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1 INTRODUCTION

CEO (CRI): Vaughan Hattingh

This annual research report encompasses progress achieved within the CRI Group of research alliance partner organisations over the period January 2003 to December 2003. Unfortunately a report on the ARC-ITSC contributions to the Cultivar and Rootstock Development Programme was not supplied for inclusion in the report because an agreement on the ownership of cultivar intellectual property was not concluded. Hopefully this matter will be resolved in the near future and these contributions can be included in the next report.

The overall structure of industry research and technical services was retained as initiated in 2001, with Research, Extension and the Citrus Improvement Programme making up the three CRI Divisions. The structure of the research portfolio was maintained in the form of four Programmes, namely Disease Management, Integrated Pest Management, Crop Load and Fruit Quality Management and Cultivar and Rootstock Development. A set of projects were retained under each Programme, with each project consisting of a set of experiments aimed at addressing a specific problem or opportunity. Research proposals were again vetted by Programme Committees with each Committee consisting of specialist advisers and chaired by a grower nominee member of the CRI Board.

Although Market Access has been an overarching priority for all of CRI activities in the past, the 2003 cycle was marked by an intensified focus on certain aspects of research aimed at achieving, maintaining or enhancing access to export markets. Whereas research priorities had been thoroughly debated in preparation for the previous research cycle, the 2003 portfolio was based on an assessment of the need for changes to existing priorities. In addition to the consequently intensified focus on various market access issues, there was also a strong emphasis placed on research required to address various rind condition problems and CRI consequently appointed a post-harvest fruit physiologist. The need for impartial evaluation of cultivars, regardless of their ownership status, was also highlighted by growers as an urgent need and the vacant CRI post of cultivar evaluator was consequently filled by transferring a CRI Horticultural technician into this post.

The southern African citrus growers, the CRI and CGA Directors and the industry's research and technical service partners, are thanked for making it possible to continue progressing towards fulfilling the CRI Mission: "To maximise the long term global competitiveness of the southern African citrus growers through the development, support, coordination and provision of Research and Technical services by combining the strengths of all CRI Group partners".

INLEIDING

Hierdie navorsingsjaarverslag omvat die vordering wat gemaak is deur die CRI-Groep van navorsingsverbonde vennootorganisasies oor die tydperk Januarie 2003 tot Desember 2003. Ongelukkig is 'n verslag oor die LNR-ITSG se bydraes tot die Kultivar en Onderstamontwikkelingsprogram nie voorgelê vir insluiting nie aangesien 'n ooreenkoms oor die eienaarskap van intellektuele eiendom nie bereik kon word nie. Hopelik sal die aangeleentheid in die nabye toekoms opgeklaar word en die bydraes in die volgende verslag ingesluit wees.

Die algehele struktuur van bedryfsnavorsing en tegniese dienste is behou soos onderneem in 2001, met Navorsing, Voorligting en die Sitrusverbeteringsprogram as die drie CRI-Afdelings. Die struktuur van die navorsingsportefeulje is gehandhaaf in die formaat van vier programme, naamlik Siektebestuur, Integreerde Plaagbestuur, Oeslading en Vrugkwaliteitsbestuur en Kultivar en Onderstamontwikkeling. 'n Aantal projekte is onder elke Program behou, elke projek bestaande uit 'n reeks eksperimente gerig op 'n spesifieke probleem of geleentheid. Navorsingsvoorstelle is weereens deur Programkomitees gekeur, met elke komitee bestaande uit spesialisraadgewers onder voorsitterskap van 'n kweker-genomineerde lid van die CRI-Raad.

Hoewel Marktoegang in die verlede nog altyd 'n oorkoepelende prioriteit was by alle CRI-aktiwiteite, is die 2003 siklus gekenmerk deur verskerpte klem op aspekte van navorsing gerig op die bereiking, handhawing of uitbreiding van toegang tot uitvoermarkte. Waar navorsingsprioriteite deeglik beredeneer is ter voorbereiding tot die vorige navorsings siklus, is die 2003 portefeulje baseer op 'n raming van die noodsaaklikheid vir veranderings aan bestaande prioriteite. Bykomend tot die gevolglike verskerpte fokus op die onderskeie aspekte van marktoegang, is ook sterk klem gelê op navorsing gerig op verskeie probleme wat met skilgehalte ondervind word en het CRI derhalwe 'n na-oes vrugfisioloog aangestel. Die noodigheid vir onbevooroordeelde evaluasie van kultivars, ongeag van hulle eienaarskapstatus, is ook deur die kwekers na vore gebring as 'n dringende behoefte en die vakante CRI pos van kultivar-evalueerder is gevolglik gevul deur die oorplasing daarin van 'n CRI tuinboutegnikus.

Die Suider-Afrikaanse sitruskwekers, die CRI en SKV Direkteure, en die bedryf se navorsing- en tegniese diensvennote, word bedank dat hulle dit moontlik maak om vol te hou met die vordering ten opsigte van die verwesenliking van die CRI Missie: "Om die langtermyn globale mededingendheid van Suider-Afrikaanse sitruskwekers te maksimeer deur die onwikkeling, ondersteuning, koördinerende en voorsiening van Navorsing en Tegniese dienste deur kombinerende van die kragte van alle CRI-Groep vennote".

2 PROGRAMME: MARKET ACCESS TECHNICAL CO-ORDINATION

Co-ordinator: Vaughan Hattingh (CEO: CRI)

2.1 PROGRAMME SUMMARY

In accordance with World Trade Organisation (WTO) agreements, in particular the International Plant Protection Convention (IPPC), signatory countries regulate Market Access by official government to government agreements and the implementation of officially endorsed treatment and procedure protocols. Assurances that the particular trade does not constitute unacceptable levels of Sanitary and Phytosanitary (SPS) risk to the importing country are always central considerations regulating access of fresh produce to an export market. It is an internationally accepted principle that such considerations must be based on sound scientific evidence. Industry Research and Technical services therefore form a critical and central component of any successful market access programme. Pursuing, maintaining and enhancing access to markets for southern African citrus exports is consequently an overarching priority for the entire CRI Group research portfolio. This programme covers specific aspects of research and technical support that have been applied to Market Access. Co-ordination of the technical aspects of Market Access is the responsibility of the CEO CRI, Vaughan Hattingh. Market Access is also a key objective of the Citrus Growers Association of Southern Africa (CGA) and grower representation on matters of Market Access is the responsibility of the CEO of the CGA, Justin Chadwick. The official Governmental components of Market Access are the responsibility of the Department of Agriculture, Directorate Plant Health. Close collaboration has been maintained between these parties to ensure success of the industry's Market Access endeavours.

Europe

Citrus Black Spot remained a key phytosanitary concern for citrus exports to this market. As reflected in earlier annual research reports, there have been protracted deliberations between the EU and SA since 1992. SA formulated a comprehensive Pest Risk Analysis that was submitted to the EU in May 2000. This Pest Risk Analysis supported SA's objection to the EU phytosanitary restrictions relating to CBS, on the grounds that these regulations are more restrictive to trade than can be justified on the basis of scientific evidence. A response from the EU was received by SA in December 2001. A workshop of specialists was convened to consider the questions posed by the EU. A response addressing the majority of the EU concerns was returned to the EU in June 2002. Outstanding technical questions posed by the EU were incorporated into the research portfolio as priorities.

The most critical component of additional data required, was a climate matching study that would evaluate the climatic suitability of Europe for potential establishment of the CBS organism. This study was initiated in 2001 with a post-graduate student (Ms Ida Paul) at the Centre for Environmental Studies, University of Pretoria. The study was completed in 2003 and the report was officially submitted to the EU in December 2003 (2.2). The remaining study called for by the EU is aimed at establishing whether *Guignardia citricarpa* pycnidiospores from CBS-infected fruit, may be able to colonise fallen citrus leaves and produce ascospores. It is expected that this study will be completed in 2004 for submission to the EU.

The highly intensive and restrictive CBS risk management system, previously implemented in the industry, was maintained and notifications of CBS interceptions in the EU continued to decline. In contrast to southern Africa's successful management of CBS in exports, Argentinian and Brazilian citrus exports to Spain were suspended towards the end of 2003 on the basis of numerous interceptions of Argentinian and Brazilian citrus infected with *Xanthomonas campestris* (*axonopodis*) *pv citri* (citrus canker), *Elsinoe* spp. (scab) and *Guignardia citricarpa* (CBS). It is anticipated that Argentina and Brazil may be granted permission to reinstate their exports to Spain next year, once adequate risk management systems have been implemented in these industries.

The quarantine status of non-Mediterranean fruit fly species was emphasized last year (2002) when SA received a request from the Spanish phytosanitary authorities for assurances of adequate risk mitigation being applied to SA citrus exports. Revised fruit fly control recommendations were communicated to growers in 2002. Nonetheless, Spain reported an interception of a citrus consignment infested with a non-

Mediterranean fruit fly in 2003. A second workshop was convened with industry technical specialists and stricter standard fruit fly control measures were devised for the 2003 production season. Hennie le Roux and Tony Ware addressed grower groups throughout the production regions to make them aware of the importance of diligently implementing these revised fruit fly control measures. The technical validity of applying the CBS "strike and disqualification" system to fruit fly interceptions was evaluated. It was concluded that this system would be technically appropriate as a means of regulating fruit fly interceptions, but that it would first be necessary to get a better understanding of the potential risk posed by non-Mediterranean fruit flies, before the implementation of such a system could be justified. This situation will continue to be monitored in the next year.

