tarocco Scire	3	0	2	1
<b>Total Midseasons</b>	<b>10</b>	<b>0</b>	<b>5</b>	<b>5</b>
Lane Late California	6	0	3	3
Fukumoto	6	0	0	6
Gleo Ora Late	3	0	2	1
Royal Late	3	0	3	0
Lina	6	0	5	1
Cara Cara	6	0	3	3
NWI	6	1	3	2
<b>Total Navels</b>	<b>36</b>	<b>1</b>	<b>19</b>	<b>16</b>
Delta	6	2	4	0
Late	6	1	3	2

McClellan	6	0	4	2
Midknight	6	0	3	3
VG5	5	2	3	0
Bend 8A	3	0	3	0
Limpopo seedless	6	0	5	1
Rietspruit	6	0	0	6
Du Roi	6	0	3	3
VBE2	6	0	4	2
<b>Total Valencias</b>	<b>56</b>	<b>5</b>	<b>32</b>	<b>19</b>
Miho Wase	6	0	2	4
<b>Total Satsumas</b>	<b>6</b>	<b>0</b>	<b>2</b>	<b>4</b>
Tardif de Janvier II	3	0	0	3
Oronules	3	0	0	3
Nules	6	0	0	6
Primosole	6	0	4	2
<b>Total Clementines</b>	<b>18</b>	<b>0</b>	<b>4</b>	<b>14</b>
Nartia	6	2	4	0
<b>Total Grapefruit</b>	<b>6</b>	<b>2</b>	<b>4</b>	<b>0</b>
<b>Grand Total</b>	<b>151</b>	<b>8</b>	<b>76</b>	<b>67</b>

\* Stem pitting.

**Table 4.2.2.5.** Citrus viroid status of pre-immunised mother trees of different cultivars at the CFB.

Cultivar	Number of selections	Number of mother trees	Results
Mandarin hybrid	6	12	Negative
Midseasons	4	9	Negative
Navels	9	23	Negative
Valencias	2	8	Negative
Satsumas	1	3	Negative
Clementines	4	9	Negative
Grapefruit	6	12	Negative
<b>Total</b>	<b>32</b>	<b>76</b>	<b>All negative</b>

**Table 4.2.2.6.** Pre-immunised mother trees of different cultivars at the CFB currently under index for CVd's.

Cultivar	Number of selections	Number of mother trees	Results
Mandarin hybrid	3	9	await results
Midseasons	3	9	await results
Navels	6	28	await results
Valencias	9	44	await results
Satsumas	1	6	await results
Clementines	4	18	await results
Grapefruit	1	6	await results
<b>Total</b>	<b>27</b>	<b>120</b>	

**C. Nucleus Block:** Indexing of a large number of cultivars and selections for ASGV, CPV and CID were outstanding. The ASGV indexing takes 6 months and the 80 cultivars in Table 4.2.2.7 were all negative for the disease. The CPV and CID indexing of 54 cultivars in Table 4.2.2.8 are continuing.

**Table 4.2.2.7.** The number of selections in the Nucleus Block that were indexed for ASGV.

Cultivars	Number of selections indexed	Indexing Result
Navels	11	Negative
Midseasons	14	Negative
Valencias	14	Negative
Reticulatas	12	Negative
Grapefruit	1	Negative
Lemons	4	Negative
Clementines	11	Negative
Fortunella	2	Negative
Pommelit	5	Negative

Satsumas	6	Negative
<b>Total</b>	<b>80</b>	

**Table 4.2.2.8.** The number of Nucleus Block plants that were indexed for CPV and CID.

<b>Cultivars</b>	<b>Number of selections indexed</b>	<b>Indexing Result</b>
Navels	9	awaiting results
Midseasons	14	awaiting results
Valencias	10	awaiting results
Reticulatas	7	awaiting results
Grapefruit	1	awaiting results
Lemons	2	awaiting results
Clementines	6	awaiting results
Pommelit	1	awaiting results
Satsumas	4	awaiting results
<b>Total</b>	<b>54</b>	

**D. Change of the cross-protecting source from LMS 6 to GFMS 12 for soft Citrus:** Problems were encountered with the pre-immunisation of soft citrus with the LMS 6 source. Re-indexing (ELISA and biological) indicated that LMS 6 is not a suitable CTV source to pre-immunise soft citrus. At the CIS meeting in 2006 it was agreed that a change should be made to another CTV source that is compatible with mandarin types. Currently a glasshouse trial has been initiated to evaluate additional CTV sources. This will take a year whereafter a field trial can be initiated that will take at least 8 years to get a definitive answer. During August 2007 it was approved that GFMS 12 would be re-introduced as the pre-immunising source until a new suitable CTV pre-immunising source for soft citrus has been identified. Marisol Clementine trees with the GFMS 12 source performed well at Addo Research station over an 8-year period.

Pre-immunisation of the GFMS 12 source has been initiated and 8 Clementine, 4 Satsuma and 5 Reticulata selections were inoculated. Positive pre-immunisation by ELISA has not been confirmed.

**E. Cross protection of soft citrus:** The difficulty to pre-immunise soft citrus with LMS 6 has been reported in 2006 at the Cultivar Committee Meeting. An instruction was received to investigate the matter and also to find an alternative CTV source to pre-immunise soft citrus. A glasshouse trial is currently in progress. Four new CTV sources are evaluated as cross-protecting agents for soft citrus. After inoculation of the CTV sources, ELISA was done at intervals to assess the movement and titre of the CTV sources. During the first assessment 106 samples were tested. A new proposal for funding of this research will be submitted in 2008.

**F. General indexing:** Citrus material that was sent in by growers or collected during visits, was indexed for specific diseases. One sample from a Citrusdal grower is being indexed for CPV and a second sample from Mistkraal nursery are indexed for CVd.

## **Conclusion**

The diagnosis of GTD is a continuous service and results are reported to the parties involved.

## **Future Research**

- Annual re-indexing of mother trees at the CFB (every year for CTV severity and every third year for CVd).
- Indexing of STG plants for CTV, CVd, CPV, ASGV and CID.
- Indexing of suspected budwood from growers and institutions using ELISA, PCR, Dot Blot and biological indicators.

## **Technology transfer**

None.

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#### 4.2.3 PROGRESS REPORT: Citrus virus-free gene source

Experiment 790 (2005 - 2025) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

#### Opsomming

Groeipunt enting word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene. Virusvrye boompies van verskillende cultivars en seleksies word in 'n insekvrue tunnel by CRI bewaar. Sewe nuwe seleksies is ingedien vir GPE deur verskillende kliente en 'n verdere 35 is in die pyplyn vir GPE. Virusvrye materiaal word gepreïmuniseer met 'n toepaslike STV bron voordat dit vrygestel word aan die Grondvesblok by Uitenhage. Die virusvrye bron bestaan tans uit 235 cultivars en seleksies.

#### Summary

Shoot tip grafting is used to eliminate graft transmissible pathogens from citrus material. Seven new selections were introduced by various clients and a further 35 are in the pipeline. Virus-free material is pre-immunised with a suitable CTV isolate before it is supplied to the Citrus Foundation Block at Uitenhage. Virus-free trees of different cultivars and selections are maintained in an insect-free tunnel at CRI. The nucleus block has a total of 235 virus free cultivars and selections.

#### Introduction

The overall objective of the southern African Citrus Improvement Scheme is to enhance the productivity of the industry by making the highest quality propagation material available. Graft transmissible diseases (GTD) have detrimental effects on the growth and production of citrus trees since they are responsible for stunting, decline and small fruit. Shoot tip grafting (STG) is the standard method for the elimination of pathogens (Navarro *et al.*, 1975). Some pathogens are more difficult to eliminate and heat therapy should be incorporated with the STG process (Roistacher, 1977). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). Some cultivars and selections of the virus-free gene source at the ARC-ITSC have been duplicated at CRI Nelspruit as a safety measure. Shoot tip grafting (STG) facilities were established at CRI and new virus-free cultivars and selections will be added to the gene source after STG and indexing.

#### Materials and methods

*In vitro* cultured rootstocks: The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the seed coat of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile distilled water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige & Skoog, 1962). Germination takes place at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4°C in darkness.

Scion preparation: Method 1; buds of the plant that should go through STG (source plant) are budded on a standard rootstock in the glasshouse. After the buds have grown and matured (approximately 3–4 months), the source plant is defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested

and surface sterilised on a flow bench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile distilled water. Method 2; bud sticks from the source plant are cut in 50 mm lengths and surface sterilised by immersion for 10 minutes in 1% NaOCL containing a wetting agent. After 3 rinses in sterile distilled water the bud sticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32°C and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

**STG:** The seedling rootstock is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth point with primordia is placed on the horizontal cut of the incision on the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28°C exposed to 16 h light/day.

**STG plant care.** The shoot tip will start growing 3 to 4 weeks after STG. The growing shoot tip is micro-grafted with the seedling rootstock onto a vigorous-growing virus-free rootstock in the glasshouse. After micro-grafting, it is closed by a plastic bag for 8 days.

**Virus indexing.** Elimination of graft transmissible pathogens is confirmed by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Virus-free plants are multiplied and kept in an aphid-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunised (van Vuuren and Collins, 1990) with suitable CTV sources prior to release to the Citrus Foundation Block at Uitenhage.

## Results and discussion

STG:

Seven new cultivars and selections were submitted by clients and STG initiated (Table 4.2.3.1). Unsuccessful STG from previous years has been repeated (Table 4.2.3.1). Thirty successful STGs have been indexed of which 15 were negative, 5 were found positive and of 10 the results are outstanding (Table 4.2.3.2). Seven plants that were found negative for CTV, CVd and ASGV are currently being indexed for CPV and CID (Table 4.2.3.3). Seven cultivars that were found free of CTV, CVd and ASGV were pre-immunised successfully and budwood was supplied to the CFB (Table 4.2.3.4).

**Table 4.2.3.1.** Cultivars and selections in the pipeline for graft transmissible disease elimination by STG.

Cultivar	Previous introductions (2004)	Previous introductions (2005)	Previous introductions (2006)	New introductions (2007)
Clementine	3	-	3	-
Grapefruit	-	-	1	-
Lemon	-	-	-	1
Midseason	2	1	-	-
Navel	2	6	4	4
Reticulata	1	1	-	1
Valencia	1	3	1	1
Ornamental	-	-	6	-
<b>Total</b>	<b>9</b>	<b>11</b>	<b>15</b>	<b>7</b>

**Table 4.2.3.2.** STG'd plants indexed biologically for CTV, ASGV and CVds.

Cultivar	Number of plants	Negative	Positive	Awaiting result
Navel	11	7	1	3
Midseason	4	2	-	2
Mandarin	1	1	-	-
Clementine	3	-	1	2
Valencia	1	1	-	-
Grapefruit	2	-	1	1

Lemon	2	-	-	2
Ornamental citrus	6	4	2	-
<b>Total</b>	<b>30</b>	<b>15</b>	<b>5</b>	<b>10</b>

**Table 4.2.3.3.** STG'd plants indexed biologically for CPV and CID.

<b>Cultivar</b>	<b>Number of plants</b>
Navel	3
Midseason	2
Valencia	1
Reticulata	1
<b>Total</b>	<b>7</b>

**Table 4.2.3.4.** The number of new additions to the gene source where pre-immunised material with an approved CTV source was submitted to the CFB.

<b>Cultivars</b>	<b>Budwood supplied to the CFB</b>
Navel	3
Midseason	2
Valencia	1
Reticulata	1
<b>Total</b>	<b>7</b>

Maintaining the virus-free gene source. As limited space was available to maintain the virus-free gene source, a new tunnel was built at CRI-Nelspruit. The tunnel was completed and all virus-free plants were moved into this facility. Prior to the move, all plants were thoroughly treated with a broad-spectrum insecticide to prevent possible virus transmission. The cultivars and the number of selections of each cultivar are listed in Table 4.2.3.5. New additions are made continuously when STG plants test virus-free (Table 4.2.3.4). Some of the older selections have become pot-bound, and will be replanted in due course.

**Table 4.2.3.5.** The number of virus-free selections of different cultivars maintained at the Nucleus Block at CRI.

<b>Cultivar</b>	<b>Number of selections</b>
Clementine	23
Diverse (Citron, Sour orange, etc.)	2
Ellendale	4
Grapefruit	17
Kumquat	1
Lemon	20
Lime	4
Midseason	23
Navel	40
Pummelo	7
Reticulata	32
Rootstock	20
Satsuma	8
Valencia	38
<b>Total</b>	<b>235</b>

Indexing: Indexing of some cultivars and selections for ASGV, CPV and CID was outstanding. The ASGV indexing for 80 cultivars and selections are completed and they were all negative for the disease. The CPV and CID indexing of 54 cultivars and selections are continued.

## Conclusion

- During the reporting year, 7 new selections were added to the virus-free source. Additions to the source are a continuous process.
- Seven selections were received for the elimination of GTD and are in the process of STG.
- The outstanding indexing for ASGV, CPV, and CID is nearly completed.

## Future research

- Apply STG and indexing on the current introductions by CRI and clients.
- Receive, establish and maintain new additions.
- Maintain the virus-free source in the insect-free tunnel.
- Pre-immunise virus-free sources that are required by the Citrus Foundation Block.
- Introduce new information to the data-base.

## Technology transfer

None.

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### 4.2.4 PROGRESS REPORT: Dynamics of *Citrus tristeza virus* mild and severe strains in mild strain cross-protection strategies

Experiment 885 (2007 – 2010) by Katherine Stewart and Gerhard Pietersen (CRI-UP)

## Opsomming

In hierdie studie word die oorsake van die onvermoë wat soms voorkom in die strategie van *Sitrus tristeza virus* (CTV) milde ras, kruis-beskerming op pomelos in Suid-Afrika ondersoek. Vorige studies het gewys dat die milde bron van CTV wat algemeen gebruik word vir kruis-beskerming van pomelos (GFMS 12 bron), 'n strawwe ras komponent bevat. Om die virus populasie van GFMS 12 ten volle te karakteriseer, is PKR metodes waarvan twee gemik is teen 'n gekonserveerde area en twee teen 'n variërende area van die genoom, gevestig en ge-optimeer. Amplikone verkry vanaf die variërende areas is ge-kloneer, en die sekvens daarvan is bepaal. Daar is gevind dat drie hoof sekvens variante (rasse) met verskeie kleiner variante van CTV voorkom. Om te bepaal of die kruisbeskerdings falings verband hou met veranderings in die GFMS 12 mild ras populasie, moet suiwer isolate van die rasse verkry word en dan onder gekontroleerde toestande saam geïnkuleer word om die dinamiek tussen die rasse te kan bepaal. Sulke isolasie is gedoen met 550 enkel plantluis oordragings waarvan daar ten minste vyf suiwer bronne verkry is. Daar word ook ondersoek of wilde-tipe CTV rasse die GFMS 12 milde bron kruis-beskerming kan oorkom in die veld. GFMS 12 geïnkuleerde plante wat nogtans strawwe simptome wys is vanaf Malelane en Nelspruit versamel en gevestig op Meksikaanse lemmetjie, nadat vestiging van bronne deur beworteling van lote nie suksesvol was nie. Die virus populasie binne hierdie bronne sal ten volle gekarakteriseer word deur virus klonering en nukleotied volgorde bepalings.

## Summary

This study attempts to gain more insight into the cause of mild strain cross-protection failure in grapefruit in South Africa. The viral population of commonly used citrus tristeza virus grapefruit mild strain 12 (GFMS 12) has been shown to contain severe variants. In order to characterise the GFMS 12 viral population fully, PCR systems targeting 2 conserved and 2 variable regions of the viral genome were established and optimised. The amplicons from the variable regions were cloned and sequenced and shown to consist of 3 main sequence types with numerous minor variants. To determine whether the dynamics of CTV strains within the GFMS 12 source are altered under different conditions, resulting in cross protection failure, it is necessary to isolate the individual variants within the population in pure form and then re-inoculate them under different controlled conditions to study the dynamics between the two. Isolation of variants was done during the past year using 550 single aphid transmissions, with at least 5 putatively pure sources being obtained. An alternate cause of mild strain protection failure is also being studied, namely whether wild-type CTV sources overcome GFMS 12 protection. To determine this, samples have been collected from Malelane and Nelspruit areas of grapefruit trees showing severe symptoms after cross-protection breakdown of GFMS 12 and budded onto Mexican lime plants, after establishment by rooting of cuttings failed. Virus populations in these sources will be characterised through cloning and sequencing.

## Introduction

*Citrus tristeza virus* (CTV) is an aphid-borne closterovirus; and has ranked as one of the most important citrus diseases for the last 60 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 South African Citrus Improvement Scheme (CIS) all citrus is pre-immunised with one of a few mild strain population sources of CTV. These protect the plants in most cases from infection by severe forms of CTV. However, occasionally trees with 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 to severe forms within the mild population or; 4) selection, under specific host and environmental conditions for severe forms inherently present in the population; or 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 possibly the introduction of BrCA 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-immunised grapefruit trees in South Africa (van der Vyver *et al.*, 2002). Certain grapefruit cultivars pre-immunised with South African CTV sources GFMS (Grapefruit mild strain) 12 and GFMS 35 exhibited changes in the level of protection conferred by producing high percentages of small fruit (van der Vyver *et al.*, 2002). These sources were biologically evaluated on CTV sensitive plants and examined by SSCP analysis (van der Vyver *et al.*, 2002). The biological data indicated that the cross-protecting source had not retained their original status since a seedling yellows (CTV-SY) as well as severe stem-pitting components were recorded (van der Vyver *et al.*, 2002). Other strains were also found in infected trees, which were not part of the original pre-immunising source (van der Vyver *et al.*, 2002). It was postulated that super-infection occurred by other CTV strains introduced (van der Vyver *et al.*, 2002). There was no evidence in the SSCP patterns that segregation of strains within the original pre-immunising sources occurred. The SSCP patterns of the additional severe strains introduced did not correspond to the SSCP profiles of the strains within the cross-protecting sources. 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 sources, 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 CTV symptom expression and the benefits of mild strain protection (Broadbent *et al.*, 1991). 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-immunised with mild strains showed no deterioration or 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).

In this study, we attempt to understand the cause of GFMS 12 mild strain cross-protection failure in grapefruit in South Africa. Each of the potential causes discussed above will be assessed.

## Materials and methods

### *Single aphid transmissions (SAT's):*

Aphids (*Toxoptera citricida*) were allowed a 24-hour acquisition access period on a GFMS 12 infected source plant. Individuals were carefully transferred in leaf cages to Mexican lime seedlings and allowed a 24-hour inoculation access period, where after they were killed, and the seedlings maintained in an insect-free glasshouse. CTV ELISA was done after 8 weeks to confirm CTV infection. SAT sub-isolates 12-5, 12-7, 12-9 of GFMS 12 obtained from S.P. van Vuuren were subjected to further round of aphid transmission using 20 individuals. ELISA tests must still be conducted on these to confirm the transmission of the virus.

### *Severe forms of CTV:*

Field sources infected with severe strains were budded onto Mexican lime.

### *PCR systems:*

Four PCR systems have been optimised to target 2 variable regions in ORF 1a (Rubio *et al.*, 2001) and 2 conserved regions: Coat protein gene (Huang *et al.*, 2004) and the p23 gene (Sambade *et al.*, 2002).

### *Cloning of variable regions of GFMS 12 source:*

Amplicons from two variable regions of ORF1a of the GFMS 12 source were amplified and cloned into pGEM-T Easy vector system (Promega, USA). Thirty clones of each of the 2 regions were selected and sequenced in the forward and reverse directions. Phylogenetic analyses were done using BioEdit and Mega 3.1 software.

## Results and discussion

### *Single aphid transmissions:*

A total of 550 single aphid transmissions (SAT's) of the GFMS 12 source plant have been completed. Of the 550 SATs completed, 450 have been tested with ELISA and results have shown that 5 plants are infected with CTV, with a further 3 showing a very low titer, which would need to be re-tested once the plants are older. Sub-isolates 12-5, 12-7, 12-9 of GFMS 12 have undergone a round of 20 aphid transmissions each to further separate CTV into homogenous forms. ELISA results still have to confirm infection. Performing them in a slightly cooler more sheltered greenhouse-space, increased the efficiency of the aphid transmission process. This has prevented aphid deaths at the steps of feeding on the infected source as well as transmission to virus-free seedlings.

### *Severe forms of CTV:*

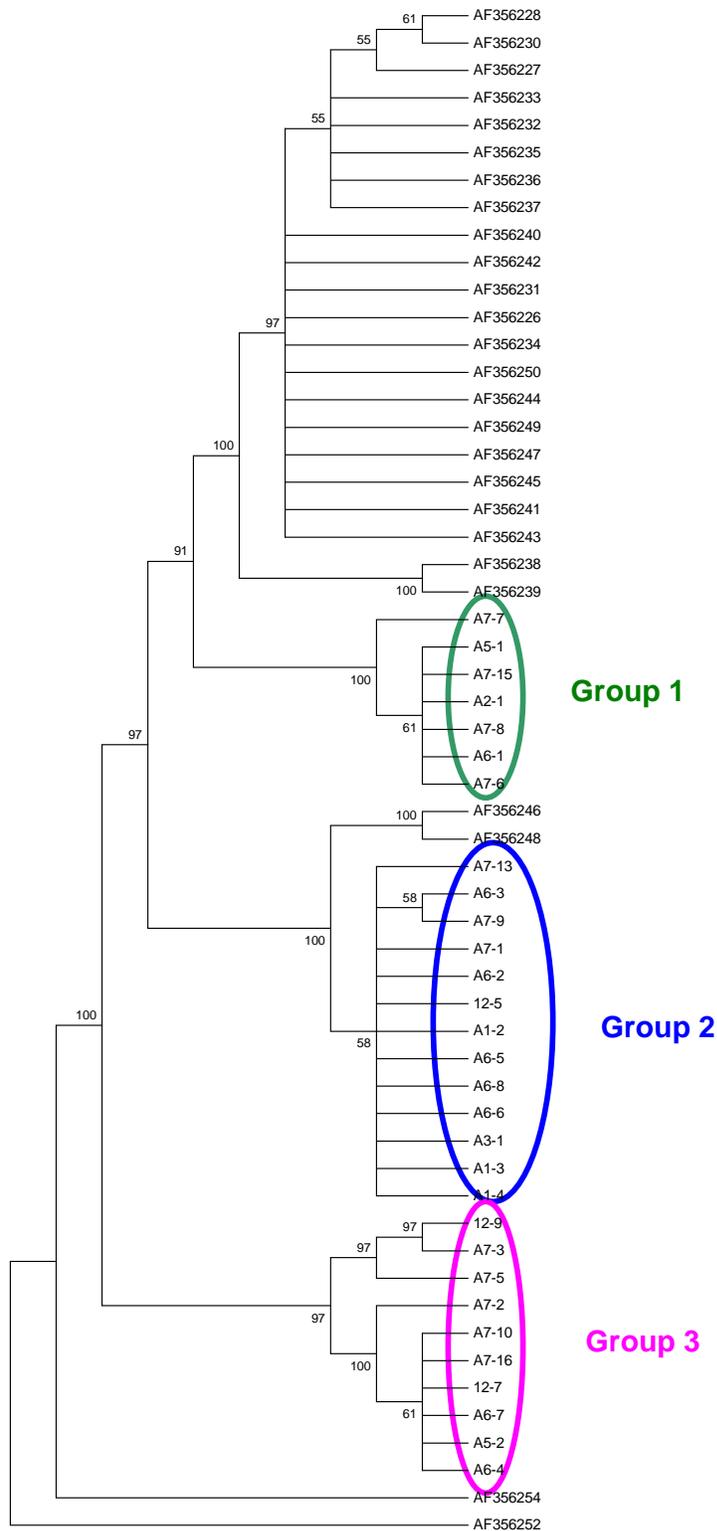
Field sources were collected in the Malelane and Nelspruit areas. Trees showing cross-protection breakdown as well as stem pitting and reduced fruit size of GFMS 12 on Marsh and Star Ruby grapefruit were sampled and budded onto Mexican lime, after initial attempts to establish the plants through rooting failed. ELISA tests must still be conducted to confirm CTV infection.

### *Cloning of GFMS 12 source:*

Amplicons from two variable regions of GFMS 12 were amplified and cloned. Thirty clones of each of the 2 regions were selected and sequenced. Region A has shown 3 main groups of variants (Fig. 4.2.4.2) with various minor variants. This region has shown more variability than region F. Sequences shown in the 3 groups from GFMS 12 population, are clearly distinct from isolates sequenced from Spain and California. Region F has shown only 2 distinct groups of variants (Fig. 4.2.4.1). The GFMS 12 population has at least 8 variants selected with cloning. There could be other variants not selected with cloning that were in a low titer within the plant but could still represent important variants. Sub-isolates of GFMS 12 referred to as 12-5, 12-7 and 12-9 were identical to variants found with cloning (Fig. 4.2.4.2).



**Figure 4.2.4.1.** Neighbourhood phylogenetic tree of Region F constructed using Mega 3.1. Isolates from GFMS 12 are indicated in circles.



**Figure 4.2.4.2.** Neighbourhood phylogenetic tree of Region A constructed using Mega 3.1. Isolates from GFMS 12 are indicated in circles. The accession numbers of Genbank reference isolates are included.

*PCR systems:*

The CTV p23 gene-specific PCR (Sambade *et al.*, 2003) protocol has been further optimised. Changes include; an annealing temperature of 57 or 55°C, 1.5 mM MgCl<sub>2</sub>, increased amounts of cDNA used as template in PCR (10 ul) and 35 cycles of denaturation, annealing and elongation. This has now allowed the successful amplification of samples with which limited success was obtained previously. The Coat protein gene-specific PCR protocol of Huang *et al.* (2004), capable of detecting all CTV sources has been

established. It is currently working on DNA clones (T36 and T30 strains) and CTV infected plant samples. Two primer sets (Rubio *et al.*, 2001) targeting two variable regions of 528 bp and 438 bp respectively of the 5' end of the CTV genome have been optimised. There are now 4 PCR systems targeting 2 variable and 2 conserved regions available for use in this study.

## Conclusion

Initial infrastructural tasks are being conducted in order to conduct experiments regarding the dynamics of strains within the mild strain cross-protection population. The very low efficiency of the aphid *Toxoptera citricida* single aphid transmission (SAT's) requires that large numbers of SAT's need to be performed, in order to ensure that pure sources of GFMS 12 viral components are obtained for further studies. A second round of aphid transmission on the sub-isolates is also planned. Obtaining these pure sources of CTV strains is the rate-limiting step in this study, and studies on the dynamics of the strains cannot be conducted until this has been achieved. In subsequent serial aphid transmission tests the use of more than one individual to increase the efficiency of transmission may be considered. The establishment and optimisation of 4 PCR systems has enabled partial characterisation of the original GFMS 12 source to take place. Cloning of 2 variable regions of GFMS 12 has shown 3 major sequence types with a number of minor variants, confirming the heterogeneous nature of this mild strain cross protecting source. Samples of plants, pre-immunised with GFMS 12 but nevertheless showing severe symptoms, i.e. instances of mild strain cross-protection failure, have been taken from Mpumalanga and budded onto Mexican lime plants. Cloning of a severe source showing cross-protection breakdown of GFMS 12 will determine whether additional strains of CTV to the GFMS 12 population occur in these plants.

## Further objectives (milestones) and work plan

- Perform 200-400 additional single aphid transmissions from GFMS 12 sources.
- Clone and characterise isolates derived from sources showing severe symptoms but originally pre-immunised with GFMS 12 (April – July 2008).
- Partially and fully sequence selected isolates from single aphid transmissions performed on GFMS 12 (April – Dec 2008).
- Design primers for quantitative (real-time) PCR to detect specific strains obtained (April – Sept 2008).
- 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 (July - Sept 2009).

## Technology transfer

- Talk was presented at the UP Plant Pathology's citrus discussion group in Feb 2008.
- Defence of PhD proposal done at UP on 9<sup>th</sup> April 2008.
- Oral presentation at the Molecular Cell Biology Group (MCBG) conference - 17<sup>th</sup> Oct 2007.

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#### 4.2.5 **PROGRESS 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) and B.Q. Manicom (ITSC)

#### **Opsomming**

Nege-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red op Swingle citrumelo onderstam, het baie eenvormig gereageer met vier ligte *Citrus tristeza virus* (CTV) bronne (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskerdings-agente. Bome met die vier bronne verskil nie betekenisvol van mekaar ten opsigte van boom grootte, produksie (kg/boom), produksie doeltreffendheid (kg/m<sup>3</sup> kroon volume), kumulatiewe produksie oor 'n tydperk van drie jaar, oes waarde (die produksie van klein vrugte) of die voorkoms van stamgleuf nie. Bome wat met die ligte CTV bronne gepreïmmuniseer was, is in alle opsigte betekenisvol beter as bome wat met 'n bekende strawwe CTV bron gepreïmmuniseer was.

#### **Summary**

Nine-year-old trees of seven red grapefruit selections, viz. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben and Oran Red reacted very similar to four mild CTV sources (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as cross-protecting agents. Trees with these four sources did not differ significantly from each other regarding growth, yield (kg/tree), yield efficiency (kg/m<sup>3</sup> canopy volume), cumulative production over a period of 3 years, crop value (production of small fruit) or the occurrence of stem pitting. Trees that were pre-immunised with these mild CTV sources were in all respects significantly better than trees that were pre-immunised with a known severe CTV source.

#### **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 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 (da Graça *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 (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 50-year-old Nartia (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 on an annual basis 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 showed the presence of a severe strain in the original source and that segregation of the strains, where the severe strain became dominant, which might be 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 no trees older than 15 years existed 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 is to evaluate new CTV sources in different red grapefruit selections.

## Materials and methods

Seven red grapefruit selections *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 are evaluated in each scion and compared to the standard (GFMS 12) and a severe source (GFSS 5). ELISA confirmed infection before they were planted in a randomised split plot with five replications at Malelane during December 1998.

The following data are taken annually: 1) Tree sizes are 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 mean radius of the tree and H = the height of the fruit bearing part; 2) The fruit are harvested, graded into the different export sizes and weighed; 3) Tree health is rated according to the occurrence of stem pitting and decline. A rating of 1 is given when the trunk appears smooth and no twig decline occurs; rating 2 when occasional mild dents occur on the trunk with no twig dieback; rating 3 is when moderate pitting occurs all over the trunk and no twig decline; rating 4 when many pits (large and/or small) occur on the trunk and decline starts; and rating 5 when severe pitting occurs and severe decline occurs.

The following calculations are 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 ( $\text{kg/m}^3$  canopy volume) is calculated; 3) The average value of the crop per tree in relation to fruit size is determined by calculating the average value per 15kg export box for each size over a period of ten years. The highest price equals a value of 10 while the other values are calculated accordingly.

## 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 4.2.5.1, Table 4.2.5.2, Table 4.2.5.3, Table 4.2.5.4, Table 4.2.5.5 and Table 4.2.5.6, respectively.

Tree size: Overall, canopy volumes of trees that were pre-immunised with the different mild sources did not differ from each other but they were significantly larger than the trees inoculated with a severe source. Of the selections, trees of Nel Ruby, Flame and Ruben were the largest and were significantly larger than the Henderson and Oran Red trees. The Oran Red trees were the smallest and it is possible that Oran Red has a genetic dwarfing characteristic (Table 4.2.5.1). The results show some interactions between selections and some of the mild CTV sources (Rio Red with GFMS 12 and GFMS 73). It also appears that Ruben has some tolerance to CTV since the severe source affected tree size to a lesser extent (Table 4.2.5.1).

Production: Trees with the different mild CTV sources produced very similar. There is only 7kg difference between the highest yield (trees with GFMS 73) and the lowest (trees with GFMS 35). With the grapefruit selections, the yield of Rio Red, Nel Ruby, Flame and Ruben did not differ from each other and was significantly better than that of Henderson and Oran Red. The production of Star Ruby was between these two groups (Table 4.2.5.2). The body of the table shows only three cases where the production was significantly negatively affected by mild CTV sources; Rio Red with GFMS 12, Henderson with GFMS 35 and Ruben with GFMS 73. This may be a normal seasonal fluctuation.

The yield efficiency of the trees with the different mild CTV sources did not differ from each other (Table 4.2.5.3). 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 (see tree size of Oran Red cultivar, Table 4.2.5.1) and vigour where it is decreased (see large trees of Nel Ruby and Ruben in Table 4.2.5.1).

The cumulative yield over the last 3 years shows no difference between trees that were pre-immunised with the four mild CTV sources (Table 4.2.5.4). They were significantly better than trees with the severe source (GFSS 5). Of the selections, Nel Ruby and Flame trees produced significantly better than the Star Ruby, Henderson and Oran Red trees. The lower production of the latter two selections may be ascribed to the smaller tree size (Table 4.2.5.1). The lower production of the Star Ruby trees may be due to slightly lower yield efficiency (Table 4.2.5.3).

Apart from tree life, the symptom that makes CTV such an important disease is the reduction in fruit size that has a direct affect on the market value. Table 4.2.5.5 shows the calculated market value of the 2007 crop. There was no significant difference between trees that were pre-immunised with the different mild CTV sources but they all had significantly higher market values than the trees 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. To a lesser extent, the crop value was also reduced in Flame by GFMS 35, and Ruben by GFMS 73.

**Stem pitting:** Overall the stem pitting did not differ among trees pre-immunised with the different mild CTV sources (Table 4.2.5.6). The Ruben trees had the least stem pitting and displayed some tolerance to CTV. Generally the trunks of the trees are smooth with occasional pits.

**Table 4.2.5.1.** Tree size (canopy volume = m<sup>3</sup>) of 9-year-old red grapefruit selections that were pre-immunised with different CTV sources.

Grapefruit selections	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	26.4 ab	34.9 a	27.5 ab	26.5 ab	20.7 b	<b>27.2 xy</b>
Rio Red	23.6 bc	35.0 a	30.1 ab	26.2 b	16.4 c	<b>26.3 xy</b>
Henderson	25.8 a	21.5 a	21.7 a	24.1 a	21.1 a	<b>22.8 yz</b>
Nel Ruby	35.6 a	35.9 a	32.7 a	37.5 a	19.0 b	<b>32.1 w</b>
Flame	33.5 a	24.5 ab	28.9 ab	33.6 a	21.3 b	<b>28.4 wx</b>
Ruben	39.7 a	34.2 ab	32.6 ab	29.9 ab	26.7 b	<b>32.6 w</b>
Oran Red	18.8 a	20.4 a	22.3 a	21.4 a	15.9 c	<b>19.8 z</b>
Mean	<b>29.1 u</b>	<b>29.5 u</b>	<b>28.0 u</b>	<b>28.5 u</b>	<b>20.2 v</b>	

Figures in each row 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 4.2.5.2.** Production (kg/tree) of 9-year-old red grapefruit selections that were pre-immunised with different CTV sources.

Grapefruit selections	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	197.4 a	208.2 a	206.1 a	186.9 a	108.2 b	<b>181.3 xy</b>
Rio Red	198.8 b	244.7 a	213.2 ab	217.9 ab	143.5 c	<b>203.6 x</b>
Henderson	193.9 a	140.7 b	182.6 a	192.7 a	165.7 ab	<b>175.1 y</b>
Nel Ruby	191.0 ab	214.2 ab	209.5 ab	236.1 a	156.9 b	<b>201.6 x</b>
Flame	227.3 a	178.5 a	194.7 a	230.8 a	175.3 a	<b>201.3 x</b>
Ruben	226.0 a	207.8 ab	200.8 ab	162.9 b	202.0 ab	<b>199.9 x</b>
Oran Red	163.5 ab	174.0 ab	193.6 ab	185.1 ab	142.4 b	<b>171.7 y</b>
Mean	<b>199.7 u</b>	<b>195.5 u</b>	<b>200.1 u</b>	<b>202.8 u</b>	<b>156.3 v</b>	

Figures in each row 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 4.2.5.3.** Yield efficiency (kg/m<sup>3</sup> canopy) of 9-year-old red grapefruit selections that were pre-immunised with different CTV sources .

Grapefruit selections	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	7.8 a	6.6 a	7.6 a	7.8 a	5.6 a	<b>7.1 yz</b>
Rio Red	8.6 ab	7.1 b	7.2 ab	8.6 ab	8.9 a	<b>8.1 y</b>
Henderson	7.5 a	6.8 a	8.7 a	8.6 a	8.5 a	<b>8.0 y</b>
Nel Ruby	5.4 b	6.1 b	6.4 b	6.5 b	8.9 a	<b>6.7 z</b>
Flame	7.0 ab	7.6 ab	6.7 b	7.2 ab	8.4 a	<b>7.4 yz</b>
Ruben	5.8 b	6.1 ab	6.5 ab	5.8 b	7.8 a	<b>6.4 z</b>
Oran Red	10.3 a	8.8 a	8.7 a	8.6 a	9.4 a	<b>9.2 x</b>
<b>Mean</b>	<b>7.5 uv</b>	<b>7.0 v</b>	<b>7.4 uv</b>	<b>7.6 uv</b>	<b>8.2 u</b>	

\* Figures in each row 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 4.2.5.4.** Cumulative yield over a 3-year period of 9-year-old red grapefruit selections that were pre-immunised with different CTV sources .

Grapefruit selections	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	551.8 ab	492.8 b	586.7 a	571.5 ab	376.6 c	<b>515.9 yz</b>
Rio Red	577.6 a	638.2 a	585.7 a	678.4 a	404.5 b	<b>576.9 wx</b>
Henderson	541.3 a	440.0 b	599.3 a	561.9 a	463.7 b	<b>521.2 xyz</b>
Nel Ruby	558.5 ab	642.6 a	618.5 ab	646.5 a	464.1 b	<b>586.0 w</b>
Flame	625.4 a	516.7 b	607.8 ab	635.2 a	513.6 b	<b>579.8 w</b>
Ruben	646.4 a	591.9 ab	596.2 ab	453.4 c	494.9 bc	<b>556.6 wxy</b>
Oran Red	462.7 bc	511.7 ab	542.1 a	549.2 a	414.7 c	<b>796.1 z</b>
<b>Mean</b>	<b>566.2 u</b>	<b>547.7 u</b>	<b>590.9 u</b>	<b>585.2 u</b>	<b>447.5 v</b>	

\* Figures in each row 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 4.2.5.5.** The effect of different CTV sources on the average crop value per tree for the 2007 season of red grapefruit selections .

Grapefruit selections	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	106.7 a	116.9 a	122.0 a	108.4 a	51.4 b	<b>101.1 xyz</b>
Rio Red	104.4 b	135.1 a	110.7 ab	123.8 ab	61.9 c	<b>107.2 wxy</b>
Henderson	107.7 a	77.4 bc	90.9 abc	103.0 ab	72.2 c	<b>90.2 z</b>
Nel Ruby	107.3 ab	124.0 a	128.4 a	127.3 a	72.8 b	<b>111.9 wx</b>
Flame	134.8 a	96.3 bc	111.6 abc	123.0 ab	80.3 c	<b>109.2 wx</b>
Ruben	141.5 a	125.6 ab	122.5 ab	102.0 b	110.8 ab	<b>120.5 w</b>
Oran Red	82.2 b	90.9 ab	108.8 a	108.3 a	68.7 b	<b>91.8 yz</b>
<b>Mean</b>	<b>112.1 u</b>	<b>109.5 u</b>	<b>113.6 u</b>	<b>113.7 u</b>	<b>74.0 v</b>	

\* Figures in each row 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 4.2.5.6.** The effect of different CTV sources on stem pitting rating\*\* of 9-year-old red grapefruit selections .

Grapefruit selections	CTV sources					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	2.3 ab	2.2 ab	1.8 a	2.5 ab	3.4 b	<b>2.4 y</b>
Rio Red	2.6 ab	2.2 a	2.1 a	2.6 ab	3.1 b	<b>2.5 y</b>
Henderson	2.0 a	2.2 a	2.9 a	2.5 a	2.3 a	<b>2.4 y</b>
Nel Ruby	2.5 b	2.5 b	1.2 a	2.6 b	2.0 b	<b>2.2 xy</b>

Flame	2.0 a	2.4 a	2.4 a	2.3 a	2.5 a	<b>2.3 y</b>
Ruben	2.2 a	1.7 a	1.6 ab	1.7 a	2.0 a	<b>1.8 x</b>
Oran Red	2.8 c	2.4 bc	2.1 bc	1.8 a	2.1 ab	<b>2.2 xy</b>
Mean	<b>2.3 uv</b>	<b>2.2 uv</b>	<b>2.0 u</b>	<b>2.3 uv</b>	<b>2.5 v</b>	

Figures in each row 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).

\*\* Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

## Conclusion

The reaction of the different grapefruit selections to the different CTV sources was very similar and it appears that any of these mild CTV sources can be used as a pre-immunising source for any of the red grapefruit selections that were used in the trial. The trees with GFMS 12, which was replaced with GFMS 35 as the commercial pre-immunising source, performs well at this stage. This situation may be ascribed to favourable climatic conditions for tree growth since the trees with a known severe source produced only 23% less than those with the mild CTV sources after 9 years in the field.

## Future research

Measure trees, rate disease occurrence, harvest, grade and weigh fruit.

## Technology transfer

None.

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#### 4.2.6 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)

##### Opsomming

Daar is gevind dat die *Nartia tristeza* bron (GFMS 12) wat voorheen gebruik is vir pre-immuisering in die suider Afrikaanse Sitrus Verbeteringskema, gekontamineer is met 'n strawwe *Citrus tristeza virus* (CTV) ras. Twintig sub-isolate van twee afsonderlike *Nartia* bronne (A=GFMS 12, C=GFMS 14) en die Mouton bron is in Beltsville MD, VSA, deur middel van enkel plantluis oordragings voorberei. Ses van die 20 sub-isolate wat 'n 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. Virusvrye Star Ruby en Marsh pomelo boompies is met die ses Beltsville sub-isolate gepreïmmuniseer, twee enkel plantluis oordraging sub-isolate van die LNR-ITSG (GFMS 12/7, GFMS 12/9), GFMS 12 en GFMS 35. Boompies is virusvry 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 4 jaar na plant. In die vroeë stadium is die volgende aanduidings: 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-isolat B390/3 die beste in Marsh en B389/4 die beste in Star Ruby. Met betrekking tot produksie ( $\text{kg/m}^3$ ), presteer sub-isolate B390/5 en B389/4 die beste in Marsh en GFMS 12/7 en B389/4 die beste in Star Ruby. Virusvrye kontrole bome presteer goed in die vroeë stadium maar mag verander sodra hulle geïnfekteer 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. 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.

##### Summary

It was found that the *Nartia* source (GFMS 12), which is currently used to pre-immunise white grapefruit and pummelos, is contaminated by a severe strain of CTV. Twenty 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 protection abilities against severe strains should 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. ELISA confirmed pre-immunisation. ELISA revealed that two of the Beltsville sub-isolates did not comply with traits of a good cross-protecting isolate because they transmitted poorly, 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 is known to carry a severe strain. According to 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. According to production (efficiency  $\text{kg/m}^3$ ), trees with sub-isolates B390/5 and B389/4 performed the best in Marsh grapefruit and trees with GFMS12/7 and B389/4 performed the best in Star Ruby grapefruit. The Marsh (white grapefruit) trees with GFMS 35, which is the red grapefruit pre-immunising source, are better than trees with GFMS 12, 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 12 or GFMS 35, the present pre-immunising sources for grapefruit.

##### 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 propagative 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 to 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 virus-free. 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, production and tree health are monitored on an annual basis.

## Results and discussion

The data of the two grapefruit cultivars cannot be compared since they are grown under different climatic conditions.

Tree size: 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 =  $R^2(\text{PIH}-1.046R)$ , where R = the radius of the tree and H = the height of the fruit bearing part.

*Marsh grapefruit:* The canopy volumes of the Marsh trees are presented in Table 4.2.6.1. There are significant differences between the original sources and sub-isolates. At this stage, trees with sub-isolate B390/3 and the virus-free trees grew the best. Some of the sub-isolates (B389/4, B390/5) retarded growth 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 4.2.6.3. The Star Ruby tree sizes are more even indicating less difference 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.

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: During harvesting, the replicates of some treatments were mixed by accident and therefore statistical analysis could not be performed.

*Marsh grapefruit:* The average production per tree for each treatment for the Marsh trees is presented in Table 4.2.6.2. Trees with sub-isolate B389/1, GFMS 35 (control source) and those that were planted virus-free produced the highest yield. Cumulatively trees with GFMS 35 yielded the best followed by trees that were planted virus-free. Trees with sub-isolate B390/3 produced the poorest.

*Star Ruby grapefruit:* The average production per tree for each treatment for the Star Ruby trees is presented in Table 4.2.6.4. Trees with sub-isolate B389/4 had the highest yield, 34% more than trees with GFMS 35, which is the standard mild source to protect grapefruit. Cumulatively trees with B389/4 also yielded the highest followed by trees with GFMS 12. Trees with sub/isolate B389/1 yielded the worst.

This was the second 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 1 to 3, where, 1 is a smooth trunk with no visible pits and 3 severe stem pitting accompanied by decline of twigs. The results are presented in Table 4.2.6.1 and Table 4.2.6.3 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 4.2.6.1.** Tree size (canopy volume in m<sup>3</sup>) and stem pitting rating of Marsh grapefruit trees pre-immunised with different CTV sources and sub-isolates, 4 years after planting at Riversbend\*.

Treatment	Canopy volume (m <sup>3</sup> )	Stem pitting rating**
B389/1	13.5 cd	0 a
<b>B389/4</b>	10.0 de	0 a
B390/3	19.1 a	0 a
B390/5	8.0 e	0 a
GFMS 12/7	10.7 de	0.3 a
GFMS 12/9	11.5 de	0.2 a
GFMS 12 (previous cross-protector)	9.4 e	1.4 b
GFMS 35 (current cross-protecting source)	15.3 bc	0 a
Virus-free (Control)	18.6 ab	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: 1 = Smooth trunk; 2 = Mild pitting; 3 = Severe pitting.

**Table 4.2.6.2.** Average yield (kg/tree), the yield efficiency and cumulative yield over 2 years of Marsh grapefruit pre-immunised with different CTV sources and sub-isolates, 4 years after planting at Riversbend\*.

Treatment	Yield Kg/tree	Efficiency Kg/m <sup>3</sup>	Cumulative yield
B389/1	72.1	5.3	85.9
<b>B389/4</b>	59.0	5.9	<b>79.7</b>
B390/3	21.6	1.1	49.8
B390/5	49.0	6.1	56.3
GFMS 12/7	41.5	3.9	64.7
GFMS 12/9	38.0	3.3	59.5
GFMS 12 (previous cross-protector)	48.2	5.1	68.5
GFMS 35 (current cross-protecting source)	72.9	4.8	103.9
Virus-free (Control)	71.5	3.8	99.7

\* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

**Table 4.2.6.3.** Tree size (canopy volume in m<sup>3</sup>) and stem pitting rating of Star Ruby grapefruit trees pre-immunised with different CTV sources and sub-isolates, 4 years after planting at Tambuti\*.

Treatment	Canopy volume (m <sup>3</sup> )	Stem pitting rating**
B389/1	29.7 a	0 a
<b>B389/4</b>	33.0 a	0 a
B390/3	29.8 a	0 a
B390/5	26.2 a	0 a
GFMS 12/7	27.6 a	0.8 b
GFMS 12/9	24.8 a	0.3 a
GFMS 12 (previous cross-protector)	23.7 b	2.1 c
GFMS 35 (current cross-protecting source)	29.0 a	0 a
Virus-free (Control)	32.5 a	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: 1 = Smooth trunk; 2 = Mild pitting; 3 = Severe pitting.

**Table 4.2.6.4.** Average yield (kg/tree), the yield efficiency and cumulative yield over 2 years of Star Ruby grapefruit pre-immunised with different CTV sources and sub-isolates, 4 years after planting at Tambuti\*.

Treatment	Yield Kg/tree	Efficiency Kg/m <sup>3</sup>	Cumulative yield
B389/1	38.9	1.3	42.4
<b>B389/4</b>	61.2	1.8	<b>72.5</b>
B390/3	49.7	1.7	57.3
B390/5	43.8	1.7	49.1
GFMS 12/7	39.2	1.4	43.5
GFMS 12/9	49.3	2.0	63.5
GFMS 12 (previous cross-protector)	48.5	2.0	65.8
GFMS 35 (current cross-protecting source)	40.1	1.4	46.4
Virus-free (Control)	41.1	1.3	43.2

### Conclusion

The trees are still young and therefore all differences should be seen as 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;
- The Marsh grapefruit trees with GFMS 35 are better than trees with GFMS 12. This confirms previous findings;
- Sub-isolate B389/4 gave contradictive results where trees with this sub-isolate performed well in the Star Ruby trees but poorly in the Marsh trees. 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 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;
- According to production (efficiency kg/m<sup>3</sup>), trees with sub-isolates B390/5 and B389/4 yielded the best in Marsh grapefruit and trees with GFMS12/7 and 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.

### Future research

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

None.

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#### 4.2.7 PROGRESS REPORT: Cross-protection of Marsh and 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)

##### Opsomming

As gevolg van die teenwoordigheid van 'n strawwe ras in die *Nartia Citrus tristeza virus* (CTV) bron (GFMS 12) was dit nodig om bronne 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 indeksing 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 groottes is gemeet 3 jaar na uitplant. Die boompies groei heelwat stadiger as boompies in die ander pomelo produserende streke. Stamgleuf evaluasies is gedoen op die boompies en op die stadium is geen stamgleuf gevind nie, wat toegeskryf kan word aan die hoë somer temperature wat die CTV kan onderdruk gedurende daardie periode. Dit is egter nog te vroeg om afleidings te maak vanaf die vroeë resultate.

##### Summary

As a result of the presence of a severe strain in the *Nartia* (GFMS 12) CTV source, it was essential to separate the strains in isolates into 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 discriminate 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 36 months after planting. Trees grow much slower than other grapefruit production areas. Stem pitting evaluations were done and no stem pitting was found, which can be due to higher summer temperatures that can suppress the *tristeza* virus during that period. It is still too early to draw any conclusions from these early results.

##### 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, 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 and 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 3-year-old trees were measured and the canopy volumes (m<sup>3</sup>) calculated. The results are presented in Table 4.2.7.1. 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 very young. 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 1 to 3, where, 1 is a smooth trunk with no visible pits and 3 severe stem pitting accompanied by decline of twigs. The results are presented in Table 4.2.7.1. At this stage there were no symptoms of stem pitting, which can be due to very high summer temperatures that will suppress the CTV during the hot and dry summer months.

**Table 4.2.7.1.** Canopy volumes of Star Ruby trees pre-immunised with different CTV sources and sub-isolates, 3 years after planting in the Orange River Valley.

Treatment	Canopy volume (m <sup>3</sup> )	Stem pitting rating**
B389/1	4.9 abc	1
<b>B389/4</b>	3.5 bc	1
B390/3	3.7 abc	1
B390/5	4.1 abc	1
GFMS 12/7	5.3 ab	1
GFMS 12/9	4.6 abc	1
GFMS 12 (previous cross-protector)	5.7 a	1
GFMS 35 (current cross-protecting source)	3.0 c	1
Virus-free (control)	3.2 c	1

\* Figures in the columns followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

\*\* Stem pitting rating: 1 = Smooth trunk; 2 = Mild 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 (kg/tree and fruit size) and tree health (stem pitting and decline).

## Technology transfer

None.

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#### 4.2.8 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)

##### 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 gebruik vir verdere evaluering. Hierdie 19 bronne is 'n tweede keer geïnkuleer op Meksikaanse lemmetjie en vergelyk 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). Na 'n tydperk van 6 maande is die geïnkuleerde plante ge-evalueer vir groei en voorkoms van stamgleuf asook die virus titer d.m.v. ELISA. Die 4 mees belowendste bronne, wat vry is van viroïede (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; Tshipise 19/5 – versamel vanaf Marsh in Tshipise), word verder gebruik om virusvrye Marsh en Star Ruby boompies mee vir boord evaluasie te preïmmuniseer. 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.

##### Summary

Budwood was cut in the different grapefruit production areas of southern Africa from 108 outstanding 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 CTV mildness. 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), that were 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 Citrus in the Letsitele area during February 2007, while the Marsh trees were planted at Riverside in the Malelane area during March 2007. The trees will be evaluated annually for growth, production, fruit size, as well as tree health.

##### 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 benefit of optimum growth and production of virus-free trees cannot be used because of the abundance of the aphid insect vector 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 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 Sitrus 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 trees were successfully established at two sites.

## **Conclusion**

No data has been taken yet.

## **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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### **4.2.9 PROGRESS 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)

## **Opsomming**

Vruggrootte is 'n groot probleem by Clementines in die Oos- en Wes Kaap. Om die invloed van pre-immunisering op vruggrootte te bepaal, word nie-gepre-immuniseerde en gepre-immuniseerde bome van

sewe Clementine seleksies (Clemlate, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) en een Satsuma seleksie (Miho Wase) op Addo Navorsingstasie vergelyk. Die boomgrootte en produksie resultate van die 4-jaar-oue bome was wisselvallig en ELISA het getoon dat die bome in proef 1 wat virusvry geplant is, besmet geraak het met CTV. Boomgroottes van gepreïmmuniseerde Clemlate, Guillermina en Clemenpons in proef 1 is betekenisvol kleiner as die bome wat virusvry geplant is. Hierdie resultaat is nie bevestig in die tweede proef nie wat aandui dat die verskille wisselvallig is en mag varieer van jaar tot jaar. Die seleksies verskil ook tussen mekaar ten opsigte van hul reaksies tot CTV besmetting. Die doel om vruggrootte te verbeter deur die uitsluiting van CTV kan nie bereik word nie en dit sal na die vergelyking van CTV preïmmunisering en natuurlike CTV besmetting deur plantluise moet verander. Daar was geen verskille tussen die virusvrye geplante en gepreïmmuniseerde Satsuma bome nie.

## Summary

Fruit size is a great 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 selections (Clemlate, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) and a Satsuma selection (Miho Wase) are compared at Addo Research Station. The tree growth and yield results of the 4-year-old trees are very erratic and ELISA showed that the virus-free planted trees of trial 1 became CTV infected. The canopy volumes of pre-immunised Clemlate, Guillermina and Clemenpons in trial 1 were significantly smaller than the trees that were planted virus-free. This was not apparent with the same selections in the second trial, which indicates that the differences are erratic and may vary from year to year. The selections also differ among each other regarding their reactions to CTV infection. The objective of the experiment to increase fruit size by eliminating CTV cannot be achieved and the aim should be changed to a comparison of CTV pre-immunisation with mild sources and natural infection by aphids. There was no difference between the virus-free planted and pre-immunised Satsuma trees.

## 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. This 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 (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, 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 Clemlate, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated four times. Trial two consisted of the selections Clemlate, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated five times. Trial three was Miho Wase Satsuma and each treatment was replicated eight times.

Growth (canopy size), production and fruit size are measured annually. In August 2007 leaf samples were taken from each quadrant of each of the trees that were planted virus-free in trial 1. The 4 samples of each tree were pooled and ELISA was performed to assess if the trees were still free of CTV.

## Results and discussion

The canopy volumes of the Clementine and Satsuma trees were determined according to Burger *et al*, (1970) and 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. The canopy volumes of pre-immunised Clemlate, Guillermina and Clemenpons in trial 1 were significantly smaller than the trees that were planted virus-free. This was not apparent with the same selections in trial 2 (Table 4.2.9.2), which indicates that the differences are erratic and may vary from year to year. Also, the pre-immunised Esbal trees in trial 2 were significantly larger than the trees that were planted virus-free. The erratic results may be due to the fact that ELISA confirmed that all trees but 2 of those that were planted virus-free in trial 1 were infected by CTV. The effect of the natural CTV infection will become apparent in the years to follow. The objective to increase fruit size by eliminating CTV cannot be achieved and the aim should change to a comparison of CTV pre-immunisation with mild sources and natural infection by aphids. There was no difference between the virus-free planted and pre-immunised Satsuma trees (Table 4.2.9.3).

**Table 4.2.9.1.** Canopy volumes (m<sup>3</sup>) of trees planted virus-free and pre-immunised Clementine selections in trial 1, 4 years after planting<sup>1</sup>.

Cultivar or selection	Treatment		Mean
	Virus-free	Pre-immunised	
Clemlate 1163	8.9 a	6.9 b	7.7 x
Orogrande 1300	5.6 a	5.7 a	5.6 y
Guillermina 1331	8.9 a	3.7 b	6.3 xy
Nour Tardif V15 1561	7.1 a	5.6 a	6.3 xy
Oronules 1570	6.3 a	6.9 a	6.6 xy
Esbal 1571	6.9 a	5.8 a	6.3 xy
Clemenpons 1581	6.1 a	3.1 b	4.6 y
<b>Mean</b>	<b>7.1 v</b>	<b>5.4 w</b>	

<sup>1</sup> Figures in each row 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 (m<sup>3</sup>) of trees planted virus-free and pre-immunised Clementine selections in trial 2, 4 years after planting<sup>1</sup>.

Cultivar or selection	Treatment		Mean
	Virus-free	Pre-immunised	
Clemlate 1163	8.1 a	6.5 a	7.3 x
Orogrande 1300	7.0 a	6.3 a	6.6 xy
Guillermina 1331	6.2 a	6.8 a	6.5 xy
Nour Tardif V15 1561	7.0 a	5.6 a	6.3 xy
Esbal 1571	5.3 b	7.7 a	6.5 xy
Clemenpons 1581	5.5 a	5.0 a	5.3 y
<b>Mean</b>	<b>6.5 v</b>	<b>6.3 v</b>	

<sup>1</sup> Figures in each row 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 (m<sup>3</sup>) of trees planted virus-free and pre-immunised Miho Wase Satsuma trees in trial 3, 4 years after planting<sup>1</sup>.

Scion	Treatment	
	Virus-free	Pre-immunised
Miho Wase 983	6.8 a	5.4 a

<sup>1</sup> Figures followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

The fruit of the 3 trials were weighed and are presented in Table 4.2.9.4, Table 4.2.9.5 and Table 4.2.9.6 respectively. Overall, the trees that were planted virus-free produced significantly better than the pre-immunised trees in trial 1 but not in trial 2. A significant variation occurred among the Clementine selections with the lowest production by Nour in trial 1 and trial 2. Clemlate (trial 1 and 2) and Clemenpons (trial 2) also yielded significantly lower. 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 4.2.9.4.** Average yield per tree (kg) of virus-free planted and pre-immunised trees of Clementine selections in trial 1, 4 years after planting<sup>1</sup>.

Cultivar or selection	Treatment		Mean
	Virus-free	Pre-immunised	
Clemlate 1163	24.7 a	20.4 a	22.5 yz
Orogrande 1300	40.1 a	29.8 a	34.9 xy
Guillermina 1331	28.9 a	25.9 a	27.4 xyz
Nour Tardif V15 1561	19.8 a	15.3 a	17.5 z
Oronules 1570	39.3 a	9.2 b	24.3 yz
Esbal 1571	51.4 a	28.0 a	39.7 x
Clemenpons 1581	30.3 a	25.1 a	27.7 xyz
<b>Mean</b>	<b>31.7 v</b>	<b>23.8 w</b>	

<sup>1</sup> Figures in each row 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 Clementine selections in trial 2, 4 years after planting<sup>1</sup>.

Cultivar or selection	Treatment		Mean
	Virus-free	Pre-immunised	
Clemlate 1163	36.2 a	26.3 a	31.2 y
Orogrande 1300	48.4 a	42.5 a	45.4 x
Guillermina 1331	45.2 a	50.2 a	47.7 x
Nour Tardif V15 1561	26.0 a	22.8 a	24.4 y
Esbal 1571	68.5 a	48.3 b	58.5 x
Clemenpons 1581	36.3 a	26.3 a	31.3 y
<b>Mean</b>	<b>43.5 v</b>	<b>36.1 v</b>	

<sup>1</sup> Figures in each row 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, 4 years after planting<sup>1</sup>.

Scion	Treatment	
	Virus-free	Pre-immunised
Miho Wase 983	46.0 a	42.5 a

<sup>1</sup> Figures followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

## Conclusion

In comparison with 2006, there is no set trend to differentiate between the treatments. It appears that the selections each differ on their reactions to CTV infection. Since CTV naturally infected the trees that were planted virus-free, the original aim of comparing virus-free and pre-immunised trees can no longer be done. The trees in all 3 trials should be sampled and tested for the presence of CTV.

## Future research

- Measure tree size, harvest, grade and weigh fruit.
- Take leaf samples from all the trees and do ELISA to determine their CTV status.

## Technology transfer

None.

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#### 4.2.10 **PROGRESS REPORT: Identification of suitable *Citrus tristeza virus* sources for pre-immunising Turkey Valencia**

Experiment 789 (2005 - 2015) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

#### **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) geïnokuleer om die beste ligte CTV bron te identifiseer vir kruisbeskermingsdoeleindes. Bome wat met die GFMS 12 bron geïnokuleer is en bome wat virusvry gelaat is, dien as positiewe en negatiewe kontroles, onderskeidelik. Pre-immunisasie is bevestig deur middel van ELISA en die bome is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant.

#### **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 part of the citrus industry and therefore it is a high priority to identify a suitable CTV source for pre-immunisation. 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 will be evaluated annually for growth, production, fruit size, as well as tree health.

#### **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 4.2.10.1). 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 4.2.10.1.** 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

Successful pre-immunisation was confirmed by means of ELISA. Trees were planted during March 2007 at Riverside in the Malelane area.

### Conclusion

No data has been taken yet.

### 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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#### 4.2.11 **PROGRESS REPORT: Evaluation of CTV sources in Valencia**

Experiment 788 (2000 - 2009) by S.P. van Vuuren, J.H.J. Breytenbach (CRI) and B.Q. Manicom (ITSC)

#### **Opsomming**

Die effek van drie *Citrus tristeza virus* (CTV) bronne (LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49) word in drie Valencia bostamme (McClean, McClean Saadloos and Delta) op Troyer citrange onderstam vergelyk. Boomgrootte van McClean Saadlose Valencia was betekenisvol kleiner as die van McClean en Delta Valencia maar hul produksie was 50% beter as laasgenoemde twee. Die kontrole bome wat virusvry geplant is, groei en produseer baie swak. Die produksie van bome met CTV bronne SM 41 en SM 49 is hoogs betekenisvol beter as die kontrole bome. Dit is 'n aanduiding dat die twee CTV bronne beskerming aan die bome teen die introduksie van CTV rasse, wat vir die swak toestand van die kontrole bome verantwoordelik is, gebied het. Oor die algemeen was produksie swak met 'n groot persentasie klein vrugte.

#### **Summary**

The effect of CTV isolates (LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49) are being compared in three Valencia scions (McClean, McClean Seedless and Delta) on Troyer citrange rootstock. Tree size of McClean Seedless Valencia was significantly smaller than those of McClean and Delta Valencias but their production was more than 50% better than the latter two scions. The control trees that were planted virus-free are growing and yielding very poorly. There is a trend that trees with the SM 41 and SM 49 CTV sources perform better than those with the other CTV sources and highly significantly better than trees that were planted virus-free. It also shows that these two CTV sources protected the trees from the introduction of CTV strains that caused the reduction in growth and poor yield of the trees that were planted virus-free.

#### **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 (Oberholzer, 1959; Webber, 1925). 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 no 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 use of tolerant rootstocks in the South African citrus industry, it was generally accepted that CTV has no effect on sweet oranges and mandarins. This situation can be partly 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 (Barkley, 1991; Roistacher, 1988) 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 (Barkley, 1991; Calavan *et al.*, 1980; Müller *et al.*, 1968). 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

McCleean, McCleean Seedless and Delta Valencia trees on Troyer citrange rootstock were grown according to normal nursery practices under aphid-free conditions. When the scions have developed to approximate 5 mm in diameter, they were inoculated with CTV sources, which were derived from sweet orange and showed promise in glasshouse tests. The sources are LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49. Trees with these sources are compared to trees with a severe source (SOSS 2) as well as un-inoculated virus-free trees. The trees were planted in 2000 according to a split plot design with five replications at the ARC-ITSC Experimental farm, Malelane. The effect of the CTV sources on growth, production, fruit size and tree health is determined.

## Results and discussion

**Tree size.** 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 =  $R^2(\text{PIH}-1.046R)$ , where R = the mean radius of the tree and H = the height of the fruit bearing part (**Table 4.2.11.1**). In general, the canopy volumes of McCleean Seedless Valencia were significantly smaller than that of the Delta and McCleean Valencia trees. With the CTV sources, trees with the SM 36 source and those that were planted virus-free were significantly smaller than trees with sources SM 41, SM 45 and SM 49. The effect of the CTV sources on the scions individually (body of the table), followed the same trend as the CTV means.

**Production.** The yield was generally poor and variable with the majority of fruit small (**Table 4.2.11.2**). This situation was more severe in the two previous years and it appears that the trees are recovering slowly to whatever caused the poor condition of the trees in 2005. Overall, trees with the SM 36 source and those that were planted virus-free yielded the poorest followed by those with LMS 6. Of the scions, the McCleean Seedless trees outperformed the McCleean and Delta trees with more than 50%. In the body of the table trees with SM 36 were poor in all three cultivars. The poor performance of the trees that were planted virus-free confirms earlier findings and shows how important it is to pre-immunise tolerant cultivars as well. The uneconomic yield efficiency in **Table 4.2.11.3** may still be a result of the 2005 situation, however, it is unacceptable low. The longterm effect of the CTV sources that are shown in **Table 4.2.11.4**, reveals little difference among the CTV sources, with the exception of SM 36. The McCleean Seedless trees yielded the best over a 4-year period. The most variation caused by the CTV sources is also more apparent in this scion. The best CTV sources at this stage are SM 41 and SM 49.

**Table 4.2.11.1.** Tree size (canopy volume = m<sup>3</sup>) 7 years after planting of three Valencia selections that were pre-immunised with different mild CTV sources, a severe source and trees that were planted virus-free.

CTV source	Scion**							
	McC		McC SL		Delta		Mean	
LMS 6	27.7	ab	23.8	ab	28.2	ab	<b>26.5</b>	<b>xy</b>
SM 34	28.3	ab	25.1	ab	30.2	ab	<b>27.8</b>	<b>xy</b>
SM 36	14.8	c	11.2	c	15.5	c	<b>13.8</b>	<b>z</b>
SM 41	26.6	ab	25.0	ab	32.5	ab	<b>28.0</b>	<b>x</b>
SM 45	30.9	a	25.1	ab	33.5	ab	<b>29.8</b>	<b>x</b>
SM 49	30.4	a	22.3	ab	34.6	a	<b>29.1</b>	<b>x</b>
SOSS 3	27.4	ab	26.7	a	27.1	b	<b>27.1</b>	<b>xy</b>
VF	25.2	b	20.3	b	26.9	b	<b>24.1</b>	<b>y</b>
<b>Mean</b>	<b>26.4</b>	<b>u</b>	<b>22.4</b>	<b>v</b>	<b>28.6</b>	<b>u</b>		

Figures in each column in the body of the table followed by the same letter do not differ significantly at the 5% level. Figures in the mean row and column that are followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

\*\* Scions: McC = McCleean Valencia; McC SL = McCleean Seedless Valencia; Delta = Delta Valencia.

**Table 4.2.11.2.** The production (kg/tree) 7 years after planting of three Valencia selections that were pre-immunised with different mild CTV sources, a severe source and trees that were planted virus-free\*.

CTV source	Scion**						Mean	
	McC		McC SL		Delta			
LMS 6	26.8	abc	72.6	bc	19.0	c	<b>39.5</b>	<b>xyz</b>
SM 34	22.6	c	94.2	a	29.5	bc	<b>48.8</b>	<b>wxy</b>
SM 36	24.4	bc	30.6	d	19.0	c	<b>24.7</b>	<b>z</b>
SM 41	36.5	abc	76.7	ab	70.0	a	<b>61.1</b>	<b>w</b>
SM 45	13.8	c	77.1	ab	55.0	ab	<b>48.6</b>	<b>wxy</b>
SM 49	48.5	ab	90.4	ab	40.6	abc	<b>59.9</b>	<b>wx</b>
SOSS 3	49.3	a	86.9	ab	29.7	bc	<b>55.3</b>	<b>wx</b>
VF	33.4	abc	56.0	c	11.7	c	<b>33.7</b>	<b>yz</b>
<b>Mean</b>	<b>31.9</b>	<b>v</b>	<b>73.1</b>	<b>u</b>	<b>34.3</b>	<b>v</b>		

Figures in each column in the body of the table followed by the same letter do not differ significantly at the 5% level. Figures in the mean row and column that are followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

\*\* Scions as in Table 4.2.11.1.

**Table 4.2.11.3.** Yield efficiency (kg/m<sup>3</sup>) 7 years after planting of three Valencia selections that were pre-immunized with different mild CTV sources, a severe source and trees that were planted virus-free\*.

CTV source	Scion**						Mean	
	McC		McC SL		Delta			
LMS 6	1.0	ab	3.3	abc	0.7	bc	<b>1.7</b>	<b>z</b>
SM 34	0.8	ab	3.8	ab	1.0	abc	<b>1.9</b>	<b>z</b>
SM 36	1.6	a	2.8	c	1.3	abc	<b>1.9</b>	<b>z</b>
SM 41	1.3	ab	3.2	abc	2.2	a	<b>2.2</b>	<b>z</b>
SM 45	0.5	b	3.1	bc	1.8	ab	<b>1.8</b>	<b>z</b>
SM 49	1.7	a	4.1	a	1.2	abc	<b>2.3</b>	<b>z</b>
SOSS 3	1.8	a	3.3	abc	1.2	abc	<b>2.1</b>	<b>z</b>
VF	1.3	ab	2.8	c	0.4	c	<b>1.5</b>	<b>z</b>
<b>Mean</b>	<b>1.3</b>	<b>v</b>	<b>3.3</b>	<b>u</b>	<b>1.2</b>	<b>v</b>		

Figures in each column in the body of the table followed by the same letter do not differ significantly at the 5% level. Figures in the mean row and column that are followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

\*\* Scions as in Table 4.2.11.1.

**Table 4.2.11.4.** Cumulative yield (kg) over a 4-year period of 7-year-old Valencia selections that were pre-immunised with different mild CTV sources, a severe source and trees that were planted virus-free.

CTV source	Scion**						Mean	
	McC		McC SL		Delta			
LMS 6	133.8	ab	205.8	bc	100.6	bc	<b>146.7</b>	<b>xy</b>
SM 34	90.7	b	250.3	a	107.3	bc	<b>149.5</b>	<b>xy</b>
SM 36	89.5	b	99.5	e	73.0	c	<b>87.3</b>	<b>z</b>
SM 41	125.0	ab	217.8	abc	166.3	a	<b>169.7</b>	<b>x</b>
SM 45	94.0	b	199.8	cd	132.0	ab	<b>142.0</b>	<b>xy</b>
SM 49	144.9	a	241.6	ab	131.0	ab	<b>172.5</b>	<b>x</b>
SOSS 3	128.7	ab	212.6	abc	102.7	bc	<b>148.0</b>	<b>xy</b>
VF	89.7	b	165.2	d	70.9	c	<b>108.6</b>	<b>yz</b>
<b>Mean</b>	<b>112.1</b>	<b>v</b>	<b>199.1</b>	<b>u</b>	<b>110.5</b>	<b>v</b>		

\* Figures in each column in the body of the table followed by the same letter do not differ significantly at the 5% level. Figures in the mean row and column that are followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

\*\* Scions as in Table 4.2.11.1.

#### Conclusion

- The trees are recovering slowly from whatever problem was encountered during 2005.
- The McClean Seedless trees yielded more than 50% better than the McClean and Delta trees.
- There is a trend that trees with the SM 41 and SM 49 CTV sources perform better than those with the other CTV sources and highly significant better than trees that were planted virus-free. It also shows that these two CTV sources protected the trees from the introduction of CTV strains that caused the reduction in growth and poor yield of the trees that were planted virus-free.

#### Future research

Determine tree size, harvest, assess fruit size and weigh fruit.

#### Technology transfer

None.

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4.2.12 **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)

**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 vergelyk met LMS 6 (standard vir soetlemoene) en boompies wat virusvry geplant is. Pre-immunisering is bevestig deur middel van ELISA waarna die boompies gedurende September 2007 by Karsten Boerdery in die Kakamas omgewing geplant is. Jaarlikse evaluasies word gedoen vir boomgrootte, vruggrootte, oes opbrengs, sowel as van hul gesondheidstoestand.

**Summary**

CTV is host and climate specific and 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 -, Midkinight -, 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.

**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 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

Successful pre-immunisation was confirmed by means of ELISA and the trees successfully established in the orchard at Kakamas.

## Conclusion

No data was collected yet.

## 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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### 4.2.13 FINAL REPORT: Screening of rootstocks for Citrus Blight tolerance Experiment 32 (1992 - 2008) by J.H.J. Breytenbach (CRI)

## Opsomming

Die inokulasie van sitruskroei in Delta Valencia bome op 18 verskillende onderstamme induseer 'n afname in boomgrootte en produksie in vergelyking met ongeïnokuleerde bome. Ongeïnokuleerde bome het 'n gemiddelde oesopbrengs van 103.5 kg/boom gelewer terwyl geïnokuleerde bome 89.6 kg/boom gelewer het, met beide 'n opbrengs effektiwiteit van slegs 2 kg/m<sup>3</sup>. Dit is baie laag in vergelyking met 'n normale boom van dieselfde ouderdom wat 8 kg/m<sup>3</sup> lewer. Serologiese analises van die 12-kd proteïen, wat slegs in sitruskroei-besmette bome voorkom, is gebruik om bome te identifiseer wat met sitruskroei besmet is. 'n Totaal van 52% van die geïnokuleerde bome het positief getoets vir die 12-kd proteïen terwyl die ongeïnokuleerde bome 35% positief getoets het. Dit bevestig die visuele simptome in die boord maar identifiseer ook die siekte in 'n vroeë stadium wanneer geen simptome nog waargeneem kan word nie. Die resultate van die boomvolumes, produksie, die water-opnametoets en die voorkoms van die 12-kd proteïen komplimenteer mekaar nie en maak die interpretasie van die data moeilik. Slegs Swingle citrumelo het in al vier kriteria nl. boomvolumes, opbrengs, water opname en proteïen toetse as die beste onderstam uitgestaan. Onderstamme Empress mandarin, Zhu luan en C35 citrange is die meeste ge-afekteer deur sitruskroei. Bome op Swingle citrumelo, X639, Gou Tou en Sun Chu Sha toon die meeste toleransie teen die siekte. Indien dië bome na verloop van tyd ook geïnfekteer word deur sitruskroei, beteken dit dat die onderstamme net die boom se leeftyd kan verleng en nie weerstandbiedend is nie.

## Summary

The inoculation of citrus blight (CB) in Delta Valencia trees on 18 different rootstocks showed a reduction in tree size and yield in comparison with un-inoculated trees. Un-inoculated trees had a mean yield of 103.5 kg/tree, while inoculated trees had a mean yield of 89.6 kg/tree, both with a yield efficiency of 2 kg/m<sup>3</sup>. This, however, is very low comparing to a normal tree that will produce 8 kg/m<sup>3</sup> at the age of 15 years. Serological analyses of the 12-kd protein, which can only be found in citrus blight trees, were used to identify trees that are infected with citrus blight. A total of 52% of the inoculated trees tested positive for the 12-kd protein, while 35% of the non-inoculated trees tested positive. Results confirmed the visual symptoms in the orchard but also identified the disease in an early stage when no symptoms were visible. The results of the canopy volumes, yield, water uptake test and the presence of the 12-kd protein do not compliment each other and makes the interpretation of the data difficult. Only Swingle citrumelo performed the best in the four criteria

used to evaluate the trees, i.e. tree size, yield, water up-take and protein tests. Rootstocks that were the most affected by CB are Empress mandarin, Zhu luan and C35 citrange. Rootstocks that showed the most tolerance to CB are Swingle citrumelo, X639, Gou Tou and Sun Chu Sha. If any of these trees get infected after time with CB it means that rootstocks can only prolong tree life since it is in fact not resistant to the disease.

## Introduction

Citrus blight (CB) affects most commercially grown scion cultivars in the citrus production areas of the world where this disease occurs. CB is primarily a disease affecting the rootstock and the most sensitive rootstock cultivars appear to be Rough lemon, Volckameriana and Rangpur lime. These are followed by trifoliolate orange and its citrange hybrids, Cleopatra mandarin, sweet orange and sour orange.

The symptoms of trees with CB are similar to those of a number of other declines of citrus. The finding of distinctive proteins in leaves and roots of infected trees has led to the development of serological tests that are useful in distinguishing trees with CB from those declining from other disorders. Two CB-associated proteins (35 and 12-kd) were purified by preparative electrofocusing and SDS-PAGE. Polyclonal antisera were produced to both proteins, and a monoclonal antibody was produced to the 12-kd protein. Both proteins were readily detected in crude extracts from CB trees by immuno spot and western blot assays. In several experiments, trees with symptoms of CB that were positive by water uptake tests and zinc wood analyses were also positive in the serological tests. Some bearing trees were found to contain the two proteins up to one year before CB symptoms developed. The 12-kd protein was detected in young trees 3 months after root-graft inoculations (Derrick *et al.*, 1993).

Until the inception of the Citrus Improvement Scheme in South Africa in 1973, practically all commercial citrus orchards were established on Rough lemon rootstock. Rough lemon remained the most popular rootstock until 1990 and in 1991 was superseded by Volckameriana, Swingle citrumelo, Carrizo citrange and Troyer citrange. The presence of CB in South Africa has influenced the rootstock choice in affected areas. Rootstocks such as X639 (*Poncirus trifoliata* x Cleopatra mandarin), M&T (Minneola tangelo x *P. trifoliata*) and Swingle citrumelo are being used to establish new plantings. Trees on Swingle citrumelo are being used to replace trees that have succumbed to CB in existing orchards.

This investigation is to identify rootstocks that are the least affected by CB and can be used successfully in CB areas.

## Materials and methods

A rootstock experiment to evaluate the tolerance of various rootstocks to CB has been established in Letsitele at Bosveld Sitrus. The trial comprises of virus-free Delta Valencia scions on Carrizo citrange, Empress mandarin, *P. trifoliata*, Swingle citrumelo, Volckameriana and Samson tangelo that were planted in 1990. Gou Tou, Orlando tangelo, M&T, X639, Marsh grapefruit, Zhu Luan and Sweet Orange were planted in 1992. In 1995, trees on Cleopatra mandarin, C35 and Sun Chu Sha were added, and during 1996 trees on Benton citrange and Sunki mandarin were included. The execution of the trial was split over several years because of the extent of the execution of the treatments and the availability of rootstocks.

Trees on the different rootstocks were planted in pairs as receptor trees equidistant from a CB infected donor tree. Three to four roots, 5-6 mm in diameter, of one of the pair of receptor trees were approach grafted to the roots of the donor tree. Six pairs of each rootstock were planted and grafted. The roots of the donor trees were opened, grafted to the receptor trees that were planted bare rooted. They were planted normal depth. The non-grafted trees constituted as the un-inoculated controls and were also planted bare rooted. The donor trees were selected using standard diagnostic techniques such as water uptake and zinc accumulation in the xylem. The donor trees were severely cut back and removed 3 years after inoculation since they started to interfere with the growth of the young trees. The young trees were treated with granular formulations of Temik and Ridomil and trunk paint applications of Aliette, every 3 months for the first three years to exclude the effects of *Phytophthora* and citrus nematode infections.

The following data were taken each year:

- Tree sizes are measured;
- Yield and fruit size are determined;
- Water uptake tests;
- The presence of the 12-kd protein is determined.

## Results and discussion

For unknown reasons, trees on the Sweet orange rootstock were stunted and therefore were terminated from the experiment.

Since CB is a disease that develops mostly 8 years or more after planting, the results of the canopy size and yield are presented according to their planting dates (Table 4.2.13.1 and Table 4.2.13.2). It is expected that data of inoculated and un-inoculated trees of CB tolerant rootstocks will not differ, since both the inoculated and uninoculated trees will not show any detrimental effects of the disease.

### Tree size.

In the early stages (first 5-10 years), of this experiment the un-inoculated trees were generally larger than the inoculated trees, which showed that CB inoculation reduced tree growth. However, as the trees grew older and the disease spread naturally into the un-inoculated trees the average difference in trees size between un-inoculated and inoculated trees of the 1990 planting were 19.1% after 15 years, the 1992 planting were only 0.5% after 15 years and in the 1995/96 planting 8.7% after 12 years. There are, however, contradictory results in two rootstocks that are not commercially used in South Africa, i.e. 22% of inoculated trees on Zhu Luan rootstock were smaller than the un-inoculated trees, which are understandable, but 20% un-inoculated trees on Marsh grapefruit rootstock were smaller than the inoculated trees. However, Marsh grapefruit is sensitive to *Citrus tristeza virus* and the virus could have had an influence on this rootstock's performance. The tree sizes of the 1990 planting showed that Swingle citrumelo were the least affected. In the 1992 planting, X639 and Gou Tou were the least affected. Results of the 1995/96 planting indicate that tree sizes of, Sun Chu Sha and Cleopatra mandarin rootstocks were the least affected. Trees on Benton Citrange were generally much smaller than trees that were also planted during 1995/96. Un-inoculated trees were 65% smaller and inoculated trees were 58% smaller, compared to the mean of the other four rootstocks. This may be due to a sensitivity of the rootstock to re-plant soil. To summarise, trees on Swingle citrumelo, X639 Orlando tangelo, Gou Tou, Sun Chu Sha and Cleopatra mandarin rootstocks showed the most tolerance in a CB situation, while trees on C35 citrange and Zhu Luan rootstocks were the most susceptible (Table 4.2.13.1).

### Yield

Overall, the un-inoculated trees yielded slightly higher, with a mean yield of 103.5 kg per tree and un-inoculated trees 89.6 kg per tree, but there was no difference in yield efficiency ( $\text{kg/m}^3$ ) (Table 4.2.13.2). Growth stress factors usually increase production, which results in a high percentage small fruit. However, there is virtually no difference in the % small fruit of the inoculated and un-inoculated trees. This may be due to the low overall production of the trees, i.e. the un-inoculated trees on Gou Tou rootstock had a canopy volume of  $85.1 \text{ m}^3$  and a yielded of 101.2 kg. That gives a production efficiency of  $1.2 \text{ kg/m}^3$ . Normal production is approximately  $8 \text{ kg/m}^3$ . Trees on Swingle citrumelo rootstock had the highest yield of all the trees on the other rootstocks. In the 1992 planting, 33% of the inoculated trees produced a higher yield than the un-inoculated trees, which are now 67% naturally infected according to the 12-kd protein test (Table 4.2.13.4). Comparing the production of inoculated and un-inoculated trees show that Empress mandarin in the 1990 planting, Zhu Luan in the 1992 planting and Benton citrange rootstock in the 1995/96 planting, are the least tolerant overall.

### Water uptake

Water uptake ability of CB infected trees is significantly reduced due to the presence of occlusions by amorphous plugs in the xylem. Water uptake was the quickest in trees on Swingle citrumelo (1990), Gou Tou (1992) and Sun Chu Cha (1995/1996) rootstocks. The poorest uptake occurred in trees on Carrizo citrange (1990), X639 (1992) and Cleopatra mandarin (1995/1996) (Table 4.2.13.3)

### CB protein

A higher number of inoculated trees showed the presence of the 12-kd protein (52%) than in the un-inoculated trees (35%) (Table 4.2.13.4). Some of the latter group of trees probably got infected by natural means. None of the trees on Sun Chu Sha (1995/1996) rootstock showed the presence of the 12-kd protein. Next best were trees on Swingle citrumelo (1990) and Orlando tangelo (1992) with two trees out of 12 with the 12-kd protein. The presence of the 12-kd protein was the highest in trees on C35 citrange (1995/1996) rootstock (12/12 positive). In the 1992 planting MxT performed the worst (10/12 positive) and in the 1992 planting Carrizo citrange (9/12 positive) performed the worst.

### Visual symptoms

The trees were visually evaluated for decline symptoms i.e. twig die back, wilting of leaves and zinc deficiency symptoms associated with CB (Table 4.2.13.5). Of the inoculated trees, 40% showed symptoms while 20% of the un-inoculated trees showed symptoms. The presence of symptoms showed a lower

infection rate than the 12-kd protein test, which indicates that the protein test is more sensitive and identifies CB infection before decline symptoms can be observed.

#### Comparison

To make a meaningful conclusion of the results obtained, using the different criteria, the best and worst rootstocks for each criterion are summarised in (Table 4.2.13.6).

**Table 4.2.13.1.** Tree sizes of CB inoculated and un-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Tree volume (m <sup>3</sup> )		% Difference
		Un-inoculated	Inoculated	
<i>Poncirus trifoliata</i>	1990	64.9 a	48.2 a	-26
Swingle citrumelo	1990	73.7 a	75.8 a	3
Empress mandarin	1990	59.1 a	26.1 b	-56
Carrizo citrange	1990	42.1 a	29.3 a	-30
Volckameriana	1990	67.3 a	58.1 a	-14
Sampson tangelo	1990	73.2 a	79.2 a	-8
<b>Average 1990</b>		<b>50.7</b>	<b>38.8</b>	<b>-19.1</b>
MxT	1992	62.4 a	57.5 a	-8.5
X639	1992	56.8 a	56.3 a	-1
Gou Tou	1992	85.1 a	89.7 a	5.1
Orlando tangelo	1992	72.1 a	69.6 a	-3.6
Zhu Luan	1992	35.9 a	29.4 a	-22.1
Marsh grapefruit	1992	47.4 a	59.4 a	20.2
<b>Average 1992</b>		<b>60.0</b>	<b>60.3</b>	<b>0.5</b>
Cleopatra mandarin	1995	43.4 a	43.2 a	-0.5
Sun Chu Sha	1995	65.0 a	75.0 a	13.3
C35 citrange	1995	44.6 a	25.5 a	-75
Sunki mandarin	1996	34.7 a	33.5 a	-3.6
Benton citrange	1996	16.6 a	18.3 a	9.2
<b>Average 1995/96</b>		<b>40.9</b>	<b>39.1</b>	<b>-4.6</b>
<b>Mean</b>		<b>50.5</b>	<b>46.1</b>	<b>-8.7</b>

Figures in each row followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

**Table 4.2.13.2.** Yield (kg), yield efficiency (kg/m<sup>3</sup>) and % small fruit (count <105) of un-inoculated and CB inoculated Delta Valencia trees on different rootstocks

Rootstock	Year planted	Yield		Yield Efficiency	
		Un-inoculated	Inoculated	Un-inoculated	Inoculated
<i>P. trifoliata</i>	1990	128.4 a	160.1 a	2.0	3.3
Swingle citrumelo	1990	133.5 a	139.7 a	1.8	1.9
Empress mandarin	1990	139.2 a	61.5 b	2.4	2.4
Carrizo citrange	1990	130.4 a	80.2 a	3.1	2.8
Volckameriana	1990	178.5 a	124.9 a	2.7	2.1
Sampson tangelo	1990	81.6 a	62.3 a	1.1	0.8
<b>Average 1990</b>		<b>131.9</b>	<b>104.8</b>	<b>2.6</b>	<b>2.6</b>
MxT	1992	95.2	105.6 a	1.5	1.8
X639	1992	117.0 a	107.0 a	2.1	1.9
Gou Tou	1992	101.2 a	117.9 a	1.2	1.3
Orlando tangelo	1992	101.8 a	110.9 a	1.4	1.6
Zhu Luan	1992	75.7 a	62.8 a	2.1	2.1
Marsh grapefruit	1992	70.9 a	116.2 a	1.5	2.0
<b>Average 1992</b>		<b>109.5</b>	<b>103.4</b>	<b>1.6</b>	<b>1.8</b>

Cleopatra mandarin	1995	46.6 a	32.1 a	1.1	0.7
Sun Chu Sha	1995	78.8 a	94.6 a	1.2	1.3
C35 citrange	1995	77.8 a	49.6 a	1.7	1.9
Sunki mandarin	1996	78.2 a	90.5 a	2.3	2.7
Benton citrange	1996	63.4 a	36.7 a	3.8	2.0
<b>Average 1995/96</b>		<b>69.0</b>	<b>60.7</b>	<b>2.0</b>	<b>1.7</b>
<b>Mean</b>		<b>103.5</b>	<b>89.6</b>	<b>2.1</b>	<b>2.0</b>

Figures in each row followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

**Table 4.2.13.3.** Comparison of water-uptake (seconds/10 ml) through the trunk xylem of CB inoculated and un-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Un-inoculated	Inoculated	% Difference
<i>P. trifoliata</i>	1990	35 a	41 a	17
Swingle citrumelo	1990	23 a	21 a	-8
Empress mandarin	1990	27 a	24 a	-12
Carrizo citrange	1990	24 a	58 a	141
Volckameriana	1990	24 a	36 a	52
Sampson tangelo	1990	35 a	38 a	8
MxT	1992	75 a	78 a	4
X639	1992	65 a	45 a	-31
Gou Tou	1992	66 a	39 a	-41
Orlando tangelo	1992	67 a	50 a	-25
Zhu Luan	1992	69 a	53 a	-23
Marsh grapefruit	1992	76 a	59 a	-24
Cleopatra mandarin	1995	78 a	90 a	15
Sun Chu Sha	1995	57 a	37 a	-35
C35 citrange	1995	68 a	76 a	12
Sunki mandarin	1996	67 a	53 a	-20
Benton citrange	1996	90 a	64 a	-29

Figures in each row followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

**Table 4.2.13.4.** Comparison of 12-kd protein serological tests of CB inoculated and un-inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Number of trees with 12-kd protein present (/6)		
		Inoculated	Un-inoculated	Total (/12)
<i>P. trifoliata</i>	1990	2	1	3
Swingle citrumelo	1990	2	0	2
Empress mandarin	1990	3	0	3
Carrizo citrange	1990	6	3	9
Volckameriana	1990	4	2	6
Sampson tangelo	1990	1	1	2
MxT	1992	6	4	10
X639	1992	5	4	9
Gou Tou	1992	1	3	4
Orlando tangelo	1992	1	1	2
Zhu Luan	1992	4	2	6
Marsh grapefruit	1992	2	2	4
Cleopatra mandarin	1995	2	2	4
Sun Chu Sha	1995	0	0	0
C35 citrange	1995	6	6	12
Sunki mandarin	1996	6	4	10
Benton citrange	1996	4	1	5

**Table 4.2.13.5.** Presence of decline symptoms on CB inoculated and un-inoculated Delta Valencia trees.

Rootstock	Year Planted	Un-inoculated (/6)			Inoculated (/6)		
		Visually Positive	Visually +/-	Visually Negative	Visually Positive	Visually +/-	Visually Negative
<i>P. trifoliata</i>	1990	0	0	6	3	0	3
Swingle citrumelo	1990	0	0	6	1	2	3
Empress mandarin	1990	0	0	6	2	0	4
Carrizo citrange	1990	1	0	5	4	0	2
Volckameriana	1990	0	0	6	3	0	3
Sampson tangelo	1990	1	0	5	1	0	5
MxT	1992	0	3	3	2	2	2
X639	1992	3	2	1	1	2	3
Gou Tou	1992	1	1	4	1	1	4
Orlando tangelo	1992	0	0	6	0	0	6
Zhu Luan	1992	1	0	5	2	1	3
Marsh grapefruit	1992	0	0	6	0	0	6
Cleopatra mandarin	1995	0	1	5	2	1	3
Sun Chu Sha	1995	0	1	5	0	1	1
C35 citrange	1995	1	0	5	3	2	1
Sunki mandarin	1996	0	1	5	1	0	5
Benton citrange	1996	0	3	3	0	2	4

+/- Doubtful

**Table 4.2.13.6.** Summary of best (least difference between inoculated and un-inoculated) and worst rootstocks according to the CB evaluation criteria.

Criterion	Rootstock	
	Best	Worst
Tree size	Swingle citrumelo (1990) X639 (1992) Cleopatra mandarin (1995)	Empress mandarin (1990) Zhu luan (1992) C35 citrange (1995)
Yield	Swingle citrumelo (1990) X639 (1992) Sun Chu Sha (1995)	Empress mandarin (1990) Marsh grapefruit (1992) Benton citrange (1995)
Water uptake	Swingle citumelo (1990) Gou Tou (1992) Sun Chu Sha (1995)	Carrizo citrange (1990) X639 (1992) Cleopatra mandarin (1995)
12-kd protein presence	Swingle citrumelo (1990) Gou Tou (1992) Sun Chu Sha (1995)	Carrizo citrange (1990) MxT (1992) C35 citrange (1995)

## Conclusion

Trees on Swingle citrumelo, X639 and Sun Chu Cha rootstocks appear to exhibit the most tolerance and growers should consider these rootstocks in CB areas. Empress mandarin, Carrizo citrange, C35 citrange and Benton citrange rootstocks appear to be the most sensitive.

An Idaeovirus was detected in citrus trees with CB and the researchers hypothesised that it may be the cause of CB (Derrick *et al.*, 2006). Since this experiment is terminated, it is a good opportunity to collect leaf samples from all the trees. The samples will be submitted to Prof. Gerhard Pietersen who will test the samples to see if there is any involvement of an Idaeovirus. There is a 15-year history of the trees that will give some additional information. The samples will be taken as soon as possible because the trees may be removed by the grower.

## Future research

A final evaluation was done during the 2007/2008 season. The objective of the experiment has been achieved and will therefore be terminated.

Leaf samples will be collected for tests to determine the possible involvement of an Idaeovirus in CB trees in South Africa.

## Technology transfer

None.

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### 4.2.14 PROGRESS REPORT: Evaluation of citrus material for greening resistance Experiment 815 (2006 - 2015) by S.P. van Vuuren (CRI) and G. Pietersen (CRI-UP)

## 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 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 PKR getoets om te bevestig dat hulle besmet was met die vergroeningsbakterie en sodoende die plante blootgestel het aan die organisme. Na 3 maande word die plante vir die voorkoms van vergroeningsimptome ge-evalueer. Klone wat 'n hoë persentasie simptomeelose plante het word d.m.v. polimerase ketting reaksie (PKR) getoets om te bepaal of hulle vry van die vergroenings-organisme is (weerstandbiedend) of die organisme huisves sonder dat simptome ontstaan (verdraagsaamheid of toleransie). Twee klone, E2 en T2, is in 2006 as simptomeelose na blootstelling aan die vektor geïdentifiseer. PKR het verder getoon dat hulle vry van die organisme is. Dit is ook bevestig deur PKR dat die sitrus bladvlooië wat gebruik is vir oordraging van die organisme, besmet was. Die twee klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* isolate ge-preïmmuniseer en in die boord vir verdere evaluasie uitgeplant. Vier klone wat in 2006 verkry is, is op onderstamme vermeerder vir blootstelling aan sitrus bladvlooië. As gevolg van lae 'n populasies van die insek vektor, is blootstellings nog uitstaande. Van 65 embryo oordragings in 2007, kon geen plante genereer word nie.

## Summary

Attempts are made to obtain greening resistance by rescuing embryos from healthy chimeras on greening infected fruit and growing them on artificial medium. The little plants 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 PCR to establish if they were infectious. After 3 months, the plants are also evaluated for the presence of greening by visual inspections. Clones with high percentage symptomless plants are tested by PCR to establish resistance or tolerance. Two clones, GTC-E2 and GTC-T2, were identified symptomless after exposure to the vector. PCR confirmed that they were free of the greening organism. It was also confirmed that some batches of psylla that were used for challenging the plants were infected with the greening organism. These two clones have been multiplied on virus-free rootstocks and separately pre-immunised with two CTV sources where after they were planted in an orchard for further evaluations. The 4 clones that were obtained in 2006 were multiplied on rootstocks but were not challenged due to low populations of the insect vector. From 65 embryo transmissions during 2007, no plants could be generated.

## Introduction

Huanglongbing (HLB) (syn. Greening disease) in South Africa, caused by the gram negative bacterium "*Candidatus Liberibacter africanus*" (Garnier *et al.*, 2000), remains the most destructive disease in the cooler production areas of South Africa. In the past, crop losses of 30-100% were recorded in some areas due to the unmarketability 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 vector (Oberholzer *et al.*, 1965; Schwarz, 1968). In the Western Cape, 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 former 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 HLB resistant citrus cultivars by conventional breeding were unsuccessful (de Lange *et al.*, 1985). One clone showed some resistance but the internal quality of the fruit was unacceptable for consumption. Breeding to attain acceptable quality, without losing resistance, was regarded as having a low chance of success and the attempts were terminated (unpublished data). In another attempt, clones were collected by Dr. C.H. Buitendag from “healthy” twigs growing out from infected branches in heavily infected orchards (personal communication). None of these showed tolerance or resistance in subsequent field evaluations (unpublished data).

Chimera development on citrus fruit is a genetic disorder and occurs quite often. Some cultivars are more prone to this disorder than others. HLB 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. Immature seeds can be removed aseptically and regenerated on artificial medium, the process referred to as embryo rescue (Button *et al.*, 1974). Possibly resistant plants may be generated from the “abortive” seeds in these healthy fruit sections by means of embryo rescue. Artificial challenging of regenerated plants with the HLB bacterium by means of the insect vector *Trioza erytreae*, and using molecular techniques (Hocquellet *et al.*, 1999; Irey *et al.*, 2006; Li *et al.*, 2007; Planet *et al.*, 1995; Villachanoux *et al.*, 1992) 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 HLB infected fruit and to screen the recovered clones for genetic resistance or tolerance to HLB before planting promising clones in the field for evaluation under commercial conditions.

## Materials and methods

**Embryo rescue.** Delta Valencia and Olinda Valencia fruit with greening symptoms, displaying healthy chimeras, were collected in various 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” seeds were dissected aseptically from the healthy sectors of diseased fruit and cultured on modified Murashige & Tucker (M&T) (1969) medium containing 500 mg/l malt extract. Cultures were allowed to develop for 4 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 and artificial challenge of embryo rescued plants with the HLB organism by the insect vector.** When shoots of a clone had developed to 1-2 cm on the artificial medium, they were micro-grafted to healthy rough lemon rootstocks in the greenhouse. When the grafts had developed approximately 15-20 mature buds, each clone was multiplied on healthy rootstocks. Ponkan mandarin was used as a susceptible control. After the buds have grown for 1-2 cm, the clones were challenged with Liberibacter by means of psylla collected in infected orchards. The plants were exposed to psylla caught during December 2005 and January 2006. 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 psyllids, alive and dead, were recovered from the challenged plants and stored separately at -20°C until polymerase chain reaction (PCR) was performed.

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 HLB symptom development was monitored and then PCR was done to confirm HLB infection.

## PCR

**DNA isolation detection of HLB in plants:** 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  $\beta$  ribosomal protein operon was carried out with primers A2/J5 (Hocquellet *et al.*, 2000) with the following programme: Initial denaturation at 94° for 60 sec, 35 cycles of 92°C for 20 sec, 62°C for 20 sec, 72°C for 40 sec, extending the extension time by 2 sec per cycle, and a final 5 min at 72°C, using Taq DNA polymerase and buffer from Promega.

## DNA isolation detection of HLB in psylla:

Psyllids collected from each plant as alive or dead were processed separately. One to five psylla were placed in a 500  $\mu$ l eppendorf tube and squashed with a sterile pipette tip after which 50  $\mu$ l 5X SSPE buffer (0.9 M NaCl, 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 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).

**Field trial for natural challenge.** Clones that showed potential resistance or tolerance to the HLB Liberibacter 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 [GXII] (Van Vuuren *et al.*, 2000) previously reported to give some protection against HLB (Table 4.2.14.3). ELISA was done on all the plants before they were planted in the field in a HLB area according to a randomised block design. Normal orchard practices are being followed to control psylla and HLB infection will be monitored on a regular basis and confirmed by PCR.

## Results and discussion

Embryo rescue. Sixty-five embryos were transferred to M&T medium of which none generated into plants. The solid medium with embryos turned liquid when they were transferred to the growth room. The situation cannot be explained.

Artificial challenge of embryo rescued plants with the HLB organism by the insect vector. The clones that were generated during 2006 (O/03/30-RA2, O/03/30-GB, OC4/10-RA, OC4/11-RA, OC4/15-RA2) were multiplied to 5 replications for the Liberibacter challenge. Low populations of the vector throughout the year resulted that the challenge is outstanding. As soon as insects are available, the clones will be challenged.

HLB leaf symptoms of plants that were challenged during 2006. Yellow veins and mottling started to develop on the leaves of some plants 6 weeks after the challenge with Liberibacter using psylla. Assessment of infection by symptom expression of plants was terminated 3 months after the challenges. One out of 6 of the challenged Ponkan control plants developed symptoms. No symptoms were observed in plants of clones GTC-E2 and GTC-T2. Generally the infection rate was lower in this challenge than in a previous challenge (2005) and varied between 2/6 and 3/6 among the symptomatic clones. The difference may be due to the time of year when field psylla were collected for the challenges. In 2005, it was done during October, just after the spring flush when the insect population was in abundance. With this challenge it was done during the middle of summer (December to January) of the following year. "Ca" *L. africanus* is suppressed by high temperatures and it is possible that the titre of the organism was low during this time due to heat suppression (Schwarz & Green, 1972).

PCR primers. DNA bands of the correct size (669 bp) were obtained with primers A2/J5. The A2/J5 primer detected the presence of HLB at higher sensitivity from plant extracts than the primers OI1, OI2 (which detect the 16S ribosomal RNA gene) previously used. This is in contradiction to the report of Li *et al.* (2007) who found that the OI1 and OI2 primers were more sensitive for "Ca. *L. africanus*".

Detection of HLB in plants and psylla. Liberibacter were detected by PCR in Wizard extracts from plants infected with HLB and agreed with the presence of leaf symptoms in all cases (Table 4.2.14.1). The results also show that the two clones (GTC-E2 and GTC-T2) that did not get infected by HLB were challenged by Liberibacter-infected psylla (Table 4.2.15.1). The PCR results of the plants were not always complimented by PCR results of the psylla (Table 4.2.14.2). It may be possible that the Liberibacter levels were below the detectable levels in psylla that tested negative but positive transmissions occurred. On the other hand, where plants tested negative but were challenged by positive psylla, that the plants had resistance to the Liberibacter. Batching will distort the true numbers, but it can be surmised that around 35% of field psylla were infected with the Liberibacter, which agrees with other observations (unpublished data).

**Table 4.2.14.1.** PCR results of embryo rescued clones challenged for 7 days with groups of 5 psylla each. Surviving and dead psylla collected at the end of the challenge period were tested to confirm positive challenges.

Clones	PCR	
	Psylla Positive/Tested	Plants Positive/Tested
Ponkan	2/6	1/6
GTC-6	2/6	2/6
GTC-7	3/6	2/6
GTC-9	3/6	3/6
GTC-E2	2/6	0/6
GTC-E3	2/6	3/6
GTC-E4	4/6	3/6
GTC-E6	2/6	3/6
GTC-T2	2/6	0/6
Control	1/2 <sup>a</sup>	1/2 <sup>a</sup>

<sup>a</sup> Known negative and positive controls.

**Table 4.2.14.2.** Comparing the presence of “*Ca. L. africanus*” in plants with the presence of the organism in batches of psylla used for challenging the plants.

PCR reaction on plants	PCR reaction on psylla	Number
-	-	29
+	-	3
-	+	8
+	+	14

Field evaluation. Initial evaluations of the potential greening tolerant (GT) clones in the laboratory look promising as apparently HLB resistant or tolerant clones were obtained. However, the ultimate test will be in the field where the plants will be repeatedly challenged for several years with natural occurring HLB *Liberibacter*.

Plants of three GT 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 4.2.14.2). Currently no results regarding their resistance or tolerance under natural conditions are available as they have been in the field for less than a year. Their horticultural properties will also be evaluated.

**Table 4.2.14.3.** 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 <sup>a</sup>	SM 49
GTC-CV	Carrying original CTV source

<sup>a</sup> This clone had a low % infection in the laboratory test during 2005. CTV source SM 49 may increase its HLB tolerance.

## Conclusions

A total of five embryos that were rescued developed into suitable material for micro-grafting or planting. Four of these were from the healthy sectors of chimeras while only one was derived from a greening infected sector. For clone O/03/30, a plant from the greening as well as the healthy sector developed. This provides material that may be suitable for comparison of genes.

Clones from 2006 were grafted on rootstocks and pre-immunised with suitable CTV isolates. After pre-immunisation is confirmed by ELISA, they will be planted in the field.

## Acknowledgement

I would like to thank Gerhard Pietersen and Aletta Kotzé who did the PCR of the psylla.

## Future research

- Do embryo rescue when fruit are available;
- Establish plants when embryos develop into plantlets;
- Challenge plants that have been multiplied;
- Do PCR on challenged plants and psylla used for challenging
- Measure trees of field trial to assess growth.

## Technology transfer

S.P. van Vuuren and B.Q. Manicom, 2007. Initial attempts to Obtain Huanglongbing Resistant or Tolerant Sweet Orange by Embryo Rescue from Healthy Chimeras of Diseased Fruit. XVII<sup>th</sup> Conf. Intern. Org. of Citrus Virologists. 22-26 October, 2007. Adana, Turkey.

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**4.2.15 PROGRESS REPORT: Eradication of citrus greening infections in existing orchards**  
Experiment 818 (2006 – 2009) by M.C. Pretorius (CRI)

**Opsomming**

Huanglongbing, wat meer algemeen as sitrusvergroening bekend staan, is 'n ernstige bakteriese siekte wat alle sitrus kultivars affekteer. "*Candidatus Liberibacter africanus*" is die siekte veroorsakende spesie. Algemene siektesimptome is die vergeling van blaarnerwe en omliggende plantselle, gevolg deur die vergeling en "mottling" van die blare. Siektesimptome verskil tussen kultivars. Bekende effektiewe beheermaatreëls sluit die voorkoming van beweging van plantmateriaal van besmette gebiede na onbesmette gebiede in, sowel as die voorsiening van siekte-vrye plantmateriaal aan die sitrusbedryf, die effektiewe beheer van die vektor, en die vermindering van die inokulum deur verwydering van die besmette bome en takke. Die doel van hierdie proef was om 'n nuwe benadering tot sitrusvergroenings- beheer te evalueer deur die bakterie inokulum in reeds besmette plantmateriaal (in die veld asook in potte) met lowerbespuitings met 'n sistemiese produk te verminder. Die effektiwiteit van 'n hittebehandeling teen die bakteriese infeksie, asook die kombinasie met die sistemiese produk is ook ge-evalueer. Twee pot proewe en een veld proef is uitgelê. Bome in potte is met plastiek bedek en die 'n gelyke aantal is met die sistemiese produk gespuit. Die veldproef is eenmalig teen verskillende dosisse toegedien. In die hittebehandeling was die totale grade/uur bo 30°C meer as 4000 (D/H 30°C ) en het effektief die vergroeningsbakterie verlaag, wat vorige resultate bevestig. Die blaarbespuitings met die sistemiese produk het belowende resultate in die potte gelewer. Die veldproef se resultate was teleurstellend en meer gereelde toedienings word voorgestel eerder as 'n eenmalige toediening.

**Summary**

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. Heat treatments were also applied on their own and in combination with one of the foliar applied systemic products. Two pot trials were laid out at CRI: 1) To evaluate the effect of the combination of heat treatments and the combination of one of the foliar applied systemic product to reduce the *Liberibacter* inoculum in greening infected trees for a period of 6 months; 2) To determine the effectiveness of applying both the foliar applied systemic products at different times and rates during the season for a period of 6 months. A field trial was laid out at Crocodile Valley Citrus Co. and infected trees were sprayed once only in spring with one of the foliar applied systemic products to determine the effect on *Liberibacter* inoculum in infected trees. The heat treatment confirmed previous results that it is possible to inhibit the bacterium if the total Centigrade degree hours above 30°C (DH/30) can be raised to at least 1 900 (DH/30) and maintained for at least 2½ months. The results of the foliar applied systemic products in the pot trials are promising and should be repeated to confirm the data obtained in the trials. The field trial data was disappointing and the pot trial data will be used to lay out an extended field trial that will include more sprays at different dosages.

**Introduction**

Citrus Huanglongbing (HLB), commonly called citrus greening, 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 Huanglungbin, 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 to be similar, 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 Graca, 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. The species were named "*Ca. Liberibacter africanus*", (causing the disease in Africa), "*Ca. Liberibacter asiaticus*" and "*Ca. Liberibacter americanus*" (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 America's and *Trioza erytreae* in Africa (Bové, 2006). Only the African greening, "*Ca. L. africanus*" 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 dieback. 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 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 is to evaluate a new approach to effectively control the greening bacteria in citrus trees in greening infected regions by possibly reducing the bacterium with a single systemic foliar application and to confirm Schwarz's heat treatment results.

## **Materials and methods**

Three trials, consisting of two pot trials and one field trial, were conducted during the 2006 and 2007 seasons. The two pot trials were laid out at CRI. Delta Valencia trees in 10 l pots were used for both trials. In the first trial, the combined effect of heat treatment and the application of a foliar applied systemic product (for confidentiality reasons, this product will only be referred to as "Product A") to inhibit the greening organism was evaluated. In the second trial, an additional foliar-applied systemic product (for confidentiality reasons, this product will only be referred to as "Product B") were evaluated together with Product A. In the field, greening-infected trees at Crocodile Valley Citrus Co. were only sprayed with Product A as a single foliar application to determine the effect of this product on the Liberibacter in infected trees.

### CRI pot trials

Pot trial 1 consisted of seven treatments that included the combination of heat treatments and foliar applications of Product A. Six single trees served as replications. Two treatments consisted of covering the trees with plastic only. Four treatments were sprayed with Product A and thereafter covered with plastic. 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 4.2.15.1. The potted trees were covered with handmade plastic dome structures except for the untreated control treatment representing the untreated, standard control. The temperatures inside and outside the domes were logged on an hourly basis, 24 hours per day, with a Squirrel data logger (SQ 1025). Two periods were planned for the heat treatments,

the first for 3 months and the second for six months, commencing November 2006 until the end of April 2007. The total Centigrade degree hours above 30°C (DH/30) inside and outside the domes was recorded. Leaf samples were collected during the cooler months at the end of winter in 2006, prior to the first applications of the sprays and heat treatments. The trial was sampled again in the winter months in 2007. Samples consisted of thirty leaves per tree of each treatment and were sent to the University of Pretoria for PCR analysis.

**Table 4.2.15.1.** The evaluation of a combination of systemic foliar applications and heat treatments in pot trial 1, conducted at CRI for the control of HLB for the period November 2006 to April 2007.

Treatment	Dosage/10 ℓ water	Period
Untreated Control	-	-
Heat treatment	Control	Full period - ± 6 months
Heat treatment	Control	Half period - ± 2½ months
Product A + Heat	1mℓ + heat	Full period
Product A + Heat	2mℓ + heat	Full period
Product A + Heat	1mℓ + heat	Half period
Product A + Heat	2mℓ + heat	Half period

Pot trial 2 consisted of nine treatments with five single tree replications per treatment. Product 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 4.2.15.2. 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. Thirty leaves per tree of each treatment were collected and sent to the University of Pretoria for PCR analysis.

**Table 4.2.15.2.** The evaluation of two foliar applied systemic products applied at different times and rates conducted at CRI as the second pot trial, for the control of HLB for the period 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

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.

#### Field trial

Seven-year-old greening-infected Delta Valencia interplant trees at Crocodile Valley Citrus Co. were used as an initial screening trial. Product A was applied at three different dosages (100 mℓ, 200 mℓ & 400 mℓ/100 ℓ water) as a single foliar application in the spring of 2006. The product was 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. Leaf samples were collected prior to the application of the products and sent to UP for PCR analysis to confirm the presence of HLB. Three single trees per treatment were used in the trial. The treatments, dosages and time of applications are shown in Table 4.2.15.3. 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 weekly basis from November 2006 to April 2007. The occurrence of fallen fruit after the normal November drop could be due to the presence of greening bacterium in the trees. Leaves were sampled in the winter of July 2007 to determine the status of the greening organism in the treated trees.

**Table 4.2.15.3.** The evaluation of a single spring application, applied in September 2006 of Product A applied at three different rates at Crocodile Valley Citrus Co. as a field experiment on interplant greening-infected Delta Valencia trees for the control of HLB.

Treatment	Dosage/100 ℓ of water	Time of Application
1. Untreated control	-	-
2. Product A	100 mℓ	September 2006
3. Product A	200 mℓ	September 2006
4. Product A	400 mℓ	September 2006

The leaf samples from all the trials were tested with species-specific primers for “Ca.” *L. africanus* 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, petiole and young bark scrapings of the samples submitted. The PCR was performed twice on the DNA extract of each sample. Positive controls and negative controls (using water instead of template DNA) were included.

## Results and discussion

### CRI pot trials

Temperatures in Pot trial 1 were logged on an hourly basis inside the plastic domes as well as the ambient temperatures outside the plastic domes, for a period of 3 months. Initially a second treatment was planned to heat-treat the plants for 6 months as well. However, this was not feasible because of very high temperatures being recorded inside the plastic domes during the 2½-month period. It was visibly clear that after 3 months it would be fatal for the trees to be exposed to these extreme heat conditions for longer periods, therefore, the plastic covers were removed. Maximum temperatures inside the plastic dome covers in excess of 50°C were recorded twice during the recorded period. The results of the data logger’s total DH/30 for the logged period are presented in Table 4.2.15.4.

All the leaf samples collected prior to the commencement of the first applications in the trials tested positive for HLB. No phytotoxic reaction was visible on leaves of any of the foliar sprayed trees. The PCR results obtained from the samples taken after the treatments in pot trials 1 and 2 are presented in Tables 5 and 6 respectively. The August 2007 PCR results of pot trial 1 clearly indicate that the heat treatments and the foliar application of Product A were able to reduce the greening bacteria in the treated trees, whereas the untreated control treatment’s status remained unchanged as positive (Table 4.2.15.5). The results of the heat treatment obtained by the data logger recorded a total of 4001.5 DH/30 inside the plastic domes compared to the ambient recordings of 190.43. The total of 4001.5 DH/30 clearly had a positive effect in reducing the greening bacteria in the heat treated trees (Table 4.2.15.4). These results correlate with data from Schwarz and Green (1972) where it was reported that heat applications in excess of 1900 DH/30 successfully inhibited greening symptoms. Heat treatments in mature orchards will be impractical but could be used as an alternative method of the control on young trees if the DH/30 is closely monitored and temperatures do not rise to extreme levels inside the covers. The combination of the foliar applied Product A and the heat treatments were also effective in reducing the greening bacteria. These results are promising and further research is necessary to confirm the effectiveness of Product A.

In Pot trial 2, Product A and B were applied at two different dosages and different times. The PCR results (Table 4.2.15.6), indicate that only Product A, applied at double the dosage, was effective in reducing the greening bacterium to an undetectable level. With the exception of two treatments, viz. Product A applied on a monthly and bi-monthly basis, all the other treatments were unable to substantially reduce the the bacterium below undetectable levels. When compared to the untreated control, the PCR test clearly indicates that all five trees in the untreated control were positive, whereas in the treated trees, results of only 2 and 3 positive samples out of 5 were recorded, indicating a reduction of the bacterium levels in the treated plants. Although Products A and B in the other treatments did not reduce the greening bacterium to undetectable levels, the results are still very promising and it is suggested that the trial should be repeated for another season.

No phytotoxic leaf symptoms could be found on any of the trees treated in these trials.

**Table 4.2.15.4.** Total monthly Celcius degree-hours above 30°C inside and outside the plastic domes collected with a Squirrel data logger for a period of three months commencing November 2006 to the end of January 2007 in Pot trial 1.

Date	Celcius degree-hours above 30°C*	
	Ambient	Inside Plastic dome
November 2006	0.03	573.6
December 2006	88.7	1560.4
January 2007	101.7	1867.5
<b>Total</b>	<b>190.43</b>	<b>4001.5</b>

\* One degree above 30°C for one hour = 1 DH.

**Table 4.2.15.5.** Results of the PCR analysis conducted on leaf samples collected prior to the commencement of pot trial 1 and one year later.

Treatment	Dosage / 10 ℓ water	Heat exposure (months)	Aug 06 # Tested / # +	Aug 07 # Tested / # +
Untreated Control	-		6/6	6/6
Heat treatment	-	6 mo	6/6	6/2
Heat treatment	-	2 ½ mo	6/6	6/0
Product A + Heat	1 ml	6 mo	6/6	6/0
Product A + Heat	2 ml	6 mo	6/6	6/1
Product A + Heat	1 ml	2 ½ mo	6/6	6/0
Product A + Heat	2 ml	2 ½ mo	6/6	6/0

**Table 4.2.15.6.** Results of the PCR analysis conducted on leaf samples collected prior to the commencement of pot trial 2 and one year later.

Treatment	Dosage / 10 ℓ water	Time of application	Aug 06 # Tested / # +	Aug 07 # Tested / # +
Untreated Control	-	-	5/5	5/5
Product A	1 ml	1 application only	5/5	5/2
Product A	2 ml	1 application only	5/5	5/0
Product A	1 ml	Every two weeks	5/5	5/2
Product A	1 ml	1 x month	5/5	5/5
Product A	1 ml	1 x every 2 month	5/5	5/5
Product B	1 ml	Every 2 weeks	5/5	5/3
Product B	1 ml	1 x month	5/5	5/3
Product B	1 ml	1 x every 2 month	5/5	5/3

#### Field trial

The results obtained from the PCR analysis of the field trial during spring in September 2006 at Croc Valley Citrus Co. are presented in Table 4.2.15.7. All the leaf samples collected in the trial tested positive for greening. The fallen fruit that was collected on a weekly basis are presented in Table 4.2.15.8.

The results of the field trial (Table 4.2.15.7) were very disappointing as it is clear that none of the different dosages were successful in reducing the greening bacterium. This can possibly be attributed to infection levels of the greening bacterium that were too high to justify only one application per season. The product was only applied only once according to its withholding period on the registered label, which only allows a single spray during spring. No phytotoxic reaction was visible on leaves of any of the sprayed trees. No conclusive results could be obtained with the fruit drop evaluation (Table 4.2.15.8). It was anticipated that less fruit would have fallen from treated trees if the foliar applications were successful. The high number of fruit collected when the trial started (Row 1, 8/11, Table 4.2.15.8) could be attributed to the normal November fruit and leaf drop. If these fruit is not included in the total of fruit at the end of the season, the results indicated that less fruit were collected under the treated trees. It is recommended that an extended field trial with a different approach is planned for the next season.

**Table 4.2.15.7.** Results of the PCR analysis conducted on leaf samples collected prior to the commencement of the field trial as well as a year later.

Treatment	Dosage/100 ℓ water	Aug 06 # Tested / # +	Aug 07 # Tested / # +
1. Untreated control	-	3/3	3/3
2. Product A	100 ml	3/3	3/2
3. Product A	200 ml	3/3	3/3
4. Product A	400 ml	3/3	3/3

**Table 4.2.15.8.** Results of fallen fruit collected weekly in the field trial at Croc Valley Citrus Co. for a six month period from November 2006 to April 2007 after a single foliar application of Product A was applied in September 2006.

Date	Dosage/100 ℓ water			
	Control	100 ml	200 ml	400 ml
8/11	125	178	301	228
14/11	31	28	22	28
21/11	17	20	14	15
27/11	11	11	15	13
5/12	3	4	5	7
12/12	0	2	2	4
18/12	0	0	1	0
25/01	150	16	6	6
1/02	2	3	3	4
8/02	1	0	0	1
13/02	0	2	0	0
20/02	1	1	0	0
27/02	0	0	0	0
6/03	0	3	2	0
13/03	2	0	0	0
11/04	7	16	1	8
TOTAL	350 (225)*	283 (105)	372 (71)	314 (86)

\* The November 8/11 # of fruit subtracted from total.

### Conclusion

If it is found that these products were to be effective to control or eradicate the greening bacterium in the plant, this control measure would have tremendous benefits for growers in the cooler production areas. These products are safe to use, easy to apply and will therefore be a viable economic option to producers for utilization in greening infected orchards.

### Future research

Results of the samples collected (both the pot and field trial) during the winter months of 2008 will indicate whether the products were effective in reducing the greening bacterium in infected citrus trees. If proven to be effective it is suggested to do a commercial trial by spraying the products on a bigger scale on infected trees.

### Technology transfer

The following study groups were visited:

MC Pretorius	10/07/07	Katrivier	Vergroening
MC Pretorius	11/07/07	Kirkwood	Vergroening
MC Pretorius	11/07/07	Addo	Vergroening
MC Pretorius	12/07/07	Patensie	Vergroening
MC Pretorius	12/07/07	Baviaans	Vergroening

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### 4.2.16 PROGRESS REPORT: Epidemiology of greening disease – alternate hosts and spread Experiment 886 (2007 - 2008) by Baby Phahladira and Gerhard Pietersen (CRI-UP)

## Opsomming

Inligting oor die moontlike voorkoms van "*Candidatus Liberibacter africanus*" in inheemse plante in Suid-Afrika sal help dat 'n geïntegreerde beheer strategie vir die vergroening siekte ontwikkel kan word. In hierdie studie word verskeie inheemse bome van die Sitrus familie (*Rutaceae*) ge-evalueer vir hul potensiaal as gashere vir "*Ca. L. africanus*". Monsters van verskeie inheemse plante van die *Rutaceae*, met of sonder simptome, is versamel in natuurlike bos naby sitrus boorde of in botaniese tuine. Die teenwoordigheid van "*Ca. L. africanus*" in die monsters is bepaal deur DNS ekstraksie en konvensionele PCR met 'n gepubliseerde metode sowel as met 'n nuut-ontwikkelde multipleks PCR metode met 'n interne plant-spesifieke kontrole. Een *Calodendrum capensis* (Kaapse kastaiing) vanaf Nelspruit en twee vanaf Pretoria was positief vir *Liberibacter* DNS. DNS nukleotid volgorde bevestigings het bevestig dat die DNS die van "*Ca. L. africanus*" spp. *capensis* is. Die gasheer reeks van "*Ca. L. africanus*" is ook bepaal deur verskeie inheemse lede van die *Rutaceae* te inokuleer met die bakterieë deur enting. Hoë Ct waardes met "real-time" PCR dui op moontlike lae konsentrasies van die patoog in van die ge-inokuleerde plante, maar die resultate moet bevestig word na verdere verloop van tyd na inokulasie. Indien bevestiging verkry word, is dit moontlik dat van die gashere 'n rol in die epidemiologie van "*Ca. L. africanus*" in Suid Afrika speel. Ruimtelike analyses van verspreiding van vergroening in drie boorde het nie toegelaat dat gevolgtrekkings oor die rol van aangrensende inheemse plante gemaak kan word nie. Dit was aangesien die een boord 'n gradient gehad het as gevolg van bome van verskillende ouderdomme in die twee helftes van die boord, en die ander twee boorde te lae vlakke van vergroening gehad het om statisties te analiseer.

## Summary

The identification of alternate hosts to citrus of "*Candidatus Liberibacter africanus*" amongst the indigenous plants of South Africa will aid in the development of an integrated control strategy for greening disease. In this study, indigenous plants, mainly of the citrus family (*Rutaceae*), were evaluated for their capability to host the pathogen. 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 "*Ca. Liberibacter africanus*" was investigated by DNA extraction and a published conventional PCR protocol, and a newly developed multiplex PCR containing an internal control. One *Calodendrum capensis* (Cape chestnut) plant from Nelspruit and two from Pretoria tested positive for the presence of *Liberibacter* DNA. DNA sequencing confirmed the DNA to be that of "*Ca. Liberibacter africanus*" subspecies *capensis*. The host range for "*Ca. L. africanus*" was also studied amongst a number of plants of

the family *Rutaceae* by graft-transmission tests. High Ct values in real-time PCR tests on inoculated plants were obtained, and might be indicative of low concentrations of the bacterium, but the results need confirmation after a longer period post-inoculation. If the results confirm the presence of “*Ca. L. africanus*” from these Rutaceous plants, this might indicate the possibility that these plants host the bacteria and may play a role in its epidemiology locally. Spatial analysis of three citrus orchards for greening did not allow conclusions regarding spread from surrounding indigenous vegetation as one orchard had a gradient due to age differences in trees in the two halves and the remaining two orchards had relatively low incidences of the disease.

## Introduction

Citrus greening is a destructive disease of citrus (Bové, 2006) and is caused in South Africa by a fastidious bacterium “*Candidatus Liberibacter africanus*”. 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. The perpetuation of the disease may be due to the presence of hosts other than citrus, which may serve as reservoirs of the disease. We propose to study 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 require graft or vector transmission of the bacterium and which do not allow the analysis of large numbers of samples. 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. In the current study we plan to determine if alternate hosts to citrus exist by using a combination of PCR to detect the bacterium in field-collected plants in regions of high greening infection pressure, as well as by grafting indigenous Rutaceous species with known sources of bacteria. The PCR will be modified to include an internal control directed at an ubiquitous plant gene. This will serve as an indicator of the success of DNA extraction from various plant species and the lack of endogenous plant inhibitors from these indigenous plants being tested for the first time by PCR. The spread of greening will also be monitored at orchards in which indications of primary spread from the direction of natural (non-citrus) vegetation may appear. Intensive sampling of the natural vegetation in areas where such gradients exist will be done. Should *Liberibacter* variants be obtained in indigenous hosts, some sequence data will be generated, transmission studies will be performed to assess whether they are able to infect *Citrus* sp. and whether they are transmissible by *Trioza erytreae*.

## Materials and methods

### *Samples.*

A total of 167 indigenous non-citrus Rutaceous hosts were collected during the past two seasons at Pretoria from Vredefort; at Nelspruit from the Lowveld National Botanical Gardens, ARC-ITSC, Crocodile Valley Citrus and Fredenheim Experiment station; at Cape Town from Kirstenbosch National Botanical Gardens; and at Alma from Rhenosterpoort. The samples were composed of *Calodendrum*, *Vepris*, *Clausena*, *Zanthoxylum*, *Agathosma*, *Acmadenia*, *Coleonema*, *Diosma*, *Adenandra* and *Euchaetis* species, and include two *Zanthoxylum capense* plants with clear blotchy mottle symptoms.

### *Conventional and Multiplex PCR.*

Multiplex PCR with an internal control was developed with *rpl* A2 and *rpl* J5 primer A2/J5 (Hocquellet *et al.*, 1999) and a second primer pair RBCL-C705/RBCL-H535 targeting the ubiquitous ribulose biphosphate carboxylase oxygenase (Rubisco) gene (Nassuth *et al.*, 2000). The multiplex PCR was developed using infected citrus material as models. The Rubisco gene were first amplified using PCR conditions similar to those described by Hocquellet *et al.* (1999) to assure the amplification of the targeted gene under conditions similar to those used for greening bacterium. Conventional PCR (Hocquellet *et al.*, 1999) was used together with the multiplex PCR to test samples for the presence of *Liberibacter* DNA to ensure that the sensitive, specific detection of “*Ca. L. africanus/asiaticus*” under conditions free of the competition between the two primer sets used in the multiplex PCR.

### *Graft transmission.*

During September 2007, a graft transmission experiment was done on 10 Rutaceae seedlings of *Agosthema capensis*, *A. ciliaris*, *Calodendrum capensis*, *Clausena anasita*, *Vepris lanceolata* and *Zanthoxylum capense*. The seedlings were inoculated with three bark patches of confirmed “*Ca. Liberibacter africanus*” infected material (UPCRI 06-0150, 06-0195 and 06-0280). Two citrus seedlings were inoculated with an infected source to serve as positive controls while one seedling of each Rutaceous and citrus host were not inoculated and are used as negative controls. Symptom expressions were monitored over the inoculation period. Samples from inoculated plants were tested for “*Ca. L. africanus*” or *asiaticus* 3 months and 6 months after inoculation.

#### Total DNA extraction.

Total DNA extraction was performed using the CTAB extraction method by Doyle and Doyle (1991) with modifications according to Fundecitrus.

#### Quantitative real-time PCR:

Quantitative real-time PCR using Taqman probe HLBpr and primers HLBafr/HLBr specific for “*Ca. L. africanus*” (Li *et al.*, 2006) was used to test three *C. capensis* plants from Rhenosterpoort in the Waterberg mountains and all the inoculated seedlings.

### Results and discussion

The “*Ca. L. asiaticus/africanus*” primer system *rpl* A2 and *rpl* J5 (Hocquellet *et al.*, 1999) was utilised in a multiplex PCR along with primers RBCL-C705 and RBCL-H535 (Nassuth *et al.*, 2000) directed to a segment of the gene coding for ribulose-biphosphate carboxylase oxygenase (Rubisco), a protein universally found in plants and involved in the Calvin cycle. This system was first optimised and assessed for its ability to detect “*Ca. L. africanus*” and/or Rubisco simultaneously from various plant species. The multiplex PCR was able to simultaneously amplify expected products of 169 bp and 500 bp for the Rubisco and “*Ca. L. africanus*” respectively. It was used to test total DNA extracts of all samples to indicate successful DNA extraction, “*Ca. L. africanus*” presence and non-inhibited reactions.

Amongst the 167 indigenous Rutaceous samples collected, 3 *C. capensis* plants tested positive by conventional PCR. Two of the plants were from Pretoria while one was from the Lowveld National Botanical Gardens in Nelspruit. The PCR products obtained, which were slightly larger than those expected for “*Ca. L. africanus*” were sequenced directly and analyses thereof revealed high similarity to that of “*Ca. L. africanus*” subspecies *capensis*. This subspecies of Liberibacter has been detected previously on *C. capensis* only in the Stellenbosch region (Garnier *et al.*, 2000), but could not be studied biologically due to the failure to establish a culture of it. Dr. van Vuuren grafted tissue from the infected *C. capensis* sample to a healthy *C. capensis* seedling supplied to him as well as to a number of citrus seedlings. The *C. capensis/C. capensis* graft took and tests to confirm the transmission of the bacteria must still be conducted. A further 3 samples of *C. capensis* from the Waterberg yielded positive Ct values using real-time PCR, possibly indicative of a low concentration of the bacteria. The Ct value results were confirmed by subjecting the completed real-time PCR reaction to electrophoresis, where the expected 100 bp products were observed. Amplicon concentrations obtained from these samples thus far have been too low for sequencing studies. This subspecies has now been detected on *C. capensis* in the Stellenbosch region (original report), Pretoria (this study), Waterberge (this study) and Nelspruit (this study) and it may be endemic in this host. To gain insight into the possible endemic nature of “*Ca. L. africanus*” spp. *capensis* in *C. capensis*, it is necessary to collect samples from larger numbers of trees spread over various geographic regions. Nine *C. capensis* trees were identified during a survey for the tree in Pretoria in late November, 2007. The sites where they grow were recorded and samples of these trees will be collected in winter 2008 to test for “*Ca. L. africanus*”, and the spp. *capensis*. None of the remainder of non-citrus, mainly Rutaceous plants, collected thus far from various sites has yielded Liberibacter specific amplicons following total DNA extraction and tests for the presence of “*Ca. L. africanus/asiaticus*” using either the A2/J5 primer based PCR (Hocquellet *et al.*, 1999) or the multiplex PCR recently developed to detect both “*Ca. L. africanus/asiaticus*” (using A2/J5 primers) and the ubiquitous Ribulose bisphosphate carboxylase oxygenase (Rubisco). The two symptomatic *Zanthoxylum capensis* samples must be re-collected and tested for Liberibacters using very low stringency conditions or with primers to more conserved regions in the Liberibacters, in order to detect potentially differing species of Liberibacter.

Samples of both *Agosthema* species inoculated with “*Ca. L. africanus*” died during the course of the experiment. This may be due to the Liberibacter pathogen but is more likely associated with root pathogens, as plants displayed the rapid wilting and death responses typical of root diseases. None of the *Calodendrum capensis*, *Clausena anasita*, *Vepris lanceolata* and *Zanthoxylum capense* samples pre-tested to ensure their “*Ca. L. africanus*”-free status, inoculated with “*Ca. L. africanus*” showed symptoms associated with greening over the inoculation period. 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 and robust nature quantitative real-time PCR was then used to test DNA extracts of these plants. High Ct values were obtained for some inoculated samples that could indicate possible low concentration of bacterium. This data still requires confirmation.

Two citrus orchards, one in Schoemanskloof (6494 trees, Navels and Valencias) the other at Nelspruit (692 trees of various Navel selections) were monitored on a tree-for-tree basis for greening symptoms by Dr. van Vuuren, Ms. Baby Phahladira, Mr. M. Schwerdtfeger and G. Pietersen on the 9<sup>th</sup> to the 11<sup>th</sup> of July 2007. Trees were assigned a row/plant position coordinate and infected and missing trees were plotted and

analysed to establish greening spread. A further grove near Nelspruit was similarly monitored and analysed. The Schoemanskloof orchard yielded an unexpected high infection level of greening of 29%. A clear gradient within this block existed, but was due to one half of the block being considerably older than the other half, with concomitant larger numbers of infected plants. This compounding effect made it difficult to make conclusions regarding the presence of gradients due to surrounding indigenous vegetation. The two Nelspruit orchards yielded relatively low greening incidences and could not be statistically analysed for gradients.

Unsubstantiated reports of the negative effect of guava having a negative effect on greening or Huanglongbing (HLB) spread were investigated during winter, 2007. This was done in the Rustenburg district, an area known to have high greening disease pressure. Ten-year-old citrus trees directly adjoining a more than 30-year old guava plantation were monitored individually for greening symptoms, with symptoms confirmed by PCR. An unexpectedly low incidence of greening infected trees, suggested that either a low disease pressure actually exists in the area during the past years (possibly the very hot dry past few years or regular insecticide applications had a dramatic effect on psylla numbers), or the guavas were serving as an effective repellent over a greater spatial scale than that monitored. However, one greening affected tree amongst only 4 found amongst the initial 1092 plants monitored was directly next to the guava plantation, possible evidence that the repellent theory was not correct. To test the possibility that the repellent action may have a greater spatial influence, increasingly distant citrus blocks were monitored to determine whether a more gradual infection gradient could be observed. During this monitoring (done rapidly with a vehicle due to time constraints) no evidence of a gradient could be noted and very few additional greening infected citrus trees could be found. While the lack of greening in this area, known to have high greening pressure is very unusual, it is not possible to draw reliable conclusions from our observations. However, the presence of a greening infected tree, one of only a few such trees, found directly next to the guava plantation, led us to decide that the investigation into control of greening by some exploitation of guava was not worth pursuing. However, during the IOCV conference held in Turkey, 2007 a presentation titled "Investigations of the Effect of Guava as a Possible Tool in the Control/Management of HLB" by T. Gottwald *et al.* displayed some convincing laboratory evidence of a negative effect of guava on psylla (*Diaphorina citri*) incidence. These studies will be followed on in order to gain insight into the potential use of compounds derived from guava for the use of psylla control.

## Conclusion

This study has found *Calodendrum capensis* trees from widely distributed areas infected with the "Ca. Liberibacter africanus" spp. capensis variant, and may indicate that this host is endemically infected with this bacteria. Its role in the epidemiology of citrus must still be assessed. The potential of a number of other Rutaceous plants to serve as hosts of "Ca. L. africanus" must still be confirmed.

## Further objectives (milestones) and work plan

- Real-time PCR reactions need to be repeated on inoculated plants as well as on the *C. capensis* samples from the Waterberg and the resulting products to be sequenced to confirm the presence of "Ca. L. africanus" or "Ca. L. africanus" spp. capensis.
- Transmission of "Ca. L. africanus" and "Ca. L. africanus" spp. capensis to *C. capensis* and Citrus respectively must be demonstrated.
- Transmission of "Ca. L. africanus" spp. capensis by *Trioza erytreae* must be demonstrated.
- *Z. capensis* plants with greening-like symptoms must be evaluated for other Liberibacter species.

## Technology transfer

A poster was presented at the Molecular Cell Biology Group meeting at the University of Pretoria.

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#### 4.2.17 **PROGRESS REPORT: Epidemiology of greening disease-variability** Experiment 887 (2007 – 2008) by Aletta Kotze and Gerhard Pietersen (CRI-UP)

##### **Opsomming**

Slegs "*Candidatus Liberibacter africanus*" is gevind in 197 sitrus monsters versamel uit al die hoof sitrus produksie areas van Suid-Afrika. "*Ca. L. asiaticus*" en "*Ca. L. americanus*" word dus nog as eksoties tot Suid-Afrika beskou en moet uit die land gehou word deur fitosanitêre vereistes en kontroles. Rasse van "*Ca. L. africanus*" mag egter hier voorkom, en indirekte bewyse hiervoor is reeds in die verlede gekry. Dit is belangrik om rasbepalings van patogene te doen sodat doeltreffende beheerstrategieë ontwikkel kan word, veral die gebruik van weerstandbiedende cultivars. In hierdie studie is die variasie in nukleotied volgorde bepaal binne die *rpl* geen en 'n gedeelte van die "outer membrane protein" (*omp*) geen van die 197 "*Ca. L. africanus*" bronne. Geen volgorde verskille in die *rpl* of *omp* geen is onderskeidelik in 104 en 65 bronne wat tot dusvêr getoets is, gevind nie. Die gebruik van SSCP om bronne vooraf te evalueer voor volgorde bepalinge gedoen word, sal met die oorblywende bronne geskied.

##### **Summary**

Only "*Candidatus Liberibacter africanus*" was found in 197 citrus samples with greening or greening-like symptoms collected throughout citrus production areas in South Africa. "*Ca. L. asiaticus*" and "*Ca. L. americanus*" are therefore still considered exotic diseases that must be excluded from South Africa by phytosanitary regulations and controls. Strains of "*Ca. L. africanus*", however, may exist with some indirect evidence of this having been reported in the past. It is therefore important to identify strains of this pathogen in order to affect efficient control strategies, especially the use of resistant cultivars. In this study the sequence variability within the *rpl* gene and the outer membrane protein (*omp*) gene of the 197 "*Ca. L. africanus*" sources are being determined. No sequence differences have been found within the *rpl* and *omp* genes of 104 and 65 respective sources sequenced thus far. The use of SSCP to pre-screen the samples prior to sequencing will be employed on the remaining sources.

##### **Introduction**

Citrus greening is probably the most severe disease of citrus caused by a vectored pathogen, affecting a large portion of the citrus growing countries (Halbert & Manjunath, 2004). Citrus greening is also known as yellow shoot, likubin, huanglongbing, leaf mottling and yellow dragon (Gottwald *et al.*, 1989). The disease severely affects the number of marketable fruit that can be harvested from infected plants. The disease is caused by a phloem-limited gram-negative bacterium (Batool *et al.*, 2007). Three citrus greening pathogens have been discovered, namely "*Candidatus Liberibacter asiaticus*", "*Ca. L. africanus*" and "*Ca. L. americanus*". A sub-species of "*Ca. L. africanus*", namely "*Ca. L. africanus*" subsp. *capensis*, has been described only in the Western Cape region of South Africa from an indigenous Rutaceous host, *Calodendrum capensis*. Only "*Ca. L. africanus*" and "*Ca. L. africanus*" spp. *capensis* are known to occur in South Africa. Both "*Ca. L. asiaticus*" and "*Ca. L. americanus*" have recently been detected in regions of the world previously free of any Liberibacters, and it has become important to unequivocally determine the presence of only "*Ca. L. africanus*" and its *capensis* subspecies in South Africa. A collection of samples of greening and greening-like symptomatic citrus was done in October - November, 2006 and tested by PCR whether exotic "*Ca. L. americanus*" and "*Ca. L. asiaticus*" occur in South Africa. While no "*Ca. L. americanus*" amplicons were obtained, amplicons of "*Ca. L. africanus*" and "*Ca. L. asiaticus*" 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.

Furthermore, in South Africa greening has been controlled to manageable levels through the use of clean stock, elimination of inoculum via voluntary and regulatory means, use of pesticides to control psylla vectors in the citrus crop, and biological control of psylla vectors in non-crop reservoirs. To increase the efficiency of control strategies it is necessary to characterise the bacterium and to determine if variants with a differing epidemiology exist. Furthermore, as resistance to the disease 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 their 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. Bastianel *et al.* (2005) demonstrated that variants of “*Ca. L. asiaticus*” can be differentiated based on the sequence obtained from the Omp gene. Tests, using monoclonal antibodies in ELISA tests in the past, failed to detect all sources of the bacterium, an indication of variability of the epitope to which the monoclonal antibody was directed (Garnier *et al.*, 1991). Furthermore, van Vuuren (1993) demonstrated differences in efficiency of transmission of greening from different “*Ca. L. africanus*” sources, possibly further evidence that a number of biological variants may exist. The sources of “*Ca. L. africanus*” identified above can be used to determine the variability of “*Ca. L. africanus*” in South Africa. The omp Gene, shown by Bastianel *et al.* (2005), to differentiate several variants of “*Ca. L. asiaticus*” will be used to determine the variability of South African sources of “*Ca. L. africanus*”, and allow selection of variant for use as challenge sources in the resistance trails.

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 recurrence of the disease after treatment is 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 “*Ca. L. africanus*” 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 African during an October - November, 2006 collection trip. Amplicons indicative of “*Ca. L. africanus*” or “*Ca. L. asiaticus*” were confirmed in 197 sources. Total DNA extracts from each of these sources were used 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 “*Ca. L. africanus*” or “*Ca. L. asiaticus*” 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 20s at 92°C, 20s at 62°C and 45s at 72°C. The reagents used in the amplification were prepared according to the protocol of Hocquellet *et al.* (1999). A 15 µl of sample was loaded into the wells after mixing 15 µl of sample with 2 µl liter of loading buffer. The Promega 100bp ladder was used as a marker. The samples were run on a 1% gel for 30 minutes at 100V constant voltage.

Samples positive in the A2/J5 PCR were also subjected to a nested PCR system (Bastianel *et al.*, 2005). 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). Controls included a positive plant, negative control (buffer) and a healthy plant. 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). 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).

### Sequence analysis

To determine if variation occurred in the region amplified, 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 kit<sup>TM</sup>). 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

A total of 104 citrus sources amongst the 197 sources collected, yielded amplicons in the A2/J5 –GB1/GB3 multiplex PCR utilised in 2006 during tests for “*Ca. L. africanus*”, “*Ca. L. asiaticus*” and “*Ca. L. americanus*”. These were used as templates to generate further amplicons for sequencing using the *rpl* A2 and *rpl* J5 (A2/J5) primer pair of Hocquellet *et al.* (1999). These were amongst 249 samples collected during a recent countrywide survey of greening disease. Samples were selected further, based on having yielded high (three stars) to medium yield (two or one stars) amplicons indicative of either “*Ca. L. asiaticus*” or “*Ca. L. africanus*” infections during the initial screening done of all collected samples. These were then subjected to un-replicated forward- and reverse-primer mediated direct sequencing reactions and analysed using an automated sequencer at the sequencing facility of UP or at Inqaba, Pretoria. Sequence data generated from all sources indicate the presence of “*Ca. L. africanus*” only. Multiple alignment of these sequences suggest that very little variation exists within this region of the genome of the local sources. 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. In view of the lack of variability in sequence detected in amplicons of the A2/J5 primer system it was decided to employ another PCR system to identify possible sequence variants. This PCR was directed at the outer membrane protein (*omp*) gene, known to be variable in “*Ca. L. asiaticus*” (Bastianel *et al.*, 2005). The relevant primers were synthesised (IDT Technologies) and the PCR implemented using a positive control (DNA amplicon of the *omp* gene specific PCR on “*Ca. L. asiaticus*”), received from Prof. J. Bové. While clear bands could be obtained using the positive control, the PCR failed to amplify any product from known “*Ca. L. africanus*”-infected, South African DNA extracts. PCR using the A2/J5 primer set, however, confirmed the viability of the South African DNA. Different template amounts used to initiate the *omp* PCR reactions were tested. A number of MgCl<sub>2</sub> and annealing temperatures in thermal gradient matrices were tested to determine conditions for detection of the South Africa samples. No significant improvement of the PCR was achieved. PCR reagents from different companies were therefore assessed for their usefulness in this PCR. Ultimately completely different PCR reagents (Fermentas, Hanover, MD) were required to get the PCR to work against the South African DNA samples. Amplification of “*Ca*”*L. africanus*” from known infected extracts was readily obtained. Sequencing was performed on the amplified product. A total of 45 sequences with the OMP8inv seq (forward) and 20 sequences using the HP1inv seq (reverse) primers have been generated to date. Multiple alignments performed on the 45 forward sequences (with the OMP8inv seq primer) have shown no sequence variation thus far.

## Conclusion

Sequence data generated thus far confirms the presence in South Africa of only “*Ca. L. africanus*”. The lack of variation demonstrated thus far with both the A2/J5 primers as well as within the *omp* gene suggests that local sources of “*Ca. L. africanus*” may be very homogenous. However, it must be noted that only a very small portion of the genome of the bacterium is being evaluated, and as the whole genome sequence of the Liberibacters have not been determined, and variable regions not yet identified. The regions characterised within this study may be relatively conserved and not reflect variant sequences. A more rapid and cheap method to confirm the homogeneity in the genes targeted, single-strand conformational polymorphism (SSCP), will be utilised as a pre-screening method on the remaining samples before determining the sequence of potential variants.

## Further objectives (milestones) and work plan

- May-Jun, 2008: Establish and optimise SSCP system to *omp* gene PCR system in laboratory.
- June-Dec, 2008: Prepare concentration standard for real-time PCR. Optimize for quantitative use.

- Plan and initiate glasshouse trial to establish effect of Harpin compound on “*Ca. L. africanus*” concentration in plants. Perform glasshouse trial on Harpin compound. Grow plants, inoculate with Greening. Do treatments. Monitor symptoms, test for greening by real-time PCR.
- Jul-August, 2008: Use omp specific PCR followed by SSCP to analyse remaining South African “*Ca. L. africanus*” sources for variability to amplify and sequence target region of “*Ca. L. africanus*” sources collected countrywide. Do sequence analysis of any variants detected.
- July-Dec, 2008: Initiate field trial for Harpin compound. Identify orchard with sufficient greening infected trees. Confirm infection by PCR. Plan treatments.
- Dec, 2008- July, 2009: Monitor field trails. Continue with treatments.
- Design primers for PCR to target newly sequenced region of “*Ca. L. africanus*”. Optimise PCR.
- Use PCR followed by SSCP to detect potential new variants amongst “*Ca. L. africanus*” sources collected countrywide (149 sources). Determine sequence of sources which differ in their SSCP banding patterns. Do sequence analysis.
- Jan-Mar, 2009: Challenge putatively resistant plants with *Liberibacter* strains (after having determined variability). If obtained, sequence variants, develop variant specific PCR/real-time PCR.

### Technology transfer

Schwerdtfeger, M., and Pietersen, G., 2007. Survey for “*Candidatus*” *Liberibacter* species on citrus in South Africa. XVII<sup>th</sup> Conference of the International Organization of Citrus Virologists. 22-26 October, 2007. Adana, Turkey.

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#### 4.3 PROJECT: CITRUS BLACK SPOT Project coordinator: G.C. Schutte (CRI)

##### 4.3.1 Projekopsomming

Vier bespuitings van Oktober tot Januarie bestaande uit DPX (koperhidroksied), C40 (kopersulfaat), C30 (kopersulfaat plus mankoseb) en Pennfluid (vloeibare mankoseb) was effektief vir die beheer van sitruswartvlek (SSV) en kan vir registrasie aanbeveel word. Geen fitotoksisiteit of koperstippelvorming met enige van die koperbehandelings is waargeneem nie. Tenkmengsels bestaande uit C40 en C30 met Citrole 100 minerale spuitolie het ook goed gewerk. Tenkmengsels van Pennfluid met beide Benlate of Cabrio plus Citrole 100 minerale spuitolie, kan ook as twee bespuitings tydens November en Januarie aanbeveel word. Verskeie toedienings of spuitprogramme bestaande uit ’n nuwe swamdoder, BAS600F, is getoets. Ongelukkig is fitotoksisiteit in die vorm van konsentriese ringe aan die onderkant van vrugte waargeneem. Die benatters, Breakthrough en Wetcit, in tenkmengsels met mancozeb teen onderskeie dosisse van 2.5 tot 5 ml/100 l water en 75 ml/100 l water, en hoër dosisse in tenkmengsels met Ortiva en mancozeb, was effektief vir die beheer van SSV. Geen fitotoksisiteit is in beide gevalle waargeneem nie. ’n Ander benatter, Solitaire, in tenkmengsels met mancozeb was ook effektief in spuitprogramme vir die beheer van SSV teen dosisse van 50 tot 75 ml/100 l water, maar fitotoksisiteit is waargeneem (4.3.2). Verskeie swamdoders en kombinasies van produkte is as ’n enkel blaarbespuitings gedurende Augustus 2007 vir die beheer van die primêre inokulum (askospore in perithecia) op die boom toegedien. Die meeste askospore is op blaarafval gedurende Desember en Januarie geproduseer. Die minste askospore is op blare wat slegs met water (kontrole) behandel is, geproduseer, gevolg deur difenoconazole (Score) en spuit-urea met ’n fosfonaat, terwyl die fosfonaat en dodine (Syllit) van die hoogste getalle askospoorproduksie opgelewer het. Geen van

die swamdoders wat as blaarbespuitings in hierdie projek getoets is, kon askospoorproduksie op die blaarafval konstant inhibeer nie (4.3.3). *Guignardia* isolate wat op strobilurien-bevattende media met 20 dpm azoxystrobin of trifloxystrobin, of 5 dpm pyraclostrobin kon groei, is geselekteer. Daar is egter gevind dat miseliumgroei op suiwer strobilurien-bevattende agar nie 'n aanduiding van bestandheid nie, omdat die swam moontlik 'n alternatiewe respirasieweg *in vitro* kan gebruik (4.3.4).

Volwasse blare is uit boorde met 'n geskiedenis van *Guignardia* besmetting versamel en aan mikro- en dripbesproeiing onderwerp om te bepaal watter besproeiingsmetode dra die meeste tot askospoorvrystelling by. Resultate het getoon dat betekenisvol meer askospore op blare geproduseer is wat in die boord met drupbesproeiing geïnkubeer is, in vergelyking met blare in 'n boord met mikrobesproeiing. Meer askospore is naby besproeiingspunte onder die boom, as in vol son weg van besproeiingspunte geproduseer. Verskille in aantal askospore getel op verskillende tye van evaluasie, is moontlik geassosieer met die stadium van blaarkompostering, en was dus afhanklik van klimatoriese faktore soos reënval en temperatuur (4.3.5). Jong saailinge is gebruik om die moontlike infeksies van *Guignardia citricarpa* en *G. mangiferae* te bepaal. Blaarinokulasies deur piknidiospore op blare te plaas, asook deur miselium binne in plante te plaas, is uitgevoer waarna isolasies na verskillende periodes uit weefsel gemaak is. Geen infeksie kon na die blaarinokulasies bewys word nie en slegs een staminokulasie met *G. mangiferae* miselium het tot infeksie gelei (4.3.6).

Die SSV-vrye akkreditasie van sitruskwekerye en -boorde is van groot belang aangesien die vroeë opsporing van hierdie kwarantynpatogeen die risiko van inokulum verspreiding van siekte-vrye areas in suidelike Afrika kan verminder. 'n Polimerase ketting reaksie (PKR) metode is ontwikkel vir die opsporing van *Guignardia* spesies vanaf blaarafval, groen blare met simptome asook simptoomblose groen blare in die boorde en kwekerye. Hierdie metode is ge-optimeer en kan nou as deel van 'n akkreditasie skema vir die roetine opsporing van *Guignardia citricarpa* in die sitrusindustrie geïmplimenteer word (4.3.7). As deel van 'n vroeë opsporingsmetode vir *Guignardia* spp. en meer spesifiek *Guignardia citricarpa*, is 'n nuwe direkte PKR metode ontwikkel en ge-optimeer vir die isolasie en amplifikasie van *Guignardia* piknidiospore vanaf takkies in boorde. Die PKR metode blyk suksesvol te wees alhoewel die isolasie en kultivering van die piknidiospore nie effektief genoeg is nie. Verskillende isolasie metodes van piknidiospore vanaf takkies sal in die toekoms ondersoek moet word. Die PKR metode sal help met die vroeë opsporing van sitrus swartvlek (CBS) in boorde (4.3.8). 'n Oorsigartikel van navorsing spruitend uit Universiteit Pretoria is tans in finale fases van afhandeling. Hierdie artikel sal baie waardevol vir marktoegangsonderhandelinge wees.

## Project summary

Four applications from October to January of DPX (copper hydroxide), C40 (copper sulphate) and C30 (copper sulphate plus mancozeb) and Pennfluid (liquid mancozeb) was effective in controlling CBS and can be recommended for registration. No phytotoxicity or copper stippling was observed with any of the copper treatments. Tank mixtures of C40 and C30 with Citrole 100 mineral oil can also be recommended for registration. Tank mixtures of Pennfluid with Benlate and Cabrio plus Citrole 100 mineral oil, can also be recommended as two applications applied in November and January. No phytotoxicity was observed at the highest rate (200 ml/100 l water) of Pennfluid tested. Various applications or spray programmes consisting of a new fungicide, BAS600F, were successfully tested for the control of CBS. However, phytotoxicity was observed in the form of concentric rings on the bottom side of the fruit. Tank mixtures of mancozeb with the surfactants, Breakthrough and Wetcit, at respective rates between 2.5 to 5 ml/100 l water and 75 and 100 ml/100 l water, were also effective in controlling CBS. No phytotoxicity was evident in any of the treatments. Another surfactant, Solitaire, in tank mixtures with mancozeb was effective in a spray programme for the control of CBS if sprayed at rates between 50 to 75 ml/100 l water but phytotoxicity was observed on the fruit (4.3.2). Targeting the inoculum source on the tree is a relatively new concept in citrus black spot control as most ascospores were produced on leaf litter during December and January of the growing season. However, the least amount ascospores was produced on leaves sprayed with water only (control), followed by difenoconazole (Score) or spray urea plus a phosphonate. A phosphonate alone and dodine (Syllit) allowed the highest number of ascospores to be produced on leaf litter. None of fungicides evaluated in this trial, applied as foliar applications, could consistently reduce the production of ascospore inoculum on citrus leaf litter (4.3.3). *Guignardia* isolates that grew on strobilurin-amended agar with 20 ppm azoxystrobin or trifloxystrobin, or 5 ppm pyraclostrobin were selected as tolerant reference cultures. However, results in this trial suggest that the observed strobilurin-tolerance was probably associated with the ability of *G. citricarpa* isolates to utilise an alternative respiration pathway *in vitro*, rendering this technique unsuitable for resistance monitoring (4.3.4).

Mature leaves were sampled from orchards with a history of high levels of *Guignardia* infection to determine the contribution of the different irrigation methods towards ascospore release. Results showed significantly more ascospores were produced on leaves incubated in an orchard with drip irrigation, compared to leaves

incubated in an orchard with micro irrigation. More ascospores were produced close to the irrigation source under the canopies of trees, than in open sunlight at a distance from an irrigation source. Differences in numbers of ascospores observed at different times of evaluation were probably related to the stage of leaf litter decomposition and therefore, dependant on climatic conditions such as rainfall and temperature (4.3.5). Seedlings were used to determine the possible infections of *Guignardia citricarpa* and *G. mangiferae*. Plants were inoculated by placing pycnidiospores on the leaves or with mycelium by placing it underneath the bark. Isolations were then made after a period of time from the leaves and stems. No infection of the leaves could be proven. Only one stem inoculation with *G. mangiferae* resulted in infection (4.3.6).

The *Guignardia citricarpa*-free accreditation of citrus nurseries and orchards is of great importance since early detection of this quarantine pathogen may reduce the risk of spreading the inoculum to disease-free areas in Southern Africa. A polymerase chain reaction (PCR) method was developed to detect *Guignardia* spp. from leaf litter, green leaves with symptoms as well as symptomless green leaves in the orchards and nurseries. This method was optimised and can now be implemented as part of an accredited scheme for routine screening of *Guignardia citricarpa* in the citrus industry (4.3.7). As part of an early detection method for *Guignardia* spp. and in particular *Guignardia citricarpa*, a new direct-PCR method was developed and optimised for the isolation and amplification of *Guignardia* pycnidiospores from twigs in orchards. This PCR method proves to be very successful, but the isolation and culturing of the pycnidiospores has proved to be ineffective. Different means of isolation of pycnidiospores from twigs on culture media should be explored in future. The PCR method will assist in the early screening and detection for citrus black spot (CBS) in orchards (4.3.8). A review article of research from University of Pretoria is currently in its final stages completion. This article will prove invaluable during market access negotiations.

- 4.3.2 **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 2007): by G.C. Schutte (CRI)

### Opsomming

Vier bespuitings toegedien tussen Oktober tot Januarie bestaande uit DPX (koperhidroksied), C40 (kopersulfaat) en C30 (kopersulfaat plus mancozeb) was effektief vir die beheer van sitruswartvlek (SSV) en kan aanbeveel word vir registrasie. Geen fitotoksiteit of koperstippelvorming was waargeneem met enige van die behandelings nie. Tenkmengsels bestaande uit C40 en C30 met Citrole 100 minerale spuitolie het ook goed gewerk en kan ook aanbeveel word vir registrasie. Geen fitotoksiteit was waargeneem in enige van die behandelings nie. Vier bespuitings van Pennfluid teen dosisse van 150 ml/100 l water en hoër kan aanbeveel word vir registrasie. Tenkmengsels van Pennfluid met beide Benlate of Cabrio plus Citrole 100 minerale spuitolie kan ook as twee bespuitings aanbeveel word tydens November en Januarie. Geen fitotoksiteit was waargeneem nie, selfs teen die hoogste dosis (200 ml/100 l water) van Pennfluid. Verskeie toedienings of spuitprogramme bestaande uit BAS600F vir die beheer van SSV is getoets. Die belowenste pogram was mancozeb / BAS600F + mancozeb + olie / BAS600F + mancozeb + olie / mancozeb (4 toedienings) en BAS600F (50 ml/100 l water) + mancozeb + oil (3 toedienings). Laasgemoemde spuitprogram sal kwekers een spuitronde spaar. Fitotoksiteit was egter waargeneem in die vorm van konsentriese ringe aan die onderkant van die vrug. Die benatter, Breakthrough, in tenkmengsels met mancozeb was effektief in 'n spuitprogram saam met mancozeb vir die beheer van SSV teen dosisse van 2.5 tot 5 ml/100 l water. Die ander benatter wat getoets is nl. Wetcit, teen dosisse van 75 ml/100 l water en hoër in tenkmengsels met Ortiva en mancozeb, was effektief vir die beheer van SSV. Waar Wetcit toegedien was teen dosisse van 75 en 100 ml/100 l water in 'n tenkmengsel met mancozeb, was dit ook effektief teen SSV. Geen fitotoksiteit is in beide gevalle waargeneem nie. 'n Ander benatter, Solitaire, in tenkmengsels met mancozeb was ook effektief in spuitprogramme vir die beheer van SSV teen dosisse van 50 tot 75 ml/100 l water, maar fitotoksiteit was waargeneem.

### Summary

Four applications applied between October to January of DPX (copper hydroxide), C40 (copper sulphate) and C30 (copper sulphate plus mancozeb) was effective in controlling CBS and can be recommended for registration. No phytotoxicity or copper stippling was observed even at the highest rate of DPX tested (225 g/100 l water). Tank mixtures of C40 and C30 with Citrole 100 mineral oil can also be recommended for registration. No phytotoxicity was observed in any of the treatments. Four applications of Pennfluid at rates higher than 150 ml/100 l water can be recommended for registration for CBS control. Tank mixtures of Pennfluid with both Benlate and Cabrio plus Citrole 100 mineral oil, can also be recommended as two applications applied in November and January. No phytotoxicity was observed at the highest rate (200 ml/100 l water) of Pennfluid tested. Various applications or spray programmes for BAS600F exist for the

successful control of citrus black spot. The most promising spray programmes are mancozeb / BAS600F + mancozeb + oil / BAS600F + mancozeb + oil / mancozeb (4 applications) and BAS600F (50 ml/100 l water) + mancozeb + oil (3 applications). The latter spray programme will save growers one spray round. Phytotoxicity was, however, observed in the form of concentric rings on the bottom side of the fruit. The surfactant, Breakthrough, in tank mixtures with mancozeb was effective in a spray programme for the control of CBS if sprayed at rates between 2.5 to 5 ml/100 l water. No phytotoxicity was evident on any fruit in any of the treatments. The other surfactant tested, Wetcit, at rates of 75 ml/100 l water and higher in tank mixtures with Ortiva and mancozeb, was effective in a spray programme for the control of CBS. Wetcit was effective in controlling CBS when applied at rates of 75 and 100 ml/100 l water in a tank mixture with mancozeb. No phytotoxicity was evident in any of the treatments. Another surfactant, Solitaire, in tank mixtures with mancozeb, was effective in a spray programme for the control of CBS if sprayed at rates between 50 to 75 ml/100 l water. Phytotoxicity was evident on the fruit in all the treatments where Solitaire was included in the spray mixture.

## Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa), affects all commercial citrus cultivars. 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 (Kotze, 1963). 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 (Kotze, 1963; Kellerman et al., 1977). Ascospores are dependent on conversion currents and favourable environmental conditions to reach a suitable host substrate, since the maximum vertical distance of ascospore ejection from a pseudothecium is 10-12 mm (Kiely, 1948; Kotze 1963) and the horizontal disease dispersion occurs at distances below 24.7 m (Sposito et al., 2007). 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 a newly developed Quest volumetric spore trap and sampler are used to determine the first onset of ascospore release in South Africa.

A 4-spray programme of copper fungicides used for CBS control can result in rind stippling and darkening of blemishes (Brodrick, 1970; Schutte et al., 1997). However, alternating copper fungicides with mancozeb in a 4-spray programme, solved this problem. Protective fungicides became less popular after the release of postinfection 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 4-spray protective schedule (Kellerman & Kotze, 1977). However, 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 (Schutte, 1996). Two applications of kresoxym-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 (Schutte et al., 2002). Resistance development of *Venturia inaequalis* towards the strobilurins, compel the incorporation of two additional mancozeb sprays; viz. before and after the strobilurin applications. Since the registration of strobilurins in South Africa, no new fungicides were registered for use against CBS. Moreover, restrictions are implemented on the use of dithiocarbamates on citrus fruits exported to Canada and the USA. This restriction is also valid for citrus producing countries in the southern hemisphere that export fruit to the USA and Canada.

There is an urgent need for new chemicals to control citrus black spot. The registration of new fungicidal groups are extremely difficult and we therefore need to look at new formulations of copper and mancozeb and how they can fit in with existing spray programmes without causing phytotoxic effects such as copper stippling. There are also numerous new surfactants and wetters on the market. The aim is also to see if one can replace mineral spray oil with these surfactants.

## Materials and methods

Three orchards (Brits 1, 2A and 2B) were selected at Croc Valley Citrus Co. 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 but all trees were sprayed to the point of run-off. Certain treatments commenced 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. Each treatment was replicated six times in

single-tree plots arranged in a randomised complete block design. Neighbouring trees were used as guard trees between plots and within rows. 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: DPX 524 g/kg WG (copper hydroxide test product from Du Pont), Copstar 180 g/l SC (copper hydroxide, Agchem, L 7026), Du Pont Kocide 2000, 538 g/kg WG (copper hydroxide, Du Pont), Dithane M45, 800 g/kg WP (mancozeb, Dow AgroSciences, L 2914), C40 (copper sulphate, WG test product from Total), C30 (copper sulphate and mancozeb test product from Total), Pennfluid 420 g/l SC (test product from Total), Benlate 500 g/l WP (benomyl, Villa Crop Protection, L 6909), BAS600F 200 g/l SL (new experimental fungicide from BASF), Cabrio 250 g/L EC (pyraclostrobin, BASF, L 6818), Ortiva 250 g/l SC (azoxystrobin, Syngenta, L 5968) and Demildex, 850 g/l WP (copper oxychloride, Delta Chemicals, L 5094). Specific spray programmes are described below.

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 were analysed by ANOVA, using Fisher's LSD test ( $P = 0.05$ ).

## Results and discussion

### a) New fungicides

#### DPX (Copper hydroxide)

Results (Table 4.3.2.1) show that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb, Copstar and Kocide 2000 treatments and the four different rates of copper hydroxide tested with regards to the criterion clean exportable fruit. Although not significant different from each other, was there a 5% difference in the amount of clean exportable fruit between the best copper hydroxide treatment applied at a rate of 225 g/100 l water and the lowest rate of the same fungicide evaluated at a rate of 125 g/100 l water, which is significant in a zero tolerance export market. There was, however, a significant difference in percentage clean exportable fruit between the copper hydroxide rates of 150 and 175, Kocide 2000 and the lowest rate of copper hydroxide with Nu-Film 17 with regards to the criterion. There was also a 0.6% difference in the amount of clean exportable fruit between the copper hydroxide tank mixtures with either Sporekill or Nu-film 17. All the treatments were significant different from the control. Disease pressure was high as the untreated control resulted in only 25.2% clean exportable fruit. No statistical significant differences were observed between any of the fungicide treatments in the other two criteria, but they were significant different from the untreated control.

#### C40 (Copper sulphate)

Results (Table 4.3.2.2) show that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb and Copstar treatments and all the C40 treatments. The C40 rates (75, 100 and 150 g/100 l water) evaluated did not differ significantly. For example, C40 evaluated at 150 g/100 l water had a 6.4% difference from the C40 rate of 75 g/100 l water. All these C40 rates show a dosage response in terms of efficacy as the rates are lowered (75 g < 100 g < 150 g). Where C40 was evaluated a rate of 75 g/100 l water in a tank mixture with Citrole 100 mineral oil (250 ml/100 l water), it resulted in 94.0% clean exportable fruit which was not significant different from the C40 also 75 g/100 l water but without Citrole 100 oil. These treatments were also not significant different from the two standard registered treatments consisting of mancozeb and Copstar with regards to all criteria used for evaluation. With regards to the criterion fruit with 1-3 CBS lesions, four mancozeb (200 g/100 l water) and C40 applications evaluated at a rate of 150 g/100 l water were significant different from the C40 rate evaluated a 75 g/100 l water, also a four applications. The untreated control resulted only in 25.2% clean exportable fruit. All the treatments were significant different from the control with regards to all the criteria.

#### C30 (copper sulphate plus mancozeb)

Results (Table 4.3.2.3) show that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb and Copstar treatments and all the C30 treatments. Although the C30 (100 g/100 l water) tank mixture with Citrole 100 mineral oil (250 ml/100 l water) resulted in 96.8% clean exportable fruit, it was not significant different from the C30 (also 100 g /100 l water) tank mixture with Sporekill (100 ml/100 l water) that had 93.2% clean exportable fruit, a difference of 3.6%. Both these treatments had 50 g less product than that recommended for C30 alone (150 g/100 l water), showing that if either Citrole 100 or Sporekill are added to the spray mixture, you can lower the C30 rate to 100 g/100 l water and maintain effective control of CBS. These treatments were also not significant different from the two standard

registered treatments consisting of mancozeb and Copstar with regards to all criteria used for evaluation. The untreated control resulted only in 25.2% clean exportable fruit. All the treatments were significant different from the control with regards to all the criteria.

#### Pennfluid

Results (Table 4.3.2.4) show that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb treatment and the three rates of Pennfluid (100, 150 and 200 ml/100 l water) that have been tested; in all cases four applications according to the mancozeb or Copstar labels. These treatments were also not significantly different from the two standard registered treatments consisting of mancozeb and Copstar. Pennfluid tested at a rate of 200 ml/100 l water was the best treatment resulting in the highest amount of clean exportable fruit, viz. 98.6% in comparison with the untreated control that resulted only in 25.2% clean exportable fruit. All the treatments were significant different from the control with regards to all criteria.

Two applications (in November and January) where tank mixtures of Pennfluid (150 ml) or mancozeb (150 ml (registered standard) plus Citrole 100 (oil) with Cabrio (10 ml), were also not significant different ( $P < 0.05$ ) from each other. These two treatments were, however, significantly different from the same tank mixtures where Cabrio was replaced with Benlate and only resulted in 89.2% clean exportable fruit. This lower disease control with Benlate can possibly be attributed to benzimidazole resistance in the pathogen population in this experimental orchard. This treatment would have performed better if these resistant strains were absent in this orchard and can therefore also be used for CBS control in those regions of South Africa where resistant strains are absent.

#### BAS600F

Results (Table 4.3.2.5) show that the standard treatment consisting of mancozeb / Cabrio+mancozeb+oil / Cabrio+mancozeb+oil / mancozeb (4 applications) was significantly ( $P > 0.05$ ) better than the two lowest rates of BAS600F (25 and 50 ml/100 l water) plus mineral spray oil (3 applications) with regards to criterion clean exportable fruit. There was a 17.6% difference in the amount of clean exportable fruit between these respective treatments. On the other hand, BAS600F (50 ml/100 l water) in a tank mixture with mancozeb and mineral spray oil (150 g + 250 ml/100 l water) resulted in the next best treatment with 98.6% clean exportable fruit. Although the latter treatment is not significantly different from the same BAS600F rate where it was evaluated in a tank mixture with mineral spray oil only, a difference of 16.6% is significant especially when fruit are exported to a zero tolerance market. In this case, the difference of 16.7% can be attributed to the contribution of mancozeb in this tank mixture with BAS600F and mineral spray oil for the control of CBS.

Where multiple applications of BAS600F were done (6 applications every three weeks), the highest rate of 75ml/100 l water performed the best, resulting in 97.6% clean exportable fruit. This treatment was, however, not significantly different from 6 applications at rates of 25 and 50ml/100 l water using the same intervals, although they resulted in 94.6 and 86% clean exportable fruit respectively. There were also no significant differences ( $P < 0.05$ ) between the standard treatment consisting of mancozeb / Cabrio+mancozeb+oil / Cabrio+mancozeb+oil / mancozeb (4 applications) and a similar spray programme where Cabrio was replaced with BAS600F at a rate of 50 ml/100 l water (mancozeb / BAS600F+mancozeb+oil / BAS600F+mancozeb+oil / mancozeb (4 applications)). This spray programme resulted in 97% clean exportable fruit. No significant differences were observed between the standard mancozeb treatment and all the different treatments where BAS600F was included. Disease pressure was high as the untreated control resulted in only 22.6% clean exportable fruit, which was significant different from all the treatments.

The same scenario was experienced with the criterion, fruit with 1-3 CBS lesions. Here the three applications of BAS600F in a tank mixture with mineral spray oil as well as six applications of BAS600F evaluated at a rate of 50 ml/100 l water, were not significant different from the control. With the criterion 4 and more CBS lesions, all the treatments were significant different from the control. The latter had 66% fruit with four and more CBS lesions (Table 4.3.2.1).

### **b) Adjuvants**

#### BreakThru

Results (Table 4.3.2.6) show that there were no significant differences ( $P < 0.05$ ) between all the criteria used for evaluation and the standard registered mancozeb and Copstar (4 applications) and all the spray programmes consisting of BreakThru in tank mixtures with mancozeb. The best treatment with BreakThru was at a rate of 5 ml Breakthrough /100 l water, followed by 2.5 ml/100 l water and the highest rate of 10

mℓ/100 ℓ water. Although there were no significant differences ( $P < 0.05$ ) between the 5 and 10 ml/100L water rates, the 5% difference in efficacy can be attributed to the good spreading properties of BreakThru that caused extensive run-off of the fungicides at the spray volumes applied. 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 25.2% clean exportable fruit. No statistically significant differences were observed between any of the fungicide treatments in the other two criteria, but they were significant different from the untreated control.

#### Wetcit

Results (Table 4.3.2.7) show that there were no significant differences ( $P < 0.05$ ) between all the criteria used for evaluation and the standard registered standards, mancozeb and Copstar (4 applications) as well and spray programmes consisting of two applications of Ortiva+mancozeb+oil and the new spray programmes that consisted of Wetcit at two rates (75 or 100 mℓ) viz. Ortiva+mancozeb+Wetcit / Ortiva+mancozeb+Wetcit (2 applications). Where four applications were applied on a monthly basis during the susceptible period from October to January for CBS, all Wetcit rates performed well in tank mixtures with mancozeb and were also not significant different from each other ( $P < 0.05$ ). These treatments were also not significant different from the two standard registered treatments (mancozeb and Copstar) that were included in this trial. Disease pressure was high as the untreated control resulted in only 25.2% clean exportable fruit. No statistical significant differences were observed between any of the fungicide treatments in the other two criteria, but they were significant different from the untreated control. Although there was no significant differences between all the treatments, the mancozeb+Wetcit (50 mℓ) treatment (4 applications) resulted in 9.8% fruit with CBS lesions, which is not acceptable for export markets that have a zero tolerance towards CBS.

#### Solitaire

Results (Table 4.3.2.8) show that there were no significant differences ( $P < 0.05$ ) for any of the criteria used for evaluation and the standard registered mancozeb and Copstar (4 applications) and all the spray programmes consisting of Solitaire in tank mixtures with mancozeb. All treatments were significant different from the untreated control. Solitaire tested best at a rate of 50 mℓ/100 ℓ water, followed by 75 mℓ/100 ℓ water. Although there were little between the Solitaire rates, the difference in efficacy can be attributed to the good spreading properties of Solitaire that caused extensive run-off of the fungicides at the spray volumes applied. The standard mancozeb treatment resulted in 3.4 and 4.4% more clean fruit than the reduced rates of mancozeb (150 g/100 ℓ water) in tank mixtures with Solitaire. 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 25.2% clean exportable fruit.

#### **Conclusion**

DPX (copper hydroxide) evaluated at rates of 150 g/100 ℓ water and higher was effective in controlling CBS and can be recommended for registration. No phytotoxicity or copper stippling was observed even at the highest rate tested of 225 g/100 ℓ water (Fig. 4.3.2.1). Four applications of C40 (copper sulphate) at rates of 100 g/100 ℓ water can be recommended for registration for CBS control. Tank mixtures of C40 at a rate of 75 g/100 ℓ water with Citrole 100 mineral oil can also be recommended applied as four applications from October to January. No phytotoxicity was observed in any of the treatments. Four applications of C30 (copper sulphate plus mancozeb) at rates of 150 g/100 ℓ water can be recommended for registration for CBS control. Tank mixtures of C30 with either Citrole 100 mineral oil or Sporekill can also be recommended as four applications from October to January. No phytotoxicity was observed in any of the treatments.

Four applications of Pennfluid at rates higher than 150 mℓ/100 ℓ water can be recommended for registration for CBS control. Tank mixtures of Pennfluid with both Benlate and Cabrio plus Citrole 100 mineral oil, can also be recommended as two applications applied in November and January. The latter tank mixtures of Pennfluid and Benlate can be recommended in those regions where the benzimidazole resistant strains are absent. No phytotoxicity was observed at the highest rate (200 mℓ/100 ℓ water) of Pennfluid tested.

Various potential applications or spray programmes for BAS600F exist for the successful control of citrus black spot. The most promising spray programmes are mancozeb /BAS600F + mancozeb + oil /BAS600F + mancozeb + oil /mancozeb (4 applications) and BAS600F (50 mℓ/100 ℓ water) + mancozeb + oil (3 applications). The latter spray programme will be economical as growers will be saving on one spray round. Phytotoxicity was observed in the form of concentric rings on the bottom side of the fruit due to some compound in the formulation, and should be sorted out by the manufacturer before this product can be safely recommended for registration (Fig. 4.3.2.1).

The surfactant, Breakthrough, in tank mixtures with mancozeb was effective in a spray programme for the control of CBS if sprayed at rates between 2.5 to 5 ml/100 l water. No phytotoxicity was evident on any fruit in any of the treatments. The other surfactant tested, Wetcit, at rates of 75 ml/100 l water and higher in tank mixtures with Ortiva and mancozeb, was effective in a spray programme for the control of CBS. Wetcit (75 and 100 ml/100 l water) in a tank mixture with mancozeb was also effective in controlling CBS. All these spray programmes where Wetcit was used as a surfactant, can be recommended for registration. No phytotoxicity was evident on any fruit in any of the treatments. Another surfactant, Solitaire, in tank mixtures with mancozeb was effective in a spray programme for the control of CBS if sprayed at rates between 50 to 75 ml/100 l water. Phytotoxicity was evident on the fruit in all the treatments where Solitaire was included in the spray mixture (Fig. 4.3.3.1), and at this stage this product cannot safely be recommended for use in citrus.

### 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. Likewise, there is a continuous search for new fungicides or fungicides with new characteristics, new ideas on how we can alter old fungicidal spray programmes to be included in effective spray programmes and how to cope with fungicide resistance strategies. 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 and results will be presented on the bi-annual CRI Symposium in August 2008.

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**Table 4.3.2.1.** Evaluation of copper hydroxide applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment <sup>y</sup>		Concentration (g/ml product/100l water)	Percentage of fruit in each class <sup>x</sup>		
			Lesions/fruit		
			0	1-3	≥4
9	Mancozeb	200 g	96.2 ab	1.4 a	2.4 a
7	Kocide 2000	200 g	99.4 a	0.6 a	0.0 a
8	Copstar	350 ml	93.4 ab	1.4 a	5.2 a
1	Copper hydroxide	125 g	93.2 ab	3.8 ab	3.0 a
3	Copper hydroxide	150 g	99.2 a	0.8 a	0.0 a
2	Copper hydroxide	175 g	99.6 a	0.4 a	0.0 a
4	Copper hydroxide	225 g	98.2 ab	1.4 a	0.4 a
5	Copper hydroxide + Sporekill	125 g + 100 ml	91.8 ab	3.4 ab	4.8 a

6	Copper hydroxide + NuFilm 17	125 g + 25 ml	91.2 b	6.0 b	2.8 a
10	Control		25.2 c	12.2 c	62.6 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 2 January 2007.

**Table 4.3.2.2.** Evaluation of C40 (copper sulphate) alone and in tank mixtures with Citrole 100 applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Brits 1, Nelspruit, South Africa.

Treatment <sup>y</sup>		Concentration (g/ml product/100 l water)	Percentage of fruit in each class <sup>x</sup>		
			Lesions/fruit		
			0	1-3	≥4
6	Mancozeb	200 g	96.2 a	1.4 a	2.4 a
5	Copstar	350 ml	93.4 a	3.6 ab	3.0 a
1	C40	75 g	88.8 a	7.0 b	4.2 a
2	C40	100 g	93.6 a	2.8 ab	3.6 a
3	C40	150 g	95.2 a	0.8 a	4.0 a
4	C40 + Citrole 100	75 g + 250 ml	94.0 a	4.2 ab	3.8 a
7	Control		25.2 b	12.2 c	62.6 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.

**Table 4.3.2.3.** Evaluation of C30 (copper sulphate + mancozeb) alone and in tank mixtures with Citrole 100 or Sporekill applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Brits 1, Nelspruit, South Africa.

Treatment		Concentration (g/ml product/100 l water)	Percentage of fruit in each class <sup>x</sup>		
			Lesions/fruit		
			0	1-3	≥4
8	Mancozeb	200 g	96.2 a	1.4 a	2.4 a
6	Copstar	350 ml	93.4 a	3.6 a	3.0 a
3	C30 + Citrole 100	100 g + 250 ml	96.8 a	2.6 a	0.6 a
4	C30 + Sporekill	100 g + 100 ml	93.2 a	4.6 a	2.2 a
5	C30	150 g	95.0 a	4.2 a	0.8 a
9	Control		25.2 b	12.2 b	62.6 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.

**Table 4.3.2.4.** Evaluation of Pennfluid (mancozeb) alone and in tank mixtures with Benlate and Cabrio applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Brits 1, Nelspruit, South Africa.

Treatment		Concentration (g/ml product/100 l water)	Percentage of fruit in each class <sup>x</sup>		
			Lesions/fruit		
			0	1-3	≥4
7	Mancozeb <sup>y</sup>	200 g	96.2 ab	1.4 a	2.4 a
6	Copstar <sup>y</sup>	350 ml	93.4 ab	3.6 a	3.0 a
1	Pennfluid <sup>y</sup>	100 ml	94.6 ab	3.0 a	2.4 a
2	Pennfluid <sup>y</sup>	150 ml	95.8 ab	3.2 a	1.0 a
3	Pennfluid <sup>y</sup>	200 ml	98.6 a	1.0 a	0.4 a
8	Mancozeb + Cabrio + Citrole 100	150 g + 10 ml + 250 ml	98.0 a	1.0 a	1.0 a
5	Pennfluid + Cabrio + Citrole 100 <sup>z</sup>	150 ml + 10 ml + 250 ml	97.8 a	1.8 a	0.4 a
4	Pennfluid + Benlate + Citrole 100 <sup>z</sup>	150 ml + 50 g + 250 ml	89.2 b	5.4 a	5.4 a
9	Control		25.2 c	12.2 b	62.6 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.

<sup>z</sup> Spray dates were 7 November 2006 and 3 January 2007.

**Table 4.3.2.5.** Evaluation of BAS600F applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment	Concentration (g/ml product/ 100 l water)	Percentage of fruit in each class <sup>v</sup>		
		Lesions/fruit		
		0	1-3	≥4
Mancozeb <sup>x</sup>	200 g	95.0 ab	1.0 a	4.0 a
Mz/Cabrio+Mz+oil/Cabrio+Mz+oil/Mz <sup>w</sup>	200 g/ 10 ml + 150 g + 250 ml/10 ml + 150 g + 250 ml/ 200 g	99.6 a	0.4 a	0.0 a
Mz/BAS600F+Mz+oil/BAS600F+Mz+oil/Mz <sup>w</sup>	200 g/50 ml + 150 g + 250 ml/10 ml + 150 g + 250 ml/ 200 g	97.0 ab	2.6 ab	0.4 a
BAS600F (6x) <sup>z</sup>	25 ml	94.6 ab	3.3 ab	2.1 a
BAS600F (6x) <sup>z</sup>	50 ml	86.0 ab	5.3 abc	8.7 a
BAS600F (x6) <sup>z</sup>	75 ml	97.6 ab	2.0 ab	0.4 a
BAS600F + oil (x3) <sup>y</sup>	25 ml + 250 ml	82.0 b	10.3 c	7.7 a
BAS600F + oil (x3) <sup>y</sup>	50 ml + 250 ml	82.0 b	7.6 bc	10.4 a
BAS600F+oil (x3) <sup>y</sup>	75 ml + 250 ml	88.0 ab	5.3 abc	6.7 a
BAS600F+Mz+oil (x3) <sup>y</sup>	50 ml + 150 g + 250 ml	98.6 ab	1.4 ab	0.0 a
Control		22.6 c	11.0 c	66.4 b

<sup>v</sup> 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.

<sup>w</sup> Spray dates were 10 October 2006, 7 November 2006, 19 December 2006 and 23 January 2007

<sup>x</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.

<sup>y</sup> Spray dates were 10 October 2006, 21 November 2006 and 3 January 2006

<sup>z</sup> Spray dates were 10 October 2006, 31 October 2006, 21 November 2006, 12 December 2006, 3 January 2007 and 23 January 2007

**Table 4.3.2.6.** Evaluation of Breakthrough in tank mixtures with mancozeb applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment <sup>y</sup>	Concentration (g/ ml product/100 l water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-3	≥4
Copstar	350 ml	93.4 a	3.6 a	3.0 a
Mancozeb	200 g	96.2 a	1.4 a	2.4 a
Breakthrough+mancozeb	2.5 ml + 150 g	91.0 a	6.2 ab	2.8 a
Breakthrough+mancozeb	5 ml + 150 g	93.6 a	2.0 a	4.4 a
Breakthrough+mancozeb	10 ml + 150 ml	88.6 a	1.6 a	9.8 a
Control		25.2 b	12.2 b	62.6 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.

**Table 4.3.2.7.** Evaluation of Wetcit in tank mixtures with mancozeb and Ortiva and mancozeb applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment	Concentration (g/ ml product/100 l water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-3	≥4
Copstar <sup>x</sup>	350 ml	93.4 a	3.6 a	3.0 a
Mancozeb <sup>x</sup>	200 g	96.2 a	1.4 a	2.4 a
Wetcit+mancozeb <sup>x</sup>	50 ml + 150 ml	90.2 a	4.6 a	9.8 a
Wetcit+mancozeb <sup>x</sup>	75 ml + 150 g	95.2 a	2.0 a	2.8 a
Wetcit+mancozeb <sup>x</sup>	100 ml + 150 g	95.8 a	2.2 a	2.0 a
Ortiva+mancozeb+oil <sup>z</sup>	20 ml + 150 g + 250 ml	97.8 a	1.4 a	0.8 a

Ortiva+mancozeb + Wetcit <sup>z</sup>	20 ml + 150 g + 75 ml	97.6 a	1.6 a	0.8 a
Ortiva+mancozeb + Wetcit <sup>z</sup>	20 ml + 150 g + 100 ml	93.4 a	3.6 a	3.0 a
Control		25.2 b	12.2 b	62.6 b

<sup>x</sup> 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.

<sup>x</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.

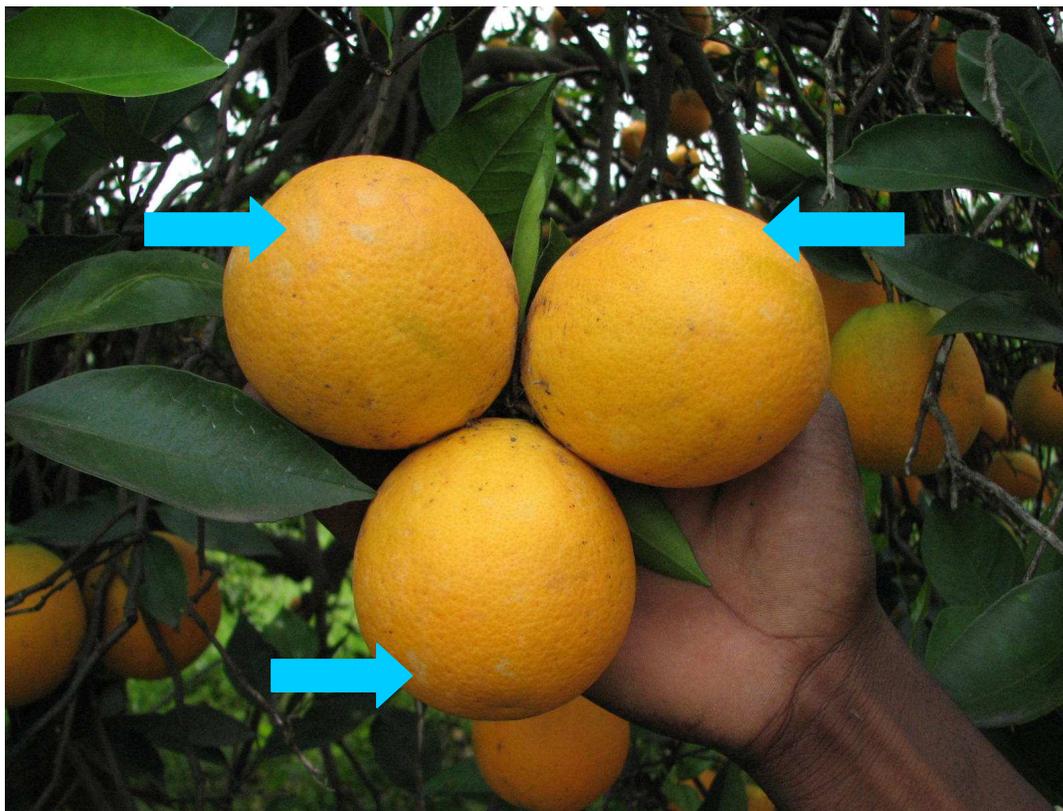
<sup>z</sup> Spray dates were 7 November 2006 and 3 January 2007

**Table 4.3.2.8.** Evaluation of Solitaire in tank mixtures with mancozeb applied from October and January during 2006 and 2007 for the control of *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment <sup>y</sup>	Concentration (g/ml product/100 l water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-3	≥4
Mancozeb	200 g	96.2 a	1.4 a	2.4 a
Copstar	350 ml	93.4 a	3.6 a	3.0 a
Solitaire + mancozeb	50 ml + 150 g	92.8 a	2.8 a	4.4 a
Solitaire + mancozeb	75 ml + 150 g	91.8 a	4.0 a	4.2 a
Control		25.2 b	12.2 b	62.6 b

<sup>x</sup> 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.

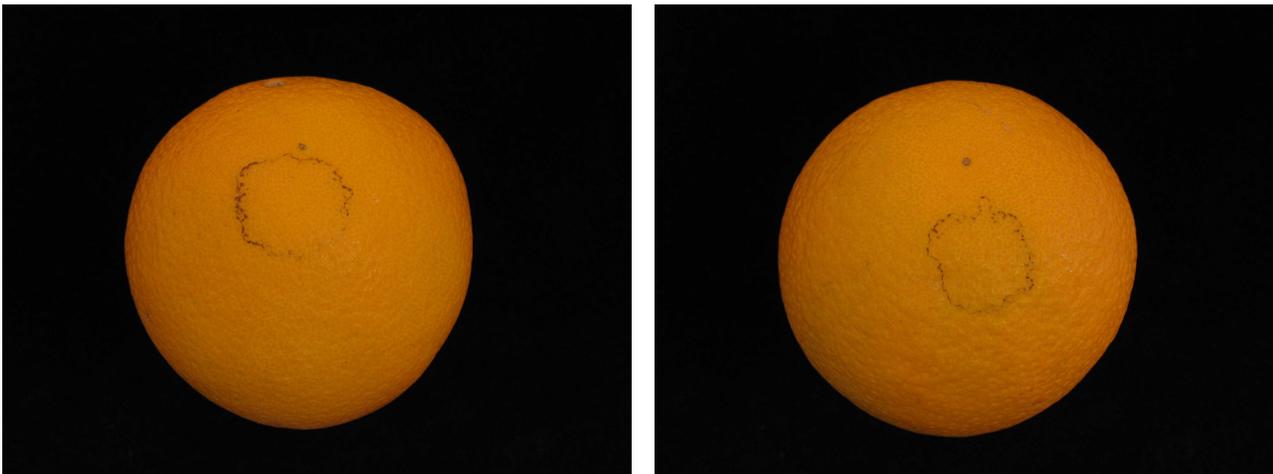
<sup>y</sup> Spray dates were 10 October 2006, 7 November 2006, 5 December 2006, 3 January 2007.



**Fig. 4.3.2.1.** Copper hydroxide evaluated at rates of 150 g/100 ml water and higher was extremely effective in controlling CBS and can be recommended for registration. No phytotoxicity or copper stippling was observed even at the highest rate tested of 225 g/100 ml water (Fig.4.3.2.1).



**Fig. 4.3.2.2.** Concentric ring-burn due to the application of BAS600F on 'Valencia' oranges as sprayed for the control of citrus black spot during the susceptible period from October to January



**Fig. 4.3.2.3.** Phytotoxicity on 'Valencia' oranges after foliar application of Solitaire in a tank mixture with mancozeb as sprayed for the control of citrus black spot during the susceptible period from October to January.

#### 4.3.3 FINAL REPORT: Reducing the inoculum potential in leaves by applying fungicides to citrus trees in the orchard

Project 07 CRI FS 2 (August 2007 – April 2008): S.H. Swart (QMS Agri Science)

##### Opsomming

Die vermindering van inokulum deur inokulumbronne te teiken, is 'n relatief nuwe konsep in die beheer van sitrus swartvlek in Suid-Afrika waar kommersiële spuitprogramme hoofsaaklik fokus op die beskerming van vrugte met behulp van swamdoder applikasies tussen Oktober en Februarie. Die doel van hierdie projek was om inokulum te verminder deur die patogene in lewende blare op die sitrusboom te teiken, voordat blare na die boordvloer val. Verskeie swamdoders en kombinasies van produkte is as 'n enkel toediening gedurende Augustus 2007 deur middel van blaarbespuitings aangewend. Die meeste askospore is op blaarafval gedurende Desember en Januarie geproduseer. Die minste askospore was geproduseer op blare wat slegs met water (kontrole) behandel is, gevolg deur Score en daarna spuit urea + Phytex. Op blare wat met Phytex alleen en Syllit toedienings behandel is, is van die hoogste getalle askospore produksie waargeneem. Geen van die swamdoders wat as blaarbespuitings in hierdie projek getoets is, kon veroorsaak dat minder askospore op die blaarafval geproduseer word nadat blare vir 'n tydperk op die boordvloer gelaat is nie. Sommige produkte het moontlik 'n effek op die komposteringsproses gehad met die gevolg dat inokulum produksie vermeerder het.