Japan

Large scale validation of fruit fly cold sterilization procedures for Clementines, had previously been conducted by A.B. Ware (1999, 2000, 2001 and 2002) and submitted to Japan in support of a request to commence exporting Clementines to Japan. Further explanation of technical details was requested by Japan and supplied by SA in 2003. Japan accepted these applications and indicated a need for Japanese authorities to visit SA to inspect the Clementine industry, before permission to commence exporting Clementines to Japan would be considered. Such a visit has been scheduled to take place during the 2004 harvest season.

A technical motivation was compiled in support of an application from SA to Japan, for acceptance of less stringent cold sterilization conditions for fruit fly disinfestation. The request was based on a call for equivalency with the cold sterilization requirements imposed by Japan on citrus imported from other countries. The motivation was submitted to Japan in 2002. SA was advised by Japan in 2003 that Japan would require SA to conduct a large scale validation trial for such revised treatment conditions and that this trial would need to be conducted in the presence of Japanese scientists. Such a validation trial has been scheduled for execution in 2004.

The potential inclusion of lemons and limes from Swaziland was investigated. The Japanese authorities indicated that a separate application from the Swaziland National Plant Protection Organisation, with supportive validation data would be required. The compilation of a motivation for the inclusion of lemons and limes based on literature relating to the fruit fly host status of lemons and limes is to be considered.

USA

USDA implemented a maximum permissible phytosanitary rejection rate of 25% for pre-shipment inspections in the SA fruit export programme to USA. Should this threshold be exceeded at any point, the programme would be halted for that particular fruit type. The threshold is to decline to 20% in 2004. The phytosanitary rejection rates experienced in 2003 were consistently retained below the permissible threshold.

On commencement of the 2003 export season, USDA advised SA that they were reducing the tolerance for interception of FCM-infested citrus during pre-shipment inspection. A threshold of two FCM-infested fruit per inspected carton was applied in previous seasons. This was reduced to a maximum of two infested fruit within the entire inspection lot on commencement of the 2003 season. The industry lodged an objection to this on the basis that the existence of a validated cold sterilization treatment, applied after inspection, made the reduced inspection tolerance implemented by USDA technically unjustifiable and thus contrary to the spirit of the IPPC. A statistical model was compiled by Hattingh (CRI) and Pringle (University of Stellenbosch), to enable the determination of a technically justifiable tolerance for interception levels during inspections prior to the implementation of a disinfestation treatment that had been shown to comply with the probit 9 level of efficacy. On the basis of the industry objection and supportive arguments, USDA reverted to implementation of the tolerances applied in previous seasons (2.3).

The principal reason for rejections in the USA export programme remained the presence of immature unidentifiable mealybugs. A molecular identification technique for rapid and reliable identification of the mealybug species, regardless of their life stage, had previously been developed by A. Severn-Ellis (ITSC) and V. Hattingh (CRI). USDA advised SA that they accepted implementation of this technique in the phytosanitary inspection process in 2003. Severn-Ellis, being one of the developers of the technique, was authorised by SA Directorate Plant Health to conduct official identifications within the export programme (2.4).

The technical validity of applying a zero tolerance for interception of fruit fly-infested fruit during pre-shipment and pre-disinfestation inspection within the USA export programme, was also challenged on the basis of the statistical model referred to above. A ruling from USDA on this matter is still awaited.

USDA advised SA that they were embarking upon a research project to map the temperature distribution within ships that were applying cold sterilization treatments. A delegation of USDA scientists was sent to SA in 2003 to obtain information required to initiate this study. A task team of southern African industry role players, consisting of scientists, regulators and shippers was convened to host the delegation. On completion of the visit, SA was advised by USDA that their delegation had been impressed by the SA systems, that the study was continuing and would initially focus on the development of a simulation model and that the model would later be validated through monitoring of actual shipping conditions.

Citrus producers from several regions where Citrus Black Spot is not known to occur, requested that disease surveys be conducted to determine whether these regions could potentially be recognised as being CBS-free, as a first step in gaining access to the USA market. CBS surveys were conducted in parts of the Limpopo Province, Eastern Cape and Northern Cape in 2002. Additional surveys were conducted in parts of the Limpopo Province, Eastern Cape and eastern regions of the Western Cape in 2003. Conclusive identification of the last field samples is still awaited.

The potential application of a quantitative risk assessment approach for areas with low CBS incidence in pursuit of approval to export to the USA, was evaluated by a task team of CRI and Department of Agriculture Scientists. It was concluded that the development of a system, whereby CBS-free production sites within areas of low incidence, would be a more technically sound approach in terms of existing IPPC guidelines. A framework system was compiled and the validity of the system evaluated in a workshop with participation by industry representatives, scientists and Department of Agriculture regulators. A revised framework document was subsequently developed and will be further evaluated in workshops with growers and other relevant parties in 2004, before preparing a final document to be submitted to USDA for consideration.

Since the requisite cold treatment for FCM disinfestation of citrus has deleterious effects on fruit quality, there is an urgent need to find a less damaging alternative treatment. Evaluation of irradiation as a potential post-harvest FCM disinfestation treatment was initiated. Research was conducted by Hendrik Hofmeyr in collaboration with scientists from the International Atomic Energy Agency and the United States Agricultural Research Services. This research is still at an early stage, but initial efficacy results are promising. Once the minimum effective dosage has been more accurately determined, fruit quality evaluations will be undertaken. Discussions have been initiated with USDA officials to establish mutually acceptable procedures for the execution of an efficacy validation trial.

South Korea

High rejection levels for unidentifiable immature mealybugs remained a constraint on exports to this market. South Africa had previously requested S Korea to accept the use of the molecular mealybug identification technique referred to under the report on USA. A South Korean scientist was sent to SA for three weeks in 2003 to oversee the execution of a trial aimed at validating the identification technique. The joint trial was successfully concluded with the assistance of five South African scientists (V Hattingh from CRI, A Severn-Ellis from ARC ITSC, W Wakgari from University of Stellenbosch, I Millar from the National Collection of Insects and Bruce Tate from CRI) and three officials from the Department of Agriculture (M Bolton, C Hattingh and W Pieterse). A comprehensive report was compiled and submitted to the South Korean Plant Health authorities in support of an application to commence using the ID technique in phytosanitary inspections. A response from the S Korean authorities is pending.

The technical justification for not implementing a tolerance for interceptions of Red Scale, FCM and Fruit Flies during pre-shipment inspection was contested by the industry, on the basis that such inspections are followed by a validated disinfestation treatment for these organisms. The objection has been lodged with the Department of Agriculture for referral to the South Korean Plant Health Authorities for consideration. A response from S Korea is awaited.

The market opportunity for inclusion of lemons and grapefruit in the South Korean export programme, was verified with exporters. The need for a large-scale validation of the FCM cold sterilization protocol, with the participation of a South Korean scientist, had been established previously. The potential viability of inclusion of lemons and grapefruit under the requisite cold sterilization protocol is to be evaluated from a fruit quality perspective, before embarking on the costly large-scale validation trial.

Thailand

A draft protocol for citrus exports from SA to Thailand was received in 2001. In 2002 SA provided scientific evidence in support of removing certain restrictions that would have seriously compromised the potential

profitability of the export programme under the conditions proposed in 2001. A significantly improved protocol was returned by Thailand in 2002. A motivation for a less demanding cold sterilization treatment was compiled by industry scientists in 2002 and provided to the Department of Agriculture for submission to Thailand. In 2003 it transpired that the electronic documentation of this SA counter-proposal had been lost during a computer network failure at the Department of Agriculture and had not been submitted to Thailand. A follow up workshop with participation of industry scientists, technical shipping specialists and regulators was held and a replacement proposal compiled for submission to the Thailand Plant Health Authorities. A response from Thailand is pending.

China

Opening of the Chinese market for export of southern African citrus has been identified by citrus growers as a top priority. Data on the presence of citrus pests and diseases in SA was submitted to China in December 2001, with an application to open the Chinese market for exports of citrus from SA. The CEO CGA was invited to accompany the Minister of Agriculture to meet with the Chinese Minister of Agriculture in 2003, where the importance of advancing the citrus application was emphasized. The Chairman of the CGA Board visited the Chinese citrus industry and initiated discussions regarding research and technical collaboration between the Chinese and SA citrus industries. CRI (CFB) sold large quantities of seed to China in 2002 and 2003. Two delegations of high-ranking Chinese officials visited SA in 2003 and were hosted by the citrus industry for part of the time. The Chinese officials indicated that a provisional assessment of the citrus pest list had identified three organisms of phytosanitary concern, namely Mediterranean Fruit Fly, False Codling Moth and Carob Moth. The last Chinese delegation to visit SA in December 2003, advised that SA should send a delegation to China to provide impetus to SA's application for access to the market.

Other markets

The citrus pest and disease data package had previously been submitted to Israel with an application to open this market for citrus exports. Israel responded in 2003, by requesting additional scientific data on several pests and diseases. SA has not as yet supplied this information. The requirements for reinstatement of citrus exports to Iran were officially requested of Iran by the SA Department of Agriculture. An application to commence exports of citrus to Australia is still pending at the stage of Pest Risk Analysis by Australia. The requirements for potentially commencing exports to the Canary Islands were investigated and the Market Access Working Group, with industry and Department of Agriculture representation, advised the industry not to pursue this matter further.

Residues

The southern African citrus industry has been taking steps since 1992 to pro-actively adjust pesticide usage practices in the industry and thereby remain compliant with changes to the residue requirements of its export markets. A document entitled "Recommended Usage Restrictions for Plant Protection Products on Southern African Export Citrus" is periodically updated as a guideline to the industry. Prior to commencement of each export season, the Department of Agriculture also issues a list of updated MRLs in market countries.

Withdrawal of MRLs for 2,4D on citrus in the EU during the 2003 season, disrupted exports to this market and necessitated the allocation of considerable resources to resolving the crisis. The industry became aware of the impending problem late in 2002. Intensive deliberations between the southern African citrus industry and the relevant regulatory authorities in various EU member states and the European Commission, failed to avert the withdrawal of EU MRLs for 2,4D on citrus in April 2003. The southern African citrus industry formed a trade delegation with representation from most of the major citrus industries exporting citrus to the EU, including Mediterranean countries. The delegation met with relevant parties in the European Commission and an emergency plan of action was agreed upon. CRI conducted various residue trials, compiled a supportive data package and acquired additional data from the Californian citrus industry, to support an application to the EC for reinstatement of an import MRL for 2,4D. While this legislative process was underway, temporary import MRLs were established in key EU member states. The process was concluded with the EC approving the establishment of a harmonised EU import MRL for 2,4D on citrus.

The MRLs for several other products used on citrus in southern Africa changed in the EU during 2003, mostly requiring the absence of detectable residues, but in a few cases resulting in less restrictive residue requirements. The "Recommended Usage Restrictions" document was periodically amended in accordance with these changes.

The southern African citrus industry identified proposed changes to the EU MRL framework legislation, as constituting the risk of having highly disruptive consequences for the future supply of fresh produce

exports to the EU market. At an international level, various potentially affected parties were informed of the situation and were encouraged to lodge objections with the European Commission, including an official objection from SA. The proposed amendments to the relevant EU legislation have consequently been taken under reconsideration and the outcome of this process is pending.

One of the implications of the proposed changes to the EU MRL legislation framework would have been particular disruption to fresh produce trade in the UK. The UK authorities consequently requested all affected parties to supply comprehensive information on various aspects of pesticide usage on produce traded in the UK. To minimise the potential for confusion that such a request may have had on the industry, a meeting was held with the relevant officials in the UK, the necessary data compiled and provided to the UK authorities as a consolidated industry position.

It was previously reported that discussions had been initiated between the SA Citrus Industry, COLEACP and the European Commission, to investigate the possibility of procuring financial support for SA fresh produce industries to address challenges posed by changing EU residue legislation. The consequent SA-EU Pesticide Initiative Programme (PIP) proposal was accepted by the EC in 2003, with a budget of approximately R40m for the four-year programme. The formation of implementation structures and procedures in SA were largely completed in 2003 and the programme is expected to commence functioning in 2004.

It was previously reported that there had been interceptions of SA citrus in Japan in 2002 with unacceptably high levels of imazalil. The identification of the source of the transgression and the implementation of appropriate risk mitigation procedures within the industry in 2003, ensured that this incident did not result in significant disruption to this important export programme.

There were reports of a SA citrus consignment with dithiocarbamate residues in excess of the Japanese MRL. The assistance of the affected chemical manufacturer and importer was obtained and the matter was addressed with the Japanese authorities in a manner that avoided significant disruption of the programme. Residue data were acquired from the chemical manufacturer and additional residue trials were conducted by Tian Schutte. Appropriate amendments were consequently made to the "Recommended Usage Restrictions" document.

SA was notified of a proposed revision of the Japanese MRL legislation and invited to comment. The implications of the proposed changes were evaluated and an appropriate response compiled for official communication through the Department of Agriculture. Implementation of the proposed changes to the Japanese MRL legislation are expected to result in a considerable simplification of compliance criteria and be considerably less restrictive than the current situation.

Other Market Access issues

Maintenance of the phytosanitary security of the southern African citrus industry is a top priority for CRI. Preventing the incursion of new pests and diseases into the industry is a key function of the CIP in collaboration with the Plant Health Authorities. One of the high-risk avenues for potential new pest and disease incursions is through the southerly spread of pests and diseases from northern neighbouring countries. Swaziland citrus growers expressed special concern regarding the uncertain status of citrus pests and diseases in Mozambique. A team of industry scientists consequently undertook a survey of pests and diseases from Maputo to central Mozambique and across to central Zimbabwe. No indications of significant new threats were identified, other than the potential for neglect of orchards in parts of Zimbabwe, with the associated risk of higher incidence and spread of diseases of phytosanitary concern that had been well contained under good agricultural practices (2.5). A programme of ongoing trap monitoring for the potential risk of exotic fruit flies was initiated in the Maputo corridor area. A joint industry – Department of Agriculture working group was established to assist in the management of foreign pest and disease incursions.

PROGRAMOPSOMMING

In ooreenstemming met Wêreld-handelsorganisasie (WHO) ooreenkomste, in besonder die Internasionale Plantbeskermingskonvensie (IPBK), reguleer ondertekenende lande Marktoegang by wyse van amptelike regering-tot-regering ooreenkomste en die toepassing van amptelik-geëndosseerde behandeling- en prosedure-protokolle. Versekering dat die bepaalde bedryf nie onaanvaarbare vlakke van Sanitêre en Fitosanitêre (SFS) risiko inhou vir die invoerende land nie is altyd 'n hoofoorweging by die bepaling van toegang van varsprodukte tot 'n uitvoermark. Dit is 'n internasionaal-aanvaarde beginsel dat sulke oorwegings baseer moet wees op grondige wetenskaplike gegewens. Nywerheidsnavorsing en Tegnieese dienste verteenwoordig daarom 'n kritiese en sentrale komponent in enige suksesvolle marktoegang-program. Nastrewing, handhawing en

uitbouing van toegang tot markte vir Suider-Afrikaanse sitrusuitvoere is gevolglik 'n oorkoepelende prioriteit vir die CRI-Groep navorsingsportefeulje in sy geheel. Hierdie program dek spesifieke aspekte van navorsing en tegniese ondersteuning wat toegespits is op Marktoegang. Koördinerings van die tegniese aspekte van Marktoegang is die verantwoordelikheid van die HUA CRI, Vaughan Hattingh. Marktoegang is ook 'n sleuteldoelwit van die Sitruskwekersvereniging van Suider-Afrika (SKV) en verteenwoordiging van kwekers by aangeleenthede van Marktoegang is die verantwoordelikheid van die Bestuurshoof van SKV, Justin Chadwick. Die amptelike Regeringskomponente betrokke by Marktoegang berus by die Departement van Landbou, Direkoraat Plantgesondheid. Nieuwe samewerking word gehandhaaf tussen hierdie partye om sukses van die bedryf se Marktoegang-ondernemings te verseker.

Europa

Sitruswartvlek bly 'n fitosanitêre probleem van sleutelbelang vir uitvoer na hierdie mark. Soos aangedui in vorige navorsingsjaarverslae, het langgerekte beraadslaging sedert 1992 plaasgevind tussen die EU en SA. SA het 'n omvattende Plaag-risiko Analise geformuleer wat aan die EU voorgelê is in Mei 2000. Hierdie Plaag-risiko Analise ondersteun SA se beswaar teen die EU fitosanitêre beperkings ten opsigte van SSV op grond daarvan dat die bepaling meer beperkend is op handel as wat geregtig word deur wetenskaplike feite. Terugvoering van die EU aan SA is in Desember 2001 ontvang. 'n Werkgroep van spesialiste is byeengeroep om die vrae wat deur die EU gestel is te oorweeg. 'n Uiteensetting waarin meeste van die EU se bekommernisse aangespreek is, is in Junie 2002 aan die EU voorsien. Uitstaande tegniese vrae wat deur die EU gestel is, is as prioriteite ingesluit in die navorsingsportefeulje.

Die mees kritiese komponent van die bykomende inligting wat benodig is, was 'n klimaatpassingstudie om die klimaatgeskiktheid van Europa vir potensiële vestiging van die SSV patoog te bepaal. Hierdie studie is in 2001 van stapel gestuur by die Sentrum vir Omgewingstudies, Universiteit van Pretoria, met 'n nagraadse student (Me Ida Paul). Die ondersoek is in 2003 afgehandel en die verslag amptelik voorgelê aan die EU in Desember 2003 (2.2). Die oorblywende studie wat deur die EU versoek is, behels 'n ondersoek na die moontlikheid dat konidiums van *Guignardia citricarpa* op SSV-geïnfekteerde vrugte in staat is om afgevalde sitrusblare te koloniseer en askospore daarop te produseer. Na verwagting sal hierdie studie in 2004 afgehandel wees vir voorlegging aan die EU.