##### Summary

The reduction of inoculum by targeting the inoculum source is a relatively new concept in citrus black spot control in South Africa, where commercial trends are to protect fruit from infection by applying fungicide programmes between October and February. The aim of this trial was to reduce inoculum by targeting the pathogen in living foliage on the tree, before leaves drop to the ground. Several fungicides and combinations of products were applied to foliage during a single spray application in August 2007. Most ascospores were produced on leaf litter during December and January of the growing season. The least ascospores was produced on leaves sprayed with water only (control), followed by Score or spray urea + Phytex. Phytex alone and Syllit applications to the foliage allowed for the highest number of ascospores to be produced on leaf litter. None of fungicides evaluated in this trial, applied as foliar applications, could consistently reduce the production of ascospore inoculum on citrus leaf litter. Some products possibly affected the compostation process negatively and therefore, enhanced inoculum production.

##### Introduction

The major source of citrus black spot inoculum is ascospores of *Guignardia citricarpa* Kiely, which are produced on decomposing leaf litter on the orchard floor. In order to break the life cycle of citrus black spot, or to reduce inoculum levels, leaves on the tree were targeted during the 2006/2007 season by applying several fungicides as a single foliar application in August (Project 2006/CBS 7). The number of ascospores produced on leaves picked in a specific month, and the total number of ascospores produced on leaves picked over three consecutive months, were determined with a Kotzé Inoculum Monitor (KIM) and compared for different fungicide treatments. Variance between replicates was large and no statistical differences between treatments could be found for any of the parameters evaluated. However, results showed that the majority of the inoculum was produced between 2 and 4 months after leaves were picked and left on the orchard floor to decompose. Some pre-blossom fungicide treatments seemed to have reduced ascospore inoculum production on fallen leaf litter while others enhanced the process. The lowest number of ascospores was produced on leaves treated with triadimefon/carbendazim (Rambo), spray urea and where no fungicide was applied (control). Relatively low numbers of ascospores were also produced on leaves treated with benomyl, compared to other fungicides. The highest number of ascospores was produced on leaves treated with mancozeb. Results obtained during this preliminary trial were unexpected, especially the fact that leaves from the untreated control produced the least ascospore inoculum (Project 2006/CBS 7). The fact that mancozeb, a fungicide used extensively to protect fruit from citrus black spot, enhanced inoculum production on leaf litter, might also be reason for concern if inoculum sources must be reduced (Swart *et al.*, 2005, Project 2006/CBS 7).

Several reports on the reduction of inoculum, by targeting leaf litter on the orchard floor, especially with regards to the use of urea in apple orchards to reduce ascospore inoculum of *Venturia inaequalis*, showed promising results (Beresford *et al.*, 2000, Sutton *et al.*, 2000, Mondal and Timmer, 2003). However, no reference could be found where foliage was targeted before leaves drop to the orchard floor in order to reduce inoculum. There is also no reference that some fungicide applications to the foliage might enhance ascospore inoculum on leaf litter. The aim of this project was to re-evaluate the effect of some of the fungicides, and test the efficacy of other fungicides and fungicide mixtures on ascospore production on citrus

leaf litter on the orchard floor. Some changes were made in the protocol in order to reduce variability amongst replicates.

### Material and methods

Several chemicals were applied with hand-held lances at 20 bar pressure until run-off (30 l / tree) on the 21<sup>st</sup> of August 2007 to 9-tree-blocks, randomly selected in a thirty-year-old Valencia orchard in the Letsitele area, Limpopo Province (Table 4.3.3.1). Each treatment was replicated twice. Sixty days after treatment (middle October 2006), approximately 300 mature leaves were picked from each treatment block. Sampling of leaves was repeated in November and December 2007. On each occasion, leaves from a specific treatment were thoroughly mixed and 4 grids per replicate were prepared by fixing approximately 25 leaves between two plastic grids. Grids were placed on the orchard floor and one grid per replicate was retrieved from the orchard on a monthly basis for 4 consecutive months, starting 30 days after being placed in the orchard. To determine the level of *Guignardia* ascospores produced on leaf litter samples, grids with leaves were dipped in hot water (50°C) for 5 min and placed in a Kotzé-inoculum-monitor (KIM). *Guignardia* ascospores, discharged over a period of 2 hours, were deposited on a Vaseline® coated microscope slide. After staining the slide with lacto-phenol-cotton-blue, ascospores in four lanes (45 mm long) on each microscope slide, representing approximately 180 mm<sup>2</sup> surface area, were counted, using a light microscope at 400x magnification and the total number of ascospores was recorded.

**Table 4.3.3.1.** Treatments applied to reduce inoculum production

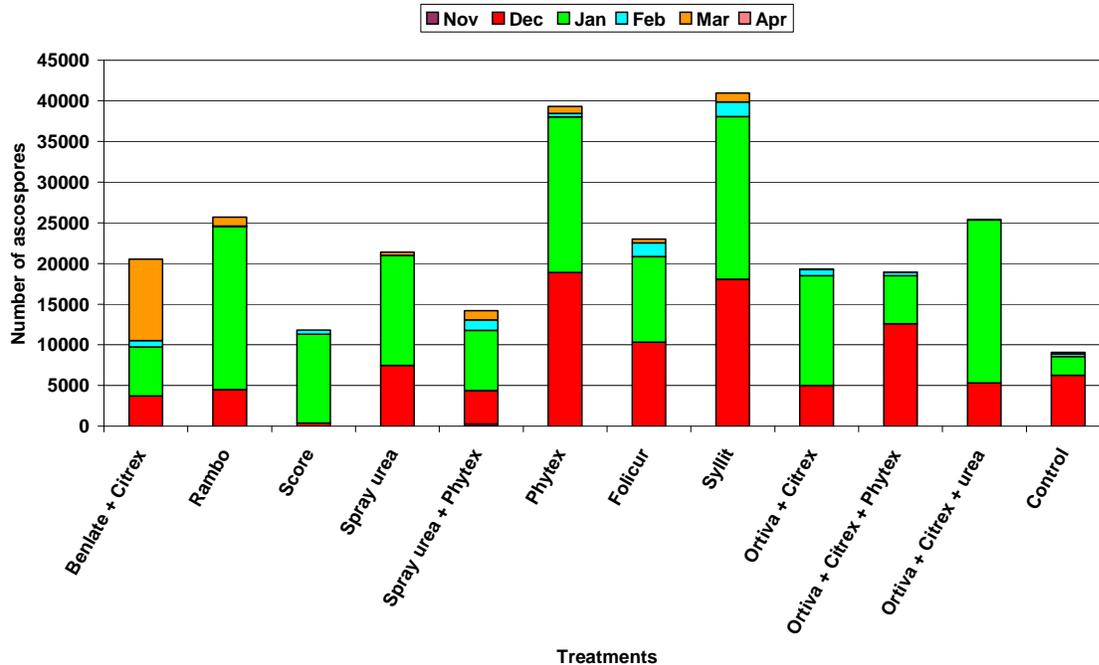
Trt #	Products	Active ingredient(s)	Formulation	Dosage (g or ml /100 l)
1	Benlate + Citrex	Benomyl + oil	500 g / kg, WP	50 g + 300 ml
2	Rambo	Carbendazim/triadimefon	200/165 g/l, SC	25 ml
3	Score	Difenoconazole	250 g/l, EC	25 ml
4	Spray urea	Urea	-	1010 g
5	Spray urea + Phytex	-	-	1010 g + 1000 ml
6	Phytex	Potassium phosphonate	200 g/l, SL	1000 ml
7	Folicur	Tebuconazole	250 g/l, EW	25 ml
8	Syllit	Dodine	400 g/l, SC	240 ml
9	Ortiva + Citrex	Azoxystrobin + oil	250 g/l, SC	25 ml + 300 ml
10	Ortiva + Citrex + Phytex	-	-	25 ml + 300 ml + 1000 ml
11	Ortiva + Citrex + urea	-	-	25 ml + 300 ml + 1000 g
12	Control	Water	-	-

### Results and discussion

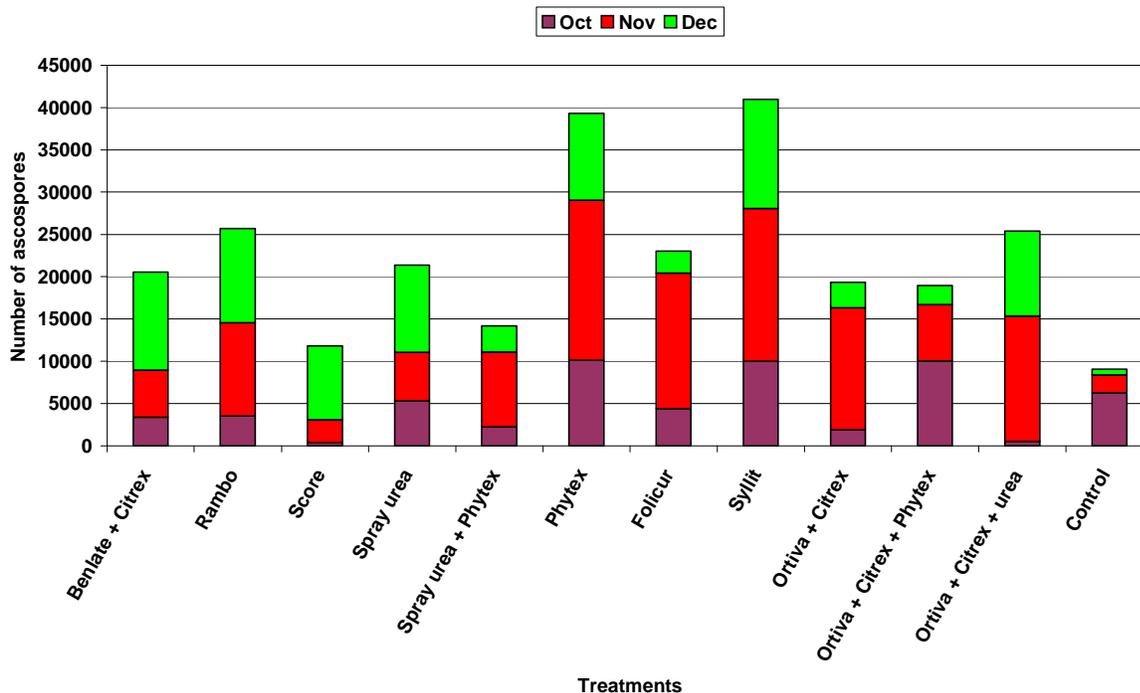
Results from leaves picked in October 2007 showed the majority of ascospores were produced 2 months after leaves were picked (Fig. 4.3.3.1). Leaves sprayed with Score or Ortiva + citrex + urea produced the least ascospores over the 4 months of evaluation. Results from leaves picked in November 2007 showed the majority of ascospores were again produced 2 months after leaves were picked, but a fair number were already produced after 1 month (Fig. 4.3.3.1). Leaves sprayed with Score or water (control) produced the least ascospores over the 4 months of evaluation. Results from leaves picked in December 2007 showed the majority of ascospores were produced 1 month after leaves were picked and that leaves sprayed with water (control) produced the least ascospores over the 4 months of evaluation (Fig. 4.3.3.1).



**Figure 4.3.3.1.** The total number of *Guignardia* ascospores (n = 2), as determined with a Kotzé Inoculum Monitor, produced over 4 months on leaves picked in October, November and December 2007 after foliar fungicide treatment in August.



**Figure 4.3.3.2.** The total number number of *Guignardia* ascospores, as determined with a Kotzé Inoculum Monitor, produced over 6 months on leaves picked in October, November and December 2007 after foliar fungicide treatment in August.



**Figure 4.3.3.3.** The total number of *Guignardia* ascospores, as determined with a Kotzé Inoculum Monitor, produced on leaves picked in October, November and December 2007 and evaluated between November 2007 and April 2008.

Results taking into account the total number of ascospores produced over 6 months on leaves picked at specific periods (October, November or December), showed that more ascospores were produced on leaves picked during November and December (Figure 4.3.3.2). This might be an indication that most fungicides applications to the foliage might only have an effect on ascospore production should leaves drop within one month after application. The fact that the number of ascospores produced on leaf litter, obtained from the control treatment was less on leaves, picked further from the application date, might indicate that other factors apart from chemical applications also can play a significant role on the number of ascospore produced. Results taking into account the total number of ascospores produced at specific times (November to April), are shown in Figure 4.3.3.3. Most ascospores were produced on leaves during December and January. The least ascospores was produced on leaves sprayed with water only (control), followed by Score and Spray urea + Phytex. Phytex alone and Syllit applications to the foliage allowed for the most ascospores to develop on leaf litter. Rambo and Spray urea was some the best treatments and Phytex one of the worst treatments during a previous evaluation (Project 2006/CBS 7).

## **Conclusions**

The reduction of inoculum by targeting the inoculum source is a basic concept of disease management. However, this is a relatively new concept in citrus black spot control in South Africa, where commercial trends are to protect fruit from infection by applying fungicide programs between October and February during the growing season. In this trial, the strategy was to reduce inoculum by targeting the pathogen in living foliage on the tree, before leaves drop to the ground. Results so far were not very successful, since few fungicides showed the ability to reduce inoculum, and some even enhanced the production of inoculum on leaf litter. Similar results were also found in previous trials (Swart *et al.*, 2005, Project 2006/CBS 7).

Several factors can influence ascospore inoculum production on citrus leaf litter. Swart (2005) showed that climatic conditions, especially rain and temperature, influence the deterioration process of leaf litter and, therefore, the time and level of inoculum production. The fact that more ascospores were produced on leaves picked in November and December, compared to October might be related to a reduction in fungicide activity over time, since an opposite trend was observed where foliage was sprayed with water alone. It can also be argued that fungicides can affect biological organisms involved in the decomposition processes of the leaf litter, since larger numbers of inoculum were produced in some cases where foliage was treated with fungicide applications.

It can be concluded that none of fungicides evaluated in this trial, as foliar applications, could consistently reduce the production of ascospore inoculum on citrus leaf litter effectively. Previous trials showed that Spray urea and Rambo reduced inoculum levels compared to the untreated control (Project 2006/CBS 7). Therefore the search for a systemic fungicide, with activity to keep leaves pathogen free over an extended period must continue. The possibility that some fungicides affect the decomposition process negatively and therefore, enhance inoculum production, should be investigated further, since mancozeb, which is a major fungicide used in citrus black spot control programs, showed the highest tendency to cause enhanced levels of ascospore production.

Indications are that a holistic systems approach, based on several strategies for managing inoculum levels, should be followed. The effect of production practices such as the removal of inoculum sources (pruning and removal of leaf litter, either manually, chemically or biologically), reducing inoculum levels in the remaining foliage (fungicide application in August), using fungicides to protect citrus fruit against infection by *G. citricarpa* that are less prone to enhanced inoculum production (benomyl, carbendazim, strobilurine) and applications to enhance the decomposition process before peak inoculum production can occur, should be investigated.

## **Technology transfer**

Results in this project and in the previous associated project (Project 2006/CBS 7) have been discussed at Letsitele and Hoedspruit study groups and at QMS citrus black spot monitoring meetings. Results will also be presented on the bi-annual CRI Symposium in August 2008.

## **Future research**

Future work will include a project to study the effect of a holistic approach of managing inoculum in a commercial orchard. The effects of pruning, removal of leaf litter, pre-flower fungicide applications, standard fungicide applications and enhancing the compostation process will be studied in a.

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### 4.3.4 FINAL REPORT: The status of strobilurin resistance in citrus orchards Project 2007/FS1 (August 2007–April 2008): by S.H. Swart (QMS Agri Science)

## Opsomming

*Guignardia* isolate is op strobilurien-bevattende media geselekteer wat by 20 dpm azoxystrobin of trifloxystrobin, of 5 dpm pyraclostrobin kon groei. Hierdie isolate is geberg en in verdere toetse as tolerante verwysings isolate gebruik. *G. citricarpa* isolate is in verskeie boorde in die Letaba en Letsitele areas versamel waar strobilurieni gedurende die vorige groeiseisoen gebruik is. Toleransie is *in vitro* op strobilurien-bevattende mout-ekstrak-agar gemeet en ook vergelyk met laboratorium vervaardigde tolerante isolate. Groei van miselium is meer onderdruk word op media wat pyraclostrobin bevat en toetse is by 0.1, 1 en 5 dpm uitgevoer, terwyl groei op azoxystrobin en trifloxystrobin by 5, 10 en 20 dpm bestudeer kon word. Teenstrydige resultate is verkry wanneer toetse herhaal is. Laboratorium vervaardigde tolerante isolate het dieselfde gereageer as isolate wat nog nooit in aanraking met strobilurien swamdoders was nie. In die algemeen kon isolate op media met hoë konsentrasies van azoxystrobin en trifloxystrobin groei (20 dpm), terwyl groei op media met pyraclostrobin net by konsentrasies tot by 0.1 dpm waargeneem is. Wisselvallige resultate dui daarop dat *in vitro* strobilurien weerstand wat waargeneem is, moontlik verband hou met die vermoë van sommige *G. citricarpa* isolate om alternatiewe respiratoriese weë in die teenwoordigheid van strobilurien swamdoders te volg, eerder as genetiese mutasie wat 'n meer permanente verandering met betrekking tot swamdoder weerstand het.

## Summary

*Guignardia* isolates were selected on strobilurin-amended agar and isolates that grew on 20 ppm azoxystrobin or trifloxystrobin, or 5 ppm pyraclostrobin were placed in storage to use as tolerant reference cultures in further studies. *G. citricarpa* isolates were obtained from several orchards in the Letaba and Letsitele areas where strobilurin fungicides were sprayed. Growth of isolates were measured on strobilurin-amended malt-extract-agar and also compared with strobilurin-tolerant laboratory produced cultures. Results showed that pyraclostrobin could inhibit mycelial growth at much lower dosages when incorporated into agar, compared to azoxystrobin and trifloxystrobin. Several isolates obtained from citrus orchards could also grow on media amended with strobilurin fungicides. However, conflicting results were obtained during different tests. Laboratory manufactured tolerant isolates grew similarly to wild strain isolates, that has never been in contact with strobilurin fungicides. In general, isolates could grow on media amended with higher levels of azoxystrobin and trifloxystrobin (20 ppm), compared to growth on media amended with pyraclostrobin (0.1 ppm). Conflicting results in this trial suggest that the observed *in vitro* strobilurin-tolerance was probably associated with the ability of *G. citricarpa* isolates to utilize an alternative respiration pathway rather than site mutations which would cause more permanent changes with regards to fungicide resistance.

## Introduction

Azoxystrobin (Ortiva), pyraclostrobin (Cabrio) and trifloxystrobin (Flint) are used extensively for control of *Guignardia citricarpa* Kiely, the causal agent of citrus black spot in South Africa. These products have been used commercially for more than 8 years in the Letsitele, Letaba and Hoedspruit areas. Field resistance to a fungicide occurs when populations of the target pathogen are not controlled adequately by registered commercial applications. The strobilurin fungicides are included under Code 11, the QoI (Quinone outside inhibitors) group in the Fungicide Resistance Committee's (FRAC) list of fungicides. The mode of action is to inhibit the respiration process of fungal mitochondria, causing reduction in aerobic energy production and inhibition of growth by fungi (Becker *et al.*, 1981; Godwin *et al.*, 1994). This highly specific mode of action

increases the potential for resistant pathogen individuals to develop. Powdery mildew pathogens resistant to the Qol fungicides were reported in 1998, and since then several other pathogens, but according to the 2006 list, no resistant *Guignardia* isolates have been reported yet (FRAC list of plant pathogenic organisms resistant to disease control agents, December 2006).

In order to prolong the effectiveness of fungicide prone to developing resistance, managed strategies must be followed strictly. One of the requirements of such management is to monitor populations regularly in order to spot the development of tolerant populations at an early stage. The ideal strategy should be to base these monitoring efforts upon comparisons with baseline sensitivity data (i.e., sensitivity distributions determined before the widespread use of the new chemistries) and the techniques must be suitably precise and reliable to detect relevant shifts in sensitivity within the population (Olaya *et al.*, 1998; Avila-Adame *et al.*, 2003; Hoffman and Wilcox, 2003; Mondal *et al.*, 2005).

The aim of this study was to produce strobilurin-tolerant *Guignardia* isolates, that could be used as reference isolates for testing isolates obtained from citrus orchards, treated with strobilurin fungicides, for detection of populations with elevated levels of fungicide resistance.

## Materials and methods

Fungicide stock solutions with Ortiva (250 g azoxystrobin/l; SC), Flint (500 g trifloxystrobin/kg; WG) and Cabrio (250 g pyraclostrobin/l; EC) were prepared in sterile distilled water in order to give final concentrations of 0.01, 0.1, 1, 5, 10 and 20 ppm of the respective active ingredients when added to 250 ml of pre-cooled autoclaved malt-extract-agar media (5 g malt extract + 15 g agar agar/l distilled water, pH = 5.6). Twenty-five ml of fungicide-amended agar was decanted into 95 mm plastic petri-dishes and allowed to solidify.

### Production of strobilurin-tolerant isolates

*G. citricarpa* isolates were cultured repeatedly on malt-extract-agar ameliorated with a range of concentrations for azoxystrobin, pyraclostrobin and trifloxystrobin. Cultures were incubated at 25±1°C for 10 days. Isolations were taken from colonies growing at the highest fungicide concentration in each batch and plated on media with concentrations equal or higher than the media from where it was obtained. This process was repeated several times. Reference cultures were stored on PDA slants under mineral oil.

### Collection and isolation of Guignardia isolates

Citrus orchards, sprayed with strobilurin fungicides during the 2006/2007 growing season, were inspected for fruit with typical citrus black spot symptoms in July, August and September 2007. Isolations were made from black spot lesions and plated on potato-dextrose-agar (15 g PDA/l of water, pH = 5.6) amended with 250 ppm chloramphenicol to retard bacterial growth. Five mm plugs from 7-day-old cultures were inoculated in the middle of 2-day-old fungicide amended malt-extract-agar plates and incubated at 25±1°C. *Guignardia* isolates showed a higher sensitivity towards pyraclostrobin, compared to azoxystrobin and trifloxystrobin during initial *in vitro* tests, therefore isolates were compared on malt-extract-agar ameliorated with 5, 10 and 20 ppm for azoxystrobin or trifloxystrobin, or with 0.1, 1 and 5 ppm for pyraclostrobin. Throughout this report dosage 1 (D1) refers to 5 ppm for azoxystrobin or trifloxystrobin and 0.1 ppm for pyraclostrobin, dosage 2 (D2) refers to 10 ppm for azoxystrobin or trifloxystrobin and 1 ppm for pyraclostrobin, and dosage 3 (D3) refers to 20 ppm for azoxystrobin or trifloxystrobin and 5 ppm for pyraclostrobin.

### Data collection and comparison of isolates

*In vitro* growth of individual *Guignardia* isolates were variable between trials and isolates, therefore data was expressed as the diameter of growth for a specific isolate as a percentage of the diameter of growth of the same isolate on media with no fungicide added. Data sets of different trials were subjected to analysis of variance and statistical differences between means were determined with Fisher's t-test at a 5% level of significance using Statistica 8.0 by Statsoft.

## Results and discussion

### Evaluation of laboratory produced strobilurin-tolerant isolates

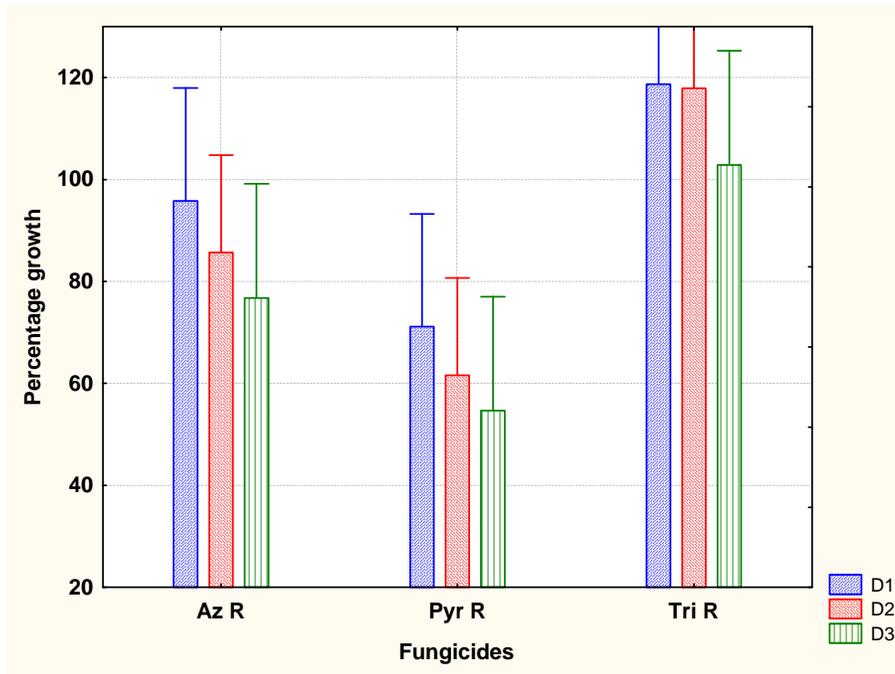
During the process of selecting laboratory produced strobilurin-tolerant isolates, and when the first *in vitro* comparisons in terms of mycelial growth for these isolates were made in October 2007, it was observed that colony diameters of *Guignardia* isolates were larger on strobilurin-amended agar at higher concentrations of

azoxystrobin and trifloxystrobin (5, 10 and 20 ppm), compared to pyraclostrobin-amended agar (0.1, 1 and 5 ppm) (Fig. 4.3.4.1). The mean colony diameter of strobilurin-tolerant isolates was more than 50% larger than colony diameter of respective isolates on agar free of fungicides. Variation in growth between tolerant isolates of the same group was large, and no statistical differences could be shown. In general results also showed that the mean diameter of tolerant isolates for each test fungicide decreased as fungicide concentration in the agar increased.

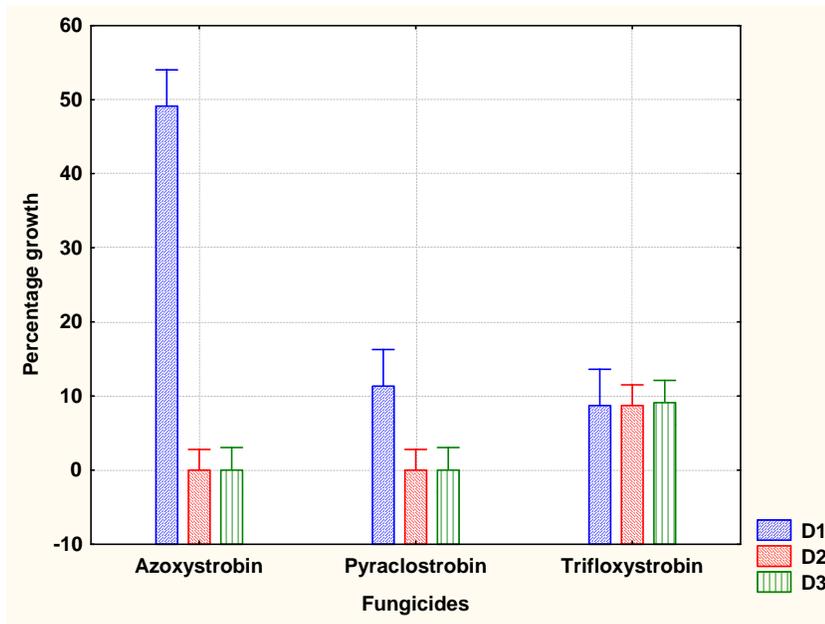
Evaluation of isolates obtained from an orchard in the Letaba area showed that the percentage growth (mean for ten isolates) on media with 5 ppm azoxystrobin was nearly 50%, compared to that of growth on media with no fungicide incorporated (Fig. 4.3.4.2). Variation amongst isolates was small thus results suggested that the sample had a population of *Guignardia* isolates that could tolerate low levels of azoxystrobin. The same isolates could not grow well on agar amended with the lowest concentrations of pyraclostrobin or trifloxystrobin tested.

Evaluation of isolates obtained from orchards in the Letsitele area showed that isolates grew better on agar amended with azoxystrobin and the percentage growth (mean for four isolates) on media with 20 ppm azoxystrobin was more than 50%, compared to that of growth on media with no fungicide incorporated (Fig. 4.3.4.3). Variation amongst isolates was large, therefore data suggests that the sample consisted of sensitive and tolerant *Guignardia* isolates. The same isolates could also tolerate media with 5 and 10 ppm trifloxystrobin, while media amended with pyraclostrobin at 0.1, 1 and 5 ppm inhibited growth. Different results were observed when isolates from another orchard in the Letsitele area was studied (Fig 4.3.4.4). In this study, variation in growth between isolates were much smaller than for the former sample and growth on azoxystrobin-amended agar was less inhibited than on trifloxystrobin at 20 ppm or pyraclostrobin at 0.1, 1 or 5 ppm.

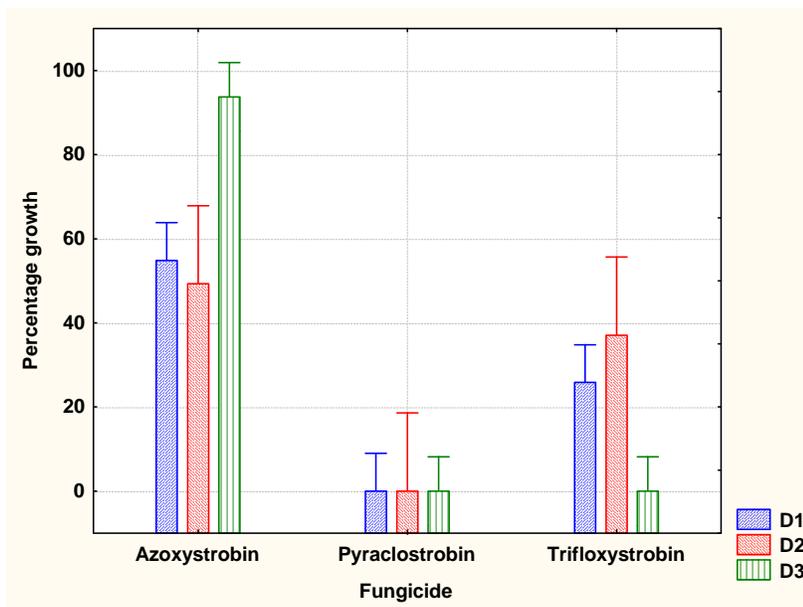
Results of isolates obtained from three orchards in the Letaba area showed different patterns of strobilurin tolerance in a single study with seven isolates obtained from each of orchards O71 and O72 and six isolates from O73 (Fig. 4.3.4.5). Variation between isolates in a sample was large. *Guignardia* isolates from all three orchards showed elevated tolerance to azoxystrobin at all three dosages evaluated. In this case, isolates from all three orchards grew better on media with the highest azoxystrobin concentrations tested. Similar results were observed for trifloxystrobin for isolates obtained from O73, but not for isolates obtained from O71 and O72. Isolates evaluated on pyraclostrobin-amended media showed some tolerance at 0.1 ppm, especially for isolates obtained from orchard O72. At pyraclostrobin concentrations of 1 and 5 ppm, growth of isolates from all three orchards was inhibited, although some individuals could grow at these levels.



**Figure 4.3.4.1.** Comparison of the percentage growth, in terms of growth on media free of fungicides, of respective laboratory produced strobilurin-tolerant isolates. Az R = azoxystrobin-tolerant isolates, Pyr R = pyraclostrobin-tolerant isolates, and Tri R = trifloxystrobin-tolerant isolates, tested on respective fungicide-amended agar.



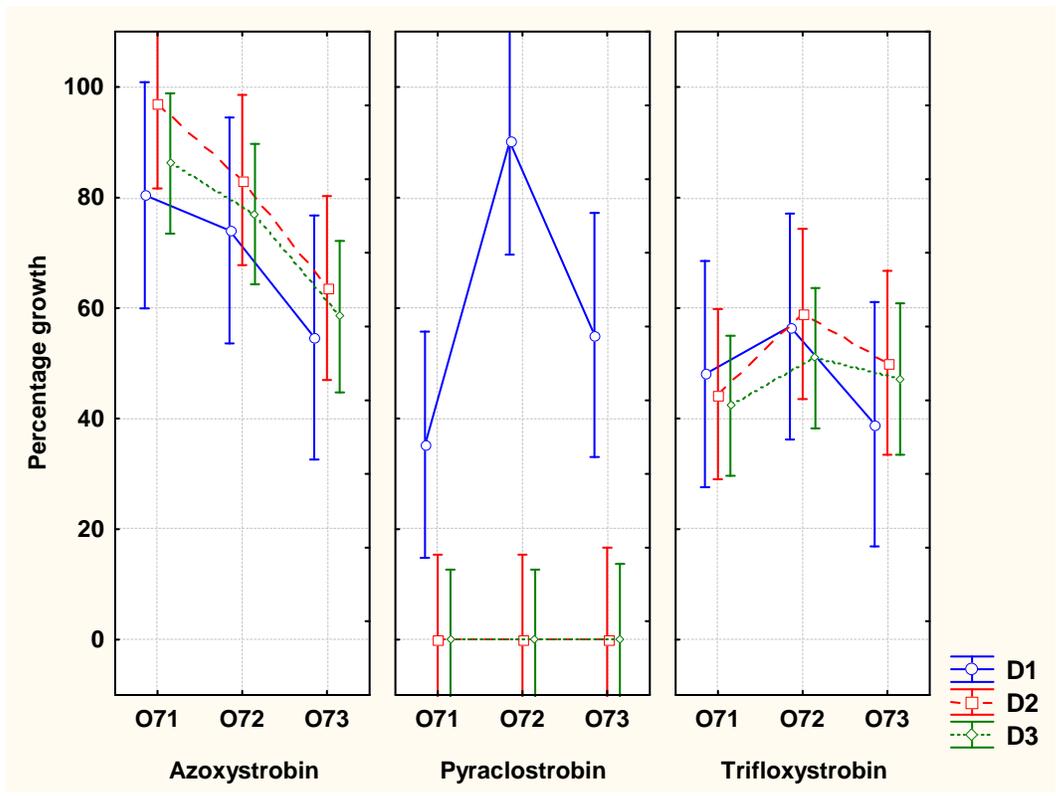
**Figure 4.3.4.2.** Comparison of the percentage growth (mean for 4 isolates), of isolates obtained from orchard A in the Lesitele area



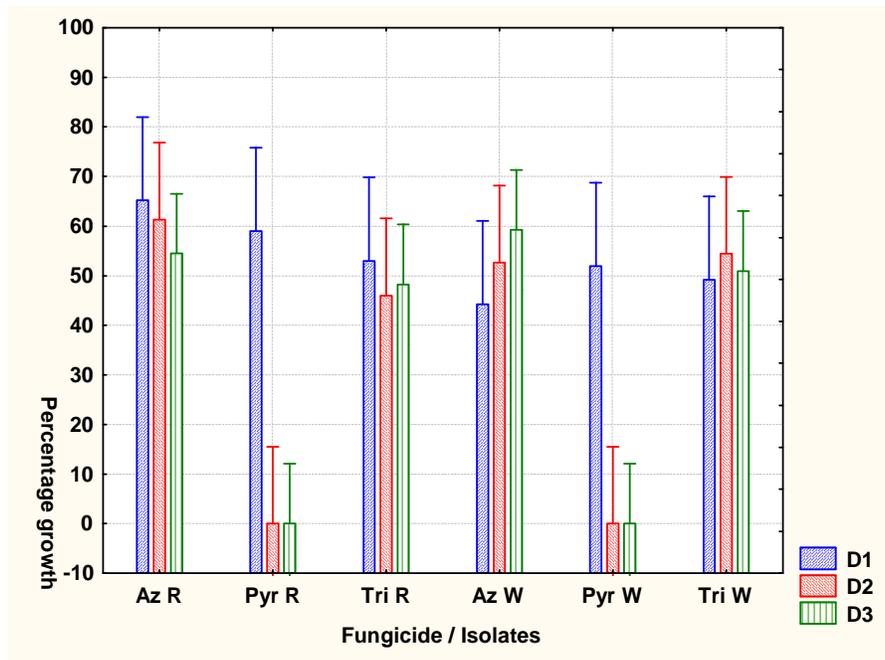
**Figure 4.3.4.3.** Comparison of the percentage growth (mean for 5 isolates), of isolates obtained from orchard B in the Letsitele area

During the latter evaluation, laboratory produced strobilurin-tolerant isolates, as well as wild strain isolates (obtained from orchards not sprayed with strobilurin fungicides) were included as reference cultures. There was large variation in growth for all isolates tested on all concentrations tested for the three strobilurin-amended media. The growth of tolerant isolates (mean for 3 isolates of each strobilurin fungicide), expressed as a percentage of the growth on media with no fungicide was determined after 7 days incubation (Fig. 4.3.4.6). Results showed that azoxystrobin tolerant isolates grew well on media amended with azoxystrobin with a slight decrease in growth with increasing fungicide concentrations. Similarly, trifloxystrobin-tolerant isolates could grow well on media amended with trifloxystrobin, also with a slight decrease in growth with increasing fungicide concentrations. Pyraclostrobin-tolerant isolates could only grow at 0.1 ppm, while growth was inhibited at concentrations of 1 and 5 ppm. Growth of wild strain isolates on strobilurin-amended media was similar to laboratory produced strobilurin-tolerant isolates. Therefore, results indicate that isolates, that previously displayed the ability to grow on media amended with high levels of strobilurin (Fig. 4.3.4.1), apparently have lost this ability, especially with regards to media amended with pyraclostrobin. Results with the wild strain isolates also put a question mark on results obtained with isolates obtained from orchards in the Letsitele and Letaba areas sprayed with strobilurin programs during the growth season.

The ability of *Guignardia* isolates, to grow on strobilurin-amended media in this trial, can not be considered to be indications of resistant individuals caused by site-specific mutations due to contradictory and variable results obtained during different tests. One would expect that isolates that are tolerant to a specific strobilurin fungicide, should also show similar tolerance to the other strobilurin fungicides evaluated in this trial due to the specific mode of action associated with QoI fungicides.



**Figure 4.3.4.4.** Comparison of the percentage growth (mean for 7, 7 and 6 isolates for orchards O71, O72 and O73, respectively), in terms of growth on media free of fungicide, of isolates obtained from orchards in the Letaba area.



**Figure 4.3.4.5.** Comparison of the percentage growth (mean for 3 isolates), in terms of growth on media free of fungicide, of strobilurin-tolerant isolates and wild strain isolates obtained from orchards not treated with strobilurin fungicides.

## Conclusion

Qoi fungicides (i.e. strobilurins) inhibit respiration by binding to the Qo center of cytochrome *b*, functioning as a crucial component of the mitochondrial complex III. Resistance to Qo inhibitors can be based on the expression of an alternative respiration pathway, circumventing electron transport through the cytochrome *b* target site of Qols, or alternatively, mutation of cytochrome *b* target sites (Avila-Adame *et al.*, 2003). Strobilurin fungicides can be tested for efficacy and resistance on amended solid agar media (Mondal *et al.*, 2005; Avila-Adame *et al.*, 2003; Gutierrez *et al.*, 2006), however, fungicide sensitivity to strobilurin fungicides determined *in vitro* may not be an accurate reflection of their sensitivity *in vivo* (Olaya and Köller, 1999). The reason is that in addition to target site mutations, strobilurin sensitivities can also be diminished by the induction of alternative respiration pathways, especially for fungal mycelia.

For DMI fungicides *in vivo* and *in vitro* sensitivities of isolates are correlated (Köller, *et al.*, 1997). The mechanism of strobilurin resistance observed *in vitro*, however, is often not correlated with *in vivo* levels of disease control (Olaya *et al.*, 1998; Avila-Adame, *et al.*, 2003). The discrepancy between strobilurin sensitivities of spore germination in the absence or presence of their hosts complicates the development of monitoring procedures (Olaya *et al.*, 1998; Olaya and Köller, 1999; Avila-Adame *et al.*, 2003). Indications are that sensitivities determined under *in vitro* conditions might also require *in vivo* sensitivity tests. However, *in vivo* tests are laborious, more prone to variable results, and not easily standardized.

Results in this trial suggest that the observed *in vitro* strobilurin-tolerance was probably associated with the ability of *G. citricarpa* isolates to utilize an alternative respiration pathway, more easily achieved in the presence of azoxystrobin and trifloxystrobin than pyraclostrobin, which could be present in some isolates and absent in others, rather than site mutations which would cause more permanent changes with regards to fungicide resistance.

## Technology transfer

Results will be presented on the bi-annual CRI Symposium in August 2008.

## Future objectives (milestones) and work plan

We propose that continuous testing of *Guignardia* isolates, obtained from orchards subjected to strobilurin programs, should be carried out. A reliable test procedure must still be developed and future research should be focused on *in vitro* and *in vivo* sensitivity tests with conidia obtained from at least fifty distinct disease lesions. Sensitivity tests should be done in the presence and absence of salicylhydroxamic acid (SHAM, Sigma) as an inhibitor of the alternative respiration pathway. PCR or other methods for determining tolerance to Qol fungicides should also be evaluated. Proposals to address these subjects will be conveyed to CRI.

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Olaya, G., and Köller, W. 1999. Baseline sensitivities of *Venturia inaequalis* populations to the strobilurin fungicide kresoxim-methyl. *Plant Disease* 83:274 – 278.

#### 4.3.5 **FINAL REPORT: Evaluation of leaf litter inoculum potential on the orchard floor as affected by the irrigation systems**

Project 2006/CBS 1 (September 2006 – March 2007): by S.H. Swart (QMS Agri Science)

#### **Opsomming**

Vanaf twee boorde op Letaba Oranje, Letsitele, is volwasse blare met 'n geskiedenis van *Guignardia* besmetting in September 2006 versamel wat aan mikro- en dripbesproeiing onderwerp asook van onbehandelde 42-jarige Navel bome op Letaba landgoed om te bepaal watter besproeiingsmetode dra die meeste by tot askosporvystelling. Resultate het getoon dat betekenisvol meer askospore op blare geproduseer is wat in die boord met drupbesproeiing geïnkubeer is, in vergelyking met blare in 'n boord met mikrobeproeïing, indien blare in September gepluk is en in Desember geëvalueer is. Meer askospore is in posisies onder die boom, naby besproeiingspunte geproduseer as in vol son op 'n afstand van besproeiingspunte. Verskille in aantal askospore getel op verskillende tye van evaluasie, is moontlik geassosieer met die stadium van blaar kompostering, en was dus afhanklik van klimatoriese faktore soos reënval en temperatuur.

#### **Summary**

The study was conducted in two orchards at Letaba Oranje, Letsitele, to include micro and drip irrigation systems. Mature leaves, from 42-year-old, unsprayed Navel trees at Letaba Estates, with a history of high levels of *Guignardia* infection, were sampled in September 2006 to determine the contribution of the different irrigation methods towards ascospore release. Results showed significantly more ascospores were produced on leaves incubated in an orchard with drip irrigation, compared to leaves incubated in an orchard with micro irrigation, when picked in September and evaluated in December. More ascospores were produced under the canopies of trees, close to the irrigation source than in open sunlight at a distance from an irrigation source. Differences in numbers of ascospores observed at different times of evaluation were probably related to the stage of leaf litter compostation and therefore, dependant on climatic conditions such as rainfall and temperature.

#### **Introduction**

Citrus black spot is traditionally controlled by protecting fruits with fungicide applications from early October until the end of February. This is a labour intensive and costly process that cannot ensure 100% lesion free fruit, especially under high disease pressure conditions. Fungicide applications are very effective, however, occasionally some orchards produce fruits with lesions in spite of sound spraying programs. With the current phytosanitary status of citrus black spot in the EU, USA and Japan, we need to assess alternative methods that can contribute to disease control and that can possibly play an important role in a systems approach for improved disease management strategies.

Inoculum is produced on fallen, decomposing citrus leaves on the orchard floor (Kotzé, 1988). Mature ascospores can develop within 40 – 180 day, depending on frequency and length of wetting periods and prevailing temperatures (McOnie, 1964, Kotzé, 1988). The reduction of inoculum by targeting the inoculum source is a relatively new concept in citrus black spot control strategies that can be incorporated in a systems approach strategy. Results of the project where ascospore inoculum potential was studied over time, indicated that the macro-climate in an area, but also the micro-climate in an orchard can have an effect on the rate at which leaf litter deteriorate and therefore on production and availability of ascospore inoculum (Swart, 2005).

The aim of this project was to study the effect of microclimate under tree canopies, as influenced by the type of irrigation system that are used, on the production and availability of ascospore inoculum. The trials were designed to evaluate the effect of micro and drip irrigation systems and to determine the areas under the canopy where the majority of ascospore inoculum is produced.

## Materials and methods

Mature leaves, from 42-year-old, unsprayed Navel trees at Letaba Estates, with a history of high levels of *Guignardia* infection, were sampled in September 2006. Enough leaves were picked, mixed thoroughly, and placed between two plastic grids (30 leaves / grid-set) for 30 grid sets per picking time. Grids were randomly selected and placed in two orchards, representing micro- and drip irrigation systems respectively (15 grid sets / orchard), placed at three different positions under tree canopies. Grids were placed next to irrigation points in the row, halfway between the trunk and the drip zone between rows, and in full sunlight at a distance from the irrigation source (where trees had been removed). Each position was replicated five times in each orchard. The first set of grids was retrieved after 3 months (December 2006) and the last set after 5 months (February 2007).

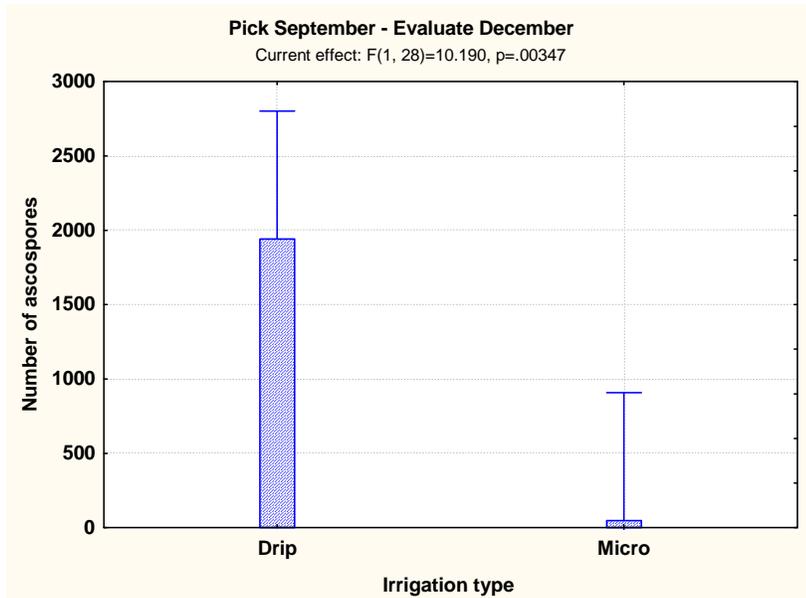
Leaves were also sampled in November 2006, and treated in a similar manner to the leaves sampled in September. The first retrieval of grids for leaves sampled in November 2006 was in January 2007 and the last in March 2007. Leaves were processed by dipping grids with partly decomposed leaves in hot water (50°C) for 5 min. Grids were shook in order to remove excess water prior to placing them in a Kotzé Inoculum Monitor (KIM) in order to trap released ascospores of *Guignardia* spp. on a Vaseline® coated microscope slide. Four lanes (45 mm long) on each microscope slide were counted on a light microscope at 400x magnification and the total number of ascospores, on approximately 180 mm<sup>2</sup> surface area, was recorded.

## Results and discussion

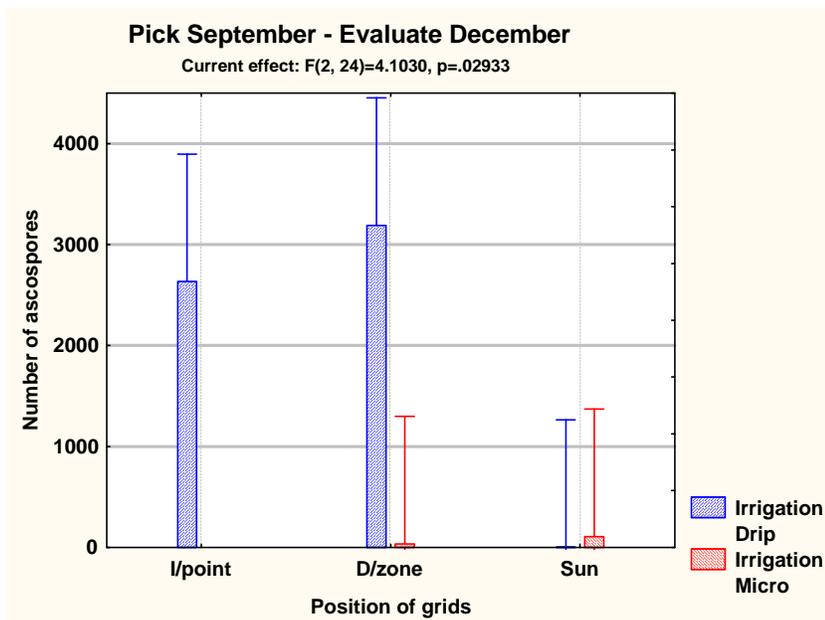
The number of ascospores trapped from leaf litter, incubated in orchards with different irrigation types, and in different positions with regards to the irrigation point, gave variable results for the different times when leaf samples were placed in the orchards and when samples were recovered for analysis (Table 4.3.5.1). The most ascospores were trapped on leaves picked in September and evaluated in December 2006. During this evaluation, the type of irrigation system (Fig. 4.3.5.1) and the positions of incubation (Fig. 4.3.5.2) played a significant role regarding the number of ascospores produced on leaf litter on the floors of orchards used in this study (Fig. 4.3.5.1). The most abundant number of ascospores was produced on leaves incubated in an orchard with drip irrigation, while leaves incubated in an orchard with micro-irrigation, produced relatively low numbers of ascospores at the time of evaluation (Table 4.3.5.1, Fig. 4.3.5.1). However results clearly showed that this was only the case for leaves incubated within the microclimate of tree canopies. The effect of irrigation type was reversed for ascospores produced on grids at a distance from irrigation points in full sunlight, where more spores were observed on leaves incubated in orchards with micro-irrigation than orchards with drip irrigation during the December evaluation (Figs. 4.3.5.2 and 4.3.5.3). Leaves incubated under tree canopies was in a more advanced stage of compostation, especially in orchards with micro-irrigation, than leaves in orchards with drip irrigation systems, and the closer to the irrigation point, the more advanced compostation was. Leaves incubated in direct sunlight were hardly composted at all.

**Table 4.3.5.1.** Number of ascospores observed at 3 different locations in orchards with drip and micro-irrigation systems (mean number / grid for 5 replicates)

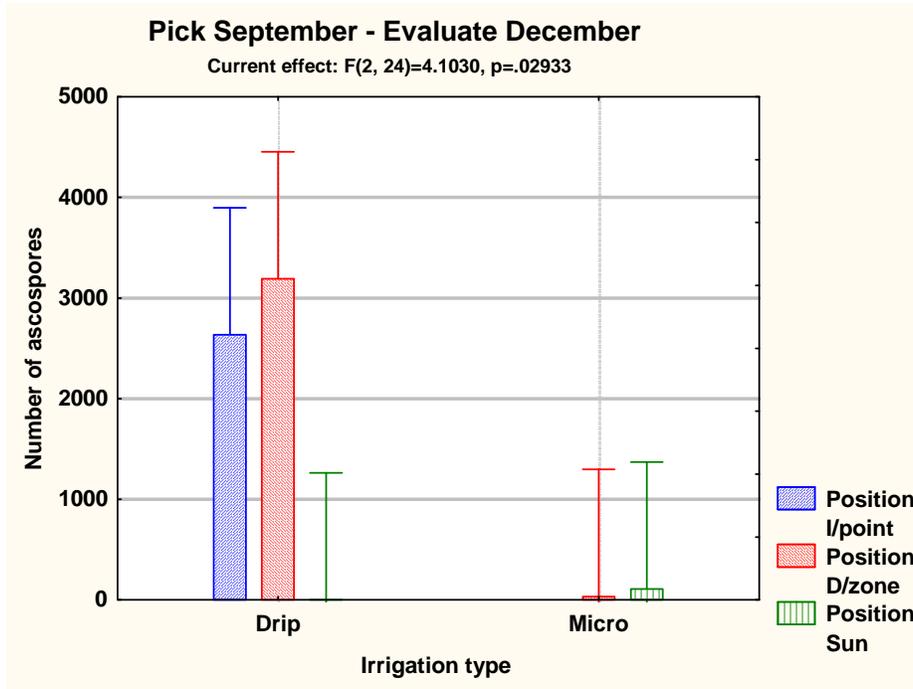
Irrigation type	Position of grids	Time picked – Time evaluated			
		Sep - Dec	Sep - Feb	Nov - Jan	Nov - Mar
Drip	Irrigation point	2635 a	357.6	1.0	0
Drip	Drip zone	3191 a	21.8	22.0	0
Drip	Full Sun	1 b	0.6	0.2	0
Micro	Irrigation point	0 b	0	0.2	0.2
Micro	Drip zone	35 b	0	0	0
Micro	Full Sun	108 b	0.2	0	0



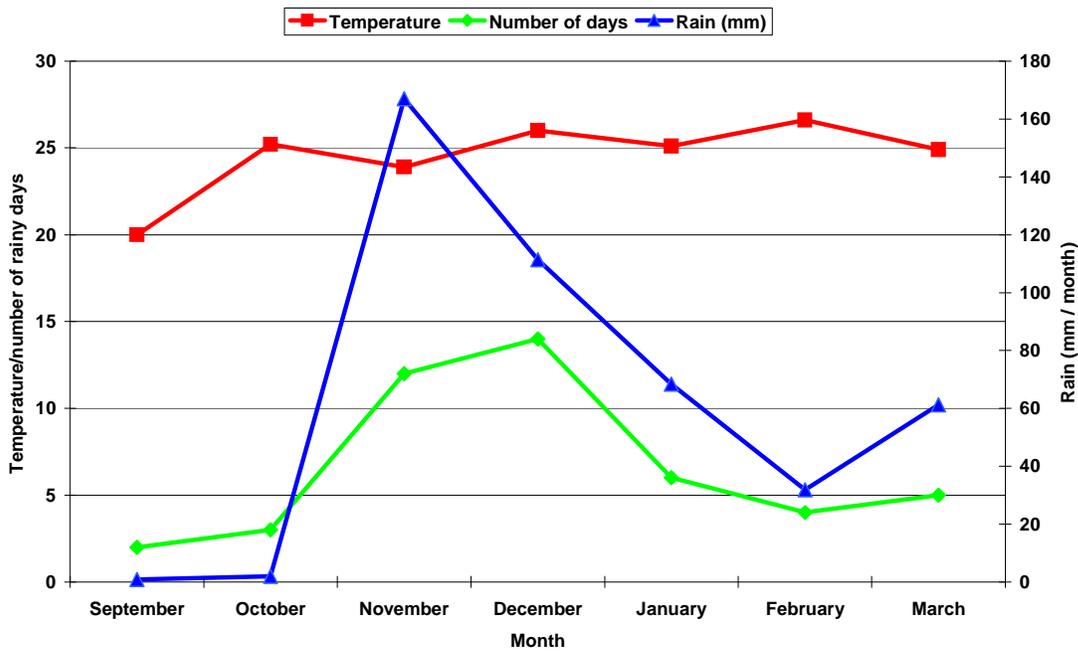
**Figure 4.3.5.1.** Number of ascospores produced in December on fallen leaves (September) in orchards with drip or micro irrigation systems.



**Figure 4.3.5.2.** Number of ascospores produced in December on leaves dropped in September in orchards with drip or micro irrigation systems, at different incubation points.



**Figure 4.3.5.3.** Number of ascospores produced in December on leaves dropped in September in orchards with drip or micro irrigation systems, at different incubation points.



**Figure 4.3.5.4.** Monthly climatic conditions as recorded by Letaba Oranje weather station.

The absence of ascospores on the advanced composted leaves from orchards with micro-irrigation, can probably be due to ascospore release at a much earlier stage than December, when evaluation took place,

while leaves from the orchard with drip irrigation were at a slightly less composted stage, and at optimum ascospore production. Leaves, at a distance from irrigation sources, were not composted sufficiently to promote the production of large numbers of ascospores.

Leaves picked in November and left in orchards until January or March, were also well decomposed at the time of evaluation and visibly more than leaves picked in September. This was probably due to more frequent rainy periods especially during November, December and January. Weather data show 2 dry months, followed by 2 wet months for leaves picked in September and 3 wet months followed by 1 dry month for leaves picked in November. No difference in ascospore production on leaves incubated in orchards with drip, or orchards with micro irrigation systems could be detected during this evaluation period, probably because the effect of irrigation systems was influenced by climatic conditions to some extent, when leaves were evaluated in January 2007 and later (Table 4.3.5.1, Fig. 4.3.5.3). Leaves incubated in full sunlight produced noticeably low numbers of ascospores, irrespective of irrigation type and time of evaluation, except for the September – December evaluation where leaves were incubated in orchards with micro-irrigation.

It appears that the irrigation method and general wetness in the microclimate of trees are major factors affecting the compostation process of leaves on the orchard floor, but it also seems that other factors, such as micro-organisms, especially within the microclimate of tree canopies can play an equally important role on the compostation process and the production of reproductive structures on fallen citrus leaves, by *Guignardia* spp.

## Conclusions

The irrigation type has a direct influence on micro-climate underneath the canopies of trees in a citrus orchard and therefore, can influence the number of ascospores produced on decaying leaves. It was obvious in this trial that irrigation type played a more important role with regards to the compostation process and ascospore production in periods of relatively dryness. Schutte (2005) reported that approximately 1000 leaves per m<sup>2</sup> dropped over a 6 months period and that 40% more decomposed leaves occurred under micro-irrigation systems than under drip irrigation or with no irrigation. In this study the number of ascospores produced on leaves that dropped in September was evaluated in December and February, and those that dropped in November was evaluated in January and March. Therefore, only a few sections of this 6 months period was covered and although it was determined that significantly more ascospores were available on leaves incubated in a orchard with drip irrigation, the trial could not reveal information regarding the period when and at what level ascospores were discharged from leaves in the orchard under micro irrigation. It is quite possible that ascospores were released at an earlier stage in orchards with micro-irrigation due to enhancement of the compostation process. Studies conducted to determine the time between leaf drop and ascospore production showed that the process can occur within 30 days and continue for 90 days during periods of frequent rain but will take up to 180 days under dry conditions (Swart, 2005, Kotzé, 1988, McOnie, 1964).

## Technology transfer

CRI symposium.

## Future work

A more elaborate trial will have to be conducted in order to make assessments on shorter intervals (1 month) and over a longer period of time. Therefore, we suggest that this project should be repeated and that between 7 and 2 grids for each replicate be prepared, depending of the month when leaves are picked. Leaves should be picked in September (7 grids / rep), October (6 grids / rep), November (5 grids / rep), December (4 grids / rep), January (3 grids / rep), and February (2 grids / rep) and grids should be retrieved from the orchard floor on monthly intervals. A total of 270 grids should, therefore, be assessed in order to reveal the true effect irrigation type on ascospore production on fallen leaves during the period when citrus fruits are susceptible to infection by ascospores of *G. citricarpa*.

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#### 4.3.6 **FINAL REPORT: Determining the role of *Guignardia citricarpa* and *Guignardia mangiferae* in the CBS complex**

Experiment QMS 2007/SS 2 (2007-2008): by S. Serfontein and S.H. Swart (QMS Agri Science)

#### **Opsomming**

Jong saailinge is in 'n groeikabinet by 12 ure lig en 12 ure donker, temperature van 25-26°C en relatiewe humiditeit bo 60%, gehou en in studies om die moontlike infeksies van *Guignardia citricarpa* en *G. mangiferae* te bepaal, gebruik. Blare is geïnokuleer met 'n suspensie van *G. citricarpa* pycnidiospore of *G. mangiferae* askospore, terwyl stamme met miselium onder die bas geïnokuleer is. Isolasië is dan na verskillende periodes vanuit die weefsel gemaak. Geen infeksie kon bewys word van die blaarinokulasië nie. Slegs een staminokulasië met *G. mangiferae* miselium het tot infeksie gelei. Dit kan afgelei word dat die pycnidiospore van *G. citricarpa* en die askospore en myselium van *G. mangiferae* nie geredelik plante by die toestande waar ons hulle gehou het, infekteer nie.

#### **Summary**

Seedlings, which were grown in a growth chamber with 12 hours light and 12 hours dark at 25-26°C and relative humidity above 60%, were used to determine the possible infections of *Guignardia citricarpa* and *G. mangiferae*. Leaves were inoculated with a suspension of *G. citricarpa* pycnidiospores or *G. mangiferae* ascospores, while stems were inoculated with mycelium underneath the bark. Isolations were then made, after a period of time, from the leaves and stems. No infection of the leaves could be proven. Only one stem inoculation with *G. mangiferae* resulted in infection. It can be concluded that the mycelium and pycnidiospores of *G. citricarpa* and the ascospores and mycelium of *G. mangiferae* do not readily infect plants under the conditions they were kept during this trial.

#### **Introduction**

In order to understand the lifecycle of *Guignardia citricarpa* (Gc) it is important to determine if systemic infections can take place and to determine if both Gc and *G. mangiferae* (Gm) can infect, and thus survive in plant tissue, without causing any symptoms.

#### **Materials and methods**

Seedlings, cultivar Rough Lemon, at six a leaves stage was obtained from a nursery. The seedlings were produced in a tunnel in steam-sterilized bark. Seedlings were replanted in virgin sandy soil in pots with a 10 cm diameter. Seedlings were kept in a growth chamber, 12 hours dark, 12 hours light. Light source were fluorescent lights from the side. Temperature and humidity was controlled as indicated.

#### Leaf inoculations:

Twenty seedlings were inoculated with pycnidiospores of Gc and ascospores of Gm by spraying leaves with spore suspensions obtained from a 21-day-old potato dextrose agar (PDA) culture of Gc and 4 week old Med 5 (Serfontein & Swart, 2005) cultures of Gm. Spores were counted with a Petroff-Hauser counting chamber and set at a concentration of ca 60 000 spores per ml. Control plants were sprayed with water. Plants were incubated at 85% humidity, and 26°C. Isolations were made on PDA supplemented with 250 mg chloramphenicol per l (PDA +) after 7 and 14 days, from one leaf per plant, after surface disinfection with 70% ethanol.

In a repeat experiment, 10 seedlings each were inoculated with Gc and with Gm. Leaf inoculations were repeated by adding the spore suspension onto cotton wool balls about 1 cm in diameter, after soaking and expressing the excess fluid. Cotton wool balls were taped to the adaxial and abaxial leaf surfaces of three young leaves per plant. Ten control plants were inoculated with water. Plants were covered with plastic bags for 7 days to increase humidity. Isolations were made after 21 days. Nine seedlings each were also inoculated with pycnidiospores of Gc and ascospores of Gm by placing a drop of 30 µl, containing ca 10<sup>9</sup> spores per ml in the centre of a leaf, after marking the inoculation area with a circle using a permanent marker. Each plant was gently covered with a plastic bag for 5 days to increase humidity. Plants were kept in the growth chamber with an average temperature of 25°C and 60% humidity. Isolations were made from inoculated leaves after 10 weeks.

### Stem inoculations:

Nine seedlings each were inoculated by lifting a 5 mm piece of bark from the stem using a sterile scalpel. Small pieces of mycelium from 10-day-old cultures of Gc (PDA) and Gm (Med 5) were placed under the bark. Control plants were treated the same, but no mycelium were placed under the bark. Inoculations were done 2 cm above the soil level as well as 4 cm below the growth tip of the plants. Wounds were wrapped with Parafilm, which were left on for the duration of the trial. Plants were kept in the growth chamber with an average temperature of 25 °C and 60% humidity. After 6 weeks, 5 pieces of tissue from the inoculation site as well as 5 pieces 5 mm above and 5 mm below the inoculation site were placed on PDA+ and incubated. Inoculations were repeated on 9 seedlings with each of Gc and Gm and isolations made 10 weeks after inoculation.

## **Results and discussion**

### Leaf inoculations:

No *Guignardia* could be isolated from any of the leaf inoculations. During the first trial the water source used for the humidifier had a high Ec of 1 200 µs. A visible layer of salt was observed on the leaves and could have influenced infection by the spores. This water was then replaced with water with an Ec of 40 µs. The repeated inoculations were also not successful.

### Stem inoculations:

Four of the 9 inoculated seedlings died. Gc was isolated 6 weeks after inoculation from the inoculation sites just beneath the growth tip from 4 of the surviving 5 plants. From these 4 inoculation sites, 18 out of 20 isolations yielded Gc. No Gc could be isolated above or beneath the inoculation site. No Gc could be isolated from any of the inoculations on the older tissue. Gm was isolated from one inoculation site just below the growth tip. The tissue had light brown discoloration. It was also isolated from above and below the inoculation site. From the 15 isolations (above, on and below the inoculation site), all yielded Gm. Gm was also isolated, with one isolation from each of two inoculation sites on the older tissue. After the six weeks, plants were covered in a white layer due to the high Ec of the water source. The stem inoculations were close to the humidity source and due to the salt, some plants did not survive. No Gm or Gc was isolated from any of the seedlings from the repeated inoculations. The seedlings' young growth, were not as soft as during the first round of inoculation. It seems that the inoculations should be done on tissue just after plants have flushed. The cause of low inoculations success on the stems was not due to the salt in the chamber, but maybe due to the physiological age of growth.

## **Conclusion**

Inoculations on the leaves were not successful. The reason for this could not be determined. In previous work, leaf inoculations on young *in vitro* plants were successful (Serfontein & Swart, 2006). It can be speculated that leaves are only susceptible to infections at a very young stage. The only inoculations, which were successful with Gc, was the stem inoculations, but the infection did not become systemic. Gc could only be re-isolated from the inoculations on very young and soft tissue. As Gc was isolated only from the inoculation site, it can also be speculated that the isolations only yielded the original inoculated mycelium. In our previous work, systemic infections were found on the *in vitro* seedling inoculations. This could not be repeated with any of our infections on bigger seedlings. Gm was able to infect one plant systemically. The infection spread at least 5 mm from the inoculation site. This inoculation was also on young tissue. Gm was also re-isolated from only two inoculation sites on the older tissue. After the repeated stem inoculations, isolations were made after 10 weeks whereas re-isolations were made 6 weeks after the first set of inoculations. The mycelium used for the inoculations could have naturally died after the longer incubation period. Under the conditions we kept the plants, no systemic infections by Gc could be induced, neither by pycnidiospore nor by mycelium inoculations. Gm also did not readily infect plants.

## **References cited**

- Serfontein, S. and S.H. Swart. 2005. *In vitro* production of *Guignardia citricarpa* ascospores. CRI Group Annual Research Report 2005:267-270.
- Serfontein, S. and S.H. Swart. 2006. *In vitro* infection of plants. CRI Group Annual Research Report 2007 *in press*

#### 4.3.7 FINAL REPORT: Development of a protocol for the accreditation of nurseries and orchards Experiment PPL 4B (Start date–End date): by Linda Meyer and Lise Korsten (UP)

### Opsomming

Die *Guignardia* vrye akkreditasie van sitruskwekerie en -boorde is van groot belang aangesien die vroeë opsporing van hierdie kwarentynpatogeen die risiko van inokulumverspreiding na siektevrye areas in Suidelike Afrika kan verminder. 'n Polimerase ketting reaksie (PKR) metode is ontwikkel vir die opsporing van *Guignardia* spesies vanaf blaarafval, groen blare met simptome asook simptoomlose groen blare in die boorde en kwekerie. Hierdie metode is geoptimeer en kan nou geïmplimenter word as deel van 'n akkreditasie skema vir die roetine opsporing van *Guignardia citricarpa* in die sitrusindustrie.

### Summary

The *Guignardia citricarpa*-free accreditation of citrus nurseries and orchards is of great importance since early detection of this quarantine pathogen may reduce the risk of spreading the inoculum to disease free areas in Southern Africa. A polymerase chain reaction (PCR) method was developed to detect *Guignardia* spp. from leaf litter, green leaves with symptoms as well as symptomless green leaves in the orchards and nurseries. This method was optimised and can now be implemented as part of an accredited scheme for routine screening of *Guignardia citricarpa* in the citrus industry.

### Introduction

The PCR technique greatly enhances our ability to detect and understand the survival of *Guignardia* spp. on fruit, leaves and litter. Current molecular biology offers various tools for the characterisation of mixed communities of microorganisms in the plant systems and for obtaining a better understanding of host pathogen interactions. The objective of this study is to test the PCR-based *Guignardia* species detection method and develop a protocol for the industry that can result in the screening of symptomless nursery and orchard leaf material for the presence of CBS. The epidemiological significance and colonisation rate of *Guignardia* species naturally infecting leaves can also be determined.

### Materials and methods

This technique has been optimised for regular leaf litter screening in nurseries with great success (PPL 4, 2005 CRI Annual Report). Various attempts to amplify *Guignardia* spp. from green symptomless leaves and leaves with symptoms were made and *G. citricarpa* was amplified directly from leaves in both instances with a high amplicon yield. The optimised protocol as well as protocol for artificial leaf wilting (PPL 17, 2006 CRI Annual Report) has been presented to a team of biometricians (ARC-Stellenbosch) in order to determine the required sampling strategy that will allow detection of  $\geq 2\%$  infected trees at 95% confidence level for an infinite number of trees.

The protocols and sampling strategy will be validated in repeated spiked laboratory experiments.

### Results

#### 1. Leaf sampling

- a. **Sample size:** 801 leaves should be sampled from the total number of trees in the structure or consignment (combined batches). According to statistical sampling procedures for an infinite number of trees/leaves, this amount of leaves will allow detection of  $\geq 2\%$  infected trees at 95% confidence level (Snedecor and Cochran, *Statistical methods*, 8<sup>th</sup> edition).
- b. **Sampling strategy:** Collect only the oldest leaves from the scion. Select the senescent leaves, especially those showing necrotic and CBS-like symptoms, if present. In order to ensure a representative sample is drawn, a systematic sampling approach is recommended: divide the total number of trees by 801 (=  $X$ ), and pick a leaf from every  $X^{\text{th}}$  tree. For example, if the batch consists of 16,000 trees, a leaf should be picked from every 20<sup>th</sup> tree (16,000 divided by 801 = 19.98).
- c. **Packing and labelling:** Leaves should be carefully packed in paper bags and kept cool at 4-10°C from picking until processing in the laboratory. Each sample should be clearly labelled with an adhesive white label, on which the following detail is recorded in pencil: 1) Permit application reference number, 2) Date of picking, 3) Nursery name, 4) Cultivar / rootstock combination, 5) Number of trees in batch, 6) Nursery's batch number. Samples should be picked, labelled and delivered to the processing laboratory within 24 hours.

## 2. Laboratory processing of leaves

- a. Good Laboratory Practices should be maintained at all times.
- b. Record should be kept of all samples received and processed. Aspects that should be noted include label detail (as in 1.c.), dates received and processed, condition of sample, results.
- c. Leaf samples in paper bags can be stored at 20°C for no longer than 7 days before processing.
- d. Processing of leaves involves artificial leaf wilting, followed by inspection and selection of leaves with typical *Guignardia* fruiting bodies (pycnidia or perithecia) from which DNA will be extracted and *G. citricarpa*-specific PCR will be conducted.

### i. Protocol for artificial leaf wilting

1. Wash leaves in running tap water to remove dirt and drain to remove excess water.
2. Air-dry leaves for 12 hours out of direct sunlight OR air-dry leaves for 2 to 4 hours in direct sunlight.
3. Soak air-dried leaves in tap water for 30 minutes, drain to remove excess water and place in a 20 micron clear plastic bag. Use 20 to 50 leaves per bag, depending on size of leaves and bag.
4. Close bag, including as much as possible air within the bag, and place bag with leaves in an incubator at 42°C for 6 h.
5. After 6 h, remove the bag from the incubator and mix leaves by shaking the bag.
6. Open the bag to allow leaves to air-dry and incubate under fluorescent and near-UV light for 18 h.
7. Repeat steps 3 to 6 for at least 21 days or until ample fructification of *Guignardia* is visible on the leaf surface.
8. Note: It is important to monitor the moisture within the bag closely, since no fruiting bodies will develop if the leaves are too dry and the leaves will rot if it is too wet. Unfortunately the correct moisture levels are only known through experience.

### ii. Protocol for isolation and PCR of fungal DNA from dry citrus leaf litter

1. Care should be taken to prevent cross-contamination of samples and these steps should be conducted in a laminar flow cabinet, one batch processed at a time, followed by sterilisation of all equipment and flow cabinet work surface with 100% ethanol.
2. Select 5-8 leaves with visibly developed pycnidia or perithecia from the bulk sample.
3. Using the Unicore 1.2 mm diameter punch, remove 20-25 (maximum) leaf pieces from the selected leaves.
4. When no perithecia/fungal structures are visible, select 10-12 leaves and remove no more than 2 punches from each. Preference is given to typical black spot lesions if at all present.
5. Add leaf sections to a 1.5 ml Eppendorf tube and grind leaf sections to a very fine powder in the Eppendorf tube.
6. Using the DNeasy Plant Mini DNA extraction kit (Qiagen), complete the DNA extraction process.
7. PCR amplification:
  - Prepare 24 µl PCR mix by adding optimised volumes of the following :
    - dH<sub>2</sub>O (adjusted volume to a final volume of 25µl total product);
    - 2.5µl 10x buffer (or according to manufacturers specifications);
    - 200µM of each dNTP or dNTP mix (according to manufacturers specifications);
    - (10 pmol) CITRIC1 primer (5'-GAA AGG TGA TGG AAG GGA G-3');
    - (60 pmol) CAMEL2 primer (5'-AGT ATA CAA AAC TCA AGA ATT C-3');
    - (15 pmol) ITS4 primer;
    - 0.5U Taq polymerase, (according to manufacturers' specifications).
  - Add 1 µl DNA of each sample.
  - Positive controls: DNA from *Guignardia citricarpa* and *G. mangiferae*.
  - Negative controls: DNA from *Colletotrichum gloeosporioides* and dH<sub>2</sub>O.
8. PCR cycle:

- Initial denaturation: 95°C for 2 min
  - 35 cycles of: 93°C for 30 s, 56°C for 45 s, 72°C for 90 s
  - Final extension: 72°C for 7 min
9. Visualisation:
- 1.25% agarose gel in 10% TBE buffer.
  - Band sizes to be observed: 620 bp for *Guignardia citricarpa*, and 430 bp for *G. mangiferae*.
10. Identification of putative *Guignardia citricarpa* PCR products.
- Sequencing analyses will be used to determine whether all 620 bp amplicons amplified from DNA samples were indeed *G. citricarpa*.
  - 620 bp amplicons will be cut from agarose gels and purified using a QIAquick Gel Extraction kit (Qiagen, Valencia, CA, USA).
  - The resulting product will be sequenced using the ITS4 or CITRIC1 primer according to the sequencing reaction and cycle conditions as recommended by the manufacturer.
  - The identity of the sequences will be determined by BLAST analyses.

## Discussion

The molecular PCR detection technique is sensitive and specific for the detection of target organisms in symptomless leaves and is cost and time effective. The optimised PCR protocol can therefore assist in future accreditation of nursery and orchard material, since the presence of *G. citricarpa* in an orchard or nursery may become a quarantine issue. With this technology, the presence of the quarantine pathogen can be detected at a very early stage of infection and the transportation of infected material can be limited as far as possible.

## Conclusion

With this research, a standard sampling and detection method for *Guignardia citricarpa* was developed for routine screening of possible infected leaves and symptomless nursery and orchard material. This method will reduce the risk of disease development and establishment in disease free citrus production regions of South Africa. By screening orchards for *Guignardia* spp. presence, an early indication of citrus black spot (CBS) presence can be established and a nursery can be certified CBS free or not.

## Future research

CBS infected leaves has been used in a spiking trial to determine optimum detection threshold values. Infected leaves were mixed in different ratios of 1: 100; 2: 100; 10: 100; 20: 100. A standard PCR is currently being done on the leaf mixes. These results should be available early June. By spiking and testing the probability of detecting one *Guignardia citricarpa* infected leaf in a hundred non-infected leaves, the established sampling protocol for nursery screening for *Guignardia* infected trees in orchards and nurseries can be validated.

## Technology transfer

The sampling technology will be transferred to the nursery industry for inclusion in the nursery certification scheme. The methodology will provide a more sensitive approach to screen nurseries for CBS presence or absence.

### 4.3.8 PROGRESS REPORT: Epiphytic and endophytic occurrence of *Guignardia citricarpa* on twigs

Experiment PPL 11 (2007-2009): by Linda Meyer and Lise Korsten (UP)

## Opsomming

As deel van 'n vroeë opsporing metode vir *Guignardia* spp. en meer spesifiek *Guignardia citricarpa*, is 'n nuwe direkte PKR metode ontwikkel en geoptimeer vir die isolasie en amplifikasie van *Guignardia* piknidiospore vanaf takkies in boorde. Die PKR metode blyk suksesvol te wees alhoewel die isolasie en kultivering van die piknidiospore nie effektief genoeg is nie. Verskillende isolasie metodes van piknidiospore vanaf takkies sal in die toekoms ondersoek moet word. Die PKR metode sal help met die vroeëtydige opsporing van sitrus swartvlek (CBS) in boorde.

## Summary

As part of an early detection method for *Guignardia* spp. and in particular *Guignardia citricarpa*, a new direct-PCR method was developed and optimised for the isolation and amplification of *Guignardia* pycnidiospores from twigs in orchards. This PCR method proves to be very successful, but the isolation and culturing of the pycnidiospores has proved to be ineffective. Different means of isolation of pycnidiospores from twigs on culture media should be explored in future. The PCR method will assist in the early screening and detection for citrus black spot (CBS) in orchards.

## Introduction

Previously, the citrus black spot (CBS) research group at the University of Pretoria have developed a quick and efficient method of detecting *Guignardia* spp. from plant material without prior culturing of the isolates. With this technology, very small amounts of fungal DNA can easily be detected. This is important since *G. citricarpa* is a quarantine pathogen of citrus. The early detection of this species on citrus fruit and in the orchard is therefore essential to reduce the risk of fruit being discarded during export to foreign countries. The objective of this study is therefore to isolate pycnidiospores from citrus twigs through direct isolation and determining the presence of *Guignardia* on the twigs by direct PCR from the plant material. This method may assist in the screening of orchard and nursery material for early detection of *Guignardia* spp.

## Materials and methods and results

For the detection and isolation of *Guignardia* spp. from twigs in the orchard and nurseries, a few attempts have been made to isolate pycnidiospores from twigs on various types of media. The media tested included carrot agar media amended with different furfural or lavender oil concentrations in comparison to control carrot agar plates. These concentrations are specified in Table 4.5.x.1. This media is semi-selective for *Guignardia* spp.

**Table 4.3.8.1.** Carrot agar amended with furfural and lavender oil concentrations for the isolation of pycnidiospores from citrus twigs.

Furfural concentrations	Lavender oil concentration
1%	-
2%	2µl
3%	5µl
4%	10µl

Although carrot agar sustains the growth of *Guignardia* spp., using carrot agar amended with furfural or lavender oil at different concentrations did not prove highly successful in supporting pycnidiospore germination. *Cladosporium*, *Penicillium* and *Aspergillus* species were frequently isolated from this media. Two of the forty eight 2% furfural amended plates supported the growth of *Guignardia* spp.

Direct PCR from plant material is a commonly used method. With this method, DNA from plant material is extracted together with the DNA of the fungal species to be studied. We have attempted to amplify the *Guignardia* directly from the plant material various times with great success. *Guignardia citricarpa* was easily isolated and identified with the PCR method. New symptomatic twigs were received from CRI but contained no pycnidiospores, isolations and PCR was unsuccessful. Material was also received from QMS and was also negative in isolation and PCR. Another round of sample collection should be done.

## Discussion

Several attempts to isolate pycnidiospores from twigs collected from the orchard were not successful. This may be due to the fact that other organisms which are epiphytic or endophytic may have overgrown the slower growing *Guignardia* culture, or that the pycnidiospores were not viable at the time of isolation. If another means of isolating the pycnidiospores on a more selective media is successfully developed, this technique should be repeated.

The direct PCR of pycnidiospores from twigs is showing great promise for future early detection of *Guignardia citricarpa* presence in the orchard. With the direct-PCR method, the use of culture media to grow the isolates prior to identification becomes unnecessary since only a presence or absence of the pathogenic species is required. This method should, however, be repeated since positive results could not be obtained

with new material received. This should be repeated once more for confirmation purposes by using twigs containing pycnodiospores for isolation during the current citrus season.

#### Future research

It is proposed that infected twigs should be assessed during different periods of the season using the direct-PCR protocol to conclude this project facet. This work should be completed by mid June 2008.

#### Technology transfer

This work facet should be reported to industry via the national citrus symposium

#### 4.3.9 PROGRESS REPORT: Development of a Citrus Black Spot (CBS) Disease Forecasting model Experiment PPL 15 (2005-2008): by C.M. van Ginkel and Lise Korsten (UP)

CRI Disease Management Committee made the following recommendation at their 2006 meeting: 'Last year for funding but funding only to be released if considered suitable for a PhD'. Subsequently, communication between Mr van Ginkel and his promoters, Prof Korsten and Dr Jacque van der Waals broke down to the extent that they resigned themselves from this study. Funding was not approved. Presently, the CBS project coordinator, Dr Tian Schutte, has approached Mr van Ginkel for collaboration on a similar project to be proposed for 2009/10. Mr van Ginkel is also continuing to write his PhD dissertation and has also been incentivised by CRI to scientifically publish the research associated with this study.

#### 4.4 PROJECT: SOIL BORNE DISEASES Project coordinator: M.C. Pretorius (CRI)

##### 4.4.1 Projekopsomming

Alternatiewe beheermaatreëls teen die sitrusaalwurm is 'n prioriteit vir navorsers wêreldwyd. 'n Geïntegreerde beheerbenadering sal in die toekoms meer van toepassing wees, eerder as die bekende tradisionele beheermetodes. Internasionale druk om die gebruik van die uiters toksiese aalwurmdoders te verminder, raak al hoe groter. Die grondgedraagde siekteprojek se strategie was dus hoofsaaklik gekonsentreer op die evalueer van die effek van alternatiewe produkte op hul eie of in kombinasie met huidige geregistreerde aalwurmdoders en biologiese beheerprodukte vir die beheer van die sitrusaalwurm in sitrus in Suid-Afrika. 'n Kontrakproef vir VSA-gebaseerde maatskappy met 'n organiese produk word tans ge-evalueer (4.4.2). 'n Voor-plant proef op herplant grond is beplan om die effektiwiteit van nuwer generasie berokingsmiddels met mekaar te vergelyk vir die effektiewe beheer van die sitrusaalwurm voordat boorde op herplant gronde herplant word. 'n Geskikte proefperseel sal voor die einde van die 2008 seisoen uitgelê word (4.4.3). Die eierstimulasie proef is ge-inisieer omdat geen geregistreerde aalwurmdoder 'n effek op die sitrusaalwurmeiers nie. 'n Nuut geformuleerde eierstimulant wat op sy eie en in kombinasie met 'n aalwurmdoder gebruik kan word se resultate het getoon dat die eierstimulant wel die aalwurmtellings kon verhoog (met tot 225%). Geen langtermyn eierstimulasie-effek was moontlik met die middel nie. Die gekombineerde eierstimulant + aalwurmdoder het wyfietellings uiters effektief verminder (74% en 90%). Die positiewe resultaat wat met die middels behaal is, stimuleer toekomstige navorsing in hierdie veld (4.4.4). Die evalueer van produkte soos bv. Abamectin, Crop Guard (furfural) op sy eie en in kombinasie met Sustain (byvoegmiddel, nie-ioniese "sticker – spreader"), Wetcit (benatter), Nontox – Silica (silica), Bio-Neem (*Azadirachta indica*), Bio-Tode (Natuurlike produk) plus Bio-Neem, Mocap korrels (ethoprophos) asook 'n generiese ethoprophos en Rugby Gr (cadusafos) is ge-evalueer. Belowende resultate is behaal met van die nie-toksiese middels maar opvolgproewe is nodig om resultate te bevestig (4.4.5).

*Phytophthora arecae* en *Phytophthora palmivora* is die eerste keer op ornamentele plante in 2005 in Suid Afrika gerapporteer en die patogene hou 'n moontlike gevaar in vir sitrusproduksie. Die doelwitte van hierdie studie was om te bepaal (1) of 'n laventel kwekery in die Wes Kaap die hoofbron is van *P. palmivora*, (2) of kultuur-gebaseerde isolasie metodes *P. palmivora* sal kan opspoor, (3) of molekulêre tegnieke *P. palmivora* kan onderskei van *P. arecae* en (4) watter *Phytophthora* spesies kom voor in verskillende sitrusproduksie areas. Isolasiestudies het getoon dat dit onwaarskynlik is dat *P. palmivora* met kultuur-gebaseerde tegnieke opgespoor sal kan word wat die status van *P. palmivora* by die laventel kwekery onseker laat. *Phytophthora palmivora* spesies-spesifieke "primers" is ontwikkel vir gebruik in "Real-time PCR" (RT-PCR). Die molekulêre karakterisering van vier geenareas van *P. palmivora* en *P. arecae* isolate kon nie verskille tussen die spesies uitwys nie, alhoewel morfologiese verskille wel teenwoordig was. *Phytophthora* isolate (154) is versamel in 37 sitrus boorde en kwekerye. Die isolate is geïdentifiseer as *P. nicotianae*, *P. citrophthora*, *P. cryptogea/drechsleri* en *P. citricola* (4.4.6). Stamkanker op Clementines word hoofsaaklik veroorsaak deur *Phytophthora citrophthora*. 'n Spuitprogram bestaande uit 'n blaarbespuiting met die fosfonaat, Fighter, asook

drie stambespuittings (elke twee maande) van twee algisied behandelings met Acrobat en 'n tankmengsel van Captan en Sporekill is as kommersiële bespuittings getoets teen stamkanker. Slegs die Captan en Sporekill tankmengsel het beheer van tak- en stamkanker gegee en ook slegs waar die stamme en takke goed bedek was. Acrobat 690 (dimethomorph (90 g) met mancozeb (600 g)) het geen beheer opgelewer nie. Raamtakke moet ook goeie bedekking kry, anders sal die siekte verder ontwikkel. Die rol van duineslakke wat in boorde teenwoordig is se moontlike rol as vektore moet ondersoek word (4.4.7). Clementine kultivars soos 'Marisol', 'Clemlate', 'Oroval', 'Tardino' en 'Oroblanco' is almal positief getoets vir vatbaarheid vir *Phytophthora citrophthora*. Die siekte is ook op suurlemoene in Tucuman, Argentinië geïdentifiseer (4.4.8). Die effektiwiteit van kalium fosfonaat, toegedien deur die besproeiingstelsel, vir die beheer van wortelrot is geëvalueer. Voorlopige resultate het nie die effek van kalium-fosfonaat as grondtoediening op vrug grootte en opbrengs getoon nie. Residu analises het wel gelyke kalium-fosfonaatvlakke in die wortels vir beide konvensionele blaarbespuiting en grondtoediening getoon (4.4.9).

### Project summary

Alternative control methods for the effective control of nematodes are a worldwide priority. An integrated control approach rather than the traditional chemical approach will be the standard control measure for the control of citrus nematode in future. The strategy of the soil borne disease project was to evaluate the effect of alternative products on their own or in combination with registered nematicides and biological control products for the control of the citrus nematode in South African citrus orchards. A contract trial for an USA based company was laid out to evaluate an organic product for the control of the citrus nematode (4.4.2). Pre-plant treatments consisting of bio-fumigants, soil solarisation and the incorporation of non-toxic nematicides and safer soil fumigants, was planned on a nematode infested replant orchard. No suitable trial site could be found during the previous season. The trial will commence at the end of the 2008 season (4.4.3). Promising results were obtained with a newly formulated product with egg hatching abilities and a combination of the product with a nematicide. The egg hatching product was able to increase the female counts on the roots by 225%. No long-term nematode egg stimulation effect was visible. The combined product treatments were highly effective, reducing the female population counts by 74 to 90%. More research in this field is necessary (4.4.4). Alternatives to chemical nematicides is essential and therefore initiated the evaluation of the following products: Abamectin, Crop Guard (furfural) on its own and in combination with Sustain (adjuvant, non-ionic sticker-spreader), Wetcit (surfactant), Nontox-Silica (silica), Bio-Neem (*Azadirachta indica*), Bio-Tode (natural product) plus Bio-Neem, Mocap (ethoprophos), a generic ethoprophos and Rugby Gr (cadusafos). The results showed that the chemical nematicides are still the most effective means of reducing citrus nematode populations. Most of the alternative products did show potential and were able to reduce the female populations to acceptable levels. Further research to confirm these results are necessary (4.4.5).

*Phytophthora arecae* and *Phytophthora palmivora* was for the first time detected in an ornamental nursery in South Africa during 2005. The objectives of the study were to determine (1) whether a lavender nursery in the Western Cape has been the primary source of *P. palmivora*, (2) if culture-based isolation methods will be able to detect *P. palmivora*, (3) if molecular methods can distinguish between *P. palmivora* and *P. arecae* isolates and (4) which *Phytophthora* species are present in different citrus production regions. Culture-based isolations from the nursery material revealed the presence of *Pythium* species and two *Phytophthora* species. *Phytophthora palmivora* was not detected. *Phytophthora palmivora* species-specific primers for use in Real-time PCR (RT-PCR) were developed, and will be evaluated further for detection of *P. palmivora* within soil containing fast growing oomycetes. Molecular characterisation of *P. palmivora* and *P. arecae* isolates using four different gene areas showed no differences between the species. One hundred and fifty four *Phytophthora* isolates were collected from 37 citrus orchards and nurseries across South Africa. The isolates were identified to the species level using PCR-RFLP, which identified *P. nicotianae*, *P. citrophthora*, *P. cryptogea/drechsleri* and *P. citricola* (4.4.6). *Phytophthora* trunk and branch canker is caused by *Phytophthora citrophthora* on Clementines in the Western Cape. Spray programmes consisting of a leaf application with a phosphonate Fighter, as well as three trunk applications (every 2 months) with two algicides viz. Acrobat and a tank mixture of Captan and Sporekill were all tested in a commercial spray programme. Captan and Sporekill tank mixtures were effective in controlling the *Phytophthora* trunk and branch canker if properly applied. Acrobat 690 (dimethomorph (90 g) with mancozeb (600 g)) resulted in no control. Spray operators should focus on proper applications otherwise the disease will keep on developing. Dune snails were also observed during the evaluations and their role as potential vectors should be investigated (4.4.7). Clementine cultivars such as 'Marisol', 'Clemlate', 'Oroval', 'Tardino' and 'Oroblanco' all tested positively for susceptibility to *Phytophthora citrophthora*. The disease was also identified on lemons in Tucuman, Argentina (4.4.8). The application of phosphonates as a foliar spray or trunk paint is problematic due to the potential phytotoxic risk involved and the intensive labour requirements of the latter. Potassium phosphonate was applied through the irrigation system. No effect of the applied phosphonates through the irrigation systems on the fruit size and production was recorded during the 2006 / 2007 growing season.

Residue analyses showed that potassium phosphonate levels in the roots for conventional foliar application were similar compared to levels obtained for potassium phosphonate applied through the irrigation system. The trial will be finalised during the 2008 growing season (4.4.9).

#### 4.4.2. **PROGRESS REPORT: Evaluation of a new organic product for the control of the citrus nematode**

Experiment 894 (2006 - 2009): by M.C. Pretorius (CRI)

##### **Opsomming**

Registrasie proewe is vir Desert King, 'n VSA gebaseerde maatskappy, uitgevoer op 'n kontrakbasis om 'n nuwe biologiese beheerprodukt te evalueer vir die beheer van die sitrusaalwurm. 'n Proef is op Friedenheim Sitrus Landgoed in 'n 12 jaar oue Delta Valencia boord uitgelê, asook in die Wes Kaap by ALG Boerdery, Citrusdal. Toedienings is op beide proewe gedurende die seisoen gedoen en 3 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, an 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  $\pm$  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 3 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.

#### 4.4.3 **PROGRESS REPORT: To evaluate alternative nematode control products as part of an integrated nematode control approach in citrus replant situations**

Experiment 762 (2007 - 2014): by MC Pretorius (CRI)

##### **Opsomming**

CRI het 'n reeks van voor-plant behandelings vir aalwurbeheer voorgestel om die effek van die produkte oor 'n lang termyn op herplantgronde te evalueer,. Die voordeel van so 'n effektiewe behandeling is dat die herhaaldelike gebruik van toksiese aalwurmdoders, wat huidiglik ook baie duur is, verminder word. Geen geskikte boord kon gedurende die vorige seisoen gevind word nie. 'n Advertensie op die CRI web het 'n goeie reaksie ontlok en verskeie produsente is gevra om monsters in te stuur sodat die aalwurmstatus in hierdie boorde bepaal kan word, waarna die geskikste perseel geskies sal word. Daar word dus beplan om die proef teen die einde van die 2008 seisoen te begin.

##### **Summary**

The search for alternative control methods for the effective control of nematodes is worldwide a priority by all researchers. 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 bio-fumigants, soil solarisation and the incorporation of non-toxic nematicides and safer soil fumigants. No suitable trial site could be found during the previous season. An advert was posted on the CRI net and 12 producers responded. Samples will be collected from each farm and analysed to determine the status of the nematode populations, whereafter a suitable site will be chosen. The trial will therefore commence during the end of the 2008 season.

##### **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, Bio-control agents, rootstock choices and cultural practices should be implemented as a new approach of pest control in the soil against known soilborne diseases. This trial was initiated due to the high nematicide treatment

costs, especially when applied after planting. 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. The aim of this study is to evaluate pre-plant treatments of different products at different rates and times for sustained control of nematodes in newly planted citrus orchards.

## Materials and methods

The trial layout will consist of 8 treatments (10 trees per treatment), and will be repeated 4 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. The effect of the pre-plant treatments will then be monitored by means of soil and root analysis 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.

## Results and conclusion

No replant situation that happened during the 2007 season was suitable for CRI to lay out an pre-plant trial. The producers that replanted replant with tolerant rootstocks or had already removed the trees and no soil and root counts were possible to determine the nematode status in those orchards. The problem was advertised on the CRI net explaining exactly what is needed and what is expected from the farmers. 12 producers responded positively. It was recommended to them that they send in sample to determine the nematode status where after the most suitable site would be chosen.

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### 4.4.4 FINAL PROGRESS REPORT: Synchronizing egg hatching in citrus nematodes Experiment 860 (2003 - 2008): by M.C. Pretorius and L. Huisman (CRI)

## Opsomming

'n Kenmerk van plantparasitiese nematodes is hul vermoë om teen 'n hoë tempo te kan reproduseer wat die beheer van aalwurms bemoeilik. Eiertjies van die sitrusaalwurm, *Tylenchulus semipenetrans*, kan tot 9 jaar in die grond oorleef en indien gunstige toestande teenwoordig is sal hul lewensiklus voortgaan. Geen geregistreerde aalwurmdoder het 'n effek op die sitrusaalwurmeiers nie en eiers sal dus konvensionele behandelings oorleef. Die doelwit van hierdie projek is om produkte wat uitbroeiing van eiers sal stimuleer, te ondersoek en sodoende die effektiwiteit van aalwurmbeheer te verbeter. Aanvanklike proewe het getoon dat eierstimulante wel effektief gebruik kan word om eiertjies te stimuleer om uit te broei. Die effek van 'n nuut geformuleerde eierstimulant op sy eie en in kombinasie met 'n aalwurmdoder is op Crocodile Valley geëvalueer. Die produkte is op verskillende tye en teen verskillende dosisse toegedien. Resultate het getoon dat die eierstimulant, DL-P (2 toedienings teen 30 g), wel die wyfietellings met 98% tot 225% kon verhoog. Daar was geen langtermyn effek op eierstimulasie met ander produkte sigbaar nie. Die behandelings met die gekombineerde produk (eierstimulant + aalwurmdoder) het wyfietellings die effektiwiteit verminder (74% tot 90%). Hierdie resultaat was selfs beter as die geregistreerde produkte wat toegedien is. Die positiewe resultaat van hierdie produk blyk belowend te wees en verdere navorsing is nodig om hierdie middel in 'n moontlike aalwurmstrategie te inkorporeer.

## Summary

The high reproductive capacity of most plant parasitic nematodes is one of the features that make them such significant pests, and therefore it makes them difficult to control. The eggs of the citrus nematode, *Tylenchulus semipenetrans*, can survive for up to 9 years in the soil and during favourable conditions the eggs will hatch and the life cycle continues. None of the registered nematicides have any effect on eggs. The aim of this study is to evaluate various selected products for the ability to stimulate egg hatching and thereby improve efficiency of nematode control. Initial trial results with potential egg hatching products appear to be very promising, but the variability of the results in follow up trials was not acceptable and required more research. A new formulation of the most promising product and a combined product (egg stimulant + nematicide) were evaluated further. A trial was laid out at Crocodile Valley Citrus Co. where the products were applied at different dates and dosage. The results indicated that DL-P (2 applications at 30 g) were able to increase the female counts on the roots by 98% to 225%. No long-term nematode egg stimulation effect was visible in the results obtained with regard to the other stimulant treatments. The results of the combined treatments obtained during November 2007 were the most effective control treatments, reducing the female population counts by 74% to 90%. These results were even more effective than the two standard nematicide applications. The combined product (DL-P Plus) proved to be very effective in reducing the nematode numbers and it is therefore suggested that more research with this product should be conducted as part of the nematode control strategy.

## Introduction

The body of the nematode is protected by a multi-layered, proteinaceous cuticle, which functions as a flexible skeleton and as a barrier to undesirable elements in the environment. The cuticle is freely permeable to water but differentially permeable to various ions and other chemicals, thus providing nematodes with a selective barrier, which can prevent the entry of some chemicals (Bird, 1971). It is also a relatively resistant structure and is not readily destroyed by chemical or biological agents. Although they are aquatic animals, plant parasitic nematodes have evolved protective structures and metabolic adaptations that allow them to survive and flourish in what is often a harsh and competitive soil environment.

The high reproductive capacity of most plant parasitic nematodes is one of the features that make them such significant pests, and it also makes them difficult to control. The life cycle of many of the most important species takes only a few weeks at optimum temperatures and each female has the capacity to produce hundreds, and in some cases thousands, of progeny resulting in significant yield losses to growers. On a susceptible crop under ideal conditions for the nematode, populations that are virtually non-detectable at planting can increase to damaging levels in less than 3 months. This tremendous capacity for multiplication tends to negate the effects of antagonists as high levels of parasitism and predation may do little to diminish final nematode numbers (Stirling, 1990).

In addition to the structural features, which provide protection against antagonism, the physiological capacity of many plant parasitic nematodes to survive adverse conditions (Cooper & Van Gundy, 1971) may give them an advantage over some of their parasites and predators. For example, nematodes are the most successful anhydrobiotic animals (Womersly, 1987) and are less likely to be affected by dry conditions than many of the organisms that prey on them. Also, the behavioural modifications that occur in the anhydrobiotic state (e.g. coiling) possibly reduce the susceptibility of nematodes to parasitism and predation. However, it is important to recognise that the capacity of nematodes to survive adverse conditions does not give them an advantage over all their antagonists.

The eggs of the citrus nematode, *Tylenchulus semipenetrans* can survive for up to 9 years in the soil and during favourable conditions the eggs will hatch and the life cycle continues. It is therefore essential to evaluate alternative control methods that are effective and economically viable for the citrus producers to control nematodes on citrus in South Africa.

Initial trial results with potential egg hatching products appear to be very promising, but the variability of the results in the follow up trials was not acceptable and requires more research. The purpose of this trial was to determine if a newly formulated product with egg hatching abilities will be effective.

## Materials and methods

A field trial was laid out on 8-year-old Delta Valencia orchard at Crocodile Valley Citrus Co. Root samples collected prior to the commencement of the trial and analysed by CRI's diagnostic centre resulted in female population counts in excess of 6000 females per 10 g roots. One square meter plots were laid out under the trees representing the area to be applied. The company that formulated the stimulating product and the

combination of this product with a nematicide only had a restricted amount of product available for experimental purposes. It was therefore decided only to apply 1m<sup>2</sup> plots under each tree. The products, rates evaluated and time of application are presented in Table 4.4.4.1. The egg stimulating product (D-LP), the standard nematicides (ethoprophos and cadusafos) and the combined egg stimulating and nematicide product are all granular formulations and were all applied by hand.

The trial consisted of randomly selected single tree plots replicated 3 times. Four sets of soil and root samples were collected at 2-week intervals, with the first samples collected 2 weeks after the first applications and the last at 8 weeks after the first applications were done. A final set of samples were collected 2 months (November 2007) after the fourth set of samples were collected in August 2007. The nematode population analyses in the soil and roots were conducted by CRI's Diagnostic Centre in Nelspruit. The second stage larvae in the soil were 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).

## Results and discussion

The purpose of this trial was to investigate the effect of a new egg stimulating formulation as well as a product D-LP Plus (combination of the egg stimulating product and a nematicide). The results obtained are presented in Tables 4.4.4.2 & 4.4.4.3. The larvae counts collected in August 2007 (Table 4.4.4.2), clearly indicate that the D-LP treatments, with the exception of treatment 10, were all able to increase the larvae counts in the soil. Although not statistically different, if the treatments are compared to each other and to the untreated control or the chemical treatments, a percentage increase compared to the untreated control of between 76% to a maximum of 363% of larvae counts collected in the soil were recorded. The August results were collected 3 weeks after the last set of applications were done and the high percentage increase of nematode larvae could be attributed to that.

The November 2007 set of results showed a different tendency if compared to the August results. It is clear that the effect of the stimulating product was less effective when compared to the previous results. In some of the treatments a reduction of the larvae counts in the soil was evident rather than an increase in the populations as was expected. The decrease can not be explained, but it is known that the environmental conditions (air temperatures, soil temperatures and rainfall), which are experienced during that time of the year, viz. mid summer, normally enhance the fluctuation of the larvae population counts in the soil. The chemical standards and the combined products showed an overall higher percentage decrease of the populations in the soil, which can be attributed to the long residual effect of the nematicides in soil.

The data collected (9 July, 23 July and 6 August) and analysed (Table 4.4.4.3) to determine the effect of the applied products on the female populations in the roots indicate huge variations between the different egg stimulating treatments (Treatments 2–12). No correlation could be drawn between the different egg stimulating treatments. This cannot be explained although it is important to rather analyse the final set of results collected in November 2007. The tendency of the results should be considered rather than an individual set of results, however, the huge variations that occurred within the treatments justify the more in-depth discussion of the results. The results were expressed in the percentage de/increase of nematode populations compared to the untreated control treatment to enable a more meaningful discussion and conclusion of the results rather than comparing the results statistically.

The August 2007 results indicate that treatments with the stimulating products alone (Treatments 2-12) were able to stimulate the female population counts in the roots, although variation between the different treatments was evident. Huge percentage increases of female counts were recorded although no significant differences between the different treatments occurred. Treatment 5 (DL-P, 2 x 30 g) were able to increase the female counts on the roots by 225% and was the most effective treatment recorded at this stage when compared with the untreated control treatment. The treatment with the least increase potential recorded, at this stage, was Treatment 8 (DL-P, 3 x 30 g) with only an 11% increase of female population counts on the roots. Variable results between the different egg stimulating treatments were obtained within the November 2007 results. Again the most effective egg stimulating treatment was treatment 5 (DL-P, 2 x 30 g) and was able to increase the female populations by 98% when compared with the untreated control treatment. The treatment with the least stimulating effect was treatment 11 (DL-P, 1 x 60 g) where only an 11% increase was recorded. A decrease in some of the egg stimulating treatments (Treatments 4, 6, 9 and 12) was recorded. No long-term nematode egg stimulation effect was visible in the results obtained during the evaluation of this trial.

The combined egg stimulating and nematicide treatments (Treatments 13 and 14) initially increased the female populations (9, 23 July and 6, and 24 August) by between 5–154%. The results obtained during 24

August 2007 indicate a 76% increase of female counts on the roots for treatment 13 (DL-P Plus, 1 × 7.5 g) and no change in female counts at treatment 14 (DL-P Plus, 2 × 7.5 g) when compared with the untreated control treatment. For treatment 13, it is believed that the stimulating part of the combined product was still active, since a 76% increase was recorded, whereas when a second application was done (Treatment 14) the nematicidal effect of the product was still active in the soil because no increase of female population counts was recorded. The results of the combined treatments (Treatments 13 & 14) obtained during November 2007 were the most effective treatments, reducing the female population counts by 74 and 90%, respectively although no significant differences were recorded. These results were even more effective than the two standard nematicide applications (Treatments 15 & 16) where the female counts were only reduced by 56% by both products. The reason for the poor performance of the two nematicide treatments cannot be explained.

## Conclusion

The results obtained during this trial confirmed previous findings that the egg stimulating product was able to stimulate citrus nematode eggs to hatch. Many questions, however, still arise from this work and more research should be done with potential stimulating products. The combined product (DL-P Plus) proved to be the most effective treatment in this trial. It is therefore suggested that more research with this product should be conducted as part of the nematode control strategy.

## Technology transfer

Talks at study groups and results will be presented on the bi-annual CRI Symposium in August 2008.

## Future objectives (milestones) and work plan

More research is necessary into promising products able to stimulate citrus nematode eggs to hatch. The DL-P Plus product (egg stimulant + nematicide) should be evaluated in a nematicide control programme to determine its effectiveness and long term effect.

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**Table 4.4.4.1.** Dosages and the different times of application (weeks) of the different products applied to determine the stimulation effect of citrus nematode eggs as well as the control of the citrus nematode with the products on their own and in combination with each other.

Treatment	Dosage/m <sup>2</sup>	Time of application (weeks)				
		1	2	3	4	5
1. Untreated control	-	-	-	-	-	-
2. D-LP	30 g <sup>x1</sup>	X				
3. D-LP	60 g	X				
4. D-LP	90 g	X				
5. D-LP	30 g <sup>x2</sup>	X		X		
6. D-LP	60 g	X		X		
7. D-LP	90 g	X		X		
8. D-LP	30 g <sup>x3</sup>	X		X		X
9. D-LP	60 g	X		X		X
10. D-LP	90 g	X		X		X
11. D-LP	60 g <sup>x4</sup>	X				

12.	D-LP	90 g	X				
13.	D-LP Plus	7.5 g <sup>x5</sup>	X				
14.	D-LP Plus	7.5 g <sup>x6</sup>	X				X
15.	Ethoprophos	7.5 g <sup>x7</sup>	X				X
16.	Cadusafos	15 g	X				X

<sup>x1</sup> Application date for treatments 2,3 and 4 is 25 July 2007.

<sup>x2</sup> Application dates for treatments 5,6 and 7 are 25 July 2007 and 9 July 2007.

<sup>x3</sup> Application dates for treatments 8,9 and 10 are 25 July 2007 and 9 July 2007 and 6 August 2007.

<sup>x4</sup> Application date for treatments 11 and 12 is 25 July 2007.

<sup>x5</sup> Application date for treatment 13 is 25 July 2007.

<sup>x6</sup> Application dates for treatment 14 are 25 July 2007 and 20 August 2007.

<sup>x7</sup> Application dates for treatments 15 and 16 are 25 July 2007 and 20 August 2007.

**Table 4.4.4.2.** The effect of the applied products for the control of citrus nematode larvae in the soil as well as the stimulating effect on citrus nematode eggs as reflected in the larvae counts obtained in citrus soil from a trial site at Crocodile Valley Citrus Estate.

Treatment	Dosage / 1m <sup>2</sup>	L2 / 250 cc soil									
		9 July <sup>x</sup>	% Change <sup>z</sup>	23 July <sup>x</sup>	% Change	6Aug <sup>x</sup>	% Change	20 Aug <sup>x</sup>	% Change	7 Nov <sup>x</sup>	% Change
1. Untreated control	-	11167de <sup>y</sup>	-	4800a-d <sup>y</sup>	-	5933abc <sup>y</sup>	-	4766ab <sup>y</sup>	-	5400a-d <sup>y</sup>	-
2. D-LP	30 g	4100a-e	-63	11566def	140	3233abc	-46	12300a-d	158	900abc	-83
3. D-LP	60 g	6600a-e	-40	14300efg	197	10833c	83	18733a-d	293	3033a-d	-44
4. D-LP	90 g	10800b-e	-3	6400a-e	33	9433abc	59	15833a-d	232	1966a-d	-64
5. D-LP	30 g	5666a-e	-49	5566a-d	16	6166abc	4	11400a-d	139	1300abc	76
6. D-LP	60 g	4900a-e	-56	9333b-f	94	3800abc	-36	15433a-d	224	2666a-d	-51
7. D-LP	90 g	13200e	18	16633fg	247	9166abc	54	13933a-d	192	7766d	44
8. D-LP	30 g	4766a-e	-57	10500c-f	119	10233bc	72	22066bcd	363	3433a-d	-36
9. D-LP	60 g	5833a-e	-47	21833g	355	5933abc	-	12200a-d	156	5066a-d	-62
10. D-LP	90 g	11933e	7	6133a-d	28	10466bc	76	3900ab	-18	6366bcd	18
11. D-LP	60 g	2633a-d	-76	5500a-d	15	1366abc	-77	8366abc	76	6533cd	21
12. D-LP	90 g	11000cde	-1	10600c-f	121	2933abc	-51	16133a-d	238	3333a-d	-38
13. D-LP Plus	7.5 g	1933abc	-83	1300a	-73	2866abc	-52	4866ab	2	1966a-d	-64
14. D-LP Plus	7.5 g	2100a-d	-81	3100abc	-35	766ab	-87	2866ab	-40	433ab	-92
15. Ethoprophos	7.5 g	1766ab	-84	266a	-94	233a	-96	866a	-82	1600abc	-70
16. Cadusafos	15 g	1233a	-89	2466ab	-49	300a	-95	5400ab	13	366a	-93

<sup>x</sup> Nematode sampling dates

<sup>y</sup> Means in a column followed by the same letter are not significantly different (P>0.05) according to Fisher's LSD test.

<sup>z</sup> Percentage change relative to untreated control.

**Table 4.4.4.3.** The effect of the applied products for the control of citrus nematode females in the roots as well as the stimulating effect on citrus nematode eggs as reflected in the female population counts obtained on citrus roots from a trial site at Crocodile Valley Citrus Estate.

Treatments	Dosage per 1m <sup>2</sup>	♀ 10 g roots									
		9 July <sup>x</sup>	% Change <sup>z</sup>	23 July <sup>x</sup>	% Change	6Aug <sup>x</sup>	% Change	20 Aug <sup>x</sup>	% Change	7 Nov <sup>x</sup>	% Change
1. Untreated control	-	5866a <sup>y</sup>	-	7733a <sup>y</sup>	-	8466ab <sup>y</sup>	-	5666a <sup>y</sup>	-	6733abcde <sup>y</sup>	-
2. D-LP	30 g	20533b	250	6733a	-13	7400ab	-13	13400ab	136	6733abcde	0
3. D-LP	60 g	12466ab	113	20066c	159	12400abc	46	15133ab	167	10200de	51
4. D-LP	90 g	6733a	15	3100a	-60	15266bc	80	12866ab	127	3200abcd	-52
5. D-LP	30 g	9066a	55	6800a	-12	11800abc	39	18400b	225	13333e	98
6. D-LP	60 g	6800a	16	5933a	-23	4266ab	-50	8600ab	52	2333abc	-65
7. D-LP	90 g	20200b	244	14333bc	85	14400abc	70	14600ab	158	9533cde	42
8. D-LP	30 g	5600a	-5	7600a	-2	6133ab	-28	6266a	11	8600bcde	28
9. D-LP	60 g	8400a	43	8733ab	13	8666ab	2	11000ab	94	5733abcd	-15
10. D-LP	90 g	14533ab	148	7800ab	1	13333abc	57	6666a	18	9933de	48
11. D-LP	60 g	8466a	44	6200a	-20	3333ab	-61	8566ab	51	7466abcde	11
12. D-LP	90 g	16066ab	174	6000a	-22	11266abc	33	6733a	19	4666abcd	-31
13. D-LP Plus	7.5 g	13533ab	131	7200a	-7	21466c	154	10000ab	76	1733ab	-74
14. D-LP Plus	7.5 g	12066ab	106	8133ab	5	13266abc	57	5666a	0	666a	-90
15. Ethoprophos	7.5 g	6866a	17	2600a	-66	2533a	-70	4200a	-26	2933abcd	-56
16. Cadusafos	15 g	8266a	41	9133ab	18	4600ab	-46	6533a	15	2933abcd	-56

<sup>x</sup> Nematode sampling dates

<sup>y</sup> Means in a column followed by the same letter are not significantly different ( $P>0.05$ ) according to Fisher's LSD test.

<sup>z</sup> Percentage change relative to untreated control.

4.4.5 **FINAL REPORT: Evaluation of a combination of different chemical compounds, a biological control product, silicon and non-toxic products for the control of nematodes in citrus**  
Experiment 893 (2006 – 2007) by M.C. Pretorius (CRI)

### Opsomming

Die sitrusaalwurm, *Tylenchulus semipenetrans*, is wêreldwyd die mees algemene aalwurm wat ekonomiese verliese in sitrusboorde veroorsaak. Karbamate en organofosfaat aalwurmdoders word gebruik om hierdie pes te beheer, maar moet op 'n jaarlikse basis toegedien word. 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 vir die beheer van sitrusaalwurm ge-evalueer: Abamectin, Crop Guard (furfural) op sy eie en in kombinasie met Sustain (byvoegmiddel, nie-ioniese “sticker–spreader”), Wetcit (benatter), Nontox–Silica (silica), Bio-Neem (*Azadirachta indica*), Bio-Tode (natuurlike produk) plus Bio-Neem, Mocap korrels (ethoprophos), asook 'n generiese ethoprophos en Rugby Gr (cadusafos). Resultate het getoon dat sekere van die alternatiewe produkte wel aalwurmtellings tot aanvaarbare vlakke verlaag het, maar dat opvolgproewe nodig is om die resultate te bevestig alvorens die produkte kommersieël aanbeveel kan word. Die chemiese standaardde het weereens getoon dat dit uiters effektief is.

### Summary

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus worldwide and is the most abundant plant-parasitic nematode in citrus groves. Non-fumigant post-plant nematicides (carbamate or organophosphate acetylcholinesterase inhibitors) are used to control this pest, but should be applied every year. Due to safety, environmental concerns and political pressure, only a few registered chemical nematicides remain to be utilised by farmers and the use of these products is highly restricted. Developing alternatives to chemical nematicides is essential and a priority for researchers worldwide. The following products were evaluated in this trial: abamectin, Crop Guard (furfural) on its own and in combination with Sustain (adjuvant, non-ionic sticker-spreader), Wetcit (surfactant), Nontox–Silica (silica), Bio-Neem (*azadirachta indica*), Bio-Tode (natural product) plus Bio-Neem, Mocap (ethoprophos), a generic ethoprophos and Rugby Gr (cadusafos) all being granular formulations. The results showed that the chemical nematicides are still the most effective means of reducing citrus nematode populations in both the soil and roots of citrus trees. Most of the alternative products did, however, show potential and were able to reduce the female populations to acceptable levels. Further research to confirm these results and a better understanding of the products mode of action and residual activity are necessary before these products could be introduced into an integrated nematode management strategy 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/ 250cc 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 are currently registered on citrus in South Africa: 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 political pressure, only a few registered chemical nematicides remain to be utilised by farmers and the use of these products is highly restricted. Developing alternatives to chemical nematicides is essential and a priority for 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 study was to evaluate (screen) a range of alternative products such as non-toxic, organic compounds and biological products for the control of the citrus nematode. International pressure from various organisations and Governments to reduce the use of highly toxic and environmentally unfriendly products justify this approach. This approach, if found to be effective, would form part of the integrated pest management approach already being followed by the South African citrus growers.

## Materials and methods

A nematode infested citrus orchard with nematode female counts in excess of 5000 females per 10g of roots was identified. This was regarded as a suitable trial site, as the standard threshold value of 1000 females per 10g of root was exceeded. The 12-year-old citrus orchard with a 10m<sup>2</sup> drip zone is situated north of Nelspruit at Friedenheim Estate. The liquid formulated products were applied by means of a 10-litre watering can and an even distribution of the products under the drip zone of the trees was ensured. The registered granular cadusafos treatment, which served as the standard chemical control, was applied by hand. Protective clothing was used to protect the researcher and staff when applying these products. Single tree plots in a random block design replicated 6 times were utilised. The following products were evaluated in the trial: Abamectin, Crop

Guard (furfural) on its own and in combination with Sustain (adjuvant, non-ionic sticker-spreader), Wetcit (surfactant), Nontox-Silica (silica), Bio-Neem (*Azadirachta indica*), Bio-Tode (Natural product) plus Bio-Neem, Mocap (ethoprophos) and a generic ethoprophos both were granular formulations) and Rugby Gr(cadusafos). The different dates of applications and dosages are presented in Table 4.4.5.1. All the applications were executed in good weather conditions with day temperatures in excess of 32°C.

The trees were sampled before the January 2007 and March 2007 applications were applied and two months later during June 2007, after the final applications were applied in April 2007. A final set of samples were collected in December 2007, to determine the long term effect if any of the products evaluated. The nematode population analyses in the soil and roots were conducted by the Diagnostic Centre in Nelspruit. The second stage larvae in the soil were 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).

## Results and discussion

Tables 4.4.5.2 and 4.4.5.3 present the results obtained from the root and soil samples that were collected during the season from January to December 2007. The final set of samples was collected in December 2007 to determine the long term effect of the soil applied products, while the second last set of data (June 2007) reflects the effectiveness of the products applied for that specific season.

The variation of the results between the different treatments of the larvae counts in the soil (Table 4.4.5.2) varied tremendously and it is therefore difficult to draw conclusions from the results obtained throughout the 2006/2007 growing season. It is known that environmental conditions such as temperatures and rainfall are only but a few factors that can have an effect on the larvae population status of the citrus nematodes in the soil of citrus orchards. The citrus nematode's female population counts in the roots of citrus trees are not affected to the same extent as the larvae populations in the soil. The results presented in Table 3 will therefore be discussed in detail.

The first set of results (Table 4.4.5.3), obtained from the samples collected two months after the trial commenced, indicate no significant differences between the different treatments. To some extent, this reaction was expected because it is known that it is normally too early to expect a result at such an early stage. The direct effect of the different applications applied during the 2006/07 season is visible in data collected during June 2007 (Table 4.4.5.3). The results were discussed as the percentage de/ increase of nematode populations compared to the untreated control treatment to enable a more meaningful discussion and conclusion of the results rather than comparing the results statistically.

It is clear from the results (June 2007), two months after the final applications were done in April 2007 (Table 4.4.5.1) that all the treatments were able to reduce the female population counts when compared with the untreated control treatment. Although the differences between the treatments did not all differ significantly, the percentage decrease indicate the effect of the different treatments. The treatments that were able to reduce the female counts by more than 80% at this stage were the Furfural treatment applied on its own once a month for 5 consecutive months (50 ml /10m<sup>2</sup> tree canopy), the ethoprophos (70 g, granular formulation) applied 3 times during the season at a 2-month interval, and the best treatment was the generic ethoprophos (70 g, granular formulation) applied 3 times during the season also at a 2-month interval. The following treatments were able to reduce the female population counts between 70 and 80%: both abamectin treatments (40 & 10 ml /10m<sup>2</sup> tree canopy) applied on a monthly basis for a maximum of 4 applications, both the Silica treatments (400 g/l & 800g / 1l) applied at a 2-weekly basis commencing in November 2006 to the end of January 2007 and then again three 2-weekly applications that commenced at the end of March to April 2007, the Wetcit (10 ml/ 10m<sup>2</sup> tree canopy) applied on a monthly basis for a maximum of 5 months and the standard chemical control treatments of cadusafos (15 g/10m<sup>2</sup> tree canopy) applied 3 times during the season at a two month interval. The lower dosage treatment of abamectin (10 ml/10m<sup>2</sup> tree canopy) appeared to be effective and sufficient in reducing the female counts by 75% if compared to the higher dosage treatment. Both the Silica treatments that were applied at different rates were effective at this point in reducing female counts. It appeared that the furfural treatment applied on its own was more effective than the furfural and Sustain combination. The remaining treatments were able to reduce the female population counts by at least 50% up to 69%. The cadusafos results were disappointing (-75%). In the past, three cadusafos applications were able to reduce the female counts by > 85% at the end of the season and were able to maintain the control for most of the following season. At present, these results cannot be explained, but will be investigated. The results of most of the non-registered products

were very promising when compared with the registered standard, cadusafos and the other nematicide, ethoprophos.

The result of the long term effect of the applied products is reflected in the December 2007 analysis (Table 4.4.5.3). Although these samples were collected in the new growing season, it is clear from these results that most of the treatments were not able to maintain the high level of control 8 months after the final applications were done. Only the two ethoprophos treatments were superior and able to keep the female population numbers at acceptable levels. The abamectin (10 ml/10m<sup>2</sup> tree canopy), the Furfural (50 ml/10m<sup>2</sup> tree canopy) applied on its own and the furfural and Sustain (50 ml + 10 ml/10m<sup>2</sup> tree canopy) combination were able to maintain a population reduction of a 66 and 60%, respectively. The standard chemical control treatment, cadusafos, failed to keep the female counts at lower levels. These results were disappointing. The BioTode + Neem and Silica treatments were also less effective 8 months after the final applications were made. The results clearly indicate that the residual effect of some of these products was very short and that more frequent applications would be necessary. The Bio-Neem treatment, on its own, especially the 15 ml dosage, was not successful in effectively reducing the female population counts.

## Conclusion

In order to reduce the use of highly toxic chemical compounds, it is essential to evaluate a range of alternative products for the control of the citrus nematode. It is clear from the results that the chemical nematicides are still the most effective means of reducing citrus nematode populations in the soil and roots of citrus trees. Most of the alternative products evaluated did, however, show potential and were able to reduce the female populations of the citrus nematode to acceptable levels. Further research to confirm these results and to obtain a better understanding of the products regarding its mode of action, residual activity and treatment strategy are necessary before these products could be utilised with confidence by the South African citrus growers. It is anticipated that a different treatment strategy must be adopted by researchers and the grower community to ensure the successful implementation of these less toxic compounds. For example, more frequent applications and better management practices will be necessary. These products does offer a safer and hopefully more economical option for nematode control, but should be thoroughly tested to clearly demonstrate consistent and effective levels of control. It is therefore recommended that the most effective products be re-evaluated.

## Future research

Specific dosages, activity and residual activity of these less toxic products were evaluated and promising results were obtained. It is therefore suggested that this work should continue.

## Technology transfer

Results will be presented at the next CRI Symposium in August 2008.

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**Table 4.4.5.1.** Dosages and dates of application of the different products applied to determine the effect of these treatments on the citrus nematode populations at Friedenheim Estate.

Treatments		Dosage/tree/ 10m <sup>2x</sup>	Nov 2006		Dec 2006		Jan 2007		Feb 2007		March 2007		April 2007	
			21/11	29/11	5/12	19/12	11/1	25/1	1/2	14/2	1/3	14/3	11/4	25/4
1	Untreated control	-	-	-	-	-	-	-	-	-	-	-	-	-
2	BioTode + Neem	1 mℓ + 15 mℓ	X		N				X		N		X	X
3	Abamectin	40mℓ			X		X		X		X			
4	Abamectin	10 mℓ			X		X		X		X			
5	Bio-neem	15 mℓ	X				X				X			
6	Bio-neem	30 mℓ	X				X				X			
7	Silica (400 g/ℓ)	25 mℓ	X	X	X	X	X	X	X			X	X	X
8	Silica (800 g/ℓ)	25 mℓ	X	X	X	X	X	X	X			X	X	X
9	Furfural	50 mℓ	X		X		X		X		X			
10	Furfural + Sustain	50 mℓ + 10 mℓ	X		X		X		X		X			
11	Furfural + Sustain	50 mℓ + 10 mℓ	X	X			X	X			X	X		
12	Cadusafos	150 g	X				X				X			
13	Ethoprophos	70 g	X				X				X			
14	Wetcit	10 mℓ	X		X		X		X		X		X	
15	Ethoprophos	70 g	X				X				X			

<sup>x</sup> Products applied as a soil application by means of a 10 ℓ watering can.

**Table 4.4.5.2.** The effect of various soil - applied toxic, non - toxic, organic and a biological control range of products applied at different rates and dates at Friedenheim Estate for the control of citrus nematode larvae populations in the soil for the period from January to December 2007.

No.	Treatments	Dosage/10m <sup>2</sup> tree canopy	January 07 <sup>y</sup>		March 07 <sup>y</sup>		June 07 <sup>y</sup>		December 07 <sup>y</sup>	
			L2/250 cc soil	% Change <sup>x</sup>	L2/250 cc soil	% Change	L2/250 cc soil	% Change	L2/250 cc soil	% Change
1	Untreated control	-	2600 a <sup>z</sup>	0	2883defg <sup>z</sup>	0	4450bcd <sup>z</sup>	0	3333d <sup>z</sup>	0
2	BioTode + Neem	1 ml + 15 ml	2616 a	1	5133g	78	5166d	16	1716abcd	-49
3	Abamectin	40ml	1933 a	-35	3216cdefg	12	2116abc	-52	2800cd	-16
4	Abamectin	10 ml	2733 ab	-5	2616abcde	-9	3216abcd	-28	1816abcd	-46
5	Bio-neem	15 ml	2616 a	1	2550abcde	-12	4050bcd	-9	2200bcd	-34
6	Bio-neem	30 ml	2533 a	-3	2350abcde	-18	4416bcd	-1	1650abc	-50
7	Silica (400 g/l )	25 ml	4516 b	42	4200defg	46	3866bcd	-13	1716abcd	-49
8	Silica (800 g/l )	25 ml	2483 a	-5	4333efg	50	3116abcd	-30	2200bcd	-34
9	Furfural	50 ml	2216 a	-17	1916abcd	-34	1900ab	-57	766ab	-77
10	Furfural + Sustain	50ml + 10ml	3016 ab	14	5016fg	74	4466cd	0	2866cd	-14
11	Furfural + Sustain	50ml + 10ml	2666 ab	2	1216abc	-58	3400abcd	-24	700ab	-79
12	Cadusafos	150 g	2733 ab	5	2683bcde	-7	2866abcd	-36	1433abc	-57
13	Ethoprophos	70 g	2816 ab	8	850ab	-71	866a	-81	700ab	-79
14	Wetcit	10 ml	1583 a	-64	2716bcdef	-6	3100abcd	-30	1416abc	-58
15	Ethoprophos (Generic)	70 g	1283 a	-103	350a	-88	900a	-80	233a	-93

<sup>x</sup> Percentage change relative to untreated control.

<sup>y</sup> Nematode sampling dates

<sup>z</sup> Means in a column followed by the same letter are not significantly different (P>0.05) according to Fisher's LSD test.

**Table 4.4.5.3.** The effect of various soil - applied toxic, non - toxic, organic and a biological control range of products applied at different rates and dates at Friedenheim Estate for the control of citrus nematode female populations in the roots for the period from January to December 2007.

No.	Treatments	Dosage/10m <sup>2</sup> tree canopy	January 07 <sup>y</sup>		March 07 <sup>y</sup>		June 07 <sup>y</sup>		December 07 <sup>y</sup>	
			♀ / 10g roots	% Change <sup>x</sup>	♀ / 10g roots	% Change	♀ / 10g roots	% Change	♀ / 10g roots	% Change
1	Untreated control	-	3866a <sup>z</sup>	0	4166e <sup>z</sup>	0	6050e <sup>z</sup>	0	5933e <sup>z</sup>	0
2	BioTode + Neem	1 ml + 15 ml	2566a	-34	2800bcde	-33	2300bcd	-62	3833bcde	-35
3	Abamectin	40ml	3133a	-19	3900de	-6	1766abcd	-71	2800abcd	-53
4	Abamectin	10 ml	4566a	18	2933bcde	-30	1533abc	-75	2000ab	-66
5	Bio-neem	15 ml	3200a	-17	4166e	0	2366bcd	-61	4366cde	-26
6	Bio-neem	30 ml	3800a	-2	3566cde	-14	3000d	-50	2966abcd	-50
7	Silica (400 g/l )	25 ml	4366a	13	3000bcde	-28	1700abcd	-72	3550bcd	-40
8	Silica (800 g/l )	25 ml	3200a	-17	2500bcd	-40	1766abcd	-71	4633de	-22
9	Furfural	50 ml	3300a	-15	1966abc	-53	866a	-86	2400abc	-60
10	Furfural + Sustain	50ml + 10ml	4333a	12	3166cde	-24	2300bcd	-62	2766abcd	-53
11	Furfural + Sustain	50ml + 10ml	3700a	-4	1400ab	-66	2933cd	-52	2366abc	-60
12	Cadusafos	150 g	3433a	-11	2600bcde	-38	1533abc	-75	2566abcd	-57
13	Ethoprophos	70 g	2433a	-37	566a	-86	733a	-88	1300a	-78
14	Wetcit	10 ml	2833a	-27	2100abc	-50	1400ab	-77	3000abcd	-49
15	Ethoprophos (Generic)	70 g	2933a	-24	733a	-82	633a	-90	1300a	-78

<sup>x</sup> Percentage change relative to untreated control.

<sup>y</sup> Nematode sampling dates

<sup>z</sup> Means in a column followed by the same letter are not significantly different (P>0.05) according to Fisher's LSD test.

#### 4.4.6 PROGRESS REPORT: Characterization of *Phytophthora* species from various South African citrus production regions

Experiment US1/07 (October 07 – December 2009): by J. Meitz (SU), M.C. Pretorius, T. Schutte, L. Huisman, E. Carstens (CRI), W.J. Botha (ARC) and A. McLeod (SU)

##### Opsomming

*Phytophthora arecae* en *Phytophthora palmivora* is die eerste keer in 2005 in Suid Afrika gerapporteer, slegs op ornamentele plante in 'n beperkte geografiese area. Hierdie patogene hou 'n moontlike gevaar in vir sitrus produksie. Die doelwitte van hierdie studie was om te bepaal (1) of 'n laventel kwekery in die Wes Kaap die hoofbron is van *P. palmivora*, (2) of kultuur gebaseerde isolasie metodes *P. palmivora* sal kan opspoor, (3) of molekulêre tegnieke *P. palmivora* kan onderskei van *P. arecae* en (4) watter *Phytophthora* spesies kom voor in verskillende sitrus produksie areas. 'n Uitgebreide *Phytophthora* opname was by die laventel kwekery uitgevoer, wat vermoedelik die bron is van *P. palmivora* in die Wes Kaap. Kultuur gebaseerde isolasies uit die kwekery materiaal het die teenwoordigheid van vinnig-groeiende *Pythium* spesies en twee *Phytophthora* spesies aangedui op die *Phytophthora* selektiewe isolasie medium. *Phytophthora palmivora* was nie geïsoleer nie. 'n Aantal van die *Pythium* en *Phytophthora* spesies wat uit die kwekery geïsoleer is, sowel as *P. palmivora*, is kunsmatig in grond geïnokuleer. Isolasiestudies uit die grond het getoon dat dit onwaarskynlik is dat *P. palmivora* met kultuur gebaseerde tegnieke opgespoor sal kan word. Gevolglik is die status van *P. palmivora* by die laventel kwekery onseker. *Phytophthora palmivora* spesie-spesifieke "primers" is ontwikkel vir gebruik in "Real-time PCR" (RT-PCR), en sal verder vir die opsporing van *P. palmivora* in grond, waar vinnig groeiende oomcyete spesies voorkom, geëvalueer word. Die molekulêre karakterisering van vier geen areas van *P. palmivora* en *P. arecae* isolate kon nie verskil tussen die spesies uitwys nie, alhoewel morfologiese verskille teenwoordig was. *Phytophthora* isolate (154) is versamel in 37 sitrus boorde en kwekerye in Suid Afrika. Die isolate is tot op spesie-vlak deur PCR-RFLP as *P. nicotianae*, *P. citrophthora*, *P. cryptogea/drechleri* en *P. citricola* geïdentifiseer.

##### Summary

*Phytophthora arecae* and *Phytophthora palmivora* was reported for the first time in South Africa in 2005, and are a potential threat to citrus production. Currently, these pathogens have only been detected on ornamental plants, in a restricted geographical area. The objectives of the study were to determine (1) whether a lavender nursery in the Western Cape has been the primary source of *P. palmivora*, (2) if culture-based isolation methods will be able to detect *P. palmivora*, (3) if molecular methods can distinguish between *P. palmivora* and *P. arecae* isolates and (4) which *Phytophthora* species are present in different citrus production regions. An extensive *Phytophthora* survey was done at the lavender nursery thought to be the primary source of *P. palmivora* in the Western Cape. Culture based isolations from the nursery material revealed the presence of many fast growing *Pythium* species and two *Phytophthora* species that grew on the *Phytophthora* semi-selective medium. *Phytophthora palmivora* was not detected. A few of the *Pythium* and *Phytophthora* species isolated from the nursery, as well as *P. palmivora* were artificially inoculated into soil, which showed that it is unlikely that *P. palmivora* will be detected with culture based techniques. Consequently, the status of *P. palmivora* at the lavender nursery remains uncertain. *Phytophthora palmivora* species specific primers for use in Real-time PCR (RT-PCR) were developed, and will be evaluated further for sensitive detection of *P. palmivora* within soil containing fast growing oomycetes. Molecular characterization of *P. palmivora* and *P. arecae* isolates using four different gene areas showed no differences between the species, although morphological differences were evident. *Phytophthora* isolates (154) were collected from 37 citrus orchards and nurseries across South Africa. The isolates were identified to the species level using PCR-RFLP, which identified *P. nicotianae*, *P. citrophthora*, *P. cryptogea/drechleri* and *P. citricola*.

##### Introduction

New genetic variants of soilborne citrus pathogens such as *Phytophthora citrophthora* and *Phytophthora nicotianae* (syn. *P. parasitica*), and the emergence of other species such as *Phytophthora palmivora* and *Phytophthora arecae* present a potential threat to the citrus industry (Zitko *et al.*, 1991; Timmer *et al.*, 1990; Erwin and Ribeiro, 1996; Graham *et al.*, 1998a, Graham *et al.*, 1998b). These *Phytophthora* species have different temporal and climatic requirements, and therefore often have different distribution patterns (Graham *et al.*, 1998b, Matheron *et al.*, 2002; Cohen *et al.*, 2003). Research into the species composition, genetic diversity and population genetics of citrus *Phytophthora* species is required to develop effective control and detection methods of these pathogens. The genetic diversity within pathogen populations can provide an indication of the

potential of these populations to adapt to control measures, including cultivar resistance and fungicide applications (McDonald *et al.*, 2003). Furthermore, knowledge of the specific *Phytophthora* species present on certain host plants in a country is very important for the compilation of phytosanitary lists required for import and export markets.

A few *Phytophthora* species of citrus can cause root, foot and crown rot, gummosis (rotting of bark anywhere on the tree) as well as fruit decay. *P. nicotianae* is best known for causing foot rot and root rot, whereas *P. citrophthora* is known for causing fibrous root rot as well as gummosis that extend high up on scions and can girdle and eventually kill trees (Timmer *et al.*, 1998; Timmer, 2005; Cohen *et al.*, 2003). Less is known about *P. palmivora*, but it has been shown to be more virulent than *P. nicotianae* on fibrous roots of sweet orange, sour orange and Swingle citrumelo. Furthermore, *P. palmivora* can outcompete *P. nicotianae* on roots of rough lemon seedlings, suggesting that it could cause serious damage on citrus should it become widespread (Ziko *et al.*, 1991; Ziko *et al.*, 1994). Under stress conditions such as attack by Diaprepes weevils, *P. palmivora* has also been found to break the resistance of normally resistant rootstocks (Swingle citrumelo) (Graham *et al.*, 1998). *P. palmivora* is thought to, similar to *P. citrophthora*, invade the entire root cortex and most likely do not depend on the availability of root tips for infection and reproduction as do *P. nicotianae* (Graham 1990; Widmer *et al.*, 1998; Ziko *et al.*, 1994). *Phytophthora palmivora*, *P. nicotianae* and *P. citrophthora* can all infect citrus fruit causing brown rot. *P. nicotianae* causes the least economical losses of these species with regard to brown rot, since it does not produce abundant sporangia on infected fruit, compared to abundant sporangial production on fruit by *P. citrophthora* and *P. palmivora* (Ziko *et al.*, 1994). *P. palmivora* has been found to cause 30 to 90% fruit losses in Florida with fruit infections occurring above 1m in the canopy, whereas *P. nicotianae* infections are confined to below 1m in the canopy (Ziko *et al.*, 1991; Graham *et al.*, 1998, Timmer *et al.*, 2000).

In South Africa, *P. nicotianae* and *P. citrophthora* have been reported on citrus (Maeko & Couthin, 2002; Thompson *et al.*, 1995; Whener *et al.*, 1986). However, *Phytophthora palmivora* has not yet been reported on citrus in South Africa, and is currently listed as a quarantine pathogen in South Africa. The first report of *P. palmivora* in South Africa was in 2005, when the pathogen was only found on ornamental plants in a restricted geographical region. In addition to *P. palmivora*, another *Phytophthora* species, *P. arecae*, was also found for the first time in South Africa in 2005 (Stellenbosch University, unpublished data). The pathogenicity of *P. arecae* on citrus is mainly unknown, since it is primarily known as a pathogen of palms. Unfortunately the species status of *P. palmivora* and *P. arecae* is currently uncertain, since these pathogens can not be distinguished at a molecular level, yet there are morphological differences. Consequently, some authors have suggested that these species may in fact be the same species (Erwin & Ribeiro, 1996; Kroon *et al.*, 2004; Mchau & Coffey, 1994). This fact complicates surveys conducted for the presence of *P. palmivora*.

Culture based isolation of different *Phytophthora* species can sometimes be ineffective, depending on the material from which isolations are made. Isolations from soil or plant material that contain *Pythium* species, such as *P. vexans*, that grow on *Phytophthora* semi-selective media (PARPH) can prevent the isolation of *Phytophthora*. This is often the case in diseased plant and soil samples, since *Pythium* species are aggressive secondary invaders that colonized plant tissue subsequent to attack by *Phytophthora*. In general, most *Pythium* species grow much faster than *Phytophthora* species on culture media. Furthermore different *Phytophthora* species also have differential growth rates on culture media. The selective isolation of fast growing *Pythium* and *Phytophthora* species on culture media may result in some slow growing *Phytophthora* species not being detected. This problem can possibly be circumvented by using molecular methods that can detect specific species directly from plant or soil baiting material.

## Materials and methods

Investigating whether a lavender nursery in the Western Cape has been the primary source of *P. palmivora*. In the lavender nursery, plant and soil material were collected from 6 nursery blocks containing different ornamental plants (lavenders, rosemary and malva). Isolations were made from surface sterilized plant roots and crowns by plating onto *Phytophthora* semi-selective media (PARPH). Isolations from soils were made by using the leaf disk baiting technique (Tsao *et al.*, 1983). Hyphal growth from all *Phytophthora* isolations was subcultured for DNA extraction. *Phytophthora* species were identified using PCR amplification with *Phytophthora* specific primers, followed by restriction fragment length polymorphism (RFLP) analyses (PCR-RFLP) (Drenth *et al.*, 2006).

Determine if current isolation methods will be able to detect *P. palmivora*. The probability of isolating *P. palmivora* from mixed oomycete inoculated soil samples was evaluated by artificially inoculating soil samples

with several oomycete species grown on V8 agar plates (Table 4.4.6.1). Subsequently, a leaf disk baiting method (Tsao et al., 1983) was used for culture based isolation of the different oomycetes onto *Phytophthora* semi-selective media. *Phytophthora palmivora* specific RT PCR primers were developed from the ITS region, and Real-time PCR using SYBR Green was performed on a Rotor-Gene 600 machine (Corbett Research) using DNA from pure cultures of *P. palmivora*, *P. nicotianae* and *P. dreschleri*.

Determine if molecular methods can distinguish between *P. palmivora* isolates found on lavender in the Western Cape and *P. arecae* isolate from a palm nursery. Two *P. palmivora* isolates obtained from two lavender farms in the Western Cape as well as two *P. arecae* isolates obtained from a palm nursery were characterized using four gene sequence areas (internal transcribed spacer region,  $\beta$ -tubulin, alfa elongation factor and cox genes) as well as morphology. These cultures are the only cultures that we currently have available for testing.

Determine which *Phytophthora* species are present in different citrus production regions. Soil samples were collected from 37 citrus orchards. *Phytophthora* isolations from citrus soils, culturing, DNA extraction and species identification were done as described above. A group of isolates representing the different PCR-RFLP groups were sequenced in order to confirm the PCR-RFLP identifications.

## Results and discussion

Investigating whether a lavender nursery in the Western Cape has been the primary source of *P. palmivora*. Isolations from ornamental plant and soil material yielded 30 putative *Phytophthora* isolates. Three of the isolates that grew on PARPH were morphologically identified as *Pythium* sp. and also did not amplify with *Phytophthora* specific primers (Drenth et al., 2006), confirming their identity as *Pythium*. One isolate was identified as *P. vexans* through sequence analyses. The *Pythium* samples were collected mainly from the Abriali lavender mother block of the nursery where most die-back of lavenders was observed, indicating high secondary infections. This is very problematic for the isolation of *Phytophthora*. Most of the *Phytophthora* isolates were identified as *P. drechleri* and *P. nicotianae* based on their RFLP patterns. No *P. palmivora* isolates were obtained from the samples.

Determine if current isolation methods will be able to detect *P. palmivora*. The results of the soil inoculation experiment showed that *P. palmivora* could not be detected when other fast growing oomycete species were present in soil samples (Table 4.4.6.1). The lack of detection of *P. palmivora* in mix inoculations is most likely due to its slow growth on PARPH. In order to try and solve this problem in the future, *P. palmivora* specific primers were developed. Real-time PCR with these primers, using Sybr Green, was able to distinguish *P. palmivora* from *P. nicotianae* and *P. dreschleri* when DNA isolated from pure cultures were used. The lowest detection level of *P. palmivora* was shown to be 0.0001 ng/ul, which is approximately 10 000 times more sensitive than conventional PCR detection.

Determine if molecular methods can distinguish between *P. palmivora* isolates found on lavender in the Western Cape and *P. arecae* isolate from a palm nursery in Mpumalanga. *P. arecae* and *P. palmivora* could not be distinguished at a molecular level, since there were no differences in the sequences of the four gene areas, even though the isolates could be distinguished morphologically.

Determine which *Phytophthora* species are present in different citrus production regions. In total, 154 *Phytophthora* isolates were collected from 37 citrus orchards and nurseries across South Africa (Table 4.4.6.1). The *Phytophthora* specific ITS primers, along with the PCR-RFLP method were effective in identifying *Phytophthora* isolates to species level. Sequencing analyses confirmed the PCR-RFLP identifications. The PCR-RFLP of the ITS region identified four different species, including 91 isolates of *P. nicotianae* (Pn), 32 isolates of *P. citrophthora* (Pc), one isolate of *P. cryptogea* (Pcry) and two isolates of *P. citricola* (Pcit) (Table 4.4.6.1). *Phytophthora citricola*, which can be aerially dispersed, has previously been reported as causing fruit rot of grapefruits and shoot tip blight of lemons in South Africa (Von Malitz & Von Broembsen, 1985; Wagner, 1940).

**Table 4.4.6.1** Inoculation of soil with different oomycete (*Pythium* and *Phytophthora*) species in order to determine whether a culture based soil baiting technique was able to recover each species when co-inoculated with other species.

Species used for inoculation	Species baited (morphology)	Total # of leaf discs	# leaf discs infected	Colony diameter after 4 days [cm]*
<i>P. palmivora</i>	<i>P. palmivora</i>	24	12	0.7-1.3
<i>P. drechsleri</i> + <i>P. nicotianae</i> + <i>P. palmivora</i>	Mixed = <i>P. drechsleri</i> + <i>P. nicotianae</i>	37	37 (mixed)	2.0
<i>P. drechsleri</i> + <i>P. palmivora</i>	<i>P. drechsleri</i>	22	22	1.9-2.2
<i>P. nicotianae</i> + <i>P. palmivora</i>	<i>P. nicotianae</i>	12	12	0.8-1.9
<i>Pythium vexans</i> + <i>P. palmivora</i>	<i>P. vexans</i> , <i>P. palmivora</i>	8	7 1	1.6-2.0 1.0

\* Colony diameter was determined by measuring hyphal growth from the edge of the leaf disk up to the terminal end of hyphae growing from the leaf disk.

**Table 4.4.6.2.** Distribution of different *Phytophthora* species in six citrus production regions of South Africa from 2005 to 2008.

Province	Area	# Orchards	# Isolates 2005	# Isolates 2006	# Isolates 2007	# Isolates 2008	Total # Isolates
Eastern Cape	Fort Beaufort, Kirkwood, Patensie	6	1 Pc	1 Pc	2 Pc, 1 Pcry, 4 Pn, 2 ND	7 Pn	18
Kwazulu-Natal	Melmoth	1	-	-	-	4 Pn	4
Limpopo	Baltimore, Hoedspruit, Mokopane, Letsitele	5	-	7 Pn, 2 Pc	11 Pn	6 ND	26
Mpumalanga	Barberton, Karino, Malelane, Marblehall, Nelspruit, Ohrigstad	11	1 Pcit	-	27 Pn, 1 Pc	16 Pn, 5 ND	50
North West	Brits	1	-	-	4 Pn	-	4
Western Cape	Buffelsjagrivier, Citrusdal, Swellendam, Knysna, Wildernis	14	18 Pc	1 Pc	1 Pcit, 1 Pc	5 Pc, 15 Pn, 10 ND	51

Pc = *Phytophthora citrophthora*, Pn = *Phytophthora nicotianae*, Pcit = *Phytophthora citricola*, Pcry = *Phytophthora cryptogea/drechsleri*, ND= not determined

## Conclusion

The putative source of *P. palmivora* in the Western Cape could not be confirmed. Therefore, the source that distributed the pathogen still remains uncertain until more effective detection methods for *P. palmivora* can be developed. A first step towards this goal was made by developing Real-time PCR primers for the specific

detection of *P. palmivora*, which will not require culturing of oomycetes on synthetic media. The method will be evaluated and developed further in order to determine whether the method will be able to detect *P. palmivora* in mixed oomycete infected soil and plant material. The real-time detection of *Phytophthora* directly from plant tissue and leaf disks used in soil isolations, could hold potential for future use in the testing of citrus nurseries for the presence of *Phytophthora* in a sensitive and high throughput manner. The most abundant *Phytophthora* species isolated from South African citrus orchards between 2006 and April 2008 was *Phytophthora nicotianae*. *Phytophthora citrophthora* was mainly found in the Eastern- and Western Cape, with isolated incidences in Limpopo and Mpumalanga. *Phytophthora palmivora* was not isolated from any citrus nursery or orchard.

### Future research

1. Continuation of the *Phytophthora* survey of citrus orchards and nurseries.
2. Identification of all *Phytophthora* isolates through PCR-RFLP of ITS region, and further characterization of a subset of isolates through sequence analyses (Stellenbosch University).
3. A total of 150 to 200 isolates of *P. nicotianae* and 50 to 70 isolates for *P. citrophthora* will be selected for a population study where isolates will be characterized using either (Random amplified microsatellites (RAMS) or Amplified Fragment Length Polymorphisms (AFLP) and mating type characterization.
4. Characterization of *P. palmivora* and *P. arecae* isolates from lavender and palm seedlings, using isozyme analyses, mating type and pathogenicity of citrus seedlings and fruits.

### Technology transfer

Meitz, J., Coerze, S. Bester, W. and McLeod, A. 2008. Characterization and detection of *Phytophthora* sp. on Lavender. 1<sup>st</sup> regional Symposium Western Cape Branch, 8 May 2008, Stellenbosch.

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**4.4.7 PROGRESS REPORT: Control of *Phytophthora* trunk and branch canker on Clementines in the Western Cape**  
Experiment 836 (2006 – 2008) by G.C. Schutte (CRI)

**Opsomming**

'n Suijtprogram bestaande uit 'n blaarbespuiting met die fosfonaat, Fighter, (beginnende laat-winter) asook drie 2-maandelikse stambespuitings van twee algiesidiese behandelings (met Acrobat en 'n tenkmengsel van Captan en Sporekill beginnende in die winter (aanvang van die eerste reën)) is as kommersiële bespuitings getoets. Slegs die Captan en Sporekill tankmengsel het beheer van *Phytophthora* tak- en stamkanker gegee en ook slegs in gevalle waar die stamme en takke goed bedek was. Acrobat 690 (dimethomorph (90g) met mancozeb (600g)) het geen beheer gelever nie. Tien geselekteerde bome is met beitels gekrap om die buitenste rant van die letsels te ontbloot, en hierdie aksie het inderdaad verdere ontwikkeling van die siekte gekeer. In die kroon van die boom waar daar nie met die beitels gekrap was nie en waar daar gesukkel word om die stam en kroon goed te bedek, het die siekte verder ontwikkel. Suijtoperateurs moet oplet dat die raamtakke ook goed te bedek word, anders sal die siekte verder ontwikkel. Duisende duineslakke is ook waargeneem tydens die finale evaluasie en hulle moontlike rol as vektore sal ondersoek word.

**Summary**

A spray programme consisting of a foliar application with a phosphonate Fighter (in late winter), as well as three 2-monthly trunk applications with two algicides (viz. Acrobat and a tank mixture of Captan and Sporekill with the onset of winter (when the first rain falls)) were tested in a commercial spray programme. Only the Captan and Sporekill tank mixtures resulted in control of *Phytophthora* trunk and branch canker if properly applied. Acrobat 690 (dimethomorph (90g) with mancozeb (600g)) resulted in no control. All 10 selected trees used for initial evaluation were scratched with a chisel to expose the outer margins of the lesions. This evaluation method failed as this actually prevents the disease from further development. In the crown of the trees, the disease was not controlled on lesions that were not scratched or where no proper algicide treatments were applied. Spray operators should focus on proper applications otherwise the disease will keep on developing. Thousands of dune snails were also observed during the last evaluation and their role as potential vectors will be investigated.

## 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 2 289 ha of these plantings or 1.7 million trees with an average of 743 trees /ha (Anonymous, 2007). 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, 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). *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 to infect through wounds or growth cracks and produce lesions that 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 a similar syndrome 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), were later shown to be highly susceptible to other pathogens such as the citrus tristeza virus (CTV) (Bar-Jospeh, 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 *P. 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 is 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

### Isolations

During all the visits to Swellendam, soil samples were taken close to the trunks and placed into a plastic bag and maintained at 20–25°C during transport to the laboratory and storage prior to processing. After 5 days,  $\pm$  6-7 g of soil was placed into small containers (3x5 cm), diluted with  $\pm$  5 ml water and floating citrus leaf discs were used as bait. After 3 days, these leaf discs were placed onto PARP selective media and incubated at 24°C. Of these isolates, one sub-culture of each isolate was sent to the Plant Protection Research Institute in Pretoria for identification.

### Fungicidal treatments

Two rows in a 'Nules' orchard at Frankenhof Estates east of Swellendam were selected (21 May 2007). Ten trees within each row with visible infections were selected for treatments. The trunks were surface scratched with a chisel to expose the outer borders of the infection and marked. 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 February 2008). The following treatments were applied at the following rates:

- a) Fighter (570 ml/h $\ell$  water) – foliar application
- b) Captan + Sporekill (200 g + 100 ml/h $\ell$  water) – trunk application
- c) Acrobat (dimethomorph = 90 g + mancozeb = 600 g) (120 g/h $\ell$  water) – trunk application

The timing of each treatment is presented in Fig. 4.4.7.1.

General tree health of the trunks, the tree canopy, as well as the branches of the first 10 selected trees infected with *P. citrophthora* with visible infections were rated when the 'Nules' orchard was assessed on 21 May 2007 (before the application of the autumn algicides) and on 6 February 2008 by rating the tree canopy to the following scale: 0 = healthy tree and 10 = dead tree. The region from the scion upwards to just below the first branches ( $\pm$  50 cm in length) and the branches were rated to the following scale: 10% = where 10% of total outside circumference of the trunk (and branches) showed infection; 90% = where 90% of the total outside circumference of the trunk (and branches) showed infection. Trees that showed 100% were already dead at that stage.

## Results and discussion

### Isolates

All the soil isolates tested positive for *Phytophthora citrophthora*.

### Algicidal treatments

The trees in the 'Nules' orchard at Frankenhof recovered from further die-back in all the treatments where the whole lesion was exposed after scratching the outer regions of the lesions (Tables 4.4.7.1 and 4.4.7.2). No callus formation took place in any of the treatments. In certain cases, the bark peeled from the trunk created ideal protection for snails such as the brown snail [*Helix aspersa* (Müller)] and the tower snail (Fig. 4.4.7.2). On Acrobat-treated trees where the lesions were not surface scratched, all lesions developed further showing typical lesion formations such as excessive gumming and loosening of the bark (Fig. 4.4.7.3). Trees in the same row that received the same algicide treatment but were not surface scratched with a chisel did not result in any callus formation showing that Acrobat is not an effective treatment. The opposite was observed for the Captan (Merpan) + Sporekill treatment. The surface scratching on the outer edge of the lesion in fact controlled the disease (Fig 4.4.7.4), which confirmed anecdotal reports of this control measure in Spain (Antonio Vicent, personal communication). 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 type of trees were excluded from our initial tree selection before treatments commenced.

## Conclusion

From our field observations, Acrobat at the rate prescribed by BASF was not an effective treatment against *Phytophthora citrophthora* branch and trunk cankers. This might be attributed to the low rate of dimethomorph

(9%) in the formulation of Acrobat (69%). It will be retested in glasshouse trials to confirm these findings (Exp 888).

Captan in tank mixtures with Sporekill again gave effective control, but not on branches within the canopy that were not protected following hand lance spraying. Captan+Sporekill treated trees showed callus formation, indicating that this is an effective treatment. It is critical that spray operators thoroughly apply the algicides to the trunks and the lower funnel shaped branches to limit further spreading of the disease up into the main branches (Fig. 4.4.7.5).

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 tower snails were identified in Clementine mandarin trees and their role as possible vectors will be studied in future research.

### Technology transfer

Talks at study groups and results will be presented on the bi-annual CRI Symposium in August 2008.

### 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 4.4.7.1.** Tree ratings before (21 May 2007) and after (6 February 2008) the trunk application of Acrobat treatments.

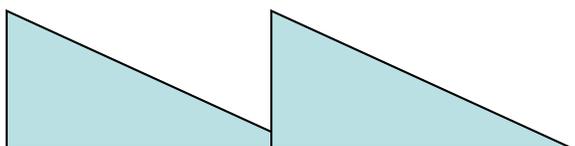
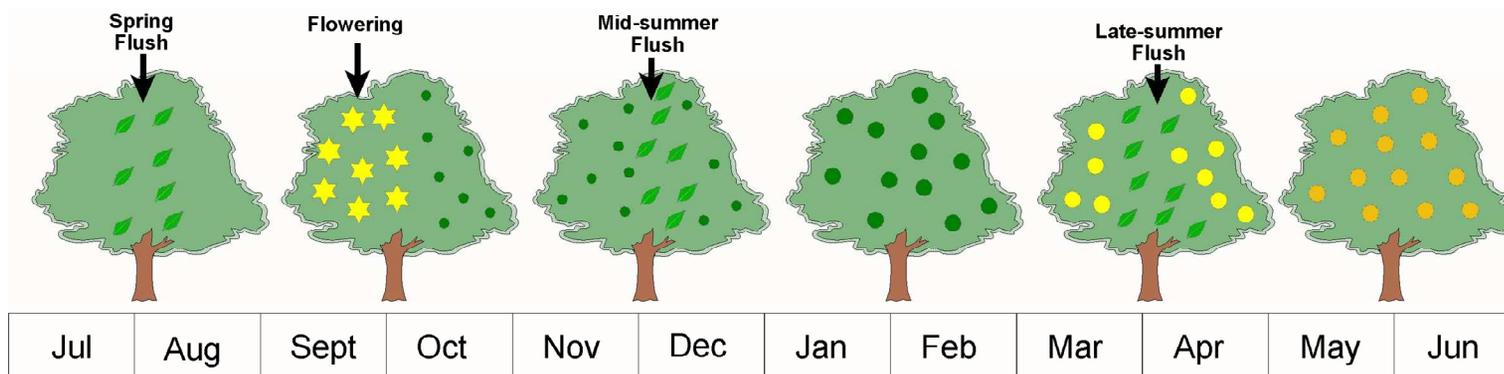
Tree number	Trunk rating*		Tree canopy rating*		Branch rating*	
	21 May 2007	6 February 2008	21 May 2007	6 February 2008	21 May 2007	6 February 2008
1	80	80	10	10	60	50
2	80	80	10	10	60	50
3	80	70	10	10	70	70
4	90	90	10	10	90	90
5	40	40	10	9	30	30
6	100	100	9	9	60	20
7	70	70	9	9	60	60
8	100	90	10	9	80	50
9	40	40	7	7	40	40
10	90	90	10	10	100	100

\*'Nules' tree trunks and branches affected by *Phytophthora citrophthora* was rated on a scale from 0 – 10 (where 10 = healthy and 0 = dead). If the tree trunk was girdled by the disease, a % scale (where 100% = healthy and 0% = totally infected) based on the lesion circumference was used.

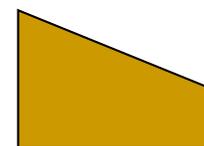
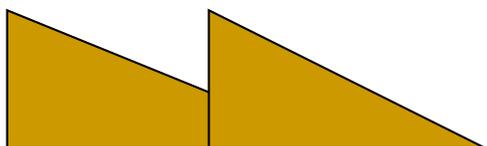
**Table 4.4.7.2.** Tree ratings before (21 May 2007) and after (6 February 2008) the trunk application of Captan (Merpan) plus Sporekill treatments.

Tree number	Trunk rating*		Tree canopy rating*		Branch rating*	
	21 May 2007	6 February 2008	21 May 2007	6 February 2008	21 May 2007	6 February 2008
1	50	50	10	10	40	40
2	20	20	10	10	60	60
3	90	90	10	10	70	70
4	90	90	10	10	80	50
5	100	100	10	10	90	90
6	60	60	10	10	40	40
7	40	40	8	8	40	40
8	100	100	10	10	40	40
9	60	60	10	10	10	10
10	70	70	9	9	40	40

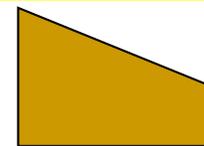
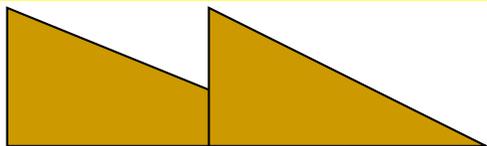
\*'Nules' tree trunks and branches affected by *Phytophthora citrophthora* was rated on a scale from 0 – 10 (where 10 = healthy and 0 = dead). If the tree trunk was girdled by the disease, a % scale (where 100% = healthy and 0% = totally infected) based on the lesion circumference was used.



**Foliar application: Fighter (570 ml/hl water)**



**Trunk application: Captan + Sporekill (200 g + 100 ml/hl water)**

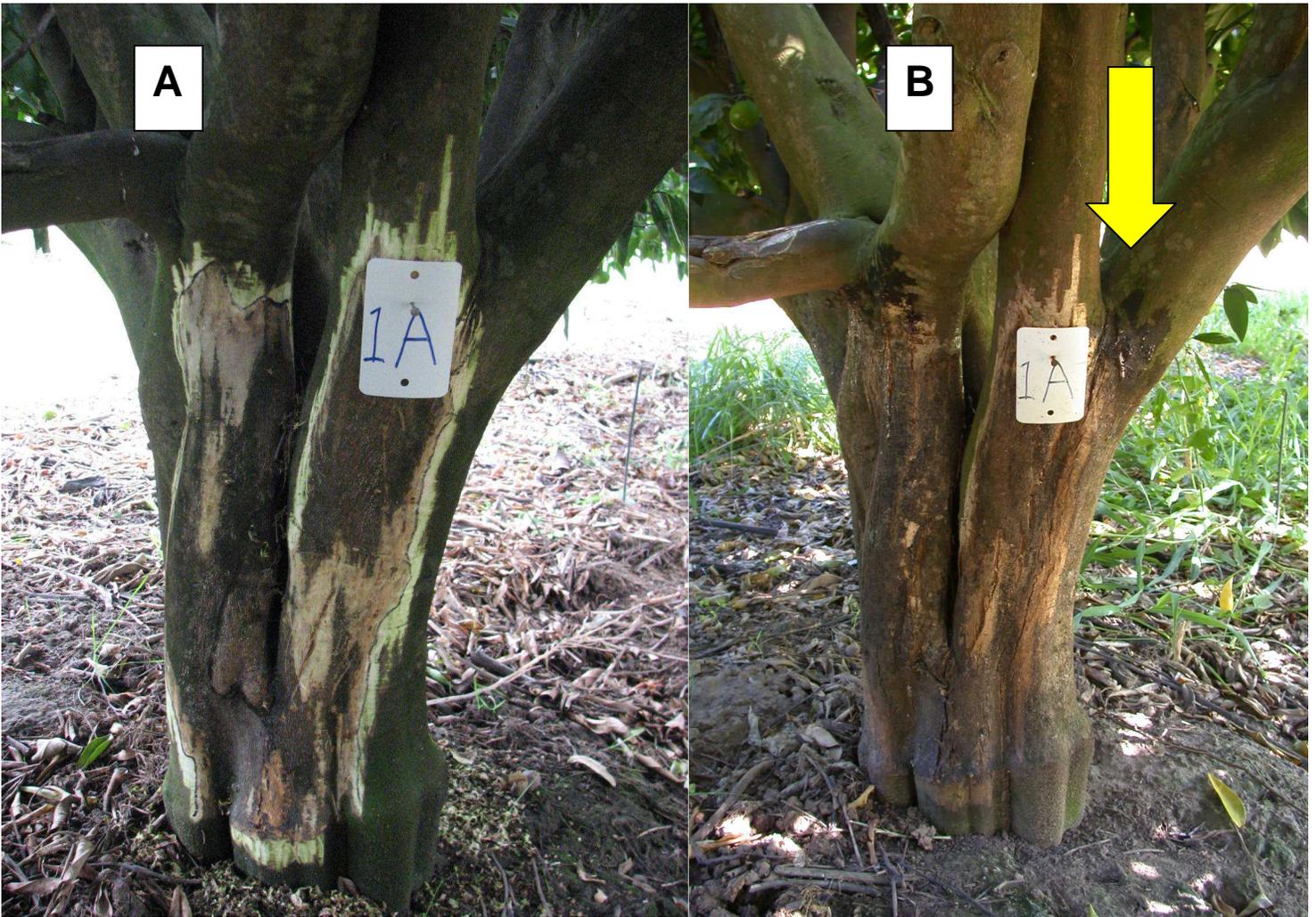


**Trunk application: Acrobat (120 g/hl water)**

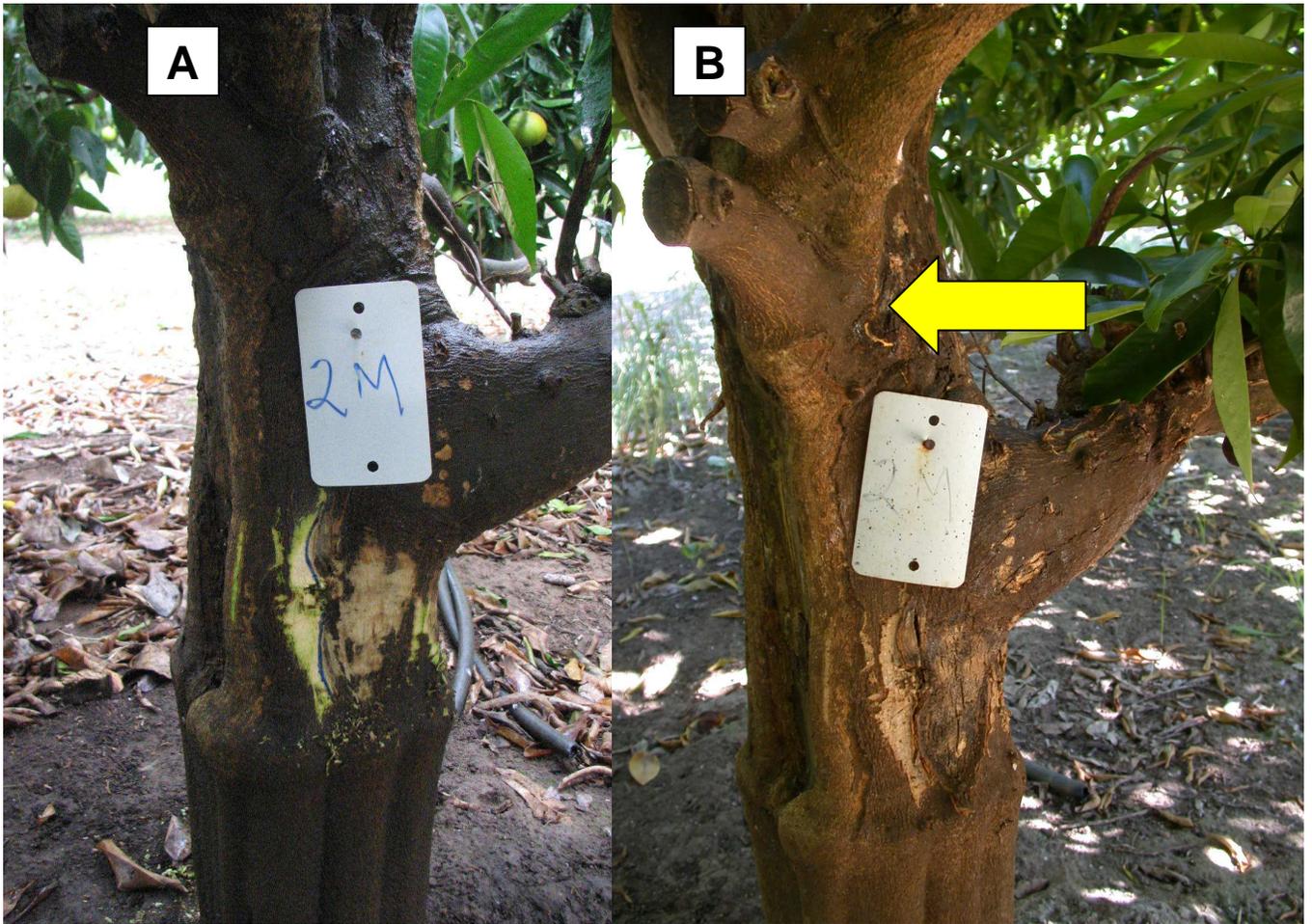
Fig. 4.4.7.1. Spray programmes evaluated on 'Nules' trees for the control of *Phytophthora citrophthora* during 2007-2008.



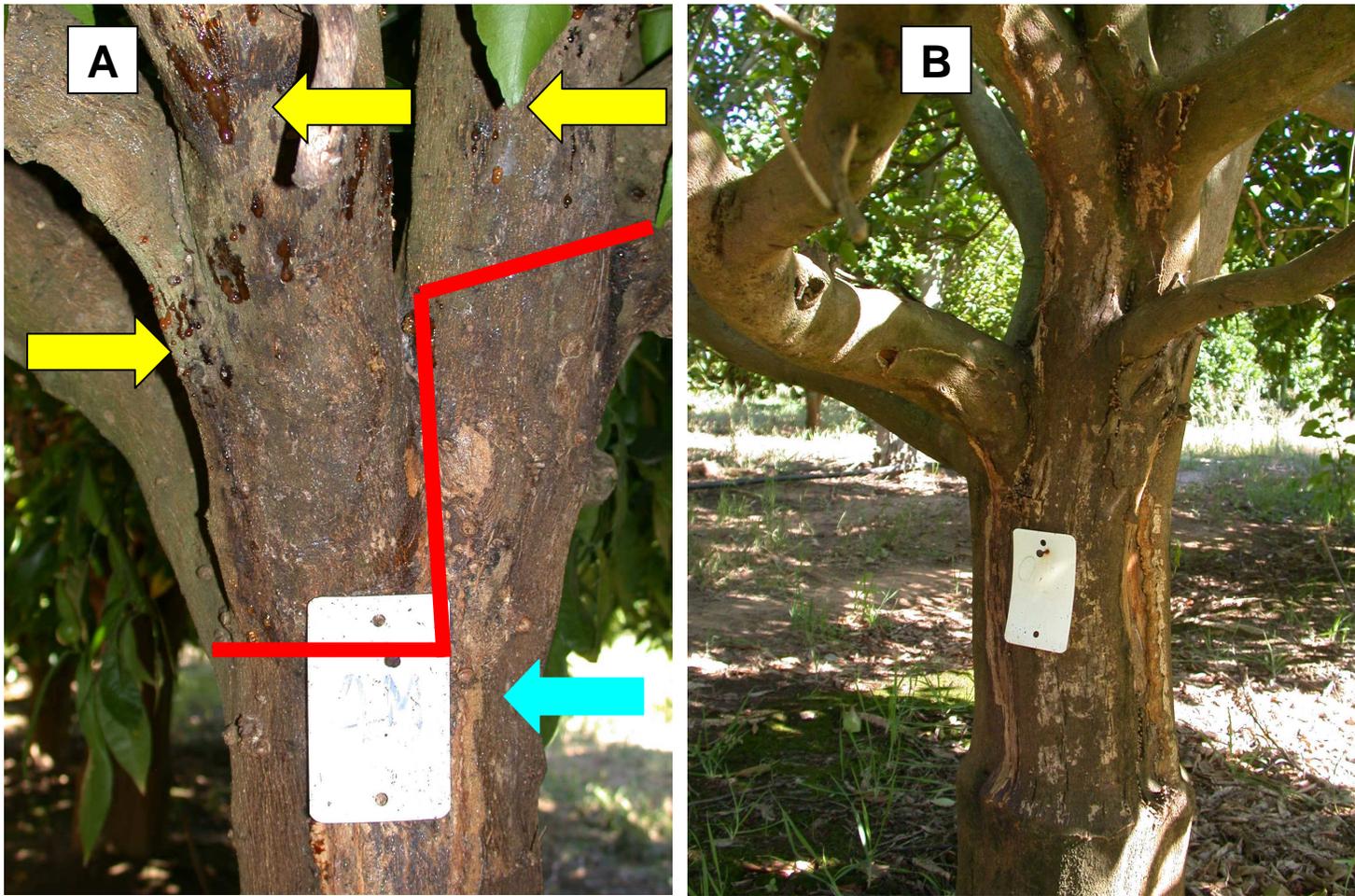
Fig. 4.4.7.2. Tower snails hiding under bark fragments on the trunk of 'Nules' Clementine mandarin.



**Fig. 4.4.7.3.** Lesion exposure with a chisel (21 May 2007) before the trunk treatment with Acrobat (120 g/hl water)(A) and the same tree on 6 February 2008 showing no callus formations. New lesions develop beyond the scratched area on the branches (Arrows) (B).



**Fig. 4.4.7.4.** Lesion exposure with a chisel (21 May 2007) before the trunk treatment with Captan plus Sporekill (100 ml + 100 ml/h<sub>l</sub> water) (A) and the same tree on 6 February 2008 showing no callus formations on the scratched areas. Callus formation did occur on areas at the top of the unexposed areas showing that Captan plus Sporekill is an effective treatment (B).



**Fig. 4.4.7.5.** Callus formation on the trunk of 'Nules' Clementine mandarin after Captan + Sporekill tree trunk spray applications (blue arrow) and further development of *P. citrophthora* on the branches as a result of poor trunk spray application (yellow arrows)(A). Callus formation on the trunk and branches of 'Nules' Clementine mandarin of the same treatment as a result of good spray coverage (B).

4.4.8 **UPDATE: Susceptibility of citrus cultivars to *Phytophthora citrophthora***  
by G.C. Schutte (CRI)

**Opsomming**

Clementine kultivars soos 'Marisol', 'Clemlate', 'Oroval' en 'Oroblanco' is almal al positief getoets vir vatbaarheid vir *Phytophthora citrophthora*. 'n Nuwe ingevoerde Clementine kultivar van Italië, nl. 'Tardino' was ook vatbaar vir *P. Citrophthora* getoets. Die siekte is ook op suurlemoene in Tucuman, Argentinië geïdentifiseer.

**Summary**

Clementine cultivars such as 'Marisol', 'Clemlate', 'Oroval' and 'Oroblanco' all tested positively for susceptibility to *Phytophthora citrophthora*. A new imported Clementine cultivar, 'Tardino' from Italy was also susceptible for *P. citrophthora*. The disease was also identified on lemons in Tucuman, Argentina.

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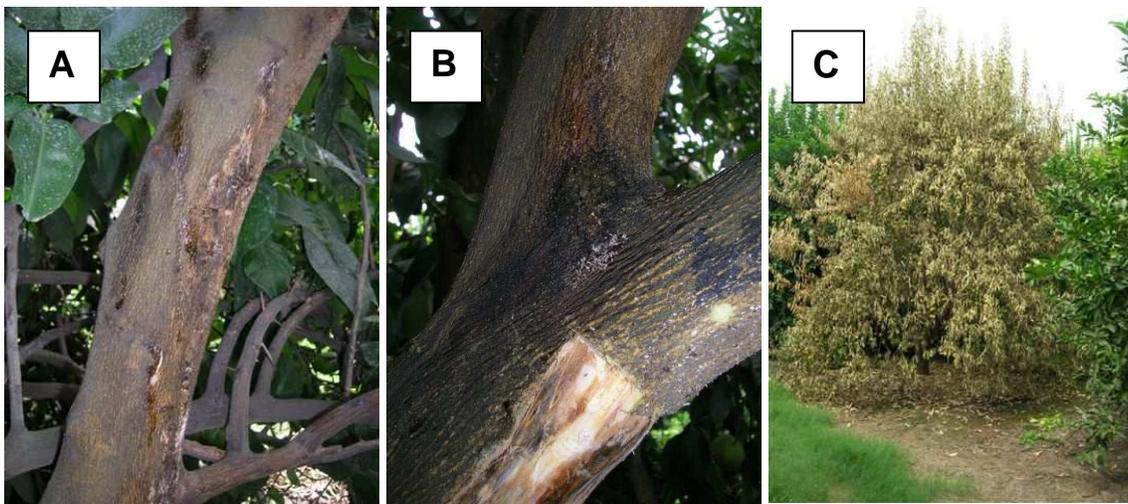
**Table 4.4.81.** World wide distribution of citrus hosts susceptible to *Phytophthora citrophthora* trunk and branch canker.

Host	Cultivar	Country	Reference
<i>Citrus limon</i>	Lemon	USA New Zealand Argentina Australia Italy France Corsica	Fawcett, 1936 Matheron & Matjeka, 1988 Cockayne & Cunningham, 1921 Fawcett, 1915; Schutte 2008 Darnell-Smith, 1918 Traverso, 1921 Cohen, 2003 Cohen, 2003
<i>Citrus paradisi</i>	Grapefruit	South Africa USA Congo Puerto Rico India Australia New Zealand Israel Corsica	Wager, 1931 Fawcett, 1915 Staner, 1929 Guiscafre-Arrillaga, 1931 Sharangapani, 1930 Fraser, 1942 Brien, 1946 Schiffman-Nadel & Cohen, 1968 Cohen, 2003
<i>Citrus reticulata</i>	Clementine: Nules Oroval Clemenpons  Clemlate Oroblanco Tardivo	South Africa South Africa & Spain Corsica, South Africa & Spain South Africa & Spain South Africa South Africa	Schutte, 2007; Alvarez, 2008 Alvarez, 2006 Cohen, 2003 Schutte, 2007; Alvarez, 2008 Schutte, 2007; Alvarez, 2008 Schutte, 2008
<i>Citrus reticulata</i> hybrids	Mandarins: Nova Fortune Orlando tangelo	Spain Spain USA	Alvarez, 2006 Alvarez, 2008 Matheron & Matjeka, 1988
<i>Citrus sinensis</i>	Sweet oranges: Salustiana	Spain USA Taiwan South Africa Congo Philippines Australia Argentina New Zealand Morocco	Alvarez, 2006 Fawcett, 1915 Sawada, 1919 Doidge, 1925 Staner, 1929 Anon. 1938 Fraser, 1942 Frezzi, 1950 Fletcher, 1957 Cohen, 2003
<i>Citrus</i> spp.	Citrus	Brazil Sardinia Spain Sicily Egypt Puerto Rico Tunisia Mozambique	Averna-Sacca, 1912 Petri, 1925 Fawcett, 1930 Fawcett, 1930 Fawcett, 1930 Guiscafre, 1932 Chabrolin, 1932 Cardoso, 1934

		Australia Argentina South Africa Mauritius	McLennan, 1936 Frezzi, 1940 Loest, 1950 Orian, 1951
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**Fig. 4.4.8.1.** Die-back of the Clementine mandarin, 'Tardivo' at the CFB, due to *Phytophthora citrophthora* trunk and branch canker.



**Fig. 4.4.8.2.** Gum exudation (A), trunk infection (B) and eventual die-back of lemons trees in Tucuman, Argentina due to *Phytophthora citrophthora* trunk and branch canker.

#### 4.4.9 **PROGRESS REPORT: Determine the efficacy of phosphonates applied through the irrigation system on citrus for control of *Phytophthora* root rot**

Experiment QMS 07 / WvdP 2 (August 2007 – September 2008): by W. van de Pypekamp and S.H. Swart (QMS Agri Science)

##### **Opsomming**

Die toediening van fosfonate as 'n blaarbespuiting of stam-verf is problematies as gevolg van die potensiele fitotoksiese risiko 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 toedieningsmetode se tekortkoming kan oorkom. Die effektiwiteit van die verskillende toedieningsmetodes, die effek van boomouderdom, besproeiingsstelsel en grondtipe rakende die toediening van kalium-fosfonaat deur die besproeiingstelsel is ook bepaal. Die proef is uitgevoer in die Letsitele-area. Resultate verkry gedurende die 2de seisoen (2006 / 2007) is in die verslag saamgevat, gevolgtrekkings of besprekings sal eers gemaak word indien finale resultate beskikbaar is in Augustus / September 2008. Voorlopige resultate het nie duidelik die effek van kalium-fosfonaat as grondtoediening op vruggrootte en opbrengs getoon nie. Residu analyses het gelyke kalium-fosfonaatvlakke in die wortels vir beide konvensionele blaarbespuiting en grondtoediening getoon.

##### **Summary**

The application of phosphonates as a foliar spray or trunk paint is problematic due to the potential phytotoxicity risk involved 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. Trials to determine the effect of tree age, irrigation type, soils types, and type of application (foliar, trunk or irrigation) were conducted in the Letsitele area. Results obtained during the 2<sup>nd</sup> growth season (2006/2007) are included in this report. However, no conclusions can be made, pending final results that will be available in August / September 2008. Preliminary results did not clearly indicate the effect of applying potassium phosphonates through the irrigation systems on fruit size and production. Residue analyses showed that potassium phosphonate levels in the roots for conventional foliar application were similar compared to levels obtained for potassium phosphonates applied through the irrigation system.

##### **Introduction**

Production cost, 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 phytotoxicity risk on fruit and the cost and labour intensiveness of applying enough trunk paints to be effective. Anecdotal evidence suggests that the application of potassium phosphonate through the irrigation system on commercial scale seems to be very effective for the control of *Phytophthora* root and collar rot. Applying phosphonate products through the irrigation system reduced the cost component of labour and also greatly reduced dosages for some tree age groups compared to current registered application methods.

The aims of this project were to compare the efficacy, and determine optimal dosages when phosphonates were applied through different types of irrigation systems (drip vs. micro) compared with foliar and trunk applications and to determine the effect of soil texture and different dosages when phosphonates are applied through the irrigation system.

##### **Materials and methods**

###### The effect of tree age, dosages and type of irrigation system

Trials were conducted in the Letsitele area, Limpopo Province, on trees under drip and micro irrigation systems. Table 4.4.9.1 show specific potassium phosphonate (Phytex 200 SL) dosages used on different tree age groups and for different types of irrigation systems (micro and drip). Three different phosphonate rates, ½x, x and 2x were evaluated. Tree age groups included in the trial were, 0 - 2 years (micro and drip irrigation), 3 - 6 years (micro and drip irrigation), 7 - 10 years (micro irrigation only) and > 15 years (micro and drip irrigation). Each treatment consisted of 3-tree plots replicated 10 times in a randomised block design.

**Table 4.4.9.1.** Treatments applied to determine effects of tree age, irrigation types and dosages of potassium phosphonates applied through the irrigation system.

Orchard number	Tree age group	Irrigation type	Treatment number	Dosage active ingredient mL / per tree*
RS-22	0-2	Micro	1/2 x	10.5
			x	21
			2x	42
			Control	-
RS-11, E-7 E-10	3-6	Micro	1/2 x	21
			x	42
			2x	84
			Control	-
D-18	7-10	Micro	1/2 x	35.5
			x	71
			2x	142
			Control	-
RS-1	>15	Micro	1/2 x	35.5
			x	71
			2x	142
			Control	-
RS-21	0-2	Drip	1/2 x	10.5
			x	21
			2x	42
			Control	-
J-40	3-6	Drip	1/2 x	21
			x	42
			2x	84
			Control	-
G-1.11	>15	Drip	1/2 x	21
			x	42
			2x	84
			Control	-

\*The first application started in October 2005, with 3 applications per season (between September and March), except for age group > 15 years, which received an extra application per season.

The effect of soil texture (sand vs. clay) and dosage

In the second trial, the effect of soil type, clay (orchard E-10) and sandy soils (orchard E-7) and phosphonate dosages applied, was determined. The trial was carried out in the Letsitele area on two similar Valencia orchards (age group 7 – 10 years, cultivar, tree spacing, irrigation system and proximity), with the only observed difference being soil type. Treatments at 17, 34 and 70 l/ha (1/2 x, x and 2 x) were compared with an untreated control (Table 4.4.9.1). The first application started in October 2005, with 3 applications per season (between September and March), except for age group >15 years, which received an extra application per season.

The effect of application methods on efficacy

Potassium phosphonate (Phosguard 400 SL) was applied as foliar, trunk or soil applications in an old Valencia orchard showing signs of decline (age group >15 years). The effect of different applications methods of potassium phosphonate was also studied on trees in the age group 7-10 years. The first application started in October 2005, with 3 applications per season (between September and March) except for age group >15 years, which received an extra application per season. Dosages applied are depicted in Table 4.4.9.2. The dosages for trunk and foliar application are as per product registration label and dosages for irrigation application as per Tables 4.4.9.1 and 4.4.9.2.

**Table 4.4.9.2.** Treatments applied in the trial to determine the efficacy of different application methods of potassium phosphonates.

Treatment number	Treatment Description	Application method	Dosage	Volume applied per tree
1	Phosguard 400 SL FL	Foliar application	500 ml / 100 l	3.8 l
2	Phosguard 400 SL SP	Trunk paint application	1:1 diluted	Paint trunk from ground to graft joint, approx. 250 ml
3	Phosguard 400 SL IR	Irrigation application	350 ml / 100 l	10 l
4	Untreated control	-	-	-

#### Data collection

The effect of treatments on production was determined by harvesting 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 fruit from each replicate. A representative sub-sample was taken, consisting of approximately 20 kg fruit for each replicate, pooled, and sized with a commercial “rope-and-roller” sizer to determine size distribution. Residue levels were determined by taking samples approximately 3 weeks after the last 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). Root samples for the final season (2007 / 2008) will be taken 3 weeks after the last phosphonates applications (March 2008) and sent to SABS for residue analyses. The final harvesting of fruits will be conducted in September 2008.

#### Results and discussion (2006 / 2007 seasons' preliminary results)

Results of residue analyses in roots, conducted to determine the effects of tree age, irrigation system and soil types for different application methods of potassium phosphonates to control *Phytophthora* root and collar rot, are depicted in Table 4.4.9.3. Residue analyses regarding the effect of different application methods of potassium phosphonates in controlling *Phytophthora* root and collar rot are depicted in Table 4.4.9.4. The average yield for all orchards in this trial, as determined in August 2007, is depicted in Table 4.4.9.5. Orchard LQ-21 was mistakenly harvested by orchard manager before samples could be taken, thus no production values are available for this orchard. Fruit size distribution of all orchards as determined in August 2007 is depicted in Figs. 4.4.9.1 to 8. Size distribution for each treatment is depicted as the weight of fruit in each size category, based on the percentage fruit in each size range as determined for the sub-sample.

**Table 4.4.9.3.** Residue analyses in roots 3 weeks after final application of potassium phosphonates through the irrigation (2006 / 2007 season).

Number	Orchard number	Tree age group	Treatment number	Sample Type	Type of irrigation	Soil type	Phosphonate Levels (mg / kg)
1	RS-21	0-2	2. x	Roots	Drip	Sandy	4.3
2	RS-22	0-2	2. x	Roots	Micro	Sandy	45
3	J-40	3-6	2. x	Roots	Drip	Sandy	36
4	RS-11	3-6	2. x	Roots	Micro	Sandy	6.6
5	D-18	7-10	2. x	Roots	Micro	Sandy	17
6	G-1.11	>15	2. x	Roots	Drip	Sandy	17
7	RS-1	> 15	2. x	Roots	Micro	Sandy	91
8	E-7	7-10	2. x	Roots	Micro	Sandy	50
9	E-10	7-10	2. x	Roots	Micro	Clay	48
10	J-31	-	Control	Roots	Micro	Sandy	No detection

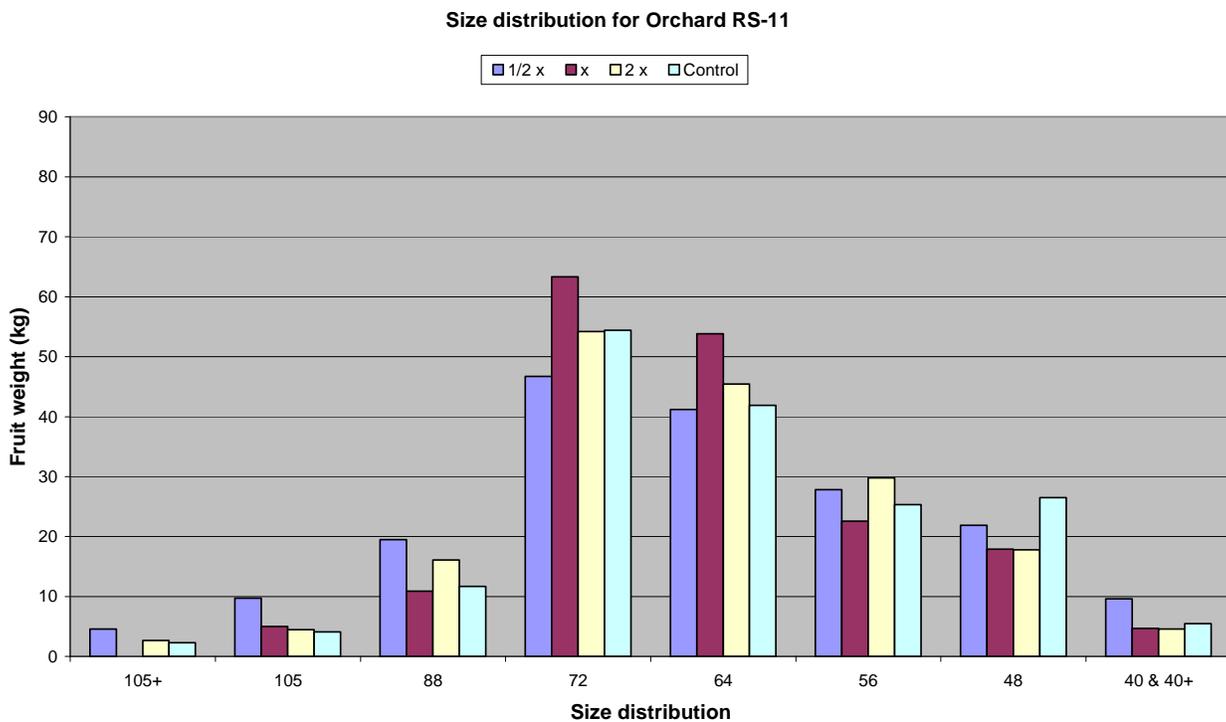
**Table 4.4.9.4.** Residue levels in roots and fruit, 3 weeks after final application of potassium phosphonates after sampling in May / June 2007.

#	Orchard number	Tree age group	Treatment type	Sample type	Type of irrigation	Soil type	Application method	Phosphonate Levels (mg / kg)
1	LQ-28	7-10	Foliar	Roots	Micro	Sandy	Foliar	46
2	LQ-28	7-10	Trunk paint	Roots	Micro	Sandy	Trunk paint	41
3	LQ-28	7-10	Irrigation	Roots	Micro	Sandy	Irrigation	6.7
4	LQ-21	> 15	Foliar	Roots	Micro	Sandy	Foliar	70
5	LQ-21	> 15	Trunk paint	Roots	Micro	Sandy	Trunk paint	28
6	LQ-21	> 15	Irrigation	Roots	Micro	Sandy	Irrigation	52
7	J-31	-	Control	Roots	Micro	Sandy	-	ND
9	LQ-28	7-10	Foliar	Fruit	Micro	Sandy	Foliar	36
10	LQ-28	7-10	Trunk paint	Fruit	Micro	Sandy	Trunk paint	4.4
11	LQ-28	7-10	Irrigation	Fruit	Micro	Sandy	Irrigation	2.1
12	LQ-28	7-10	Control	Fruit	Micro	Sandy	-	ND
13	LQ-21	> 15	Foliar	Fruit	Micro	Sandy	Foliar	70
14	LQ-21	> 15	Trunk paint	Fruit	Micro	Sandy	Trunk paint	6.7
15	LQ-21	> 15	Irrigation	Fruit	Micro	Sandy	Irrigation	ND

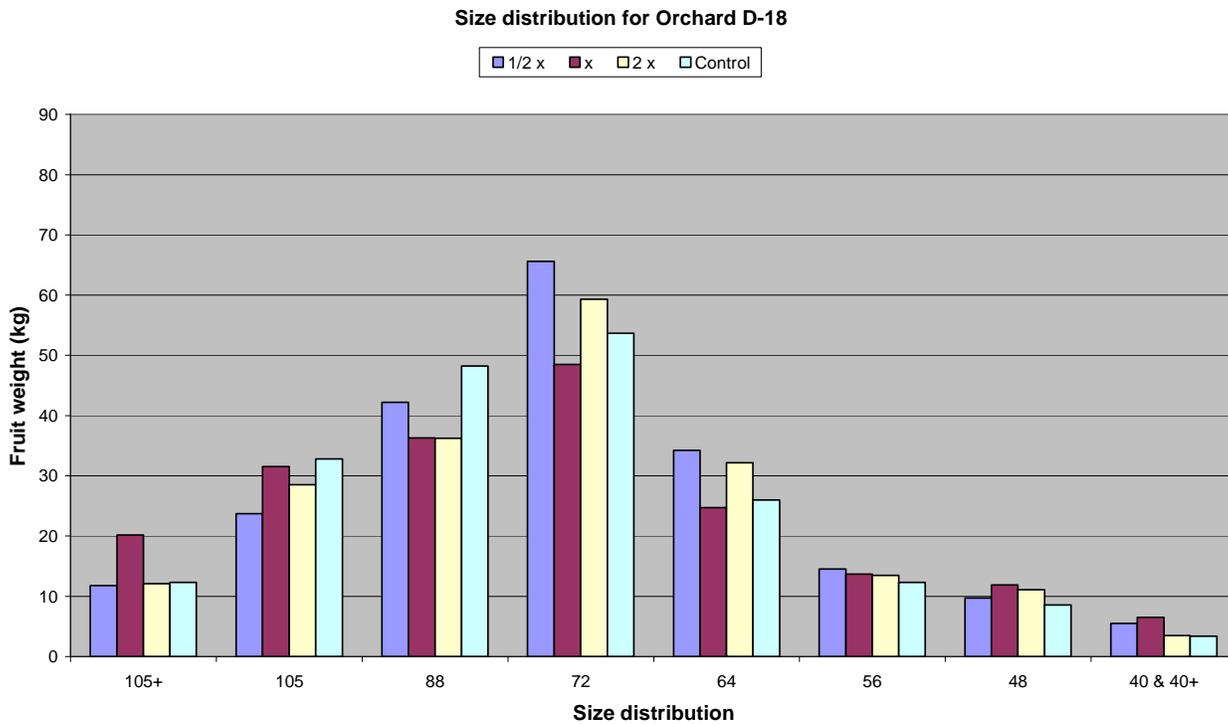
**Table 4.4.9.5.** The average yield (kg fruit) per treatment of all producing orchards as determined in August 2007.