Die hoogs-intensiewe en -beperkende SSV risikobestuursisteme wat reeds toegepas word is gehandhaaf en het 'n volgehoue afname in SSV-onderskeppings in die EU tot gevolg gehad. In teenstelling met die suksesvolle Suider-Afrikaanse bestuur van SSV met uitvoere, is die Argentynse en Brasiliaanse sitrusuitvoere na Spanje teen die einde van 2003 opgehef op grond van verskeie onderskeppings van vrugte besmet met *Xanthomonas campestris* (*axonopodis*) pv. *citri* (sitruskanker), *Elsinoe* spp. (skurfsiekte) en SSV. Na verwagting behoort Argentinië en Brasilië toestemming verkry om volgende jaar uitvoere na Spanje voort te sit mits voldoende risiko-bestuursisteme in werking gestel word.

Die kwarantynstatus van nie-Mediterreense vrugtevliespesies is in die vorige jaar (2002) beklemtoon toe SA 'n versoek ontvang het van die Spaanse fitosanitêre owerhede vir versekering dat voldoende risiko-beperkings toegepas word by SA sitrusuitvoere. Hersiene aanbevelings vir vrugtevliesbeheer is aan kwekers bekend gestel in 2002. Spanje het nietemin 'n onderskepping van 'n besending sitrus besmet met 'n nie-Mediterreense vrugtevlies in 2003 aangemeld. 'n Tweede werkgroep bestaande uit tegniese bedryfspecialiste is byeengeroep en strengere standaard vrugtevlies-beheermaatreëls is vir die 2003 produksieseisoen opgestel. Hennie le Roux en Tony Ware het produsentegroepe in al die produksiegebiede toegesprek om hulle bewus te maak van die belangrikheid om hierdie hersiene vrugtevlies-beheermaatreëls noudeset toe te pas. Die tegniese geldigheid om die SSV "staak en diskwalifikasie" sisteem toe te pas op vrugtevliesonderskeppings, is ondersoek. Daar is tot die gevolgtrekking gekom dat die sisteem tegnies toepaslik is vir die regulering van vrugtevliesonderskeppings, maar 'n beter begrip van die potensiële risiko wat nie-Mediterreense vrugtevlies inhou moet eers verkry word voordat implementering van so 'n sisteem geregtig kan word. Monitoring van die situasie word in die komende jaar voortgesit.

Japan

Grootskaalse staving van koue-sterilisasiëprosedures vir vrugtevlies by Clementines is voorheen onderneem deur A B Ware (1999, 2000, 2001 en 2002) en voorgelê aan Japan ter ondersteuning van 'n versoek om met die uitvoer van Clementines na dié land te begin. Verdere verduideliking van tegniese besonderhede is deur Japan versoek en in 2003 deur SA voorsien. Japan het dit aanvaar en aangedui dat Japanse owerhede SA moet besoek om ondersoek in te stel na die Clementinebedryf alvorens toestemming oorweeg kan word om 'n aanvang te neem met die uitvoer van Clementines na Japan. Die besoek is geskeduleer om plaas te vind tydens die 2004 plukseisoen.

'n Tegnieuse motivering is opgestel ter ondersteuning van 'n aansoek van SA aan Japan vir aanvaarding van minder streng koue-sterilisasiestoelstande vir vrugtevlug-ontsmetting. Die versoek is gegrond op 'n beroep vir gelykwaardigstelling met die koue-sterilisasiereëls wat deur Japan ingestel is vir sitrus ingevoer van ander lande. Die motivering is in 2002 aan Japan voorgelê. SA is in 2003 deur Japan in kennis gestel dat dit van SA vereis word om 'n omvattende proef te onderneem ter staving van die hersiene behandelingsstoelstande en dat hierdie uitvoer moet word in die teenwoordigheid van Japanse wetenskaplikes. Die stawingsproef is geskeduleer vir 2004.

Die moontlike insluiting van suurlemoene en lemmetjies vanaf Swaziland is ondersoek. Die Japanese owerhede het aangedui dat 'n afsonderlike aansoek van die "Swaziland National Plant Protection Organisation", met ondersteunende stawingsgegevens, benodig sal word. Opstelling van 'n motivering vir die insluiting van suurlemoene en lemmetjies gegrond op literatuur wat verband hou met die vrugtevlug-gasheerstatus van suurlemoene en lemmetjies, word oorweeg.

VSA

'n Maksimum toelaatbare fitosanitêre afkeursyfer van 25 % vir voorverskeping inspeksies in die SA vrugte-uitvoerprogram na die VSA is deur die USDA in werking gestel. Indien hierdie drempel op enige tydstip oorskry word, sal die program vir die bepaalde vrugsoort stopgesit word. Die drempel word in 2004 verlaag na 20 %. Fitosanitêre afkeursyfers is in 2003 deurgaans benede die toelaatbare drempel gehandhaaf.

Met die aanvang van die 2003 uitvoerseisoen is SA deur die USDA in kennis gestel dat hulle die toleransie vir onderskepping van VKM-besmette sitrus tydens voorverskeping inspeksie verlaag. 'n Drempel van twee VKM-besmette vrugte per inspeksiekarton is die vorige seisoene toegepas. Dit is met aanvang van die 2003 seisoen verminder na 'n maksimum van twee besmette vrugte in die hele inspeksielot. Die bedryf het hierteen beswaar aangeteken op grond daarvan dat die bestaan van 'n geldige koue-sterilisatiebehandeling, toegepas ná inspeksie, die verlaagde inspeksietoleransies van die USDA tegnies onregverdigbaar maak en dus teenstrydig is met die gees van die IPBK. 'n Statistiese model is deur Hattingh (CRI) en Pringle (Universiteit van Stellenbosch) opgestel vir die bepaling van tegnies-regverdigbare toleransies wat voldoen aan die probit 9 vlak van doeltreffendheid, vir onderskeppingsvlakke tydens inspeksies voor implementering van 'n ontsmettingsbehandeling. Gegrond op die bedryf se beswaar en ondersteunende argumente, het die USDA teruggekeer na implementering van die toleransies wat in vorige seisoene van toepassing was (2.3).

Die teenwoordigheid van onvolwasse onidentifiseerbare wolluise was steeds die hoofrede vir afkeurings in die VSA uitvoerprogram. 'n Molekulêre identifikasietegniek vir die vinnige en betroubare identifisering van van wolluisspesies, ongeag van hulle lewensfase, is vroeër ontwikkel deur A Severn-Ellis (ITSG) en V Hattingh (CRI). SA is deur die USDA in kennis gestel dat hulle die implementering van hierdie tegniek in die fitosanitêre inspeksieproses in 2003 aanvaar. Severn-Ellis, as een van die ontwikkelaars van die tegniek, is deur die SA Direktoraat van Plantgesondheid goedkeuring verleen om amptelike identifikasies in die uitvoerprogram te onderneem (2.4).

Die tegnieuse geldigheid van die toepassing van zero-toleransie by die onderskepping van vrugte tydens voorverskeping- en voorontsmetting-inspeksie in die VSA uitvoerprogram is ook betwis op grond van die statistiese model hierbo na verwys. Uitsluitel hieroor deur die USDA is nog nie verkry nie.

SA is deur die USDA verwittig dat hulle begin het met 'n navorsingsprojek om die temperatuurverspreiding binne skepe wat koue-sterilisatiebehandelings toepas, te bepaal. 'n Afvaardiging van USDA wetenskaplikes is in 2003 na SA gestuur om die nodige inligting te bekom vir die studie. 'n Werkgroep van Suider-Afrikaanse rolspelers in die bedryf, bestaande uit wetenskaplikes, reguleerders en verskepers, is saamgestel om as gasheer vir die afvaardiging te dien. Na afloop van die besoek is SA deur die USDA meegedeel dat hulle afvaardiging beïndruk was met die SA sisteme en dat die studie voortgesit word. Klem sal aanvanklik gelê word op die ontwikkeling van 'n simulasiemodel en die bruikbaarheid daarvan sal mettertyd bevestig word deur monitering van werklike verskepingstoelstande.

Sitrusprodusente van verskeie gebiede waarin Sitruswartvlek nie bekend is om voor te kom nie, het versoek dat opnames onderneem word om te bepaal of hierdie gebiede moontlik erken kan word as SSV-vry, as eerste stap om toegang te verkry tot die VSA-mark. SSV-opnames is in 2003 uitgevoer in dele van Limpopoprovinsie, Oos-Kaap en die oostelike gedeeltes van die Wes-Kaap. Bevestigende identifikasie van die laaste veldmonsters word nog ingewag.