Orchard number	Tree age group	Irrigation type	Treatment number	Average production value (kg fruit)
RS-11	3-6	Micro	½ x	58.4
			x	61.5
			2x	64.4
			Control	56.8
D-18	7-10	Micro	½ x	95.7
			x	95.5
			2x	103
			Control	89.2
RS-1	>15	Micro	½ x	146.3
			x	144.1
			2x	134.5
			Control	141.2
E-7	7-10	Micro	½ x	113.3
			x	132
			2x	122.5
			Control	116.6
E-10	7-10	Micro	½ x	138

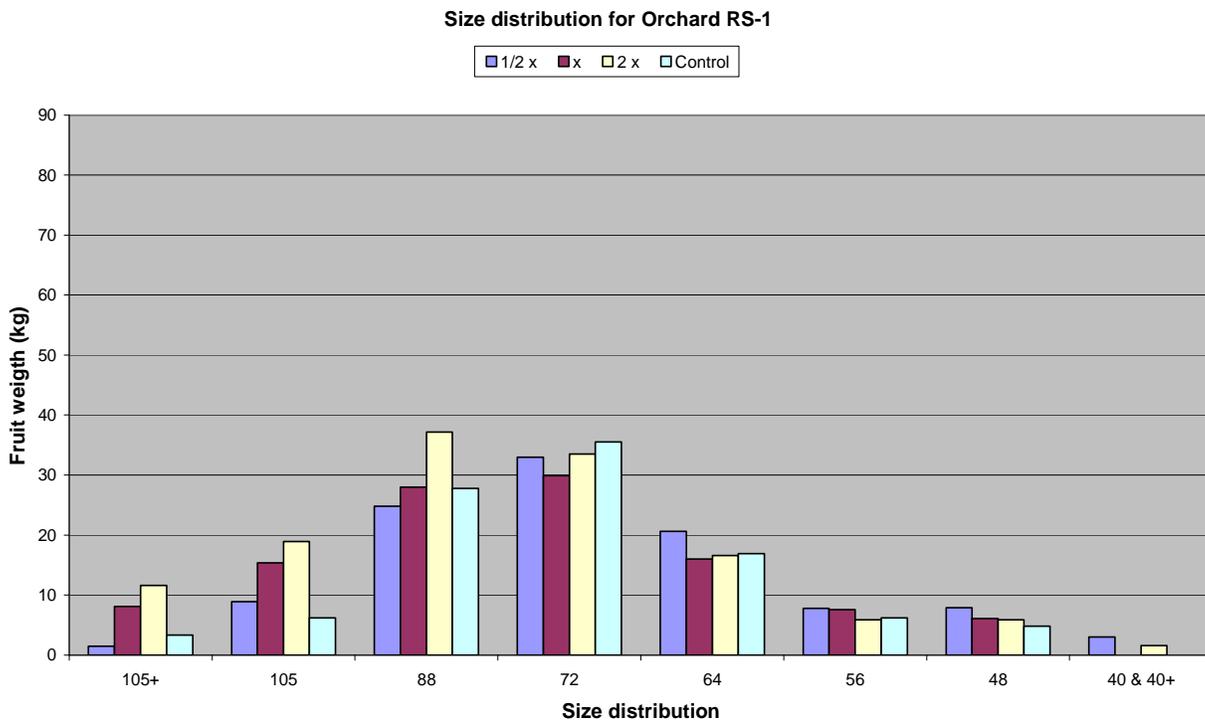
			x	141
			2x	134.3
LQ-28	7-10	Micro	Control	152.6
			Foliar	86.3
			Trunk paint	97.5
			Irrigation	105.4
J-40	3-6	Drip	Control	102.8
			½ x	88.1
			x	82.5
			2x	77.5
G-1.11	>15	Drip	Control	73.1
			½ x	262.7
			x	277.3
			2x	260.7
			Control	244.9



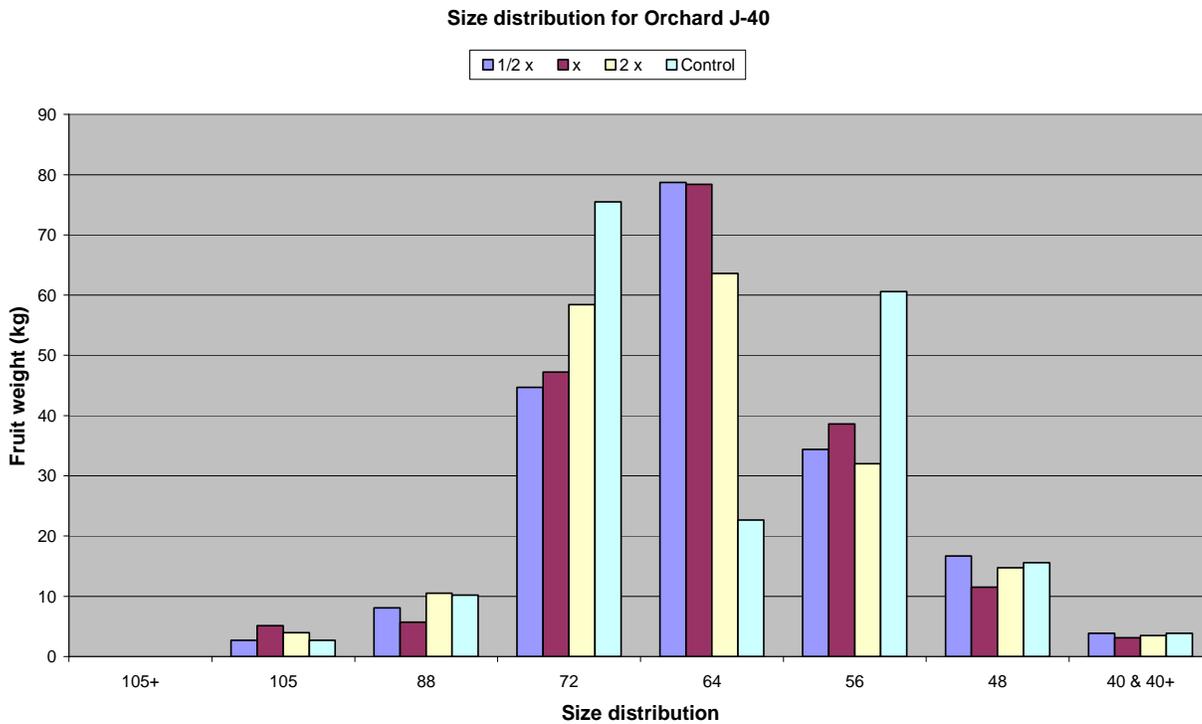
**Fig 4.4.9.1.** Size distribution for orchard RS-11, treated with potassium phosphonates at different dosages (applied through a micro irrigation system).



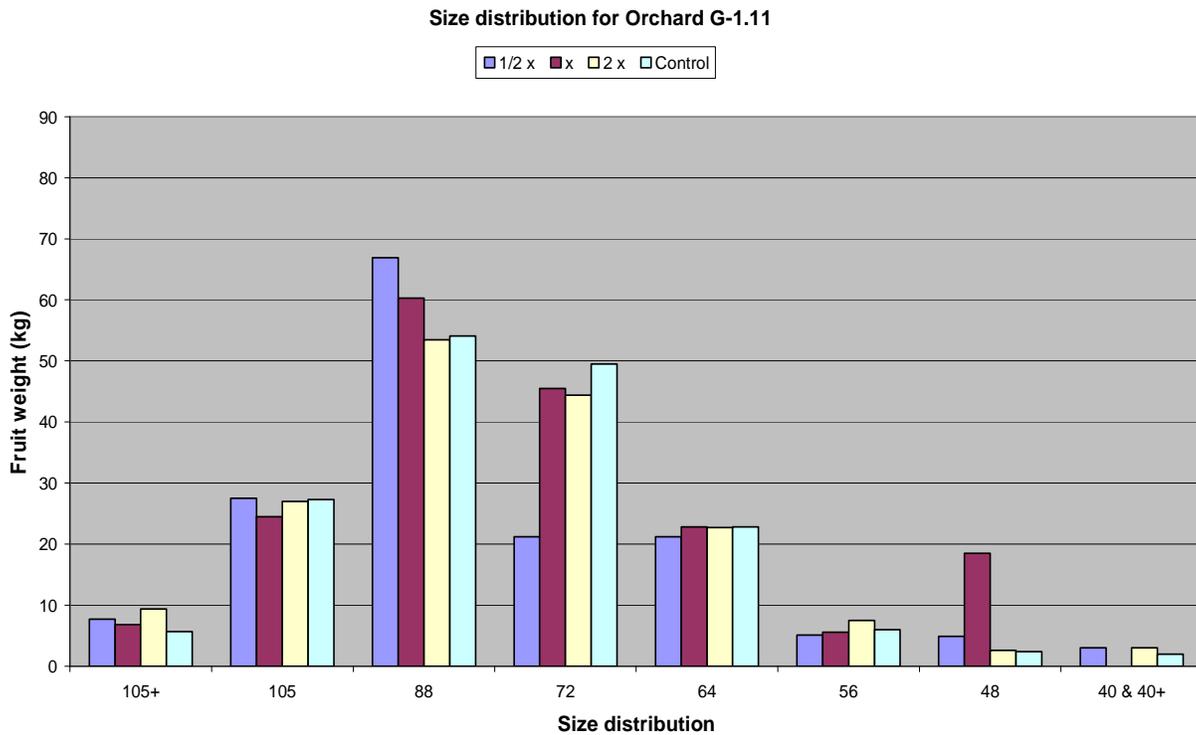
**Fig. 4.4.9.2.** Size distribution for orchard D-18, treated with potassium phosphonates at different dosages (applied through a micro irrigation system).



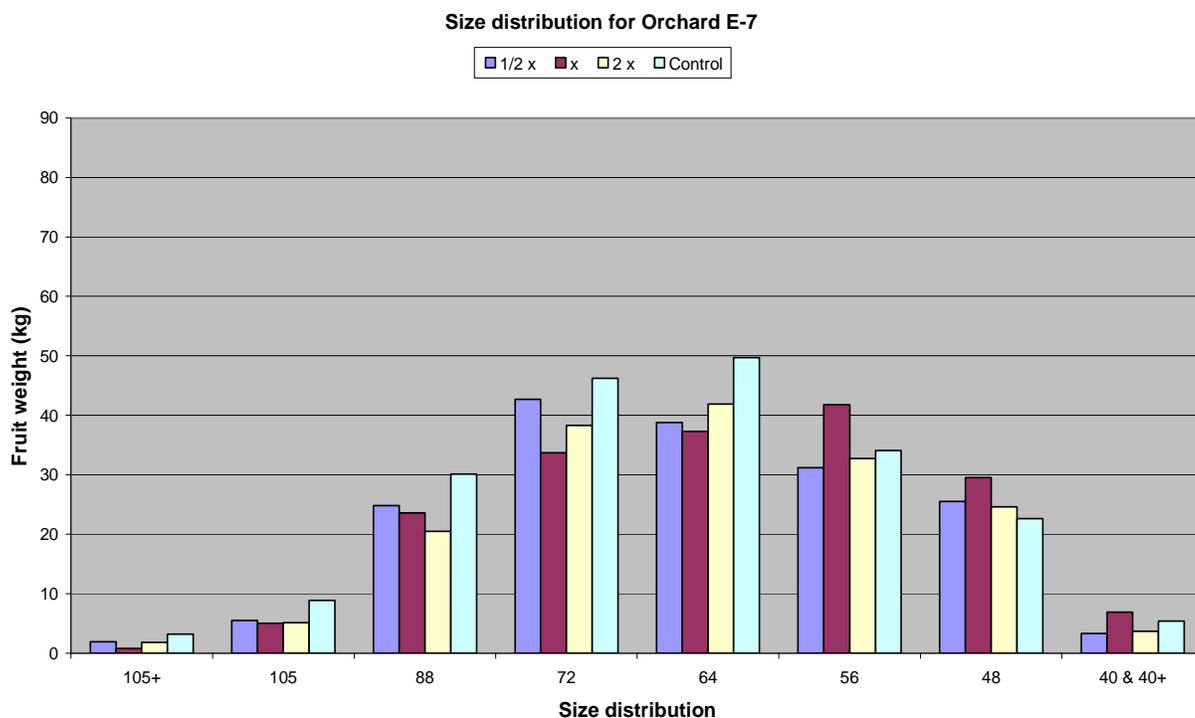
**Fig 4.4.9.3.** Size distribution for orchard RS-1, treated with potassium phosphonates at different dosages (applied through a micro irrigation system).



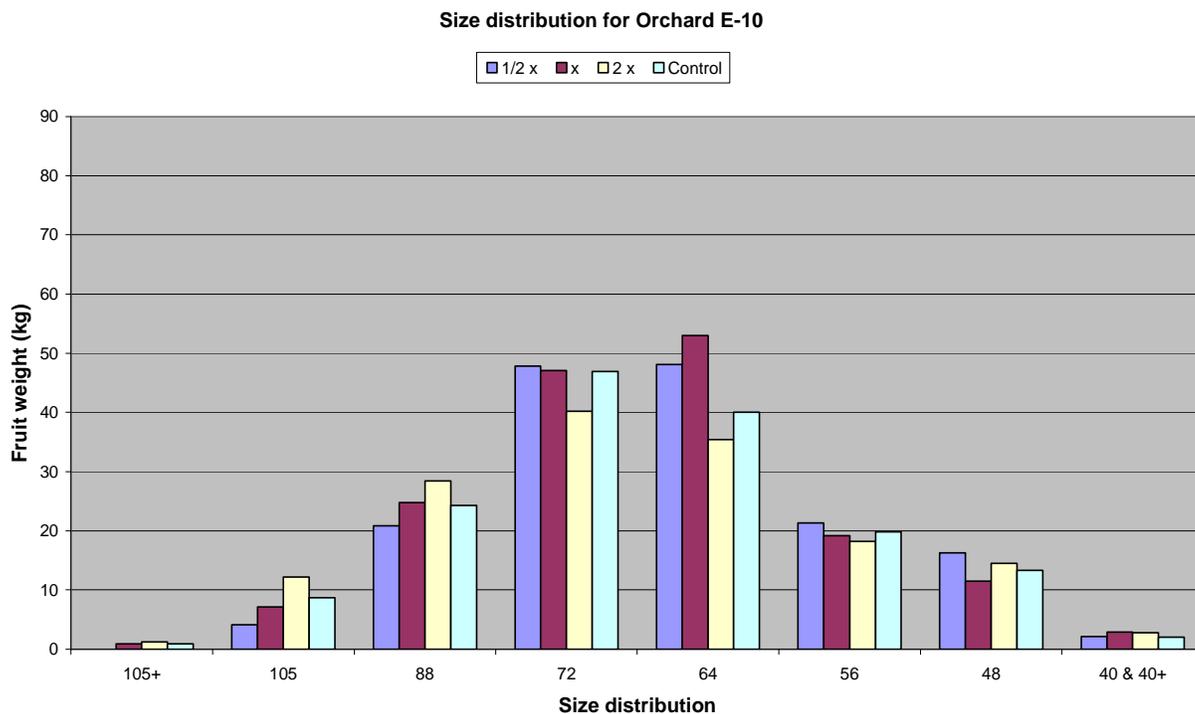
**Fig 4.4.9.4.** Size distribution for orchard J-40, treated with potassium phosphonates at different dosages (applied through a drip irrigation system).



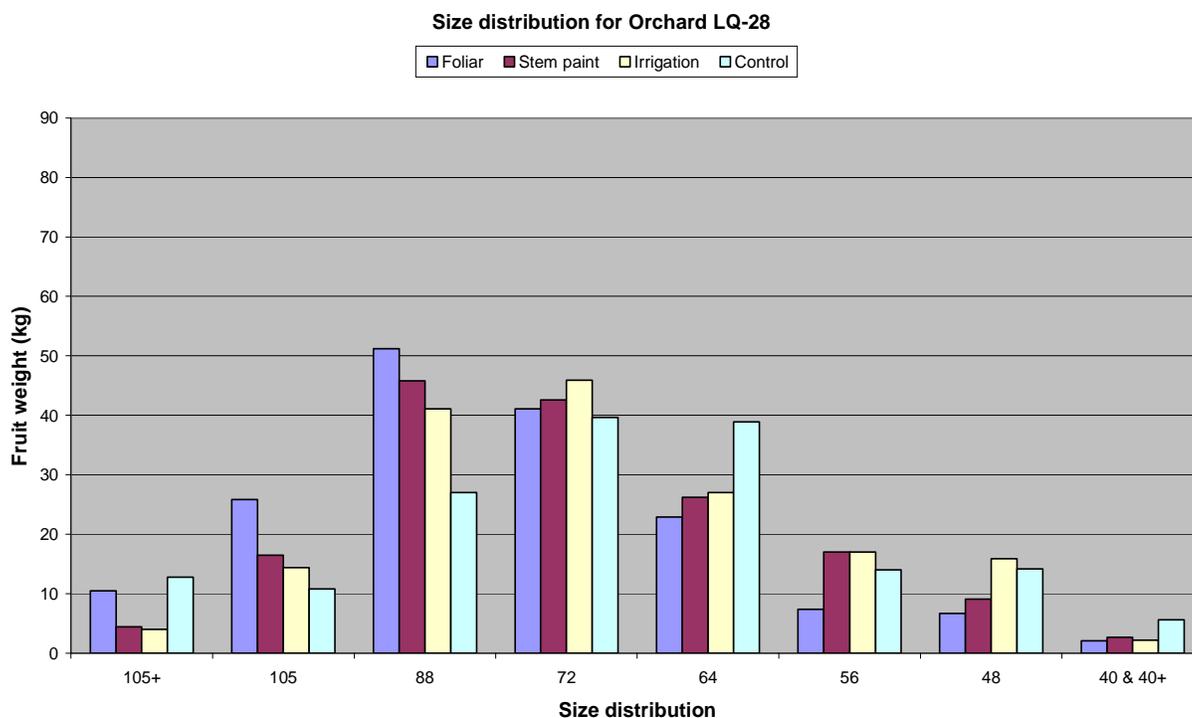
**Fig 4.4.9.5.** Size distribution for orchard G-1.11, treated with potassium phosphonates at different dosages (applied through a drip irrigation system).



**Fig 4.4.9.6.** Size distribution for orchard E-7, treated with potassium phosphonates at different dosages (applied through a micro irrigation system, sandy soils).



**Fig 4.4.9.7.** Size distribution for orchard E-10, treated with potassium phosphonates at different dosages (applied through a micro irrigation system, clay soils).



**Fig 4.4.9.8.** Size distribution for orchard LQ-28, treated with potassium phosphonates, applied as different application methods.

### Conclusion

Results compiled in this report are preliminary results, final discussions and conclusions can only be made once all the data of the 3 seasons have been evaluated and compared. However, results obtained thus far did not clearly indicate that the application of potassium phosphonates through the irrigation improved fruit size or production significantly compared to untreated controls. Residue samples indicated that potassium phosphonates applied through the irrigation system under certain conditions and tree age groups was detectable in the plant at levels similar to potassium phosphonates applied as foliar application (Table 4.4.9.3 and 4.4.9.4). Residue analyses showed that detectable potassium phosphonate levels was similar for sandy and clay soils, thus indicating that soils types possibly did not affect the uptake via the roots of potassium phosphonates by plants (Table 4.4.9.3). A final report will be compiled pending evaluations at harvest in September 2008.

### Further objectives (milestones) and work plan

Further objectives and future work will be included in final report.

### Technology transfer

Technology transfer will take place at biannual CRI symposium and presented at relevant study groups.

### References cited

Will be included in final report in September 2008.

#### 4.5 PROJECT: POST-HARVEST PATHOLOGY

Project coordinator: K.H. Lesar (CRI)

##### 4.5.1 Project summary

Philabuster (from Janssen Pharmaceutica) demonstrated good control of the citrus pathogen *P. digitatum* (**sensitive and resistant strains**) infection on navel and Valencia oranges and Star Ruby grapefruit in aqueous treatments, compared to the standard imazalil treatment. The inhibition of the same infections on Valencia oranges by Philabuster applied in the wax treatments was not as effective as the dip treatments. The *in vitro* screening of Philabuster against the latent pathogens, *Diplodia* stem-end rot and Anthracnose indicated that Philabuster was not as effective in inhibiting pathogen growth on PDA growth medium, compared to the standard recommended thiabendazole (4.5.2).

The GRAS chemicals, sodium carbonate and bicarbonate and lime sulphur, demonstrated good control of green mould and sour rot infections on lemons in hot water dip treatments, compared to the standard imazalil and guazatine. Effective synergistic activity between sodium carbonate and guazatine, at reduced rates, was observed against the control of the two pathogens on Valencia oranges (4.5.2).

Two formulations of the post-harvest fungicide ortho-phenylphenate (OPP), Ortocil and Cerexagri, demonstrated reasonable control of green mould (*P. digitatum*) and sour rot (*Geotrichum candidum*) infections on navel oranges compared to the standard imazalil and guazatine. A small degree of phytotoxicity was observed on sensitive (green) parts of the rinds. Good control of *P. digitatum* infection on Delta Valencias by a double application of imazalil (Fungazil) at 500 mg/kg in a dip treatment and at 2000 mg/kg in wax, and single applications of these two concentrations was observed. Residue levels of 3.2 and 3.4 mg/kg, below the MRL were recorded, and this indicates acceptable levels to ensure sporulation inhibition and better decay control (4.5.2).

Sporekill was applied at concentrations of 1ml/l and 2 ml/l in combination with reduced rates of imazalil (250 mg/kg) and guazatine (500 mg/kg), compared to the standard rates of the 500 and 1000 mg/kg. These combinations were applied in aqueous dip treatments for efficacy against infections by imazalil sensitive and resistant *P. digitatum* biotypes. Sporekill in combination with the reduced rates of imazalil and guazatine demonstrated good synergistic activity in the control of *P. digitatum* infections (4.5.2).

The screening of PGR's, Retain, Agromos, Bioboost, Croplife and Califix (2,4-D sodium salt) as possible alternatives to 2,4-D, was repeated on lemons. Good calyx retention by Retain at the higher concentration (500 ppm) was evident. Calyx retention at both rates of Califix was equivalent to both Deccomone (2,4-D sodium salt) rates. The lower and higher concentration of Retain and Bioboost respectively were the only other two treatments that demonstrated reasonable calyx retention (4.5.3).

The citrus export supply chain extends from local packhouses to international distribution centres. Preliminary studies by the University of Pretoria determined that postharvest contamination often occurs further down the export chain. This creates an optimal environment for the onset of postharvest decay and in particular postharvest decay caused by *Penicillium* spp. In this study various contamination points along the supply chain were sampled. Seventeen *Penicillium* spp. involved in the supply chains were identified. The presence of these species can serve as an indication of substandard hygiene conditions and can be a health and safety risk and serve as inoculum for the onset of postharvest diseases (4.5.4).

A bacterial antagonist, originally selected from isolates found on citrus fruit surfaces, has previously shown promising activity against postharvest fungal fruit pathogens. In this study, the isolate was identified as *Bacillus subtilis* and its inhibitory activity was confirmed on three postharvest citrus pathogens. Further research is planned on characterisation of the phenolic free acids and volatile compounds produced, as well as mutation studies and *in vivo* trials will also be conducted on citrus fruit (4.5.5).

Pathogen resistance development to postharvest fungicides used regularly in various industries has always been a threat. Two of the most commonly used fungicides in the citrus industry are imazalil and guazatine. Due to the overzealous use of these products, *Penicillium* populations found in the citrus industries of South Africa should be screened for any resistance to these fungicides. *Penicillium* isolates were isolated from various packhouses in the major citrus producing regions of South Africa (4.5.6).

## Projekopsomming

Philabuster (vanaf Janssen Pharmaceutica) het goeie beheer van die sitruspatogeen *P. digitatum* (sensitiewe en bestande rasse) besmetting op nawel en Valencia lemoene en Star Ruby pomelos in 'n doopbehandeling, in vergelyking met die standard imazalil behandeling getoon. Die inhibisie van dieselfde besmettings deur Philabuster in waks aanwending was nie ewe effektief nie. Die *in vitro* evaluering van Philabuster teen *Diplodia* stingelent vrot en Antraknose het gewys dat Philabuster nie dieselfde mate van inhibisie van die twee patogene op ADA groeimedium, in vergelyking met die standaard aanbevole tiabendasool. Die na-oes swamdoder ortofenielfenaat (OFF) (Ortocil en die Cerexagri formulasies) het redelike goeie beheer van *P. digitatum* en *Geotrichum candidum* op Nawel lemoene in vergelyking met die standaard aanbevole imazalil sulfaat en guazatine getoon. Met die behandelings is 'n klein mate van fitotoksisiteit op sensitiewe (groen) dele van die nawels waargeneem (4.5.2).

Goeie bederfbeheer van groenskimmel en suurvrot deur die GRAS chemikalieë natrium karbonaat, natrium bikarbonaat en kalkswawel in 'n warm water doopbehandeling, in vergelyking met die standard imazalil en guazatine, is waargeneem. Doeltreffende sinergistiese werking tussen natrium karbonaat saam met guazatine, teen laer konsentrasies, is teen infeksie deur die twee patogene op Valencias waargeneem. Sporekill is teen konsentrasies van 1ml/l en 2 ml/l saam met imazalil teen 250 mg/kg en guazatine teen 500 mg/kg, laer konsentrasies as die standaard 500 mg/kg imazalil en 1000 mg/kg guazatine, in doopbehandelings teen infeksie deur imazalil sensitiewe en bestande rasse van *P. digitatum* (groenskimmel) getoets. Sporekill saam met die twee swamdoders het 'n goeie sinergistiese werking teen *P. digitatum* besmetting getoon. Die spuit van die drie kwaternêre ammonium verbindings, Sporekill, Quattrokil en Desogerm, voor-oes vir die vermindering van swamspoorlading in die boord en op die vrugoppervlak, het 'n mate van werking op spoorlading gewys. Goeie beheer van *P. digitatum* infeksies op Delta Valencias met 'n dubbele aanwending van imazalil (Fungazil) teen 500 mg/kg in 'n doopbehandeling en 2000 mg/kg in waks waargeneem. Residuvlakke van 3.2 en 3.4 dpm, minder as die MRV, is gerapporteer. Hierdie dubbele aanwending van imazalil dui aan dat aanvaarbare residu vlakke van imazalil op vrugte wat groen- en blouskimmel sporulasie en bederf sal beheer (4.5.2).

Die evaluasie van plantgroeireguleerders, Retain, Agromos, Bioboost, die nuwe organiese middel Croplife en Califix (2,4-D natrium sout) is op suurlemoene, as maontlike plaasvervangers vir 2,4-D natrium sout, herhaal. Goeie blomkelk-behoud deur Retain teen die hoër konsentrasie (500 dpm) is waargeneem. Blomkelk-behoud deur Califix teen albei konsentrasies was soortgelyk aan Deccomone. Die laer konsentrasie van Retain en die hoogste konsentrasie van Bioboost is die enigste ander twee behandelings wat 'n redelike mate van blomkelk-behoud op die suurlemoene getoon het (4.5.3).

Die sitrus uitvoerketting strek vanaf plaaslike pakhuis tot by internasionale verspreidingspunte. Voorlopige studies deur die Universiteit van Pretoria het bepaal dat besmetting gereeld verder af in die uitvoerketting plaasvind. Dit skep ideale omstandighede vir na-oes bederf, veral deur *Penicillium* spp. Tydens hierdie studie is verskeie besmettingspunte in die uitvoerketting gemonster. Sewentien *Penicillium* spp wat swak higiëniese toestande aandui, is in die uitvoerketting geïdentifiseer en die teenwoordigheid van hierdie patogene, wat 'n gesondheid risiko mag wees, kan die inokulum vir na-oes siektes voorsien (4.5.4).

'n Bakteriese antagonis, oorspronklik geselekteer uit isolate wat op sitrus-oppervlakke voorgekom het, het vroeër belowende effekte teen die groei van na-oes patogene getoon. In hierdie studie is die isolaat geïdentifiseer as *Bacillus subtilis* en sy inhiberende aktiwiteit is teen drie na-oes sitrus patogene bevestig. Verdere navorsing word beplan vir die karakterisering van die fenoliese sure wat deur hierdie bakterie geproduseer word, asook mutasie-studies en *in vivo* proewe op sitrusvrugte (4.5.5).

*Penicillium* isolate is vanaf verskeie pakhuis in die belangrikste sitrus-produiserende areas van Suid Afrika geïsoleer. Hierdie isolate het deur 'n voorlopige toetsfase gegaan waartydens die dosis reaksie getoets is by laer en hoër limiete rondom die aanbevole dosis. Die *Penicillium* isolate met besonder hoë weerstandsvlakke is geïdentifiseer om die monstergrootte te verklein. Die ander *Penicillium* isolate sal verder vir weerstand leen laer swamdoder konsentrasies getoets word waarvolgens die EC50 waardes bepaal sal word. Tydens hierdie voorlopige studie is indikasies van hoër weerstand teen guazatine gevind, alhoewel hoë-vlak weerstand wel ook teen imazalil gevind is (4.5.6).

#### 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 nuwe na-oes swamdoder Philabuster (van Janssen Pharmaceutica), bestaande uit 'n mengsel van imazalil en pyrimethanil, het in vergelyking met die standaard Fungazil sulfaat 750 BP, goeie beheer teen infeksie deur die na-oes sitrus patoogeen *P. digitatum* (groenskimmel) getoon en is vir registrasie aanbeveel.
- *In vitro* evaluering van Philabuster teen *Dipolodia natalensis* en *Colletotrichum gloeosporioides*, onderskeidelik die patogene van *Diplodia* stingelent vrot en antraknose, het egter gewys dat Philabuster in vergelyking met TBZ die twee patogene nie voldoende inhibeer nie.
- Bederfbeheer van *P. digitatum* en *G. candidum* op suurlemoene met die GRAS chemikalieë, natriumkarbonaat, natriumbikarbonaat en kalkswawel, in 'n warmwaterbad doopbehandeling was vergelykend met standaard imazalil SO<sub>4</sub> en guazatine. Verskillende konsentrasies van natriumkarbonaat in kombinasie met verskillende konsentrasies van guazatine het ook goeie bederfbeheer op Valencia lemoene gegee. Boonop is sinergistiese werking tussen die natrium karbonaat met guazatine teen laer konsentrasies as die standard waargeneem.
- Die swamdoder Ortocil en die Cerexagri formulasie van Ortofenielfenaat (OFF) het 'n redelike mate van beheer van *P. digitatum* en *Geotrichum candidum* op nawel lemoene in vergelyking met die standard aanbevole imazalil sulfaat en guazatine getoon, maar met 'n klein mate van fitotoksiteit op sensitiewe (groen) dele.
- Delta Valencia vrugte is behandel met 'n dubbel aanwending van imazalil, eerstens in 'n doopbehandeling van 500 dpm en, tweedens, 'n waksaanwending van 2000 dpm, asook enkelaanwendings van hierdie behandelings vir die beheer van *P. digitatum* infeksies. Goeie beheer van *P. digitatum* infeksies deur al die konsentrasies van imazalil is waargeneem. Residuvlakke van 3.2 en 3.4 dpm is getoon vir die dubbelbehandeling, 4.0 vir die enkel waksbehandeling, en 0.7 en 0.9 dpm vir die enkel doopbehandeling. Al die vlakke is heelwat onder die toegelate Minimum Residu Toleransie van 5.0 dpm, maar die enkel doopbehandeling het residu-vlakke van <1 dpm gehad. Hierdie dubbele aanwending van imazalil dui aan dat aanvaarbare residu vlakke van imazalil op vrugte sal sorg vir groen- en blouskimmel sporulering inhibisie en beter bederf beheer.
- Sporekill is teen konsentrasies van 1 ml/l en 2 ml/l saam met imazalil teen 250 mg/kg en guazatine teen 500 mg/kg in doop behandelings teen infeksie deur imazalil sensitiewe en bestande rasse van *P. digitatum* aangewend. Sporekill saam met die twee swamdoders het 'n goeie sinergistiese werking getoon teen die beheer van *P. digitatum* besmetting.
- Die spuit van die drie kwaternêre ammonium verbindings, Sporekill, Quattrokill en Desogerm, voor-oes vir die vermindering van swamspoorlading in die boord en op die vrugoppervlak, het 'n mate van werking op spoorlading gewys.

##### Summary

Various trials were conducted in this experiment:

- The new post-harvest fungicide Philabuster (from Janssen Pharmaceutica), made up of a mixture imazalil and pyrimethanil, demonstrated good control of the post-harvest citrus pathogen *P. digitatum* compared to the standard Fungazil 750 WSP. Philabuster was recommended for registration.
- *In vitro* screening of Philabuster against *Dipolodia natalensis* and *Colletotrichum gloeosporioides*, the pathogens of *Diplodia* stem-end rot and anthracnose respectively, demonstrated that Philabuster did not effectively inhibit these two pathogens, compared to the standard TBZ.
- Decay control of *P. digitatum* and *G. candidum* on lemons by the GRAS chemicals sodium carbonate, sodium bicarbonate and lime sulphur, in a hot water dip treatment, was comparable to the standard imazalil SO<sub>4</sub> and guazatine. Different concentrations of sodium carbonate in combination with different concentrations of guazatine also demonstrated good decay control on Valencia oranges. Effective synergistic activity between sodium carbonate and guazatine, at reduced rates, was observed.
- The two formulations of the fungicide Ortho-phenylphenate (OPP), Ortocil and Cerexagri, demonstrated reasonable control of *P. digitatum* and *G. candidum* on navel oranges, compared to the standard imazalil

sulphate and guazatine. However, a small degree of phytotoxicity was observed on sensitive (green) patches of the rinds.

- Delta Valencia oranges were treated with a double application of imazalil, firstly in a dip treatment at 500 ppm and secondly at 2000 ppm in the wax for control of *P. digitatum* infections. Good control was observed. Residue levels of 3.2 and 3.4 ppm were recorded for the double application, 4.0 ppm for the single wax application and 0.7 and 0.9 ppm for the single dip treatment. All the residue levels were well below the Minimum Residue Level of 5.0 ppm, but the single dip application had residue levels of < 1.0 ppm. This double application of imazalil demonstrates that acceptable residue levels of imazalil on fruit will ensure green and blue mould sporulation inhibition and better decay control.
- Sporekill was applied at concentrations of 1 ml/l and 2 ml/l in combination with imazalil at 250 mg/kg and guazatine at 500 mg/kg, in dip treatments against infections by imazalil sensitive and resistant biotypes of *P. digitatum*. Sporekill together with the two fungicides at reduced rates, demonstrated good synergistic activity.
- The spraying of the three quaternary ammonium compounds, Sporekill, Quattrokill and Desogerm, pre-harvest for the reduction of fungal spore load in the orchard and on fruit surfaces, prior to harvest, demonstrated a degree of activity against spore load reduction.

### **Trial 1. The evaluation of a new post-harvest fungicide Philabuster for the control of the post-harvest disease *Penicillium digitatum* (citrus green mould) after harvest (March 2006–March 2009)**

#### **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 resistant spores 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. The prime reason for formulating such a compound was for the use thereof in a resistant strategy in citrus packhouses to reduce and prevent the further build-up of *Penicillium* mould resistant biotypes against imazalil. An added advantage of formulating the two fungicides into one compound allows for a more practical and efficient application of the two post-harvest fungicides.

The aim of this study was to evaluate the efficacy of Philabuster for the control of *P. digitatum* (citrus green mould) post-harvest infections by imazalil sensitive and resistant spores. Philabuster was applied in a cold water dip/drench treatment and also a hot water dip treatment at 40°C and in citrus wax.

## Materials and methods

*In vivo* evaluation trials were conducted with the Janssen Pharmaceutica Philabuster 400 SC (200 g/l imazalil and 200 g/l pyrimethanil) on Navel and Valencia oranges that were inoculated with an imazalil sensitive strain of *P. digitatum* for the ambient dip/drench treatments. Navel and Star Ruby grapefruit were inoculated with an imazalil resistant strain of *P. digitatum* for the comparative ambient and hot water dip treatments, and Valencia oranges were inoculated with both an imazalil sensitive and resistant strain of *P. digitatum* for the wax treatments. Philabuster 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 in the dip treatments, and with Fungazil 500 EC at the recommended commercial rate of 3 g/l giving a treatment concentration of 3000 ppm imazalil in the wax treatments. Philabuster was evaluated at the rates of 0.125% (1/2x), 0.25% (1x) and 0.5% (2x), giving treatment concentrations of 250, 500 and 1000 ppm, respectively, in the dip treatments and at rates of 0.5%, 1.0% and 1.5% in the wax treatments, giving treatment concentrations of 1000, 2000 and 3000 ppm, respectively.

Spore suspensions of *P. digitatum* (imazalil sensitive and resistant spores) 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  $1 \times 10^6$  spores/ml (Eckert et al., 1986; Morris et al., 1978).

Untreated navel and Valencia oranges (from Crocodile Valley Estate) and Star Ruby grapefruit (from Strydomblock) were obtained in bulk. For the purpose of inoculation, 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 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 60-120 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35  $\mu$ l of spore suspension to each injury site using a micropipette. The inoculated fruit was incubated for 12 hours at  $\pm 23^\circ\text{C}$  (to simulate a 12-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated.

### Ambient dip/drench and hot water treatments

Inoculated fruit was divided into 3 replicates of 10 fruit per treatment for the ambient dip/drench treatments and 3 replicates of 10 fruit per treatment for the ambient/hot water dip treatments. All the treatments were immersed in a 3 minute dip at ambient (18°C) and at 40°C.

Treatments on navels and Valencias at 18°C and navels and S/R grapefruit at 18°C and 40°C :

1. Untreated control - water dip
2. Standard treated control – Fungazil WSP 67g /100 l (500 ppm imazalil)
3. Philabuster – 0.125% (250 ppm imazalil and 250 ppm pyrimethanil)
4. Philabuster – 0.25% (500 ppm imazalil and 500 ppm pyrimethanil)
5. Philabuster – 0.5% (1000 ppm imazalil and 1000 ppm pyrimethanil)

After treatment, the fruit was incubated in paper packets at 20°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. The ambient dip/drench trial was conducted twice on navel oranges and once on Valencia oranges.

### Wax treatments

Inoculated fruit was divided into 3 replicates of 20 fruit per treatment. The wax applications were done in the FMC applicator on the CRI packline. The fruit was exposed to the wax application on the brushes for 3 minutes. The fruit was waxed with a Carnuba wax (FMC Tropical 18% total solids)

Treatments on Valencias with imazalil sensitive and resistant *P. digitatum* spores:

1. Untreated control - wax only
2. Standard treated control – Fungazil 500 EC 150ml/ 25 ℓ (3000 ppm imazalil)
3. Philabuster – 0.5% (1000 ppm imazalil and 1000 ppm pyrimethanil)
4. Philabuster – 1.0% (2000 ppm imazalil and 2000 ppm pyrimethanil)
5. Philabuster – 1.5% (3000 ppm imazalil and 3000 ppm pyrimethanil)

After treatment, the fruit was incubated in paper packets at 20°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

### Ambient dip/drench and hot water treatments

Philabuster 400 SC demonstrated good control of the citrus pathogen *P. digitatum* (sensitive strain) infection on navel and Valencia oranges, compared to the standard recommended Fungazil sulphate 750 WSP (Table 4.5.2.1). In all three trials, Philabuster and Fungazil effected 100% inhibition of green mould, except for the 0.125% Philabuster treatment, which showed between 80% and 90% inhibition. Philabuster at the recommended rate of 500 ppm (0.25%) demonstrated 100% control of infection by the imazalil-resistant strain of *P. digitatum*; on navels treated at ambient and 40°C (Table 4.5.2.2) and on S/R grapefruit at the same temperatures (Table 4.5.2.3), compared to only 36.7% inhibition on navels (Table 4.5.2.2) and 40-43.3% inhibition on S/R grapefruit by the standard recommended Fungazil 750 WSP (imazalil) at 500 ppm. No phytotoxicity was evident on the fruit treated at the highest concentration (0.5%) of Philabuster in all the trials.

**Table 4.5.2.1 (Trial 1).** Percentage inhibition of green mould on navel and Valencia oranges that were wounded and inoculated with *P. digitatum* (sensitive strain) 12 hours before dip-treatment in water (18°C) with 0.125%, 0.25% and 0.5% Philabuster and 67 g/100ℓ Fungazil 750 WSP.

Treatments	% Inhibition <sup>a</sup>		
	Navels (Trial 1)	Navels (Trial 2)	Valencias
1. Untreated control	0.0 b	0.0 b	0.0 b
2. Treated control: Fungazil (67 g/100 ℓ)	100.0 a	100.0 a	100.0 a
3. Philabuster (0.125%)	90.0 a	90.0 a	90.0 a
4. Philabuster (0.25%)	100.0 a	100.0 a	100.0 a
5. Philabuster (0.5%)	100.0 a	100.0 a	100.0 a

<sup>a</sup> 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 (Trials 1).** Percentage inhibition of green mould on navel oranges that were wounded and inoculated with *P. digitatum* (resistant strain) 12 hours before dip-treatment in water (18°C and 40°C) with 0.125%, 0.25% and 0.5% Philabuster and 67 g/100ℓ Fungazil 750 WSP.

Treatments	% Inhibition <sup>a</sup>	
	Navels (18°C)	Navels (40°C)
1. Untreated control	0.0 d	0.0 d
2. Treated control: Fungazil (67 g/100 ℓ)	36.7 c	36.7c
3. Philabuster (0.125%)	80.0 b	83.3 b
4. Philabuster (0.25%)	100.0 a	100.0 a
5. Philabuster (0.5%)	100.0 a	100.0 a

<sup>a</sup> 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 4.5.2.3 (Trial 1).** Percentage inhibition of green mould on S/R grapefruit that were wounded and inoculated with *P. digitatum* (resistant strain) 12 hours before dip-treatment in water (18°C and 40°C) with 0.125%, 0.25% and 0.5% Philabuster and 67 g/100ℓ Fungazil 750 WSP

Treatments	% Inhibition <sup>a</sup>	
	S/R grapefruit (18°C)	S/R grapefruit (40°C)
1. Untreated control	3.3 d	0.0 c
2. Treated control: Fungazil (67 g/100 ℓ)	43.3 c	40.0 b
3. Philabuster (0.125%)	76.7 b	86.7a
4. Philabuster (0.25%)	96.7 a	100.0 a
5. Philabuster (0.5%)	100.0 a	100.0 a

<sup>a</sup> 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).

#### Wax treatments

Philabuster 400 SC did attain a fairly high degree of control of the citrus pathogen *P. digitatum* (sensitive strain) infection on Valencia oranges, compared to the standard recommended Fungazil 500 EC (Table 4.5.2.4). However none of the three rates achieved 100% control, as was observed with the dip treatments. Philabuster did nevertheless reduce *P. digitatum* (resistant strain) infection significantly, compared to the standard Fungazil 500 EC. The inhibition of infections by *P. digitatum* (sensitive and resistant strains) on Valencia oranges by Philabuster applied in the wax treatments was not as effective as the dip treatments.

**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 strains) 12 hours before treatment in citrus wax with 0.5%, 1.0% and 1.5% Philabuster and 150 ml/25ℓ Fungazil 500 EC.

Treatments	% Inhibition <sup>a</sup>	
	Valencias (Sensitive spores)	Valencias (Resistant spores)
1. Untreated control	0.0 d	0.0 e
2. Treated control: Fungazil (150ml/25ℓ)	98.3 a	18.3 d
3. Philabuster (0.5%)	78.3 c	55.0 c
4. Philabuster (1.0%)	91.7 b	80.0 b
5. Philabuster (1.5%)	96.7 ab	98.3 a

<sup>a</sup> 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).

#### Discussion

The above results indicate that Philabuster was more effective in aqueous solutions (warm or ambient) compared with the suspension of Philabuster in citrus waxes. In general, waxes reduce the effectiveness of citrus fungicides (Smilanick et al., 1997). It was observed that when Philabuster was mixed into the wax before application of the suspension, Philabuster was not readily miscible with the wax, resulting in a "lumpy" dispersion of the product, even after vigorous agitation for a lengthy period of time. This scenario was confirmed during recent discussions with Alain Garnier from Janssen Pharmaceutica. The mixing of Philabuster was a problem specifically in carnauba waxes, but not in the polyethylene waxes. This problem will need to be investigated further.

Philabuster demonstrated that it was a very effective compound for the management of citrus green mould infections in packhouses. The product is unique in that the pyrimethanil in the formulation has a different mode of action than any of the other post-harvest fungicides currently being used. This makes the product of particular value because it is effective in controlling *P. digitatum* isolates that are resistant to imazalil, guazatine and possibly thiabendazole as well. The imazalil resistant isolate (from Prof. John Mildenhall's *in vitro* screenings) used in these trials was also resistant to guazatine and Philabuster effectively controlled green mould infections by this isolate as indicated in a previous study.

The introduction of Philabuster, which has recently been registered in South Africa, into packhouses must be done to incorporate the product into strategies to minimise the development of resistant isolates. Imazalil is still

by far our most important fungicide in use and therefore we need to recommend the use of Philabuster in strategies where we can protect imazalil, and other fungicides for that matter, against the build up of resistant populations.

In the absence of selection pressure, the proportion of imazalil resistant spores produced from mixed infections from sensitive and resistant isolates declines rapidly after several cycles (Holmes and Eckert, 1995). Therefore, it is likely that isolates resistant to imazalil and pyrimethanil would also have this characteristic and might be easier to manage than those resistant to pyrimethanil alone (Smilanick et al. 2006).

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## **Trial 2. The *in vitro* evaluation of Philabuster against the inhibition of Anthracnose and Diplodia** (March 2007)

### Introduction

Stem-end rots of citrus have been reported from all the major citrus producing countries in the world (Brown et al., 1966). In South Africa, *Diplodia natalensis* is usually considered to be the most important, while Anthracnose can also be severe on occasions. Judging by surveys of decay of South African fruit overseas before 1965, it would appear that the severity of *Diplodia*, *Alternaria* and Anthracnose decay varies from year to year and is seldom responsible for more than 10% of the total decay. However experience has shown that these diseases can be a much more important component of the decay of fruit from certain production areas (Christ, 1964). Both *Diplodia* stem-end rot (Fig. 4.5.2.1) and Anthracnose (Fig. 4.5.2.2), caused by *Diplodia natalensis* and *Colletotrichum gloeosporioides*, respectively, are two major decays related to degreening on early harvested fruit. Degreening greatly increases the incidence and severity of both of these diseases.

Both of these pathogens grow and sporulate on dead wood in citrus trees. Water (rain or irrigation) transmits the fungal spores to the rinds and/or calyx tissue of immature fruit and the spores do not germinate but remain latent or quiescent until conditions are ideal (degreening, ageing etc.) for germination of the spores and resultant infection.



**Fig. 4.5.2.1** (Trial 2). *Diplodia* stem-end rot.



**Fig. 4.5.2.2** (Trial 2). Anthracnose.

One of the control measures for these two pathogens is the use of chemicals pre-packhouse and in the packhouse. A post-harvest drench with thiabendazole (TBZ) before degreening is probably the most common treatment. A heated imazalil solution might also be used for post-harvest drenching before degreening, but TBZ is generally more effective against *Diplodia* stem-end rot and Anthracnose. On the packing line TBZ can be applied in water wax at 2000 ppm (Zhang et al., 2002).

Janssen Pharmaceutica claimed that Philabuster effectively controls *Diplodia* stem-end rot and Anthracnose infections. The aim of this *in vitro* trial was to screen the efficacy of Philabuster, compared with TBZ, for inhibition of these two pathogens.

### Materials and methods

Isolations of both pathogens (Anthracnose and *Diplodia*) were made from infected citrus fruit onto Potato Dextrose agar (PDA) and incubated as stock cultures for the purpose of this evaluation. Stock solutions of both Philabuster (5 ml/100 ml) and the standard recommended TBZ, Tecto 500 SC (2 ml/100 ml) were made up at a concentration of 10,000 ppm (a.i.) in sterile deionised water.

Both stock solutions were then diluted further in sterile deionised water to concentrations of 0.1, 1.0, and 10.0 ppm. These concentrations of the two products were then incorporated into pour plates (petri dishes) of PDA. Three plates of each dilution of Philabuster and the standard Tecto were poured for both pathogens. PDA plates without fungicide were also poured as control plates.

The stock cultures of the two pathogens were prepared for this evaluation by plugging 9 mm agar plugs of the pathogens on the PDA culture plates, by means of a cork borer. The PDA petri dishes incorporated with Philabuster and thiabendazole were then inoculated with the two pathogens by placing a single 9 mm plug of each of the pathogens in the centre of three PDA plates of each concentration of the fungicides. Three control plates per fungicide were inoculated likewise.

The plates were then incubated at 25°C until such time as the control cultures had completely grown over the entire surface of the petri dishes. The diameter growth of the pathogens on each plate was then measured in millimetres (mm) and the average growth for the three plates, for each concentration recorded as average mm growth. Two *in vitro* evaluations were conducted for both pathogens. The % inhibition of the pathogens by the fungicides was calculated and the following results were recorded.

## Results and discussion

Tables 4.5.2.1 (Trial 2) and 4.5.2.2 (Trial 2) indicate the percentage inhibition, of the two latent pathogens, *D. natalensis* and *C. gloeosporioides*, by Philabuster and the standard thiabendazole, recorded in the two *in vitro* evaluations. The results indicate that Philabuster is much less active than thiabendazole in inhibiting the two latent pathogens. This shows that the newly registered fungicide Philabuster would not be able to serve as an alternative to thiabendazole against these pathogens.

**Table 4.5.2.1 (Trial 2).** The *in vitro* inhibition of *D. natalensis* and *C. gloeosporioides* by Philabuster compared with the standard thiabendazole.

Growth in mm				
Concentration (ppm) a.i.	Philabuster		Standard TBZ	
	Anthracnose	Diplodia	Anthracnose	Diplodia
Control x 3	51,0	54,0	52.0	58.0
0,1	50.0	50.0	41.0	44.0
0,1	50.0	49.0	43,0	40.0
0,1	51.0	54.0	43,0	48.0
<b>Ave</b>	<b>50.3</b>	<b>51.0</b>	<b>42.3</b>	<b>44.0</b>
1,0	40.0	39.0	2,0	0.0
1,0	44.0	42.0	0.0	0.0
1,0	39.0	44.0	4.0	0.0
<b>Ave</b>	<b>41.0</b>	<b>41.7</b>	<b>2.0</b>	<b>0.0</b>
10,0	12.0	10.0	0.0	0.0
10,0	14.0	18.0	0.0	0.0
10,0	16.0	16.0	1.0	0.0
<b>Ave</b>	<b>14.0</b>	<b>14.7</b>	<b>0.3</b>	<b>0.0</b>
% Inhibition <sup>a</sup>				
Concentration (ppm) a.i.	Philabuster		Standard TBZ	
	Anthracnose	Diplodia	Anthracnose	Diplodia
Control x 3	0.0 c	0.0 c	0.0 c	0.0 c
0.1	1.3 c	5.6 c	18.6 b	24.1 b
1.0	19.6 b	22.8 b	96.1 a	100.0 a
10.0	72.5 a	72.9 a	99.4 a	100.0 a

<sup>a</sup> 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 2)** The *in vitro* inhibition of *D. natalensis* and *C. gloeosporioides* by Philabuster compared with the standard thiabendazole.

Growth in mm				
Concentration. (ppm) a.i.	Philabuster		Standard TBZ	
	Anthracnose	Diplodia	Anthracnose	Diplodia
Control x 3	58.0	56.0	53.0	56.0
0,1	52.0	53.0	41.0	49.0
0,1	52.0	53.0	43.0	51.0
0,1	49.0	54.0	40.0	45,0
<b>Ave</b>	<b>51.0</b>	<b>53.3</b>	<b>41.3</b>	<b>48.3</b>
1,0	40.0	42.0	2,0	2.0
1,0	39.0	42.0	1.0	2.0

1,0	41.0	42.0	0.0	3.0
<b>Ave</b>	<b>40.0</b>	<b>42.0</b>	<b>1.0</b>	<b>2.3</b>
10,0	14.0	18.0	0.0	0.0
10,0	17.0	18.0	0.0	0.0
10,0	18.0	17.0	0.0	0.0
<b>Ave</b>	<b>16.3</b>	<b>17.7</b>	<b>0.0</b>	<b>0.0</b>
<b>% Inhibition<sup>a</sup></b>				
Concentration (ppm) a.i.	Philabuster		Standard TBZ	
	Anthracnose	Diplodia	Anthracnose	Diplodia
Control x 3	0.0 d	0.0 d	0.0 c	0.0 c
0,1	12.0 c	4.8 c	22.0 b	13.7 b
1,0	31.1 b	25.0 b	98.1 a	95.8 a
10,0	71.9 a	68.5 a	100.0 a	100.0 a

<sup>a</sup> 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;  $P \leq 0.05$ ).

### Conclusions

No further *in vitro* screening of Philabuster against inhibition of the latent pathogens is required. However, simulated shipping storage trials and possible longer storage of susceptible fruit treated with Philabuster and thiabendazole would be a worthwhile trial to conduct to confirm these *in vitro* results recorded above.

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### **Trial 3. The potential role of GRAS chemicals in the control of the major post-harvest citrus pathogens (March 2005 – March 2009)**

#### Introduction

Green mould, caused by *Penicillium digitatum* (Pers.: Fr.) and sour rot, caused by *Geotrichum candidum* (Link ex Pers.), are two of the most economically important post-harvest diseases of citrus. The primary mode of infection by both 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).



**Fig. 4.5.2.1** (Trial 3). *P. digitatum* (green mould)



**Fig. 4.5.2.2** (Trial 3). *G. candidum* (sour rot)

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. 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. It has thus become necessary to screen new chemicals, such as GRAS (generally regarded as safe) chemicals, for fungicidal properties against the citrus pathogens in order to find new, safe compounds that could assist in overall decay control as well as preventing 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 firstly to determine the efficacy of the GRAS chemicals sodium carbonate, sodium bicarbonate and lime sulphur, in a simulated citrus packhouse hot water bath, and secondly to evaluate the efficacy of sodium carbonate in combination with reduced concentrations of guazatine in a simulated pre-greening drench treatment, against post-harvest green mould and sour rot of citrus fruit.

## Materials and methods

Untreated Valencia oranges (Crocodile Valley Citrus, Nelspruit, RSA) and lemons (Larten Estate, Nelspruit, RSA) 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* and *G. candidum* were made up by suspending 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  $1 \times 10^6$  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  $\mu$ 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 long delay after harvesting prior to packhouse treatments, inoculated fruit were incubated at 23°C for 16 h until further treatment.

**Evaluation of sodium carbonate, sodium bicarbonate and lime sulphur in a simulated citrus packhouse hot water bath.** Green lemons (harvested just prior to colour break) were used in this trial and all the treatments were done 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. The 8 treatments involved an untreated control (clean water), a treated control (500 ppm imazalil SO<sub>4</sub>, in the case of inoculation with *P. digitatum*, and 1000 ppm guazatine, in the case of inoculation with *G. candidum*), sodium carbonate at 3% and 5%, sodium bicarbonate at 3% and 5%, and lime sulphur at 3% and 5%. 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 wounds.



**Fig. 4.5.2.3 (Trial 3). Hot water bath.**

Evaluation of sodium carbonate in combination with reduced concentrations of guazatine in a simulated pre-degreening drench treatment. Inoculated Valencia oranges were used in this trial and treatments simulated a pre-degreening drench as fruit was dipped for 3 minutes at ambient temperature (20°C). The 16 treatments involved an untreated control (clean water), treated control (guazatine at 125, 250 and 500 ppm), sodium carbonate alone at 3%, 4% and 6%, and sodium carbonate at these rates in combination with guazatine at the above-mentioned rates. After treatment, fruit was incubated and evaluated as described above.



**Fig. 4.5.2.4 (Trial 3). Pre-degreening drench**

## Results

In the simulated hot water bath trial, fairly good inhibition of infections of both pathogens by the three GRAS compounds was observed (Table 4.5.2.1-Trial 3). However, when statistically compared with the respective fungicide treatments of imazalil and guazatine, similar decay inhibition was observed only for 5% lime sulphur and 5% sodium carbonate for green mould and sour rot, respectively.

In the simulated pre-greening drench trial, sodium carbonate alone inhibited green mould and sour rot to a lesser extent than in the hot water bath trial (Tables 4.5.2.1 and 4.5.2.2 – Trial 3), with only the 6% rate demonstrating marginal efficacy. Guazatine at 500 ppm completely inhibited both pathogens, while reduced rates proved significantly less effective (Table 4.5.2.2 – Trial 3). However, in combination with sodium carbonate, especially with the 4% and 6% rates, a synergistic effect was observed as complete inhibition of green mould and sour rot was observed with all rates of guazatine tested.

**Table 4.5.2.1 (Trial 3).** Mean percentage wounds decayed with green mould or sour rot on lemon fruit that were wounded, inoculated with a *P. digitatum* or *G. candidum* spore suspension and subsequently dip-treated in various treatments in a simulated citrus packhouse hot water bath at 40°C.

Treatments	Infected wounds (%) <sup>a</sup>	
	Green mould	Sour rot
Untreated control (clean water)	100.0 a	96.7 a
Treated control (imazalil or guazatine)	1.7 g	0.0 e
Sodium carbonate (3%)	31.7 e	30.0 c
Sodium carbonate (5%)	30.0 e	5.0 e
Sodium bicarbonate (3%)	61.7 c	30.0 c
Sodium bicarbonate (5%)	55.0 d	38.3 b
Lime sulphur (3%)	80.0 b	20.0 d
Lime sulphur (5%)	13.3 f	20.0 d

<sup>a</sup>Means in each column followed by the same letter are not significantly different (Fisher's Unprotected LSD;  $P \leq 0.05$ ).

**Table 4.5.2.2 (Trial 3).** Mean percentage wounds decayed with green mould or sour rot on Valencia oranges that were wounded, inoculated with a *P. digitatum* or *G. candidum* spore suspension and subsequently dip-treated in various treatments in a simulated pre-greening drench at 20°C.

Treatments	Infected wounds (%) <sup>a</sup>	
	Green mould	Sour rot
Untreated control (clean water)	98.3 e	100.0 h
Guazatine (125 ppm)	61.7 d	26.7 c
Guazatine (250 ppm)	60.0 d	10.0 b
Guazatine (500 ppm)	0.0 a	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (3%)	96.7 e	80.0 g
Na <sub>2</sub> CO <sub>3</sub> (4%)	98.3 e	70.0 f
Na <sub>2</sub> CO <sub>3</sub> (6%)	60.0 d	40.0 e
Na <sub>2</sub> CO <sub>3</sub> (3%) + Guazatine (125 ppm)	21.7 b	30.0 d
Na <sub>2</sub> CO <sub>3</sub> (3%) + Guazatine (250 ppm)	33.3 c	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (3%) + Guazatine (500 ppm)	0.0 a	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (4%) + Guazatine (125 ppm)	20.0 b	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (4%) + Guazatine (250 ppm)	0.0 a	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (4%) + Guazatine (500 ppm)	0.0 a	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (6%) + Guazatine (125 ppm)	0.0 a	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (6%) + Guazatine (250 ppm)	0.0 a	0.0 a
Na <sub>2</sub> CO <sub>3</sub> (6%) + Guazatine (500 ppm)	0.0 a	0.0 a

<sup>a</sup>Means in each column followed by the same letter are not significantly different (Fisher's Unprotected LSD;  $P \leq 0.05$ ).

## Discussion

Results from the simulated hot water bath trial demonstrated that infections of green mould and sour rot on lemons that were exposed to heated solutions (40°C) of sodium carbonate, sodium bicarbonate and lime sulphur for 3 minutes were fairly effectively inhibited by these GRAS chemicals. Sodium carbonate, at a concentration of 5%, demonstrated 60% inhibition of green mould infection, while 5% lime sulphur demonstrated 87% inhibition. For sour rot, 5% sodium carbonate showed 95% inhibition, and lime sulphur 80% inhibition at 3% and 5%. Smilanick et al. (1997) found that sodium carbonate (soda ash) provided control of green mould on oranges without the need to heat the solutions to temperatures that increase the risk of injury to fruit (>50°C), even exposure as brief as 1 minute. It was furthermore found that the concentration of sodium carbonate greatly influenced efficacy, whereas differences among temperatures or between immersion periods were small. The most effective temperature and sodium carbonate combinations were 40.6 or 43.3°C with 4% or 6% sodium carbonate with an immersion time of 2 minutes (Smilanick et al., 1997).

Sodium carbonate was less effective in water at ambient temperature, as demonstrated in the simulated pre-greening trial where even the 6% rate provided poor control of both diseases on Valencia oranges. Nonetheless, when applied in combination with a reduced rate (250 ppm) or the standard recommended rate for guazatine in a pre-degreening drench (500 ppm) complete control was obtained with 4% and 6% sodium carbonate. This synergistic effect was especially pronounced as 4% sodium carbonate alone proved to be ineffective against green mould and poorly effective against sour rot.

Results from this study clearly demonstrate the potential of integrating GRAS chemicals into a successful post-harvest decay management programme. For the pre-greening drench, sodium carbonate at 4% or 6% can be used in combination with the standard rate of guazatine (500 ppm) or a reduced rate of 250 ppm. In subsequent hot water baths, the standard fungicides imazalil and guazatine, can be substituted with either 5% sodium carbonate or 5% lime sulphur. Future research needs to investigate the possible synergistic effect of combinations in the latter treatment, and importantly also the effect of reduced rates of guazatine in synergistic combinations on fungicide resistance development.

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**Trial 4. The repeat evaluation of the post-harvest fungicide Ortocil (ortho-phenylphenate) compared with the Cerexagri formulation (ortho-phenylphenate) for the control of post-harvest diseases** (March 2006 – March 2009)

### Introduction

The water-soluble post-harvest fungicide SOPP (Sodium ortho-phenylphenate) provides fairly good protection against *P. digitatum* (green mould) and *P. italicum* (blue mould), and demonstrates limited activity against Diplodia stem-end rot, Alternaria core rot and *G. candidum* (sour rot). SOPP is not compatible with TBZ and imazalil sulphate in water and incorrect usage of SOPP may lead to serious phytotoxicity (burn) on citrus rinds. SOPP concentration, pH of the mixture, temperature of the mixture and time of exposure of the fruit to the mixture must be controlled accurately. Any deviations within the recommended parameters of these variables could also lead to serious "burn" on fruit.

The standard pre-degreening drench mixture includes the use of guazatine for the protection of the fruit against green and blue mould and sour rot. Unfortunately, guazatine has to be withdrawn from the drench mixture where fruit is being treated and exported to countries without permitted MRL's, such as USA, Canada, Korea and Japan. Without guazatine in the pregreening drench, fruit will be exposed to infections by these three pathogens and full development of these infections will be compounded during degreening.

Therefore, it has become important to find a compound, with a different mode of action to guazatine, that can be alternated with guazatine in the drench for protection against the *Penicillium* organisms and sour rot and also play a role in reducing the risk of the build up of resistant green and blue mould and sour rot fungal spores to both compounds and minimise the risk of the build up of imazalil resistant *Penicillium* spores.

The Spanish formulation of ortho-phenylphenate (Ortocil) was re-evaluated and a formulation from Cerexagri in Italy was also screened, for possible inclusion in a pre-degreening drench mixture. The two products were evaluated for efficacy against infections caused by *P. digitatum* (imazalil sensitive and resistant spores) and *G. candidum*.

## Materials and methods

Spore suspensions of *P. digitatum* (imazalil sensitive and resistant spores) and *G. candidum* were prepared by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were spectrophotometrically adjusted to concentrations of  $1 \times 10^6$  spores/ml. Good sound, untreated Valencia oranges (Crocodile Valley Citrus Estate) were obtained in bulk. The fruit was divided into 3 replicates of 20 fruit per treatment and all 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.

Each fruit was wounded by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides giving 120 infection sites per treatment. Each fruit was then infected with the pathogen by applying 35  $\mu$ l of spore suspension to each injury site using a micropipette.

The inoculated fruit was treated 4 hours after inoculation with the chemical compounds by immersion in a water bath containing each treatment suspension at ambient temperature (18°C) for 3 minutes. The following treatments were conducted:

### Imazalil sensitive *P. digitatum* isolate

1. Untreated control
2. Treated control – 500 ppm imazalil sulphate
3. 1000 ppm Ortocil
4. 2000 ppm Ortocil
5. 3000 ppm Ortocil
6. 1000 ppm Cerexagri
7. 2000 ppm Cerexagri
8. 3000 ppm Cerexagri

### Imazalil resistant *P. digitatum* isolate

9. Untreated control
10. Treated control – 500 ppm imazalil sulphate
11. 1000 ppm Ortocil
12. 2000 ppm Ortocil
13. 3000 ppm Ortocil
14. 1000 ppm Cerexagri
15. 2000 ppm Cerexagri
16. 3000 ppm Cerexagri

### *G. candidum*

17. Untreated control
18. Treated control – 1000 ppm guazatine
19. 1000 ppm Ortocil
20. 2000 ppm Ortocil
21. 3000 ppm Ortocil
22. 1000 ppm Cerexagri
23. 2000 ppm Cerexagri
24. 3000 ppm Cerexagri

After treatment, the fruit was incubated in paper packets and plastic bags (sour rot) at 20°C for 7-10 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay inhibition (number of infected wounds).

## Results and discussion

Both Ortocil and Cerexagri (ortho phenylphenate) demonstrated fairly good control of infection caused the imazalil sensitive spores of the pathogen *P. digitatum* compared with the standard imazalil sulphate (Table 4.5.2.1 – Trial 4). The two products also inhibited infections caused by imazalil resistant **P. digitatum** spores effectively. However the activity on navel oranges against sour rot infections caused by the pathogen *G. candidum*, compared with the standard guazatine was not as good as in previous trials done on Valencia oranges. The best control of sour rot was 70.0% inhibition at the highest concentration of 3000 ppm by both OPP formulations. The degree of control of sour rot at the three concentration of both products was much lower than in previous trials with Valencias. One concern in these trials was that a mild “phytotoxicity” was observed on the navels (some of the navels still had patches of green rinds) treated with both products at 3000 ppm. These trials need to be repeated and the efficacy of these two compounds need to be evaluated on atleast one other citrus cultivar.

**Table 4.5.2.1 (Trial 4).** Mean percentage inhibition of infection of navel oranges following wounding, inoculation with imazalil-sensitive and -resistant *P. digitatum* and *G. candidum* and subsequent dip-treatment with various concentrations of Ortocil and Cerexagri formuations (ortho phenylphenate) and the standard fungicides imazalil sulphate and guazatine.

Treatments	% inhibition <sup>a</sup>
<b><i>P. digitatum</i> sensitive isolate</b>	
1. Untreated control	0.0 c
2. Treated control – 500 ppm imazalil sulphate	100.0 a
3. 1000 ppm Ortocil	83.3 b
4. 2000 ppm Ortocil	85.0 ab
5. 3000 ppm Ortocil	93.3 ab
6. 1000 ppm Cerexagri	81.7 b
7. 2000 ppm Cerexagri	83.3 b
8. 3000 ppm Cerexagri	91.7ab
<b><i>P. digitatum</i> resistant isolate</b>	
9. Untreated control	0.0 d
10. Treated control – 500 ppm imazalil sulphate	35.0 c
11. 1000 ppm Ortocil	73.3 b
12. 2000 ppm Ortocil	83.3 b
13. 3000 ppm Ortocil	98.3 a
14. 1000 ppm Cerexagri	75.0 b
15. 2000 ppm Cerexagri	88.3 ab
16. 3000 ppm Cerexagri	96.7 a
<b><i>G. candidum</i></b>	
17. Untreated control	6.7 e
18. Treated control – 1000 ppm guazatine	100.0 a
19. 1000 ppm Ortocil	3.3 e
20. 2000 ppm Ortocil	38.3 c
21. 3000 ppm Ortocil	70.0 b
22. 1000 ppm Cerexagri	13.3 de
23. 2000 ppm Cerexagri	35.0 cd
24. 3000 ppm Cerexagri	70.0 b

<sup>a</sup>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;  $P \leq 0.05$ ).

**Trial 5. The evaluation of a double application of imazalil in citrus packhouses for the control of post-harvest *Penicillium* infections (green and blue mould) and sporulation inhibition for registration purposes (March 2006 – March 2007)**

**Introduction**

The citrus industry has been experiencing higher than normal levels of waste on export citrus for the past 2-3 seasons. An important contributing factor to the overall problem of the higher incidence of waste (specifically the *Penicillium* wound pathogens) on export citrus over the last two seasons was the overall low residue level of imazalil (< 1.0 mg/kg) retained on export citrus after treatment in the packhouses, as observed in the majority of residue analyses recorded by the accredited test laboratories. This resulted in *Penicillium* sporulation on infected fruit and excessive spread of infections in export cartons with high waste and also an increased risk of selection for resistant spores (Fig. 4.5.2.1 – Trial 5).



**Fig. 4.5.2.1 (Trial 5).** Imazalil at < 1.0 mg/kg



**Fig. 4.5.2.2 (Trial 5).** Imazalil at > 1.0 mg/kg (ideal 2-3 mg/kg)

Residues of 2.0 and 3.5 mg/kg imazalil were needed to control the sporulation of *P. digitatum* on oranges and lemons (Fig. 4.5.2.2-Trial 5)). Residues after 30 to 60 seconds treatment in heated aqueous imazalil were

sufficient to control sporulation, but residues after 15 seconds treatments were too low and required an additional application of 1070 mg.ml<sup>-1</sup> (i.e. approx. 1000 ppm) imazalil in the wax to deposit an amount of imazalil sufficient to control sporulation. The reason packers continued to use imazalil in wax is its reliable control of mould sporulation (Smilanick et al., 1997). More importantly, imazalil in wax is used to “top-up” residue levels after non-recovery application of imazalil. Brown and co-workers suggested that about 2 ppm imazalil residues were sufficient to control green mould sporulation (Taverner, 2005.). In Florida, non-recovery sprays of imazalil are usually followed by 1000 to 2000 ppm imazalil in wax, resulting in a residue of about 4 ppm (Smilanick et al., 1997).

The aim of these trials was to investigate a means of increasing residue levels to levels that would inhibit sporulation (2.0-3.0 mg/kg) without exceeding MRL levels (5.0 mg/kg). In the first trial, Delta Valencia oranges were treated with a double application of imazalil viz. a water dip treatment of imazalil sulphate 750 WSP (standard rate of 500 mg/kg), and a dip application of Fungazil 500 EC (2000 mg/kg) in citrus wax. In a second trial the efficacy of these imazalil treatments was evaluated against the control of *P. digitatum* infections on Valencia oranges.

## Materials and methods

Trial 1. Good sound, untreated Delta Valencia oranges (Crocodile Valley Citrus Estate) were obtained in bulk. The fruit was washed in clean water and surface sterilised by dipping in a 1% NaOCl solution for 2 minutes. The fruit was then allowed to dry prior to treatment.

Treatments involved the following:

1. Untreated control (water dip)
2. 500 mg/kg imazalil sulphate (Fungazil 750 WSP) in dip treatment
3. 2000 mg/kg imazalil EC ( Fungazil 500 EC) in wax application
4. 500 mg/kg Fungazil 750 WSP (dip) and 2000 mg/kg Fungazil 500 EC (wax)

The treatments, with imazalil sulphate 750 WSP were done in a water dip at ambient temperature. After treatment the fruit was left to dry in order to retain a residue of the imazalil sulphate on the fruit prior to the second application of imazalil incorporated in the wax. All the treatments in the water and wax dips were immersed for 3 minutes. After treatment with the second imazalil application in the wax dip the fruit was allowed to dry overnight. Treated samples were placed in paper bags, labelled and stored for 2-3 days in the deep freeze until frozen and then couriered to the SABS for residue analyses.

Trial 2. A spore suspension of *P. digitatum* was made up by suspending the spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of 1×10<sup>6</sup> spores/ml (Morris et al., 1978). Untreated Valencia oranges (Crocodile Valley Citrus Estate) were obtained in bulk. For the purpose of inoculation, 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 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 4 hours at ±23°C (to simulate a 4-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated.

Ambient dip and wax treatments. Inoculated fruit was divided into 3 replicates of 10 fruit per treatment for the ambient dip and wax treatments. All the treatments were immersed in a 3 minute water and wax dip. The following treatments were conducted:

1. Untreated control (aqueous dip)
2. Untreated control (wax dip)
3. 500 mg/kg imazalil sulphate (Fungazil 750 WSP) in dip treatment (standard)
4. 2000 mg/kg imazalil EC ( Fungazil 500 EC) in wax application
5. 3000 mg/kg imazalil EC ( Fungazil 500 EC) in wax application (standard)
6. 500 mg/kg Fungazil 750 WSP (dip) and 2000 mg/kg Fungazil 500 EC (wax)

After treatment, the fruit was incubated in paper packets at 20°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.

The trial was conducted three times on Valencia oranges for registration purposes.

## Results and discussion

**Trial 1.** Residue levels of all the imazalil treatments were below the MRL of 5.0 mg/kg for imazalil in all markets (Table 4.5.2.1-Trial 1). Dip treatment with imazalil (Fungazil 750 WSP) resulted in residue levels below 1 mg/kg. The recorded imazalil residue results indicate that the double application of imazalil in a citrus packhouse can be safely recommended without exceeding the MRL of 5.0 mg/kg for imazalil in all markets. A single imazalil application by means of 2000 mg/kg imazalil 500 EC in wax also resulted in effective residue levels without exceeding MRL levels. A single dip-application of imazalil did, however, not attain residue levels of higher than the desired 1 mg/kg. The imazalil residue levels attained after double application in the packhouse will also achieve the desired inhibition of *Penicillium* sporulation and reduce the selection for resistant spores, should there be *Penicillium* infections in export cartons of citrus.

**Table 4.5.2.1 (Trial 1).** The recorded results of duplicate imazalil residue analyses conducted by SABS.

	Treatments	Imazalil residue level (mg/kg)
1	Water dip	Nil
2	500 mg/kg imazalil 750 WSP dip	0.7 ; 0.9
3	2000 mg/kg imazalil 500 EC in wax	4.0 ; 4.0
4	500 mg/kg imazalil 750 WSP + 2000 mg/kg imazalil 500 EC in wax	3.2 ; 3.4

**Trial 2.** The standard recommended imazalil aqueous dip (500 mg/kg) and wax (3000 mg/kg) treatments completely controlled *P. digitatum* infection. The lower dosage (2000 mg/kg) of the wax application demonstrated only an average of 87.8% inhibition of *P. digitatum* infection, compared to the standard treatment of 3000 mg/kg. The double application of imazalil in the aqueous dip and the wax application at 2000 mg/kg also controlled the infection.

**Table 4.5.2.2 (Trial 1).** The percentage inhibition of green mould on Valencia oranges that were wounded and inoculated with *P. digitatum* 4 hours before treatment with three imazalil applications.

Treatments	% Decay inhibition <sup>a</sup>		
	Val 1	Val 2	Val 3
1. Untreated control (water dip)	0.0 c	0.0 c	0.0 c
2. Untreated control (wax dip)	0.0 c	0.0 c	0.0 c
3. 500 mg/kg imazalil 750 WSP in water dip treatment	100 a	100 a	100 a
4. 2000 mg/kg imazalil 500 EC in wax	86.7 b	86.7 b	90 b
5. 3000 mg/kg imazalil 500 EC in wax	100 a	100 a	100 a
5. 500 mg/kg imazalil 750 WSP + 2000 mg/kg imazalil 500 EC in wax	100 a	100 a	100 a

<sup>a</sup> 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).

## Conclusion

The double application of imazalil viz. a standard aqueous application plus an additional application of 2000 mg/kg in the citrus wax can safely be recommended for registration for application in citrus packhouses for the retention of adequate residue on fruit for effective decay control and sporulation inhibition.

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**Trial 6. The evaluation of Sporekill in combination with reduced concentrations of the post-harvest fungicides imazalil and guazatine against the control of infections by *Penicillium digitatum* (citrus green mould) (March 2007)**

### Introduction

Sporekill is a liquid broad spectrum agricultural disinfectant / plant sanitiser for use in citrus packhouses in pre-degreening drenches and dumptank and recirculating spray-on/brush-on washing systems to prevent the build-up of post-harvest pathogens (green and blue mould, sour rot and others) and to disinfect citrus fruit surfaces during washing in these systems. When non-inoculated oranges are punctured and then put through a sanitiser, fruit shows little or no rot. This shows that sanitisers can kill spores washing off mouldy fruit and contaminating water. This prevents viable spores transferring onto clean fruit coming into the system (Taverner et al., 2002). Using sanitisers to sanitise wash water or fungicide tanks reduces the risk of contaminating healthy fruit with spores of sour rot (*Geotrichum candidum*) or blue and green mould (*Penicillium italicum* and *P. digitatum*) (Taverner et al., 2007).

Sporekill, a quaternary ammonium compound with active ingredient didecyl dimethyl ammonium chloride, was registered for use in the South African citrus industry in 2002. Sporekill plays a dual role in citrus packhouses, firstly, as a sanitising agent in fruit washing systems and secondly by complimenting the role of the fungicides in an integrated decay program by virtue of its fungicidal properties. The aim of these trials was to evaluate the efficacy of Sporekill in combination with reduced concentrations of the post-harvest fungicides imazalil and guazatine in the control of infections by *P. digitatum*.

### Materials and methods

*In vivo* evaluation trials were conducted with the Sporekill (120 g/l) separately and in combination with lower concentrations of imazalil (Fungazil sulphate 750 WSP) and guazatine (CitriCure 210 SL) on Valencia oranges that were inoculated with imazalil sensitive and resistant strains of *P. digitatum* for the ambient dip treatments. Imazalil was applied at the lower rate of 33.5 g/100l and guazatine at the lower rate of 240 ml/100l giving treatment concentrations of 250 and 500 ppm, respectively. These treatments were compared with the standard Fungazil sulphate 750 WSP at the recommended commercial rate of 67 g/100l giving a treatment concentration of 500 ppm imazalil and the standard CitriCure 210 SL at the recommended commercial rate of 480 ml/100l giving a treatment concentration of 1000 ppm guazatine in the dip treatments. Sporekill was evaluated at the rates of 1 ml/l and 2 ml/l giving treatment concentrations of 1000 and 2000 ppm (product, not a.i.) in the dip treatments, respectively.

Spore suspensions of *P. digitatum* (imazalil sensitive and resistant spores) 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  $1 \times 10^6$  spores/ml (Morris et al., 1978).

Untreated Valencia oranges (Crocodile Valley Citrus Estate) were obtained in bulk. For the purpose of inoculation, 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 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  $\mu$ l of spore suspension to each injury site using a micropipette. The inoculated fruit was incubated for 12 hours at  $\pm 23^\circ\text{C}$  (to simulate a 12-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated.

### Ambient dip treatments

Inoculated fruit was divided into 3 replicates of 10 fruit per treatment for the dip treatments. All the treatments were immersed in a 3 minute dip at ambient (18°C). The following treatments were done on Valencias with imazalil sensitive and resistant *P. digitatum* spores:

1. Untreated control - water dip
2. Standard treated control – Fungazil WSP 67g /100 ℓ (500 ppm imazalil)
3. Standard treated control – CitriCure SL 480 ml/100ℓ (1000 ppm guazatine)
4. Fungazil WSP 33.5g /100 ℓ (250 ppm imazalil)
5. CitriCure SL 240 ml/100ℓ (500 ppm guazatine)
6. Sporekill 1mℓ/ℓ (1000 ppm)
7. Sporekill 1mℓ/ℓ (1000 ppm) plus Fungazil WSP 33.5g /100 ℓ (250 ppm imazalil)
8. Sporekill 1mℓ/ℓ (1000 ppm) plus CitriCure SL 240 ml/100ℓ (500 ppm guazatine)
9. Sporekill 2mℓ/ℓ (2000 ppm)
10. Sporekill 2mℓ/ℓ (2000 ppm) plus Fungazil WSP 33.5g /100 ℓ (250 ppm imazalil)
11. Sporekill 2mℓ/ℓ (2000 ppm) plus CitriCure SL 240 ml/100ℓ (500 ppm guazatine)

[Please note that all concentrations designated g/ 100 ℓ or ml/100ℓ refers to the a.i. of the product. Concentrations designated ml/ℓ refer to product concentration and not a.i.]

After treatment, the fruit was incubated in paper packets at 20°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 and discussion

Sporekill, at the standard rate of 100 ppm inhibited 56.7-60.0% of the imazalil-sensitive and -resistant *P. digitatum* infections, respectively. Imazalil and guazatine, at the reduced concentrations, demonstrated fairly good control of imazalil-sensitive *P. digitatum* infections (86.7-90.0%) compared to the standard concentrations of imazalil and guazatine. The control of imazalil-resistant *P. digitatum*- infections by the reduced dosages of the two compounds, on the other hand, was very poor. However, Sporekill, at the standard rate of 1000 ppm, in combination with imazalil and guazatine at the reduced rates, demonstrated a vast improvement in control of both the imazalil-sensitive and -resistant *P. digitatum* infections. This demonstrates a very good additive effect between Sporekill and the two fungicides, at the reduced rates, in controlling the infections (Table 1.)

**Table 4.5.2.1 (Trial 6).** Percentage inhibition of green mould on Valencia oranges that were wounded and inoculated with *P. digitatum* (sensitive and resistant strains) 12 hours before treatment in a aqueous dip with Sporekill alone and in combination with imazalil and guazatine.

Treatments	% Decay inhibition <sup>a</sup>	
	Valencias (Sensitive isolate)	Valencias (Resistant isolate)
1. Untreated control	0.0 e	0.0 e
2. Standard 500 ppm imazalil	100.0 a	23.3 d
3. Standard 1000 ppm guazatine	100.0 a	70.0 ab
4. 250 ppm imazalil	86.7 bc	0.0 e
5. 500 ppm guazatine	90.0 ab	3.3 e
6. 1000 ppm Sporekill	56.7 d	60.0 bc
7. 1000 ppm Sporekill + 250 ppm imazalil	96.7 ab	63.3 bc
8. 1000 ppm Sporekill + 500 ppm guazatine	100.0 a	53.3 c
9. 2000 ppm Sporekill	76.7 c	83.3 a
10. 2000 ppm Sporekill + 250 ppm imazalil	96.7 ab	83.3 a
11. 2000 ppm Sporekill + 500 ppm guazatine	100.0 a	80.0 a

<sup>a</sup> 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).

## Conclusions

The potential for Sporekill to be used in combination with reduced rates of the post-harvest fungicides in an integrated decay control strategy looks very promising. This trial will need to be repeated with these two fungicides. Screening of Sporekill in combination with thiabendazole and Philabuster also needs to be investigated. However, prior to specific recommendation of Sporekill + reduced rates of imazalil or guazatine, the effect on resistance development should be considered as reduced residue loading with at-risk fungicides will promote resistance development, especially when the partner product used in the mixture does not provide residual protection.

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## **Trial 7. The evaluation of the effect of pre-harvest spraying of sanitisers in the orchard for the reduction of fungal spore load prior to harvesting citrus fruit (March 2007)**

### Introduction

Green mould, caused by *Penicillium digitatum* (Pers.:Fr.) Saccardo and blue mould, caused by *Penicillium italicum* Wehmer, are the most economically important post-harvest diseases of citrus fruits in South Africa, and are responsible for 90% of all the losses caused by the post-harvest pathogens (Christ, 1966). Traditionally, citrus is treated with the post-harvest fungicides, imazalil, thiabendazole (TBZ) and guazatine to control *Penicillium* and other post-harvest decay types. However, with the ongoing pressure exerted by the markets, especially niche markets, to reduce chemical residues and even discontinue the use of some and even all of the chemicals on fruit, some South African producers are already exporting chemical-free citrus to certain niche markets in Europe. With this shift away from chemical residues more and more reliance is being placed on sanitising products and there is also a call for the screening of organic products with possible fungicidal properties to fill or supplement the role of fungicides.

Sanitisers, particularly sodium and calcium hypochlorites, have been used for many years in packhouse flotation tanks as an alternative treatment for post-harvest rots (Brown et al., 1984; Taverner 2001). South African citrus packhouses are now also using quaternary ammonium compounds (quats) successfully in pre-degreening drenches and washing systems in packhouses. In 1997, SARDI conducted a trial to determine how effective sanitisers were at killing fungal spores. The results showed that when non-inoculated oranges are artificially wounded and then put through a sanitiser mixture in a packhouse washing system, little or no decay was observed on the fruit. This showed that sanitisers can kill spores washing off mouldy fruit and contaminating water and can prevent viable spores contaminating clean fruit, also with possible injuries, coming into the washing system (Cunningham, 2002).

The aim of this trial was to establish what effect, if any, the pre-harvest spraying of citrus trees with quats a week prior to harvesting of the fruit would have on the fungal spore load.

### Materials and methods

Two pre-harvest spray trials were done, firstly on cv. Satsuma mandarins at harvest maturity at “Waterval Sitrus” in the Burgersfort production area and secondly on navel oranges at Crocodile Valley Citrus Estate in the Nelspruit production area.

In the first trial, two quats (Sporekill 120g/l and QuattroKill 126g/l) were applied to Satsumas and in the second trial Desogerm was applied to navel oranges. The three quats were applied at the standard recommended concentration of 1 l/ 1000 l (1000 ppm product), and also at a double dosage of 2000 l/ 1000 l specifically to observe for any possible phytotoxicity.

In the first trial on Satsumas, the treatments were applied to three rows of 10 trees. One row served as untreated control and the other two rows were sprayed with the two concentrations of the Sporekill and QuattroKill. In the second trial, three rows of 10 navels trees were sprayed with Desogerm as in the first trial.

The chemicals were applied to individual trees to the point of runoff, 1 week before the fruit was harvested for packing. The applications in the first trial were done on 28 February 2007 and in the second trial on 12 April 2007. Four lug boxes of 200 fruit were picked randomly from the 10 trees of each treatment the following week prior to all the fruit being harvested.

The treated fruit was transported back to CRI in Nelspruit for the trials to be evaluated.

Fifty (50) fruit were randomly selected from each treatment of the 4 lug boxes per treatment. This fruit as then washed in sterile water with a surfactant Tween 20. The washings were then serially diluted (i.e.  $10^1 - 10^6$  dilution factor) aseptically, and 1ml of each dilution was transferred to a potato dextrose agar (PDA) media. The PDA media was seeded with the antibiotic chloramphenicol to prevent any bacterial contamination. The pour plates were left to set and thereafter incubated at 25°C for 7-10 days. After incubation the dilution plate with 30-300 fungal colonies was counted and the number of colonies was multiplied by the dilution factor. The results were then recorded as total plate count of fungal colonies in each quat treatment compared to the colony count from the fruit on the untreated trees. Any specific citrus fungal pathogen colonies were also counted and recorded.

## Results and discussion

The results in Table 4.5.2.1-Trial 7) indicate extremely high total counts in the untreated control samples. However, the counts dropped drastically after spraying with the three quats, even though the counts are still reasonably high. There is very little difference in the counts between the standard (1000 ppm) the higher (2000 ppm) concentrations applied. No phytotoxicity was evident on the fruit rinds. In this case only two cultivars were used, and the fruit was fairly well coloured up when the quats were sprayed. More cultivars and cultivars with greener, more sensitive rinds would have to be screened for phytotoxicity as well. Nevertheless, the results indicate that these products can be used as a “tool” to aid in the reduction of spore load prior to harvesting fruit. Perhaps the ideal situation would be to spray the trees and then harvest the fruit the next day, but this is not always feasible and practical. This would also have to be evaluated.

Work done on preharvest spaying of Satsumas with sanitising agents in New Zealand demonstrated the feasibility of applying sanitisers as a perharvest treatment to reduce the spore numbers on the surface of the Satsumas. The sanitisers sodium hypochlorite (buffered to pH 6.9) and didecyldimethyl ammonium chloride (quats) were the most effective at reducing the spore numbers of *P. digitatum* (Rheinländer et al., 2007).

**Table 4.5.2.1 (Trial 7).** PDA colony counts after serial dilutions of washings from the rinds of navels and Satsumas, sprayed pre-harvest with 3 quaternary ammonium compounds 1 week prior to harvest

Fruit sprayed	Treatments	Colony count	
		Total	Penicillium
Satsumas	Untreated control	$> 300 \times 10^6/\text{ml}$	
	Sporekill - 1000 ppm	$13 \times 10^4/\text{ml}$	$2 \times 10^1/\text{ml}$
	Sporekill - 2000 ppm	$3 \times 10^4/\text{ml}$	
	QuattroKill - 1000 ppm	$11 \times 10^4/\text{ml}$	
	QuattroKill - 2000 ppm	$4 \times 10^4/\text{ml}$	
Navels	Untreated control	$> 300 \times 10^6/\text{ml}$	
	Desogerm - 1000 ppm	$6 \times 10^4/\text{ml}$	$14 \times 10^1/\text{ml}$
	Desogerm - 2000 ppm	$14 \times 10^4/\text{ml}$	

## Conclusions

No further work is planned with the pre-harvest spraying of sanitising compounds for the reduction of fungal spore load in orchards prior to harvesting. The results from these trials will serve as an indication of some activity in reduction of spore load in orchards. Shipping trials would have to be conducted to determine whether this reduction of spore load in the field would result in a similar reduction in storage decay. However, it is not a given

that such a treatment can become a standard recommendation, but this application can be used as a “tool” by producers to supplement a well managed overall orchard sanitation program.

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### 4.5.3 **PROGRESS REPORT: The evaluation of plant growth regulators (PGRs), applied post-harvest, as possible alternatives to 2,4-D sodium salt (Deccomone) for calyx retention on citrus fruit** Experiment 754 (March 2005 – March 2009) by K.H.Lesar (CRI)

## Opsomming

Die plantgroeireguleerders, Retain, Agromos, Bioboost en die nuwe organiese middel Croplife is op suurlemoene herhaal. Califix, ook 'n 2,4-D natrium sout formulاسie, is geevalueer vir addisionele data bo en behalwe die vorige data ingedien vir registrاسie. Die produkte is in water doopbehandelings aangewend vir blomkelk-behoud op vrugte na gesimuleerde verskeping. Goeie blomkelk-behoud deur Retain teen die hoër konsentrasie (500 dpm), in vergelyking met die standaard aanbevole 2,4-D (Deccomone), is waargeneem. Blomkelk behoud deur Califix teen albei konsentrasies is gelyk aan die Deccomone blomkelk behoud. Die laer konsentrasie van Retain en die hoogste konsentrasie van Bioboost is die enigste ander behandelings wat 'n redelike mate van blomkelk behoud op die suurlemoene getoon het.

## Summary

The ongoing evaluation of plant growth regulators, Retain, Agromos and Bioboost and a new organic product Croplife (a nutrient synergist), was repeated on lemons. Califix, also a 2,4-D Sodium salt formulation, was also screened for additional data, over and above the previous data needed for registration. The products were applied to lemons at different concentrations in dip treatments and evaluated for calyx retention on the fruit after simulated shipping. Fairly good calyx retention by Retain at the higher concentration (500 ppm) was recorded compared to the standard Deccomone. Calyx retention by Califix at both concentrations was the same as those levels of retention by the standard Deccomone at the same concentrations. Retain at the lowest concentration (250 ppm) and Bioboost at the highest concentration were the only other treatments that demonstrated a reasonably high degree of calyx retention in these trials on lemons.

## Introduction

Prior to the start of the 2003 citrus season, notification was received from the EU MRL committee informing the citrus industry that the MRL for 2,4-D would be decreased from 2.0 mg/kg to 0.05 mg/kg. 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 retained the 0.05 ppm MRL for the remainder of the 2003 season until a national MRL of 1.0 ppm was set. A harmonised EU MRL of 1.0 ppm was finally set at the start of the 2004 citrus season.

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 (calyx). 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.

## Materials and methods

The following plant growth regulators (PGR's) were evaluated: Retain (aminoethoxyvinylglycine), Agromos (phytoalexin enhancer), Bioboost (phytoalexin enhancer), Croplife (a nutrient synergist) and Califix (2,4-D Sodium salt).

Good, sound, untreated lemons (Larten Estate) 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 a suitable sanitising agent. 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.

The following treatments were conducted:

1. Untreated control – water dip only
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. Califix - 250 ppm
7. Califix - 500 ppm
8. Agromos - 2.4 l/100 l
9. Agromos - 4.8 l/100 l
10. Bioboost - 1.2 l/100 l
11. Bioboost - 2.4 l/100 l
12. Croplife - 140 ml/100 l
13. Croplife - 270 ml/100 l
14. Croplife - 350 ml/100 l
15. Croplife - 500 ml/100 l

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 8°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 any stem-end infections, caused by any of the latent pathogens.

## Results and discussion

Fairly good calyx retention by Retain at the higher concentration (500 ppm) was recorded compared to the standard Deccomone. The calyx retention by Califix at both concentrations was the same as those levels of retention by the standard Deccomone at the same concentrations. This data will be submitted, together with previous data screened on oranges, for registration of the product. There has occasionally been a supply problem with the standard recommended 2,4-D sodium salt formulation, Deccomone, in the industry. Therefore it has been necessary to screen another sodium salt formulation from another source for registration.

Retain at the lowest concentration (250 ppm) and Bioboost at the highest concentration were the only other two treatments that demonstrated a reasonably high degree of calyx retention in these trials on lemons. No stem-end infections by any of the latent pathogens were observed on the fruit evaluated.

**Table 4.5.3.1.** Mean percentage button retention on lemons following dip-treatment with various concentrations of Deccomone, Retain Califix, Agromos, Bioboost and Croplife after 8-week storage at simulated shipping conditions.

Treatments	Mean Calyx Retention (%) <sup>a</sup>
1. Untreated Control	21.92 f
2. Treated Control 250 ppm 2,4-D (Deccomone)	81.25 b
3. Treated Control 500 ppm 2,4-D (Deccomone)	95.85 a
4. Retain 250 ppm	77.07 bc
5. Retain 500 ppm	83.30 ab
6. Califix 250 ppm	83.32 ab

7. Califix 500 ppm	95.82 a
8. Agromos 2.4 l/100 l	60.40 de
9. Agromos 4.8 l/100 l	66.67 cde
10. Bioboost 1.2 l/100 l	58.32 e
11. Bioboost 2.4 l/100 l	72.92 bcd
12. Croplife 140 ml/100l	56.25 e
13. Croplife 270 ml/100l	60.42 de
14. Croplife 350 ml/100l	64.57 cde
15. Croplife 500 ml/100l	62.50 de

<sup>a</sup> Means followed by the same letter are not significantly different (Fisher's Unprotected LSD;  $P \leq 0.05$ )

## Conclusion

The screening of all these products as possible alternatives to the 2,4-D has only revealed one product (Retain) with good activity against calyx retention, compared to the standard 2,4-D. Nevertheless the ongoing screening of other new products will continue.

### 4.5.4 PROGRESS REPORT: Occurrence of *Penicillium* spp. in the citrus supply chain

Experiment PPL 20 (April 2007 - March 2009): by Rene Jacobs and Lise Korsten (UP)

## Opsomming

Een voorsieningsketting is vanaf plaaslike pakhuis gedeeltelik na internasionale verspreidings sentrums gevolg. Vir die identifikasie van moontlike kontaminasiepunte in die voorsieningsketting, is monsters van verskeie oppervlaktes in die ketting geneem. Ons het reeds sewentien *Penicillium* spesies geïdentifiseer. Meeste van die spesies wat geïdentifiseer is, is wel-bekende grondgedraagde saprofiete, dus kan die teenwoordigheid daarvan dien as indikatore van substandaard hygiëniese toestande. 'n Aantal *Penicillium* spp. wat in die voorsieningsketting geïsoleer is, kan gesondheids- en veiligheidsrisikos wees. Dit kan ook dien as inokulumbrone vir die inisiering van na-oes siektes.

## Summary

One supply chain was partially followed from local packhouses to international distribution centres. To identify possible contamination points along the supply chain, various surfaces were sampled. We have identified 17 *Penicillium* spp. involved in the supply chains thus far. Most of these species obtained are well known soil-borne saprophytes, therefore the presence of these species can serve as an indication of substandard hygiene conditions. A number of *Penicillium* species isolated within the supply chain can be a health and safety risk and serve as inoculum for the onset of postharvest diseases.

## Introduction

The citrus export chain is characterised in general by several disruptions in the cold chain. This creates an optimal environment for the onset of postharvest decay and in particular postharvest decay caused by *Penicillium* spp. In preliminary studies conducted by the research group of the University of Pretoria, it became evident that postharvest contamination often occurs further down the export chain with a lack of accountability for these significant economic losses to the South African citrus industry (Jacobs and Korsten, 2004; Johnston et al, 2006).

The supply chain approach focuses on reducing inoculum levels of postharvest pathogens, especially *Penicillium* spp., by monitoring environments throughout the supply chain and developing management procedures to reduce inoculum loads through improved cleaning protocols. We will evaluate the effectiveness of the current citrus production and supply chains from farm to fork to determine critical control points that should be managed more effectively to reduce decay. *Penicillium* isolates obtained will be used as hygiene indicators for environmental and fruit postharvest contamination along the export chain and species dominance and inoculum levels will be determined. A polymerase chain reaction (PCR) diagnostic assay for regular screening of *Penicillium* decay in the citrus export industry will be developed.

## Materials and methods

The cold chain has been followed prior to the onset of this study. The first chain sampling was only focussed on international points to determine possible contamination points, the second sampling was mainly focussed on local sampling. Various environments along the cold chain were sampled to detect the presence of *Penicillium* spp. that may serve as contamination points along the supply chain. Swab samples were taken locally and internationally of surfaces such as packhouse and coldroom walls, floors, container walls and floors, fruit handlers' hands and gloves etc. as illustrated in Table 4.5.4.1. The contaminants on the swabs were dislodged in test tubes containing Ringers and were dilution plated on Malt extract agar (MEA). Air contamination testing was also conducted with an air sampler. All plates were incubated at 25°C for seven days after which pure *Penicillium* cultures were made from these plates. All cultures were preserved on MEA slants and in sterile water.

The *Penicillium* isolates obtained in the cold chain were divided into morphological groups according to their cultural characteristics such as colony texture and formation, conidial colour, secondary metabolite colour and abundance and colour formation of the colony on the underside of the plate (Fig. 4.5.4.1). These and other features evaluated are indicative of specific *Penicillium* species according to Pitt (1991) and Samson and Pitt (2000). If very slight variations were observed in cultural morphology, isolates were divided into different morphological groups; therefore more than one morphological group may represent the same *Penicillium* species. Representative isolates of each morphological group were randomly selected to be used as a representative of the group in further trials. For the morphological identification of the *Penicillium* isolates obtained in the export chain, all isolates were plated on three different species selective media according Pitt (1991). Cultural characteristics, colony growth and microscopic characteristics were evaluated after 7 days incubation at 25°C, 37°C and 5°C respectively.

For the molecular identification, single conidial isolates were obtained prior to DNA extraction of the representative isolates. The internally transcribed spacer (ITS) and the beta-tubulin (Bt-tub) gene regions were amplified for all the representative isolates after PCR optimisation. All the amplicons were sequenced to confirm morphological identification.

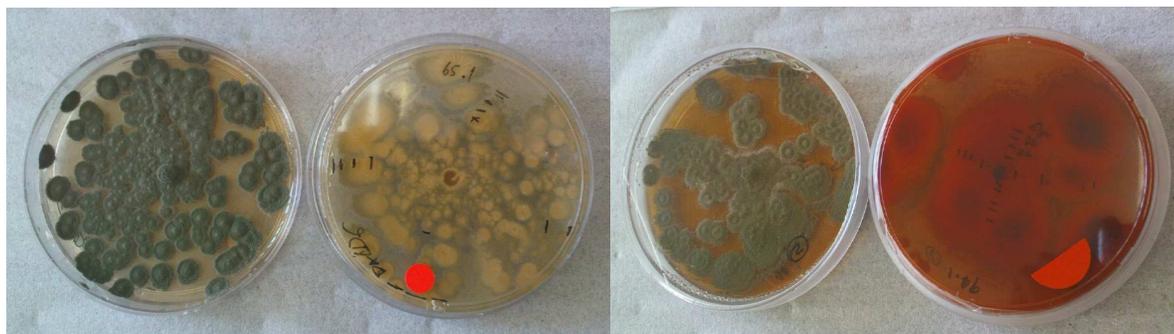
## Results and discussion

At least 264 *Penicillium* isolates were obtained (Table 4.5.4.1), and divided in groups based on colony morphology (Fig. 4.5.4.1). Sequence identities of morphological groups are displayed in table 2. Sequence identities are confirmed with the optimised PCR-RFLP identification system developed for *Penicillium* species in the cold chain.

**Table 4.5.4.1.** The number of isolates obtained locally and internationally from various surfaces in the citrus cold chain isolated during the two export seasons sampled.

Local				International				
Area Sampled	Locations sampled*			Area Sampled	Locations Sampled			
	DT	LC	SS		A	R	H	SD
Packhouse wall	0	0	26	Cold room wall	1	0	0	3
Packhouse floor	23	2	13	Cold room floor	0	0	0	4
Dirty boxes	0	0	8	Repack facility floor	3	0	1	2
Clean boxes	0	0	6	Repack facility wall	1	0	1	0
Pickers hands	10	4	0	Repack surface	18	0	4	1
Taps	0	2	0	Repack surface boxes	5	0	0	0
Sorters' gloves	0	3	0	Truck wall	0	1	0	0
Packers	3	2	0	Truck floor	0	1	0	0
Packing bins	34	15	0	Container floor	0	8	0	0
Graders' gloves	0	2	0	Container wall	0	5	0	0
Picking bags	5	6	0	Distributor	0	0	15	0
Metal rollers	0	5	0	Harbour	6	12	0	0
Grabbing bins	0	2	0					
Sorting bags	0	1	0					
<b>Isolate numbers</b>	<b>75</b>	<b>44</b>	<b>53</b>	<b>Isolate numbers</b>	<b>34</b>	<b>27</b>	<b>21</b>	<b>10</b>

\*0 - does not necessarily mean that those areas had no *Penicillium* isolates since all the surfaces sampled were not present in all areas and regions.



**Fig. 4.5.4.1.** Two *Penicillium* isolates inoculated on malt extract agar being evaluated for their cultural characteristics and exudates production on the top and bottom of the Petri dish.

**Table 4.5.4.2.** Sequence identities of *Penicillium* species isolated in the citrus export chain from local and international regions sampled.

Locally isolated <i>Penicillium</i> species	Internationally isolated <i>Penicillium</i> species
-	<i>Penicillium atramentosum</i>
<i>P. aurantiogriseum/P. commune</i> *	<i>P. aurantiogriseum/P. commune</i> *
<i>P. biourgeianum</i>	<i>P. biourgeianum</i>
-	<i>P. brevicompactum</i>
-	<i>P. citrinum</i>
-	<i>P. commune</i>
<i>P. corylophilum</i>	<i>P. corylophilum</i>
-	<i>P. crustorum</i>
<i>P. crustosum/P. commune</i> *	<i>P. crustosum/P. commune</i> *
<i>P. crysogenum</i>	<i>P. crysogenum</i>
-	<i>P. expansum</i>
<i>P. glabrum</i>	<i>P. glabrum</i>
<i>P. italicum</i>	<i>P. italicum</i>
<i>P. paneum</i>	<i>P. paneum</i>
<i>P. polonicum</i>	<i>P. polonicum</i>
-	<i>P. roqueforti</i>
-	<i>Eupenicillium tropicum</i>

\* These isolates showed inconclusive sequencing results for the two species as indicated since these species are very closely related and fall in the same *Penicillium* sub-group according to Samson and Pitt (2000).

- These isolates were not identified locally during the two seasons sampled.

From the isolation points sampled, it is evident that pickers' hands, picking bins and packhouse walls and floors may serve as contamination sources for postharvest infection of citrus fruit locally. In general on the international side, contamination could be traced back to repack surfaces and bins at the repacking facilities as well as storage facilities at the harbour. The high *Penicillium* counts encountered during these preliminary trials in these areas may be due to poor hygiene practices. Major economical losses due to poor quality fruit on these markets may be due to contamination at the end of the supply chain and not necessary from the on farm situation. These preliminary results should thus be further studied to confirm this hypothesis.

By evaluating the *Penicillium* species isolated during these trials both locally and internationally, *P. glabrum* was by far the most commonly encountered species. This species is generally known as an indoor environmental species and the presence thereof is therefore not uncommon, however, the level of contamination with this species may serve as a hygiene indicator of the indoor environment such as a repack or distribution facility.

*Penicillium* spp. that was isolated from international environments includes *P. brevicompactum*, *P. citrinum*, *P. expansum*, *P. roqueforti* and *Eupenicillium tropicum*. Some of these species are well known toxin producing *Penicillium* species and consumption of these toxins may have health and safety risks associated with it, for

instance with the toxin citrinin that is produced by *P. citrinum*. It is therefore critically important to identify all the *Penicillium* species encountered in the citrus export chain to determine the health and safety risks, source and level of *Penicillium* contamination and critical contamination control points in the cold chain.

All the species that were isolated locally were also isolated internationally. *Penicillium italicum*, which is a well known citrus fruit pathogenic species, was isolated in very low numbers both locally and internationally. Due to this finding, it may be important to evaluate the pathogenicity of these *Penicillium* species, which were isolated locally or internationally, to determine the impact that the presence of these species may have on postharvest decay of South African exported citrus fruit.

## Conclusion

We aim to deliver a greater understanding of genetic population dynamics of *Penicillium* spp. in the global and local context. There will be a better understanding of contamination and control points on both the local and international side of the supply chain. This will help in developing a more sustaining long-term integrated disease management program.

## Technology transfer

Oral presentation at the CRI Research Symposium.

## Further objectives and workplan

- Conduct the citrus supply chain in 2008 and 2009.
- 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 to name a few. Air samples will also be taken.
- Purify isolates, preserve and morphologically group according to cultural and morphological characteristics at different temperature conditions and on different selective growth media.
- Pathogenicity tests of isolated species on citrus fruit.
- Selected dominant reference groups to be amplified using PCR and sequenced for species identification purposes.
- DNA extraction, gene amplification, RFLP and sequencing.
- Primer development and optimisation of the technique.
- Compare partial gene sequences.
- Determine control points along the citrus supply chain.

## References cited

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- Johnston, CL., Jacobs, R and Korsten, L. 2006. Identification of *Penicillium* species in the litchi export chain. *South African Litchi Growers' Association Yearbook* 18: 51-57.
- Samson, R.A. and Pitt, J.I. 2000. Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. Harwood Academic Publishers. The Netherlands.

### 4.5.5 PROGRESS REPORT: Development of alternative disease control products

Experiment PPL 21 (April 2007 - March 2009): by E Arrebola, K Zeeman and L Korsten (UP)

## Opsomming

'n Bakteriese antagonis, oorspronklik geselekteer uit isolate wat op sitrus-oppervlakke voorgekom het, het vroeër belowende effekte teen die groei van na-oes patogene getoon. In hierdie studie is die isolaat geïdentifiseer as *Bacillus subtilis* en sy inhiberende aktiwiteit is bevestig op drie na-oes sitrus patogene. Die bakterie se beheermeganismes is bewys as kompetisie vir spasio en nutriënte, produksie van lipopeptiede soos Bacillomycin en fenoliese sure, asook die produksie van vlugtige stowwe. Verdere navorsing word beplan vir die verdere karakterisering van die fenoliese sure wat deur hierdie bakterie geproduseer word, asook mutasie-

studies om die gene te vind wat verantwoordelik is vir die produksie van die sekondêre verbindings. *In vivo* proewe word ook beplan op sitrusvrugte met die oog op kommersiële aanwending van die biobeheeragent.

## Summary

A bacterial antagonist, originally selected from isolates found on citrus fruit surfaces, has previously shown promising activity against postharvest fungal fruit pathogens. In this study, the isolate was identified as *Bacillus subtilis* and its inhibitory activity was confirmed on three postharvest citrus pathogens. Its mode of action was established as competition for space and nutrients, production of the lipopeptide Bacillomycin and phenolic free acids, as well as the production of volatile compounds. Further research is planned on characterisation of the phenolic free acids and volatile compounds produced, as well as mutation studies to find the genes responsible for the production of secondary compounds. *In vivo* trials will also be conducted on citrus fruit for commercial application of the biocontrol agent.

## Introduction

Several *Bacillus* strains have been considered to be natural factories of bio-active compounds such as lipopeptides, and the significance of their involvement in plant microbial disease control have been demonstrated (Asaka and Shoda 1996; Emmert and Handelsman 1999). Lipopeptides are the most frequently produced antibiotic compounds by bacilli, exhibiting a wide antimicrobial spectrum and exceptional surfactant activities (Magnet-Dana and Peypoux 1994; Vanittanakom et al. 1986; Vater et al. 2002; Vollembroich et al. 1997). These amphiphilic compounds are classified into three families: the iturin family, represented by iturin A, mycosubtilin, and bacillomycin (Thimon et al. 1995; Duitman et al. 1999; Tsuge et al. 2001; Moyne et al. 2004); the fengycin family, including the related plipastatin (Vanittanakom et al. 1986, Steller et al. 1999); and the surfactin family, which, although generally not fungitoxic, are possibly the most powerful biosurfactants described and are thought to facilitate fungitoxic activity, also exhibiting antiviral characteristics (Magnet-Dana et al. 1992). Surfactins show a strong synergistic action in combination with iturin A and also seem to be a key factor in the establishment of stable biofilms, which may inhibit the biofilm formation of other bacteria, thus contributing to their protective action.

Apart from secondary compounds excreted by bacteria into their surrounding media, some also produce volatile compounds. Isoprene, or isopentenyl diphosphate (IDP), is the general precursor of all terpenoids, representing a very diverse class of natural products (Kuzman *et al.* 1995). Bacteria use the precursors IDP and DMADP for the synthesis of several secondary compounds. Several bacterial species also use these precursors to synthesise isoprene, which is emitted into their environment. *Bacillus subtilis* typically emits isoprene in high levels compared to other bacterial species. The function of the emitted isoprene has been postulated as being a signal molecule in the natural environment of the microorganism. Another possible explanation for the emitted isoprene is the efflux as an overflow metabolite in the bacterial pathway to isoprenoid structures. The uptake of isoprene by microorganisms present in soil samples has been described as a sink of atmospheric isoprene (Cleveland and Yavitt 1998). However, there is no full evidence supporting the hypotheses mentioned.

Fungal postharvest diseases are some of the most important limiting factors for successful fruit export in South Africa. The management of these fungal diseases are strongly dependent on chemicals. The aim of this work is to evaluate the possibility of exploiting antagonistic bacteria in the post-harvest biological control of the fungal pathogens *Penicillium digitatum*, *Alternaria citri* and *Colletotrichum gloeosporioides*. Among a collection of bacterial strains isolated from citrus fruit or leaf surfaces, a potential antagonist has been selected by means of screening methods based on *in vitro* competition, and was identified as *Bacillus subtilis*. The isolate proved to be effective in the control of fungal diseases in *in vitro* assays. The following objectives were proposed:

1. Identification of the antagonistic bacterial species using *in vitro* and *in vivo* screening
2. Evaluation of its antagonistic effect/s.
  - 2.1 Analysis of lipopeptides produced by the antagonist.
  - 2.2 Analysis of phenolic free acids produced by the antagonist.
  - 2.3 Analysis of volatile compounds produced by the antagonist.
3. In packhouse screening

## Materials and methods

### 1 – Identification of the antagonistic bacterial species and evaluation of its antagonistic effect/s.

Bacteria were routinely grown on nutrient agar (NA, Biolab, Johannesburg) at 30°C for 24h. The bacterial isolate, PPCB002, which showed promising antifungal activity on PDA plate assays, was identified according to biochemical and physiological tests. The tests carried out for preliminary characterisation included Gram staining and endospore formation studies. Further characterisation was performed using the analytical profile index (API) 20E and API 20NE test strips (BioMérieux, Marcy-l'Etoile, France) (Holt *et al.* 1994; Földes *et al.* 2000; Walker *et al.* 1998), as well as 16S-rDNA sequencing.

To obtain 16S-rDNA sequences, colony polymerase chain reaction (PCR) was performed on the isolate using primers 1486R-P and 41-F (Stackebrandt and Goodfellow 1991). The resulting PCR product was purified and used directly for sequencing. Homology studies were carried out using the NCBI program BLAST. The genetic sequences of the two primers used are 1486R-P: 5'GCTACCTTGTTACGACTTCGTCCC3' and 41-F: 5'GCTCAGATTGAACGCTGGCG3'

Antagonism was evaluated *in vitro* as the ability to compete for space and nutrients on agar plates. The strain was cultivated for the assay on Standard-1 agar (STD1, Biolab) from preserved cultures, stored at -18°C, and tested against fungal postharvest citrus pathogens *P. digitatum*, *A. citri* and *C. gloeosporioides*. The fungi were spread-plated onto PDA plates, left to dry and thereafter stab-inoculated with the bacteria on the same plates. The bacterial strain PPCB002 was inoculated on fungal plates with two lipopeptide producing controls, UMAF6614 and UMAF6639, for comparative purposes. The culture plates were incubated at 25°C and evaluated weekly for formation of inhibition zones.

## **2 – Analysis of lipopeptides produced by the antagonist.**

### **2.1 Identification of lipopeptides of *B. subtilis***

In order to identify the compounds responsible for antifungal activity, methanolic fractions from the butanolic extracts of cell-free culture filtrates of the antagonist were initially separated on silica thin-layer chromatography (TLC) sheets, using as control the characterised strains UMAF6614 and UMAF6639, which were analysed using purified iturin A, fengycin, and surfactin as standards (data not shown), and the methanolic extracts were analysed by reverse-phase high-performance liquid chromatography (RP-HPLC). Three main groups of peaks were observed at elution times comparable with those observed for standard lipopeptides.

### **2.2 Lipopeptide quantification**

Quantification of lipopeptide extracts using Folin Ciocalteu's reagent was originally used in phenolic quantification, but can also be used in protein quantification. The procedure was adapted for analysis using a 96-well ELISA plate. The results were read at  $\mu = 690$  in an ELISA plate reader. These results were expressed as mg/ml gallic acid equivalent.

### **2.3 Antagonism on plates using 60 $\mu$ g of lipopeptides of each sample**

To compare the inhibition ability of antagonist PPCB002, the same amount of lipopeptides (60  $\mu$ g) was screened against the three fungal pathogens, as this was the minimal volume (5  $\mu$ l) that showed pathogen inhibition. The extract was titrated onto sterilised paper discs on Petri dishes after spread-plating each pathogen onto the agar. Methanol was used as solvent, and was therefore included as control. Statistical evaluation was performed by one-way ANOVA using SPSS 8.0 software.

## **3 – Analysis of phenolic free acids produced by the antagonist.**

Analysis of the phenolic free acids on TLC plates was originally performed in Spain and the quantification of phenolic concentrations was continued using the Folin-Ciocalteu method. The production and extraction of free phenolic acids was optimised to obtain a higher yield at a lower cost as follows: Fresh antagonist cultures were inoculated into 50 ml APM medium in a 250 ml Erlenmeyer flask and shake-incubated (100 rpm) at 37°C for 5 days. The tubes were centrifuged at 5000 rpm for 5 min. and the supernatant was filtrated using double Watman filter paper. The pH was lowered using 2% acetic acid in 10 ml volume. Extraction was performed twice with equal volumes of diethyl-ether. Finally, the solutions were left to air-dry completely and re-dissolved in 5 ml of methanol.

Using the phenolic extracts from the new optimised protocol, two types of antagonism assays were performed on the postharvest fungi using paper discs on the fungal plates: the first method assessed the activity of the same volume of phenolic extracts than lipopeptides (Ph) to maintain the relationship between lipopeptides and

phenolic production; the second using the same amount (weight) than the lipopeptides (Ph60), i.e. using 60 µg of each sample. Methanol, which was used as solvent, served as control against the antagonist extract.

High performance liquid chromatography (HPLC) was also performed on the bacterial phenolic extracts and characterised where possible, using available standards (Dept. Biochemistry, University of Pretoria).

#### 4 – Analysis of volatile compounds produced by the antagonist.

In this trial, we aimed to identify the production of fungus-inhibiting volatiles by the selected antagonist strain. The principal objective was to screen the inhibitory activity of the volatile compound/s produced by the antagonist, presumably involved in the biological control of fungal plant pathogens.

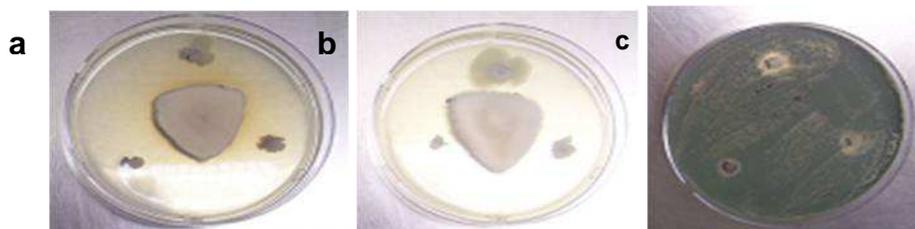
To establish the antifungal capacity of the volatile compounds a preliminary assay was done, using an *in vitro* Petri dish system. Nutrient rich media for both bacteria and fungi (Nutrient agar and PDA respectively) were used in separate Petri dishes. The bacterial strain was inoculated using sterile cotton swabs and the fungi by transferring a 3x3 mm plug from an actively growing fungal culture in the centre of the PDA plate. The plates were inverted, joined together using Parafilm and incubated at 25°C for 10 days. The results were indicated as differences in fungus growth diameter (mm) on the plates. A positive control was included, which was not inoculated with bacteria.

### Results and discussion

#### 1 – Identification of the antagonistic bacterial species and evaluation of its antagonistic effect/s.

The biochemical tests and API results both revealed that the antagonist was *Bacillus subtilis*. Furthermore, the PCR-sequencing resulted in a gene sequence size of 915 bp. Homology studies with the NCBI program BLAST, available databases and gene libraries confirmed the identity of the antagonist as *B. subtilis* (99.78% certainty). Comparison with the Nucleotide Collection of NCBI revealed

In the fungal plate studies, inhibition zones, restricting fungal growth, were clearly visible on the culture plates after one week of incubation (Fig. 4.5.5.1). Inhibition zones were present on all the plates, but the strongest inhibition was found on the plates of *A. citri* and *C. gloeosporioides*.



**Figure 4.5.5.1.** *In vitro* effect of antagonistic bacterial strain PPCB002 (bottom right colony), compared with UMAF6614 (top) and 6639 (bottom left), against citrus postharvest pathogens (a) *Alternaria citri*, (b) *Colletotrichum gloeosporioides* and (c) *Penicillium digitatum*.

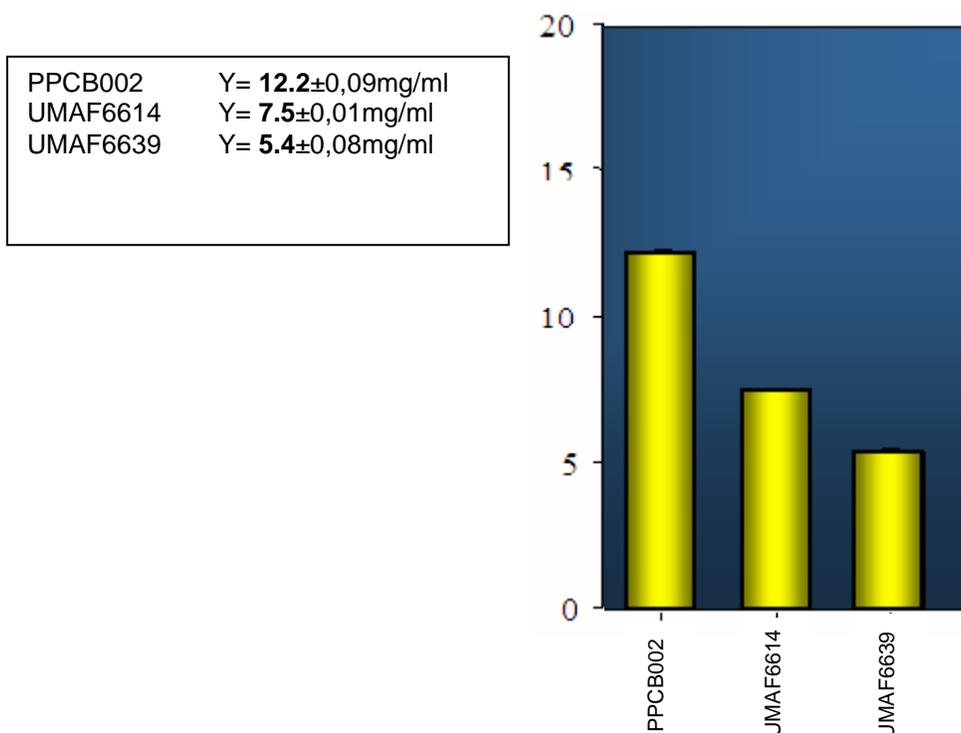
#### 2 – Analysis of lipopeptides produced by the antagonist.

Three main groups of peaks were observed at elution times comparable with those observed for standard lipopeptides. The *Bacillus* strains produced spots with Rf values similar to the standards fengycin (Rf = 0.09), iturin A (Rf = 0.3), and surfactin (Rf = 0.7).

Quantification of lipopeptide extracts using Folin Ciocalteu's reagent, when read at  $\mu = 690$ , are presented in Fig. 2. The results indicated that the antagonist is a strong lipopeptide producer compared to the two control strains. In the lipopeptide antagonism essay, the strongest lipopeptide activity was observed against *C. gloeosporioides* (Table 4.5.5.1). The statistical evaluation of the results is given in Table 4.5.5.2.

The formula used to determine the phenolic content per sample (gallic acid equivalent) is given below:

$$Y=1.3527X-0.019$$



**Fig. 4.5.5.2.** Lipopeptide quantification of *Bacillus subtilis* antagonist strain PPCB002, with UMAF6614 and UMAF6639 as control isolates.

**Table 4.5.5.1.** Bacterial lipopeptide antagonism assay (60 µg) after 1 week incubation

Lip60 (mm)	Control		PPCB002	
	<i>Alternaria citri</i>	24 <sup>a</sup>	00	26
<i>Colletotrichum gloeosporioides</i>	19	00	25	04
<i>Penicillium digitatum</i>	21	00	22	00

<sup>a</sup> For both the control and antagonist, the first column represents the distance from the fungus inoculation point to the paper disc centre, and the second column is the inhibition radius (mm).

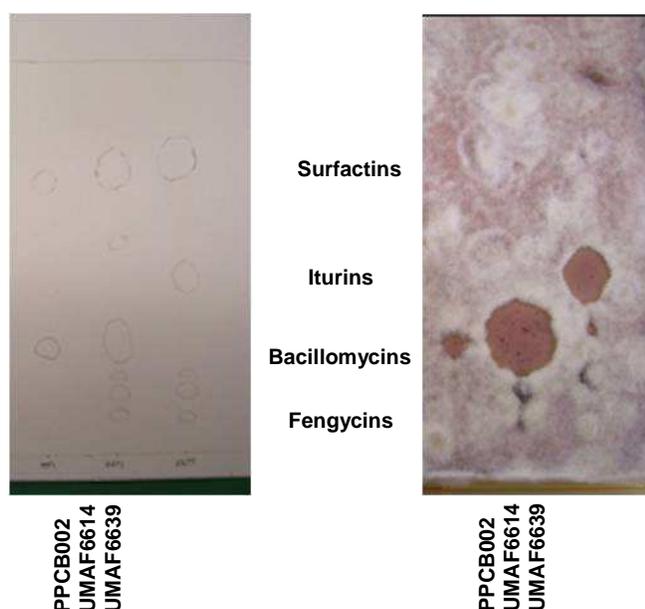
**Table 4.5.5.2.** Statistical comparison of lipopeptide analysis by means of one-way ANOVA

Lip60	Control	PPCB002
<i>Alternaria citri</i>	a	B
<i>Colletotrichum gloeosporioides</i>	a	B
<i>Penicillium digitatum</i>	No significant difference	

When the same amount (60 µg) of lipopeptide extract was used to perform TLC bioautography assays with the antagonist and the two control strains, inhibition zones were visible where fungitoxic compounds were present, e.g. for *C. gloeosporioides* the following were observed, (spots listed from the bottom of the plate upwards):

- |   |  |
|---|--|
| 1 <sup>st</sup> group of spots: 2 cm (Rf = 0.14)    | Fengycins (no visible production by isolate PPCB002) |
| 2 <sup>nd</sup> group of spots: 4 cm (Rf = 0.28)    | Bacillomycins (produced by PPCB002 and UMAF6614)     |
| 3 <sup>rd</sup> group of spots: 6.5 cm (Rf = 0.46)  | Iturins (only produced by UMAF6639)                  |
| 4 <sup>th</sup> group of spots: 10.5 cm (Rf = 0.75) | Surfactins (produced by all the isolates).           |

Antifungal activity was visibly exhibited by the Bacillomycins of isolate PPCB002, although the activity was not as high as the standards used in the trial (Fig. 4.5.5.3).



**Fig. 4.5.5.3.** Antifungal activity of antagonist and control strain lipopeptide extracts on *Colletotrichum gloeosporioides*

### 3 – Analysis of phenolic free acids produced by the antagonist.

The optimised procedure to quantify phenolic free acids yielded a concentration of  $2 \pm 0.06 \mu\text{g}/\mu\text{l}$  phenolic compounds (gallic acid equivalent) for isolate PPCB002. The inhibition zone measurements are compared in Table 4.5.5.3, which revealed that there was no significant variation between the extracts used, whether applying the extracts on volume or weight basis.

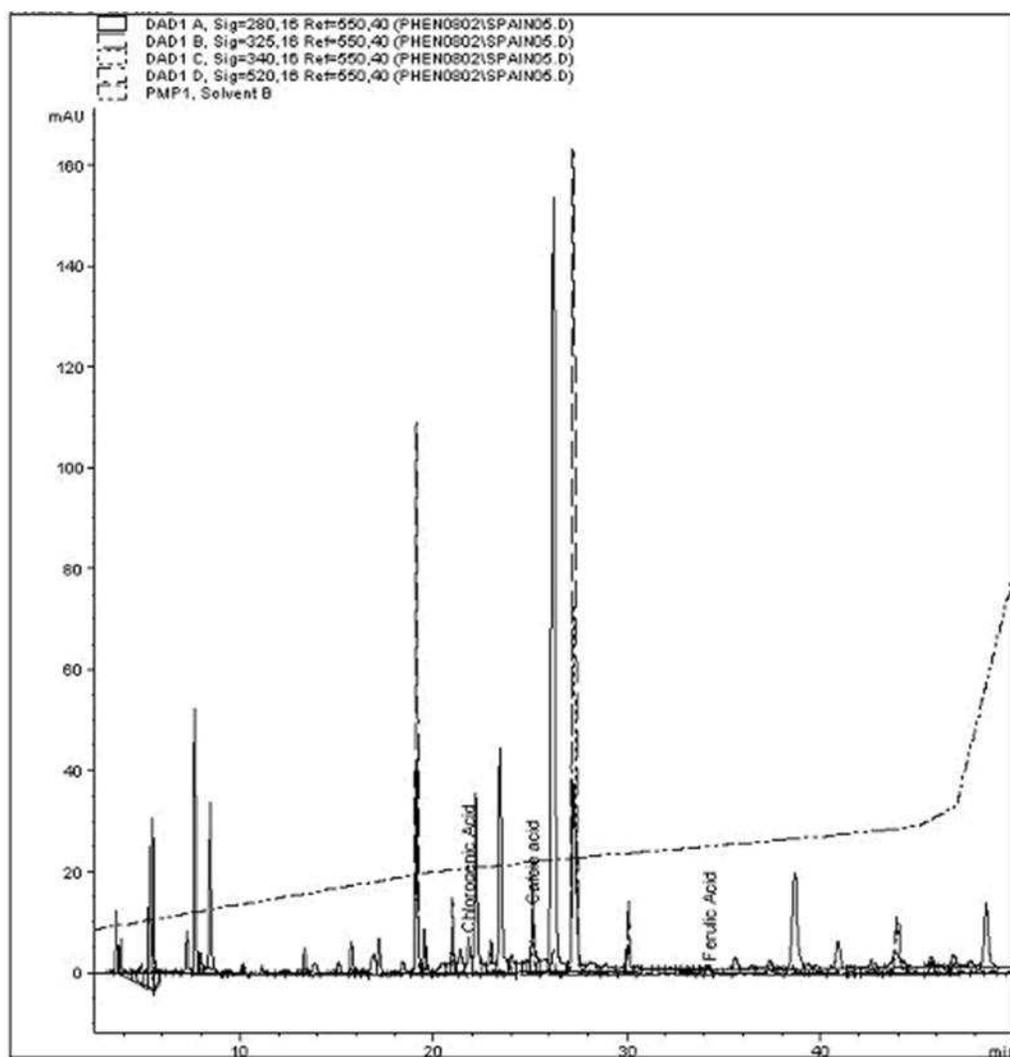
When HPLC analysis of the phenolic extracts were performed, several peaks (indicating compounds produced) were observed, although the majority were not possible to identify (Fig. 4.5.5.4), partly due to an insufficient range of standards as well as complications accessing commercially available phenolic standards. However, the HPLC spectrum clearly suggests a broad range of phenolic compounds produced. Further analyses are planned in collaboration with the Dept. Plant Sciences (University of Pretoria).

**Table 4.5.5.3a.** Antagonist assays *in vitro* on fungal plates, using the same volume of extracts (Ph) vs. the same weight of phenolics (Ph60) as used for the lipopeptide extracts

Pathogenic fungus	Ph (mm)				Ph60 (mm)			
	Control		PPCB002		Control		PPCB002	
<i>Alternaria citri</i>	24	00	26	07	22	00	29	11
<i>Colletotrichum gloeosporioides</i>	21	00	27	04	19	00	25	04
<i>Penicillium digitatum</i>	19	00	23	00	20	00	24	00

**Table 4.5.5.3b.** Statistical comparisons by means of one-way ANOVA

Ph	Control	PPCB002	Control	PPCB002
<i>Alternaria citri</i>	a	b	a	B
<i>Colletotrichum gloeosporioides</i>	a	b	a	B
<i>Penicillium digitatum</i>	No significant difference			



**Fig. 4.5.5.4.** Chromatographic analysis of phenolic compounds produced by antagonist PPCB002.

#### 4 – Analysis of volatile compounds produced by the antagonist

The measurements of the visual inhibition effect are compared in Table 4.5.5.4. The fungus inhibited best by the volatiles from PPCB002 was *C. gloeosporioides*, followed by *A. citri*. According to these results, the volatiles are not responsible for inhibition of *P. digitatum*.

**Table 4.5.5.4.** Inhibition of citrus pathogens by antagonistic volatile compounds in rich medium (nutrient agar), expressed both as fungal colony diameter ( $\emptyset$ ) and percentage (%) of fungal radial growth compared with the control.

$\emptyset$ in mm	Control	PPCB002
<i>Alternaria citri</i>	65	62
<i>Colletotrichum gloeosporioides</i>	80	54
<i>Penicillium digitatum</i>	80	80
% growth	Control	PPCB002
<i>Alternaria citri</i>	100	95,4
<i>Colletotrichum gloeosporioides</i>	100	67,5
<i>Penicillium digitatum</i>	100	100

## Conclusion

The results obtained through these studies indicated that the mode of action of the selected antagonist includes competition, lipopeptide and phenolic compound production, as well as emission of volatiles. This variety of antagonistic activity would establish a "hurdle system" in the fruit environment, which would make it more difficult for fungal pathogens to establish and cause postharvest fruit diseases, or to easily build up resistance against antagonistic strains.

## Technology transfer

Results were presented at the UP Plant Pathology's citrus biocontrol discussion group.

## Further objectives and workplan

Further studies are currently conducted with other *Penicillium* spp., which are currently found in the citrus production chain. Current studies are also being conducted to determine the genes responsible for the secondary compounds produced, and also to characterise these compounds. We are also continuously isolating new seasonal pathogens, which could potentially be sensitive to the selected antagonist or other strains. During the current season, packhouse trials will be done to test product performance *in situ*. The final aim is to implement these antagonists into an integrated pest management programme.

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#### 4.5.6 PROGRESS REPORT: Imazalil and guazatine fungicide resistance screening of dominant *Penicillium* species isolated from all citrus producing regions in South Africa Experiment PPL 23 (April 2007 - March 2010): by R Jacobs and L Korsten (UP)

### Opsomming

Patogeen weerstandsontwikkeling teen na-oes swamdoders wat gereeld in verskeie industriële gebruik word, was nog altyd 'n risiko. Twee van die mees algemene swamdoders wat in die sitrus industrie gebruik word, is imazalil en guazatine. As gevolg van die oormatige gebruik van die produkte is dit noodsaaklik dat die *Penicillium* populasies wat in die sitrus industrie vir enige weerstand teen die swamdoders getoets word. *Penicillium* isolate is vanaf verskeie pakhuse in die belangrikste sitrus-produiserende areas van Suid Afrika geïsoleer. Hierdie isolate het deur 'n voorlopige toetsfase gegaan waartydens die dosis reaksie getoets is by laer en hoër limiete rondom die aanbevole dosis. Die *Penicillium* isolate met besonder hoë weerstandsvlakke is geïdentifiseer om die monster grootte te verklein. Die ander *Penicillium* isolate sal verder getoets word vir weerstand teen laer swamdoder konsentrasies waarvolgens die EC50 waardes bepaal sal word. Tydens hierdie voorlopige studie is indikasies van hoër weerstand teen guazatine gevind, alhoewel hoë-vlak weerstand wel ook teen imazalil gevind is.

### Summary

Pathogen resistance development to postharvest fungicides used regularly in various industries has always been a threat. Two of the most commonly used fungicides in the citrus industry are imazalil and guazatine. Due to the overzealous use of these products, *Penicillium* populations found in the citrus industries of South Africa should be screened for any resistance to these fungicides. *Penicillium* isolates were isolated from various packhouses in the major citrus producing regions of South Africa. These isolates have gone through a pre-screen phase by which the dose response was tested at higher and lower limits around the recommended dose. The *Penicillium* isolates with very high resistance levels were identified to reduce the sample size. The remaining *Penicillium* isolates will be tested further for resistance at lower fungicide concentrations to determine the EC50 values. Indications of higher resistance levels to guazatine were observed during these preliminary studies.

### Introduction

*Penicillium* is one of the most commonly encountered fungal species in the atmosphere, which makes it ubiquitous. Some species are soil-borne and others prefer decaying vegetation or any wet, humid area or surface (Pitt, 1991). The main purpose of *Penicillium* in these areas is the decay of dead or dying organic matter.

Citrus fruit are undergoing various postharvest chemical and enzymatic changes with the secretion of water and cellular components, which creates the optimal acidic environment for *Penicillium* conidial attachment, germination and colonisation of fruit. Postharvest *Penicillium* decay is therefore a common phenomenon in the citrus industry. *Penicillium* postharvest decay is responsible for major economic losses and is generally controlled by chemical fungicides, of which the most important of these to the South African citrus fruit industry are imazalil and quazatine.

The objectives of this study are the identification of the *Penicillium* species associated with citrus fruit decay by screening *Penicillium* isolates obtained from infected fruit at various citrus packhouses in all the citrus producing regions of southern Africa (this aspect of the study overlaps with Exe# PPL20: Occurrence of *Penicillium* spp. in the citrus supply chain). These species will be screened *in vitro* for their possible resistance to the commercially used chemical fungicides, imazalil and guazatine. With these data, we will be able to determine the effectiveness of these chemicals in controlling *Penicillium* growth.

## Materials and methods

This project was initiated by Plant Pathology Laboratories, University of Pretoria, in July 2007. 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 Prof. Mildenhall and Lynn Trollop from KATCO. Upon arrival, 53% of the isolates received were plated on malt extract agar (MEA) (Merck) amended with chloramphenicol. The remaining 47% of the isolates could not be used due to mite or fungal contamination.

For the second facet of this project, trans-swabs (5 to 6 per packhouse) were sent in protective envelopes to 250 packhouses in all the major citrus producing regions of southern Africa. Methods of aseptic sampling from fruit lesions were included to ensure that only *Penicillium* spp. causing postharvest decay of citrus fruit were isolated and contamination from external sources could be excluded. The University of Pretoria received inoculated swabs from 34 of the citrus packhouses between July and November 2007 for fungicide resistance analysis. All the swabs received were processed by dilution plating of the -3 to -6 swab dilutions containing Ringers (Merck) onto MAE to ensure that low conidial counts could be detected and isolated as well.

The isolates from both project facets (146 from the first project facet with KATCO isolates and 342 from the second project facet with swabs) were purified and preserved on MEA culture plates, MEA slants and in sterile water (Fig. 4.5.6.1). All isolates were grouped into 94 morphological groups based on identical cultural characteristics and morphological identification of representative isolates were 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, based on the characteristics on all three species-specific media, represent a different *Penicillium* species. If a slight variation in cultural characteristics was observed, isolates were divided into another morphological group; therefore more than one morphological group can be representative of the same *Penicillium* species. A representative isolate was chosen randomly from all identical isolates of the 94 morphological groups to represent the characteristics of the group. If a morphological group contained a large number of isolates, more than one representative isolate was chosen for that group and used in further trials. Single conidial isolates were produced from the morphological group representative isolates and were used in further trails.



**Figure 4.5.6.1.** A frontal and reverse image of a *Penicillium* isolate

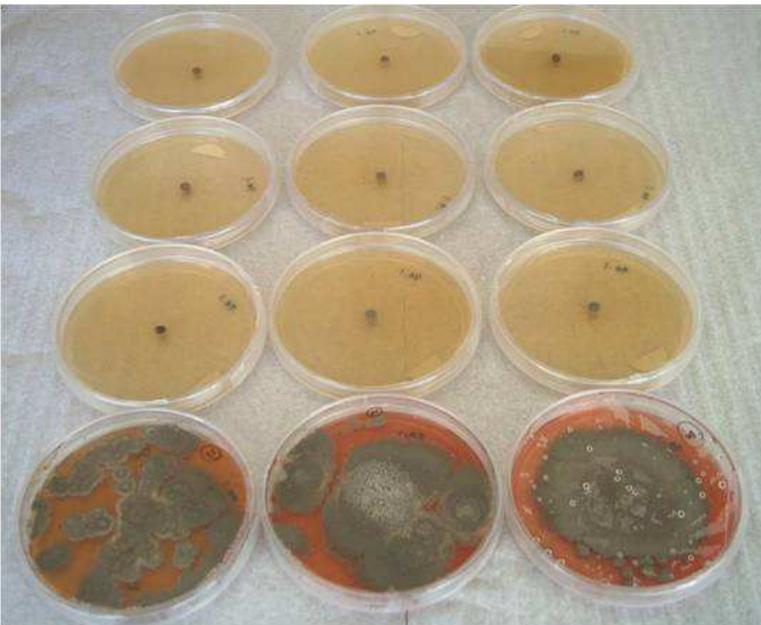
In the pre-screening phase, we have screened for *Penicillium* species that show resistance at extremely high fungicide levels to be able to reduce the sample size to a more manageable size when testing for EC50-values. For this extreme resistance testing, 5 mm disks containing cultural growth of the representative isolates were inoculated in triplicate onto the centre of Petri dishes containing MEA amended with 250 ppm (50%), 500 ppm (100%) or 1000 ppm (200%) strength of the recommended imazalil fungicide dose or 504 ppm (50%), 1008 ppm (100%) or 2016 ppm (200%) strength of the recommended guazatine fungicide dose. Three Petri dishes containing MEA without any fungicide were also inoculated with a 5 mm culture disc of each representative isolate. The 2256 cultures (for both imazalil and guazatine resistance trials) were incubated at 25°C for 8 days. Cultural growth was assessed every second day in duplicate for each culture to determine the *Penicillium* growth rate inhibition by the fungicides evaluated. Growth rate on the amended fungicide plates were compared with the control plates to determine resistance or sensitivity to the fungicides (Fig. 4.5.6.2). Isolates were regarded less

resistant at extremely high fungicide dose levels if no growth was observed on the culture plates after 8 days incubation at 25°C (Fig. 4.5.6.3).

### Results and discussion



**Figure 4.5.6.2.** Growth inhibition of a *Penicillium* isolate inoculated in triplicate on malt extract agar (MEA) amended with 50% (second from left), 100% (second from right) or 200% (far right) the recommended fungicide dose for the dose response testing after eight days incubation at 25°C. Control plates containing only MEA (far left) are also included for comparative purposes



**Figure 4.5.6.3:** Growth inhibition of a *Penicillium* isolate inoculated on malt extract agar (MEA) amended with 50% (top), 100% (second row from top) or 200% (third row from top) the recommended fungicide dose after eight days incubation at 25°C. Control plates containing only MEA (bottom row) are also included for comparative purposes

**Table 4.5.6.1.** Percentage of *Penicillium* isolates isolated from citrus packhouses displaying extremely high levels of resistance as determined by the dose response reaction at three different fungicide concentrations tested.

Isolates	Imazalil			Guazatine		
	50%	100%	200%	50%	100%	200%
UP (342)	13%	11.5%	9.2%	65.7%	55.2%	21.1%
KATCO (146)	34.8%	33.3%	30.5%	74.8%	71.7%	38.5%

Please note that isolates not identified as extremely resistant in this pre-screening trial is not necessarily sensitive to the tested fungicides. EC50 values (presently being determined) will indicate resistance of these isolates.

UP = Isolated by University of Pretoria; KATCO = Isolated by KATCO

From this first phase pre-screening for imazalil and guazatine resistance, it was clear that there are *Penicillium* isolates, that were isolated from fruit in citrus packhouses around the country, that display a very high level of resistance to the fungicides imazalil and guazatine. In various instances, some isolates showed a high level of resistance to both fungicides. A larger proportion of the *Penicillium* isolates tested were able to grow at the elevated concentrations of guazatine, compared with the situation for imazalil..

## Conclusion

Typical resistance development in DMI fungicides, such as imazalil, involves a gradual or step-wise shift in sensitivity. The findings obtained in this first phase screening of isolates should thus be viewed in this context, and therefore most probably represent an under-estimation of the imazalil and guazatine resistance development in southern African citrus packhouses. Concentrations used to discriminate between sensitive and less sensitive / resistant isolates are generally markedly lower (i.e. below 1 ppm) (Holmes and Eckert, 1999; Zhu et al., 2006; Ghosop et al., 2007; Kinay et al., 2007). However, a remarkable proportion of the isolates tested was able to grow on MEA amended with 250 to 1000 ppm imazalil or 500 to 2000 ppm guazatine, which might be indicative of advanced levels of resistance development.

## Technology transfer

Research paper and presentations to CRI.

## Further objectives and workplan

- Second phase screening of isolates at lower dosages
- Statistical analysis of tests
- Molecular species identification and confirmation.
- Screen resistant isolates further to detect variation in fungicide resistance within a morphological group and species.
- Screen some non-resistant species further to detect variation in fungicide resistance within a morphological group and species at various fungicide concentrations.
- Statistical analysis of data for growth rate inhibition in assistance of a statistician.
- Statistical analysis of data significance in resistance between morphological groups and species and between fungicides in assistance of a statistician.
- Draw various conclusions regarding *Penicillium* resistance levels for imazalil and guazatine within and between *Penicillium* spp.
- Develop an integrated control strategy for resistance management

## Project facet that will link with the CRI project (850) of K. Lesar

- National and regional (packhouse) status of imazalil and quazatine resistance of *Penicillium*.
- *In vivo* studies demonstrating whether these resistance classes constitute “practical resistance” (i.e. loss of control following packhouse treatment). It is important to note that at the present stage we have only demonstrated laboratory resistance and that the CRI should comment to growers/packhouses that this does not necessarily mean loss of control, as this aspect must first be proven in the *in vivo* studies. This aspect will be done jointly by CRI and UP.
- Formulate a protocol for routine screening of *Penicillium* species for imazalil and quazatine resistance, which encompasses sampling strategies, discriminatory fungicide concentrations that will indicate practical levels of resistance, etc.

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### 4.6 PROJECT: FRUIT AND FOLIAR DISEASES

Project coordinator: G.C. Schutte (CRI)

#### 4.6.1 Projekopsomming

Die Alternaria bruinvlek beheerproef in die winterreënvalstreek van die Wes Kaap kon nie voltooi word nie omrede die landgoed finansiële probleme ondervind het en besluit het om, nadat twee bespuitings toegedien is, die boord uit te stoot. Daarenteen is suksesvolle proewe in die somerreënvalstreek uitgevoer. Resultate toon dat drie behandelings bestaande uit drie strobilurin en mancozeb tankmengsels met óf minerale spuit olie óf Sporekill, het goeie beheer van Alternaria bruinvlek gegee. Kwekers kan dus tot 5 spuit rondtes spaar. Die resultate kon selfs beter gewees het indien daar net voor oes 'n laat mancozeb (200 g/100 l water) bespuiting toegedien was omrede daar reën geval het tot 24 April 2007. Hierdie waarneming wys op die waarde van siektevoorspelling (word in 'n nuwe projek in samewerking met Spaanse navorsers vanaf 2008 bestudeer). Beide die standaard maandelikse toedienings (8) van koperoksichloried en mancozeb het beide goed teen Alternaria bruinvlek gevaar. Alhoewel Pennfluid ('n nuwe vloeibare mancozeb formulاسie) Alternaria bruinvlek goed teen 200 g/100 l water beheer het, het dit fitotoksiteit tot gevolg gehad. Die drie nuwe WG formulاسies bestaande uit kopersulfaat en koperhidroksied viz. Cuprofix (kopersulfaat), C40 (kopersulfaat + mancozeb) and DPX (koperhidroksied) het almal goed presteer teen dosisse van 100 g, 100 g and 150 g/100 l water onderskeidelik. Dit was interessant om daarop te let dat hierdie WG formulاسies baie minder koperstippelvorming tot gevolg gehad het in vergelyking met die WP formulاسies.

Sitrus blaar- en vrugsiektes word meestal deur hoë-volume swamdodersspuite beheer. Hierdie spuite lei meestal tot groot mates van afloop. Vanaf aanvanklike resultate uit die spuittoedieningsprojek was dit duidelik dat biologiese effektiwiteit van spuite met toenemende afloop afneem. Navorsing in hierdie projek sal dus op optimisering van spuittoediening fokus om voldoende bedekking met minimale afloop te verseker. Drempelwaardes vir biologiese effektiwiteit (80% beheer van Alternaria bruinvlek) is bereken as 3 tot 4% vir kwantitatiewe en 30 tot 40 vir kwalitatiewe bedekking. Biologiese effektiwiteitstoetse sal herhaal word, maar met 'n verbeterde inokulasie-metode met beter kwantifisering van infeksie. Drempelwaardes sal dan met bedekkingwaardes na kommersiële toediening met standaard spuitpomp vergelyk word. Fotografie en beeldanalise was groot bottelnekke en hierdie stappe moes verbeter word. Nuwe toerusting is aangeskaf om die robuustheid, draagbaarheid en tydseffektiwiteit van hierdie stappe te verbeter. Dit is nou heelwat verbeter en deurvloei van monsters is nou 8 maal versnel.

### Project summary

The Alternaria brown spot trial that was executed in the winter rainfall region of the Western Cape could not be completed because the Estate ran into financial difficulties and decided to pull out the orchard after two applications have been applied. However, the trial in the summer rainfall region was more successful. Results showed that three applications consisting of three strobilurins and mancozeb tank mix applications with either mineral spray oil or Sporekill, gave good control of Alternaria brown spot, hereby saving growers 5 spray rounds. The results with all these treatments could have been better if a late mancozeb treatment (200 g/100 l water) was applied at the end of the season as rain spells still continued until the 24 April 2007. This observation demonstrates the potential of disease prediction (which is being researched in a new 2008 project in

collaboration with Spanish researchers). Both the standard copper oxychloride and mancozeb spray programmes sprayed at monthly intervals (8 applications), performed well at registered rates of 200 g/100 l water against *Alternaria* brown spot. Although Pennfluid (a new liquid mancozeb formulation) controlled *Alternaria* brown spot very well at a rate of 200 ml/100 l water, severe phytotoxicity problems were observed. Three new WG formulations of copper sulphate and copper hydroxide viz. Cuprofix (copper sulphate), C40 (copper sulphate + mancozeb) and DPX (copper hydroxide) performed well at rates of 100 g, 100 g and 150 g/100 l water respectively. Interesting to note is that copper stippling was also less with these WG formulations in comparison with the WP formulations of the same fungicides.

Fruit and foliar diseases of citrus are mostly controlled by means of high volume fungicide application, often leading to excessive levels of run-off. From initial results from the spray application project, it was clear that biological efficacy declined with increased run-off. Future experimentation should thus focus on optimising application to ensure adequate deposition of the active ingredient with minimal run-off. From the biological data obtained thus far, the benchmark deposition values for 80% control of *Alternaria* brown spot were calculated at 3 to 4% for quantitative and 30 to 40 for qualitative measurement. Biological efficacy trials will be repeated, although with an improved inoculation technique that allows better quantification of infection. Benchmark values will then be compared with deposition values following commercial application with the standard spray machines. Image capturing and analysis were major bottle-necks in the research conducted thus far, and these steps needed to be improved. New equipment was acquired to improve the robustness, portability and time-efficiency of these steps. The set-up was optimised and presents a dramatic improvement (up to 8 times faster throughput of image capturing and analysis of samples).

#### 4.6.2 PROGRESS REPORT: New spray programmes for the control of *Alternaria* brown spot in the winter rainfall region of South Africa

Experiment 749 (Sep 2004–June 2010): by G.C. Schutte (CRI)

##### Opsomming

Die proef kon nie voltooi word nie omrede die Landgoed finansiële probleme ondervind het en besluit het om die boord uit te stoot nadat slegs twee bespuitings toegedien is.

##### Summary

The trial could not be completed because the Estate ran into financial difficulties and decided to remove the orchard after two applications had been applied.

##### Introduction

Brown spot disease of citrus caused by *Alternaria alternata* is one of the most prevalent fungal diseases in all production areas in South Africa. Minneola tangelos, Novas, mandarins and their hybrids, tangors and grapefruit are the most susceptible cultivars. The disease can affect both fruit and foliage and is most prevalent under wet, humid conditions. The fruit lesions are very unsightly and readily reduce crop value. Toxin formation in foliar and twig infection also causes defoliation.

South Africa has both winter and summer rainfall areas. In both areas, wet, humid periods can occur, which favour brown spot disease. This applies particularly to the autumn in southern areas that have a Mediterranean climate. Heavy dew can also create suitable conditions for disease development (Timmer *et al.*, 2000) and again the southern areas are more susceptible to this climatic factor. Nevertheless, due to the unpredictability of seasonal climatic trends, it is necessary to annually protect the most susceptible cultivars against the disease in all areas. This typically requires a multiple spray programme to cover the possible infection periods from spring to autumn. In this regard, the strategy employed by South African growers is to use the more expensive systemic fungicides during the wet, high-disease-pressure periods (Schutte *et al.*, 1994) and the less expensive contact fungicides during dry, low-disease-pressure periods. However, not all fungicides have acceptable MRLs in the European Union.

Our aim was to evaluate a new copper hydroxide at a rate that was effective for CBS control and at a reduced rate but in a tank mixture with Sporekill (didecyl dimethyl ammonium chloride 12%), a disinfectant or plant sanitiser, that is also registered against citrus black spot.

## Materials and methods

A trial site was selected on the farm, Sovereign Estates, near Swellendam on Nova' with a high incidence of brown spot. Two spray programmes were selected comprising copper hydroxide (C40) and rates of 100 g/100 ℓ water and 50 g/100 ℓ water in a tank mixture with a disinfectant or plant sanitizer (Sporekill) at a rate of 100 ml/100 ℓ water. A randomised row design with 25 trees per row was used per treatment and sprayed with a Cima spray machine. Buffer rows were allowed between each of the treatments. Trees were thoroughly to the point of run-off. A total of 7 to 8 applications were made at monthly intervals between 27 September 2006 and 14 March 2007. All sprays were applied during good weather conditions without wind or rain. The evaluation of brown spot (200 fruit per replicate) on the fruit rind was conducted just prior to harvesting, during mid-June. Criteria used for rating the fruit were:

0 = fruit with no brown spot lesions,

1 = fruit with one to five brown spot lesions,

2 = fruit with six or more brown spot lesions.

The results were expressed as percentages and the means compared using Fisher's LSD test for significance

## Results and discussion

No results were obtained from the field trial conducted at Sovereign Estates at Buffeljachts. After three applications the farm manager informed us that they wanted to remove the orchard. However, during a visit in August 2007, they informed us that they decided not to remove the orchard. An inspection of the orchard revealed that the three applications early in the season still resulted in more than 80% clean fruit. This observation clearly shows that if a prediction model is in place, the growers do not really have to spray 8 monthly applications from the first flush until harvest. This aspect is being addressed in a new project that started in 2008.

## Conclusion

Trials will continue on the same estate or somewhere else during the 2008 -2009 season.

## Future research

Research on *Alternaria* brown spot spray programmes is needed and will continue to ensure clean exportable fruit, especially for the lucrative USA market. Prediction modelling and weather prediction will benefit growers because the summer months are dry. They will save a lot of money if they could only spray when conditions are suitable for infection. A 'Metos' automatic weather station has been placed on the farm 'Frankenhof' east of Swellendam for this purpose.

## Technology transfer

When results are available, reports in this regard will be distributed to citrus growers and will be included in various talks to citrus growers.

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#### 4.6.3 PROGRESS REPORT: Positioning and evaluation of new spray programmes consisting of strobilurins 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 (CRI)

##### Opsomming

Drie behandelings bestaande uit drie strobilurin en mancozeb tankmengsels met óf minerale spuit olie óf Sporekill, het goeie beheer van *Alternaria* bruinvlek gegee. Kwekers kan dus tot 5 spuit rondtes spaar. Die resultate kon selfs beter gewees het indien daar net voor oes dalk 'n laat mancozeb (200 g/100 l water) bespuiting toegedien was omrede daar reën geval het tot 24 April 2007. Hierdie waarneming wys op die waarde van siektevoorspelling (word in 'n nuwe projek in samewerking met Spaanse navorsers vanaf 2008 bestudeer). Beide die standaard maandelikse toedienings (8) van koperoksichloried en mancozeb het beide goed gevaar teen *Alternaria* bruinvlek. Alhoewel Pennfluid ('n nuwe vloeibare mancozeb formulاسie) *Alternaria* bruinvlek goed beheer het teen 200 g/100 l water, het dit fitotoksisiteit tot gevolg gehad. Die drie nuwe WG formulاسies bestaande uit koper sulfaat en koperhidroksied viz. Cuprofix (kopersulfaat), C40 (kopersulfaat + mancozeb) and DPX (koperhidroksied) het almal goed presteer teen dosisse van 100 g, 100 g and 150 g/100 l water onderskeidelik. Dit was interessant om daarop te let dat hierdie WG formulاسies baie minder koperstippelvorming tot gevolg gehad het in vergelyking met die WP formulاسies.

##### Summary

Three applications consisting of three strobilurins and mancozeb tank mix applicatons with either mineral spray oil or Sporekill, gave good control of *Alternaria* brown spot hereby saving growers 5 spray rounds. The results with all these treatments could have been better if a late mancozeb treatment (200 g/100 l water) was applied at the end of the season as rain spells still continued until the 24 April 2007. This observation demonstrates the potential of disease prediction (which is being researched in a new 2008 project in collaboration with Spanish researchers). Both the standard copper oxychloride and mancozeb spray programmes sprayed at monthly intervals (8 applications), performed well at registered rates of 200 g/100 l water against *Alternaria* brown spot. Although Pennfluid (a new liquid mancozeb formulation) controlled *Alternaria* brown spot very well at a rate of 200 ml/100 l water, severe phytotoxicity problems were observed. Three new WG formulations of copper sulphate and copper hydroxide viz. Cuprofix (copper sulphate), C40 (copper sulphate + mancozeb) and DPX (copper hydroxide) performed well at rates of 100 g, 100 g and 150 g/100 l water respectively. Interesting to note is that copper stippling was also less with these WG formulations in comparison with the WP formulations of the same fungicides.

##### 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 in 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 aims were to evaluate the three strobilurins using three applications only at 8 week intervals during the high disease pressure period from October to January, and to evaluate new copper hydroxide WG formulations as well as a new SC mancozeb formulation.

## Materials and methods

Ten single-tree plots per treatment were randomly selected from a 'nova' orchard at Belmont 50 km west of Nelspruit. The trees were 14 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. Neighbouring trees were used as guard trees between plots and 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 4.3.1. 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 mancozeb with either mineral spray oil or Sporekill

Three strobilurin applications sprayed at 60 day intervals gave good control of *Alternaria* brown spot (Table 4.6.3.2). Where lower than registered rate of the contact fungicide mancozeb (*viz.* 150 g/h $\ell$ ) was sprayed in a tank mixture with registered strobilurins (Flint, Cabrio or Ortiva) and Sporekill, good control was observed, but significant differences ( $P > 0.05$ ) were observed. The mixture with Cabrio resulted in 95.2% clean exportable fruit followed by the Ortiva mixture with 92.8% clean exportable fruit and the Flint mixture with only 84.2% clean exportable fruit; the latter mixture performing significantly poorer than the Cabrio mixture. The standard registered treatments consisting of either mancozeb or copper oxychloride (8 applications) also performed well and there were no significant differences between these treatments and the all treatments involving strobilurins, except for the Flint, mancozeb and Sporekill treatment. The results with all these treatments could have been better if a late mancozeb treatment (200 g/100  $\ell$  water) was applied on the 2 April 2007 as rain spells still continued until the 24 April 2007.

### Pennfluid

Results (Table 4.6.3.3) showed that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb treatment and Pennfluid evaluated at the highest rates of 150 and 200 ml/100  $\ell$  water, but there were, however, significant differences between the standard mancozeb treatment and the Pennfluid rates of 100 and 150 ml/100  $\ell$  water with regards to the criterion, clean exportable fruit. The other standard treatment included in the experiment, copper oxychloride, was not significant different from the 150 and 200 ml/100  $\ell$  water Pennfluid treatments. However, both the standard treatments were significantly different from the Pennfluid evaluated at a rate of 100 ml/100  $\ell$  water.

With regards to the criterion, fruit with one to five *Alternaria* brown spot lesions, all the standard registered treatments consisting of mancozeb and copper oxychloride as well as the highest rate of Pennfluid evaluated (200 ml/100l water), were significant different from the lowest rate of Pennfluid (100 ml/100  $\ell$  water) and the control. The criterion, fruit with six and more *Alternaria* brown spot lesions, showed that there were no significant differences between all the treatment even where the lowest rates of Pennfluid (100 and 150 ml/100  $\ell$  water) resulted in 7.6 and 7.8% fruit with six and more brown spot lesions, respectively. Although Pennfluid controlled

Alternaria brown spot very well, severe phytotoxicity problems were observed and should be investigated to see if this problem can be overcome (Fig. 4.6.3.1).

#### Cuprofix

Results (Table 4.6.3.4) show that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb and copper oxychloride treatments and all the Cuprofix treatments. Cuprofix (200 g/100 l water) and Cuprofix (150 g/100 l water) gave the highest percentage clean exportable fruit of 100.0% and 99.6%, respectively. These two treatments also resulted in no fruit with 6 and more Alternaria brown spot lesions. The Cuprofix rates of 100 g/100 l water alone and in tank mixtures with mineral spray oil (Citrole 100) were also not significant different from the treatments mentioned above. Although these treatments had between 1.0 and 1.8% fruit with one to five Alternaria brown spot lesions and 1.2% fruit in the criterion with 6 and more brown spot lesions; they were, however, not significant different from the other Cuprofix treatments.

#### C40

Results (Table 4.6.3.5) showed that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb and copper oxychloride treatments and all the C40 treatments tested at rates of 100, 150 and 200 g/100 l water as well as C40 in tank mixtures with mineral spray oil (100 g + 250 ml/100 l water). C40 tested at rates of 150 and 100 g + mineral spray oil, resulted in 100% clean exportable fruit. Disease pressure was high as the untreated control resulted in only 38.2% clean exportable fruit. With regards to the criterion, fruit with one to five Alternaria brown spot lesions, all the standard registered treatments consisting of mancozeb and copper oxychloride as well as all the C40 rates evaluated, were not significant different from each other. They were all significant different from the control. The criterion, fruit with six and more Alternaria brown spot lesions, showed that there were no significant differences between all the treatments, but they were significant different from the control.

#### DPX

Results (Table 4.6.3.6) show that there were no significant differences ( $P < 0.05$ ) between the standard registered mancozeb and copper oxychloride treatments and all the DPX treatments. Kocide (150 g/100 l water) and the highest rate DPX (225 g/100 l water) both resulted in the highest percentage clean exportable fruit of 99.6%. The DPX rates of 125 and 175 g /100 l water were also not significant different from the treatments mentioned above. DPX in tank mixtures with Sporekill and Nu Film 17 were also not significant different from the same treatments mentioned above. The standard mancozeb and copper oxychloride treatments were equally effective in control Alternaria brown spot. Disease pressure was high as the untreated control resulted in only 38.2% clean exportable fruit. In both the other criteria used for evaluation, all the treatments were also significant different from the control. With regards to the criterion fruit with one to five Alternaria brown spot lesions, the incidence ranged between 0.0 and 1.4% but there were no significant differences ( $P < 0.05$ ) between the treatments. All the treatments were however significant different from the control as the untreated control resulted in 23.8% fruit with one to five Alternaria brown spot lesions. The same scenario was experienced with fruit in the criterion with 6 and more Alternaria brown spot lesions. Copper stippling was severe with the standard copper oxychloride treatment (WP) and only faint stippling was observed with the DPX treatments (WG) (Fig. 4.6.3.2).

#### **Conclusion**

Three strobilurin and mancozeb applications in tank mixtures with either mineral spray oil or Sporekill, gave good control of Alternaria brown spot versus 8 applications with contact fungicides. This type of spray programme will save the growers 5 spray rounds if compared with the contact/preventative type of spray programme at monthly intervals. Concomitantly, the strobilurins do have a systemic or local systemic mode of action and their long lasting residual action plays an important role for the good fungicidal action against Alternaria brown spot (Häuser-Hahn, Pontzen & Baur, 2003). 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 might be necessary for future trials if these types of spray programmes will be registered in the future.

Both the registered copper oxychloride and mancozeb spray programmes sprayed at monthly intervals, performed well at registered rates of 200 g/100 l water against *Alternaria* brown spot. Pennfluid, a new liquid mancozeb formulation, only performed well at a rate of 200 ml/100 l water but phytotoxicity was also recorded at this rate of application and can not be recommended for further use on Mandarins. New WG formulations of copper hydroxide (Cuprofix, C40 and DPX) performed well against ABS, but copper stippling was observed in all the treatments. This can be overcome if copper fungicides are alternated with mancozeb. More emphasis should be placed on fungicide applications in late April at the end of the rainy season as the results with all the treatments could have been better if a late mancozeb treatment (200 g/100 l water) was applied on 2 April 2007, because rain spells continued until the 24 April 2007 resulting in late ABS infections (Fig. 4.6.3.3).

### Future objectives (milestones) and work plan

More spray programmes consisting of different mixtures with Sporekill as well as any new fungicides will be evaluated in the new season. Prediction modelling and weather prediction will benefit growers because the summer months are dry. They will save a lot of money if they could only spray when conditions are suitable for infection. A 'Metos' automatic weather station has been placed in the 'nova' orchard on the farm 'Belmont', west of Nelspruit.

### 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 will also form part of the 2008 CRI Citrus Symposium in the Drakensberg.

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**Table 4.6.3.1.** Application of copper oxychloride, copper hydroxide, copper sulphate (C30 & C40) and mancozeb as well as strobilurins (Flint, Ortiva, Cabrio) using three applications with either mineral spray oil or Sporekill during the high disease pressure period from October for *Alternaria* brown spot control on 'Novas' at Belmont, near Nelspruit in S.A. during 2006 and 2007.

18 September 2006	16 October 2006	13 November 2006	11 December 2006	8 January 2007	5 February 2007	5 March 2007	2 April 2007
MZ 200g	MZ 200g	MZ 200g	MZ 200g	MZ 200g	MZ 200g	MZ 200g	MZ 200g
Fynox 200g	Fynox 200g	Fynox 200g	Fynox 200g	Fynox 200g	Fynox 200g	Fynox 200g	Fynox 200g
	Cabrio +MZ+SK 10ml+150g+100ml		Cabrio +MZ+SK 10ml+150g+100ml		Cabrio +MZ+SK 10ml+150g+100ml		
	Cabrio +MZ+O 10ml+150g+250ml		Cabrio +MZ+O 10ml+150g+250ml		Cabrio +MZ+O 10ml+150g+250ml		
	Flint +MZ+SK 10g+150g+100ml		Flint +MZ+SK 10g+150g+100ml		Flint +MZ+SK 10g+150g+100ml		
	Flint +MZ+O 10g+150g+250ml		Flint +MZ+O 10g+150g+250ml		Flint +MZ+O 10g+150g+250ml		
	Ortiva+MZ+O 20ml + 150g + 250ml		Ortiva+MZ+O 20ml + 150g + 250ml		Ortiva+MZ+O 20ml + 150g + 250ml		
	Ortiva+MZ+SK 20ml + 150g + 100ml		Ortiva+MZ+SK 20ml + 150g + 100ml		Ortiva+MZ+SK 20ml + 150g + 100ml		
Kontrolle	Kontrolle	Kontrolle	Kontrolle	Kontrolle	Kontrolle	Kontrolle	Kontrolle
Pennfluid 100ml	Pennfluid 100ml	Pennfluid 100ml	Pennfluid 100ml	Pennfluid 100ml	Pennfluid 100ml	Pennfluid 100ml	Pennfluid 100ml
Pennfluid 150ml	Pennfluid 150ml	Pennfluid 150ml	Pennfluid 150ml	Pennfluid 150ml	Pennfluid 150ml	Pennfluid 150ml	Pennfluid 150ml
Pennfluid 200ml	Pennfluid 200ml	Pennfluid 200ml	Pennfluid 200ml	Pennfluid 200ml	Pennfluid 200ml	Pennfluid 200ml	Pennfluid 200ml
Cuprofix 100g	Cuprofix 100g	Cuprofix 100g	Cuprofix 100g	Cuprofix 100g	Cuprofix 100g	Cuprofix 100g	Cuprofix 100g
Cuprofix 150g	Cuprofix 150g	Cuprofix 150g	Cuprofix 150g	Cuprofix 150g	Cuprofix 150g	Cuprofix 150g	Cuprofix 150g
Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml	Cuprofix+Citrole 100g+250ml
Cuprofix 200g	Cuprofix 200g	Cuprofix 200g	Cuprofix 200g	Cuprofix 200g	Cuprofix 200g	Cuprofix 200g	Cuprofix 200g
C40 100g	C40 100g	C40 100g	C40 100g	C40 100g	C40 100g	C40 100g	C40 100g
C40 150g	C40 150g	C40 150g	C40 150g	C40 150g	C40 150g	C40 150g	C40 150g
C40+Citrole 100g+250ml	C40+Citrole 100g+250ml	C40+Citrole 100g+250ml	C40+Citrole 100g+250ml	C40+Citrole 100g+250ml	C40+Citrole 100g+250ml	C40+Citrole 100g+250ml	C40+Citrole 100g+250ml
C40 200g	C40 200g	C40 200g	C40 200g	C40 200g	C40 200g	C40 200g	C40 200g
DPX 125g	DPX 125g	DPX 125g	DPX 125g	DPX 125g	DPX 125g	DPX 125g	DPX 125g
DPX 175g	DPX 175g	DPX 175g	DPX 175g	DPX 175g	DPX 175g	DPX 175g	DPX 175g
DPX 225g	DPX 225g	DPX 225g	DPX 225g	DPX 225g	DPX 225g	DPX 225g	DPX 225g
DPX+SK 125+100ml	DPX+SK 125+100ml	DPX+SK 125+100ml	DPX+SK 125+100ml	DPX+SK 125+100ml	DPX+SK 125+100ml	DPX+SK 125+100ml	DPX+SK 125+100ml
DPX+NuF 125g+25ml	DPX+NuF 125g+25ml	DPX+NuF 125g+25ml	DPX+NuF 125g+25ml	DPX+NuF 125g+25ml	DPX+NuF 125g+25ml	DPX+NuF 125g+25ml	DPX+NuF 125g+25ml
Kocide 2000 150g	Kocide 2000 150g	Kocide 2000 150g	Kocide 2000 150g	Kocide 2000 150g	Kocide 2000 150g	Kocide 2000 150g	Kocide 2000 150g

DPX = new copper hydroxide formulation from DuPont; Cuprofix = new copper sulphate formulation from Total; C40 = new copper sulphate/mancozeb formulation from Total; MZ = mancozeb; SK = Sporekill; O = mineral spray oil

**Table 4.6.3.2** Evaluation of strobilurins and mancozeb in tank mixtures with either mineral spray oil or Sporekill during the high disease pressure period from September to April during 2006 and 2007 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 <sup>x</sup>		
		Lesions/fruit		
		0	1-5	≥6
Mancozeb <sup>y</sup>	200 g	98.6 a	0.6 a	0.8 a
Copper oxychloride <sup>y</sup>	200 g	98.2 a	0.8 a	1.0 a
Cabrio+mancozeb+oil	10 ml+150 g + 250ml	97.6 a	1.6 a	0.8 a
Cabrio+mancozeb+Sporekill <sup>z</sup>	10 ml+100 g+100 ml	95.2 a	2.4 ab	2.4 ab
Ortiva+mancozeb+oil <sup>z</sup>	20 ml+150 g+250ml	94.6 a	4.2 ab	1.2 a
Ortiva+mancozeb+Sporekill <sup>z</sup>	10 g+100 g+100 ml	92.8 ab	3.4 ab	3.8 ab
Flint+mancozeb+oil <sup>z</sup>	10 g+150 g+250 ml	94.4 a	3.6 ab	2.0 a
Flint+mancozeb+Sporekill <sup>z</sup>	20 ml+100 g+100 ml	84.2 b	10.8 b	5.0 c
Control		38.2 c	23.8 c	38.0 d

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 18 September 2006, 16 October 2006, 13 November 2006, 11 December 2006, 8 January 2007, 5 February 2007, 5 March 2007, 2 April 2007.

<sup>z</sup> Spray dates were 16 October 2006, 11 December 2006 and 5 February 2007.

**Table 4.6.3.3.** Evaluation of Pennfluid (mancozeb) during the high disease pressure period from September to April during 2006 and 2007 for the control of *Alternaria alternata* on 'Nova' mandarins at Belmont, Schagen.

Treatment <sup>y</sup>	Concentration (g/ml product/100 litre water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-5	≥6
Mancozeb	200 g	98.6 a	0.6 a	0.8 a
Copper oxychloride	200 g	98.2 ab	0.8 a	1.0 a
Pennfluid	200 ml	95.8 ab	3.2 a	1.0 a
Pennfluid	150 ml	86.2 bc	6.2 ab	7.6 a
Pennfluid	100 ml	79.6 c	12.6 b	7.8 a
Control		38.2 d	23.8 c	38.0 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 18 September 2006, 16 October 2006, 13 November 2006, 11 December 2006, 8 January 2007, 5 February 2007, 5 March 2007, 2 April 2007.

**Table 4.6.3.4.** Evaluation of Cuprofix (copper sulphate) during the high disease pressure period from September to April during 2006 and 2007 for the control of *Alternaria alternata* on 'Nova' mandarins at Belmont, Schagen.

Treatment <sup>y</sup>	Concentration (g/ml product/100 litre water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-5	≥6
Mancozeb	200 g	98.6 a	0.6 a	0.8 a
Copper oxychloride	200 g	98.2 a	0.8 a	1.0 a
Cuprofix	200 g	100.0 a	0.0 a	0.0 a
Cuprofix	150 g	99.6 a	0.4 a	0.0 a
Cuprofix	100 g	97.0 a	1.8 a	1.2 a
Cuprofix + mineral spray oil	100 g + 200 ml	97.8 a	1.0 a	1.2 a
Control		38.2 b	23.8 b	38.0 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 18 September 2006, 16 October 2006, 13 November 2006, 11 December 2006, 8 January 2007, 5 February 2007, 5 March 2007, 2 April 2007

**Table 4.6.3.5.** Evaluation of C40 (copper sulphate + mancozeb) during the high disease pressure period from September to April during 2006 and 2007 for the control of *Alternaria alternata* on 'nova' mandarins at Belmont, Schagen.

Treatment <sup>y</sup>	Concentration (g/ml product/100 litre water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-5	≥6
Mancozeb	200 g	98.6 a	1.4 a	0.0 a
Copper oxychloride	200 g	98.2 a	0.8 a	1.0 a
C40	200 g	99.4 a	0.2 a	0.4 a
C40	150 g	100.0 a	0.0 a	0.0 a
C40	100 g	99.4 a	0.4 a	0.2 a
C40 + mineral oil	100 g + 250 ml	100.0 a	0.0 a	0.0 a
Control		38.2 b	23.8 b	38.0 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 18 September 2006, 16 October 2006, 13 November 2006, 11 December 2006, 8 January 2007, 5 February 2007, 5 March 2007, 2 April 2007.

**Table 4.6.3.6.** Evaluation of DPX (copper hydroxide) applied alone or in tank mixtures with Sporekill and Nu Film 17 during the high disease pressure period from September to April during 2006 and 2007 for the control of *Alternaria alternata* on 'nova' mandarins at Belmont, Schagen.

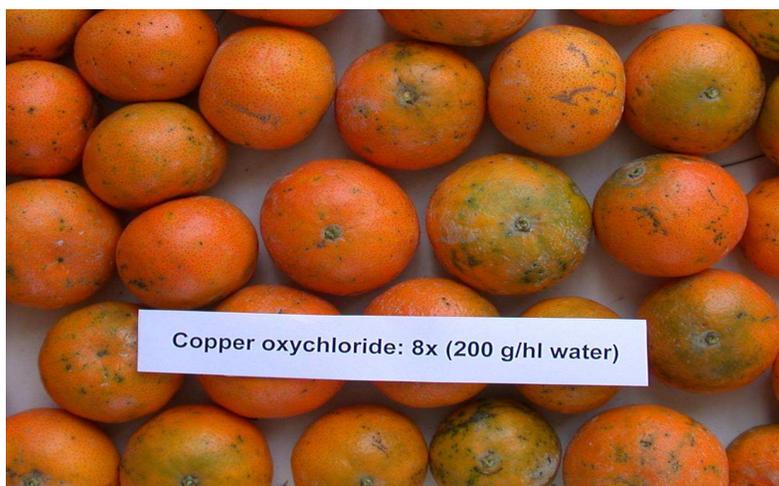
Treatment <sup>y</sup>	Concentration (g/ml product/100 litre water)	Percentage of fruit in each class <sup>x</sup>		
		Lesions/fruit		
		0	1-5	≥6
Mancozeb	200 g	98.6 a	1.4 a	0.0 a
Copper oxychloride	200 g	98.2 a	0.8 a	1.0 a
Kocide	150 g	99.6 a	0.0 a	0.4 a
DPX	225 g	99.6 a	0.4 a	0.0 a
DPX	175 g	99.0 a	0.6 a	0.4 a
DPX	125 g	99.4 a	0.6 a	0.0 a
DPX + Sporekill	125 g + 100 ml	98.8 a	1.2 a	0.0 a
DPX + Nu Film 17	125 g + 25 ml	97.4 a	1.2 a	1.4 a
Control		38.2 b	23.8 b	38.0 b

<sup>x</sup> 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.

<sup>y</sup> Spray dates were 18 September 2006, 16 October 2006, 13 November 2006, 11 December 2006, 8 January 2007, 5 February 2007, 5 March 2007, 2 April 2007.



**Fig. 4.6.3.1.** Phytotoxicity on 'Nova' mandarins experienced at harvest after 8 applications of Pennfluid at a rate of 200 ml/100 l water for the control of Alternaria brown spot.



**Fig. 4.6.3.2.** 'Nova' mandarin fruit samples taken at harvest after 8 field applications with the lowest DPX rate (125 g/100 l water) (top left) and the highest DPX rate (225 g/100 l water) (top right) showing less stippling than the standard copper oxychloride treatment (bottom left) also after 8 applications. The standard mancozeb treatment (bottom right) resulted in no stippling.

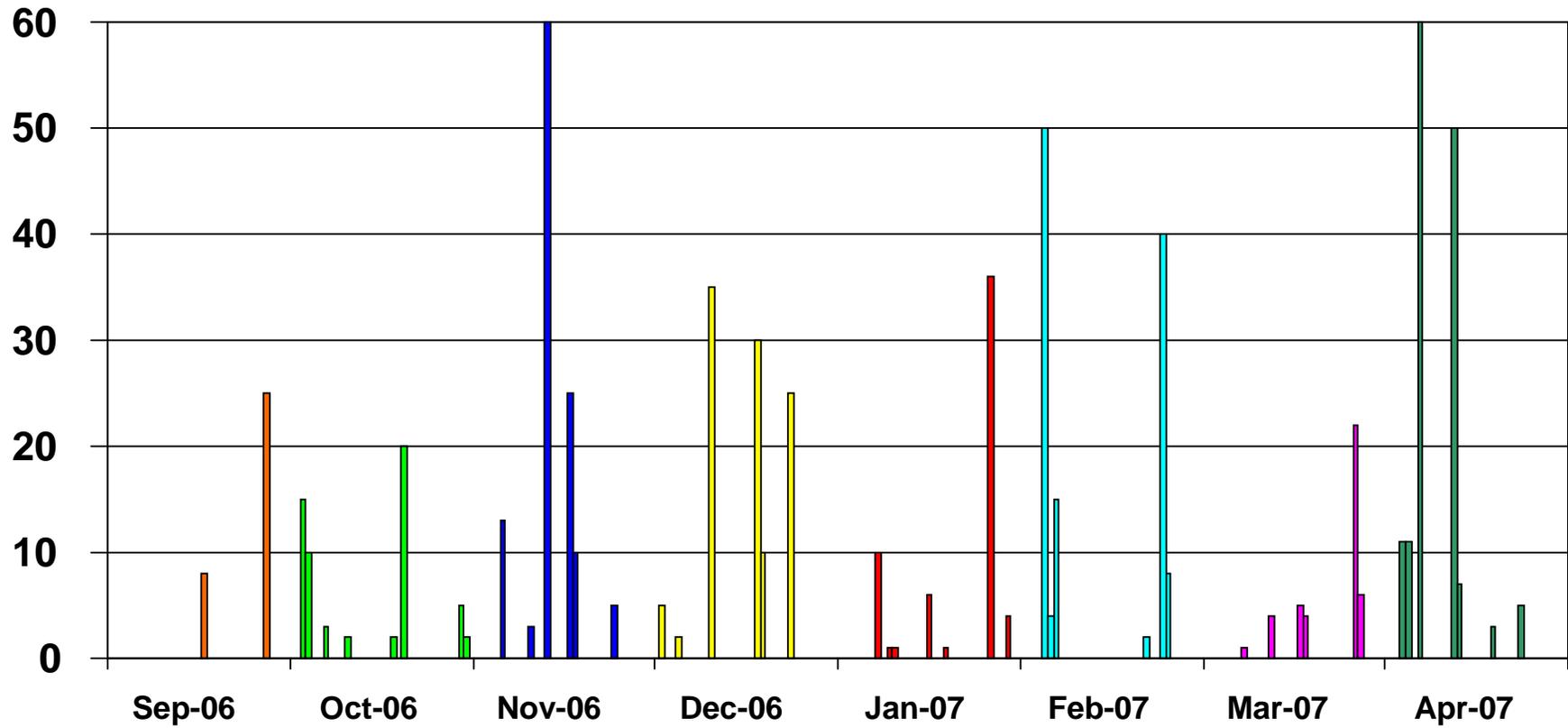


Fig. 4.6.3.3. Rainfall (mm) for the period September 2006 to April 2007 at the experimental site at Belmont, Nelspruit.

#### 4.6.4 **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 swamdoderspuite beheer. Hierdie spuite lei meestal tot groot mates van afloop. Vanaf aanvanklike resultate was dit duidelik dat 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. Drempelwaardes vir biologiese effektiwiteit (80% beheer van *Alternaria* bruinvlek) is bereken as 3 tot 4% vir kwantitatiewe en 30 tot 40 vir kwalitatiewe bedekking. Biologiese effektiwiteitstoetse sal herhaal word, maar met 'n verbeterde inokulasie-metode met beter kwantifisering van infeksie. Drempelwaardes sal dan met bedekkingswaardes na kommersiële toediening met standaard spuitpompe vergelyk word. Fotografie en beeldanalise was groot bottelnekke en hierdie stappe moes verbeter word. Nuwe toerusting is aangeskaf om die robuustheid, draagbaarheid en tydseffektiwiteit van hierdie stappe te verbeter. Dit is nou heelwat verbeter en deurvloei van monsters is nou 8 maal versnel.

##### **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. From initial results, it was clear that biological efficacy declined with increased run-off. Future experimentation should thus focus on optimising application to ensure adequate deposition of the active ingredient with minimal run-off. From the biological data obtained thus far, the benchmark deposition values for 80% control of *Alternaria* brown spot were calculated at 3 to 4% for quantitative and 30 to 40 for qualitative measurement. Biological efficacy trials will be repeated, although with an improved inoculation technique that allows better quantification of infection. Benchmark values will then be compared with deposition values following commercial application with the standard spray machines. Image capturing and analysis were major bottle-necks in the research conducted thus far, and these steps needed to be improved. New equipment was acquired to improve the robustness, portability and time-efficiency of these steps. The set-up was optimised and presents a dramatic improvement (up to 8 times faster through-put of image capturing and analysis of samples).

##### **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 spray applications to citrus trees in South Africa involve 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.*, 2006a&b; 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.

## **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 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 agricultural 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**

##### *Leaves and fruit*

Mature leaves were obtained from young Nova Clementine trees that were grown in 10 l plastic pots in a glasshouse at 25–28°C. Mature untreated Eureka lemon and Valencia orange fruit were obtained from a commercial citrus packhouse prior to any postharvest treatments.

##### *Spray application*

Spray mixtures consisted of the SARDI Yellow Fluorescent Pigment (400 g/l, EC; South Australian Research and Development Institute, Loxton SA 5333 Australia) at 0.2 l/100 l (Brink *et al.* 2004; 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). A gravity-fed mist spray gun (ITW DEVILBISS Spray Equipment Products, 195 Internationale Blvd, Glendale Heights IL 60139 USA) was used to apply the spray volumes onto leaves or fruit. Spraying was done in a spray chamber [660 × 1410 × 880 mm (h×l×w)] with leaves or fruit slanted at a 30° angle. The spray gun was mounted onto the spray chamber at a distance of 60 cm from the target with spray angle of 90° relative to the target. Spray volumes to the upper and lower surfaces of leaves were 0.5, 1, 2, 3, 3.5, 4, 4.5, 5, 6 ml, and to fruit were 0.25, 0.5, 0.75, 1, 2, 3, 5 and 9 ml. Run-off was evident at the higher volumes. As control treatments, leaves or fruit were either not sprayed, or dipped in the spray mixture. After spraying or dipping, leaves or fruit were carefully removed and placed in a dry chamber where the deposited spray mixture was allowed to dry.

Due to anticipated variation (Brink *et al.* 2004), an experimental unit consisted of 10 randomly selected leaves or fruit to which each treatment was applied separately to individual leaves or fruit, as experimental sub-units. The experiment was repeated once for each of the two leaf sides and fruit.

##### *Spray deposition analysis*

Spray cover assessment was done by means of protocols developed and validated by Brink *et al.* (2004) and Fourie *et al.* (2007). Sprayed plant material was illuminated under black light and visualised using a Nikon SMZ800 stereo microscope at 10× magnification. Digital photos were taken with a Nikon DMX 1200 camera and image analyses performed with Image-Pro Plus version 6.2 software (Media Cybernetics, www.mediacy.com). Quantitative analyses involved removal of green channels from the image, followed by quantification of the percentage area covered by the foreground elements (deposited pigment) of the binarised image (Brink *et al.* 2004, 2006). For qualitative analysis, a combined Euclidian distance map and skeleton is created on the

binarised image, with absolute white indicating the furthest distance from a particular foreground element. Subsequent analysis of grey-scale values indicates spray deposition quality. Thus, smaller values (fewer white pixels measured; i.e. particles closer together) indicate a better quality of deposition.

## **The effect of run-off on biological efficacy**

### *Fungal isolates and inoculum production*

Infected leaves, fruit and twigs were collected from *Alternaria* brown spot infected Minneola and Alandale tangerine orchards from Citrusdal (Western Cape province, South Africa). The samples were examined under the microscope and isolations were made onto potato dextrose agar (PDA) medium. Single-spore cultures from *A. alternata* isolates were prepared through plating on water agar (WA) medium. Pathogenicity tests, to identify virulent *A. alternata* pv. *citri* isolates, were subsequently performed.

Abundant conidia were obtained by growing these isolates on potato carrot agar (PCA) plates for 14 days at 25°C. Conidial suspensions were prepared by pouring sterile water on the PCA cultures and gently rubbing the surface of the medium with a glass rod. The suspension was filtered through 3 layers of cheesecloth and the concentration was fixed at  $1 \times 10^5$  spores/ml by using a hemacytometer. Drop inoculations with 10- $\mu$ l-droplets were made onto young leaves, which were obtained from flushes of the glasshouse-grown Nova trees. Leaves were incubated in plastic containers [40 × 20 × 10 cm (l×w×h)], which served as moist chambers, for 3½ days at 25°C before the number of necrotic lesions per leaf was counted.

### *Spray application*

Spray mixtures consisted of the fluorescent pigment and copper hydroxide (Kocide 2000®, 538 g/kg WG, Plaaskem, Witfield, South Africa) at 150 g/100 l dosage. Young Nova leaves were obtained from flushes of the glasshouse-grown Nova trees. Ten shoots, each containing at least 10 recently flushed leaves, were removed from the tree and placed in sterile water to preserve turgidity. In the laboratory, leaves of similar size were dissected from each shoot and leaf petioles placed into 1.5-ml-Eppendorf tubes that were filled with 3% water agar and secured with parafilm. The upper or lower leaf surfaces were sprayed with 0.25, 0.5, 0.75, 1, 2, 3 and 5 ml, as described previously. As control treatments, leaves were either not sprayed, or dipped into the spray mixture. Each set of treatments (one leaf per treatment) was conducted 10 times, and the 10 leaves from each treatment combination comprised an experimental unit as described previously. The experiment was repeated twice.

### *Inoculation and incubation*

Sprayed leaves were placed onto wet paper towels in plastic containers that served as moist chambers. Eight 10- $\mu$ l-drops from a  $1 \times 10^5$  spores/ml conidial suspension of a virulent *A. alternata* pv. *citri* isolate of each were placed onto the upper or lower surface of each leaf, followed by moist incubation for 3½ days at 25°C. The number of droplets with necrotic lesions on each leaf was rated, and the percentage infection calculated.

### *Statistical analyses*

Spray deposition and infection data were subjected to analyses of variance. Students t-Least Significance Difference were calculated at the 5% significance level to compare treatment means of significant effects. Hoerl regression analyses of quantitative ( $y = Ax^B e^{Cx}$ ) and qualitative ( $y = A(x+1)^B e^{C(x+1)}$ ) deposition values and quadratic ( $y = A+Bx+Cx^2$ ) regression analyses infection data over spray volumes (data from dip treatments not included) were done to demonstrate trends. Pearson's correlation analysis was done to compare mean infection and deposition data. All statistical analyses were done using SAS v8.2 statistical software.

### Characterisation of spray deposition with current spray application methods

[Experimentation on this objective will commence in 2008/9]

## Results and discussion

### Conduct a thorough survey of current spray application materials and methods in all major citrus growing areas

Presentations were made at 8 study group meetings (Addo, Katrivier, Hoedspruit, Groblersdal, Tshipise, Pongola, Swaziland and Nelspruit) and 32 questionnaires were received. Data were transferred to an Excel spread sheet.

This aspect is ongoing.

### Determine benchmarks for biological efficacy of copper hydroxide against *Alternaria* brown spot

#### **Spray deposition on leaves and fruit**

The addition of the fluorescent pigment to the spray mixture allowed for the clear visualisation of spray deposition on leaves and fruit (Fig. 4.6.4.1). Aggregation of pigment particles in distinct circular patterns, which are the remnants of droplets containing the fluorescent pigment that dried on the leaf surface, can clearly be seen when sprayed leaves are illuminated with black light (Fig. 4.6.4.1 A-D). When spray volume was increased, the size of droplets likewise increased (Fig. 4.6.4.1 C and D) relative to those on leaves sprayed with lower volumes (Fig. 4.6.4.1 A and B), but signs of spray run-off were clearly visible, especially on the upper surfaces of leaves (Fig. 4.6.4.1 C). On Eureka lemon (Fig. 4.6.4.1 E) and Valencia oranges (Fig. 4.6.4.1 F), the droplet-effect was not as distinct, but pigments were nonetheless aggregated in amorphous groups.

#### *Mature leaves*

Analysis of variance of quantitative deposition values on mature leaves indicated significant effects for spray volume ( $P < 0.0001$ ) and leaf side ( $P = 0.0352$ ). For the qualitative deposition values, a significant volume  $\times$  leaf side interaction ( $P = 0.0035$ ) was observed.

Hoerl regression analyses for quantitative deposition values on upper and lower leaf surfaces yielded very good fits on convex curves demonstrating deposition trends on mature leaves ( $R^2$  values of 0.969 and 0.969, respectively; Table 4.6.4.1; Fig. 4.6.4.2). There was an increase in deposition as spray volume increased until the apex of curve was reached. On upper leaf surfaces, maximum quantitative deposition (*circa* 32.8%) was obtained with 4 to 5 ml spray volume, whereas 3.5 to 4 ml spray volume resulted in maximum deposition on lower leaf surfaces (*circa* 43.9%). With further increase in spray volume the amount of deposition on leaves decreased due to the effect of run-off.

Since qualitative deposition values decrease with improved deposition, Hoerl regression analyses fitted concave curves to deposition values on upper and lower leaf surfaces ( $R^2$  values of 0.990 and 0.991, respectively; Table 4.6.4.1, Fig. 4.6.4.2). The bases of the curves were visibly flatter than the apices of the quantitative deposition curves, with best quality deposition obtained by a larger range of volumes: 2 to 4 ml on upper (*circa* 9.8) and lower (*circa* 8.2) leaf surfaces.

Quantitative deposition values on upper and lower leaf surfaces following dip treatment (means of 4.4% and 0.6%, respectively; Fig. 4.6.4.2) were significantly lower (as determined by Student's T-tests) than deposition following spray application at most volumes. Qualitative deposition following dip treatment (means of 59.3 and 100.7, respectively; Fig. 4.6.4.2) was also significantly poorer than that for all spray volumes tested.

#### *Fruit*

For Eureka lemon and Valencia orange fruit, analyses of variance of quantitative ( $P=0.0001$  and  $P = 0.0011$ , respectively) and qualitative ( $P < 0.0001$ ) deposition values indicated significant effects for spray volume. Hoerl regression analysis for quantitative and qualitative deposition values yielded very good fits demonstrating the deposition trends on fruit over a range of spray volumes ( $R^2$  values  $> 0.882$  and  $0.979$ , respectively; Table 4.6.4.2; Fig. 4.6.4.3). For both fruit types, maximum quantitative deposition was observed at a spray volume of 3 to 5 ml, although Valencia orange fruit exhibited a markedly higher mean deposition at these spray volumes (17.8%) compared with for Eureka lemon (9.8%). Qualitative deposition curves for the two fruit types were of similar shape with 2 to 5 ml yielding the best quality deposition. Mean values for qualitative

deposition at these volumes on Valencia oranges was generally better (i.e. lower values) than those on Eureka lemons (5.1 vs. 12.4).

Mean quantitative deposition values on Eureka lemon and Valencia orange fruit following dip treatment (1.4% and 6.4%, respectively; Fig. 4.6.4.3) were significantly lower (as determined by Student's T-tests) than spray application at most volumes tested. For qualitative deposition following dip treatment on Valencia orange fruit (25.6), mean values were comparable to spray application with 1 ml, whereas qualitative deposition on lemon fruit was markedly poorer (60.1).