Die moontlike toepassing van 'n kwantitatiewe risiko-raming benadering vir gebiede met 'n lae voorkoms van SSV, in die strewe na goedkeuring vir uitvoer na die VSA, is deur 'n werkgroep van CRI en Departement van Landbou wetenskaplikes evalueer. Daar is tot die gevolgtrekking gekom dat 'n sisteem waarvolgens

SSV-vry produksiegebiede binne streke met 'n lae voorkoms van die siekte geïdentifiseer word, tegnies 'n meer betroubare benadering is in terme van bestaande IPBK-ri glyne. 'n Raamwerksisteem is opgestel en die geldigheid daarvan evalueer tydens 'n werkgroep met deelname deur bedryfsvertegenwoordigers, wetenskaplikes en reguleerders van die Departement van Landbou. 'n Hersiene raamwerkdokument is vervolgens opgestel wat verder evalueer sal word in werkgroepe met kwekers en ander tersaaklike partye in 2004, alvorens 'n finale dokument voorberei word vir voorlegging aan die USDA.

Aangesien die vereiste kouebehandeling vir VKM-ontsmetting van sitrus nadelige effekte het op vrugkwaliteit, bestaan daar 'n dringende behoefte aan 'n minder-skadelike alternatiewe behandeling. Bestraling is as moontlike na-oes VKM-ontsmettingsbehandeling ondersoek. Die navorsing is onderneem deur Hendrik Hofmeyr in samewerking met wetenskaplikes van die Internasionale Atoomenergie-agentskap en die "United States Agricultural Research Services". Hierdie navorsing is nog op 'n vroeë stadium, maar voorlopige resultate lyk belowend. Sodra die minimum effektiewe dosis meer noukeurig bepaal is, sal vrugevaluasies onderneem word. Samesprekings word onderneem met amptenare van die USDA om wedersyds-aanvaarbare prosedures vir die uitvoering van 'n proef ter staving van die doeltreffendheid van die proses daar te stel.

Suid-Korea

Hoë afkeurvlakke op grond van onidentifiseerbare onvolwasse wolluise bly 'n beperking op uitvoere na hierdie mark. Suid-Afrika het Suid-Korea voorheen versoek om die molekulêre wolluis-identifiseringstegniek waarna in die verslag oor die VSA verwys is, te aanvaar. 'n Suid-Koreaanse wetenskaplike is in 2003 vir drie weke na SA gestuur om toesig te hou oor die uitvoering van 'n proef ter bevestiging van die identifiseringstegniek. Die gesamentlike proef is suksesvol afgehandel met die hulp van vyf Suid-Afrikaanse wetenskaplikes (V Hattingh van CRI, A Severn-Ellis van LNR-ITSG, W Wakgari van die Universiteit van Stellenbosch, I Millar van die Nasionale Versameling van Insekte en Bruce Tate van CRI) en drie beamptes van die Departement van Landbou (M Bolton, C Hatting en W Pieterse). 'n Omvattende verslag is opgestel en voorgelê aan die Suid-Koreaanse Plantgesondheidsowerhede ter ondersteuning van 'n aansoek om met die ID-tegniek te begin by fitosanitêre inspeksies. 'n Antwoord van die Suid-Koreaanse owerhede is hangende.

Die tegniese regverdiging om nie 'n toleransie te implementeer vir onderskeppings van Rooidopluis, VKM en Vrugtevlieë tydens voorverskeppingsinspeksies nie, is deur die bedryf betwis op grond daarvan dat hierdie inspeksies gevolg word deur 'n geldige ontsmettingsbehandeling vir bogenoemde organismes. Die beswaar is ingedien by die Departement van Landbou vir verwysing na die Suid-Koreaanse Plantgesondheidsowerhede vir oorweging. Terugvoering van Suid-Korea word afgewag.

Die markgeleentheid vir insluiting van suurlemoene en pomelos in die Suid-Koreaanse uitvoerprogram, is met uitvoerders bevestig. Die nodigheid vir grootskaalse staving van die VKM koue-sterilisasië protokol, met deelname deur 'n Suid-Koreaanse wetenskaplike, is voorheen bepaal. Die potensiële lewensvatbaarheid van insluiting van suurlemoene en pomelos onder die vereiste koue-sterilisasië protokol sal evalueer word uit 'n vrugkwaliteitsperspektief alvorens 'n duur grootskaalse stawende proef onderneem word.

Thailand

'n Konsepprotokol vir uitvoere vanaf SA na Thailand is in 2001 ontvang. In 2002 het SA wetenskaplike bewyse voorsien ter ondersteuning van die opheffing van sekere beperkings wat die potensiële winggewendheid van die uitvoerprogram onder die voorwaardes voorgelê in 2001 ernstig sou kompromitteer. 'n Beduidend verbeterde protokol is in 2002 deur Thailand teruggestuur. 'n Motivering vir 'n minder-veeleisende koue-sterilisasiëbehandeling is in 2002 deur bedryfswetenskaplikes opgestel en aan die Departement van Landbou voorsien vir voorlegging aan Thailand. In 2003 het dit aan die lig gekom dat die elektroniese dokumentasie van SA se teenvoorstel verlore geraak het tydens 'n rekenaarnetwerkonderbreking by die Departement van Landbou en gevolglik nie aan Thailand voorgelê is nie. 'n Opvolg-werkgroep met deelname deur bedryfswetenskaplikes, tegniese verskeppingspesialiste en reguleerders is gehou en 'n vervangingsvoorstel opgestel vir voorlegging aan die Thailandse Plantgesondheidsowerhede. 'n Antwoord van Thailand is hangende.

China

Oopstelling van die Chinese mark vir Suider-Afrikaanse sitrus is deur die sitruskwekers identifiseer as 'n top prioriteit. Gegewens oor die teenwoordigheid van sitrusplae en -siektes in SA is in Desember 2001 aan China voorgelê, tesame met 'n aansoek om die Chinese mark oop te stel vir uitvoere van sitrus uit SA. Die Bestuurshoof van SKV is uitgenooi om die Minister van Landbou te vergesel na 'n ontmoeting met die Chinese Minister van Landbou in 2003, waartydens die belang om die sitrusaansoek te bevorder, beklemtoon is. Die Voorsitter van die SKV-Raad het die Chinese sitrusbedryf besoek en begin met samesprekings aangaande wetenskaplike en tegniese samewerking tussen die Chinese en SA sitrusbedrywe. CRI (CFB) het groot

hoeveelhede saad aan China verkoop in 2002 en 2003. Twee afvaardigings hooggeplaaste Chinese amptenare het SA in 2003 besoek en was vir 'n gedeelte van die tyd gaste van die sitrusbedryf. Die Chinese amptenare het aangedui dat hulle voorlopig drie organismes van fitosanitêre belang kon identifiseer in die sitrusplaaglys, naamlik Mediterreense Vrugtevlug, Vals Kodlingmot en Carobmot. Die mees onlangse Chinese afvaardiging wat SA in Desember 2003 besoek het, het aanbeveel dat SA 'n afvaardiging na China stuur om stukrag te verleen aan SA se aansoek om toegang tot die mark.

Ander markte

Die sitrus pes en plaag datapaket is voorheen aan Israel voorgelê met 'n aansoek om hierdie mark vir sitrusuitvoere te open. Israel het in 2003 reageer met 'n versoek vir bykomende wetenskaplike inligting oor verskeie plae en siektes. SA het nog nie die inligting voorsien nie. Die vereistes vir herinstelling van sitrusuitvoere na Iran is amptelik deur die SA Departement van Landbou versoek van Iran. 'n Aansoek om aanvang te neem met uitvoere van sitrus na Australië is steeds hangende in die fase van Plaagrisikoanalise deur Australië. Die vereistes vir moontlike uitvoere na die Kanariese Eilande is ondersoek en die Marktoegang-werkgroep, met verteenwoordiging uit die bedryf en Departement van Landbou, het aanbeveel dat die bedryf nie verder met die aangeleentheid voortgaan nie.

Residus

Die Suider-Afrikaanse sitrusbedryf neem sedert 1992 stappe om plaagdoderpraktike in die bedryf pro-aktief aan te pas en daardeur in lyn te bly met residuvereistes van sy uitvoermarkte. 'n Dokument getiteld "Recommended Usage Restrictions for Plant Protection Products on Southern African Export Citrus" is van tyd tot tyd bygewerk as 'n riglyn vir die bedryf. Voor die aanvang van elke uitvoerseisoen reik die Departement van Landbou ook uitgewes uit van 'n lys van opgedateerde MRV'e in marklande.

Opheffing van MRV'e vir 2,4D op sitrus in die EU tydens die 2003 seisoen het uitvoere na hierdie mark ontwig en die toewysing van aansienlike hulpbronne genoodsaak om die krisis te oorkom. Die bedryf het laat in 2002 bewus geraak van die dreigende probleem. Intensiewe beraadslagings tussen die Suider-Afrikaanse sitrusbedryf en die tersaaklike regulerende owerhede in verskeie EU-lidlande en die Europese Kommissie kon nie die onttrekking van EU MRV'e vir 2,4D op sitrus in April 2003 afwend nie. Die Suider-Afrikaanse sitrusbedryf het 'n handelsafvaardiging daargestel met verteenwoordiging deur meeste van die hoof-sitrusbedrywe wat sitrus uitvoer na die EU, insluitend Mediterreense lande. Die afvaardiging het met betrokke partye in die Europese Kommissie vergader en ooreenstemming bereik oor 'n noodplan van aksie. CRI het verskeie residu-proewe onderneem, 'n ondersteunende datapaket opgestel en bykomende besonderhede bekom van die Kaliforniese sitrusbedryf ter ondersteuning van 'n aansoek aan die EK vir herinstelling van 'n MRV vir 2,4D. Terwyl hierdie wetgewende proses aan die gang was, is tydelike invoer-MRV'e daargestel in deurslaggewende EU lidlande. Die proses is afgehandel met die EU wat goedkeuring verleen het aan die daarstelling van 'n geharmonieerde EU invoer-MRV vir 2,4D op sitrus.

Die MRV'e vir verskeie ander produkte wat op sitrus in Suider-Afrika gebruik word, het in 2003 in die EU verander. In meeste gevalle word die afwesigheid van aantoonbare residu vereis, alhoewel minder-beperkende residu-vereistes in enkele gevalle ingestel is. Die "Recommended Usage Restrictions" dokument is periodiek gewysig in ooreenstemming met hierdie veranderings.

Die Suider-Afrikaanse sitrusbedryf het voorgestelde wysigings aan die EU MRV-raamwerkwetgewing identifiseer as synde 'n risiko in te hou met hoogs-ontwrigtende gevolge vir toekomstige voorsiening van varsproduktuitvoere na die EU-mark. Verskeie potensieel-benadeelde partye op internasionale vlak is in kennis gestel van die toedrag van sake en aangemoedig om beswaar aan te teken by die Europese Kommissie, insluitend 'n amptelike beswaar van SA. Die voorgestelde wysigings aan die tersaaklike EU-wetgewing word derhalwe heroorweeg en die uitslag van die proses is hangende.

Een van die implikasies wat die voorgestelde wysigings aan die EU MRV-wetgewingsraamwerk sou meebring is 'n besondere ontwrigting van die varsproduktehandel in die VK. Die VK-owerhede het gevolglik alle geraakte partye versoek om omvattende inligting te voorsien oor verskeie aspekte van plaagdodergebruik op produkte wat in die VK verhandel word. Om die moontlike verwarring wat so 'n versoek mag meebring te minimaliseer, is 'n vergadering gehou met die betrokke beamptes in die VK, en die nodige data saamgestel en voorsien aan die VK-owerhede as 'n gekonsolideerde bedryfsposisie.

Dit is voorheen berig dat daar met samesprekings begin is tussen die SA Sitrusbedryf, COLEACP en die Europese Kommissie oor die moontlikheid om finansiële steun te bekom vir SA om die uitdagings teweeggebring deur veranderende EU-residuwetgewing aan te spreek. Die voortspruitende SA-EU Plaagdoder-Inisiatiefprogram (PIP) voorstel is in 2003 deur die EK₁₀ aanvaar, met 'n begroting van ongeveer R40m vir die

vier-jaar begroting. Die daarstelling van implementeringstrukture en -prosedures in SA was grootliks afgehandel in 2003 en die program sal na verwagting in 2004 in werking gestel word.

Dit is voorheen berig dat onderskeppings van SA sitrus met onaanvaarbaar-hoë vlakke van imazalil in 2002 voorgekom het in Japan. Identifisering van die bron van die oortreding en implementering van toepaslike risiko-verminderende prosedures in die bedryf in 2003, het verseker dat die insident nie 'n betekenisvolle ontwinging van hierdie belangrike uitvoerprogram tot gevolg gehad het nie.

Daar was berigte van 'n SA sitrusbesending met ditiokarbamaatreste hoër as dié van die Japanese MRV. Die hulp van die betrokke chemiese vervaardiger en invoerder is verkry en die aangeleentheid is met die Japanese owerhede geskik op 'n wyse wat betekenisvolle ontwinging van die program voorkom het. Residu-data is bekom van die chemiese vervaardiger en bykomende residuproewe is uitgevoer deur Tian Schutte. Toepaslike wysigings is dienvolgens gemaak aan die "Recommended Usage Restrictions" dokument.

SA is in kennis gestel van 'n voorgestelde hersiening van die Japanese MRV-wetgewing en uitgenooi om kommentaar te lewer. Die implikasies van die voorgestelde verandering is evalueer en toepaslike terugvoering opgestel vir amptelike kommunikasie deur die Departement van Landbou. Implementering van die voorgestelde verandering aan die Japanese MRV-wetgewing sal na verwagting 'n aansienlike vereenvoudiging van voldoeningmaatstawwe meebring en heelwat minder beperkend wees as die huidige toedrag van sake.

Ander Marktoegang aangeleenthede

Handhawing van die fitosanitêre sekuriteit van die Suider-Afrikaanse sitrusbedryf is 'n topprioriteit vir CRI. Voorkoming van die binnekoms van nuwe plae en siektes in die bedryf is 'n sleutelfunksie van die SVP in samewerking met die Plantgesondheidsowerhede. Een van die hoë-risiko weë vir die binnekoms van nuwe plae en siektes is deur die suidelike verspreiding van plae en siektes vanaf noordelike buurlande. Swaziland se sitruskwekers het besonderse kommer uitgespreek oor die onsekere status van sitrusplae en -siektes in Mosambiek. 'n Span van bedryfswetenskaplikes het gevolglik 'n opname gemaak van plae en siektes vanaf Maputo tot in sentraal-Mosambiek en verder na sentraal-Zimbabwe. Geen aanduidings van enige beduidende nuwe bedreiging is gevind nie, behalwe vir die potensiële verwaarloosing van boorde in dele van Zimbabwe, met die gepaardgaande risiko van 'n hoër voorkoms en verspreiding van siektes van fitosanitêre belang wat goed in toom gehou is met gesonde boerderypraktyke (2.5). 'n Program vir voortgesette lokvalmonitering van die potensiële risiko van eksotiese vrugtevlieë is in die Maputo-korridor gebied van stapel gestuur. 'n Gemeenskaplike bedryf-Departement van Landbou werkgroep is tot stand gebring om te help met die bestuur van eksotiese plaag- en siekte-invalle.

2.2 Mapping the potential distribution of Citrus Black Spot caused by *Guignardia citricarpa* Kiely

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Summary

The suitability of the European climate for establishment of Citrus Black Spot (CBS), caused by *Guignardia citricarpa* Kiely, was assessed in a climate matching study. The study comprised three phases, with the first phase based on a climate envelope approach, whereas in phases 2 and 3 the software programme CLIMEX was used. CLIMEX allows the estimation of the geographic distribution of a species as determined by climate. In phase 2 of the study the Match Climates function within CLIMEX was used to compare the climatic conditions of Europe with South Africa and Australia. Although phase 2 results indicated the inability of the disease to persist in Europe, the Match Climates function within CLIMEX only allows the user to compare meteorological data from different localities, with no reference to the preferences of a given species. Applying this function only allows a rough assessment of the risk of a pest or pathogen establishing in a new location. It was, therefore, necessary to also apply the Compare Locations function within CLIMEX to predict the potential geographic distribution of CBS based on its climatic requirements (Phase III). A parameter set that describes the climatic preferences of the species, were inferred from information on the current known distribution of the species. The procedure is referred to as inverse or inferential modelling and involves the development of hypotheses regarding factors that limit the distribution, and manual adjustment of parameter values until the simulated geographical distribution coincides closely with the observed distribution. In addition to the distribution₁₁ data from South Africa, precise data on the

distribution of the disease in Australia allowed the compilation of a parameter set that accurately reflected the current known distribution of the disease. Results demonstrated that CBS is unlikely to establish itself in Europe. Similarly, the climate of the Northern Cape region of South Africa and parts of Australia were also shown to be unsuitable for disease development. A global risk map was produced that indicated that the risk of the potential establishment of the disease does exist in several citrus producing countries of the world, yet there was no such risk in Europe or the countries neighboring Europe.

2.3 Proposed rejection level for FCM infestations intercepted during pre-shipping inspection of citrus for export from South Africa to USA

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2.3.1 Introduction

A programme of export of citrus from certain regions in SA to USA has been in operation for several years. The programme includes pre-shipping inspection by USDA APHIS officials in SA. The programme also stipulates that the fruit must be exposed to in-transit (shipping) cold-sterilization as a disinfestation treatment for False Codling Moth and Fruit Flies.

The programme is based on IPPC SPS principles and therefore any rejection procedures adopted within the programme need to be science based. USDA APHIS has recently proposed amendments to the interception level at which consignments will be rejected on the basis of FCM infestation. This document investigates the issue of an appropriate rejection level based on statistical considerations.

The existing inspection and rejection procedure has been based on an agreement between SA and USA, whereby the pre-shipping (and pre-disinfestation) inspection of samples drawn from citrus consignments would ensure that a 95% confidence level of detecting (and rejecting) an infestation level in excess of 4% will be maintained when a 6% sample is inspected.

Accordingly the following sampling procedure for constituting an inspection sample was adopted in the programme and appropriate logistical procedures have been developed around this process.

SECTION 2.3.2 OF THIS REPORT IS A VERBATUM EXTRACT FROM THE USA CITRUS WORK PLAN

2.3.2 Inspection

2.3.2.1 Sampling rate requirement

The sampling procedure provides a 95% confidence level when an infestation level of 4% or higher is present when 6% of the consignment is sampled and inspected.