### The effect of run-off on biological efficacy

Twenty-three *A. alternata* isolates were obtained from the sampled material. Eight isolates caused necrotic lesions on Nova leaves with isolate 7A proving to be the most virulent (results not shown), and was therefore used in subsequent biological efficacy trials. The isolate was deposited into the Stellenbosch University culture collection.

Analyses of variance of the quantitative and qualitative deposition values on young leaves indicated significant spray volume  $\times$  leaf side interactions ( $P < 0.0001$  and  $P = 0.0007$ , respectively). Hoerl regression analyses for quantitative and qualitative deposition values and infection over spray volume yielded very good fits ( $R^2$  values from 0.907 to 0.993) demonstrating deposition trends in young leaves (Table 4.6.4.3; Fig. 4.6.4.4). On upper leaf surfaces, the quantitative deposition curve did not reach its apex within the range of spray volumes although it flattened off at 5 ml (22.4%). On the lower leaf surfaces, maximum deposition was attained at 3 ml (18.9%), with markedly lower deposition at higher volumes and dip treatment. Qualitative deposition curves on upper and lower leaf surfaces followed similar trends with bases of curves reached at 2 to 3 ml (circa 17.3%). Mean quantitative (0.50% and 0.16% for upper and lower surfaces, respectively) and qualitative (165.0 and 167.9 for upper and lower surfaces, respectively) deposition values for dip treatments, as determined by Student's T-test, were significantly poorer than those following spray application (Fig. 4.6.4.4).

The analysis of variance for the infection of *A. alternata* pv. *citri* on sprayed young leaves indicated significant effects for spray volume ( $P < 0.0001$ ) and leaf side ( $P = 0.0011$ ). As was evident from Student's T-tests, dip-treated leaves (mean 93.25%) yielded statistically lower infection levels than leaves sprayed with volumes from 0.75 to 5 ml (48.25% to 2.71%). The lowest infection levels were observed on leaves sprayed with 2 and 3 ml (2.92% and 2.71%, respectively), with 1 ml and 5 ml yielding a mean of 16.04% and 14.18% infection, respectively. Although infection levels on unsprayed upper and lower leaf surfaces were similar (100% and 99.17%, respectively), the mean infection level on lower leaf surfaces were significantly higher (55.79%) than that on upper leaf surfaces (46.30%).

Concave quadratic curves were fitted to the infection data on upper and lower leaf surfaces ( $R^2$  values of 0.69 and 0.78, respectively; Table 4.6.4.3; Fig. 4.6.4.5). Infection was almost completely inhibited (>10% infection) on upper leaf surfaces sprayed with 2 to 5 ml of the copper hydroxide mixture, and on lower leaf surfaces sprayed with 2 to 3 ml. On both leaf surfaces projected infection levels were markedly lower at lower spray volumes, as well as at 5 ml on lower leaf surfaces.

Pearson's correlation analysis indicated very good correlation between infection (data for dip-treatments included) and quantitative and qualitative deposition values on upper (-0.824 and +0.764, respectively) and lower leaf surfaces (-0.885 and +0.707, respectively). Thus, infection followed a similar trend as quantitative and qualitative deposition. Moreover, sigmoidal regression analyses of mean infection percentages against quantitative and qualitative deposition on upper and lower surfaces yielded very good fits (Table 4.6.4.4; Fig. 4.6.4.5). A larger quantity of spray deposition was required to control infections on the upper leaf surface, compared with lower leaf surfaces (Fig. 4.6.4.5 AB). On upper leaf surfaces, a predicted quantitative deposition of 2.99% was needed to reduce infection from 100% to 20%, while 4.14% was needed on lower leaf surfaces. A similar trend was observed for qualitative deposition where a better quality deposition was needed on lower leaf surfaces for similar infection levels. For 20% infection, the qualitative deposition was predicted at 39.59 on upper leaf surfaces, and circa 32 on lower leaf surfaces.

**Table 4.6.4.1.** Coefficients for Hoerl regression analyses of quantitative ( $y = Ax^B e^{Cx}$ ) and qualitative ( $y = A(x+1)^B e^{C(x+1)}$ ) deposition values following spray application with SARDI Yellow Fluorescent Pigment to upper and lower leaf surfaces of mature Nova mandarin leaves at volumes ranging from 0 to 6 ml.

Deposition analysis	Variables			R <sup>2</sup> -value
	A ± SE	B ± SE	C ± SE	
<b>Quantitative analysis</b>				
Upper leaf surface	3.33 ± 1.445	3.34 ± 0.794	-0.62 ± 0.176	0.969
Lower leaf surface	5.15 ± 2.034	3.75 ± 0.761	-0.78 ± 0.175	0.969
<b>Qualitative analysis</b>				
Upper leaf surface	42.55 ± 2.554	-5.49 ± 0.414	1.42 ± 0.149	0.990
Lower leaf surface	53.28 ± 8.137	-5.94 ± 0.434	1.56 ± 0.154	0.991

**Table 4.6.4.2.** Coefficients for Hoerl regression analyses of quantitative ( $y = Ax^B e^{Cx}$ ) and qualitative ( $y = A(x+1)^B e^{C(x+1)}$ ) deposition values following spray application with SARDI Yellow Fluorescent Pigment to Eureka lemon and Valencia orange fruit at volumes ranging from 0 to 9 ml.

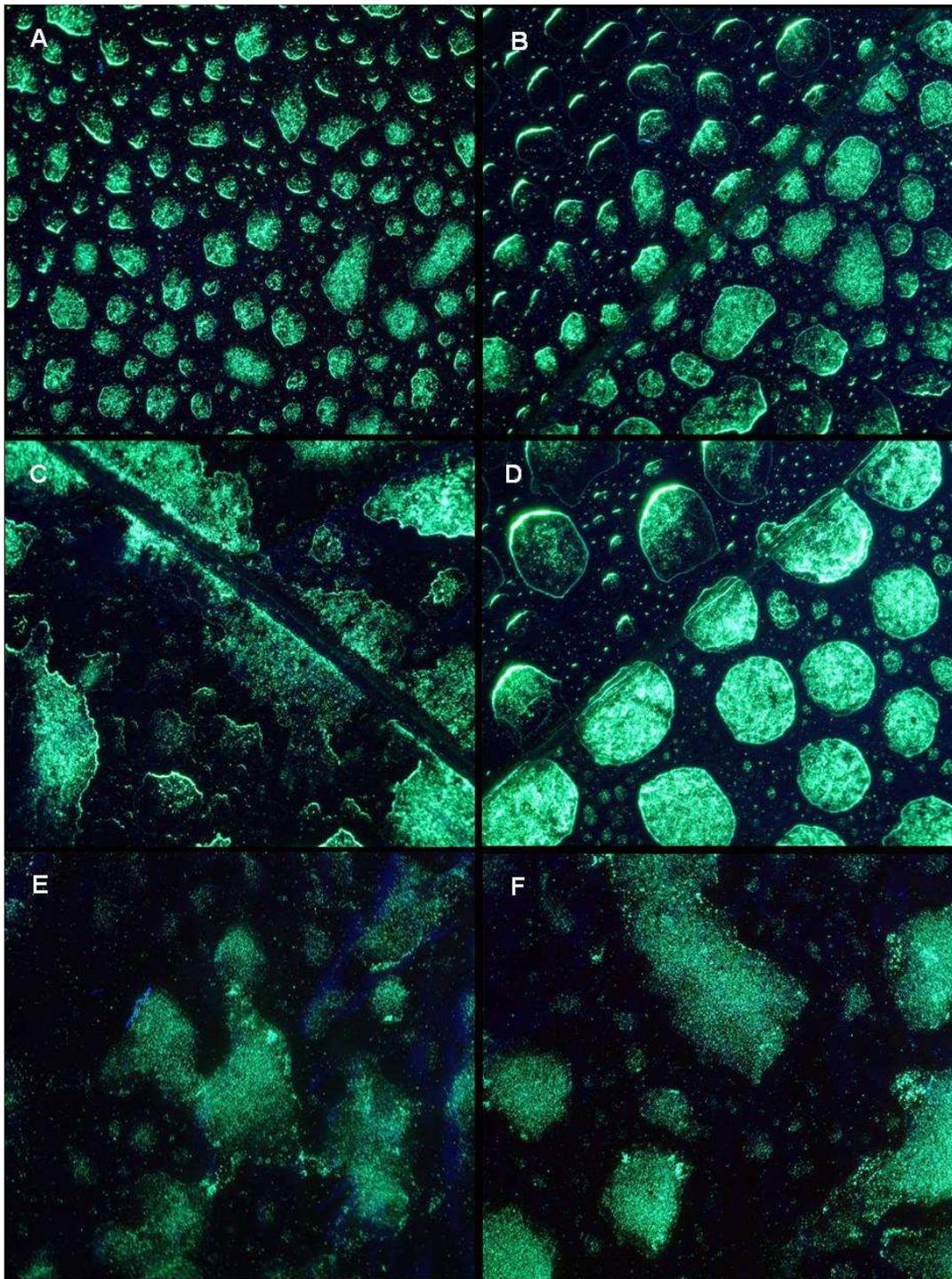
Deposition analysis	Variables			R <sup>2</sup> -value
	A ± SE	B ± SE	C ± SE	
<b>Quantitative analysis</b>				
Eureka lemon	1.86 ± 0.688	2.83 ± 0.683	-0.57 ± 0.156	0.882
Valencia orange	3.47 ± 1.164	2.84 ± 0.626	-0.57 ± 0.145	0.899
<b>Qualitative analysis</b>				
Eureka lemon	113.10 ± 7.545	-3.92 ± 0.235	0.78 ± 0.071	0.989
Valencia orange	78.41 ± 9.843	-5.07 ± 0.433	1.03 ± 0.131	0.979

**Table 4.6.4.3.** Coefficients for Hoerl regression analyses of quantitative ( $y = Ax^B e^{Cx}$ ) and qualitative ( $y = A(x+1)^B e^{C(x+1)}$ ) deposition values following spray application with a mixture of SARDI Yellow Fluorescent Pigment and copper hydroxide to upper and lower leaf surfaces of young Nova mandarin leaves at volumes ranging from 0 to 5 ml, as well as coefficients for quadratic regression analyses ( $y = A+Bx+Cx^2$ ) of infection percentages following inoculation of sprayed leaves with spore suspensions of *Alternaria alternata* pv. *citri*.

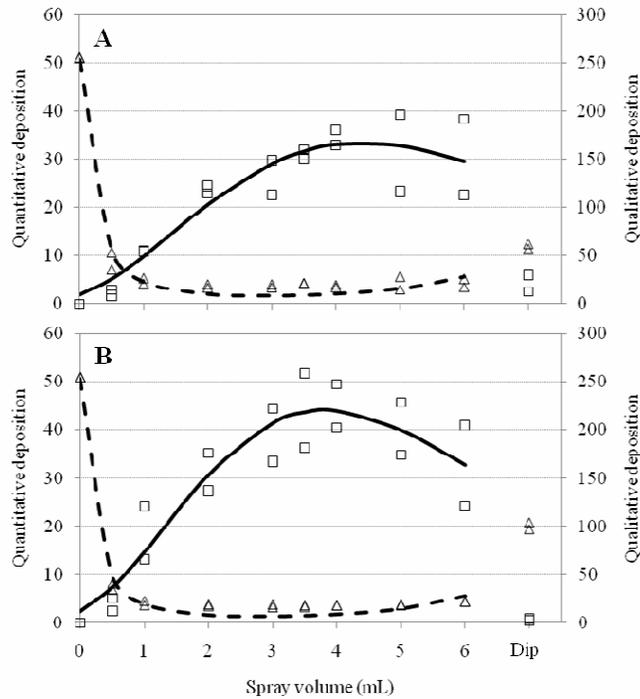
Deposition analysis	Variables			R <sup>2</sup> -value
	A ± SE	B ± SE	C ± SE	
<b>Quantitative analysis</b>				
Upper leaf surface	0.99 ± 0.378	4.59 ± 0.839	-0.85 ± 0.198	0.966
Lower leaf surface	1.67 ± 0.748	7.95 ± 1.336	-2.16 ± 0.381	0.907
<b>Qualitative analysis</b>				
Upper leaf surface	58.61 ± 5.734	-5.16 ± 0.244	1.44 ± 0.102	0.993
Lower leaf surface	43.13 ± 5.163	-5.56 ± 0.341	1.74 ± 0.127	0.984
<b>Infection</b>				
Upper leaf surface	106.438 ± 2.902	-71.140 ± 3.590	11.134 ± 0.707	0.692
Lower leaf surface	102.684 ± 2.604	-71.760 ± 3.188	10.534 ± 0.628	0.781

**Table 4.6.4.4.** Coefficients for sigmoidal regression analyses [ $y = A + B / (1 + \exp(-(x-C)/D))$ ] of mean infection percentages against quantitative and qualitative deposition on upper and lower surfaces of young Nova Clementine leaves that were sprayed with SARDI Yellow Fluorescent Pigment and copper hydroxide at volumes ranging from 0 to 5 ml and a dip-treatment and drop-inoculated with a spore suspension of *Alternaria alternata* pv. *citri*.

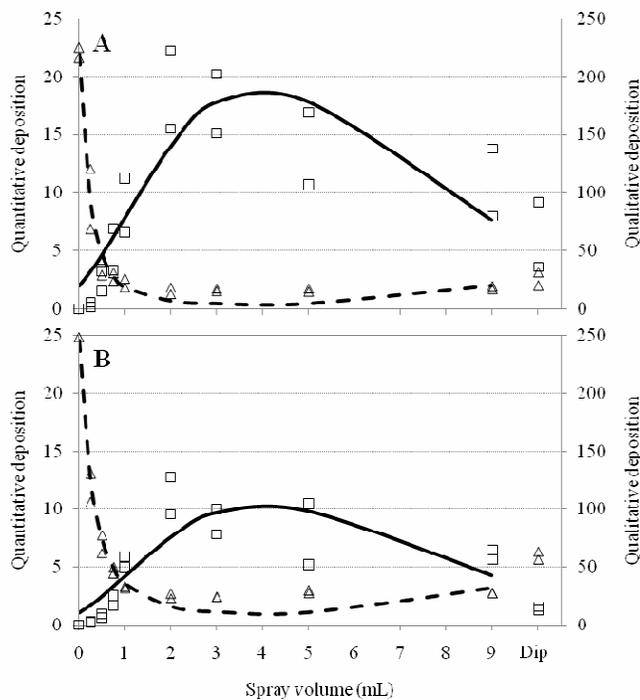
Deposition analysis	Variables				R <sup>2</sup>
	A ± SE	B ± SE	C ± SE	D ± SE	
<b>Quantitative analysis</b>					
Upper leaf surface	0.83 ± 2.096	102.67 ± 5.938	2.10 ± 0.132	-0.62 ± 0.143	0.957
Lower leaf surface	9.49 ± 1.450	93.06 ± 2.901	3.08 ± 0.095	-0.75 ± 0.091	0.978
<b>Qualitative analysis</b>					
Upper leaf surface	-0.57 ± 2.476	98.90 ± 3.612	44.91 ± 0.647	3.90 ± 0.839	0.961
Lower leaf surface	98.8 ± 10.44	-7539 ± 951480	-110 ± 4122	-31.2 ± 33.23	0.633



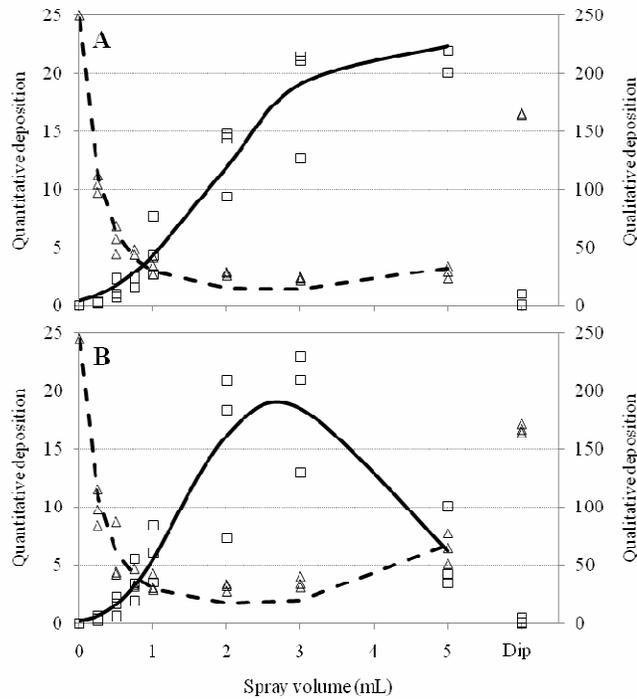
**Fig. 4.6.4.1.** Digital images of upper and lower leaf surfaces of mature Nova Clementine leaves sprayed with 2 ml (A and B, respectively) and 4 ml (C and D, respectively), and Eureka lemon and Valencia orange fruit sprayed with 5 ml (E and F, respectively) of a SARDI Yellow Fluorescent Pigment solution and visualised under black light illumination at 10x magnification.



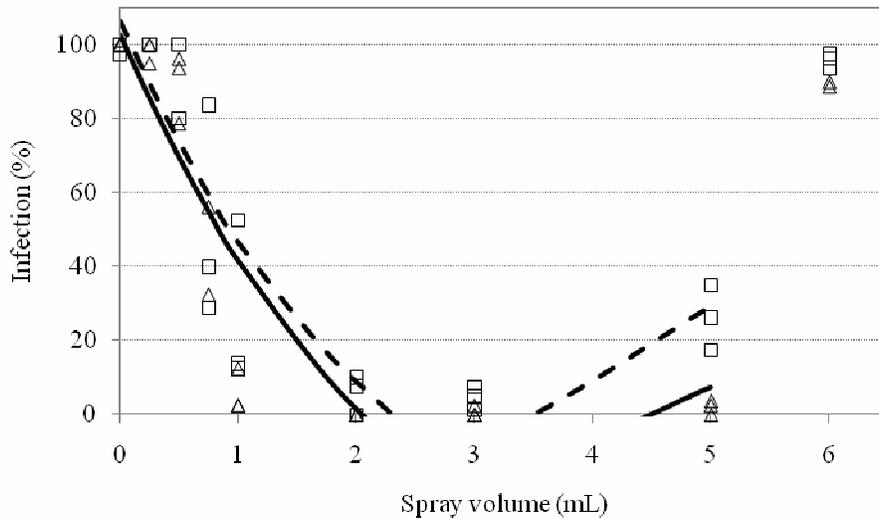
**Fig. 4.6.4.2.** Mean quantitative (percentage area covered by fluorescent pigment;  $\square$ ) and qualitative (grey-scale values of skeleton of Euclidian map of binarised images;  $\Delta$ ) deposition values and respective Hoerl regression lines (—, - -) on upper (A) and lower (B) surfaces of mature Nova Clementine leaves following spray application with SARDI Fellow fluorescent pigment at volumes ranging from 0 to 6 ml and a dip-treatment.



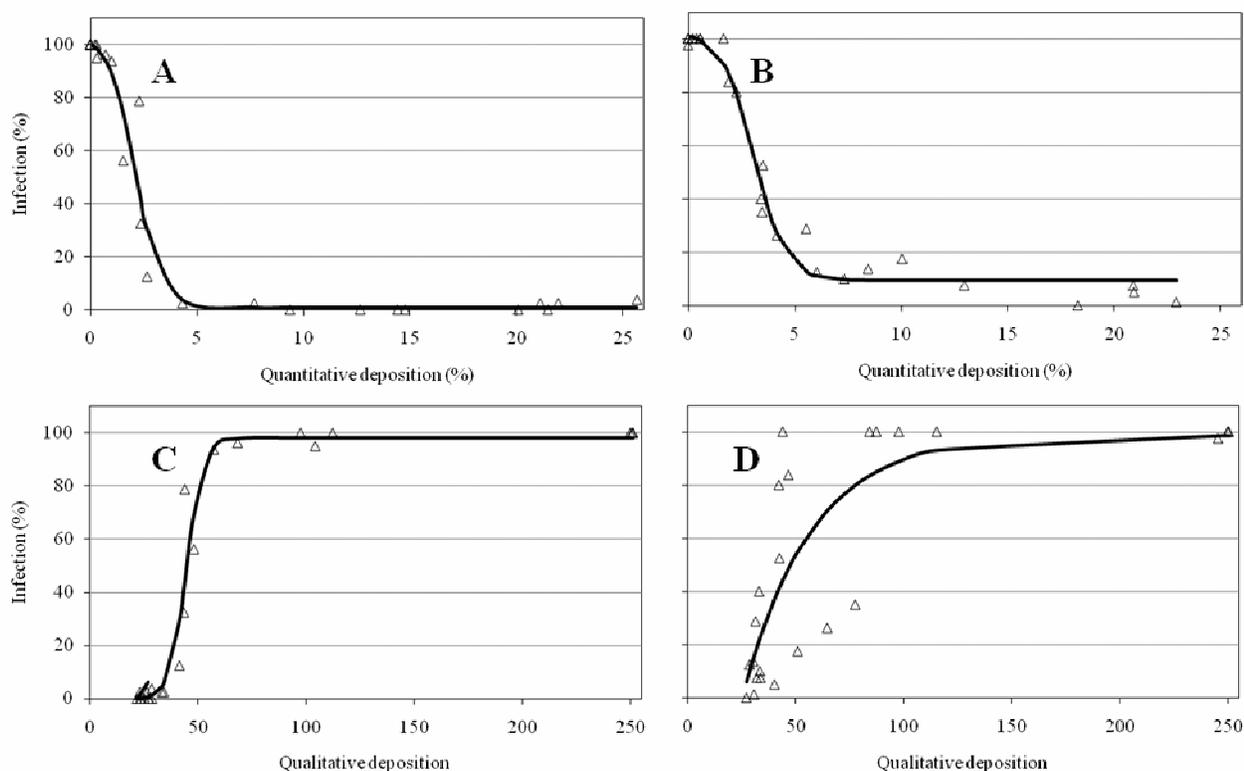
**Fig. 4.6.4.3.** Mean quantitative (percentage area covered by fluorescent pigment;  $\square$ ) and qualitative (grey-scale values of skeleton of Euclidian map of binarised images;  $\Delta$ ) deposition values and respective Hoerl regression lines (—, - -) on Valencia orange (A) and Eureka lemon (B) fruit following spray application with SARDI Fellow fluorescent pigment at volumes ranging from 0 to 9 ml and a dip-treatment.



**Fig. 4.6.4.4.** Mean quantitative (percentage area covered by fluorescent pigment;  $\square$ ) and qualitative (grey-scale values of skeleton of Euclidian map of binarised images;  $\Delta$ ) deposition values and respective Hoerl regression lines (—, - -) on upper (A) and lower (B) surfaces of young Nova Clementine leaves following spray application with a mixture of SARDI Yellow Fluorescent Pigment and copper hydroxide at volumes ranging from 0 to 5 ml and a dip-treatment.



**Fig. 4.6.4.5.** Mean percentage lesion development and respective quadratic regression lines on upper ( $\square$ , —) and lower ( $\Delta$ , - -) surfaces of young Nova Clementine leaves that were sprayed with SARDI Yellow Fluorescent Pigment and copper hydroxide at volumes ranging from 0 to 5 ml and a dip-treatment and drop-inoculated with a spore suspension of *Alternaria alternata* pv. *citri*.



**Fig. 4.6.4.6.** Mean infection percentage and respective sigmoidal regression lines plotted against quantitative (percentage area covered by fluorescent pigment; A, B) and qualitative (grey-scale values of skeleton of Euclidian map of binarised images; C, D) deposition on upper (A, C) and lower (B, D) surfaces of young Nova Clementine leaves that were sprayed with SARDI Yellow Fluorescent Pigment and copper hydroxide at volumes ranging from 0 to 5 ml and a dip-treatment and drop-inoculated with a spore suspension of *Alternaria alternata* pv. *citri*.

#### Characterisation of spray deposition with current spray application methods

[Experimentation on this objective will commence in 2008/9]

#### **Conclusion**

From the results obtained to date, it was clear that biological efficacy declines with increased run-off. Future experimentation should thus focus on optimising application to ensure adequate deposition of the active ingredient with minimal run-off. From the biological data obtained thus far, the benchmark deposition values for 80% control was calculated at 3 to 4% for quantitative and 30 to 40 for qualitative measurement. Image capturing and analysis were major bottle-necks in the research described above, and these steps needed to be improved. New equipment was acquired to improve the robustness, portability and time-efficiency of these steps. The set-up is now optimised and presents a dramatic improvement (up to 8 times faster through-put of image capturing and analysis of samples).

A new inoculation technique has also been developed based on a technique described in a recent publication by Antonio Vicent and co-authors. This involved micro-spray inoculation of leaves with a spore suspension of *Alternaria alternata* pv. *citri* and subsequent moist-incubation for 3 days. This technique is a vast improvement of the droplet-inoculation technique previously used as infection is now more accurately quantifiable. It is anticipated that infection will be quantified by means of the digital image analysis software. The first round of biological efficacy trials has been completed, photos taken of all infected leaves. Images will be analysed and data statistically analysed and correlated with deposition analyses of the fluorescent pigment used in the spray mixture.

## Technology transfer

Study group meetings (Addo, Katrivier, Hoedspruit, Groblersdal, Tshipise, Pongola, Swaziland and Nelspruit)

## 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. New objective: Use of adjuvants for improved spray deposition on citrus leaves and fruit. [Note that this objective will be subject to contractual buy-in from selected companies. Project commenced in January 2008]

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#### 4.7 CRI Diagnostic Centre (Laura Huisman and Timothy Zulu - CRI)

Analysis	Citrus nurseries	Commercial samples	Other crops	Research samples
Nematodes: Roots		415	48	1138
Soil		17	82	1110
<i>Phytophthora</i> : Soil	1239	433	106	634
Nursery water	79		2	
Black spot		9		
Red scale		1		
Citrus greening (UP)		2		50

#### Citrus Accredited Nurseries

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme to send samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. One thousand two hundred and thirty nine samples were received by the DC for *Phytophthora* analyses, of which only 1.9% tested positive.

#### Commercial samples

Samples were received from the following citrus areas: Western Cape, Mpumalanga, Limpopo, Eastern Cape, North-West and Natal. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and citrus nematodes. *Phytophthora citrophthora* was isolated in some samples received from coastal areas. Forty-nine percent of the samples analysed for citrus nematodes had counts above the threshold value of 1000 females per 10 grams of roots, and nematicide treatments were recommended. Forty-four percent of the samples analysed for *Phytophthora* tested positive.

#### Other crops

Nematode counts were done on soil or root samples of grapes, persimmons, plums, litchis, vegetables and pecan nuts. *Phytophthora* and *Pythium* analyses were done on avocado and macadamia samples. The macadamia industry started a nursery improvement scheme similar to the Citrus Improvement Scheme. The DC analysed samples from 13 macadamia nurseries and 24 samples from avocado nurseries for *Phytophthora cinnamomi*.

#### Research samples

Nematode and *Phytophthora* analysis were done on samples from experimental trials to test environmental friendlier nematicides and fungicides. *Phytophthora* isolates from all the citrus areas were sent to the University of Stellenbosch for their research project to determine which *Phytophthora* species are present in the production areas.

#### CRI Diagnostiese Sentrum (Laura Huisman en Timothy Zulu)

Analysis	Sitrus Kwekerie	Kommerciële monsters	Ander gewasse	Navorsings monsters
Nematode: Wortels		415	48	1138
Grond		17	82	1110
<i>Phytophthora</i> : Grond	1239	433	106	634
Kwekery water	79		2	
Swartvlek		9		
Rooidopluis		1		
Sitrusvergroeningsiekte (UP)		2		50

## **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-nege-en-dertig monsters is ontvang vir *Phytophthora* ontledings, waarvan slegs 1,9% positief getoets het.

## **Kommersiële monsters**

Monsters is uit die volgende sitrusverbouingsareas ontvang: Wes Kaap, Mpumalanga, Limpopo, Oos Kaap, Noord-Wes en Natal. Die meeste van die monsters wat van sitruskwekers ontvang is, is ontleed vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*. *Phytophthora citrophthora* is uit monsters afkomstig van koeler kusareas geïsoleer. Nege-en-veertig persent van die aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wopies per 10 g wortels gehad. Aalwurmdoderbehandelings is aanbeveel. Vier-en-veertig persent van die monsters wat vir *Phytophthora* ontleed is, het positief getoets.

## **Ander Gewasse**

Aalwurmtellings is op grond of wortelmonsters van duiwe, persimmons, pruime, litchis, pekanneute en groente gedoen. Avokado en makadamia monsters is vir *Phytophthora* en *Pythium* ontleed. Die makadamia industrie het met 'n kwekery verbeteringskema begin, soortgelyk aan die Sitrus Verbeteringskema. Die DC het monsters vanaf 13 makadamia kwekerye en 24 monsters van avokado kwekerye vir *Phytophthora cinnamomi* ontleed.

## **Navorsings monsters**

Aalwurm en *Phytophthora* ontledings is op monsters van navorsingsprojekte om nuwe meer omgewingsvriendelike aalwurm- en swamdoders te toets, gedoen. *Phytophthora* isolate afkomstig van verskillende sitrusareas is aan die Universiteit van Stellenbosch gestuur as deel van hul navorsingsprojek om vas te stel watter *Phytophthora* spesies in die sitrusareas teenwoordig is.

## 5 PROGRAMME: CROP AND FRUIT QUALITY MANAGEMENT

### 5.1 PROGRAMME SUMMARY

By Tim G. Grout (Manager: Research & Technical, CRI)

Crop and fruit quality research on citrus is complicated and knowledgeable researchers in this field are few. The budget for this programme is approximately half of that for the disease management programme and the integrated pest management programme but this is not a reflection of the need, because millions of Rands are being lost due to some of the problems under investigation, it is due rather to a lack of capacity in the required areas of expertise. During 2007 a new project was launched in this programme on the cold chain and packaging, and research funding was assigned to this for the first time in 2008. CRI also hopes to be able to appoint a researcher with expertise in nutrition during 2008 who will hopefully be able to address some of the needs in that field that desperately require attention. In some respects, horticultural research on citrus is similar to IPM because every problem is influenced by several other factors and there are few simple solutions. More than thirty years ago researchers discovered that gibberellic acid could reduce creasing but we still don't fully understand the reasons for this condition or how to prevent it occurring. This lack of knowledge of fundamental physiological processes also applies to research on other rind problems such as Peteca spot of lemons and rind breakdown in Clementines. The rind condition research reported on here can be likened to completing a jigsaw puzzle without knowing what it should look like. A few important pieces have been found that give us an idea of what the final picture may be but it is not yet clear enough to be sure. A few years ago, some people were of the opinion that high carbon dioxide levels were responsible for various rind problems but now it has been shown that they are not responsible for puffiness or rind breakdown in Clementines and may not even play a role in Peteca spot. Furthermore, high carbon dioxide levels were found to actually reduce chilling injury.

Encouraging progress has been made in developing practical solutions to some problems, even though they may not be fully understood. One such solution is the use of 2,4-D sprays to reduce the size of navel openings on navel oranges and another is the benefit that molybdenum provides in reducing chilling injury. Chilling injury is becoming a more important issue with increasing volumes of fruit being sent to countries that require cold disinfestation treatments. Unfortunately, Oroblancos appear to be very sensitive to the cold which makes their export to Japan very risky. An investigation of the use of gamma irradiation as a possible alternative to cold treatment gave disappointing results too because lemons which are very susceptible to chilling injury were also found to be very susceptible to irradiation damage. With the increasing cost of water and possible shortages in some areas in the future as the climate changes, the research conducted on the partial root zone drying irrigation method will be valuable. Water was also found to be valuable in the form of cold- and hot-water baths which improved fruit colour. With all costs relating to citrus export continually increasing, the development of practices that save growers money will always be a priority. The investigation of hand thinning of Clementines may save labour costs and time at harvest and future research on improvements to the cold chain and nutritional practices will hopefully provide some relief in the years to come.

### PROGRAMOPSOMMING

Deur Tim G. Grout (Bestuurder: Navorsing & Tegnieuse, CRI)

Navorsing op oesgrootte en vrugkwaliteit van sitrus is gekompliseerd en daar is min kundige navorsers op hierdie gebied. Die begroting vir hierdie program is omtrent die helfte van die Siektebestuur- en die Geïntegreerde plaagbestuurprogram, maar dit reflekteer egter nie die behoefte nie, want miljoene rande word as gevolg van party van die probleme wat ondersoek word, verloor. Dit is eerder weens 'n tekort aan kapasiteit in die verlangde areas van kundigheid. Gedurende 2007 is 'n nuwe projek in hierdie program op die koue-ketting en verpakking geloods, en fondse vir navorsing is vir die eerste maal in 2008 hiervoor toegeken. CRI hoop ook om 'n navorser, wat 'n voedingskundige is, gedurende 2008 aan te stel, wat hopelik sommige van die behoeftes op hierdie gebied, wat dringend aandag benodig, sal kan aanspreek. In sommige opsigte is navorsing op hortologiese aspekte van sitrus dieselfde as IPM, omdat elke probleem deur verskeie ander faktore beïnvloed word, en daar min eenvoudige oplossings bestaan. Meer as dertig jaar gelede het navorsers ontdek dat gibberelliensuur kraakskil kan verminder, maar ons verstaan steeds nie die redes vir die toestand en hoe om dit te verhoed nie. Die tekort aan kennis oor die fundamentele fisiologiese prosesse, is ook op navorsing van ander skilprobleme soos Peteka vlek van suurlemoene en skilafbraak by Clementines, van toepassing. Die navorsing op skildefekte waarvoor hier gerapporteer word, kan wees soos om 'n legkaart te voltooi sonder om te weet hoe dit moet lyk. 'n Paar belangrike stukke, wat ons 'n idee kan gee van hoe die finale prentjie mag lyk, is gevind, maar dit is nie duidelik genoeg om seker te wees nie. 'n Paar jaar gelede was party mense van die opinie dat hoë vlakke van koolstofdioksied vir verskeie skilprobleme verantwoordelik was, maar dit is nou bewys dat dit nie vir pofferigheid of skilafbraak in

Clementines verantwoordelik is nie, en mag ook selfs nie eens 'n rol in Peteka vlek speel nie. Verder is daar gevind dat hoë vlakke van koolstofdoksied in werklikheid koueskade verminder.

Belowende vordering in die ontwikkeling van praktiese oplossings vir van die probleme is gemaak, alhoewel dit nog nie heeltemal verstaan word nie. Een van die oplossings is die gebruik van 2,4-D bespuitings om die nawel-end te verklein, en 'n ander is die voordeel in die vermindering van koueskade deur molibdeen. Koueskade word al meer belangrik met die toename in vrugvolumes wat na lande, wat koue-behandelingsmetodes vereis, gestuur word. Dit wil voorkom asof Orobancos baie sensitief vir koue is, wat die uitvoer na Japan baie riskant maak. 'n Ondersoek na die gebruik van gammabestraling, as moontlike alternatief vir koue-behandeling, het ook teleurstellende resultate opgelewer omdat suurlemoene, wat baie vatbaar vir koueskade is, ook gevind is om baie vatbaar vir bestralingskade te wees. Met die toename in waterkoste en die moontlike tekorte daarvan in sekere areas in die toekoms soos die klimaat verander, sal die navorsing wat op die gedeeltelike wortelone-uitdroging-besproeiingsmetode uitgevoer is, waardevol wees. Water is ook gevind om in koue- en warmwaterbaddens waardevol te wees om vrugkleur te verbeter. Met die toenemende verhogings in al die kostes verbonde aan sitrus-uitvoere, sal die ontwikkeling van praktyke wat vir die produsente geld kan bespaar, altyd 'n prioriteit bly. Die ondersoek na die uitdunning van Clementines met die hand, kan arbeidsonkoste en tyd tydens oes bespaar, en toekomstige navorsing op verbeterings aan die koueketting en voedingspraktyke sal hopelik in die jare wat kom, verligting bring.

## 5.2 PROJECT: RIND CONDITION

Project coordinator: J.P. Bower (UKZNP)

### 5.2.1 Project summary

The project on rind condition comprises a number of areas relating to the type of rind disorder. The main categories comprised creasing, puffiness development, rind breakdown disorders such as Peteka spot in lemons and rind breakdown of other cultivars as well as chilling injury, with particular reference to cold sterilized lemons.

The work relating to creasing has considered a number of aspects, both as applications to decrease incidence as well as understand the physiology. A number of growth regulators and calcium formulations were applied (5.2.2). The best results were obtained with GA<sub>3</sub>, especially a double application at petal fall and early December (53% creasing reduced to 23%). All calcium applications had an effect, but not as good as GA<sub>3</sub>. Early applications of GA<sub>3</sub> also had little effect on fruit colour. Fruit on the southern sides of the trees had more creasing. No relationships could be found in this experiment between creasing and albedo mineral content at the time of harvest. Further work on various products will continue. Additional details concerning distribution of creasing within trees and mineral content profiles were also studied (5.2.3.), but no results can as yet be reported, as the mineral results are not yet available. In order to better understand the physiology of creasing, work was done on carbohydrate and mineral allocation within the trees (5.2.4), by changing leaf number, light interception and transpiration of leaves near fruit, as well as fruit thinning and girdling. Unfortunately, no creasing occurred in the orchard, and the work will have to be repeated.

Work on the effects of gas composition surrounding fruit during shipping on development of puffiness, rind breakdown and colour loss has been completed (5.2.5). The work was done on Clementine fruit shipped at low temperature (-0.5°C) for 32 days. Neither ethyl ene nor CO<sub>2</sub>, separately or in combination, appear to have any significant role to play in the development of these disorders. No further work of this type will be done, and future studies to solve the problems will need to take a different direction.

The causes of Peteka spot in lemons are still largely unknown. In the work outlined in section 5.2.6, the possible roles of CO<sub>2</sub> and ethylene were studied by manipulating gas concentrations surrounding fruit after harvest. Both application of ethylene and blocking ethylene action by 1-MCP, reduced Peteka. Anaerobic conditions also reduced the problem, but accumulation of gasses when fruit was stored in a plastic bag increased Peteka spot. The latter technique has been confirmed as useful for predicting the likelihood of Peteka development. Additionally, the role of wax type and packhouse handling was studied (5.2.7). Although picking under cold conditions, hot water treatment, brushing, and waxing with various types of waxes was tested before storage at cold sterilization temperature, no Peteka was found. Storage in plastic bags also did not result in Peteka, indicating no potential for the problem. Further work on the causative factors of Peteka will continue.

Work has been done on the various postharvest treatments for reducing chilling injury (CI), especially for cold sterilized lemons. Various gas and chemical treatments were applied in experiment 832 (section 5.2.8). 1-MCP at 1000 ppb resulted in a considerable decrease in CI, although 2000 ppb did not, indicating a dose response. High levels of CO<sub>2</sub> (5%) for 24 hours after harvest, also significantly reduced CI. The latter is promising, and will be further tested in the next season. The use of a hot water treatment was tested in

experiment 869 (5.2.9). While no results were obtained for fruit from the Nelspruit area due to a lack of chilling development, promising results were obtained from work done in KwaZulu-Natal, where two sources of fruit were used. In one, no chilling injury occurred, while in the other, hot water at 53°C did decrease damage, as did the addition of molybdenum to the water bath. The results may be related to total antioxidant capacity, which was higher in fruit from the site where no damage occurred, and was increased by the hot water and molybdenum. Further work may lead to a model for chilling sensitivity prediction, as well as a technique to decrease damage. Work on cold sterilization of Oroblancos (5.2.10) confirmed previous results, indicating that they are highly sensitive to chilling under cold sterilization temperature conditions of -0.5°C for 12 days. Although no decay was found, which was a problem in previous work.

## Projekopsomming

Die projek op skil integeteit bestaan uit verskeie navorsingsgebiede, naamlik kraakskil, pofferigheid, skilafbraak by verskillende kultivars, insluitend Peteco op suurlemoene sowel as koueskade, veral met betrekking tot kouesterilisering by suurlemoene.

Die werk op kraakskil het verskeie aspekte in ag geneem. Die navorsing het toepassings van verskeie chemikalieë sowel as werk om die fisiologie te verstaan, ingesluit. 'n Aantal groeireguleerders en kalsiumformulasies is getoets (5.2.2). Die beste resultate is met GA<sub>3</sub>, veral 'n dubbele toepassing by blomblaarval en vroeg Desember (53% kraakskil verlaag na 23%) verkry. Alle kalsium bespuitings het 'n effek gehad, maar geen so goed soos GA<sub>3</sub> nie. GA<sub>3</sub> wat vroeg gespuit was, het ook geen noemenswaardige effek op vrugkleur gehad nie. Vrugte op die suidelike kant van die boom het meer kraakskil gehad. Geen verwantskap is tussen albedo minerale inhoud en kraakskil by pluktyd gekry nie. Werk op die effek van verskillende produkte sal voortgaan. Verdere inligting m.b.t. minerale inhoud en distribusie van kraakskil binne die boom (5.2.3) het nog geen resultate gelewer nie, omdat mineraal analiese nog nie afgehandel is nie. Om die fisiologie van kraakskil beter te verstaan, is koolhidrate en minerale verspreiding binne die boom (5.2.4) as gevolg van veranderinge in aantal blare, ligintersepsie en transpirasie bestudeer. Die effek van vruguitdunning en ringuleering is bygevoeg. Ongelukkig is geen kraakskil in die boom gekry nie, en die werk sal moet herhaal word.

Werk op die effek van gas komposisie rondom vrugte tydens verskeping, op skilafbraak, pofferigheid en kleurverlies is nou afgehandel (5.2.5). Die werk is op 'Clementines' gedoen, met 'n verskepingstemperatuur van -0.5°C vir 32 dae. Beide etileen en CO<sub>2</sub> gesaamentlik of apart het geen invloed op die ontwikkeling van afwykings gehad nie. Geen soortgelyke werk op die probleem is beplan nie.

Die faktore wat Peteca in suurlemoene veroorsaak is nog nie bekend nie. In die werk by afdeling 5.2.6. is die moontlike rol van CO<sub>2</sub> en etileen bestudeer. Die gas konsentrasies rondom die vrugte na oes is verander en die effek gemeet. Beide die aanvulling van etileen en die gebruik van 1-MCP, wat etileen bewerkings blokeer, het Peteca verminder. Anaerobiese toestande het ook die probleem verminder maar die ophoping van respirasie gasse tydens opberging binne 'n plastiese sak het die probleem vermeerder. Die tegniek werk goed as 'n voorspellings metode. Die rol van waks en pakhuis behandelings is ook bestudeer (5.2.7). Al was vrugte tydens koue toestande gekluk, warm water behandelings toegepas, normale en oorborseleing en waks van verskillende tipes toegedien voor opberging onder kouesterilisering toestande, is geen peteca gevind nie. Opberging in plastiese sake het ook geen peteca veroorsaak nie, wat aangedui het dat daar geen potensiaal was vir die ontwikkeling van peteca nie. Verdere werk op die faktore wat Peteca veroorsaak, sal voortgaan.

Verskeie na-oes behandelings om koueskade te verminder, veral op suurlemoene met kouesterilisering, is gedoen. Verskeie gas en chemiese behandelings is getoets. (5.2.8). 1-MCP teen 1000dpb het 'n betekenisvolle afname in koueskade getoon, alhoewel 2000 dpb nie so 'n effek gehad het nie, wat die effek van dosis aandui. CO<sub>2</sub> van 5% vir 24 ure na pluk het ook 'n goeie effek gehad. Die behandeling is belowend en sal volgende seisoen weer getoets word. Die gebruik van warm water behandelings was in proef 869 (5.2.9) getoets. Terwyl geen resultate verkry is by vrugte vanaf die Nelspruit area as gevolg van geen koueskade, belowende resultate is vanaf die proef in KwaZulu-Natal, waar vrugte vanaf twee plekke gebruik was. By een van hulle is geen skade gekry nie maar in die ander het warm water by 53°C skade verminder. Molybdeen in die waterbad het dieselfde gedoen. Die resultate is moontlik as gevolg van totale anti-oksiderende aktiwiteit, wat hoër was in vrugte wat nie skade gehad het nie, sowel as by die warm water en molybdeen behandeling. Verdere werk kan moontlik na 'n model vir koueskade voorspelling lei, sowel as 'n behandeling vir vermindering van skade. Werk op die kouesterilisering van Oroblancos (5.2.10) het vorige werk bevestig. Hulle is hoogs sensitief vir koue teen die kouesterilisering temperatuur van -0.5°C vir 12 dae. Al is geen bederf gekry nie, was dit wel 'n probleem by vorige proewe.

## 5.2.2 PROGRESS REPORT: Evaluation of alternative means of controlling creasing (albedo breakdown)

Experiment 849 (December 2006-March 2008): by Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

### Summary

Trials were laid out in Citrusdal on Bahianinha Navels and in Addo on Palmer Navels in October 2006. The objective of this study was to determine if adding fulvic acid to  $\text{Ca}(\text{NO}_3)_2$  increases its uptake and effectivity, and to investigate the effectivity of other Ca sources in reducing creasing incidence. A further objective was to determine if earlier than normal application of  $\text{GA}_3$  would reduce creasing effectively without the negative effect on colour development. On both sites,  $\text{GA}_3$  was applied at petal drop, after physiological fruit drop or at the end of January 2007.  $\text{GA}_3$  in combination with  $\text{Ca}(\text{NO}_3)_2$  or Bioboost, respectively, were both applied after physiological fruit drop.  $\text{Ca}(\text{NO}_3)_2$  (alone or in combination with fulvic acid), amino-chelated or glucose chelated Ca-sources, were all applied 3 times; at petal drop, after physiological fruit drop and 4 weeks later. Trees at Addo were evaluated for creasing incidence at harvest on 5 June 2007. Unfortunately the trial in Citrusdal was harvested without collecting data from the trial site. All calcium spray treatments (amino- or glucose-chelated Ca or  $\text{Ca}(\text{NO}_3)_2$ ), applied alone, showed a reduction in the creasing percentage. The addition of fulvic acid to  $\text{Ca}(\text{NO}_3)_2$  had a negative effect resulting in no reduction in creasing incidence.  $\text{GA}_3$  applications applied alone or in combination with other mineral nutrients reduced creasing incidence markedly. No relationship between the albedo mineral content at harvest and creasing incidence could be established.

### Opsomming

Proewe is uitgelê in Citrusdal op Bahianinha navelbome en in Addo op Palmer navelbome in Oktober 2006. The doel van die studie was om te bepaal of fulviensuur die opname en effektiwiteit van  $\text{Ca}(\text{NO}_3)_2$ , om kraakskil te beheer, verhoog en om die effektiwiteit van ander Ca bronne om kraakskil te beheer, te bepaal. Nog 'n doel was om te bepaal of vroeër toediening van  $\text{GA}_3$  as normaal kraakskil voorkoms sal verminder sonder die negatiewe effek op kleurontwikkeling. By beide proefpersele is  $\text{GA}_3$  toegedien by blomblaarval, na fisiologiese vrugval of aan die einde van Januarie 2007.  $\text{GA}_3$  gekombineer met  $\text{Ca}(\text{NO}_3)_2$  of Bioboost onderskeidelik, is beide toegedien na fisiologiese vrugval.  $\text{Ca}(\text{NO}_3)_2$  (aleen of gekombineer met Fulviensuur), amino-gecheleerde of glucose-gecheleerde Ca-bronne, is almal 3 keer toegedien by blomblaarval, na fisiologiese vrugval and 4 weke later. Bome by Addo is geevalueer vir kraakskil voorkoms by oestyd op 5 Junie 2007. Ongelukkig is die proef by Citrusdal geoes voordat enige evaluasies gedoen kon word. Alle kalsium behandelings (amino- of glukose-gecheleerde Ca of  $\text{Ca}(\text{NO}_3)_2$ ), wat alleen toegedien is, het 'n verlaging in kraakskil voorkoms getoon. Die byvoeging van Fulviensuur by die  $\text{Ca}(\text{NO}_3)_2$  het 'n negatiewe uitwerking gehad en geen afname in kraakskil voorkoms getoon nie.  $\text{GA}_3$  toedienings wat alleen of in kombinasie met ander produkte toegedien is, het kraakskil voorkoms betekenisvol verlaag. Daar was geen verwantskap tussen albedo minerale vlakke by oestyd en kraakskil voorkoms nie.

### Introduction

$\text{GA}_3$  and calcium sprays are both established means of achieving significant reductions in creasing incidence within a season in citrus. The effectiveness of calcium as a control measure for creasing has been investigated in a series of experiments by Storey et al. (2002) and Treeby and Storey (2002). Likewise,  $\text{GA}_3$  either applied alone or in combination with other mineral nutrients, has been investigated and is used to ameliorate the incidence of creasing in most citrus producing countries (Gambetta et al., 2000). In the Eastern Cape as early as 1972 a preliminary trial showed that  $\text{GA}_3$  applied at colour break reduces creasing incidence. This was in conformity with results already reported by Embleton et al. (1973) from their  $\text{GA}_3$  trial in California (Gilfillan and Stevenson., 1974).

The role of calcium as a cementing material of cell walls may be the basic requirement for normal development of long tuberances in albedo tissues (Storey and Treeby, 1994) and stretching of the rind during fruit development (Storey and Treeby, 2002). Creasing is associated with a thinner peel (Jones et. al., 1976) and reduced levels of peel Ca (Nagy et. al., 1985) thus imposing large mechanical stresses upon the connections between albedo cells (Storey and Treeby, 2002). Calcium spray treatments, by increasing the calcium levels in the rind at a critical time of fruit development, markedly reduced creasing. Spraying of citrus fruit with 1% or 2% Ca  $(\text{NO}_3)_2$  or  $\text{CaCl}_2$  throughout fruit development increased the proportion of unaffected fruit from 30 to 65-80 % (Storey and Treeby 2002).

The effect of GA<sub>3</sub> on creasing is via a decrease in the pectin methyl activity (Jona et al., 1989), which is unusually high in fruits affected by creasing. The effectiveness of GA as a control measure for creasing is dependent on the correct concentration, spray solution pH and timing of application. Concentrations of between 5 ppm and 20 ppm have been used in different studies: 5 ppm (Bevington et al., 1973), 10 ppm (Bevington et al., 1973; Monselise et al., 1976; Gilfillan et al., 1980) and 20 ppm (Bevington et al., 1973; Monselise et al., 1976; Gilfillan et al., 1980). The effectiveness of GA<sub>3</sub> can also be increased by acidification to pH 4.0 (Greenberg and Goldschmidt, 1989). The best results were obtained when fruitlets were sprayed when they were about 30-40 mm (Bevington, 1973; Monselise et al., 1976; Gilfillan et al., 1981). GA<sub>3</sub> sprays applied in mid-January present the farmer with a greener fruit at the beginning of picking (Gilfillan et al., 1980). Embleton et al. (1973), Gilfillan and Stevenson (1974) and Bevington (1975) also reported that application of GA<sub>3</sub> when fruitlets are bigger increased green colour on fruit at picking and reduced creasing.

Creasing can render large percentages of fresh fruit unsuitable for the fresh market (Treeby and Storey, 2002). Overall losses of 15% have been observed in light creasing years and up to 50% in severe creasing years in South Africa (Gilfillan et al., 1981). It is possible that adding fulvic acid to Ca(NO<sub>3</sub>)<sub>2</sub> will increase the uptake and effectivity of the Ca source to reduce creasing and that the chelated Ca sources would increase uptake of Ca and reduce creasing more than the conventional Ca sources. Also it is possible that earlier than normal application of GA<sub>3</sub> will reduce creasing effectively without the negative effect on colour development.

The objective of this study was to test these hypotheses and to evaluate the effectiveness of different calcium spray treatments and GA<sub>3</sub> treatments, applied at different times, on alleviating the severity of creasing of Navel oranges. The different spray treatments and application timings used in this study could be used to establish an effective spray programme that will significantly reduce the severity of creasing in Navel oranges.

## Materials and methods

### Plant material

Palmer navel trees in Addo in the Eastern Cape and Bahianinha navel trees in Citrusdal were used for this study in the 2006/ 2007 season. The tree spacing in Addo was 6 m between rows and 4 m in rows and the row direction of the orchard was north to south. Tree spacing in Citrusdal was 6 m between rows and 6 m in rows and the row direction of the orchard was north to south. Both sites have a history of creasing. Unfortunately the trial in Citrusdal was harvested without collecting data from the trial site. Therefore only data from Addo is presented in this report.

### Layout

The field trial consisted of a randomized complete block design with 10 treatments and ten single tree replicates per treatment.

## Treatments

Treatments included:

1. Control.
2. Gibberellic acid (GA<sub>3</sub>) applied on 9 December 2006.
3. Gibberellic acid (GA<sub>3</sub>) applied at petal drop (24 October 2006) and on 9 December 2006.
9. Gibberellic acid (GA<sub>3</sub>) applied on 31 January 2007.
8. Bioboost at 30 ml/100 liter water applied at petal drop (24 October 2006) and Gibberellic acid (GA<sub>3</sub>) and Bioboost at 30 ml/100 liter water applied on 9 December 2006.
4. Gibberellic acid (GA<sub>3</sub>) and Ca(NO<sub>3</sub>)<sub>2</sub> applied on 9 December 2006.
5. Gluco-calcium applied at petal drop, on 9 December 2006 and 4 weeks later (2 January 2007).
6. Cal-Pro applied at petal drop (24 October 2006), on 9 December 2006 and 4 weeks later (2 January 2007).
7. Ca(NO<sub>3</sub>)<sub>2</sub> (195 g Ca /kg) applied at petal drop (24 October 2006), on 9 December 2006 and 4 weeks later (2 January 2007).
10. Fulvic acid at 300 ml/100 litres water and Ca(NO<sub>3</sub>)<sub>2</sub> applied at petal drop (24 October 2006), 2 weeks after petal drop (7 December 2006), on 9 December 2006, and 4 weeks later (2 January 2007).

All Gibberellic acid (GA<sub>3</sub>) applications were applied at 10 ppm with 5 ml Breakthru/100 litres water. Ca(NO<sub>3</sub>)<sub>2</sub> was applied at 2 kg/100 litres water. Gluco-calcium and Cal-Pro were applied at 675 ml and 825 ml/100 litres water, respectively. Gluco-calcium contains 100 g Ca/litre, 26 g amino acids/litre and 1 mg Auxins/litre.

Cal-Pro is an amino chelated product containing 84 g Ca/litre product. Bioboost is a product known to increase fruit size.

#### On tree evaluation

Ten fruit per single-tree replicate were selected on 5 June 2007 randomly from the outside of the tree at eye-level on four positions of the tree; north, south, east and west. Forty fruit per single tree were scored for creasing and colour. The first seven replicates were evaluated. 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 as a zero, if a portion or entire single sphere was creased, a one and so on. A zero meant there was zero percent creasing and a four meant 100 percent creasing. Fruit colour was determined based on the CRI colour chart for oranges, with a range between one and eight, one being completely orange and eight being green. Creasing severity and creasing percentage was also calculated for the on-tree evaluation.

#### Laboratory analysis

Ten fruits per single tree were picked on 5 June 2007 randomly from the outside of the tree at eye-level on the western side of the tree for the first eight replicates. The outside of each fruit was marked. The sampled fruit were assessed for creasing, colour and size. Creasing and colour was evaluated in the same way as the on-tree evaluation for creasing and colour. The fruit diameter of each fruit was measured using an electronic caliper. To determine the relationship between creasing incidence and albedo mineral content at harvest, the shady side of the albedo of picked fruit from the different treatments were dried and stored in small vials. The samples were then sent to the laboratory for mineral nutrient analyses (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B).

#### Statistical analysis

Analysis of variance was performed using the computer program SAS (Statistical Analysis System) enterprise guide 3.

### **Results and discussion**

#### Creasing percentage

All calcium spray treatments applied alone reduced creasing percentage significantly (Table 5.2.2.1). The addition of fulvic acid had a negative effect on the action of Ca and did not show any reduction in creasing percentage. GA<sub>3</sub> applications applied alone or in combination with other products reduced creasing percentage markedly. The best treatment was a double GA<sub>3</sub> applied alone at petal fall and on 9 December 2006. Creasing percentage was reduced almost by half from 53% to 23% (Table 5.2.2.1). Creasing severity showed a similar trend to creasing percentage (Table 5.2.2.1).

#### Fruit Colour

Fruit sprayed with calcium alone or in combination with either fulvic acid or GA<sub>3</sub> had little or no colour problem. As expected, a delay in fruit colour change was more pronounced on fruit applied with GA<sub>3</sub> at the end of January. A minimal colour problem was also prevalent in the double application of GA<sub>3</sub> at petal fall and early December. A similar response was observed in the combined applications of GA<sub>3</sub> and Bioboost (Table 5.2.2.1). However the single application of GA<sub>3</sub> in early December had no colour problem.

#### Bearing positions

Fruit harvested from the south side had significantly more creasing than fruit from the north and east, but not from the west (Table 5.2.2.2). Fruit with the worst colour were picked from the north and fruit with the best colour from the west, and fruit picked from the south and east had an intermediate colour.

#### Albedo mineral content

In general, there were no significant differences in albedo mineral (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B) content between the untreated control fruit and fruit receiving the different treatments. A relationship between the mineral content of the albedo at harvest of treated fruit vs. non-treated fruit and creasing incidence could not be established (Tables 5.2.2.3 and 5.2.2.4).

As with this study, calcium spray treatments of either CaCl<sub>2</sub> or Ca(NO<sub>3</sub>)<sub>2</sub> have been used to reduce creasing severity (Storey et. al., 2002; Storey and Treeby, 2002). It was reported that GA<sub>3</sub> application timings have a marked negative effect on colour, especially in late applications (Monselise et.al., 1976; Gilfillan and Stevenson, 1974; Bevington, 1973, Embleton et.al., 1973 and Gilfillan 1980) as was the case when GA<sub>3</sub> was applied at the end of January in this study. Fruit on the south side had a higher number of creased fruit. A similar observation was also made by Jones et.al. (1967) (northern hemisphere) and also reported by Holtzhausen (1981). This could be that the fruits are on the shaded side and are susceptible to develop a

thin peel under shade and a thin peel is associated with creasing (Jones et al., 1967; Storey et al., 2002). Fruit on the western side had no colour problem because of their exposure to the sun. Very little consistency was observed in the albedo mineral content at harvest between treated and untreated fruit.

## Conclusion

In this study calcium spray treatments of Gluco-calcium, Cal-Pro,  $\text{Ca}(\text{NO}_3)_2$  effectively reduced creasing. The combination of  $\text{Ca}(\text{NO}_3)_2$  with fulvic acid was not effective. Fulvic acid did not enhance the effectiveness of this spray treatment in this study. The best response with  $\text{Ca}(\text{NO}_3)_2$  was observed when it was sprayed in combination with  $\text{GA}_3$ . This positive effect can be attributed to the known effect of  $\text{GA}_3$  in controlling creasing. In this trial the best response was observed with a double application at petal fall and early December. Applications made at the end of January also had a good response, but a marked delay in colour development was observed at the time of picking. Interestingly, a once-off application in early December was not as effective as the double application of  $\text{GA}_3$ . Bioboost combined with  $\text{GA}_3$  had the poorest results. Bioboost reduced the effectivity of  $\text{GA}_3$ . The relationship between albedo mineral content at harvest and creasing incidence could not be established in this study.

## Future research

This study will continue to find and test different products in reducing creasing incidence. The uptake and effectivity of different Ca sources in reducing creasing incidence will also be investigated in the following season.

## Technology transfer

It is too soon to include these results in any technology transfer.

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**Table 5.2.2.1.** The effects of different calcium spray treatments and GA application timings on the incidence of creasing, colour and creasing severity on Navel oranges in the Addo area, South Africa.

Treatment	Creasing %	Colour	Creasing severity
Control	53.2 a <sup>z</sup>	2.9 de	1.1 a
GA applied on 9 December 2006	40.4 bc	3.0 cd	0.8 bc
GA applied at petal drop + 9 December 2006	23.6 e	3.2 b	0.5 e
Gibberelic acid (GA) applied end of January	25.4 de	3.5 a	0.5 e
Gibberelic acid (GA) and Bioboost applied at petal drop + 9 December 2006	42.5 ab	3.1 bc	0.9 bc
Gibberelic acid (GA) and Ca(NO <sub>3</sub> ) <sub>2</sub> applied on 9 December 2006	30.0 cde	3.0 bcd	0.6 de
Gluco-calcium applied at petal drop, on 9 December 2006+ 4 weeks later.	36.1 bcd	3.0 cde	0.7 cd
Cal- Pro applied at petal drop + on 9 December 2006 + 4 weeks later.	34.6 bcde	3.0 e	0.7 cd
Ca(NO <sub>3</sub> ) <sub>2</sub> applied at petal drop + on 9 December 2006 +4 weeks later.	37.9 bc	3.0 de	0.7 cd
Fulvic acid and Ca(NO <sub>3</sub> ) <sub>2</sub> applied at petal drop +2 weeks after petal drop + on 9 December 2006 + 4 weeks later.	45.7 ab	3.0 cde	1.0 ab
<i>P-value</i>	0.0001	0.0001	0.0001

<sup>z</sup>Means with the same letter are not significantly different at the 5% level

**Table 5.2.2.2.** Evaluation of the relationship of the bearing position of a fruit on a tree and the creasing incidence, colour and creasing severity on Navel oranges in the Addo area, South Africa.

Side	Creasing %	Colour	Creasing severity
North	32.4 b <sup>z</sup>	3.4 a	0.8 ab
South	41.9 a	3.2 b	0.9 a
East	34.1 b	3.2 b	0.6 b
West	39.3 ab	2.4 c	0.7 b
<i>P-value</i>	0.0431	0.0001	0.0052

<sup>z</sup>Means with the same letter are not significantly different at the 5% level

**Table 5.2.2.3.** Albedo mineral nutrient levels of treated and untreated Navel oranges in the Addo area, South Africa.

Treatment	N	P	K
Control	0.75	0.038 ab <sup>z</sup>	0.27
GA applied on 9 December 2006	0.73	0.037 ab	0.25
GA applied at petal drop + 9 December 2006	0.75	0.039 a	0.30
Gibberelic acid (GA) applied end of January	0.71	0.039 a	0.27
Gibberelic acid (GA) and Bioboost applied at petal drop + 9 December 2006	0.74	0.035 ab	0.26
Gibberelic acid (GA) and Ca(NO <sub>3</sub> ) <sub>2</sub> applied on 9 December 2006	0.74	0.038 ab	0.28
Gluco-calcium applied at petal drop, on 9 December 2006+ 4 weeks later.	0.71	0.039 a	0.27
Cal- Pro applied at petal drop + on 9 December 2006 + 4 weeks later.	0.75	0.039 a	0.29
Ca(NO <sub>3</sub> ) <sub>2</sub> applied at petal drop + on 9 December 2006 +4 weeks later.	0.71	0.034 ab	0.28
Fulvic acid and Ca(NO <sub>3</sub> ) <sub>2</sub> applied at petal drop +2 weeks after petal drop + on 9 December 2006 + 4 weeks later.	0.71	0.033 b	0.25
<i>P-value</i>	0.4775	0.0408	0.6041

<sup>z</sup>Means with the same letter are not significantly different at the 5% level

**Table 5.2.2.4.** Albedo mineral nutrient levels of treated and untreated Navel oranges in the Addo area, South Africa.

<b>Treatment</b>	<b>Ca</b>	<b>Mg</b>	<b>Na</b>	<b>Mn</b>	<b>Fe</b>	<b>Cu</b>	<b>Zn</b>	<b>B</b>
Control	0.51	0.014 ab <sup>z</sup>	101	2.2	17.5	2.9	7.7	25.7 a
GA applied on 9 December 2006	0.51	0.014 ab	87	1.4	22.4	2.6	6.9	26.0 a
GA applied at petal drop + 9 December 2006	0.51	0.019 a	118	1.5	19.7	2.8	7.2	26.1 a
Gibberelic acid (GA) applied end of January	0.52	0.011 ab	114	1.0	18.2	2.9	7.5	25.2 ab
Gibberelic acid (GA) and Bioboost applied at petal drop + 9 December 2006	0.52	0.014 ab	100	1.4	15.6	2.6	7.1	25.4 a
Gibberelic acid (GA) and Ca(NO <sub>3</sub> ) <sub>2</sub> applied on 9 December 2006	0.49	0.019 a	99	1.3	25.9	2.6	7.0	26.1 a
Glucocalcium applied at petal drop, on 9 December 2006+ 4 weeks later.	0.51	0.010 b	92	1.7	15.3	2.4	6.6	25.9 a
Cal- Pro applied at petal drop + on 9 December 2006 + 4 weeks later.	0.51	0.015 ab	122	1.7	9.8	2.8	8.4	25.5 a
Ca(NO <sub>3</sub> ) <sub>2</sub> applied at petal drop + on 9 December 2006 +4 weeks later.	0.49	0.015 ab	141	1.8	9.5	2.6	6.9	25.5 a
Fulvic acid and Ca(NO <sub>3</sub> ) <sub>2</sub> applied at petal drop +2 weeks after petal drop + on 9 December 2006 + 4 weeks later.	0.53	0.013 c	141	1.5	36.3	3.1	7.2	24.5 b
<i>P-value</i>	0.8169	0.0005	0.8926	0.1767	0.0523	0.5796	0.3578	0.0070

<sup>z</sup>Means with the same letter are not significantly different at the 5% level

### 5.2.3 **PROGRESS REPORT: Relationship of bearing position on a tree and the incidence and severity of creasing/albedo breakdown**

Experiment 863 (December 2006-March 2008): by Stephan Verreyne (CRI at SU), Zanele Phiri (SU)

#### **Summary**

The objectives of this study are: 1) to determine if the position of fruit on a tree has an effect on the incidence and the severity of creasing, 2) to determine if creasing is more pronounced on the inside (shaded) or outside (exposed) part of the fruit and 3) to determine if these differences in creasing incidence mentioned above are related to differences in macro- or micronutrient concentrations in the albedo tissue of fruit at harvest. Palmer Navel trees in Addo planted in a north-south row direction were used for this study. Fruit were sampled at harvest time in May 2007 from four different sub-sectors, the inside and outside top part of the tree, and the inside and outside bottom part of the tree of four sectors, viz. north, south, west and east. For outside sub-sectors the peel of fruit collected was divided into sections of the outside (sunny side) and the inside (shady side) of the fruit. Creasing incidence (%), fruit size and peel thickness were determined. Samples of albedo tissue of fruit were prepared for mineral nutrient analyses. Laboratory analyses are still awaited, and full results will be presented in the next annual research report.

#### **Opsomming**

Die doel van die studie was om: 1) te bepaal of die posisie van vrugte in 'n boom 'n effek op die voorkom en graad van kraakskil het, 2) om te bepaal of kraakskil erger in die binnekant (skadukant) of buitekant (sonkant) van vrugte voorkom en 3) om te bepaal of hierdie verskille in kraakskil verband hou met makro- of mikroelement konsentrasies in die albedo weefsel van vrugte by oestyd. Palmer Navel lemoenbome in Addo in 'n noord-suid ruyrigting is gebruik vir die studie. Vrugte is geoes tydens oestyd in Mei 2007 van vier sub-sektore, die binne en buite boonste dele van die boom, en die binne en buite onderste dele van die boom in die vier sektore, nl. noord, suid, wes en oos. Vir buite sub-sektore is die skil opgedeel in die buite- (sonkant) of binnekant (skadukant) van die vrug. Kraakskil voorkoms (%), vruggrootte en skildikte is bepaal. Monsters albedo weefsel van die vrugte is voorberei vir minerale element analises. Ons wag vir hierdie analises van die laboratorium en die volledige resultate sal in die volgende jaarlikse navorsingsverslag verskyn.

### 5.2.4 **PROGRESS REPORT: Effect of manipulation of carbohydrate and mineral nutrient allocation in the tree on creasing incidence**

Experiment 864 (December 2006-March 2008): by Stephan Verreyne (CRI at SU) , Zanele Phiri (SU)

#### **Summary**

Manipulations applied to Washington Navel trees in Citrusdal after physiological fruit drop, on 5 December 2006 or at the end of January 2007 include covering leaves behind fruit with shade cloth, covering fruit with bags, girdling scaffold branches, pruning windows to improve light distribution in the trees, removing leaves from behind fruit and hand thinning shoots to shoots bearing single fruit. Trees were harvested in May 2007. Fruit were collected from trees from each treatment and percentage creasing incidence, leaf and albedo tissue mineral nutrient analyses were determined. Unfortunately creasing incidence was very low in this orchard and therefore, none of the treatments resulted in significant differences in fruit diameter, creasing severity or creasing incidence. The treatments also had no major effect on both leaf and albedo mineral nutrient levels and no correlations or trends among treatments, creasing incidence, leaf mineral nutrients, or albedo mineral nutrients at harvest were evident. Therefore, this trial will be repeated in the current season.

#### **Opsomming**

Manipulasies toegedien op Washington Navel lemoenbome in Citrusdal na die fisiologiese vrugval periode, op 5 Desember 2006 of aan die einde van Januarie 2007 sluit in die toemaak van blare agter die vrugte met skadunet, toemaak van vrugte met sakkies, ringelering van raamtakke, snoei van vensters in bome om ligverspreiding te verbeter, verwydering van blare agter die vrugte, en handuitdun van takkies met vrugte tot enkelvrugte. Bome is geoes in Mei 2007. Vrugte is versamel van bome van die veskillende behandelings en kraakskil voorkoms, blaar- en albedo minerale vlakke is bepaal. Ongelukkig was kraakskil voorkoms in die boord baie laag en daarom het geen behandeling betekenisvolle verskille in vrugdeursnit, kraakskil voorkoms en die graad van kraakskil getoon nie. Die behandelings het ook geen effek gehad op beide blaar- en albedo

minerale vlakke by oes nie en geen korrelasies of tendense tussen kraakskil voorkoms, blaar minerale vlakke, en albedo minerale vlakke was sigbaar nie. Dus, hierdie eksperiment sal in die huidige seisoen herhaal word.

## Inleiding

Kraakskil is 'n skilkwaal by sekere sitrus vrugte. Dit is uitkenbaar as nou, ingesonne groewe wat in verskillende rigtings op die vrug voorkom en 'n onreëlmatige patroon vorm. Hierdie abnormaliteit lei tot aansienlike negatiewe gevolge vir die produsent, siende dat alle vrugte met kraakskil in die pakhuis verwyder word (Jones et al., 1967; Stanton, 1970). Dit kan tot meer as 50 persent van die oes affekteer in sekere jare en gebiede en dus lei dit tot groot dalings in winste.

Kraakskil word veroorsaak deur die albedo (wit gedeelte van skil) wat skeur. Seldeling in die albedo vind net in die eerste  $\pm 63$  dae na volblom plaas, ook genoem Fase I van vrugontwikkeling. Daarna hou dit op, maar seldeling in die endokarp (eetgedeelte) duur voort. Dit saam met selvergroting plaas dan spanning op die albedo wat gedwing word om te rek. Indien kragte tussen albedo selle nie sterk genoeg is nie breek die verbindings tussen selle en dit veroorsaak dan dat die albedo skeur. Die flavedo (oranje gedeelte) en epidermis (buitenste selle) se selverdeling het dwarsdeur vruggroei plaasgevind, so hierdie dele is nie onder stres nie, maar as gevolg van die albedo wat skeur by kraakskil val hierdie buitenste gedeelte dan in en dit lei tot die sigbare verandering aan die buitekant van die vrug in Fase III (Bain, 1958).

Die posisie van vrugte op bome het 'n groot effek op hoeveelheid kraakskil teenwoordig. Op dieselfde boom kom daar vrugte met en sonder kraakskil voor so ook op dieselfde tak. Oor die algemeen vind kraakskil ontwikkeling aan die boom-kant van die vrug plaas d.w.s aan die skadu-kant van die vrug en is minder ernstig aan die sonkant van die vrug (Fourie en Joubert, 1957; Jones et al., 1967; Beresky et al., 1977). Volgens Jones et al. (1967) is hierdie kraakskil afkomstig van die radiale temperatuurgradiënt oor die vrug, en dus 'n verskil in waterpotensiale.

Kraakskil ontwikkel wanneer selle in die albedo van mekaar wegtrek waar selwande aan mekaar vasgeheg is. Kraakskil word gekorreleer met lae pektien vlakke en lae kompleksvorming met minerale in selwande. Pektien degradasie veroorsaak dat bindings tussen selle breek soos vrugte ryp word. Tydens rypwording is daar 'n toename in water oplosbare pektiene en pektolitiese ensieme, wat die voorkoms van kraakskil bevorder. Baie minerale soos Mo, Zn, Ca, S, B en Mg vorm komplekse met pektiene en versterk die binding tussen albedo selle (Monselise et al., 1976).

Pektiene met koolhidrate as basis is afhanklik van koolhidraat metabolisme. Zn beïnvloed koolhidraat metabolisme op verskillende maniere en miskien kan Zn koolhidraat sintese bevorder, wat sal bydra tot pektiensintese (Bower, 2004).

Ferreira (1984) het waargeneem dat 'n oormaat fosfor of ongebalanseerde voedings kondisies (veral lae kalium en stikstof) tot kraakskil kan lei. In 'n aantal proewe van Jones et al. (1967) is daar gevind dat hoë fosfaat toedienings kraakskil geweldig vermeerder. Lae N word ook geassosieer met die voorkoms van kraakskil terwyl geringe voorkoms gepaard gaan met hoë N -toedienings (Le Roux en Crous, 1938). Fourie en Joubert (1957) in Suid Afrika en Sites en Deszyck (1952) in Florida het bewys dat lae kalium en hoë fosfor verantwoordelik kan wees vir die vermeerdering van kraakskil. K verbeter vruggroote en lei tot vorming van dikker skille en daarom is dit belangrik om te verseker dat daar genoeg K na vrugte getranslokeer word.

Soos uit al die boegenoemde waargeneem kan word is dit duidelik dat daar heelwat faktore is wat 'n rol speel op die voorkoms van kraakskil. Veral koolhidraatvlakke en mineraal nutriente allokasie speel 'n groot rol in die voorkoms van kraakskil. Die rede vir hierdie navorsing is om te bepaal hoe verskillende manipulasies die koolhidraat en minerale nutriente allokasie beïnvloed en sodoende dan ook die effek op kraakskil.

## Materiaal en metode

Hierdie navorsing is gedoen in 'n navel boord op die plaas Biesievlak in die Citrusdal omgewing. Tien verskillende behandelings is uitgevoer wat ook die kontrole insluit. Elke behandeling is op tien verskillende bome gedoen met tien vrugte per boom in 'n gerandomiseerde volledige blok ontwerp.

Die tien verskillende behandelings is soos volg gedoen.

1) Geen veranderinge is aangebring nie, genoem die kontrole.

- 2) Die toemaak van blare agter die vrugte met skadunet op 5 Desember 2006. Die blare is toegemaak om die effek van verminderde transpirasie naby die vrugte te bepaal.
- 3) Die wegneem van blare agter die vrug op vrugdraende lote op 5 Desember 2006. Blare is vermoedelik 'n groot sink vir sitokiniene en dus is dit gedoen om te sien of meer sitokiniene na die vrug sal beweeg en watter effek dit sal hê.
- 4) Die toemaak van blare agter die vrugte met skadunet einde Januarie 2007.
- 5) Die raamtakke is geringeleer op 5 Desember 2006. Dit is gedoen sodat al die voedingstowwe nie weg sal beweeg na die wortels nie, maar naby die vrugte gehou word.
- 6) Blare wat skadu oor die vrugte gooi is op 5 Desember 2006 gesnoei. Dit veroorsaak dan dat die hele vrug son sal kry. Die effek daarvan op kraakskil behoort positief te wees.
- 7) Handuitdunning op draende lote is gedoen. Lote met drie vrugte op is verminder na een vrug toe. Dit is op 5 Desember 2006 gedoen.
- 8) Vrugte is ook op 5 Desember 2006 met sakkies toegemaak. Dit sal moontlik transpirasie verminder en dus behoort dit te lei tot meer kraakskil. Dit vat ook lig weg en kan temperatuur van vrugte ook verander.
- 9) Vrugte is ook einde Januarie 2007 met sakkies toegemaak.
- 10) Blare wat skadu oor die vrugte gooi is einde Januarie 2007 gesnoei.

Die kleur, deursneë, kraakskil hoeveelheid aan skadukant, kraakskil hoeveelheid aan sonkant, kraakskil totaal, setpersentasie van vrugte gemerk en ook die kraakskilpersentasie van elke vrug is gemeet by oestyd in Mei 2007. Die kleur is volgens die CRI kleurkaart bepaal, dit wissel van 1 tot 8 met 1 oranje en 8 groen. Die deursneë word gemeet met 'n digitale meetinstrument (calliper). Om die kraakskil hoeveelhede te bereken is daar van nul gegee vir geen kraakskil, een effense kraakskil en twee is wanneer die meeste van die helfte skadu of sonkant vol kraakskil is. Die setpersentasie dui aan hoeveel van die oorspronklike vrugte wat gemerk was aan die eide nog op die boom was om dit te ontleed. Die kraakskilpersentasie is dan bepaal vir elke behandeling onderskeidelik. Nadat dit als gemeet word, word die blare en albedo van die skil dan onderskeidelik ingestuur vir laboratorium toetse om die mineraalkonsentrasies te bereken. Al die data word dan statisties ontleed om sodoende die effek van al die behandelings te bepaal.

## **Resultate en bespreking**

Geen behandeling het die vrugdeursnit, graad van kraakskil of die kraakskil persentasie betekenisvol beïnvloed nie (Tabel 5.2.4.1). Vrugset % is betekenisvol verlaag deur blare agter die vrugte in Desember te verwyder. Ongelukkig was kraakskil oor die algemeen baie laag in die boord en geen behandelings het kraakskil betekenisvol verhoog of verlaag. Dit maak interpretasie van die resultate baie moeilik. Met die toemaak van die vrugte op 5 Desember 2006 het die kraakskil aan die sonkant betekenisvol verhoog. Die skadukant van die vrug is meer geneig tot kraakskil (Fourie en Joubert, 1957; Jones et al., 1967; Beresky et al., 1977). Die behandelings het ook 'n geringe effek gehad op beide blaar (Tabel 5.2.4.2)- en albedo minerale vlakke (Tabel 5.2.4.3) en geen korrelasies tussen die verskillende behandelings, kraakskil voorkoms, blaar minerale vlakke en albedo minerale vlakke was duidelik sigbaar nie.

## **Gevolgtrekking**

Oor die algemeen was dit 'n jaar met nie baie kraakskil voorkoms in die Citrusdal omgewing nie. Dit het veroorsaak dat daar nie eintlik enige werklike betekenisvolle verskille was tussen die behandelings wat kraakskil aanbetref nie. Met die minerale ontledings is daar ook nie baie wat verander het nie en geen definitiewe tendense was sigbaar nie.

## **Toekomstige navorsing**

Die eksperiment sal herhaal word en in 'n jaar met 'n groter kraakskil voorkoms sal dit meer duidelikheid gee oor die effek van hierdie manipulasies op kraakskil voorkoms en meer duidelikheid verskaf oor die meganisme van kraakskil ontwikkeling.

## **Tegnologie oordrag/Technology transfer Produsente praatjies/Grower presentations**

Verreyne, J.S. Rind condition: Physiological disorders in citrus. Malelane Citrus Study Group. Malelane, 17 July 2007

Verreyne, J.S. Rind condition: Physiological disorders in citrus. Komatipoort Citrus Study Group. Komatipoort, 17 July 2007  
Verreyne, J.S. Rind condition: Physiological disorders in citrus. Swaziland Citrus Study Group. Swaziland, 18 July 2007  
Verreyne, J.S. Rind condition: Physiological disorders in citrus. Beitbridge Citrus Study Group. Beitbridge, 2 August 2007

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**Tabel 5.2.4.1.** Kraakskil voorkoms by verskillende behandelings.

Behandeling	Kleur 1 tot 8	Deursnee mm	Kraakskil skadu 0 tot 2	Kraakskil son 0 tot 2	Kraakskil total 0 tot 4	Set %	Kraakskil %
Kontrole	4.6 ab <sup>z</sup>	77.5	0.23	0.03 b	0.26	78 bcd	12
Blare bedek (Des)	4.7 ab	76.0	0.30	0.05 b	0.33	75 bcde	15
Blare verwyder (Des)	4.8 a	72.5	0.12	0.00 b	0.11	56 e	4
Blare bedek (Jan)	4.7 ab	76.8	0.21	0.00 b	0.21	94 ab	14
Ringeleer (Des)	4.7 ab	79.5	0.06	0.00 b	0.06	72 cde	4
Snoei (Des)	4.4 ab	94.4	0.16	0.00 b	0.16	76 bcd	12
Handuitdun (Des)	4.2 bc	76.8	0.13	0.02 b	0.15	69 cde	6
Vrugte bedek (Des)	3.9 cd	73.0	0.23	0.13 a	0.35	62 de	10
Vrugte bedek (Jan)	3.5 d	78.6	0.07	0.00 b	0.07	100 a	7
Snoei (Jan)	4.5 ab	80.3	0.10	0.00 b	0.10	87 abc	7
<i>P</i> -waarde	0.0001	0.1454	0.2066	0.0098	0.1094	0.0001	0.4855

<sup>z</sup>gemiddelde in 'n kolom gevolg deur verskillende letters is betekenisvol verskillend by die 5% vlak.

**Tabel 5.2.4.2.** Blaar minerale ontleding by verskillende behandelings.

Behandeling	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
Kontrole	2.37	0.10	1.05 ab <sup>z</sup>	3.21	0.36 ab	513 bc	25.9	124.7	6.0 c	74.0	78.4
Blare bedek (Des)	2.27	0.09	1.21 a	3.24	0.34 ab	402 c	28.8	143.5	6.9 c	79.1	71.0
Blare verwyder (Des)	2.28	0.09	0.74 c	3.58	0.19 d	659 ab	23.9	131.9	11.0 a	86.8	88.2
Blare bedek (Jan)	2.35	0.10	1.10 ab	3.36	0.41 a	422 c	26.7	118.3	6.6 c	86.9	86.9
Ringeleer (Des)	2.34	0.10	1.02 ab	3.28	0.34 ab	657 ab	23.4	126.2	6.6 c	70.4	85.7
Snoei (Des)	2.39	0.10	1.02 ab	3.30	0.22 cd	617 ab	20.6	204.6	6.6 c	69.5	114.7
Handuitdun (Des)	2.29	0.09	0.92 bc	3.54	0.30 bc	523 abc	24.1	124.1	7.4 bc	68.3	76.3
Vrugte bedek (Des)	2.23	0.09	0.71 c	3.73	0.28 bcd	679 ab	23.2	125.5	9.1 ab	83.6	83.2
Vrugte bedek (Jan)	2.24	0.09	0.91 bc	3.46	0.20 d	706 a	22.2	125.8	10.5 a	65.5	107.1
Snoei (Jan)	2.42	0.10	1.04 ab	3.46	0.36 ab	399 c	22.1	105.2	6.0 c	66.8	75.3
<i>P</i> -waarde	0.6428	0.4876	0.0003	0.5796	0.0001	0.0003	0.0814	0.5341	0.0001	0.0605	0.1150

<sup>z</sup>gemiddelde in 'n kolom gevolg deur verskillende letters is betekenisvol verskillend by die 5% vlak.

**Tabel 5.2.4.3.** Albedo minerale ontleding by verskillende behandelings

<b>Behandeling</b>	<b>N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>Na</b>	<b>Mn</b>	<b>Fe</b>	<b>Cu</b>	<b>Zn</b>	<b>B</b>
Kontrole	0.58 bc <sup>z</sup>	0.04	0.31	0.45	0.03 b	49.4	0.7	17.7 b	1.3 b	11.09	23.5 ab
Blare bedek (Des)	0.66 a	0.04	0.37	0.44	0.03 b	40.1	0.6	8.1 b	2.0 ab	11.47	23.8 ab
Blare verwyder (Des)	0.55 bc	0.04	0.10	0.47	0.02 b	38.2	0.6	1.8 b	1.9 ab	11.06	24.4 a
Blare bedek (Jan)	0.60 abc	0.03	0.20	0.44	0.03 b	37.6	0.8	7.8 b	1.7 ab	11.28	23.2 ab
Ringeleer (Des)	0.56 bc	0.04	0.27	0.50	0.02 b	48.9	0.5	7.0 b	1.5 b	11.32	23.9 ab
Snoei (Des)	0.56 bc	0.04	0.31	0.43	0.02 b	39.8	0.7	5.9 b	1.4 b	11.48	22.6 ab
Handuitdun (Des)	0.53 bc	0.03	0.30	0.46	0.02 b	40.6	1.4	5.1 b	1.7 ab	11.62	21.8 b
Vrugte bedek (Des)	0.62 ab	0.05	0.32	0.49	0.06 a	44.5	1.4	71.5 a	2.0 ab	11.18	24.7 a
Vrugte bedek (Jan)	0.56 bc	0.04	0.31	0.47	0.04 b	36.3	0.4	20.2 b	2.2 a	10.47	22.9 ab
Snoei (Jan)	0.53 c	0.03	0.30	0.50	0.03 b	38.2	0.5	9.6 b	2.2 a	10.91	21.8 b
<i>P-waarde</i>	<i>0.0133</i>	<i>0.1124</i>	<i>0.0617</i>	<i>0.3014</i>	<i>0.0060</i>	<i>0.8439</i>	<i>0.3264</i>	<i>0.0039</i>	<i>0.0260</i>	<i>0.9654</i>	<i>0.0244</i>

<sup>z</sup>gemiddelde in 'n kolom gevolg deur verskillende letters is betekenisvol verskillend by die 5% vlak.

5.2.5 **FINAL REPORT: Effect of gaseous conditions on rind condition of Nules Clementine mandarin**  
Experiment 780 (January 2005 - March 2007): PJR Cronje (CRI), GH Barry and M Huysamer (SU-DFPT)

### Summary

This experiment was done as the last part of a three year project to ascertain if a combination of elevated concentrations of CO<sub>2</sub> and ethylene would contribute to incidence of puffiness, rind breakdown and colour loss of Nules Clementine mandarin during shipment. The gas treatments (5% CO<sub>2</sub> + 5 ppm Ethylene and 1% CO<sub>2</sub> + 1 ppm Ethylene) were administered in a closed system for 32 days at -0.5°C, prior to 1 week shelf life and evaluation thereafter. The data from 2007 concurred with those collected in 2005/6 in that these gas treatments did not cause a significant increase in the above-mentioned rind disorders. Unfortunately, the cause of the disorder therefore still remains to be elucidated.

### Opsomming

Gedurende die proef is die effek van gastoestande (m.a.w. verhoogde CO<sub>2</sub> en etileen konsentrasies) op skil kwaliteit van 'Nules Clementine' mandaryne gedurende verskeping bepaal as deel van 'n drie jaar projek. Hierdie eksperiment is uitgevoer om te bepaal of 'n kombinasie van verhoogde etileen en CO<sub>2</sub> vlakke die voorkoms van powwerigheid, kleur verlies en skilafbraak verhoog in Nules Clementine mandaryne gedurende verskeping. Die gasbehandelings van 5% CO<sub>2</sub> + 5 ppm Etileen en 1% CO<sub>2</sub> + 1 ppm Etileen is toegedien in 'n geslote sisteem vir 32 dae teen -0.5°C. Daarna is vrugte vir 1 week teen omgewings toestande opgeberg voor evaluasie. Die ingevorderde data van 2007 ondersteun die vorige data van 2005/6 in dat die verhoogde CO<sub>2</sub> en etileen nie verantwoordelik was vir 'n drastiese of betekenisvolle verhoging in die voorkoms van bogenoemde skildefekte nie. Ongelukkig is die gevolgtrekking van die projek dat die oorsaak van die genoemde skildefekte nog onbekend is.

### Introduction

The gaseous conditions during shipment of 'Nules Clementine' mandarins were suspected to play a causative role in the increased occurrence of the following rind disorders.

*Puffiness:* An expansion of the rind that causes the rind to pull away from the pulp and in advanced stage of the disorder the segments separate from each other.

*Fruit yellowing:* Fruit complying to colour specifications prior to shipping are received in the USA as yellow fruit below colour specifications.

*Rind Breakdown:* Collapse of oil glands 4-5 weeks after harvest during shipment.

During the previous two seasons (2005-6) the separate effect of elevated ethylene (above normal degreening concentrations) and CO<sub>2</sub> (above commercial shipment levels 0.6%) were studied (CRI annual reports 2004-6). These results indicated that the separate gasses do not cause an increase in either one of the rind disorders mentioned. It was therefore necessary to determine the combined effect of the gasses on the rind condition of 'Nules Clementine' mandarins.

### Materials and methods

The 'Nules Clementine' mandarins were harvested on 16 May 2007 from Wellgevonde experimental farm, Stellenbosch area, degreened (3 days) and packed according to normal commercial practices. Eight replicates, consisting of 20 fruit each were used per treatment. Fruit were placed in a bucket with a connection to a flow board and an outlet from the cold room. Gas bottles were connected to flow boards from which tubes fed into the buckets in the cold room. Premixed gas from Afrox was used and the treatments were as follows: 0.03% CO<sub>2</sub> (normal air and control), 5% CO<sub>2</sub> + 5 ppm ethylene, and 1% CO<sub>2</sub> + 1 ppm ethylene. The fruit were kept in closed plastic buckets during the experiment. In all treatments, air made up the balance of the gas mixture. The fruit were kept in a cold room at -1°C for 32 days (to simulate the maximum commercial period). The flow rate of the treatment gases was high enough to prevent a build up of additional ethylene and CO<sub>2</sub> inside the bucket.

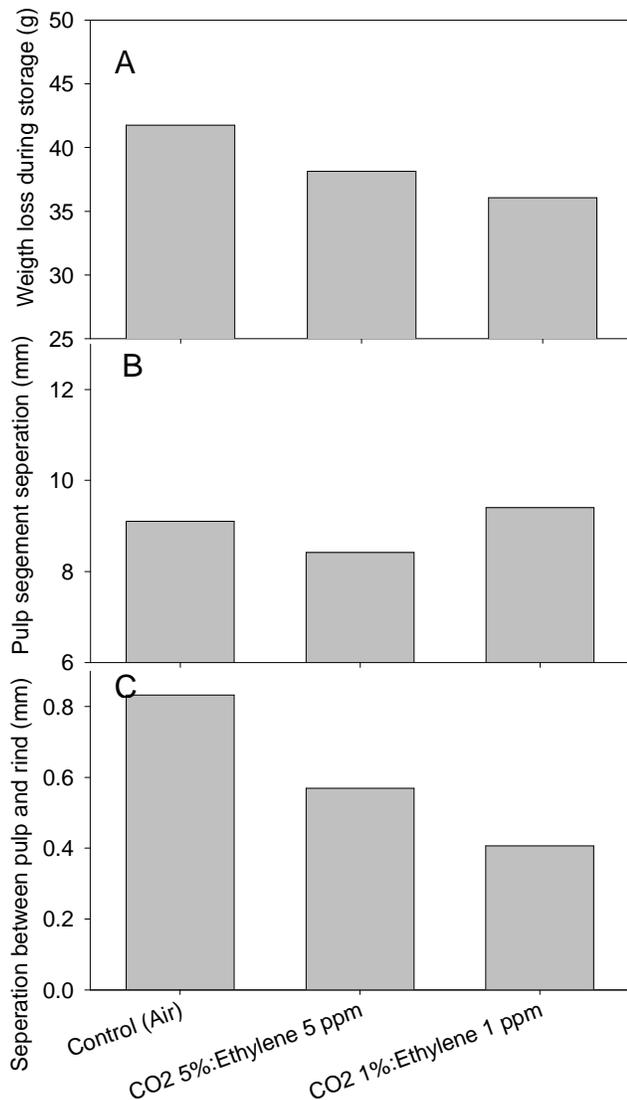
After the storage period the fruit were kept at ambient temperature (15 to 20°C) for one week, with the buckets open to prevent CO<sub>2</sub> and ethylene build-up. The fruit colour was evaluated with a chromameter (Minolta NR 4000, Osaka, Japan) after cold storage and expressed as chroma, lightness and hue°. The symptoms of rind

disorders were scored and the fruit were cut open to evaluate the degree of puffiness as well as the internal colour change of the pulp. The degree of puffiness was measured with a calliper according to the distance between the pulp and the peel and the separation of the centre of the fruit as the segments detached. The data were analysed with GLM procedures of SAS 2.

## **Results and discussion**

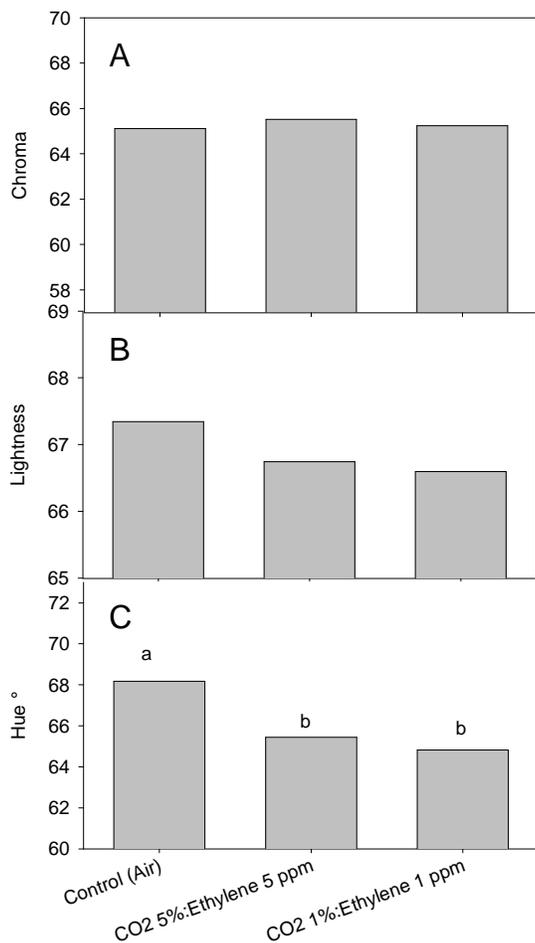
During the first year of the project (2005) the aim was to determine if high CO<sub>2</sub> levels were a cause of puffiness in 'Nules Clementine' mandarins. No significant difference was found between the control at 0.03% CO<sub>2</sub> and the treatments. The 2006 experiments, testing elevated ethylene concentrations, also resulted in no significant increase in occurrence of puffiness. The 2007 experiments were therefore planned to combine the two gasses and test the effect at elevated concentrations. No incidence of rind breakdown was recorded in the experiment and no decrease in pulp colour was seen (data not shown).

Weight loss was measured to determine if the development of puffiness occurs with a sudden loss of water as the rind defect developed. No significant differences were measured between the three treatments (Fig. 5.2.5.1.A). Pulp separation between segments in the middle of the fruit (mm) is measured as one of the indications of severity of puffiness, however, the treatments did not result in any significant increase (Fig. 5.2.5.1.B). Separation of the pulp and rind (mm) is also measured as a puffiness severity indicator. Due to the very low incidence of puffiness in the experiment only very small separations could be seen (less than 1 mm). No significant decrease in separation was seen between the control and the various concentrations of gasses (Fig. 5.2.5.1.C).



**Figure. 5.2.5.1. ABC.** The effect on 'Nules Clementine' mandarins of a combination of elevated CO<sub>2</sub> and ethylene on fruit weight loss (A) and degree of puffiness (separation between pulp (B) and rind as well as segment separation(C)). Fruit were subjected to the gas treatments for 32 days at -0.5°C. No significant differences occurred between any treatments in A, B or C (P > 0.05)

Chroma is an indicator of vividness of a colour and a higher number is equal to a more vivid colour display. However, no significant differences were recorded between treatments (Fig. 5.2.5.2.A). Lightness denotes whether a colour is more dark or light, with a higher number being a lighter (whiter) colour. In this category there were also no significant differences between treatments (Fig. 5.2.5.2.B). Hue° gives an indication of the specific colour and a lower value is equal to a redder colour (close to 50) while a number closer to 80 is more yellow. Both the two gas treatments had significantly better (less yellow, more orange) than the control. This would be expected as ethylene is known to increase the colour development in citrus fruit (Fig. 5.2.5.2.C).



**Figure 5.2.5.2. ABC.** The effect on 'Nules Clementine' mandarins of a combination of elevated CO<sub>2</sub> and ethylene on fruit colour. Fruit were subjected to the gas treatments for 32 days at -0.5°C. Different letters above the bars denote significant differences between treatments (P ≤ 0.05)

### Conclusion

Interpreting these results together with the previous seasons' (2005-6) experiments indicate that ethylene and CO<sub>2</sub> at elevated levels do not cause puffiness, rind breakdown or loss of colour during shipment at -0.5°C of 'Nules Clementine' mandarins. The CO<sub>2</sub> levels used were above those measured on board a commercial bulk reefer vessel i.e. 0.6% (Cronje 2004) and concentrations normally used in the degreening rooms (1-3 ppm).

Unfortunately the mechanism of puffiness, colour loss and rind breakdown developing during shipment therefore still remains unknown. It is suspected that maturity could play a role as more puffing occurs later in the season when some fruit are outside the optimum harvest window.

### Future research

No future research will be done along this line of enquiry into these rind disorders.

### Technology transfer

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## Acknowledgments

The researcher would like to thank the personnel of Cape Citrus in Simondium for helping with the degreening and packaging of the fruit.

### 5.2.6 PROGRESS REPORT: Peteca spot of lemons

Experiment 833 (April 2006 – July 2009): Paul Cronje (CRI at SU), Anton Gouse (SU)

## Summary

Pre and postharvest experiments to determine the effect and efficacy of various chemicals and gasses on prevention of peteca spot were done during 2007. The pre-harvest foliar application unfortunately did not result in any data due to an absence of peteca in the samples. However, the postharvest gas treatments (CO<sub>2</sub> ethylene, air and nitrogen) did result in significant differences between treatments. Ethylene at 5 ppm and 100% nitrogen applied for 7 days, resulted in zero peteca spots developing. The usage of 1-MCP also yielded surprising results and significantly decreased the incidence of peteca. These two results indicate that ethylene (externally applied as well as internally synthesised) probably plays a major role in determining the sensitivity of the oil gland to collapse in lemon fruit. During 2008, experiments will be done to confirm these results and gain more information on this problematic disorder.

## Opsomming

Gedurende 2007 was verskeie chemikalie en gasse as voor en na-oes behandelings toegedien om die effektiwiteit op voorkoming asook impak op peteka te bepaal. Ongelukkig het die voor-oes behandeling geen peteka opgelewer nie. Die na-oes behandelings was egter baie suksesvol en belangrike inligting is ingewin. Die vrugte was vir 7 dae aan gas (CO<sub>2</sub>, etileen, lug en stikstof) blootgestel om die effek van anaërobiese respirasie op peteka ontwikkeling te bepaal. Die etileen (5 ppm) en stikstof (100%) het teen verwagting geen peteka opgelewer nie. Die verwagting was dat die anaërobiese respirasie, gedurende die behandelings moes lei tot verhoogde peteka. Die betekenisvolle verlaging in peteka na 1-MCP behandelings ondersteun egter die resultate van die gas proewe. Dit dui daarop dat etileen (eksterne en interne) 'n rol speel in die bepaling van die skil se peteka sensitiwiteit. Na gelang van die resultate sal verdere proewe gedurende 2008 gedoen word om nog meer informasie oor die fisiologiese defek in te samel.

## 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 depressed or sunken areas (Cronje, 2007). Unfortunately, the occurrence of this disorder is very sporadic and has resulted in a number of experiments failing to produce data. However, the occurrence can be severe, resulting in substantial economic losses. 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 CO<sub>2</sub> conditions which lead to 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 probably being due to increased respiration and water loss at the higher temperature. Weather conditions prior to harvest were also suspected to increase the incidence and Undurraga *et al.* (2006) collected data illustrating peteca decreased if days to harvest after a rainfall event are extended. Research into this disorder during 2007 has followed two strategies viz. attempts by pre-harvest foliar treatments to increase

rind condition (and therefore reduce peteca incidence), as well as the postharvest treatments to decrease fruit susceptibility to peteca development. The pre-harvest foliar sprays were Gibberellic acid, silica and LPE and amino acid. The second part of the experiment consisted of treating fruit with postharvest treatments known to influence fruit respiration i.e. ethylene, CO<sub>2</sub>, nitrogen and 1-MCP.

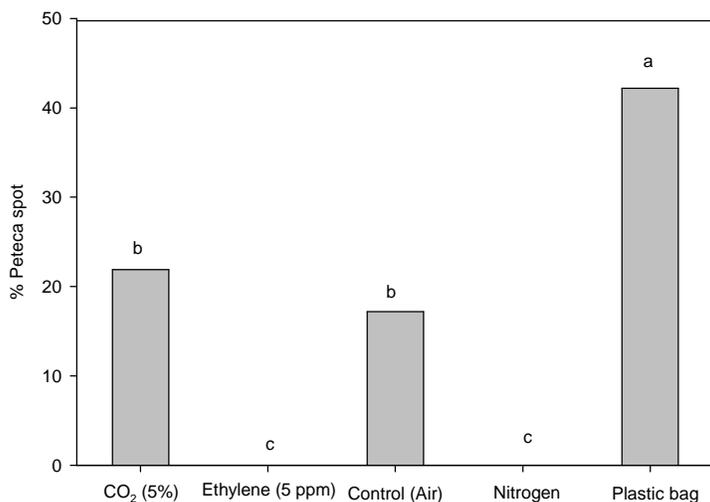
## Materials and methods

Lemon orchards in the Rawsonville and Stellenbosch areas (semi-desert and costal) were chosen for the foliar treatments due to both areas having had high peteca incidence in 2006. The chemical sprays were applied 1 month before harvest (8-10 May) and at the following concentrations: Silica (manufacturers specifications), LPE (200 and 400 ppm), GA<sub>3</sub> (10 and 20 ppm) and a water control. Ten replicates of each treatment were used. The fruit were harvested in the first week of June and taken to the Department of Horticultural Science (SU), and placed in closed plastic bags and kept for 10 days at shelf temperature conditions before visual evaluation.

The fruit for the postharvest treatments were sampled from an orchard in Simondium in July, which had high peteca at the time of harvesting. Sampling was done during a wet cold front, which is known to normally result in severe peteca. Fruit were subjected at the department to continual gas treatments for 7 days at 20°C of ethylene (5 ppm), CO<sub>2</sub> (5%), nitrogen or air as well as fruit being closed in plastic bags. The fruit were evaluated after 7 days for peteca. After the positive results from this evaluation more fruit were collected from the same orchard and treated postharvest with 1-MCP (2000 and 1000 ppb) prior to being stored in plastic bags for 10 days and subsequent evaluation.

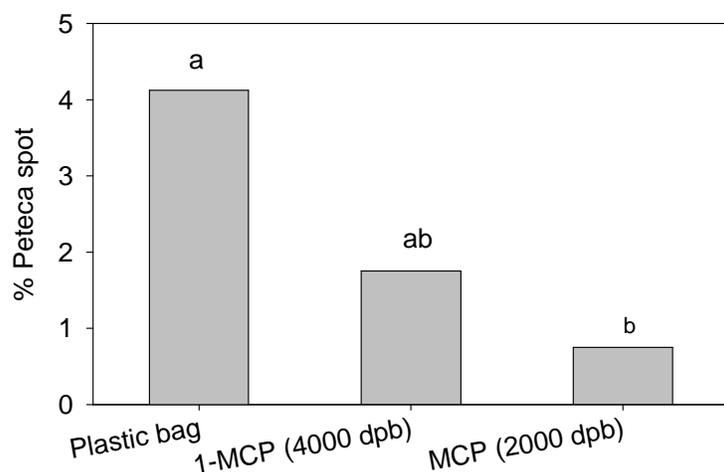
## Results and discussion

Unfortunately none of the pre-harvest foliar treatments resulted in any peteca developing on the lemons. However, the sampling strategy used in the postharvest experiments worked very well, and significant data were collected. Control (air) resulted in peteca spots (18%), with the plastic bag treatments significantly more (44%). The CO<sub>2</sub> gas treatment did not significantly increase peteca compared with the control, this was unexpected as the higher CO<sub>2</sub> concentration in the plastic bags was thought to trigger the development of peteca. However, the most surprising results were the total lack of any peteca spots in both the nitrogen and ethylene gas treatments (Fig. 5.2.6.1).



**Figure 5.2.6.1.** The effect of various gas treatments for 7 days on the occurrence of peteca spot of lemons. Different letters between bars denote significant differences ( $P \leq 0.05$ )

This positive, although surprising result lead to a follow up experiment of which the hypothesis was that if applied ethylene decreases peteca development, it would logically follow that the application of an ethylene blocker (1-MCP) would result in higher peteca incidence. Again contradictory to the argument, the results were exactly the opposite and a significant reduction was seen (Fig. 5.2.6.2).



**Figure 5.2.6.2.** The effect of 1-MCP ethylene action blocker on the occurrence of peteca spot of lemons. Different letters between bars denote significant differences ( $P \leq 0.05$ )

### Conclusion

Research on this postharvest disorder will always be problematic due to the irregular occurrence thereof. However, significant progress has been made in the past two seasons, and has resulted in a better understanding of postharvest conditions playing a role in peteca spot. The discovery and usage of the “forcing treatment” of peteca in closed plastic bags has led to a number of new questions and is currently being successfully used in the commercial packhouse to determine if fruit are peteca prone. In the bag a build up of gasses known to be produced by fruit respiration i.e.  $\text{CO}_2$  and ethylene would occur. The experiment was planned to test the individual gas effects on peteca incidence. It was thought that anaerobic respiration (brought about by nitrogen and high  $\text{CO}_2$ ) would result in the highest incidence. The zero peteca spots in the nitrogen treatments were therefore quite surprising and could indicate the anaerobic respiration at zero  $\text{O}_2$  could induce a stress response protecting the oil glands. The same argument could be made for the ethylene treatment. Ethylene as a wounding response is known to protect fruit against pathogen attack as well as during chilling injury. The result of 1-MCP (ethylene blocker) can possibly be explained by a negative feedback inhibition mechanism resulting in internal ethylene production and a protection effect on the oil glands. These possibilities need further clarification.

### Future research

During 2008 postharvest experiments using the same chemicals and treatments will be done to gather more information on peteca spot. The strategy of working with fruit currently known to be peteca prone will continue.

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## 5.2.7 **PROGRESS REPORT: The evaluation of postharvest operational issues that could promote the occurrence of peteca spot in lemons**

Experiment 795 (April 2005 - July 2009) by K.H.Lesar (CRI)

### **Summary**

The work during the last year has concentrated on the effect of waxes on the development of peteca spot. Green lemons were picked in cold conditions, which should favour development of the disorder. Before treating with a range of waxes of different solids content and storage at cold sterilisation temperatures, fruit were dipped in a water bath at temperatures of 30, 40 or 50°C, roughly handled and subjected to normal and over-brushing. No peteca was found, even after extended storage and storage in plastic bags (previously found to induce the disorder if potentially present). The causes of peteca spot have thus not been found, but these trials will be repeated.

### **Opsomming**

Die afgelope jaar se werk het of die effek van wakse op die ontwikkeling van peteca gekonsentreer. Groen suurlemoene is in koue toestande wat die ontwikkeling van die peteca behoort te bevorder, gepluk. Voor dat die vrugte met verskeie wakse van verskillende vastestowwe inhoud behandel was, was hulle in 'n water bad van 30, 40 of 50°C gedoop, rof gehanteer en normal of oorgeborsel. Na behandeling, was vrugte onder toestande van kouesterilisasie geplaas. Geen peteca is gevind nie, selfs na verlengde opberging, en opberging binne 'n plastiese sak wat voorheen bewys was as 'n aanduiding van die moontlikheid van peteca ontwikkeling. Die oorsaak van peteca is dus nie gevind nie, en die proef sal herhaal word.

### **Introduction**

Peteca spot is a physiological disorder that appears as deep pits on the peel surface of lemons, usually after packing. 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. Peteca also 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, 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.

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, with a good uniform coverage of a light wax is reported to reduce the risk of peteca development in lemons. However, 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 this trial 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. These lemons were treated with a range of citrus waxes with different solid levels to determine the effect of these 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.

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, the lemons were divided up into 6 replicates x 15 fruit each per treatment.

The lemons were then waxed, by means of a dip treatment, with the following citrus waxes.

(i)	Carnauba wax	10% total solids
(ii)	Carnauba wax	14% total solids
(iii)	Carnauba wax	26% total solids
(iv)	Carnauba wax	18% total solids
(v)	Carnauba – wood rosin	25% total solids
(vi)	Polyethylene – Shellac	19.6% total solids
(vii)	Polyethylene – wood rosin	23% total solids
(viii)	Bees wax – Shellac	20.2% total solids
(ix)	Carnauba natural	14% total solids
(x)	Polyethylene	18% total solids

The waxed fruit was left at ambient temperature to dry overnight.

## 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)
14. As in 4 then waxed with wax (ix)

15. As in 4 then waxed with was (x)

After drying, 3 replicates x 15 fruit were placed in paper bags and 3 replicates 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 CO<sub>2</sub> were measured which may have resulted in the development of peteca spot.

### **Storage**

The fruit was stored the following day under simulated cold disinfestation conditions at -0.6°C for 22 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 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 appear to be 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.

### **Future research**

As the lemons used did not appear to be pre-disposed to the disorder, further work will be necessary and these trials will be repeated during next season.

### **Technology transfer**

Nothing to report yet.

#### **5.2.8 PROGRESS REPORT: Postharvest treatments to prevent chilling injury of citrus fruit** Experiment 832 (May 2005 – August 2009): Paul Cronje (CRI at SU)

### **Summary**

During 2007, various postharvest treatments as part of an ongoing project aimed to reduce the impact of CI on citrus fruit were done. Postharvest treatments (gas and chemical drench) were used on 'Valencia' oranges prior to being stored for 32 days at -0.5°C. The gas treatments consisted of ethylene (5 ppm) and CO<sub>2</sub> (5 ppm) applied to fruit for 24 hours prior to cold storage. The results show a significant CI reduction in the CO<sub>2</sub> treatment. The postharvest chemical applications were applied as these products (Jasmonic acid, TBZ and 1-MCP) are known to influence CI severity in other fruit crops. The lower concentration of 1-MCP (1000 ppb) did result in significant reduction of CI symptoms. Contradictory to what is accepted in the citrus industry, TBZ did not reduce CI. During 2008 more experiments will be done that focus on the positive results seen after the CO<sub>2</sub> and 1-MCP treatments.

## Opsomming

Gedurende 2007 is 'n reeks eksperimente gedoen wat deel vorm van die koueskade voorkomings projek. In die seisoen is daar van na-oes gas en chemikalie gebruik gemaak om koueskade van 'Valencia' lemoene te verminder wat na behandeling vir 32 dae teen  $-0.5^{\circ}\text{C}$  opgeberg was. Die gas behandelings het bestaan uit etileen (5 ppm) en  $\text{CO}_2$  (5%) wat vir 24 uur toegedien was voor opberging. Die  $\text{CO}_2$  behandeling het in betekenisvolle verlaging teweeg gebring. Die na-oes chemikalie (Jasmonic acid, TBZ and 1-MCP) was in 'n waterbad toegedien voor koue opberging. Die 1-MCP behandeling (1000 dpb) het die koueskade betekenisvolle verlaag en dui daarop dat etileen betrokke is in die koueskade meganisme. In teenstelling met wat aanvaar word in die sitrusbedryf, het TBZ nie tot 'n verlaging van koueskade gelei nie. Hierdie positiewe resultate sal gevolg word in 2008 en suurlemoene asook valencia lemoene sal van gebruik gemaak word.

## Introduction

Chilling injury poses a severe threat to the economic viability of exporting citrus fruit to markets requiring cold sterilization protocols viz. USA, China and Japan. Citrus fruit have a high susceptibility to chilling injury (CI), probably due to their tropical and subtropical origin, and the symptoms of CI are seen in the rind as pitting or scalding patterns. Although all cultivars are susceptible, variation between cultivars exist and lemons and grapefruit are for example much more prone to CI than Clementine mandarins and Valencia oranges. For chilling injury to develop in a plant tissue, it needs to be subjected to a potentially damaging temperature (normally below  $4^{\circ}\text{C}$  in citrus), for a cultivar specific duration (Kays and Paull, 2004). The mechanism controlling chilling injury of horticultural produce is still eluding scientists even though a considerable effort has been made to unravel the mystery. The proposed model of Lyons (1973), where changes in membrane permeability, associated with a membrane-lipid physical phase transition from a flexible liquid-crystalline to a solid-gel structure is the primary event associated with CI, still has merit. These changes would cascade into secondary events such as loss in regulatory control and metabolic imbalance, cell autolysis and eventual cell death followed by a visible symptom such as pitting or scalding in the case of citrus fruit.

However, the possible high returns in these aforementioned markets open new opportunities to implement additional postharvest treatments which could ensure a lower incidence of CI. The aim of the 2007 season's experiments was to test various chemicals mentioned in literature to influence and decrease CI of other fruit crops. The treatments were applied as either a postharvest drench (Jasmonic acid, TBZ and 1-MCP) or a gas ( $\text{CO}_2$  and ethylene).

## Materials and methods

'Valencia' oranges were collected after harvest from Goede Hoop Sitrus kooperasie in Citrusdal, Western Cape on 23 August 2007. The fruit were taken to department of Horticultural Science at the University of Stellenbosch for treatment. All treatments were replicated 8 times and thereafter fruit stored at  $-0.5^{\circ}\text{C}$  for 32 days. The fruit were evaluated for visual CI symptoms (pitting or scalding) after cold storage (Eval 1) and again after 1 week at shelf life conditions (Eval 2).

The chemical used were as follows:

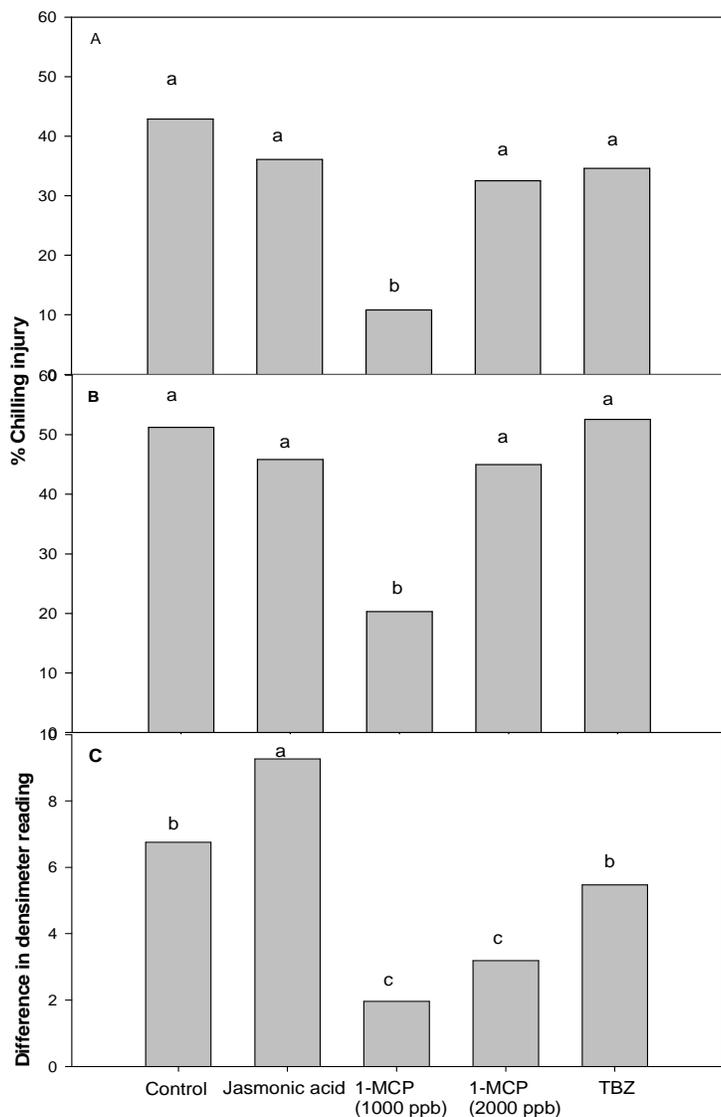
- Jasmonic acid: a plant hormone with known involvement in plant stress responses and reported to decrease CI in some horticultural crops. It was applied at 500 ppm (0.4L/5L  $\text{H}_2\text{O}$ )
- Thiabendazole (TBZ): This well know fungicide has been linked in various citrus industries in the world to decrease CI via an unknown mechanism. It was applied at 4000 ppm (1mL/5L  $\text{H}_2\text{O}$ ).
- 1-MCP: It is an aggressive blocker of ethylene action on the cellular level and the fact that ethylene is hypothesised to play a role in reducing CI of citrus makes this chemical ideal for experimental purposes. It was applied at 1000 and 2000 ppb in a chlorine free water bath.

The gas treatments were applied for 24 hours at ambient temperature ( $18-20^{\circ}\text{C}$ ) and were 5%  $\text{CO}_2$  and 5 ppm ethylene. Data were analysed with the GLM procedure of SAS.

## Results and discussion

In both experiments high levels of CI symptoms were recorded,  $\pm 30\%$ . This is higher than normal commercial CI and would mainly have been due to the lack of wax used and storing of fruit for 32 days to simulate maximum

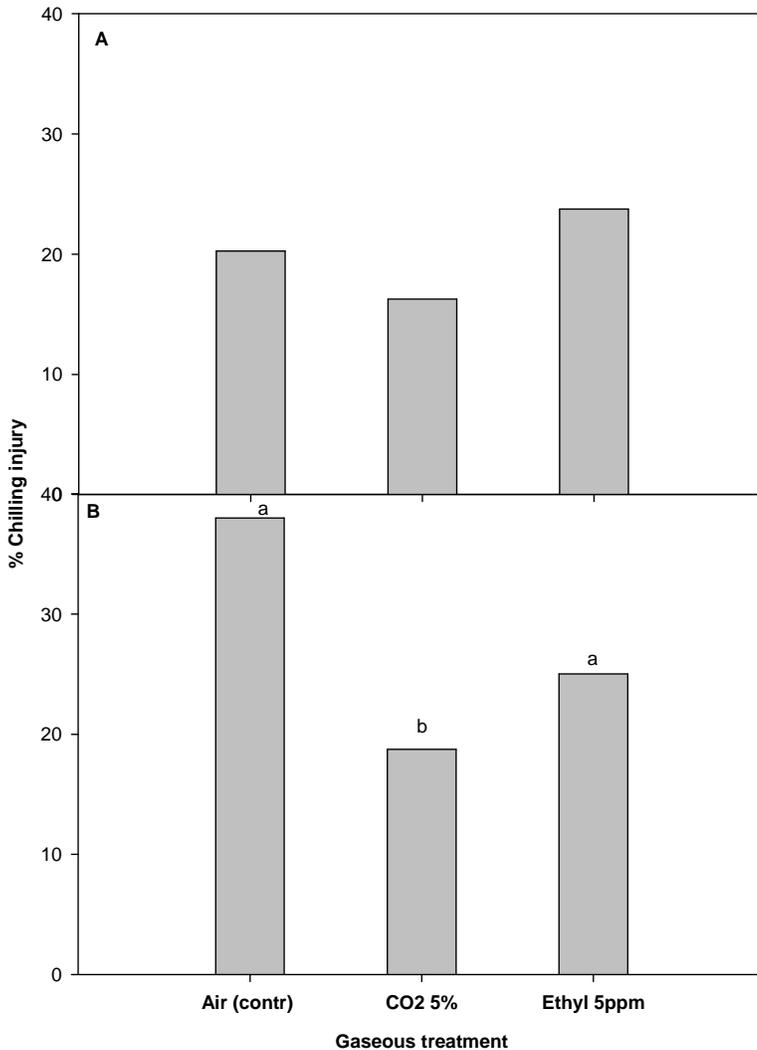
exposure, which is much longer than the normal cold sterilization protocol of 24 days at -0.5°C. All the chemical treatments showed CI symptoms after cold storage and these values increased as expected after 1 week at shelf temperatures. This increase is typical of CI and is due to water loss of the damaged tissue and an oxidation of the damaged lesions resulting in more visible symptoms. 1-MCP (1000 ppb) resulted in a dramatic decrease of CI (20% vs. 50% of the control), although higher CI seen in the 1-MCP at 2000 ppb illustrates the complexity of the possible ethylene mechanism on CI (Fig. 5.2.8.1. AB). The visual data are supported by the densimeter measurements which give an indication of firmness of the rind and therefore the amount of damage done to the cellular structure. These measurements were presented as the difference between the two evaluation dates. The lower values of the 1-MCP treatments show this chemical to have a possible protective action, preventing cellular breakdown and lesion development (Fig. 5.2.8.1. C).



**Figure 5.2.8.1 ABC.** The effect of four postharvest chemical drench applications to Valencia oranges prior to storage for 32 days at -0.5°C (A). After cold storage fruit were kept for 1 week at shelf temperature (B). The difference in densimeter measurements between Evaluation 1 and 2 is presented in C. Different letters on bars denote significant differences between the treatments ( $P \leq 0.05$ )

Initially the gas treatments did not result in any significant differences in the occurrence of CI directly after cold storage, but as expected, CI symptoms increased after 1 week shelf life and these results showed significant

differences between treatments. It is interesting to note that even though the ethylene treatment did not result in a significant difference between evaluation 1 or 2 and similar results were obtained for CO<sub>2</sub>, the latter treatment did result in significantly less CI than the control after the shelf life period. It is concluded that CO<sub>2</sub> could possibly be used to decrease the severity of CI in citrus (Fig. 5.2.8.2. A, B).



**Figure 5.2.8.2. A,B.** The effect of 24 hours postharvest gas treatments (5% CO<sub>2</sub> and 5 ppm Ethylene) at ambient temperatures on occurrence of chilling injury of 'Valencia' oranges. Fruit were stored for 32 days at -0.5°C prior to 1 week at shelf temperature. Evaluations were done cold after storage (A) and shelf life (B). Different letters on bars denote significant differences (P ≤ 0.05)

### Conclusion

Both these experiments indicate that the hypothesis of ethylene involvement in the CI mechanism could be correct, but, it remains a complex physiological process of which very little is understood and will probably involve internal ethylene production and cellular response thereupon (Lafuente *et al.*, 2001).

The lack of a positive effect after TBZ application was disappointing as it is commonly accepted to increase citrus fruit resistance to chilling. Unfortunately no explanation could be found for this result.

The positive effect of especially the CO<sub>2</sub> treatment is very encouraging. If this treatment could be confirmed in following season's experiments on a variety of cultivars, it could present a very valuable opportunity in decreasing the impact of cold sterilization treatments.

### Future research

During the 2008 season the gas application experiments (ethylene and CO<sub>2</sub>) as well as the 1-MCP treatments will be repeated on not only 'Valencia' oranges but also lemons.

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### Acknowledgments

The researcher would like to thank the personnel of Goede Hoop Sitrus Ko-operasie in Citrusdal for helping with the degreening and packaging of the fruit.

5.2.9 **PROGRESS REPORT: The use of hot water dip treatments to prevent chilling injury (CI) on lemons, Marsh grapefruit and Oroblancos exported to Japan**  
Experiment 869 (March 2006 – July 2009) by K.H. Lesar (CRI)

### Summary

Hot water dips are known to decrease cold injury on various types of stored citrus, but the effects on lemons are less well known. Green and fully coloured lemons were dipped in water at 18 and 53°C, as well as TBZ and salicylic acid before treatment with various waxes before storage at cold sterilization temperatures. In an additional experiment at the University of KwaZulu-Natal (UKZN), fruit from two sources was dipped in hot water at 47 and 53°C with and without molybdenum. In the first trial no chilling injury was noted, even after the storage was extended. This indicates the importance of orchard factors. In the case of the UKZN fruit, one site showed chilling injury while the other did not. Less chilling injury was found for the 53°C dip, especially where molybdenum was added. Biochemical analysis showed that total anti-oxidants were higher in fruit that did not have chilling injury, that hot water increased anti-oxidants and molybdenum resulted in a longer maintenance of anti-oxidant levels. It is believed that these results will lead to prediction of chilling sensitivity and optimization of treatments.

### Opsomming

Doop van verskeie sitrus tipes in warm water is bekend om koueskade te verminder, maar die effek op suurlemoene is minder bekend. Groen en volgekleurde suurlemoene is in water by 18 en 53°C gedoop, sowel as TBZ en salisiliesuur voordat hulle by kouesterilisering temperatuur opgeberg was. By nog 'n eksperiment by die Universiteit van KwaZulu-Natal (UKZN), was vrugte vanaf twee bronne in warm water by 47 en 53°C met en sonder molybdeen gedoop. In die eerste proef is geen koueskade gevind nie, selfs na verduur opberging. Dit dui aan die belangrikheid van vrugte oorsprong by sensitiwiteit tot koue. In die geval van die UKZN vrugte dié van een oorsprong het koueskade gekry, terwyl die ander, nie. Minder skade is by vrugte gedoop by 53°C, en veral waar molybdeen bygevoeg was. Biochemiese analiese het bewys dat totale anti-oksidente hoër was in vrugte wat nie koueskade gehad het nie, en dat warm water hulle verhoog het terwyl molybdeen die vlakke vir langer hoog gehou het. Dit is geglo dat dié resultate na voorspelling van koue sensitiwiteit en optimalisering van behandeling sal lei.

### 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. 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).

Factors that cause CI are still largely unknown. Methods to reduce CI, especially during cold disinfestation, must be afforded high priority.

### Materials and methods

Early (green) and late (fully coloured) lemons were harvested at Larten Estates in the Karino area for the purpose of this trial. The green and fully coloured lemons were then separately divided up into 6 replicates of 20 fruit per treatment. The lemons were treated on the packline at CRI in Nelspruit. The fruit was washed in the high pressure spray with a suitable sanitising agent (Prasin). After washing the lemons were treated in the hot water bath for 2 minutes exposure at ambient (18°C) and at 53°C and thereafter dried in the drying tunnel.

The waxed treatments were done by means of a dip treatment. The fruit was allowed to dry overnight before storage.

### Treatments

1. Untreated Control
2. Treated Control - ambient dip at 18°C for 2 minutes
3. Hot water dip at 53°C for 2 minutes **unwaxed**
4. Hot water dip at 53°C for 2 minutes **waxed**

Fruit in this treatment, after dipping at 53°C, were waxed with the following range of citrus waxes

- |     |                                      |                      |
|-----|--------------------------------------|----------------------|
| (a) | carnauba tropical - 10% total solids |                      |
| (b) | carnauba tropical - 14% total solids |                      |
| (c) | sta. fresh 890HS – 26% total solids  |                      |
| (d) | citruslustr. carnauba natural        | - 25% total solids   |
| (e) | citruslustr. Europa                  | - 19.6% total solids |
| (f) | citruslustr. special                 | - 23% total solids   |
| (g) | citruslustr. special M               | - 20,2% total solids |
5. 1000 ppm TBZ dip at 18°C for 2 minutes
  6. 1000 ppm TBZ dip at 53°C for 2 minutes
  7. Condition for 7 days + hot water dip at 53°C for 2 minutes
  8. 50 ml Sentinal (salicylic acid) at ambient (18°C) for 2 minutes
  9. 50 ml Sentinal (salicylic acid) at 53°C for 2 minutes

### Storage

After treatment the fruit was stored as follows:

- 3 replicates x 20 fruit 4 weeks at 10°C + 7 days at 20°C
- 3 replicates x 20 fruit 14 days at -0.6°C + 2 weeks at 10°C + 7 days at 20°C

In the case of fruit treated in KwaZulu-Natal, lemons were sourced from Sun Valley Estates as well as Ukulinga experimental farm. Fruit were treated with hot water and molybdenum before being waxed and stored at -0.5°C for 28 days. Every seven days, fruit was removed from the cold room for inspection, allowed to stand at room

temperature for a further five days, inspected again and the flavedo removed for total anti-oxidant determination. Hot water and molybdenum treatments were as follows:

1. Control (standard packhouse treatment)
2. Fruit dipped in water at ambient temperature
3. Hot water at 47°C
4. Hot water at 53°C
5. Molybdenum dips of 1, 5 and 10 µmol at ambient temperature
6. Molybdenum dips of 1, 5 and 10 µmol at 47 and 53°C

## **Results and discussion**

After cold disinfestation and shipping of the lemons no CI symptoms were evident on the treated lemons.

These lemons (green and coloured) were then stored, after shipping for 4 weeks, for a further 4 weeks at both 10°C and 2°C to possibly induce CI. The lemons were evaluated after 2 weeks extended storage and then stored a further 2 weeks and evaluated again.

After a total period of 8 weeks the lemons were looking a bit withered because of moisture loss (especially the unwaxed fruit). I would have stored the fruit for 2 weeks longer if all the fruit was waxed, but decided to terminate the trial.

Fruit from Ukulinga showed no chilling injury, but had higher total anti-oxidant capacity than fruit from Sun Valley, which did show chilling injury. This was reduced by hot water, especially 53°C, as well as molybdenum. Total antioxidants were higher in hot water treated fruit, and molybdenum treatments, which also resulted in longer maintenance of the anti-oxidant capacity.

The lemons used here were obtained from peteca prone orchards at Larten Estate, from the same orchards where a high incidence of peteca spot was experienced in the 2004 and 2005 citrus seasons. These same lemons were used for the 2006 peteca spot/wax trials and this CI trial.

Good peteca spot results were recorded in the 2005 trials with lemons from the same source after shipping and 6 weeks extended storage at 2°C, but not this time even after 6 weeks extended storage, and no results were recorded in the CI trial either, also after extended storage.

The fruit in the UKZN trial also showed differences with respect to origin. However, if fruit is sensitive to cold injury, hot water treatment, especially 53°C does appear to result in some protection, which is further enhanced by addition of molybdenum in the water bath. The physiology behind this appears to be the total anti-oxidant activity in the rind cells, which protects them from the free radicals produced by the stress caused during low temperature storage.

## **Conclusion**

The sensitivity of the fruit rind to low temperature storage appears to be clearly established before harvest while the fruit is still green and fairly immature, it is possibly related to the total anti-oxidant capacity and is probably related to environmental conditions. This predisposes the fruit to peteca, CI and other rind conditions during the harvesting of the fruit and the further handling and treatments thereafter. It is unknown as to what the orchard conditions are that may pre-dispose fruit to damage. However, if the work on anti-oxidant capacity is correct, a sensitivity model may be developed, and hot water treatment and the use of molybdenum which may act as a biochemical co-factor, is promising.

## **Future research**

The work will be repeated in the coming season to confirm the use of hot water treatments, as well as molybdenum, and further lead to development of a sensitivity model for chilling risk analysis.

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### 5.2.10 PROGRESS REPORT: The influence of cold disinfestation and duration of storage on the condition of Oroblancos/Sweeties exported to Japan

Experiment 847 (March 2006 – July 2008) by K.H.Lesar (CRI)

#### Summary

The Oroblanco is said to be particularly susceptible to chilling injury when stored at cold sterilization temperatures. In view of possible export to Japan, knowledge of the sensitivity of South African fruit of this cultivar to chilling is essential. Fruit was placed under cold sterilization temperature as well as at 4.5 and 11°C, and thereafter evaluated for cold injury as well as pathological problems. A high degree of cold injury (24%) was found after shipping at -0.5°C for 12 days. Initial damage was increased by further to 24% by shipping at 4.5°C for 8 days. Although lower if shipped for 8 days at 11°C after cold sterilization, cold injury was still not acceptable (8.4%). No significant chilling injury was found in fruit shipped for 20 days at 4.5°C or 11°C. No decay was found, although high *Diplodia* was found in previous work.

#### Opsomming

Die Oroblanco is uiters sensitief vir koueskade wanneer kouesterilisasie temperature gebruik word. As gevolg van die moontlikheid van uitvoer na Japan toe, kennis van die koue sensitiwiteit van Suid-Afrikaanse vrugte is noodsaaklik. Vrugte is onder kouesterilisasie temperature sowel as 4.5 en 11°C opgeberg, en daarna vir koueskade en patologiese probleme evalueer. Koueskade was hoog (24%) by vrugte vir 12 dae teen -0.5°C gevolg met 8 dae teen 4.5°C. Al was die skade laer as die 8 dae teen 11°C was, was dit nog nie aanvaarbaar nie. Geen betekenisvolle skade is by 4.5 en 11°C vir 20 dae opberging gekry nie. Geen bederf is gekry nie, alhoewel vorige werk dit wel getoon het.

#### Introduction

Citrus in general is known to be sensitive to cold damage (chilling injury) during shipping and storage, but certain cultivars (some soft citrus cultivars, lemons and grapefruit varieties), are particularly prone to chilling injury, especially when exposed to "cold disinfestation" temperatures. It is especially the yellow pigmented citrus cultivars *viz.* lemons, Marsh grapefruit, and even the yellow areas of Star Ruby and Rose grapefruit which are the most sensitive, as they do not contain the carotenoids which act as anti-oxidants that protect the fruit against chilling injury.

At some stage there was uncertainty as to whether the Oroblanco was a grapefruit or an orange. The trade, including the Japanese trade, now recognises the Oroblanco as a low acid white grapefruit and it has gained a good reputation of being a high quality fruit in Japan.

Simulated cold disinfestation trials conducted in 1992 by Barry and Burdette demonstrated Oroblancos were particularly sensitive to chilling injury (CI). These results were reported by Barry in the 1992 Outspan Research Progress Report. 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). The USA has been exporting Oroblancos to Japan for some time, and now the South African citrus industry has received a request to export Oroblancos to Japan. The aim of this work was to determine the condition of the Oroblanco after cold disinfestation and simulated shipping to Japan.

## Materials and methods

Seventy-two 16 kg standard packhouse treated export cartons (count 45) of Oroblancos were received from TSB Hectorspruit on 13 March 2007. The fruit was divided up into 3 replicates x 6 cartons (count 45 i.e. 810 fruit) per treatment. The cartons were marked and stored under conventional shipping and cold disinfestation conditions as described in Table 5.2.10.1. After cold disinfestation and shipping storage of this fruit the fruit was evaluated for decay and chilling injury damage (CI). The following results were reported as percentage decay and chilling injury.

## Results and discussion

The results in Table 5.2.10.1. indicate a high incidence of CI damage (23.9%) of the Oroblancos after storage under cold disinfestation conditions and further shipping at 4.5°C. The further storage of the fruit at a shipping temperature of 4.5°C compounds the CI damage compared to further shipping at 11°C. However, shipping this fruit further at 11°C, after cold disinfestation, also results in an unacceptably high incidence of CI. No decay was recorded in these treatments. However results in the same trials in 2006 indicated a high incidence of Diplodia stem end rot on the 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 5.2.10.1.** Oroblancos compared for CI damage after conventional shipping conditions vs cold disinfestation conditions during export to Japan.

Treatments	% Decay	% CI (chilling injury) <sup>a</sup>
1: 20 days @ 4.5°C followed by 7 days @ 20°C	Nil	0.9 a
2: 20 days @ 11°C followed by 7 days @ 20°C	Nil	0.0 a
3: 12 days @ -0.6°C followed by 8 days @ 4.5°C, then 7 days @ 20°C	Nil	23.9 c
4: 12 days @ -0.6°C followed by 8 days @ 11°C, then 7 days @ 20°C	Nil	8.4 b

<sup>a</sup> 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).



**Figure 5.2.10.1.** Chilling Injury symptoms reported in these treatments.

## Conclusion

Consequently the work done over the last two seasons on the risk of exporting Oroblancos to Japan indicates a possible high risk of decay resulting from quiescent latent pathogens and also chilling injury damage when the fruit is exposed to cold disinfestation conditions that are required by the market.

## Future research

Final confirmation of results will be made in the coming season, and the experiment closed.

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### 5.3 **PROJECT: FRUIT PRODUCTION AND QUALITY** Project coordinator: Stephan Verreyne (CRI at SU)

#### 5.3.1 **Project summary**

In the fruit production and quality project, five studies are presented, using pre- and postharvest treatments to improve colour development, tree response to partial root zone drying and deficit irrigation, 2,4-D to reduce the size of the navel end opening, benefits of hand thinning of Nules Clementine and to determine the sensitivity of citrus fruit to a wide range of irradiation dosages.

In section 5.3.2, the problem of insufficient colour development of citrus fruit was tackled in various ways. Fruit were cold-shocked or hydro-cooled and subsequently 'hydro-warmed', resulting in a significant colour improvement by reducing flavedo chlorophyll and increasing flavedo carotenoid concentration. Postharvest tungsten (W) dips resulted in poor colour change in fruit, while W had minor or negative effects on colour development and molybdenum (Mo) improved colour development when applied 19 weeks AFB. Pigment composition of the flavedo showed that violaxanthin is the major carotenoid in 'Navel' and 'Valencia', with  $\beta$ -citraurin, playing only a minor role.

In section 5.3.3. the response of Bahianinha Navel orange trees to the partial root zone drying (PRD) irrigation method was evaluated against the current grower's practice (the control), a well-watered treatment and the regulated deficit irrigation (RDI) treatments at Mazowe Citrus Estate over four growing seasons (2005 – 2008). PRD, RDI and the control treatments caused the development of generally thin and relatively deep roots compared to the well-watered treatment. Reductions in the stomatal conductance occurred under PRD while the leaf water potential was similar to that of the well-watered treatment. As a consequence, sap flow and hence transpiration rates were significantly lower under PRD irrigation than the control treatment even with similar irrigation levels. The yield per tree was generally higher in PRD treatments than in the current grower's practice (the control) in most seasons. No significant changes in the internal quality of the fruit occurred due to the PRD and RDI treatments while fruit size was directly dependent on crop load.

In section 5.3.4 the efficacy of 2,4-D in reducing navel end size under South African conditions was determined. Robyn, Palmer and Lane Late navel trees were treated with 2,4-D (ester form) at 25 ppm at 100% petal drop in 2006. In all the cultivars, 2,4-D increased the percentage closed navels and decreased average navel end size but had no effect on fruit shape, peel thickness or internal fruit quality. However, it gave slightly coarser rinds, slightly greener fruit and greener navel ends. 2,4-D shows a lot of potential as a treatment to reduce navel end size of navel oranges.

The objective of the research presented in section 5.3.5 was to determine if handthinning on Nules Clementine trees results in any economic benefit, even if it doesn't result in an increased fruit size. Hand thinning increased fruit growth and fruit size and had no significant effect on yield. The total time taken to thin and harvest was the same for the two treatments, but harvest took longer in the unthinned control trees. Therefore, hand thinning

reduced the harvest time. This is especially important for Clementines since it is harvested at the start of the rainy season in the Western and Eastern Cape.

In section 5.3.6, a study was conducted to test Clementine mandarin, Navel orange and lemon fruit for sensitivity to a wide range of gamma irradiation dosages. Irradiation leads to a drastic increase in decay of especially lemon fruit during storage, as well as a very high incidence of rind physiological disorders. Navel orange and Clementine mandarin rind quality were both negatively affected at high dosages but lemon rind was extremely sensitive even at low dosages. Season-to-season and fruit-to-fruit variation also play a role, but cultivar choice seems to be the biggest factor in determining sensitivity to irradiation.

### **Projekopsomming**

In die vrugproduksie en kwaliteit projek word vyf studies aangebied naamlik, die gebruik van voor- en na-oes behandelings om vrugkleur ontwikkeling te bevorder, die response van lemoenbome onder gedeeltelike wortelsone uitdroging (PRD) en geregleerde stres besproeiing, toediening van 2,4-D om die navelend opening van navel lemoene te verklein, die voordele van handuitdun by Nules Clementine en om die sensitiwiteit van sitrus onder 'n wye reeks bestralings dosisse te bepaal.

In seksie 5.3.2 is die probleem van onvoldoende kleurontwikkeling op verskeie maniere aangepak. Vrugte is blootgestel aan 'n koue skok of hidro-verkoel en daarna hidro-verwarm. Dit het 'n betekenisvolle kleurverbetering tot gevolg gehad deur flavedo chlorofil konsentrasies te verlaag en flavedo karotenoid konsentrasies te verhoog. Na-oes dompelings van W het swak vrugkleurverandering tot gevolg gehad. W het klein of negatiewe effekte op kleurontwikkeling gehad terwyl Mo kleurontwikkeling verbeter het wanneer dit 19 weke na volblom toegedien is. Pigment samestelling in die flavedo het getoon dat violaxanthin die belangrikste karotenoid in 'Navel' and 'Valencia' is, maar  $\beta$ -citaurin, speel 'n klein rol in skilkleur.

In seksie 5.3.3 is die response van Bahianinha Navel lemoenbome onder die gedeeltelike wortelsone uitdroging (PRD) besproeiingsmetode vergelyk met die respons van plaaslike produsente se besproeiingspraktyke (kontrole), 'n goed benatte behandeling en geregleerde stres besproeiing (RDI) by Mazowe Sitrus Landgoed oor vier seisoene (2005-2008). PRD, RDI en kontrole behandelings het die vorming van dunner en dieper wortels veroorsaak in vergelyking met die goed benatte behandeling. Verlaging in die huidmondjie geleiding het plaasgevind onder PRD terwyl die blaar waterpotensiaal dieselfde was as die goed benatte behandeling. Dus, was die sapvloeï en transpirasietempo betekenisvol laer onder PRD as die kontrole selfs met dieselfde besproeiingsvlakke. Die oeslading per boom was oor die algemeen hoër onder PRD as in die kontrole in die meeste van die seisoene. Geen betekenisvolle veranderings in interne kwaliteit is waaargeneem as gevolg van PRD en RDI behandelings nie, terwyl vruggrootte direk afhanklik van oeslading was.

In seksie 5.3.4 is die effektiwiteit van 2,4-D om die navelend grootte onder Suid-Afrikaanse toestande te verklein, bepaal. Robyn, Palmer en Lane Late navelbome is behandel met 2,4-D (ester vorm) teen 25 dpm by 100% blombelaarval in 2006. By al die kultivars het 2,4-D die persentasie toe navelente verhoog en die gemiddelde navelend grootte verklein maar het geen effek gehad op vrugvorm, skildikte of interne kwaliteit nie, maar het bietjie growwer vrugte, bietjie groener vrugte en groener navelente tot gevolg gehad. 2,4-D toon baie potensiaal as 'n behandeling om navelend grootte van navel lemoene te verklein.

Die doel van die navorsing in seksie 5.3.5 was om te bepaal of handuitdun enige ekonomiese voordeel inhou, al veroorsaak dit nie groter vrugte nie. Handuitdun het vruggroei en vruggrootte verbeter, maar het geen betekenisvolle effek op oeslading gehad nie. Die totale tyd geneem om uit te dun en te oes was dieselfde vir die twee behandelings, maar oes van die kontrole (onuitgedunde) bome het langer geneem. Dus, handuitdun het die oestyd verminder. Dit is veral belangrik vir Clementines omdat dit in die begin van die reënseisoen in die Wes- en Ooskaap geoes word.

In seksie 5.3.6 is die effek van 'n wye reeks bestralings dosisse op die sensitiwiteit van Clementine mandaryn, Navel lemoene en suurlimoene, bepaal. Bestraling het 'n verhoogde voorkoms van bederf van vrugte in opberging veroorsaak, veral in suurlimoene. Die behandelings het ernstige skildefekte veroorsaak. Navel lemoene en Clementine mandaryne se skilkwiteit is ook negatief beïnvloed maar was minder sensitief as die suurlimoene. Variasie tussen seisoene en vrug-tot-vrug verskille in sensitiwiteit speel egter ook 'n rol in die vlakke van skade deur bestraling. Kultivar bly egter die belangrikste aspek wat die voorkoms van skildefekte bepaal.

### 5.3.2 FINAL REPORT: Improving colour of physiologically mature citrus fruit January 2004 - December 2007: I. Bertling, M. Mosoeunyane and J. Bower (UKZN)

#### Summary

The development of the typical orange peel colour of various types of citrus is often unsatisfactory, despite excellent internal fruit colour. Particularly early season fruit are less intensely coloured, making the marketing of these physiologically mature, but non-coloured, fruit difficult. This problem of insufficient colour development was tackled from various aspects. Firstly, as cold exposure improves colouring of such fruit, these were cold-shocked or hydro-cooled and subsequent 'hydro-warmed' to mimic fall environmental conditions in the orchard, resulting in a significant colour improvement by reducing flavedo chlorophyll and increasing flavedo carotenoid concentration. Secondly, as certain micronutrients are involved in carotenoid synthesis, pre-harvest applications of molybdenum (Mo) and tungsten (W) were carried out. Postharvest W dips resulted in poor colour change in fruit, while W had minor or negative effects on colour development; however, Mo improved colour development when applied 19 weeks AFB. The effect of Mo application on pigment formation was similar to cold treatment as the Mo dip increased the carotenoid and decreased the chlorophyll concentration in the flavedo. Furthermore, internal quality parameters neither improved nor worsened with the treatments. Thirdly, the pigment composition was analysed in detail in order to be able to manipulate production of certain carotenoids in the flavedo in the future. Contrary to previous reports using the same analysis technique, it was found that Violaxanthin is the major carotenoid in 'Navel' and 'Valencia', with  $\beta$ -citraurin, a pigment abundant in 'Minneola' peel, playing only a minor role in the rind colour of these types. Lastly, an investigation into the potential alteration of the protein expression brought about by the application of cold plus warmth or Mo demonstrated that after a combination of cold-shock with hot water treatment, new proteins were expressed. In the future, the formation of these proteins must be more closely examined while simultaneously a semi-commercial trial of post-harvest molybdenum dips and cold plus hot water bath exposure should be tested.

#### Opsomming

Die ontwikkeling van 'n tipiese oranje skilkleur van verskeie tipes sitrus is meestal onvoldoende, ongeag uitstekende interne vrugkleur. Veral vroeë seisoen vrugte het 'n minder intense kleur, wat die bemarking van hierdie reeds fisiologiese ryp, maar swak gekleurde vrugte, moeilik maak. Die probleem van onvoldoende kleurontwikkeling is op verskeie maniere aangepak. Eerstens, omdat koue opkleur van sulke vrugte verbeter, is vrugte blootgestel aan 'n koue skok of hidro-verkoel en daarna hidro-verwarm om herfs omgewingstoestande in die boord te simuleer. Dit het 'n betekenisvolle kleurverbetering tot gevolg gehad deur flavedo chlorofil konsentrasies te verlaag en flavedo karotenoid konsentrasies te verhoog. Tweedens, omdat sekere mikroelemente betrokke is by karotenoid sintese, is vooroes behandelings van molibdeen (Mo) and tungsten (W) toegedien. Na-oes dompelings van W het swak vrugkleurverandering tot gevolg gehad. W het klein of negatiewe effekte op kleurontwikkeling gehad terwyl Mo kleurontwikkeling verbeter het wanneer dit 19 weke na volblom toegedien is. Die effek van Mo toediening op pigment vorming was soortgelyk aan koue behandeling. Die Mo dompeling het karotenoid konsentrasies verhoog en chlorofil konsentrasies verlaag in die flavedo. Interne kwaliteit is nie deur die behandelings geaffekteer nie. Derdens, is die pigment samestelling in detail geanaliseer om in staat te wees om die produksie van sekere karotenoïde in die flavedo te manipuleer. In teenstelling met vorige resultate, is gevind dat Violaxanthin die belangrikste karotenoid in 'Navel' and 'Valencia' is, maar  $\beta$ -citraurin, 'n pigment wat volop in 'Minneola' skil is, speel 'n klein rol in skilkleur. Laastens, 'n studie oor die potensiële verandering van proteïen vorming deur koue en warmte of Mo het aangedui dat die kombinasie van koue-skok en warm water behandeling lei tot die vorming van nuwe proteïene. In die toekoms sal die vorming van hierdie proteïene bestudeer moet word asook a semi-kommersiële proef met na-oes Mo dompelings en koue plus warm water bad behandelings sal uitgevoer moet word.

#### Introduction

The South African Citrus Industry caters for the fresh fruit export market, which accounts for 54% of its production. Therefore, good internal fruit quality is essential and has to be combined with high external appeal. However, particularly the first exports of the season suffer from insufficient colour development. This development of the typical rind colour of physiologically mature citrus fruit revolves around two components. Firstly, it depends on the breakdown of chlorophylls and, secondly, on the new formation of specific carotenoids. This process occurs naturally in South Africa at the onset of winter with the reduction in temperatures, a period when mild day temperatures are combined with cold nights and cool soil temperatures (Young and Erickson, 1961). However, other parameters, such as micronutrient application, as well as exposure to stress impact on

the carotenoid biosynthesis of physiologically mature, but externally green fruit. Hence, such treatments result in physiological changes leading to the development of orange rind colour. Tisdale *et al.* (1990) observed that citrus are sensitive to Mo deficiency. However, molybdenum is a component of the sulfated molybdenum cofactor (MoCo) and involved in ABA biosynthesis (Milborrow, 2001). As ABA induces many stress-responsive genes (Rock, 2000), a link between Mo and carotenoid production has been suggested (Milborrow, 2001). Tungsten, an element closely related to Mo, is also involved in ABA metabolism (Milborrow, 2001), and could therefore play a role in citrus rind colour development. In support of this Oberholster (2001) observed rind colour changes after treatment of 'Navel' and 'Valencia' rind explants with 10 $\mu$ M Mo and 1 $\mu$ M W solutions. Furthermore, the synthesis of pigments is temperature-sensitive, with sensitivity varying from plant to plant (Gross, 1987). In South Africa, visual colour change in 'Navel' and 'Valencia' is correlated to the synthesis of  $\beta$ -citraurin, a temperature sensitive process (Stewart and Wheaton, 1973). Oberholster (2001) furthermore reported that exposing rind discs of 'Navel' and 'Valencia' to 4 $^{\circ}$ C followed by incubation at 22 $^{\circ}$ C for either 1 or 4 days results in a significant increase in the carotenoid content of rind discs. Kato *et al.* (2004) reported a relationship between carotenoid accumulation and expression of carotenoid biosynthetic genes during fruit maturation in the citrus varieties, 'Satsuma' mandarin (*C. unshiu* Marc.), 'Valencia' orange (*C. sinensis* Osbeck), and 'Lisbon' lemon (*C. limon* Burm. f.).

Based on these findings, the effect of pre-harvest application of certain micronutrients and post-harvest environmental temperature stresses (a combination of chilling and heating) on fruit colour development of early 'Navel' and 'Valencia' fruit was investigated. It was hypothesised that post-harvest exposure of *Citrus* fruit to chilling (4 $^{\circ}$ C) with subsequent heat exposure (40 $^{\circ}$ C) enhances colour development in the flavedo; similarly, application of Mo and W will stimulate carotenoid production in the flavedo. Furthermore, in order to determine which treatment enhances orange colour development best, colour measurements of oranges to which these treatments were applied were undertaken, in case it is possible for an on-line facility to be used to separate oranges, which can be coloured up by one of the treatments, from those which cannot.

## Materials and methods

### Field applications of molybdenum and tungsten

Foliar Mo and W applications were carried out on 6-year-old 'Navel' and 18-year-old 'Valencia' trees on Orangewood Farm in the Natal Midlands. Either 0.2 or 0.4g/L Mo or W were applied per tree, 19 weeks after full-bloom. T5 fruit were harvested, washed, dried and cooled in a 4 $^{\circ}$ C cold-room. Rind temperatures of these fruit dropped to 3.95 $^{\circ}$ C and pulp temperatures to 5.78 $^{\circ}$ C within the 9 hours of exposure. A further batch of T5 fruit was immersed in 1 or 10  $\mu$ M solutions of Mo, transferred to a 4 $^{\circ}$ C cold room for 9 hours followed by heat shock in a 60 $^{\circ}$ C water bath. Furthermore, T5 fruit from trees that had been foliar-sprayed with 0.2g of Mo or W as well as an untreated batch of T5 fruit were picked. They were cold shocked in a 4 $^{\circ}$ C water bath followed by a 'heat shock' in the hot water bath. Fruit were visually assessed for colour.

### Postharvest treatments

Similarly, untreated fruit at the T5 stage were picked and exposed to the cold water bath followed by a 30s or 120s in the hot water bath. A further batch of fruit was first immersed in either 1 or 10 $\mu$ M W or Mo solution and subsequently exposed to 9 hrs in the cold-room followed by a 30 s or 120 s hot water bath. The individual pigment pattern of 'Navel' and 'Valencia' fruit were analysed by HPLC combined with a DAD detector.

## Results

### Field application of molybdenum and tungsten

There was no difference in the onset of colour-break of fruit from treated or untreated trees. HPLC analysis of 3 g dry flavedo tissue indicated that violaxanthin is the predominant pigment in both citrus types and in all treatments. Although no  $\beta$ -citraurin was detected in control (untreated) 'Navel' and 'Valencia' fruit, it was detected in fruit from trees treated with W or Mo. In contrast to 'Navel',  $\beta$ -citraurin was also detected in 'Valencia' fruit that were foliar sprayed with NaPO<sub>4</sub> (Table 5.3.2.1).

### Postharvest treatments

Similarly, untreated T5 fruit were exposed to a cold water bath followed by a 30s or 120s hot water bath. A further batch of fruit was first immersed in either 1 or 10 $\mu$ M W or Mo solution and subsequently exposed to 9 hrs at 4 $^{\circ}$ C cold-room followed by a 30 s or 120 s hot water bath. The individual pigment pattern of 'Navel' and 'Valencia' fruit were analysed by HPLC combined with a DAD detector.

### Colour determination and analysis

The concentration of rind pigments (chlorophylls and carotenoids) was determined spectrophotometrically.

### Effect on chlorophyll a+b concentration

A significant ( $P < 0.001$ ) interaction between date of sampling, pre- and main-treatments as well as cultivar on the chlorophyll a+b concentration in 'Navel' and 'Valencia' peels was found. Significant differences in chlorophyll a+b concentration between pretreatments were more notable 7 days after treatment (DAT) than 14 DAT.

'Navel', when stored at room temperature, lost less chlorophyll than when exposed to a liquid treatment ( $P < 0.001$ ). The lowest chlorophyll concentration was found in Mo-treated rinds 7 and 14 DAT (Fig. 5.3.2.1A). Surprisingly, chlorophyll degradation in control and water-treated fruit was faster under coldroom conditions than under room temperature (Fig. 5.3.2.1B). With the exception of non-treated fruit at room temperature, the major chlorophyll loss in the rind occurred within 7 days after treatment and the reduction in the second week of storage was much less, particularly so in the hot water treated fruit. In all hot water treatment combinations differences between treatments were non-existent or minor 14 DAT (Fig. 5.3.2.1C-1F).

Comparison of the trend of chlorophyll a+b degradation in 'Navel', 'Valencia' 7 DAT (Fig. 5.3.2.2A-F) reveals a notable difference. There was slower chlorophyll a+b degradation in 'Valencia' than in 'Navel' fruit that were subjected to similar postharvest treatments combinations of pre- with main-treatments. This observation was evident 7 DAT (Fig. 5.3.2.2A-F). The combination of 10  $\mu\text{M}$  Mo with cold shock resulted in the lowest chlorophyll a+b concentration than the control. There was a significantly lower chlorophyll a+b concentration in fruit that were treated with the combinations of +Mo with HWT 120s than the control 7 DAT. Generally, data collected 7 DAT provided comparable results that could be used for evaluation of the effect of treatments than that recorded 14 DAT.

### Effect on total carotenoids

Pre- and main-treatment as well as sampling date affected the total carotenoid concentration of the peel significantly ( $P = 0.001$ ). The interactions of these factors on total peel carotenoids were also significant ( $P = 0.001$ ) in both cultivars. In general, the carotenoids concentrations increased from the day of treatment to 7 DAT (Table 5.3.2.2). However, while the carotenoid concentration in 'Navel' either declined or remained constant from 7 to 14 DAT, in 'Valencia' the increase in carotenoid concentration from 7 to 14 DAT was much higher. Treatment combinations with either 1 or 10  $\mu\text{M}$  Mo yielded the highest carotenoid concentration in 'Navel' and 'Valencia', particularly when in combination with 30 s HWD (Table 5.3.2.2). In 'Navel' the CR + HWD 30s treatments gave the best result 7 DAT while the HWD 30 s only seemed to have retained the carotenoids better 14 DAT. In 'Valencia' the HWD for 30 s had the highest carotenoid concentration 7 DAT, while 14 DAT the highest carotenoid concentration was determined in 30s hot water dipped with subsequent room temperature storage and 120 s hot water followed by cold room storage.

Generally, the highest carotenoid concentrations were determined in 'Navel' and 'Valencia' fruit (7 DAT) that had been treated with combinations of air with CR-HWT 30 s and immersion in 10  $\mu\text{M}$  Mo most often yielded the highest carotenoid concentration.

### Pigment composition of 'Navel' and 'Valencia' fruit analysed by HPLC

The dominant carotenoid in all treatments was violaxanthin, making up from 53 to 77% of the carotenoid profile (Table 5.3.2.3). Besides violaxanthin, zeaxanthin was found to be a major pigment in 'Navel' and 'Valencia'. Neoxanthin was only detectable in control, non-immersed 'Valencia' and 'Navel' as well as in air x CR-treated fruit. Nothing was detected in other treatments.  $\beta$ -citraurin was detected only in 1  $\mu\text{M}$  Mo x RT, 1 or 10  $\mu\text{M}$  Mo x HWT 30s, 1  $\mu\text{M}$  Mo x CR-HWT 30s and 1  $\mu\text{M}$  Mo x CR-HWT 120s -treated 'Valencia' fruit (Table 5.3.2.3), while nothing was detected in 'Navel' fruit (Table 5.3.2.4). Phytofluene and phytoene, pigments which have few conjugated double bonds, hence are colourless, were detected in all treatment combinations (Air x RT, water x RT, +Mo x RT). Interestingly, the concentration (percent of total peak area) of phytoene and phytofluene in 'Valencia' was  $\geq 1\%$  in air x RT while in other treatments combinations the concentration was less. The trend was not observed in 'Navel' fruit where about 3% of phytofluene was detected. Violaxanthin was predominant in all treated 'Navel' and 'Valencia' fruit. The lowest concentration of violaxanthin was detected in Air x RT fruit than in water x RT, and 1 or 10  $\mu\text{M}$  Mo -treated 'Valencia' fruit. That was not the case in 'Navel' fruit. Lutein concentrations that were detected in 'Navel' fruit were  $>6\%$  in all treatments combination while small amounts ( $\leq 1\%$ ) in few treatments were detected in the majority of treatments combinations in 'Valencia' fruit.

### Effect on visual colour

The pre-treatments (air, water, 1  $\mu\text{M}$  or 10  $\mu\text{M}$  Mo) did not have any significant effect on 'Navel' and 'Valencia' fruit colour ( $P = 0.450$  and  $0.248$ , respectively) (Table 5.3.2.5). On the other hand, the pre-treatments had significant effects on the parameter  $b^*$ , and chroma ( $C^*$ ) attributes of 'Navel' and 'Valencia' fruit. The main-treatments (room temperature, cold room, HWT 30 or 120s, CR-HWT 30 or 120s) had significant effects ( $P = 0.001$ ) on  $L^*$ ,  $b^*$ , and  $C^*$  of both, 'Navel' and 'Valencia' fruit. The pre- x main-treatments interaction had also a significant effect ( $P = 0.001$ ) on  $L^*$ ,  $b^*$ , and  $C^*$  of 'Navel' fruit, and  $b^*$  and  $C^*$  of Valencia\* fruit.

Fruit colour changes were visible as significantly ( $P = 0.001$ ) higher  $L^*$  values in 'Navel' fruit that were treated with water x CR, 1  $\mu\text{M}$  Mo x CR and 10  $\mu\text{M}$  Mo x CR than the control. Some fruit still had some patches of green, as indicated by negative  $a^*$  values (Table 5.3.2.5), while others were fully degreened and displayed positive  $a^*$  values. As a result of the clear difference between negative and positive values of some treatments  $a^*$  data were not analysed statistically. Air x RT or water x RT, resulted in slow visual colour change towards fully degreened 'Navel' or 'Valencia'. Similarly, air x HWT 30s, and water x HWT 30s-treated 'Navel' fruit were slow to degreen completely. This was also reflected in both 'Navel' and 'Valencia' fruit that were treated with air x CR or water x CR. The treatment combinations of 10  $\mu\text{M}$  Mo x RT or 10  $\mu\text{M}$  Mo x CR showed a speedy change of 'Navel' and 'Valencia' peel colour, as indicated by the change in  $a^*$  values, from negative to positive. This was also observed in 10  $\mu\text{M}$  Mo x HWT 30s-treated fruit. Generally, treatment combinations resulted in fast reddening displayed by an increase in  $a^*$  values.

Significantly higher  $b^*$  values were observed in 'Valencia' fruit that were treated with the combinations of 10  $\mu\text{M}$  Mo with CR, 1 or 10  $\mu\text{M}$  Mo with RT, as well as 10  $\mu\text{M}$  Mo with HWT 30s than controls. Furthermore, significantly higher  $b^*$  values (yellowing) were observed when: 'Navel' and Valencia' fruit were treated with 1 or 10  $\mu\text{M}$  Mo x RT, 'Valencia' fruit were treated with 10  $\mu\text{M}$  Mo x CR, 'Navel' fruit were treated with 1 or 10  $\mu\text{M}$  Mo x HWT 30s and 'Valencia' fruit were treated with 10  $\mu\text{M}$  Mo x HWT 30s or 1  $\mu\text{M}$  Mo x HWT 120s. The change in chroma on both cultivars followed a similar pattern to that of  $b^*$  explained above.

## **Discussion**

### Fruit colour

Fruit colour change is often used as a criterion to assess the ripening stage of fruit (Huyskens-Keil *et al.*, 2006). Colour change of citrus fruit is strongly correlated with the storage period at ambient temperature (Singh and Reddy, 2006), as it is shown in Table 5.3.2.5 where all CIE  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$  attributes in treated and untreated fruit had changed remarkably within 14 DAT. It has been reported that postharvest immersion of citrus fruit in hot water affects rind colour (Oberholster, 2001). According to Woolf (1997), Lurie (1998), and Paull and Chen (2000) heat treatments can be used to inhibit the ripening process. This is supported by the results of the present study which indicate slow colour change of 'Navel' fruit that were treated with air x HWT 30 s and also water x HWT 30s. The discrepancy in response to similar treatment combinations shown by 'Valencia' can be accounted for by difference in cultivar. We therefore tentatively conclude that the response to HWT is a result of a variety of factors working in concert. A slow colour development following HWT was noted on cherry tomato (*Lycopersicon esculentum*, cv. Coco), although fruit were also stored under low  $\text{O}_2$  (Ali *et al.*, 2004). The significant increase in  $b^*$  as a result of 10  $\mu\text{M}$  Mo x CR, 1 or 10  $\mu\text{M}$  Mo x RT as well as in heat-treated fruit contradicts findings by Sozzi *et al.* (1996) that heat inhibits colour development. However, Mo treatments showed superior  $b^*$  values suggesting, that Mo had a certain impact on colour saturation. Similarly, accumulation of yellowness following 10  $\mu\text{M}$  Mo with HWT 30s-treated 'Valencia' fruit could be attributed to Mo acting as a co-factor in the ripening process under heat stress condition (Vitor *et al.*, 2001).

### Carotenoids

Reports indicate that  $\beta$ -citaurin, a  $\text{C}_{30}$  apocarotenoid, is a major carotenoid in 'Clementine' (*C. reticulata*) (Curl, 1965), 'Navel' and 'Valencia' (*C. sinensis* L. Osbeck) (Oberholster *et al.*, 2001) 'Bonanza' and 'Cara Cara' (*C. sinensis* L. Osbeck) (Rodrigo *et al.*, 2004). In the present study the concentration of  $\beta$ -citaurin in colour-break orange peel was regulated by postharvest treatments. Although, the pigment was detected in very small concentrations (<1% of total carotenoid concentration) in 'Valencia' peel, it was however, detected mostly in fruit that were subjected to stress at colour-break. Nonetheless,  $\beta$ -citaurin was detected in fruit that were treated with 1 $\mu\text{M}$  Mo (control) and have not been subjected to cold and/or heat shock. This could be attributed to a co-factoring role on constitutive carotenogenesis process in the peel. The combination of the same (1 $\mu\text{M}$ ) Mo concentration with HWT (30s) or CR-HWT (30 or 120s) tends to have an added impact on accumulation of  $\beta$ -citaurin concentration. This suggests that  $\beta$ -citaurin concentration might be affected by postharvest

treatments. Although the exact biosynthetic pathway of citraurin is not yet known, it is believed that the compound results from a cleavage of  $\beta$ -carotene (van Vliet *et al.*, 1996),  $\beta$ -cryptoxanthin (Gross, 1981) or zeaxanthin (Yokoma and White, 1966) by carotenoid cleavage dioxygenases (CCD) (Giuliano *et al.*, 2003). The constitutive accumulation of citraurin in 1 $\mu$ M Mo-treated (stress-free treatment) fruit suggest that, in addition to stress, Mo-cofactor dependent enzymes may have enhanced the formation of citraurin, during postharvest treatment, possibly as an artefact. The pigment was not detected in 'Navel' fruit.

Compared to carotenoids occurring at the beginning of the pathway (phytoene and phytofluene), those further metabolised, were predominant. However, 9-cis-violaxanthin, reported as a dominant carotenoid in 'Valencia' fruit (Kato *et al.*, 2004), also occurred at high level. The violaxanthin concentration decreased significantly ( $P < 0.001$ ) in response to longer (120 s) heat exposure. Additionally,  $\beta$ -cryptoxanthin, which has a significant contribution to the bright-orange of the peel of 'Navelate' (Rodrigo *et al.*, 2003) was not detected in this study. This could be related to time of termination of the experiment; we might have sampled prior to  $\beta$ -cryptoxanthin accumulation in the peel.

The absence of 9-cis-neoxanthin in the peel samples may be an indication of the stage of chromoplast differentiation, as this carotenoid mostly occurs during fruit ripening (Rodrigo *et al.*, 2004). The absence of this carotenoid in the peel of 'Navelate' fruit (*C. sinensis* L. Osbeck) at colour-break has been previously reported by Rodrigo *et al.* (2004). The possible conversion of 9-cis-neoxanthin to xanthoxal, an immediate precursor of the plant stress hormone ABA (Cowan and Richardson, 1997) could provide a possible explanation for the lack of 9-cis-neoxanthin in all applied temperature stresses (Table 5.3.2.5). The concentration of lutein 14 DAT was generally in the range of 0-10% of total carotenoids. This concentration concurs with the report by Rodrigo *et al.* (2004) in colour-break 'Navelate' fruit. Additionally,  $\alpha$ -carotene, also a  $\beta, \epsilon$ -carotene, was not detected in the peel of 'Valencia' 14 DAT. This can serve as evidence of carotenoid transformation that may have occurred during the period of storage at room temperature. Contrary to Rodrigo *et al.* (2004) who did not report the occurrence of  $\beta$ -carotene in colour-break and full-colour 'Navelate' fruit, we did detect  $\beta$ -carotene in 'Navel' and 'Valencia' peel 14 DAT, even though at very low concentration ( $< 1\%$  of total carotenoids). In support of our results,  $\beta$ -carotene was reported in 'Ailsa Craig' tomato fruit 24 days after colour-break stage (Fraser *et al.*, 1994). However, the present results are supporting (Xu *et al.*) reported the presence of  $\beta$ -carotene in citrus fruit post colour-break. The occurrence of as well as the concentration of phytoene determined in our study are in line with Rodrigo *et al.* (2004). In general, the low concentrations of lutein and other 'early' carotenoids (phytoene and phytofluene) suggest that our efforts to improve carotenogenesis in *Citrus sinensis* fruit contributed chiefly towards channelling of carotenoids building-blocks to  $\beta, \beta$ -branching during peel colour evolution, a process believed to be genetically engineerable (Hirschberg, 1999).

## Conclusion

Through implementation of certain treatments, which probably exert some form of temperature stress onto the rind of citrus fruit, the colour of the flavedo can be manipulated by enhancing the violaxanthin concentration of Navel and Valencia fruit. The combination of cold and hot water baths in a semi-commercial trial will be the next step in the effort to change colour in physiologically mature, but non-coloured, citrus fruit.

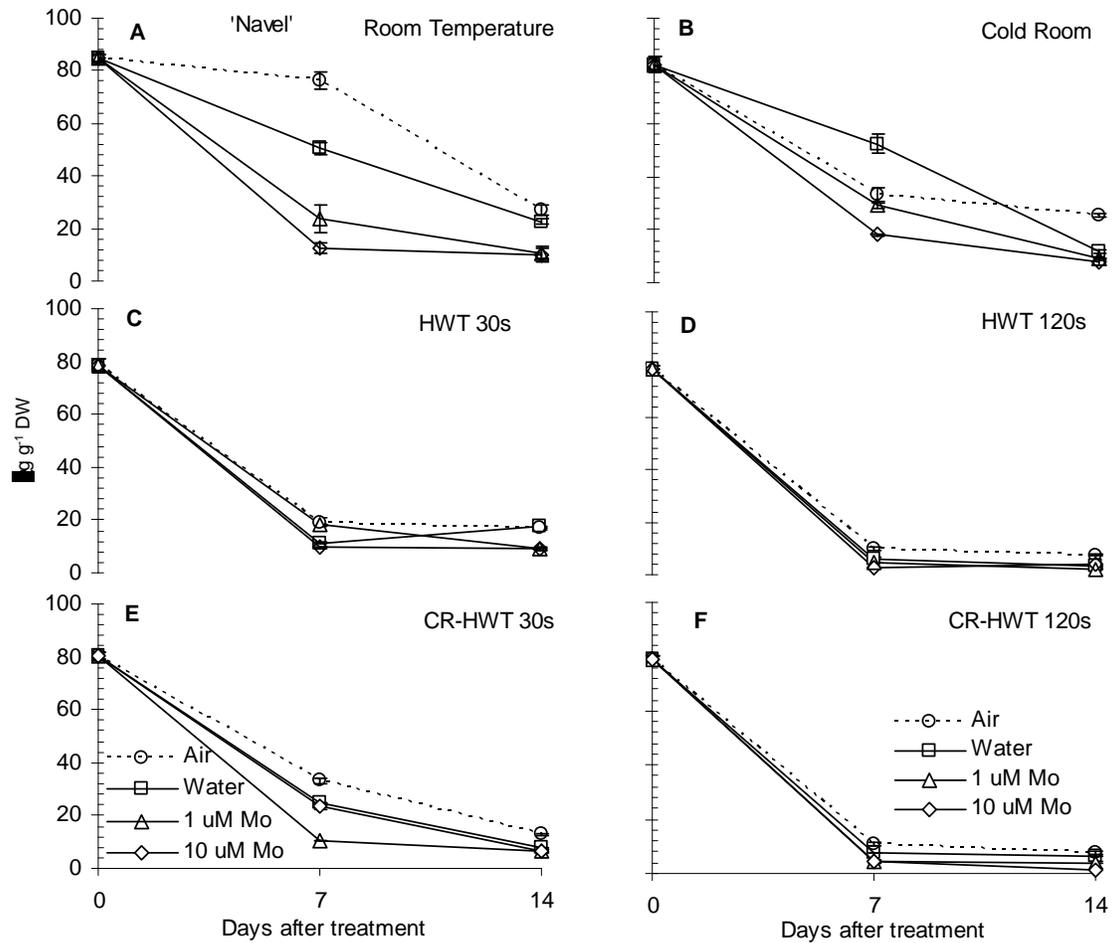
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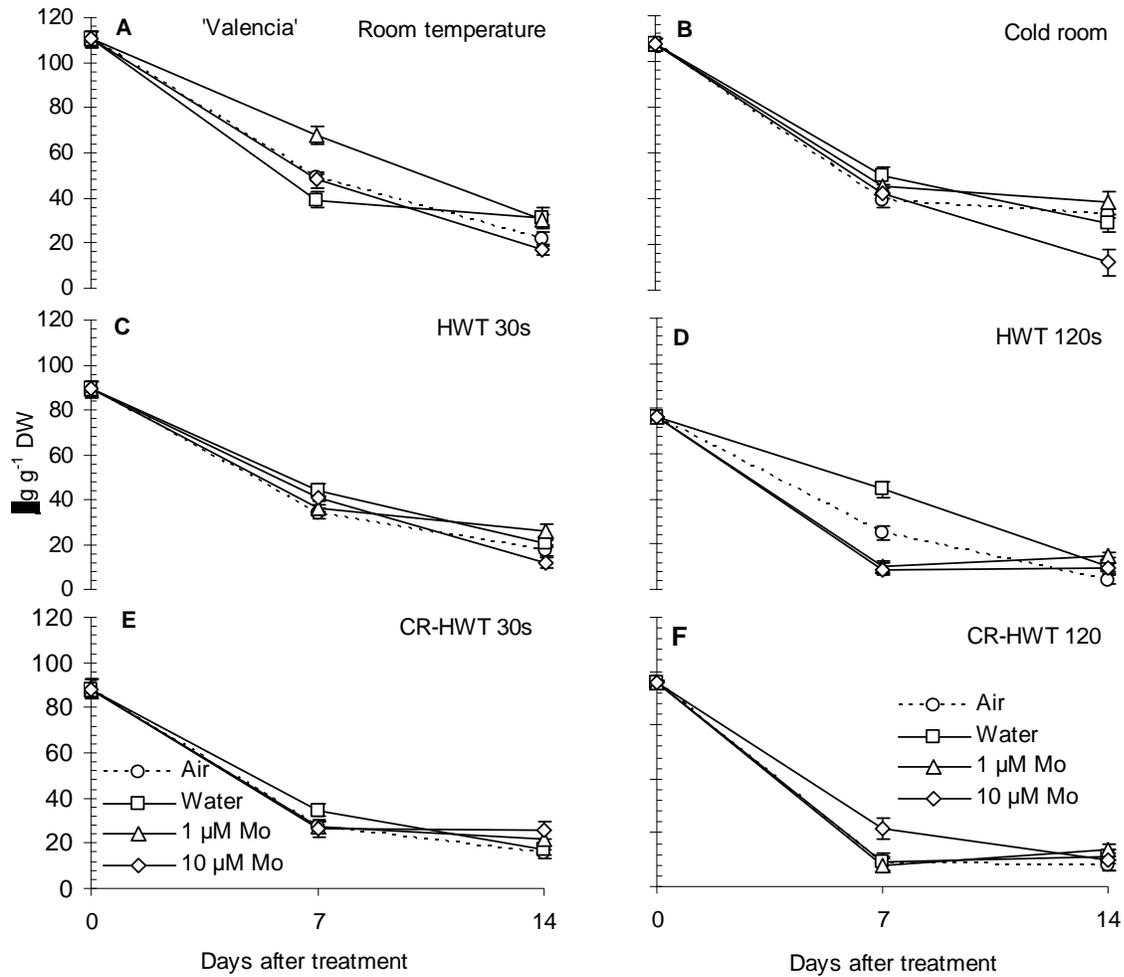
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**Table 5.3.2.1.** Carotenoid concentration (% of total peak area) in 'Valencia' and 'Navel' flavedo with xanthophylls and  $\beta$ -carotene monitored at 450 nm and  $\beta$ -citraurin at 460 nm

Treatment	'Navel'			'Valencia'		
	Violaxanthin	$\beta$ -citraurin	$\beta$ -carotene	Violaxanthin	$\beta$ -citraurin	$\beta$ -carotene
Control	72.56 <sup>a</sup>	0.00	0.87 <sup>b</sup>	70 <sup>a</sup>	0.0	1.01 <sup>b</sup>
NaPO <sub>4</sub> (0.4g/L)	65.93 <sup>b</sup>	0.00	1.71 <sup>b</sup>	63 <sup>bc</sup>	2.97 <sup>b</sup>	0.73 <sup>b</sup>
+W (0.2g/L)	74.67 <sup>a</sup>	7.13 <sup>a</sup>	0.42 <sup>c</sup>	67.7 <sup>ba</sup>	3.47 <sup>b</sup>	2.17 <sup>a</sup>
+W (0.4g/L)	65.15 <sup>b</sup>	6.01 <sup>ab</sup>	4.64 <sup>a</sup>	57.7 <sup>bc</sup>	6.57 <sup>a</sup>	1.02 <sup>b</sup>
+Mo (0.2g/L)	63.32 <sup>b</sup>	4.42 <sup>b</sup>	1.26 <sup>b</sup>	54.0 <sup>c</sup>	2.47 <sup>b</sup>	2.10 <sup>a</sup>
+Mo (0.4g/L)	65.47 <sup>b</sup>	5.86 <sup>ba</sup>	0.11 <sup>c</sup>	68.3 <sup>ba</sup>	1.36 <sup>c</sup>	0.99 <sup>b</sup>
P value	<.001	<.001	<.001	0.05	<.001	<.001
LSD	3.553	1.914	0.840	12.24	1.1	0.44
CV%	2.9	27.0	30.8	10.6	21.3	18.0



**Figure 5.3.2.1.** Change in total chlorophyll a+b of 'Navel' oranges over a period of 14 days of storage (incubation) at ambient temperature after subjecting them to various combinations of postharvest treatments. Legends are pretreatments. Types of main treatment are shown at the top-right of each graph. HWT: hot water (60°C) immersion for certain duration (seconds). CR-HWT: coldroom exposure followed by HWT. Data points are means  $\pm$  standard error (n = 3).



**Figure 5.3.2.2.** Change in total chlorophyll a+b of 'Valencia' oranges over a period of 14 days of storage (incubation) at ambient temperature after subjecting them to various combinations of postharvest treatments. Legends are pretreatments. Types of main treatment are shown at the top-right of each graph. HWT: hot water (60°C) immersion for certain duration (seconds). C R-HWT: coldroom exposure followed by HWT. Data points are means  $\pm$  standard error (n = 3).

**Table 5.3.2.2.** Changes in total carotenoids (xanthophylls & carotenes) of 'Navel' and 'Valencia' peel over a period of 14 days of storage (incubation) at ambient temperature after subjecting fruit to different combinations of molybdenum with temperature treatments

Pre-	Main-	Carotenoid concentration ( $\mu\text{g}\cdot\text{g}^{-1}\text{ DW}$ )					
		'Navel'			'Valencia'		
Treatments		0 DAT	7 DAT	14 DAT	0 DAT	7 DAT	14 DAT
Air	Room	118.64	151.90 <sup>a</sup>	134.11 <sup>a</sup>	93.34	173.85 <sup>b</sup>	177.50 <sup>b</sup>
Water	temperature	118.64	158.11 <sup>a</sup>	121.62 <sup>b</sup>	93.34	178.73 <sup>b</sup>	194.95 <sup>a</sup>
1 $\mu\text{M}$ Mo		118.64	137.97 <sup>b</sup>	139.54 <sup>a</sup>	93.34	207.75 <sup>a</sup>	161.97 <sup>c</sup>
10 $\mu\text{M}$ Mo		118.64	161.13 <sup>a</sup>	142.66 <sup>a</sup>	93.34	205.56 <sup>a</sup>	186.79 <sup>ab</sup>
Air	Cold room	118.64	123.15 <sup>c</sup>	135.99 <sup>b</sup>	93.34	167.04 <sup>c</sup>	157.10 <sup>c</sup>
Water		118.64	143.46 <sup>b</sup>	136.62 <sup>b</sup>	93.34	184.40 <sup>b</sup>	174.64 <sup>b</sup>
1 $\mu\text{M}$ Mo		118.64	178.67 <sup>a</sup>	156.81 <sup>a</sup>	93.34	201.64 <sup>a</sup>	164.65 <sup>bc</sup>
10 $\mu\text{M}$ Mo		118.64	132.47 <sup>a</sup>	136.57 <sup>a</sup>	93.34	157.03 <sup>c</sup>	206.15 <sup>a</sup>
Air	HWT 30s	118.64	135.46 <sup>b</sup>	147.93 <sup>b</sup>	93.34	179.31 <sup>b</sup>	198.28 <sup>a</sup>
Water		118.64	146.60 <sup>bc</sup>	154.31 <sup>b</sup>	93.34	211.11 <sup>a</sup>	174.04 <sup>b</sup>
1 $\mu\text{M}$ Mo		118.64	140.01 <sup>c</sup>	133.46 <sup>c</sup>	93.34	204.72 <sup>a</sup>	197.44 <sup>a</sup>
10 $\mu\text{M}$ Mo		118.64	176.30 <sup>a</sup>	177.03 <sup>a</sup>	93.34	207.05 <sup>a</sup>	156.47 <sup>c</sup>
Air	HWT 120s	118.64	158.64 <sup>a</sup>	115.90 <sup>c</sup>	93.34	160.73 <sup>b</sup>	146.94 <sup>b</sup>
Water		118.64	157.05 <sup>ab</sup>	127.69 <sup>b</sup>	93.34	144.21 <sup>c</sup>	174.23 <sup>a</sup>
1 $\mu\text{M}$ Mo		118.64	143.69 <sup>b</sup>	136.69 <sup>b</sup>	93.34	173.98 <sup>a</sup>	167.35 <sup>a</sup>
10 $\mu\text{M}$ Mo		118.64	129.03 <sup>c</sup>	150.72 <sup>a</sup>	93.34	174.15 <sup>a</sup>	150.81 <sup>b</sup>
Air	CR-HWT 30s	118.64	155.64 <sup>c</sup>	125.46 <sup>c</sup>	93.34	219.71 <sup>a</sup>	158.81 <sup>c</sup>
Water		118.64	183.56 <sup>b</sup>	161.83 <sup>a</sup>	93.34	192.81 <sup>c</sup>	170.40 <sup>b</sup>
1 $\mu\text{M}$ Mo		118.64	173.05 <sup>b</sup>	158.74 <sup>a</sup>	93.34	201.55 <sup>b</sup>	174.71 <sup>b</sup>
10 $\mu\text{M}$ Mo		118.64	192.93 <sup>a</sup>	149.37 <sup>a</sup>	93.34	199.74 <sup>c</sup>	211.37 <sup>a</sup>
Air	CR-HWT 120s	118.64	132.69 <sup>c</sup>	140.94 <sup>b</sup>	93.34	167.59 <sup>c</sup>	178.06 <sup>b</sup>
Water		118.64	147.83 <sup>b</sup>	168.70 <sup>a</sup>	93.34	162.65 <sup>c</sup>	167.89 <sup>b</sup>
1 $\mu\text{M}$ Mo		118.64	146.15 <sup>b</sup>	124.87 <sup>c</sup>	93.34	186.83 <sup>b</sup>	211.20 <sup>a</sup>
10 $\mu\text{M}$ Mo		118.64	165.36 <sup>a</sup>	125.92 <sup>c</sup>	93.34	194.47 <sup>a</sup>	178.49 <sup>b</sup>
LSD (0.05)	= 7.3						

Rind samples were collected from ten fruit from each of all three replicated and combined to form on pooled sample per treatment.

HWT: hot water immersion for 30 or 120s. CR-HWT: a combination of cold-shock and HWT.

Values followed by the same letter within a block of a main-treatment are not statistically different

**Table 5.3.2.3.** Carotenoid concentration of 'Valencia' flavedo 14 days after post harvest cold- and/or heat-shock treatments. Spectral characteristics xanthophylls and carotene were monitored at 450 nm, while an apocarotenoid was monitored at 460 nm

Pre-Treatments	Main-Treatments	Percentage of total peak area (%)							
		Xanthophylls						Carotenes	
		Neo.	Viol.	Zea.	Lut.	β-cit.	β-car.	Phyt.	Phyf.
Air	RT	2.85	55.11 <sup>c</sup>	6.91 <sup>b</sup>	ND	ND	0.53 <sup>a</sup>	1.0 <sup>a</sup>	2.99 <sup>a</sup>
Water		ND	74.29 <sup>a</sup>	2.22 <sup>c</sup>	ND	ND	0.56 <sup>a</sup>	0.24 <sup>c</sup>	0.22 <sup>c</sup>
1μM Mo		ND	68.35 <sup>b</sup>	9.11 <sup>a</sup>	0.11	0.11	0.14 <sup>b</sup>	0.06 <sup>d</sup>	0.63 <sup>b</sup>
10μM Mo		ND	76.53 <sup>a</sup>	6.87 <sup>b</sup>	ND	ND	0.23 <sup>b</sup>	0.42 <sup>b</sup>	0.60 <sup>b</sup>
Air	CR	ND	63.76 <sup>c</sup>	7.57 <sup>bc</sup>	ND	ND	0.98 <sup>a</sup>	1.05 <sup>a</sup>	2.89 <sup>a</sup>
Water		ND	68.68 <sup>bc</sup>	5.97 <sup>c</sup>	ND	ND	0.80 <sup>b</sup>	0.01 <sup>d</sup>	0.30 <sup>c</sup>
1μM Mo		ND	69.44 <sup>b</sup>	14.54 <sup>a</sup>	ND	ND	0.60 <sup>c</sup>	0.55 <sup>b</sup>	0.61 <sup>b</sup>
10μM Mo		ND	74.04 <sup>a</sup>	7.91 <sup>bc</sup>	ND	ND	0.41 <sup>d</sup>	0.20 <sup>c</sup>	0.35 <sup>c</sup>
Air	HWT	ND	65.03 <sup>a</sup>	5.4 <sup>b</sup>	ND	ND	0.26 <sup>b</sup>	0.26 <sup>a</sup>	1.15 <sup>a</sup>
Water	30s	ND	70.80 <sup>a</sup>	8.52 <sup>a</sup>	ND	ND	0.32 <sup>b</sup>	ND	ND
1μM Mo		ND	70.82 <sup>a</sup>	10.06 <sup>a</sup>	0.14	0.14	0.56 <sup>a</sup>	ND	ND
10μM Mo		ND	68.69 <sup>a</sup>	8.97 <sup>a</sup>	0.15	0.15	0.28 <sup>b</sup>	0.39 <sup>a</sup>	0.13 <sup>b</sup>
Air	HWT	ND	52.96 <sup>b</sup>	16.27 <sup>a</sup>	ND	ND	0.73 <sup>a</sup>	1.0 <sup>a</sup>	2.52 <sup>a</sup>
Water	120s	ND	55.66 <sup>b</sup>	13.21 <sup>b</sup>	ND	ND	0.48 <sup>b</sup>	0.22 <sup>c</sup>	ND
1μM Mo		ND	64.64 <sup>a</sup>	13.73 <sup>ab</sup>	ND	ND	0.26 <sup>c</sup>	0.60 <sup>b</sup>	0.51 <sup>c</sup>
10μM Mo		ND	63.42 <sup>a</sup>	14.78 <sup>ab</sup>	ND	ND	0.46 <sup>b</sup>	0.61 <sup>b</sup>	0.82 <sup>b</sup>
Air	CR-HWT	ND	72.39 <sup>a</sup>	8.60 <sup>b</sup>	ND	ND	0.77 <sup>a</sup>	0.57	0.53 <sup>a</sup>
Water	30s	ND	69.71 <sup>a</sup>	9.58 <sup>b</sup>	ND	ND	0.71 <sup>a</sup>	0.03	0.14 <sup>b</sup>
1μM Mo		ND	67.01 <sup>a</sup>	9.22 <sup>b</sup>	0.16	0.16	0.80 <sup>a</sup>	ND	ND
10μM Mo		ND	66.77 <sup>b</sup>	14.54 <sup>a</sup>	ND	ND	0.80 <sup>a</sup>	ND	0.49 <sup>a</sup>
Air	CR-HWT	ND	62.97 <sup>a</sup>	11.88 <sup>a</sup>	ND	ND	0.42 <sup>ab</sup>	0.45 <sup>a</sup>	0.77 <sup>a</sup>
Water	120s	ND	64.18 <sup>a</sup>	13.55 <sup>a</sup>	ND	ND	0.47 <sup>a</sup>	ND	0.65 <sup>a</sup>
1μM Mo		ND	63.74 <sup>a</sup>	3.98 <sup>b</sup>	0.13	0.13	0.52 <sup>a</sup>	0.07 <sup>b</sup>	0.11 <sup>b</sup>
10μM Mo		ND	68.42 <sup>a</sup>	14.08 <sup>a</sup>	ND	ND	0.33 <sup>b</sup>	0.60 <sup>a</sup>	0.66 <sup>a</sup>
P value (n = 3)			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD <sub>(0.05)</sub> (pre- x main-)			5.5	3.0	0.01	0.01	0.12	0.17	0.20
CV%			5	18.8	20.1	20.1	14	30.8	17.6
s.e.d (pre- x main-)			2.74	1.5	0.005	0.005	0.06	0.09	0.10

CR: cold room, HWT: hot water immersion, ND: not detected, RT: room temperature, Neo: Neoxanthin, Lut: lutein, Viol: Violaxanthin, β cit: β citraurin, β car: β carotene, Phyt: phytoene, Phyf: phytofluene, Zea: zeaxanthin

For the purpose of statistical analysis, 0.001 (the minimum peak area limit) was used for ND.

Values followed by the same letter within a block of a main-treatment are not statistically different

**Table 5.3.2.4.** Carotenoid concentration in 'Navel' flavedo 14 days after post harvest cold- and/or heat-shock treatments. Spectral characteristics xanthophylls and carotene were monitored at 450 nm, while an apocarotenoid was monitors at 460 nm.

Pre-	Main-	Percentages in total peak area (%)							
		Xanthophylls						Carotenes	
Treatments		Neo.	Viol.	Zea.	Lut.	β-cit.	β-car.	P.	PF.
Air	RT	4.52	66.78 <sup>c</sup>	9.77 <sup>a</sup>	3.25 <sup>b</sup>	ND	0.27 <sup>b</sup>	0.06b	0.57 <sup>a</sup>
Water		ND	68.97 <sup>b</sup>	9.46 <sup>a</sup>	7.85 <sup>a</sup>	ND	0.43 <sup>a</sup>	0.40a	0.86 <sup>b</sup>
1μM Mo		ND	73.41 <sup>a</sup>	4.76 <sup>b</sup>	7.75 <sup>a</sup>	ND	ND	0.31ab	0.69 <sup>b</sup>
10μM Mo		ND	72.30 <sup>ab</sup>	5.78 <sup>b</sup>	8.35 <sup>a</sup>	ND	ND	0.17b	3.93 <sup>a</sup>
Air	CR	3.8	70.93 <sup>ab</sup>	10.09 <sup>a</sup>	3.55 <sup>b</sup>	ND	0.56 <sup>a</sup>	0.59a	0.97 <sup>ab</sup>
Water		ND	66.60 <sup>b</sup>	8.21 <sup>a</sup>	7.47 <sup>a</sup>	ND	0.38 <sup>b</sup>	0.39a	1.60 <sup>a</sup>
1μM Mo		ND	73.04 <sup>a</sup>	8.29 <sup>a</sup>	7.05 <sup>a</sup>	ND	ND	0.21a	0.10 <sup>c</sup>
10μM Mo		ND	71.16 <sup>a</sup>	7.85 <sup>a</sup>	8.33 <sup>a</sup>	ND	ND	0.44a	0.62 <sup>b</sup>
Air	HWT	ND	75.19 <sup>a</sup>	12.31 <sup>a</sup>	1.39 <sup>c</sup>	ND	0.30 <sup>ab</sup>	0.06b	0.80 <sup>a</sup>
Water	30s	ND	64.92 <sup>b</sup>	10.30 <sup>a</sup>	7.52 <sup>a</sup>	ND	0.42 <sup>a</sup>	0.41a	0.84 <sup>a</sup>
1μM Mo		ND	64.05 <sup>b</sup>	9.47 <sup>a</sup>	9.02 <sup>a</sup>	ND	ND	0.42a	0.84 <sup>a</sup>
10μM Mo		ND	66.25 <sup>b</sup>	9.50 <sup>a</sup>	9.32 <sup>a</sup>	ND	0.25 <sup>b</sup>	0.67a	0.54 <sup>a</sup>
Air	HWT	ND	65.71 <sup>a</sup>	12.28 <sup>a</sup>	11.13 <sup>a</sup>	ND	0.21 <sup>a</sup>	0.11b	1.24 <sup>a</sup>
Water	120s	ND	63.46 <sup>ab</sup>	10.29 <sup>a</sup>	9.16 <sup>b</sup> <sup>c</sup>	ND	0.24 <sup>a</sup>	0.68a	1.14 <sup>a</sup>
1μM Mo		ND	59.75 <sup>b</sup>	12.44 <sup>a</sup>	9.90 <sup>b</sup>	ND	ND	0.87a	0.62 <sup>b</sup>
10μM Mo		ND	64.00 <sup>a</sup>	10.16 <sup>a</sup>	7.39 <sup>c</sup>	ND	0.15 <sup>a</sup>	0.70a	0.99 <sup>ab</sup>
Air	CR-HWT	ND	66.74 <sup>a</sup>	9.02 <sup>b</sup>	4.29 <sup>b</sup>	ND	0.16 <sup>a</sup>	0.38b	0.67 <sup>a</sup>
Water	30s	ND	66.10 <sup>a</sup>	8.52 <sup>cb</sup>	7.84 <sup>a</sup>	ND	0.21 <sup>a</sup>	0.62ba	0.84 <sup>a</sup>
1μM Mo		ND	61.70 <sup>b</sup>	12.42 <sup>a</sup>	6.92 <sup>a</sup>	ND	ND	0.80a	0.74 <sup>a</sup>
10μM Mo		ND	61.62 <sup>b</sup>	12.18 <sup>ab</sup>	7.61 <sup>a</sup>	ND	0.23a	0.63ba	0.57 <sup>a</sup>
Air	CR-HWT	ND	63.32 <sup>ab</sup>	8.74 <sup>a</sup>	6.71 <sup>a</sup>	ND	0.07 <sup>b</sup>	0.65a	1.85 <sup>a</sup>
Water	120s	ND	66.19 <sup>a</sup>	10.52 <sup>a</sup>	8.80 <sup>a</sup>	ND	ND	0.82a	0.72 <sup>ab</sup>
1μM Mo		ND	61.34 <sup>b</sup>	2.33 <sup>c</sup>	8.71 <sup>a</sup>	ND	ND	0.74a	0.74 <sup>ab</sup>
10μM Mo		ND	64.84 <sup>a</sup>	8.85 <sup>a</sup>	7.91 <sup>a</sup>	ND	0.25 <sup>a</sup>	0.70a	0.48 <sup>b</sup>
P value			<0.001	<0.001	<0.001		<0.001	0.017	<0.001
LSD <sub>(0.05)</sub> (pre- x main-)			4.42	3.3	1.8		0.12	0.30	0.47
CV%			4.0	21.7	14.8		42.7	36.9	29.9
s.e.d (pre- x main-)			2.2	1.7	0.89		0.06	0.15	0.23
n = 3									

CR: cold shock, HWT: hot water immersion, RT: room temperature, ND: not detected, RT: room temperature, ND: not detected, Neo: 9-cis-neoxanthin, Lut: lutein, Viol: 9-cis-violaxanthin, PF: phytofluene, Zea: zeaxanthin, β cit: β citraurin, β car: β carotene, P: phytoene.