The sampling protocol is as follows:

Inspection lot size	Biometric sample size
0 to 160 cartons	25 cartons
160 to 800 cartons	50 cartons
800 cartons or more	75 cartons

The sample cartons should be numbered as each is selected. The number should reflect the sample interval. In example number 1 (below) the cartons would be numbered 10, 52, 94, etc. There is no numbering required for non-sampled cartons from the consignment.

2.3.2.2 Sampling procedure

2.3.2.2.1 Sample selection procedure for consignments that are HOMOGENOUS (only one producer, one fruit variety):

- (a) Establish the inspection unit e.g. 6000 cartons.
- (b) Divide the inspection unit size by the biometric sample size: $6000/75 = 80$ (this is the sampling interval).
- (c) Randomly select a number between 1 and the answer in point 2 (which is 80) e.g. 10. This is the number of the first carton to be selected for inspection.
- (d) To determine the second carton, add the sampling interval (80) to the first carton number (10): $80 + 10 = 90$; 90 is then the number of the second carton.
- (e) To determine the third carton, add the sampling interval (80) to the second carton number (90): $80 + 90 = 170$; 170 is then the number of the third carton.
- (f) Continue this process until the biometric sample size (in this case 75 cartons) is reached.

2.3.2.2.2 Sample selection procedure for consignments consisting of fruit of two or more different cultivars:

For instance, a consignment can consist of 2 different cultivars of citrus. It is sampled as a unit so, if a rejection occurs due to any one of the cultivars, the whole consignment will be rejected.

2.3.2.2.3 Sample selection procedure for consignments consisting of fruit from two or more producers:

The number of pallets presented by any one producer, in relation to the number presented by the other producers contributing to a consignment, determine the number of sample cartons to be drawn from each producer.

Example 1:

Producer	Inspection Unit Size	Biometric Sample Size for 800 Cartons or more
1	10 pallets: 1,600 cartons	$1600 \div 3,200 \times 75 = 37.5$ round to 37
2	5 pallets: 800 cartons	$800 \div 3,200 \times 75 = 18.75$ round to 19
3	5 pallets: 800 cartons	$800 \div 3,200 \times 75 = 18.75$ round to 19
Total:	20 pallets: 3,200 cartons	75

1. Interval determination for all 3 producers is $3,200 / 75 = 42.66$ (always round down) = 42
2. Select number between 1-42 (example 10)
3. First sample carton is number 10
4. Second sample carton is $42 + 10 = 52$
5. Third sample carton is $42 + 52 = 94$
6. Continue until appropriate number of sample cartons are drawn (example 37 cartons for producer 1)

2.3.2.2.4 Optional sampling procedures (for citrus only): Popular Count Sampling

The Popular Count refers to the size of fruit being packed. The shipment will be composed of several counts, e.g. small, medium and large fruit. The popular count refers to that count which comprises the most of the consignment e.g. the medium fruit form 60% of the consignment, small fruit 10% and large fruit 30% - the most popular count will then be the medium fruit. An assessment of mealy bug infestation levels across the fruit size was done. The result indicated a very strong uniform distribution of infestation level across the fruit size categories. This indicates that, irrespective of where the sample is drawn from in the range of size categories, the probability of detecting mealy bug remains the same. In light of the above justification, popular count sampling will be allowed as an option for citrus.

Example 2: A single producer consignment

Count	Number of Cartons
# 1	600 popular count
# 2	300
# 3	300
Total	1200

Step 1. 1,200 cartons in consignment is greater than 800 cartons thus 75 cartons required

Step 2. $600 \div 75 = 8$, thus 8 is the sampling interval

Example 3: For multiple Producer Consignments:

Producer	Count	Number of cartons	Step 1
1	# 1	600 Popular count	
1	# 2	300	
1	# 3	300	
1	Total	1200	$1200 \div 4800 \times 75 = 18.75$ round to 19
2	# 1	300	
2	# 2	600 Popular count	
2	# 3	300	
2	Total	1200	Same as above
3	# 1	600	
3	# 2	1200 Popular count	
3	# 3	600	
3	Total	2400	$2400 \div 4800 \times 75 = 37.5$ round to 37
Total consignment		4800	Total sample 75

Step 2

- Interval for Producers 1 and 2: $600 \div 19 = 33$
- Interval for Producer 3: $1200 \div 37 = 32$

2.3.2.3 Miscellaneous

- A. The sample cartons should be numbered as each is selected. The number should reflect the sample interval. In example number 1, above, the cartons would be numbered 10, 52, 94, etc. There is no numbering required for non-sampled cartons from the consignment.
- B. If industry is estimating the shipment size, the selected interval may produce either a smaller or larger sample than required. For example:
 - a. At the conclusion of the production run too few cartons have been submitted, additional cartons should be selected from the shipment. No more than 1 sample box should be selected from a pallet. Mark these sample cartons with a double X (e.g....XX).
 - i. Required sample size is 75 cartons, at the conclusion of the production run only 72 cartons have been selected. The three cartons needed to complete the sample should be removed from 3 of the pallets composing the shipment. These three cartons should be marked with double X (e.g...XX) and added to the sample. XX cartons must be re-incorporated into their original pallets.
 - b. If during the production run the required number for sample cartons are selected before the completion of the production run, continue to use the sampling interval until production run is complete. Continue to number the sample.
 - i. For example if the required sample size is 75 but using this method 82 cartons are selected, the entire 82 carton sample will be submitted for inspection. The USDA/NDA inspector will inspect 75 of the 82 cartons. A portion of the 75 cartons will be drawn from the last cartons sampled.
- C. After the sample cartons are selected, the sample cartons are not to be opened without the presence of a plant health official (USDA and/or NDA).
- D. USDA/NDA officers are required to monitor sampling and safeguarding activities. These monitoring activities should average 4 hours per week.
- E. High Cube Pallets are used to reduce unused space in the top of the shipping containers. To lessen the unused space 3 pallets are broken down and their cartons are added to the 20 pallets used to fill a 40 foot shipping container. These 20 oversized pallets must be re-strapped. There will be a few loose cartons remaining from the 3 broken down pallets. These loose cartons must be "stickered" with USDA – passed stickers, indicating the cartons have been precleared. The APHIS 203 should indicate 20 pallets plus the number of loose/un-strapped cartons.

2.3.3 Statistical considerations

It has been assumed that the distribution of infested fruit within a consignment is random and this assumption was based on the following considerations. FCM larvae are cannibalistic, providing a selection pressure against aggregate oviposition. During the orchard picking process, picking trailers are

simultaneously filled by many pickers selecting fruit from many different trees across several rows. On arrival at the packhouse, fruit from more than one picking trailer is mixed in the dump tank and further mixing takes place as the fruit is fed out of the dump tank onto the packhouse processing line. Size sorting and inspection on the line has a further mixing effect. When filling an individual carton, a packer will take one or two fruit off the moving line at a time until the carton is filled, further adding to the mixing of fruit accumulated in each carton. The process of collecting an inspection sample is furthermore spread across the volume of cartons constituting a consignment. Therefore it is safe to assume that any particular fruit in a consignment has as much chance of being placed in a particular carton as any other. If this assumption is accepted, then individual fruit in a box can be considered as being randomly selected units, and collection of the sample on a carton basis is an issue of convenience. However, the appropriate inspection sample unit, on which to base an evaluation of the infestation level should be the individual fruit.

In situations in which the universe (consignment) is not infinitely large or sampling is not with replacement the hypergeometrical distribution should be used. However, the binomial distribution provides a good approximation of the hypergeometrical distribution if the universe (consignment) is very large and the sample is small in relation to the universe. This is the case here, since as an example the universe is 43 200 fruit (consignment of 600 boxes with 72 fruit per box) and the sample is 5 400 fruit (75 boxes with 72 fruit per box). In addition, the binomial distribution is less cumbersome to work with. In accordance with the sampling procedure described above, and the need to adhere to the 95% confidence level, it is necessary to determine how many infested fruit may be tolerated within the inspection sample (rejection level) before it may be concluded that the infestation level in the consignment exceeds the 4% infestation level. The attached Excel spreadsheet (Addendum 1) models a calculation of the appropriate rejection level on inspection of the sample, using the binomial distribution model (Table 2.3.2.1). These calculations can be repeated using the hypergeometrical distribution.

Table 2.3.2.1. Rejection levels for various fruit size counts, when a 75 carton sample is inspected, based on the binomial distribution model

Fruit types	Size count (fruit per carton)	Maximum allowable infestation level (fruit per inspection sample)
Clementines	18	41
	20	48
	24	59
	28	70
	60	159
	68	181
Oranges and navels	40	103
	48	125
	56	147
	64	170
	72	192
	88	238
Lemons	95	>250
	115	>250
	140	>250
	165	>250
Minneolas	35	80
	42	101
	54	117
	81	218
	99	>250
	117	>250

USDA APHIS has advised that the hypergeometrical distribution model was used in establishing the original rejection level. The hypergeometrical distribution may be more appropriate, but with large samples the binomial distribution approximates the hypergeometrical distribution well. When fruit are used as the sampling unit, the sample size can be considered to be large.