For the purpose of statistical analysis 0.001, which was below the minimum peak area limit was used in the place of ND.

Values followed by the same letter within a block of a main-treatment are not statistically different

**Table 5.3.2.5.** Average L\*a\*b\* attributes of Citrus fruit 14 days after treatment

Pre-Treatments	Main-Treatments	'Navel'				'Valencia'			
		L*	a*	b*	C*	L*	a*	b*	C*
Initial <sup>x</sup>	Min.	58.98	-16.00	50.04	53	58.98	-16.00	50.04	53
	Max.	65.57	-10.00	55.57	57	65.57	-10.00	55.57	57
Air	RT	73.81 <sup>a</sup>	2.21	68.13 <sup>b</sup>	68.25 <sup>b</sup>	77.39	1.34	74.75 <sup>b</sup>	74.95 <sup>b</sup>
Water		72.78 <sup>b</sup>	1.98	67.29 <sup>c</sup>	67.38 <sup>b</sup>	77.31	0.31	73.99 <sup>bc</sup>	74.08 <sup>b</sup>
1µM Mo		78.97 <sup>ab</sup>	3.53	70.91 <sup>a</sup>	71.08 <sup>a</sup>	79.46	3.85	76.93 <sup>a</sup>	77.07 <sup>a</sup>
10 µM Mo		74.43 <sup>a</sup>	3.87	72.15 <sup>a</sup>	72.29 <sup>a</sup>	79.71	4.64	77.22 <sup>a</sup>	77.43 <sup>a</sup>
<i>Main treatment mean</i>		73.75	0.80	69.22	69.75	78.46	2.53	75.72	75.89
Air	CR	72.27 <sup>b</sup>	1.82	67.87 <sup>a</sup>	67.99 <sup>b</sup>	77.46	-1.45	72.27 <sup>b</sup>	72.39 <sup>b</sup>
Water		74.05 <sup>a</sup>	1.19	69.39 <sup>a</sup>	69.48 <sup>ab</sup>	77.32	0.27	73.94 <sup>b</sup>	74.02 <sup>b</sup>
1µM Mo		74.48 <sup>a</sup>	1.03	70.62 <sup>b</sup>	70.65 <sup>a</sup>	77.13	-1.23	73.01 <sup>b</sup>	73.08 <sup>b</sup>
10 µM Mo		74.89 <sup>a</sup>	3.34	72.58 <sup>b</sup>	72.69 <sup>a</sup>	78.93	7.04	77.13 <sup>a</sup>	77.52 <sup>a</sup>
<i>Main treatment mean</i>		73.92	0.94	70.11	70.20	77.71	1.15	74.09	74.25
Air	HWT	74.66 <sup>a</sup>	1.43	71.13 <sup>ab</sup>	71.19 <sup>ab</sup>	79.13	5.33	76.09 <sup>b</sup>	76.39 <sup>b</sup>
Water	30s	74.35 <sup>a</sup>	1.32	69.65 <sup>b</sup>	69.69 <sup>b</sup>	79.59	3.83	76.91 <sup>ab</sup>	77.06 <sup>ab</sup>
1µM Mo		74.88 <sup>a</sup>	4.60	72.31 <sup>a</sup>	72.53 <sup>a</sup>	79.81	4.87	76.94 <sup>ab</sup>	77.15 <sup>ab</sup>
10 µM Mo		75.50 <sup>a</sup>	4.65	72.70 <sup>a</sup>	72.91 <sup>a</sup>	79.46	6.61	77.88 <sup>a</sup>	78.20 <sup>a</sup>
<i>Main treatment mean</i>		74.85	1.63	71.45	72.65	79.50	5.16	76.96	77.20
Air	HWT	77.66 <sup>a</sup>	4.62	73.22 <sup>a</sup>	73.39 <sup>a</sup>	79.26	6.18	75.31 <sup>b</sup>	75.66 <sup>b</sup>
Water	120s	76.88 <sup>a</sup>	4.85	73.21 <sup>a</sup>	73.40 <sup>a</sup>	79.11	8.12	77.00 <sup>a</sup>	77.48 <sup>a</sup>
1µM Mo		75.03 <sup>b</sup>	7.14	71.84 <sup>b</sup>	72.24 <sup>a</sup>	79.17	7.19	77.12 <sup>a</sup>	77.48 <sup>a</sup>
10 µM Mo		74.97 <sup>b</sup>	6.44	72.53 <sup>ab</sup>	72.85 <sup>a</sup>	78.86	7.33	75.87 <sup>ab</sup>	76.26 <sup>ba</sup>
<i>Main treatment mean</i>		76.11	5.76	72.70	71.58	79.10	7.20	76.32	76.72
Air	CR-HWT	74.43 <sup>a</sup>	4.86	70.68 <sup>a</sup>	70.89 <sup>a</sup>	78.52	2.87	75.38 <sup>a</sup>	75.53 <sup>a</sup>
Water	30s	74.71 <sup>a</sup>	5.83	71.78 <sup>a</sup>	72.06 <sup>a</sup>	79.25	1.41	75.78 <sup>a</sup>	75.87 <sup>a</sup>
1µM Mo		75.08 <sup>a</sup>	6.85	72.45 <sup>a</sup>	72.79 <sup>a</sup>	78.73	4.75	76.55 <sup>a</sup>	76.80 <sup>a</sup>
10 µM Mo		75.18 <sup>a</sup>	5.63	71.80 <sup>a</sup>	71.97 <sup>a</sup>	78.80	4.65	75.52 <sup>a</sup>	75.71 <sup>a</sup>
<i>Main treatment mean</i>		74.85	5.79	71.76	71.92	78.83	3.41	75.81	75.98
Air	CR-HWT	76.88 <sup>a</sup>	4.51	72.68 <sup>a</sup>	72.86 <sup>a</sup>	80.25	6.67	77.61 <sup>a</sup>	77.95 <sup>a</sup>
Water	120s	75.77 <sup>ab</sup>	6.10	71.94 <sup>a</sup>	72.24 <sup>a</sup>	80.80	5.79	77.65 <sup>a</sup>	77.90 <sup>a</sup>
1µM Mo		74.62 <sup>b</sup>	7.24	72.21 <sup>a</sup>	72.64 <sup>a</sup>	79.70	6.71	77.13 <sup>a</sup>	77.48 <sup>a</sup>
10 µM Mo		75.77 <sup>ab</sup>	6.12	72.59 <sup>a</sup>	72.88 <sup>a</sup>	79.97	7.05	76.51 <sup>a</sup>	76.90 <sup>a</sup>
<i>Main treatment mean</i>		75.76	5.99	72.36	72.65	80.18	6.56	77.23	77.56
P value		0.001		0.001	0.001	<sup>y</sup> 0.001		0.001	0.001
LSD <sub>(0.05)</sub>									
Pre- x Main-		1.4		1.71	1.71			1.6	1.6
Main-		0.72		0.72	0.85	0.75			
CV%		2.2		2.7	2.7	2.1		2.4	2.4
s.e.d. (Pre- x Main-)		0.73		0.9	0.9	<sup>y</sup> 0.38		0.81	0.8

L\* represent lightness, a\* represent green-red colour axis, b\* represent blue-yellow axis, C\* represent chroma. CR: Cold room, HWT: hot water immersion, Water: water sterilized with Spore-Kill. NB: stock solution of molybdenum was diluted with sterilized water to the required volume and concentration. <sup>x</sup>Initial CIE L\*a\*b\* values at the beginning of postharvest treatments. <sup>y</sup>ANOVA information of the main-treatment effect. (n = 10).

Values followed by the same letter within a block of a main-treatment are not statistically different

### 5.3.3 FINAL REPORT: Response of Bahianinha Navel orange trees to the partial root zone drying and deficit irrigation strategies in northern Zimbabwe

October 2005-March 2008: S. Dzikiti<sup>1</sup>, E. Mashonjowa<sup>1</sup>, K. Steppe<sup>2</sup>, T. Mhizha<sup>1</sup>, B. Chipindu<sup>1</sup>, R Lemeur<sup>2</sup> and J.R. Milford<sup>1</sup> (<sup>1</sup>Agricultural Meteorology Group, Physics Department, University of Zimbabwe, <sup>2</sup>Laboratory of Plant Ecology, University of Ghent, Belgium)

#### Summary

In this study the response of Bahianinha Navel orange trees to the partial root zone drying (PRD) irrigation method was evaluated against the current grower's practice (the control), a well-watered treatment and the regulated deficit irrigation (RDI) treatments at Mazowe Citrus Estate over four growing seasons (2005 – 2008). The Navel orange trees were grafted on Troyer citrange rootstock and the trees were 4 years old when the trial began. Subjecting the orange trees to the PRD, RDI and the control treatments (i.e. single drip line treatments) caused the development of generally thin and relatively deep roots compared to the well-watered treatment. This root morphological change was an apparent adaptation by the trees to seek water from deeper soil layers to meet the transpirational demand. Despite the already low average stomatal apertures in Bahianinha Navel orange trees (due to stomatal cycling), further reductions in the stomatal conductance occurred under PRD while the leaf water potential was similar to that of the well-watered treatment. As a consequence, sap flow and hence transpiration rates were significantly lower under PRD irrigation than the control treatment even with similar irrigation levels. While the average yield per tree under PRD was consistently lower than in the well-watered treatment, the yield per tree was generally higher in PRD treatments than in the current grower's practice (the control) in most seasons. No significant changes in the internal quality of the fruit occurred due to the PRD and RDI treatments while fruit size was directly dependent on crop load.

#### Opsomming

In die studie is die response van Bahianinha Navel lemoenbome onder die gedeeltelike wortelsone uitdroging (PRD) besproeiingsmetode vergelyk met die respons van plaaslike produsente se besproeiingspraktyke (kontrole), 'n goed benatte behandeling en geregleerde stres besproeiing (RDI) by Mazowe Sitrus landgoed oor vier seisoene (2005-2008). Die navel lemoenbome was geënt op Troyer citrange onderstam en die bome was vier jaar oud toe die studie begin is. Die PRD, RDI en kontrole behandelings (enkel druplyn) het die vorming van dunner en dieper wortels veroorsaak in vergelyking met die goedbenatte behandeling. Die morfologiese verandering in die wortels was 'n duidelike aanpassing van die bome om water dieper in die grond te soek om tred te hou met die verliese as gevolg van transpirasie. Ten spyte van die lae aantal huidmondjies in Bahianinha navel lemoenbome het verlagings in die huidmondjie geleiding plaasgevind onder PRD terwyl die blaar waterpotensiaal dieselfde was as die goed benatte behandeling. Dus, was die sapvloeï en transpirasie tempo betekenisvol laer onder PRD as die kontrole selfs met dieselfde besproeiings vlakke. Die gemiddelde oeslading per boom onder PRD was konstant laer as die goed benatte behandeling en die oeslading per boom was oor die algemeen hoër onder PRD as in die kontrole in die meeste van die seisoene. Geen betekenisvolle veranderings in interne kwaliteit is waargeneem as gevolg van PRD en RDI behandelings nie, terwyl vrugsgrootte direk afhanklik van oeslading was.

#### Introduction

The availability of adequate water throughout the year is a major factor in citrus production. The increasing frequency and severity of droughts in recent years (Makarau and Jury, 1997), presumably due to the effects of global climate change, require the development of efficient irrigation strategies to obtain more fruit per drop of irrigation water. Recent studies on the water requirements of fruit trees have shown that imposing controlled levels of water stress at certain phenological stages causes a shift in the allocation of assimilates in favour of reproductive development rather than vegetative growth (Bacon, 2004). Thus applying carefully managed levels

of water stress potentially has beneficial effects such as improved yield quality and quantity using less water (Davies et al., 2001).

This study evaluated the possible application of irrigation strategies that use reduced irrigation levels in citrus production such as the partial rootzone drying (PRD) and the regulated deficit irrigation (RDI) methods. The partial root zone drying (PRD) irrigation strategy is based on the premise that subjecting part of the rootzone of the trees to water stress while the other part is well watered leads to reduced stomatal conductance while the plant water status is unaffected. In this way, the transpiration rates are lowered without necessarily subjecting the trees to water stress since water is readily available on the well-watered part of the rootzone (Dry *et al.*, 1996; Loveys *et al.*, 1998). Regular switching of the wet and dry sides of the rootzone e.g. at 10 – 21 day intervals depending on the crop is needed to maintain the sensitivity of the roots to soil drying. Partial stomatal closure under PRD is achieved via the so-called root-to-shoot chemical signaling mechanisms involving the generation of stress hormones e.g. the abscisic acid (ABA) or changes in the sap pH on the dry side of the rootzone which are then transported through the xylem vessels to the leaves. High levels of the ABA signal in the leaves cause a reduction in the stomatal aperture depending on the concentration (Davies et al., 2001). Given the fact that during stomatal closure transpiration is reduced much faster than CO<sub>2</sub> uptake, it is possible that reducing the size of the stomatal aperture also reduces transpirational losses while the yield can be maintained. This difference in the transpirational and CO<sub>2</sub> uptake response arises from the fact that the water vapour gradient between the sub-stomatal cavities and the leaf exterior is much steeper than the CO<sub>2</sub> gradient (Bacon, 2004). The regulated deficit irrigation strategy on the other hand operates in such a way that rather than replacing all the water lost by evapotranspiration during irrigation, only a fraction of the water requirement is applied so that partial water stress is maintained in the root zone (Chalmers et al., 1981) with the potential benefits already mentioned above.

While progress has been made on the practical utilization of the PRD strategy, e.g. in the viticulture industry (Dry *et al.*, 1996), recent studies show that this technique cannot be applied to all crops. For example, experiments on bell pepper which has a good hydraulic connection between the fruit and the vegetative part show that the PRD strategy cannot be used on this crop as it leads to severe yield losses and fruit size reduction. In the case of the Bahianinha navel orange trees, Dzikiti et al (2006) and Steppe et al (2006) showed that the average stomatal apertures of these trees are already low under optimal environmental conditions due to the occurrence of the cyclic opening and closure of the stomata round the day. Thus, the objective of this study was to establish whether or not the PRD strategy can be beneficial to the Navel orange trees given their active regulation of the stomatal aperture. This was done by comparing, among other things, the water use efficiency (defined in this study as the fruit yield per unit mass of water transpired) of the PRD treatment with that of other irrigation regimes e.g. the current grower's practice, RDI and well-watered treatments in a typical semi-arid environment.

## Materials and methods

### Experimental site and irrigation treatments

Trials were conducted in a drip irrigated 2 ha orchard with four-year-old Bahianinha Navel orange trees [*Citrus sinensis* L. (Osbeck)] budded on Troyer citrange rootstock [*Citrus sinensis* x *Poncirus trifoliata* L. Raf] at Mazowe Citrus Estate, Zimbabwe (17°27' S, 30°59' E, 1189 m above sea level). The growing medium, in excess of 1.0 m depth, were the dark-red clayey loam soils belonging to the Banket 5E.2 series (local classification) with a high clay percentage (Hussein, 1982). Three irrigation regimes were set up in September 2004 namely, the control (current grower's practice), partial rootzone drying applying the same irrigation levels as the control (PRD100) and the well-watered treatment applying 200% of the control. Two more treatments namely, the regulated deficit at 50% of the control (RDI50) and the partial rootzone drying at 50% of the control (PRD50) were set up in August 2005. Each treatment comprised 10 single tree replicates of similar physical size excluding trees on the edges of the rows and the trees were planted on ridges approximately 15 cm high. The control treatment used a single drip line next to the tree rows with emitters delivering 2.3 litres of water per hour while the PRD treatments had two drip lines each placed at 1.1 m either side of the tree row. In the PRD treatments, irrigation was done using one line at a time while the other line was blocked. Irrigation was then switched between the two lines every ten days. A similar set up of the drip lines as in the PRD treatments was used in the well-watered treatment only that irrigation was with both lines all the time. In the RDI50 treatment, a single drip line as in the control treatment was used but with half the drippers closed.

### Environmental data collection

The orchard microclimate was monitored using an automatic weather station located to the southwest of the orchard approximately 70 m from the edge, installed in October 2004. A CM11 pyranometer (Kipp and Zonen, The Delft, Netherlands) installed horizontally, measured the solar radiation incident on the crown of the trees, while wind speed was monitored using an AL100 cup anemometer (Vector Instruments, Rhyl, UK) at 2 m height. Air temperature and relative humidity were monitored using an HMP35AC probe (Campbell Scientific Ltd, Shepshed, UK) inserted in a 12-plate Gill radiation shield (Vaisala Ltd, Finland) at ~1.5 m above the ground. Rainfall was monitored using a tipping bucket rain gauge (Delta – T Devices, Cambridge, UK) raised to approximately 20 cm above the ground. Signals from all the sensors were recorded automatically at 5 s intervals and 5 min averages (and totals for rainfall) were stored on a datalogger (CR23X, Campbell Scientific Ltd, Shepshed, UK).

In the control treatment, soil water content was monitored in the rootzone (~ 25 cm) and beyond (~ 70 cm) using theta probes (ML2x Delta – T Devices, Cambridge, UK) located next to drip emitters. An additional two theta probes measured soil water content in the rootzone of the PRD100 treatment on either side of the tree row. All sensors were connected to the second datalogger (CR23X, Campbell Scientific Ltd, Shepshed, UK) situated in the orchard with signals averaged every 15 min. Measurement of soil water content in the other treatments was done using the gravimetric method at selected intervals due to equipment limitations.

### Physiological measurements

A model tree was selected in November 2005 in each of three treatments, namely the control, PRD100 and the well-watered treatments and instrumented with one SGB19 heat balance sap flow gauge (Dynamax, Inc, Houston, USA) on an exposed branch. More trees could not be sampled because of equipment and logistical constraints. Installation of the gauges was done according to the manufacturers' recommendations and spurious signals due to thermal effects were eliminated by wrapping additional aluminium foil around the gauges and parts of the branches. The sap flow gauges were connected to the third datalogger and measured sap flow every 5 min for several days before being removed just before the onset of the rains.

To establish the treatment effects on the transpiration rates, the sap flow measurements were normalized with the transpiring leaf area of each branch ( $F_{LA}$ , in  $m^3 m^{-2}$  leaf area  $time^{-1}$ ). The branch leaf area was obtained by stripping all the leaves from each branch and a leaf area meter (Delta –T Devices, Cambridge, UK) connected to a PC was used for quantifying the leaf area. Scaling up  $F_{LA}$  in treatment 'i' to transpiration by the whole orchard ( $E_{FL}$ , in  $m^3 m^{-2}$  soil  $time^{-1}$  or  $10^3$  mm  $time^{-1}$ ) when subjected to that treatment, the leaf area index (LAI) weighted by the proportion of the trees in age category 'j' was used such that:

$$E_{FL} = \sum_{i,j} F_{LA} (i, j) LAI (j)$$

The leaf area index was measured on trees of different canopy dimensions using a Sunscan Ceptometer (Delta-T Devices, Cambridge, UK). Scaling up the whole orchard transpiration rate from one day to the whole season was done by correlating the scaled up transpiration rates for days with uninterrupted data with the product of the key driving climatic variables for transpiration namely the solar radiation and the vapour pressure deficit of the air.

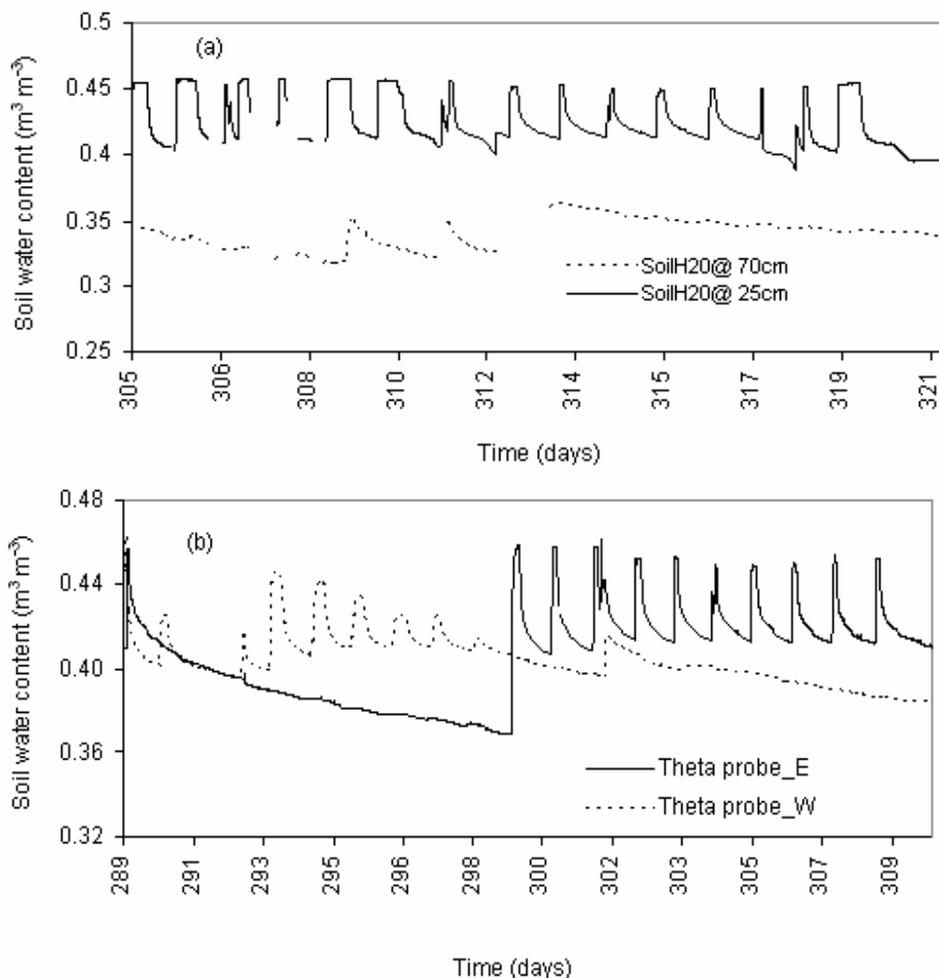
Several campaigns were conducted between 2005 and 2008 to compare the stomatal conductance and leaf water potential between the different irrigation treatments. Stomatal conductance was measured on three healthy and fully expanded leaves in three trees per treatment using the diffusion porometer (Delta-T Devices, Cambridge, UK, model AP4) while the leaf water potential was measured using a thermocouple psychrometer (Wescor Inc, Logan, UT, USA) with equilibration intervals of at least 40 min for each sample. Because of the limited number of the C-52 sample chambers (only 4), water potential measurements were not replicated in all the campaigns.

Average yield at Mazowe was determined from three trees of similar size per treatment and 10 fruit of similar size were selected for internal quality assessment by experts in the fruit processing factory at Mazowe Citrus Estate.

## Results and discussion

### Soil water regimes and root development

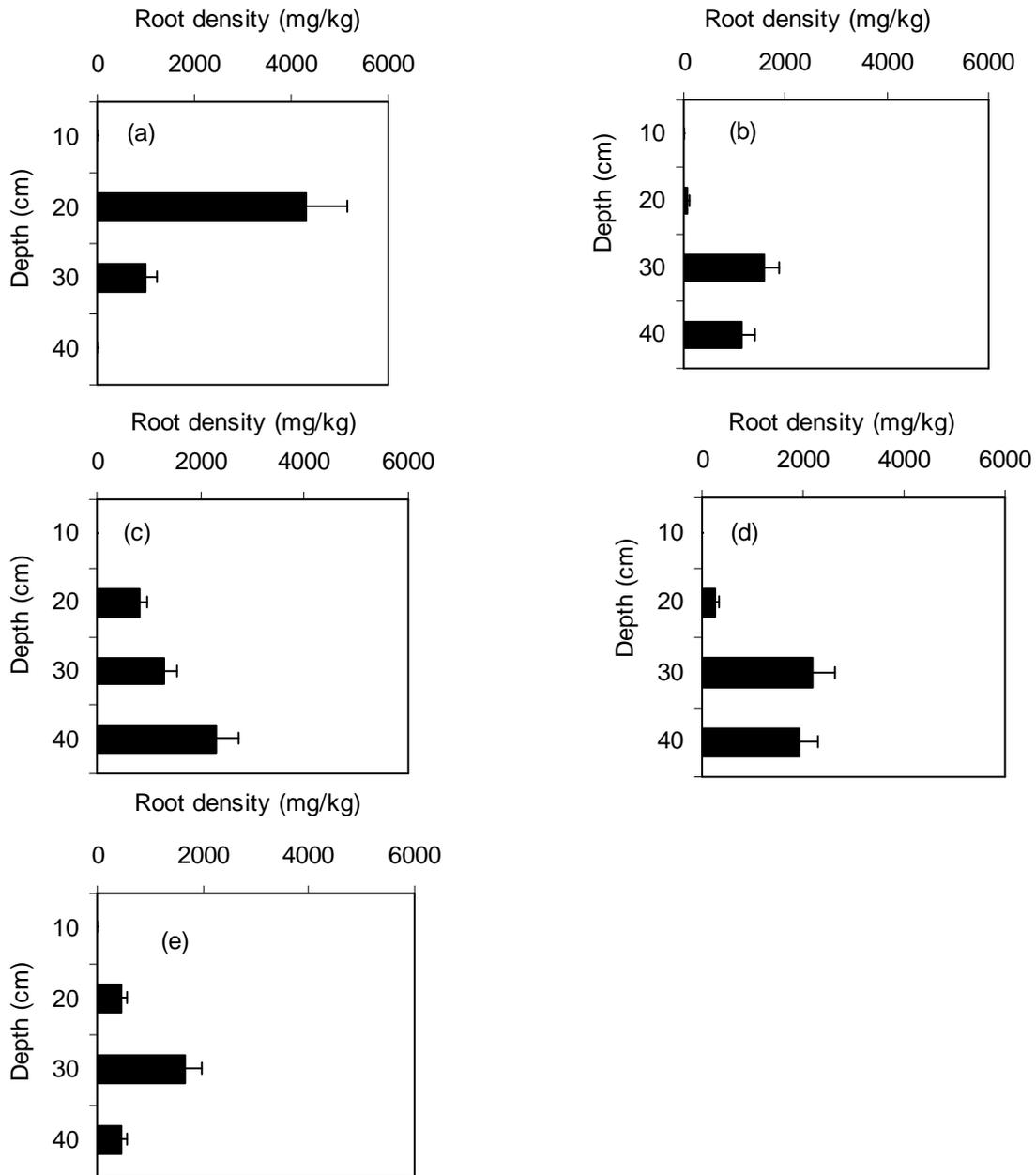
Fig. 5.3.3.1 shows the typical evolution of the soil water content measured in the control (a) and the PRD100 treatment (b), respectively. Given the daily irrigation schedule implemented at Mazowe, soil water content in the rootzone of the control treatment stayed near field capacity ( $\sim 44\%$  v/v) with deep percolation recorded by the probe at 70 cm depth occurring due to rainfall or extended irrigation events. Typical ten day wetting and drying cycles in the PRD treatments are clearly evident in Fig. 5.3.3.1 (b) with the soil water content on the dry side approaching the permanent wilting point of the soils ( $\sim 28\%$  v/v). This illustrated that under PRD each individual tree had two distinct soil water regimes in its root zone.



**Figure 5.3.3.1.** (a) Soil water content in the rootzone of the control treatment (current grower's practice). The continuous line depicts the soil water content in the root zone ( $\sim 25$  cm depth) while the dotted line is the water content beyond the rootzone ( $\sim 70$  cm depth). (b) The soil water regimes in the rootzone of the PRD treatments being measured by two theta probes, one to the east of the tree row (Theta probe\_E) and the other to the west (Theta probe\_W).

As expected, the development of the active feeder roots responsible for water uptake ( $< 4$  mm diameter, according to Barry et al., 2004) was strongly dependent on the soil water regimes. In all treatments, no roots were found in the range 0 – 10 cm (Fig. 5.3.3.2). In the well-watered treatment (Fig. 5.3.3.2a), most roots occurred in the 15 – 25 cm depth range while most roots occurred between 30 and 40 cm in all other treatments (Fig. 5.3.3.2 b – e). Average diameter of the roots tended to be thicker (data not shown) for trees in the well-watered treatment while thinner roots occurred in the other treatments which was an apparent adaptation to the

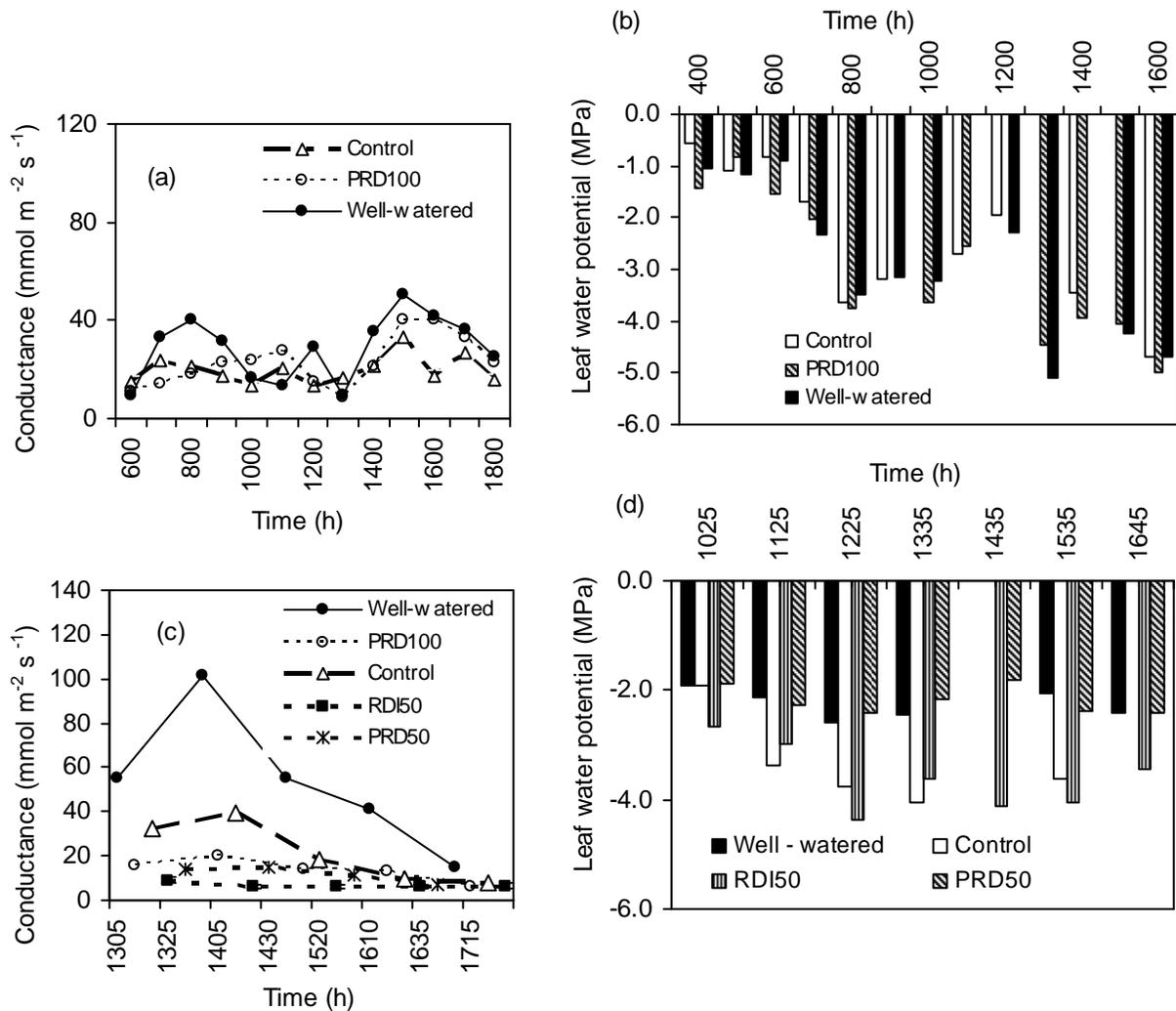
limited water availability in these treatments. Thinner roots have a larger surface area for the absorption of water and nutrients compared to thicker ones (Bacon, 2004).



**Figure 5.3.3.2.** Root density distribution (mg of roots per kg of soil) in the well watered treatment (a), PRD100 treatment (b), the control treatment (c), the RDI50 treatment (d) and the PRD50 treatment (e). Each bar is an average of two readings per depth from two pits per treatment. Error margins of 20% were included for the root density at each depth.

Effect of irrigation regimes on tree water status and sap flow

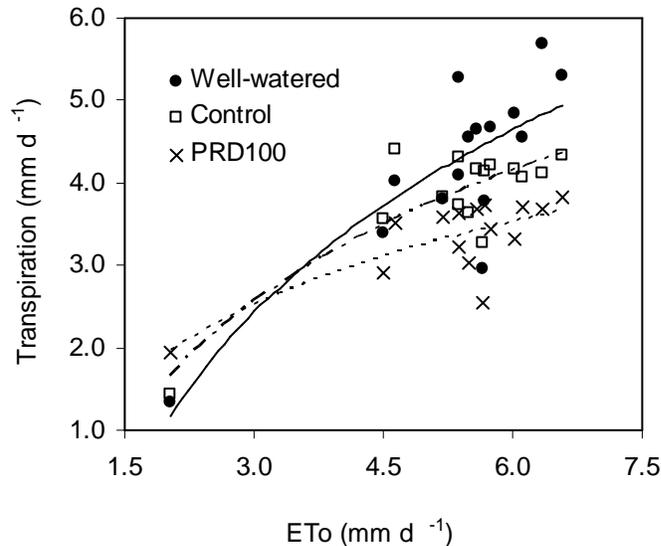
To ascertain whether PRD effects indeed occurred in the Navel orange trees, various campaigns were conducted to measure simultaneously the stomatal conductance and the leaf water potential of the trees in the period 2005 to 2008 and typical results are shown in Fig. 5.3.3.3. The reference dataset for these two variables was collected during the rainy season on 5 February 2005 when irrigation had stopped. No significant differences were found in both the stomatal conductance (Fig. 5.3.3.3a) and the leaf water potential (Fig. 5.3.3.3 b).



**Figure 5.3.3.3.** (a) Hourly measurements of the stomatal conductance in three treatments namely the control, PRD100 and the well-watered treatment during a dry spell in the rainy season on 5 February 2005; (b) Leaf water potential in three treatments on 5 February 2005. Differences in the stomatal conductance between treatments were not significant and no PRD effects were detected. (c) Stomatal conductance in five treatments namely the control, PRD100, PRD50, RDI50 and the well-watered treatment on 14 May 2006; (d) Leaf water potential of four treatments namely the control, PRD50, RDI50 and the well watered on 14 May 2006. PRD effects, namely, a reduction in stomatal conductance while the leaf water potential was maintained is clearly observed.

The very negative water potentials up to  $-5$  MPa indicated stress conditions since there was an extended dry spell during this period and irrigation had still not resumed because of logistical problems at the estate. Fig. 5.3.3.3c shows the course of the stomatal conductance when the different treatments were imposed. It is apparent that trees under the well-watered treatment had higher stomatal conductances reaching a peak of approximately  $100 \text{ mmol m}^{-2} \text{ s}^{-1}$  and less negative leaf water potentials (Fig. 5.3.3.3d) than all the other treatments. While the stomatal conductance of e.g. the PRD50 treatment was much lower than that of the well-watered, the leaf water potential under the PRD50 treatment was not significantly different from that of the well-watered treatment (Fig. 5.3.3.3d) indicating typical PRD effects. This trend was confirmed by other subsequent campaigns (data not shown).

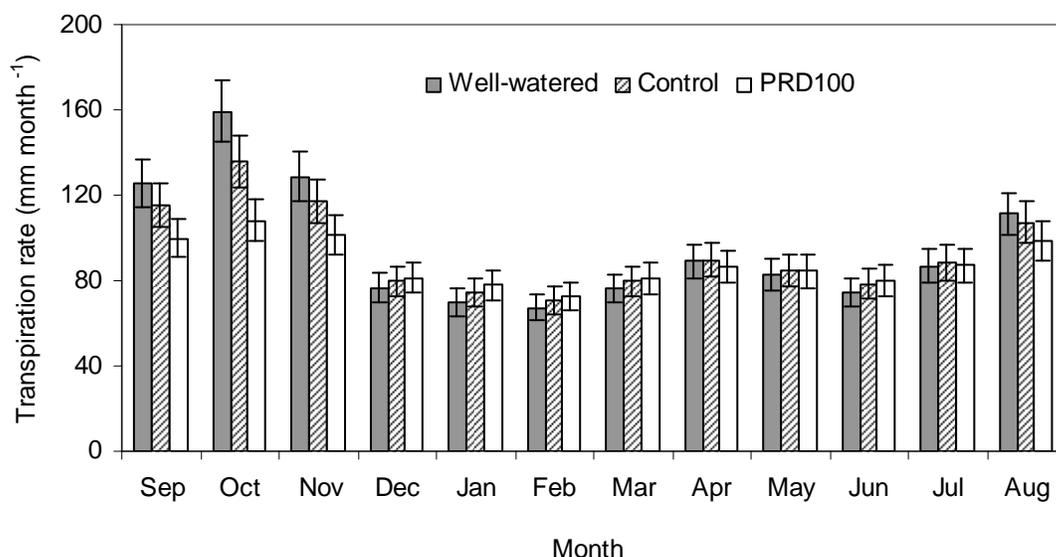
The fact that under PRD, transpiration rates were reduced was further confirmed by the independent results of the sap flow measurements (Fig. 5.3.3.4). In many studies the potential evapotranspiration (ET<sub>o</sub>) is considered as a measure of the atmospheric evaporative demand (Rana et al., 2005). The response of the Navel orange trees under different irrigation regimes to the same atmospheric evaporative demand shows that transpiration rates were suppressed by greater margins at higher ET<sub>o</sub> values illustrating that the PRD effects were indeed sustained over long periods and tended to be more pronounced at higher values of ET<sub>o</sub>.



**Figure 5.3.3.4.** Effect of the atmospheric evaporative demand represented by the daily total potential evapotranspiration (ET<sub>o</sub>) on the transpiration rates (daily total sap flow scaled from single tree to orchard level) under three different irrigation treatments.

#### Seasonal dynamics of water use, yield and water use efficiency

Of practical importance in citrus production is the long term (e.g. monthly, seasonal or annual) response of the trees to the different irrigation regimes in terms of overall water use and the yield response. Since sap flow could not be measured over long periods due to persistent power cuts at the trial site and also because of the physical damage to the trees caused by these gauges, empirical relationships were developed between the sap flow rates presented in Fig. 5.3.3.4 and the key climatic driving variables for transpiration namely, the solar radiation and the VPD of the air for three treatments on which sap flow was measured. Temporal up scaling of the transpiration data from a few days to seasonal and annual scales was done on the basis of the climatic data only assuming that the functional form of these empirical relationships remained the same throughout the year. Fig. 5.3.3.5 shows the monthly transpirational losses from the well-watered, control and the PRD100 treatments from September 2005 to August 2006 based on these empirical relationships. It is evident the PRD treatment gave lower monthly total transpirational losses only during the warm and dry months and it is not clear whether this is a direct result of the up-scaling assumptions.



**Figure 5.3.3.5.** Monthly total transpiration dynamics for the well-watered treatment (grey columns), the control treatment (checked columns) and the partial rootzone drying (PRD) treatment (open columns) during the 2005 – 2006 growing season.

Table 5.3.3.1 shows the effects of the different irrigation regimes on fruit size, mean fruit number per tree and the average yield of each tree over at least three growing seasons. No significant differences in fruit size were obtained due to the different treatments and rather fruit size tended to be affected more by crop load. When crop load was high, fruit size tended to be smaller and vice versa. It should be remarked that during the 2007 – 2008 season, fruit size at Mazowe was uncharacteristically small due to operational problems e.g. persistent power cuts and equipment failure e.g. frequent pump break downs. Average yield per tree was generally higher in the well-watered treatment in most seasons except 2007 – 2008 compared to the other treatments which used single drip lines. However, the yield under the PRD100 and indeed the PRD50 was also generally higher than that of the current grower’s practice (the control). More interestingly, treatments receiving 50% less water than the control (i.e. the PRD50 and the RDI50) gave consistently higher yields than the current grower’s practice over three seasons suggesting that it is possible to reduce the irrigation levels by 50% and still achieve high yields.

As shown in Table 5.3.3.2, no significant differences in fruit internal quality occurred due to the different irrigation treatments and typical estimates of the water use efficiency when the orchard was irrigated by the different treatments in 2005 – 2006 season are shown in Table 5.3.3.3.

**Table 5.3.3.1.** Effects of the irrigation regimes on mean fruit mass, fruit number and average yield per tree for the period 2005 to 2008. The average number of fruit per tree was not determined at harvest in 2005.

Treatment	Fruit mass (g)				Fruit no.				Average yield (kg/tree)			
	05	06	07	08	05	06	07	08	05	06	07	08
Control	239.7	236.2	223.4	207.4	—	137	217	192	52.0	28.8	46.8	39.6
PRD100	272.2	256.0	241.4	205.5	—	183	242	187	41.9	34.2	48.1	38.5
Well-watered	271.8	226.2	212.0	220.4	—	243	279	226	47.4	52.0	55.1	43.6
RDI50	—	229.6	242.6	189.4	—	221	227	318	—	44.5	45.7	60.2
PRD50	—	264.0	251.2	202.8	—	209	214	270	—	42.3	49.2	54.8

**Table 5.3.3.2.** Effect of the irrigation regimes on internal fruit quality including the average juice yield per tree. Ten fruit of the same size obtained from the exposed parts of the south-western quadrant of the canopy were used for internal quality assessment.

Treatment	Juice (%)			Acid (%)			TSS (%)			TSS:TA Ratio			Juice yield (kg/tree)		
	05	06	07	05	06	07	05	06	07	05	06	07	05	06	07
Control	49 <sup>a</sup>	47 <sup>a</sup>	46 <sup>a</sup>	0.78	0.73	0.76	9.7 <sup>a</sup>	9.0 <sup>a</sup>	9.8 <sup>a</sup>	12.4 <sup>a</sup>	12.3 <sup>a</sup>	12.9 <sup>a</sup>	25.5	13.5	21.5
PRD100	49 <sup>a</sup>	45 <sup>a</sup>	46 <sup>a</sup>	0.84	0.75	0.81	9.6 <sup>a</sup>	9.0 <sup>a</sup>	9.6 <sup>a</sup>	11.4 <sup>a</sup>	12.0 <sup>a</sup>	11.9 <sup>a</sup>	20.7	15.4	22.1
Well-watered	48 <sup>a</sup>	51 <sup>a</sup>	45 <sup>a</sup>	0.81	0.71	0.79	9.2 <sup>a</sup>	9.2 <sup>a</sup>	10.3	11.4 <sup>a</sup>	13.0 <sup>a</sup>	13.1 <sup>a</sup>	22.6	26.5	24.8
RDI50	–	48 <sup>a</sup>	51 <sup>a</sup>	–	0.78	0.64	–	9.2 <sup>a</sup>	9.2 <sup>a</sup>	–	11.8 <sup>a</sup>	14.8 <sup>a</sup>	–	21.4	23.3
PRD50	–	44 <sup>a</sup>	47 <sup>a</sup>	–	0.77	0.71	–	9.2 <sup>a</sup>	9.0 <sup>a</sup>	–	11.9 <sup>a</sup>	12.7 <sup>a</sup>	–	19.5	23.1

Values within each year followed by different letters are significantly different from the control at ( $P \leq 0.05$ )

**Table 5.3.3.3.** Estimates of the water use efficiency obtained from the scaled up branch sap flow data in three irrigation treatments during the 2005/06 growing season (September 2005 to May 2006). Total yield in each treatment was calculated by assuming that all the trees (1191) in the 2 ha orchard had the same yield while the total transpiration is also calculated for the 2 ha.

### Conclusions and Recommendations

Treatment	Total seasonal transpiration (m <sup>3</sup> )	Average yield (kg/tree)	Total yield of orchard (kg)	Water use efficiency (g L <sup>-1</sup> )
Well-Watered	21140	52.0	61932.0	2.93
PRD	17160	34.2	40732.2	2.37
Control	19640	28.8	34300.8	1.75

Treatments irrigated by the PRD and RDI strategies did not seem to reduce citrus yield at Mazowe Citrus Estate compared to the current grower's practice although even higher yields can be obtained by adopting the well-watered treatment (i.e. irrigation with double lines). This experiment also demonstrated that it is possible to maintain the same yield levels using up to 50% less water than the current irrigation levels. While calculations of the water use efficiency above based on transpirational losses suggest that the water use efficiency is higher under the well-watered treatment, in practice more water will have been used by irrigating with the double lines. Consequently, water use efficiency calculated on the basis of the water applied is much lower for the well-watered treatment than the figures presented in Table 5.3.3.3. Based on this trial, the partial rootzone drying and regulated deficit irrigation treatments appear to have a potential to improve the water use efficiency in the irrigation of Navel orange trees and probably other cultivars as well.

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#### 5.3.4 PROGRESS REPORT: Application of 2,4-D to reduce the size of the navel end opening of navel oranges

Experiment 935 (October 2006-March 2008): Stephan Verreyne (CRI at SU), Giverson Mupambi (SU)

##### Summary

Work in Chile on Lane Late Navels suggests that 2,4-D sprayed during full bloom reduces the size of the navel end. In Chile 5 to 20 ppm was evaluated with 20 ppm giving the best results. The objective of this study was to determine the efficacy of 2,4-D in reducing navel size under South African conditions as well as to determine any detrimental effects. A smaller navel end would not only reduce the percentage of culled fruit due to large navels, but would possibly lead to reduced insect infestation, such as mealybug, mites and false codling moth. Robyn, Palmer and Lane Late navel trees were treated with 2,4-D (ester form) at 25 ppm at 100% petal drop in 2006. At harvest approximately 20 kg per tree replicate was used to measure fruit diameter and the size of the navel end. A subsample was used to measure external and internal fruit quality. In all the cultivars, 2,4-D at 25 ppm increased the percentage closed navels and decreased average navel end size but had no effect on fruit shape, peel thickness or internal fruit quality. However, it caused slightly coarser rinds, slightly greener fruit and greener navel ends. No granulation or thicker pedicels were observed. Negative treatment effects include curled and distorted spring flush leaves. 2,4-D shows lots of potential as a treatment to reduce navel end size of navel oranges, but the negative effects should be minimized. A second season of data needs to be collected (on vs. off year) in different areas on different cultivars before a commercial recommendation can be made.

##### Opsomming

Werk in Chile op Lane Late Navel lemoene wys dat 2,4-D toegedien tydens volblom die navelend grootte verklein. In Chile is 5 tot 20 dpm toegedien en 20dpm het die beste resultate getoon. Die doel van die studie was om die effektiwiteit van 2,4-D om die navelend grootte te verklein, te bepaal onder Suid-Afrikaanse toestande asook om enige nadelige effekte vas te stel. 'n Kleiner navelend grootte sal nie net die % uitgooivrugte verlaag nie, maar ook moontlik lei tot minder inseksskade soos bv. witluis, knopmyt en valskodlingmot. Robyn, Palmer en Lane Late navelbome is behandel met 2,4-D (ester vorm) teen 25 dpm by 100% blomblaarval in 2006. By oestyd is ongeveer 20 kg per boom replikaat gebruik om vrugdeursnit en die grootte van die navelend te meet. 'n Submonster is gebruik vir eksterne en interne kwaliteit bepalings. By al die kultivars het 2,4-D teen 25 dpm die persentasie toe navelente verhoog en die gemiddelde navelend grootte verklein maar het geen effek gehad op vrugvorm, skildikte of interne kwaliteit nie, maar het bietjie growwer vrugte, bietjie groener vrugte en groener navel ente tot gevolg gehad. Geen granulasie of dikker vrugstele is waargeneem nie. Negatiewe effekte sluit in gekrulde lente "flush" blare. 2,4-D toon baie potensiaal as 'n behandeling om navelend grootte van navel lemoene te verklein, maar die negatiewe effekte moet verminder

word. 'n Tweede seisoen van data moet versamel word (aan- vs. af-jaar) in verskillende areas op verskillende kultivars voor 'n kommersiele aanbeveling gemaak kan word.

## Introduction

Work in Chile on Lane Late Navels suggests that 2,4-D sprayed during full bloom reduces the size of the navel end. In Chile 5 to 20 ppm was evaluated on Lane Late with 20 ppm giving the best results. 20 ppm resulted in 49% closed navels compared to 3% in the control and a navel size of 0.48 cm compared to 1,2 cm in the control. 20 ppm had the greatest percentage of closed navels and the smallest navel diameter. 2,4-D had no effect on the number of fruit per tree and yield. In the following season, 15 ppm on Lane Late resulted in smaller navels (0.25 vs. 0.76 cm), and a greater percentage closed navel (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 27 mm) and 3,5,6-TPA (15 ppm at 26 or 27 mm), only 2,4-D increased the percentage fruit with closed navels, decreased the percentage fruit with split navels and reduced navel size, with no differences in yield, fruit size, fruit shape compared to the control, with the other auxins giving no significant results. 2,4-D decreased juice % (38.2 vs. 41.3) and titratable acidity (TA), had no effect on total soluble solids (TSS), and increased the TSS:TA ratio. All auxins increased peel roughness, but had no effect on external colour, fruit shape or rind thickness (Saavedra, 2006).

In California, 2,4-D was sprayed at 18 ppm at 5 to 6 weeks after full bloom and it seemed to reduce navel end size. 2,4-D resulted in 20% fruit  $\geq 4$  rating compared to the control with 31% fruit  $\geq 4$  (1=no visible navel and 6=large navel end) (E. Rabe, personal communication). Fruit were not sent through the packinghouse or juiced due to the freeze in January 2007.

No work on the effect of 2,4-D on navel end size is published, but a lot of work has been done on the effect of 2,4-D on fruit size improvement or late hang of fruit. 2,4-D has a slight thinning effect that normally results in no effect on yield due to a reduced number of larger fruit. 2,4-D at 20 ppm applied at full bloom, 2WAFB and 6 WAFB on Esbal mandarins reduced fruit number, with no effect on yield due to a lower number of larger fruit (Duarte et al., 1996). Only 2,4-D applied at 2WAFB reduced juice percentage (2.1%) with none of the treatments having an effect on TSS, acidity, TSS:TA ratio or colour. A similar response has been reported for Nova mandarin (Guardiola, 1996). Best results were obtained for Valencia and Shamouti when 2,4-D (20 ppm) was applied 6-8 weeks after full bloom (Erner et al., 1993). 2,4-D applied at full bloom on Washington Navels resulted in decreased juice percentage, a decrease or no effect on TSS, increase or no effect on TA, an increased percentage of green fruit, an increase in fruit-stem diameter in relation to fruit diameter, with a greater effect at higher concentrations (8 to 72 ppm) (Stewart et al., 1951). At very high concentrations (25 to 225 ppm), 2,4-D on Navels resulted in cylindrical fruit with thick and pebbled rinds, curling of leaves, protruding navels, develop seedlike structures, reduced juice percentage and TA and has no effect on TSS (Stewart and Klotz, 1947). A delay in colour development, thicker pedicels, a thinning effect or reduced fruit numbers, a reduction in splitting on Washington Navels was also reported. In grapefruit, high concentrations resulted in cylindrical fruit, thick, coarse rinds and dry, hard juice vesicles (Stewart and Parker, 1954).

Previous studies using 2,4-D at 16 ppm for fruit size improvement on Valencia orange, reported no effect on yield, juice %, TSS, TA, TSS:TA ratio or granulation, with an increase in fruit size and a decrease in fruit number (Erickson and Richards, 1955). No effect on internal fruit quality was also reported on Clementine (24 ppm). Stewart et al. (1952) observed only small, inconsistent differences on Valencia orange in internal fruit quality at up to 48 ppm. Treated fruit had thicker fruit stems (pedicel) in proportion to fruit diameter and a thinning effect was observed.

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 size under South African conditions as well as to determine any detrimental effects on internal fruit quality, external fruit quality and vegetative growth. A smaller navel end would not only reduce the percentage culled fruit due to large navels, but would possibly lead to reduced insect infestation, such as by mealybug, mites and false codling moth.

## Materials and methods

### Treatments and plant material

Rob Paterson of the farm Twaktuin in Clanwilliam sprayed Robyn, Palmer and Lane Late navel trees with 2,4-D (ester form) at 25 ppm at 100% petal drop in 2006. The trial was a semi-commercial trial with two rows sprayed and two rows left unsprayed for each cultivar.

### Measurements

At harvest of each cultivar the following measurements were done: Approximately 20 kg from the east side of 20 sprayed and 20 non-sprayed uniform trees with a good crop load were picked for analyses. Leaf samples (for mineral leaf analyses) were taken and pooled into 5 samples from sprayed and non-sprayed trees, respectively. Fruit diameter and the size of the navel were measured on each picked fruit. There was no obvious difference in fruit colour from the two treatments. Two subsamples of 12 fruit each from treated and non-treated trees were taken to the lab for the following measurements: One to measure external and internal fruit quality. External fruit quality was determined by measuring fruit diameter, fruit height, colour, colour at navel end, rind coarseness and peel thickness. Internal fruit quality was determined by measuring total soluble solids (TSS), titratable acidity (TA), juice percentage and visible symptoms of granulation were scored. A second sample was peeled for albedo and flavedo samples for mineral analyses.

## Results and discussion

Throughout the season the following observations were made for all the cultivars. Some of the styles were still attached to fruit at a late stage of fruit development. A lot of spring flush leaves. Leaf damage comprising of curled and distorted leaves occurred on the fruit-bearing flush (spring flush). The curled and distorted leaf flush on sprayed trees observed after treatment was not obviously visible at harvest time. Affected leaves probably dropped by this time or recovered from the damage. No thick stems or pedicels or any attached styles were observed in fruit from treated trees.

### Palmer Navels

Treated fruit were smaller than control fruit (Table 5.3.4.1). There were however, no differences in fruit weight and navel size of only open navels. 2,4-D increased the percentage of closed navels significantly by 30% and decreased average navel size of all fruit. In fruit used for quality determinations, 2,4-D had no effect on diameter, height, fruit shape (D/H), rind coarseness or peel thickness (Table 5.3.4.2). Although not significant, treated fruit had slightly coarser rinds. Treated fruit had greener navel ends as well as greener colour. The green navel ends observed in treated fruit may be eliminated by slight degreening. There were no significant differences in internal fruit quality, although the reduced TSS (0.3) and juice percentage (1.3%) in treated fruit may be of commercial importance (Table 5.3.4.3). Granulation was not visible in any of the fruit sampled. Regarding the relationship between fruit size and navel end size, only 10% of the variation in navel size is explained by the variation in fruit size (Fig. 5.3.4.1). Therefore fruit size does not influence navel end size. No large differences were observed in mineral nutrient concentrations between treated and non-treated fruit albedo, flavedo or leaves, except for lower Fe and Zn concentrations in the albedo and lower concentration of leave P of treated trees (Table 5.3.4.4). The packout was also increased with 10% compared to the control (data not shown).

### Robyn Navels

2,4-D had no effect on fruit diameter, fruit weight, navel end size and stem thickness behind the fruit (Table 5.3.4.5). 2,4-D increased the percentage of closed navels significantly by 24% and decreased average navel size of all fruit. In fruit used for quality determinations, 2,4-D had no effect on external fruit quality, therefore no effect on fruit diameter, height, fruit shape (D/H), colour, colour at the navel end, rind coarseness or peel thickness (Table 5.3.4.6). There were no significant differences in internal fruit quality (Table 5.3.4.7). Granulation was not visible in any of the fruit sampled.

### Lane Late Navels

2,4-D had no effect on fruit diameter, fruit weight and stem thickness behind the fruit (Table 5.3.4.8). 2,4-D increased the percentage of closed navels significantly by 39%, decreased average navel size of all fruit and had a negative effect on fruit colour. 2,4-D significantly increased the average navel end size of fruit with open navels. In fruit used for quality determinations, 2,4-D increased fruit diameter and fruit height and resulted in greener fruit, but had no effect on fruit shape (D/H), colour at the navel end, rind coarseness or peel thickness

(Table 5.3.4.9). There were no significant differences in TSS, TA or juice percentage, but 2,4-D significantly increased the TSS:TA ratio (Table 5.3.4.10). Granulation was not visible in any of the fruit sampled.

## Conclusion

In all the cultivars, 2,4-D at 25 ppm increased the percentage closed navels and decreased average navel end size but had no effect on fruit shape, peel thickness or internal fruit quality. However, it gave slightly coarser rinds, slightly greener fruit and greener navel ends. No granulation or thicker pedicels were observed. Negative treatment effects include curled and distorted spring flush leaves. 2,4-D shows lots of potential as a treatment to reduce navel end size of navel oranges, but the negative effects should be minimized. A second season of data needs to be collected (on vs. off year) in different areas on different cultivars before a commercial recommendation can be made.

## Future research

In the following season the optimal timing and concentration of application will be determined on different cultivars and in different areas. The negative effects on fruit quality will also be minimized. The effect of other synthetic auxins on navel end size reduction will also be determined and the effect on return bloom will also be determined.

## Acknowledgements

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## Technology transfer

Verreyne, J.S. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end of navel oranges-a preliminary study. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Grahamstown, 21-24 January 2008: 152.

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**Table 5.3.4.1.** Effect of 2,4-D on fruit size, the percentage of closed navels and navel end size of Palmer Navel

	Diameter	Weight	% closed navels	Navel size (open navels)	Navel size (all fruit)
	----mm----	----g----		----mm----	----mm----
Control	85.25±1.13 a	314±11.72	36.8±3.55 b	10.26±0.80	6.57±0.53 a
25 ppm 2,4-D	81.96±1.07 b	282±11.41	67.2±3.36 a	8.80±0.76	2.91±0.50 b
LSD	3.16	33.2	9.91	2.22	1.47
P-value	0.0418	0.0552	0.001	0.1915	0.001

**Table 5.3.4.2.** Effect of 2,4-D on external fruit quality of Palmer Navel

	Diameter	Fruit height	D/H	Colour <sup>z</sup>	Colour at navel end <sup>y</sup>	Rind coarseness <sup>x</sup>	Peel thickness
	----mm----	----mm----					----mm----
Control	82.35±0.99	82.10±1.16	1.005±0.01	1.3±0.08 b	1.6±0.09 b	1.5±0.46	6.01±0.32
25 ppm 2,4-D	80.35±0.94	79.81±1.10	1.012±0.01	1.8±0.08 a	1.9±0.09 a	2.6±0.43	5.84±0.31
LSD	2.77	3.25	0.02	0.23	0.26	1.28	0.90
P-value	0.1517	0.1630	0.3712	0.0001	0.0172	0.0839	0.7169

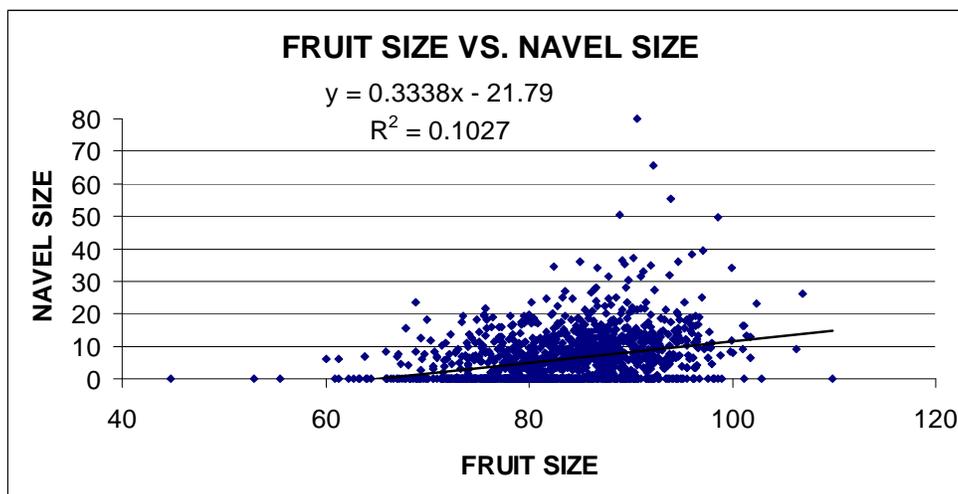
<sup>z</sup>1-8 on colour chart, 1-orange, 8-green

<sup>y</sup>1-4

<sup>x</sup>rind coarseness chart, 1-smooth, 8 coarse

**Table 5.3.4.3.** Effect of 2,4-D on internal fruit quality of Palmer Navel

	TSS	TA	TSS:TA ratio	Juice %
		----%----		
Control	10.7±0.13	1.19±0.02	8.8±0.25	43.1±0.66
25 ppm 2,4-D	10.4±0.12	1.14±0.02	9.0±0.24	41.8±0.63
LSD	0.35	0.07	0.70	1.86
P-value	0.0939	0.1459	0.6845	0.1625



**Figure 5.3.4.1.** Relationship between fruit size and navel end size for Palmer Navel.

**Table 5.3.4.4.** Albedo, flavedo and leaf mineral nutrients in 2,4-D treated and control Palmer Navel fruit

<b>Albedo</b>	<b>N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>Na</b>	<b>Mn</b>	<b>Fe</b>	<b>Cu</b>	<b>Zn</b>	<b>B</b>
	%					mg/kg					
Control	0.52	0.036	0.33	0.73	0.052	63.62	2.10	20.20 a	1.96	7.19 a	20.81
25 ppm 2,4-D	0.53	0.036	0.33	0.70	0.056	50.64	1.09	15.88 b	2.05	6.08 b	21.90
LSD	0.06	0.01	0.06	0.19	0.01	30.93	2.02	3.69	0.59	0.89	1.45
P-value	0.8233	1.000	1.000	0.7970	0.4860	0.3616	0.2828	0.0269	0.7467	0.0207	0.1215
STDERR	0.018	0.003	0.018	0.059	0.004	9.49	0.619	1.131	0.182	0.272	0.445
<b>Flavedo</b>	<b>N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>Na</b>	<b>Mn</b>	<b>Fe</b>	<b>Cu</b>	<b>Zn</b>	<b>B</b>
Control	0.96	0.082	1.22	1.18	0.14	74.54	12.41	54.54	4.23	8.33	23.75
25 ppm 2,4-D	0.94	0.080	1.28	1.12	0.14	92.27	9.38	47.59	3.66	7.20	23.71
LSD	0.10	0.01	0.22	0.39	0.03	36.49	3.75	13.07	1.17	3.41	2.22
P-value	0.5770	0.6938	0.5532	0.7136	0.8619	0.2952	0.0997	0.2550	0.2913	0.4652	0.9678
STDERR	0.032	0.003	0.066	0.119	0.008	11.19	1.150	4.007	0.359	1.046	0.679
<b>Leaves</b>	<b>N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>	<b>Na</b>	<b>Mn</b>	<b>Fe</b>	<b>Cu</b>	<b>Zn</b>	<b>B</b>
Control	2.12	0.12 a	1.12	4.65	0.28	1096	136.4	130.2	12.0	57.6	93.4
25 ppm 2,4-D	2.09	0.11 b	1.10	4.81	0.33	1061	123.6	124.2	12.0	55.8	93.2
LSD	0.10	0.006	0.10	0.53	0.07	179.67	16.2	15.47	9.37	7.07	6.48
P-value	0.5421	0.0400	0.7221	0.4874	0.1319	0.6634	0.1061	0.3971	1.0000	0.5733	0.9450
STDERR	0.031	0.002	0.031	0.161	0.021	55.09	4.971	4.742	2.872	2.168	1.988

**Table 5.3.4.5.** Effect of 2,4-D on fruit size, the percentage of closed navels and navel end size of Robyn Navel

	Diameter	Weight	% closed navels	Navel size (open navels)	Navel size (all fruit)	Stem thickness
	----mm----	----g----		----mm----	----mm----	--mm--
Control	79.73	257	45.3 b	10.67	4.25 a	4.05
25 ppm 2,4-D	81.15	270	69.0 a	7.39	2.35 b	4.83
STDerr	0.73	6.54	5.92	2.45	0.56	0.36
LSD	2.12	18.96	17.16	7.08	1.62	1.05
P-value	0.1815	0.1802	0.0086	0.3510	0.0229	0.1371

**Table 5.3.4.6.** Effect of 2,4-D on external fruit quality of Robyn Navel

	Diameter (D)	Fruit height (H)	D/H	Colour <sup>z</sup>	Colour at navel end <sup>y</sup>	Rind coarseness <sup>x</sup>	Peel thickness
	----mm----	----mm----					----mm----
Control	81.43	82.34	0.99	2.58	1.9	1.7	5.94
25 ppm 2,4-D	80.52	81.67	0.99	2.37	1.8	1.7	5.94
STDerr	1.01	1.32	0.01	0.14	0.09	0.06	0.11
LSD	2.93	3.81	0.017	0.41	0.27	0.18	0.33
P-value	0.5299	0.7180	0.6234	0.2996	0.3938	0.8249	0.9967

<sup>z</sup>1-8 on colour chart, 1-orange, 8-green

<sup>y</sup>1-4 1

<sup>x</sup>rind coarseness chart, 1-smooth, 8 coarse

**Table 5.3.4.7.** Effect of 2,4-D on internal fruit quality of Robyn Navel

	TSS	TA	TSS:TA ratio	Juice %
		----%----		
Control	9.83	0.90	10.89	46.99
25 ppm 2,4-D	9.83	0.96	10.40	47.06
STDerr	0.15	0.02	0.26	0.40
LSD	0.43	0.07	0.75	1.14
P-value	1.000	0.1292	0.1951	0.9059

**Table 5.3.4.8.** Effect of 2,4-D on fruit size, the percentage of closed navels and navel end size of Lane Late Navel

	Diameter	Weight	% closed navels	Navel size (open navels)	Navel size (all fruit)	Colour	Stem thickness
	----mm----	----g----		----mm----	----mm----		--mm--
Control	81.93	287.85	15.4 b	8.79 b	7.53 a	1.27 b	4.15
25 ppm 2,4-D	83.04	298.85	54.0 a	12.17 a	5.25 b	2.18 a	4.20
STDerr	0.44	4.74	3.43	0.88	0.58	0.13	0.05
LSD	1.27	13.72	2.05	2.55	1.68	0.36	0.14
P-value	0.0824	0.1118	0.0001	0.0112	0.0095	0.0001	0.4314

**Table 5.3.4.9.** Effect of 2,4-D on external fruit quality of Lane Late Navel

	Diameter (D)	Fruit height (H)	D/H	Colour <sup>z</sup>	Colour at navel end <sup>y</sup>	Rind coarseness <sup>x</sup>	Peel thickness
	----mm----	----mm----					----mm----
Control	81.92 b	85.25 b	0.97	1.56 b	0.55	1.33	5.76
25 ppm 2,4-D	84.15 a	87.64 a	0.97	1.93 a	0.85	1.27	6.03
STDerr	0.51	0.76	0.01	0.08	0.12	0.09	0.19
LSD	1.47	2.21	0.04	0.23	0.35	0.27	0.54
P-value	0.0042	0.0351	1.000	0.0029	0.0992	0.6851	0.3086

<sup>z</sup>1-8 on colour chart, 1-orange, 8-green

<sup>y</sup>1-4

<sup>x</sup>rind coarseness chart, 1-smooth, 8 coarse

**Table 5.3.4.10.** Effect of 2,4-D on internal fruit quality of Lane Late Navel

	TSS	TA	TSS:TA ratio	Juice %
		----%----		
Control	10.32	1.15	9.09 b	42.62
25 ppm 2,4-D	10.33	1.08	9.69 a	44.13
STDerr	0.09	0.03	0.19	0.63
LSD	0.26	0.08	0.54	1.83
P-value	0.9170	0.0630	0.0281	0.1012

**5.3.5 PROGRESS REPORT: Economic benefit of hand thinning of Nules Clementine**  
Experiment 865 (December 2006-March 2008): Stephan Verreyne (CRI at SU)

**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. Fruit thinning usually causes a certain reduction in total fruit yield, although the smaller yield may be of higher commercial value. The objective of this study was to determine if handthinning results in any economic benefit, even if it doesn't result in an increased fruit size. Nules Clementine trees on Troyer citrange rootstock in Porterville were used for the study. On 7 December 2006, 12 single trees in a complete randomized block design were left unthinned or all fruit <21 mm were handthinned from 12 trees, respectively. Hand thinning increased fruit growth and fruit size and caused no significant improvement in 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. Therefore, hand thinning by removing small unmarketable fruit reduced the harvest time. This is especially important for Clementines since it is harvested at the start of the rainy season in the Western and Eastern Cape.

## Opsomming

'n Premie word betaal vir groter vrugte en die inkomste uit klein vrugte is meestal minder as die pluk en transport kostes. Vruguitdunning veroorsaak gewoonlik 'n verlaging in oesgrootte, maar die kleiner oes kan 'n groter ekonomiese waarde hê. Die doel van die studie was om te bepaal of handuitdun enige ekonomiese voordeel inhou, al veroorsaak dit nie groter vrugte nie. Nules Clementine bome op Troyer citrange onderstam in die Porterville area is gebruik vir die studie. Op 7 Desember 2006 is 12 enkelbome in 'n volledige gerandomiseerde blok ontwerp onuitgedun gelaat of 12 bome is uitgedun deur vrugte <21 mm te verwyder. Handuitdun het vruggroei en vruggrootte verbeter maar het geen betekenisvolle effek op oeslading gehad nie, alhoewel dit 'n 11% oesverlaging tot gevolg gehad het. Die totale tyd geneem om uit te dun en te oes was dieselfde vir die twee behandelings, maar oes van die kontrole (onuitgedunde) bome het 17 minute langer geneem. Dus, handuitdun, deur die klein, onbemarkbare vrugte te verwyder, het die oestyd verminder. Dit is veral belangrik vir Clementines omdat dit in die begin van die reënseisoen in die Wes- en Ooskaap geoes word.

## 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, (which leads to alternate bearing) and therefore reduce the amount of unmarketable fruit to be picked 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 for increasing 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 is to determine the economic benefit of hand thinning. The cost of thinning and the cost of harvesting for the different treatments will be determined to ascertain whether handthinning results in any economic benefit, even if it doesn't result in an increased fruit size.

## Materials and methods

### Plant material and treatments

Nules Clementine trees on Troyer citrange rootstock in Porterville were used for 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 complete 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.

### 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

Thinning removed on average 494 fruit per tree and took on average 21 minutes for one person per tree (Table 5.3.5.1). 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 5.3.5.2). Handthinning resulted in 8% more marketable fruit >55mm, but only 3% more marketable fruit >51mm (Table 5.3.5.3). Hand thinning also resulted in a major shift towards larger fruit sizes compared to the control (Fig. 5.3.5.1). The control trees peaked at size 2 (59-64mm) while thinned trees peaked at X (68-72mm) to 2X (72-78mm). Thinning also increased fruit growth significantly from the 2<sup>nd</sup> measuring date until harvest as indicated by the significant P-values at the 5% level for each measuring date after thinning was applied (Fig. 5.3.5.2).

## Conclusion

Hand thinning increased fruit growth and fruit size and had no significant effect on yield, although it resulted in an 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. This is the time taken to remove small unmarketable fruit, that was removed already earlier in the season for the thinned trees. Thinning by removing small unmarketable fruit reduced the harvest time. This is especially important for Clementines since it is harvested at the start of the rainy season in the Western and Eastern Cape. Hand thinning can also be considered where synthetic auxins didn't have the desired thinning effect on a heavy crop.

## Future Research

Future research would include a more detailed cost benefit analysis to determine if there is any economic benefit in handthinning, even if it doesn't result in an increased fruit size. The cost of thinning and the cost of harvesting for the thinned and unthinned trees will be determined at harvest.

## Technology transfer

- Verreyne, J.S. Fruit size and yield effects of time and severity of hand thinning on 'Nules' Clementine mandarin. SASCP, SSSSA, SASHS Combined Congress, Badplaas, 22-25 January 2007.
- Verreyne, J.S. Crop manipulation in citrus. Malelane Citrus Study Group. Malelane, 17 July 2007
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- Verreyne, J.S. Crop manipulation in citrus. Marble Hall/Groblersdal Citrus Study Group. Moosrivier, 30 July 2007
- Verreyne, J.S. Crop manipulation in citrus. Burgersfort/Ohrigstad Citrus Study Group. Ohrigstad, 30 July 2007
- Verreyne, J.S. Crop manipulation in citrus. Hoedspruit Citrus Study Group. Hoedspruit, 31 July 2007
- Verreyne, J.S. Crop manipulation in citrus. Constantia/Letsitele Citrus Study Group. Letsitele, 31 July 2007
- Verreyne, J.S. Crop manipulation in citrus. Waterberg Citrus Study Group. Potgietersrus, 1 August 2007

Verreyne, J.S. Crop manipulation in citrus. Tshipise Citrus Study Group. Tshipise, 1 August 2007  
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**Table 5.3.5.1.** The effect of hand thinning on total yield and the time taken to thin and harvest Nules Clementine trees

	Removed	Time taken to thin	Yield	Time taken to harvest	Total time taken for thin and harvest	Yield reduction
	-no./tree-	--minute <sup>y</sup> --	-kg/tree	--minute--	--minute--	--%--
Treatment						
Control	--	--	187.6	137	137	--
Handthin, 7 Dec 2006, ≤ 21 mm	494	21	167.2	120	141	11%
<i>P</i> -value			0.1433	0.2153	0.8423	

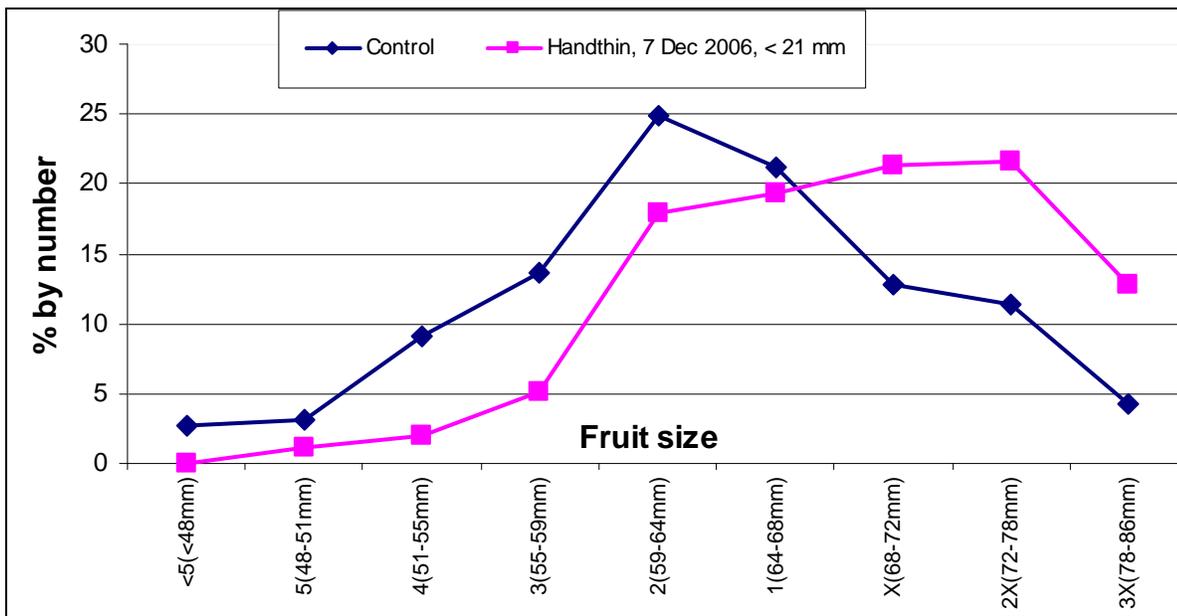
<sup>y</sup>minute indicate the time taken for one person to thin or harvest one tree.

**Table 5.3.5.2.** Effect of hand thinning on the shift in harvest time of Nules Clementine trees

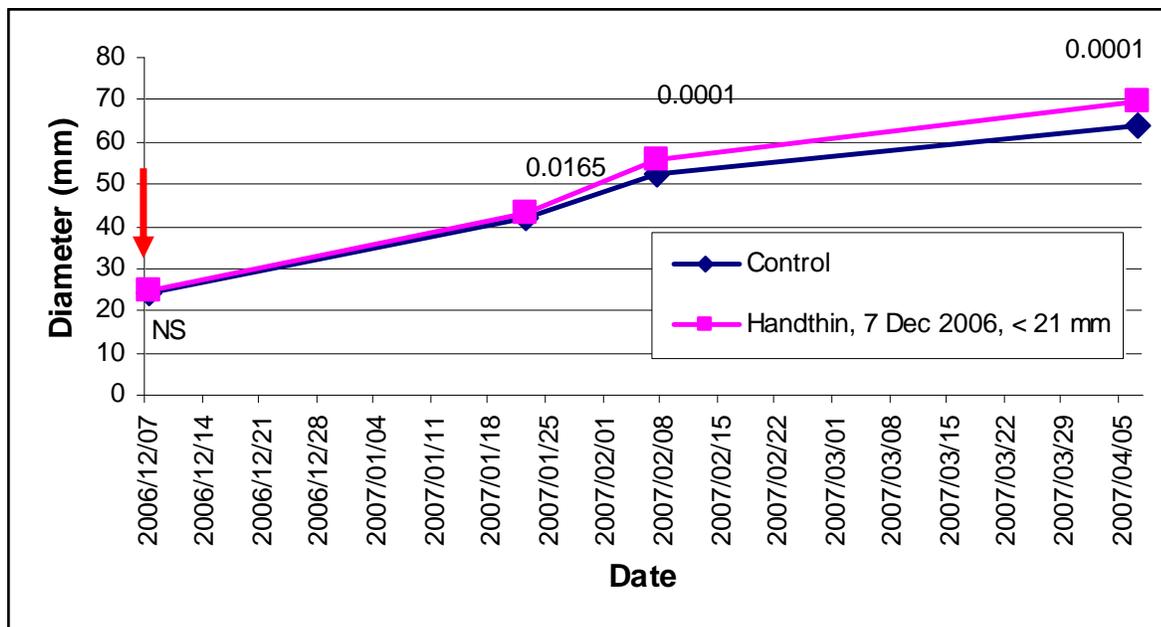
	1 <sup>st</sup> harvest	% of total yield	2 <sup>nd</sup> harvest	Total yield
	-kg/tree-	--%--	-kg/tree-	-kg/tree
Treatment				
Control	119.6	63.4	62.1	187.6
Handthin, 7 Dec 2006, ≤ 21 mm	97.3	58.5	69.9	167.2
<i>P</i> -value	0.1620	0.3578	0.8280	0.1433

**Table 5.3.5.3.** The effect of hand thinning on the percentage of marketable fruit > count 3 (55 mm) or > count 4 (51 mm) on Nules Clementine trees

Treatment	1 <sup>st</sup> harvest	% of total yield	2 <sup>nd</sup> 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
<i>P</i> -value	0.1620	0.3578	0.8280	0.1433



**Figure 5.3.5.1.** The effect of handthinning on the fruit size distribution at harvest of Nules Clementine trees.



**Figure 5.3.5.2.** The effect of handthinning on fruit growth throughout the season of Nules Clementine fruit.

### 5.3.6 FINAL REPORT: Effects of irradiation on product quality of citrus, with emphasis on rind and sensory quality

Experiment: IRR 01/05 and 02/05 (June 2005–August 2007): Graham Barry (Private), Paul Cronje (CRI) and Willem van Kerwel (SU)

#### Summary

During the 2005 and 2007 seasons, citrus fruit were tested for sensitivity to a wide range of irradiation dosages. Three cultivars were used *viz.* Clementine mandarin, Navel orange and lemon and their rind conditions as well as internal quality aspects were evaluated. The irradiation dosages used in 2005 were as follows; 0, 75, 100, 225, 300, 450, 600, 750 and 900 Gy and in 2007; 0, 300, 400, 500, 600 and 700 Gy. The irradiation treatments were administered at Hepro's BP1 cobalt pallet irradiator in Montague Gardens, Cape Town. Thereafter the fruit were stored at 4°C for a maximum period of 10 weeks. During this period samples were taken every 2 weeks for the visual rind and internal quality evaluations. In both seasons, irradiation affected internal quality aspects, e.g. the total sugar content was increased, while juice content decreased in some varieties. Irradiation leads to a drastic increase in decay of especially lemon fruit during storage, as well as a very high incidence of rind physiological disorders. Navel orange and Clementine mandarin rind quality were both negatively affected at high dosages but lemon rind was extremely sensitive even at low dosages. Off-taste also developed in the irradiated fruit as the time in storage increased (probably due to anaerobic respiration setting in). On the whole, irradiation at 300 Gy seems to be the limit at which certain citrus species could tolerate this treatment. However, there are other aspects also playing a role, such as season-to-season and fruit-to-fruit variation, but cultivar choice seems to be the biggest factor in determining sensitivity to irradiation. Type of irradiation equipment is also very important and the whole pallet system used in this experiment does not seem to be a practical solution. The reason being that those fruit positioned in the outside cartons of the pallet are exposed to very high dosages (3-4 times required) in order to realise the desired dose in the middle of the pallet.

#### Opsomming

Gedurende die 2005 en 2007 sitrus seisoene was die effek van 'n wye reeks bestralings dosisse op die interne en eksterne kwaliteite aspekte van sitrus vrugte bepaal. Drie kultivars, nl. Clementine mandaryn, Navel lemoene en suurlemoene is in albei seisoene gebruik. Die behandeling is gedoen in die BP1 pallet bestralings eenheid van Hepro in Kaapstad met kobalt as bron. Gedurende 2005 was die volgende dosisse gebruik: 0, 75, 100, 225, 300, 450, 600, 750 en 900 Gy en gedurende 2007 die volgende reeks 0, 300, 400, 500, 600 en 700 Gy. Na die behandeling is die vrugte vir tot 'n maksimum van 10 weke teen 4°C opgeberg waartydens daar elke

2 weke monsters getrek is vir evaluasie. Die evaluasie het 'n visuele skilkwaliteit/skildefek asook interne sapkwaliteite ingesluit. In beide die seisoene het die bestraling die interne kwaliteit beïnvloed m.a.w. tot byvoorbeeld hoër suiker inhoud of verlies van sap inhoud gelei. Die bestraling het ook 'n verhoogde voorkoms van bederf soos die opbergings periode verleng teweeg gebring, veral in suurlemoene. Die behandelings het tot ernstige agteruitgaan van skilkwaliteite gelei en dit was veral sigbaar in die suurlemoene al was dit teen lae vlakke. Nawel lemoene en Clementine mandaryne se skilkwaliteite is ook negatief beïnvloed maar was minder sensitief as die suurlemoene. Die bestraling het ook die smaak van die sap beïnvloed en gedurende opberging het afsmake ontwikkel (heelmoontlik a.g.v. anaerobiese respirasie). In 'n geheel gesien blyk 300 Gy die maksimum dosis waaraan die meeste sitrus kultivars blootgestel kan word. Aspekte soos variasie tussen seisoene en vrug-tot-vrug verskille in sensitiwiteit sal egter ook 'n rol speel in die vlakke van skade deur bestraling. Kultivar bly egter die belangrikste aspek wat die insidensie van skildefekte sal bepaal. Die manier waarop die vrugte bestraal word is egter ook krities en die heel pallet tegniek soos hier gebruik is nie prakties uitvoerbaar nie a.g.v. uiters hoë dosisse (3-4 keer die verlangde dosis) waaraan die buitenste vrugte blootgestel moet word om die vrugte binne die pallet ook aan die verlangde dosis bloot te stel.

## Introduction

Citrus fruit exported to certain markets, especially those with a citrus industry of their own, have to undergo strict commercial insect quarantine treatments in order to comply with phytosanitary trade barriers. There are a few options currently available and used in horticultural exports, *viz.* postharvest methyl bromide application as well as in-transit cold sterilisation treatments. However, citrus fruit react negatively to methyl bromide and develop off-tastes during storage. Therefore, export programmes of citrus fruit to special markets (prescribing insect sterilisation treatments, i.e. USA, Japan, China, Iran etc.) use cold sterilisation treatments during shipment. The level of chilling injury due to the prolonged exposure (e.g. 22 days at  $-0.6^{\circ}\text{C}$  for the USA and China) can be severe and economically damaging. This loss of quality as well as questions regarding the efficacy of the cold sterilisation treatments on insect larvae has led to irradiation of fruit being studied as an alternative to cold sterilisation for the control of false codling moth and fruit fly larvae.

Irradiation was approved in 1986 by the Food and Drug Administration (FDA) in the USA for use on fruit and vegetables at up to 1 kGy (100 krad). Even though most research has focussed on extending fruit and vegetable life by reducing decay organisms, irradiation has been shown to have a high efficacy of killing, sterilising or preventing further development of various insect species. The dosages required have also been illustrated to be significantly lower for insect sterilisation than necessary for effective decay control (Mitcham, 1999). In various studies this low dose of gamma irradiation as a quarantine treatment has been shown to be effective for various insect species, for example Hall and Martinez (2001) reported a minimum dose for Mexican fruit fly of 58-69 Gy to be effective even though 3 times higher could be absorbed by the citrus fruit without damage to the fruit.

Reports on dose levels detrimental to fruit quality (internal and external) vary. This is probably due to the irradiation equipment and experimental set-up used. The Floridian researchers (Miller and McDonald, 1996) applied 0.3 or 0.6 kGy to 15 fruit packed in a commercial fibreboard citrus box. The minimum and maximum deviations were 0.23 and 0.33 kGy and 0.49 and 0.67 kGy for the 0.3 and 0.6 kGy treatments, respectively. From the report it seems that these fruit were irradiated in single cartons and not palletised. They concluded that grapefruit used in the experiment tolerate dosage of 0.3 kGy without serious damages, however at 0.6 kGy severe rind damage developed in 12.5% of treated fruit during storage. Ladaniya *et al.* (2003) in India, irradiated 24 fruit (not describing the container or if palletised) of three cultivars, *viz.* 'Nagpur' mandarin, 'Mosambi' orange and 'Kagzi' acid lime at 0.25, 0.5, 1 and 1.5 kGy at the rate of 2.5 kGy/h with the  $D_{\max}/D_{\min}$  ratio of 1:21. Treatments were done in such a manner that 90% of fruit received the target dosage, which suggests the use of non-palletised irradiation equipment. They reported effective control of *Penicillium* rot at 1.5 kGy, but no positive result on *Alternaria citri* and *Bortyodiplodia theobromae*. Rind damage was recorded on oranges as well as a reduction in firmness, acidity and Vitamin C content at most dosages. They also found higher TSS in all irradiation-treated fruit as well as a significant increase in respiration.

Irradiation of 'Rio Red' grapefruit with 70, 200, 400 and 700 Gy by Patil *et al.* (2004) in what seems to be non-palletised conditions, showed that not only does irradiation dosage play a role in eventual quality but also fruit maturity (time of harvest). Early season fruit were more sensitive than later hanging fruit. They also showed at lowered dosages ( $\leq 200$  Gy) an enhancement of health-promoting compounds (flavonone, Vitamin C, limonin glycoside), but treatments  $\geq 400$  Gy increased the incidence of rind disorders. Canopy position of grapefruit also

had an effect on irradiation sensitivity and interior fruit had 27% compared with 15% pitting (zero in control fruit) (McDonald *et al.*, 2000).

The objective of this study was to determine at which dosage three citrus types could be irradiated in a pallet system without negative impact on their fruit quality (internal and external). The second object was to determine the irradiation dose distribution in a pallet of fruit.

## **Materials and methods**

The study to determine the effect of irradiation on internal and external (rind condition) quality of citrus fruit was done during the 2005 and 2007 seasons. The same cultivars and evaluation parameters were used in both seasons, although the dosages differed between the two years. The irradiation treatments were administered at Hepro's BP1 cobalt pallet irradiator in Montague Gardens, Cape Town.

The dose response study included three citrus types, namely Clementine mandarin, Navel orange and lemons. All the fruit received normal commercial postharvest packhouse treatments, i.e. chlorine bath, drying tunnels, fungicide treatment, waxing and packing before being irradiated. The Clementine mandarin fruit were packed in 400 x 300 mm 10 kg cartons and were calibre 2 fruit. The Navel orange and lemons were packed in 400 x 300 mm 15 kg telescopic cartons. The Navel oranges were count 64 and the lemons 115.

### Irradiation treatments

The 2005 target dosages were 0 (control), 75, 100, 150, 225, 300, 450, 600, 700 and 900 Gy. For replication purposes 12 cartons per variety were used. One dosimeter was placed in the middle of each carton for a total number of twelve dosimeters per variety per dosage. The 2007 target dosages were 0 (control), 300, 400, 500, 600, 700 Gy. The dosage selections were based on the results from the 2005 data and were a narrower dose range with the dosages below and above the sensitivity dosages determined in 2005. During both seasons the cartons were stacked in a pallet with normal commercial dimensions prior to irradiation treatments. In the irradiation chamber the pallets were placed on rotation tables to ensure all sides received the same dosage.

### Dose distribution

As a separate experiment in 2005, the dose distributions in a pallet of fruit were determined by placing dosimeters in positions representing all positions in separate pallets of Clementine mandarins and Navel oranges. The fruit was packed onto a pallet of 1.2 m x 1.0 m x 1.57 m (height taken from the pallet). The dosimeters were attached to rigid boards (3 mm thick) so as to provide a stable height per layer and exact positions for the dosimeters placement. An example of dosimeter distribution can be seen in Figure 5.3.6.1.

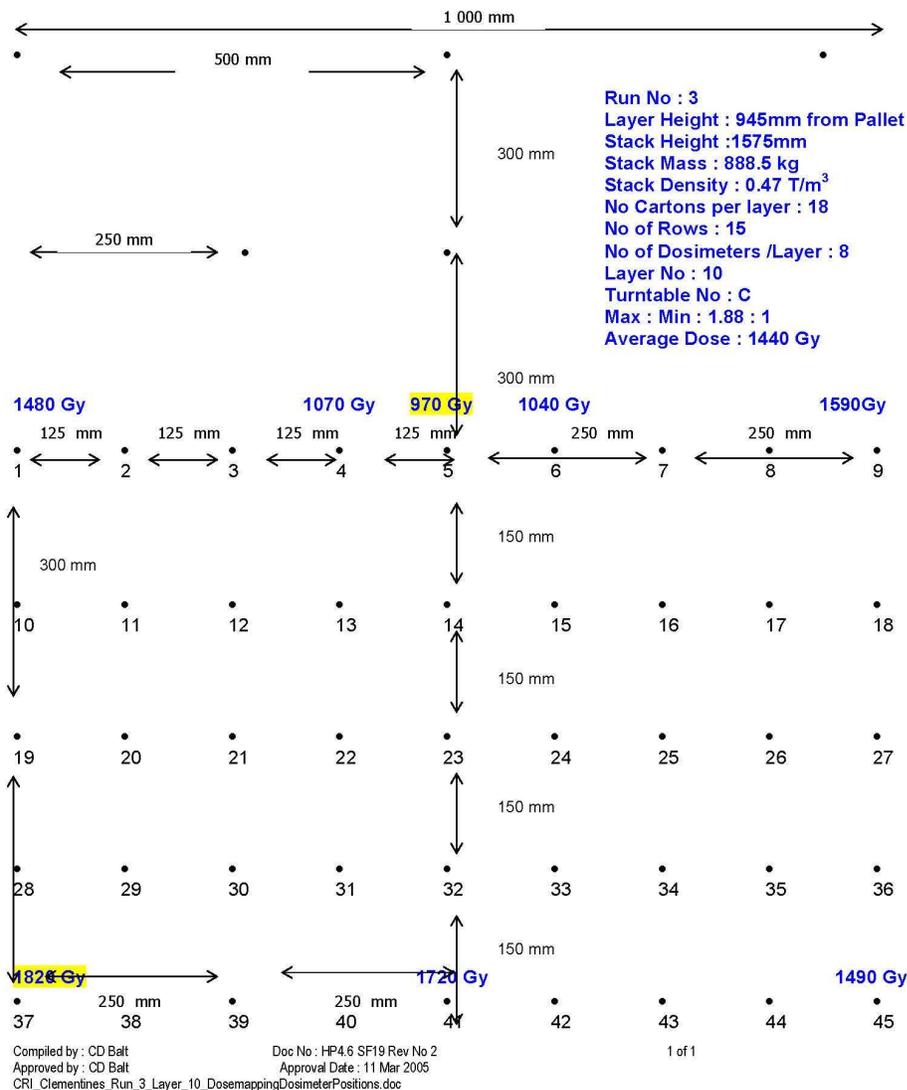
- The Clementine mandarin cartons were stacked to a 15 layer pallet consisting of 18 cartons per layer. The stack density was  $0.51 \text{ T/m}^3$ . The dosimeters were placed at the bottom and top of the pallet as well as in every third layer (4, 7, 10 and 13).
- The Navel orange cartons were stacked into 6 layers of 10 cartons per layer and the stack density was  $0.47 \text{ T/m}^3$ . The dosimeters were placed at the bottom, top and in the middle of the pallet.

After a total run time of 30 minutes the dosimeters were removed from the stack and read in the calibrated spectrophotometer at a wavelength of 530 nm as well as the measurements of the dosimeters taken by calibrated micrometer.

HEPRO Cape (Pty) Ltd  
 HP4.6 Dosimetry  
 Dosemapping : Dosimeter Positions  
 Incorporating both Test A and Test B positions

HP4.6 SF19 Rev No 2

Company Name : CRI  
 Product Description : Clementines  
 Date of Trial : 28 June 2005  
 GRV No : 35621  
 Batch No : 28614



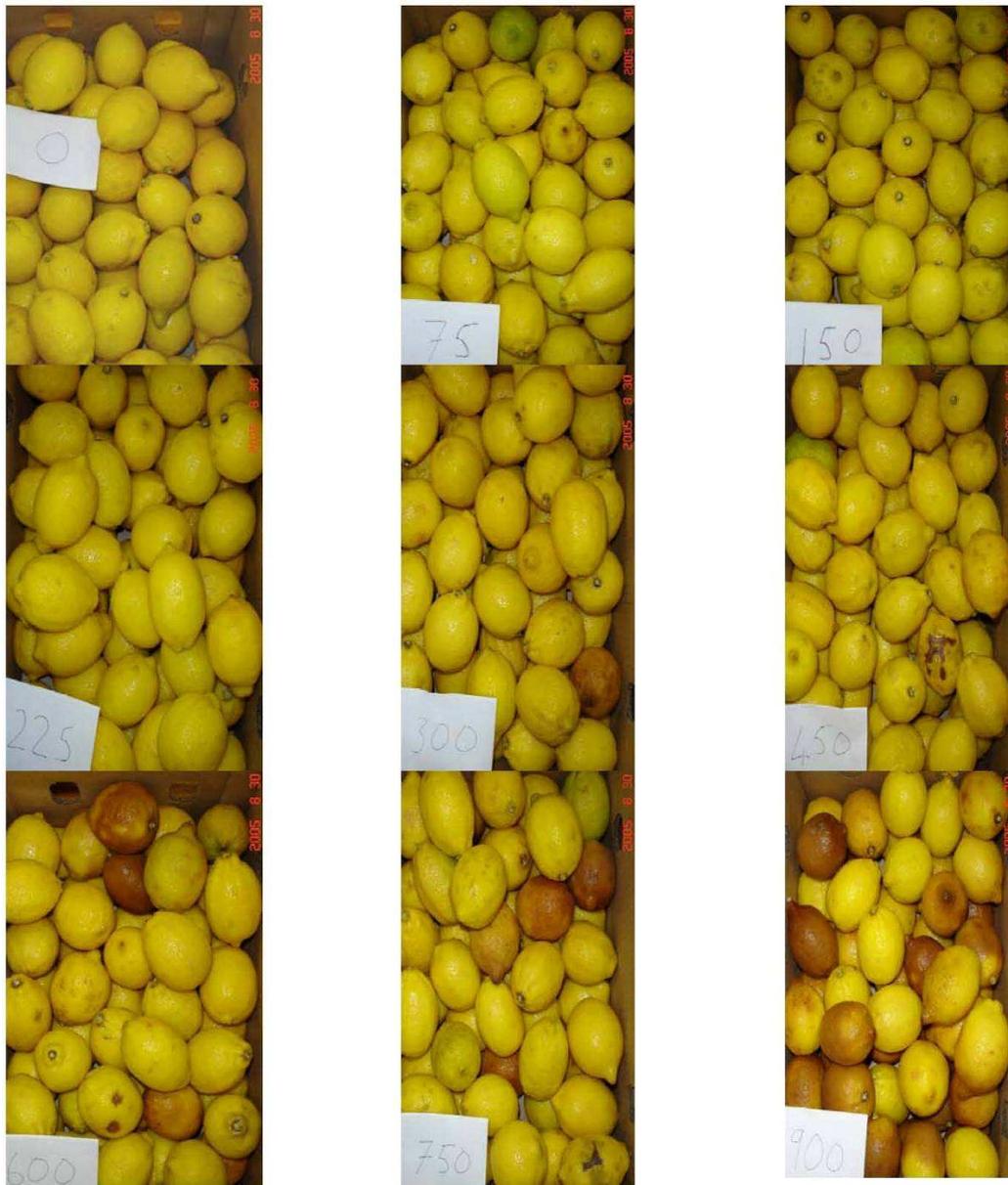
**Figure 5.3.6.1.** Example of dosimeter placement in one layer of a pallet of Navel orange or Clementine mandarin during the dose mapping experiment. Dosimeters were only placed in the half of each measured layer as pallets were rotated during irradiation and all sides of the pallet receive even doses. In the Navel orange pallet this configuration was repeated at the bottom, top and in the middle of the pallet. In the Clementine mandarin pallet the dosimeter pattern was repeated every 3<sup>rd</sup> layer (bottom, 4, 7, 10, 13 and top). The data collected from these readings is summarised in Tables A and B. The maximum dose measured can be seen in cartons on the side of the pallet and the minimum levels were consistently measured in the middle of the pallet (position 5).

**Measurements**

After irradiation, fruit were stored at 4°C for a maximum of 10 weeks to simulate commercial handling, shipping and storage duration. Fruit were removed for evaluation every 2 weeks. For each sampling date eight replications of 10 fruit each were removed from the cold store of each variety. Severely decayed fruit were removed from the cartons and not used in the visual or internal evaluation. The first evaluation date was the day

after treatment to give a baseline for changes in the internal quality evaluations. At each evaluation the fruit were first visually evaluated for various rind disorders and appearance before being destructively sampled for internal quality, i.e. Brix, acidity, juice content and taste. The rind condition evaluations were done according to known rind disorders specific to each variety, as well as some general classes for all varieties, viz. browning (more internal discoloration occurring in the flavedo), scalding (a discoloration of the epicuticular cells of the rind as seen in chilling injury), rind collapse (total softness of the rind followed by decay). The specific evaluation parameters per cultivar are as follows (Figures 5.3.6.1 and 5.3.6.2):

- **Clementine mandarin:** Puffiness (separation of the pulp and rind), rind collapse.
- **Navel orange:** Pitting (dark brown depressions in the flavedo, similar to postharvest pitting and chilling injury), rind collapse, including stem end rind breakdown (SERB).
- **Lemons:** Peteca spot (oil gland leaking into flavedo/albedo resulting in a collapse of the tissue).



**Figure 5.3.6.2.** Effect of irradiation at 0, 75, 150, 225, 300, 450, 600, 750, and 900 Gy on lemons during 2005. Severe incidence of scalding, peteca spot and total rind collapse can be seen as the irradiation dose increases.



**Figure 5.3.6.3.** Effect of irradiation on rind condition of Navel orange (top two photos) and Clementine mandarin during 2007. Incidence of scalding, browning and SERB can be seen.

## Results

### *Fruit quality*

#### **2005**

##### Navel orange

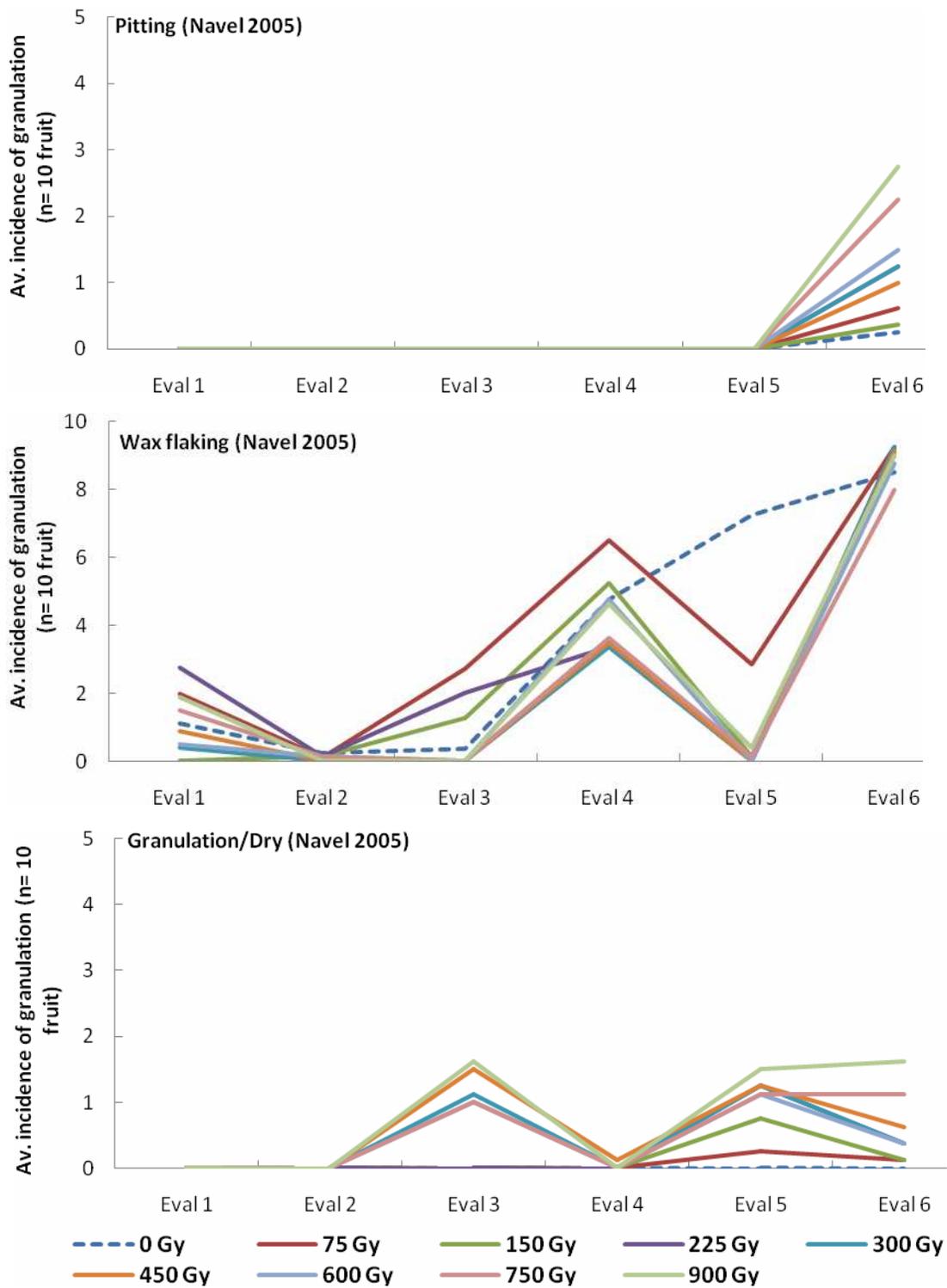
Higher incidence of granulation/dryness of the pulp was seen in the irradiated fruit. The high incidence of wax flaking can more likely be attributed to application than irradiation treatment. Internal quality of the control Navel fruit had on average less variation in Brix, acidity (TA), and juice content. The data indicate that above 75 Gy the internal quality could be affected in Navel orange fruit (Fig. 5.3.6.4 and 5.3.6.5).

##### Clementine mandarin

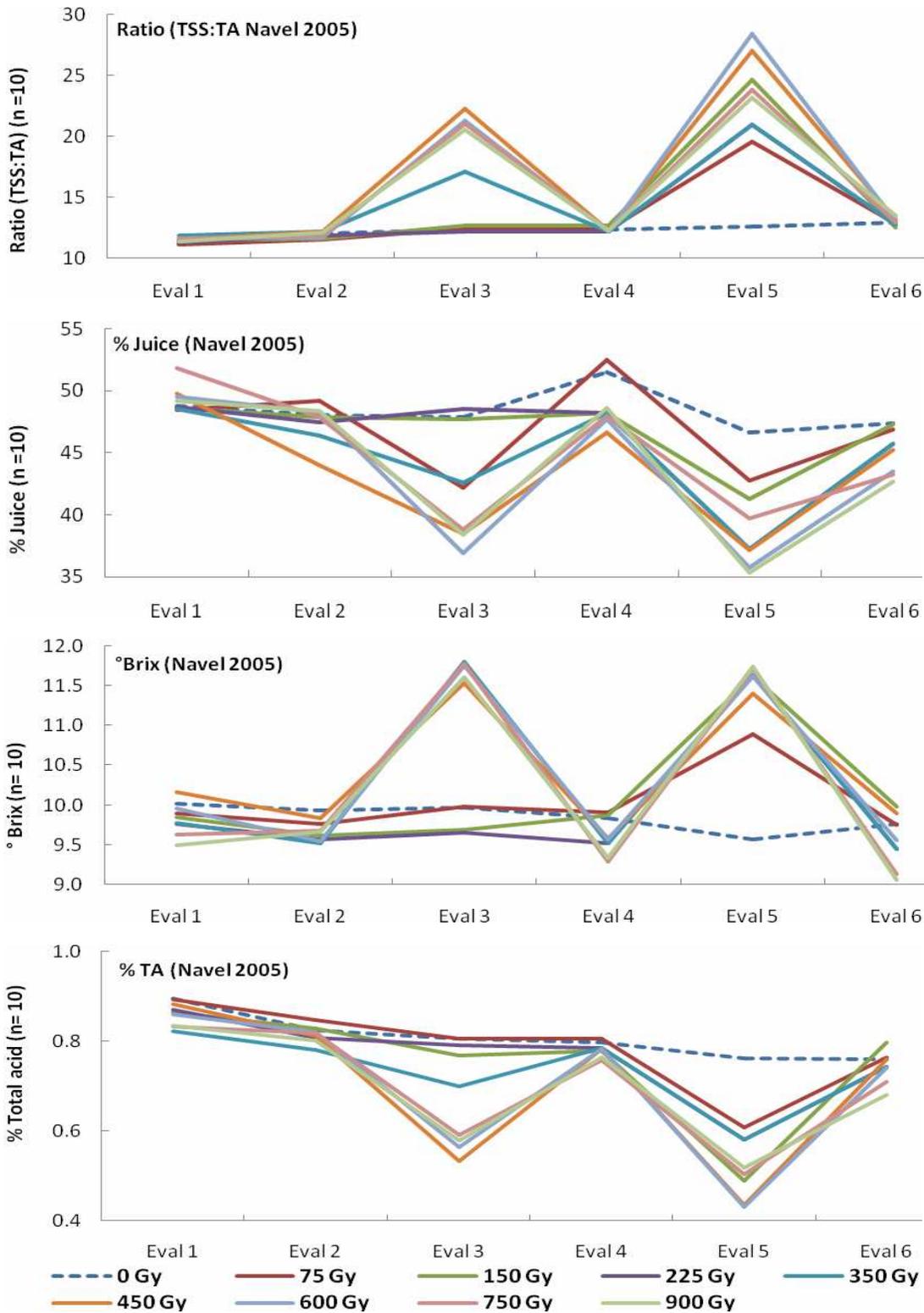
All the fruit in the experiment (including the control) showed a high incidence of puffiness, the most prevalent disorder in the experiment. Therefore, irradiation did not play a causative role in this disorder as well as in granulation. The rind condition was acceptable during most of the experiment and only showed signs of deterioration after evaluation 4-5 (i.e. 6-8 weeks storage). The internal quality showed a detrimental dosage response and juice content, Brix and TA all changed on average. The result from these changes was an increase in ratio (TSS:TA) above the control at most dosages (Fig. 5.3.6.6 and 5.3.6.7).

##### Lemons

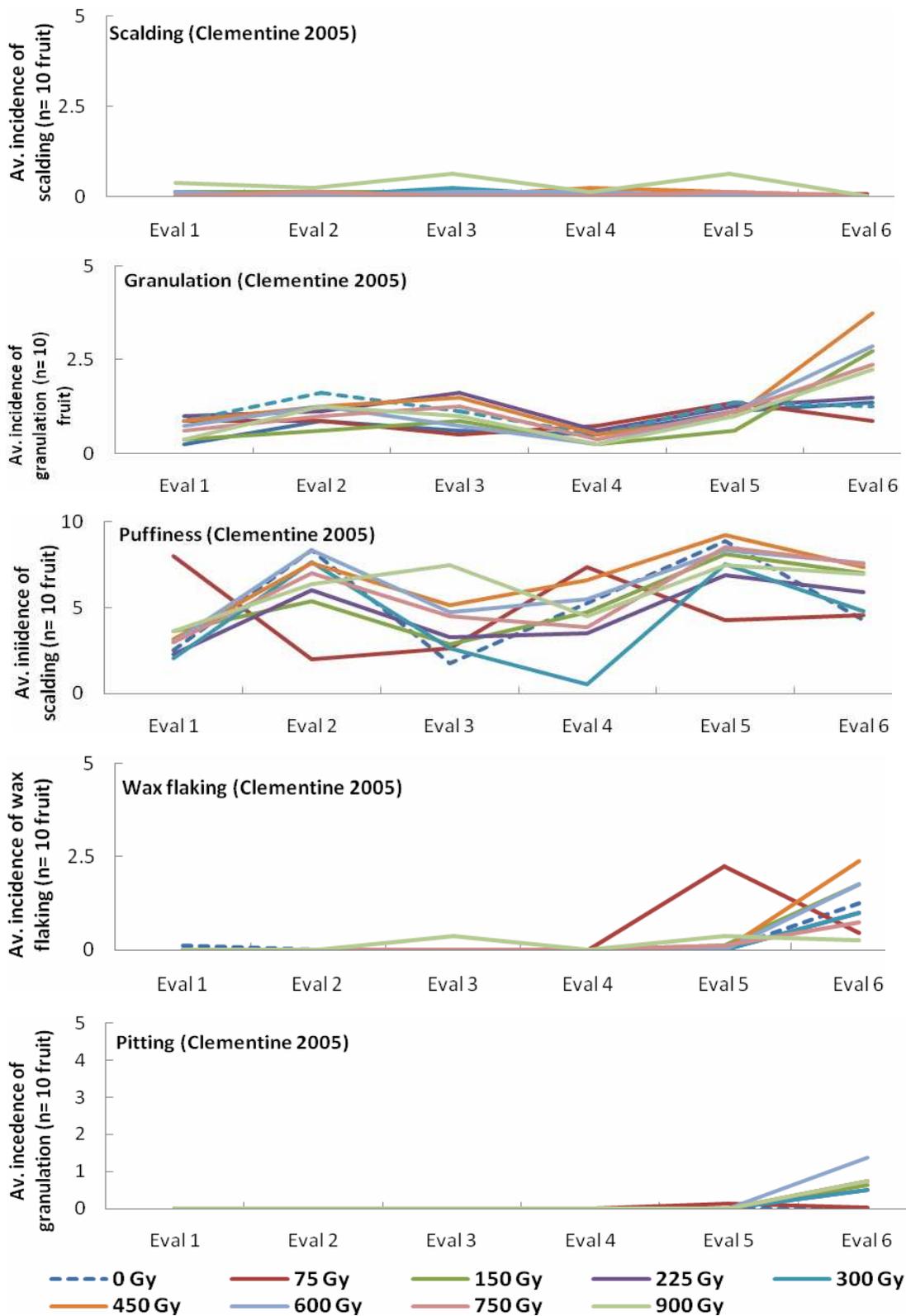
Irradiation had the biggest negative impact on the external quality of lemon fruit even though the peteca spot incidence only dramatically increased after evaluation 4, i.e. 6 weeks storage. Visually the fruit had a "tired" look and a high number of fruit were lost even before evaluation due to the high decay in the cartons (data not shown). Juice content was negatively affected after evaluation 3, i.e. 4 weeks storage, by high irradiation dosage (Fig. 5.3.6.8).



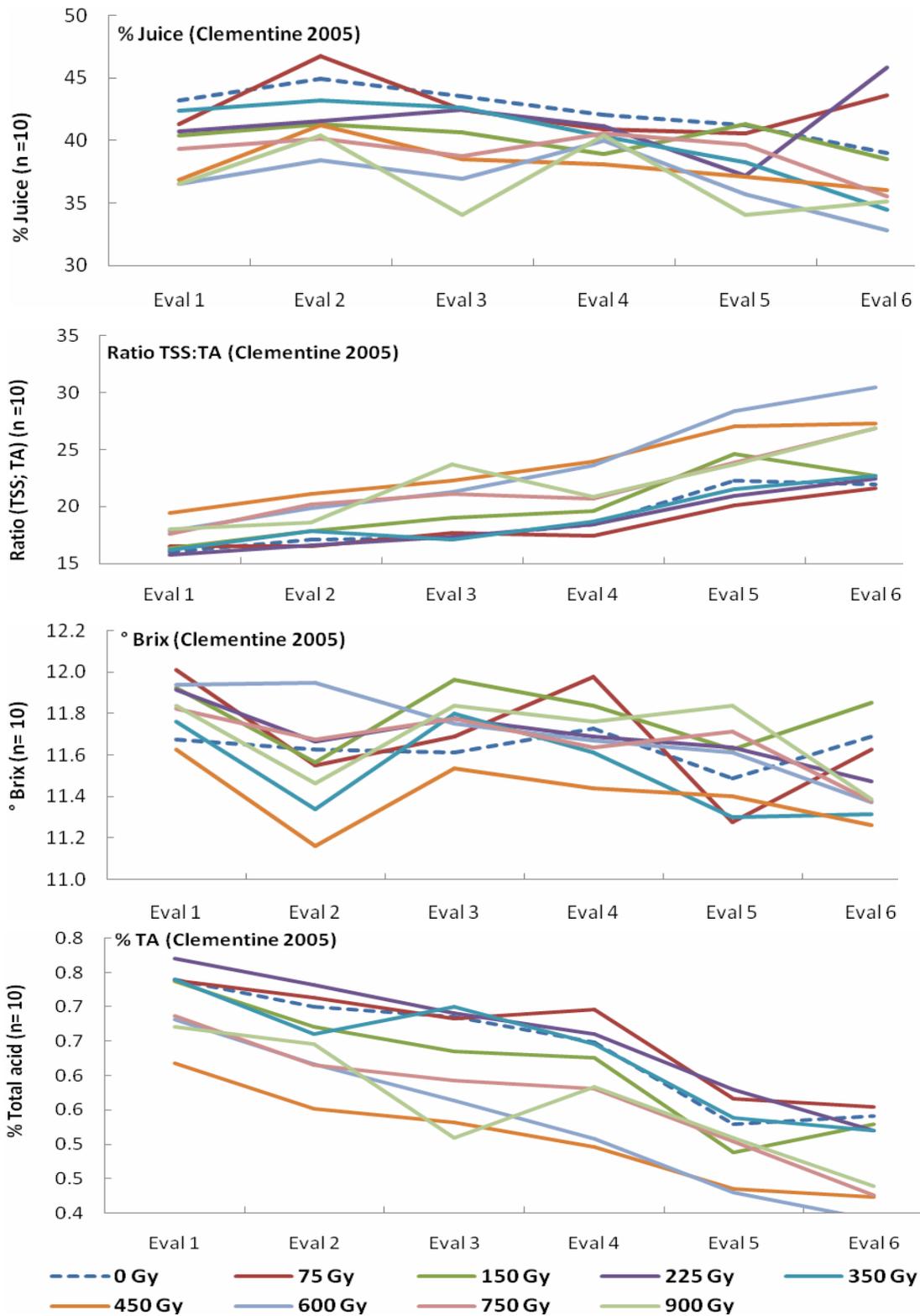
**Figure 5.3.6.4.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2005 season on rind condition and incidence of physiological disorders of Navel orange fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



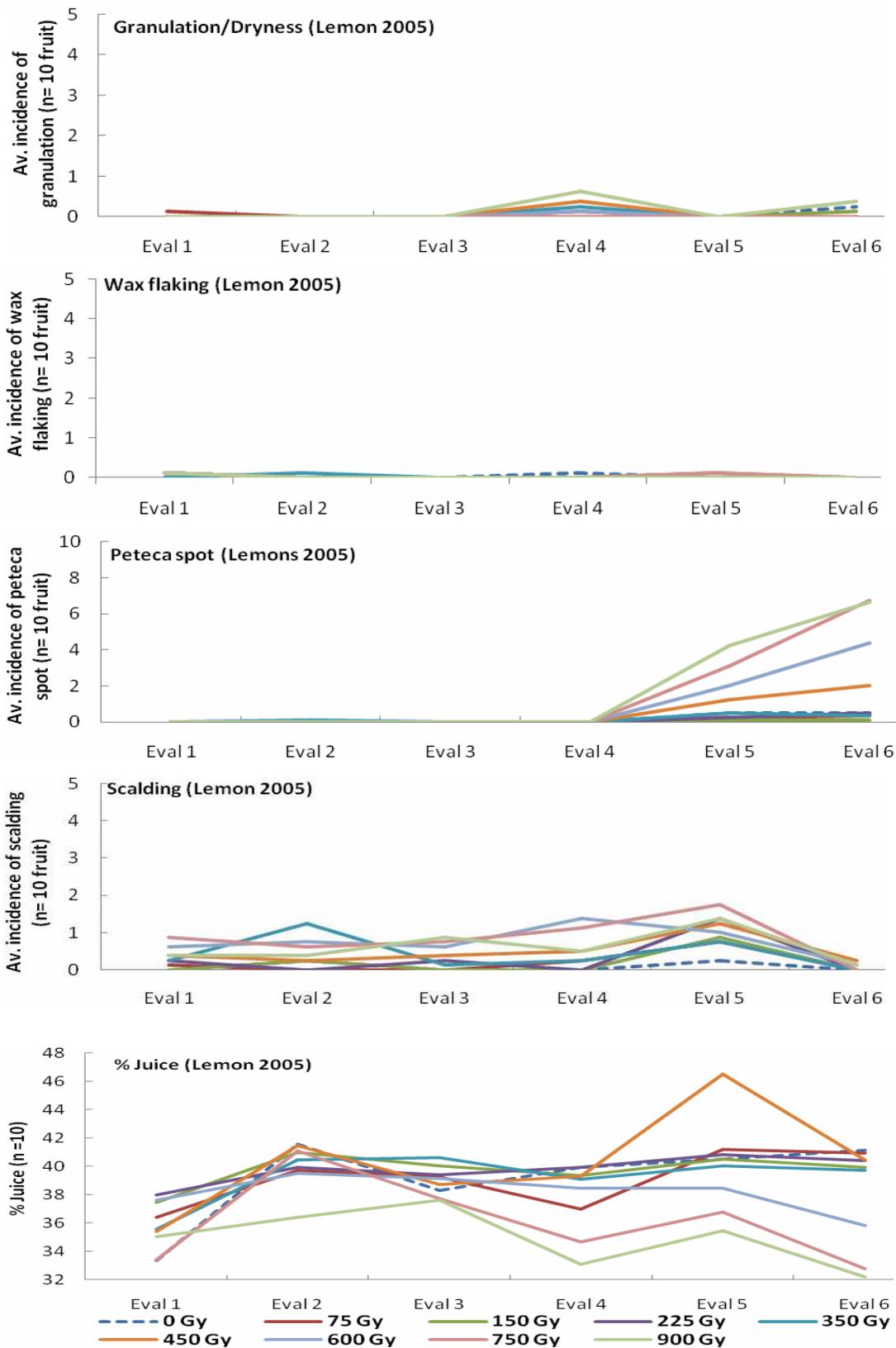
**Figure 5.3.6.5.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2005 season on Navel orange fruit internal quality. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



**Figure 5.3.6.6.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2005 season on rind condition and incidence of physiological disorders of Clementine mandarin fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



**Figure 5.3.6.7.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2005 season on internal quality of Clementine mandarin fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



**Figure 5.3.6.8.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2005 season on rind condition, incidence of physiological disorders and % juice of lemon fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.

### **Dose distribution**

The average maximum dose reading in the Clementine mandarin pallet (Table 5.3.6.1) was 1748 Gy and the minimum dose was 832 Gy. The Max / Min Ratio was 2.21:1 for the average values and 3.32:1 for the worst case scenario (highest and lowest overall dose readings) in the Clementine mandarin pallet. The highest dose readings were in the outside carton position, whereas the minimum in the middle of the pallet. This pattern was the same for both cultivars.

The average maximum dose in the Navel orange pallet (Table 5.3.6.2) was 1687 Gy with a minimum average dose of 728 Gy. This data gave a Max / Min Ratio of 2.32:1 for the Navel oranges and a ratio of 3.93:1 for the worst case scenario (highest and lowest values in pallet).

**Table 5.3.6.1.** Summary of dose mapping in the Clementine mandarin pallet.

Layer Number	Maximum Dose (Gy)	Maximum Position Number	Minimum Dose (Gy)	Minimum Position Number	Maximum: Minimum Ratio
1	1870	41	730	5	2.56 : 1
4	1930	41	930	4	2.08 : 1
7	1880	45	960	4	1.96 : 1
10	1960	42	910	5	2.15 : 1
13	1650	41	870	9	1.9 : 1
15	1200	37	590	5	2.03 : 1
<b>Average</b>	1748		832		2.1 : 1
<b>Overall</b>	1960	Layer 10 Position 42	590	Layer 15 Position 5	3.32 : 1

**Table 5.3.6.2.** Summary of dose mapping in the Navel orange pallet.

Layer Number	Maximum Dose (Gy)	Maximum Position Number	Minimum Dose (Gy)	Minimum Position Number	Maximum: Minimum Ratio
1	1760	41	700	4	2.51 : 1
4	2050	45	960	5	2.14 : 1
6	1280	37	520	5	2.46 : 1
<b>Average</b>	1687		728		2.32 : 1
<b>Overall</b>	2050		520		3.94 : 1

### **Fruit quality**

#### **2007**

During the 2007 season, juice of the mandarin and orange fruit were tasted and casual observations made on the tastes during internal quality evaluations. This is presented in Table 5.3.6.3 and illustrates a decrease in quality soon after the second evaluations and even more so at higher dosages. However, only after 6 to 8 weeks of storage (Eval. 3-4), when anaerobic respiration was probably setting in and resulting in the associated off flavours. It is also evident that Clementine mandarin developed these off flavours on average quicker than Navel orange fruit.

The incidence of decay per carton was noted to give an indication of un-evaluable fruit during storage (Table 5.3.6.4). As seen here it was especially the lemon fruit that had a severely negative reaction following irradiation.

**Table 5.3.6.3.** Tasting and observations of Navel orange and Clementine mandarin juice (only 2007).

<b>Evaluation date</b>	<b>Irradiation dose (Gy)</b>	<b>Navel orange</b>	<b>Clementine mandarin</b>
Evaluation 1 28 June	0	Sweet	Very sweet
	300	Sweet	Juice sweet
	400	Sweet	Sweet
	500	Sweet	Very sweet
	600	Sweet	Very sweet
	700	Sweet	Very sweet
Evaluation 2 11 July	0	Sweet	Sweet
	300	Off taste/milky	Milky
	400	Acidic	Very sweet
	500	Off taste	Off taste
	600	Off taste	Very sweet/milky
	700	Off taste	Very sweet
Evaluation 3 25 July	0	Sweet	Sweet
	300	Milky	Very sweet
	400	Sweet/acidic	Off taste
	500	Off taste	Off taste
	600	Milky/acidic	Off taste
	700	Milky	Off taste
Evaluation 4 8 August	0	No observations	Milky
	300		Milky
	400		Off aftertaste
	500		Sweet/acidic
	600		Sweet
	700		Off aftertaste
Evaluation 5 22 August	0	Sweet/pleasant taste	Sweet/pleasant taste
	300	Sweet/milky	Sweet
	400	Sweet/milky	Sweet
	500	Bitter after taste	Sweet/decaying taste
	600	Milky/bland	Sweet/decaying taste
	700	Sweet	Sweet/milky
Evaluation 6 2 September	0	Sweet/bland	Sweet
	300	Sweet/decaying taste	Watery
	400	Sweet/decaying taste	Unpalatable/unappetising
	500	Bland	Unpalatable/unappetising
	600	Unpalatable/unappetising	Very unpalatable/unappetising
	700	Unpalatable/unappetising	Very unpalatable/unappetising

**Table 5.3.6.4.** Incidence of decayed fruit in cartons in 2007 that had to be removed/discarded and could not be used in evaluation due to unidentifiable rind defect/blemish.

<b>Evaluation date</b>	<b>Irradiation dose (Gy)</b>	<b>Lemon</b>	<b>Navel orange</b>	<b>Clementine Mandarin</b>
Evaluation 1 28 June	0	Zero decay	Zero decay	Zero decay
	300			
	400			
	500			
	600			
	700			
Evaluation 2 11 July	0	4	2	2
	300	4	4	3
	400	6	3	0
	500	6	3	0
	600	4	1	1

	700	0	2	1
Evaluation 3 25 July	0	7	1	0
	300	4	1	0
	400	2	3	0
	500	6	0	1
	600	3	1	1
	700	8	2	1
Evaluation 4 8 August	0	0	5	2
	300	3	1	2
	400	1	2	4
	500	1	3	1
	600	11	1	2
	700	8	0	0
Evaluation 5 22 August	0	8	3	0
	300	1	1	2
	400	1	4	1
	500	11	10	1
	600	27	1	2
	700	38	1	0
Evaluation 6 2 September	0	25	3	1
	300	35	2	3
	400	22	9	2
	500	44	4	3
	600	31	0	3
	700	78	0	1

#### Navel orange

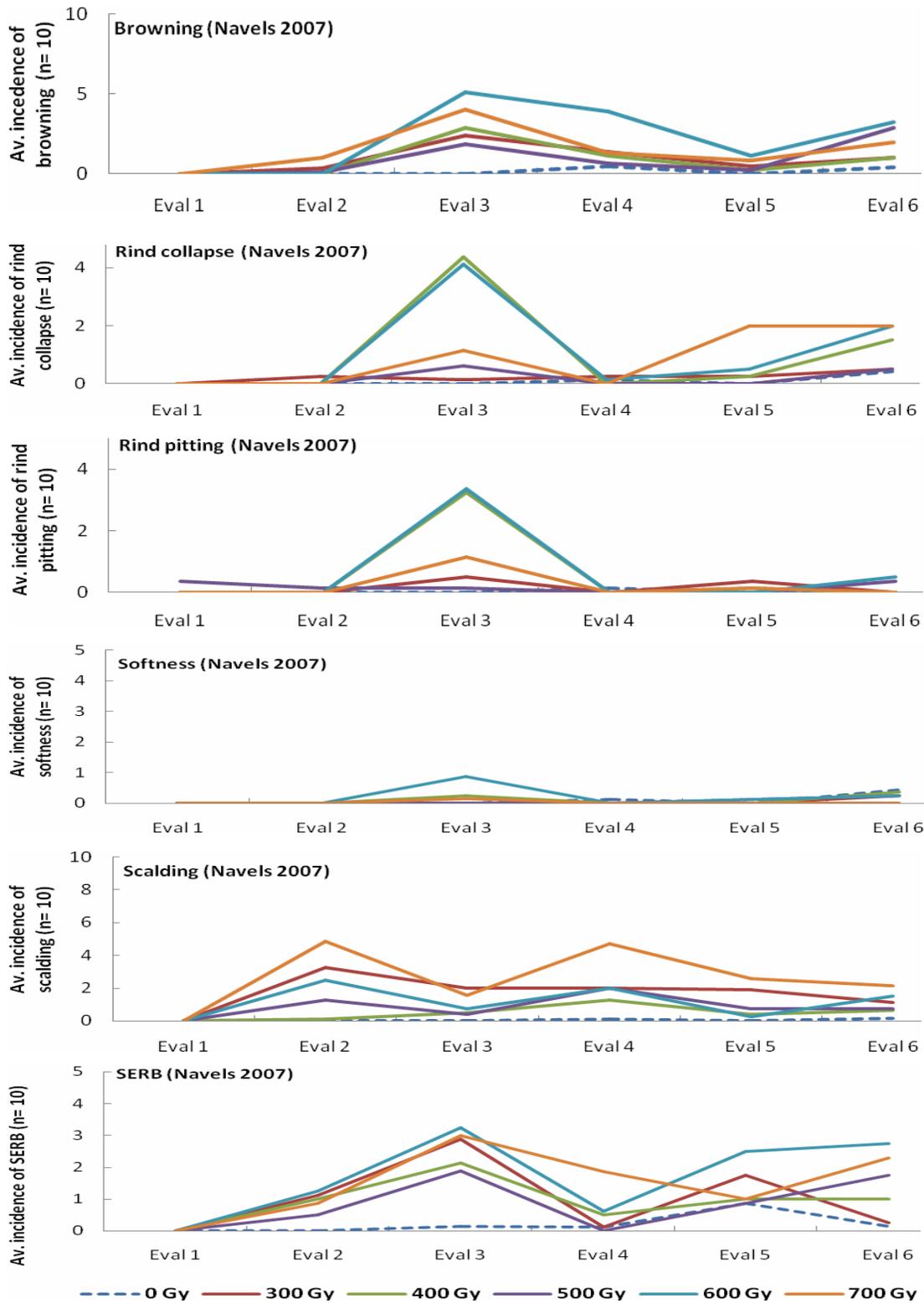
During this season, much more pronounced rind damage occurred than during 2005 illustrating a year-to-year variation in rind sensitivity. Rind browning, as well as scalding, increased at almost all irradiation dosages to high levels. The probable reason for these categories decreasing after evaluation 4, i.e. 6 weeks storage, was the higher decay development and resultant discarding of fruit before evaluation (Fig. 5.3.6.9). The only internal quality aspect that differed noticeably between treatments and control was the increase in Brix of almost all irradiation dosages (Fig. 5.3.6.10).

#### Clementine mandarin

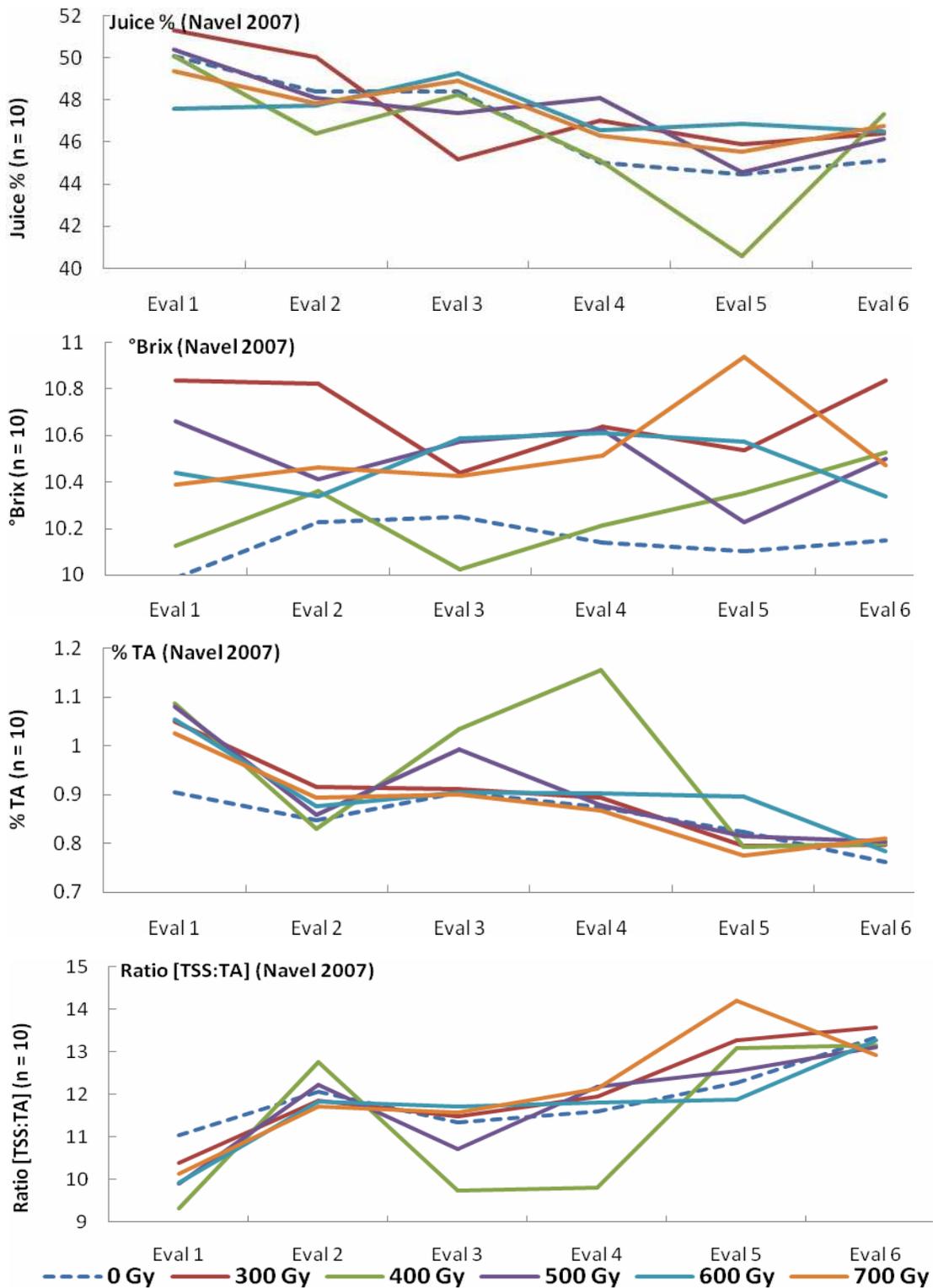
Roughly the same pattern repeated itself in incidence of physiological disorders during 2007 (Fig. 5.3.6.11) compared with 2005, especially in puffiness showing no real difference between treatments and control. The total acid (TA) of the Clementine mandarin during this season was reduced by the irradiation in comparison with the control, as well as what seems to be an increase in the Brix during storage. The ratio (TSS:TA) as a result increased as seen in 2005, which could indicate a higher rate of metabolism due to irradiation (Fig. 5.3.6.12).

#### Lemons

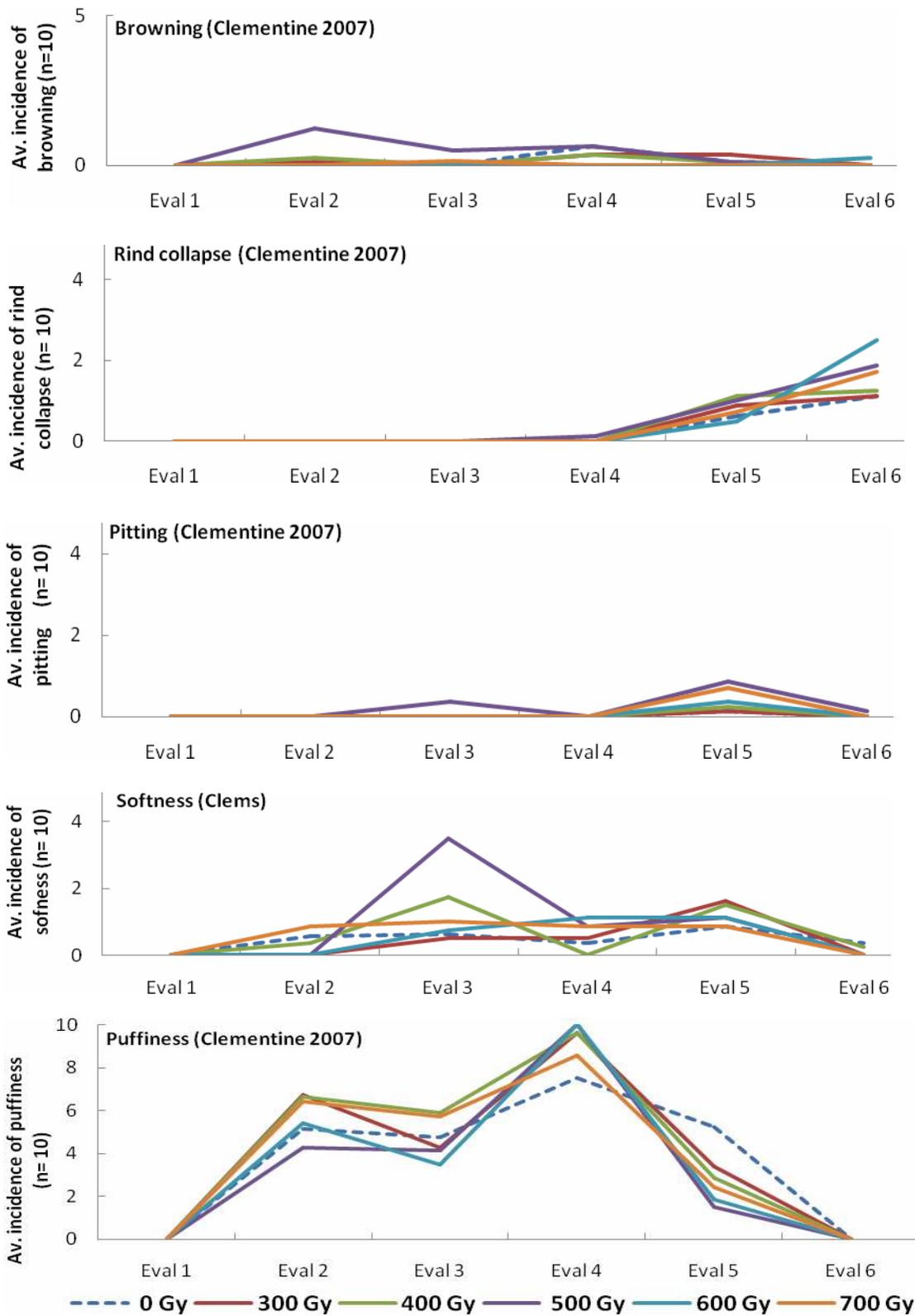
Of the three varieties used, lemons had the highest degree of sensitivity to irradiation in both seasons. Even only after 2 weeks storage nearly 100% of the fruit had one or the other rind disorder, making it unmarketable. Most fruit also showed more than one symptom of rind disorder at the same time, i.e. peteca spot and scalding (Fig. 5.3.6.13). The late loss in juice content of the lemons after evaluation 4, i.e. week 6 of storage, could be due to total loss of rind integrity and a resulting loss of moisture for the rind and pulp (Fig. 5.3.6.14).



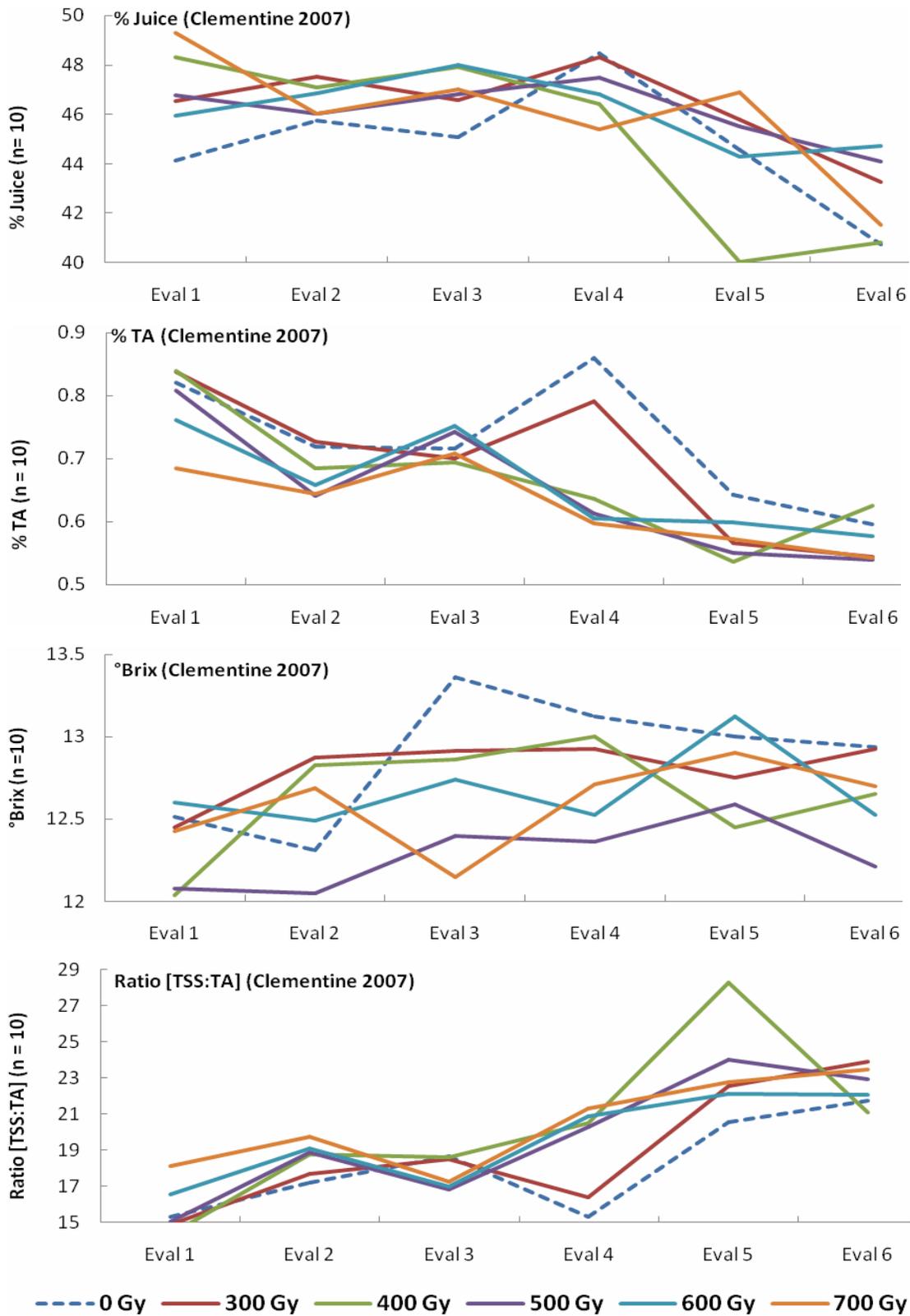
**Figure 5.3.6.9.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2007 season on rind condition and incidence of physiological disorders of Navel orange fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



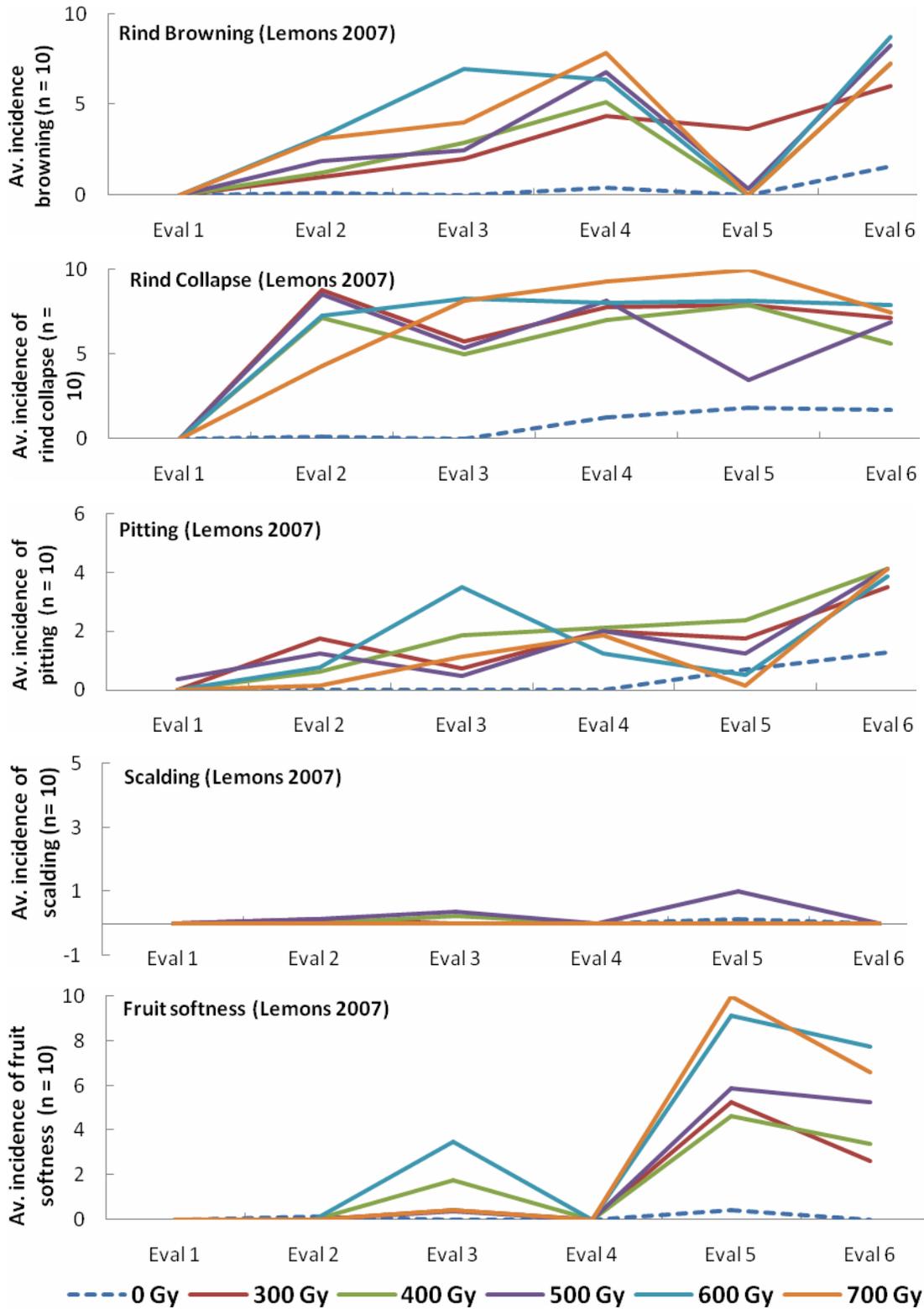
**Figure 5.3.6.10.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2007 season on internal quality of Navel orange fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



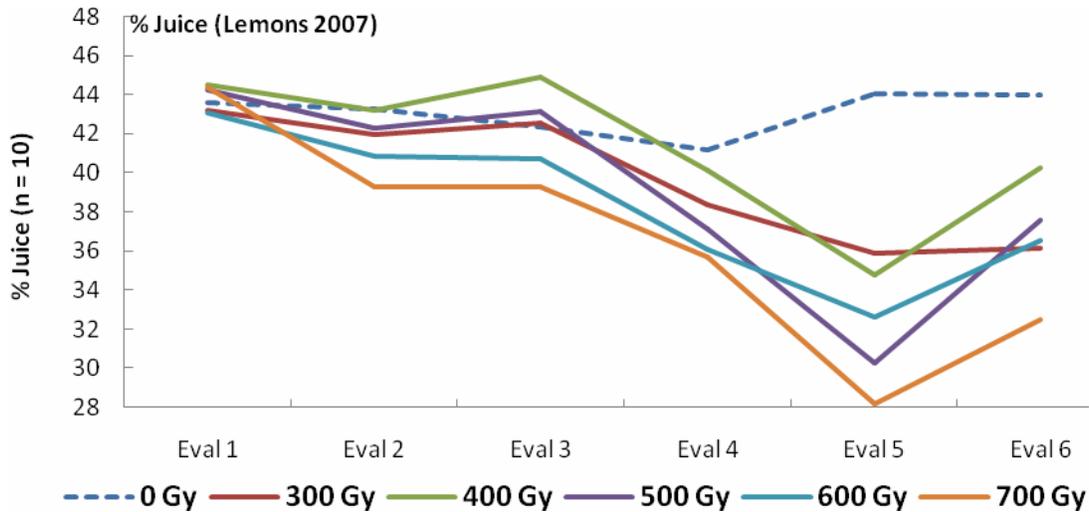
**Figure 5.3.6.11.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2007 season on rind condition and incidence of physiological disorders of Clementine mandarin fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



**Figure 5.3.6.12.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2007 season on internal quality of Clementine mandarin fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



**Figure 5.3.6.13.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2007 season on rind condition, incidence of physiological disorders of lemon fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.



**Figure 5.3.6.14.** Effect of various dosages of irradiation and subsequent storage at 4°C during the 2007 season on % Juice of lemon fruit. Evaluations (2-6) were done every second week with evaluation 1 being done the day after irradiation treatment.

### Conclusions

Irradiation could potentially be a valuable tool to replace, or use in combination with, cold sterilisation treatments for false codling moth and fruit fly control in export citrus fruit. However, this technology will require low dosages, i.e. <300 Gy, before practical solutions can be found to administer the correct dose to all fruit in the shortest time without being detrimental to fruit quality.

Dosages above 300 Gy will probably be detrimental during most seasons in not only damaging rind quality but also internal quality aspects of all three citrus types tested. In comparing these results with literature the obvious difference is the application equipment, viz. in a pallet in contrast to loose fruit or individual cartons being irradiated. The second option will allow lower dosages due to the gradient from the source to the point furthest away being very short due to less hindrance by packaging and other fruit in the pallet system.

The dose distribution values, especially the minimum values indicate there are some differences between cultivars in dose distribution. This could be due to fruit physical characteristic such as rind thickness and sugar content. To address this variation the cartons in the pallet could be differently staked, e.g. leaving a central column in the stack open.

The dose ratios show that in a pallet irradiating system, such as used here, in order to reach the desired levels in each and every position a dose 4 times above the required minimum dose would have to be administered by the radiation source to ensure adequate levels in a worst case scenario, i.e. the carton in the middle of the pallet.

The variation seen between seasons also concurs with other studies and shows that if this technology is to be used commercially, serious consideration should be given to horticultural aspects such as maturity, cultivar selection and canopy management.

### Future research

The only research that is planned involves an entomological study.

### Technology transfer

Nothing has been communicated further at this stage.

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## 6 PROGRAMME: CULTIVAR AND ROOTSTOCK EVALUATION

### 6.1 PROGRAMME SUMMARY

By Andrew T.C. Lee (Manager: Cultivar Development)

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.

### 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 early and later. Satsuma x Nova looks promising as an early maturing selection. Primosole is early maturing but shows variable potential at this early stage. The commercial Dobashi Beni trees are not yet in production. A single trial site with all the late maturing selections has 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. Clemensons 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. Tardif de Janvier I bore well but with only medium fruit size and lowish acid. The Tardif de Janvier II also has medium fruit size and low acid and does not appear to be late maturing. Information on both selections is limited. Tardivo had poor production and medium small fruit size, good quality and green styler ends, maturing mid May to mid June. Nour production was not good with poor fruit size. Fruit quality was good with similar maturity to Tardivo. Rind colour is retarded and the selection does not look promising. Final evaluations are necessary on some of the selections.

A trial was laid out to ascertain whether certain Clementine mandarins could be produced commercially for export in the intermediate and cool inland citrus production areas of the country in accordance with market needs, as well as finding superior selections in terms of internal fruit quality, colour and fruit size. The first selection to harvest was Ain Toujdate, with only the juice content on SC below export minimum. The trees produced a good crop with damage to the branches because of the heavy crop loads. There was less cross pollination between the selections and less seeds per fruit this season.

*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. M37 had good quality, maturing in June. The Murcott x Clem semi commercial block bore its first small crop with large fruit size, quite acceptable quality and seedy fruit, maturing late June. Bay Gold had good production and fruit size but does not look promising in the cooler production areas due to high acid. 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. Cami had fair yields, good fruit size and high acid. Empress mandarin had poor production and fruit size and does not look promising. Some selections need to be evaluated further.

*Mandarins (Burgersfort):* To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap. Primosole was the earliest selection to mature this season. Unfortunately this selection seems to develop major problems in the cooler areas. Granulation, large fruit size, coarse rinds and low juice content occurred in this area. The Orighstad area may be more suitable to test Primosole. Hadass will be evaluated in Swaziland at Tambuti Estate the next season when the trees produce sufficient fruit for evaluations. Cami produced good yields and internal quality this season. The fruit was sweet with a nice juicy taste.

*Mandarins (Marble Hall):* Primosole is not suitable for the Marble Hall area because of too high heat units. Orighstad and other cooler areas may be more appropriate for this selection. Hadas is also not suitable for this area because of too low heat units. Hot areas may be more appropriate like Swaziland.

*Navels:* The aim is to find cultivars to spread the navel season both earlier and later and to minimise production peaks, also with more advanced rind colour, particularly at the commencement of the season and with improved fruit set potential in the desired fruit size range. Fukumoto is early maturing, similar, to slightly earlier than Lina/Newhall, developing a deep orange/red rind colour. There appears to be no direct evidence of incompatibility of Fukumoto on citrange hybrid rootstocks although Swingle citrumelo and Koethan citrange had some indications of possible abnormality. More clarity is needed on rootstock choice. Maturity of Letaba Early appears to be later than Lina/Newhall. Atwood had slightly later maturity than the Lina/Newhall and has delayed rind colour. Dream matures after Lina/Newhall and can be considered as a mid maturing selection. Cliff Early looks promising as an early maturing navel. Fenix and Sundays River Early do not appear to be early maturing and Krajewski Early not so early. Washington (CFB material) performed well with good production, fruit size and quality. Santa Catarina 1 and 3 are large and vigorous trees and bore few fruit. Cambria (CFB material) had good production and fruit size, round to elongated fruit shape and good quality but acid can tend to get low. Summer Gold is late maturing. Renken Late, Coetzee Late and Mouton Late 1 and 2 improved over last season and warrant further evaluations. The Witkrans (old selection) has similar characteristics to Royal Late. Glenora Late is late maturing and a vigorous tree and had good quality but some seed in a mixed block. A comparison between the Autumn Gold, Powell, Chislett and Californian Lane Late showed little difference between the selections. Fruit size tends to be on the large side in Citrusdal. Juice percentage can be low on Rough lemon/Rangpur lime rootstocks on sandy soil. Further evaluations of all the selections are necessary as some of the trees are still young.

*Navels (Burgersfort):* To optimise profitability by improving productivity (fruit set and size); pack out percentage (creasing and oleo resistance, smaller navel ends to counter mealy bug, *Alternaria* infection, less wind prone – time of flowering and inside bearing), fruit quality (rind colour early in the season, juice quality, granulation, low acidity) and extend the harvest and marketing season (early- mid and late maturing selections). Most of the selections evaluated did not comply with the minimum export standards. Low acid levels seem to be one of the common problems and reasons for this scenario. The trial will be evaluated for one more season that might foresee some explanations.

*Navels (Marble Hall):* All the selections evaluated in this trial did not comply with the minimum export standards. The large quantities of rainfall measured late in the season might be part of the problem for the decrease in fruit quality. The trial will be evaluated for one more season.

*Midseasons:* The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless. Tarocco production varied between sites and fruit size good. Quality was generally good but can be masked by high acid which could delay harvest. Tarocco Gallo and 57/1E/1 both had reasonable production with acceptable to good fruit size. Both had high acid. Gallo colour was behind Tarocco. All three older Tarocco selections have thorns of varying degrees and appear to lessen with ageing. There were only slight difference between the three commercially planted Maltaise selections. Maltaise Half II was slightly ahead of Maltaise Half, while Maltaise Barlerin the highest acid. Of concern with all three are the high acid levels. Raratonga had acceptable production, good fruit size, fair quality and tart. The trees are vigorous with large thorns. Further evaluations on all the Tarocco selections, Raratonga, Clara, Tacle and commercial Maltaise are necessary.

*Valencias:* The aim of the Valencia project is to find early, mid and late maturing Valencia selections that are seedless, have large fruit size and with improved fruit set ability compared to the existing range of selections. Various new selections were evaluated at the CFB. Limpopo seedless is the earliest to mature with acceptable quality off young trees and seedless without cross pollination. G5 also seedless where not cross pollinated and acceptable to good quality. Portsgate had borderline quality and not outstanding in any way except for virtual seedless in a mixed block. McClean Seedless looks similar to Valencia Late with fairly high acid and 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. Kleinhans was characteristic of the selection.

*Valencias (Onderberg):* 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). 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 by time, including the average fruit size. Alpha seems promising, with most of the other selections not complying with the minimum export standards.

*Knysna area:* The purpose of the trial is to find suitable, high quality, especially late maturing soft citrus cultivars for the Knysna area. Young Aoshima had poor quality. Bay Gold does not look promising due to high acid levels even when overmature. Sweet Spring bore a good crop of good fruit size but lack flavour. The Nouvelle was riddled with *Alternaria brown spot*. Kiyomi had excessive acid levels and performs better, although not outstanding in the Heidelberg area. Thoro Temple is not outstanding in any way and not recommended. The late maturing CELL, Clementarde and Clemlate clementine selections are similar and have certain production drawbacks, including small fruit size and low acid levels. Evaluations to continue on the satsuma and mandarin hybrids.

*Lemons:* To develop cold hardy, thornless, seedless (with acceptable fruit size) lemon selections which are compatible with a wider rootstock range; 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; 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 produce 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 151kg, followed by Fino 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 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. 'n Aantal nuwe Satsuma hibriede, bv. Satsuma x Nova en Primasole lyk belowend, maar is steeds in 'n eksperimentele stadium of geskikte voortplantings materiaal is nog nie beskikbaar nie.

*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. Buiten proewe in die Kaap areas, is daar ook verskeie proewe in die koel- en intermediere sitrus produksie areas van die land uitgelê om te verseker dat Clementine mandaryne kommersieel vir uitvoer aangeplant kan word, sowel as uitstekende nuwe seleksies in terme van interne kwaliteit te verseker. Verskeie Clementine seleksies soos byvoorbeeld Clemenpons, Tardif de Janvier I, Tardif de Janvier II, Tardivo, Nour, Sidi Aissa en Ain Taoujdat word tans ge-evalueer. Die Burgersfort laat Clementine proef is afgehandel en gereed vir publikasie.

*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. Koue, intermediere en warm sitrus areas word ingesluit in hierdie projek. Die Murcott x Clementine semi kommersieel blok het 'n goeie oes geproduseer hierdie seisoen en selfs vrugte met 'n lae saadtelling het goeie pryse in die Verre Ooste verdien as gevolg van die groot vruggrootte. Verskeie ander Mandaryn hibriede, soos byvoorbeeld Winola, Nectar, Hadas, Bay Gold, Cami en Tacle word ge-evalueer, maar tot op datum was resultate wisselvallig en verdere evaluasies oor 'n langer tydperk word vereis om hierdie kultivars deeglik te toets.

*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 effens vroeër ryp as Lina/Newhall en ontwikkel 'n diep oranje-rooi skilkleur. Daar is egter tekens van onverenigbaarheid by Fukumoto op citrange hibried onderstamme soos die geval is in California. 'n Nuwe seleksie is by kwarantyn ingehandig wat by die program ingesluit sal word so spoedig moontlik in 'n poging om die probleem op te los. Daar is verskeie Nawe seleksies wat ge-evalueer word soos Letaba Early, Atwood, Dream en Painter Early. Hierdie seleksies word vergelyk met Lina en Newhall. Ander kultivars soos bv. Fenix, Sundays River Early, Krajewski Early, Santa Catarina 1 en 3, Witkrans, Cambria, Powell, Autumn Gold, Glen Ora, Summer Gold, Chislett en Californian Lana Late word vergelyk met Washington en Palmer. Renken Late, Coetzee Late, Royal Late en Mouton Late 1 en 2 is ook seleksies wat verder ge-evalueer sal word. Verdere evaluasies van al hierdie seleksies is noodsaaklik, omdat baie van die bome nog

jonk is. Tot op datum het meeste van die seleksies in die proewe by Burgersfort en Marble Hall areas nie aan die minimum uitvoer vereistes voldoen nie. Lae suurvlakke is die hoofrede vir hierdie probleem. Hierdie proewe sal voortgaan.

*Midseisoene:* Die doel van die proef is om midseisoen seleksies, wat beter in die koeler streke sal aard in terme van vruggrootte, gepigmenteerde vleis en saadloosheid, te vind. 'n Aantal Tarocco seleksies word ge-evalueer insluitend Tarocco Gallo en 57/E/1. Daar was geringe verskille tussen die drie kommersieel aangeplante Maltaise seleksies, m.a.w. Maltaise Demi Sanguine II, Maltaise Demi Sanguine en Maltaise Barlerin. Raratonga was nie in hierdie proewe ingesluit nie.

*Valencias:* Die doel van die Valencia proef is om vroeër, mid en laat seleksies met groot vruggrootte, saadloos en verbeterde vrugset as alternatiewe vir die huidige seleksies te soek. Verskeie nuwe seleksies word in die hoof Valencia areas ge-evalueer. Ingesluit in hierdie proewe is Limpopo saadloos, G5, Portsgate, McClean saadloos, Rietspruit, Bend 8A 1 en 2, Delicia, Kleinhans, Alpha en Ruby. Evaluasies gaan voort.

*Knysna area:* Die doel van die proef is om geskikte, hoër gehalte, veral laat mandaryn kultivars te vind vir die Knysna area. Aoshima Satsuma, Bay Gold, Sweet Spring en Nouvelle word in hierdie area ge-evalueer.

*Heidelberg area:* Kiyomi, Thoro Temple en 'n aantal Clementine, Satsuma en Mandaryn hibriede word in hierdie proef ge-evalueer.

*Suurlemoene:* Kouegeharde, doring- en saadlose suurlemoenseleksies (met aanvaarbare vruggrootte), wat met 'n wye reeks onderstamme verenigbaar is, moet ontwikkel word. Die oes seisoen 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 (kleur, skildikte, sapinhoud) moet behou word. Die boomeienskappe en prestasie van nuwe kultivars soos Villafranca, Limoneira, Eureka SL (saadloos), Fino 49 en Verna word vergelyk met die kommersieel geproduseerde Eureka om die doelwitte te bereik.

#### 6.2.2 Evaluation of Mandarin hybrids in the cool inland areas

Experiment 73 A by J. Joubert (CRI)

#### Opsomming

Geskikte Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul. Primosole het weer groot, growwe vrugte geproduseer met 'n redelike goeie oes op die bome, maar hierdie seisoen was daar feitlik geen granulasie probleme nie. Daar word weereens beklemtoon dat hierdie seleksie in die koeler streke aangeplant word, bv. Orighstad. Bay Gold en Cami het 'n baie swak oes geset en daar was nie voldoende vrugte vir evaluasie doeleindes nie. Roma het hierdie seisoen as die beste opsie van al die betrokke seleksies gepresteer, en kan vir aanplantings oorweeg word. Die interne kwaliteit lyk baie belowend met goeie suur % (1.23) tot en met T1 kleurbreek.

#### Summary

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap. Primosole produced large, coarse fruit and a fairly good yield, but this season there was a very low incidence of granulation. This selection should do well in even cooler areas like Orighstad. Bay Gold and Cami produced a very light crop without sufficient fruit numbers to evaluate. Roma performed very promising and might be worthwhile to plant. Internally the acid content (1.23) remained fairly high towards peak maturity with external colour T1.

#### Introduction

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

#### Materials and methods

Field evaluations were conducted. Internal fruit analysis was conducted for the Burgersfort area during the 2007 season.

**Table 6.2.2.1.** List of mandarin selections evaluated during the 2007 season.

Selection	Site	Rootstock	Tree age	No. of trees
Bay Gold	Zalo Citrus	CC	2001	5
Cami	Zalo Citrus	CC	2001	3
Primosole	Zalo Citrus	CC	2001	5
Roma	Zalo Citrus	CC	2001	5

## Results and discussion

### Bay Gold

Trees were evaluated at Zalo Citrus (Burgersfort) in Mpumalanga during the 2007 season.

Alternative bearing resulted in a very light crop produced on the trees and by the time of evaluations there were insufficient fruit numbers to evaluate.

### Cami

With the visual evaluations early in the season, it was evident that the yield on the trees was very light and there would not be enough fruit for further evaluations.

### Primosole

This season Primosole produced a higher juice% (55.5%) in comparison to the 2006 season when granulation was a major problem. The fruit size varied from 1X to 1XXX, which is slightly on the large side. There was less granulation this season, resulting in higher juice content internally. The ideal climatic zone for this cultivar would be Orighstad and other cooler areas. Maturity middle to end of March.

### Roma

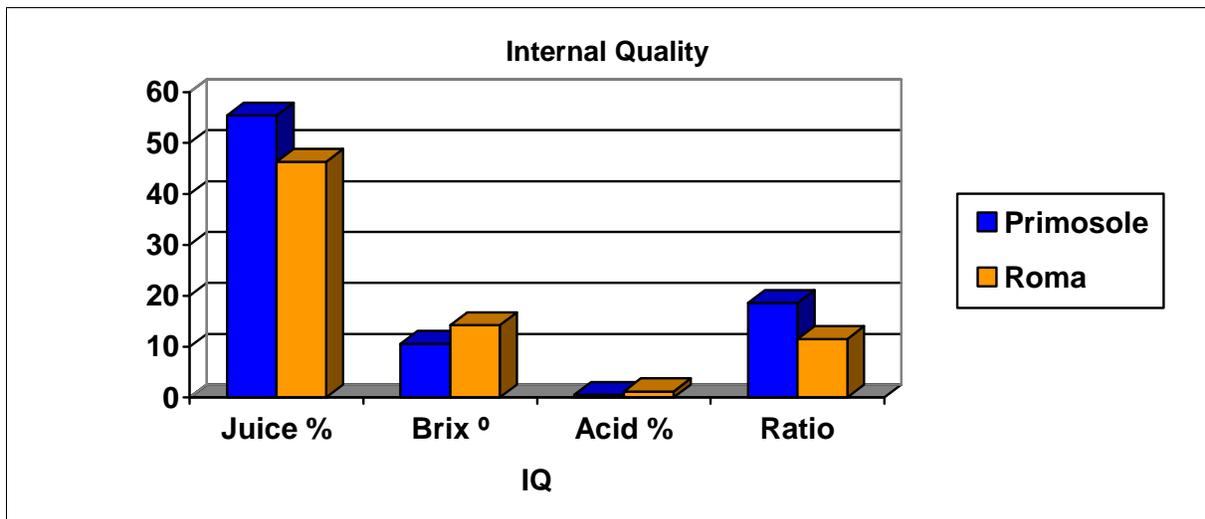
The average seed count per fruit was 9.2, with fruit size being larger than the previous season (1-1XXX). The number of seeds per fruit was much lower than last year probably due to lower pollination pressure. The fruit had a juicy sweet taste in comparison to the other selections. The internal quality was good, with the Brix° impressively high (14.2). Maturity middle to the end of May.

## Conclusions and recommendations

Primosole should be planted in cooler areas as coarse rinds, large fruit and granulation were a problem in the Burgersfort area. Roma performed well in this area and should also perform well in the cooler areas, based on the internal and eating quality of the fruit. This was the final evaluation.

**Table 6.2.2.2.** Internal fruit quality data for Mandarin selections for the cool inland areas during the 2007 season.

Selection	Root-Stock	Date harvested	Size mm	Count	Juice %	Brix Brix °	Acid %	Ratio	Ave. seed	Colour
Primosole	CC	16/04/2007	68-86	1X-1XXX	55.4	10.60	0.57	18.60	0.0	T1
Roma	CC	16/04/2007	59-72	2-1XX	47.9	13.80	1.82	7.6	8.6	T6
Roma	CC	24/05/2007	64-86	1-1XXX	46.3	14.20	1.23	11.5	9.2	T1-3



### 6.2.3 Evaluation of Mandarin hybrids in the cool inland areas Experiment 73 B by J.Joubert (CRI)

#### Opsomming

Geskikte Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul.

Primosole het hierdie seisoen weer bewys dat hierdie area nie geskik is vir die seleksie nie. Die kwaliteit van die vrugte sal baie verbeter in 'n koeler area. Cami het 'n swakker oes geproduseer in vergelyking met die vorige seisoen, maar die interne kwaliteit was belowend gewees. Intern was die vrug gereed vir oes, terwyl die eksterne kleur die proses vertraag het. Roma het goed presteer, maar die suur vlakke het vroeg relatief laag gedaal.

#### Summary

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

Primosole will produce better quality fruit when planted in a cool production area, for example Orighstad. Cami decreased in production in comparison with the previous season, but the internal quality remained promising. The external colour was delayed, although internally the fruit complied with the export qualities. The acid levels in the fruit produced by Roma dropped too low early in the season, shortening the shelf life of the fruit.

#### Introduction

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

#### Materials and methods

Field evaluations and internal fruit analyses were conducted in the Marble Hall area during the 2007 season.

**Table 6.2.3.1.** List of mandarin selections evaluated during the 2007 season.

Selection	Site	Rootstock	Tree age	No. of trees
Bay Gold	Moosrivier Estate	CC	2001	9
Cami	Moosrivier Estate	CC	2001	5
Imamura	Moosrivier Estate	???		7
Primosole	Moosrivier Estate	CC	2001	9
Roma	Moosrivier Estate	CC	2001	10

## Results and discussion

### Bay Gold

Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2007 season.

The trees produced a good yield, with medium to large fruit size (1X-1XXX). This season granulation was almost non-existent. The external skin texture was coarse, and the fruit shape similar to a Minneola. The acid content remained on the high side till harvest time, resulting in peak internal quality, but external colour was delayed. Maturity appears to be the end of May.

### Cami

Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2007 season. Internally the fruit matured well before external colour, which was delayed, and was evaluated for a third time to determine the peak maturity time. The trees produced a poor yield with medium to large fruit size (1-1XXX). The poor yield and lighter crop were probably the reason for the fruit size increase in comparison to the previous season when yields were good. This selection matures later and the internal quality was optimum by end of May, beginning of June.

### Imamura

This year was the first evaluation of the selection. There was not enough fruit to complete a fourth evaluation. After completing the third evaluation, the acid content remained above 1.8%, and the external colour varied from T6 to T7. Evaluations will continue, but this selection seems to be fairly late maturing at approximately the end of June.

### Primosole

Trees were evaluated at Moosrivier Estate (Marble Hall) in Mpumalanga during the 2007 season. Similar problems occurred in comparison with the previous season. Fruit size was large and rinds were coarse. The granulation problems were less than the previous season, but still unacceptable. Sunburn was a major problem this season, due to the higher number of heat units. This climatic zone is too warm for Primosole production; cooler areas might produce better fruit quality. Maturity end of March.

### Roma

The yield on the trees varied from poor to good with medium to large (3-1XXX) fruit size. Internally the fruit complied with the minimum export standards, but the external colour was delayed. By the time the external colour was between T3 and T4, the acid% was on the low side. Optimum time to harvest this selection appears to be in the beginning of May for this climatic zone.

## Conclusions and recommendations

Primosole will perform better in a cooler area where granulation and coarse rinds should not be a problem. The fruit size will also decrease with better yields on the trees.

Imamura was evaluated for the first time, and the selection seems to be late maturing (end of June). The Brix and juice content was acceptable, but the acid % remained high.

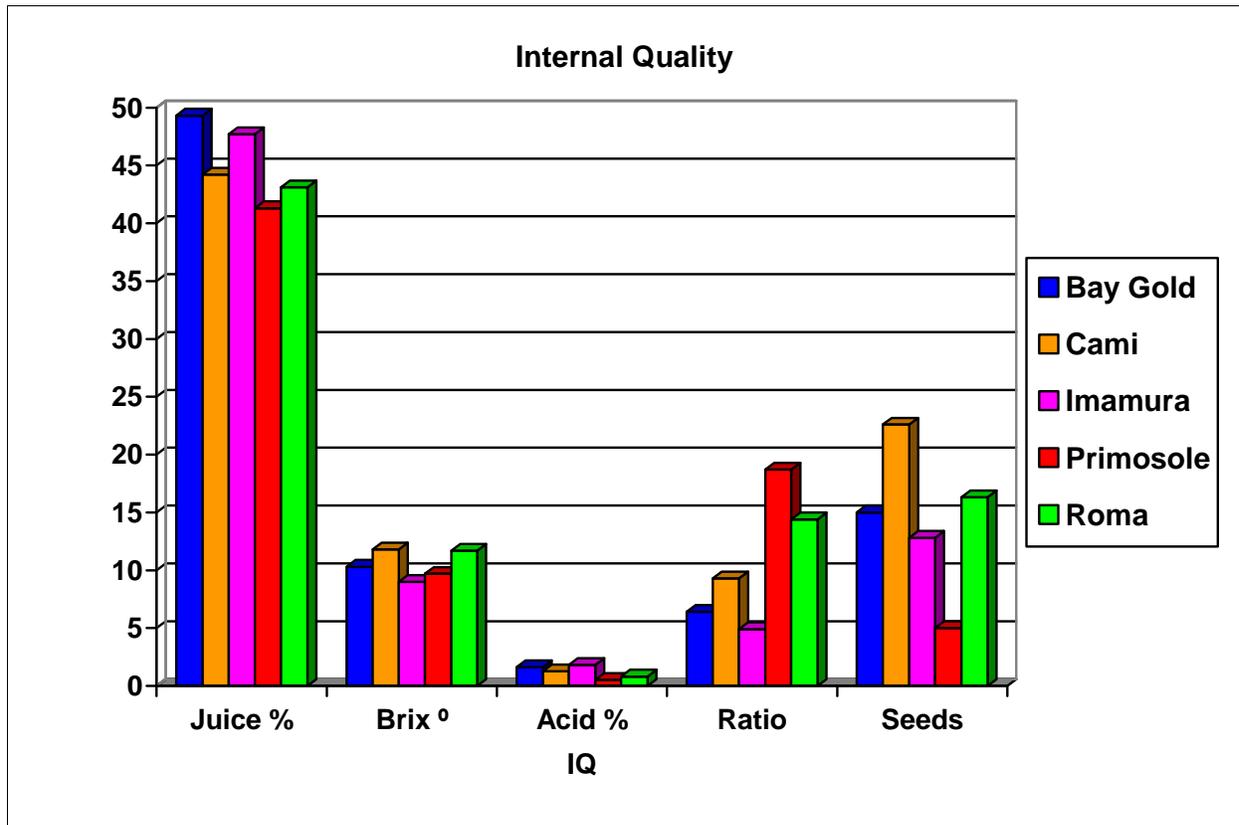
Roma seems to have a problem with low acid contents, but the Brix° and juice % were acceptable.

Last season Cami performed well in comparison to this season when yields were low, but the internal quality was acceptable.

**Table 6.2.3.2.** Internal fruit quality data for Mandarin selections for the cool inland areas during the 2007 season.

Selection	Root-Stock	Date harvested	Site	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Bay Gold	CC	11/04	Moosrivier	1-1XX	50.8	10.50	1.85	5.7	16.3	T7-8
Bay Gold	CC	07/05	Moosrivier	1X-1XXX	49.3	10.30	1.62	6.4	15.0	T4-5
Cami	CC	11/04	Moosrivier	3-1	51.1	11.20	1.71	6.5	18.9	T8
Cami	CC	07/05	Moosrivier	2-1XX	50.3	11.10	1.34	8.3	21.2	T7-8
Cami	CC	31/05	Moosrivier	1-1XXX	44.2	11.80	1.27	9.3	22.6	T4-5
Imamura	CC	11/04	Moosrivier	3-1X	51.9	8.70	3.02	2.9	13.1	T8
Imamura	CC	07/05	Moosrivier	1-1XX	52.8	9.00	2.09	4.3	14.4	T7-8
Imamura	CC	31/05	Moosrivier	2-1XX	47.7	9.00	1.82	4.9	12.8	T6-7
Primosole	CC	11/04	Moosrivier	1X-1XXX	41.3	9.70	0.52	18.7	5.0	T6-7

Roma	CC	11/04	Moosrivier	3-1X	45.0	10.30	1.20	8.6	16.8	T7-8
Roma	CC	07/05	Moosrivier	1-1XX	48.6	11.20	0.92	12.2	14.3	T5-7
Roma	CC	31/05	Moosrivier	1-1XXX	43.1	11.70	0.81	14.4	16.3	T3-4



#### 6.2.4 Evaluation of Navels in the cool inland areas Experiment 74 A by J. Joubert (CRI)

##### Opsomming

Wingsgewendheid moet verhoog word deur vrug gehalte en produksie te verbeter. Die klem moet gelê word op oes en vruggrootheid, pakpersentasies, kraakskil en oleo weerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, binnedrag en vruggehalte. Skilkleur vroeg in die seisoen, uitstekende sappehalte, geen granulasie, moet verbeter word, asook om die oes- en bemarkingseisoen deur vroeë-, middel- en laatrypwordende seleksies te verleng. Meeste van die seleksies het hierdie seisoen aan die minimum uitvoer standaard voldoen, met die uitsondering van Bahianinha en Fukumoto met lae sap %. Die vorige seisoen was hierdie tendens die teenoorgestelde gewees, met meeste seleksies wat nie uitvoer standaard gehaal het nie. Dream en Powell Summer het besonder goed gevaar, en kan sterk oorweeg word met nuwe nawel aanplantings vir die toekoms.

##### Summary

Profitability needs to be optimised by improving productivity and size, raising pack out percentages by reducing creasing and oleo, selecting for smaller navel ends to counter mealy bug and *Alternaria* infection, and selecting for less wind prone cultivars. In addition improved fruit quality is required with earlier rind colour development, excellent juice quality, no granulation and acceptable acid levels. Extension of the the harvest and marketing season must be addressed by these early- mid and late maturing selections. This season the opposite scenario occurred to 2006, with most of the cultivars complying with the minimum export standards, except for Bahianinha and Fukumoto which produced low acid levels. Dream and Powell Summer performed very well, producing very good quality fruit. These selections must be considered when planning new orchard plantings.

## Introduction

To find suitable Navel selections for the cool inland citrus production areas to fill the early, mid and late season gap.

## Materials and methods

Field evaluations and laboratory analysis were conducted on Atwood, Autumn Gold, Bahianinha, Cara Cara, Dream, Fukumoto, Powel Summer and Tulegold selections at Zalo Citrus (Burgersfort), a site in the cool inland production area.

Internal quality data were compared with the minimum average export requirements for navels: 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

**Table 6.2.4.1.** List of Navel selections evaluated at Zalo Citrus (Burgersfort) during the 2007 season.

Selection	Rootstock	Tree age	No. of trees
Atwood	CC	2001	5
Autumn Gold	CC	1999	5
Autumn Gold	SC	1999	5
Bahianinha	SC	2001	4
Cara Cara	CC	2001	8
Dream	CC	2001	9
Fukumoto	CC	2001	3
Powel Summer	CC	1999	5
Powel Summer	SC	1999	5
Tulegold	CC	2001	9

## Results and discussion

### Atwood

The selection produced an average yield for this season, with medium to large fruit size. By the time of harvest the fruit complied with internal minimum export standards. Atwood matured well with a Brix° of 13 and juice content of 52.3%. Fibre content was fairly high, but not unacceptable. Maturity was end of April, beginning of May.

### Autumn Gold (Late maturing Navel)

The yield produced on both CC and SC were average, with medium to large (count 40-88) fruit size on the trees. SC produced a higher Brix° of 13.2 and acid content of 1.39 in comparison to CC with a Brix of 12.6 and acid content of 1.09. The external colour on both was similar by the time of internal maturity, between T1 and T3. Maturity appears to be middle to end of June.

### Bahianinha

There was adequate fruit on the trees for one evaluation, with small to medium fruit size. The juice % was below export standard, but the Brix content improve to a 13.5 reading. Bahianinha fruit contained high acid levels during the first evaluation, which may well have been too early for this selection. Externally the colour varied between T5 and T6, explaining probably why the juice content was still on the low side. Maturity might be end of May.

### Cara Cara

Cara Cara produce fruit with low acid levels, reducing the shelf life of the fruit after harvest. It will be wise to pick the fruit fairly early in the season before acid levels drop. The internal colour was not that intense in comparison to the warmer areas, where a deeper red internal colour was achieved. Cara Cara produced an excellent yield on the trees, with medium to large fruit. Maturity middle of May.

### Dream

Dream produced a good yield on the trees, with medium to large (count 48-72) fruit size. This selection appears very promising, with good internal quality and exceptional flavour. Internally the fruit complied with minimum export standards. The fruit shape on average was round, but at least 10% was elongated. Maturity middle to end of May.

### Fukumoto

The trees produced an average yield, with medium to large (48-72) fruit size. Internally the juice, Brix and acid content were on the low side, below the minimum export standards required. There are better selections in the pipeline. CRI is presently cleaning new material through shoot tip grafting, and evaluations will then continue. Maturity middle of April for this selection.

### Powel Summer (Late maturing Navel)

Powell Summer performed very similar on both CC and SC, with a poor to average yield produced on the trees. Internally the fruit complied with the minimum export standards, with reasonable high Brix values ranging from 13.0 to 13.6. The Brix values exceeded those of Autumn Gold at the same time, giving Powell Summer a more acceptable flavour. There was no marked difference between the external colour of the fruit by the time of harvest between CC and SC. Maturity middle to end of June for this climatic area.

### Tulegold

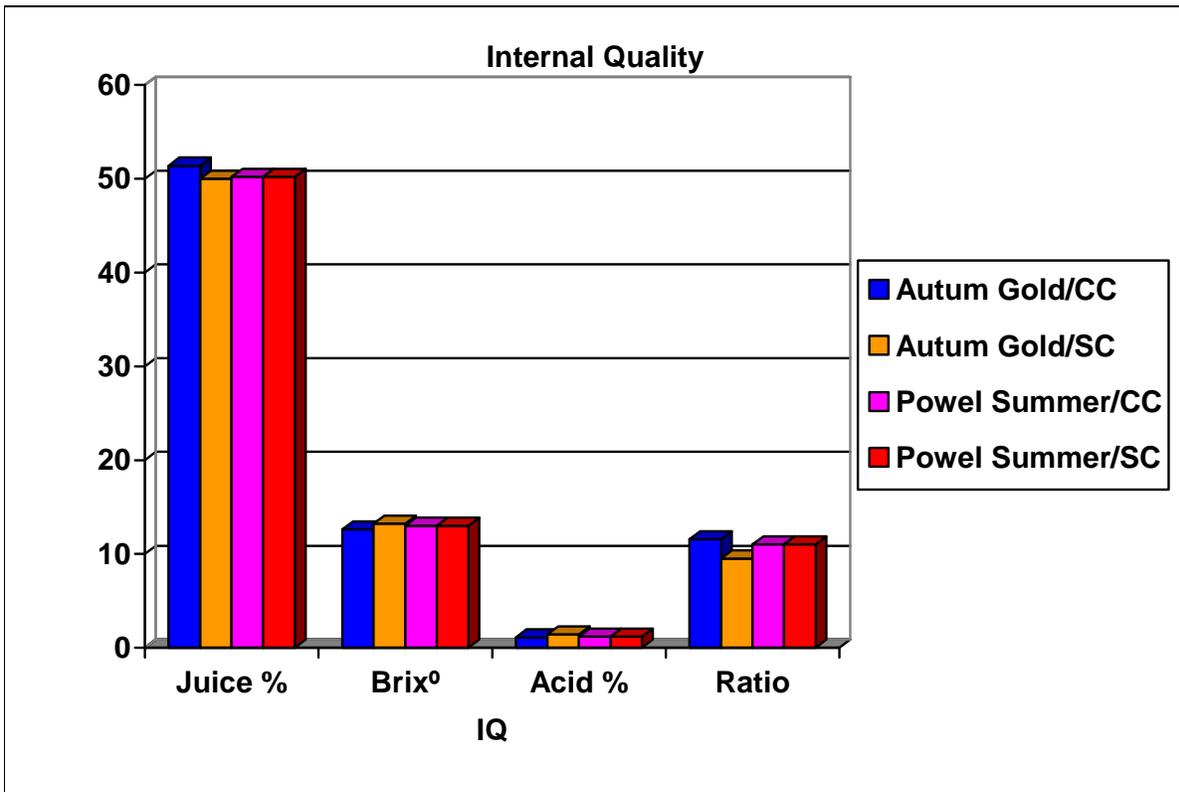
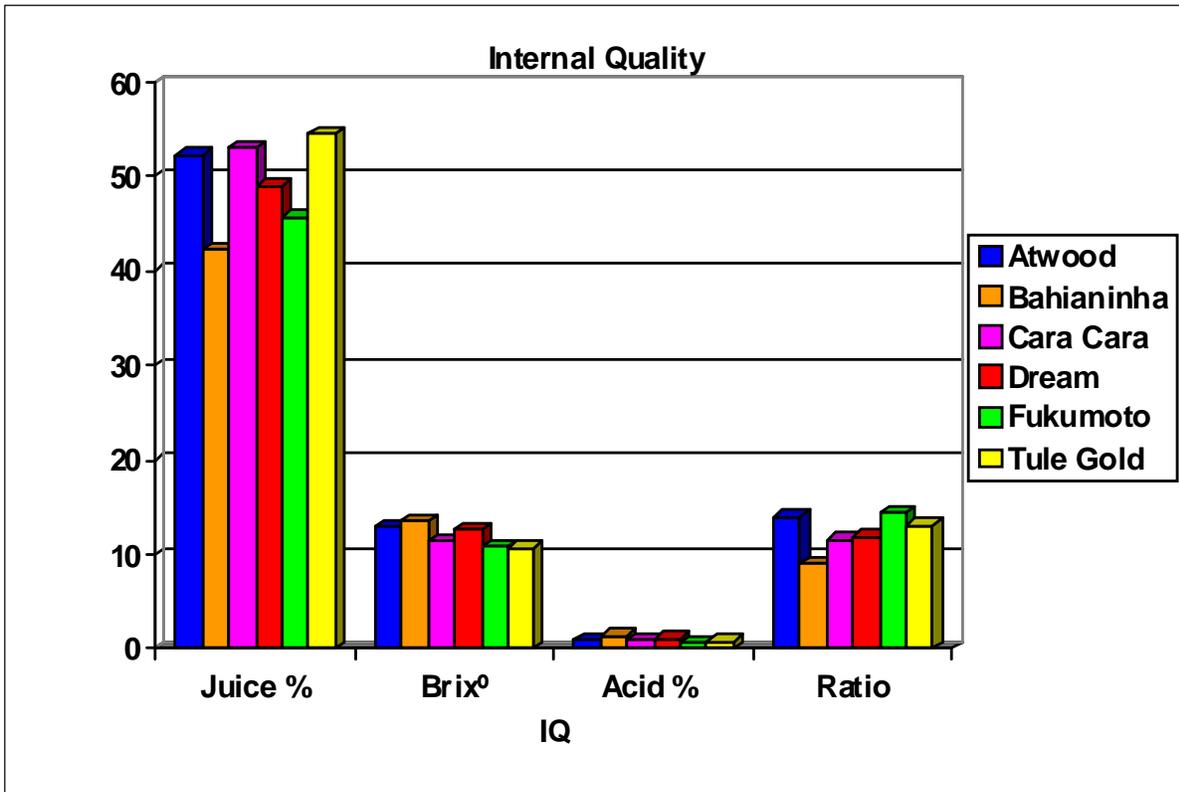
Trees were evaluated at Zalo Citrus, Burgersfort in Mpumalanga during the 2007 season. Tulegold produced a good yield on the trees, with medium to large (count 40-88) fruit size. The trees are semi dwarfed in this trial at Burgersfort, in comparison to the trees at Moosrivier with a medium tree size, similar to the other mid-maturing navel selections such as Atwood and Dream. The acid and Brix content internally was on the low side, with the highest juice % (54.5%) tested for this trial. Maturity middle to end of May.

## **Conclusions and recommendations**

The low acid content problem from last season improved dramatically this season, with only Cara Cara, Fukumoto and Tulegold testing on the low side. In some cases timing might be the reason for this occurrence, although all selections complied with the minimum export standards for acid content. The incompatibility status of Fukumoto on CC and SC has not yet been resolved and this selection remains experimental. There were no changes in the condition of the trees at Zalo Citrus as the green tips above the bud union remain similar in size and numbers to the previous season. The evaluations were completed this season and new selections will be planted or top worked in this trial site for future evaluations

**Table 6.2.4.2.** Internal fruit quality data for Navel selections at Zalo Citrus (Burgersfort), a cool inland production area, during 2007.

<b>Selection</b>	<b>Root-stock</b>	<b>Date harvested</b>	<b>Count</b>	<b>Juice %</b>	<b>Brix °</b>	<b>Acid %</b>	<b>Ratio</b>	<b>Ave. seed</b>	<b>Colour</b>
Atwood	CC	16/04	72-48	48.0	11.50	1.01	11.39	0.0	T5-6
Atwood	CC	24/05	72-40	52.3	13.00	0.93	13.98	0.0	T2
Autum Gold	CC	24/05	72-48	46.3	11.80	1.04	11.35	0.0	T4-6
Autum Gold	CC	06/06	88-40	48.2	12.00	1.08	11.11	0.0	T3-4
Autum Gold	CC	28/06	64-48	51.4	12.60	1.09	11.56	0.0	T1-3
Autum Gold	SC	24/05	88-48	47.3	12.50	1.47	8.50	0.0	T5-6
Autum Gold	SC	06/06	88-48	47.1	12.50	1.46	8.56	0.0	T3-5
Autum Gold	SC	28/06	88-48	50.0	13.20	1.39	9.50	0.3	T1-2
Bahianinha	SC	16/04	88-56	42.3	13.50	1.50	9.00	0.0	T5-7
CaraCara	CC	16/04	72-56	47.2	10.40	0.98	10.61	0.0	T1-2
CaraCara	CC	24/05	72-48	53.0	11.50	0.99	11.62	0.0	T2-4
Dream	CC	16/04	88-56	46.2	11.90	1.17	10.17	0.0	T5-6
Dream	CC	24/05	72-48	49.1	12.60	1.06	11.89	0.0	T1-4
Fukumoto	CC	16/04	72-48	45.7	10.90	0.75	14.53	0.1	T4-5
Powel Summer	CC	24/05	72-48	48.9	12.30	1.22	10.08	0.3	T5
Powel Summer	CC	06/06	72-48	48.4	12.70	1.30	9.77	0.0	T4-5
Powel Summer	CC	28/06	72-48	50.2	13.00	1.18	11.02	0.0	T1-2
Powel Summer	SC	24/05	72-40	45.8	12.30	1.30	9.46	0.0	T5-6
Powel Summer	SC	06/06	72-56	48.2	12.80	1.44	8.89	0.0	T3-6
Powel Summer	SC	28/06	72-48	50.5	13.60	1.36	10.00	0.0	T1-2
Tule Gold	CC	16/04	88-40	52.9	9.80	0.86	11.40	0.0	T6-7
Tule Gold	CC	24/05	88-40	54.5	10.70	0.82	13.05	0.0	T2-4



## 6.2.5 Evaluation of Navels in the intermediate inland area

Experiment 74 B by J. Joubert (CRI)

### Opsomming

Wingsgewendheid moet verhoog word deur vrug gehalte en produksie te verbeter. Die klem moet gelê word op oes en vruggrootte,, pakpersentasies, kraakskil en oleo weerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, binnedrag en vruggehalte. Skilkleur vroeg in die seisoen, uitstekende sappehalte, geen granulasie, moet verbeter word, asook om die oes- en bemarkingseisoen deur vroeë-, middel- ae laatrypwordende seleksies te verleng. Die suur vlakke van meeste seleksies het te laag gedaal en nie aan die uitvoer standaardte voldoen nie. Dream toon goeie potensiaal, en met gunstige klimaatstoestande kan goeie kwaliteit vrugte geproduseer word.

### Summary

Profitability needs to be optimised by improving productivity and size, raising pack out percentages by reducing creasing and oleo, selecting for smaller navel ends to counter mealy bug and *Alternaria* infection, and selecting for less wind prone cultivars. In addition improved fruit quality is required with earlier rind colour development, excellent juice quality, no granulation and acceptable acidlevels. Extension of the the harvest and marketing season must be addressed by tese of early- mid and late maturing selections. The acid levels on most of the selections dropped below the minimum requirements. Dream seems promising, and with favourable climatic conditions will produce good quality fruit on the trees.

### Introduction

To find suitable Navel selections for the intermediate inland citrus production areas to fill the early, mid and late season gap.

### Materials and methods

Field evaluations and laboratory analysis were conducted on Atwood, Cara Cara, Dream, Fukumoto and Tulegold selections at Moosrivier Estate (Marble Hall), an intermediate production area.

Internal quality data were compared with the minimum average export requirements for navels: 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

**Table 6.2.5.1.** List of Navel selections evaluated at Moosrivier Estate (Marble Hall) during 2007.

Selection	Rootstock	Tree age	No. of trees
Atwood	CC	2001	5
Cara Cara	CC	1997	Semi-Com.
Dream	CC	2001	9
Fukumoto	CC	2001	3
Tule Gold	CC	2001	9

### Results and discussion

#### Atwood

Atwood produced fruit with a low acid content from early in the season. The juice content (44.6%) by the time of harvest was below the minimum export standards, with the external colour ranging from T2 to T4. Yield production on the trees varied from poor to good, probably caused by alternative bearing patterns. Maturity seems to be middle to end of May.

#### Cara Cara

All the trees were harvested well before the peak maturity time, probably to set a better crop next season. Unfortunately there were no fruit to evaluate for this season.

#### Dream

Dream produced an average yield on the trees. The low acid content seems to be a general problem on all the selections, with Dream being no exception on the rule. The juice and Brix content tested below the minimum export standards for this season. Harvest time for Dream in this climatic area is end of April.

### Fukumoto

The fruit size on the trees varied from large to extra large (count 64-40). Internally the juice and Brix content comply with the minimum export standards, but the acid content (0.55%) was below the minimum standard. There was a delay on the external colour by the time of harvest measuring between T3 and T5. There are better selections in the pipeline that will be evaluated for future recommendations. Maturity middle of April.

### Tulegold

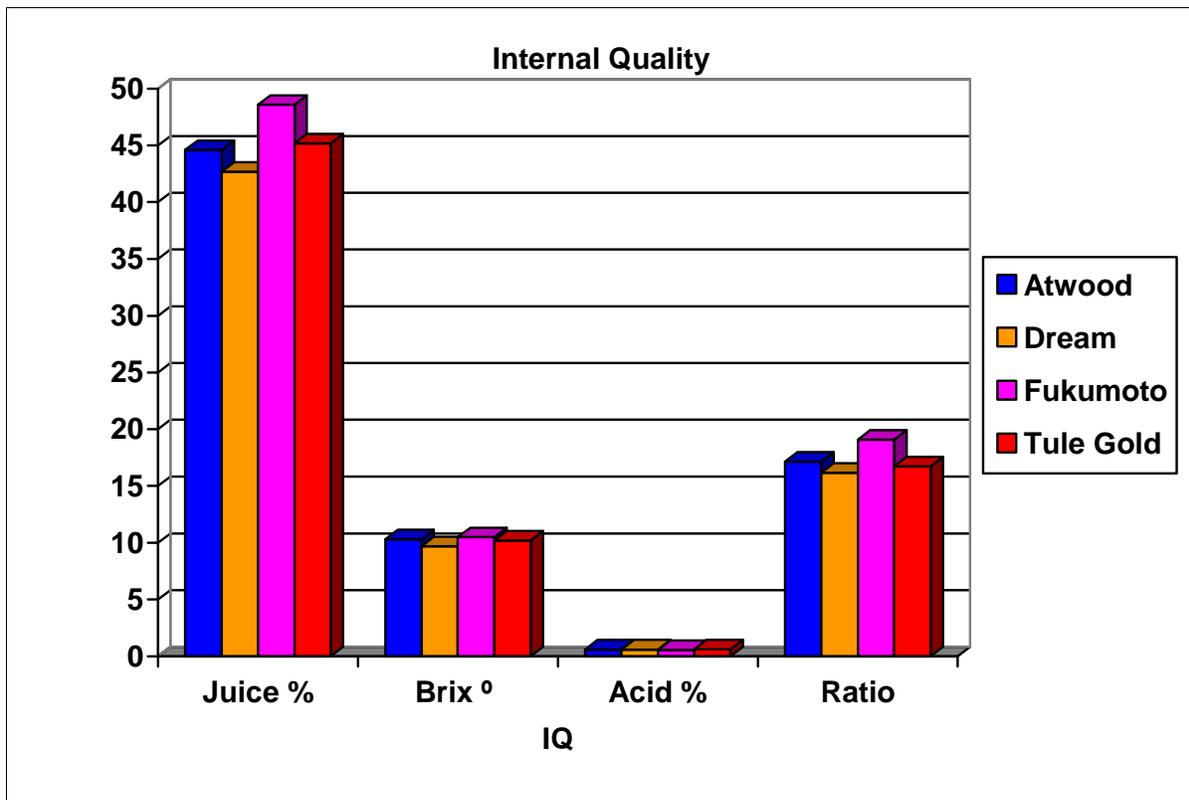
Brix (10.2) was the only internal factor to comply with the minimum export standards, with acid (0.61) and juice (45.2%) levels well below minimum. The trees produced a good yield with medium (count 72-56) fruit size. The trees in the Burgersfort area performed better in comparison to the trees in the Marble Hall area. Maturity middle to end of May.

### **Conclusions and recommendations**

No selection complied with the minimum export standards this season, similar to the previous season. The fruit size varied from medium to large. In comparison with the trial at Burgersfort, the internal quality decreased. Dream seems promising, bearing in mind the internal quality problems, but had acceptable fruit size. By managing the selection carefully, good yields and production will be possible. This was the last evaluation of the trial, and in future new selections will be planted.

**Table 6.2.5.2.** Internal fruit quality data for Navel selections in the intermediate inland area (Moosrivier Estate, Marbel Hall) during the 2007 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Atwood	CC	11/04	73-88	72-48	51.7	10.10	0.83	12.17	0.0	T6-7
Atwood	CC	07/05	80-91	64-40	50.4	10.00	0.64	15.63	0.0	T6
Atwood	CC	31/05	78-99	64-36	44.6	10.30	0.60	17.17	0.0	T2-4
Dream	CC	11/04	76-88	72-48	49.1	8.90	0.69	12.90	0.0	T6-7
Dream	CC	07/05	79-96	64-40	50.7	9.70	0.67	14.48	0.0	T5-7
Dream	CC	31/05	78-92	64-40	42.7	9.70	0.60	16.17	0.0	T3-4
Fukumoto	CC	11/04	77-89	72-48	48.6	9.40	0.62	15.16	0.0	T5-6
Fukumoto	CC	07/05	78-93	64-40	48.6	10.50	0.55	19.09	0.0	T3/4/5
Tule Gold	CC	11/04	74-79	72-64	48.1	9.30	0.63	14.76	0.0	T5-6
Tule Gold	CC	07/05	76-82	72-56	49.5	9.60	0.55	17.45	0.5	T5-6
Tule Gold	CC	31/05	76-82	72-56	45.2	10.20	0.61	16.72	0.0	T2-3



#### 6.2.6 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 vruggroottesverspreiding (telling 48, 56 en 72); beter skilkleur, uitwendige voorkoms en skikbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke). Alpha het weer baie potensiaal hierdie seisoen getoon met goeie interne kwaliteit vrugte. Van die belangrike vrug eienskappe was dun skille, hoë sap inhoud en intense geel intern kleur. Glen Ora Late het ook goed presteer, met goeie interne kwaliteite en optimum vruggroote. 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 selections (for hot areas). Alpha performed very well this season and produced good internal quality fruit. The fruit contained a high juice %, produced thin rinds with intense yellow internal colours. Glen Ora Late once again performed very well, with good internal qualities and optimum fruit size. Evaluations will continue the next season.

##### Introduction insert

To find suitable Valencia selections for the hot inland citrus production areas with superior qualities.

##### Materials and methods

Field evaluations and laboratory analysis were conducted on Alpha (Rietspruit), Glen Ora Late, Maritz Early, McClean SL, Midnight, Ruby Valencia and Turkey (control) at Esselen Nursery, Malelane.

**Table 6.2.6.1.** Internal fruit quality data was compared with the 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
Midknight	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34
Delta Seedless	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 6.2.6.2.** List of Valencia selections evaluated at Esselen Nursery (Malelance) during 2007.

Selection	Rootstock	Tree Age	No. of trees
Alpha (Rietspruit)	CC	1996	1
Glen Ora Late	CC	2000(Top)	3
Ruby Val	CC		1
Turkey	C35	1998	1

## Results and discussion

### Alpha

Alpha produced a good yield with medium to large (count 88-48) fruit size on the trees. The internal quality of the fruit was excellent and complied to the minimum export standards, with 61.6% juice, 11.5° Brix and 1.33 acid. Qualities associated with the fruit were thin rind, dark yellow internal colour and high juice content. Alpha produced 0.6 seeds per fruit on average. Maturity middle to end of June.

### Glen Ora Late

Trees were evaluated at Esselen nursery (Malelane) in Mpumalanga during the 2007 season. The seed quantity was similar to Alpha, also counting 0.6 seeds per fruit on average. Glen Ora produced a good yield, with medium to large (count 40 to 72) fruit size. Internally the quality was excellent, complying to the minimum export standards with 60.7% juice, 10.5° Brix and 1.37 acid. Maturity end of June.

### Ruby

There were insufficient numbers of fruit on the tree to evaluate. Evaluations will continue next season.

### Turkey

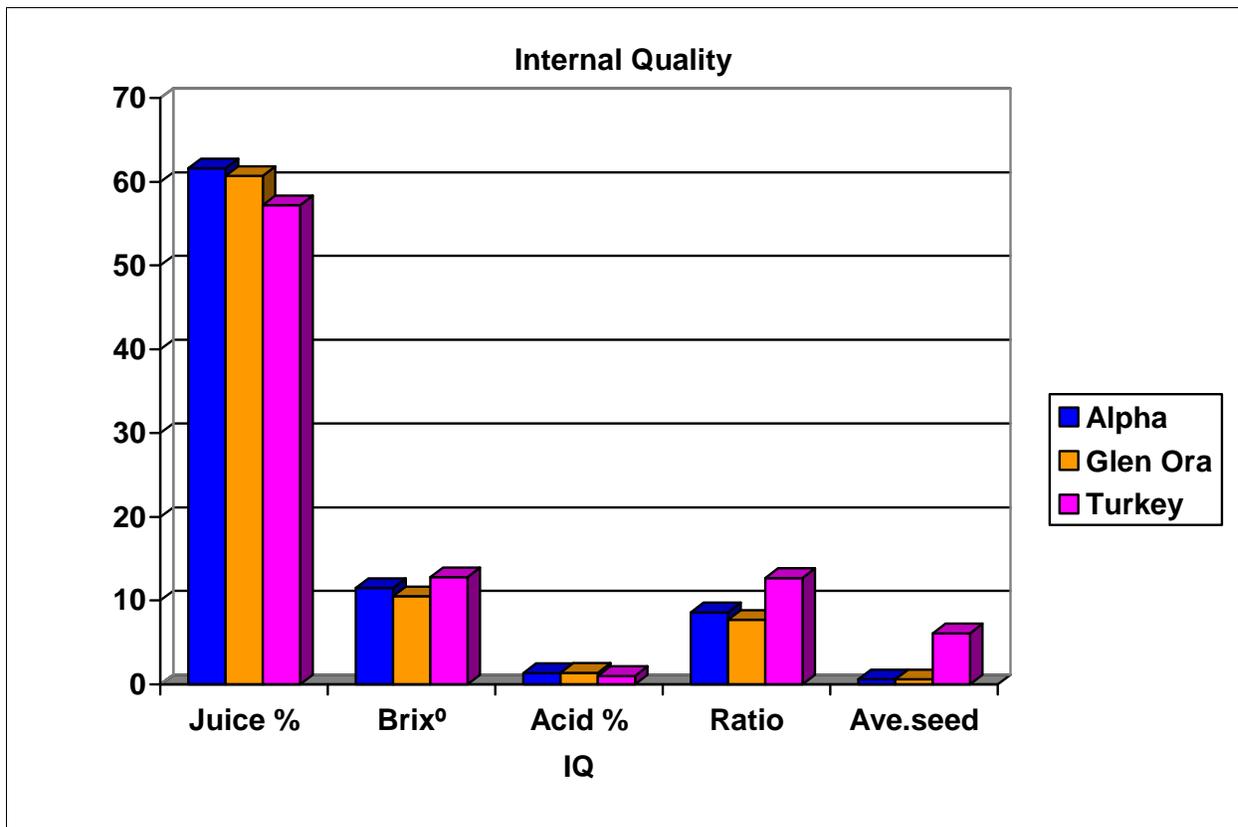
Internally the fruit complied with the minimum export standards, and the trees produced a good yield. The fruit size varied from small to medium, and the external rind texture was very smooth. Peelability of the fruit is fairly easy, and the internal texture soft. Maturity from end of May to middle of June.

## Conclusions and recommendations

All the selections evaluated complied with the export standards, and Alpha performed the best for this trial site, followed by Glen Ora Late and Turkey. Hopefully there will be enough fruit to evaluate the next season on Ruby, because the new seedless selection appears to be very promising. Evaluations will continue.

**Table 6.2.6.3.** Internal fruit quality data for Valencia orange selections at Esselen Nursery (Malelane) during the 2007 season.

Selection	Root-stock	Date harvested	Size mm	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Alpha	CC	27/06	72-86	88-48	61.6	11.50	1.33	8.6	0.6	T1-2
Glenora Late	CC	27/06	75-91	72-40	60.7	10.50	1.37	7.7	0.6	T1-3
Turkey	CC	27/06	74-82	72-56	57.2	12.80	1.01	12.7	6.1	T1-2



**6.2.7 Evaluation of Lemon selections in the inland areas**  
Experiment 79 by J. Joubert (CRI)

**Opsomming**

Kouegeharde, doring- en saadlose suurlemoenseleksies met aanvaarbare vruggrootte, wat met 'n wye reeks onderstamme verenigbaar is, 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 in die koers van 40% sap geproduseer. Villafranca produseer steeds die laagste saad telling per vrug (0 sade per vrug), gevolg deur Verna met 8.6 sade per vrug.

Die hoogste produksie per boom was op Eureka saadloos (Israel) geproduseer (127.11kg/boom), en die laagste produksie op Verna met 24.65 kg/boom.

**Summary**

To develop cold hardy, thornless, seedless lemon selections with acceptable fruit size which are compatible with a wider rootstock range; 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). Tree characteristics and performance of new cultivars were compared with the commercially grown Eureka to meet these objectives.

All the selections evaluated in this trial produced juice content in the region of 40%. Villafranca still produced the lowest seed count (0 seeds/fruit) followed by Verna with 8.6 seeds per fruit.

The best production per tree was on Eureka SL (Israel) (127.11kg/tree), and the lowest on Verna with 24.65kg per tree.

## Introduction

To find suitable Lemon selections for the hot inland citrus production areas with superior qualities.

## 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 various rootstocks.

**Table 6.2.7.1.** List of lemon cultivars evaluated at Tekwane (Karino area) during the 2007 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

\* Esselen Nursery, Malelane

## Results and discussion

### Eureka SL (ARC)

Good production of seedless fruit at Esselen Nursery.

### Tekwane Estates

Fino 49 produced the highest juice content (40.5%) for this season, followed by Villafranca (39.7%) and Eureka SL (39%). The highest average seed count per fruit was on Fino 49 (21.4 seeds/fruit), and the lowest seed count on Villafranca (0 seeds/fruit). The fruit size on all the selections peaked at count 144, except for Villafranca between count 105 and 125. Eureka seedless (Israel) produced the best crop (127.11 kg/tree) per tree, followed by Fino 49 (98.09 kg/tree) and Limoneira (84.72 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 choosing the best option for your personal situation.

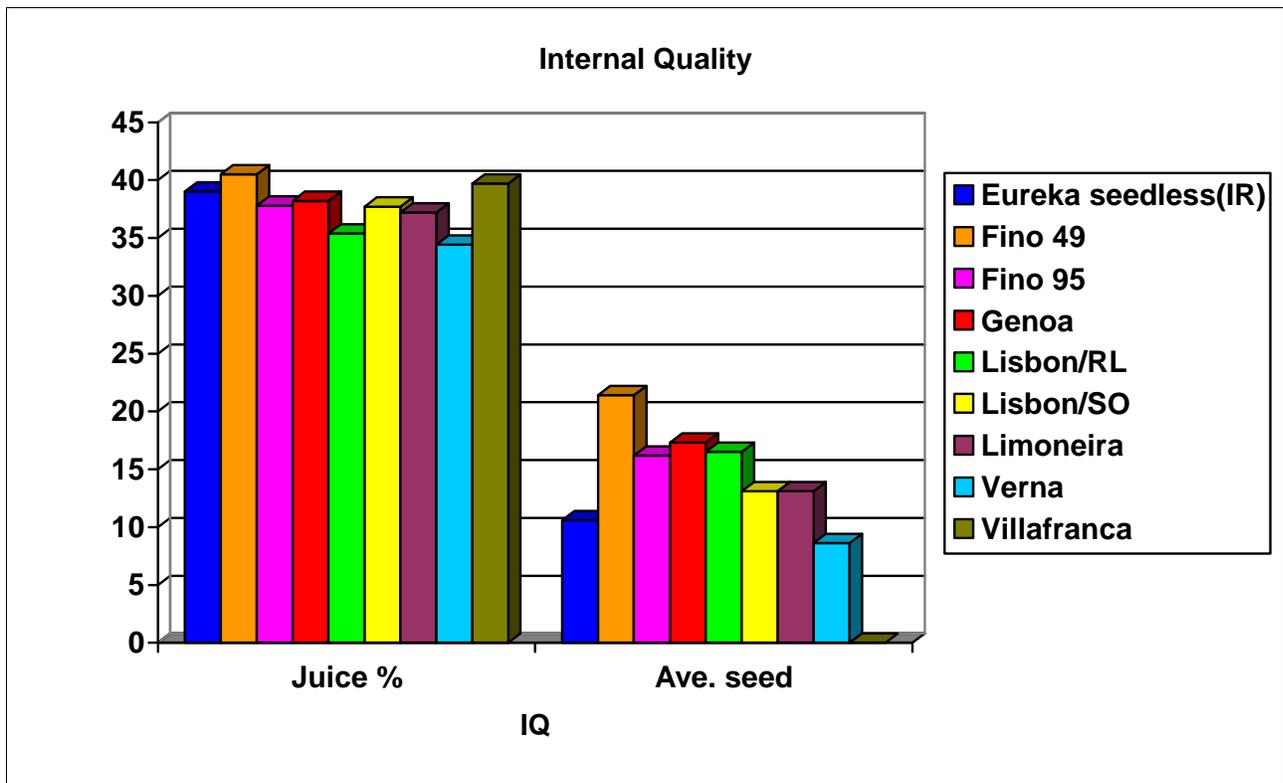
**Table 6.2.7.2.a.** Internal fruit quality data for Lemons from Tekwane Estate (Karino) during the 2007 season.

Selection	Root-stock	Date harvested	Juice %	Ave. seed
Eureka seedless(IR)	RL	10/04	41.4	6.7
Eureka seedless(IR)	RL	04/05	41.0	9.3
Eureka seedless(IR)	RL	19/06	36.1	9.6
Eureka seedless(IR)	RL	26/07	37.6	16.9
Fino 49	RL	10/04	40.1	18.1
Fino 49	RL	04/05	42.4	19.8
Fino 49	RL	19/06	38.7	26.3
Fino 49	RL	26/07	40.5	21.3
Fino 95	RL	10/04	38.3	15.5

Fino 95	RL	04/05	38.5	15.8
Fino 95	RL	19/06	38.7	13.7
Fino 95	RL	26/07	35.6	19.6
Genoa	RL	10/04	41.0	14.2
Genoa	RL	04/05	40.5	16.3
Genoa	RL	19/06	35.0	22.0
Genoa	RL	26/07	36.1	16.5
Lisbon	RL	10/04	37.3	12.8
Lisbon	RL	04/05	37.2	17.7
Lisbon	RL	19/06	32.9	13.9
Lisbon	RL	26/07	34.1	21.6
Lisbon	SO	10/04	39.5	12.2
Lisbon	SO	04/05	39.7	12.9
Lisbon	SO	19/06	35.0	14.1
Lisbon	SO	26/07	36.5	13.2
Limoneira	RL	10/04	39.4	7.7
Limoneira	RL	04/05	37.6	17.8
Limoneira	RL	19/06	36.4	17.4
Limoneira	RL	26/07	35.5	9.3
Verna	RL	10/04	34.4	8.6
Verna	RL	04/05	32.9	3.9
Verna	RL	19/06	32.5	6.6
Verna	RL	26/07	37.9	15.4
Villafranca	RL	10/04	42.9	0.0
Villafranca	RL	04/05	38.3	0.0
Villafranca	RL	19/06	38.9	0.1
Villafranca	RL	26/07	38.6	0.0

**Table 6.2.7.2.b.** Average internal fruit quality data for Lemons from Tekwane Estate (Karino) during the 2007 season.

<b>Selection</b>	<b>Root-stock</b>	<b>Juice %</b>	<b>Ave. seed</b>
Eureka seedless(IR)	RL	39.0	10.6
Fino 49	RL	40.5	21.4
Fino 95	RL	37.8	16.2
Genoa	RL	38.2	17.3
Lisbon	RL	35.4	16.5
Lisbon	SO	37.7	13.1
Limoneira	RL	37.2	13.1
Verna	RL	34.4	8.6
Villafranca	RL	39.7	0.0



**Table 6.2.7.3.** Fruit size distribution of Lemons on different rootstocks at Tekwane Estate (Karino) during the 2007 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Eureka seedless(IR)	RL	48	0.00	Lisbon	SO	48	0.00
Eureka seedless(IR)	RL	56	0.69	Lisbon	SO	56	0.00
Eureka seedless(IR)	RL	72	3.94	Lisbon	SO	72	0.18
Eureka seedless(IR)	RL	88	9.40	Lisbon	SO	88	1.41
Eureka seedless(IR)	RL	115	40.24	Lisbon	SO	115	23.41
Eureka seedless(IR)	RL	144	45.72	Lisbon	SO	144	75.00
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Fino 49	RL	48	0.03	Limoneira	RL	48	0.00
Fino 49	RL	56	0.10	Limoneira	RL	56	0.19
Fino 49	RL	72	1.51	Limoneira	RL	72	0.62
Fino 49	RL	88	4.83	Limoneira	RL	88	1.42
Fino 49	RL	115	35.36	Limoneira	RL	115	25.91
Fino 49	RL	144	58.16	Limoneira	RL	144	71.86
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Fino 95	RL	48	0.00	Verna	RL	48	0.00
Fino 95	RL	56	0.09	Verna	RL	56	0.13
Fino 95	RL	72	0.68	Verna	RL	72	1.25
Fino 95	RL	88	2.97	Verna	RL	88	3.76
Fino 95	RL	115	24.84	Verna	RL	115	29.20
Fino 95	RL	144	71.42	Verna	RL	144	65.66
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Genoa	RL	48	0.00	Villafranca	RL	48	0.28
Genoa	RL	56	0.22	Villafranca	RL	56	3.58



6.2.8 **Evaluation of Valencia selections in the hot inland areas (Swaziland)**  
Experiment 740A 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 bome het hierdie seisoen baie beter presteer, met Alpha, McClean SL en Tambuti Early wat uitgestaan het bo die ander seleksies. Delta as kontrole het nie so 'n goeie oes op die bome geproduseer in vergelyking met die ander drie seleksies nie. Mouton Early is verwyder a.g.v. Appelstam groef virus, want verdere besmetting van die res van die boorde kan meganies plaasvind.

**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 trees performed very well this season, with Alpha, McClean SL and Tambuti Early outperforming the other selections. Delta as the control did not perform as well as the other three selections. Mouton Early was removed from the trial site, because of Apple stemgroef virus. The risk of infecting to the rest of the orchards by mechanical farming methods was too high.

**Introduction**

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 hot production region.

**Materials and methods**

Field evaluations and laboratory analysis were conducted on Alpha (Rietspruit), Delta, McClean SL, Mouton Early, Portsgate, Ruby Valencia and Tambuti Early at Tambuti Esatate, Swaziland.

**Table 6.2.8.1.** Internal fruit quality data was compared with the minimum export requirements for Valencia types.

Variety	% Juice	Brix <sup>o</sup>	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 Seedless	52	10.5	0.85	1.5%	7.5:1	Colour plate 3 of set no. 34

**Table 6.2.8.2.** List of Valencia selections evaluated at Tambuti Estate (Swaziland) during 2007.

Selection	Rootstock	Tree Age	No. of trees
Alpha (Rietspruit)	CC	2002	19
Delta	CC	2002	20
McClean SL	CC	2002	20
Portsgate	CC	2002	20
Ruby	CC	2002	16
Tambuti Early	CC	2002	4

**Results and discussion**

Alpha

This season Alpha produced a good crop on the trees with medium to large (count 88 to 48) fruit size. Internally the selection appears to be promising and complies with the minimum export standards. The juice content was one of the highest figures tested for this trial. Alpha produced a good flavour in the fruit and could be compared to McClean seedless. Maturity middle of June for this climatic region.

Delta

This selection produced a medium to large (count 88 to 56) fruit size on the trees, and bore an average to good crop this season. The internal quality complied with the minimum export standards, with fairly high juice

contents (54.1%). There were round and elongated fruit on the trees with smooth rind texture. Maturity end of June.

#### McClellan SL

When the external colour of the fruit was between T3 and T6, the acid content was already below 1%, although still above minimum requirements. The trees produced a good yield, and the fruit size on average was medium (count 72 to 88). McClellan produced an excellent Brix° of 13.8, the best of all the selections evaluated. The fruit internally matured to an intense dark yellow colour, adding value to the quality of this selection. Maturity appears to peak by middle to end of June.

#### Mouton Early

Trees were removed due to Apple stemgroove virus symptoms on this selection. Selection was readmitted for shoot tip grafting, and after the cleaning process evaluations will continue.

#### Portsgate

The acid content this season improved in comparison with the previous season, and higher levels were tested at peak maturity. Internally the fruit complied with the minimum export standards and was similar to the other selections evaluated. The fruit size count was between 72 and 88 (medium), with a good crop on the trees. The fruit shape on the trees was elongated. Maturity middle to end of May.

#### Ruby Valencia

There is a better selection available for evaluation, which has lower seed counts. The trees will be top worked in the future to the new improved selection and evaluations will continue.

#### Tambuti Early

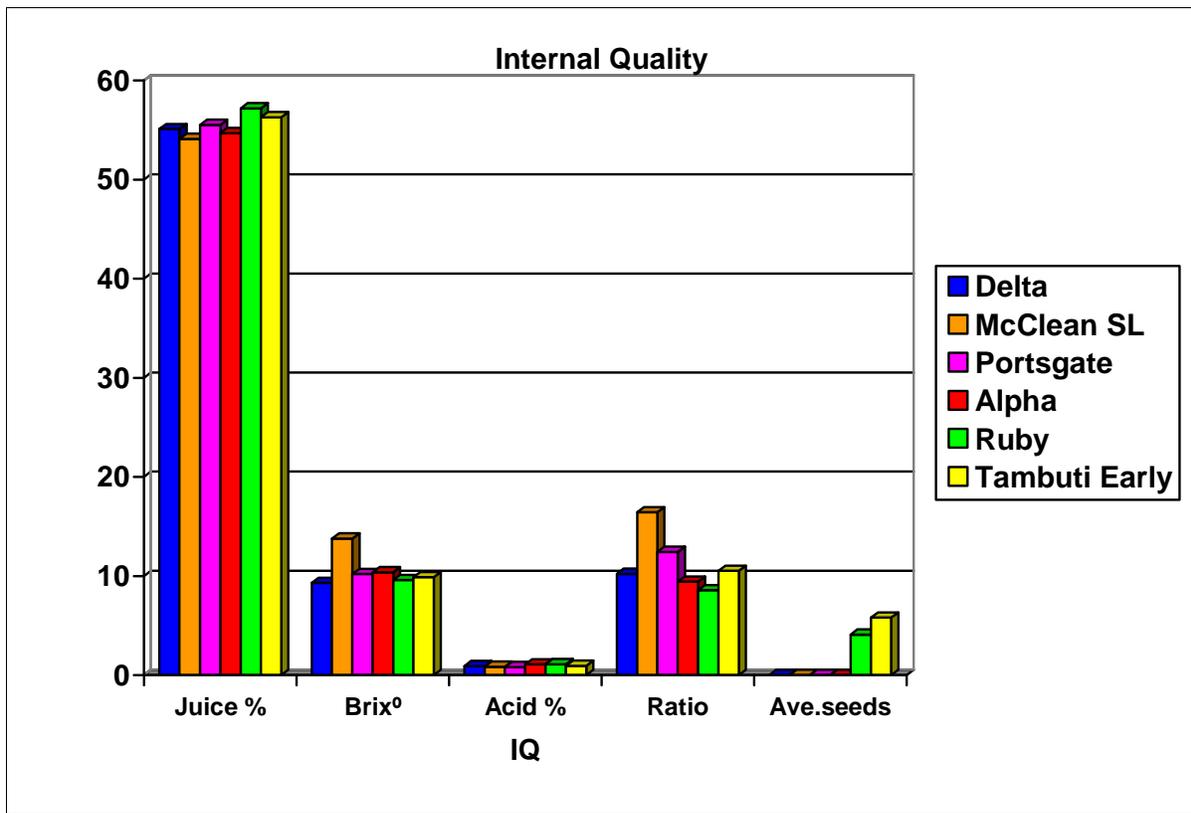
Trees were evaluated at Tambuti Estate (Swaziland) during the 2007 season. Tambuti Early produced medium (count 56-88) sized fruit on the trees, and the internal quality complied with the export standards by the time of harvest. The highest number of seeds, 5.8 per fruit was counted on this selection. Maturity middle of June.

### **Conclusions and recommendations**

Alpha, McClellan SL and Tambuti Early performed well this season, with good internal quality and optimal fruit size for export. Note that Tambuti Early developed some seeds, in comparison to Alpha and McClellan SL not developing any seeds. Delta as control performed well on average, and will always be a good option for new plantings. The yield produced on the three selections outperformed the yield produced on Delta, which struggles with fruit set problems in certain years. Evaluations will continue.

**Table 6.2.8.3.** Internal fruit quality data for Valencia orange selections at Tambuti Estate (Swaziland) during the 2007 season.

Selection	Root-stock	Date harvested	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Delta	CC	14/06	88-64	53.8	9.8	1.00	9.80	0.0	T4,5,6
Delta	CC	19/07	72-56	55.1	9.3	0.91	10.22	0.0	T1-2
McClellan Seedless	CC	14/06	88-64	51.9	10.9	0.87	12.53	0.0	T3-6
McClellan Seedless	CC	19/07	88-72	54.1	13.8	0.84	16.43	0.0	T1-2
Mouton Early	CC	14/06	88-48	50.0	9.5	0.60	15.83	0.2	T2-3
Portsgate	CC	14/06	88-72	55.3	10.6	1.09	9.72	0.0	T4-6
Portsgate	CC	19/07	88-72	55.5	10.2	0.82	12.44	0.0	T2-4
Alpha (Rietspruit)	CC	14/06	88-48	54.7	10.4	1.10	9.45	0.0	T3-5
Alpha (Rietspruit)	CC	19/07	72	56.8	11.2	1.12	10.00	0.0	T1-2
Ruby	CC	14/06	125-56	53.6	9.5	1.21	7.85	5.2	T5-7
Ruby	CC	19/07	105-64	57.2	9.6	1.12	8.57	4.1	T4-6
Tambuti Early	CC	14/06	88-56	52.3	9.1	0.89	10.22	5.5	T2-6
Tambuti Early	CC	19/07	88-56	56.3	9.9	0.94	10.53	5.8	T2-4



### 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 and production and marketing requirements. The development of a new rootstock is inherently 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.

#### Projekopsomming

Kommersiële onderstam keuses is relatief beperk, en die beste beskikbare opsie is nie altyd geskik om die perseël se beperkinge aan te spreek nie, sowel as produksie en bemarkings vereistes. Die ontwikkeling van 'n nuwe onderstam is 'n lang en ingewikkelde proses, en dit is onwaarskynlik dat enige nuwe ondestam al die ideale eienskappe sal besit.

Een van die hoof doelwitte van onderstam evaluasies is om betroubare onderstamme te vind wat vrugsgroote beheer. Belangrike eienskappe soos goeie produksie, bemerkbare vrugsgroote en interne kwaliteit, asook siekte en insek weertandbiedendheid. Aanpasbaarheid by 'n wye reeks bostamme en grondtipes is 'n belangrike vereiste.

Die onderstam navorsing programme van die 1980's en 1990's, het tot aansienlike verandering in eksklusiewe onderstam keuse gelei. Die fokus het van Growwe skil na Carrizo, Troyer citrange en Swingle citrumelo verskuif. Daar bestaan steeds 'n groot behoefte vir nuwe generasie onderstamme.

### 6.3.2 Evaluation of Delta Valencia rootstock trial at Moosrivier Estates Experiment 94 by J. Joubert (CRI)

#### Opsomming

Die prestasie van Delta Valencia op 30 verskillende onderstamme in herplant gronde, moet oor 'n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word. Die bome se interne kwaliteit het verbeter, alhoewel die oesproduksie nie noodwendig baie toegeneem het nie. Die onderstam kombinasies wat hierdie seisoen beter oeste geproduseer het, kan nie noodwendig met die vorige seisoen vergelyk word nie. Alternatiewe drag patrone kan moontlik hiervoor verantwoordelik wees. Smooth Flat Seville het hierdie seisoen die hoogste produksie gelewer, en die interne kwaliteit van die vrugte het aan minimum uitvoer standaarde voldoen. Al die onderstam kombinasies het in vruggroote telling gedaal van hoofsaaklik telling 72/88 na telling 105/125, effens aan die klein kant.

#### Summary

To evaluate the performance of Delta Valencia on 30 different rootstocks in replant soils, over an eight-year production cycle in the Marble Hall area. The internal quality on the fruit improved, although the yield production was similar with comparison to the 2006 season. Different rootstock combinations performed better this season, and alternative bearing patterns might be the main reason. Smooth Flat Seville produced the highest yield on the trees, and the internal quality complied with the minimum export standards. All the rootstock combinations decreased in fruit size count from average 72/88 to count 105/125, on the smaller side.

#### Introduction

To evaluate the performance of Delta Valencia on 30 different rootstocks in replant soils, over an eight-year production cycle in the Marble Hall area.

#### Materials and methods

A randomised block design comprising of 22 rootstocks of two replicates of five trees each, the other 20 rootstocks were planted in a non-randomised design comprising of 10 trees per rootstock. 30 of the 42 rootstocks was selected and evaluated for the 2007 season.

Delta Valencia on the following rootstocks are being evaluated: F80/8, F80/3, C32, C35, X639, RL-C, RL-S, RL-W, PT, HRS812, R xT, Sunki 1113, CM, CC, TC, Volk, KC, TB, ML, RC, JT, RT, BC, Sunki 1112, ST, SC, RP, SM, SFS, Sunki 1116. The trees were planted in 1998. Trees were evaluated at Moosrivier Estates (Marble Hall), in Mpumalanga during the 2007 season. Full names for these abbreviations appear below.

#### Results and discussion

##### Internal fruit quality analysis (Table 6.3.2.1)

- Juice%: The highest juice content was produced by C32 (56.9%), followed by Sunki 1112 (55.6%) and Sunki 1113 with 55.5%. Eight of the rootstocks evaluated (C35, X639, RL-S, SM, CC, Volk, F80/3, SFS and RL-W) tested below 52%, not complying with the minimum export standards. The lowest juice content was produced by SM (50%).
- Brix<sup>o</sup>: This season the Brix content increased, with 21 of the 30 rootstock combinations evaluated producing a Brix<sup>o</sup> higher than 10.5. Sunki 1113 produced the highest Brix content of 11.8, followed by HRS 812 with 11.6 and RC with 11.4. Volk and ML tested the lowest sugar content for this trial with 9 Brix<sup>o</sup>. During the 2006 season, Volk produced a 7.9 Brix<sup>o</sup> and remained the lowest sugar content for this trial.
- Acid: Sunki 1112 rootstock provided the highest acid content (1.64%) for this season, followed by C32 (1.51%) and Sunki 1113 (1.48%). The lowest acid content measured 0.97% (Volk) and complied with the minimum export standards (above 0.85%). In 2006 Volk was below the minimum acid % for export quality; all the other combinations internal quality was acceptable.

#### Fruit size distribution (Table 6.3.2.2)

- The fruit size evaluation shows the largest peak at count 105/125 on all 30 rootstock combinations. There was a decrease in fruit size in comparison to the 2006 season, where the fruit size count peaked at 72, followed by count 56 and count 88.

#### Production per tree (Table 6.3.2.3)

- SFS rootstock set the best crop on the trees (115.4 kg/tree) in comparison with the other 29 rootstocks. CM was the second highest producer with 104.6 kg/tree, followed by Volk (101.1 kg/tree). JT produced the lowest yield on the trees at 35.8 kg/tree.
- There was no major increase in production between year 2006 and year 2007, although the bearing varied between the rootstock combinations.

### **Conclusions and recommendations**

Thirty of the forty-two rootstocks were selected on a production and fruit quality basis and evaluated. The internal quality improved this season, and only one rootstock was below the minimum acid level for export requirements. The fruit size on all the combinations decreased and peaked at count 105/125, with Deltas on the small side. Normally fruit size decreases with high yields and large numbers of fruit set on the trees, but the yields did not increase drastically to make this conclusion in this trial.

<b>Abbreviation</b>	<b>Rootstock</b>
F80/8	F80 citumelo 8
PT	Pomeroy trifoliolate
C32	Citrango 32
HRS 812	Sunki 812
CM	C.macrophylla
C35	Citrango 35
X639	Trifoliolate x Cleopatra
ML	Milam Lemon
RC	Rusk citrango
JT	Jacobsen trifoliolate
RL-S	Rough lemon Schaub
SC	Swingle citumelo
RP	Rangpur lime
SM	Shekwasha mandarin
RxT	Rangpur x Troyer
RL-C	Rough lemon Cairn
Sunki 1113	Flying dragon x Sunki(1113)
CC	Carrizo citrango
TC	Troyer citrango
Volk	Volckameriana
KC	Koethen citrango
TB	Terrabella citumelo
RT	Rubidoux trifoliolate
BC	Benton citrango
F80/3	F80 citumelo 3
Sunki 1112	Flying dragon x Sunki(1112)
ST	Sampson tangelo
SFS	Smooth flat seville
Sunki 1116	Flying dragon x Sunki(1116)
RL-W	Rough lemon Wallace

**Table 6.3.2.1.** Internal fruit quality of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) on 11 July 2007.

Root-Stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
F80/8	88-64	55.0	10.8	1.35	8.00	0.0	T1-3
PT	105-72	53.5	11.1	1.29	8.60	0.0	T1-2
C32	88-72	56.9	10.8	1.51	7.15	0.0	T1-2
HRS 812	88-64	54.0	11.6	1.30	8.92	0.0	T1-3
CM	88-72	52.2	9.4	1.20	7.83	0.0	T3-5
C35	88-48	51.0	10.9	1.45	7.52	0.0	T1-3
X639	88-64	50.6	10.2	1.24	8.23	0.0	T1-3
ML	105-72	52.6	9.0	1.09	8.26	0.0	T1-4
RC	125-64	54.7	11.4	1.42	8.03	0.0	T1-3
JT	125-72	52.7	10.5	1.24	8.47	0.0	T1-4
RL-S	105-64	51.8	10.5	1.07	9.81	0.0	T1-2
SC	88-64	54.7	10.0	1.29	7.75	0.0	T1-4
RP	125-64	52.9	9.5	1.13	8.41	0.0	T1-4
SM	105-64	50.0	9.7	1.25	7.76	0.0	T2-4
RxT	88-64	53.4	9.7	1.25	7.76	0.0	T1-4
RL-C	88-56	50.0	9.5	1.10	8.64	0.0	T1-3
Sunki 1113	88-64	55.5	11.8	1.48	7.97	0.0	T1-3
CC	88-72	51.3	10.9	1.31	8.32	0.0	T1-3
TC	88-72	52.9	10.5	1.25	8.40	0.0	T1-3
Volk	105-64	50.7	9.0	0.97	9.28	0.0	T1-3
KC	105-64	54.1	10.8	1.43	7.55	0.0	T1-3
TB	88-64	54.5	9.8	1.36	7.21	0.0	T1-3
RT	105-72	55.3	10.5	1.43	7.34	0.0	T1-4
BC	88-56	55.3	10.5	1.26	8.33	0.0	T1-3
F80/3	88-72	51.5	9.7	1.32	7.35	0.0	T1-3
Sunki 1112	105-72	55.6	11.1	1.64	6.77	0.0	T1-4
ST	125-64	53.9	10.7	1.36	7.87	0.0	T1-2
SFS	88-64	51.9	10.1	1.32	7.65	0.0	T1-3
Sunki 1116	105-64	53.5	10.0	1.35	7.41	0.0	T2-4
RL-W	88-56	50.4	10.5	1.38	7.61	0.0	T1-3

**Table 6.3.2.2.** Fruit size distribution of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2007 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/8	48	0.21	RL-S	48	0.04	KC	48	0.64
F80/8	56	1.99	RL-S	56	1.31	KC	56	4.61
F80/8	72	8.07	RL-S	72	8.88	KC	72	17.16
F80/8	88	22.91	RL-S	88	23.52	KC	88	31.13
F80/8	105/125	55.66	RL-S	105/125	53.93	KC	105/125	41.35
F80/8	144	11.16	RL-S	144	12.31	KC	144	5.11
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
PT	48	0.00	SC	48	0.00	TB	48	0.09
PT	56	0.83	SC	56	2.65	TB	56	1.71
PT	72	6.25	SC	72	10.35	TB	72	10.28
PT	88	19.67	SC	88	23.75	TB	88	28.60
PT	105/125	57.54	SC	105/125	49.29	TB	105/125	52.41
PT	144	15.72	SC	144	13.97	TB	144	6.90

<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
C32	48	0.11	RP	48	0.37	RT	48	0.11
C32	56	2.65	RP	56	2.98	RT	56	0.84
C32	72	13.07	RP	72	13.91	RT	72	8.64
C32	88	30.39	RP	88	23.42	RT	88	23.47
C32	105/125	46.99	RP	105/125	48.25	RT	105/125	55.69
C32	144	6.78	RP	144	11.07	RT	144	11.26
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
HRS812	48	0.43	N	48	0.00	BC	48	0.08
HRS812	56	6.57	N	56	1.91	BC	56	2.20
HRS812	72	20.57	N	72	8.93	BC	72	11.92
HRS812	88	31.35	N	88	21.57	BC	88	29.36
HRS812	105/125	37.30	N	105/125	54.04	BC	105/125	50.12
HRS812	144	3.79	N	144	13.56	BC	144	6.31
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
CM	48	0.03	RxT	48	0.29	F80/3	48	0.50
CM	56	2.26	RxT	56	2.43	F80/3	56	1.68
CM	72	16.16	RxT	72	10.34	F80/3	72	8.74
CM	88	32.95	RxT	88	26.57	F80/3	88	19.64
CM	105/125	43.74	RxT	105/125	54.19	F80/3	105/125	50.67
CM	144	4.85	RxT	144	6.18	F80/3	144	19.21
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
C35	48	0.28	RL-C	48	0.12	Sunki 1112	48	0.00
C35	56	2.65	RL-C	56	0.98	Sunki 1112	56	1.47
C35	72	15.46	RL-C	72	8.63	Sunki 1112	72	7.29
C35	88	33.18	RL-C	88	23.00	Sunki 1112	88	22.71
C35	105/125	44.81	RL-C	105/125	53.40	Sunki 1112	105/125	58.14
C35	144	3.61	RL-C	144	13.88	Sunki 1112	144	10.38
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
X639	48	0.00	Sunki 1113	48	0.30	ST	48	0.00
X639	56	0.88	Sunki 1113	56	2.95	ST	56	1.55
X639	72	5.54	Sunki 1113	72	13.84	ST	72	10.56
X639	88	17.98	Sunki 1113	88	26.78	ST	88	27.42
X639	105/125	58.59	Sunki 1113	105/125	47.46	ST	105/125	53.68
X639	144	17.01	Sunki 1113	144	8.67	ST	144	6.79
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
ML	48	0.19	CC	48	0.13	SFS	48	0.00
ML	56	2.52	CC	56	2.43	SFS	56	4.19
ML	72	11.20	CC	72	13.45	SFS	72	15.67
ML	88	25.67	CC	88	30.67	SFS	88	29.01
ML	105/125	50.73	CC	105/125	46.46	SFS	105/125	44.20
ML	144	9.70	CC	144	6.85	SFS	144	6.93
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
RC	48	0.19	TC	48	0.04	Sunki 1116	48	0.07
RC	56	6.02	TC	56	1.14	Sunki 1116	56	1.96
RC	72	16.30	TC	72	8.30	Sunki 1116	72	10.26
RC	88	24.88	TC	88	23.69	Sunki 1116	88	21.41

RC	105/125	44.41	TC	105/125	52.95	Sunki 1116	105/125	47.99
RC	144	8.20	TC	144	13.88	Sunki 1116	144	18.32
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
JT	48	0.34	Volk	48	0.03	RL-W	48	2.27
JT	56	2.35	Volk	56	1.91	RL-W	56	10.08
JT	72	10.29	Volk	72	10.90	RL-W	72	20.67
JT	88	20.81	Volk	88	23.04	RL-W	88	30.42
JT	105/125	50.78	Volk	105/125	49.86	RL-W	105/125	32.18
JT	144	15.44	Volk	144	14.27	RL-W	144	4.37

**Table 6.3.2.3.** Production of Delta Valencia on different rootstocks at Moosrivier Estate (Marble Hall) during the 2007 season.

<b>Rootstock</b>	<b>Kg/tree</b>	<b>Kg/tree</b>	<b>Kg/tree</b>	<b>Kg/tree</b>	<b>Kg/tree</b>
<b>Selection</b>	<b>2003</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>
F80/8	48.4	33.4	49.4	71.9	75.5
PT	43.1	32.5	47.6	75.6	66.9
C32	54.9	25.4	38.6	73.5	61.0
HRS 812	50.7	24.0	25.4	81.8	75.5
CM	57.4	51.0	56.5	112.9	104.6
C35	58.4	27.4	39.6	80.9	61.1
X639	47.2	26.3	48.1	77.6	72.5
ML	60.5	35.4	42.3	93.9	87.3
RC	50.1	37.4	40.1	78.2	72.9
JT	26.3	30.8	43.0	96.5	35.8
RL-S	54.3	25.1	52.0	66.8	75.2
SC	40.0	10.9	27.3	80.2	79.3
RP	59.4	45.7	79.8	85.0	89.0
SM	28.8	13.3	23.9	45.4	86.3
RxT	36.2	22.5	41.6	75.6	57.0
RL-C	55.7	26.8	43.7	82.0	77.6
Sunki 1113	48.4	28.7	45.8	83.8	78.5
CC	37.0	22.0	45.2	95.6	77.6
TC	50.6	11.1	52.7	75.2	77.6
Volk	56.7	28.1	42.8	82.4	101.1
KC	39.0	32.7	50.8	75.1	49.5
TB	49.5	16.9	50.0	90.8	72.7
RT	36.8	35.7	30.0	79.0	56.9
BC	61.7	47.7	66.5	89.5	80.1
F80/3	49.9	27.1	53.8	79.8	65.0
Sunki 1112	43.9	22.9	37.4	101.8	88.0
ST	18.5	10.6	25.4	78.2	73.7
SFS	51.4	40.1	37.8	73.2	115.4
Sunki 1116	32.5	25.8	43.1	74.7	92.4
RL-W	34.8	14.2	46.1	76.3	44.8

### 6.3.3 Evaluation of Midnight and Delta Valencia rootstock trial at Letaba Estates Experiment 137 A by J. Joubert (CRI)

#### Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer.

Die Delta seleksie in kombinasie met verskeie onderstamme het belowende interne kwaliteite opgelewer en al die kombinasies, behalwe RL-C, het aan die minimum uitvoer standaard voldoen. Vruggrootte het aansienlik kleiner geword, en die algemene telling was tussen 105 en 125. Die vorige seisoen was die vruggrootte tellings meer in die omgewing van 72 to 88 gewees, baie meer ideaal vir Valencia uitvoere. Die produksie het soortgelyk gebly, wat die moontlikheid van meer vrugte op die bome met kleiner vruggrootte dan uitsluit. Die Deltas in hierdie proef het redelik swak geset, en die oes produksie data moet korrek benader word. Daar is verskeie metodes wat gevolg kan word om beter vrugset op die bome te bevorder.

Die teenoorgestelde situasie het by die Midnight bome se vruggrootte vorming voorgekom. Die vruggroote het toegeneem van gemiddeld telling 105/125 in 2006 tot telling 88 en 72 vir hierdie seisoen. Intern het die vrugte uitstekend gevaar, en meeste van die onderstam kombinasies het aan die uitvoer standaard voldoen. Die produksie het hierdie seisoen swakker vertoon, maar daar was heelwat vrugval by die bome betrokke. Gedeeltelik kan hierdie tendens toegeskryf word aan die feit dat die vrugte vroeër gereed was om te oes, ongeveer twee weke.

## Summary

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area.

The Delta selection in combination with a variety of rootstocks produced a promising internal quality in the fruit, except for RL-C, all the other selections complied with the export standards. Fruit size decreased considerably this season, with the average between count 105 and 125. The previous season all combinations peaked between count 72 and 88, very promising for export. Fruit production remained similar, cancelling the possibility of more fruit on the trees and smaller fruit size. The Deltas in this trial experience a fruit set problem, and there are numerous methods to improve set and fruit production.

There was an opposite situation on the Midnight trees, with regards to fruit size. The fruit size increased on average from count 105/125 to count 88/72 for this season. Internally the fruit performed well, and most of the combinations complied with the export standards. Yield production was lower for 2007 due to the high number of fruit that dropped before harvesting the trial. The peak maturity time for harvesting the fruit was two weeks earlier, explaining the extent of the fruit drop.

## Introduction

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Midnight Valencia and Delta Valencia. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system.

## Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Midnight Valencia and Delta Valencia trees were budded onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates near Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per replicate (total of 20 trees per rootstock).

**Table 6.3.3.1.** List of cultivar and rootstock combinations planted at Letaba Estates.

Selection	Rootstock	No. of trees
Delta Valencia	F80/9	10
Delta Valencia	SC	10
Delta Valencia	CC	10
Delta Valencia	F80/3	10
Delta Valencia	C35	10
Delta Valencia	KC	10
Delta Valencia	MxT	10
Delta Valencia	BC	10
Delta Valencia	X639	10
Delta Valencia	RL-C	8
Midnight Valencia	RL-C	9
Midnight Valencia	F80/9	6

Midnight Valencia	BC	8
Midnight Valencia	MxT	10
Midnight Valencia	SC	10
Midnight Valencia	F80/3	10
Midnight Valencia	CC	9
Midnight Valencia	C35	8
Midnight Valencia	KC	10
Midnight Valencia	X639	10

## Results and discussion

### Delta Valencia

#### Internal fruit quality analysis (Table 6.3.3.1.a)

- Juice %: CC produced the highest juice content (60.3%) followed by X639 (59.7%) and SC (59.5%). All the rootstocks complied with the export standards, with BC producing the lowest juice content (57.1%). The average juice content on all the fruit produced in this trial increased this season.
- Brix<sup>o</sup>: The highest Brix<sup>o</sup> was produced by C35 (12.0) followed by F80/9 (12.0) and BC (11.6). The only rootstock below the minimum requirement was RL-C with 9.7.
- Acid%: RL-C produced the highest acid content (1.37%) followed by SC (1.31%) and BC (1.28%). All the combinations were below the maximum and above the lowest acid standards for export standards.

#### Fruit size distribution (Table 6.3.3.1.b)

- The fruit size evaluation shows the largest peak at count 105/125 on all the combinations. The next highest count in fruit size was count 88 with F80/9, KC, SC, MxT, CC, BC, F80/3, C35 and RL-C.

#### Production per tree (Table 6.3.3.1.c)

- X639 produced the highest yield per tree (91.1 kg), followed by RL-C with 73.3 kg/tree and SC with 68.6 kg/tree. F80/9 produced the lowest yield on the trees of 32 kg/tree. Bear in mind that F80/9 provides a medium tree size in comparison to X639, RL-C and SC.

### Midnight Valencia

#### Internal fruit quality analysis (Table 6.3.3.2.a)

- Juice %: F80/3 produced the highest juice content (60.4%) followed by CC (59.8%) and KC (59.5%). The juice content on all the rootstocks complied with the minimum export standards; rootstock RL-C tested the lowest. (53.6%).
- Brix<sup>o</sup>: C35 produced the highest Brix<sup>o</sup> (12.9) followed by F80/9 (12.2) and M xT (11.9). BC and F80/3 were the only two selections below the minimum standard with 10.0 Brix.
- Acid: RL-C produced the highest acid content (1.55) by the time of harvest, followed by C35 (1.42) and MxT (1.41). The lowest acid content was produced by KC (1.05). All the selections complied with the minimum export standards (0.85).

#### Fruit size distribution (Table 6.3.3.2.b)

- The optimal fruit size for Valencias is between count 72 and 88. The fruit size evaluation shows the largest peak at count 72 on RL-C, F80/3, F80/9, CC, BC, C35, KC and SC. The next highest count in fruit size was count 88. Considering that count 88, followed by count 72 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.3.3.2.c)

- Midnight Valencia on F80/3 produced the highest yield (80.5kg/tree). BC produced 74.0 kg/tree, followed by X639 with 72.2 kg/tree and SC with 58.7 kg/tree. C35 produced the lowest yield in this trial evaluated (37.1 kg/tree). F80/3, BC and C35 produce a medium tree size in comparison to X639 and SC with large tree sizes.

**Conclusions and recommendations**

Delta Valencia:

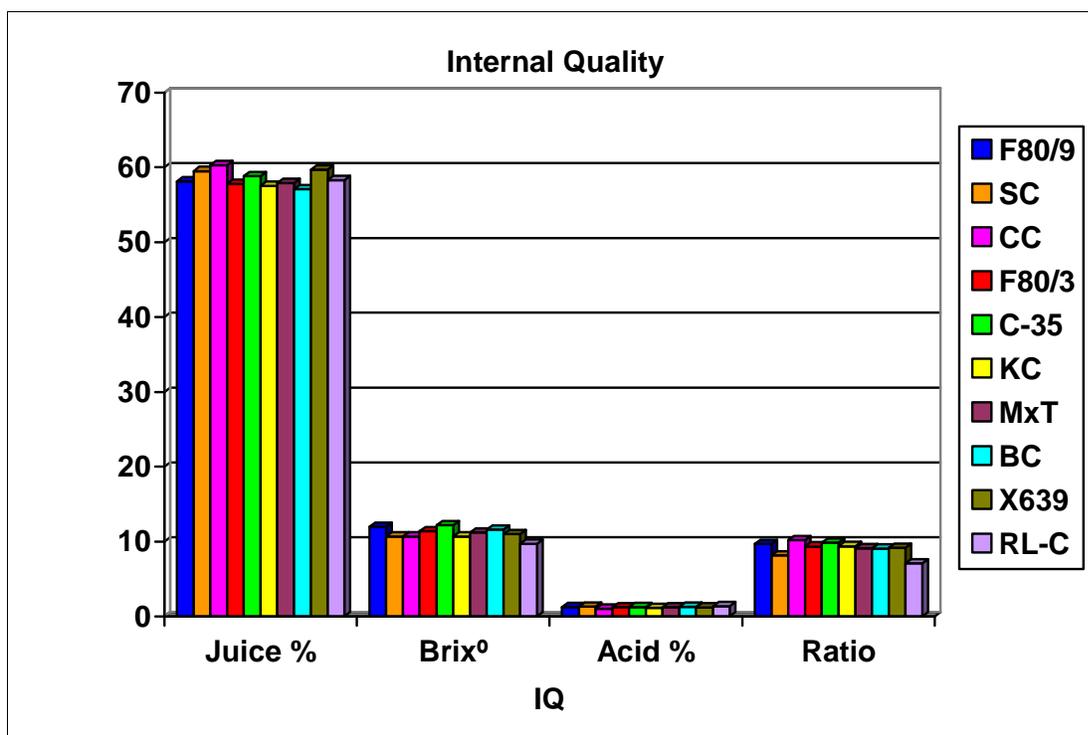
The internal quality on all the combinations proved promising this season, with only RL-C not complying with the export standard with a Brix content of 9.7. Comparing the fruit size data with the 2006 season, there was a considerable decrease in size on all combinations from count 72 to count 105/125. Some yield production on the trees increased, but not that significantly to influence fruit size so drastically.

Midnight Valencia:

All the combinations complied with the minimum export standards for juice content, with 60.4% being the highest juice % measured. The sugar content on all the selections increased to as high as 12.9 Brix produced on C35. The fruit size for this season increased from count 105/125 to count 72, followed by count 88. There was a slight decrease in yield on the trees due to substantial fruit drop during 2007. This situation might explain the severe decrease in yield on the C35 trees. The peak harvesting time was two weeks earlier in comparison to the 2006 season which could have influenced the fruit drop.

**Table 6.3.3.1.a.** Internal fruit quality data of Delta Valencia on different rootstocks at Letaba Estates (Letsitele) on 24 July 2007.

Root-Stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
F80/9	125-56	58.1	12.00	1.24	9.68	0.0	T1-2
SC	88-64	59.5	10.70	1.31	8.17	0.0	T1-2
CC	88-64	60.3	10.70	1.05	10.19	0.0	T1-2
F80/3	88-72	57.8	11.40	1.22	9.34	0.0	T1-2
C-35	88-64	58.8	12.20	1.24	9.84	0.0	T1-2
KC	88-56	57.5	10.70	1.14	9.39	0.0	T1-2
MxT	72-56	57.9	11.20	1.23	9.11	0.0	T1
BC	88-56	57.1	11.60	1.28	9.06	0.0	T1-2
X639	105-72	59.7	11.00	1.20	9.17	0.0	T1-2
RL-C	88-72	58.3	9.70	1.37	7.08	0.0	T1-2



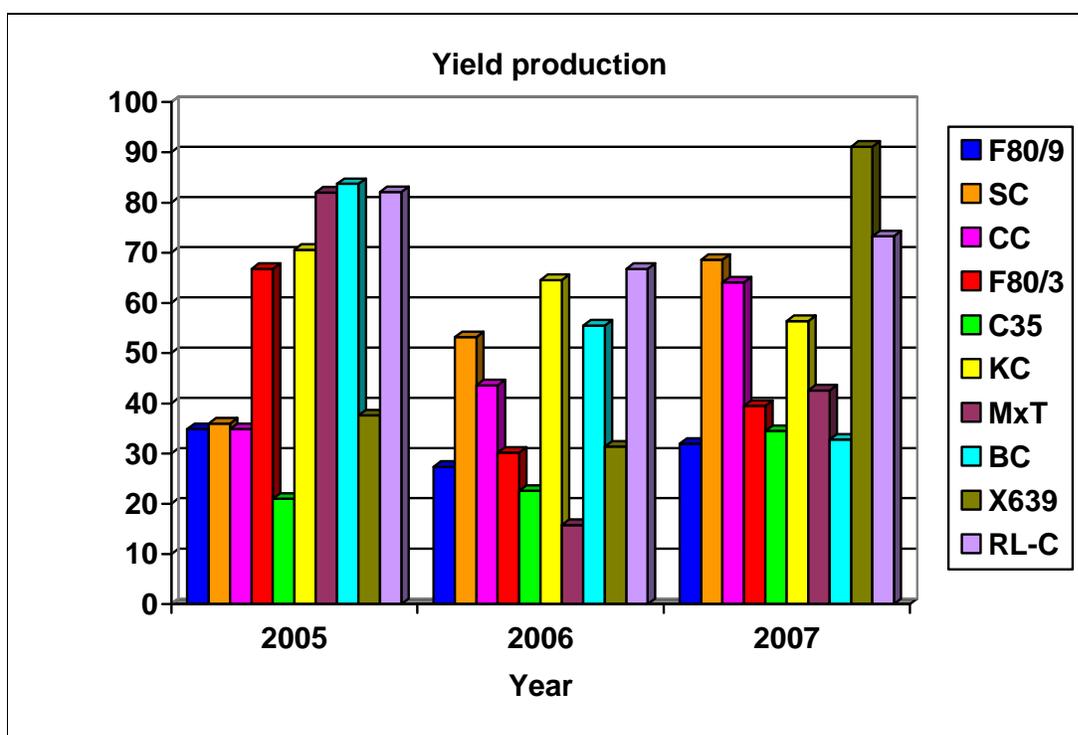
**Table 6.3.3.1.b.** Fruit size distribution of Delta Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2007 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/9	48	0.26	KC	48	0.19
F80/9	56	3.53	KC	56	4.26
F80/9	72	11.31	KC	72	20.43
F80/9	88	22.62	KC	88	30.83
F80/9	105/125	49.33	KC	105/125	38.66
F80/9	144	12.95	KC	144	5.64
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
SC	48	0.00	MxT	48	0.25
SC	56	1.96	MxT	56	4.67
SC	72	16.47	MxT	72	20.00
SC	88	36.25	MxT	88	32.42
SC	105/125	40.74	MxT	105/125	36.83
SC	144	4.58	MxT	144	5.83
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
CC	48	0.18	BC	48	1.82
CC	56	2.01	BC	56	13.28
CC	72	11.47	BC	72	19.64
CC	88	29.59	BC	88	22.47
CC	105/125	47.40	BC	105/125	33.37
CC	144	9.36	BC	144	9.42
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/3	48	0.00	X639	48	0.00
F80/3	56	1.18	X639	56	0.36
F80/3	72	14.14	X639	72	2.73
F80/3	88	28.87	X639	88	15.80
F80/3	105/125	48.40	X639	105/125	62.45
F80/3	144	7.41	X639	144	18.66

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
C35	48	0.87	RL-C	48	0.00
C35	56	4.73	RL-C	56	1.41
C35	72	11.48	RL-C	72	9.99
C35	88	22.08	RL-C	88	27.37
C35	105/125	48.12	RL-C	105/125	51.59
C35	144	12.73	RL-C	144	9.64

**Table 6.3.3.1.c.** Production per tree of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2007 season.

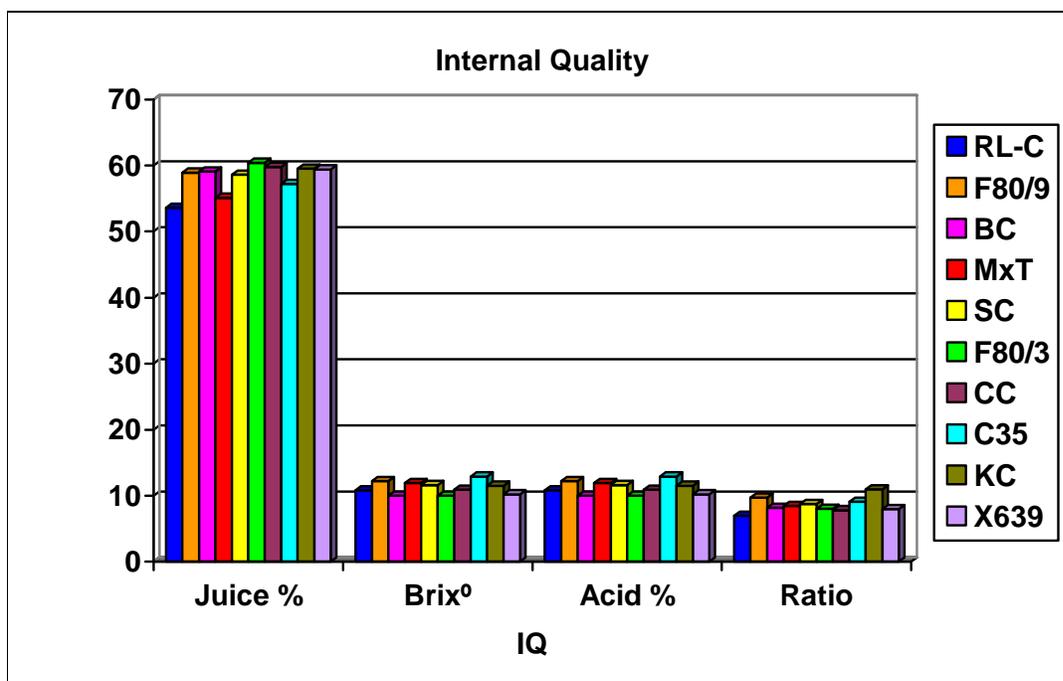
Rootstock	Kg/tree (2005)	Kg/tree (2006)	Kg/tree (2007)
F80/9	34.9	27.4	32.0
SC	36.0	53.2	68.6
CC	34.9	43.6	64.1
F80/3	66.8	30.2	39.5
C35	21.0	22.6	34.5
KC	70.6	64.6	56.4
MxT	82.0	15.8	42.6
BC	83.7	55.5	32.8
X639	37.6	31.4	91.1
RL-C	82.1	66.8	73.3



**Table 6.3.3.2.a.** Internal fruit quality data of Midnight Valencia on different rootstocks at Letaba Estates (Letsitele) on 24 July 2007.

Root-Stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
RL-C	72-56	53.6	10.80	1.55	6.97	0.0	T1-2
F80/9	72-48	58.9	12.20	1.26	9.68	0.2	T1-2
BC	72-48	59.1	10.00	1.23	8.13	0.3	T1

MxT	72-64	55.1	11.90	1.41	8.44	0.5	T1
SC	72-56	58.6	11.60	1.33	8.72	0.0	T1
F80/3	88-64	60.4	10.00	1.25	8.00	0.3	T1-2
CC	72-56	59.8	10.90	1.40	7.79	0.0	T1-2
C35	72-64	57.2	12.90	1.42	9.08	0.8	T1
KC	72-48	59.5	11.50	1.05	10.95	0.0	T1-2
X639	88-56	59.4	10.20	1.28	7.97	0.3	T1-2



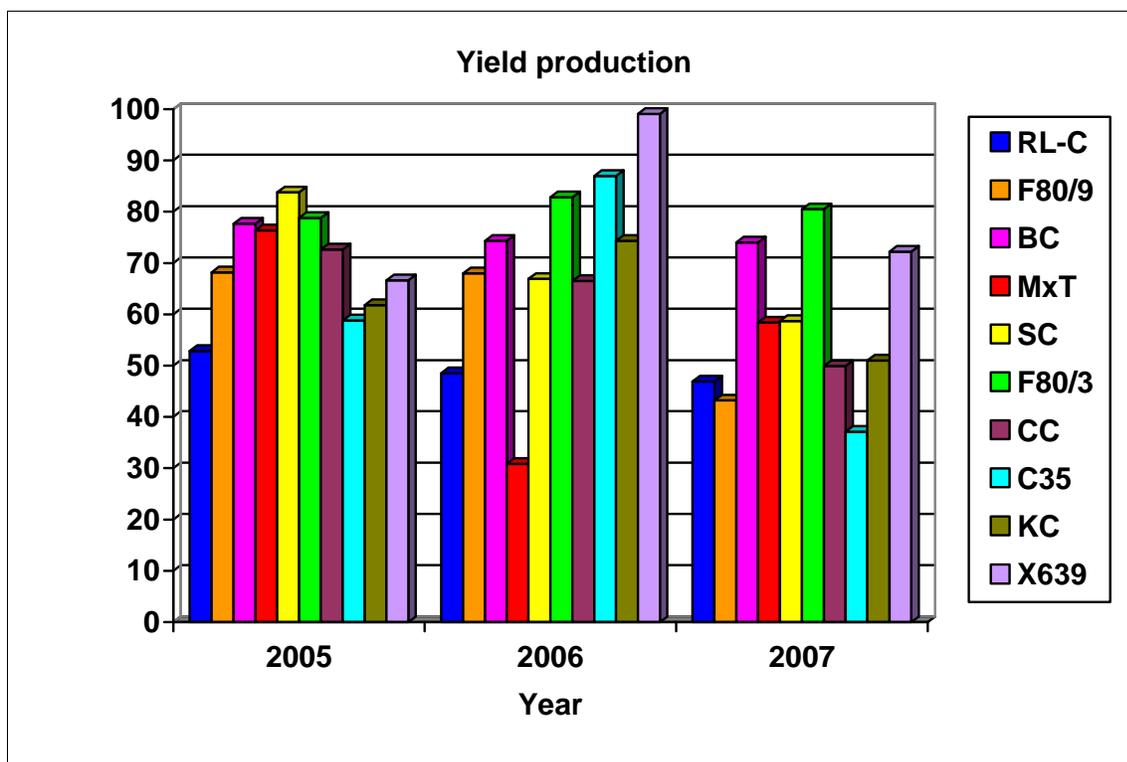
**Table 6.3.3.2.b.** Fruit size distribution of Midnight Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2007 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
RL-C	48	1.23	F80/3	48	0.77
RL-C	56	19.50	F80/3	56	15.67
RL-C	72	34.77	F80/3	72	36.25
RL-C	88	24.51	F80/3	88	32.94
RL-C	105/125	16.99	F80/3	105/125	13.41
RL-C	144	3.00	F80/3	144	0.95
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/9	48	1.32	CC	48	1.15
F80/9	56	24.81	CC	56	16.93
F80/9	72	35.40	CC	72	37.18
F80/9	88	23.82	CC	88	26.25
F80/9	105/125	11.50	CC	105/125	16.79
F80/9	144	3.14	CC	144	1.71
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
BC	48	1.76	C35	48	1.84
BC	56	25.19	C35	56	22.54
BC	72	42.57	C35	72	32.30
BC	88	23.76	C35	88	22.33
BC	105/125	5.89	C35	105/125	17.74
BC	144	0.83	C35	144	3.25
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit

MxT	48	0.46	KC	48	3.60
MxT	56	9.15	KC	56	32.64
MxT	72	30.45	KC	72	37.90
MxT	88	34.43	KC	88	19.29
MxT	105/125	23.85	KC	105/125	6.21
MxT	144	1.66	KC	144	0.36
<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
SC	48	1.79	X639	48	0.19
SC	56	25.98	X639	56	8.20
SC	72	36.17	X639	72	32.84
SC	88	24.86	X639	88	38.25
SC	105/125	10.01	X639	105/125	18.98
SC	144	1.19	X639	144	1.54

**Table 6.3.3.2.c.** Production of Midnight Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2007 season.

Rootstock	Kg/tree (2005)	Kg/tree (2006)	Kg/tree (2007)
RL-C	52.9	48.5	46.9
F80/9	68.2	68.0	43.2
BC	77.7	74.3	74.0
MxT	76.4	30.8	58.4
SC	83.8	66.9	58.7
F80/3	78.8	82.8	80.5
CC	72.7	66.5	49.9
C35	58.8	87.0	37.1
KC	61.8	74.3	51.0
X639	66.6	99.1	72.2



#### 6.3.4 Evaluation of Star Ruby rootstock trial at Letaba Estates Experiment 137 B by J. Joubert (CRI)

##### Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermedieë sitrusproduksie gebied geëvalueer.

Hierdie produksie area het meer as die gemiddelde jaarlikse reënval ontvang vir die seisoen, en die besproeiing kon optimum plaasvind. Al die onderstam kombinasies het 'n beter oes geproduseer in vergelyking met die 2006 seisoen. Die hoogste oes is deur F80/3 op die bome geset, met 161.7kg/boom. Die interne kwaliteit van die vrugte het baie verbeter en die meeste van die onderstam kombinasies het aan die uitvoerstandaarde voldoen. BC en KC presteer besonder goed, as die kleiner boomvolumes in ag geneem word. BC en KC vorm medium grootte bome en groei nie so aggresief nie, wat groot voordele inhou vir laer oeskostes, insek beheer kostes ens. CC word algemeen as onderstam in die sitrusbedryf gebruik, en het weer baie belowend presteer met goeie interne kwaliteit vrugte.

##### Summary

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. This production area received a higher rainfall average in comparison to the 2006 season, resulting in optimal moisture levels being attained when coupled with irrigation applications. All the rootstock combinations produced a better yield on the trees. The best yield was produced by F80/3, weighing 161.7 kg/tree. Internally the fruit quality improved and most of the combinations complied with the minimum export standards. BC and KC performed very well, taking the smaller tree size in considerations. Both rootstocks do not grow vigorously, and produce medium size trees, decreasing harvest and spray costs. CC performed well, and is one of the best quality inducing rootstock options for replant soils.

##### Introduction

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Star Ruby grapefruit. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system.

##### Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Star Ruby grapefruit were budded onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates near Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per replicate (total of 20 trees per rootstock).

##### Results and discussion

###### Internal fruit quality analysis (Table 6.3.4.1)

- Juice %: SC produced the highest juice content (58.3%), followed by RL-C (55.3%) and X639 (54.8%). In 2006 RL-C produced the highest juice content (58.8%) followed by X639 (58.3%) and BC (56.8%). The lowest juice content was produced on MxT with 50.9%. CC and F80/3, in 2006 produced the lowest juice content of 47.9%. This season all the combinations complied with the minimum of 50% juice content, better in comparison to the previous season.
- Brix<sup>o</sup>: This season F80/9 produced the highest Brix<sup>o</sup> of 11.0, followed by MxT (10.9) and KC with 10.8. Last season CC and C35 produced the highest Brix<sup>o</sup> (9.1) for this Star Ruby trial, and the second highest Brix<sup>o</sup> was produced by SC (9.0) followed by F80/3 with 8.9. For 2007 the lowest Brix content was produced by RL-C (9.4), a similar situation to 2006 when RL-C tested 7.3<sup>o</sup>.
- Acid: The average acid content on all the combinations tested lower in comparison to the 2006 season. F80/3 once again produced the highest acid content of 1.79, followed by MxT (1.69) and RL-C with 1.67. The e lowest acid content was measured on KC with 1.46, slightly higher than 2006.

#### Fruit size distribution (Table 6.3.4.2)

- All the combinations peaked at count 64, except for BC, KC and F80/9 which peaked at count 48. The second highest average count in fruit size was 48 on RL-C, SC, MxT, F80/3, C35, CC and X639. KC and BC produced the highest number of count 40 fruit, developing the smallest average fruit size for the trial.

#### Production per tree (Table 6.3.4.3)

- In comparison with the 2006 season all the selections produced a better yield, with F80/3 increasing from 63.1 kg/tree to 161.7 kg/tree and SC increasing from 106.2 kg to 189 kg/tree . SC produced the highest yield (189.0 kg/tree), followed by RL-C with 162.8 kg/tree and CC (158.8 kg/tree). The lowest yield production was on the KC scion/rootstock combination (102.5 kg/tree).

### **Conclusions and recommendations**

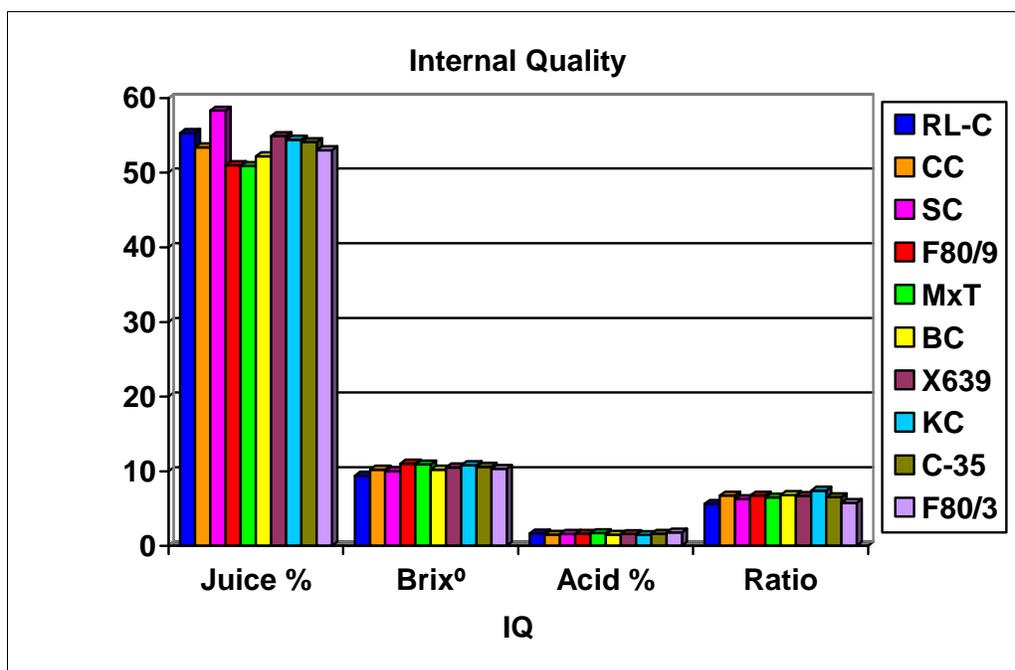
This season a different situation occurred as this production area received more than their average annual rainfall for the year.

The trees recovered well after the previous dry season and inadequate rainfall. Internally all the combinations complied with the minimum export standards, with the fruit size development similar to 2006. Yield production recovered well, although the recovery rate differs between the rootstock combinations. X639 recovered the slowest in comparison to the other combinations..

BC, KC and F80/3 showed substantial promise this season, and should be kept in mind when planning to plant or replant an existing orchard. These rootstocks do not grow vigorously and developed a medium size tree with good to excellent yield. There are numerous advantages with the smaller tree size, for example easy harvesting, spray methods and quantities of chemicals used.

**Table 6.3.4.1.** Internal fruit quality of Star Ruby grapefruit on different rootstocks at Letaba Estates (Letsitele) on 2 May 2007.

Root-Stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
RL-C	48-36	55.3	9.40	1.67	5.63	0.7	T1-2
CC	56-40	53.4	10.20	1.51	6.75	0.2	T1-2
SC	49-36	58.3	10.00	1.60	6.25	0.9	T1-2
F80/9	48-32	51.0	11.00	1.63	6.75	0.9	T1,2,3
MxT	48-40	50.9	10.90	1.69	6.45	1.4	T1-2
BC	40-32	52.2	10.20	1.50	6.80	1.4	T1
X 639	48-36	54.9	10.50	1.57	6.69	0.8	T1-2
KC	56-32	54.4	10.80	1.46	7.40	1.1	T1-2
C-35	48-40	54.1	10.60	1.63	6.50	0.7	T1-2
F80/3	48-36	53.0	10.30	1.79	5.75	0.5	T1



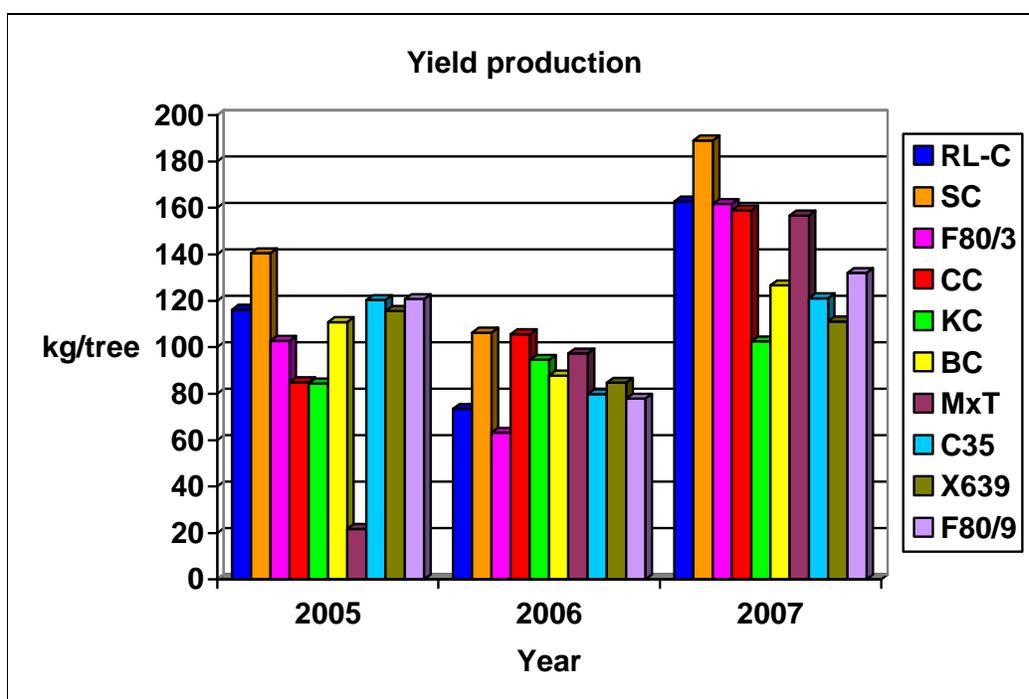
**Table 6.3.4.2.** Fruit size distribution of Star Ruby grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2007 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
RL-C	27	0.55	BC	27	0.68
RL-C	32	0.77	BC	32	1.57
RL-C	36	2.83	BC	36	5.94
RL-C	40	10.83	BC	40	17.38
RL-C	48	42.14	BC	48	49.52
RL-C	64	42.89	BC	64	24.92
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
SC	27	0.01	MxT	27	0.03
SC	32	0.07	MxT	32	0.12
SC	36	0.56	MxT	36	0.95
SC	40	2.86	MxT	40	5.10
SC	48	27.23	MxT	48	39.76
SC	64	69.26	MxT	64	54.03
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/3	27	0.19	C35	27	0.39
F80/3	32	0.19	C35	32	0.55
F80/3	36	1.37	C35	36	2.68
F80/3	40	7.25	C35	40	6.77
F80/3	48	45.42	C35	48	35.07
F80/3	64	45.59	C35	64	54.55
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
CC	27	0.17	X639	27	0.06
CC	32	0.40	X639	32	0.39
CC	36	1.32	X639	36	2.02
CC	40	6.74	X639	40	7.53
CC	48	38.21	X639	48	39.63
CC	64	53.15	X639	64	50.37
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
KC	27	0.41	F80/9	27	0.11

KC	32	0.79	F80/9	32	0.21
KC	36	5.06	F80/9	36	2.25
KC	40	17.76	F80/9	40	9.20
KC	48	47.79	F80/9	48	44.63
KC	64	28.18	F80/9	64	43.60

**Table 6.3.4.3.** Production of Star Ruby Grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2007 season.

Rootstock	Kg/tree (2005)	Kg/tree (2006)	Kg/tree (2007)
RL-C	116.1	73.4	162.8
SC	140.5	106.2	189.0
F80/3	102.8	63.1	161.7
CC	84.9	105.6	158.9
KC	84.4	94.6	102.5
BC	110.7	87.7	126.6
MxT	21.7	97.3	156.7
C35	120.3	79.8	121.0
X639	115.6	84.7	111.1
F80/9	120.7	77.9	132.0



### 6.3.5 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 vruggroottes moet toeneem.

Hierdie seisoen het al die kombinasies uitstekend gevaar in vergelyking met die vorige seisoen. Die opbrengste het met meer as 50 % toegeneem, en die interne kwaliteit het aan alle minimum standaarde vir

uitvoere voldoen. Midnight op Sunki 812 het baie belowende vuggrootte geproduseer, tussen telling 72 en 56. Die beste Delta kombinasie was op FF-6 gewees, met baie goeie oes produksie, interne kwaliteit en vruggrootte.

## Summary

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.

This season all the different combinations performed well in comparison with the previous season. Yields increased with more than 50 %, and the internal quality complied with all the minimum export standards. Midnight on Sunki 812 developed excellent fruit size, between count 72 and 56. Delta on FF-6 outperformed the other combinations, with good yield production, internal quality and fruit size.

## Introduction

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 was imported and propagated in 1996 by Esselen Nursery, an 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 6.3.5.1.** 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 discussions

### Midnight Valencia

Internally (Table 6.3.5.2) the fruit complied with the minimum export standards and the Brix (12.7) content increased every season. The acid (1.15) content remained fairly high and increased the shelf life of the fruit after harvesting. The fruit size peaked at count 72, followed by count 56, producing the optimum fruit size for Midnight (Table 6.3.5.3). Midnight on Sunki 812 produced a 50% higher yield in comparison with the previous season, from 46.8 kg/tree to 98.7 kg/tree (Table 6.3.5.4).

### Delta Valencia

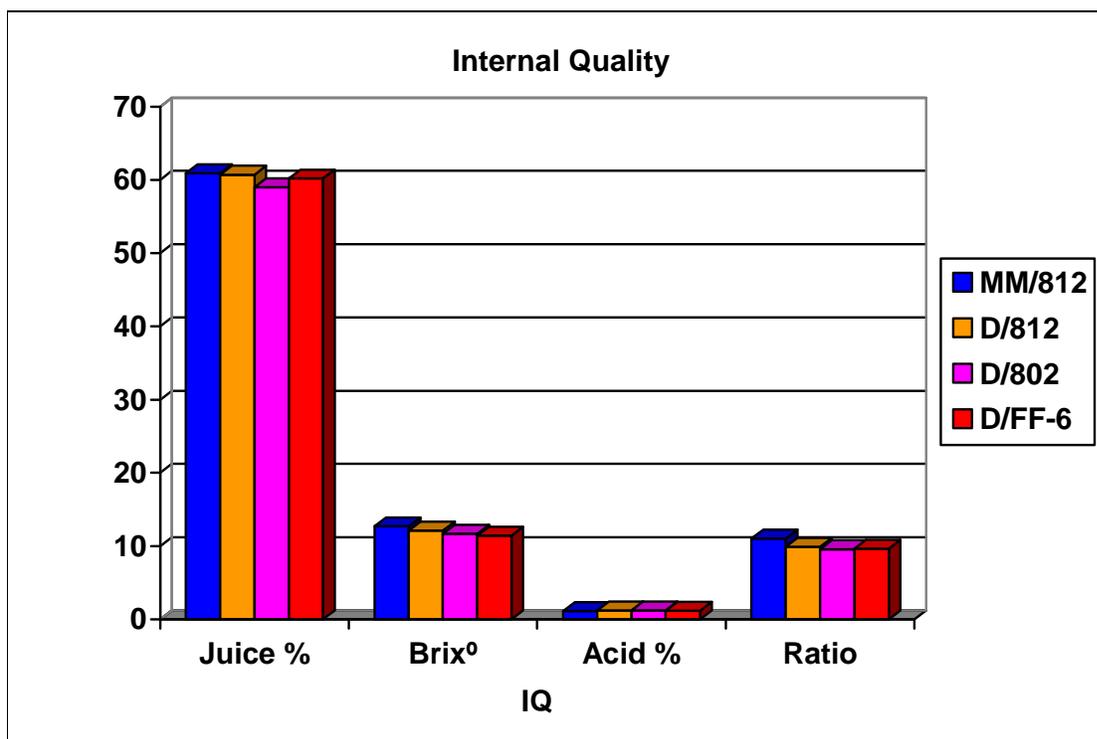
Delta on all three rootstocks produced fruit with good internal quality and complied with the export standards. Sunki 812 tested 12.1 Brix and 1.22 acid by the time of harvest, the highest contents for this season (Table 6.3.5.2). All three rootstocks peaked at count 105/125, followed by count 88 (Table 6.3.5.3). Delta on FF-6 set the best crop on the trees with 134.1 kg/tree, followed by Sunki 812 and 802 with 120 kg/tree. The yield production increased by 50 % in comparison to the previous season (Table 6.3.5.4).

## Conclusions and recommendations

Midnight and Delta on the different rootstock selections produced better quality fruit, complying with the minimum export standards for Valencias. The fruit set on all the combinations increased by more than 50%, producing an excellent yield on the trees.

**Table 6.3.5.2.** Internal fruit quality of Midnight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 27 July 2007.

Selection	Root-stock	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Midnight	Sunki 812	60.9	12.70	1.15	11.04	0.2	T1
Delta	Sunki 812	60.7	12.10	1.22	9.92	0.0	T1-2
Delta	Sunki 802	59.0	11.70	1.22	9.59	0.0	T1
Delta	FF-6	60.2	11.40	1.18	9.66	0.0	T1

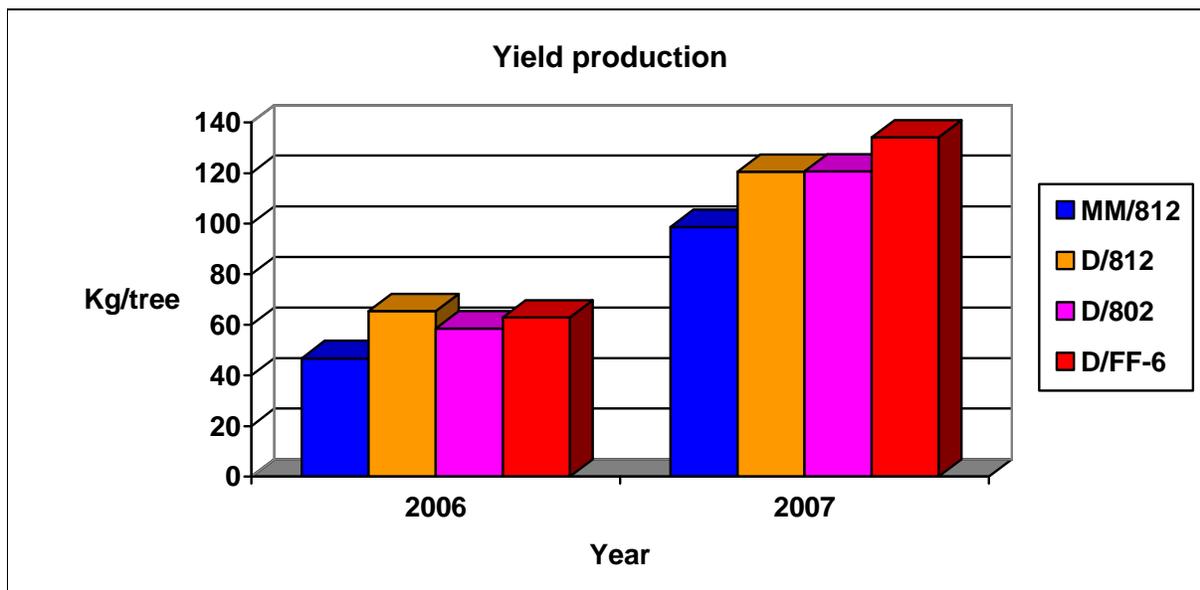


**Table 6.3.5.3.** Fruit size distribution at Esselen nursery during the 2007 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	Sunki 812	48	4.04	Delta	Sunki 802	48	0.03
Midnight	Sunki 812	56	34.01	Delta	Sunki 802	56	0.87
Midnight	Sunki 812	72	34.47	Delta	Sunki 802	72	8.83
Midnight	Sunki 812	88	17.09	Delta	Sunki 802	88	27.85
Midnight	Sunki 812	105/125	8.55	Delta	Sunki 802	105/125	52.66
Midnight	Sunki 812	144	1.85	Delta	Sunki 802	144	9.76
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 812	48	0.19	Delta	FF-6	48	0.03
Delta	Sunki 812	56	8.80	Delta	FF-6	56	1.08
Delta	Sunki 812	72	23.18	Delta	FF-6	72	12.86
Delta	Sunki 812	88	30.99	Delta	FF-6	88	31.01
Delta	Sunki 812	105/125	33.26	Delta	FF-6	105/125	48.27
Delta	Sunki 812	144	3.58	Delta	FF-6	144	6.75

**Table 6.3.5.4.** Production per tree of Midnight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2007 season.

Cultivar	Rootstock	Kg/tree (2006)	Kg/tree (2007)
Midknight	Sunki 812	46.8	98.7
Delta	Sunki 812	65.4	120.5
Delta	Sunki 802	58.5	120.6
Delta	FF-6	62.9	134.1



### 6.3.6 Evaluation of Grapefruit varieties on new imported rootstocks in the Swaziland area Experiment 416 B by J. Joubert (CRI)

#### Opsomming

Die prestasie van pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplant grondtipes moet ondersoek word. Die produksie, vrugsgroote, interne gehalte en skilkleur moet verbeter word.

Star Ruby en Marsh het 'n beter produksie gelewer (vestig goed) nadat die bome herplant is vanaf Tambankulu Estate (2000). Die Marsh en Star Ruby kombinasies het albei lae suiker inhoude geproduseer, soortgelyk aan die 2006 seisoen en onder die minimum uitvoer standaarde. Sap en suurinhoud was aanvaarbaar, en die produksie was baie belowend gewees. In albei gevalle het Sunki 809 as onderstam die beste produksie gelewer. Die vrugsgroote by Star Ruby het gepiek by telling 48, maar vir Marsh het die groottes gevarieer tussen telling 36 en 48.

Die nuwe aanplantings het goed gevestig en is vir die tweede seisoen ge-evalueer. Intern het die verskillende kombinasies baie goed gevaar en aan al die uitvoer spesifikasies voldoen. Die oes produksie het baie verbeter in vergelyking met 2006, en hier gaan baie waardevolle resultate verkry word.

## Summary

The performance of grapefruit cultivars on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved.

Star Ruby and Marsh produced a good crop after being transplanted from Tabankulu Estates (2000). The Marsh and Star Ruby combination both produced low sugar content, similar to the 2006 season and below the minimum export requirements. Juice and acid levels were acceptable, and production was very promising. Sunki 809 as rootstock on Marsh and Star Ruby, produced the best yield for this season. The fruit size for Star Ruby peaked at count 48, but with Marsh the fruit sizes varied between count 36 and 48.

The new plantings are well established and the trees produced enough fruit for evaluations. The internal quality was very promising and all the combinations comply with the packing specifications. The yield production on these trees improved in comparison with the 2006 season.

## Introduction

The performance of grapefruit cultivars on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved.

## Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 was imported and propagated in 1996 by Esselen Nursery, an accredited nursery in Malelane.

Star Ruby grapefruit was budded onto the four rootstock hybrids, Marsh grapefruit onto three rootstocks, and Oroblanco onto one rootstock in 1997. The newly imported rootstock hybrids include: Pummelo x trifoliolate orange HRS 802, Changsa x English large flowered trifoliolate orange HRS 809, Sunki x Beneke HRS 812 and Sunki x macrophylla C61. The trees were planted at Tambankulu Estates, Swaziland, in 1999. Experimental trees at Tambankulu Estates were transplanted at Tambuti Estates in Swaziland during November 2000 as certain orchards were to be removed from Tambankulu Estates. The trees were cut back and painted with white PVA. Making use of an excavator, the trees were uprooted and transplanted immediately at the new site. The trees were well watered and in good condition at the time of transplanting.

There was a second planting in 2003, 10 trees Marsh, NelRuby and Star Ruby all on C35, MxT, SC, and X639.

**Table 6.3.6.1.** Number of trees per rootstock in the grapefruit trial at Tambuti, Swaziland.

<b>Planted 2000</b>		
<b>Selection</b>	<b>Rootstock</b>	<b>No.of trees</b>
Marsh	812	1
Marsh	809	2
Marsh	C61	4
Star Ruby	C32	4
Star Ruby	802	2
Star Ruby	809	1
Star Ruby	812	2
Star Ruby	C61	5
Star Ruby	C35	4
Star Ruby	SC	8
<b>Planted 2003</b>		
<b>Selection</b>	<b>Rootstock</b>	<b>No.of trees</b>
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

## Results and discussions

Planted 2000

### Marsh

Marsh C61 (Table 6.3.6.2) produced the highest juice content (58.5%) followed by Sunki 812 (58.3%) and Sunki 809 (54.6%). The Brix<sup>o</sup> values (Table 6.3.6.2) were below the export minimum of 9 for the Japan markets, ranging from 7.8 to 8.6<sup>o</sup>. The fruit size production (Table 6.3.6.3) on C61, peaked at count 48 followed by Sunki 809 with count 40 and Sunki 812 with count 36. Marsh in combination with rootstock Sunki 809 (Table 6.3.6.4) produced the best yield (120.5 kg/tree) followed by C61 (112.4 kg/tree) and Sunki 812 (103.7 kg/tree).

### Star Ruby

The highest juice content (Table 6.3.6.2) was produced on Sunki 812 (62.3%) followed by C32 (60.7%) and Sunki 802 (60.4%). All the combinations complied with the juice content export standards, but unfortunately only C32 (10.1), C35 (9.5) and Sunki 802 (9.9) was above the minimum Brix<sup>o</sup> for Japan requiring minimum 9 Brix (Table 6.3.6.2). The highest fruit size production peaked at count 48 (C61, C35, Sunki 812) followed by count 40 and count 36 (Table 6.3.6.3). Star Ruby on Sunki 809 produced the best yield on the trees with 169.7 kg followed by C32 (146.2 kg/tree) and Sunki 802 (145.6 kg/tree) (Table 6.3.6.4).

Planted 2003

### Marsh

All the combinations produced a Brix<sup>o</sup> above 9.0, complying with the export standards. MxT outperformed the rest of the rootstocks with 11 Brix<sup>o</sup>. X639 produced the best juice content (56.0%) followed by SC (53.8%) and C35 (52.1%) (Table 6.3.6.5). Marsh peaked at count 48, followed by count 40 and count 36 (Table 6.3.6.6). Marsh on SC outperformed the rest of the combinations and produced 67.3 kg/tree in comparison 2006 with 41.5 kg/tree. The other rootstocks varied between 30.4 and 37.6kg/tree (Table 6.3.6.7). All the combinations increased fruit production at least double in comparison to 2006.

### NelRuby

Internally NelRuby was very promising this season and complied with all the minimum export standards. The highest juice content was produced on MxT with 59.9% and highest Brix<sup>o</sup> also on MxT at 10.8 (Table 6.3.6.5). Fruit size peaked at count 48 followed by count 36 and count 40. Considering that count 48, followed by count 40 and count 36 was ranked from the highest percentage fruit per rootstock to the lowest percentage (Table 6.3.6.6). NelRuby on SC produced 37.5 kg/tree (2006-19.4 kg/tree) by the time of harvest, followed by X639 (31.6 kg/tree) and MxT (25.9 kg/tree) (Table 6.3.6.7).

### Star Ruby

Star Ruby on all the rootstocks complied with the export standards. The fruit produced on SC resulted in the best juice content (58.8%) and Brix<sup>o</sup> (11.1) for this trial (Table 6.3.6.5). All the fruit size counts peaked at count 48, followed by count 40 and 36 (Table 6.3.6.6). Production peaked at 49.2 kg/tree on MxT, followed by SC (48.8 kg/tree) and C35 (33.3 kg/tree) (Table 6.3.6.7).

## Conclusions and recommendations

Planted 2000:

The Brix content on all the Marsh combinations was below 9.0, not complying with the minimum export standards for Japan. Marsh on Sunki 809 rootstock outperformed the other combinations, producing the best yield on the trees with good internal quality, except for the slightly low Brix content.

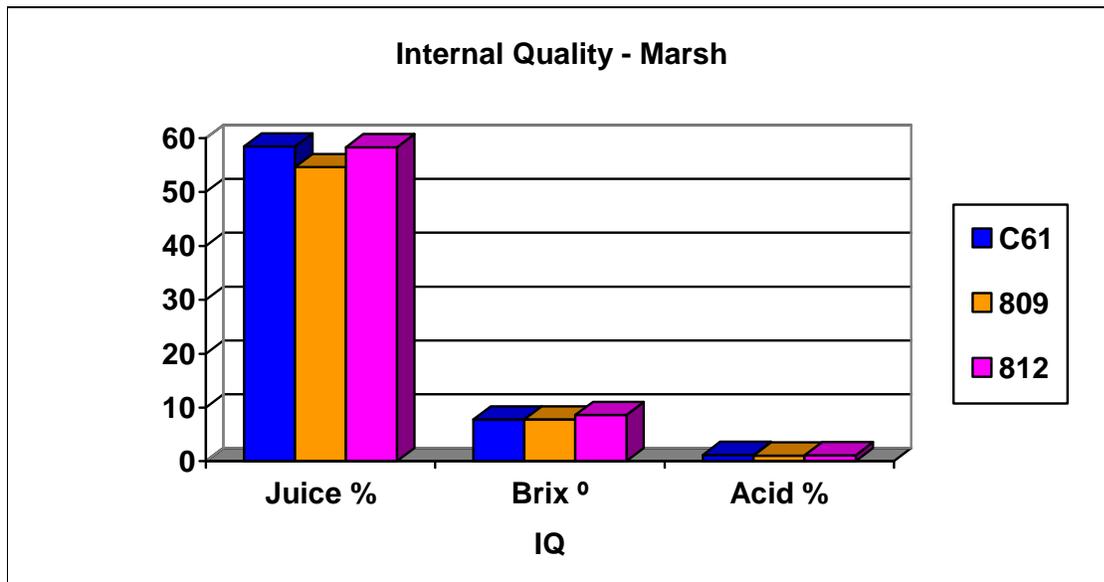
The fruit size peaked at count 48, all the combinations tested below 9.0 Brix for this trial, not complying with the export standards. Star Ruby on Sunki 809 performed similar to the Marsh selection, outperforming the rest with the highest yield production of 169.7 kg/tree.

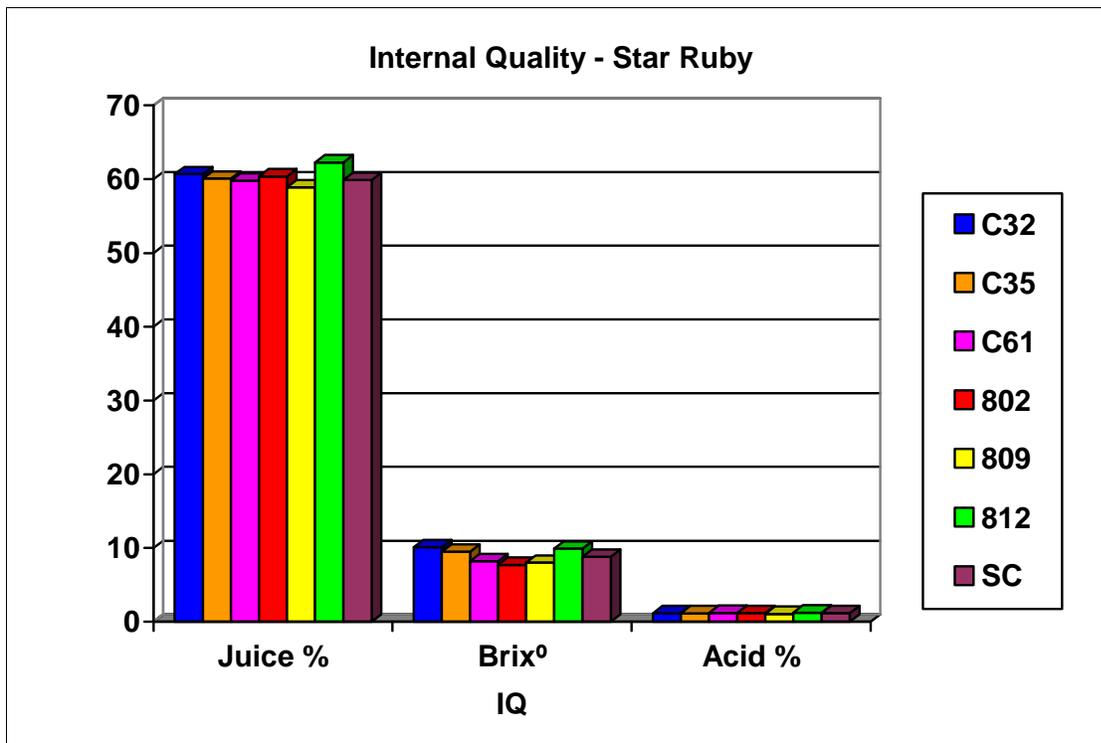
Planted 2003:

These combinations were evaluated for the second time this season, and the production increased substantially in comparison with the 2006 season. The internal quality on all three selections was very promising and complied with the export standards. Marsh, NelRuby and Star Ruby peaked at count 48, followed by count 36/40. The yield on the trees increased by up to 50 %, evaluations will continue.

**Table 6.3.6.2.** Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 22 May 2007 (Planted 2000).

Selection	Root-stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. Seed	Colour
Marsh	C61	40-23	58.5	7.8	1.21	6.45	2.8	T1-4
Marsh	809	40-27	54.6	7.8	1.08	7.22	2.1	T1-2
Marsh	812	36-27	58.3	8.6	1.12	7.68	2.1	T1-3
TSR	C32	40-27	60.7	10.1	1.15	8.78	0.1	T1
TSR	C35	40-27	60.1	9.5	1.15	8.26	0.3	T1-2
TSR	C61	40-32	59.8	8.2	1.19	6.89	0.3	T1
TSR	802	40-23	60.4	7.7	1.16	6.64	0.0	T1-2
TSR	809	36-27	58.9	8.0	1.06	7.55	0.3	T1
TSR	812	40-27	62.3	9.9	1.23	8.05	0.3	T1
TSR	SC	36-27	59.9	8.8	1.16	7.59	0.0	T1





**Table 6.3.6.3.** Fruit size distribution per rootstock at Tambuti Estate during the 2007 season (Planted 2000).

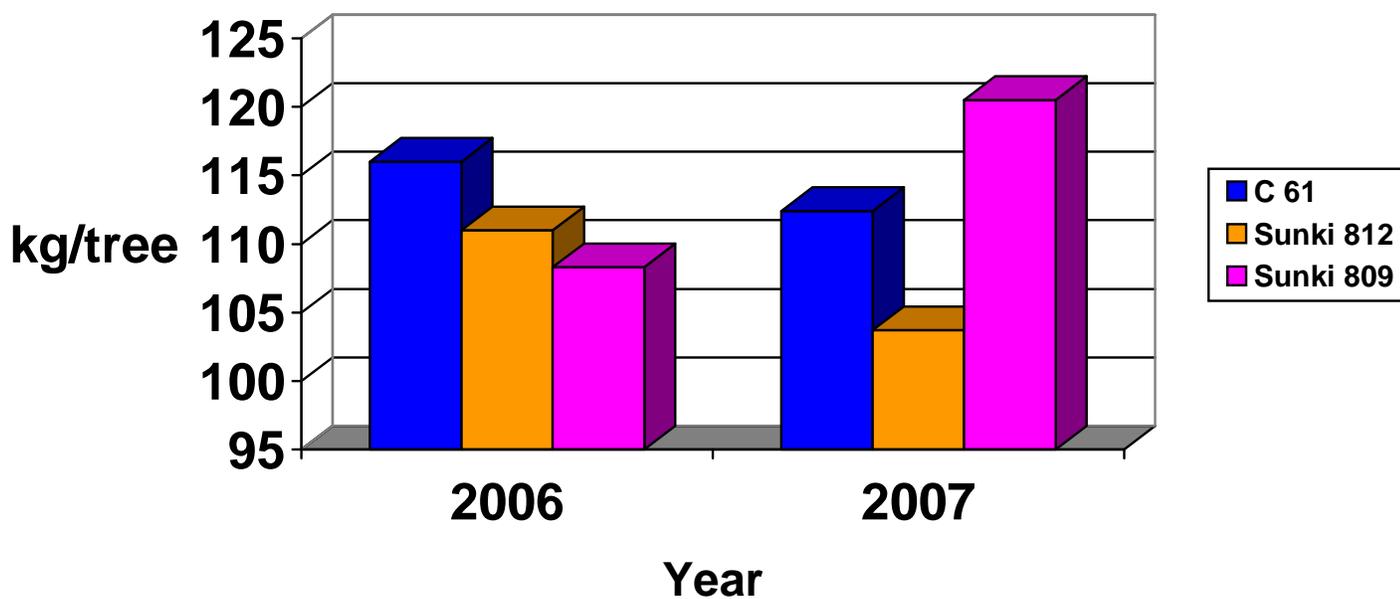
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 61	27	9.72	Star Ruby	C 35	27	10.88
Star Ruby	C 61	32	4.89	Star Ruby	C 35	32	7.60
Star Ruby	C 61	36	19.98	Star Ruby	C 35	36	20.33
Star Ruby	C 61	40	26.30	Star Ruby	C 35	40	25.19
Star Ruby	C 61	48	31.37	Star Ruby	C 35	48	25.67
Star Ruby	C 61	64	7.75	Star Ruby	C 35	64	10.34
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 812	27	8.47	Star Ruby	SC-C1	27	18.92
Star Ruby	Sunki 812	32	7.84	Star Ruby	SC-C1	32	13.50
Star Ruby	Sunki 812	36	15.47	Star Ruby	SC-C1	36	29.75
Star Ruby	Sunki 812	40	19.07	Star Ruby	SC-C1	40	22.53
Star Ruby	Sunki 812	48	36.02	Star Ruby	SC-C1	48	11.55
Star Ruby	Sunki 812	64	13.14	Star Ruby	SC-C1	64	3.75
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 809	27	7.16	Marsh	C 61	27	8.70
Star Ruby	Sunki 809	32	6.95	Marsh	C 61	32	6.47
Star Ruby	Sunki 809	36	22.32	Marsh	C 61	36	19.63
Star Ruby	Sunki 809	40	29.89	Marsh	C 61	40	27.39
Star Ruby	Sunki 809	48	23.58	Marsh	C 61	48	31.20
Star Ruby	Sunki 809	64	10.11	Marsh	C 61	64	6.61
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 802	27	10.82	Marsh	Sunki 812	27	13.41
Star Ruby	Sunki 802	32	13.17	Marsh	Sunki 812	32	10.87
Star Ruby	Sunki 802	36	28.29	Marsh	Sunki 812	36	23.91
Star Ruby	Sunki 802	40	26.47	Marsh	Sunki 812	40	21.74
Star Ruby	Sunki 802	48	15.25	Marsh	Sunki 812	48	23.19
Star Ruby	Sunki 802	64	6.00	Marsh	Sunki 812	64	6.88
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit

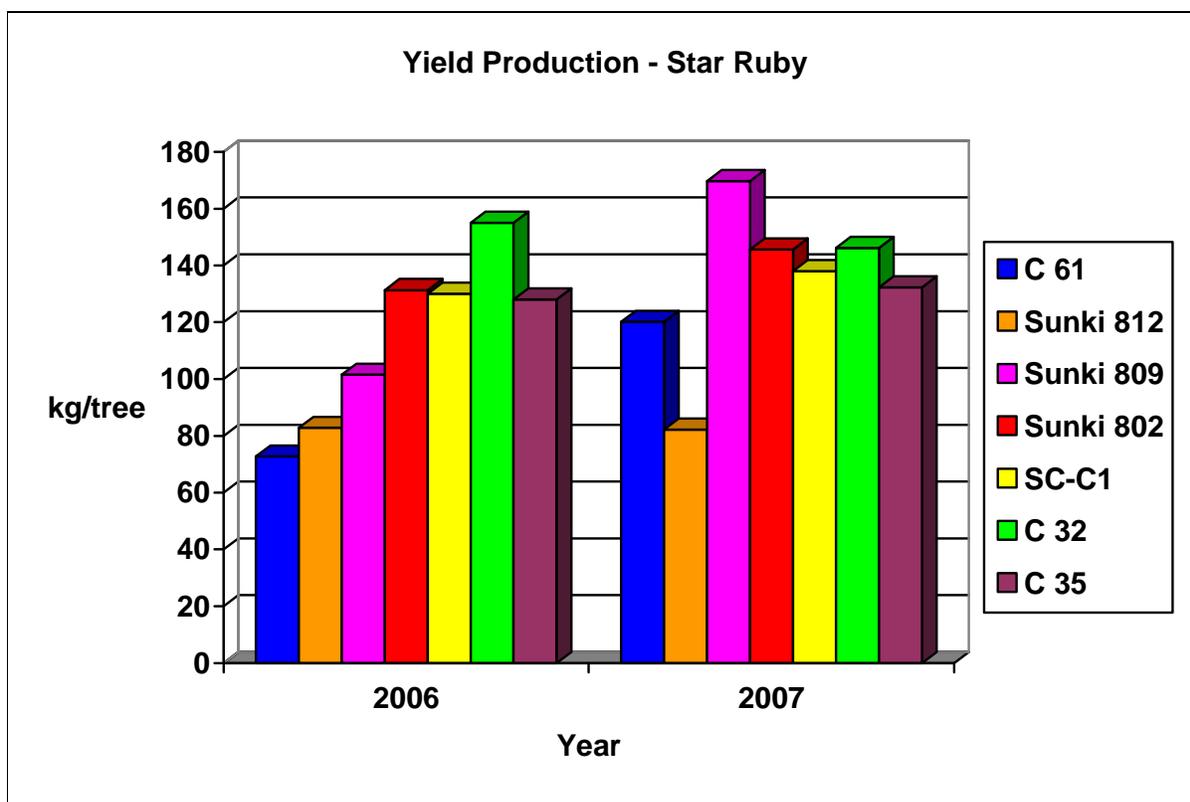
Star Ruby	C 32	27	22.32	Marsh	Sunki 809	27	15.81
Star Ruby	C 32	32	10.58	Marsh	Sunki 809	32	11.18
Star Ruby	C 32	36	23.74	Marsh	Sunki 809	36	23.64
Star Ruby	C 32	40	23.41	Marsh	Sunki 809	40	25.72
Star Ruby	C 32	48	15.26	Marsh	Sunki 809	48	19.33
Star Ruby	C 32	64	4.68	Marsh	Sunki 809	64	4.31

**Table 6.3.6.4.** Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2007 (Planted 2000).

Cultivar	Rootstock	Kg/tree(06)	Kg/tree(07)
Marsh	C 61	116.0	112.4
Marsh	Sunki 812	111.0	103.7
Marsh	Sunki 809	108.3	120.5
Star Ruby	C 61	72.7	120.1
Star Ruby	Sunki 812	82.8	82.2
Star Ruby	Sunki 809	101.5	169.7
Star Ruby	Sunki 802	131.3	145.6
Star Ruby	SC-C1	130.0	138
Star Ruby	C 32	155.0	146.2
Star Ruby	C 35	128.0	132.3

### Yield production - Marsh





**Table 6.3.6.5.** Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 22 May 2007 (Planted 2003).

Selection	Root-stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. Seed	Colour
Marsh	C35	40-27	52.1	10.1	1.04	9.71	4.3	T1-6
Marsh	MxT	40-23	51.6	11.0	1.29	8.53	4.4	T4-5
Marsh	SC	40-27	53.8	10.7	1.32	8.11	3.9	T4-5
Marsh	X639	40-27	56.0	9.5	1.14	8.33	4.6	T2-4
NelRuby	C35	40-27	56.4	10.2	0.90	11.33	1.8	T1-2
NelRuby	MxT	40-27	59.9	10.8	1.08	10.00	3.6	T1-2
NelRuby	SC	36-27	55.1	10.3	1.08	9.54	3.4	T1-2
NelRuby	X639	40-32	57.3	10.1	1.06	9.53	3.2	T2
TSR	C35	36-27	57.8	9.7	1.08	8.98	1.1	T1
TSR	MxT	36-23	58.7	10.9	1.40	7.79	0.8	T1
TSR	SC	40-32	58.8	11.1	1.45	7.66	0.3	T1
TSR	X639	40-32	57.2	10.1	1.33	7.59	0.9	T1-2

**Table 6.3.6.6.** Fruit size distribution per rootstock at Tambuti Estate during the 2007 season (Planted 2003).

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	C 35	27	11.66	Nelruby	SC	27	0.00
Marsh	C 35	32	11.32	Nelruby	SC	32	0.08
Marsh	C 35	36	26.21	Nelruby	SC	36	3.58
Marsh	C 35	40	26.21	Nelruby	SC	40	14.49
Marsh	C 35	48	19.86	Nelruby	SC	48	55.49
Marsh	C 35	64	4.73	Nelruby	SC	64	26.35

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	MxT	27	0.83	Nelruby	X639	27	0.00
Marsh	MxT	32	1.48	Nelruby	X639	32	0.28
Marsh	MxT	36	6.59	Nelruby	X639	36	2.26
Marsh	MxT	40	21.99	Nelruby	X639	40	14.06
Marsh	MxT	48	50.74	Nelruby	X639	48	58.49
Marsh	MxT	64	18.37	Nelruby	X639	64	24.91
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	SC	27	0.63	Star Ruby	C 35	27	3.14
Marsh	SC	32	1.36	Star Ruby	C 35	32	4.26
Marsh	SC	36	14.41	Star Ruby	C 35	36	15.20
Marsh	SC	40	26.87	Star Ruby	C 35	40	27.56
Marsh	SC	48	46.01	Star Ruby	C 35	48	38.20
Marsh	SC	64	10.71	Star Ruby	C 35	64	11.65
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Marsh	X639	27	0.32	Star Ruby	MxT	27	1.16
Marsh	X639	32	1.58	Star Ruby	MxT	32	2.65
Marsh	X639	36	9.38	Star Ruby	MxT	36	12.33
Marsh	X639	40	25.61	Star Ruby	MxT	40	24.83
Marsh	X639	48	49.95	Star Ruby	MxT	48	46.27
Marsh	X639	64	13.17	Star Ruby	MxT	64	12.75
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Nelruby	C 35	27	2.35	Star Ruby	SC	27	0.91
Nelruby	C 35	32	3.86	Star Ruby	SC	32	1.23
Nelruby	C 35	36	18.62	Star Ruby	SC	36	9.94
Nelruby	C 35	40	32.38	Star Ruby	SC	40	22.92
Nelruby	C 35	48	36.91	Star Ruby	SC	48	47.14
Nelruby	C 35	64	5.87	Star Ruby	SC	64	17.86
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Nelruby	MxT	27	0.00	Star Ruby	X639	27	0.50
Nelruby	MxT	32	0.47	Star Ruby	X639	32	1.67
Nelruby	MxT	36	3.27	Star Ruby	X639	36	7.01
Nelruby	MxT	40	14.84	Star Ruby	X639	40	19.70
Nelruby	MxT	48	50.12	Star Ruby	X639	48	46.08
Nelruby	MxT	64	31.31	Star Ruby	X639	64	25.04

**Table 6.3.6.7.** Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2007 (Planted 2003).

Cultivar	Rootstock	Kg/tree (2006)	Kg/tree (2007)
Marsh	C35	19.1	32.7
Marsh	MxT	16.1	37.6
Marsh	SC	41.5	67.3
Marsh	X639	7.6	30.4
Nelruby	C35	12.4	20.5
Nelruby	MxT	10.6	25.9
Nelruby	SC	19.4	37.5
Nelruby	X639	8.6	31.6
Star Ruby	C35	4.6	33.3
Star Ruby	MxT	12.9	49.2
Star Ruby	SC	19.9	48.8
Star Ruby	X639	5.9	18.4

6.3.7 **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 kommersiële 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. Ongelukkig het die plukspanne Midnight en Portsgate verkeerdelik saam met die res van die kommersiële bome geoes, en kon die produksie inligting nie hierdie seisoen verkry word nie. Slegs Delta en McClean SL kon dus geevalueer word vir 2007, maar gelukkig is die bome nog jonk. Evaluasies sal voortgesit word.

**Summary**

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. This rootstock trial was harvested for the third time this season and keep in mind the trees are still young. The difference in yield production escalated in comparison with 2005 and valuable information is available. The capital investment to establish the orchard will bring in returns via early production. By mistake the picking teams harvested Midnight and Portsgate with the rest of the commercial trees, and no production results were available for the 2007 season. Only Delta and McClean SL were evaluated for 2007, fortunately the trees are still young and future evaluations will continue.

**Introduction**

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 1998. Evaluate visually to determine production per tree, trueness to type and compatibility with scion and harvest each tree with the sizer to determine production per tree as well as fruit size distribution per tree. Samples will be taken and internal quality tested and analysed. Fruit colour will be evaluated and analysed.

**Table 6.3.7.1.** List of cultivar x rootstock combinations in the Valencia trial at TSB Hectorspruit in the Komatipoort area.

<b>Selection</b>	<b>Rootstock</b>
Delta (Control)	C35
Delta (Control)	CC
Delta (Control)	KC
Delta (Control)	MxT
Delta (Control)	SC
Delta (Control)	Terrabella
Delta (Control)	X639
McClean SL	C35
McClean SL	CC
McClean SL	KC
McClean SL	MxT
McClean SL	SC
McClean SL	Terrabella
McClean 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 6.3.7.2)

- Juice %: All the selections comply with the export standards above 52% juice content. X639 produced the highest level (60.8%) followed by SC (59.4%) and C35 (58.2%). The lowest juice content was produced on TB with 57.8%.
- Brix<sup>o</sup>: TB and KC produced the highest Brix<sup>o</sup> (12.1) followed by X639 (12.0) and SC/C35 (11.7). This season all the other combinations were above the minimum levels for packing, with MxT producing the lowest sugar content of 11.3° Brix.
- Acid: All the rootstock combinations produced a too low acid level below 0.85 and did not comply with export standards.

#### Fruit size distribution (Table 6.3.7.3)

- The fruit size evaluation shows the largest peak between counts 72, 88 and 105/125. C35 and KC peaked at count 88, MxT and TB peaked at count 105/125 and both SC as well as X639 peaked at count 72.
- Production per tree (Table 6.3.7.4)
- MxT produced the highest yield per tree (71.5 kg), followed by SC with 43.4 kg/tree and TB with 38.2 kg/tree. All three selections develop into a large tree by the time of maturity.

### McClellan SL

#### Internal fruit quality analysis (Table 6.3.7.2)

- Juice %: X639 produced the highest juice content (59.8%) followed by TB (59.2%) and SC (58.5%). All the selections comply with the export standards above 48% juice content.
- Brix<sup>o</sup>: CC produced the highest Brix content (12.2), followed by X639 with 12.1 and TB with 12 Brix. The lowest Brix content was produced on MxT with 11.2°.
- Acid: C35 was below the minimum Valencia export standard with 0.66. All the other selections comply with the minimum standards.

#### Fruit size distribution (Table 6.3.7.3)

- The fruit size evaluation shows the largest peak at count 105/125 on CC, KC, MxT, SC, TB and X639. The next highest count in fruit size was count 88. The third highest count evaluated in fruit size was count 72.

#### Production per tree (Table 6.3.7.4)

TB produced the highest yield per tree (98.1 kg), followed by C35 with 72.3 kg/tree and X639 with 69.9 kg/tree

## Conclusions and recommendations

The fruit set and crop production on the trees increased considerably with better internal qualities and fruit size. Due to unforeseen circumstances, Portsgate and Midnight were accidentally harvested with the rest of the commercial trees, and no results are available for 2007. Delta complied with the minimum export standards for juice and Brix content, but the acid content was too low. Delta on TB and KC produced the highest Brix content, and MxT set the best crop on the trees.

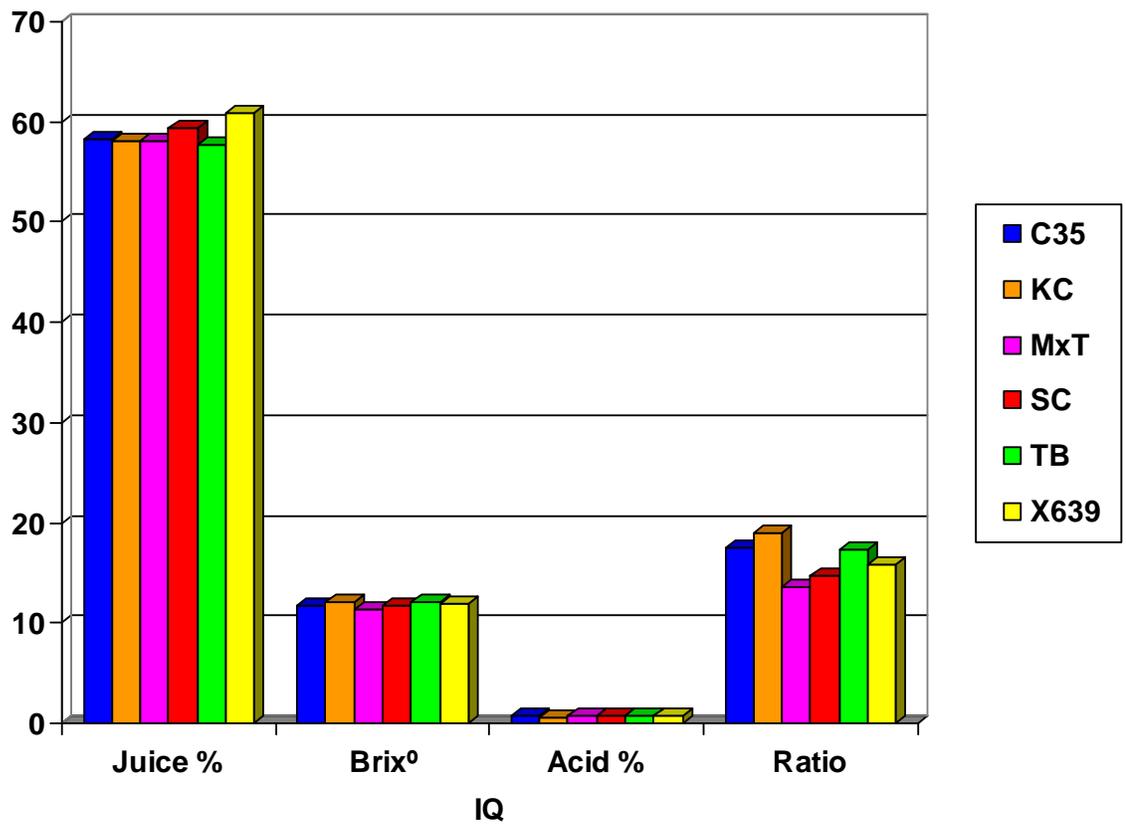
McClellan SL on all the rootstock selections complied with the export standards, except for C35 developing too low acid content by the time of harvest. Terrabella set the best yield on the trees, producing 98.1 kg/tree.

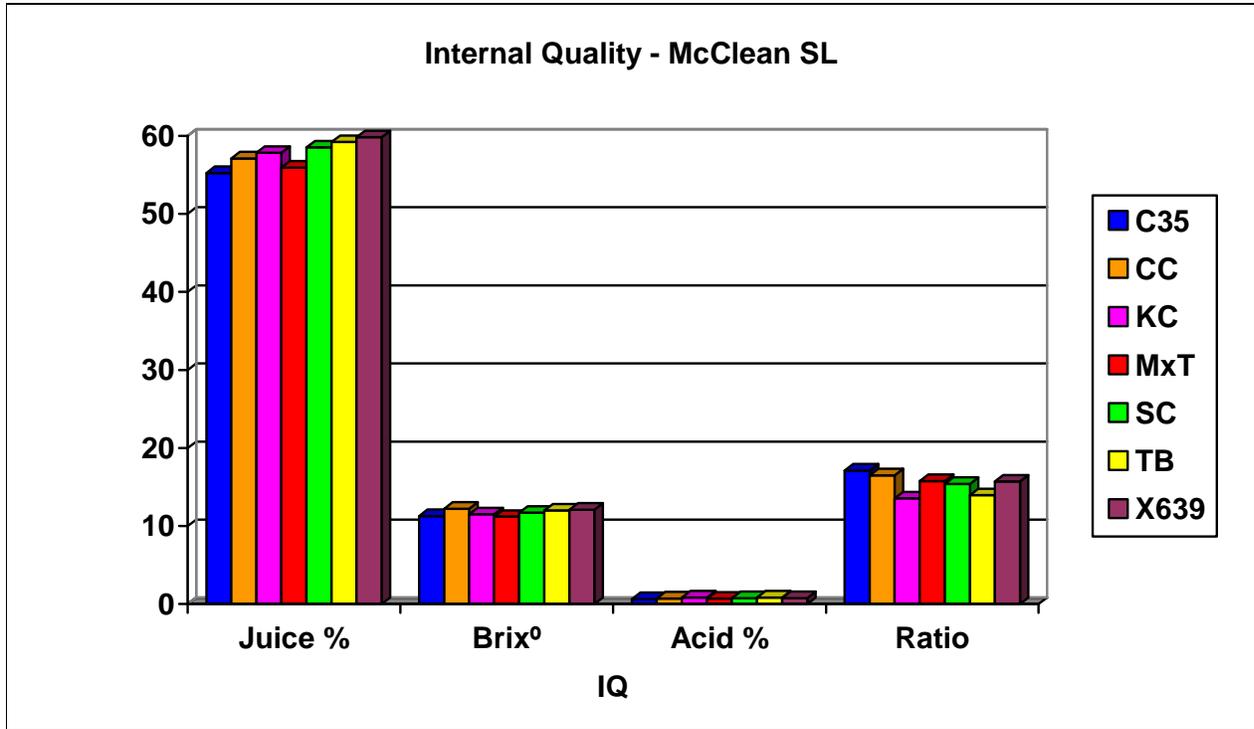
The trial looks promising at this stage. It will be very valuable to evaluate the production increase on the young trees. Over the long term this will give an indication of the performance of the combinations.

**Table 6.3.7.2.** Internal fruit quality data for Valencias on different rootstocks at TSB Hectorspruit on 29 August 2007.

Selection	Root-Stock	Count	Juice %	Brix °	Acid %	Ratio	Ave. seed	Colour
Delta	C35	88-48	58.2	11.70	0.67	17.46	0.0	T1-2
Delta	KC	105-64	58.1	12.10	0.64	18.91	0.0	T1
Delta	MxT	88-48	58.1	11.30	0.83	13.61	0.0	T1-5
Delta	SC	88-48	59.4	11.70	0.80	14.63	0.0	T1-2
Delta	TB	72-64	57.8	12.10	0.70	17.29	0.0	T1-2
Delta	X639	88-56	60.8	12.00	0.76	15.79	0.0	T1
McClellan SL	C35	72-40	55.2	11.30	0.66	17.12	0.0	T1-2
McClellan SL	CC	88-48	57.1	12.20	0.74	16.49	0.0	T1-2
McClellan SL	KC	125-48	57.8	11.50	0.85	13.53	0.0	T1-2
McClellan SL	MxT	88-40	55.9	11.20	0.71	15.77	0.0	T1-2
McClellan SL	SC	125-72	58.5	11.70	0.76	15.39	0.0	T2-4
McClellan SL	TB	105-64	59.2	12.00	0.86	13.95	0.0	T1-2
McClellan SL	X639	105-48	59.8	12.10	0.77	15.71	0.0	T1-3

### Internal Quality - Delta





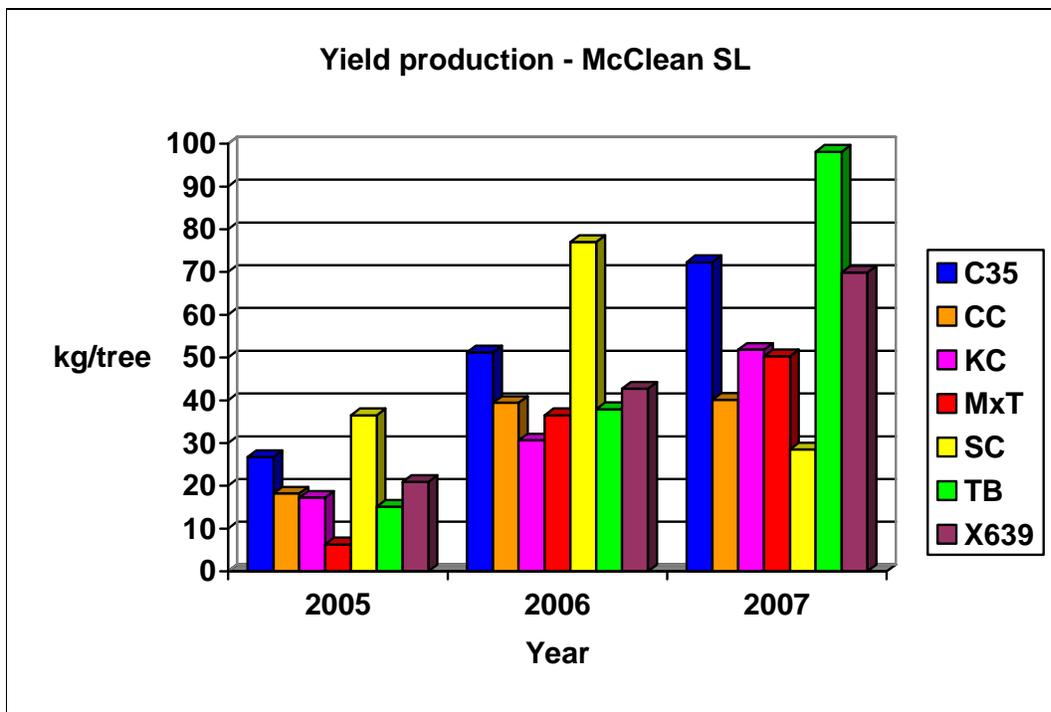
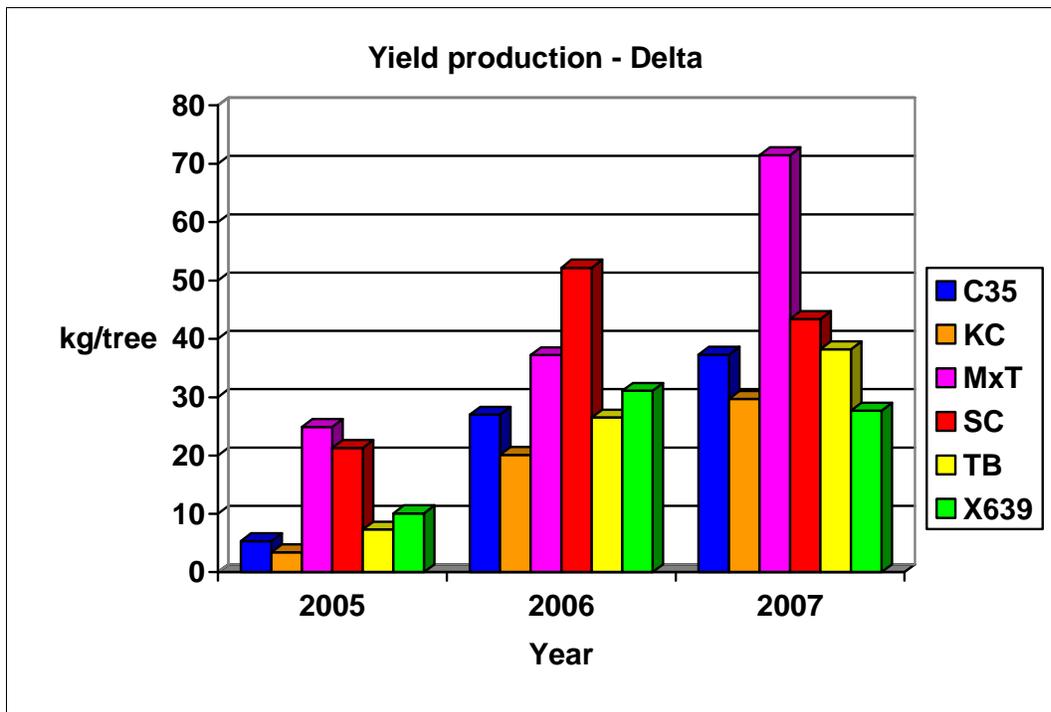
**Table 6.3.7.3.** Fruit size distribution per rootstock at TSB Hectorspruit during the 2007 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	C35	48	0.31	Delta	C35	48	0.30
McClean SL	C35	56	7.76	Delta	C35	56	5.52
McClean SL	C35	72	22.55	Delta	C35	72	23.87
McClean SL	C35	88	33.93	Delta	C35	88	35.31
McClean SL	C35	105/125	30.20	Delta	C35	105/125	32.74
McClean SL	C35	144	5.26	Delta	C35	144	2.27
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	CC	48	0.17	Delta	KC	48	0.24
McClean SL	CC	56	4.46	Delta	KC	56	5.24
McClean SL	CC	72	15.40	Delta	KC	72	25.00
McClean SL	CC	88	23.40	Delta	KC	88	31.22
McClean SL	CC	105/125	45.96	Delta	KC	105/125	30.98
McClean SL	CC	144	10.61	Delta	KC	144	7.32
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	KC	48	0.20	Delta	MxT	48	0.19
McClean SL	KC	56	3.65	Delta	MxT	56	3.75
McClean SL	KC	72	16.71	Delta	MxT	72	16.63
McClean SL	KC	88	28.65	Delta	MxT	88	27.60
McClean SL	KC	105/125	43.30	Delta	MxT	105/125	46.35
McClean SL	KC	144	7.49	Delta	MxT	144	5.48
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
McClean SL	MxT	48	0.49	Delta	SC	48	1.25
McClean SL	MxT	56	6.60	Delta	SC	56	11.27
McClean SL	MxT	72	18.89	Delta	SC	72	29.79
McClean SL	MxT	88	27.32	Delta	SC	88	27.73
McClean SL	MxT	105/125	37.15	Delta	SC	105/125	25.85

McClean SL	MxT	144	9.55	Delta	SC	144	4.11
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
McClean SL	SC	48	0.00	Delta	TB	48	0.09
McClean SL	SC	56	0.87	Delta	TB	56	6.02
McClean SL	SC	72	7.03	Delta	TB	72	21.80
McClean SL	SC	88	22.08	Delta	TB	88	31.48
McClean SL	SC	105/125	50.54	Delta	TB	105/125	32.24
McClean SL	SC	144	19.48	Delta	TB	144	8.36
<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>
McClean SL	TB	48	0.00	Delta	X639	48	5.29
McClean SL	TB	56	2.45	Delta	X639	56	24.57
McClean SL	TB	72	17.45	Delta	X639	72	29.55
McClean SL	TB	88	31.75	Delta	X639	88	21.31
McClean SL	TB	105/125	40.59	Delta	X639	105/125	16.64
McClean SL	TB	144	7.76	Delta	X639	144	2.64
	<b>Cultivar</b>	<b>Rootstock</b>	<b>Size</b>	<b>% Fruit</b>			
	McClean SL	X639	48	0.27			
	McClean SL	X639	56	9.60			
	McClean SL	X639	72	25.54			
	McClean SL	X639	88	29.83			
	McClean SL	X639	105/125	29.94			
	McClean SL	X639	144	4.83			

**Table 6.3.7.3.** Production per tree of Valencia selections on different rootstocks at TSB Hectorspruit during the 2007 season.

<b>Cultivar</b>	<b>Rootstock</b>	<b>Kg/tree(05)</b>	<b>Kg/tree(06)</b>	<b>Kg/tree(07)</b>
Delta Valencia	C35	5.3	27.0	37.3
Delta Valencia	KC	3.4	20.1	29.7
Delta Valencia	MxT	24.9	37.2	71.5
Delta Valencia	SC	21.3	52.2	43.4
Delta Valencia	TB	7.3	26.5	38.2
Delta Valencia	X639	10.1	31.1	27.7
McClean SL	C35	26.7	51.2	72.3
McClean SL	CC	18.2	39.4	40.1
McClean SL	KC	17.2	30.6	51.8
McClean SL	MxT	6.3	36.5	50.3
McClean SL	SC	36.5	77.0	28.5
McClean SL	TB	15.1	37.9	98.1
McClean SL	X639	20.9	42.7	69.9



**6.3.8 Colour charts for Star Ruby, Rosé and Lemons. Sheepnose charts for Star Ruby and rind quality charts for Lemons**  
 Experiment 903 by A.T.C. Lee, J. Joubert, Kim Stoltz and Peter Stephen (CRI)

A meeting was held in Nelspruit during October 2007 to draft colour charts for Star Ruby Grapefruit, Rose Grapefruit and Lemons as well as to draft sheepnose charts for Star Ruby and rind quality for lemons. Representatives of grapefruit and lemon production areas were present as well as CRI personnel to discuss these charts.

The photographs used had been taken by Peter Stephens using fruit collected by Johan Joubert and Kim Stoltz during the 2007 season.

The proposed Rose colour charts were rejected and it was requested that fruit be collected the following season to redo this chart.

The Star Ruby colour chart was provisionally accepted with certain changes and is now awaiting final approval.

The Star Ruby sheepnose chart was accepted with certain changes.

The lemon colour chart is still under discussion.

### **Kleurplate van Star Ruby, Rosé en Suurlemoene. Skaapneus plate van Star Ruby en skil kwaliteit plate van Suurlemoene**

'n Vergadering is in Nelspruit gedurende Oktober 2007 gehou om die voorlopige kleurplate vir Star Ruby pomelo, Rosé pomelo en Suurlemoene te bespreek. Die voorlopige skaapneus plate vir Star Ruby en die skil kwaliteit plate vir suurlemoene is ook voorgelê. Die verteenwoordigers van die Pomelo en Suurlemoen produksie areas was teenwoordig, so wel as die CRI personeel om hierdie plate te bespreek.

Peter Stephens het die foto's geneem, Johan Joubert en Kim Stoltz het die vrugte versamel gedurende die 2007 seisoen.

Die voorgestelde Rosé kleur plate was nie aanvaar nie en daar is versoek om weer vrugte die volgende seisoen te versamel om die plate te verbeter.

Die Star Ruby skaapneus plate word aanvaar met geringe aanpassings.

Die Suurlemoen kleur plate is steeds onder bespreking.

#### **6.3.9 Other Activities**

##### Blemish standards

The new blemish standards book was finalised for reprinting. Furthermore final proposals for the Star Ruby sheepnose and colour charts were prepared and forwarded to the Grapefruit Focus Group for approval. In addition Lemon colour charts were submitted to the Lemon Focus Group for further attention.

##### Cultivars

The first evaluations and internal quality tests of the Patensie Early Navel, a branch mutation of Palmer navel, were initiated on the property of C. Malan, Patensie. In addition internal fruit quality tests were carried out on early maturing cultivars.

Trial sites throughout the Western Cape, Eastern Cape, KwaZulu-Natal, Letsitele, Rustenburg, Marble Hall, Burgersfort, Swaziland and Mpumalanga areas were visited to meet the cooperators and discuss the continuation and evaluation of these trials during 2008.

The establishment of a semi commercial planting of Clemcotts (Murcott x Clementine hybrid) was arranged in association with P. Nortje, Kirkwood.

The owners of new local mutants in numerous citrus areas were visited to discuss the future of their cultivars and arrange for evaluations during 2008.

In the Western Cape the Lemon rootstock trial at Citrusdal was evaluated and harvested; the Late Navel trial at Hexriver citrus was evaluated during the same visit. This data has not yet been processed.

Meetings were held with technical personnel of other citrus organizations to discuss cooperation in cultivar development projects.

##### General

The first workshop to be held with all companies involved in cultivar development in South Africa was held to improve cooperation and understanding on cultivar issues.

Cultivar study group meetings were arranged by CRI's Extension Representative in the southern areas of South Africa; these were attended by Extension, Cultivar Development and the Private Cultivar Companies.

Legal

Discussions were held with G. Gess on CRI's non propagation and ownership agreements with other organizations.

Procedures for the release of propagation material of CRI cultivars to growers and nurserymen were drafted and formalised.

Publications

An article on the Burgersfort Late Clementine trial at L. Lotter as well as an article to motivate growers to find new local mutations were prepared and finalised for publication.

7 **CITRUS IMPROVEMENT SCHEME (CIS) 2007**  
By Thys du Toit and Louise Jackson (CRI)

7.1 **PROGRAMME SUMMARY**

*Citrus Foundation Block:* A total of 2 009 235 buds were supplied by the Citrus Foundation Block during 2007 which is 67 616 less than in 2006. Star Ruby, followed by Midnight, are the 2 most popular cultivars over the past 3 years. An increase in seed sales from 951 litres in 2006 to 1990 litres in 2007 points to an increase in the demand for nursery trees. The second insect controlled green house is now in full production with the exception of 2700 seedlings which are available for black spot (BS) sensitive cultivars. Shade-house 1 has been filled with 10 600 seedlings which can be budded early in 2008. This shade-house will be replaced during 2008 by a third insect controlled green house, which will be erected over the newly budded trees.

*Tree Certification:* During 2007, 1 341 312 trees were certified, compared to 1 613 914 trees during 2006.

*Nursery Certification:* In May and November 2007, 19 nurseries were audited and certified.

*Statutory Improvement Scheme:* Exploratory discussions were held with the Department of Agriculture to investigate if the Citrus Improvement Scheme as currently operated in the citrus industry can be accommodated as a statutory scheme under the Plant Improvement Act. The Plant Improvement Act, in its current form, requires a National Variety List, which would prevent cultivars/varieties from being commercialised immediately upon release. Negotiations are continuing to find a way to accommodate the scheme within the terms of the Act.

*Protective zone around the Citrus Foundation Block:* Notice of intention to declare the area within a 5km radius outside the Citrus Foundation Block as a citrus free- zone has been issued. It is now the Department of Agriculture's responsibility to have this protective zone approved by the Minister for implementation.

*Shoot tip grafting and Gene bank:* Twenty new cultivars were released to the Citrus Foundation Block for establishment, evaluation and multiplication by the Shoot Tip Grafting facility at the ITSC, and a further seven cultivars were received from the CRI Shoot Tip Grafting facility in Nelspruit. During 2007/8, 13 cultivars were submitted to the ITSC and 38 to the CRI for shoot tip grafting. There are currently 413 cultivars in the ITSC's gene bank, while the CRI backup gene bank has 235 cultivars.

**Citrus Foundation Block**

Budwood supply during 2007 compared to the 2 preceding years, 10 most popular cultivar selections.

2007			2006			2005		
Selection	Buds	%	Selection	Buds	%	Selection	Buds	%
<b>TOTAL</b>	<b>2009235</b>		<b>TOTAL</b>	<b>2076851</b>		<b>TOTAL</b>	<b>2627528</b>	
Star Ruby	317045	15.8%	Star Ruby	371140	17.9%	Star Ruby	533913	20.3%
Midnight	259195	12.9%	Midnight	268973	13.0%	Midnight	337970	12.9%
Mor 26	97325	4.8%	Palmer	121493	5.8%	Bahianinha	229710	8.7%
Delta	89900	4.5%	Bahianinha	104820	5.0%	Delta	156165	5.9%
Du Roi	89875	4.5%	Late	95550	4.6%	Turkey	138150	5.3%
Nova	82900	4.1%	Turkey	81700	3.9%	Palmer	126696	4.8%
Bahianinha	77320	3.8%	Du Roi	78170	3.8%	Du Roi	84610	3.2%
Cambria	76880	3.8%	Washington	75692	3.6%	Autumn Gold	83200	3.2%
Benny 2	70775	3.5%	Nadorcott 1	69500	3.3%	Cal.Lane Late	77500	2.9%
Eureka	66750	3.3%	Delta	60030	2.9%	Washington	73410	2.8%

Budwood supply per area during 2007 compared to 2 preceding years.

<b>Area</b>	<b>2007</b>	<b>%</b>	<b>2006</b>	<b>%</b>	<b>2005</b>	<b>%</b>
Eastern Cape	364310	18.1%	469452	22.6%	478079	18.2%
Western Cape	465685	23.2%	311494	15.0%	383089	14.6%
Northern Cape	49000	2.4%	34940	1.7%	46850	1.8%
Kwazulu-Natal	46800	2.3%	31550	1.5%	15500	0.6%
Limpopo	821460	40.9%	777085	37.4%	1252503	47.7%
Mpumulanga	159180	7.9%	312240	15.0%	312552	11.9%
North-West	98800	4.9%	135090	6.5%	82780	3.2%
African States	4000	0.2%	3000	0.1%	13950	0.5%
Swaziland	0	0.0%	2000	0.1%	42225	1.6%
<b>Total</b>	<b>2009235</b>		<b>2076851</b>		<b>2627528</b>	

Budwood supply per area and variety during 2007 compared to the 2 preceding years.

Variety	Year	Eastern Cape	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Swaziland	Western Cape	Other African States	Total 2007	Total 2006	Total 2005
Clementine	2007	3300		1500	1150	2200			21390		<b>29540</b>		
Clementine	2006	900		1000	1800	1400	1700		25050	100		<b>31950</b>	
Clementine	2005	7700				1000	10100		19200	100			<b>38100</b>
Ellendale	2007								500		<b>500</b>		
Ellendale	2006					200			200	100		<b>500</b>	
Ellendale	2005					1600			1000	100			<b>2700</b>
Grapefruit	2007	34300		155845	53250	550	30000		52950		<b>326895</b>		
Grapefruit	2006	23600	4000	153620	157500	3030	17500		15680	200		<b>375130</b>	
Grapefruit	2005	21000		396863	111550	1050	8850		1690	1050			<b>542053</b>
Grapefruit Hybrid	2007				150	100			65		<b>315</b>		
Grapefruit Hybrid	2006	300		1000	3900					100		<b>5300</b>	
Grapefruit Hybrid	2005			5100	1800	100				100			<b>7100</b>
Kumquat	2007		2000	5800	2300	3500			6500		<b>20100</b>		
Kumquat	2006	600	1000	4000	3200	2230			1100			<b>12130</b>	
Kumquat	2005		500		3100	3050			2800				<b>9450</b>
Lemon	2007	30100	2500	16100	3870	17600	1000		22600		<b>93770</b>		
Lemon	2006	10000	4000	4900	15900	10030	1000		11850	200		<b>57880</b>	
Lemon	2005	30120	1000	28400	37900	14400	500		8160	500			<b>120980</b>
Lime	2007		2500	15850	2000	2500	1000		7300		<b>31150</b>		
Lime	2006	300	6500	6800	8300	4760			2910	100		<b>29670</b>	
Lime	2005	300		1800	800	30	400		3200	100			<b>6630</b>
Mandarin Hybrid	2007	99050	5500	51600	6020	24750			172160		<b>359080</b>		
Mandarin Hybrid	2006	89900		26070	8100	23400	200		53581	400		<b>201651</b>	
Mandarin Hybrid	2005	24398	2000	37950	2200	9550	3000		59914	400			<b>139412</b>
Midseason	2007					750			6100		<b>6850</b>		

Variety	Year	Eastern Cape	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Swaziland	Western Cape	Other African States	Total 2007	Total 2006	Total 2005
Midseason	2006								9970			9970	
Midseason	2005								1830				1830
Navel	2007	104160	22300	120320	13200	38000	8000		128445	2000	436425		
Navel	2006	160402	10250	190430	46400	51160	6540		115895	300		581377	
Navel	2005	223017	7000	328090	69218	23900	15700		212795	5000			884720
Satsuma	2007	11100	6000		2700	2850	3000		5500		31150		
Satsuma	2006	42050	2000	2000	5100	1000			385	300		52835	
Satsuma	2005	3344		40300	3800	4600			8250	400			60694
Valencia	2007	82300	6000	454445	74540	6000	6000		42175	2000	673460		
Valencia	2006	141400	3800	387265	62040	37880	8000	2000	74873	1200		718458	
Valencia	2005	168200	5000	414000	82184	23500	8300	42225	64250	6200			813859
<b>Total per annum</b>											<b>2009235</b>	<b>2076851</b>	<b>2627528</b>

#### Summary of budwood supply during 2007

A higher demand for budwood was anticipated after the good fruit season, but this did not materialize 67 616 less buds were supplied than during 2006 and 618 299 less than during 2005. Nevertheless we continue to expect an escalation in demand. Star Ruby and Midnight are the two most popular cultivars for 3 years running now. The other cultivars ranking amongst the top 10 vary from year to year.

Seed supplied per rootstock selection, in South Africa during 2007 compared to the 2 preceding years.

Area Name	Year	Australian Trifoliolate	C35 Citrange	Carrizo Citrange	Minneola X Trifoliolate	Rough Lemon	Rough Lemon (Schaub)	Swingle Citrumelo	Troyer Citrange	Volckameriana	X639	Yuma Citrange	Total 2007	2007 (%)	2006 Total	2006 (%)	2005 Total	2005 (%)
Eastern Cape	2007		42	82	1	25		21	7	1.5	10	1	190.5	9.6%				
Eastern Cape	2006		27	125		12	2	10	2	5	7				190	20.0%		
Eastern Cape	2005		43	101		24		16	40	9	13						246	15.5%
KwaZulu-Natal	2007				8			8	8	8			32	1.6%				
KwaZulu-Natal	2006				2			5	3	3					13	1.4%		
KwaZulu-Natal	2005				2			8	4	2	2						18	1.1%
Limpopo	2007		38	448	63	104	2	421		62	38	5	1181	59.3%				
Limpopo	2006		40	170	20	40	20	142	10	1	15				458	48.2%		
Limpopo	2005		47	265	25	84		311	110	2	38	4					886	55.7%
Mpumalanga	2007		7	18		1		10					36	1.8%				
Mpumalanga	2006			5		6									11	1.2%		
Mpumalanga	2005		3	20		8		16	9		2						58	3.6%
North-West Province	2007		5					27		4	2		38	1.9%				
North-West Province	2006		5	6		6		5		6	4				32	3.4%		
North-West Province	2005		6	6		12		4		6	4						38	2.4%
Northern Cape	2006						4								4	0.4%		
Northern Cape	2005										16						16	1.0%
Western Cape	2007	15	73	234	1	27	10	32	38	8	75		513	25.8%				
Western Cape	2006		21	33		45	7	32	59	36	10				243	25.6%		
Western Cape	2005		50	155		75		20	10		20						329.5	20.7%
<b>Total per year</b>													<b>1991</b>		<b>951</b>		<b>1592</b>	

Seed exported per rootstock selection during 2007 compared to the 2 preceding years.

Area Name	Year	Australian Trifoliolate	C35 Citrange	Carrizo Citrange	Cleopatra Mandarin	Flying Dragon	Minneola X Trifoliolate	Rough Lemon	Swingle Citrumelo	Troyer Citrange	Volckameriana	X639	Total 2007	2007 (%)	2006 Total	2006 (%)	2005 Total	2005 (%)
Australia/NZ	2007					10				20	2		32	2.4%				
Australia/NZ	2006					12			12	40					64	11.0%		
Australia/NZ	2005								8	30							38	5.0%
Carribbean	2007		25	13					32		5		75	5.6%				
Carribbean	2006	2	30	4							4				38	6.5%		
Carribbean	2005								7								7	0.9%
China	2007	35		460		40			78	424			1037	76.9%				
China	2006			235					22						257	44.0%		
China	2005		20	65			20		70			150					325	42.7%
Europe	2006		30												30	5.1%		
Europe	2005			100													100	13.1%
Other African States	2007			6				1	6	3		4	20	1.5%				
Other African States	2006			1			1	12	6	13	1	1			35	6.0%		
Other African States	2005			2	2				50	7	1						62	8.1%
South America	2007								10				10	0.7%				
Thailand	2007		24										24	1.8%				
Thailand	2005				30												30	3.9%
USA	2007		150										150	11.1%				
USA	2006		160												160	27.4%		
USA	2005			200													200	26.2%
<b>Total per year</b>													<b>1348</b>		<b>584</b>		<b>762</b>	

## Seed Supplied per rootstock 2005-2007.

<b>Rootstock</b>	<b>2007 (litres)</b>	<b>%</b>	<b>2006 (liters)</b>	<b>%</b>	<b>2005 (litres)</b>	<b>%</b>
Carrizo Cintrange	1261	37.8%	579	37.7%	914	38.8%
Swingle Citrumelo	645	19.3%	234	15.2%	510	21.7%
Troyer Citrange	500	15.0%	127	8.3%	210	8.9%
C35 Citrange	364	10.9%	313	20.4%	169	7.2%
Rough Lemon (Chain)	158	4.7%	121	7.9%	202.5	8.6%
X639	129	3.9%	37	2.4%	245	10.4%
Volckameriana	90.5	2.7%	56	3.6%	20	0.8%
MXT	73	2.2%	23	1.5%	47	2.0%
Australian Trivoliata	50	1.5%	2	0.1%	0	0.0%
Flying Dragon	50	1.5%	12	0.8%	0	0.0%
Rough Lemon (Schaub)	12	0.4%	33	2.1%	0	0.0%
Yuma Citrange	6	0.2%	0	0.0%	4	0.2%
Cleopatra Mandarin	0	0.0%	0	0.0%	32	1.4%
<b>Total per year</b>	<b>3338.5</b>		<b>1537</b>		<b>2353.5</b>	

## Seed supplied, local and export 2005-2007.

<b>Location</b>	<b>2007</b>	<b>%</b>	<b>2006</b>	<b>%</b>	<b>2005</b>	<b>%</b>
South Africa	1990.5	59.6%	951	62.0%	1591.5	67.6%
Export	1348	40.4%	584	38.0%	762	32.4%
<b>Total</b>	<b>3338.5</b>		<b>1535</b>		<b>2353.5</b>	

## Summary of Seed Supply During 2007

A big increase in demand for seed was experienced in South Africa, where seed supplied in 2007 was 1039 litres more than in 2006 and 399 litres more than in 2005, supporting an optimistic expectation for increased budwood demand in the future. Carrizo continues to be the most popular rootstock cultivar choice. The increase in seed exports is as a result of a large order received from China. We were unable to supply China's full needs, as seed demand for seed by South African nurseries receives preference, and only surplus seed is exported. The seed export market is very volatile and we must therefore take full advantage of this market before it dries up altogether.

## Tree Certification

<b>Area</b>	<b>2007</b>		<b>2006</b>		<b>2005</b>	
	<b>Trees</b>	<b>%</b>	<b>Trees</b>	<b>%</b>	<b>Trees</b>	<b>%</b>
Botswana	13755	1.0%	2400	0.1%	270	0.0%
Eastern Cape	214726	16.0%	306167	19.0%	401207	21.5%
Gauteng	22710	1.7%	19000	1.2%	23955	1.3%
KwaZulu-Natal	64720	4.8%	47382	2.9%	54601	2.9%
Limpopo	421818	31.4%	237932	14.7%	432375	23.2%
Mozambique	0	0		0.0%	600	0.0%
Mpumalanga	257833	19.2%	477365	29.6%	666730	35.7%
Namibia	1950	0.1%	6895	0.4%		
North-West Province	127092	9.5%	20097	1.2%	30616	1.6%
Northern Cape	7024	0.5%	112966	7.0%	-	
Orange Free State	0	0	2000	0.1%		
Other African States	12500	0.9%				

Area	2007		2006		2005	
	Trees	%	Trees	%	Trees	%
Swaziland	20800	1.6%	32120	2.0%	39329	2.1%
Western Cape	122804	9.2%	315727	19.6%	197493	10.6%
Zimbabwe	53580	4.0%	33863	2.1%	19000	1.0%
<b>Total</b>	<b>1341312</b>		<b>1613914</b>		<b>1866176</b>	

Tree Certificate percentage per variety per annum during 2007 compared to 2 preceding years.

Variety	2007 Trees	2007%	2006 Trees	2006%	2005 Trees	2005%
Valencia	518246	39%	409209	25%	574795	31%
Navel	447106	33%	575819	36%	552847	30%
Grapefruit	270384	20%	313421	19%	346620	19%
Mandarin Hybrid	51220	4%	110014	7%	76267	4%
Lemon	20319	2%	70012	4%	198728	11%
Satsuma	17452	1%	57037	4%	49756	3%
Clementine	8310	1%	73892	5%	63719	3%
Lime	3415	0%	1260	0%	0	0%
Grapefruit Hybrid	2600	0%	1669	0%	1260	0%
Kumquat	2260	0%	100	0%	450	0%
Ellendale	0	0%	500	0%	250	0%
Midseason	0	0%	981	0%	1484	0%
<b>Total per year</b>	<b>1341312</b>		<b>1613914</b>		<b>1866176</b>	

Tree Certificate percentage per rootstock per annum during 2007 compared to 2 preceding years.

Rootstock	2007 Trees	%	2006 Trees	%	2005 Trees	%
Carrizo Citrange	486565	36%	764684	47%	713959	38%
Swingle Citrumelo	417852	31%	339860	21%	512792	27%
Rough Lemon	243739	18%	221790	14%	310039	17%
C35 Citrange	80539	6%	112969	7%	119903	6%
X639	40066	3%	92333	6%	83644	4%
MXT	35417	3%	60107	4%	71710	4%
Troyer Citrange	30241	2%	9164	1%	38631	2%
Other	6893	1%	13007	1%	15498	1%
	<b>1341312</b>		<b>1613914</b>		<b>1866176</b>	

Trees registered per area and variety during 2007 compared to the 2 preceding years.

Variety	Year	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Orange Free State	Swaziland	Western Cape	Other African States <sup>1</sup>	Total 2007	Total 2006	Total 2005
Clementine	2007										8310		8310		
Clementine	2006	343						10000			63549			73892	
Clementine	2005	8972				1200	3000				50547				63719
Ellendale	2006							500						500	
Ellendale	2005					250									250
Grapefruit	2007	15478		40257	110349	89960	340	2300		11700			270384		
Grapefruit	2006	19369		33700	102089	125831	260	14152		18000		20		313421	
Grapefruit	2005	5358	3535	32843	99279	171806	6000			27269	330	200			346620
Grapefruit Hybrid	2007				1150	1450							2600		
Grapefruit Hybrid	2006				59	1610								1669	
Grapefruit Hybrid	2005				1010	250									1260
Kumquat	2007				1800	460							2260		
Kumquat	2006										100			100	
Kumquat	2005										450				450
Lemon	2007	14911	1800	2725	374	379					130		20319		
Lemon	2006	29172	1600		1526	33429					4285			70012	
Lemon	2005	92643	2700	16448	8625	72842	2000				3470				198728
Lime	2007				650	2765							3415		
Lime	2006	250			450						560			1260	
Mandarin Hybrid	2007	8460	1000	1725	700	6950	12630				19405	350	51220		
Mandarin Hybrid	2006	21059		512	7786	15104	2130	500			62623	300		110014	
Mandarin Hybrid	2005	14619			9549	40770	9995				1334				76267
Midseason	2006	900									81			981	
Midseason	2005	383									1101				1484
Navel	2007	91004	9610	12246	34031	95362	79019	3142			64062	58630	114106		
Navel	2006	141928	8000	3850	16380	176074	12832	65672	2000		146258	2825		575819	

<sup>1</sup> Includes Botswana, Mozambique, Namibia and Zimbabwe

Variety	Year	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mpumalanga	North-West Province	Northern Cape	Orange Free State	Swaziland	Western Cape	Other African States <sup>1</sup>	Total 2007	Total 2006	Total 2005
Navel	2005	197711	20	4800	23952	216216	5690				103988	470			552847
Satsuma	2007	4551			3222	449	8980					250	17452		
Satsuma	2006	25015			5	23515	700	3750			4017	35		57037	
Satsuma	2005	21075				15270					13411				49756
Valencia	2007	80322	10300	7767	269542	60058	26123	1582		9100	30897	22555	518246		
Valencia	2006	68131	9400	9320	109637	101802	4175	18392		14120	34254	39978		409209	
Valencia	2005	60446	17700	510	289960	148126	3931			12060	22862	19200			574795
<b>Total per year</b>													1341312	1613914	1866176

#### Summary of Tree Certification During 2007

During 2007, 272 602 less trees were certified than during 2006 and 524 864 less than during 2005. Growers must regularly be informed of the purpose of tree certification and reminded that they should insist on tree certification from the nurseries.

The following nurseries were accredited during 2007.

Nursery	Address	Telephone
Apapanzi	P O Box 147, Kirkwood, 6120	042 2301483
BF Joubert	P O Box 193, Kirkwood, 6120	042 2300309
Casmar	P O Box 3 Mooinooi, 0325	0145 743152
Du Roi	P O Box 66, Letsitele, 0885	015 3451650
Esselen	P O Box 100, Malelane, 1320	013 7900160
H J Joubert	P O Box 207, Montagu, 6720	0236 142237
La Rhyn	P O Box 111, Citrusdal, 7340	022 9213541
Letsitele	P O Box 1, Letsitele, 0885	015 3451600
Mistkraal	P O Box 16, Kirkwood, 6120	042 2301461
Ngwenya	P O Box 36, Malelane, 1320	013 7903004
Cedarberg - Citrusdal #	P O Box 69, Simondium, 7670	021 8741033
Paksaam	P O Box 16, Patensie, 6335	042 2830201
Sondagsrivier	P O Box 304, Kirkwood, 6120	042 2300349
Stargrow	P O Box 189, Citrusdal, 7340	022 9212232
Tweeling	P O Box 190, Kirkwood, 6120	042 230 1408
Vaalharts	P O Box 317, Hartswater, 8570	053 4740565
Waterfall	P O Box 339, Adelaide, 5760	046 6840738
Westfalia	P O Box 14, Duiwelskloof, 0835	015 309 0050
Witkrans	P O Box 17, Boshhoek, 0301	014 5733036

# New Nursery

### Production at the Citrus Foundation Block

The second insect controlled greenhouse is now in full production with 45 700 increase trees, of which 2 700 seedlings have not yet been budded as they are being reserved for black spot (BS) sensitive cultivars, as this greenhouse is rain proof. Cultivars sensitive to BS are also pro-actively treated with chemicals.

Shade-house 1 has been filled with 10 600 seedlings which can be budded early in 2008 to cultivars not sensitive to BS, as rain cannot be kept out of this site at the moment. This shade-house will be replaced during 2008 by a third insect controlled greenhouse, which will be erected over the newly budded trees.

The phasing out of open ground increase trees is being carried out as and when sufficient increase trees become available in the insect controlled greenhouses. The ideal being strived for is to establish all increase trees within insect controlled greenhouses, and to eradicate all open ground evaluation and increase trees, so that only seed source trees remain outside.

### Nursery Certification

In 2007, 19 nurseries were visited in May and November to be audited and certified according to CIS guidelines. One new nursery, "Cedarberg Tree Nursery", has been established in Citrusdal and is in production. A list of certified nurseries was published in the SA Fruit Journal in April/May 2007. In general, nursery standards are high. A call is made on growers to visit various nurseries before choosing a nursery to order trees from, as there are differences between the nurseries. Orders must be placed in writing, and the grower must stipulate his requirements. No below-standard trees should be accepted and planted.

### Statutory Citrus Improvement Scheme

Exploratory discussions were held with the Department of Agriculture to investigate if the Citrus Improvement Scheme as currently operated in the citrus industry can be accommodated as a statutory scheme under the Plant Improvement Act. The Plant Improvement Act, in its current form, requires a National Variety List, which means that all varieties/cultivars must first be evaluated before being placed on the list, causing a delay of up to 5 years before new cultivars can be commercially planted. Currently a proposed amendment to the Plant Improvement Act is being communicated to all fruit and wine industries for their approval. Negotiations will continue in pursuit when this process has been completed.

### Protective Zone Around the Citrus Foundation Block

Notice of intention to declare the area within a 5 km radius outside the Citrus Foundation Block as a citrus free-zone has been issued. It is now the Department of Agriculture's responsibility to have this protective

zone approved by the Minister for implementation. The Department of Agriculture invited all residents in the affected area to attend a meeting at the Citrus Foundation Block on 31 July 2007. A representative from the Department explained the proposed notice of intention to all those who attended the meeting and a 2-week period was allowed for objections to be submitted. To date no objections have been received.

**Shoot Tip Grafting and Gene Bank**

There are currently 2 institutions which carry out shoot tip grafting. The Agricultural Research Council's (ARC) Institute for Tropical and Sub-tropical Crops (ITSC) in Nelspruit handle all imported cultivars, which are received under quarantine in South Africa, as well as local cultivars. The Citrus Research International's (CRI) Virological Department in Nelspruit handle only local cultivars. Full reports will be submitted separately by both these institutions.

**1/03/2007 – 31/03/2008**

<b>Service Delivery</b>	<b>ARC-ITSC</b>	<b>CRI</b>
Cultivars received for shoot tip grafting	13	38
Cultivars supplied to the CFB for establishment	20	7
Cultivars in the gene bank	413	235

## 8 INTERNATIONAL VISITS

### 8.1 G.C. SCHUTTE

Tour to Limeira, Brazil to present a talk on Alternaria brown spot – 13-17 June 2007

#### Summary

I was invited by the "Governo do Estado de Sao Paulo, Instituto Agronomico" to present a talk during the "29<sup>th</sup> Semana da Citricultura" held at Limeira. At the seminar all the talks were in Portuguese except for mine and I had to make use of an interpreter. The tour was sponsored (as before), by a chemical company. Before and after the seminar, various citrus growers were visited to get an impression of what the severity of their various diseases such as CBS, Alternaria, CVC and citrus canker were. Spray machines in operation were also investigated. Various researchers and laboratories were also visited.

#### Itinerary

Sunday 10 June	Travel: Johannesburg – Sao Paulo - Jaboticabal
Monday 11 June	Visit to Fundecitrus Institute at Araraquara; visit citrus groves: Cutrale as well as Citrovita all in the Araraquara region
Tuesday 12 June	Meeting was held with Dr Arlindo de Salvo, a citrus consultant; visited Antonio Carlos Baraldi at Citrolandia. The same morning, Roberto Fukugauti, owner of Santa Eliza Estates, a large mandarin farm, was also visited. After lunch, Evaldo and Irineu Fortes, owners of Agua Branca, a large mandarin and orange farm in the Aguai region, were visited.
Wednesday 13 June	Presented a talk at the Citrus Expo at Limeira. Visited a post harvest laboratory at the same venue and had meetings with researchers.
Thursday 14 June	Field visits to JF Citrus north east of Limeira in the morning and to Sonia Maria Farm west of Sorocaba, the largest mandarin grower in the world.
Friday 15 June	Meetings with researchers at the Citrus Expo. Visited the Hortitec Trade show at Holambra.
Saturday 16 June	Day off
Sunday 17 June	Travel: Sao Paulo – Johannesburg

#### Visit to Fundecitrus

A meeting was held with with Dr. Marcel Sposito, a research plant pathologist. In his introduction he mentioned that Brazil produce 350 million tons of citrus fruit with an average of 22 tons per hectare. Four hundred thousand jobs are created by their citrus industry. About 90% of all their orchards are not under irrigation and 90% of their fruit goes for juice. Fundecitrus is also funded by their industry (@ 35c/40,8 kg box).

Their main research is focused on black spot, citrus canker and CVC. A new disease called "citrus sudden death" (CSD), is their main concern at the moment as 4 million trees in the north east of Sao Paulo State are dying. Interesting to note is that 80% of their root stocks are Rangpur lime and Volckameriana and that CSD only attack these rootstocks. They have a huge problem on hand! There are 9 researchers and 45 co-workers working on this problem at the moment.

Spray programmes recommended by Fundecitrus for citrus diseases in Brazil at a rate of 8 litres per tree.

**Table of a spray programme where citrus canker is a problem:**

Date	Fungicide	Rate	Disease
October (60% petal fall)	Copper	180g/100L water	Scab, CBS, canker, post bloom fruit drop (antraknose)
November	Copper + oil	180g + 0.25%/100L water	CBS, melanose, canker
December	Copper + oil	180g + 0.25%/100L water	CBS, melanose, canker
January	Copper + oil	180g + 0.25%/100L water	CBS, melanose, canker
February	Copper + oil	180g + 0.25%/100L	CBS, melanose, canker

		water	
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### Spray programme where citrus canker does not exist:

Date	Fungicide	Rate	Disease
October (60% petal fall)	Copper	180g/100L water	Scab, melanose, CBS, post bloom fruit drop (antracnose)
November	Copper + oil	180g + 0.25%/100L water	CBS, melanose
December	Carbendazim+ copper + oil <b>or</b> Strobilurin + copper + oil	50 ml + 180g + 0.25%/100L water <b>or</b> (as listed below) + 180g + 0.25%/100L water	CBS, melanose
End-January/February	Carbendazim+ copper + oil	50 ml + 180g + 0.25%/100L water	CBS, melanose

### Information obtained from Syngenta, Bayer and BASF brochures

Interesting information was obtained from brochures regarding the registered rates of strobilurin fungicides for the control of CBS:

Strobilurin	Brazilian rate	South African rate	Intervals
Amistar / Ortiva (250 SC)	2 applications @ <b>16 ml</b> / 100 L water with 0.5% oil only	2 applications @ <b>20 ml</b> / 100L water with 150 g mancozeb plus 0.3% oil	42 days for both countries
Comet / Cabrio (250 EC)	2 applications @ <b>15 ml</b> / 100 L water with 0.2% oil	2 applications @ <b>10 ml</b> / 100 L water with 150 g mancozeb plus 0.5% oil	45 days interval for Brazil 42 days interval for SA
Flint (500 WG)	2 applications @ <b>7.5 g</b> / 100 l water	2 applications @ <b>10 g</b> / 100 L water with 150 g mancozeb plus 0.3% oil	None given on Brazilian brochure 42 days interval for SA

### New saprophyte in citrus nurseries near Limeira

During a visit to a nursery (I could not recall the name) a basidiomycete was noticed that grew in the plastic bags that deprived the citrus trees from water in such a manner that they sometimes die (Fig. 8.1.1). According to the nursery manager, it was identified as *Leucocoprinus spp.*



**Fig. 8.1.1.** Growth features of the *Leucocoprinus spp.* fungus in plastic pockets of citrus nursery trees.

These visits were my first ever (after 2 previous visits) that the Brazilian spray equipment could be seen in operation. I came to an exciting conclusion that the whole Brazilian citrus industry rely on an average of 2 300 liters / ha which is even lower than our 4 000 liters/ ha which we consider to be low volume spraying. They were surprised that I told them that we spray up to 12 000 liters / ha and even 8 000 liters / ha is sometimes the cut-off point for citrus black spot control. According to Eduardo Feichtenberger, they accepted an average of 2 300 liters / ha which is the industry standard for the USA! That is why they cannot control CBS and canker. Due to the size of their estates, they also do not have fill-up points in the orchards and they send tankers to the spray machines to be filled with water. In all three visits we went to the orchards and could detect spray residues on the outside 30-50 cm of the trees but nothing on the inside of the trees. Trees are also not skirted and the bottom and inside fruit are riddled with CBS.



**Fig. 8.1.2.** FMC spray machine in operation on a “Cutrale” farm in the Araraquara region (left) and the spray coverage just after application (right). The blue arrows show the outside fruit are covered on the one cheek, while fruit and all the inside foliage were not covered (white arrow).



**Fig. 81.3.** A new “Jacto” spray machine demonstrating its capabilities at the Citrus Expo (left) and in operation on the “Sonia Maria Farm” west of Sorocaba (right).



**Fig. 8.1.4.** Spray deposit on Navel leaves and fruit after the being sprayed with the new “Jacto” spray machine (left) and the lack of spray coverage on the inside fruit and leaves on the same tree (right).

**Conclusion**

The Brazilians will never be able to control CBS with any of their spray machines. The answer will be to change over to oscillating booms and to get penetration into the tree canopy.

**Citrus Expo**

Interesting new machines were exhibited such as a low volume spray applicator and a harvester.



**Fig. 8.1.5.** A low volume spray applicator used to spray small trees (left) and a mobile “automatic” harvester (right).

**New leaf raking machine to remove leaf litter from citrus trees to eliminate the source of CBS inoculum**

At the Citrus Expo I met an MSc student from the University of Jaboticabal, Jose Antonio Bellotte, who developed a machine (Fig. 8.1.7) (as part of his MSc) for the raking of leaf litter from under the canopies. In principal it looks good, but after the machine did its work, roots were exposed (Fig. 8.1.8). On flat surfaces it performed well, but where trees are planted on ridges (as in South Africa) it will be impractical to use this technique. In Brazil less than 10% of the trees are under irrigation and it will work well there, but in South Africa where nearly all the orchards are under irrigation, these irrigation lines will be destroyed. It was also noted that there were some leaves left behind within the rows in line with the trunks. These leaves can only be removed manually (Fig. 8.1.9).



**Fig. 8.1.7.** Leaf raking machine in operation.



**Fig. 8.1.8.** Exposed citrus roots after the raking operation.



**Fig. 8.1.9.** Removal of fallen citrus leaves from a Brazilian orchard. Notice the absence of micro-irrigation and ridges in these orchards.

#### **Search for the Brazilian nursery that exported trees to Angola**

I was tasked to find which nursery exported trees to Angola. We had to find out who they are in order for DoA to provide the Angolan government with evidence to prohibit or ban the further importation of trees from Brazil due to diseases such as CVC and citrus canker. An informant whom had spoken to the director/manager of **Citrograf, Christiano Caesar Graf**, confessed that they have exported a number of trees to Angola. The growth medium removed from the trees, they were bundled together, put in plastic bags and flown by air to Angola. In a brochure obtained on the nursery, they claim that their trees are free from diseases such as CVC, Asiatic greening, citrus canker, nematodes, Phytophthora and Tristeza. Further details regarding this nursery group can be found on the brochure below.

Viveiro do Rochedo

Em 2007 a Citrograf Mudas conclui sua 3ª unidade de produção de mudas de citros, demonstrando ainda mais confiança e credibilidade no mercado citrícola brasileiro.

Citrograf Mudas, a melhor decisão.

Conchal Rio Claro Ipeúna

Unidade Conchal  
Rod. SP 191 Km 21,4  
T/F (19) 3866-2285 - Caixa Postal 41  
13.835-000 - Conchal - SP

Viveiro do Horto  
T/F (19) 3534-9981  
Bairro Camaquã - Caixa Postal 226  
13.500-000 - Rio Claro - SP

Viveiro do Rochedo  
Estrada Municipal de Ipeúna s/n  
Bairro Rural - Caixa Postal 34  
13.537-000 Ipeúna - SP

**Citrograf**  
mudas  
A Melhor Decisão

www.citrograf.com.br • mudas@citrograf.com.br

Copies of various theses on CBS as a topic were obtained:

**a) Temporal and spatial dynamics of citrus black spot (*Guignardia citricarpa*) and quantification of the damages caused to citrus culture**

Sposito, Marcel Bellato

The fungus *Guignardia citricarpa* is the causal agent of citrus black spot (CBS), which is a disease that makes the fruits unsightly and unsuitable for the fresh fruit market. Besides, premature fruit drop may occur, reducing the productivity. A diagrammatic scale for the two symptoms of CBS (hard spot and false melanose) was developed to evaluate the severity and help in epidemiological studies of this disease. The susceptibility level of 'Hamlin', 'Pera' and 'Valencia' sweet orange to CBS was evaluated in commercial orchard, under natural infection. The monomolecular model was fitted to the incidence and severity progress curves of the disease for the three sweet oranges. According to the progress curves, there was not significant difference among cultivars, showing that 'Hamlin', 'Pera' and 'Valencia' sweet orange have similar susceptibility level to CBS. *G. citricarpa* in the epidemic phase produce ascospores and conidia. The

ascospores, produced in leaves on soil, are disseminated by wind, while conidia, produced in plant twigs and fruits, are disseminated by water. The pattern of spatial distribution of CBS plants was evaluated by dispersion index and Ripley K function. The groups of symptomatic plants were distributed independently of disease incidence, suggesting that short distance dispersion of the inoculum could be the most important factor in distribution of the disease. The binary form of Taylor's power law and dispersion index were used to evaluate the distribution of symptomatic fruits in the plant. According to dispersion index 84% of the evaluated plants showed aggregation of symptomatic fruits, while by the Taylor's power law the symptomatic fruits aggregation occurred independently of the incidence of plant disease, suggesting that the increase of disease in field conditions is related to conidia production. The effect of ascospore suppression on intensity of CBS was evaluated by removing citrus leaves from the orchard soil surface, while the conidia suppression was evaluated by early harvesting of late maturation fruits. These treatments, carried out during two years in a high inoculum incidence area, reduced the disease severity in the second year, but the satisfactory control was not observed. However, these treatments could be important in CBS control whether used in association with chemical control. Damages caused by yield reduction and losses of financial return were evaluated in two experiments. In relation to non-treated orchards, those treated orchards where the production/ha increased more than hundred boxes (40.8 kg each box) were considered profitable. The destination of the orange production (juice of fresh fruit market) are important for determine the size of samples to assess the CBS incidence. Since the *G. citricarpa* fungus is considered a quarantine organism that causes qualitative losses, in areas used for production of fresh fruit market whole orchard has to be evaluated. However, in areas used for production of fruits to concentrated orange juice, 285 plants sampled are enough to determinate the CBS incidence superior to 15%, in average orchards containing 2200 plants.

#### **b) Control of *Guignardia citricarpa*, causal agent of Citrus Black Spot**

Rodrigues, Maria Beatriz Calderan

Citriculture is an extreme important rural activity in social and economical national context in Brazil. Oranges are 49% of total Brazilian fruit production. However, thousands of tons are lost due to the action of phytopathogens annually. The Black Spot of Citrus (BSC) is responsible for great lost in various citrus producers regions all around the world, being already designed as a phytosanitary barrier, mainly in European market. For in nature fruit consuming, the fruit esthetic is a limiting factor, where BSC compromises the market of fresh affected fruits. Chemical control of plant pathogens is the most commonly way used to minimize damages in citriculture by BSC, although the application of such products implies in high costs, not only for farmers but also for environment, causing soil and water contamination and increasing the selection pressure on pathogen population. On this way, the biocontrol became an attractive way, as a strategy that permits a minor environmental impact besides the plant protection against phytopathogens. For this application, are necessary researches based on utilization of biocontrol techniques, as for example, the using of micro-organism producers of hydrolytic enzymes. Such enzymes, like quitinases, endoglicanases and  $\beta$ -glucosydases are able to digest the fungal and bacterial cell wall. In this work, 24 strains of *G. citricarpa* were evaluated about the sensibility to fungicides used in field for BSC control: pyraclostrobin and carbendazim, in dosages of 0,5, 1,0 and 2,0 mg a.i./mL, aiming to verify the effect of selection pressure caused by continuous use of this compounds. Two of these strains presented resistance to carbendazim in all evaluated dosages, showing that the use of this agrochemical may select resistant individuals, resulting a non-efficiency of this compound for BSC control. An alternative to minimize this kind of effect must be the application of this compound in combination to others active principles. The cellulolitic and chitinolitic activities of 96 fungi strains widely spread phylogenetically were evaluated for selection of potential biological control agents. Four strains that presented major activity of each enzyme, besides two *Trichoderma* lineages, were tested as potential biological control of *G. citricarpa* in an experiment with 'Valencia' orange leaves, comparing the action of these biocontrollers with commercial fungicides. Although a better pathogen control was achieved in leaves treated with piraclostrobin, two fungi strains revealed to have the similar efficiency to fungicide, inhibiting the development of *G. citricarpa*, suggesting the possible future utilization of biocontrol methods to Black Spot of Citrus.

#### **c) Effect of chitosan and UV-C on the control of *Guignardia citricarpa* on postharvest oranges**

Rappussi-da-Silva, Maria Cristina Canale

Brazil is the biggest producer and exporter of orange juice, and this is one of the most important economical activities in the country. The fruits can be affected by the citrus black spot, disease caused by the fungus *Guignardia citricarpa*, which depreciates them commercially, causes premature fall and increases the production cost. Alternative measures to the chemical control are being studied and, in this context, resistance induction can be considered. The present work had as objective evaluate the *in vitro* effects of chitosan and UV-C radiation on mycelial growth, germination and appressorium formation by *G. citricarpa*

and the action of the abiotic agents on controlling the disease on post harvest oranges, under room temperature and refrigeration storage, also studying the mechanisms of resistance in the plant tissue in response to the better treatment. The chitosan concentrations were 0, 0.5, 1.0, 1.5; 2.0 and 3.0% and the UVC doses were 0.52, 1.04, 3.13, 10.44 and 15.66 kJ.m<sup>-2</sup>. Chitosan inhibited mycelial growth and stimulated the germination and the appressorium formation that were morphologically abnormal. UV-C did not inhibit mycelial growth, but reduced it at the highest dose used. For the *in vivo* experiments, oranges were collected, sanitized with hypochlorite and treated. Chitosan concentrations of 0.5, 1.0 and 2.0% and the UV-C dose of 7 kJ m<sup>-2</sup> exhibited better results in Valencia oranges. Analyses of peel colour of irradiated fruits revealed a light browning. The fungicides thiabendazole and imazalil did not control the disease in Pêra Rio oranges, but fewer lesions appeared on fruits treated with the fungicides in association with chitosan, under room temperature and refrigeration. Colour analysis of peel indicated yellowing and no significant differences among soluble solids, titratable acidity, pH vitamin C and ratio. In the chitosan, thiabendazole and UV-C assays, there was a better control of lesion appearing by treatment with chitosan, applied alone or in association with fungicide and UV-C, at room temperature or refrigeration. Chitosan and the harpin protein were similar on the controlling of the disease and, in comparison to the citric acid, chitosan presented better control on Valencia oranges. For biochemical analysis, flavedo samples were homogenized in acetate buffer, centrifuged, and the supernatant collected. The reagents used were CM-chitin-RBV, CM-Curdlan-RBB, guaiacol, cathecol and L-phenylalanine for chitinase, glucanase, peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase, respectively. For phenol determination, flavedo was homogenized in acidified methanol and the evaluation was made with Folin-Ciocalteu. Chitosan increased enzyme activities in the first 24 h after treatment, with the highest activity in that time. Activity of phenylalanine ammonia-lyase was not detected, well as absent of phenolic compounds accumulation. Chitosan and UV-C exhibited *in vitro* effect on *G. citricarpa*, however, only chitosan showed potential on the control of black spot in postharvest oranges.

**d) Diagrammatic scale for assessment of citrus black spot in leaves and effect of temperature and wetness duration in the pre-penetration conidia of *Guignardia citricarpa* Kiely [*Phyllosticta citricarpa* (McAlp.) Van der Aa]**

Noronha, Marissônia de Araujo

Citrus black spot caused by *Guignardia citricarpa* Kiely [*Phyllosticta citricarpa* (McAlp.) van der Aa] presents two infection forms, conidia and ascospores. Information regarding the importance of the conidia in the epidemiology of the disease is scarce and controversial. Seeking a better understanding of the pathosystem citrus -*G. citricarpa* (*P. citricarpa*), the objectives of this dissertation were: elaborate and validate a diagrammatic scale for assessments of the citrus black spot; verify the effect of the temperature and of the wetness duration in the appressorium formation; observe through scanning electron microscopy the germination and formation of appressorium on outstanding lemon 'Siciliano' leaves submitted to different temperatures and wetness duration. The diagrammatic scale with severity levels of 1; 3; 6; 12; and 24% of diseased leaf area was validated by two groups of raters, with experience and without experience in the quantification of diseases. The scale provided better precision and accuracy for both experienced and inexperienced raters, considering the estimates average of them. In the majority of cases, the bias between estimated and actual disease severity were more evident for disease severity levels between 5 and 15%. The reproducibility of assessments resulted in R<sup>2</sup> with more uniform values for the majority of the experienced raters, considerable differences of precision were observed among inexperienced raters. The effect of the temperature (10°C - 40°C) and of the wetness duration (4 - 48 h) in the germination of conidia and appressoria formation of *G. citricarpa* (*P. citricarpa*), was assessed "in vitro" and on the surface of lemon 'Siciliano' leaves. The appressoria formation occurred in all the temperatures starting from 12 hours of wetness. The extreme temperatures (10°C and 40°C) were less favourable to the appressorium formation. The minimum temperature for appressorium formation, estimated by generalized beta function was of 3°C and the maximum of 48,4°C, both for 48 hours of wetness. The appressorium formation was favored considerably by the wetness duration period, with the maximum of appressoria formed at 24 hours of wetness, for majority of the temperatures. The wetness duration period constituted of 48 hours was essential so that the spores submitted to temperatures of 10°C and 40°C, formed appressorium. The response surface obtained by the multiplication of the generalized beta and monomolecular functions provided a close fit to observed data in the estimate of the relative percentage of formed appressorium (R<sup>2</sup> =0,75). The samples observed in scanning electron microscopy made possible the acquisition of images of conidia and appressoria on the surface of lemon 'Siciliano' leaves in all the temperature combinations and wetness evaluated.

**e) *In vitro* effect of *Saccharomyces cerevisiae* on *Guignardia citricarpa*, causal agent of citrus black spot**

Fialho, Mauricio Batista

Due to the consumers perception about the impact caused by pesticides utilization over the environment and human health, besides the acquisition of resistance for part of the phytopathogens, the society has exercised pressures that had led to the establishment of governmental politics that restrict the use of fungicides leading agriculturists and researchers to consider the application of techniques of biological control of plant pathogenic fungi. *Guignardia citricarpa* is the causal agent of citrus black spot that has a great economic importance, therefore interfering in production and causing aesthetic depreciation of the fruits that can interfere with commercialization of fresh-fruit in the external market. In this context, the aim of this work was to evaluate *in vitro* the potential of *Saccharomyces cerevisiae* strains, used in fermentative process, as biocontrol agents against *G. citricarpa*. Through plate assay it was evidenced that among the tested strains of *S. cerevisiae* (BG-1, CR-1, CAT-1, KD-1, K-1 and PE-2), the strain CR-1 was the one that demonstrated the greatest antagonistic activity against the phytopathogen, causing 73% of micelial growth inhibition. It was also demonstrated that the strains were able to produce volatile compounds with fungistatic action inhibiting up to 83% the development of the pathogen. The autoclaved and not autoclaved culture filtrate, as well as the thermal inactivated cell obtained from the growth of strain CR-1 in YEPD medium for 24 h, did not cause reduction in the fungal vegetative growth. The production of extracellular hydrolytic enzymes (chitinases,  $\beta$ -1,3-glucanases and proteases) by the yeast was not detected in YEPD medium with glucose or cell wall preparation of *G. citricarpa* at the evaluated times. Based upon the obtained information it was possible to evidence that the strains of *S. cerevisiae*, specially the strain CR-1, are potentials antagonists for the control of *G. citricarpa*. The possible mechanism used for inhibition by yeast is the volatile production however other mechanisms cannot be discarded. Thus, the present work shows the potential of *S. cerevisiae* to control *G. citricarpa* in orange fruits in postharvest.

**f) The effect of orange (*Citrus sinensis*) albedo extracts the resistance inducers with salicylic acid, acilbenzolar-s-methyl and *Saccharomyces cerevisiae* on the control of *Phyllosticta citricarpa* (teleomorph: *Guignardia citricarpa*)**

Cardoso Filho, Julio Alves

Black spot of citrus (CBS) has been a limiting factor in the export of brazilian oranges to Japan and European countries and Japan. Except for *Citrus aurantium* and its hybrids, all commercially growing *Citrus* spp. are susceptible to the pathogen. The fungus *Guignardia citricarpa*, discovered by Kiely in 1948 in New South Wales, is the sexual stage of the causal agent of CBS and *Phyllosticta citricarpa* is the imperfect stage. An important characteristic of CBS is the long latent period after infection. The infection is carried out by ascospores and pycnidiospores. The fungicidal application is the most important method of control of CBS. The CBS lesion in citrus fruits is limited to the flavedo, since *P. citricarpa* does not infect the albedo. The albedo is rich in cellulose, soluble carbohydrates, pectin, phenolic compounds, amino acids and vitamins. The phenolics present in the plants are secondary metabolic products and are believed to be produced as a result of the plant interaction with the environment and synthesized as a response to attempted phytopathogen attacks. The phenolics that occur in *Citrus* include flavonoids, anthocyanins, coumarins and psorolens. These compounds may exhibit antiviral and antimicrobial activities, and may contribute to the control of CBS disease. An another possibility to the CBS control is the activation of factors resistance by the use of abiotics (Bion and salicylic acid) and biotics (*Saccharomyces cerevisiae*) "plant defence activator" (inducers). Therefore, the objectives of this paper were to study the *in vitro* effects of aqueous, ethanolic and methanolic albedo orange extracts on the germination, appressorium formation and mycelial growth of *P. citricarpa* as well as to evaluate the use of the *S. cerevisiae*, Bion and salicylic acid as "plant defence activator" at post and preharvest conditions in fruit of 'Pêra-Rio' and leaves of 'Siciliano' lemon. The results showed that the use of albedo extracts, 10 and 100 mg per mL of water, inhibited 100 % the germination, appressorium formation and mycelial growth of *P. citricarpa*. It was also observed that the extracts of albedo, depending upon the concentration, exhibited fungicidal or fungistatic activity. The use of *S. cerevisiae*, Bion and salicylic acid at postharvest conditions did not affect the development of new lesions of CBS in 'Pêra-Rio' orange fruit. It was also observed that the use of *S. cerevisiae* and Bion at preharvest conditions, did not induce resistance against *P. citricarpa* in leaves of 'Siciliano' lemon naturally infected with *G. citricarpa* under field conditions. Thus, it is suggested that other studies be carried out, mainly regarding the potential of orange albedo's extracts as an alternative method for CBS control.

## 8.2 G. PIETERSEN & S.P. VAN VUUREN

Attendance of the 17<sup>th</sup> Conference of the International Organization of Citrus Virologists in Adana, Turkey, 22-26 October, 2007

### Introduction

The conference was held in the Seyhan Hotel, Adana, in the centre of the Mediterranean citrus-growing region of Turkey. This city is also home to the Çukurova University, where the local organizing committee, headed by Dr. Nüket Önelge are employed. The conference was well organized and relatively informal. Delegate numbers were lower than usual due to a large number of potential delegates citing personal safety concerns for not attending. The conference consisted of 55 oral presentations, 45 posters, and some 70 delegates from 17 countries attended it. A pre-conference tour from 18-21 October, 2007 was also provided in order to demonstrate a number of citrus diseases occurring in Turkey, of interest to delegates. Nearly half of the conference delegates participated in the pre-conference tours.

### Objectives

1. Participating in the pre-conference tour observing diseases that are common in orchards of Turkey.
2. Attending the oral and poster presentations during four days of the Conference.
3. To present oral presentations and submit papers;  
Gerhard: Short communication with the title "Survey for '*Candidatus*' Liberibacter species on citrus in South Africa";  
Fanie: Full length paper with the title "Initial attempts to obtain Huanglongbing resistant or tolerant sweet orange by embryo rescue from healthy chimeras of diseased fruit".
4. Chaired oral sessions;  
Gerhard: Session V: *Citrus tristeza virus*, Citrus Sudden Death and Citrus Variegated Chlorosis;  
Fanie: Session IX: Citrus Huanglongbing.

### Pre-conference and conference field trip

The program for the pre-conference tour is attached (Appendix 1). Citrus graft transmissible diseases that were observed during the tours are divided in two categories (i) diseases occurring in South Africa; and (ii) diseases not occurring in South Africa.

#### Diseases that occur in South Africa

***Citrus tristeza virus (CTV)***: CTV does occur in Turkey but the citrus brown aphid *Toxoptera citricida* is absent. The CTV isolates are mild and do not induce stem pitting, seedling yellows or sweet on sour reaction on biological indicators. Spread of the disease by other aphid species is very slow. Serological, some isolates react with the "severe" monoclonal antibody MCA13 that was developed in Florida (USA) to detect severe strains in that country. However, this antibody reacts with the mild isolates used for cross-protection in South Africa. The main rootstock in Turkey is the CTV sensitive sour orange. It was interesting to see forty-year-old CTV infected sweet orange on a sour orange rootstock (Fig. 1). Because of the sour orange rootstock this is a particularly susceptible combination, leading to citrus rapid decline. However, the strains in this orchard are so mild that severe stunting occurs but not the sweet on sour reaction. The use of sour orange rootstocks usually phased out of a citrus-production area once CTV becomes established and spreads with an efficient vector such as *T. citricida*. The most severe isolates in Turkey were collected from Satsuma which was imported from Japan.



**Fig. 8.2.1.** Dr. Pedro Moreno (IVIA, Spain), Dr. Silvio Lopes (Fundecitrus, Brazil) and other IOCV delegates inspect a *Citrus tristeza virus* affected sweet orange on sour orange rootstock showing severe stunting.

**Citrus cachexia (CCa):** This disease is caused by citrus viroids in the Hop stunt group but only CVd-IIb and CVd-IIc are involved. Mandarins are affected by this disease and the symptom is gumming in the bark of the mandarin only and not on the sour orange rootstock (Fig. 8.2.2). The symptom does not occur in sweet orange or grapefruit although they are symptomless carriers. Other citrus viroids such as CEVd, Groups CVd-I, CVd-II and CVd-III as well as CVd-IV also occur in Turkey. Since sour orange rootstock, which is tolerant to these viroids, is the main rootstock, the effects of these viroids can not be seen. When the brown citrus aphid establishes itself in Turkey, the spread of CTV will be quick and the CTV sensitive sour orange rootstock will have to be abandoned. When the rootstock switch is to the trifoliolate types, major problems with citrus viroid infection will be experienced on these sensitive rootstocks.



**Fig. 8.2.2.** Cachexia symptoms in a mandarin tree. Note the gumming in the bark of the mandarin tree but not in that of the sour orange rootstock.

#### Diseases that do not occur in South Africa

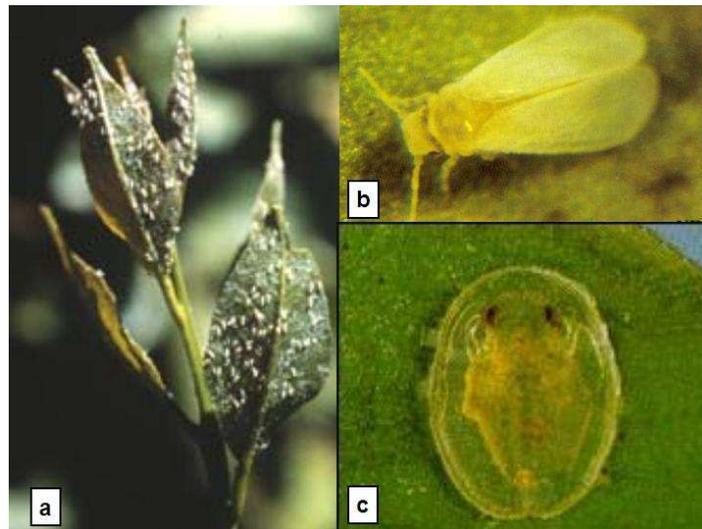
**Citrus yellow vein clearing (CYVC):** During the pre-conference tour, delegates visited a Kütdiken Eureka lemon orchard on sour orange rootstock on the grounds of the University of Çukurova where CYVC had infected the entire orchard. Symptoms of this disease are striking, apparently primarily so in the spring and

autumn flushes, but also persists on mature leaves. Symptoms start as yellow flecking of various lengths on the lateral veins of leaves progressing to general yellow vein clearing (Fig. 8.2.3). There is some distortion of the young leaves giving them a crinkling appearance. The disease has been shown by the conference organisers (N. Önelge and co-workers) to be mechanically transmissible with slash inoculation and graft transmission to other lemon varieties and sour orange, but did not find any symptoms when sweet orange, mandarin, grapefruit, Mexican lime or rough lemon were graft inoculated. This disease was seen for the first time in Turkey in 2000, and no recorded data regarding natural spread has been made. The cultivar was imported from Italy. Similar symptoms, possibly the same disease, have also been recorded in Pakistan, where it is prevalent in lemon orchards. The economic importance of the disease is not known and although tree growth is not affected, the crop and fruit size is reduced. Symptoms have similarities to those induced by *Citrus tristeza virus*, *Citrus variegation virus* and the causal organisms of Citrus ringspot and Citrus chlorotic dwarf disease (CCD). However, CCD causes distinct symptoms on rough lemon, while the causal agent of CYVC does not. While unidentified flexuous rod-shaped particles were detected in some infected trees in Pakistan, no pathogen has been found in Turkey.



**Fig. 8.2.3.** Typical leaf symptoms of Citrus yellow vein clearing diseases on lemon.

**Citrus chlorotic dwarf (CCD):** The “highlight” of new diseases observed in Turkey was seeing CCD infected trees. We were taken to these trees, which grow at Mersin, quite close to Adana, during the pre-conference tour. The disease is unique to Turkey and has not been seen elsewhere. It was first seen in 1980 and has subsequently spread rapidly within the Mediterranean citrus-growing region of Turkey. The disease is transmitted by the bayberry whitefly (*Parabemesia myricae*) (Fig. 8.2.4), which was introduced to Turkey some years before the disease was noted. The disease affects most species and cultivars of Citrus, being especially severe in lemons, grapefruit and some mandarins and tangelos. The causal agent of this disease has all the characteristics of a virus but it has not been identified yet. Purifications are infectious and affect navel, grapefruit, lemon and Minneola tangelo. The symptoms are short internodes, leaf flecking as well as small and twisted leaves, often also with a notch at the tip of the leaf (Fig. 8.2.5). Trees that get infected at an early age are dwarfed. No fruit symptoms develop.



**Fig. 8.2.4.** Images of bayberry whitefly (*Parabemisia myricae*) on: a) individuals on citrus (members.tripod.com), b) adult (H. Browning, U. Florida), c) nymph (H. Browning, U. Florida).



**Fig. 8.2.5.** Typical leaf symptoms of citrus chlorotic dwarf disease are leaf distortion, leaf tip notches and chlorotic leaf laminar patterns.



**Fig. 8.2.6.** Leaf and twig symptoms of citrus chlorotic dwarf disease showing leaf distortion, leaf tip notches and chlorotic leaf laminar patterns typical of this disease.

**Citrus stubborn disease:** Citrus stubborn disease, caused by *Spiroplasma citri*, has been detected in Turkey since the mid 1980s and delegates to the pre-conference tour were shown a few trees infected by this disease. However, casual observations of orchards in passing, suggest that the disease is both common and widespread in the areas travelled. This disease constitutes one of the citrus diseases which must be prevented at all costs from reaching our shores. It is important primarily in hot arid citrus-growing areas where it occurs, but less so in cool areas and areas with hot, humid conditions. The spiroplasma is transmitted by several species of leafhopper. It has not been transmitted mechanically and seed-transmission has not been observed. The disease affects most citrus species and cultivars as well as a wide range of non-Citrus hosts. Sweet orange, grapefruit, mandarin and mandarin hybrids are particularly severely affected. The disease is rarely lethal, but trees affected when young are stunted. Such a tree was observed during the pre-conference tour (Fig. 8.2.7). Foliage is often dense and abnormally upright. Leaves may be cupped and abnormally thick, and have variable chlorotic patterns resembling those of nutritional deficiencies. Off-season flowering often occurs, and was observed in Turkey, and this leads to fruit of various developmental stages being on the tree at the same time. Fruits are usually few and small and lop-sided or acorn shaped (see image in Fig. 8.2.7 taken in Turkey, illustrating the thickened albedo at the stem end). Fruits often do not colour at the stem ends as they mature, and seeds are often aborted. These symptoms may be confused with Citrus greening.



**Fig. 8.2.7.** Symptoms of Citrus stubborn disease. Clockwise from top left: 1) Discoloured vascular bundles at the stem end of fruit; 2) Section of fruit from infected tree illustrating the thickened albedo at the stem end, resulting in “acorn-shaped” fruit. Note the stained vascular bundles in the columella; 3) Stunted, “round” tree, dense foliage; 4) out-of-season flowering.

**Citrus gummy bark (CGB):** A number of sweet orange trees (Washington navel) displaying CGB in an orchard close to Dörtöyl, Turkey were inspected during the pre-conference tour (Fig. 8.2.8). The CGB of sweet orange is considered to have a viroid etiology, based on the similarity of symptom expression to cachexia disease of mandarins and tangelos caused by the hop stunt viroid (HSVd), related citrus viroids IIb and IIc (CVd-IIb and CVd-IIc). However, the symptoms of gummy bark develop in sweet orange and not mandarins while that of cachexia, in mandarins and not sweet orange. Typically the symptoms revealed by scraping the bark, are localized spots or a line of reddish-brown, gum-impregnated tissue around the scion circumference especially visible near the bud union. The discoloration and gumming may extend from above the bud union to the main branches of the sweet orange while in severe infection, dark streaks of gum-impregnated tissue may also be observed in longitudinal sections. Symptoms are not observed below the bud union when the rootstock used is sour orange. In Turkey, a number of CVd-II variants have been detected in CGB infected Washington navel and Dörtöyl sweet orange, a Turkish cultivar, with the predominant viroid detected being closely related to CVd-IIc, a known cachexia inducing viroid together with CVd-IIb.



**Fig. 8.2.8.** Bark symptoms of Washington navel on sour orange rootstocks detected in Dörtyol region, Turkey. Image on the left illustrates the discolouration detected on the sweet orange with the lack of symptoms under the bud-union with the sour orange rootstock. The image on the right illustrates how high above the bud-union the symptoms can be found.

### Conference Oral and Poster presentations

The programme for the conference is attached (Appendix 2). The majority of presentations were on *Citrus tristeza virus* of which more than half were on the detection and characterization of the virus. It was followed by Greening with a very wide range of subjects, the most important being the report of a new greening-like disease.

While most talks were of excellent quality and of interest, the following were selected for specific discussion because of the direct relevance to projects currently underway within the graft-transmissible disease group of CRI. If any other subjects are of interest to you, abstracts can be supplied (see programme).

### *Citrus tristeza virus*

#### 1) Quantitative Detection of *Citrus Tristeza Virus* By Direct Tissue-Print And Squash Real-Time RT-PCR Procedures

E. Bertolini, A. Moreno, E. Vidal, M.C. Martínez, N. Capote, A. Olmos, M.T. Gorris, and M. Cambra

In this presentation the authors demonstrated the usefulness of a technique for the detection of CTV which does not require any plant tissue extract preparation or nucleic acid purification, is extremely sensitive and reliable, and is ideally suited for large-scale testing. In the technique, leaf petioles are broken off the tree by hand and pressed against various membrane types. Ten such imprints from a single tree (5 shoots) are made on the same position on the membrane. A piece of the imprint is cut out with a micro-punch, placed in a microtiter plate and a small amount of releasing buffer added. This is incubated at 95°C for 10 minutes, vortexed and placed on ice. A small amount of this is then utilized in a real-time PCR for CTV. The technique was useful for detection of CTV in plants, on imprints already subjected to conventional tissue-imprint immuno-blotting and to CTV in aphids. We believe this technique is ideally suited for use in the CIP as a replacement for the current ELISA tests and will be establishing it in the laboratory over the next few months. Being a real-time PCR it will be difficult to perform at the CRI diagnostic centre in Nelspruit but, because of the apparent ease of sample preparation and the known storage lifetime and stability of the imprinted membranes (in other virus systems) it is feasible to have Kobus Breytenbach do the tissue imprints at the Citrus Foundation blocks and have the real-time PCR's done at CRI @ UP or to do it himself over here. A large-scale real-time, Faculty-use PCR apparatus is present at UP in the Genetics Department capable of 96 or 384 parallel tests. Furthermore the most expensive component of real-time PCR (glass capillaries) is obviated using that particular apparatus and would probably make the test cheaper than ELISA, given savings in labour due to the lack of onerous sample preparation. Hand-on tips were obtained during discussions with Dr. Mariano Cambria himself.

## **2) Cross Protection Against *Citrus Tristeza Virus* - a review**

Invited presentation by C.N. Roistacher, J. V. da Graça and G.W. Müller

This review placed the importance of *Citrus tristeza virus* in excellent perspective, and highlighted the need for further research on the cross-protection strategy utilised. It re-affirmed the need for projects such as those currently underway at CRI on CTV cross-protection and has made the authors more excited that we are on the right track, but need to intensify our efforts.

## **3) Two Distinct Evolutionary Pathways for *Citrus Tristeza Virus*: Recombination Defines two Gene Modules and Provides for Increased Genetic Diversity in a Narrow Host Range Plant Virus**

M. E. Hilf

This provocative presentation was excellent, and tries to address the unusual sequence relationship existing in CTV where the 5'-"half" (the replication module) has considerable greater variation amongst CTV strains than the 3'-"half" (having various other functions eg. host range, assembly, movement, virulence, suppression, and which may be summed up as the movement module). Dr. Hilf presented a credible hypothesis suggesting that a recombination took place between a closterovirus-like progenitor only relatively recently (in evolutionary terms) introduced to citrus which donated a replication module which is permissive as far as host range is concerned, and a CTV-like virus already present a long time in citrus and strictly adapted to this host, donating the movement module to produce a chimeric genome similar to modern day CTV's.

### **Citrus viroids**

## **4) Identification and Characterization of a Variant of *Citrus Viroid V* (CVd-V) in Orlando Tangelo.**

P. Serra, J.A. Pina and N. Duran-Vila

It is important to note the tentative naming of a new citrus Viroid (CVd-V), reported previously only on a citrus relative (*Atalantia citroides*) has now also been noted on a Citrus cultivar (Orlando tangelo), and may be more prevalent than previously thought. sPAGE analysis showed the presence of viroid-like RNA with an electrophoresis mobility close to that of CVd-II (Hop stunt viroid) and CVd-III. With biological indexing on Etrog citron, the viroid caused bend leaf and the shoots loose apical dominance resulting in multiple developments of shoots.

## **5) Transmissible small nuclear RNA dwarfing of commercial citrus on *Carrizo citrange* rootstock**

G. Vidalakis, J. A. Bash, and J. S. Semancik

The authors use the term "Transmissible small nuclear RNA" (Tsn-RNA) for citrus viroids that induce citrus dwarfing but cause no disease. This is done mainly with the aim to use these viroids commercially and detach them from the stigma of citrus viroids which are involved with diseases such as exocortis. They indicated that a mixture of Tsn-RNA IIIb (Cvd-IIIb), IIa (Cvd-IIa) and Ia (Citrus bent leaf viroid) are necessary to reduce tree size of Parent navel and Clementine on Carrizo citrange rootstock by 33-37%. The critical Tsn-RNA is apparently Ia since a mixture of Tsn-RNA IIa and IIIb did not reduce tree size. The presence of Cvd-Ia has not been confirmed in South Africa while Cvd-IIIb induces gum pocket disease symptoms on trifoliolate rootstocks but not on the trifoliolate hybrids.

### **Procaryote diseases (Huanglongbing (Greening), Phytoplasma, Citrus stubborn disease, Citrus variegated chlorosis)**

## **6) Investigations of the Effect of Guava as a Possible Tool in the Control/Management of HLB.**

T. Gottwald, D. Hall, G. McCollum, K. Ichinose, M. N. Chau, M. Bar-Joseph, S. Lapointe, and M. Hilf

This presentation was of particular interest to us, as the rumour of guava having a negative effect on HLB spread had reached us some months ago, and we had done a preliminary investigation into this in the past winter months. Our investigation was done in the Rustenburg district, an area known to have high disease pressure. Ten-year old citrus trees directly adjoining a more than 30-year old guava plantation were monitored individually for greening symptoms, with symptoms confirmed by PCR. An unexpectedly low incidence of greening infected trees, suggested that either a low disease pressure actually exists in the area during the past years (possibly the very hot dry past few years or regular insecticide applications had a dramatic effect on psylla numbers), or the guavas were serving as an effective repellent over a greater spatial scale than that monitored. However, one greening affected tree amongst only 4 found in the initial 21 X 52 row block monitored was directly next to the guava plantation, possible evidence that the repellent theory was not correct. To gain more insight, increasingly distant citrus blocks were monitored to determine

whether a more gradual infection gradient could be observed, suggestive of longer distance repellent effect. During this monitoring (done rapidly with a vehicle due to time constraints) no evidence of a gradient could be noted and very few additional greening infected citrus trees could be found. Conclusions cannot be reliably drawn from our observations, but the presence of a greening infected tree, one of only a few such trees, found directly next to the guava plantation led us to decide that the investigation into control of greening by some exploitation of guava was not worth pursuing. However, the authors of this presentation presented some convincing evidence of a negative effect of guava on psylla (*Diaphorina citri*) incidence. A collaborative project in the Mekong Delta in Vietnam showed that near non-detectable levels of psylla were demonstrated in citrus/white guava inter-plantings when compared to citrus monocultures. This study was followed up by glasshouse studies that demonstrate that high mortality rates of psylla are experienced when the insect is confined to guava in non-choice situations. Furthermore psylla found citrus more rapidly when caged on its own rather than when in cages contained both citrus and guava, with lower numbers of adults found when both the plant species were present. This is suggestive of volatile compounds emitted by guava, deleterious to psylla. Field experiments with guava and citrus inter-planting compared to citrus monocultures are currently underway at the University of Florida, USA to determine the dynamics of the HLB epidemiology.

#### **7) Distribution and Quantification of *Candidatus Liberibacter Americanus* in Various Leaves from a Huanglongbing-Affected Westin Sweet Orange tree in São Paulo State, Brazil**

D.C. Teixeira, C. Saillard, C. Couture, E.C. Martins, N.A. Wulff, S. Eveillard-Jagoueix, P.T Yamamoto, A.J. Ayres, and J.M. Bové

In this study the authors of the presentation compared the sensitivity of detection of *Liberibacter americanus* by conventional 16s rDNA PCR (the same techniques used by CRI @ UP when testing for *L. americanus* presence), with that of a nested 16s rDNA PCR and a SYBR-Green real time PCR. The real-time PCR protocol and primers differ from the Taqman-real-time PCR system used at CRI @ UP to detect *L. africanus*. The real time PCR proved to be the most sensitive, capable of detecting 10 *Liberibacter* cells per gram of leaf midrib tissue. The nested PCR was only slightly less sensitive whereas the conventional PCR was almost a 1000 times less sensitive. Individual leaves of a tree were tested by all three systems. A main branch showing no symptoms yielded no positives, suggesting that the whole branch was still uninfected or that *Liberibacter* cell levels were below even those of the real-time PCR method. Conventional PCR tests on leaves from branches yielded the following; of 111 leaves with blotchy mottling, all 111 were positive (with high *Liberibacter* cell concentrations). Of Zn deficiency symptoms, 61 of the 77 yielded conventional PCR-positive results, while 81 of 234 asymptomatic leaves were also positive. With the more sensitive nested PCR a further 4 samples with Zn deficiency and 30 without symptoms also tested positive. The real-time PCR added only 5 additional positive samples, all asymptomatic. Use of real-time or nested PCR is therefore critical for reliable detection of *Liberibacter*. These results also expose the fallacy that visual assessment of symptoms is the most reliable test....it just shows that symptoms are only present on leaves with the highest concentration of *Liberibacter*. Distribution of the leaves on a branch containing different concentrations of *Liberibacter* was also studied by the authors of the presentation, but appears quite erratic and difficult to characterise. They concluded that blotchy mottle leaves, often on the distal part of shoots were the best to test for *Liberibacter* followed by those showing Zn deficiencies.

#### **8) *Candidatus Liberibacter Spp.* Frequency in HLB-Infected Plants from Southwest Region of São Paulo, Brazil**

H.D. Coletta-Filho, E.F. Carlos, S.O. Dorta, K.C.S. Alves, M.A.R. Pereira, M.L.P.N. Targon, and M.A. Machado.

The interesting take-home message in this presentation also supported observations made within another oral presentation "Differential responses to temperature of citrus plants affected by *Candidatus Liberibacter americanus* and *Ca. L. asiaticus* " by S. A. Lopes, G. F. Frare, N. G. Fernandes and A. G. Andrade. Both sets of authors recorded the increase in the relative incidence of samples testing positive for *L. asiaticus* above that of *L. americanus*, suggesting that *L. asiaticus* was probably spreading more rapidly and more efficiently than *L. americanus*. This may be as a result of temperature factors, *L. americanus* has little tolerance for higher temperatures whereas *L. asiaticus* has a tolerance for higher temperatures, or it may be due to more efficient transmission of *L. asiaticus* than *L. americanus*, differing symptoms expression or differing concentrations in the plant. These aspects are under investigation currently.

#### **9) Additional Huanglongbing Agent in São Paulo State, Brazil**

D.C. Teixeira, N.A. Wulff, A.G. Mariano, E C. Martins, S. Eveillard-Jagoueix, C. Saillard, A.J. Ayres, and J.M. Bové

This presentation was the one with the greatest immediate impact on the research done at CRI. In essence what the authors of this oral presentation discovered is that citrus samples displaying greening symptoms do

not always test positive in conventional or real-time PCR with the Liberibacter primers currently utilised. After testing for the presence of unusual Liberibacters using the universal 16s ribosomal protein gene primers and finding no additional new Liberibacters, the authors, by deductive reasoning, decided to test such samples for phytoplasma using universal phytoplasma-detecting primers in PCR. In the process of so doing, got amplicons, sequenced these and discovered the presence of a phytoplasma closely related to pigeonpea witchesbroom phytoplasma in a large number of the Liberibacter negative/HLB symptomatic samples.

#### **10) Quantitative Detection of *Spiroplasma citri* by Real Time PCR**

R. K. Yokomi, A.F.S.Mello, J. Fletcher, and M. Saponari; and

#### **Assessment of Stubborn Disease Incidence in Citrus**

A. F.S. Mello, R. K. Yokomi and J. Fletcher

Primers to *S. citri* are available in the literature, and its effectiveness illustrated on a poster presented during the conference by Yokomi *et al.*, with an improved real-time PCR version also presented by Mello *et al.* Implementation of these PCR's in South Africa to detect this pathogen would be very useful to 1) confirm the absence of this disease in South Africa, 2) to be able to test for this pathogen in imported material (interestingly seed coats from infected fruit are excellent sources from which to culture the Spiroplasma), and 3) to identify the disease rapidly, should it be accidentally introduced to South Africa.

#### **11) Navelina ISA 315 sweet orange: a Citrus Variegated Chlorosis (CVC) resistant cultivar**

E.S. Stuchi, S.R. Silva, H.D. Coletta-Filho, Danilo Franco, S.A. Carvalho, O.R. Sempionato, L.C. Donadio, K.C.S. Alves

The Navelina ISA 315 cultivar was recovered by *in vitro* culture of undeveloped ovules and was introduced from Italy for CVC resistant studies. It was shown by indexing to carry cachexia disease (CVd-IIb and/or CVd-IIc). The cultivar was established in the field by top working onto diseased CVC trees as well as by approach grafting to CVC infected nursery trees. The top worked and approach grafted trees were evaluated for seven years for the presence of CVC leaf symptoms. No symptoms developed although PCR results were positive and the bacteria (*Xylella fastidiosa*) recovered from the top worked trees. Studies to investigate the effect of the cachexia viroids on CVC symptom expression are in progress.

#### Networking/Collaborations

Because of the relatively small number of delegates, of whom a large number also attended the pre-conference program (33) and a relatively high number of organised dinners, the opportunity to meet and interact with most delegates was ample and numerous contacts were made and friendships initiated. It will be extremely easy in future to contact a relevant researcher; 1) for primer sequences, protocols or positive controls, 2) for advice, 3) to exploit bilateral funding opportunities and 4) for collaboration, on most graft-transmissible diseases of Citrus. Of direct importance was the undertaking by Dr. Mark Hilf to facilitate collaboration between CRI @ UP with Dr. Z. Xiong, University of Arizona. Dr. Xiong's group have designed a microarray chip in a much more sophisticated manner than the one designed at CRI @ UP which will directly yield sequence data of any given CTV strain (See abstract presented at the 16<sup>th</sup> IOCV conference in Mexico in 2004, attached as Appendix 3, in which his development of the microarray chip is presented), and he is apparently looking for partners prepared to utilise the microchip for the purpose of CTV strain characterisation. Unfortunately Dr. Xiong did not attend the meeting in Turkey. As this exact objective is given very high priority in our research program it would be hugely beneficial for use to forge ties with Dr. Xiong and to collaborate around the use of his microarray chip, and primarily design our own microarray chip to expand on the ability to differentiate local isolates, especially around the grapefruit cross-protection problem.

During discussions with Dr. Moshé Bar-Joseph of Israel, he revealed that he has developed a multi-probe to detect all citrus viroids. He indicated that he will share this probe with South Africa in exchange for a visit to South Africa.

#### Recommendations

1. The importance to comply with the phytosanitary regulations of South Africa is emphasised. Importing citrus material from a "safe" country does not mean the material is free from diseases occurring in the 'unsafe' country from where it originated. Agents in South Africa are also importing citrus cultivars from the East at an increasing rate. It is important to establish which graft transmissible diseases occur in that country and apply the necessary indexing procedures for those diseases despite that it was declared free from them.

2. During the survey for Liberibacters conducted in 2006 and 2007 a number of samples were obtained which tested negative for Liberibacter despite the presence of clear greening symptoms. It may be possible that these samples represent instances where the greening-like disease is actually caused by a phytoplasma. This needs to be tested. A universal phytoplasma PCR already is operational within CRI @ UP and has been discussed with Aletta Kotze and will be scheduled for execution in the next few months as part of her PhD studies.

3. It is suggested that Dr. Moshé Bar-Joseph of Israel is invited as a key-note speaker on citrus viroids at the Citrus Symposium in 2008.

## APPENDIX 1

### PRE-CONFERENCE

#### Programme

#### **18 October 2007, Thursday**

Hotel check in and registration

20:00 Evening; welcome cocktail at Seyhan hotel

#### **19 October 2007, Friday**

09:00-10:00 Visit citrus areas to see Yellow vein clearing symptoms

10:00-12:30 Coffee break

10:30-11:00 Visit to Çukurova University

11:00-13:00 Visit to citrus orchards at near Adana

13:00-14:30 Lunch at the garden of packing house of Mr. Bülent Özler

14:30-17:00 Panoramic city tour

(Visit old Adana, little clock, big clock tower, Stone Bridge, Seyhan River)

19:00-21:00 Dinner and overnight at the hotel

#### **20 October 2007, Saturday**

09:00-12:00 Visit to a packing house of Mr. Bülent Özler

12:00-12:45 Transfer to Erzin and Dörtyol

12:45-14:00 Lunch

14:00-17:00 Visit to see infected orchards with gummy bark, stubborn and Citrus cachexia viroids to Erzin and Dörtyol region

17:00-18:00 Transfer to Adana

19:00-21:00 Dinner and overnight at the hotel

#### **21 October 2007, Sunday**

09:00-10:00 Transfer to Mersin

10:00-13:00 Visit citrus orchards with infected citrus chlorotic dwarf, *Citrus psorosis virus* and *Citrus tristeza virus* near Mersin city

13:00-14:30 Lunch

14:30-17:30 Drive to Kanlidivane. The name means "bloody like hell" in Turkish because it was believed that the criminals were thrown away to the deep pit to be torn by wild animals.

Visit to heaven and hell which are deep Casms one of which has chapel.

Visit Kizkalesi ("Maidens Castle"), the romantic name for the crusader castle floating in the blue water 150 meters offshore from this eastern Mediterranean resort town.

17:30-18:30 Transfer to Adana

19:00-21:00 Dinner and overnight at the hotel Seyhan, Adana

## APPENDIX 2

### PROGRAM OF XVIIth CONFERENCE OF THE INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

Adana, Turkey - October 22-26, 2007

#### Sunday, October 21, 2007

13:00-18:00 Registration at the Hotel Seyhan

#### Monday, October 22, 2007

08:00-10:00 Registration at the Hotel Seyhan (Continued)

#### OPENING CEREMONY

10:00-11:00 Welcome address by the Chairmen of the Organizing Committee, Dr. Nüket Önelge  
Address by the Chairman of the International Organization of Citrus Virologists, Dr. John da Graça  
Address by the Adana Yüreğir Citrus Growers Association (AYTUB), Uğur Paksoy  
Address by the Dean of the Çukurova University, Faculty of Agriculture, Dr. Ayzin Küden  
Opening address by the Rector of the Çukurova University, Dr. Alper Akinoğlu

11:00-11:30 Coffee break

#### SCIENTIFIC PROGRAM

##### ORAL AND POSTER SESSIONS

##### Oral session I: *Citrus tristeza virus*

Presiding: Ray Yokomi, Lochy Batista

11:30-12:30 Invited presentation by J.M. Bové: “50 years of IOCV: from graft-transmitted citrus agents to viroids, viruses and endogenous bacteria”

12:30-12:45 **Quantitative detection of *Citrus tristeza virus* by direct tissue-print and squash real-time RT-PCR procedures**  
E. Bertolini, A. Moreno, E. Vidal, M.C. Martínez, N. Capote, A. Olmos, M.T. Gorris, and M. Cambra

12:45-13:00 **Occurrence of genetic bottlenecks during *Citrus tristeza virus* acquisition by *Toxoptera citricida* in field conditions**  
Nolasco, G., Fonseca, F. and G. Silva

13:00-13:15 **A comparison between a coat protein gene targeting system and dispersed genome markers for strain discrimination of *Citrus tristeza virus***  
Siva, G., Fonseca, F. and G. Nolasco

13:15-13:30 **Toward characterizing stem pitting determinants of *Citrus tristeza virus* (CTV)**  
S. Ruiz-Ruiz, P. Moreno, J. Guerri, S. Ambrós

13:30-15:00 Lunch

##### Oral session II: *Citrus tristeza virus*

Presiding: Mark Hilf, Gustavo Nolasco

15:00-15:15 **Characterization of a severe isolate of *Citrus tristeza virus* (CTV) in commercial citrus varieties**  
S. Ruiz-Ruiz, S. Ambrós, J. Guerri, P. Mereno

15:15-15:30 **Survey for *Citrus tristeza virus* in southern Italy**  
G. Albanese, E. Ragozzino, S. Davino, R. Schimio, M. Barba

15:30-15:45 **Sequence analysis of the coat protein and the RNA-dependent RNA polymerase genes of a *Citrus tristeza virus* isolate from Turkey**  
B. Çevik, S Korkmaz

15:45-16:00 **Rapid assessment of the *Citrus tristeza virus* isolates detected at the Lincove research and extension center, EXETER, CA in spring 2007**  
R.K. Yokomi, M. Polek, E.E. Grafton-Cardwell and N. O'Connell

16:00-16:15 **Detection and identification of *Citrus tristeza virus* isolates from different citrus growing regions of Turkey**  
S. Korkmaz, B. Çevik, S. Önder, N.K. Koç

- 16:15-16:30 **Purification and secondary structure characterization of the *Citrus tristeza virus* coat protein**  
Peroni, L.A., Rosselli, L.K., Saraiva, A.M., Souza, A.P., Machado, M.A., Stach-Machado, D.R.
- 16:30-17:00 Coffee break
- 17:00-19:00 **Poster session I: *Citrus tristeza virus***
- A01 **Molecular characterization of *Citrus tristeza virus* isolates from Epirus (Greece)**  
L. Barbarossa and C. Vovlas
- A02 **Preliminary evaluation of *Citrus tristeza virus* from Apulia (Southern Italy)**  
L. Barbarossa and V. Savino
- A03 **Tristeza associated decline in a citrus area of Santiago de Cuba province and its possible cause**  
I. Peña, D. López, A. Peralta, L. Batista, J.C. Casín, M. Acuña and Y. Méndez
- A04 **Molecular marker analysis of *Citrus tristeza virus* (CTV) isolates from the Dominican Republic**  
L. Matos, M.E. Hilf and J. Borbon
- A05 **Effectiveness of antibodies developed to the recombinant coat protein of *Citrus tristeza virus***  
M.M. Iracheta-Cárdenas, P. Metheney, M.L. Polek, K.L. Manjunath, R.F. Lee, M.A. Rocha-Peña
- A06 **Use of the CP and CPm Intergene Sequences to Discriminate *Citrus tristeza virus* Strains**  
M. Saponari and R. K. Yokomi
- A07 **Characterization of *Citrus tristeza virus* Isolates by Single-Strand Conformation Polymorphism Analysis of the Coat Protein Gene**  
M. Saponari and R. K. Yokomi
- A08 **Epitope mapping of *Citrus tristeza virus* capsid proteins recognized by monoclonal antibodies.**  
Peroni, L.A., Rosselli, L.K., Saraiva, A.M., Souza, A.P., Machado, M.A. and Stach-Machado, D.R.
- A09 **Tissue print-ELISA<sup>®</sup> complete kit for screening of severe *Citrus tristeza virus* isolates at large scale testing**  
A. Abad, M. Colomer, M.T. Gorris, J.A. Pina, and M. Cambra
- A10 **Identification of Turkish strains of *Citrus tristeza virus* (CTV) by analysis of double stranded RNA methods**  
E. Ince
- A11 **Biological characterization of *Citrus tristeza virus* strains in lemon in Tucumán, Argentina**  
J. Figueroa, L. Foguet, A. Figueroa and B. Stein
- A12 **Serological and Molecular Variability in a Collection of Mediterranean *Citrus Tristeza Virus* (CTV) Isolates**  
Daden M., K. Djelouah, M. Zemzami, R. Milano, A.M. D'Onghia
- A13 **First Monitoring and Characterization of *Citrus Tristeza Virus* (CTV) and Relative Vectors in Syria**  
Abou Kubaa R, K. Djelouah, R. Addante, M. Jamal, A. M. D'Onghia
- A14 **Development of transgenic Mexican lime plants for resistance to *Citrus tristeza virus* through post-transcriptional gene silencing**  
M. Melzer, H. Mauch, D. Gonsalves, L. Peña, S. Ferreira and J. Hu

**Tuesday, October 23, 2007**

**Oral Session III: *Citrus Tristeza Virus***

- Presiding: *Pedro Moreno, Mithat Özsan*
- 08:30-09:15 Invited presentation by C.N. Roistacher, J.V. da Graça and G.W. Müller: **“Cross protection against *Citrus tristeza virus* - a review”**
- 09:15-09:30 **Present situation of *Toxoptera citricida* and *Citrus tristeza virus* in northern Spain**  
A. Álvarez, A. Hermoso de Mendoza, M. Braña, S. Méndez, A. Moreno, J.M. Llorens, and M. Cambra
- 09:30-09:45 **Elevated background in DAS-I ELISA for the detection of *Citrus tristeza virus* in mandarin varieties**  
R. K. Yokomi and Marylou Polek

- 09:45-10:00 **Replication, synergism of components and symptoms of *Citrus tristeza virus* Capão Bonito complex in Key lime plants**  
Francisca Alves dos Santos ; Alessandra Alves de Souza ; Maria Luísa P. N. Targon ; Luís Antonio Peroni e Marcos Antonio Machado
- 10:00-10:15 **Incidences of long term cross protection in the evolution of *Citrus tristeza virus* symptoms in Peru**  
K. Bederski , C.N. Roistacher , G.W. Muller , and O. P. Silvestre
- 10:15-10:30 **Detection of *Citrus tristeza virus* and citrus viroids associated with citrus in Oman**  
A.J. Khan, N.A. Al-Saady, H. Dietz, M. Kinawy, A.W. Al-Saady, Y. Al-Hinai, K. Al-Maamary, M. Cambra
- 10:30-10:45 **Occurrence, distribution and characterization of *Citrus tristeza virus* (CTV) and relative vectors in Apulia the region of South-East Italy**  
Djelouah K., F. Valentini, N. Birisk, D.Yahiaoui, A.Percoco, R. Addante, A.M. D'Onghia
- 10:45-11:00 **Biological and molecular characterization of two virulent *Citrus tristeza virus* isolates found in central California**  
R. K. Yokomi and M. Saponari
- 11:00-11:30 **Coffee break**

#### Oral Session IV: *Citrus Tristeza Virus*

- Presiding: *Georgios Vidalakis, Mani Skaria*
- 11:30-11:45 **Characterization of additional *Citrus tristeza virus* isolates in a highly citrus infected area of Sicily**  
A. Catara, A. Lombardo, G. Nobile, S. Rizza
- 11:45-12:00 **Genetic variability of Croatian *Citrus tristeza virus* isolates**  
S. Černi, G. Nolasco, M. Krajačić, and D. Škorić
- 12:00-12:15 **Differential expression of *Citrus tristeza virus* genes in tolerant and resistant hosts**  
M.L.P.N. Targon, E.F. Carlos, S.A. de Carvalho, H.D. Coletta Filho, A.A. de Souza, M.A. Takita, F.A. Santos, G.W. Muller and M.A. Machado
- 12:15-12:30 **Two distinct evolutionary pathways for *Citrus tristeza virus*: recombination defines two gene modules and provides for increased genetic diversity in a narrow host range plant virus**  
M. E. Hilf
- 12:30-12:45 **Rapid diffusion of *Citrus tristeza virus* Seedling Yellows severe isolate by *Aphis gossypii* glover**  
G. Sorrentino , S. Davino , M.Davino , M. Guardo and A. Caruso
- 12:45-13:00 ***Citrus tristeza virus* survey in Tanzania**  
G.M. Rwegasira, G.K. Kahwa and C.M. Herron
- 13:00-14:30 **Lunch**
- 14:30-16:30 **Business Meeting**
- 16:30-17:00 **Coffee break**
- 17:00-19:00 **Poster Session II: *Citrus Psorosis Virus*, *Citrus Leprosis Virus* and Citrus Variegated Chlorosis**

- B01 **Studies on the possible causes of spread of *Citrus psorosis virus***  
J. Figueroa, L. Foguet , A. Figueroa , C. Escobar , C. Mansilla and B. Stein
- B02 **Characterization of some psorosis and concave gum isolates from northwestern Argentina**  
J. Figueroa , L. Foguet , A. Figueroa , C. Escobar , B. Stein and C.N. Roistacher
- B03 **Influence of the *Brevipalpus phoenicis* endosymbiont *Cardinium* sp. in the transmission of *Citrus leprosis virus*.**  
V.M. Novelli, J. Freitas-Astúa, D.F.S. Guidotti, M.E. Hilf, T.R. Gottwald, and M.A. Machado
- B04 **Response of Mandarin Cultivars and Hybrids to *Citrus Leprosis***  
M. Bastianel, F. Nicolini, J. Freitas-Astúa, V. Rodrigues, N. Segatti, C.L. Medina, V.M. Novelli, M.A. Machado
- B05 **Haplotype characterization and genetic variability of two genes of *Citrus leprosis virus-C* through SSCP in Brazilian citrus orchards**  
Locali-Fabris, E.C., Freitas-Astúa, J., Coletta-Filho, H.D., Souza, A.A., Antonioli-Luizon, R. and Machado, M.A..
- B06 **Evidences suggesting that *Brevipalpus phoenicis-Citrus leprosis virus* interaction may not be of circulative propagative type**  
Nicolini, F., Bastianel, M.; Freitas-Astúa, J.; Kitajima, E.W.; Kubo, K.S.; Antonioli-Luizon, R.; Schons, J.; Machado, M.A.

- B07 **Initial responses of sweet orange to *Citrus leprosis virus* detected by ESTs**  
J. Freitas-Astúa, M. Bastianel, E.C. Locali-Fabris, V.M. Novelli, A.C. Silva-Pinhati, A.C. Basílio-Palmieri, M.L.N.P. Targon, K.S. Kubo, M.A. Machado
- B08 ***Xylella fastidiosa* multiplication into Pera sweet orange x Murcott tangor citrus hybrids**  
H.D. Coletta-Filho, E.O. Pereira, A.A. Souza, MA Takita, M Cristofani, and M. A. Machado
- B09 **Multidrug resistance in *Xylella fastidiosa* biofilm**  
Souza, A.A., Takita, M.A. Rodrigues, C.M., Olivato, J.C, Coletta-Filho, H.D., and Marcos A. Machado.
- B10 **Diagnosis of *Xylella fastidiosa* of Citrus Variegated Chlorosis by immunomolecular techniques**  
Peroni, L.A., Reis, J.R.R., Coletta Filho, H.D., Souza, A.A. de, Machado, M.A, Stach-Machado, D.R.
- B11 **Behavior of six sweet oranges varieties under high inoculum pressure of citrus variegated chlorosis (CVC)**  
D. Franco, E.S. Stuchi, S.R. Silva, and A.B.G. Martins
- B12 **Behavior of five Valencia sweet oranges selection under high inoculum pressure of citrus variegated chlorosis (CVC)**  
F. Tomasetto. E. S. Stuchi, S. Rodrigues-Silva, and A. B. G. Martins

**Wednesday, October 24, 2007**

08:00-19:00 **Field Tour**

**Thursday, October 25, 2007**

**Oral Session V: *Citrus Tristeza Virus*, Citrus Sudden Death and Citrus Variegated Chlorosis**

- Presiding: *Gerhard Pietersen, Changyong Zhou*
- 08:30-09:15 Invited presentation by İ. Tekin: **"A snapshot of Turkish citrus industry"**
- 09:15-09:30 **Influence of climatic variability on *Citrus tristeza virus* epidemiology in two regions of Cuba**  
L. Batista, K. Velázquez, A. Rivero, I. Peña, D. López, I. Estévez, F.F. Laranjeira and P.L. Ortiz.
- 09:30-09:45 **Spatial diffusion of two different isolates of *Citrus tristeza virus* in Sicily**  
M.Guardo' G. Sorrentino, S. Davino, , M. Davino and A. Caruso.
- 09:45-10:00 **Cloning, expression and polyclonal antiserum production of recombinant capsid protein of Citrus Sudden Death (CSD)**  
Peroni, L.A., Lorenzi, M.S., Colletta, H.D., Saraiva, A.M., Souza, A.P., Machado, M.A. and Stach-Machado, D.R.
- 10:00-10:15 **Transmission of Citrus Sudden Death associated symptoms, a summary of dates of field and greenhouse assays**  
Coletta-Filho, H.D., Müller, G.W., Borges, N., Targon, M.L.P.N., Machado, M.A.
- 10:15-10:30 **Twelve rootstocks effects on the intensity of Citrus Variegated Chlorosis (CVC) in 'Folha Murcha' sweet orange in Bebedouro, SP, Brazil**  
T. Cantuarias-Avilés, E.S. Stuchi, F.A.A. Mourão Filho, S.R.Silva
- 10:30-10:45 **Navelina ISA 315 sweet orange: a Citrus Variegated Chlorosis (CVC) resistant cultivar**  
E.S. Stuchi, S.R. Silva, H.D. Coletta-Filho, Danilo Franco, S.A. Carvalho, O.R. Sempionato, L.C. Donadio, K.C.S. Alves
- 10:45-11:15 **Coffee break**

**Oral Session VI: Miscellaneous Diseases, Virus Indexing and Survey**

- Presiding: *Timothy Williams, Klaus Bederski*
- 11:15-11:30 **An immunocapture RT-PCR procedure using *Apple stem grooving virus* antibodies facilitates molecular genetic characterization of Citrus tatter leaf virus from the original Meyer lemon host**  
M. E. Hilf
- 11:30-11:45 **Improved biological indexing of the main Citrus viruses and viroids**  
D'Onghia A. M., H. Fahmy, R. Brandonisio and K. Djelouah
- 11:45-12:00 **Pilot survey of Citrus mother trees in Greece for the presence of viruses and viroids**  
I.N. Boubourakas, G. Vidalakis, A. E. Voloudakis, T. Agorastou, G. Magripis and P. E. Kyriakopoulou

- 12:00-12:15 **Host plant-viroid interaction in Troyer citrange, sour orange and Alemow rootstocks infected by by Citrus Viroid IIIb (CVD-IIIb)**  
S. Rizza , C. Capasso, G. Catara, A. Capasso, E. Conte and A. Catara
- 12:15-12:30 **Virus and virus-like diseases in Turkish citriculture**  
N. Önelge, A. Çınar
- 12:30-14:30 **Lunch**

#### Oral Session VII: Citrus Viroids

- Presiding: *Marcos Machado, AnnaMaria D'Onghia*
- 14:30-14:45 **Citrus viroids in Colombia**  
 N. Murcia, L. Bernad, and N. Duran-Vila
- 14:45-15:00 **Identification and characterization of a variant of Citrus viroid V (CvD-V) in Orlando tangelo**  
 P. Serra, J.A. Pina and N. Duran-Vila
- 15:00-15:15 **Viroids in Tahiti limes showing bark cracking symptoms**  
 N. Murcia, K. Bederski, N.A.Wulff, C.J. Barbosa, J.M. Bové and N. Duran-Vila
- 15:15-15:30 **Desert lime (*Eremocitrus glauca*) appears to be resistant to viroid infection**  
 S. M. Bani Hashemian, C. J. Barbosa, J.A. Pina and N. Duran-Vila
- 15:30-15:45 **Transmissible small nuclear RNA dwarfing of commercial citrus on Carrizo citrange rootstock**  
G. Vidalakis, J. A. Bash, and J. S. Semancik
- 15:45-16:00 Viroids and rootstocks effects on field performance of Tahiti lime in Brazil  
E.S. Stuchi, S.R. Silva, O.R. Sempionato, and E.T. Reiff
- 16:00-16:30 **Coffee break**
- 16:30-18:45 **Poster session III: Miscellaneous diseases, virus indexing and survey**
- C01 **Effect of viroid on resistance to *Phytophthora* infection of Citrus**  
 T. P. Thomas, J. V. daGraça, A. Bhattacharya, M. Kunta, M. Sétamou, and M. Skari
- C02 **Citrus viroids in Turkey**  
 N. Onelge
- C03 **A rapid greenhouse assay to evaluate viroid-induced dwarfing.**  
 R.A. Owens, S.M.Thompson and M.E. Hilf
- C04 **Confirmation of the presence of citrus viroids in citrus orchards in North Western Argentina**  
 J. Figueroa , A. Figueroa , L. Foguet , C. Escobar and B. Stein
- C05 **An analysis of threats to the modern Brazilian citrus industry**  
 Orlando S. Passos & Chester N. Roistacher
- C06 **Quantitative Detection of *Spiroplasma citri* by Real Time PCR**  
 R. K. Yokomi A.F.S.Mello, J. Fletcher, and M. Saponari
- C07 **Assessment of Stubborn Disease Incidence in Citrus**  
 A. F.S. Mello, R. K. Yokomi and J. Fletcher
- C08 **Susceptibility of Rangpur lime rootstock selections to Citrus Sudden Death**  
 J. Pompeu Junior and S. Blumer
- C09 **Detection of virus and virus-like disease in Citrus in the Turkish Republic of Northern Cyprus**  
 N. Önelge, R. Çaluda and O. Bozan
- C10 **Yellow Vein Clearing of lemons in Turkey**  
 N. Önelge, O. Bozan, M. Gök and S. Satar
- C11 **Health status testing in the Auscitrus budwood and seed scheme**  
 G. A. Chambers, T. Herrmann, and N. J. Donovan
- C12 **A model system for measuring citrus propagation risk mitigation based on a Hazard Analysis and Critical Control Point (HACCP) methods**  
 L.G. Brown
- C13 **The Citrus Sanitation Center of the Obispo Colombres Experimental Station, Tucumán, Argentina**  
 B. Stein, J. Figueroa , L. Foguet, A. Figueroa and C.Escobar
- C14 ***Diaphorina citri* Kuw. (Hemiptera: Psyllidae), behavior and natural enemies in Cuban citriculture**  
 C. González, M. Gómez, M. Fernández, D. Hernández, J. L. R. Tapia and L. Batista
- C15 **Early detection of incompatibility between citrus scions and rootstocks by biochemical and anatomical methods**

- C16 S. Blumer ; B. Apezzato-da-Gloria; P. Mazzafera and J. Pompeu Junior  
**Sanitary characterization of “Quebra-Galho” Tahiti acid lime and selection of candidate mother trees**
- C17 S.R. Silva, A.B.G. Martins, E.S. Stuchi, S.A. Carvalho, M.L.P.N. Targon, D. Franco  
**Juvenility and Genetic Fidelity in Citrus Sanitized Plants through Stigma/Style Somatic Embryogenesis**
- C18 Carimi F., M. Siragusa , L. Abbate , A. Carra , F. De Pasquale , M. Meziane, A.M. D’Onghia  
**Preliminary observations on the phytosanitary status of the Croatian Satsuma mandarin (*Citrus unshiu* Marc.) collection**
- C19 K. Hančević, S. Černi, J. Rošin and D. Škorić  
**Survey for Citrus diseases in French Guiana**
- C20 Thermo, J.P.  
**Survey of Citrus virus and viroids diseases in Hunan Province, China**
- C21 S. Rizza, X.F. Ma, J. Han, G. Nobile, P. Bella, Z.N. Deng and A. Catara  
**Twelve years management of a high density Clementine orchard inoculated with pathogenic and non-pathogenic viroids**
- S. Rizza, G. Nobile, M. Tessitori, G. Albanese, R. la Rosa and A. Catara

**Friday, October 26, 2007**

**Oral Session VIII: Citrus Huanglongbing**

- Presiding: *Antonio Catara, Magally Williams*
- 08:30-09:15 **Spato-temporal analysis of an HLB epidemic in Florida and implications for future spread**  
T. Gottwald, M. Irey, T. Gast, and M. Hilf
- 09:15-09:30 **Investigations of the effect of guava as a possible tool in the control/management of HLB**  
T. Gottwald, D. Hall, G. McCollum, K. Ichinose, M.N. Chau, M. Bar-Joseph, S. Lapointe, M. Hilf.
- 09:30-09:45 **Surveys for Citrus Huanglongbing and its Asian citrus psyllid vector in Texas**  
J. V. da Graça, J. V. French, P. S. Haslem, M. Skaria, M. Sétamou and B. Salas
- 09:45-10:00 **Growing Huanglongbing-free Citrus trees on well-adapted rootstocks: a biotechnological challenge for better food security in Nepal**  
C. Regmi, R. P. Devkota, K. P. Paudyal, S. Shrestha, A. J. Ayres, N. Murcia, J. M. Bové and N. Duran-Vila.
- 10:00-10:15 **Additional Huanglongbing agent in São Paulo state, Brasil**  
D.C. Teixeira , N.A. Wulff, A.G. Mariano, E.C. Martins, S. Eveillard-Jagoueix, C. Saillard, A.J. Ayres, and J.M. Bové
- 10:15-10:30 **Current status of Citrus Huanglongbing (HLB) in São Paulo state, Brazil, based on molecular and visual diagnosis**  
E.F. Carlos; H.D. Coletta-Filho; L.L. Lotto; L.F. Coerini; M.T. Vitorino; & M.A. Machado
- 10:30-10:45 **Attempts to obtain Huanglongbing resistant or tolerant sweet orange by embryo rescue from healthy chimeras of diseased fruit**  
S.P. van Vuuren and B.Q. Manicom
- 10:45-11:15 **Coffee break**

**Oral Session IX: Citrus Huanglongbing**

- Presiding: *Fanie van Vuuren, Caroline Herron*
- 11:15-11:30 **Distribution and quantification of *Candidatus Liberibacter americanus* in various leaves from a Huanglongbing-affected Westin sweet orange tree in São Paulo state, Brazil**  
D.C. Teixeira, C. Saillard, C. Couture, E.C. Martins, N.A. Wulff, S. Eveillard-Jagoueix, P.T. Yamamoto, A.J. Ayres, and J.M. Bové
- 11:30-11:45 **Differential responses to temperature of citrus plants affected by *Candidatus Liberibacter americanus* and *Ca. Liberibacter asiaticus***  
S.A. Lopes, G.F. Frare, N.G. Fernandes and A.G. Andrade
- 11:45-12:00 ***Candidatus Liberibacter* spp. frequency in HLB-infected plants from southwest region of São Paulo, Brazil**  
H.D. Coletta-Filho, E.F. Carlos, S.O. Dorta, K.C.S. Alves, M.A.R. Pereira, M.L.P.N Targon M.A. Machado.

- 12:00-12:15 **Graft transmission efficiencies of *Candidatus Liberibacter americanus* and *Ca. Liberibacter asiaticus* to citrus plants**  
S.A. Lopes and G.F. Frare
- 12:15-12:30 ***Murraya paniculata* as an alternate host of *Candidatus Liberibacter americanus* and *Ca. Liberibacter asiaticus* in Brazil**  
S.A. Lopes and G.F. Frare
- 12:30-12:45 **Survey for "*Candidatus*" liberibacter species on Citrus in South Africa**  
M. Schwerdtfeger and G. Pietersen
- 12:15-12:30 **The *rpIKAJLI-rpoBC* operon of the Liberibacters: further proof that *Candidatus Liberibacter americanus* is a distinct species**  
D.C. Teixeira, S. Eveillard-Jagoueix, N.A. Wulff, C. Saillard, A.J. Ayres, and J.M. Bové
- 12:30 **Closing of the Meeting**

### APPENDIX 3

#### Rapid Analysis of *Citrus tristeza virus* Genomes Using a Resequencing Oligonucleotide Microarray

Z. Xiong<sup>1,2</sup>, R. Barthelson<sup>2</sup>, Z. Weng<sup>1,2</sup>, and D.W. Galbraith<sup>2</sup>

<sup>1</sup> Division of Plant Pathology and Microbiology

<sup>2</sup> Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721, USA

*Citrus tristeza virus* (CTV) has a large and extremely variable RNA virus genome that results in enormous variations in pathogenicity and aphid transmissibility. The ability to rapidly characterize CTV genomes is thus imperative. We have designed and are evaluating a resequencing microarray capable of querying 117,088 nucleotides for rapid CTV genomic analysis. The array tiles 25-mer oligonucleotides corresponding to all four variations (A,C,G,T) for each individual base on both strands, making it possible to sequence each strand and to identify single nucleotide polymorphisms. Full-length genomic sequences from T30, T36, Israeli VT, T3, and T68 isolates and unique sequences from H33, SY568, Japanese NUagA, Spanish T385, and Egyptian Qaha isolates were tiled on the microarray. The unique sequences were determined by pairwise comparisons with the most similar, fully-tiled genome, and subsequent analysis with an algorithm that maximizes the probability of resolving each nucleotide. Currently, cDNA clones covering the entire genomes of several representative CTV isolates are being used to validate the microarray. Further testing of the microarray using cDNA fragments amplified by RT-PCR from various CTV isolates will be carried out. The microarray is projected to be able to decipher the complete genome of any CTV strain within a week. It is expected to work for most CTV isolates, since the microarray covers the genomes of CTV isolates diverse in both biology and geographic origins. With its high capacity and speed, the microarray should prove ideal for the genomic analysis and discovery of genes governing CTV biological trait.

#### 8.3 S.D. MOORE

Report on visit to Lucerne, Switzerland, in his capacity as General Manager River Bioscience (RB)

##### Introduction

This visit took place from 20-28 October 2007 and was sponsored by River Bioscience. The second annual meeting of the International Biocontrol Manufacturers Association (IBMA) took place from 22-23 October in Lucerne. I also visited with Andermatt AG, manufacturers of biological control products. As this trip was sponsored by River Bioscience, and a large element of the trip was commercial, certain confidential aspects of the trip (including the full visit with Andermatt) have been excluded from this report.

##### Itinerary

Date/s	Destination	Institution/venue	Activity	Mode of travel
19-20 October	Lucerne, Switzerland	-	Travel via Johannesburg, Paris and Zurich	Air & train
22-23 October	Lucerne, Switzerland	KKL conference centre	IBMA meeting	-
25 October	-	Andermatt AG	Business meeting	-
26-29 October	Port Elizabeth	-	Travel via Zurich, Paris and Johannesburg	Train & air

##### Purpose of trip

Through participation in the second annual meeting of the IBMA, the purpose of the trip was:

1. To identify markets for RB's existing products, elsewhere in the world, and potential partners who could commercialise RB's products in these other regions.
2. To build beneficial and potentially beneficial relationships with other role players in the biocontrol industry.
3. To glean any information on the commercial development of biocontrol in the world, which might have any benefit for RB.
4. To identify products, manufactured elsewhere in the world, with potential for use in southern African agriculture (particularly citrus), that could be commercialised in the region by RB.

### **Programme: Sessions, papers and workshops**

22 October

**Welcome** (Chair: Lucius Tamm)

- Michel Guillon (President IBMA): Objectives of IBMA and ABIM 2007

Lucius Tamm (FiBL): Welcome on behalf of the organizing committee

Plenary session 1 (Chair: Michel Guillon)

#### **New developments in regulation and policy**

- Ralf-Udo Ehlers (University Kiel): [The outcome of REBECA](#)
- Jeroen Meeussen(CTB): [OECD-Biopesticides Steering Group: past, present and future](#)
- Lisa Moakes (PSD): [The UK biopesticide scheme](#)
- Thomas Jaekel (GTZ): [Harmonisation of registration requirements in South East Asia](#)

Plenary session 2 (Chair: Bernard Blum)

#### **Market developments for biocontrol**

- Bernhard Blum (Vice president IBMA): [How to fill the gap?](#)
- Roberto Kron-Morelli: [Biocontrol in Italy: present reality and hopes](#)
- Melvyn Fidgett (Syngenta Bioline): [It started here. Where we are now? The UK biocontrol market](#)
- Richard Ward (Biobest Canada): [Invertebrate IBCAs: market and opportunities in North America](#)
- Michael Braverman (Rudgers University): [Biopesticides market and opportunities in North America](#)

Plenary session 3 (Chair: Karel Bolckmans)

#### **Macrobials**

- Sami A. Elawad (Hamraniah Agri. Res. Stat.): [Novel Preparation and Application Method of Entomopathogenic Nematodes to Control Insect Pests](#)
- Bernhard Blum(Agrometrix): [Development of EPNs against the hazelnut borer](#)
- Guido Sterk (Biobest) and Veerle Mommaerts (University Brussels): [Effect of microbial control agents on the beneficial pollinator \*Bombus terrestris\*](#)
- Karel Bolckmans (Koppert): [Biological Control in Southern Europe, a breakthrough](#)
- Guido Sterk (Biobest): Film on *Amblyseius swirskii*

#### **Meetings of National IBMA Associations**

- IBMA Italy (Chair: Roberto Kron Morelli)
- IBMA UK (Chair: NN)
- IBMA France (Chair: NN)
- IBMA/IVB Germany, Switzerland, Austria (Chair: Hubertus Kleeberg)
- Further National IBMA Associations (Chair: NN)

23 October

Plenary session 4 (Chair: Willem Ravensberg)

#### **Microbials**

- Sean Moore (River Bioscience): [Microbial and biorational control of moth pests and fruit flies on fruit crops](#)
- Philip Kessler (Andermatt Biocontrol): [New baculovirus products tested in the field](#)
- Bruce Kirkpatrick (Valent BioSciences): [Cost benefit of using Bt-based products in IPM programs](#)
- Edith Ladurner (Intrachem Bio Italia): [NATURALIS \(Beauveria bassiana\): an effective bioinsecticide for the control of a wide range of arthropod pests](#)
- Massimo Benuzzi (Intrachem Bio Italia): [AQ 10, biofungicide based on Ampelomyces quisqualis isolate M-10: a valuable tool for powdery mildew control](#)
- Manuele Ricci (AgraQuest): [SERENADE: pre-harvest applications for post-harvest disease control in fruit crops](#)
- Stefan Kunz (Bio-Protect): [BoniProtect forte \(Aureobasidium pullulans\) – a new biocontrol agent for use in stone fruit](#)
- Mordechai Keren-Zur (AgroGreen): [Effective Biopesticides addressing market needs](#)
- Olivier Besnard (Biophytech): [Trichoderma atroviride application in grapevine nursery](#)

Plenary session 5 (Chair: Owen Jones)

### **Botanicals, natural substances and semiochemicals**

- Frank Lehnhof (Biofa). [Milsana – a Reynoutria sachalinensis based plant extract for preventive control of powdery mildew](#)
- Hubertus Kleeberg (Trifolio-M): [NeemAzal against chestnut leafminer](#)
- Walter Leipold (Proagro): [ProNet-Alfa, a wetting and bonding agent based on milk protein](#)
- Vittorio Veronelli (CBC Europe): [Application and development of pheromones in modern IPM](#)

### **IBMA Annual General Assembly Meeting**

Key points on key papers:

Ralf-Udo Ehlers (University Kiel): [The outcome of REBECA](#)

In the future, the registration of biocontrol agents in Europe will be easier than has been the case until now. Most EU countries are now regulating the use of microbial (arthropod) biocontrol agents.

Lisa Moakes (PSD): [The UK biopesticide scheme](#)

Registration fees in the UK for microbial control products is £22500 per product. In order to include the EU in the registration, an additional £7500 would be due. Although still expensive, this is more affordable than in the past and less than the cost of registering a chemical pesticide. (See [www.pesticides.gov.uk](http://www.pesticides.gov.uk)).

Bernhard Blum (Vice president IBMA): [How to fill the gap?](#)

The main reasons why biocontrol agents are rejected by growers are: their high cost; their lack of efficacy; and they are often complicated to use.

Roberto Kron-Morelli: [Biocontrol in Italy: present reality and hopes](#)

The total value of the Italian pesticide market is €16.5 billion. Biocontrol agents make up only 2% of this. There are 51 companies marketing biocontrol agents in Europe.

Melvyn Fidgett (Syngenta Bioline): [It started here. Where we are now? The UK biocontrol market](#)

The use of biocontrol in the UK is driven by a lack of chemical solutions and by supermarket protocols. The lack of registered products over the last 20 years has resulted in the “grey” usage of biocontrol products. This has resulted in many “snake oils” and grower distrust. Efficacy and use recommendations are still lacking. Nematodes are considered to be the most widely used biocontrol agent for slug control in home gardens.

Michael Braverman (Rudgers University): [Biopesticides market and opportunities in North America](#)

Biocontrol products make up only 3% of the pesticide market in North America. This is predicted to increase to 4% by 2010. 75% of all biocontrol sales are Bt products. 3% of the biocontrol market is organic. Amongst other things, the IR4 project assisted with preparations for the importation of *Chenopodium* based insecticides into the USA. *C. ambrosioides* is a Mexican plant with insecticidal properties against soft bodied insects, including thrips. *Chenopodium* spp. exist in South Africa and should be investigated for similar usage.

Guido Sterk (Biobest) and Veerle Mommaerts (University Brussels): Effect of microbial control agents on the beneficial pollinator *Bombus terrestris*

Subsequent to determining the non-target effects of microbial control agents on bumble bees, bumble bees are used as vectors to disperse such biocontrol agents in green houses.

Philip Kessler (Andermatt Biocontrol): New baculovirus products tested in the field

Due to “resistance” problems to the codling moth granulovirus, Andermatt have tested another CpGV isolate against “resistant” codling moth populations and found it to work well. On pears, 88-93% efficacy is reported. Results later in the season are not as good.

Bruce Kirkpatrick (Valent BioSciences): Cost benefit of using Bt-based products in IPM programs

Tank-mix treatments with DiPel (*Bacillus thuringiensis* var *kurstaki*) or XenTari (*Bt* var *aizawai*) with “soft” chemicals were generally more effective than rotational treatments between the Bt products and “soft” chemistry.

Edith Ladurner (Intrachem Bio Italia): NATURALIS (*Beauveria bassiana*): an effective bioinsecticide for the control of a wide range of arthropod pests

NATURALIS was 88% effective against the cherry fruit fly, *Rhagoletis cerasi*, in open fields, if applied five times at 5-7 day intervals. Med fly, *Ceratitis capitata*, infestation of peaches in Italy was reduced by 75.3% by five applications 7 days apart. Other pests against which efficacy is claimed are: white flies, spider mites, thrips, wireworms, aphids, tingids, leafhoppers and weevils.

Massimo Benuzzi (Intrachem Bio Italia): AQ 10, biofungicide based on *Ampelomyces quisqualis* isolate M-10: a valuable tool for powdery mildew control

AQ 10 is apparently registered in South Africa. It is also registered in Europe on grapes, brinjals, tomatoes, peppers, cucumbers, strawberries and roses.

Stefan Kunz (Bio-Protect): BoniProtect forte (*Aureobasidium pullulans*) – a new biocontrol agent for use in stone fruit

BoniProtect is used primarily for post-harvest disease control in apples.

Mordechai Keren-Zur (AgroGreen): Effective Biopesticides addressing market needs

BioNem WP (*Bacillus firmus*), BioSafe WP and Chancellor are bionematicides, which are patented. Shemer (*Metschnikowia fruticola*) is a biological product specifically for organic production, for pre- and post-harvest disease control. It apparently controls *Penicillium* on citrus and *Botrytis* and *Aspergillus* on grapes and *Rhizopus* on sweet potatoes. Reportedly, BioNem works better on tomatoes than does Rugby. Suppression of nematodes is recorded even up to 140 days after application. It is also possible to apply BioNem in autumn i.e. before the rainy season. This is earlier than is possible with a chemical nematicide, which explains why better results can be achieved with BioNem (as was shown against the root knot nematode on peaches). The shelf-life of BioNem is 2 years +. It is applied to the soil in the same way as a chemical nematicide would be applied. It works by puncturing eggs and larvae and is effective against a range of nematode species.

Vittorio Veronelli (CBC Europe): Application and development of pheromones in modern IPM

In South Tyrol, Italy, mating disruption is employed in 27000 ha of the total 46000 ha under apple and grape cultivation. This has led to a dramatic reduction in the use of insecticides in this area.

**Formal contribution by Sean Moore to the programme:**

I presented a paper in the Microbial Control session, as well as two posters. The paper and the posters were aimed at soliciting interest in River Bioscience’s three products from companies elsewhere in the world. As follows is the abstract of the paper:

**Microbial and biorational control of moth pests and fruit flies on fruit crops**

S.D. Moore<sup>1,2</sup>, H. Le Roux<sup>2</sup>, A.B. Ware<sup>2</sup> and D. Kriek<sup>3</sup>

<sup>1</sup>River Bioscience, PO Box 20388, Humewood 6013, South Africa; seanmoore@cri.co.za

<sup>2</sup>Citrus Research International, PO Box 20285, Humewood 6013, South Africa

<sup>3</sup>Green Trading, Plaas Bokfontein 164, District Brits 0250, South Africa

The false codling moth (FCM), *Thaumatotibia leucotreta*, is endemic to sub-Saharan Africa but now also occurs in Israel. It is a serious pest of a number of crops, such as citrus, avocados, macadamias,

persimmons, grapes, peaches and plums. It is also known to attack maize, cotton, banana, coffee, olives and pomegranates, amongst several others. CRYPTOGRAN™ contains the *Cryptophlebia leucotreta* granulovirus (CrLeGV-SA), which is a highly virulent pathogen of FCM. In semi-commercial trials on navel oranges, one application of CRYPTOGRAN™ reduced FCM infestation of fruit by between 60 and 70% for as long as 17 weeks. However, multiple applications of the product can be made in one season, depending on pest levels. CRYPTOGRAN™ has been widely and successfully used on citrus, avocados, grapes and persimmons throughout southern Africa for three years now.

Bollworm, *Helicoverpa armigera*, ranks as one of the most important lepidopteran pest in Europe, Africa, Asia and Australasia. It attacks various crops including cotton, legumes, maize, sorghum, sunflower, tobacco, tomato, oats and citrus. HELICOVIR™ is a formulation of the *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV), which is used for control of bollworm. In semi-commercial trials on navel oranges, damage to fruit was reduced by up to 84% and yield increased by almost 100%. Trials have also been conducted on cotton, tomatoes and peppers.

The M3® bait station for control of fruit flies – particularly Mediterranean fruit fly, *Ceratitidis capitata* – has been used commercially on citrus, deciduous fruit crops and peppers in South Africa, Morocco, Spain and Peru for several years now, with good success.

Research and development is currently being conducted on a number of other potential products, such as entomopathogenic nematodes, entomopathogenic fungi and new *Bacillus thuringiensis* isolates, for use against pests on fruit crops.

Valuable discussions held:

#### Roma Gwynn

Roma Gwynn is an IPM and biopesticide consultant, working for Rationale Biopesticide Consultants, out of Scotland. Most of her consultancy work currently takes place in Kenya. I have invited Roma to be a keynote speaker in a symposium on biopesticides in the International Congress of Entomology in Durban in 2008. I spent some time informing her of what is expected of her. Roma was interested to investigate with me, issues surrounding the registration of biopesticides in South Africa. Roma provided me with names of a number of researchers who might be helpful in some of the microbial projects which I am involved in.

#### Willem Ravensberg

Willem Ravensberg works for Koppert in the Netherlands. We discussed their lactoperoxidase product, which is a natural anti-microbial agent for use against bacterial and fungal pathogens in agriculture. It is promoted particularly as a post-harvest treatment and might fill a niche for use on chem.-free fruit. Willem expressed that they planned to work through Du Roi IPM in South Africa, due to their existing collaboration with this company. Both Felix Hacker (GM of Du Roi IPM) and I told Willem that Du Roi IPM and RB have a good cooperation with one another and that we would be keen to cooperate with one another on the development and commercialisation of the product in South Africa.

#### Carlos Frescata

Carlos Frescata represents a Portuguese company called Biosani ([www.biosani.com](http://www.biosani.com)). Biosani is apparently the leading distributor of biological and IPM products in Portugal. Biosani are very interested in the M3 for control of medfly on citrus, apples, pears, peaches, grapes, persimmons and kiwis in Portugal. They claim that much pressure is being applied by the government to introduce safe and environmentally friendly means of controlling fruit fly and they would therefore receive good government support in importing the M3 and getting it registered. Biosani also operate in Brazil and could therefore also provide an opportunity of getting the M3 into that country.

#### Bernard Charlot

Bernard Charlot was President of Syngenta, France, and now works for the European company, Belchim ([www.belchim.com](http://www.belchim.com)), which has 3 shareholders, Dirk Putteman, Ishihara Sangyo K, and FMC. The company has representation all over western Europe and turns over more than €150 million per annum. Five years ago the company started a biological range, the first such product being Contans WG (*Coniothyrium minitans*). This is a product active against *Sclerotinia*, a root pathogen of various crops, particularly vegetables. It is claimed that this is the most successful biopesticide in Europe, after Bt. Bernard stated that Belchim wish to extend their "green" range and were interested to know more about our intentions with our products in Europe. I informed Bernard of the limited opportunities for Cryptogran and Helicovir in Europe, but stated that if Belchim had an interest in the M3s, they should express this to us.

#### Mordecai Keren-Zur

Dr Keren-Zur is the Chief Scientific Officer with an Israeli company called Agro Green. Agro Green specialise in biological control of nematodes. BioNem WP (*Bacillus firmus*), BioSafe WP and Chancellor are their bionematicides. Discussions were held with Dr Keren-Zur regarding the potential of their products for use against the citrus nematode in South Africa. Dr Keren-Zur stated that he did not believe that their products had ever been tested against *Tylenchulus semipenetrans*. It is also unclear whether it will be possible to obtain permission to import an exotic strain of the pathogen. Subsequently, Doron Yonay, their Marketing and Development Manager has been in touch with me to investigate possibilities in South Africa. Information has been forwarded to CRI's nematologists for their opinions.

#### Frank Volk

Frank Volk works Biofa Bio-Farming-Systems, a German company which distributes biological and biorational agricultural products. He expressed strong interest in the M3, for control of the cherry fruit fly. I will be following up with him in this regard.

#### Claudia Daniel

Claudia Daniel is a Swiss entomology student, employed by FiBL, an institute dedicated to research on organic production. Claudia is conducting her PhD on attractants for management of the cherry fruit fly and was very excited to include the M3 in her studies. I will be supplying her with samples for trials.

#### **Value of visit**

The visit to Lucerne and participation in the meeting was of significant value. The opportunity was taken to establish and build relationships with researchers and commercial people (in particular), with whom valuable collaboration can be fostered. The opportunity was also taken to identify products (and ideas for products), which have potential for RB to commercialise in South Africa. RB's paper and poster presentations solicited a lot of interest, particularly in the M3. A number of opportunities have been created to expand markets for the M3, particularly in Europe.

#### **Acknowledgements**

River Bioscience is thanked for funding and facilitating the trip. River Bioscience's Directors are thanked for authorising the trip. CRI is thanked for supporting the application.

#### **8.4 KEITH LESAR**

##### Report on attending an International Post-Harvest Congress in Bologna, Italy from 3-5 May 2007

I attended an International Post-Harvest Congress in Bologna, Italy from 3-5 May 2007. The Congress focused on "Novel approaches for the control of post-harvest diseases and disorders". The main goal of the Congress was to bring together researchers and specialists involved in post-harvest protection, packaging and storage of fresh fruit and vegetables.

The aim of the Congress was to develop an integrated disease management program to reduce post-harvest losses and improve food safety.

My reasons for attending Congress was firstly to present the results of my work I conducted on GRAS chemicals in combination with post-harvest fungicides for the control of post-harvest diseases and secondly to gain pertinent knowledge in current research being conducted in post-harvest diseases worldwide.

The Congress provided a forum for sharing of information and fostered constructive interaction between participants not only from the European countries but also from countries further afield (USA, China, Israel, South Africa etc.).

The state of the art, as well as the results of fundamental and applied research concerning the control of post-harvest diseases and disorders by biological agents, natural compounds, GRAS (generally regarded as safe) compounds and physical treatments were presented at this Congress.

This Congress offered a selected program of internationally renowned invited speakers and selected oral contributions, supplemented by posters, detailing the latest research findings.

I attended all the presentations, however a number of oral presentations were of interest and pertinent to the post-harvest research I am currently conducting at CRI on citrus cultivars. A number of these presentations are indicated as follows:

### **Biological Control**

Commercial applications and future prospects for the use of biological control after harvest

**Janisiewicz, W.J.**

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Biological control of postharvest diseases celebrated its first decade of commercial use last year. In the United States, BioSave™ is the only product that is currently being used. The original registration for postharvest application to pome and citrus fruits was expanded to include cherries, potatoes, and recently sweet potatoes. This product has been formulated as frozen pellets and as a wettable powder (WP). It can be applied to produce in various ways including drenching entire bins of fruit, a bath dip on a packing line, as a drip with brushes, in flooding boxes, or a mist spray during pileup in the case of potatoes. It is also compatible with waxes. Quality control and the continuous search for new applications have been the keys to the success of this product. Current expansion of postharvest biocontrol is focused on adaptation to small orchard operations, and on broadening its use by combining with GRAS substances and other non-fungicidal methods. Potential uses also include application to mechanically harvested fruit, and use as a precautionary measure against the growth of foodborne human pathogens on intact and fresh cut produce. Genetic manipulation of biocontrol agents can improve biocontrol and may provide valuable insights into the potential of biocontrol agents. In addition, yeasts or bacteria, able to colonize fruit wounds, can be converted to biocontrol agents by transforming with foreign gene(s) responsible for antifungal activity. Although this approach is still controversial, it reveals the potential of this biotechnology to address various limitations of biocontrol agents.

Enhancement of biocontrol against postharvest rots of apples and table grapes: recent advances

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Apples and table grapes are important world-wide produced and commercialized fruit. However, postharvest storability, transportation and shelf-life of these fruit can be seriously affected by postharvest decays due to wound-invading fungi such as *Botrytis cinerea* and *Penicillium expansum*. The latter is also responsible for the production of mycotoxin patulin. Control of these pathogens is mainly based on extensive use of synthetic fungicides. However, these compounds frequently involve risks as well as restrictions. Research on alternative methods suggests biocontrol agents may be valuable alternatives to chemicals. However, under commercial conditions, biocontrol agents often not only fail to effectively control decays, but their performance is also generally lower than synthetic fungicides. For this reason, research is currently aimed at optimizing the performance of selected biocontrol agents by providing rates of disease control comparable to or better than those of chemical methods. Over the years, several biocontrol agents have been selected and characterized for their high activity against fungal pathogens by our research team. More recently, integrated approaches focused on enhancing and stabilizing the antagonistic efficacy of biocontrol agents have been investigated. Here, we report results of recent experiments aimed at optimizing the activity of biocontrol yeast isolates against grey mould (*B. cinerea*) and blue mould (*P. expansum*) on apples and/or table grapes in order to reduce the application rate of synthetic fungicides. The combined application of antagonists with compatible compounds or alternative strategies has additive or synergistic effects on biocontrol and paves the way to the implementation of biocontrol agents for a more effective and safer control of postharvest fungal pathogens.

## Synergistic effects of combining microbial biocontrol agents with silicon against postharvest diseases of fruit

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Biological control utilizing antagonistic microorganisms as a promising alternative to synthetic fungicides has achieved considerable success. Many biological control agents have been used to effectively reduce various postharvest diseases of fruits. However, these antagonists of fungal pathogens, especially under semicommercial conditions, usually are not as effective as chemical fungicides because they are affected by many factors. To enhance the biocontrol efficacy of these antagonists, various strategies, such as combining exogenous chemical compounds with microbial biocontrol agents, have been proposed.

Our results indicated exogenous application of silicon (Si) could reduce disease development caused by *Penicillium expansum* and *Monilinia fructicola* in sweet cherry fruit at 20°C. The inhibition of fruit decay was correlated closely with Si concentrations. Silicon at concentrations of 1%, in combination with the biocontrol agent *Cryptococcus laurentii* at  $1 \times 10^7$  cells per ml, provided synergistic effects against both diseases. Silicon strongly inhibited spore germination and germ tube elongation of *P. expansum* and *M. fructicola* in vitro. Based on results with scanning electron microscopy, growth of both pathogens was significantly inhibited by Si in the wounds of sweet cherry fruit. Compared with the wounded water control, Si treatment induced a significant increase in the activities of phenylalanine ammonia-lyase, polyphenoloxidase, and peroxidase in sweet cherry fruit. In addition, combinations of *C. laurentii* and *R. glutinis* with 2% silicon (Si) was most effective in controlling the diseases caused by *A. alternata* and *P. expansum* on jujube fruit stored at 20°C. When fruits were stored at 0°C, combining *C. laurentii* and *R. glutinis* with Si was as effective against *P. expansum* as was Si or the yeasts applied alone and was more effective in controlling *A. alternata*.

Based on our studies, the improvement in biocontrol efficacy of antagonistic yeast when combined with Si may be associated with the increased population density of antagonistic yeast by Si, the direct fungitoxicity property of Si to the pathogens, and the elicitation of biochemical defense responses in fruit.

## Isolation of yeast strains for postharvest control of *Penicillium digitatum* (green mold) on citrus fruits in South Africa

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Synthetic fungicides have been widely used to control *Penicillium digitatum* Sacc., the causal agent of green mold of citrus. However, in many countries, fungicides are no longer effective due to resistance, or have been rejected due to residue concerns. Biological control has been proposed as an alternative. The objectives of the study were: (1) to isolate yeasts and *Bacillus* strains antagonistic to *P. digitatum*; (2) to investigate their efficacy in controlling infection by *P. digitatum* in vivo. A total of 60 yeasts and 92 *Bacillus* were isolated from various citrus fruits from different orchards in South Africa, and screened for antagonism to *P. digitatum*. Fifteen yeast and three *Bacillus* isolates significantly reduced fruit surface area of visible *P. digitatum* growth ( $\geq 50\%$ ), when applied 3h before inoculation with the pathogen. Two yeast isolates applied 48h prior to inoculation with *P. digitatum* significantly limited the average lesion diameter to zero on navels and lemons, and almost zero on Valencias (respective controls  $\geq 50\%$  infected area). None of the yeasts or *Bacillus* isolates produced a curative action against *P. digitatum* inoculated three hours before treatment, on lemon and Valencia fruits. The yeasts provided better protection than the *Bacillus* isolates. In a packhouse trial, conducted with natural inoculum on Valencia oranges, the best yeast isolate reduced *P. digitatum* levels to 2%, versus 12% after treatment with imazalil, the standard fungicide. An electron microscope study showed a delayed or inhibited germination of *P. digitatum* when the yeast was applied pre-inoculation.

In summary, the best yeast strains were superior to the *Bacillus* strains, and provided excellent control of *P. digitatum* if applied to citrus fruit prior to inoculation (artificial or natural) by the pathogen. Critically, the best yeast strain was superior to imazalil in a realistic packhouse trial.

## Effectiveness of peracetic acid in integrated control strategies of *Penicillium* decay in Tarocco orange fruit

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The most common and serious diseases which occur in Italy during storage and marketing of citrus fruit are incited by *Penicillium digitatum* Sacc. and *P. italicum* Weh. Current decay control procedures have always relied on the application of synthetic chemical fungicides. Growing consumer concern about pesticide residue along with the development of pathogen resistance to approved pesticides are prompting the development of alternative methods of maintaining produce quality in postharvest.

This experiment was performed in an attempt to enhance decay control by combined application of peracetic acid followed by a low rate of fludioxonil or by massive application of biocontrol yeast.

Effects of combined applications on *P. digitatum* incidence in inoculated fruits. "Tarocco" orange fruit holding 24 hrs. *P. digitatum* incipient infections were submitted to the following treatments: 1) 1 min. dipping in 800 ppm of peracetic acid; 2) 1 min. dipping in 800 ppm of peracetic acid followed by Fludioxonil at 500 ppm applied as spray; 3) Fludioxonil at 500 ppm applied as spray; 4) 1 min. dipping in water (control). Green mold incidence was assessed after 2 weeks of storage at 16°C.

Effects of combined applications on decay incidence in stored fruits. "Tarocco" orange fruit were submitted to the following treatments: 1) 1 min. dipping in 800 ppm of peracetic acid; 2) 1 min. dipping in 800 ppm of peracetic acid followed by massive spray application of the biocontrol yeast *Metschnikowia fructicola*; 3) biocontrol yeast; 4) 1 min. dipping in water (control). Decay incidence was assessed after 20 and 40 days storage at 11°C.

All combined treatments resulted in a significant reduction in decay when compared to the treatments applied alone and control.

## Physical Treatments

Thermal regimes to affect inactivation of the postharvest pathogens *Penicillium digitatum* and *Geotrichum citri-aurantii* for sanitation purposes in citrus packinghouses

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Tests were conducted to support development of a simple method of thermal disinfection that could be used to sanitize ethylene de-greening rooms, storage rooms, harvest bins, and other equipment used by citrus packinghouses, and to determine how long conidia of *P. digitatum* survive in groves. Viable conidia of *P. digitatum* from decayed fruit were exposed to ambient summer conditions in central California or to combinations of controlled temperature and relative humidity (RH). Days or hours to inactivate 99% of the conidia (LT<sub>99</sub>) were estimated by probit analysis. The LT<sub>99</sub> was 30 and 42 days, respectively, for conidia of *P. digitatum* under ambient conditions at two outdoor locations in the summer of 2005, which was an exceptionally warm period. In addition to conidia of *P. digitatum*, laboratory tests included arthrospores of *G. citri-aurantii* from colonies cultured on potato dextrose agar. Longevity at low relative humidity (RH) was longer than at high RH. Hours to inactivate 99% of the conidia (LT<sub>99</sub>) of nine isolates of *P. digitatum* 50°C with 75% or 95% RH were 24.9 and 4.9 hours, respectively (Figure 1). The LT<sub>99</sub> of arthrospores of *G. citri-aurantii* was briefer than that of conidia of *P. digitatum*. At 45°C and 75% or 95% RH, the LT<sub>99</sub> was about 4 and 2 hours, respectively, while at 50°C, none were viable after 1 hour at either humidity. Since there was little or no survival of either fungus after one day at a temperature of 50°C and 75% RH or higher, we conclude commercial sanitation could employ a similar regime. This temperature is too high for citrus fruit to tolerate without a significant loss in quality, but the treatment can be a useful, non-chemical approach for room and equipment sanitation, particularly to minimize the distribution of fungicide-resistant isolates of *P. digitatum*, which are found primarily only in packinghouses, to groves or other packinghouses.

## Prestorage hot water rinsing and brushing technology to reduce decay development in fresh produce

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In a time of increased awareness among consumers that many of the chemical treatments of fruit and vegetables to control insects, diseases, and physiological disorders are potentially harmful to humans, there is an urgent need to develop effective, non-damaging physical treatments for insect disinfection and disease control in fresh horticultural products.

Hot water rinsing and brushing (HWRB) technology was first introduced in 1996. The technology cleans and disinfects the fresh harvested produce at temperatures of 48 to 62°C, for about 15 to 25 s, depends upon the fresh produce. The machine contains parallel brushes, all of which are controlled by a single motor. All components in the machine, including the hot water tank, are made from stainless steel materials. The produce is pre-washed by non-recycled pressurized tap water (ambient temperature) for about 5-10 s while revolving on cylindrical brushes. A speed-adjustable conveyor belt is connected to the simultaneous cleaning and disinfecting stage, and controls the duration of exposure to hot water, which is heated with a thermostatically-controlled gas or electric heating element. Fruits or vegetables are rinsed with the pressurized hot water, from nozzles that point down either vertically or at predetermined angles onto the produce, which rolls on brushes made from medium-soft synthetic bristles. Before sorting and packing, fruits are dried by several forced-air fans.

The overall quality of fresh produce that were treated with HWRB was significantly better, as determined by reduced weight loss, greater firmness and a sharp reduction in decay incidence, than that of untreated. Employing HWRB resulted in a 3-4 log reduction of the total microbial colony forming units (CFU) of the epiphytic microorganism population, compared to untreated. Therefore, the epiphytic microorganism population counted on HWRB-treated fruit was below or at the threshold needed for decay development on the fruit. Scanning electron microscopy (SEM) showed that HWRB removed not only soil and dust, but also fungal spores from the fruit surface. Natural openings in the epidermis of treated fruit were partially or entirely sealed with rearranged natural wax components present on the cuticle. Sealing the cracks not only reduced water loss through the epidermis, thus maintaining fruit firmness after prolonged storage, but also reduced decay incidence by limiting sites of fungal penetration into the fruit.

HWRB was found to enhance resistance to several decay causing agents in citrus fruits and tomatoes. This is probably due to activation or induction of pathogenesis related (PR) proteins in the fruit peel, as shown by accumulation of glucanases and chitinases proteins. This short prestorage heat treatment was found to enhance fruit resistance against chilling injury in fresh harvested fruits and vegetables when stored below its optimal temperatures, probably due to accumulation of heat shock proteins (HSP) in HWRB-treated fruit.

Today, this technology is used commercially on sweet peppers, melons, mangoes, sweet corn, kumquats, tomatoes, organically-grown citrus fruits, cherry tomatoes and bunch tomatoes and sweet potatoes.

## GRAS Treatments

### Prospects for postharvest elicitors to suppress postharvest disease

**Terry, L.A.**

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Increasing loss of conventional fungicides due to pathogen resistance and general unacceptability in terms of public and environmental risk has favoured the introduction of integrated pest management (IPM) programmes. Induction of natural disease resistance in harvested horticultural crops using physical, biological and/or chemical elicitors has received increasing attention over recent years, it being considered a preferred strategy for disease management. This article reviews the enhancement of constitutive and inducible antifungal compounds and suppression of postharvest diseases through using elicitors. The effect of timing of pre- and/or postharvest elicitor treatment and environment on the degree of elicitation and the potential for inducing local acquired resistance, systemic acquired resistance and/or induced systemic resistance to reduce postharvest disease is discussed.

### Modified ethanol atmosphere to control decay of table grapes during storage

**Lurie, S., Pesis, E., Gadiyeva, O., Feygenberg, O., Ben-Arie, R., Kaplonov, T., Zutahy, Y., Lichter, A.**

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The ability of three methods of applying ethanol to prevent storage decay was tested on two cultivars of table grapes, 'Superior' and 'Thompson Seedless'. Ethanol was applied by: 1) dipping grapes in 50% ethanol for 10 sec followed by air drying before packaging; 2) placing a container with a wick and 4 or 8 ml ethanol per kg grapes inside the package; 3) applying 4 or 8 ml ethanol per kg grapes to paper and placing this paper above the grapes in the package. The grapes were stored for 6 or 8 weeks at 0°C and assessed after an additional 3 days at 20°C. All methods of application controlled decay as well as or better than a SO<sub>2</sub>-releasing pad. The ethanol impregnated paper caused high levels of berry browning, perhaps because of high levels of acetaldehyde inside the package. However, the taste of the berries was not impaired by any of the ethanol applications. The taste of 'Thompson Seedless' grapes stored for 8 weeks in modified atmosphere storage was affected by CO<sub>2</sub> levels above 7%. The methods of applying ethanol used here show promise as alternatives to SO<sub>2</sub> to prevent decay of grapes during storage while maintaining fruit quality.

## Advances in the use of chitosan to control postharvest decay of table grapes

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Gray mold, caused by *Botrytis cinerea*, and blue mold, induced by *Penicillium expansum*, are the most important postharvest diseases of table grapes. Gray mold in particular is responsible for significant losses of table grapes both before and after harvest, and it is a major threat to long-distance transport and storage. Control of the disease is especially important in storage because it develops at cold temperatures (-0.5°C) and spreads quickly among berries (phenomenon called nesting). In order to meet a growing consumer demand for food without chemical preservatives, efforts have been focused on the reduction of fungicide application by discovering new natural antimicrobials, and the use of antimicrobial films. Chitosan, an N-acetylated derivative of the polysaccharide chitin, is a natural biopolymer with a disease-suppressive effect resulting from both physical and biochemical mechanisms. The physical properties of the biopolymer allow it to produce a film on the surface of treated fruits. The biochemical activity of chitosan is dual: it inhibits the growth of decay causing fungi and induces defence responses in several plant parts, included table grape berries. The goal of the research was to explore ways to further increase chitosan effectiveness for control of gray and blue mold of table grapes and to better understand mechanisms of action involved in the disease reduction. The combination of chitosan with ethanol, which is another common food additive with antifungal properties, was also evaluated. Chitosan treatment resulted effective in the reduction of gray and blue molds of table grapes in storage, either with preharvest and postharvest applications. The integrated use of reduced doses of chitosan and ethanol improved the control of gray mold of table grapes compared to their application alone, and the effect was at least additive and at times synergistic.

## Postharvest treatment with salicylic acid effectively controls pear fruit diseases and disorders during cold storage

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Salicylic Acid(SA) as a safe and natural compound has shown high potential in plant protection against diseases and different stresses. To understand the effects of postharvest SA treatment on decay extension and storage life of a an Iranian local pear cultivar (*Pyrus Communis* Cv. Gishlik) fruit, the harvested fruit were dipped in hot solution of 1mmol L<sup>-1</sup> SA and stored for 5 months at 1°C ± .5°C temperature and 85-90% relative humidity. Quality assessments during storage period and at the end of 5 month cold storage showed that hot solution of SA effectively controls fruit decay organisms and reduces fruit storage disorders. Fruit dipped only in hot water of 45°C without SA treatment were severely attacked by decay organisms and suffered from storage disorders especially of storage scald and softening. We found that SA treatment in combination with hot water dip is capable of retaining fruit quality and retarding storage diseases and disorders in pear fruit without leaving any chemical residue and with no adverse effects on fruit.

**Keywords:** Salicylic acid, Decay index, Storage life, Blue mold, Marketability.

## Posters

A number of interesting and relevant posters were also presented at the Congress.

## Acknowledgements

The author would like to take this opportunity to thank the CRI Board of Directors and CRI Management for approving my attending this Congress and for their financial support.

### 8.5 PAUL FOURIE

#### Attendance of the 16<sup>th</sup> biennial congress of the Australasian Plant Pathology Society, Adelaide, Australia (24-27 September 2007)

Prior to my appointment at CRI, this trip was specified in a THRIP grant application complementing my grapevine research projects at University of Stellenbosch. An important research collaborator on the grapevine spray application projects, Geoff Furness, was situated near Adelaide in South Australia, and since my research focus in citrus is also on spray application, it was motivated that I attend this congress to initiate collaboration with Furness on spray application in citrus. As part of this objective, I arranged a research visit for my PhD student, Jan-Cor Brink, with Furness prior to the congress. At that stage, Brink had indicated his availability to continue postdoctoral spray application research on citrus as part of my CRI funded projects.

A second important motivation was to establish research networks with the Australasian plant pathologists; thirdly, I presented an overview talk on our grapevine spray application research, and also used the opportunity to promote my new research focus on citrus; and finally to peruse relevant oral and poster presentations at the congress.

- **Collaboration with Geoff Furness**

Jan-Cor spent the week prior to the congress in Furness's labs, getting first-hand experience on citrus orchard spraying techniques and Furness's spray assessment protocol. Jan-Cor was introduced to the Labino black light, which we have subsequently acquired to use with the SARDI Yellow Fluorescent Pigment that Furness developed. Moreover, Furness has done a lot of development research on the Quantum Mist multi-fan sprayer in citrus, and has demonstrated equal coverage at reduced water volumes and increased tractor speeds compared with standard oscillating boom sprayers.

In a meeting at the Waite Campus in Adelaide, I presented our research to Furness and we brain-stormed our impending spray application research on citrus. As Jan-Cor and I are currently employed in CRI-projects, this collaboration proved to be fruitful.

- **Establish citrus research networks in Australasia**

This aspect was deemed important as my longstanding previous engagements with this society (since 1995) were focussed on grapevines. Although I found representation in citrus pathology relatively sparse, important links were made with researchers and technicians directly involved with citrus pathology in Australia: Drs Andrew Miles and Nerida Donovan both based in Queensland in the newly established Tree Pathology Research group of Prof Andre Drenth. Miles presented a paper on the eradication of citrus canker from Queensland and was recently appointed by Australia Citrus in a technical and research capacity. Donovan is responsible for citrus improvement scheme and associated diagnostic processes. Drenth has a proven research expertise on *Phytophthora* diseases and given his favourable disposition toward South Africa, might be a valuable research collaborator.

Links were refreshed with Prof David Guest from University of Sydney. David Guest has a proven research record on *Phytophthora* diseases in the tropics.

From the New Zealand researchers, I engaged with Mike Manning and Bob Fullerton about citrus research conducted at HortResearch in Auckland. Fullerton was co-author on a recent paper on the effect of preharvest sanitiser sprays on postharvest decay.

- **Present talk on spray application research on grapevine**

This talk, of which the abstract is attached as Appendix A, was presented in a concurrent session. A good number of people attended the talk, and a lively discussion followed the presentation. Following the presentation, I was approached by Gerry MacManus, a Zimbabwean expatriate working on wheat in Australia. Gerry gave me chapters from his Masters study, which studied the microscopic surface effects of adjuvants on onion leaf surfaces.

- **Perusal of other oral and poster presentations**

The complete programme and extended abstracts of all presentations are available in hard-copy format from Paul Fourie. The programme was circulated to CRI pathologists and requested abstracts were forwarded.

- **Oral presentations**

**“Effects of metalaxyl and phosphonate on Phytophthora root rot of Wollemi pine” by E.C.Y. Liew, C. A. Offord, A. Pinaria, C. Pavich and B. A. Summerell** – This talk was presented by Dr Rose Daniel of University of Sydney. An interesting observation from this study, was the variable efficacy of foliar phosphonate sprays to control Phytophthora root rot of potted Wollemi pine. Later discussions of this variability with Daniel and David Guest, a pioneer in the use of phosphonates for Phytophthora control, led to the hypothesis that, in order to be most effective, these sprays should be timed at a period when the source-to-sink transport pathway in the phloem tissue is from leaves to roots (i.e. synchronised with root flush). On the other hand, the phosphonate might be bound by clay particles when applied in soil. These aspects should be considered in recommendation of phosphonates for root rot and brown rot control.

**“The impact of organic amendments and mulch on root-knot nematode and Pythium root rot of capsicum” by G.R. Stirling and L M. Eden** – This keynote address by one of the APPS’s fellows, Graham Stirling, highlighted successes in management of nematodes and root rot in arable crops with organic amendments. Recently, APPS published a review by Stirling in a special issue of the journal Australasian Plant Pathology. This issue was forwarded to MC Pretorius, Coordinator for the Soilborne Diseases project. Stirling also presented a paper on “Biological suppression of *Pratylenchus* in northern grain-growing soils”. Stirling measured free-living nematodes as a measure of soil health. Tillage reduced plant parasitic nematodes, but only in short term, as he found that disturbance of soil impacted on the soil suppressiveness to parasitic nematodes. Fertilisation with a high carbon : low nitrogen fertilizer reduced nematodes, and in this case observed an unknown predatory fungus attacking the nematodes; the fungus was probably sustained by the C-inputs. A trash blanket improved labile C-content in soils, cooled soil temperature and reduced moisture loss. Soil solarisation was unsuccessful as it was unable to pasteurise soils to an adequate depth.

**“The use of compost to suppress soil-borne disease in horticultural crops of the Northern Adelaide plains” by M.R. Ayres, R.A. Ballard, T.J. Wicks, S.J. Barnett and K.M. Ophel-Keller** – Compost showed some effect against root diseases of tomatoes, capsicum and cucumber, but results were variable and were dependent on compost type and age.

**“The effect of silicon and phosphorus amendment on severity of *Fusarium* wilt of cotton” by L.J. Smith and D.F. Carrick** – Magnesium silicate, potassium silicate and acidified calcium silicate reduced infection significantly at low disease pressure situations, but were not effective at high disease pressure. High phosphorous levels increases wilt.

**“Management of mango postharvest diseases using plant activators” by C.N. Akem, G. MacManus, Z. Baron, and P. Boccalatte** – Plant activators stimulate natural host defence mechanisms, and 4 applications of Kasil (liquid silicone formulation), Mangocote (kaolin-based product) and Bion (acibenzolar-S-methyl) from bloom to preharvest decreased postharvest stem-end-rots and anthracnose of mango. Bion was most effective with significant reductions, similar to those obtained by the fungicide standard (mancozeb + Amistar).

**“Sudden Oak Death/*Phytophthora ramorum* in the US: A management challenge” by Susan Frankel** – This devastating disease, with striking similarities to *Phytophthora citrophthora* trunk and branch cankers of Clementines, has wreaked havoc in the USA by killing off thousands of oak trees in natural forests in California and other states. The USA’s management plan was discussed, and mostly involved eradication of infected plant material in nurseries and limited movement of host material.

**“Managing plant diseases offshore” by Jeanne van Dersal** – The presenter is employed by USDA APHIS and presented the USA’s strategy toward ensuring biosecurity. This particular initiative involves collaboration with governments that export commodities to the USA. Offshore Pest Information System (OPIS) is a real-time web-based tool for information about changing pest and pathway conditions, which can be used to assist the USA in research assistance, suppression of outbreaks, strategy development and maintenance of on-going trade.

**“The contribution of epidemiological research to plant disease management” by Dani Shtienberg** – Dani presented an inspiring talk on the relevance of epidemiological research in disease management,

highlighting the integral role that knowledge of the pathosystem is vitally important for effective control of the disease. Several case studies were presented from his own research in grapevines and greenhouse crops. He concluded his presentation with the lament that research, study and training in epidemiology is being neglected, and that this aspect needs urgent attention to ensure balanced international research portfolios.

**“Evaluation of potential citrus canker inoculum reservoirs in Emerald, Queensland”** by C.F. Gambley, M. Benham, A.K. Miles, I. Smith and P.J.I. Whittle – This presentation was one of only a few focused on citrus. An incursion of the citrus canker pathogen *Xanthomonas smithii* subsp. *citri* pathotype A (an Asiatic strain) was detected on a single property in Emerald, Queensland, in July 2004. A Pest Quarantine Area (PQA) and National Citrus Canker Eradication Program were established and in the following 10 months an additional two infested properties were identified. Within 2 months of the first report, 156 000 trees (375 ha) were destroyed in the first PQA. A further 207 000 trees (430 ha) and 6 393 trees (12 ha) were destroyed in the subsequently identified PQAs by mid-late August 2005. In July 2007, after at least 18 months of a host-free period (the canker bacterium survives <1 month in soil, and 2-3 months in debris), replanting of citrus was started. In this particular study, fruit, stem and root samples were collected from previously infested sites and subjected to PCR detection of the canker bacterium. No evidence of the presence of the pathogen was found.

**“Morphological, physiological & biological variation in *Phytophthora palmivora* on cocoa in Papua New Guinea”** by J. Saul Maora, E.C.Y. Liew and D.I. Guest – This talk was presented by Jose Saul Maora, a PhD student of David Guest, and was awarded as the best student presentation at the congress. A very innovative technique for photo-micrography of morphological structures was discussed in her presentation: the organism is grown on agar medium, whereafter a section of agar + mycelium is transferred to a microscope slide; a cover slip is placed over the agar section, and the slide moderately heated on a warm plate to melt the agar; once melted, the slide is microscopically studied. This study identified that *P. palmivora* was the sole *Phytophthora* species causing disease in Papua New Guinea, which is invaluable information needed to develop management strategies, such as resistance breeding.

**“Inoculum and climatic factors associated with epidemics of grape botrytis in New Zealand”** by R.M. Beresford – In this study, inoculum and weather factors were identified and modelled against Botrytis incidence. Both factor types were shown to be important in predicting disease severity and will be used in a decision support framework including additional inoculum-based measurements. This presentation was backed by a poster presentation **“Predicting the risk of botrytis bunch rot in cool climate viticulture”** by K.J. Evans, R.M. Beresford and J. Edwards.

**“Irrigating with reclaimed water: Impact on soil microbes under grapevines”** by B. Rawnsley – Australia was experiencing the so-called “big dry”, and this talk was particularly relevant since it investigated the use of reclaimed water (i.e. treated black water) for vineyard irrigation. No adverse effects were observed on soil microbes or grape plants. The study concluded that this is a safe and viable option for irrigation of grapevines.

- **Poster presentations**

**“A *Tylenchulus semipenetrans* population with elevated virulence to a resistant citrus rootstock”** by G.E. Walker – Carizzo citrange (CC) and Sweet orange (SO) rootstocks were compared in pot trials with two distinct soil types obtained from beneath declining citrus trees. CC was more resistant against nematodes and *Phytophthora citrophthora*, but varying effects were observed for nematode virulence, which might be attributed to biotype or population differences. CC was, however, not resistant to other nematodes present in the soils, nor to *Pythium ultimum*.

**“Comparison of high throughput PCR and tissue blot immunoassay for large-scale virus diagnostics”** by M.E. Spackman, M. Aftab, M.H. Loh, B.C. Rodoni, A.J. Freeman, J. Van Leur – Both techniques compared favourably, and the authors recommended using PCR detection on composite samples and tissue blot assays for follow-up testing of PCR-positive samples.

**“The potential use of hyperspectral imaging in surveillance for emergency plant pathogens”** by A.E. Mackie, S.D. Hetherington and A. Robles-Kelly – Hyperspectral imagery could distinguish between healthy and inoculated plants, and trials are continuing to determine its robustness and potential use.

**“Fungal biosecurity risks of contaminated footwear carried by international passengers arriving at New Zealand airports”** by F.A. Shah and M.R. McNeill – An alarming diversity of fungal genera, although common, could be isolated from soil obtained from footwear of international travellers. Recommendations were made to New Zealand biosecurity personnel to decontaminate all potential sources of plant pathogens.

**“Potential distribution of *Neonectria galligena* (European canker) in Australia using the models climate and Climex<sup>®</sup>”** by **J. Edwards, O.N. Villalta and R. Powney** – A climate matching model CLIMATE, and the simulation model CLIMEX were compared in the prediction of potential distribution of *Neonectria galligena* in Australia. Potential distribution predicted by CLIMEX was more extensive than CLIMATE.

**“Huanglongbing (citrus greening): a major threat to citrus in Pakistan”** by **S. Chohan, R. Qamar, I. Sadiq, P. Holford and A. Beattie** – Citrus greening was not known to occur in Pakistan prior to this study. Using PCR technology, the authors confirmed the presence of “*Ca. Liberibacter asiaticus*” in symptomatic plants as well as in psyllids.

**“Predicting disease risk and storage potential of table grapes (cv. Thompson seedless) using an integrated systems approach”** by **O.N. Villalta, J. Lopresti, J. Edwards, R. Holmes, B. Tomkins R. Emmett, M. Welsh, S. Salib, G. Hale and D. Partington** – The authors used the preharvest criterion disease pressure in fruit batches to predict postharvest rot. This and other quality criteria are used to develop an integrated model for prediction of storage potential of fruit batches.

### **Conclusions and recommendations**

- Continue collaboration with SARDI research on spray application.
- Acquire Labino UV lamp, as is successfully used by Furness, for improvement of our assessment protocol.
- Maintain existing research networks with Australasian pathologists, such as David Guest, Andre Drenth, Geoff Furness, Rob Beresford and Bob Fullerton, who can make valuable contributions to citrus pathology in South Africa.
- Consider collaboration with Rob Beresford (HortResearch, New Zealand) on disease prediction modelling.
- Foster new relations established with Australian citrus pathologists, Nerida Donovan and Andrew Miles.
- Learn from the Australians exemplary quick reaction needed for successful eradication of exotic pathogens.
- Potential research concepts for consideration:
  - Evaluation of plant activators for postharvest control of latent pathogens.
  - Identify, validate and model preharvest risk indicators of postharvest decay.
  - Use of hyperspectral imagery for disease surveys (such as citrus greening).
  - Develop high-throughput diagnostic protocols for use in disease surveys, research and Citrus Improvement Scheme.

## Appendix A

### OPTIMISATION OF FUNGICIDE SPRAY APPLICATION IN SOUTH AFRICAN TABLE AND WINE GRAPE VINEYARDS

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#### Introduction

Practical management of foliar and fruit diseases of grapevine relies almost exclusively on well-timed fungicide applications. Label recommendations for these fungicides mostly specify dilute application with high water volumes (500 to 1500 L/ha) depending on growth stage and/or disease pressure. These sprays are mostly applied with air blast or air shear applicators. Despite adherence to spray schedules and label recommendations, producers invariably suffer crop losses due to grapevine downy mildew, powdery mildew and grey mould when environmental conditions are conducive to disease development. Control failure is often attributed to improper spray techniques and resultant poor spray coverage of susceptible plant tissue.

In 2003, a research programme was initiated to focus on optimisation of fungicide spray application in grapevine vineyards. The aims of this programme were firstly to develop a technique to assess quantitative and qualitative spray deposition on small 3-dimensional target sites in grape bunches and leaves; secondly, to determine benchmark values for biologically effective spray deposits; thirdly, to determine spray deposition resulting from industry best-practice application; and finally, to improve spray deposition through optimal use of spray applicators or surfactants.

#### Methodology and results

**Spray assessment protocol.** In order to visualise spray deposition on target surfaces, Yellow Fluorescent Pigment® (400 g/L, EC; South Australian Research and Development Institute) at 0.2 L/100 L are added to the spray mixture. Sprayed plant material is illuminated under black light and visualised using a Nikon SMZ800 stereo microscope at 10-30x magnification. Digital photos are taken with a Nikon DMX 1200 camera and image analyses performed with Image-Pro Plus software.

Quantitative analysis involved removal of green channels from the image, followed by quantification of foreground elements (deposited pigment) of the binarised image (1). For qualitative analysis, a combined Euclidian distance map and skeleton is created on the binarised image, with absolute white indicating the furthest distance from a particular foreground element. Subsequent analysis of grey-scale values indicates spray deposition quality.

**Benchmark values for biologically effective spray deposits.** Bunches and leaves of table (Waltham Cross) and wine (Chenin blanc) grapes were sampled and precision-sprayed at various stages with different volumes of a mixture of fenhexamid (Teldor® 500 SC, Bayer) at the recommended dose (75 mL/100 L) and the fluorescent pigment. One day after spray application, leaves or bunches were dusted with dry airborne conidia of *Botrytis cinerea* in a settling tower and incubated for 24 h at high relative humidity (98%). The amount of *B. cinerea* infections was determined by means of isolations onto paraquat medium (2). Quantitative spray deposition was determined by means of the described protocol. For each cultivar, part and stage combination, biological efficacy curves were plotted for coverage vs. percentage *B. cinerea* incidence. The fluorescent pigment coverage needed for 75% control of *B. cinerea* infection (benchmark values) was subsequently calculated.

**Spray deposition from industry best-practice application.** The fungicide + pigment mixture was applied with commercial air blast and air shear spray applicators in table (Waltham Cross) and wine (Chenin blanc) grape vineyards during set, pea size, bunch closure and preharvest according to best practise and calibration. Quantitative spray cover assessment was done of front and back sides of bunches (pedicels) and leaves in various positions in the canopy, and subsequently compared with the respective benchmark values. Coverage values were generally lower than the respective benchmarks values.

**Improved spray deposition through optimal use of spray applicators or surfactants.** In order to study means of improving spray cover with existing application technology, a series of spray trials were conducted at best-practice recommendations, but with a range in spray volumes of which the spray mixture concentrates were amended accordingly. In terms of quantitative and qualitative deposition, the air shear sprayer performed markedly better at low volume (250-500 L/ha), whereas the air blast sprayer performed more consistently over a range of volumes; most likely due to correct nozzle selection.

Selected commercial surfactants were also evaluated in laboratory and vineyard trials to determine whether spray deposition quality and biological efficacy could be improved. Results clearly showed that improved quality of spray deposition led to increased control of *B. cinerea* on grape leaves. However, a

definite interaction between surfactant dosage and spray volume was observed and confirmed in subsequent vineyard trials.

### **Discussion**

A protocol was developed to accurately assess quantitative and qualitative spray deposition on grape bunches and leaves. Subsequent spray trials indicated that current best-practice spray application in vineyards resulted in sub-optimal deposition, which would most likely lead to control failure under high disease pressure conditions.

Spray deposition was remarkably improved through optimal use of spray applicators (with regard to volume delivery) or addition of correct dosages of surfactant to the spray mixture.

### **References**

1. Brink, J.C. *et al.* (2004). Development of a protocol to quantify spray deposits on grape bunches. *Proceedings of the 7th International Symposium on Adjuvants for Agrochemicals ISAA 2004*: 230-236.
2. Brink, J.C. *et al.* (2006). Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. *South African Journal of Enology and Viticulture* **27**: 51-56.

9 **TECHNOLOGY TRANSFER** (H.F. le Roux, A. Mbedzi [Nelspruit] en J.J. Bester [Port Elizabeth])

#### 9.1 **NAVORSINGSPRIORITEITE / RESEARCH PRIORITIES - 2008**

Die navorsingsprioriteite van die verskillende sitrusproduserende streke in Suider-Afrika vir 2008 is gedurende Julie en Augustus 2007 bepaal deur Hennie le Roux en Hannes Bester. Drie afsonderlike partye betrokke in die sitrusbedryf is geraadpleeg om die navorsingsbehoefes van die bedryf te bepaal. Hierdie drie partye is:

- A. Produsente deur 'n netwerk van 29 Tegnologie Oordraggroepe (Sitrusstudiegroepe)
- B. Sitrussuitvoerdersforum d.m.v. die Uitvoerders Tegnieese Paneel
- C. Pakhuisstudiegroepe, 'n onderafdeling van die CCCF (Sitrus Kouekettingforum)

#### 1. **TEGNOLOGIE OORDRAGINGS-GROEPE**

Die navorsingsprioriteite is in wese dieselfde as in die afgelope drie jaar. Sitrus swartvlek en VKM bly steeds bo aan die lys van navorsingsprioriteite. In die geval van swartvlek word daar met verwagting uitgesien na die dag dat hierdie patoëen se status in die EU verander sal word van fitosanitêr na kosmeties. Tot tyd en wyl hierdie patoëen sy fitosanitêre status in Europa verloor sal dit die hoogste prioriteit bly vir gebiede soos Letsitele en die Onderberg. Skildefekte bly steeds die belangrikste prioriteit binne die Oesopbrengs en Vrugkwaliteitsprogram met Kraakskil en Peteca wat in meer as een gebied steeds die hoogste navorsingsprioriteit vir die gebied is.

Soos in die verlede toon Tabele 1-3 die prioriteite van die verskillende areas op 'n skaal van 0-3. Aspekte waaraan 'n 0-waarde toegeken is benodig nie verdere navorsing nie terwyl 'n 3 beteken dat navorsing op daardie betrokke gebied die hoogste prioriteit moet ontvang. Elke navorsingsaspek se prioriteit word vir die verskillende streke aangetoon. Op versoek van sitrusprodusente is 'n addisionele kolom by die Oesopbrengs en Vrugkwaliteitsprogram gevoeg n.l. Plantvoeding. Die hoogste navorsingsprioriteit vir elke area word ook aangetoon. Navorsers word daaraan herhinner dat hoewel die geweegde belangrikheid ook gegee word dit misleidend kan wees aangesien daar sekere prioriteite is soos *Pseudocercospora angolensis* wat, in die geheel gesien, 'n lae gewig mag hê, maar wat vir die sitrusprodusente in die noorde van Zimbabwe een van die hoogste prioriteite is.

In die geval van die Siektebestuurprogram val die klem steeds op Swartvlek, Na-oes bederf, Vergroening en Tristeza navorsing. Die klem op *Alternaria* bruinvlek het oor die afgelope jaar gedaal in die Noordelike streke maar die behoefte is steeds daar in die Suid. 'n Effektiewe beheerprogram vir *Phytophthora citrophthora* vir die Suid-Kaap is nog nie gefinaliseer nie en moet dringend aandag kry. Bruinvrot het die afgelope seisoen tot heelwat verliese gelei omdat produsente bang was vir fosfaat fitotoksiteit en het gevolglik nie voorkomende beheer toegepas nie.

In die Geïntegreerde Plaagbestuur In die Geïntegreerde Plaagbestuur Program geniet valskoddingmot en vrugtevlug (veral Natalvlug) die hoogste prioriteit. Die bedryf het groot verwagtinge van Xsit en alhoewel Cryptogran in die meeste gebiede goed gewerk het, wil dit voorkom of dit in sekere areas vroeër gespuit moet word. Verdere voorligting om die produk effektief in hierdie areas te gebruik is nodig. Effektiewe vrugtevlugbeheer bly 'n belangrike besprekingspunt by die verskillende sitrusstudiegroepe. Die feit dat daar 'n nuwe navorser, dr Aruna Manrakhan deur CRI aangestel is beteken dat die intensiteit van vrugtevlugnavorsing in die toekoms sal toeneem. Grysmyt is deur twee van die sitrusproduserende gebiede as 'n hoë prioriteit aangedui. Die sogenaamde graan "Chinch bug" bly vir die Wes Kaap 'n bedreiging.

Die Oesopbrengs en Vruggehalte-program se klem val veral op skilprobleme soos Kraakskil, Peteca, Skilafbraak, Gepokte skil en Vruggrootte manipulerings. 'n Oplossing vir kruisbestuiving van laat mandaryne is nog nie in plek nie en moet gevind word. Die behoefte vir die aanstelling vir 'n voedingskundige om die verskillende bemestingsmiddels en wortelgroeistimulante onafhanklik te kan evalueer is krities nodig. Wat Kultivars betref is daar steeds 'n behoefte vir groot laat Valencias in die Noorde sowel as vroeë pomelos vir Japan. Die produsente is erg teleurgesteld omdat die bedanking van die Bestuurder: Kultivarontwikkeling die program tydelik ontwig het. Hulle is egter opgewonde oor die feit dat Andrew Lee intussen aangestel is om hierdie pos te vul aangesien hy oor jare se kultivarondervinding beskik Die behoefte is steeds daar vir onafhanklike evaluering van kultivars sowel as die verkryging van nuwe kultivars.

Die aspekte waaraan daar steeds aandag gegee moet word ten opsigte van elk van die verskillende navorsingsprogramme is in die volgende:

## PROGRAM: SIEKTEBESTUUR

### Projek: Swartvlek

Kennis word geneem van die EU se houding ten opsigte van die Suid Afrikaanse argumente rakende die risiko wat Suid-Afrikaanse sitrus inhou vir sitrusprodusente binne die EU. Daar is ook kennis geneem van Prof Vaughan Hattingh en sy span Swartvlekspesialiste se teenargumente en dat die saak op die hoogste vlak aandag ontvang. Geaffekteerde produsente sou graag wou sien dat hierdie saak ssm op die spits gedryf en afgehandel word.

In die geval van Weipe, Tshipise en die Benede Oranje-rivier is die hoogste prioriteit steeds toegang tot die VSA. Die produsente is op hoogte gebring van beide die CRI en die Departement van Landbou se pogings om dit goedgekeur te kry. Hulle het waardering vir die vordering wat gemaak is maar versoek dat hierdie aspek die hoogste prioriteit sal geniet totdat dit afgehandel is. Daar word aanbeveel dat die huidige Swartvlekprojek sal voortgaan met al die verskillende fasette wat nog nie afgehandel is nie.

#### 1. *Piknidiospore as bron van inokulum op suurlemoene:*

Piknidiospore kan in kultivars met meervoudige vrugsette soos suurlemoene as bron van inokulum optree. Wat is die klimaatsvereistes wat nodig is vir infeksie om plaas te vind? Op watter stadium is die blare vatbaar vir piknidiospore infeksie? Van hierdie vrae is reeds beantwoord maar die werk moet nog gepubliseer word. Dit is belangrik dat hierdie werk voltooi sal word en gepubliseer sal word.

#### 2. *Epidemiologie:*

- Evaluering van verskillende biologiese-beheeragente om die dooie blare op die grond vinniger af te breek en sodoende askospoorvrystelling te voorkom. Publikering van vordering is belangrik.
- Evaluering van die stofsuiers wat in die Oos-Kaap ontwikkel sou word om dooie blare te verwyder. Alternatiewelik: Die makadamiabedryf in Levubu gebruik 'n stofsuiers om blare te verwyder voordat hulle die neute optel. Ondersoek die moontlikheid om hierdie stofsuiers in sitrus te gebruik.
- Evaluering van grondbewerking (disc) om blare wat in die rye in gevee is effektief te bedek.

#### 3. *Na-oesbeheer:*

- 'n Studie om vas te stel wat gedoen kan word om die ontwikkeling van latente infeksies na verpakking in transito te onderdruk. Dit sluit in 'n audit van wat huidige met vrugte gebeur vanaf die pakhuis onderweg na die hawe, by die hawens voordat dit verkoel word, tydens die laaiproses en tydens verskeping.
- Effek van warmwaterbehandelings op simptomeontwikkeling.
- Chemiese / biologiese onderdrukking van simptomeontwikkeling.
- Metodes om simptomeontwikkeling in boorde te stimuleer sodat latente infeksies kan wys voordat vrugte verpak word.
- Metode om vrugte op die paklyn te kan "scan" vir latente infeksies.

#### 4. *Kwekerie:*

- Protokol vir akkreditering van kwekerie vir lewering van Swartvlek-vry kwekerie-boompies aan areas wat gesertifiseer is as areas waarin daar 'n lae voorkoms van swartvlek is (Messina & Soutpansberg magistraatsdistrikte as 'n model).
- Swartvlekbeheerprogramme wat in die kwekerie gebruik kan word wat nie 'n gevaar inhou vir die ontwikkeling van weerstand teen swamdoders wat in kommersiële boorde gebruik word nie.

#### 5. *Chemiese beheer:*

- Evaluering van nuwe chemiese middels asook beheerstrategie wat meer bekostigbaar is.
- Herimplimentering van karbendazim in swartvlekbeheerprogram.
- Herevaluering van die terugwerkende aksie van die strobiliriene om vas te stel of die terugwerkende aksie langer as 14 dae kan wees.
- Registrasie van strobiliriene op suurlemoene.
- Ondersoek om die verspreiding van Benlate weerstandbiedendheid te bepaal.

#### 6. *Weerstandbiedendheid:*

- 'n Toets om *Guignardia* weerstandbiedendheid teen die strobiliriene te bepaal soos wat tans vir die benzimidazole gedoen word.

#### 7. *Nuwe PCRs en 'n Diagnostiese "kit"*

- Verfyning van die PCRs om die teenwoordigheid van CBS op simptomelose blare en enthout te bepaal.
- Die ontwikkeling van 'n toetsapparaat wat deur produsente / pakhuis / PPECB gebruik kan word om te onderskei tussen *G. citricarpa* en *G. mangiferae*.

8. *Selektiewe medium:*
  - 'n Selektiewe medium om *Guignardia* op te kweek.
9. *Genetiese weerstand:*
  - Onderzoek die moontlikhede om geneties gemodifiseerde weerstand in sitrusplantmateriaal te bewerkstellig.
10. *Voorspellingsmodel:*
  - Voorspellingsmodel wat kan voorspel wanneer die klimatologiese toestande gunstig sal wees vir spoorontkieming en infeksie vir inokulumbestuur (Katrivier)
  - Automatisering van spoortellings.
11. Oorsigsartikel in Plant Disease om die afgelope 30 jaar se swartvleknavoring saam te vat voordat die Brasilië dit doen.

Van die navorsingsversoeke wat deur die produsente gestel is ten opsigte van swartvlek is inderdaad reeds nagevors en sal dmv Voorligting hanteer word. Sekere van die ander versoeke is bloot onprakties en sal daarom waarskynlik deur die Siektebestuur programkomitee afgekeur word, maar hulle is almal waardevolle bydraes tot die ontwikkeling van 'n sterk reeks navorsing.

### **Projek: Entoendraagbare siektes**

#### **Vergroening**

1. Vektorbeheer: Ontwikkeling van lok- en dood middels om bladvlooi op 'n soortgelyke wyse te beheer as wat die M3 vrugtevlug beheer.
2. Vektorbeheer: Ontwikkeling van alternatiewe bladvlooi-beheer strategieë soos bv. Predatore, parasiete of paringsontwriging.
3. Genetiese weerstand: Weerstandsteling deur gebruik te maak van chimeras met vergroeningsverdraagsame sektore (Embryo rescuing).
4. Genetiese weerstand: Weerstandsteling d.m.v hoëvlak biotegnologie en geneties gemanipuleerde weerstand.
5. Korrektiewe middels: Alle moontlike middels moet hier geevalueer word ongeag aanvaarbaarheid vir die markte. Dit sluit in antibiotikas, middels wat plantweerstand (SAR) verhoog en middels wat vergroeningsimptome onderdruk.
6. Daarstel van 'n kommersiële PCR diagnostiese diens om te toets vergroening.

#### **Tristeza**

1. Identifisering en karakterisering van die deel van die genoom van Tristeza wat verantwoordelik is vir kruisbeskerming en die mate van strafheid.
2. Onderzoek die teorie dat die saamstel van 'n super CTV kruisbeskermingsras nie nodig is nie, maar slegs die gedeelte van 'n kruisbeskermingsras se DNA wat die sein vir kruisbeskerming gee.
3. Evaluering van verbeterde kruisbeskermingsrasse vir elk van die verskillende pomeloproducerende areas (Tshipise, Letsitele, Hoedspruit, Malelane, Komatipoort/Swaziland, Nkwaleni, Benede-Oranje). Veral Letsitele voel sterk hieroor.
4. Verdere evaluering van verskillende CTV populasies op TSR. Maak seker dat die kruisbeskermingsras wat huidiglik by die Grondvesblok gebruik word wel die regte een is.

#### **Onverenigbaarheid**

- Onderzoek onverenigbaarheid op kumkwarte.

#### **Projek: Na-oes patologie**

1. Evaluering van nuwe wakse, oppervlak steriliseerders en swammiddels soos dit beskikbaar raak.
2. Ontwikkeling van strategieë om alternatiewe in plek te hê indien weerstand opbou teen die bestaande na-oes swammiddels (GRAS chemikalie, Fisiese behandelings soos osoon en warmwaterstrategieë).
3. Na-oes strategieë vir organies geproduseerde sitrus.
4. Monitoring van die insidensie en regstelling van imazilweerstandbiedendheid in pakhuis (Landswyd).
5. Alternatiewe beheer deur gebruik te maak van antagoniste en ander biologiese beheer agente vir "chem-free" markte.

## Projek: Grondgedraagde siektes

### Sitrusaalwurm

1. Evaluering van talle biologiese beheeragente wat in die handel beskikbaar is. (Bv produkte deur Monterey en Micro Life in Amerika)
2. Evaluering van Enzone vir aalwurmbesluit in bestaande boorde.
3. Voorplantbehandeling van herplantgronde deur middel van beroking (Telone/ Vapam) .
4. Effek van aalwurms indien enige in OHS boorde. Evaluering van *Paecilomyces lilanicus* deur drupbesproeiingstelsels.

### Phytophthora

1. Registrasie van die gebruik van fosfonate deur drupstelsels.
2. Effektiewe beheer van *P. citrophthora*.
3. Bepaling van faktore wat vrugte meer gevoelig maak vir koper en fosfonaat fitotoksiteit.

### Fusarium/Blight

1. Die rol wat *Fusarium*- wortelvrot op alternatiewe drag veroorsaak, soos wat tans met die laat mandaryne ondervind word, moet ondersoek word.
2. Die Blight Onderstam-evalueringsoef in Letsite moet gemonitor word.
3. Nuwe onderstamme se gevoeligheid vir isomartisien (*Fusarium*-toksien) moet bepaal word. Hoe vergelyk C35 met die huidige kommersiële kultivars?

## Projek: Vrug en blaarsiektes

### *Pseudocercospora angolensis* (P.a.)

1. Herevaluering van die status van *P.a.* in Zimbabwe sodra daar weer politieke stabiliteit is.
2. Residu ontledings van chemikalië wat vir die laaste *P.a* bespuiting in Februarie / Maart aanbeveel word (Hierdie werk kan nou reeds in SA gedoen word).
3. Klimatologiese kartering van *P.a.*

### Alternaria

1. Evaluering van nuwe kultivars teen *Alternaria* soos wat hulle in die land ingebring word.
2. Voorspellingsmodel vir die sitrusindustrie.
3. Evaluering van meer koste-effektiewe spuitprogramme en nuwe chemiese produkte.

### Botrytis

1. Evaluering van chemiese beheer op suurlemoene. Registrasie van produkte sodat wettige aanbevelings op sitrus gedoen kan word.
2. Bepaling van toedieningstye. Hier moet veral gelet word op *Botrytis* wat die vruggies aanval en laat val of mumifiseer vanaf blom tot albastergrootheid.

## PROGRAMME: INTEGRATED PEST CONTROL

### Project: False Codling Moth (FCM)

1. Optimising the use of Cryptogran in all the different citrus producing areas.
2. Commercialization of Xsit. Evaluation of the efficacy of area wide release of sterile FCM.
3. Evaluation of commercial biocontrol of FCM larvae using nematodes.
4. Packhouse in-line detection of infected fruit.
5. Irradiation of fruit prior to export as an alternative to cold steri.

### Project: Fruit fly

1. Re-evaluation of the Sensus traps and the recommended thresholds.
2. A model to reduce the number of M3 traps in larger orchards to reduce cost.
3. Malathion replacement for fruit fly bait spray programmes.
4. Combining M3 female (90%) and male (10%) traps to reduce both females and males.

5. Survey for exotic fruit fly along the South African borders.
6. Confirmation of of marula fruit fly status .
7. Creation of fruit fly free areas.

**Project: Mealybug**

1. Evaluate alternative chemicals to replace the OPs.

**Project: Cosmetic pests**

**Thrips**

1. Evaluation of alternative chemicals.

**Grain Chinch bug (GCB)**

1. Evaluate alternative pre-harvest chemical control options.
2. Determine GCB control in areas around the packhouse.
3. Isolate GCB attractants for monitoring and possible control purposes.
4. Determine the timing of the movement of GCB into the orchards and the risk of fruit being infested post packing.

**Leafhopper**

1. Thresholds on thrips traps.
2. Timing and control of control sprays. Registration on citrus.
3. Host range
4. Effect of intercropping.

**Project: Production pests**

**Psylla**

1. Alternative chemicals to replace OP spray treatments in older trees.
2. Develop biological control options. (Guavas?)

**Ants**

1. Develop strategies / chemicals to keep ants out of the trees but in the orchard.
2. Donor with alternative active ingredient.

**Red scale**

1. Timing of the release of *Aphytis*-based on scientific principles (monitoring of male flights?).
2. Rearing of *Aphytis africanus*.

**Soft Green scale**

1. Why more prominent in IPM orchards?

**Grey mite**

1. Effective control strategies.

**Budmite**

1. Alternative for Acarol.
2. Late miticide especially on lemons.

### **Lemon moth**

1. Alternative control options.

### **Snails**

1. Effective, affordable control measures.

### **Bollworm**

1. Registration of alternative control strategies e.g. viruses.

### **Stinkprinkaan**

1. Beheermaatreëls.

### **Project: Biocontrol disruption**

1. Keep data base of non-target effects of the different chemicals updated.

### **PROGRAM: OESOPBRENGS EN VRUGKWALITEITSBESTUUR**

#### **Projek: Oesopbrengs**

1. Kritiese evaluering van interne kwaliteit van verskillende kultivars onder Oop Hidroponiese Stelsels.
2. Riglyne vir die bestuur van besproeiing en bemesting deur OHS om maksimum suikers en kleurontwikkeling te verseker (Produksie Riglyne).
3. Evaluering van puls-besproeiing teenoor minder gereelde drup.
4. Formulering van 'n vrugset-strategie vir nawels in die Oos-Kaap waar groot temperatuur skommelings veroorsaak dat die vrugte afspeen.
5. Vrugsetstrategie vir saadlose suurlemoene.
6. Snoei van suurlemoene (Pongola) en Valencias (Beitbrug) in warm areas .

#### **Projek: Vruggehalte**

##### **Vruggroote**

1. Finalisering van vruggroote model.
2. Verfyning van die gebruik van Corasil en Maxim op vruguitdunning van Valencia Late.
3. Oesskattingmetode vir vruguitdunning.
4. Evaluering van fulviensuur, humiensuur, TopGroeï en ander soortgelyke produkte wat tans in die handel verkrygbaar is vir oes en vruggroote verbeterings.
5. Onderstamevaluering vir onderstamme wat groter vrugte gee op swaarder gronde.
6. Is die bemestingsnorme wat in die sewentigerjare op lemoene op growweskijsuurlemoene ontwikkel is steeds relevant vir vandag se kultivars op 'n wye verskeidenheid onderstamme in verskillende klimaatstreke?

##### **Interne vrugkwaliteit**

1. Verlaging van suur in sekere areas. Chemies sowel as bestuurspraktyke.
2. Ontwikkeling van 'n plaasvervanger van kalsiumarsenaat om sure te verminder.
3. Metodes om vastestowwe (TSS) te verhoog.
4. Effek van ringelering op interne kwaliteit.

##### **Oesbestuur**

1. Som strategië vir vrugset op Deltas en Midnights op.
2. Snoeivideo – opdatering van bestaande video.

### **Eksterne vrugkwaliteit**

1. Ondersoek die fisiologie van "stippeling" wat sekere tye van die jaar veroorsaak word deur bespuitings van bv. Koper, fosfonate en vrugtevlieglokase. Stel die verband tussen hierdie verskynsel (necrostoma), Melanose en "Swazi-spot" vas.
2. Indusering van langer vrugte by suurlemoene.

### **Voorspellingsmodelle**

1. Ontwikkel 'n toets om oorrypheid van vrugte te bepaal.

### **Projek: Skildefekte**

#### **Peteca**

1. Bepaal die bydraende oorsake.
2. Formuleer strategië om dit te voorkom.
3. Voorspellingsmodel. (Is vrugte in plastiese sakkies met koolsuurgas voldoende?)
4. Effek van wakse en waksaanwending. Herbevestig dat dit wel die swaarder wakse is wat skilafbraak verhoog.
5. Effek van verwelking van vrugte sowel as ontgroening.
6. Effek van warmwaterbaddens.
7. Effek van hoë stikstofvlakke.
8. Effek van die spoed en intensie waarmee vrugte geborsel word.

#### **Skilafbraak**

1. Bepaal die faktore wat 'n bydrae lewer.
2. Formuleer strategië om dit te voorkom (bestuur die fisiologie van skilafbraak).
3. Die rol wat die koueketting speel in skilafbraak. Die temperatuur / tyd protokolle van die verskillende kultivars moet opgegradeer word.
5. Bepaal die effek van snoei-intensiteit en die bestuur van sonlig op skilprobleme (karotenoides se rol).
6. Rol wat ontgroening speel in skilafbraak.

#### **Gepokte skil (Rindpitting)**

1. Bepaal die bydraende faktore. Formuleer strategië om dit te voorkom.

#### **Kraaskil**

1. Bepaal die faktore wat aanleiding gee tot kraaskil (na al die jare se navorsing is die probleem steeds nie opgelos nie en lei Sondagsrivier tot R15milj. se verliese per jaar).
2. Ontwikkel 'n betroubare model om kraaskil te voorspel.
3. Onpartydige evaluasie van die bestaande boorde onder die OHS in gebiede waar kraaskil voorkom en stel vas of daar 'n afname in kraaskil in hierdie boorde is.

#### **Oleo**

1. Voorkomingstrategie moet weer 'n slag gepubliseer word in die SA Vrugtejoernaal.

#### **Koueskade**

1. Ontwikkel verskepingsprotokolle vir nuwe kultivars soos die laat mandaryne.
2. Ontwikkel riglyne om koueskade te voorkom.

#### **Projek: Koueketting-bestuur**

1. Bepaal effektiwiteit van verkoeling van nuwe 'supervent' karton.
2. Stel effek van plastiek 'wrapping' op verkoeling van palette vas.
3. Bepaal optimum verkoeling- en verskepingstemperatuur om skildefekte te beperk.
4. Stel tyd- en temperatuurprotokolle vir nuwe variëteite vas en hersien bestaandes.
5. Screen alle wakse teen effek op skildefekte, verkoeling, vrugkleur en rակlewe.
6. Stel effek van geforseeerde lugverkoeling op skildefekte vas.

7. Bepaal invloed van kamertemperatuur-laaï op bederf en raklewe.
8. Stel tyd- en temperatuurprotokolle op vir vrugte gelaai teen kamertemperatuur.
9. Bepaal effektiwiteit van verkoeling en ventilasie per kartontipe.
10. Bepaal effek van toedraai van vrugte op verkoeling en raklewe.
11. Bepaal optimum voorverkoelingstemperatuur om oormatige kondensasie tydens hantering in hawe te verhoed.
12. Ondersoek die variasie en invloed van temperatuur en humiditeit tydens vervoer met Totliners vs platbak.
13. Die tempo van verkoeling op skildefekte moet vasgestel word.
14. Stel die effek van koue-sterilisasie op Star Ruby pomelo's na die VSA vas.

## **PROGRAMME: CULTIVAR DEVELOPMENT AND EVALUATION**

### **Project: Cultivar and Rootstock Evaluations**

#### Cultivars

##### *South*

1. Determine the distances needed to prevent cross pollination of late mandarins.
2. Determine the effect of bees on cross pollination.
3. Investigate the alternative bearing of the late mandarins.
4. Evaluate the late mandarins in the Knysna area.
5. Evaluation of Satsumas, Early and Late navels in Ohrigstad.
6. Determine the current status of the Fukomoto in SA with regard to incompatibility.
7. Vaalharts are looking for an earlier navel and Clementine variety for their area and asked that it should also include evaluations for cold hardiness.
8. Continued evaluation of cultivar trials at Richmond, KwaZulu-Natal.
9. Confirm that Impietratura did not pass through shoot-tip grafting into the material that went to the CFB. Test both trees at the CFB and in Kat River.
10. Cultivar workshop in Nkwaleni.
11. Determine the true-to-typeness of the Cambria.
12. Confirm that there is no unidentified organism involved with the Clemenpons that could pose a threat to the industry.

##### *North*

1. Letsitele Study group requested that Late navels, Late mandarins, a large Late Valencia and sweeter grapefruit to be sourced.
2. The Groblersdal area is also looking for a large Late Valencia to be picked in August and September and an early maturing navel.
3. Weipe is looking for an early Valencia that ripens before the Bennie for the Chinese market as well as a large late Valencia.
4. Evaluation of Satsumas, early- and late navels in the Ohrigstad area.
5. Swaziland would like to have the Late navels evaluated at Ngonini.
6. Burgersfort wants an early navel as well as a late Valencia.
7. Nelspruit is looking for a navel that will colour up earlier as well as an earlier Valencia.
8. Hoedspruit is looking for a late Valencia.
9. Tshipise and Hoedspruit are looking for an early grapefruit which can be used for Japan if the Florida citrus industry collapses.
10. Letsitele, Hoedspruit and the Onderberg would appreciate grapefruit selections less susceptible to sheeppnose.
11. All new cultivar trials should also be evaluated for pests and diseases as well as its susceptibility for rind problems, e.g. Oleo.

#### Rootstocks

1. Evaluation of rootstocks for drought tolerance.
2. Evaluation of rootstocks to be used where the water quality is deteriorating. High sodium, chlorine and boron (Letsitele).
3. Cold hardy rootstocks (Vaalharts and Marble Hall).

### **Project: Breeding**

1. Cultivar improvement using chimeras and *in vitro* ovule rescue.
2. Develop the capacity to do inhouse genetic manipulation of citrus cultivars (overseas)

training of CRI personnel).

**B. UITVOERDERS TEGNIESE PANEEL**

Die behoeftes van die Uitvoeragente is bepaal en sodanig verander dat vooroesaangeleenthede nie weer hierdby ingesluit is nie aangesien dit reeds by die Tegnologie Oordragingsgroepe aangespreek word. Fisiologiese skildefekte en na-oes bederf is van groot belang vir die uitvoerders, asook die werk wat deur die CCCF gedoen word, aangesien dit tot die grootste verliese aanleiding gee.

**C. PAKHUISSTUDIEGROEPE**

Die navorsingsbehoeftes van die Pakhuisstudiegroepe is in drie afdelings onderverdeel:

'Verpakking en Palettisering' sluit die behoeftes in wat deur die Verpakkingsforum aangespreek moet word, 'Koueketting-bestuur' is die navorsing wat binne die Koueketting-bestuur navorsingsprojek val en onder die Oes-opbrengs en Vruggehaltebestuursprogram inskakel, en 'Na-oes bederfbeheer' is behoeftes wat binne die Siektebestuursprogram aangespreek moet word.

DISEASE MANAGEMENT RESEARCH PRIORITIES – ALL AREAS - 2007/2008

TABLE 1

Citrus Area	CBS		Alternaria		Melanose		Diplodia		P. angolensis		Rhizopus		Botrytis		CTV		Greening		Phytophthora		Fusarium		Armillaria		Tylenchulus		Sheath nem.		Post Harvest	
	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08
Baviaans	0	3	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0	0	0	3	0	1	0	1	0	3	
Beitbridge	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	2	0	0	0	0	0	0	0	0	0	3	
Breederivier	0	0	2	2	0	0	0	0	0	0	1	1	1	1	0	0	*3	*3	1	2	0	0	0	0	1	2	0	0	3	3
Burgersfort/Ohrig	3	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	2	0	1	0	0	2	2	0	0	3	3
Citrusdal	0	0	3	3	0	0	0	0	0	0	3	3	0	0	0	0	1	1	2	3	0	0	0	0	3	3	0	0	3	3
Grobldersdal	3	3	2	2	1	1	0	0	0	0	0	0	1	1	0	0	3	3	2	2	0	1	0	0	2	2	0	0	2	3
Hoedspruit	3	3	1	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3	3	3	0	0	0	1	2	2	0	0	3	3
Katrivier	2	3	2	3	0	0	0	0	0	0	2	3	0	2	0	0	0	1	1	3	0	0	0	0	1	1	0	0	3	*3
Knysna	2	2	1	1	2	2	3	3	0	0	0	0	0	0	0	0	1	0	2	3	0	0	0	0	0	0	0	0	2	2
Komatipoort	3	3	1	0	1	1	0	0	0	0	0	0	0	0	3	3	0	0	1	1	0	0	0	0	1	1	0	0	3	3
Letsitele	3	*3	0	0	0	0	0	0	0	0	0	0	0	0	2	2	3	3	2	3	1	1	0	0	2	2	0	0	3	3
Limpopo	3	*3	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	1	0	0	0	0	2	2	0	0	2	2
Malelane	3	3	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	0	1	1	0	0	0	0	1	1	0	0	2	2
Nelspruit	3	3	2	2	0	0	0	0	0	0	0	0	1	1	1	1	3	*3	1	1	0	0	0	0	1	1	0	0	3	3
Nkwaleni	*3	3	2	2	2	2	0	0	0	0	0	0	0	0	3	3	2	2	3	3	0	0	0	0	2	2	2	2	3	3
Oranjerivier	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	1	0	0	0	0	0	0	0	0	2	2
Patensie	3	3	3	3	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	3	3	3	2	1	1	3	3
Pongola	3	3	0	0	1	1	1	1	0	0	0	0	0	0	3	3	2	2	2	2	0	0	0	0	0	0	0	0	3	3
Rustenburg	3	3	2	2	1	1	0	0	0	0	0	0	1	1	0	0	3	3	1	3	2	2	0	0	2	2	0	0	3	3
Sondagsrivier	3	3	2	2	0	0	0	0	0	0	0	0	1	2	0	0	0	1	1	1	0	0	0	0	1	1	0	0	3	3
Stellenbosch	0	0	2	2	0	0	0	0	0	0	3	3	0	0	0	0	3	3	1	3	0	0	0	0	1	1	0	0	3	3
Suid-Natal	1	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	2	2	2	2	0	0	0	0	2	2	0	0	3	3
Swartland	0	0	2	2	0	0	0	0	0	0	2	2	0	0	0	0	1	3	1	3	0	0	0	0	1	1	0	0	3	3
Swaziland	3	*3	0	0	0	0	0	0	0	0	0	0	0	0	3	3	2	2	1	1	0	0	1	1	1	1	0	0	2	2
Swellendam	2	2	3	3	0	0	0	0	0	0	0	0	0	1	0	0	*3	*3	3	3	0	0	0	0	0	0	0	0	2	3
Vaalharts	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	2	2
Waterberg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3
Weipe	3	*3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	2	0	0	0	0	0	0	0	0	2	2
Zimbabwe	3	3	0	0	3	3	0	0	3	3	0	0	1	1	0	0	3	3	1	1	1	1	0	0	1	1	0	0	3	3
<b>Weight</b>	<b>55</b>	<b>60</b>	<b>33</b>	<b>33</b>	<b>12</b>	<b>12</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>11</b>	<b>12</b>	<b>7</b>	<b>14</b>	<b>26</b>	<b>27</b>	<b>42</b>	<b>49</b>	<b>41</b>	<b>58</b>	<b>4</b>	<b>6</b>	<b>4</b>	<b>8</b>	<b>33</b>	<b>34</b>	<b>3</b>	<b>4</b>	<b>69</b>	<b>80</b>
<b>Average</b>	<b>1.9</b>	<b>2.06</b>	<b>1.34</b>	<b>1.34</b>	<b>0.4</b>	<b>0.4</b>	<b>0.13</b>	<b>0.13</b>	<b>0.1</b>	<b>0.1</b>	<b>0.4</b>	<b>0.4</b>	<b>0.2</b>	<b>0.5</b>	<b>0.8</b>	<b>0.9</b>	<b>1.4</b>	<b>1.7</b>	<b>1.4</b>	<b>2</b>	<b>.13</b>	<b>0.2</b>	<b>0.13</b>	<b>0.27</b>	<b>1.13</b>	<b>1.17</b>	<b>0.1</b>	<b>0.13</b>	<b>2.4</b>	<b>2.75</b>

\*Highest Priority for area

**INTEGRATED PEST MANAGEMENT RESEARCH PRIORITIES** **TABLE 2**

Citrus Area	FCM		Fruit Fly		Thrips		Red scale		Ants		Grey mite		Chinch bug		Mealy-bug		Psylla		Leaf-hopper		Rust mite		Bud-mite		Boll-worm		Waxy scale		Lemon moth	
	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	06	07	07	08	07	08	07	08
Baviaans	0	*3	0	2	0	1	0	1	0	2	0	0	0	0	0	1	0	1	0	2	0	1	0	2	0	3	0	0	0	2
Beitbridge	0	3	0	2	0	2	0	2	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	
Breederivier	3	3	2	2	0	0	1	1	1	1	0	0	1	1	3	3	3	3	1	1	0	0	2	2	2	2	0	0	0	1
Burgersfort/Ohrig	3	3	3	3	2	3	2	2	1	1	3	3	0	0	2	2	3	3	1	1	1	1	3	3	1	1	0	0	0	0
Citrusdal	*3	*3	2	3	2	3	2	2	1	1	0	0	3	3	3	3	0	0	1	2	2	2	3	3	2	3	0	0	3	3
Groblersdal	3	*3	3	3	2	3	1	1	1	1	3	1	0	0	2	3	3	3	0	1	1	1	1	2	1	1	0	1	2	2
Hoedspruit	3	*3	3	3	2	2	1	1	1	2	0	0	0	0	0	0	3	3	1	1	1	2	2	2	2	2	1	1	0	0
Katrivier	3	3	3	3	2	2	1	1	3	3	0	0	0	0	2	3	0	0	2	1	1	0	2	3	1	1	0	0	2	2
Knysna	3	3	3	3	1	1	1	1	1	1	0	0	0	0	1	1	2	2	2	2	0	0	0	0	0	0	0	0	0	0
Komatipoort	3	*3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	3	1	0	0
Letsitele	3	3	3	3	2	2	1	1	2	0	0	0	0	0	2	3	3	2	0	0	1	1	0	0	1	1	2	2	0	0
Limpopo	3	3	3	3	3	3	1	1	1	1	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Malelane	3	*3	3	3	2	2	1	1	2	2	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	2	2	0	0
Nelspruit	3	3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	3	3	1	1	2	3	1	2	0	0	0	0	2	2
Nkwaleni	3	*3	3	3	3	3	2	2	2	2	0	0	0	0	3	3	2	2	2	2	2	2	1	1	1	1	1	1	0	0
Oranjerivier	*3	*3	3	3	0	0	3	3	2	2	0	0	0	0	1	1	0	0	2	2	2	1	0	0	0	0	0	0	0	0
Patensie	3	3	2	2	1	1	1	1	2	2	0	0	0	0	2	2	1	1	1	1	1	1	2	2	0	3	0	0	2	2
Pongola	3	*3	3	3	2	2	2	2	3	3	0	0	0	0	3	3	2	2	0	0	1	1	1	1	1	0	2	2	1	0
Rustenburg	3	3	3	3	2	2	2	2	1	1	3	*3	0	0	1	2	3	3	0	0	0	0	1	1	1	1	0	0	0	0
Sondagsrivier	3	*3	2	3	2	2	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	2	2
Stellenbosch	*3	*3	3	3	1	1	1	1	1	2	0	0	3	3	3	3	3	3	1	1	0	0	2	1	1	1	1	1	2	2
Suid-Natal	3	3	3	2	2	2	1	2	1	1	0	0	0	0	2	2	1	2	2	3	1	*3	2	2	2	2	0	0	2	2
Swartland	*3	*3	3	3	1	1	1	1	2	2	0	0	3	3	3	3	2	3	1	1	0	0	2	1	1	1	0	0	2	2
Swaziland	3	3	3	3	2	3	1	1	1	1	0	0	0	0	1	1	2	2	0	0	1	1	2	1	0	0	0	0	0	0
Swellendam	*3	3	3	3	1	1	1	1	3	3	0	0	0	0	3	3	3	3	0	0	0	0	0	3	0	0	2	2	0	0
Vaalharts	3	3	3	2	1	1	2	2	1	1	0	0	0	0	*3	*3	0	0	2	2	2	1	1	1	1	1	1	0	0	0
Waterberg	0	3	0	2	0	2	0	1	0	2	0	3	0	0	0	1	0	3	0	1	0	3	0	3	0	2	0	1	0	0
Weipe	3	3	3	3	1	3	2	2	0	0	0	0	0	0	1	1	0	0	2	2	2	2	0	0	0	0	0	0	0	0
Zimbabwe	3	3	3	3	1	1	1	1	0	0	3	*3	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0
<b>Weight</b>	<b>78</b>	<b>87</b>	<b>74</b>	<b>80</b>	<b>42</b>	<b>53</b>	<b>35</b>	<b>40</b>	<b>35</b>	<b>39</b>	<b>12</b>	<b>13</b>	<b>10</b>	<b>10</b>	<b>47</b>	<b>54</b>	<b>42</b>	<b>48</b>	<b>22</b>	<b>28</b>	<b>23</b>	<b>29</b>	<b>29</b>	<b>37</b>	<b>18</b>	<b>27</b>	<b>14</b>	<b>14</b>	<b>20</b>	<b>22</b>
<b>Average</b>	<b>2.7</b>	<b>3</b>	<b>2.5</b>	<b>2.7</b>	<b>1.4</b>	<b>1.8</b>	<b>1.2</b>	<b>1.4</b>	<b>1.2</b>	<b>1.3</b>	<b>0.4</b>	<b>0.4</b>	<b>0.34</b>	<b>0.34</b>	<b>1.6</b>	<b>1.8</b>	<b>1.4</b>	<b>1.6</b>	<b>0.75</b>	<b>1.0</b>	<b>0.79</b>	<b>1</b>	<b>1</b>	<b>1.3</b>	<b>0.62</b>	<b>0.93</b>	<b>0.48</b>	<b>0.48</b>	<b>0.68</b>	<b>0.75</b>

\* Highest Priority for area

**CROP & FRUIT QUALITY MANAGEMENT RESEARCH PRIORITIES** **TABLE 3**

Citrus Area	Flower Manip		Fruit set		Fruit size		Internal Quality		Colour		Creasing		Rind Pitting		Rind breakdown		Peteca		Pruning		Girdling		Nutrition		Replacem of Ca-ars.		CULTIVAR & ROOTSTOCK DEVELOP- MENT	
	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08
Baviaans	0	3	0	0	0	2	0	1	0	3	0	3	0	3	0	3	0	0	0	0	0	0	0	0	3	0	3	
Beitbridge	0	0	0	1	0	0	0	0	0	1	0	0	0	3	0	3	0	0	0	3	0	0	0	3	0	0	0	1
Breederivier	2	2	2	3	2	2	1	1	1	1	1	1	2	2	2	2	2	2	1	2	2	2	2	2	0	0	2	2
Burgfort & O	1	1	1	2	1	2	1	2	0	0	3	*3	3	1	3	3	0	0	1	0	1	2	0	3	3	3	3	3
Citrusdal	0	0	2	2	1	1	3	3	3	3	3	3	3	3	3	3	1	1	1	1	0	0	2	2	3	3	3	3
Groblersdal	0	0	0	1	1	3	2	2	1	2	3	3	1	1	0	2	3	3	1	1	1	1	0	3	1	0	2	2
Hoedspruit	1	1	3	2	2	2	1	2	3	3	3	3	1	1	0	0	3	3	1	1	0	0	0	3	1	1	2	3
Katrivier	0	0	1	1	0	0	0	0	1	3	2	3	3	3	3	3	3	3	0	2	0	1	0	0	0	1	3	3
Knysna	0	0	0	0	3	3	0	1	0	1	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	*3	*3
Komatipoort	0	0	2	2	2	2	2	3	2	2	3	2	3	3	3	3	1	0	0	0	0	0	0	3	1	1	2	2
Letsitele	0	0	0	2	3	3	2	2	0	2	3	3	3	3	3	3	0	0	0	3	0	2	0	3	1	1	3	3
Limpopo	0	0	0	*3	0	0	0	0	0	0	0	0	2	2	2	2	0	2	0	0	0	0	0	3	0	2	3	3
Malelane	0	0	0	0	2	2	3	3	0	2	3	2	3	3	3	2	1	0	0	0	0	0	0	3	0	0	2	2
Nelspruit	0	0	3	2	1	1	0	0	2	2	1	1	3	3	0	0	3	3	0	0	0	0	0	3	3	3	3	3
Nkwaleni	0	0	2	3	2	3	2	2	1	1	0	1	3	3	3	3	2	2	2	2	1	1	2	2	3	2	3	3
Oranjerivier	3	2	3	3	3	3	0	0	1	1	1	1	1	1	1	1	3	3	3	1	2	1	2	2	0	0	0	3
Patensie	0	3	2	3	1	2	1	1	*3	3	3	3	2	3	2	3	3	3	0	0	2	2	2	2	3	3	3	3
Pongola	0	0	3	3	3	3	1	1	1	1	2	1	0	0	1	1	1	1	2	2	0	0	0	3	1	1	2	2
Rustenburg	1	1	2	3	1	1	1	1	0	0	1	1	1	1	0	0	2	2	1	1	1	1	0	3	2	0	2	2
Sondagsrivier	0	0	1	1	1	1	0	0	1	1	3	3	1	1	2	2	*3	3	0	0	1	1	2	2	2	2	3	3
Stellenbosch	2	2	2	2	2	2	1	3	1	1	3	3	3	3	3	3	3	3	0	0	0	2	2	2	1	3	3	3
Suid-Natal	0	2	3	3	3	3	1	0	0	0	2	2	1	1	1	1	2	2	1	1	0	0	0	0	0	0	3	3
Swartland	2	2	2	2	2	2	2	3	1	1	3	3	3	3	3	3	3	3	1	1	1	2	2	2	2	3	3	3
Swaziland	2	0	2	3	3	3	0	0	0	0	0	2	1	1	2	0	1	0	1	1	0	0	0	3	2	2	2	2
Swellendam	0	0	3	3	0	0	0	2	0	2	3	*3	2	3	2	3	3	3	0	0	1	1	3	3	0	3	3	3
Vaalharts	0	0	0	0	1	1	0	2	0	0	2	2	1	1	1	1	3	3	1	1	1	1	0	0	2	3	3	3
Waterberg	0	2	0	2	0	1	0	1	0	1	0	2	0	0	0	0	0	2	0	2	0	0	0	3	0	0	0	3
Weipe	3	3	3	3	3	3	1	2	3	3	2	2	2	2	2	2	0	0	1	1	0	0	0	3	1	1	0	2
Zimbabwe	0	0	2	2	1	1	0	0	3	3	0	0	0	0	3	3	2	2	0	0	2	2	0	3	0	0	0	0
<b>Weight</b>	<b>17</b>	<b>24</b>	<b>44</b>	<b>57</b>	<b>44</b>	<b>52</b>	<b>25</b>	<b>38</b>	<b>28</b>	<b>43</b>	<b>50</b>	<b>56</b>	<b>48</b>	<b>54</b>	<b>48</b>	<b>55</b>	<b>48</b>	<b>52</b>	<b>20</b>	<b>28</b>	<b>16</b>	<b>22</b>	<b>19</b>	<b>64</b>	<b>32</b>	<b>41</b>	<b>61</b>	<b>74</b>
<b>Average</b>	<b>0.58</b>	<b>0.82</b>	<b>1.5</b>	<b>1.9</b>	<b>1.5</b>	<b>1.8</b>	<b>0.86</b>	<b>1.3</b>	<b>0.96</b>	<b>1.48</b>	<b>1.72</b>	<b>1.93</b>	<b>1.65</b>	<b>1.86</b>	<b>1.65</b>	<b>1.89</b>	<b>1.65</b>	<b>1.79</b>	<b>0.68</b>	<b>0.96</b>	<b>0.55</b>	<b>0.75</b>	<b>0.65</b>	<b>2.2</b>	<b>1.1</b>	<b>1.4</b>	<b>2.1</b>	<b>2.5</b>

\*Highest Priority for area

**RESEARCH PRIORITIES - NATIONAL AVERAGES FOR ALL AREAS - 2008**

DISEASE MANAGEMENT		Table 1																												
	CBS		Alternaria		Melanose		Diplodia		P. angolensis		Rhizopus		Botrytis		CTV		Greening		Phytophthora		Fusarium		Armillaria		Tylenchulus		Sheath aalw.		Post Harvest	
	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08
29 NATIONAL AREAS																														
National Weight	55	60	33	33	12	12	4	4	3	3	11	12	7	14	26	27	42	49	41	58	4	6	4	8	33	34	3	4	69	80
National Average	1.9	2.06	1.34	1.34	0.4	0.4	0.13	0.13	0.1	0.1	0.4	0.4	0.2	0.5	0.8	0.9	1.4	1.7	1.4	2	.13	0.2	0.13	0.27	1.13	1.17	0.1	0.13	2.4	2.75

INTEGRATED PEST MANAGEMENT		Table 2																												
Citrus Area	FCM		Fruit Fly		Thrips		Red scale		Ants		Grey mite		Chinch bug		Mealy-bug		Psylla		Leaf-hopper		Rust mite		Bud-mite		Boll-worm		Waxy scale		Lemon moth	
	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08
29 NATIONAL AREAS																														
National Weight	78	87	74	80	42	53	35	40	35	39	12	13	10	10	47	54	42	48	22	28	23	29	29	37	18	27	14	14	20	22
National Average	2.7	3	2.5	2.7	1.4	1.8	1.2	1.4	1.2	1.3	0.4	0.4	0.34	0.34	1.6	1.8	1.4	1.6	0.75	0.10	0.79	1	1	1.3	0.62	0.93	0.48	0.48	0.68	0.75

CROP & FRUIT QUALITY MANAGEMENT		Table 3																												CULTIVAR & ROOTSTOCK DEVELOPMENT	
Citrus Area	Flower Manip		Fruit set		Fruit size		Internal Quality		Colour		Creasing		Rind Pitting		Rind breakdown		Peteca		Pruning		Girdling		Shelf life		Replacem of Ca-ars.		07	08			
	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08	07	08			
29 NATIONAL AREAS																															
National Weight	17	24	44	57	44	52	25	38	28	43	50	56	48	54	48	55	48	52	20	28	16	22	19	64	32	41	61	74			
National Average	0.58	0.82	1.5	1.9	1.5	1.8	0.86	1.3	0.96	1.48	1.72	1.93	1.65	1.86	1.65	1.89	1.65	1.79	0.68	0.96	0.55	0.75	0.65	2.2	1.1	1.4	2.1	2.5			

**PAKHUISSTUDIEGROEPE**

<b>Studiegroep</b>	<b>Onderwerpe vir Navorsing</b>
<b>Wes-Kaap Pakhuisstudiegroep</b>	<p><b>Verpakking en palettisering</b>                      Stel minimum spesifikasies vir kartonne op                      Stel minimum spesifikasies vir palette op                      Vind alternatiewe materiaal vir hout om palette te vervaardig                      Gebruik van plastiek 'wrapping' om hoekstukke te vervang                      Stel minimum spesifikasies vir hoekstukke op                      Ontwerp 'non-slip' papier vir kartonne                      Bepaal effek van 'artwork / printing' op sterkte van kartonne                      Bepaal mees effektiewe posisie en lengte van hoekstukke                      Stel beste stapelingpatroon op palet vas                      Ondersoek sterker bordkombinasie om entstukke te vervang</p>
	<p><b>Koueketting-bestuur</b>                      Bepaal effektiwiteit van verkoeling van nuwe 'supervent' karton                      Stel effek van plastiek 'wrapping' op verkoeling van palette vas                      Bepaal optimum verkoeling- en verskepingstemperatuur om skildefekte te beperk                      Stel tyd- en temperatuurprotokolle vir nuwe variëteite vas en hersien bestaandes                      Screen alle wakse teen skildefekte, verkoeling, vrugkleur en raklewe                      Stel die effek van koue-sterilisering op Star Ruby pomelo's na die VSA vas.</p>
	<p><b>Na-oes bederfbeheer</b>                      Alternatiewe swamdoders om weerstandbiedendheid teen te werk                      Strategie vir bederfbeheer laat in oesperiode</p>
<b>Oos-Kaap Pakhuisstudiegroep</b>	<p><b>Verpakking en palettisering</b>                      Stel hanteringsriglyne vir alle skakels in die koue-ketting op                      Stel minimum spesifikasies vir kartonne op                      Stel minimum spesifikasies vir palette op                      Vind alternatiewe materiaal vir vervaardiging van palette                      Stel riglyne en spesifikasies vir palettisering op ('strapping, scuring sheets, corner pieces, etc)</p>
	<p><b>Koueketting-bestuur</b>                      Stel effek van geforseerde lugverkoeling op skildefekte vas                      Bepaal invloed van kamertemperatuur-laaie op bederf en raklewe                      Stel tyd- en temperatuurprotokolle op vir vrugte gelaai teen kamertemperatuur                      Bepaal effektiwiteit van verkoeling en ventilasie per kartontipe                      Screen alle wakse teen invloed op verkoeling, uitdroging en skildefekte                      Bepaal die effek van toedraai van vrugte op verkoeling en raklewe</p>
	<p><b>Na-oes bederfbeheer</b>                      Standaardiseer metodes waarvolgens residue van swamdoders getoets word                      Screen effektiwiteit van alle bestaande en nuwe swamdoders                      Screen alle wakse teen invloed op bederf</p>
<b>Mpumalanga Pakhuisstudiegroep</b>	<p><b>Verpakking en palettisering</b>                      Evalueer palettisering met hoekstukke vs gom sonder hoekstukke                      Evalueer verskillende groottes en tipes hoekstukke                      Stel minimum spesifikasies op vir kartonne, palette en hoekstukke                      Stel riglyne vir mees effektiewe palettisering op                      Bepaal die invloed van hi-cube palette op fisiese verliese regdeur die koueketting                      Ondersoek die moontlikheid om toedraaipapier met 'interleaves' te vervang (ETP)                      Hersien merk van kartonne – bv aandui van TBZ op karton (ETP)</p>
	<p><b>Koueketting-bestuur</b>                      Bepaal optimum voorverkoelingstemperatuur om oormatige kondensasie tydens laai van skepe te verhoed                      Screen alle wakse teen effek op skildefekte en verkoeling                      Bepaal die effek van toedraaipapier op verkoeling en skildefekte                      Bepaal optimum tempo van verkoeling om skildefekte te beperk</p>

	<p><b>Na-oes bederfbeheer</b>  Vind alternatiewe swamdoders om weerstandbiedendheid teen Imazalil te oorkom  Vind uit wat is situasie tov TBZ-behandeling van vrugte vir versapping  Stel beheermetodes vir behandeling van houtverouderingsswamme op palette vas  Kry alternatief vir Guazatine om suurvrot te beheer  Stel tempo van residu-afbraak van alle na-oes bederfbeheermiddels vas  Standaardiseer motodes om residue van na-oes swamdoders te toets  Bepaal beste waks vir gebruik op vrugte na vir Japan  Bepaal effektiwiteit van Sporekill vs chloor  Nuuiste bevindinge oor effektiwiteit van Imazalil in die waks moet deurgegee word</p>
<p><b>Limpopo  Pakhuisstudiegroep</b></p>	<p><b>Verpakking en palettisering</b>  Stel minimum paletspesifikasies op, ingesluit swam- en plaagbehandeling.  Stel minimum spesifikasies vir kartonne op  Stel palettiseringsspesifikasies met foto's op  Minimum spesifikasies vir hoekstukke moet opgestel word.  Stel minimum spesifikasies vir 'strapping' sterktes en gehalte op.  Ondersoek die gebruik van 'non-slip' papier vir die vervaardiging van kartonne  Bepaal die effektiwiteit van 'strapping' vs 'netting' tydens vervoer  Akkreditasieproses vir pakmateriaalvervaardigers en diensverskaffers in die koueketting moet ingestel word  Versoek dat kartonvervaardigers spesifikasies op kartonne druk.  Kostes moet so laag moontlik gehou word sonder om gehalte in te boet.  Stel hanteringsriglyne op sodat daar nie op palette geloop word tydens laai van skepe en toemaak van trokke nie.  Versoek dat die besigheids-ent van kartonne gestandaardiseer word.</p> <p><b>Koueketting-bestuur</b>  Ondersoek die variasie en invloed van temperatuur en humiditeit tydens vervoer met Totliners vs platbak  Die tempo en effektiwiteit van verkoeling vir alle kartontipes moet ondersoek word  Die invloed van verkoelingstemperatuur en tempo van verkoeling op skildefekte moet vasgestel word  Stel oorsake en oplossings van 'rind pitting' vas.</p> <p><b>Na-oes bederfbeheer</b>  Bepaal die lewensvatbaarheid om vrugte onder sekere omstandighede 'chem free' te verpak.</p>
<p><b>KZN  Pakhuisstudiegroep</b></p>	<p><b>Verpakking en palettisering</b>  Minimum spesifikasies vir kartonne moet opgestel word.  Spesifikasies vir hoekstukke moet opgestel word.  Posisie van hoekstukke moet ondersoek word.  Minimum spesifikasies moet vir palette opgestel word.  Gebruik van kort hoekstukke ipv endstukke in open tops.</p> <p><b>Koueketting-bestuur</b>  Verkoeling en ventilasie van die verskillende kartonne moet ondersoek word.  Tempo van verkoeling van variëteite moet bepaal word.</p> <p><b>Na-oes bederfbeheer</b>  Maak seker dat Fungasil of alternatiewe Imazalil vir waksaanwending beskikbaar bly.  Aangeleentheid met CBS na Europa moet uitgeklaar word.</p>

**EXPORTERS TECHNICAL PANEL**

<b>Research Topic</b>	<b>2007</b>	<b>2008</b>
<b>Waste / Decay</b>		
Rhizopus	3	2
Resistance to Imazalil survey	3	3
Replacement to Imazalil	3	3
Post-harvest decay ID and control manual (photos, descriptions)	2	2
Optimum shipping temperature to control waste	2	2
Method to quantify over-maturity and puffiness	3	3
<b>Physiological Rind Disorders (*Priority No 1)</b>		
Rind Pitting	3	3
Peteca	3	3
Rind breakdown (mainly soft citrus)	3	3
Creasing	3	3
Optimum shipping / cooling temperature to control rind disorders	3	3
Screening all waxes against rind disorders and decay	2	3
Effect of wrappers on rind disorders		2
<b>Shipping Conditions</b>		
Temperature profiles / regimes / protocols for new varieties	2	3
Maximum CO2 levels	0	3
New container technology	2	3
<b>New Technology</b>		
Replacement of cold steri	3	3
Ozone to control decay	3	2
<b>Cold Chain Management</b>		
Cooling and ventilation of different carton types / designs	2	3
Minimum specs for cartons		3
Minimum specs for pallets		3
Alternative material to wood for pallet manufacturing		3
Revise time and temperature protocols per variety		2
Effect of ambient loading on decay and shelf-live		2
Cooling and ventilation of hi-cube pallets		2
Stronger and better ventilated pallets		3
Procedures for palletizing and handling throughout the chain		3
Effect of wrappers on cooling and shelf-live		3

## NAVORSINGSPRIORITEITSVERGADERINGS Julie & Augustus 2008

Die navorsingsprioriteite wat vir 2008 bepaal is, weerspieël weereens die probleme wat met fitosanitêre vereiste en marktoegang gepaard gaan, asook die probleme wat die grootste ekonomiese verliese tot gevolg het. VKM het by al die studiegroepe weereens nommer drie prioriteit gekry en almal is dit eens dat meer effektiewe beheeropsies gevind moet word. Vrugtevlieglokvalle se werking is wyd bevestigteken en alternatiewe opsies moet gevind word. 'n Alternatiewe produk in die plek van Malathion is belangrik. Die beheer van witluis is ook problematies in die meeste areas en alternatiewe vir OP's moet daarvoor gevind word. Die beheer van psylla in die vergroeningsareas moet aangespreek word.

In die areas waar dit voorkom, heers daar groot kommer oor die beheer van CBS en die versoek is dat baie klem op navorsing en voorligting gelê moet word. *Alternaria* was in die meeste areas vanjaar 'n groter probleem as gewoonlik. *Phytophthora* geniet hoë prioriteit. Aanbevelings om *P. citrophthora* effektief te beheer en veiliger opsies vir die beheer van bruinvrot is nodig. Na-oes bederf is uit die aard van die saak groot prioriteit in alle areas.

Kultivarontwikkeling bly 'n hoë prioriteit vir almal. Die spesifieke rol van CRI in hierdie verband is bevestigteken en die behoefte vir onafhanklike kulture- en evalueringsevalueringe deur CRI is beklemtoon. Die gebrek aan voldoende vordering om oplossings vir fisiologiese skildefekte te vind lei tot groot ekonomiese verliese en meer aandag sal hieraan gegee moet word. Die nou verband wat bestaan tussen fisiologiese skildefekte en plantvoeding het weereens die aanstelling van 'n bemestingskundige ter sprake gebring, asook die behoefte aan onafhanklike objektiewe bemestings- en besproeiingsadvies. 'n Alternatief vir Ca-arsenaat sal vinnig gevind moet word.

Verkoeling en ventilasie van sitrus is een aspek wat onlangs eers begin aandag geniet het. Heelwat navorsing en ontwikkeling sal nog op karton- en paletvervaardiging gedoen moet word. As gevolg van die ontploffing in die boubedryf het die vraag na hout so toegeneem dat die beskikbaarheid van palette 'n kritiese probleem in die nabye toekoms gaan raak en alternatiewe opsies sal dringend ondersoek moet word.

### AANVULLENDE NOTAS:

#### CITRUSDAL

##### Uitstaande issues

- Kapasiteitskepping tov kunsmisaanbevelings en onafhanklike navorsing en aanbevelings.
- *Alternaria*: Kernverrotting problematies. Kosmeties op laat manderyne.
- Kultivarontwikkeling: Saad op laat manderyne, veral Afourers.
- Alternatiewe drag op laat manderyne.
- Drempelwaarde vir FCM-lokvalle moet hersien word.
- Beheer van GCB rondom pakhuis – 'hitchhikers'.

##### 2008

- No 1 prioriteit = FCM
- *Phytophthora citrophthora* op Clems en nawels raak probleem, asook ander opsies vir bruinvrot, en humiene vir wortelvrot.
- Vrugtevlieg: Vervanging van malathion. Alternatiewe beheeropsies in GF120 se plek.
- Blaaspootjie: Swak werking van abamectin – soek alternatiewe opsies.
- Bladspringer: Bruin en groen kom meer voor – soek beheeropsies.
- Bolwurm: Raak al groter probleem. Produkte se aanwending word meer beperk. Soek alternatiewe opsies.
- Bemesting: Onafhanklike navorsing en aanbevelings !!

#### SWARTLAND

##### Uitstaande issues

- FCM-beheer steeds groot probleem

##### 2008

- No 1 prioriteit = FCM
- Vergroening hou groot bedreiging in vir verspreiding na die area – moet beheer word.

- *Phytophthora* bruinvrot en wortelvrot, asook *P. citrophthora* op clemms – soek alternatiewe, sagte beheeropsies.
- Psylla-beheer om vergroening te voorkom.
- Interne gehalte: Mark vereis hoër suiker, veral Midnight. Metode om sure op Midnight te verlaag.
- Ringelering op jong laat manderyne veroorsaak alternatiewe drag.
- Soek alternatief vir Ca-arsenaat om sure op Midnight aan te spreek.
- OP-vervanging vir mercaptothion en rooimyt-beheer belangrik.
- Soek 'n blom- en vrugsetstrategie en volledige bestuursprotokolle vooroes en na-oes per kultivar vir laat manderyne.
- Soek onafhanklike besproeiing en bemestings advies.
- Klubs vir laat manderyne is vol – soek alternatiewe kultivars asook vroeë nawels en Valencias waarvan die toelaatbare aanplantings op 'n ewekansige basis bepaal word.
- Onafhanklike evaluasie van alle bestaande kultivars van alle rolspelers is belangrik.

## STELLENBOSCH

### Uitstaande issues

- Effektiewe beheer van VKM
- Effektiewe beheer van *Rhizopus* op Satsumas
- Effek van klimaatsverandering op alle fasette van sitrusverbouing moet ondersoek word.
- Saadprobleme op laat manderyne.
- Verfyn Gibb aanbevelings vir laat hang van kultivars en bepaal effek op raklewe.
- Plaas Produksieriglyne op website en hou opgedateer.
- Onafhanklike evaluering van Maxim

### 2008

- No 1 prioriteit = FCM
- Alternatiewe beheer vir *Phytophthora* bruinvrot, *P. citrophthora* op clemms en wortelvrot.
- Soek effektiewe maniere om miere uit boom te hou.
- Interne gehalte: Mark vereis hoër suiker, veral Midnight. Metode om sure op Midnight te verlaag.
- Ringelering op jong laat manderyne veroorsaak alternatiewe drag.
- Soek alternatief vir Ca-arsenaat om sure op Midnight aan te spreek.
- OP-vervanging vir mercaptothion en rooimyt-beheer belangrik.
- Soek 'n blom- en vrugsetstrategie en volledige bestuursprotokolle vooroes en na-oes per kultivar vir laat manderyne.
- Soek onafhanklike besproeiing en bemestings advies.
- Klubs vir laat manderyne is vol – soek alternatiewe kultivars asook vroeë nawels en Valencias wat vir die breë sitrusbedryf ewe beskikbaar is.
- Onafhanklike evaluasie van alle bestaande kultivars van alle rolspelers is belangrik.

## BREEDERIVIER

### Uitstaande issues

- Effektiewe beheer van vergroening

### 2008

- No 1 prioriteit = Vergroening en psylla-beheer
- *Phytophthora citrophthora* op clemms, alternatiewe vir fosfonate / bruinvrotbeheer.
- Effektiewe, goedkoper aalwurmdoders.
- Effektiewe beheer van psylla om vergroening te beheer.
- Lemon moth kom sporadies voor – gedragstrategie en beheer.
- Vrugset en alternatiewe drag op laat manderyne moet aangespreek word.
- Die effek van snoei op beheer van insekte en vergroening moet ondersoek word.

## SWELLENDAM

### Uitstaande issues

- Beheer van vergroening.
- Vergelyk aktiewe bestandele van fosfonate – kyk ook na onsuiverhede.

- Bevestig of Surround maklik afwas in pakhuis – ondervinding teenstrydig met Graham se opinie.
- Onderzoek effek van ringelering op stam vs raamtakke op boomagteruitgang en alternatiewe drag van laat manderyne.
- Onderzoek effek van koolhidraatvlakke en *Fusarium* wortelvrot op boomagteruitgang van laat manderyne.
- Onderzoek kruisbestuiwing van alle nuwe cultivars.
- Soek protokol tov verskepingstemperature vir alle cultivars.

#### 2008

- No 1 prioriteit = Kraakskil / vrugsplit
- *Botrytis* kom sporadies voor en daar is geen beheer. Registrasie van middels vir die beheer van *Botrytis* op sitrus nodig.
- Soek alternatiewe na-oesbederf beheeropsies om weerstandbiedendheid te oorkom.
- Knopmyt raak ernstige probleem in area – soek alternatiewe beheeropsies wat effektief werk.
- OP's: soek alternatiewe vir beheer van witluis en sagte-bruindopluis.
- Vrugset op Midnights 'n probleem in die area.
- Hoë sure op Midnights moet aangespreek word.
- Soek strategie vir beter kleurontwikkeling veral vroeg in seisoen.
- Vervang Ca-arsenaat om sure te verlaag.
- Kraakskil, gepokte skil en skilafbraak was groot probleem in area vanjaar – soek dringend vordering op die navorsing.
- Onafhanklike bemestingskundige moet aangestel word om Hannes Coetzee op te volg.

#### KNYSNA

##### Uitstaande issues

- Geskikte kultivars vir die area moet gevind word.
- Effek van Sporekill op melanose (Swazi spot)

#### 2008

- No 1 prioriteit = Kultivarontwikkeling
- Soek kultivars geskik vir die area.
- Alternatiewe produkte vir Malathion moet gevind word om vrugtevlug te beheer.
- Gebied moet vry van swartvlek verklaar word om toegang tot VSA te verkry.
- Satumas se suikers is geneig om laag te wees – soek oplossings daarvoor.
- Metode om beter kleurontwikkeling vroeg in seisoen te kry, moet ondersoek word.

#### PATENSIE

##### Uitstaande issues

- Effektiewe VKM-beheer
- Effektiewe beheerprogram vir *Armillaria*
- Oplossing vir kraakskil
- Beheer van suurlemoenmot
- Tipe-egtheid van Cambria moet bewys word.
- Galle en 'bulbome' van Clemenpons moet ondersoek word, asook fitosanitêre status en risiko's.
- Tipe-egte seleksie van Robyn moet skoongemaak en by CFB gevestig word.
- China-invoerheffings moet heronderhandel word.
- Effek van humiditeit tydens verkoeling op raklewe moet ondersoek word.
- Kleurontwikkeling op vroeë kultivars moet verbeter word.
- Soek alternatiewe goedkoper behandelings vir aalwurmbeheer.
- Vrugset by Midknight en Mor is 'n probleem in die area.

#### 2008

- No 1 prioriteit = FCM
- Soek beheermaatreels vir *Botrytis*
- Soek goedkoper alternatiewe middels om aalwurm te beheer.
- Stel omvang van vergroening vas en sit beheerstrategie in plek.
- Veilige produkte vir beheer van bruinvrot moet ondersoek word.
- Effektiwiteit van osoon om pakhuis en koelkamers te steriliseer moet ondersoek word.

- Alternatiewe beheermiddels vir vrugtevlieg moet gevind word met korter PHI.
- OP's: alternatiewe en biologiese middels vir witluis en bolwurm moet gevind word.
- Die monitering en beheer van lemoenmot moet ondersoek word.
- 'n Tipe bladspringer kom voor wat geïdentifiseer moet word.
- Blom en vrugset op Mor moet aangespreek word.
- Die oorsaak van klein vruggrootte op Midnights moet vasgestel word.
- Beter kleurontwikkeling op veral vroeë variëteite moet ondersoek word.
- Soek 'n beter nawel as Robyn vir dieselfde periode.
- Soek lemoen- en sagtesitruskultivars wat eers in Aug / Sept geoes kan word.

## BAVIAANS

### **Uitstaande issues**

Nuwe studiegroep – het afgestig van Patensie. Uitstaande issues dus dieselfde.

- Effektiewe VKM-beheer
- Effektiewe beheerprogram vir *Armillaria*
- Oplossing vir kraakskil
- Beheer van suurlemoenmot
- Tipe-egtheid van Cambria moet bewys word.
- Galle en 'bulbome' van Clemenpons moet ondersoek word, asook fitosanitêre status en risiko's.
- Tipe-egte seleksie van Robyn moet skoongemaak en by CFB gevestig word.
- China-invoerheffings moet heronderhandel word.
- Effek van humiditeit tydens verkoeling op rակlewe moet ondersoek word.
- Kleurontwikkeling op vroeë kultivars moet verbeter word.
- Soek alternatiewe goedkoper behandelings vir aalwurmbeheer.
- Vrugset by Midnight en Mor is 'n probleem in die area.

### **2008**

- No 1 prioriteit = FCM
- Veilige produkte vir beheer van bruinvrot moet ondersoek word.
- Ondersoek 'chemfree' / biologiese opsies vir pakhuisbehandelings.
- Residu-ontledings verskil baie tussen labs – stel standaard-metodes vas vir gebruik deur alle labs.
- FCM-lokvalle se werking moet verbeter word.
- OP's: alternatiewe middels vir witluisbeheer moet gevind word.
- Alternatiewe, aanvullende middels tot Acarol vir beheer van knopmyt moet gevind word.
- Klein vruggrootte op Midnights moet aangespreek word.
- Kleur op vroeë kultivars, asook Robyne moet verbeter word.
- Soek laat manderyne en laat nawels vir die area.

## SONDAGSRIVIER

### **Uitstaande issues**

- Oorsaak en oplossings vir peteca
- Probleme met kraakskil moet opgelos word.
- Alternatiewe middels vir Acarol vir beheer van knopmyt moet gevind word.
- Effektiewe beheerprogram vir *Alternaria*-kernverrotting moet gevind word.
- Produkte en praktyke om grondstruktuur en wortelontwikkeling te verbeter moet gevind word.
- Metode om die kwaliteit van humiene en fulviene te bepaal moet ontwikkel word.
- Alternatiewe produk om sure te verlaag moet gevind word om Ca-arsenaat te vervang.
- Oorake en beheer van *Botrytis* moet ondersoek word.
- Monitering en beheer van lemoenmot moet aangespreek word.

### **2008**

- No 1 prioriteit = FCM.
- Hoewel Cryptogran en Isomate die beste beheer van FCM gee, moet meer effektiewe opsies gevind word.
- Behandelingen vir CBS en tyd van toediening moet spesifiek vir die area aangepas word.
- Peteca is 'n baie groot probleem en oplossings moet gevind word.
- Kraakskil is ook 'n groot probleem wat aangespreek moet word. Skille raak al dunner elke jaar.
- Voorkoms en verspreiding van vergroening moet gemonitor word.

- Effektiewe beheer van psylla moet aandag kry om vergroening te voorkom.
- Oorsake en beheer van *Botrytis* moet ondersoek word.
- Vervanging van OP's in die algemeen is belangrik, maar veral vir beheer van witluis.
- Die vangste van Natal vrugtevlug verhoog jaarliks en monitering en beheer moet verbeter word.
- Effektiewe beheer van *Alternaria* op Novas moet ondersoek word.
- Metode om die gehalte van humiensure en fulviensure te bepaal, moet ontwikkel word.

## KATRIVIER

### **Uitstaande issues**

- Oplossing vir alle fisiologiese skildefekte moet gevind word.
- Korrelasie tussen *Alternaria*-kernverrotting en knopmyt en bolwurm moet ondersoek word.
- Identifiseer die mot wat skade op suurlimoene aanrig.
- Vrugsetprobleme op Lisbon suurlimoene en nawels moet opgelos word.
- Katrivier data van retensiemonsters moet verwerk word om oorsake van bederf te evalueer.
- Ondersoek kulturele praktyke soos grondbewerking in die beheer van FCM.
- Ontwikkeling van 'n wetenskaplike analise om die gehalte van produkte met 'n koolstofbasis te kwantifiseer!! (Was no 1 in 2007)
- Benodig natuurlike produkte vir na-oes gebruik.
- Benodig beheermaatreëls voor- en na-oes.
- Soek alternatiewe metodes of middels vir mierbeheer.
- Benodig effektiewe lokmiddels vir vrugtevlug-wyfies en drempelwaardes vir beheer.
- Moet alternatiewe middels vir Acarol kry om myte te beheer.
- Kruisbestuwing van laat manderyne moet uitgesorteer word.
- Basies navorsing moet gedoen word om die oorsake van alle fisiologiese defekte vas te stel en op te los.
- Benodig vroeë clementines en nawels vir die area.
- 'n Onderstam wat koue en siekteverdraagsaam moet gevind word vir die area.

### **2008**

- No 1 prioriteit = Na-oes bederf
- No 2 = Kwantitatiewe bepaling van organiese karboksiel / koolstof bemestingstowwe.
- Swartvlek groot bron van kommer vir die area – verlang effektiewe strategie vir beheer in die area. Reël CBS werkwinkel SSM !
- CBS moet hoë prioriteit by CRI Voorligting wees.!
- Soek effektiewe beheerstrategie vir *Alternaria*.
- Beheer van *Botrytis* op suurlimoene moet ondersoek word.
- Beter beheer van *Phytophthora citophthora*, bruinvrot en wortelvrot word verlang.
- Benodig 'n beter lokmiddel vir vrugtevlug-wyfies.
- Verlang beheermiddels vir witluis laat in die seisoen agv residue.
- Benodig alternatiewe vir OP's vir beheer van witluis, blaaspootjie, bolwurm en knopmyt.
- Rooimyt moet 'n 'rating' van 2 kry en op tabel geplaas word.
- Navorsing moet op mytdoders gedoen word met die oog op die MRL situasie.
- Gedragpatrone, monitering en beheer van lemoenmot moet aangespreek word.
- Manipulasie vir vroeër kleurontwikkeling op alle cultivars moet ondersoek word.
- Nuwe ringelering en snoeimetodes vir manipulasiedoeleindes moet ondersoek word, bv 'cable ties' om groeikrag te beperk.
- Alternatiewe opsies vir Ca-arsenaat moet ondersoek word.
- Identifikasie van 'carbon footprints' vir vrugte na verskillende markte en bestemmings vir alle produksie-areas.

## BENEDE-ORANJERIVIER

### **Uitstaande issues**

- Vrugset op huidige kultivars moet verbeter word.
- Toegang tot VSA belangrik vir die area.
- Oplossing vir sonbrand moet gevind word.
- Voorkoms en omvang van knopmyt moet ondersoek word.
- Effektiewe begeer van FCM moet gevind word.

- Vrugtevlug is groot probleem in die area agv die natrossies op die druiwe – benodig effektiewe beheerstrategie.
- Kleurontwikkeling vroeg in die seisoen moet verbeter word.

#### 2008

- No 1 prioriteit = FCM
- Oplossing vir die ernstige vrugtevlugprobleem moet gevind word.
- Metode om miere uit die bome te hou moet gevind word.
- Bladspringer kom sekere jare voor – effektiewe sagte middels moet gevind word vir beheer.
- Knopmyt raak 'n groot probleem – alternatiewe vir Acarol moet gekry word.
- Benodig kultivars wat aangepas is in die area om te oes van April tot September.
- Soek meer onderstamkeuses vir die gebied.
- Siekte- en plaagbeheer vir die organiese produsente moet aandag kry.
- Benodig 'n bemestingskundige vir objektiewe advies.
- Koueskade op veral suurlemoene is 'n probleem wat aandag moet kry.

### VAALHARTS

#### Uitstaande issues

- Effektiewe witluisbeheer
- Alternaria kernverrotting op nawels – soek voorspellingsmodel en beheer.
- Vrugte vergroot nie na Mei nie – soek metode om vrugte vinniger te laat groei voor Mei.
- Valencia-tipes se sure bly te hoog – benodig oplossing.
- Benodig alternatiewe vir Ca-arsenaat om sure van Valencia-tipes te verlaag.
- Kouebestuur is groot probleem in die area en meer werk moet daarop gedoen word.
- Produsente wat nie meer uitvoer nie, beheer nie hul fitosanitêre plae nie – risiko moet aangespreek word.

#### 2008

- No 1 prioriteit = Witluis
- FCM was erger hierdie jaar en beter beheer moet verkry word.
- Nuwe lokval vir vrugtevlug moet gevind word wat effektief werk.
- Alternatiewe middels vir OP's vir witluis en dopluis moet gevind word.
- Behoeftes bestaan vir onafhanklike bemestings-aanbevelings.

### SUID-NATAL

#### Uitstaande issues

- Vrugset en vrugsgroottesverbetering van Rustenburg.
- Beheer van *Botrytis* op suurlemoene.
- Benodig beheermetode vir Fullers rose weevil.
- Vrugsgroottes op Midknights en suurlemoene moet aangespreek word..
- Benodig beheer van rooimyt – moet in tabel gelys word.

#### 2008

- No 1 prioriteit = Roesmyt (Uitskot 15 – 20% agv roesmyt) Soek alternatiewe vir Dithane in Januarie.
- Effektiewe beheermiddels vir psylla moet gevind word.
- Beheer van bladspringer moet ondersoek word.
- Blom en vrugset op Deltas, Midknights en Rustenburg nawels moet aangespreek word.
- Vrugsgroottesprobleem op Rustenburg nawels en Valencias moet aangespreek word.
- OP's vir blaaspootjiebeheer moet vervang word.

### NKWALENI

#### Uitstaande issues

- FCM steeds groot probleem vir area.
- Skilafbraak op pomelo's na Japan is erger as na ander markte – is dit agv koue-sterilisering of geforseerde lugverkoeling.?
- Hoofstuk oor kultivars in die Produksieriglyne moet opgedateer word met foto's en omskrywings.

- Produksieriglyne moet op website geplaas en gereeld opgedateer word waar produsente nutste inligting kan trek.
- Moet oorsaak van swart letsel op skil van suurlemoene kry – dalk 'snite beetle' of 'carob moth' ?
- Vrugset op pomelo's en Deltas moet ondersoek word.
- Benodig metode om suiker te verhoog en sure te verlaag om beter verhouding te kry.
- Oplossing vir vrugsplit op Deltas moet gekry word.
- 'n Sterk behoefte vir leiding bestaan vir die regte balans tussen 'n chemiese en biologiese benadering tov bemesting.
- Daar is 'n groot leemte vir bemesting- en besproeiings-aanbevelings wat gevul moet word.
- CBS is steeds 'n baie groot prioriteit.

## 2008

- No 1 prioriteit = FCM
- Alternatiewe middels om OP's te vervang vir witluis- en blaaspootjiebeheer.
- Versoek kundigheid vir bemestings- en besproeiings-aanbevelings en navorsing binne CRI.
- Vrugset op pomelo's, nawels en Valencia lates moet verbeter word.
- Vruggrootte op veral pomelo's moet aangespreek word.
- Kraaskil op Valencias is nog nie opgelos nie en oplossings moet gevind word.
- *Phytophthora* bruinvrot en wortelvrot: meer effektiewe en veiliger middels word verlang.

## BURGERSFORT / OHRIGSTAD

Hoogste prioriteit: Kraaskil

### Program: Siektebestuur

- Vergroening: Nuwe middels om bladflooi te beheer.
- Vergroening: Korrektiewe behandelings.
- *Phytophthora* beheer: Alternatiewe produkte.

### Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Kraaskil: Effektiewe beheer.
- Bemesting: Oplei van 'n persoon om dr Hannes Coetzee te vervang. Bedryf moet 'n onpartydige persoon hê vir bemestingsaanbevelings.

### Program: Geïntegreerde Plaagbestuur

- FCM: Meer effektiewe beheermaatreëls.
- Vrugtevlieg: Alles dui daarop dat daar op sekere plase (Elbert de Kock, Willie en PLM Boerdery) weerstandbiedendheid ontwikkel het teen huidige lokase. Bevestig dat dit die geval is al dan nie.

### Algemeen

- Opdatering van Produksieriglyne

## GROBLERSDAL/MARBLE HALL

Hoogste prioriteit: VKM beheer

### Program: Geïntegreerde Plaagbestuur

- VKM: Effektiewe beheer van VKM.
- Grysmyt: Voorkomende beheermaatreëls moet ontwikkel word.
- Roesmyt: Voorkomende beheermaatreëls en middels.
- *Prays citri*: Monitoring en drempelwaardes vir bespuiting nodig.
- Bladspringer: Spuitaanbevelings is en registrasies op sitrus is nodig.
- Biologiese spuitprogramme: Die evaluering van die gebruik van biologiese beheer produkte soos EM en ander tees vir insek en siektebeheer.
- Knopmyt: Alternatiewe vir Acarol
- Rooimyt: Beheer veral waar meer Confidor toegedien word.

#### Program: Siektebestuur

- CBS: Toets vir strobilierenweerstandbiedendheid.
- Verandering van CBS status in EU van fitosanitêr na kosmeties

#### Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Voedingskundige: Dringende skep van 'n pos vir 'n onafhanklike voedingskundige om nuwe produkte te evalueer en bemestingsaanbevelings te doen.
- Peteca: Riglyne aan podusente om dit te beheer.
- Kraakskil: Probleem op Bahianinas veral op Troyer. Vind oplossings.
- Vrugkleur: Vroeër kleur op vroeë vrugte.
- Stresverligters (Hitte, droogte, koue).

#### Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkeling: Bepaal die beste laat nawel vir die area.
- Kultivarontwikkeling: Vind 'n goeie vroeë nawel vir die area.
- Kultivarontwikkeling: Vind 'n goeie laat Valencia vir die area.

### HOEDSPRUIT

Hoogste prioriteit: VKM beheer.

#### Program: Geïntegreerde Plaagbestuur

- Hoogste prioriteit: Beheer van VKM sodat toegang tot China 'n kommersieel realiteit kan word.
- Vrugtevlug: Alternatiewe vir Malathion in vrugtevluglokase.
- Beheer van stinksprinkaan.
- Evaluering van abamectin bespuitings gekombineer met ander SC formulasies om vas te stel wat ringbrand op vrugte veroorsaak.

#### Program: Siektebestuur

- Vergroening: Meer effektiewe middels om organofosfate te vervang.
- Fasiliteite om diagnostiese toetse vir Liberibacter te laat doen.

#### Program: Oesopbrengs en Vrugkwaliteitsbestuur

- Vrugset: Vrugset van saadlose suurlemoene, Deltas, Midnights en pomelos.
- Vrugkwaliteit: Turkeys en Bennies word sag in die mark. Hoe om dit te vermy .
- Alternatiewe drag: Maniere om dit uit te skakel.

#### Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkeling: Goeie kwaliteit laat Valencias.
- Kultivarontwikkeling: Vroeër pomelos vir Japanese mark sou Florida se sitrus tot niet gaan.

### KOMATIPOORT

Hoogste prioriteit: VKM beheer

#### Program: Siektebestuur

- *Sitrus Swartvlek*: 'n Diagnostiese toets vir weerstandbiedendheid teen die strobiliriënes.
- *Sitrus Swartvlek*: Evaluering van alternatiewe vir olie bv Wettcit.
- *Na-oes Patologie*: TBZ probleme met vrugte wat na die sapfabrieke toe gestuur word.

#### Program: Oesopbrengs en vrugkwaliteitsbestuur

- Ringbrand: Effek van die verskillende buffers, kleefmiddels, benatters en abamectin formulasies op die ringbrand letsels wat die afgelope seisoen gevind is (generiese produkte). Toets verskillende SC formulasies wat saam met verskillende abamectin formulasies gespuit word.

- Skilgebreke: Kraakskil is 'n probleem op die Valencias en vrugsplit op die Deltas.
- Vastestowwe: Verhoging van vastestowwe op pomelos is nodig. Vastestowwe is baie wisselvallig. Na een reenbui val vastestowwe van 10 na 'n 8 en herstel nie weer.

Program: Geïntegreerde Plaaqbestuur

- VKM: Vervolmaak die Cryptogran spuitprogram vir uitvoere na China. Hoe beïnvloed temperatuurskommelings VKM populasies?
- Witluis: Ontwikkel 'n metode om te onderskei tussen verskillende witluis spp. met die oog op effektiewe parasietvrylating.
- Roidopluis: Ontwikkel 'n diagnostiese toets vir weerstandbiedendheid teen Nemesis.
- Blaaspootjie: Ontwikkel 'n diagnostiese toets vir weerstandbiedendheid teen abamectin.

Program: Kultivar en Onderstamontwikkeling

- *Kultivarontwikkeling:* Pomelokultivars wat minder gevoelig vir skaapneus is.

Algemeen

- Databank met afbraakkurwes vir alle chemiese produkte tot op 0.01 dpm. Sodat krissese nie ontstaan wanneer MRL valke verlaag word nie.
- Verpakkingsnavorsing (Ifco kratte is R1-30/karton goedkoper as karton verpakking). Watter ander moontlikhede bestaan?
- (a) Palletmakers se monopolie moet gebreek word.
- (b) PPECB is nie konsekwent nie en hulle koste is te hoog.

<b>LETSITELE/CONSTANTIA</b>
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Hoogste prioriteit: Om CBS se fitosanitêre status in die EU te verander sodat die siekte weer slegs 'n kosmetiese probleem sal wees

Program:Siektebestuur

*CBS:* Jaarlikse terugvoering oor marktoegang. Hoe beïnvloed die aansluiting van Oos-Europese lande by die EU ons marktoegang tot hierdie lande.

Citrus Tristeza Virus: Evaluering van nuwe kruisbeskermingsrasse vir pomelos in Letsitele.

*Na-oes patologie:*

- Ontwikkel alternatiewe beheerstrategiee en nuwe chemiese produkte insluitende "GRAS chemicals".
- Monitoring van imazaliel en guazitien bestandheid in pakhuisse.

*Vergroening:*

- Manupilasie van genetiese materiaal om plantweerstand teen Liberibacter te bewerkstellig.
- Korrektiewe beheer van die siekte.
- Opdatering van gasheerlyns deur die voorkoms in alternatiewe gasheer met PCR te bevestig.

Phytophthora: Evaluering van biologiese beheer middels, middels wat sistemies die weerstand in die plant verhoog (SAR), die humiensure en fulviensure op *Phytophthora* wortelvrot.

*Tylenchulus semipenetrans:* Evaluering van biologiese beheermaatreels.

Program: Geïntegreerde plaagbeheer

*VKM:* Effektiewe beheer. Toedieningstye via VKM in Letsitele.

*Vrugtevlug:*

- Alternatiewe middels vir gebruik saam met lokase. (Plaasvervangers vir Malathion en Dipterex.)
- Ondersoek Hymlyre fitotoksiteit op Nadorcot. (Hymlyre + Koper maar ook Hymlyre op sy eie wat stippling veroorsaak)
- Evaluering van 'n vermindering in die aantal M3s wat per ha gebruik word namate die blokke groter word.

*Blaaspootjie:* Alternatief vir abamectin.

*Witluis:*

- Maklike metode om tussen Sitrus en Oleander witluis te onderskei.

- Parasietnavorsing.
  - Nuwe chemiese middels om OPs te vervang.
- Psylla*: Alternatiewe gashere bo en behalwe sitrus.  
*Miere*: Ontwikkel metodes om miere op grond maar uit die boom te hou.  
*Vrugte steekmot* (Fruit piercing moth)

#### Program: Oesopbrenge en Vrugkwaliteitsbestuur

- *Interne kwaliteit*: Voorspellingsmodel.
- *Vruggrootte*: Voorspellingsmodel.
- *Opbrenge*: Voorspellingsmodel.
- *Bymiddels*: Evaluering van blaarbespuitings en grondtoedienings wat buite die registrasie van misstowwe vereniging val.
- *Skilafbraak*: Vind oorsake en oplossings.
- *Gepokte skil*: Vind oorsake en oplossings.
- *Vrugset*: Som riglyne vir Deltas en Midnights op. Stresvermindering tydens vrugsetperiode.
- *Snoei*: Opdatering van snoei video.
- *Ringelering*: Takringelering ipv stamringelering moet met mekaar vergelyk word om te verseker dat bome nie in 'n alternatiewe dragpatroon in gaan nie.
- *OHS*: Riglyne vir die gebruik van die stelsel moet voltooi word. Dit moet riglyne insluit wat sal verseker dat die vastestowwe nie nadelig beïnvloed word nie.
- *Grondverbetering*: 'n Onafhanklike persoon met 'n grondkundige en bemestingsagtergrond moet kyk na die opbou van die organise komponent in die grond sowel as die interaksie tussen die biologiese en chemiese komponente in die grond.
- *Voeding*: Aanstel van 'n grondkundige.

#### Kultivarevaluering

- Plukdatum, hantering en verskeppingsprotokol van Turkey. (Pap vrugte is 'n probleem)
- Evaluering van onderstamme vir Cl, B en Na gevoeligheid. (Benton / Sunki) Die kwaliteit van die besproeiingswater is besig om al swakker te word.
- Groot laat Valencia (met en sonder saad).

<b>LIMPOPO/TSHIPISE</b>
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Hoogste prioriteit: Marktoegang tot die VSA.

#### Program: Geïntegreerde plaagbestuur

- VKM: Oorkom die fitosanitêre bedreiging wat hierdie organisme vir die sitrusindustrie inhou.

#### Program: Siektebestuur

- Na-oes patologie: Pakhuisbestuursprogram (Bv Chloor by warmwaterbad, Opbou van *Penicillium* spore in imazililbad. Pakhuisbehandelingsstrategiesessie aan begin van pakseisoen – Keith).
- Na-oes patologie: Terugvoer van ondersoek na *Penicillium* weerstandbiedendheid teen imazalil en guazatien in die verskillende pakhuisse.

#### Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Opdatering van vrugsetstrategieë van saadlose kultivars insluitende Eureka! Saadloos in warmer areas.
- Bemestingsaanbevelings: Die bedryf benodig weer soos in die verlede 'n onafhanklike persoon om bemestingsaanbevelings te maak. Veral dringend in die lig van die feit dat dr Hannes Coetzee nie meer ver van aftrede is nie.
- Bemestings/voedingskundige om onafhanklike opinie te lewer oor kunsmisstowwe en middels wat veronderstel is om die wortelomgewing (rhisosfeer) te verbeter.
- Skilafbraak: Bepaal oorsake en oplossings. TBZ se invloed. Is daar 'n verskil tov skilafbraak op vrugte wat op verskillende kleurvlakke gepluk is.
- Peteca: Bepaal oorsake en oplossings. Verhoog swaarder wakse die gevoeligheid van suurlemoene vir peteca. (Botsende resultate).
- Skildikte van pomelos. Hoe om dit uit te skakel in jare met 'n lae oes.

- Skaapneus. Bestaande standaard gegrand op produksie in Tropiese areas. Daar is 'n periode in die mark wanneer die mark bereid is om vrugte uit dreeër gebiede soos Tshipise met skaapneus te aanvaar. (Pomelo forum. Nie navorsing).

Program: Kultivar en Onderstambestuur

- Kultivarontwikkeling: Laat Valencia (Saad en saadlose kultivar).
- Kultivarontwikkeling: Vroeer rooi pomelos om die opening te vul wat na verwagting gaan ontstaan omdat die Florida uitvoere na Japan gaan afneem a.g.v. sitrus kanker en vergroening.

**MALELANE**

Hoogste prioriteit: VKM beheer.

Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Lae vastestowwe (Advance Cap, K<sub>2</sub>SO<sub>4</sub> bespuitings se effek om dit te verbeter)
- Kleurverbetering op valencias.
- Skilgebreke: Die effek van anorganiese en organiese bemesting op skilgebreke soos kraakskil, vrugsplit, vrugbars en vrugset.
- Bemesting: 'n Onafhanklike persoon om organiese bemesting en die verbetering van die grondstruktuur te ondersoek.

Program: Geïntegreerde plaagbeheer

- Steekmot (suigmot?): Monitoring en beheer.

Algemeen:

- "Scout" kursesse.
- "Bench marking" van die industrie.

**NELSPRUIT**

Hoogste prioriteit: Vergroening

Program: Siektebestuur

- Vergroening: Korrektiewe behandelings om siek bome gesond te dokter.
- Vergroening: Die ontwikkeling van biotegnologie om die teenwoordige weerstand te oorskakel wat die plant se weerstand kan aanskakel die oomblik wat die patogeen die plant infekteer in bestaande sitrusgenemateriaal in te bou.
- Vergroening: Genetiese manipulasie van sitrusvoortplantingsmateriaal om weerstand in kommersiële sitruskultivars in te bou.
- CBS: EU aanvaarding van skrywe aan hulle gerig essensieel om te verseker dat die patogeen sy fitosantêre status verloor.
- Evalueer die gebruik van Smartfresh op sitrus vir rakkewe.
- Endokserose: Oorsake en oplossings.

Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Gepokte skil: Bepaal die oorsake en stel ondersoek in na paklyne waar borsels nie gebruik word nie. Simptome word gedokter nie oorsake.
- Skilafbraak: Is die Benny Valencia meer gevoelig as ander kultivars?
- Ca arsenaat vervanger. Dringend nodig voordat hierdie produk verbied word.
- Kraakskil: Het Corasil enige effek op kraakskil en vrugsplit?
- Bemesting: 'n Spesifieke bemestingsprogram is nodig vir Turkeys om vrugte wat sag word in die mark uit te skakel.
- Bemesting/verbeterde grondstruktuur: Voordele van humate en fulviensure moet wetenskaplik ge-evalueer word.
- Kleur: Ondersoek die gebruik van natuurlike kleurstowwe soos paprika olie in wakse (word algemeen in Mexico gedoen).

Program: Geïntegreerde Plaagbestuur

- VKM: Verfyning van toedieningsaanbevelings op laat kultivars.
- Sitrusbladvlooi: Alternatiewe beheermaatreëls. Poog om Citrimet te behou.
- Myte: Verskeie myte word nie meer deur abamectin beheer nie. Nuwe middels moet gevind word (toename in myte waar Confidor gebruik word).

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkeling: 'n Nawel wat vroeër kleur moet vir die area gevind word. 'n Vroeër Valencia moet ook gevind word wat die gebruik van Ca arsenaat onnodig sal maak.

**PONGOLA**

Hoogste prioriteit: VKM beheer

Program: Geïntegreerde Plaagbestuur

- VKM beheer.
- Witluisbeheer.

Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Evaluering van grondverbeteringsprodukte insluitende die humiensure en fulviensure.
- Organiese bemestingsriglyne.

Program: Siektebestuur

- Na-oespatologie: Ontwikkeling van alternatiewe beheerstrategie.
- Evaluering van onderskeie buffers.
- Evaluering van onderskeie kleefmiddels.
- Na-oesbeheer. Alternatiewe beheerstrategie.

Algemeen

- Databank vir afbraakurwes van chemiese produkte.
- Produksieriglyne oor Oorwerking insluitende 'n ekonomiese oorsig.

**RUSTENBURG**

Hoogste prioriteit: Grysmyt.

Program: Geïntegreerde Plaagbestuur

- Grysmyt beheer.

Program: Siektebestuur

- Vergroening: Korrektiewe beheermaatreëls.
- Vergroening: Alternatiewe beheermaatreëls vir bladvlooi.
- Toetse om die vlakke van die verskillende na-oes behandelings in die diptenks in die pakhuis te bepaal (Imazilil & Sporekill).
- TBZ in saphabrieke.

Program: Kultivar en Onderstamontwikkeling

- Kultivarontwikkeling: Monitor die verskynsel waar Bahianina nawels pitte ontwikkel.

Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Kumkwat skildefek: Oorsaak? Skilafbraak of grysmyt?
- Ontwikkel metodes om koolhidraat vlakke in die wortelstelsel te bepaal en hoe om dit vinnig te verhoog.

## SWAZILAND

Hoogste prioriteit: Bemestingsnavorsing

### Programme: Disease Management

- *Citrus Black Spot*: Alternatiewe beheermaatreëls en die verandering van die status van CBS van fitosanitêr na kosmeties.
- *Tylenchulus semipenetrans*: Enzone moet ge-evalueer word vir aalwurm en *Phytophthora* beheer.

### Programme: Crop Load and Fruit Quality Management

- Bemestingsnavorsing (onafhanklike evaluering van humiensure, fulviensure en ander middels wat veronderstel is om die wortelomgewing te verbeter).
- Bemestingsnavorsing: Sproeibemesting aanbevelings (Produksieriglyne).
- Vrugsplit: Voorkomingsmaatreëls.

### Programme: Integrated Pest Management (IPM)

- FCM: Beheermaatreëls wat toegang tot markte soos China sal verseker.
- Fruit sucking moth: Control on Clanors.

## WEIPE

Hoogste prioriteit: Marktoegang tot die VSA.

### Program: Geïntegreerde Plaagbestuur

- Alternatiewe blaaspootjiebeheermaatreëls.
- Alternatiewe dopluisbeheermaatreëls.

### Program: Oesopbrenge en Vrugkwaliteitsbestuur

- Kleurverbetering.
- Skilafbraak: Bepaal oorsake en maniere om dit te voorkom.
- Skaapneus: Her-evalueer standaarde vir afkeurings. Kyk na ander faktore soos karton gewig, skildikte en sappersentasie.

### Program: Kultivar en Onderstambestuur

- Valencia kultivars wat vroër kleur as die Bennie Valencia vir China.
- Later Valencia kultivars.

## BEITBRIDGE

Highest priority: Cold chain management

### Programme: Disease Management

- Control of blue and green mould (Resistance management).

### Programme: Crop load and Fruit Quality Management

- Pruning in the hotter areas (Light management).

### Program: Siektebestuur

- Verwerkingsvrugte: Wat is die stand t.o.v. produkte soos Tecto, imazalil en guazatien? TBZ verminder skilafbraak en moet gebruik word. Aankopers van sitruskonsentraat vereis TBZ-vry vrugte al is dit nie in ooreenstemming met EU regulasies nie.

### Program: Geïntegreerde plaagbestuur

- Biologiese maniere om blaaspootjie te beheer.

Programme: Integrated Pest Control

- Highest priority: Grey mite control.
- Red mite: Alternative chemicals to control this pest.
- Thrips: Development of more chemicals to control this pest.
- FCM: Registration of Cryptogran in Zimbabwe.

Programme: Disease Management

- *Pseudocercospora angolensis*: Its control and possible eradication in neglected or abandoned orchards once the situation in Zimbabwe has normalised.
- Greening: Alternative chemicals to replace the organophosphates.

Programme: Crop Load and Fruit Quality Management

- Confidor: Does this product stimulate fruit size and yield?
- Fruit colour: Early colour development is an issue and must be addressed.

General

*Pseudocercospora angolensis*: The SA Department of Agriculture must pressurize the Zimbabwe Department of Research and Specialist Services to remove neglected citrus orchards that pose a phytosanitary threat as a result of *P. angolensis*.

The SA Citrus industry must compile an action plan to determine the spread and eradication of this disease in a post-Mugabe era. This will include finding the financial resources to assist in eradicating the disease.

CRI must, via the NDA, establish negotiations with the Angolan government to ensure that no citrus planting material from Brazil enters Angola. Angola must source its citrus budwood and seed from the CFB. Diseases that can be introduced from Brazil into Africa includes Citrus canker, Citrus Variegated Chlorosis, Sudden Death, Leprosis, Rubiloses, *Liberibacter asiaticus* and *Liberibacter americanus* to name a few.

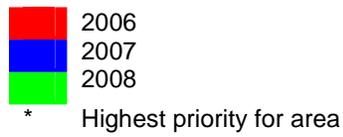
**Kommentaar (nie navorsing)**

Die volgende kommentaar wat nie direk met navorsing verband hou nie maar waarvan die CRI moet kennis dra is deur die onderskeie studiegroepe gelewer. Van die kommentaar soos punt een is vir die eerste keer gelewer terwyl van die ander punte by herhaling gelewer is. Dit is steeds geldig:

1. Daar is tans 'n groot aantal sitrusplase in die Noorde waar die eienaars a.g.v. die huidige regeringsbeleid hulle plase waarskynlik gaan verloor. Hierdie plase is meesal in die Strydom Blok, Komatipoort, Malelane, Hoedspruit, Letsitele, Tshipise en Weipe omgewings en verteenwoordig sowat 10 miljoen uitvoerkartonne. Van die vooruitgeskatte 100 miljoen uitvoerkartonne wat geskat word vir 2010, verteenwoordig dit meer as 12,5 miljoen kartonne. Indien daar nie met initiatief te werk gegaan word om te verseker dat hierdie plase vorentoe effektief bestuur word nie, kan hierdie vrugte afgeskryf word vir uitvoere. Die enigste manier waarop hierdie plase wel volhoubaar bestuur kan word in die mediumtermyn is as die bestaande produsente nadat hulle uitgekoop is sou voortgaan om hierdie besighede (boerderye) te bestuur. Sou dit nie gebeur nie sal al hierdie plase waarskynlik vyf jaar vorentoe vir die sitrusprosesseringsbedryf vrugte produseer. Dit sal 'n redelike gedeelte van die uitvoervrugte uit die mark verwyder wat goed kan wees vir die pryse van die vrugte wat wel uitgevoer sal word. Met die huidige vooruitskouing vir versappingsvrugte wat goed behoort te presteer oor die volgende 5-8 jaar kan dit ook goeie inkomstes vir die nuwe eienaars verseker. CRI moet egter nouer kontak maak met die prosesseringsbedryf sodat ons in die toekoms ook 'n heffing op verwerkte vrugte kan probeer daarstel.
2. Die behoefte aan "Scout" en Sitruskorkkursusse bestaan steeds.
3. Meganisering van sitrusverbouing. Die uitwerking van HIV word in feitlik al die streke as 'n realiteit ervaar. Die gemiddelde plukvermoë per plukker is aan die afneem.
4. CRI moet betrokke raak by navorsing van verpakkingsmateriaal.
5. Die CRI web moet meer gereeld opgedateer word.
6. Die PPECB se rol moet heroorweeg word. Hulle moet 'n groter rol speel om te help met stelsels soos EUREPGAP, Natures Choice, ICMS en BRC eerder as net gehalte inspeksies.

7. 'n Dringende versoek is gerig dat PPECB se inspeksiemetode moet verander sodat 'inlyn' inspeksies gedoen word om tyd en kostes te spaar, ipv palette weer af te breek en op te bou vir inspeksiedoeleindes soos tans die geval is.

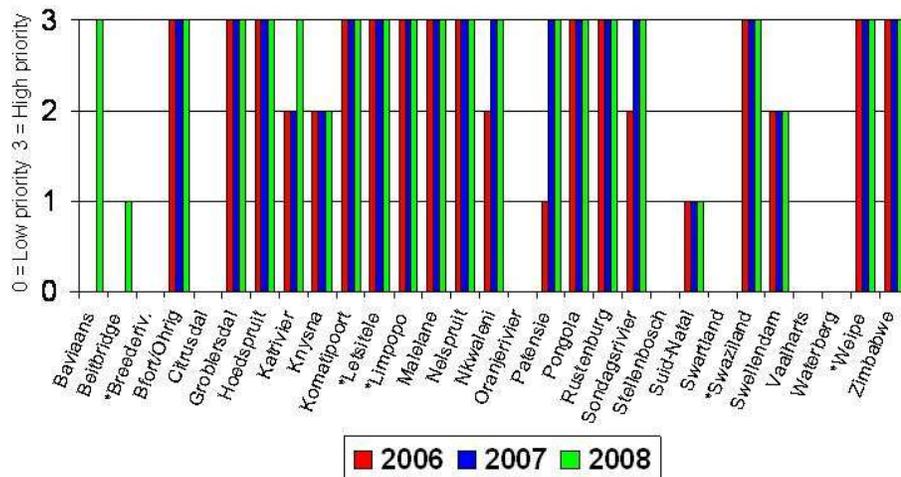
Research priorities representing CRI's four research programmes in the different study group areas of southern Africa



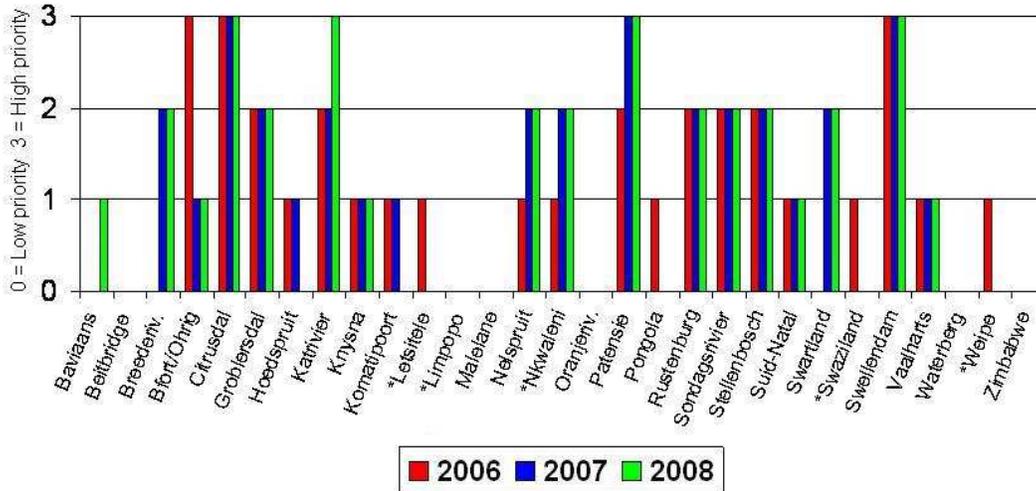
ALL AREAS

**DISEASE MANAGEMENT**

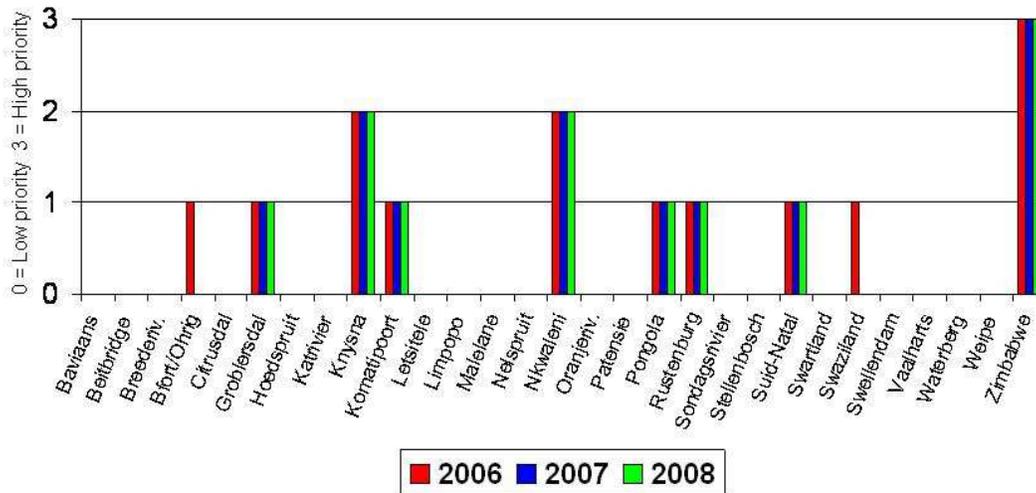
**CITRUS BLACK SPOT**



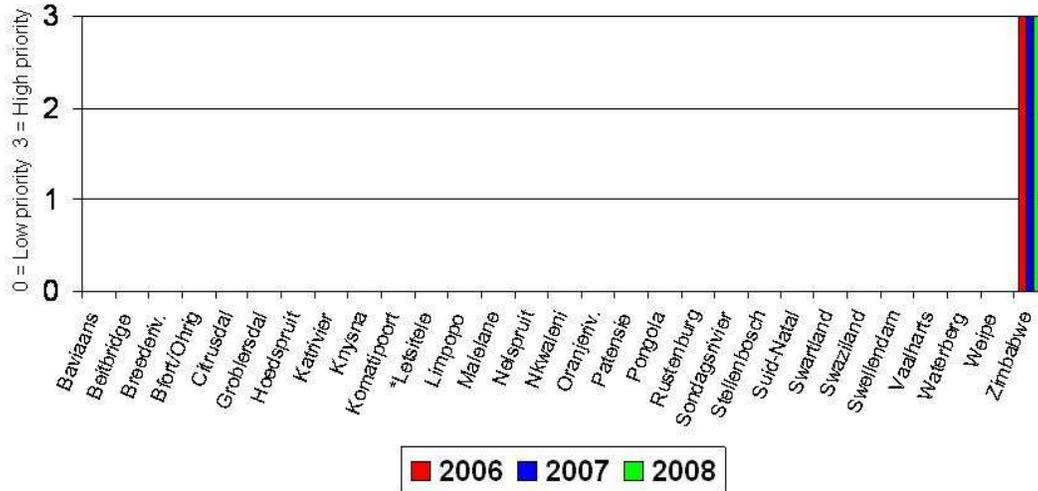
## ALTERNARIA



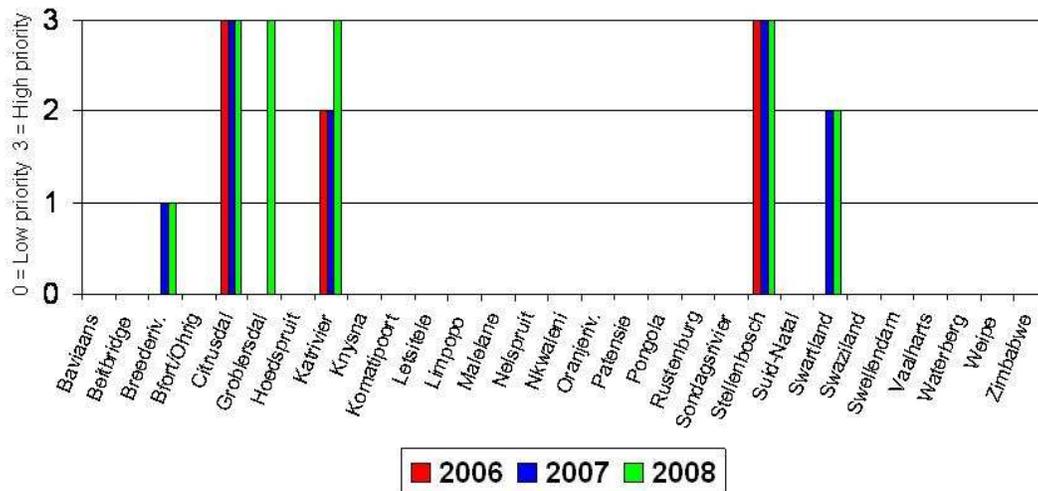
## MELANOSE



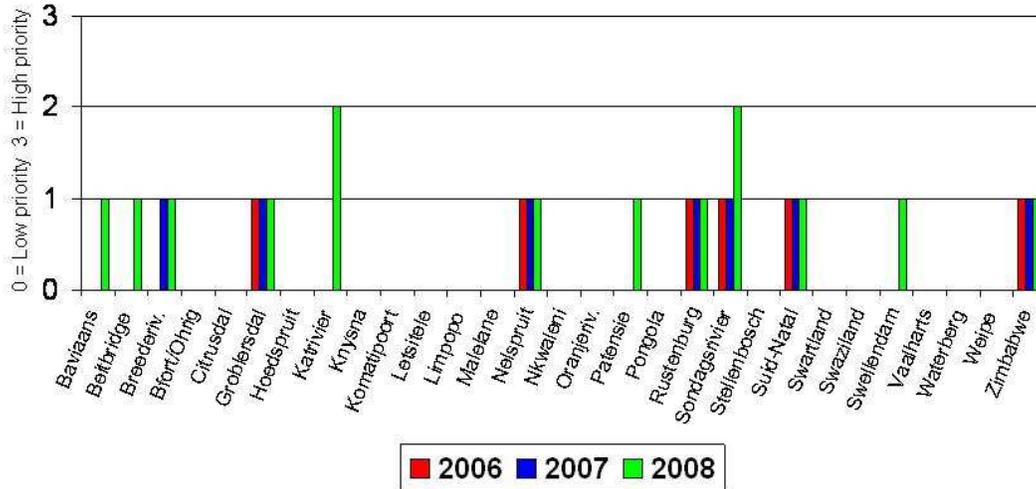
## P. ANGOLENSIS



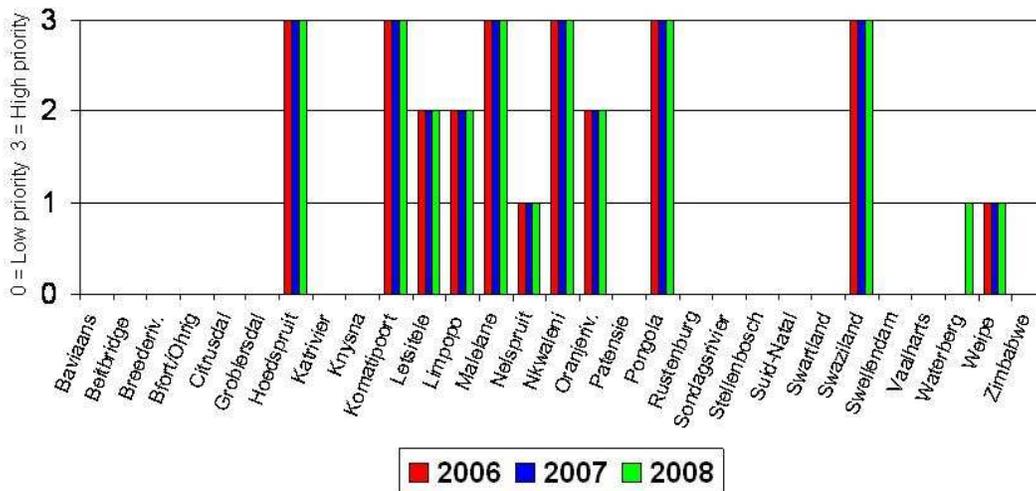
## RHIZOPUS



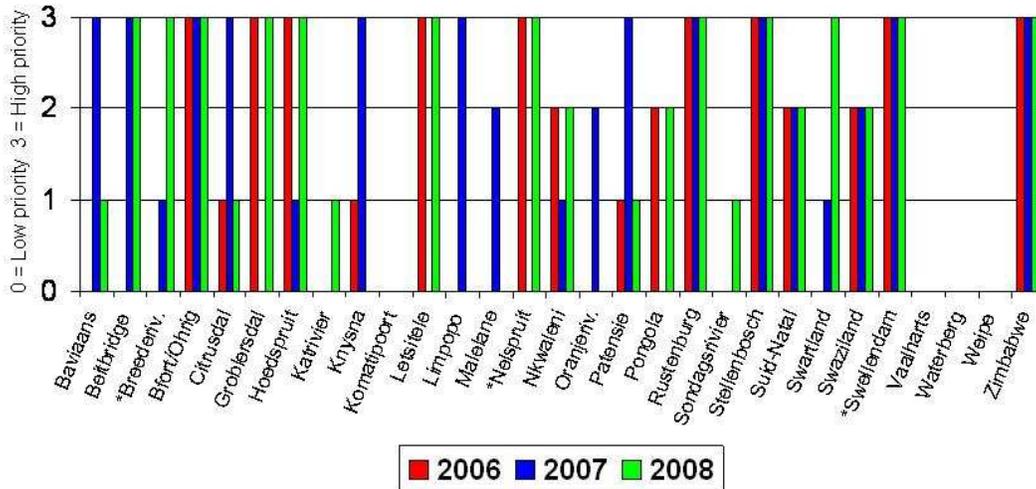
## BOTRYTIS



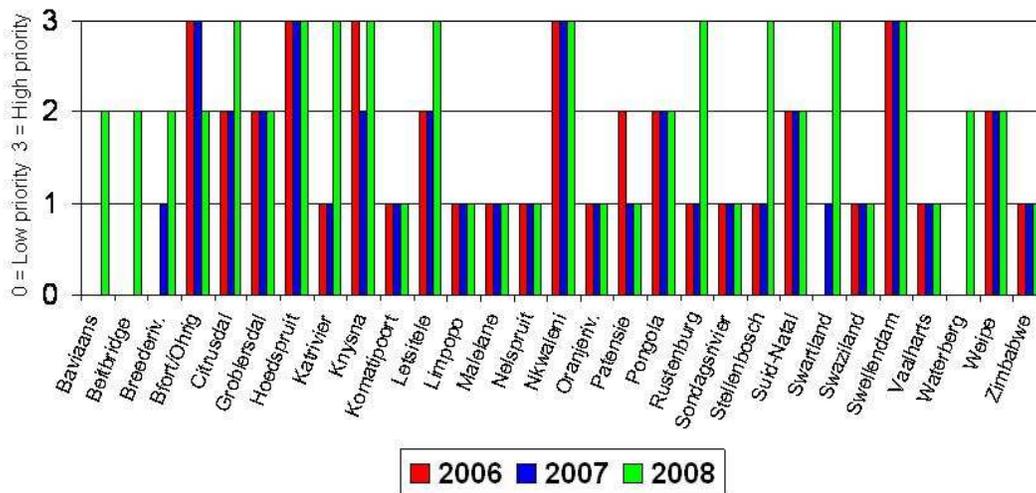
## CITRUS TRISTEZA VIRUS



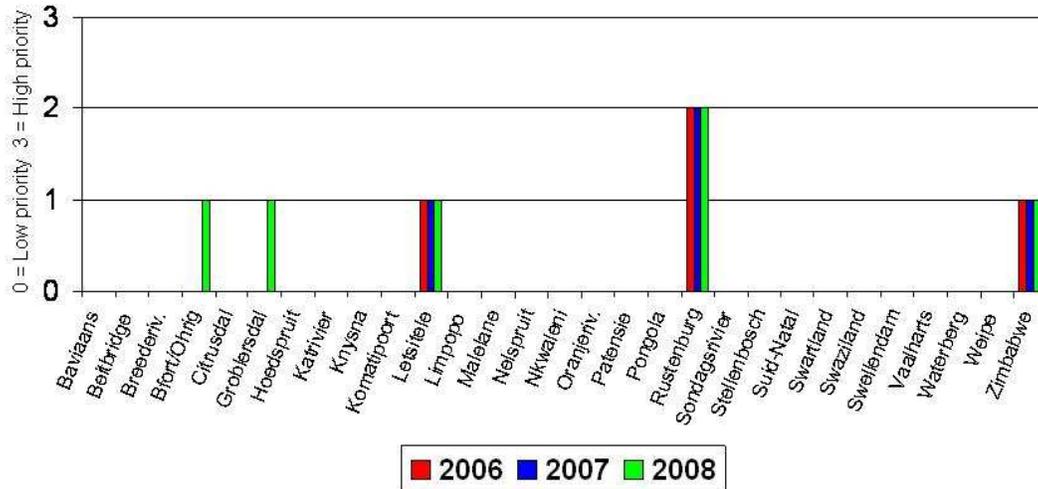
## GREENING



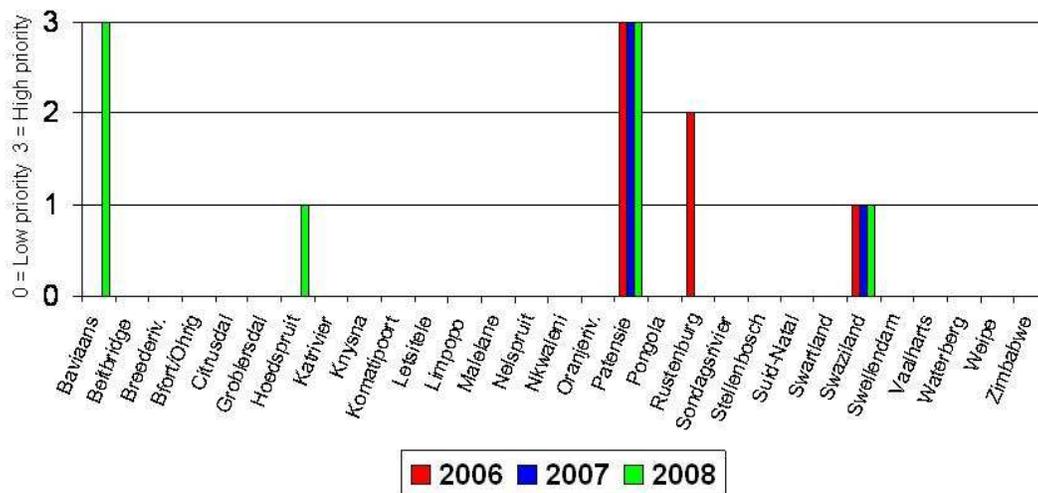
## PHYTOPHTHORA



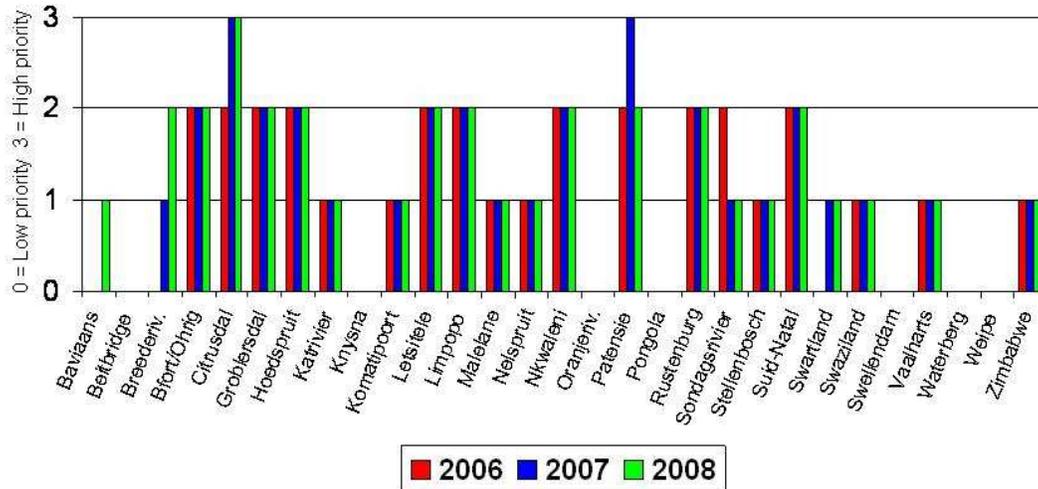
## FUSARIUM



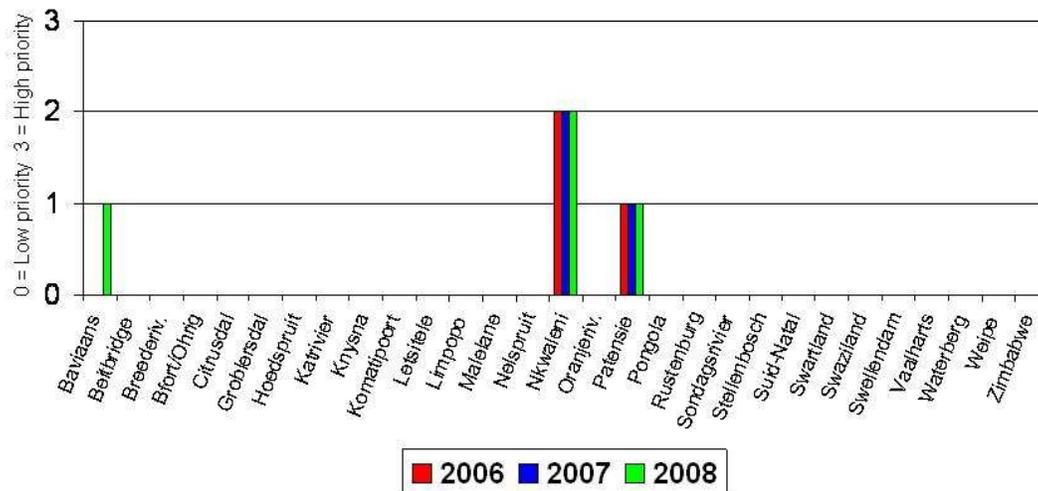
## ARMILLARIA



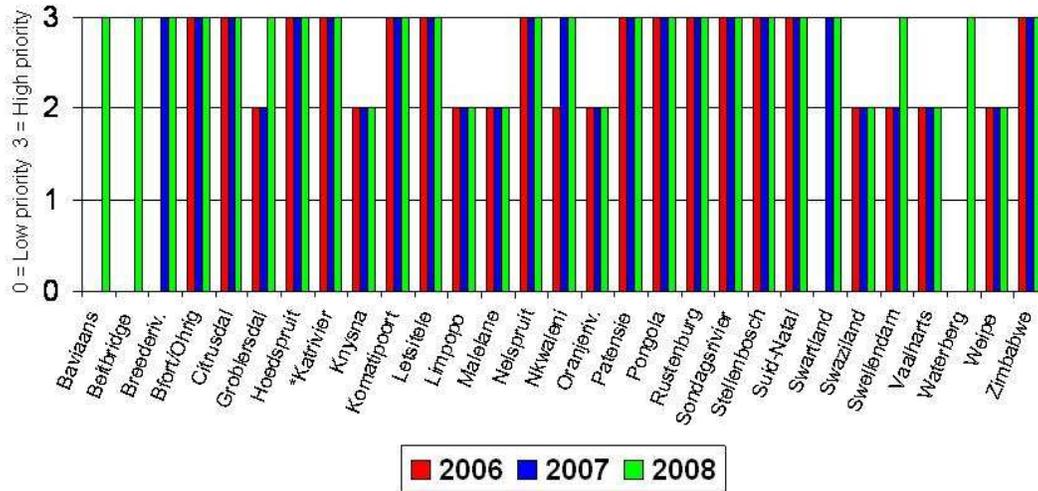
## TYLENCHULUS SEMIPENETRANS



## SHEATH NEMATODE

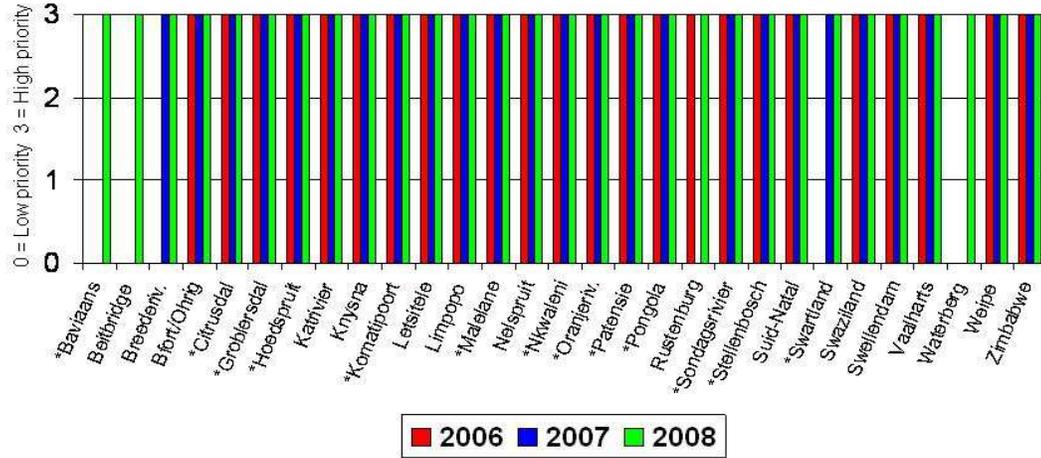


## POST-HARVEST PATHOLOGY

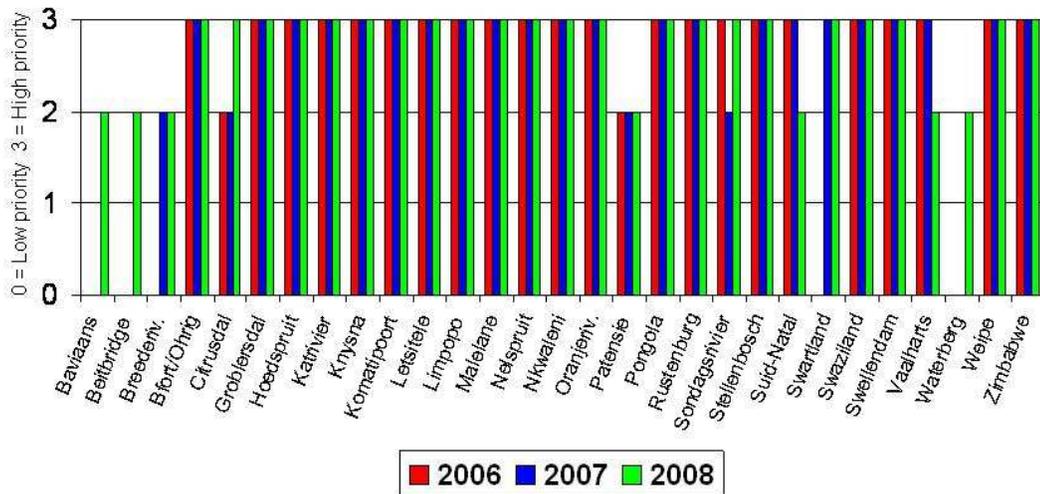


INTEGRATED PEST MANAGEMENT

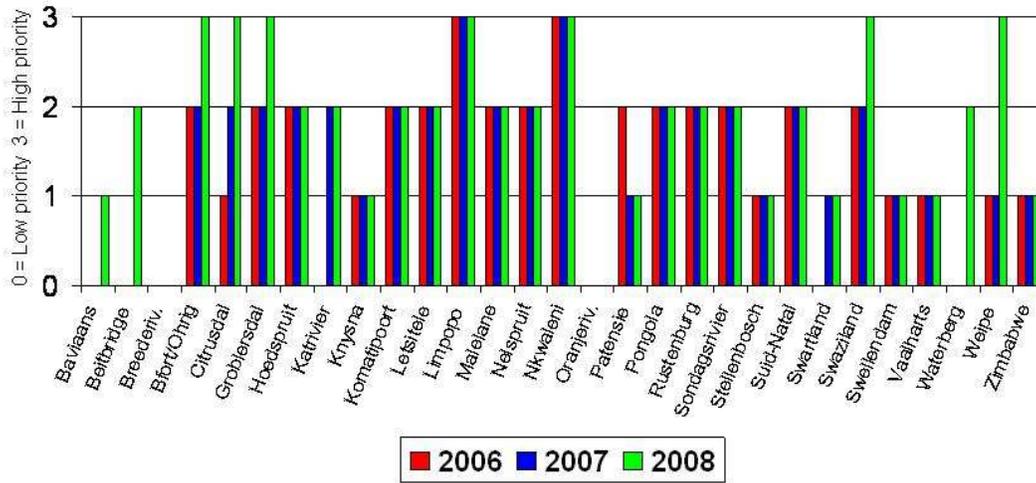
FALSE CODLING MOTH



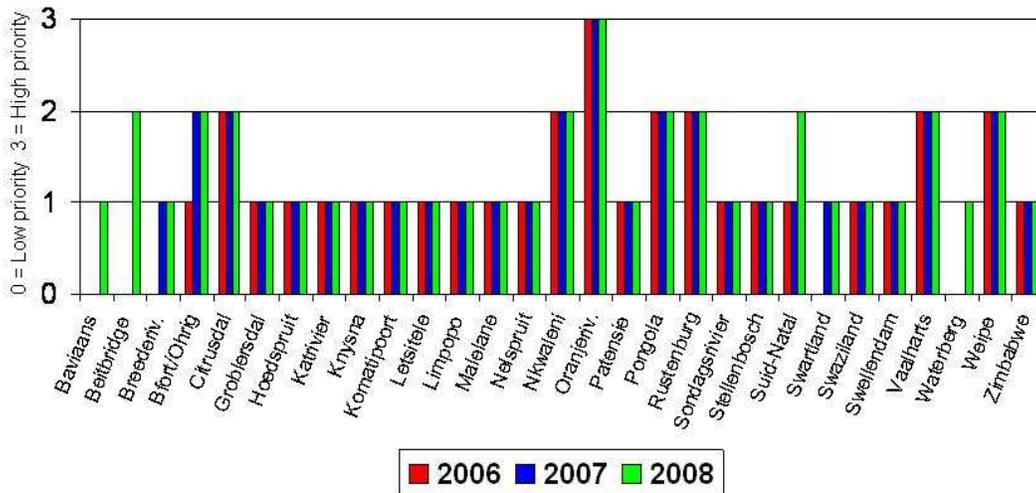
FRUIT FLY



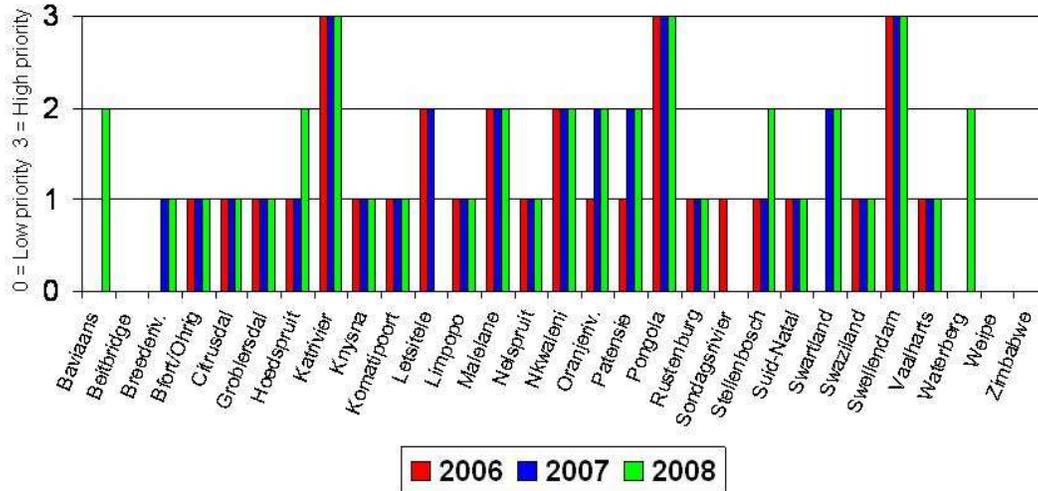
## THRIPS



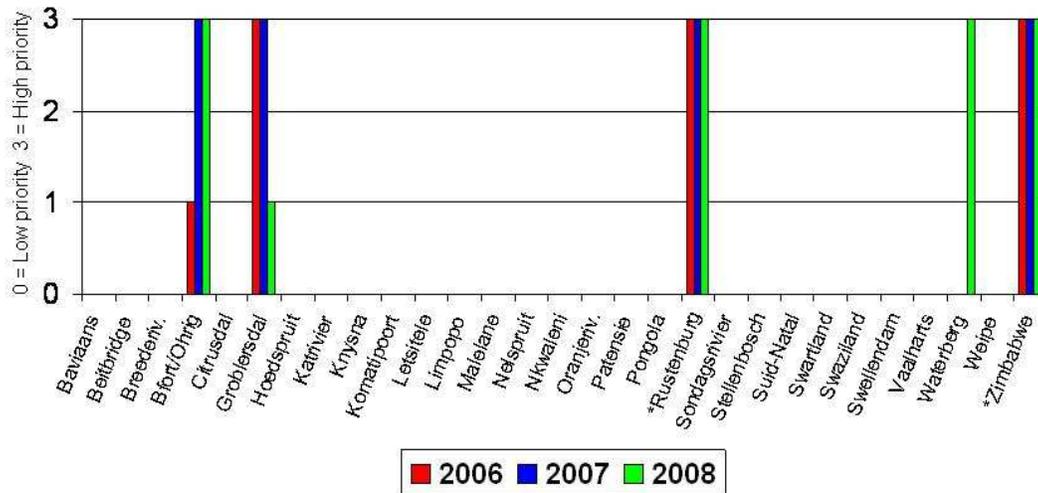
## RED SCALE



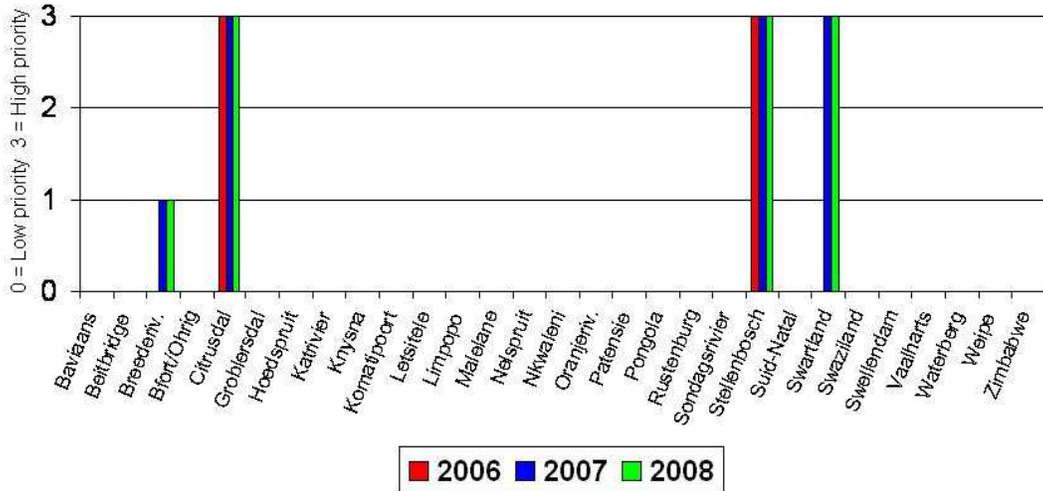
## ANTS



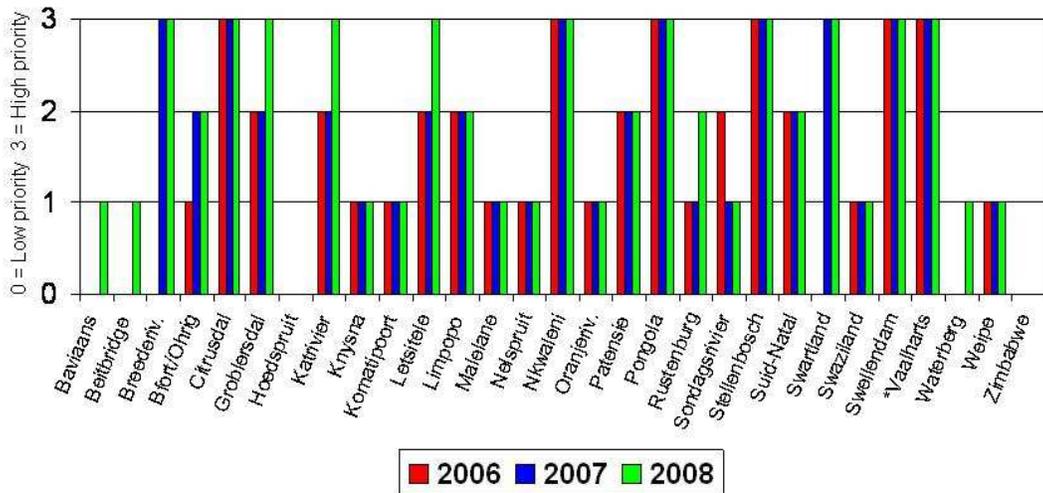
## GREY MITE



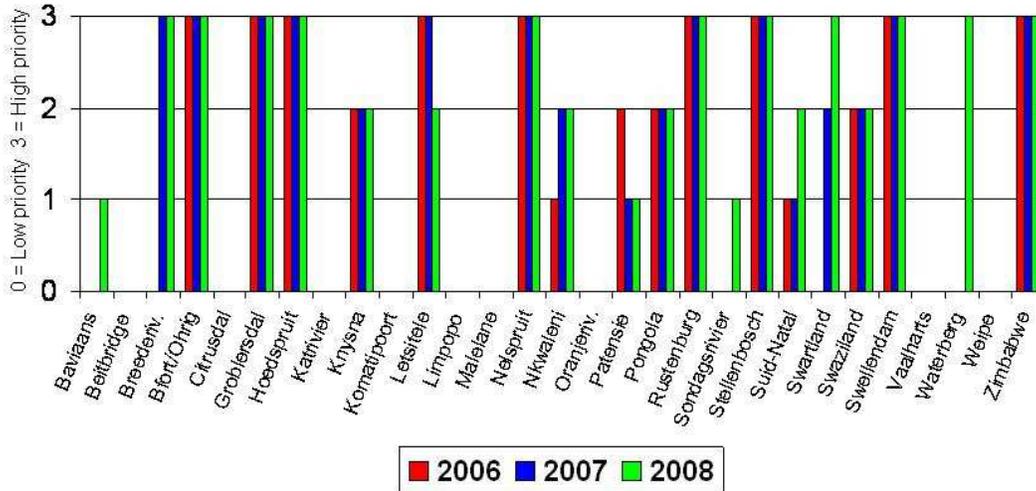
## CHINCH BUG



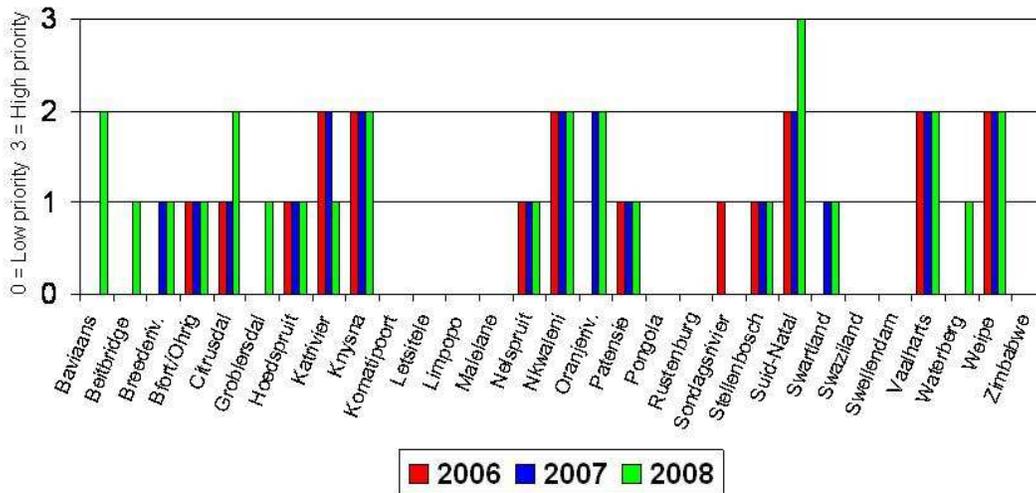
## MEALYBUG



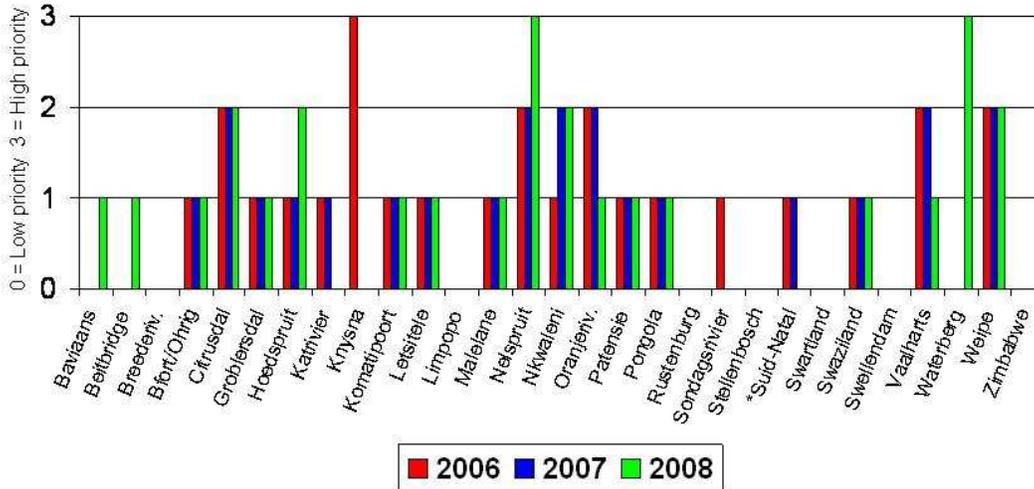
## PSYLLA



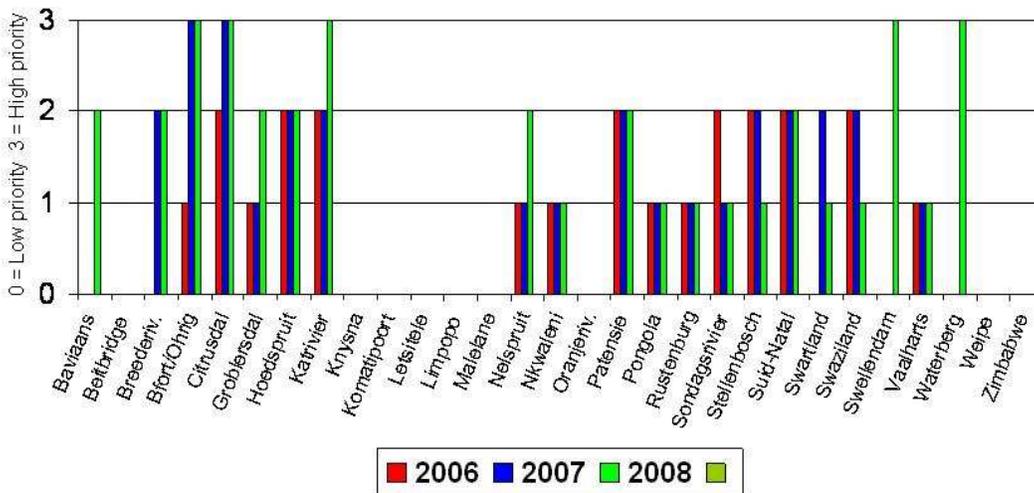
## LEAFHOPPER



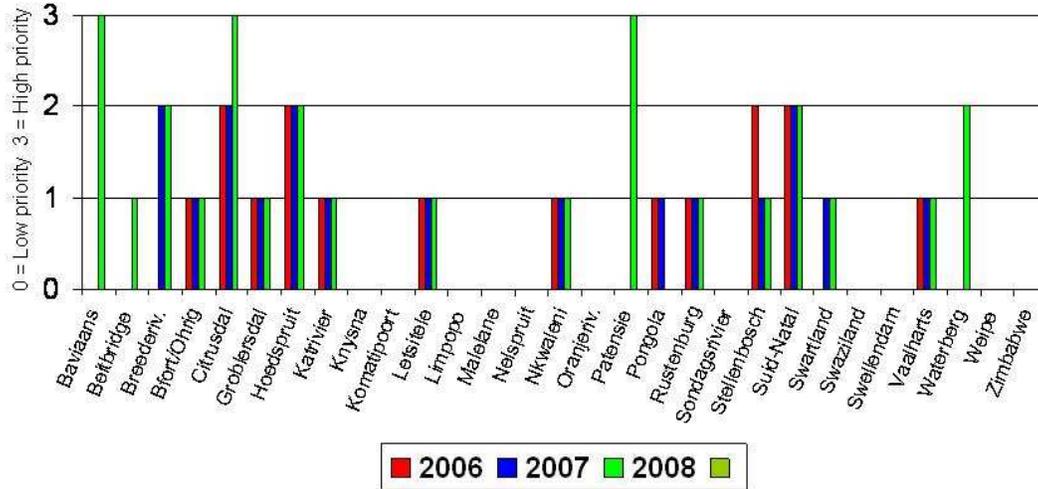
## RUST MITE



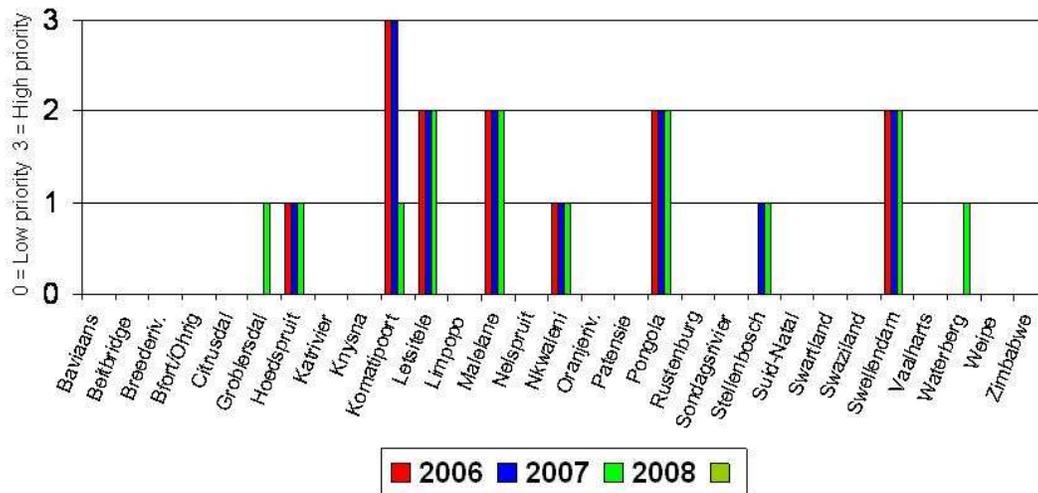
## BUDMITE



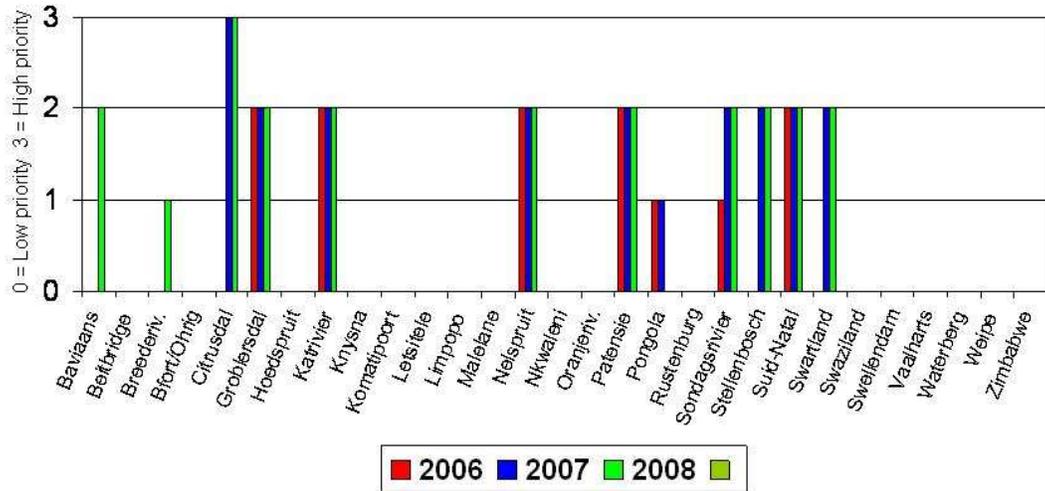
## BOLLWORM



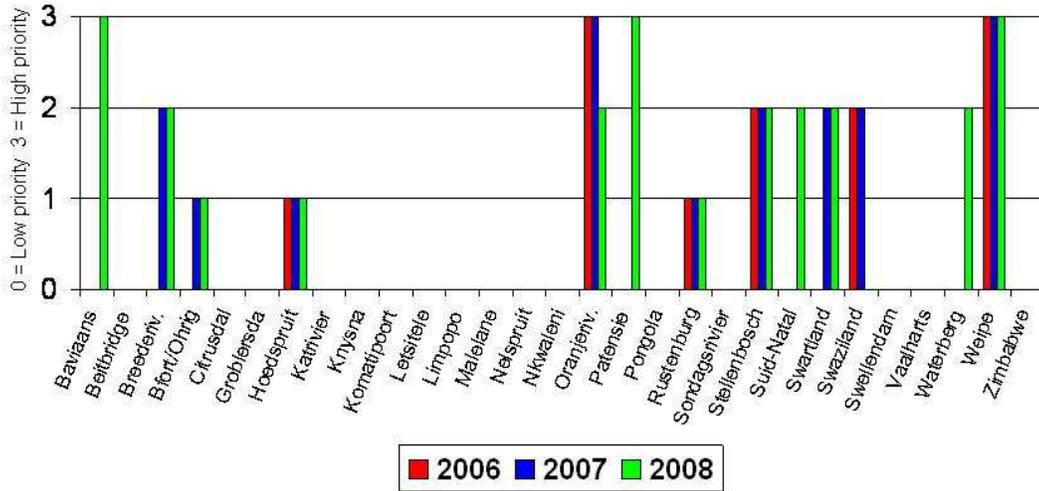
## WAXY SCALE



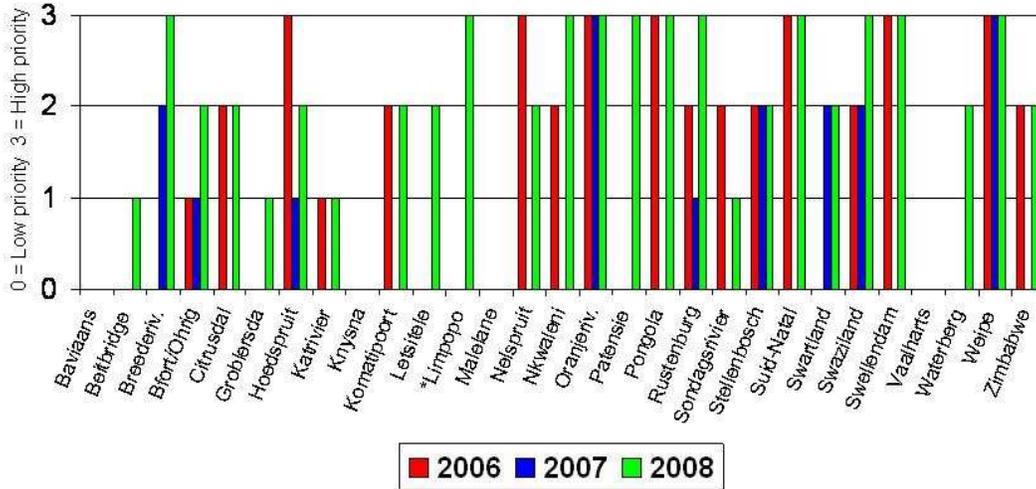
# LEMON MOTH



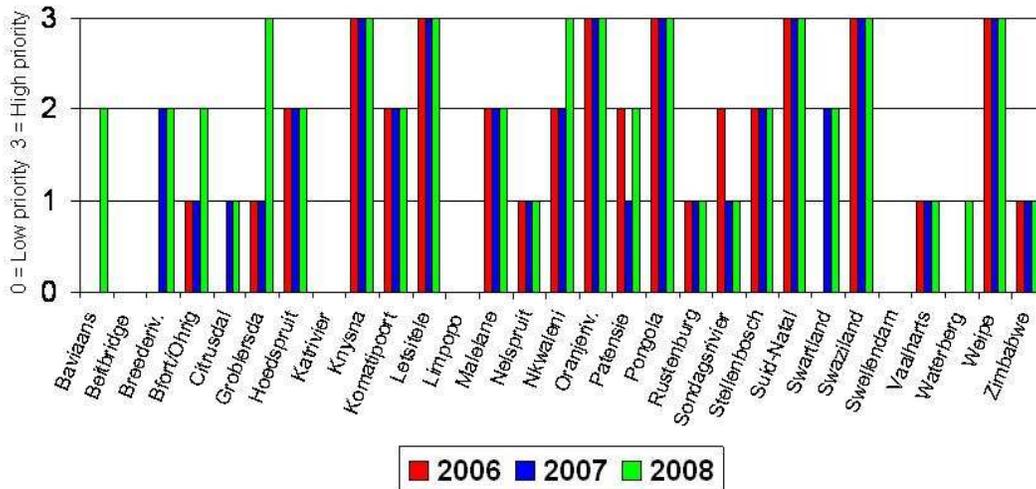
FLOWER MANIPULATION



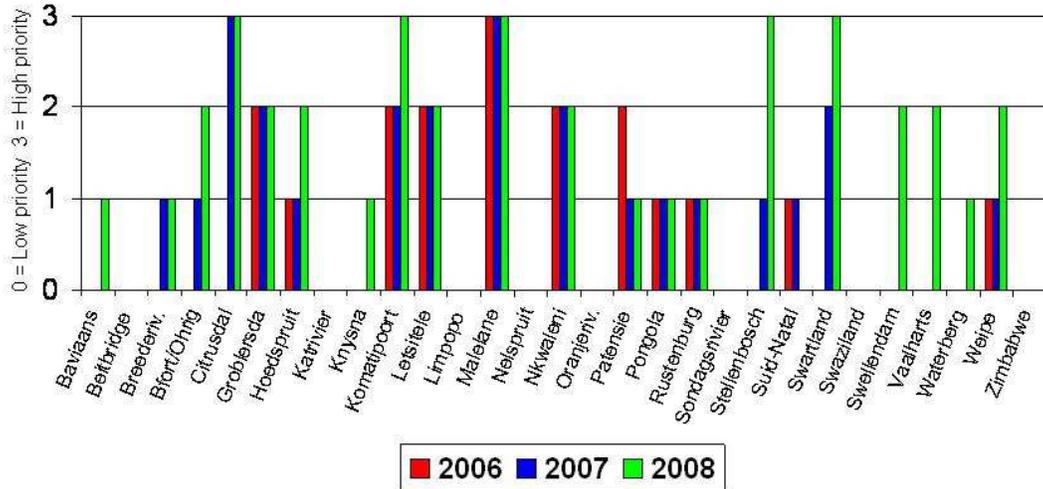
## FRUIT SET



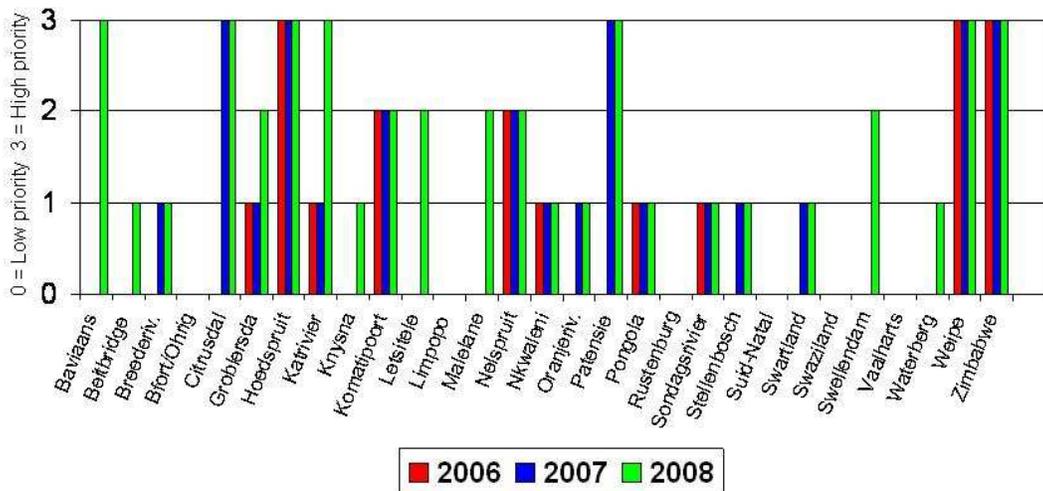
## FRUIT SIZE



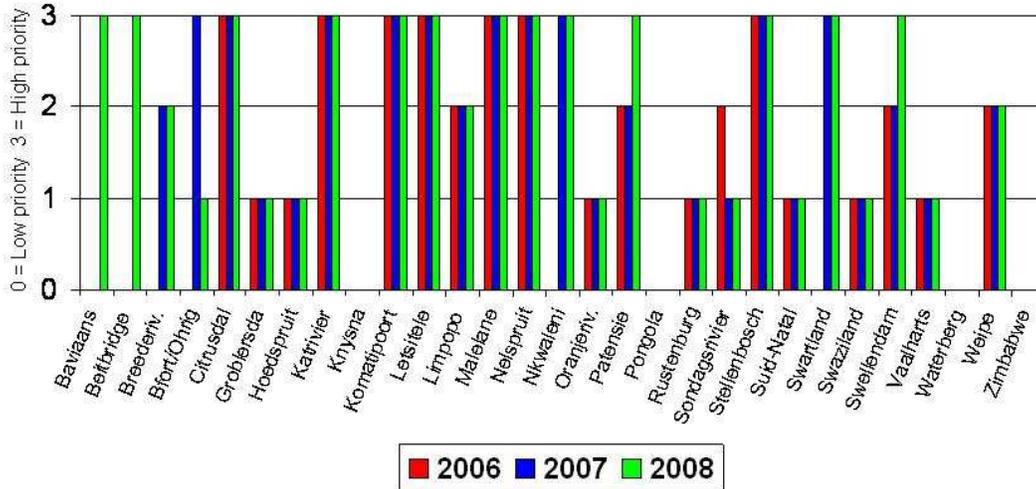
## INTERNAL QUALITY



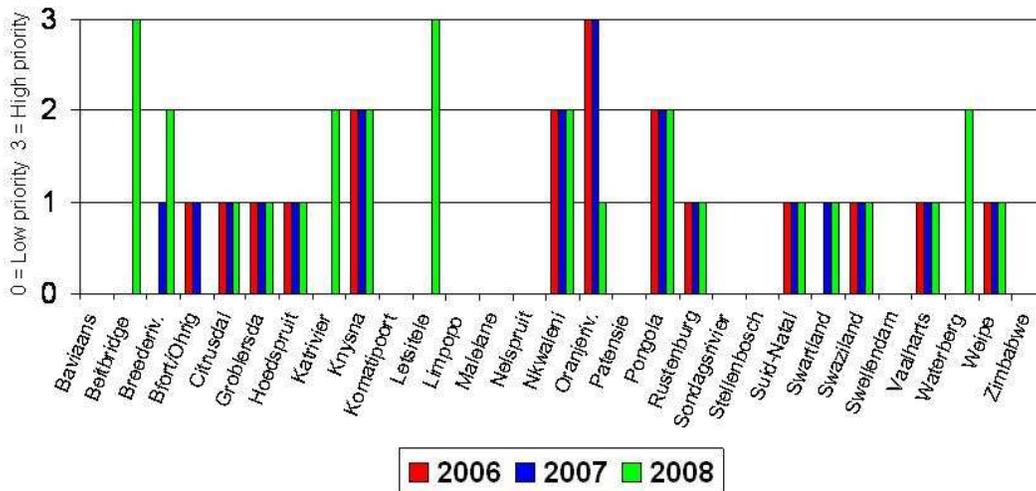
## COLOUR



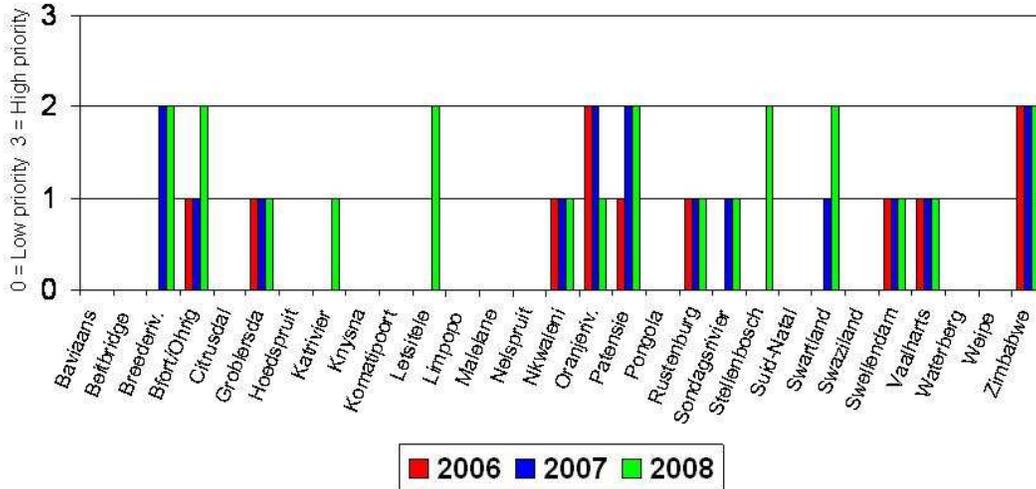
## RIND PITTING



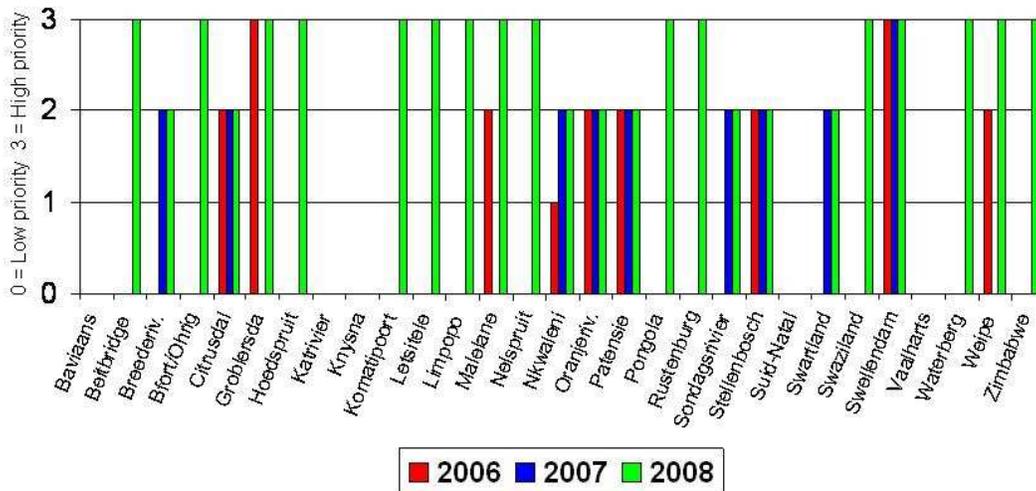
## PRUNING



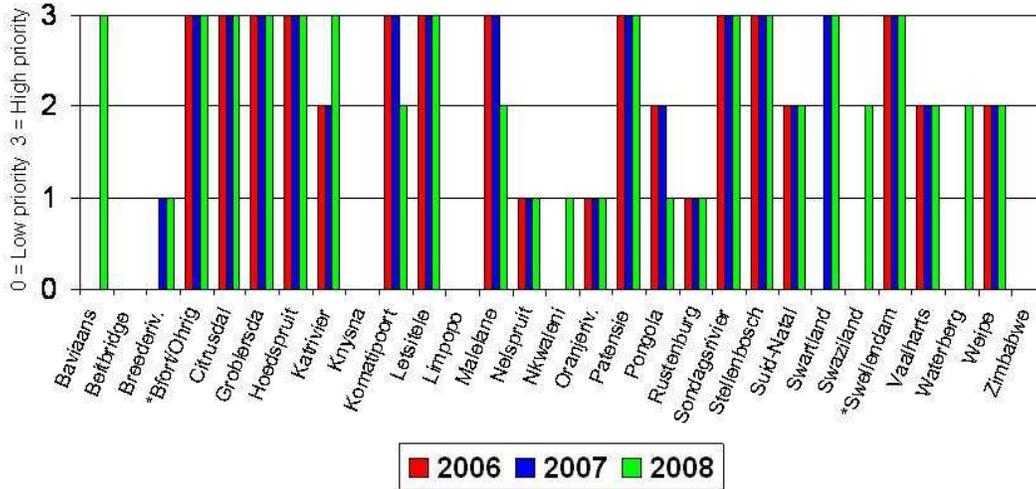
## GIRDLING



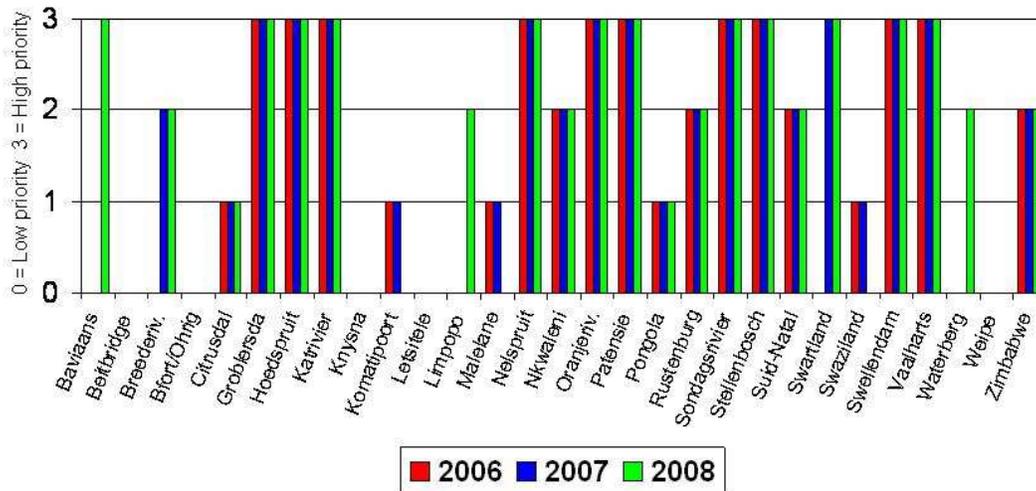
## NUTRITION



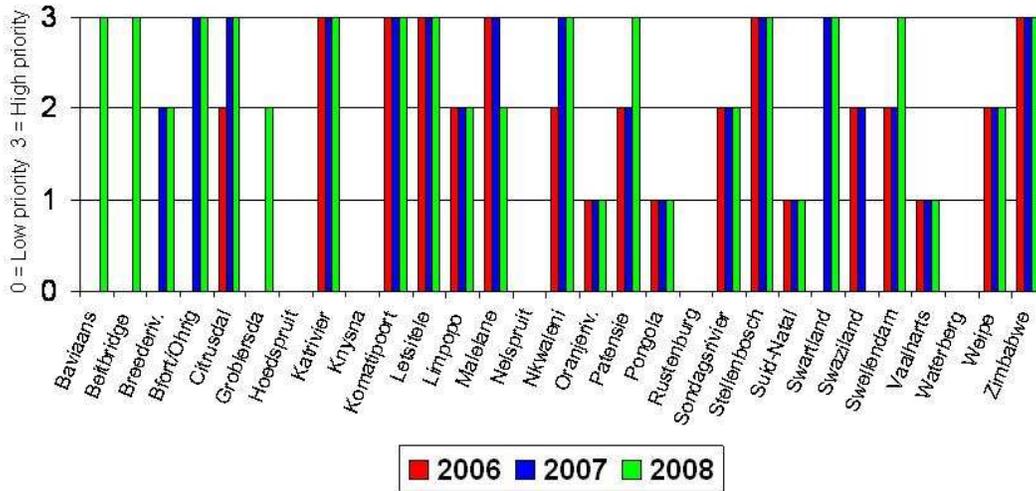
## CREASING



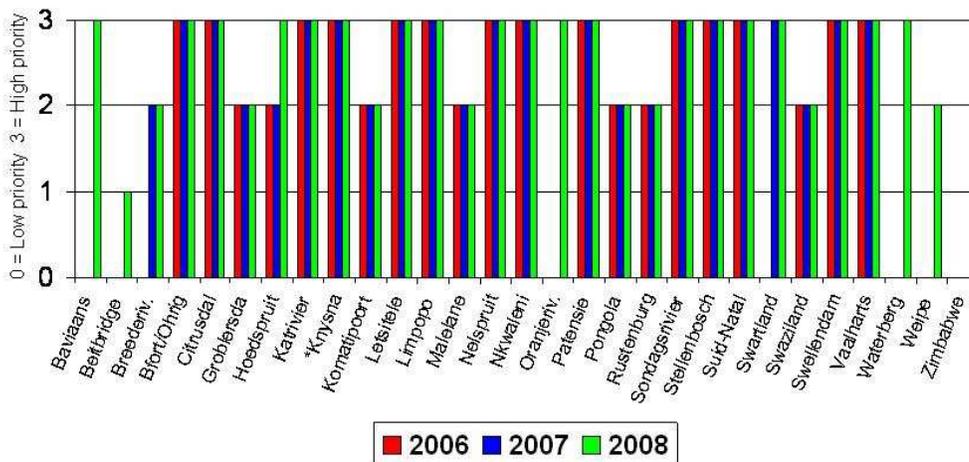
## PETECA



## RIND BREAKDOWN

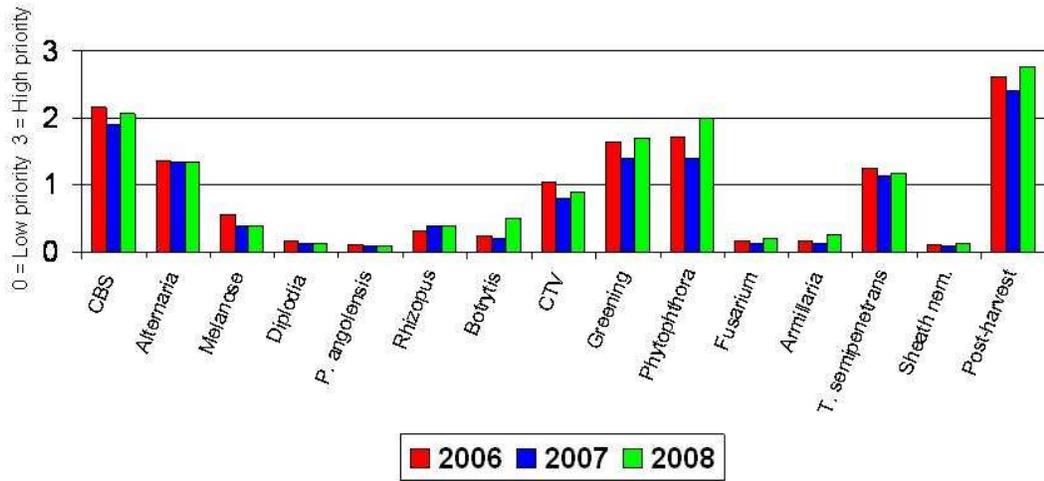


## CULTIVAR & ROOTSTOCK DEVELOPMENT

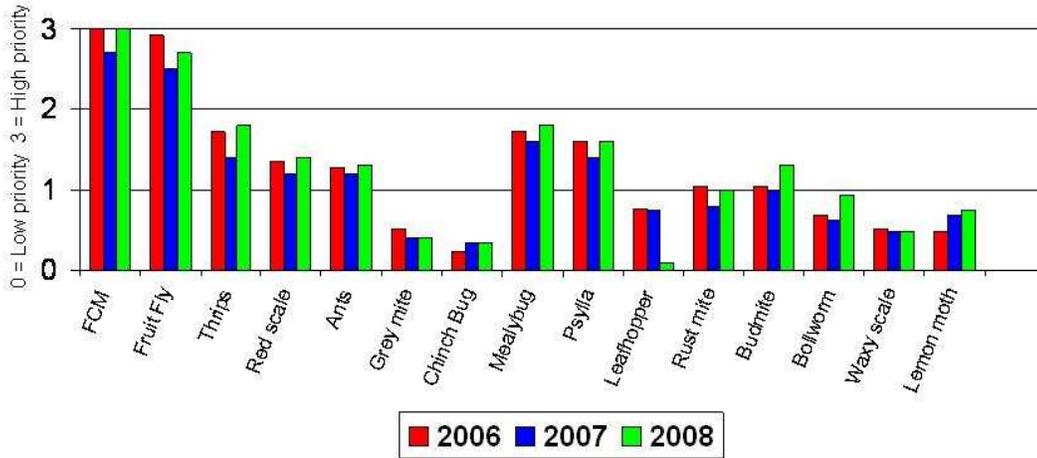


RESEARCH PRIORITIES - NATIONAL AVERAGES FOR THE RESEARCH PROGRAMMES

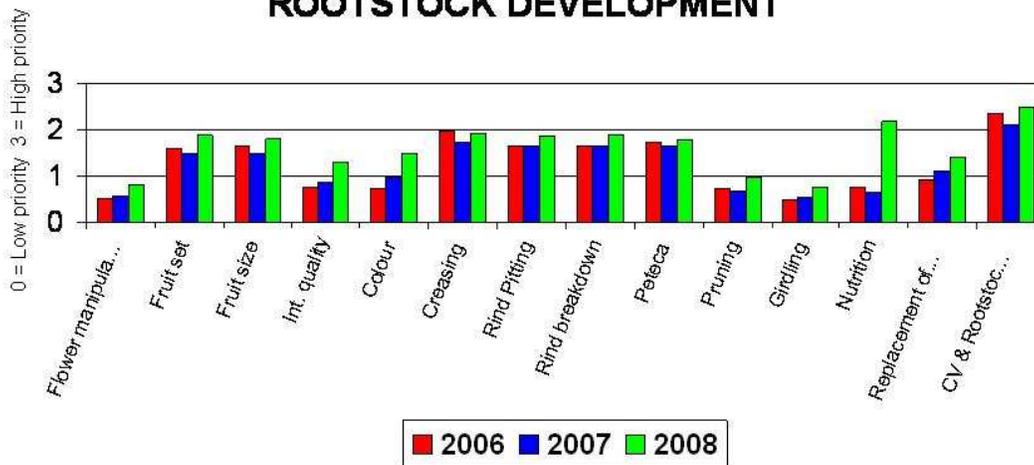
NATIONAL AVERAGES FOR DISEASE MANAGEMENT



## NATIONAL AVERAGES FOR INTEGRATED PEST MANAGEMENT



## NATIONAL AVERAGES FOR CROP & FRUIT QUALITY MANAGEMENT & CULTIVAR & ROOTSTOCK DEVELOPMENT



## 9.2 **TEGNOLOGIE OORDRAGINGS-GROEPE (TOG'e)**

CRI het gedurende 2007 sy Voorligtingsnetwerk verder uitgebrei deur Chris Maggs in Zimbabwe amptelik by die Tegnologie-oordragings-groepe in die noordelike deel van Zimbabwe betrokke te maak by die Voorligtingsnetwerk. Sy betrokkenheid is op dieselfde basis as die van die SASCCON lede wat in Suid Afrika betrokke is by die TOGs. Die Chegutu en Harare areas word nou as twee afsonderlike TOGs hanteer om die bywoning van vergaderings meer haalbaar te maak vir produsente.

Daar is 137 studiegroepvergaderings deur die twee CRI Voorligters en die SASCCON konsultante in 2007 belê. Waar nodig is gebruik gemaak van CRI navorsers om tegnologieoordraging te doen. Aangesien daar 'n groot behoefte was om die jongste ontwikkelings tov snoei aan te spreek, is dr Andy Krajewski uit Australië gekontrakteer om drie snoei werksinkels in Suid Afrika te hou. Een daarvan was in die Limpopo provinsie gehou, die tweede in die Oos Kaap en die derde in die Wes Kaap. Al drie die werksinkels is goed bygewoon so ook die studiegroepvergaderings. Al die onderwerpe waarvoor die TOGs 'n versoek geplaas het om te bespreek is hanteer. Gedurende Januarie tot Maart 2008 is 'verdere 33 vergaderings gehou saam met die bestaande sitrusstudiegroepe en met die Limpopo se Departement van Landbou om hulle by te staan om hulle Voorligtingsnetwerk tov sitrus te aktiveer.

Gedurende 2006 was daar verskeie versoeke van produsente dat sekere van die na-oesaktiwiteite en aspekte rakende die koueketting gekoördineer moes word om verliese wat ondervind word vanaf die pakhuis tot by die verbruiker te identifiseer en uit te skakel. Na verskeie vergaderings met die betrokke rolspelers is die Sitruskouekettingforum (CCCF) op 15 Februarie 2007 in Pretoria gestig. Tydens hierdie vergadering is besluit dat die CCCF deur die CRI se Voorligtingsdepartement bestuur moes word. Daar is besluit dat die CCCF oop lidmaatskap sal hê en uit vier werks-groepe sal bestaan. Hannes Bester is as voorsitter van die Forum aangewys. Die Verpakkingswerks-groep akkomodeer die rolspelers betrokke by die vervaardiging van verpakkingsmateriaal (papier, kartonne, pallette, houers), verpakking, palletisering, vervoer, verkoeling, laai en verskeping. Die Navorsingsprojek vir Kouekettingbestuur en Verpakking sal by die bestaande Oesgrootte en Vruggehalte Bestuursprogram van CRI inskakel en sluit die navorsing op vrugfisiologie, na-oeshantering, verkoeling en ventilasie in. Die projek-koördineerder is dr Malcolm Dodd van PPECB. Die Uitvoerders Tegnieese Paneel is die verteenwoordigers van die Uitvoeragente wat na die tegnieese behoeftes van die Uitvoerders moet omsien. Hannes Bester tree as sameroeper van hierdie groep op. Die Pakhuis- en Logistieke paneel bestaan uit pakhuisbestuurders en logistieke diensverskaffers sowel as die PPECB. Daar is vyf pakhuisstudiegroepe in die onderskeie streke nl. Die Wes Kaap, Oos Kaap, KZN & Swaziland, Mpumalanga en Limpopo. Onder leiding van Dawid Groenewald van SAPPI is daar heelwat werk op verpakking en palletisering gedoen deur die Verpakkingsforum. Terugvoer hieroor is aan die Uitvoerders Tegnieese Paneel en die Pakhuisstudiegroepe gegee.

Hoogs suksesvolle voorseisoen werksinkels is in Februarie 2008 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 fondse te ontsluit wat vir tegnieese ondersteuning in die koueketting aangewend kan word. Die rolspelers het onderneem die beginsel met hul onderskeie maatskappye uit te klaar, maar geen uitsluitel is tot dusver verkry nie.

Daar sal in die afsienbare toekoms kapasiteit gebou moet word om die CCCF volhoubaar te kan bestuur. Tans is daar feitlik geen hulpbronne om dit te doen nie. Die tweede probleem waarmee die sitrusbedryf sit, is dat die meeste kundiges in hierdie gebied minder as vyf jaar het voor hulle aftrede.

Koueskade het voorgekom in die Vaalharts, Vaalwater, Groblersdal, Marble Hall en Ohrigstad gebiede en sowat 2 miljoen uitvoerkartonne het verlore gegaan. Hierdie onderwerp is in die verlede afgeskeep en sal in die toekoms hanteer moet word.

In die geheel gesien het die bedryf 'n uitstekende uitvoerseisoen gehad. In Februarie 2007 is die aantal uitvoerkartonne nog op 76 miljoen geskat en is daar voorspel dat dit 'n kleinvrugjaar sou wees. Laat somerreëns in Februarie het die prentjie totaal verander en die vuggroottes was idiaal terwyl die interne gehalte uitstekend was. Uitpakpersentasies was bogemiddeld hoog en sowat 90 miljoen kartonne is uitgevoer. Behalwe vir die laat valencias was die uitvoerpryse ook gunstig.

Die betrokkenheid van konsultante by die vergaderings is puik en hulle doen uitstekende werk rakende tegnologie-oordraging en om die belangrikheid van die CRI navorsing, veral ten opsigte van fitosanitêre aspekte onder produsente se aandag te bring.

Die struktuur van die Tegnologie Oordragingsgroepe sien tans as volg daaruit:

<b>VOORSITTERS VAN TEGNOLOGIE OORDRAGINGSGROEPE 2007/8</b>				
<b>TOG</b>	<b>NAAM</b>	<b>TEL. NR.</b>	<b>FAKS NO.</b>	<b>EPOS</b>
Baviaans (Patensie)	Phillip Dempsey	082 498 2778		phillipdempsey@southernfruit.co.za
Beitbridge	Paul Bristow	072 701 9227	09263 862434	pbristow@iwayafrica.com
Benede-Oranjerivier	Francois Reyneke	082 771 6758	054-4310780	francois@karsten.co.za
Breederivier	Sakkie Bruwer	083 226 2540		izakbruwer@netactive.co.za
Burgersfort	Elbert de Kock	013-2317757	013-2318334	moronesitrus@telkomsa.net
Citrusdal	Otto Frielingsdorf	082 804 9054	022-9212511	otto@ghcitrus.com
Groblersdal/ Marble Hall	Gerda Burger	082 388 1041	013 262 6602	gerda@moosrivier.co.za
Hoedspruit	Pierre Malherbe	084 517 3378		driehoek@lantic.net
Katrivier	Bruce Knott	082 877 1164	046-6452345	j&bcitrus@bosberg.co.za
Komatipoort	Dirk Horn	013-7937536 / 083 259 3359	013-7937536	sommerreg@soft.co.za
Knysna	John Stanwix	082 789 5051	044-3884611	knycit@mweb.co.za
Letsitele	Pieter Vermaak	015 386 8718 082 491 7743	015-386 8718	nic@mweb.co.za
Limpopo	Bennie Nicholson	015-5390763 / 083 306 0552	015-5390718	alicedale@lantic.net
Malelane	Leon Esselen	013-7900160	013-7900492	esselenk@mweb.co.za
Nelspruit	Graham Piner	013-7538000 072 804 6495	013-7522560	crocval@mweb.co.za
Nkwalen	Shane Dellis	083 256 3650	035-4600634	valfarm@corpdial.co.za
Paarl/ Stellenbosch	Stephan Venter	083 670 8030	021-8733078	stephan@insectscience.co.za
Patensie	Ilze du Plessis	082 926 8086	042-2830893	ilzed@gamnet.co.za
Pongola	André Barnard	083 229 8539	034-4351083	mhlathi@idhweb.com
Richmond	Peter Button	082 488 8537		pbutton@futurenet.co.za
Rustenburg	Johan-Chris Grobler	082 922 1579	014-5733036	witkrans1@mweb.co.za

Sondagsrivier vallei	Dave Gerber	072 495 3162	042-2331037	technical@srcc.co.za
Swartland	Wietse Post	082 804 9054		wietse@clearsky.co.za
Swaziland	Gerd Höppner	09268-3232311	09268-3232317	ghoppner@swazican.co.sz
Swellendam	Sarel Neethling	028-5123606 / 082 551 2357	028-5123659	sarel@thornlands.net
Vaalharts	Tom Fouché	053-4710277 / 082 783 4842	053-4710277	marithaminnie@mweb.co.za
Waterberg	Peter Pullinger	082 322 0964 014-7432850	014-7432850	prp@netactive.co.za
Weipe	Danie Erasmus	083 236 7798	015 5330056	depoweipe@lantic.net
Zimbabwe	Chris Maggs	09263 11419624	09263 11419624	technical@interspan.co.za

9.3 **DIE RELATIEWE BEFONDSINGSONDERSTEUNING VIR NAVORSINGSPROGRAMME EN PROEKTJE VIR 2007**  
Deur Tim G Grout (CRI)

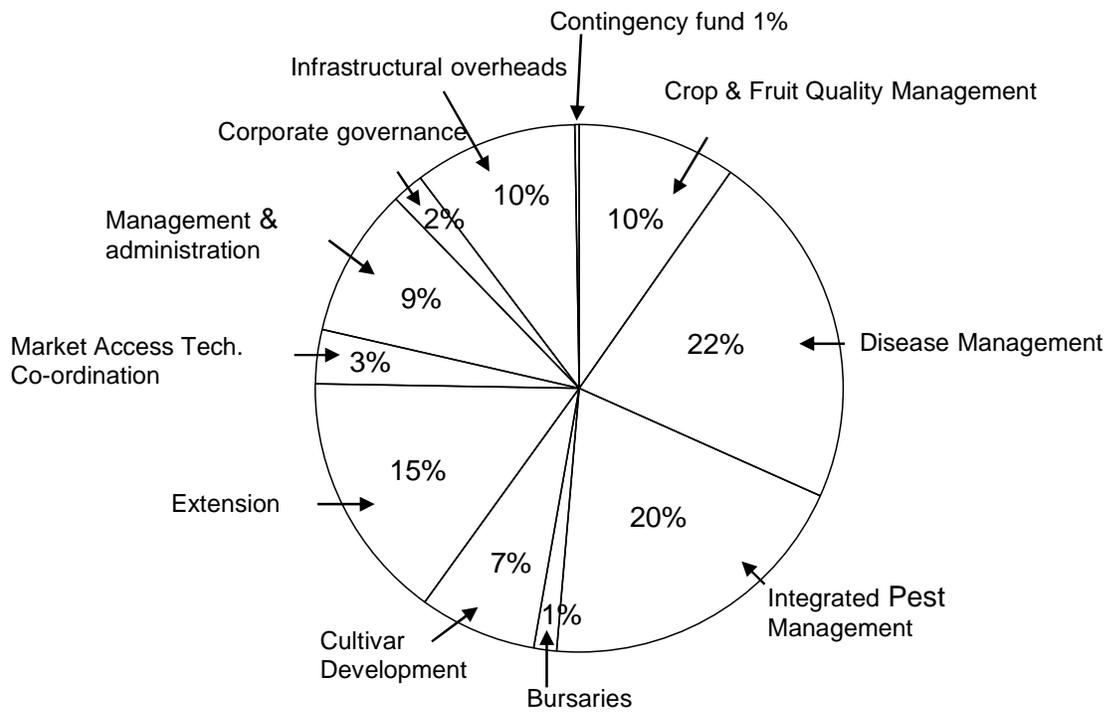
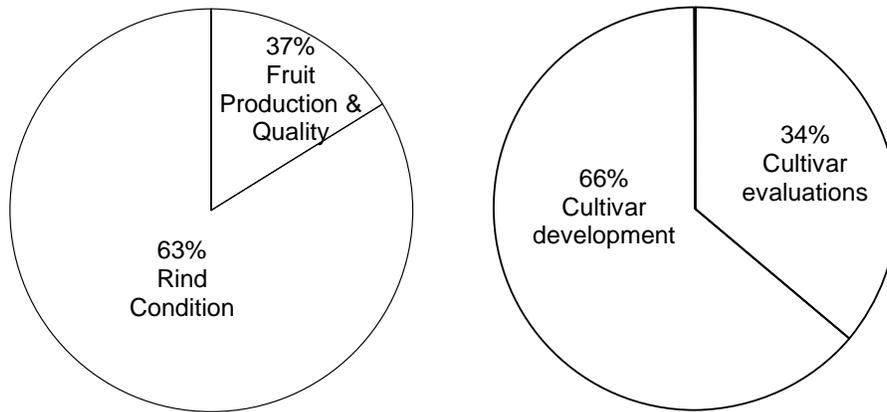
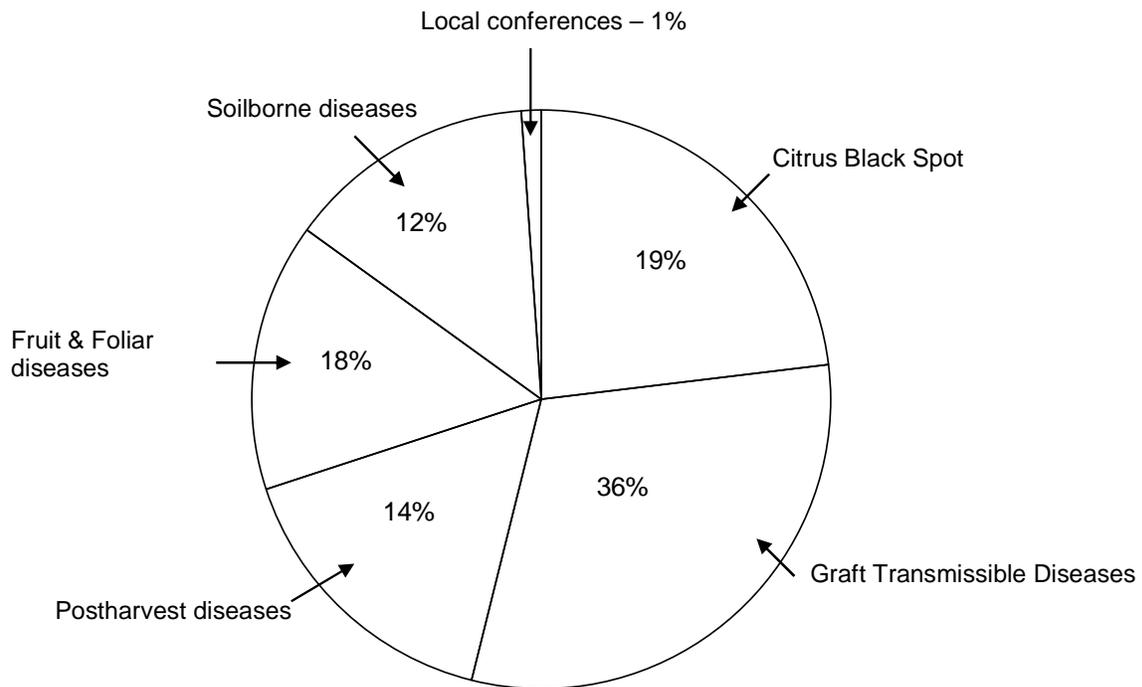


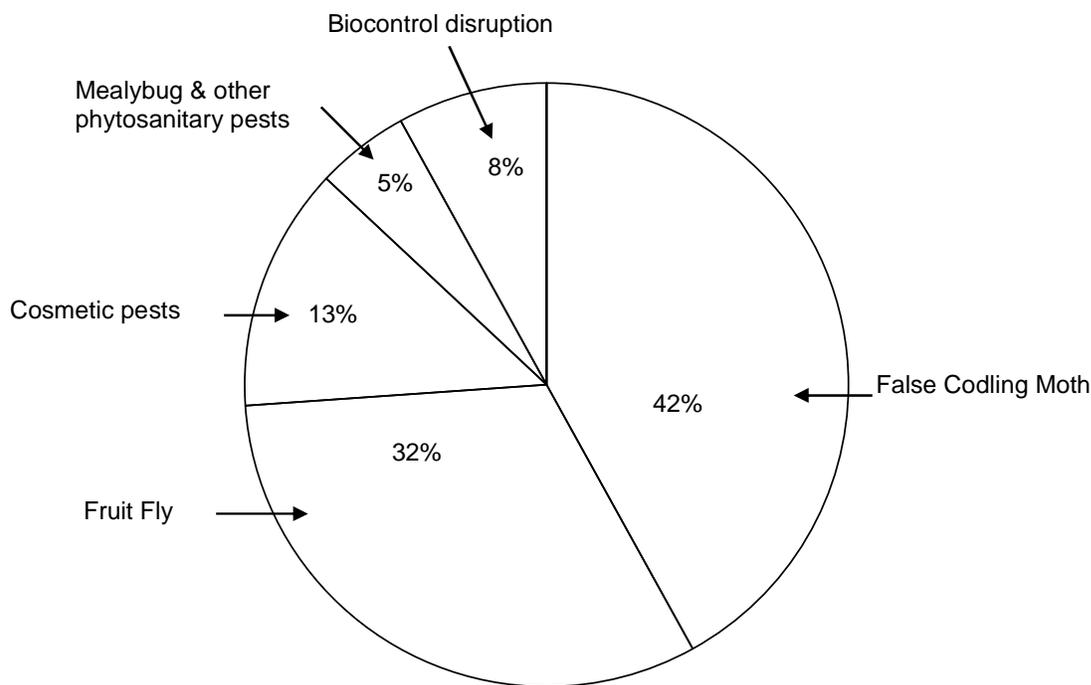
Fig. 9.3.1. Persentasie befondsing in elke CRI program en die res van die begroting vir 2007-8.



**Fig. 9.3.2.** Persentasie befondsing aan projekte in die CRI Navorsingsprogramme: Oes en Vrugkwaliteitsbestuur (links) en Kultivar en Onderstam Ontwikkeling (regs) vir 2007-8.



**Fig. 9.3.3.** Persentasie befondsing aan projekte in die CRI Navorsingsprogram: Siektebestuur vir 2007-8.



**Fig. 9.3.4.** Persentasie befondsing an projekte in die CRI Navorsingsprogram: Geïntegreerde Plaagbestuur vir 2007-8.

#### 9.4 VOORLIGTINGSAANBIEDINGS DEUR CRI GROEP NAVORSERS IN 2007

Naam	Datum	Plek	Onderwerp
Cronjé, P.J.R. (CRI)	07/03/07	Porterville	Na-oes hanteering van Mandaryn sitrus met spesiale fokus op vogverlies. Premium Mandarin Club.
	28/03/07	Sondagsrivier, Addo	Na-oes hanteering van Mandaryn sitrus met spesiale fokus op vogverlies. Premium Mandarin Club.
	29/03/07	Ohrigstad	Na-oes hanteering van Mandaryn sitrus met spesiale fokus op vogverlies. Premium Mandarin Club.
	10/04/07	Citrusdal	Skilkwaliteit: Fisiologiese defekte
	11/04/07	Paarl	Skilkwaliteit: Fisiologiese defekte
	25/04/07	Sondagsrivier, Addo	Skilkwaliteit: Fisiologiese defekte
	24/04/07	Fort Beaufort	Rind condition: Physiological defects
	Fourie, Paul (CRI)	11/05/07	Stellenbosch
05/06/07		Swaziland	Spuitbenaderings
06/06/07		Pongola	Spuitbenaderings
04/09/07		Hoedspruit	Spuitbenaderings
05/09/07		Tshipise, Weipe & Beit Bridge	Spuitbenaderings
06/09/07		Groblersdal, Marble Hall & Senwes	Spuitbenaderings
07/09/07		Nelspruit, Burgersfort, Ohrigstad, Malelane & Komatipoort	Spuitbenaderings
11/09/07		Katrivier	CBS Boordbesoeke
Grout, T.G. (CRI)	7 Feb 2007	White River	Villa Crop Protection workshop on citrus
	16 May 2007	Mazowe, Chegutu in Zimbabwe	Citrus IPM issues and Citrus spray machine overview
	5 Jun 2007	Letsitele	Managing pesticide residues on citrus

			(emergent farmers)
	4-6 Sep 2007	Hoedspruit, Tshipise, Groblersdal	Lente, die donker kant
	7 Sep 2007	Nelspruit	Spring, the dark side
	10-11 Sep 2007	Richmond, Nkwaleni	Spring, the dark side and Spraying citrus
	18 Sep 2007	Letsitele	Lente, die donker kant
	19-20 Feb 2008	Tambuti, Hectorspruit, Nelspruit	CGA levy talks
	29 Feb 2008	Hoedspruit	CGA levy talk
	13 Mar 2008	Burgersfort	Fruit fly and FCM management
Kirkman, W. (CRI)	24/04/07	Kat River	FCM, Fruit Fly
	25/04/07	Sundays River Valley	FCM, Budmite
Lesar, K.H. (CRI)	20/03/07	Tshipise	Packhouse Meeting Presentation Waste issues, treatments, waxing, new products, new research, etc.
	21/03/07	Tshipise	Alicedale Pachouse Meeting/Visit Advisory
	21/03/07	Tshipise	Maswiri Packhouse Meeting/Visit Advisory Waste issues, treatments, waxing, etc.
	21/03/07	Letsitele	C.P Minnaar Packhouse Meeting and Packhouse advisory visits As Above
	22/03/07	Letsitele	Packhouse Meeting Presentation Waste issues, treatments, waxing, new products, new research, etc.
	22/03/07	Hoedspruit	Portsmouth Packhouse Meeting/Visit Waste issues, treatments, waxing, new products, new research, etc.
	22/03/07	Hoedspruit	Packhouse meeting Presentation Waste issues, treatments, waxing, new products, new research, etc.
	23/03/07	Hoedspruit	Unifrutti Packhouse Meeting/Visit Waste issues, treatments, waxing, new products, new research, etc.
	23/03/07	Nelspruit	Packhouse meeting Presentation Waste issues, treatments, waxing, new products, new research, etc.
	27/03/07	Moosrivier	Packhouse Forum meeting Presentation Packhouse issues. Treatments etc.
	03/04/07	Stellenbosch	Packhouse Meeting Infruitec Packhouse issues. Treatments etc.
	10/04/07	Limpopo	Packhouse Forum Meeting Letsitele All issues related to Packhouses
	10/04/07	Hoedspruit	Laeveld Agrochem training meeting Waste issues, treatments, waxing, new products, new research, etc.
	1-10/05/07	Italy	Post-Harvest Congress –Presentation
	23/07/07	Citrusdal W.Cape	Packhouse Advisory Visits Five Packhouses Packhouse waste issues, treatments, waxing, new products, new research, etc.
	24/07/07	Piketberg W.Cape	Packhouse Advisory Visits Three Packhouses Packhouse waste issues, treatments, waxing, new products, new research, etc.
	25/07/07	Robertson Swellendam	Packhouse Advisory Visits Six Packhouses

		Ashton	Packhouse waste issues, treatments, waxing, new products, new research, etc.
	26/07/07	Stellenbosch Franschoek	Packhouse Advisory Visits Two Packhouses Packhouse waste issues, treatments, waxing, new products, new research, etc.
	04/09/07	Patensie E. Cape	Packhouse Advisory Visits Four Packhouses Packhouse waste issues, treatments, waxing, new products, new research, etc.
	05/09/07	SRCC E. Cape	Packhouse Advisory Visits Six Packhouses SRCC Meeting Packhouse waste issues, treatments, waxing, new products, new research, etc.
	06/09/07	Fort Beaufort E. Cape	Packhouse waste issues, treatments, waxing, new products, new research, etc.
Moore, S.D. (CRI)	20/02/07	Nelspruit	FCM management
			Corrective control of mealybug
	21/02/07	Malelane & Komatipoort	FCM management
			Corrective control of mealybug
		Swaziland & Pongola	FCM management
			Corrective control of mealybug
	27/02/07	Marble Hall	FCM management
		Burgersfort & Ohrigstad	Corrective control of mealybug
			FCM management
	28/02/07	Hoedspruit	FCM management
			Corrective control of mealybug
		Letsitele/Constantia	FCM management
			Corrective control of mealybug
	01/03/07	Tshipise, Weipe & Beit Bridge	FCM management
			Corrective control of mealybug
	01/08/07	Citrusdal	FCM management
	10/09/07	Nkwalini	FCM management
			Mealybug management
			Bollworm management
	11/09/07	S. KZN	FCM management
			Mealybug management
			Bollworm management
			Leafhopper management
12/09/07	Kat River	FCM management	
		Spring pest complex management Lemon borer moth management	
17/09/07	Sundays River	FCM management	
		Spring pest complex management	
18/09/07	Breederiver	FCM management	
		Spring pest complex management	
	Paarl, Stellenbosch, Swartland	FCM management	
		Spring pest complex management	
	Citrusdal	FCM management	
Spring pest complex management			
19/09/07	Benede Oranje-rivier	FCM management	
		Spring pest complex management	
20/09/07	Vaalharts	FCM management	
		Spring pest complex management	
Pietersen, G. (CRI-UP)	05/06/07	Swaziland	Greening
	06/06/07	Pongola	Greening
	11/06/07	Rustenburg	Greening
	12/06/07	Marble Hall/Groblersdal	Greening

		Pietersburg	Greening
	14/06/07	Burgersfort	Greening
		Nelspruit	Greening
	03/07/07	Nkwaleni	Greening
	04/07/07	Richmond	Greening
	16/07/07	Citrusdal	Greening
	17/07/07	Paarl/Stellenbosch/Swartland	Greening
	18/07/07	Breederivier	Greening
		Swellendam	Greening
Pretorius, M.C. (CRI)	10/04/07	Citrusdal	Bruinvrot Aalwurm en <i>Phytophthora</i> beheer
	11/04/07	Paarl / Stellenbosch	Bruinvrot Aalwurm en <i>Phytophthora</i> beheer
	11/04/07	Swartland	Bruinvrot Aalwurm en <i>Phytophthora</i> beheer
	12/04/07	Breederivier	Bruinvrot Aalwurm en <i>Phytophthora</i> beheer
	12/04/07	Swellendam	Bruinvrot Aalwurm en <i>Phytophthora</i> beheer
	10/07/07	Katrivier	Vergroening
	11/07/07	Kirkwood	Vergroening
		Addo	Vergroening
	12/07/07	Patensie	Vergroening
		Baviaans	Vergroening
Schutte, G.C. (CRI)	28/03/07	Citrusdal	Alternaria & Phytophthora
	19/04/07	Nelspruit (Avello)	Alternaria & Swartvlek
	30-31/05/07	Tshipese	Swartvlek
	01/06/07	Pretoria	Swartvlek werkwinkel
	01/08/07	Patensie	Alternaria kernvrot
	2&3/08/07	Paarl	Nexus opleiding
	04/09/07	Hoedspruit	Swartvlek
	05/09/07	Groblersdal	Swartvlek
	07/09/07	Nelspruit	Swartvlek
	11/09/07	Fort-Beaufort	Swartvlek werkwinkel
	12/09/07	Fort-Beaufort	Swartvlek
	12/09/07	Addo	Swartvlek & Alternaria
Verreynne, S. (CRI)	17/07/07	Malelane	Crop manipulation in citrus Rind condition: Physiological disorders in citrus
	17/07/07	Komatipoort	Crop manipulation in citrus Rind condition: Physiological disorders in citrus
	18/07/07	Swaziland	Crop manipulation in citrus Rind condition: Physiological disorders in citrus
	30/07/07	Marble Hall/Groblersdal	Crop manipulation in citrus
		Burgersfort/Ohrigstad	Crop manipulation in citrus
	31/07/07	Hoedspruit	Crop manipulation in citrus
		Constantia/Letsitele	Crop manipulation in citrus
	01/08/07	Potgietersrus	Crop manipulation in citrus
	01/08/07	Tshipise	Crop manipulation in citrus
	02/08/07	Beitbridge	Crop manipulation in citrus Rind condition: Physiological disorders in citrus
		Weipe	Crop manipulation in citrus
	12/10/07	Radio Sonder Grense (RSG)	Citrus research
<b>VOORLIGTING</b>			
<b>Datum</b>	<b>Vergadering</b>		<b>Onderwerpe en Sprekers</b>
18 Jan 2007	Na-oes en Verpakking		Bedryfsagtergrond (Vaughan Hattingh) Cooling and Ventilation (Malcolm Dodd)

		Verpakkingsprobleme (Dawid Groenewald) Packaging Forum
22-24 Jan 2007	SAVPP ,Benoni	Plantpatologie kongres
25 Jan 2007	Oos-Kaap CTA	Agenda
29 Jan 2007	Rustenburg Sitrusstudiegroep	Vrugset op Midknights & Deltas (J. Warrington) Gebruik van Pro-Gibb vir vrugset (I. Garden)
6 Feb 2007	Constantia Sitrusstudiegroep	Vergroening (Hennie le Roux)
07 Feb 2007	Villa-werkswinkel	Agenda
08 Feb 2007	Chinese afvaardiging	Besoek TSB en Neos saam met Chinese inspekteurs vir uitvoere na China.
13 Feb 2007	Andy Krajewski	Snoei
14 Feb 2007	Baviaans Sitrusstudiegroep	CGA Feedback (Justin Chadwick) Market access (Justin Chadwick) Transformation (Maxwell Hawes) Variety focus groups representatives CRI Aanwending v fondse (Hannes Bester)
	Patensie Sitrusstudiegroep	CGA Feedback (Justin Chadwick) Market access (Justin Chadwick) Transformation (Maxwell Hawes) Variety focus groups representatives CRI Aanwending v fondse (Hannes Bester)
15 Feb 2007	Katrivier Sitrusstudiegroep	CGA Feedback (Justin Chadwick) Market access (Justin Chadwick) Transformation (Maxwell Hawes) Variety focus groups representatives CRI Aanwending v fondse (Justin Chadwick)
	Verpakkingsforum	Bedryfsagtergrond (Vaughan Hattingh) Cooling and Ventilation (Malcolm Dodd) Verpakkingsprobleme (Dawid Groenewald) Model for CCCF (Vaughan Hattingh) Stigting van CCCF (Hannes Bester)
20 Feb 2007	Nelspruit Sitrusstudiegroep	CGA Terugvoer (Justin Chadwick) Transformation (Maxwell Hawes) Sitrusnavorsingsbefondsing (Hennie le Roux) Korrektiewe beheer van witluis (Sean Moore) VKM beheer (Sean Moore) CAL Dienste (Hannes Coetzee) Besproeiing & Plantvoeding (Hannes Coetzee) Vrugtevlug (Hennie le Roux)
21 Feb 2007	Malelane & Komatipoort Sitrusstudiegroep	Do
21 Feb 2007	Swaziland & Pongola Sitrusstudiegroep	Do
22 Feb 2007	Citrusdal & Swartland Sitrusstudiegroep	CGA Feedback (Paul Hardman) Variety focus groups representatives CRI Aanwending v fondse (Hannes Bester) Citrus Academy (Jacomien De Klerk)
	Paarl / Stellenbosch Sitrusstudiegroep	CGA Feedback (Paul Hardman) Variety focus groups representatives CGA representative CRI Aanwending v fondse (Hannes Bester) Citrus Academy (Jacomien De Klerk)
23 Feb 2007	Breederivier & Swellendam Sitrusstudiegroep	CGA Feedback (Paul Hardman) Variety focus groups representatives CGA representative CRI Aanwending v fondse (Hannes Bester) Citrus Academy (Jacomien De Klerk)
27 Feb 2007	Marble Hall	CGA Feedback (Paul Hardman)

	Sitrusstudiegroep	Sitrus Akademie Korrektiewe beheer van witluis VKM beheer CAL Dienste Besproeiing & Bemesting Vrugtevlieg	(Jacomien de Klerk) (S. Moore) (Sean Moore) (Hannes Coetzee) (H. Coetzee) (Hennie le Roux)
27 Feb 2007	Burgersfort & Ohrigstad Sitrusstudiegroep	Do	
28 Feb 2007	Hoedspruit Sitrusstudiegroep	Do	
28 Feb 2007	Letsitele/ Constantia Sitrusstudiegroep	Do	
28 Feb 2007	Exporters Technical Panel	Waste Control Verkoeling en Ventilasio Verpakkingsprobleme CCCF Behoeftes van Uitvoerders	(Hannes Bester) (Hannes Bester) (Dawid Groenewald) (Hannes Bester)
01 Mrt 2007	Tshipise & Weipe & Beitbrug Sitrusstudiegroepe	CGA Feedback Citrus Academy Korrektiewe beheer van witluis VKM beheer CAL Dienste Besproeiing & Bemesting Vrugtevliegbeheer	(Paul Hardman) (Jacomien de Klerk) (S. Moore) (Sean Moore) (Hannes Coetzee) (H. Coetzee) (Hennie le Roux)
02 Mrt 2007	Sondagsrivier Pakhuisstudiegroep	Waste Control Verkoeling en Ventilasio Verpakkingsprobleme CCCF Stigting van studiegroep Verpakkingsbehoefes	(Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester)
08 Mrt 2007	SASCCON Jaarvergadering	Agenda	
14 Mrt 2007	PSB Pakhuise	Waste Control Verkoeling en Ventilasio Verpakkingsprobleme	(Hannes Bester) (Hannes Bester) (Hannes Bester)
	Baviaans Sitrusstudiegroep	Boord- en oespraktyke Rypheidsindeksering Phytophthora Aalwurms Waste Control Verkoeling en Ventilasio Verpakkingsprobleme	(Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester)
20 Mrt 2007	KZN / Swaziland Packhouse Study Group	Waste Control New fungicides Cooling and Ventilation CCCF Logistical losses Formation of study group	(Hannes Bester) (Hygrotech) (Hannes Bester) (Hannes Bester) (Hannes Bester)
27 Mrt 2007	Mpumalanga Pakhuis - Sitrusstudiegroep	Pakhuisbehandelings Verkoeling en Ventilasio Sitrus Koueketting Forum Pakhuisstudiegroep	(Keith Lesar) (H. Le Roux) (H. Le Roux) (H. le Roux)
29 Mrt 2007	CMF	Agenda	
10 April 2007	Citrusdal	Boord- en Oespraktyke XSIT VSA GAP's VKM Monitoring Vrugtevliegbeheer GF 120 Fisiologiese Skildefekte Bruinvrot Phytophthora en Aalwurms	(Hannes Bester) (Piet Smit) (Sakkie Bruwer) (Rob Stotter) (Sakkie Bruwer) (Otto Frielingsdorf) (Paul Cronje) (MC Pretorius) (MC Pretorius)

	Letsitele	CCCF: Limpopo Pakhuisstudiegroep Stigtingsvergadering (Hennie le Roux) (Dawid Groenewald)
11 April 2007	Paarl / Stellenbosch	Boord- en Oespraktyke (Hannes Bester) VSA GAP's (Sakkie Bruwer) VKM-beheer (Sakkie Bruwer) Vrugtevliegbeheer (Sakkie Bruwer) Fisiologiese Skildefekte (Paul Cronje) Bruinvrot (MC Pretorius) Phytophthora en Aalwurms (MC Pretorius)
	Swartland	Boord- en Oespraktyke (Hannes Bester) VSA GAP's (Sakkie Bruwer) VKM-beheer (Sakkie Bruwer) Vrugtevliegbeheer (Sakkie Bruwer) Bruinvrot (MC Pretorius) Phytophthora en Aalwurms (MC Pretorius)
12 April 2007	Breederivier	Boord- en Oespraktyke (Hannes Bester) VSA GAP's (Sakkie Bruwer) VKM-beheer (Sakkie Bruwer) Vrugtevliegbeheer (Sakkie Bruwer) Fisiologiese Skildefekte (Hannes Bester) Bruinvrot (MC Pretorius) Phytophthora en Aalwurms (MC Pretorius)
	Swellendam	Boord- en Oespraktyke (Hannes Bester) VSA GAP's (Sakkie Bruwer) VKM-beheer (Sakkie Bruwer) Vrugtevliegbeheer (Sakkie Bruwer) Witluisbeheer (Sakkie Bruwer) Fisiologiese Skildefekte (Hannes Bester) Bruinvrot (MC Pretorius) Phytophthora en Aalwurms (MC Pretorius)
17 April 2007	Kakamas	Bederfbeheer (Hannes Bester) Verkoeling en ventilasie (Hannes Bester) CCCF (Hannes Bester) Sitrus Industrie (Hannes Bester) Boord- en Oespraktyke (Hannes Bester) Rypheidsindeksing (Hannes Bester) Phytophthora en aalwurms (Hannes Bester) VSA en Spesiale markte (Hannes Bester)
19 April 2007	Vaalharts	Pakhuisvergadering: Bederfbeheer (Hannes Bester) Verkoeling en ventilasie (Hannes Bester) CCCF (Hannes Bester) Studiegroep: CGA (Paul Hardman) Citrus Academy (Paul Hardman) CRI – Verdeling van Fondse (Hannes Bester) Boord- en Oespraktyke (Hannes Bester) Rypheidsindeksing (Hannes Bester) Phytophthora en Aalwurms (Hannes Bester)
	Hoedspruit	Phytophthora (Hennie le Roux)
24 April 2007	Katrivier	Orchard and harvesting practices (Hannes Bester) Brown rot (Hannes Bester) Phytophthora and nematodes (Hannes Bester) Physiological rind disorders (Paul Cronje)

		FCM control Fruitfly control Budmite control	(Wayne Kirkman) (Wayne Kirkman) (Wayne Kirkman)
25 April 2007	Sondagsrivier	Orchard and harvesting practices Brown rot Phytophthora and nematodes Physiological rind disorders FCM control Budmite control	(Hannes Bester) (Hannes Bester) (Hannes Bester) (Paul Cronje) (Wayne Kirkman) (Wayne Kirkman)
25 April 2007	Oos-Kaap CTA	Agenda	
30 April 2007	Rustenburg	Japanese Produsente Groep	(Hennie le Roux)
3 Mei 2007	PPECB Pre-season meeting	Agenda CCCCF	(Hannes Bester)
6-9 Mei 2007	Port Elizabeth	Nematologie simposium	(Hennie le Roux)
10 Mei 2007	Stellenbosch	CBS Marktoegangvergadering rakende Wes Kaapdistrikte, nog nie CBS vry verklaar, met NDA	(Hennie le Roux)
11 Mei 2007	Stellenbosch	Entomofage nematode bespreking met Prof L. Duncan (Univ. Florida) en Paul Fourie.	(Hennie le Roux)
16 Mei 2007	Baviaans	Snoei Sitrusverbeteringskema Vergroening Ontgroening	(Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester)
17 Mei 2007	Bothaville	NAMPO oesdag. Werwing van borge vir volgende Sitrusnavorsingsimposium.	(Hennie le Roux & Henry Skinner)
21 Mei 2007	Packaging Forum beplanning	Dawid Groenewald Hennie le Roux Hannes Bester	
22 Mei 2007	Natal Middellande	Orchard and harvesting practices Pruning Workshop	(HB en HIR) (HB en HIR)
23 Mei 2007	Suid-Natal	Orchard and harvesting practices Pruning Workshop	(HB en HIR) (HB en HIR)
28 Mei 2007	SRCC	Skilprobleme: Dun skille Antrachnose Diplodia Bemestingtekorte	
	Capespan	Karton spesifikasies Palet spesifikasies Hi-cube containers Warmlaai van vrugte	(DG / RK / HB) (DG / RK / HB) (DG / RK / HB) (DG / RK / HB)
	Dole	Karton spesifikasies Palet spesifikasies Hi-cube containers Warmlaai van vrugte	(DG / RK / HB) (DG / RK / HB) (DG / RK / HB) (DG / RK / HB)

	Colors	Karton spesifikasies Palet spesifikasies Hi-cube containers Warmlaai van vrugte	(DG / RK / HB) (DG / RK / HB) (DG / RK / HB) (DG / RK / HB)
	Afrifresh	Karton spesifikasies Palet spesifikasies Hi-cube containers Warmlaai van vrugte	(DG / RK / HB) (DG / RK / HB) (DG / RK / HB) (DG / RK / HB)
30 Mei 2007	Weipe	CBS Marktoegang vir Messina & Soutpansberg (V. Hattingh, H. Le Roux, T. Grout, P. Fourie, T. Schutte & NDA)	
31 Mei 2007	Citrus Academy strategic planning	Agenda	
1 Junie 2007	CBS (Terugvoer aan EU vergadering) Pretoria	CBS/EU Werkswinkel (V. Hattingh, JM Kotze, H le Roux, T. Grout, P. Fourie, T. Schutte, L. Korsten, I. Paul, C. Kellerman & NDA)	
5 Junie 2007	Swaziland	Spuitbenaderings Vergroening Snoei Bemesting	(Paul Fourie) (Gerhard Pietersen) (Hannes Bester) (W. Van Rooijen)
6 Junie 2007	Pongola	Spuitbenaderings Vergroening Snoei	(Paul Fourie) (Gerhard Pietersen) (Hannes Bester)
7 Junie 2007	Hoedspruit	Vergroening Snoei	(Hennie le Roux) (Hannes Bester)
8 Junie 2007	Bayer Vergadering	Agenda	
11 Junie 2007	Rustenburg	Snoei werkswinkel Vergroening	(Hannes Bester ) (Gerhard Pietersen)
12 Junie 2007	Malelane/Groblersdal	Snoeiwerkswinkel Vergroening	(Hannes Bester) (Gerhard Pietersen)
	Waterberg (Vaalwater)	Snoeiwerkswinkel Kouebestuur Vergroening	(Hannes Bester) (Hannes Bester) (Gerhard Pietersen)
13 Junie 2007	Burgersfort	Snoeiwerkswinkel Vergroening	(Hannes Bester) (Gerhard Pietersen)
14 Junie 2007	Komatipoort	Snoei Vergroening	(Hannes Bester) (Gerhard Pietersen)
	Nelspruit	Snoei Vergroening	(Hannes Bester) (Gerhard Pietersen)
18 Junie 2007	M3 vergadering (Pretoria)	M3 bemarkingsvergadering met Green Trading) (V. Hattingh, H le Roux)	
19 Junie 2007	M3 vergadering (Pretoria)	M3 bemarkingsvergadering met I. Bruwer en Green Trading (H le Roux).	
20 Junie 2007	Malelane	Kultivardag by Esselen Kwekery (F Veldman, Leon Esselen, Hennie le Roux, Johan Joubert en Fanie v Vuuren)	

25 Julie 2007	CRI Raads-vergadering (Jhb)	Agenda.	(H le Roux)
26 Junie 2007	Packaging Forum vergadering	Agenda (H le Roux, D Groenewald, H Bester): Palette Vervoer Hi-cube containers Depots	
27 Junie 2007	Benede-Oranjerivier	Bemesting Sitrusverbeteringskema Snoei Rypskade Navorsingsprioriteite	(Willie v Rooijen) (Hannes Bester) (Hannes Bester) (Hannes Bester) (Hannes Bester)
3 Julie 2007	Nkwalini	Greening Pruning Research priorities	(Gerhard Pietersen) (Hannes Bester)
4 Julie 2007	Suid-Natal	Greening CIP Research priorities	(Gerhard Pietersen) (Hannes Bester)
5 Julie 2007	Constantia	Snoeiwerkswinkel	(Dr. A. Krajewski)
6 Julie 2007	Vaalharts	Bemesting SVS Snoei Navorsingsprioriteite	(Willie van Rooijen) (Hannes Bester) (Hannes Bester)
7-14 Julie 07	Mosselbaai, George & Knysna distrikte	Swartvlekondersoek (Hennie le Roux, Elma Carstens & NDA)	
10 Julie 2007	Katrivier	Fertilization Greening Pruning Research Priorities	(Willie van Rooijen) (MC Pretorius) (Hannes Bester)
11 Julie 2007	Sondagsrivier (Kirkwood)	Bemesting Vergroening Snoei	(Willie van Rooijen) (MC Pretorius) (Hannes Bester)
	Sondagsrivier (Addo)	Bemesting Vergroening Snoei	(Willie van Rooijen) (MC Pretorius) (Hannes Bester)
12 Julie 2007	Patensie	Bemesting Snoei Vergroening Navorsingsprioriteite	(Willie van Rooijen) (Hannes Bester) (MC Pretorius)
	Baviaans	Bemesting Fisiologiese gebreke Vergroening Navorsingsprioriteite	(Willie van Rooijen) (Hannes Bester) (MC Pretorius)
13 Julie 2007	Oos-Kaap CTA	Snoeiwerkswinkel	(Dr A. Krajewski)
	Citrusdal	Vergroening Snoei Bemesting Navorsingsprioriteite	(Gerhard Pietersen) (Hannes Bester) (Willie van Rooijen)

	Nelspruit	Droogtebestuurwerkswinkel (Hennie leRoux, Steve Burdette & Australiërs)
	Nelspruit	Skildefekte (S Verreynne) Oesmanipulasie (S Verreynne) Navorsingsprioriteite
17 Julie 2007	Paarl / Stellenbosch en Swartland	Vergroening (Gerhard Pietersen) Snoei (Hannes Bester) Bemesting (Willie van Rooijen) Navorsingsprioriteite
	DFPT	CCCF presentation (Hannes Bester) Packaging Forum (Dawid Groenewald)
	Malelane	Skildefekte (S Verreynne) Oesmanipulasie (S Verreynne) Navorsingsprioriteite
	Komatipoort	Skildefekte (S Verreynne) Oesmanipulasie (S Verreynne) Navorsingsprioriteite
18 Julie 2007	Swaziland	Rind disorders (S Verreynne) Crop manipulation (S Verreynne) Research Priorities
18 Julie 2007	Breederivier	Vergroening (Gerhard Pietersen) Snoei (Hannes Bester) Bemesting (Willie van Rooijen) Navorsingsprioriteite
	Swellendam	Vergroening (Gerhard Pietersen) Snoei (Hannes Bester) Bemesting (Willie van Rooijen) Navorsingsprioriteite
23 Julie 2007	Polokwane	Signing of MoU with Limpopo DA (Hennie le Roux, Maxwell Hawes, Bigman Maloa)
24 Julie 2007	Nelspruit	Lowveld Lemon: Suurlemoen produksie vir prosesering & Residue (Izak Bruwer)
25 Julie 2007	Rustenburg	Vrugset op Midnights (J Warrington) Pro-gibb (Ian Garden) Navorsingsprioriteite
30 Julie 2007	Marble Hall/ Groblersdal	Skildefekte (P Cronjé) Oesmanipulasie (S Verreynne) Navorsingsprioriteite
	Burgersfort/ Ohrigstad	Skildefekte (P Cronjé) Oesmanipulasie (S Verreynne) Navorsingsprioriteite
31 Julie 2007	Hoedspruit	Skildefekte (P Cronjé) Oesmanipulasie (S Verreynne) Navorsingsprioriteite
	Constantia/ Letsitele	Skildefekte (P Cronjé) Oesmanipulasie (S Verreynne) Vergroening (Hennie le Roux) Navorsingsprioriteite
1 Aug 2007	Fitosanitiere Komitee	VSA GAP's SIT
	Citrusdal	VSA Bestuursprotokolle (Otto Frielingsdorf) Witluisbeheer (Sakkie Bruwer) FCM (Sean Moore) SIT update (Sampie Groenewald)
1 Aug 2007	Waterberg	Skildefekte (P Cronjé) Oesmanipulasie (S Verreynne) Navorsingsprioriteite

	Tshipise	Skildefekte Oesmanipulasie Navorsingsprioriteite	(P Cronjé) (S Verreyne)
2 Aug 2007	Beitbridge	Rind disorders Crop manipulation Research priorities	(S Verreyne) (S Verreyne)
	Weipe	Skildefekte Oesmanipulasie Navorsingsprioriteite	(P Cronjé) (S Verreyne)
	Wes-Kaap Pakhuis-studiegroep	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
	Exporters Technical Panel	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
6 Aug 2007	Mpumalanga Pakhuis studiegroep	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
7 Aug 2007	Limpopo Pakhuis-studiegroep	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
17 Aug 2007	Nelspruit	<i>Alternaria</i> <i>Phytophthora</i> Swartvlek	(Spanjaard) (Spanjaard) (Tian Schutte)
14 Aug 2007	Oos-Kaap Pakhuis-studiegroep	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
20 Aug 2007	Onderberg & Swaziland	Organiese sitrusverbouing	(Jannie Spannenberg, Deon Begemann en Gerd Höppner)
	KZN en Swaziland Pakhuisstudiegroep	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
21 Aug 2007	Limpopo-Hoedspruit Pakhuistudiegroep	Packaging Forum terugvoer Navorsingsprioriteite	(Dawid Groenewald) (Hannes Bester)
28 Aug 2007	Patensie	FCM Vrugtevlug Witluis	(Sakkie Bruwer) (Sakkie Bruwer) (Sakkie Bruwer)
	Baviaans	FCM Vrugtevlug Witluis	(Sakkie Bruwer) (Sakkie Bruwer) (Sakkie Bruwer)
30 Aug 2007	Brits	Vergroening/Koejawel ondersoek	(Gerhard Pietersen) (Hennie le Roux)
4 Sept 2007		Agri BEE Workshop (Hennie le Roux, Maxwell Hawes)	
	Hoedspruit	Lentepaagkompleks Swartvlek Spuitsbeginsels Vrugsetstrategie	(Tim Grout) (Tian Schutte) (Paul Fourie) (Ian Garden)
5 Sept 2007	Tshipise insluitende Weipe & Beitbrug	Lentepaagkompleks Spuitsbeginsels Vrugsetstrategie	(Tim Grout) (Paul Fourie) (Ian Garden)
6 Sept 2007	Grobbersdal & Marble Hall & SENWES	Lentepaagkompleks Swartvlek Spuitsbeginsels	(Tim Grout) (Paul Fourie) (Ian Garden)
7 Sept 2007	Nelspruit insluitende Burgersfort, Ohrigstad, Malelane & Komatipoort	Lentepaagkompleks Swartvlek Spuitsbeginsels	(Tim Grout) (Tian Schutte) (Paul Fourie)
10 Sept 2007	Nkwalini	Red scale	(Tim Grout)

		Thrips Fruit fly Spraying FCM Bollworm Mealybug	(Tim Grout) (Tim Grout) (Tim Grout) (Sean Moore) (Sean Moore) (Sean Moore)
11 Sept 2007	Southern Natal	Red scale Thrips Fruit fly Spraying FCM Bollworm Mealybug Red mite Leafhopper Psylla	(Tim Grout) (Tim Grout) (Tim Grout) (Tim Grout) (Sean Moore) (Sean Moore) (Sean Moore) (Tim Grout) (Tim Grout) (Sean Moore)
11 Sept 2007	Katrivier	CBS Boordbesoeke	(BK/HIR/TS/PF/HB)
	Katrivier	CBS Workshop	(BK/HIR/TS/PF/HB)
12 Sept 2007	Katrivier	CBS FCM Spring pest complex Lemon borer moth Bollworm Spray application in Citrus	(Tian Schutte) (Sean Moore) (Sean Moore) (Sean Moore) (Sean Moore) (Paul Fourie)
	Sondagsrivier	Spuittoediening op Sitrus CBS Alternaria FCM Lenteplaagkompleks	(Paul Fourie) (Tian Schutte) (Tian Schutte) (Sean Moore) (Dave Gerber)
13 Sept 2007	Patensie	CBS FCM Lenteplaagkompleks	(Hennie le Roux) (Sakkie Bruwer) (Sakkie Bruwer)
	Baviaans	CBS FCM Lente-plaagkompleks	(Hennie le Roux) Sakkie Bruwer) (Sakkie Bruwer)
17 Sept 2007	Swellendam	Lenteplaagkompleks <ul style="list-style-type: none"> <li>• Psylla</li> <li>• Bolwurm</li> <li>• Blaaspootjie</li> <li>• Witluis</li> <li>• Roodopluis</li> <li>• Roomyt</li> <li>• Knopmyt</li> <li>• Bladspringer</li> </ul> FCM VSA Protokolle	(Sean Moore)         (Sean Moore) (Sakkie Bruwer)
18 Sept 2007	Letsitele	Lenteplaagkompleks Swartvlek	(Tim Grout) (Tian Schutte)

18 Sept 2007	Breederivier	<p>Lenteplaagkompleks (Sean Moore)</p> <ul style="list-style-type: none"> <li>• Psylla</li> <li>• Bolwurm</li> <li>• Blaaspootjie</li> <li>• Witluis</li> <li>• Rooidopluis</li> <li>• Rooimyt</li> <li>• Knopmyt</li> <li>• Bladspringer</li> </ul> <p>FCM (Sean Moore) VSA Protokolle (Sakkie Bruwer)</p>
	Paarl / Stellenbosch en Swartland	<p>Lenteplaagkompleks (Sean Moore)</p> <ul style="list-style-type: none"> <li>• Psylla</li> <li>• Bolwurm</li> <li>• Blaaspootjie</li> <li>• Witluis</li> <li>• Rooidopluis</li> <li>• Rooimyt</li> <li>• Knopmyt</li> <li>• Bladspringer</li> </ul> <p>FCM (Sean Moore) VSA Protokolle (Sakkie Bruwer)</p>
	Citrusdal	<p>Lenteplaagkompleks (Sean Moore)</p> <ul style="list-style-type: none"> <li>• Bolwurm</li> <li>• Blaaspootjie</li> <li>• Witluis</li> <li>• Rooidopluis</li> <li>• Rooimyt</li> <li>• Knopmyt</li> <li>• Bladspringer</li> </ul> <p>FCM (Sean Moore) Alternaria (Otto Frielingsdorf) VSA Protokolle (Sakkie Bruwer)</p>
19 Sept 2007	Benede Oranje-rivier	<p>Lenteplaagkompleks (Sean Moore)</p> <ul style="list-style-type: none"> <li>• Bolwurm</li> <li>• Blaaspootjie</li> <li>• Witluis</li> <li>• Rooidopluis</li> <li>• Rooimyt</li> <li>• Knopmyt</li> <li>• Bladspringer</li> </ul> <p>FCM (Sean Moore) Vrugtevlieg (Sean Moore) VSA Protokolle (Hannes Bester)</p>
20 Sept 2007	Vaalharts	<p>Lenteplaagkompleks (Sean Moore)</p> <ul style="list-style-type: none"> <li>• Bolwurm</li> <li>• Blaaspootjie</li> <li>• Witluis</li> <li>• Rooidopluis</li> <li>• Rooimyt</li> <li>• Knopmyt</li> <li>• Bladspringer</li> </ul> <p>FCM (Sean Moore) Vrugtevlieg (Sean Moore) VSA Protokolle (Hannes Bester)</p>
25 Sept 2007	Winterveldt	Besoek Winterveld BEE projek (Hennie le Roux)



23 Jan 2008	Beitbrug	Studiegroepvergadering: Sitrusverbeteringskema, VKM, Myte, Wolluis	HIR, TdT, SM
24 Jan 2008	Chegutu	Studiegroepvergadering: VKM ea peste en plae, Eksotiese sitrussiektes	HIR, TdT, SM
	Mazoe	Studiegroepvergadering: VKM ea peste en plae, Eksotiese sitrussiektes	HIR, TdT, SM
25-26 Jan 2008	Produsol Kwekery	Gee riglyne vir 'n geakkrediteerde sitruskwekery	TdT, HIR
30-31 Jan 2008	CRI Management Meeting	Agenda	VH/HIR/TG/TdT/AL/HB
4-5 Feb 2008	Wes-Kaap Pakhuisstudiegroep Werkswinkel	Rypheidsindeksering Pakhuispraktyke Verkoeling en Ventilasioe 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	Rypheidsindeksering Pakhuispraktyke Verkoeling en Ventilasioe 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
8 Feb 2008	Verpakkingswerkgroep	Strategiese Sessie	VH/HIR/HB
11-12 Feb 2008	Limpopo Pakhuisstudiegroep Werkswinkel	Rypheidsindeksering Pakhuispraktyke Verkoeling en Ventilasioe 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 Feb 2008	Mpumalanga Pakhuisstudiegroep Werkswinkel	Rypheidsindeksering Pakhuispraktyke Verkoeling en Ventilasioe 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
20 Feb 2008	Logistics meeting	Logistics	HB/LG
	5 <sup>de</sup> Sitrusnavorsings-simposium	Beplanningsvergadering	Champagne Sports Resort, Bayer, HIR, HS, JdG.
21-22 Feb 2008	KZN Packhouse Study Group Workshop	Maturity Indexing Packhouse Practices	Hannes Bester Keith Lesar

		Cooling and Ventilation Physiological Disorders Time and temp protocols Packaging Work Group PPECB Logistics	Hannes Bester Paul Cronje Hannes Bester Dawid Groenewald Cyril Julius Lynette Grobler
26 Feb 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 Feb 2008	Katrivier CGA meeting	Statutory Levies	PH/VH/HB
28 Feb 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 Feb 2008	Magalies	Winterveldt BEE	HIR
4 Mrt 2008	Benede-Oranjerivier CGA vergadering	Statutêre Heffings	JC/HB/MvN
5 Mrt 2008	Vaalharts CGA vergadering	Statutêre Heffings	JC/HB/MvN
6 Mrt 2008	VSA Werkswinkel	Agenda	HB
10 Mrt 2008	Limpopo Extension	MoU discussions & organogram	AM
11 Mrt 2008	Southern Fruit Growers	Fruitnet	HB/PD
12 Mrt 2008	CMF	Agenda	HB
13 Mrt 2008	Studiegroepvergadering: Burgersfort & Ohrigstad	VKM. Vrugtevlieg, Myte. Phytophthora bruinvrot, Post harvest	HIR, TG, KL. MCP
18 Mrt 2008	Verpakkings-vergadering	Min specs en protokolle	HB/DG/RK
19 Mrt 2008	Cultivar meeting	Agenda	AL/TdT/HB/JJ
26 Mrt 2008	Angolese ambassade	Bespreek fitosanitêre aangeleenthede	HIR
27 Mrt 2008	Southern Fruit Growers	Fruitnet	HB/PD
	Soetdoring Boerdery Dendron	Yster chlorose/ Swingle probleme	HIR

## 9.5 ANDER MANIERE VAN TEGNOLOGIE OORDRAGING

### 9.5.1 SA Vrugtejoernaal deur 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 2007/8 are listed in Table 9.5.1.1. 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.

**Tabel 9.5.1.1.** S.A. Vrugtejoernaal artikels geduring 2007-8.

Uitgawe	Artikel	Skrywer
Des/Jan 07	4 <sup>th</sup> Citrus Research Symposium, Port Elizabeth	H.F. le Roux
	River Bioscience receives top awards	S.D. Moore
	CRI award winners at Eskom Science Expo's	T.G. Grout
Feb/Maart	Gerhard Pietersen re-appointed	G. Pietersen
April/Mei	A study of alternative hosts for the false codling moth, <i>Thaumatotibia</i> (=Cryptophlebia) <i>leucotreta</i> in the Eastern Cape	W. Kirkman S.D. Moore
Junie/Julie	Stigting van Citrus Cold Chain Forum/ Formation of a Citrus Cold Chain Forum	J.J. Bester & V. Hattingh
	Vergroening: Gee nou aandag aan die probleem!	H. le Roux, G. Pietersen, S.P. van Vuuren, J.J. Bester & M.C. Pretorius
Aug/Sept	Post-harvest control of grain chinch bug (Heteroptera: Lygaeidae) on citrus using pyrethrum	T.G. Grout & B. Tate
	Alternate bearing in citrus	J.S. Verreyne
Okt/Nov	Preliminary evaluation of 'Turkey' orange on various rootstocks in Citrusdal, Western Cape Province	C. Alexander (Consultant to CRI)
Des/Jan 08	Horticultural mineral oils for citrus: an update	T.G. Grout
	The potential role of GRAS chemicals in the control of the major post-harvest citrus pathogens	K.H. Lesar
Feb/Maart	Citrus Black Spot management in the Eastern Cape under the spotlight	P. Fourie & G.C. Schutte
	Vordering binne die sitrus-kouekettingforum	J.J. Bester & D. Groenewald
	Vestig nuwe aanplantings op gesonde beginsels	J.J. Bester & M.N. du Toit
	Market Access Update	V. Hattingh

#### 9.5.2 CRI webwerf deur Tim G Grout (CRI)

The usage of the website remains fairly stable with only slight fluctuations from month to month (Table 9.5.2.1). Apart from requests from dot-com and dot-net domains South African domains were the next highest. Other countries in order of decreasing visits to our website were Germany, Argentina, Australia, Netherlands, Italy and Pakistan. Access to technical information such as the Integrated Production Guidelines remains limited to residents of southern Africa and currently stands at 450 usernames.

**Tabel 9.5.2.1.** Maandelikse bladsy aansoeke op [www.cri.co.za](http://www.cri.co.za) vanaf Januarie 2007.

Month	Unique visitors	Number of visits	Pages	Hits
Jan 2007	339	446	1602	5748
Feb 2007	280	361	990	3621
Mar 2007	312	462	1208	4130
Apr 2007	326	529	1923	6550
May 2007	340	519	1653	5867
Jun 2007	262	365	1489	5340
Jul 2007	234	352	1644	4788
Aug 2007	302	466	1798	6217
Sep 2007	251	337	1076	4015
Oct 2007	322	541	1396	4967
Nov 2007	352	461	2077	6625
Dec 2007	181	273	1095	3534
Jan 2008	256	360	1353	4073

Feb 2008	265	357	1920	6748
Mar 2008	359	553	2399	7807
Total	4381	6382	23623	80030
Mean/month	292.1	425.5	1574.9	5335.3

### 9.5.3 CRInet deur Tim G Grout (CRI)

The number of messages circulated on CRInet during 2007 continued to decline compared to the previous years (Table 9.5.3.1). This decline 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 296.

**Tabel 9.5.3.1.** Nommer boodskappe gesirkuleer op CRInet per maand.

Jaar	Jan	Feb	Mar	Apr	Mei	Jun	Jul	Aug	Sep	Okt	Nov	Des	Total
2008	3	6	1										
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
2003	1	4	6	14	22	4	3	6	5	6	11	3	85

### 9.5.4 Snykant deur Tim G Grout (CRI)

During 2007/8, issues 52 to 60 were circulated via email and made available on the CRI website. The titles covered during this period are given in Table 9.5.4.1. Most were involved with residue issues.

**Tabel 9.5.4.1.** Snykant uitgawes gedurende 2007/8.

Nr.	Titel	Uitgawe	Skrywer
52	Fenpropathrin (meothrin) import tolerance for citrus in the UK	January 07	P. Hardman
53	Cautionary Notice Regarding the UK Fenpropathrin MRL.	March	P. Hardman
54	Revised EU Temporary MRL set for Carbendazim on Citrus	March	P. Hardman
55	<i>Phytophthora</i> brown rot control	March	M.C. Pretorius
56	Pre-packhouse and Packhouse chemical treatments for the 2007 season	March	K.H. Lesar
57	Frost and freeze damage of citrus fruit	June	P. Cronjé
58	Buprofezin (Applaud) and Profenofos (Selecron)	September	P. Hardman
59	Update on Carbendazim (Benomyl) EU MRL	January 08	P. Hardman
60	Commercially available granulovirus products for false codling moth control	February	S.D. Moore & V. Hattingh

### 9.5.5 5de Sitrus Navorsingsposium deur H.F. le Roux en Hannes Bester (CRI)

Die reëlings vir die 5<sup>de</sup> Sitrusnavorsingsposium vorder fluks. Die Simposium sal vanaf 3-6 Augustus 2008 by die Champagne Sports Resort in die Drakensberge aangebied word. Die hoof borgskap is opgeneem deur Bayer Cropscience terwyl SAPPI aangedui het dat hulle die formele ete sal borg, River Bioscience die verwelkomingsfunksie en Avello die golfdag. As gassprekers sal optree Prof Etienne Rabe (Wêreldwye sitrustendense), Prof Jose Bovè (Huanglongbing), Dr Joe Smilanick (Na-oesbederf), Dr Zacarias (Skilafbraak) en Dr French (Byvoegmiddels). Al vyf hierdie persone is wêreldleiers op hulle onderskeie gebiede. Daar sal voorsiening gemaak word vir 360 afgevaardigdes.

### 9.6 Industrie-verwante Vergaderings deur Hennie le Roux en Hannes Bester (CRI)

Die vergaderings wat met die Uitvoerders Tegnieese Paneel gehou is, is goed bygewoon en is baie positief ervaar. Die behoefte aan 'manuals' om na-oes bederf saam met fisiologiese skildefekte te kan identifiseer, is ook weer beklemtoon. Die fokus was die afgelopen jaar ook sterk op die werksaamhede van die CCCF gewees, aangesien daar elke jaar beduidende verliese agv foute in die koueketting gelei word.

Bywoning van beide die Citrus Exporters Forum en Citrus Marketing Forum vergaderings is steeds belangrik om ondersteuning aan die bedryf te verleen soos dit benodig mag word en is ook 'n bron van inligting om onself beter te posisioneer ten opsigte van navorsingsprioriteite en tegnologie oordraging, bv. kultivar navorsing en aanbevelings vir nuwe aanplantings, oesmanipulasie t.o.v. vruggrootheid, interne gehalte en kleur om aan markvereistes te voldoen, temperatuur protokolle, ens.

'n Versoek van die CMF, Exporters Technical Panel en verskeie studiegroepe dat alle navorsing op na-oes verwante onderwerpe gekoördineer word, het uiteindelik gelei tot die stigting van die Citrus Cold Chain Forum (CCCF) gedurende Februarie. Verskeie vergaderings met die afsonderlike belangegroepe van die CCCF is gehou met die doel om minimum standaarde, hanteringsprotokolle en tyd- en temperatuurprotokolle vir die bedryf op te stel.

Voorseisoen werksinkels is met elk van die pakhuisstudiegroepe in al die areas gehou. Die bywoning was uitstekend en het getoon hoe groot die behoefte vir hierdie inligting is.

#### 9.7 **Siekte- en plaagbeheer** deur Hennie le Roux en Hannes Bester (CRI)

*Phytophthora citrophthora* bly 'n probleem in die Suid Kaap en bevredigende beheermaatreëls word tans deur Tian Schutte ontwikkel. Fighter, die enigste kaliumfosfonaat wat geregistreer was teen *Phytophthora* bruinvrot het fitoprobleme op Satsumas in die Gamtoosriviervallei gegee. Daar is besluit om die registrasie vir bruinvrot te onttrek. Die spuit van fosfonaatprodukte teen *Phytophthora* bruinvrot op sagteskil sitruskultivars word ontmoedig omdat hierdie kultivars se skille soveel meer gevoelig is as die res van die sitruskultivars. Groen en opgekleurde sitrus (uitgesonder die sagtesitruskultivars) is nie gevoelig vir fosfonaattoksitasie wanneer die etiket gevolg word nie. Tydens kleurbreek kan daar egter 'n mate van gevoeligheid wees.

Verskeie probleme in die Wes-Kaap, wat op die Wes-Kaap CTA vergadering geïdentifiseer is, is saam met Hennie Le Roux opgevolg. Simptome van vergroening is wyd opgemerk en dis kommerwekkend dat die omvang daarvan reeds in so gevorderde stadium is. 'n Vergroeningswerksinkel is vir Vrydag 26 Mei 2006 geskeduleer om 'n strategie uit te werk om die siekte onder beheer te kry. *Phytophthora citrophthora* kom ook wydverspreid op Clementines in die Wes-Kaap voor. Verskeie boorde met hierdie probleem is besoek en aanbevelings is gemaak. Dit is tydens die studiegroepvergaderings aangespreek. Verskeie gevalle van boomagteruitgang is ook opgevolg en dis duidelik dat wortelsorg in verskeie gebiede die afgelope paar jaar verwaarloos is. Dit is ook met die studiegroepvergaderings weer aangespreek.

Weerstandbiedendheid teen na-oes swamdoders is steeds 'n probleem in verskeie areas. 'n Strategie om dit te bestuur is in plek gesit en alle pakhuis is versoek om monsters te neem om te laat toets vir weerstandbiedendheid. Die produsente en uitvoerders is weereens deeglik van die probleem verwittig en versoek om 'n rol te speel deur druk op hul pakhuis te sit om uit te vind wat hul status ten opsigte van weerstandbiedendheid is. Die samewerking van die pakhuis om monsters na die Universiteit van Pretoria toe te stuur was met enkele uitsonderings na swak.

#### 9.8 **Tuinboukundige en kultivar aspekte** deur Hennie le Roux en Hannes Bester (CRI)

Fisiologiese skildefekte bly 'n groot kopseer. Peteca is die enkele probleem wat tot die grootste verliese onder suurlemoene lei en hoewel daar wel vordering in die voorspelling van peteca is, is daar nie werkbare oplossings nie. Skilafbraak op nawels was besonder baie en 'n nuwe benadering tot die probleem sal gevolg moet word. Koueskade op veral Clementines onder koue-sterilisasie was meer as in ander jare. Navorsing met silika toon op 'n baie vroeë stadium belofte en behoort meer intensief ondersoek te word op alle skildefekte.

Daar bestaan baie wanpersepsies in die bedryf oor die effektiwiteit van organiese bemesting en die gebruik van organiese produkte wat 'wortelgroei stimuleer en opname van voedingselemente verhoog'. Die versoeke om 'n bemestingskundige aan te stel, beklemtoon weereens die behoefte aan onafhanklike advisering op hierdie vakgebied.

Daar is 'n groot vraag na die ontwikkeling van vroeë en laat nawels en Valencias, laat manderyne en niskultivars, soos bloedlemoene en nuwe variëteite. Daar is ook groot behoefte aan nawels met toe of baie klein nawelente, wat infestasië deur witluis en FCM, asook *Alternaria*, kan beperk en barsies om die nawelente voorkom en sodoende bederf beperk.

Die feit dat daar verskeie rolspelers by kultivarontwikkeling betrokke is, maak tegnologie oordraging problematies, aangesien Voorligting nie toegang tot al die inligting het nie en ook omdat daar nie altyd

objektief aanbevelings deur sekere rolspelers gemaak word nie. Die behoefte bestaan vir samewerking deur al die rolspelers om inligting aan die bedryf deur bestaande kanale beskikbaar te maak.

#### 9.9 **Fitosanitêr** deur Hennie le Roux en Hannes Bester (CRI)

Fitosanitêre bedreigings tov sitruswartvlek in Europa is steeds die grootste bedreiging wat daar vir die bedryf bestaan. Die saak word op die hoogste vlak hanteer en die uiteindelige doel is om te bewys dat vrugte nie die siekte sal versprei nie en dat die siekte nie in Europa kan vestig nie. Natalvrugtevlug hou 'n ernstige bedreiging in terwyl valskodlingmot nie alleen 'n bedreiging vir Amerika inhou nie maar ook vir die res van die markte.

Tydens die 45<sup>ste</sup> Suid Afrikaanse Plantpatologieskongres gehou vanaf 22-24 Januarie 2007 is daar 'n aanbieding gedoen met die titel: A proposed new system for citrus to gain access to international markets" deur HF le Roux, JM Kotzè, E Carstens en V Hattingh. Tydens die aanbieding is daar verduidelik op watter gronde daar aansoek gedoen is om sekere uitpakkodes as swartvlekvry verklaar te kry binne 'n magistraatsdistrik wat nie noodwendig vry van die siekte is nie.

Hennie le Roux en Elma Carstens was betrokke saam met die Departement van Landbou om 'n ondersoek te doen in die Vanrhynsdorp en Vredendaldistrikte om die distrikte swartvlekvry verklaar te kry.

'n Opname deur Prof Gerhard Pietersen en Prof Bovè het getoon dat slegs Afrika vergroening veroorsaak deur *Liberibacter africanus* in suider Afrika voorkom en nie *L. asiaticus* of *L. americanus* nie.

Sitrusaanplantings uit Brasilië is op minstens twee landgoedere in Angola gevestig. Hierdie materiaal hou 'n potensiele bedreiging in tov sitruskanker, Asiatiese en Amerikaanse vergroening, "Sudden Death", "Citrus Variegated Chlorosis", Leprose virus, Rubilose virus en "Post Bloom Fruit Drop". Alle pogings deur die amptelike kanale om kontak met die Angolese Ministerie van Landbou te maak was tevergeefs. Daar sal nou gepoog word om direk met die Angolese regering te skakel. Dit is egter duidelik dat daar steeds 'n antagonisme van die Angolese kant af teenoor Suid Afrikaners bestaan.

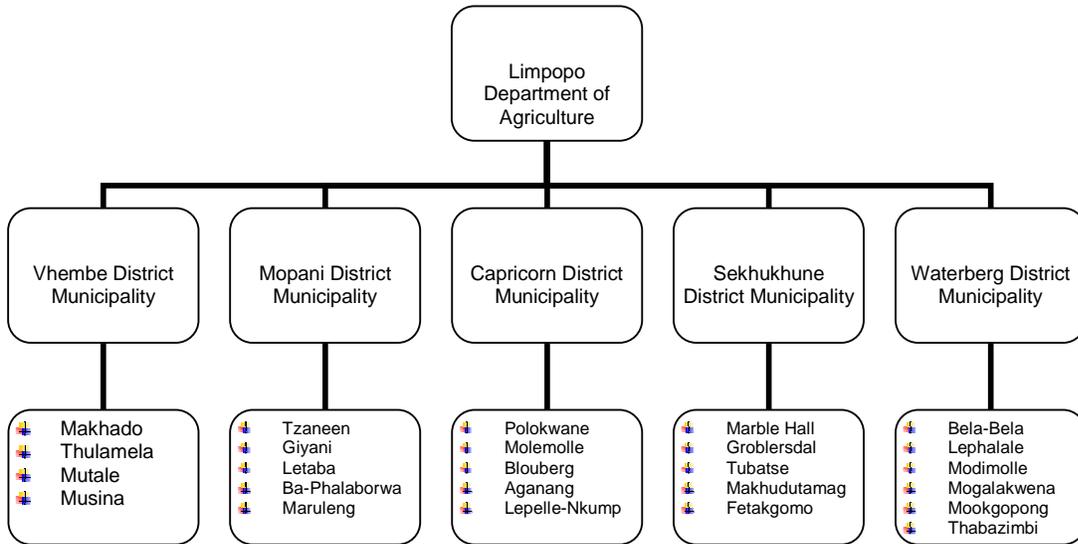
Hennie le Roux en Thys du Toit het Mosambiek besoek. 'n Sitruskwekery, Produsol, is langs die Chicamba meer in die Beira korridor begin. Samewerking tussen Produsol en die Sitrusverbeteringskema is van die allergrootste belang aangesien daar situsuitbreidings in Mosambiek te wagte is. Hierdie uitbreidings sal waarskynlik om die Gorongoza wildtuin plaasvind en saad is reeds van Brasilië ingevoer vir hierdie doel. 'n Versoek is gerig om te verseker dat hierdie saad vernietig sou word.

#### 9.10 **Transformasie** deur Hennie le Roux en Andrew Mbedzi (CRI)

Tot op hede is R1,5 biljoen se blanke plase in die Onderberg in Mpumalanga, uitgekoop. Van die sitrusplase wat sowat 'n jaar gelede oorgeneem is, het intussen feitlik ten gronde gegaan soos wat die geval ook was met die sitrusplase in die Babertondistrik. Die huidige wyse waarop plase oorgeneem word sonder om dit as 'n lopende saak oor te neem is besig om op 'n ramp af te stuur. Die tydperk wat verloop tussen die ontruiming van die vorige eienaar en die besetting deur die grondeisers lei daartoe dat die plase gestroop is deur diene teen die tyd die nuwe eienaars die plase beset. Die meeste van die gronde bly in besit van die staat terwyl die gemeenskappe wat dit ge-eis het slegs 'n gebruikreg het. Dit lei daartoe dat die plase nie as kolateraal by die banke gebruik kan word nie. Dit is dus nie moontlik om finansiering vir produksiekoste te bekom nie. Die regering se transformasiebeleid is suksesvol wat betref grondrestitusie maar dit is 'n totale mislukking tov welvaartskepping.

'n Ooreenkoms is met die Limpopo Departement van Landbou gesluit waarvolgens die CGA/ CRI hulle sal bystaan om hulle voorligters op te lei met betrekking tot sitrusproduksie. Andrew Mbedzi is vir hierdie taak aangestel. Hy is op Nelspruit gestasioneer en het vanaf Januarie 2008 sy pos aanvaar. Die eerste vergadering wat met die Limpopo regering gehou is, is gehou by die Madzivhandilla Kollege in Vhembe. Die doel was om die strukture tov die verskillende distrikte en hulle subdistrikte uit te sorteer. In 'n opvolgvergadering is die sitruskommoditeitskoördineerders uitgesorteer en is 'n werkswinkel vir April beplan om die opleidingsbehoefte van die onderskeie voorligters te bepaal. Die Limpopo Departement van Landbou is terdeë bewus van die feit dat plase wat tans miljoene uitvoerkartonne produseer in die transformasieproses is en dat die rol van hulle voorligters gaan verander van voorligters wat verantwoordelik was vir bestaansboere na voorligters wat betrokke sal moet raak by uitvoersitrus. Die bestaande voorligters het nie die nodige opleiding en ondervinding om hierdie rol oor te neem nie. Andrew sal hulle hiermee moet bystaan.

Limpopo Distrikte waarby Andrew Mbedzi (CRI) en die Limpopo Departement van landbou betrokke sal wees mbt sitrus:



Districts	District Coordinators	Municipalities	Coordinators/Officers
<b>Vhembe District Municipality</b>	Muthala K.S. (082 882 5114) (015 963 1260) (MuthalaKS@agric.limpopo.gov.za)	<b>Thulamela Municipality</b>	Ngwalungwalu N.A. (082 218 6003) (015 962 1021)
			Phuluwa R.A. (083 389 1467) (015 962 1021) phuluwara@webmail.com
			Ramukhuba N.S. (078 546 5761)
		<b>Mutale Municipality</b>	Nengovhela (079 471 0363)
			Mulaudzi A.A. (072 238 6648)
		<b>Makhado Municipality</b>	Mudau M.M. (073 153 6113)
		<b>Musina Municipality</b>	Nemutavhanani A (072 423 1004)
<b>Mopani District Municipality</b>	Sikhipha N. (076 488 3818) (015 812 3210) SikhiphaNM@agric.limpopo.gov.za	<b>Greater Tzaneen Municipality</b>	Maake L.P. (082 805 8911)
		<b>Greater Giyani Municipality</b>	Ngobeni S.R. (084 012 7526)
		<b>Greater Letaba Municipality</b>	Chauke T.S. (073 652 1631)
		<b>Ba-Phalaborwa Municipality</b>	Hlungwani S.S. (082 541 1656)

		<b>Maruleng Municipality</b>	Moriri S.C. (082 261 9105) MoririS@yahoo.com
<b>Sekhukhune District Municipality</b>	Kgopa M.P. (082 883 6748) (KgopaM@agric.limpopo.gov.za)	<b>Elias Motsoaledi Municipality</b>	Matimatjatji R.S. (0823321969)
			Lentsoane M.P. 078 225 5120
		<b>Fetakgomo Municipality</b>	Sefoka B.T. (072 397 4064)
			Lekgoro K.W. (072 527 9241)
		<b>Greater Marble Hall Municipality</b>	Thabang S.M. 072 240 0834
			Nkadimeng S.B. (072 152 6376)
		<b>Greater Tubatse Municipality</b>	Napo T.R. 073 231 5880 ttrn@webmail.co.za
			Mothoa M.P. 082 851 7465
<b>Makhudutamaga Municipality</b>	Sefala M.F.		
	Mello M.K 072 538 3810		
<b>Waterberg District Municipality</b>	Marlise Bornman (082 889 3813) (014 717 2523) Bornmanme@agric.limpopo.gov.za	<b>Bela-Bela Municipality</b>	No Citrus
		<b>Lephalale Municipality</b>	Tjano M.S. (073 886 9581) tjanoms@agric.limpopo.gov.za
		<b>Modimolle Municipality</b>	No Citrus
		<b>Mogalakwena Municipality</b>	Kgafela M.P. (073 648 2517)
		<b>Mookgopong Municipality</b>	No Citrus
		<b>Thabazimbi Municipality</b>	Rabothatha M.F. (079 188 2099) RabothataF@agric.limpopo.gov.za
<b>Capricorn District Municipality</b>	Mathabatha (082 903 9407)	<b>Polokwane Municipality</b>	Tlolane Frans 079 7310087
		<b>Molemolle Municipality</b>	Molepo Z.M. 082 751 6731 015 397 4306

		<b>Blouberg Municipality</b>	No Citrus
		<b>Aganang Municipality</b>	No Citrus
		<b>Lepelle-Nkumpi Municipality</b>	No citrus

Vergaderings en werksinkels bygewoon deur die Transformasie Voorligter:

<b>Date</b>	<b>Venue</b>	<b>Reason for the Meeting</b>
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
11-15/02/2008	Stellenbosch	Training workshop on the facilitation of Citrus Production Management Learning Materials (NQF Level 1-5)
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
05/03/2008	Limpopo/ Easy Farm / Lungane	Assessment of Mentorship Programme
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
12-13/03/2008	Eastern Cape/ Peter Trust Family Farm	Assessment of Mentorship Programme with dr Richard Bates.
20/3/2008	Limpopo	Agriseta Skills Planning Roadshow.
26/03/2008	Winterveldt	Assessment of Technical Advice given by Magalies to Winterveldt.

CRI was die afgelope jaar ook saam met Magalies Sitrusmaatskappy betrokke by die Winterveldt ontwikkeling. Hierdie ontwikkeling gaan goed aan. Daar word egter voorsien dat hulle in die toekoms besproeiingsprobleme kan ontwikkel. Magalies gaan voort om hulle op 'n twee-weeklikse basis met tegniese raad te bedien.

## 9.11 Algemeen

Rekord-volumes en pryse het die 2007 seisoen gekenmerk en word deur baie as die beste seisoen in die geskiedenis van die sitrusbedryf beskryf. Dis net die laat Valencias wat in Europa swak pryse behaal het agv die laat volumes wat daarheen verskeep is. Dis veral die Valencias wat tot die rekord uitvoervolumes bygedra het.

Die produsente is gevolglik positief oor die toekoms van die bedryf. Wat wel 'n bepalende rol in die toekoms kan speel, is die voortdurende styging in insetkoste. Die effek daarvan is nie net op plaasvlak nie, maar deur die hele voorsienings- en logistieke ketting. Transformasie gaan ook oor die medium termyn 'n invloed op die uitvoervolumes hê.

Die gehalte van die vrugte was deurgaans goed en is gerugsteun deur die feit dat die vrugte vinnig verkoop het agv gunstige marktoestande. As daar egter twee aspekte is wat uitgesonder kan word om meer aandag te kry, is dit palettisering in die pakhuis en hantering in die hawens. Beide hierdie aspekte veroorsaak groot probleme in die logistieke ketting en het 'n nadele effek op gehalte en raklewe.

Groot waardering word op baie vergaderings uit verskeie oorde uitgespreek vir die kwaliteit en kwantiteit werk wat deur CRI verrig word. Dit beklemtoon net weereens hoe belangrik die voorligtings-aksie is wat deur alle betrokke rolspelers binne CRI uitgevoer word. Voorligting deur elke spesialis-navorsers is die belangrikste aksie om die bedryf op hoogte te hou van sy of haar resultate en die belangrikheid wat dit vir die bedryf inhou en moet dus nie geringskat word nie.

Die 2008 seisoen hou ook goeie vooruitsigte in met uitvoervolumes wat baie na aan die van 2007 behoort te wees. Die pomelos se vruggroottes is kleiner as in 2007 en daarom kan die uitvoervolumes effens laer wees. Die suurlemoene beleef 'n uiters gunstige seisoen en die vooruitskouing is dat die goeie pryse tot minstens die helfte van die jaar sal voortduur omdat Argentinië se oes sowat 20 % laer is agv koueskade. Die Europese oes is ook deur koue negatief beïnvloed.

## 10 PUBLICATIONS IN 2007/8

### 10.1 Refereed publications (or ISI ranked journals)

- Bester, W., P.W. Crous and P.H. Fourie. 2007. Evaluation of fungicides as potential grapevine pruning wound protectants against *Botryosphaeria* species. *Australasian Plant Pathology* 36: 73-77.
- Damm, U., P.H. Fourie and P.W. Crous. 2007. *Aplosporella prunicola*, a novel species of anamorphic Botryosphaeriaceae. *Fungal Diversity* 27: 35-43.
- Damm, U., P.W. Crous and P.H. Fourie. 2007. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* spp. nov. *Mycologia* 99(5): 664-680.
- Grout, T.G. and K.C. Stoltz. 2007. Developmental rates at constant temperatures of three economically important *Ceratitis* spp. (Diptera: Tephritidae) from southern Africa. *Environmental Entomology* 36: 1310-1317.
- Koopman, T., C.C. Linde, P.H. Fourie and A. McLeod. 2007. Population genetic structure of *Plasmopara viticola* in the Western Cape Province of South Africa. *Molecular Plant Pathology* 8(6): 723-736.
- Manrakhan, A. and S.A. Lux. 2008. Effect of food deprivation on attractiveness of food sources, containing natural and artificial sugar and protein, to three African fruit flies: *Ceratitis cosyra*, *Ceratitis fasciventris*, and *Ceratitis capitata*. *Entomologia Experimentalis et Applicata* 127: 133-143.
- Ueckermann, E.A. and T.G. Grout. 2007. Tydeoid mites (Acari: Tydeidae, Edbakerellidae, Iolinidae) occurring on *Citrus* in Southern Africa. *J. Nat. History* 41 (37-40): 2351-2378.

### 10.2 Semi-scientific publications

- Carpenter, J., Bloem, S. and Hofmeyr, H. 2007. Area-wide control tactics for the false codling moth *Thaumotobia leucotreta* in South Africa: a potential invasive species. pp. 351-359. In: M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.), Area-wide control of insect pests. US Government.
- Cronjé, P.J.R. 2007. Postharvest Rind Disorders of Citrus Fruit. Citrus Research International, Nelspruit, South Africa. ISBN 0-7972-1169-1.
- Grout, T.G. and B.A. Tate. 2007. Postharvest control of grain chinch bug (Heteroptera: Lygaeidae) on citrus using pyrethrum. *SA Fruit J.* 6(4): 61-62.
- Kirkman, W. and Moore, S.D. 2007. A study of alternative hosts for the false codling moth, *Thaumotobia* (= *Cryptophlebia*) *leucotreta* in the Eastern Cape. *S.A. Fruit J.* 6(2):33-38.

Moore, S.D., Kirkman, W., Stephen, P.R. & Fourie, J. 2007. Bollworm on citrus: Its pest status and a threshold for intervention. S.A. Fruit J. 6(5):59-64.

Verreyne, J.S. 2007. Alternate bearing in Citrus. S.A. Fruit J. 6(4): 72-73, 75, 77.

## 11 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

Brink, J.C., G. Holz and P.H. Fourie. 2007. Low-volume concentrate application for improved spray cover in grapevine. Oral presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).

Brink, J.C., and Paul Fourie. 2007. Fungicide spray cover in grapevine canopies and control of *Botrytis cinerea*. Oral presentation at the 14<sup>th</sup> International Botrytis Symposium, Cape Town (21-26 Oct. 2007).

Brink, J.C. Sybrand van Zyl, Riaan Rossouw and Paul Fourie. 2007. Optimisation of fungicide spray application in South African table and wine grape vineyards. Oral presentation at the 5<sup>th</sup> International Table Grape Symposium, Somerset West (14-16 Nov. 2007).

Carstens, E., J.M. van Niekerk, A. Smit, P.H. Fourie and P.W. Crous. 2007. Resolving the status of *Neonectria galligena* in South Africa. Poster presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).

Cronjé, P.J.R. 2007. Improving citrus rind quality for improved postharvest storage: with special reference to mineral nutrients and carbohydrates. Rind Conditions Workshop, Casablanca, Morocco, 21-25 May 2007.

Fourie, P.H., J.C. Brink, S.A. van Zyl, R. Rossouw. 2007. Optimisation of fungicide spray application in South African table and wine grape vineyards. 16th biennial Conference of the APPS, Adelaide (24-27 Sept. 2007).

Kotze, C., J.M. van Niekerk, F. Halleen and P.H. Fourie. 2007. *In vitro* antagonism of potential biocontrol agents against grapevine trunk disease pathogens. Poster presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).

Kotze, Charl, Jan M. van Niekerk, Francois Halleen and Paul H. Fourie. 2007. Identifying potential biocontrol agents for grapevine pruning wound protection against trunk pathogen infection. Oral presentation at the 5<sup>th</sup> International Table Grape Symposium, Somerset West (14-16 Nov. 2007).

Lamprecht, Sandra, C., Johan A. Brand, Chris F.J. Spies and Paul H. Fourie. 2007. Integrated management of Botrytis grey mould of rooibos (*Aspalathus linearis*) seedlings in nurseries. Oral presentation at the 14<sup>th</sup> International Botrytis Symposium, Cape Town (21-26 Oct. 2007).

Malan, A.P. & Moore, S.D., 2007. Potential of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae for the control of false codling moth. 16th Symposium of the Nematological Society of Southern Africa 6-10 May (oral presentation).

Malan, A. P. & Moore, S.D., 2008. Entomopathogenic nematodes for the control of false codling moth. Deciduous Fruit Producers Trust IPM meeting (oral presentation).

McLean, T., P.H. Fourie, A. McLeod. 2007. Reporter gene transformation of two grapevine trunk pathogens and a potential biocontrol agent, *Trichoderma harzianum*. Poster presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).

Moore, S.D., Le Roux, H., Ware, A.B. & Kriek, D. 2007. Microbial and biorational control of moth pests and fruit flies on fruit crops. In: 2<sup>nd</sup> International Biocontrol Manufacturers Association Meeting, Lucerne, Switzerland, 20-23 October 2007.

Mostert, L., Fourie P.H., Halleen F., M.V. Jaspers, Crous, P.W. 2007. Aetiology of black foot disease of grapevines in New Zealand. Oral presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).

Phahladira, Baby and Gerhard Pietersen. A poster was presented at the Molecular Cell Biology Group meeting at the University of Pretoria.

Pretorius, M.C., Huisman, L. 2007. Evaluation of a new Ethoprophos formulation as part of the "Hard Approach" and furfural as a "Soft Approach" for the control of the citrus nematode. Nematologiese Vereeniging van Suidelike Afrika se tweejaarlikse symposium, Boardwalk Konferensie sentrum, Port Elizabeth, 6-9 Mei 2007.

Schutte, G.C. 2007. Alternaria brown spot control. 29<sup>th</sup> Semana da Citricultura, Limeira, Brazil, 13 June 2007.

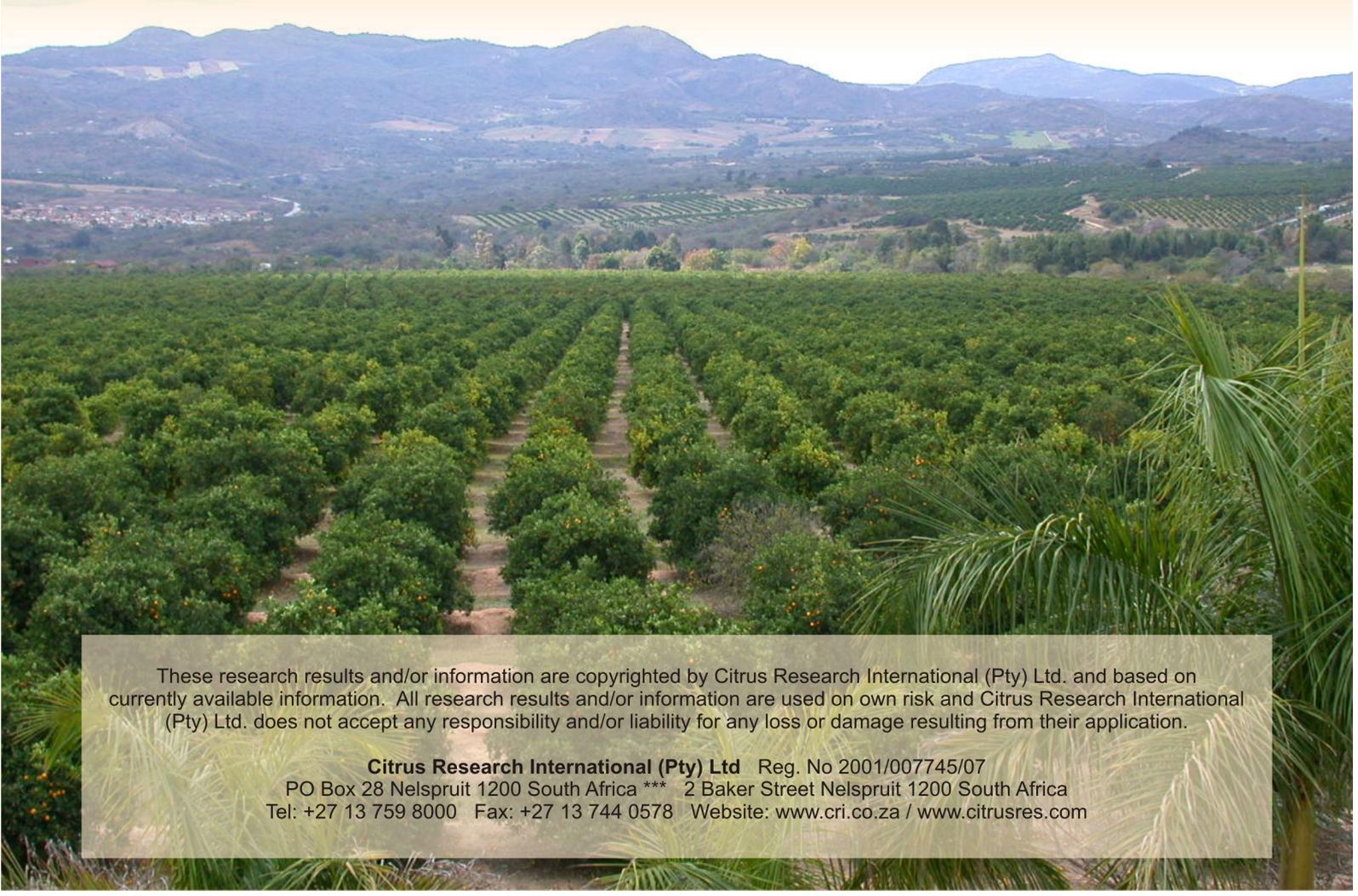
Schwerdtfeger, M. and G. Pietersen. 2007. Survey for "Candidatus Liberibacter" species on citrus in South Africa. XVII<sup>th</sup> Conference of the International Organization of Citrus Virologists. 22-26 October, 2007. Adana, Turkey.

Stewart, Katherine and Gerhard Pietersen. Defence of PhD. Proposal done at UP on 9 April 2008.

Stewart, Katherine and Gerhard Pietersen. Oral presentation at the Molecular Cell Biology Group (MCBG) conference – 17 Oct 2007.

Van Niekerk, J.M., F. Halleen and P.H. Fourie. 2007. Temporal grapevine pruning wound susceptibility and spore dispersal patterns of trunk disease pathogens. Oral presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).

- Van Niekerk, J.M., A.E. Strever, P.G. du Toit, F. Halleen and P.H. Fourie. 2007. Water stress predisposition of grapevines to infection by *Botryosphaeriaceae* trunk pathogens. Oral presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).
- Van Niekerk, J.M., A.E. Strever, P.G. du Toit, F. Halleen and P.H. Fourie. 2007. Water stress as predisposing factor of grapevines to infection by Botryosphaeriaceae trunk pathogens. Oral presentation at the 5<sup>th</sup> International Table Grape Symposium, Somerset West (14-16 Nov. 2007).
- Van Vuuren, S.P. and B.Q. Manicom. 2007. Initial attempts to obtain Huanglongbing resistant or tolerant sweet orange by embryo rescue from healthy chimeras of diseased fruit. XVII<sup>th</sup> Conference of the International Organization of Citrus Virologists. 22-26 October, 2007. Adana, Turkey.
- Van Zyl, S.A., J.C. Brink and P.H. Fourie. 2007. The use of surfactants to improve control of *Botrytis cinerea* on grape leaves. Poster presentation at 45<sup>th</sup> Congress of the SASPP, Benoni (21-24 January 2007).
- Van Zyl, Sybrand, A., Jan-Cor Brink and Paul H. Fourie. 2007. The use of surfactants to improve control of *Botrytis cinerea* on grape leaves. Oral presentation at the 14<sup>th</sup> International Botrytis Symposium, Cape Town (21-26 Oct. 2007).
- Verreynne, J.S. Fruit size and yield effects of time and severity of hand thinning on Nules Clementine mandarin. SASCP, SSSSA, SASHS Combined Congress, Badplaas, 22-25 January 2007.
- Verreynne, J.S. Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end of navel oranges - a preliminary study. SASCP, SSSSA, SASHS, SAWSS Combined Congress, Grahamstown, 21-24 January 2008: 152.



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