2.3.4 Implementation

Until recently a maximum of 2 infested fruit per individual carton in the inspection sample was used as a rejection threshold. It was more recently proposed¹⁵ that the rejection level be reduced to allow a

maximum of 2 infested fruit per inspection sample. The analysis reported above indicates that both of these levels are considerably more conservative than the requirement posed by the 95% confidence level of reacting to a 4% infestation level when a 6% sample is inspected, as agreed between SA and USA to provide the basis for inspection decisions in this programme.

It is therefore recommended that in future the figures in Table 1 be adopted as appropriate rejection levels in the inspection process and that the current sampling procedure remain unchanged.

2.4 Molecular identification technique for mealy bugs on citrus exported from South Africa to South Korea

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Report on a joint trial between the South African Directorate Plant Health and Quality and the South Korean National Plant Quarantine Services - 11 February 2003 – 27 February 2003

Introduction

South Africa has been exporting citrus (sweet oranges: Navel and Valencia varieties) to South Korea since 1999. The programme relies on pre-shipment inspection by South Korean inspectors stationed in South Africa. High levels of rejection for unidentifiable mealy bugs have occurred each year, thereby seriously jeopardising the viability of this export programme.

There are seven mealy bug species known to be associated with citrus in South Africa. Six of these species are considered to have a quarantine status in South Korea. The only non-actionable species in the South African complex is *Planococcus citri*. *P. citri* is by far the most abundant species in the complex. However, it is not feasible to distinguish between *P. citri* and the other six species when immature life stages are intercepted and on this basis such consignments are rejected.

The southern African citrus industry has been developing a molecular technique to facilitate the identification of immature stages of mealy bugs. South Korean NPQS sent an official to South Africa to observe a demonstration of this identification technique. This report documents the joint trial conducted under the supervision of the SA DPHQ and NPQS overseeing all procedures throughout.

Materials and methods

Specimen collections

Specimens were collected by Dr. Vaughan Hattingh in Somerset West on 09 February 2003, in Nelspruit on 13 February 2003, in Stellenbosch on 14 February 2003 and in Nelspruit on 24 February 2003 (Tables 2.4.1, 2.4.2 & 2.4.3). Both a South Korean NPQS and SA DPHQ official oversaw the collection of samples on 13 and 14 February 2003. The South Korean NPQS official witnessed the sample collection on 24 February 2003. Individual mealy bug specimens were selected from field samples and cultures using a magnifying eyepiece. Each collection was assigned a provisional species identification (ID) by the collector, based on gross morphological characteristics that could be observed during collection, or on the basis of having been collected from apparently monospecific cultures. The specimens were placed into small sample bottles filled with 70% ethanol.

Table 2.4.1. Mealy bug samples collected at Citrus Research International, Nelspruit, on 13 February 2003.

Sample number	Sub-samples	PCR sample number	Sample details	Origin of culture or collection	Tentative species assigned during collection	ID assigned by morphological inspection
1	A, B, C, D	PCR1	One adult or sub-adult per sub-sample	Field collection from citrus orchards (Weni - Don Leetch), Nkwaleni Valley, Kwazulu-Natal, collected by B Tate, 28 January 2003, held in paper bags in laboratory (Nelspruit) until 13 February 2003.	<i>Delottococcus elisabethae</i>	<i>D. elisabethae</i>
2	A, B, C, D		Approximately 10 x I/II instar individuals per sub-sample	Nelspruit laboratory culture on sprouted potatoes, original collection from citrus orchards (Marsh Grapefruit, Tambankulu Estates, Swaziland, March 2002).	<i>Nipaecoccus viridis</i>	<i>N. viridis</i>
2	E, F, G, H		Approximately 5 x adult or sub-adult individuals per sub-sample	Same as 2 A, B, C & D.	<i>N. viridis</i>	<i>N. viridis</i>
3	A, B, C, D	PCR4	Approximately 7 x II/III instar individuals per sub-sample	Nelspruit laboratory culture on sprouted potatoes, original material collected from Schoeman Boerdery, Grobblersdal, January 2002, from an ornamental plant <i>Cestrum borantiacum</i> .	<i>Ferrisia virgata</i>	<i>Ferrisia malvastra</i>
3	E, F, G, H		Approximately 5 x adult individuals per sub-sample	Same as 3 A, B, C & D.	<i>F. virgata</i>	<i>F. malvastra</i>
4	A, B, C, D		Approximately 5 x adult individuals per sub-sample	Nelspruit culture occurring naturally on citrus seedlings.	<i>Paracoccus burnerae</i>	<i>P. burnerae</i>
4	E, F, G, H	PCR7	Approximately 10 x I/II instar individuals per sub-sample	Same as 4 A, B, C & D.	<i>P. burnerae</i>	<i>P. burnerae</i>
5	A, B, C, D		Approximately 5 x adult specimens per sub-sample	Nelspruit culture occurring naturally on citrus seedlings.	<i>Pseudococcus longispinus</i>	<i>P. longispinus</i>
5	E	PCR9	Approximately 10 x I/II instar individuals per sub-sample	Same as 5 A, B, C & D.	<i>P. longispinus</i>	Same as samples 5 A, B, C & D (<i>P. longispinus</i>)
6	A, B, C, D		Approximately 5 x adult specimens per sub-sample	Nelspruit laboratory culture on butternuts. Original collection from Mangoes, Laughing Waters Farm, Kaalrug, 5 December 2002.	<i>P. citri</i>	<i>P. citri</i>

6	E, F, G, H	PCR11	Approximately 20 x I/II instar individuals per sub-sample	Same as 6 A, B, C & D.	<i>P. citri</i>	<i>P. citri</i>
7	A, B, C, D	PCR12	Approximately 5 x adult specimens per sub-sample	Field collection from citrus (Grapefruit, Nkwaleni Valley, KwaZulu-Natal, Don Leetch's farm Weni), collected by Mr. B. Tate 28 January 2003, held in paper bags in laboratory in Nelspruit until 13 February 2003.	<i>P. longispinus</i>	<i>P. longispinus</i>
8	-	PCR13	Approximately 2 x adult or sub-adult specimens	Field collection from citrus (Grapefruit, Nkwaleni Valley, KwaZulu-Natal), collected by Mr. B. Tate 28 January 2003, held in paper bags in laboratory in Nelspruit until 13 February 2003.	<i>N. viridis</i>	- (insufficient material, field ID of this sp is reliable)
9	A, B, C, D	PCR14	Approximately 5 x adult specimens per sub-sample	Field collection from citrus (Grapefruit, Nkwaleni Valley, KwaZulu-Natal), collected by Mr. B. Tate 28 January 2003, held in paper bags in laboratory in Nelspruit until 13 February 2003.	<i>P. citri</i>	<i>P. citri</i>
10	-	PCR15	Approximately 30 x I/II instar <i>P. citri</i> individuals (same as sample 6) + 3 x I/II <i>Nipaecoccus viridis</i> instar individuals (same as sample 2)	As for samples 6 & 2.	<i>P. citri</i> + <i>N. viridis</i>	Same as samples 6 & 2 (<i>P. citri</i> and <i>N. viridis</i>)
11	-	PCR16	Approximately 30 x I/II instar <i>P. citri</i> individuals (same as sample 6) + 3 x I/II instar <i>Pseudococcus longispinus</i> individuals (same as sample 5)	As for samples 6 & 5.	<i>P. citri</i> + <i>P. longispinus</i>	Same as samples 6 & 5 (<i>P. citri</i> + <i>P. longispinus</i>)
12	-	PCR17	Approximately 30 x I/II instar <i>P. citri</i> individuals (same as sample 6) + 2 x I/II instar <i>F. virgata</i> individuals (same as sample 3)	As for samples 6 & 3.	<i>P. citri</i> + <i>F. virgata</i>	Same as samples 6 & 3 (<i>P. citri</i> + <i>F. malvastra</i>)
13	-	PCR18	Approximately 30 x I/II instar <i>P. citri</i> individuals (same as sample 6) + 3 x I/II instar <i>P. burnerae</i> individuals (same as sample 4)	As for samples 6 & 4.	<i>P. citri</i> + <i>P. burnerae</i>	Same as samples 6 & 4 (<i>P. citri</i> + <i>P. burnerae</i>)

14	-	PCR19	Approximately 30 x I/II instar <i>P. citri</i> individuals (same as sample 6) + 1 x I/II instar <i>P. calceolariae</i> individual (same as sample 18)	As for samples 6 & 18.	<i>P. citri</i> + <i>Pseudococcus calceolariae</i>	Same as samples 6 & 18 (<i>P. citri</i> + <i>P. calceolariae</i>)
15	-	PCR20	Approximately 30 x I/II instar <i>P. citri</i> individuals (same as sample 6) + 1 x adult / sub-adult <i>D. elisabethae</i> individual (same as sample 16)	As for samples 6 & 16.	<i>P. citri</i> + <i>D. elisabethae</i>	Same as samples 6 & 16 (<i>P. citri</i> + <i>D. elisabethae</i>)
16	-	PCR21	1 x III/IV instar specimen	Reference collection held in Nelspruit in 70% ethanol in an icebox, CRI accession number Ac167, sub-sampled from material identified as <i>D. elisabethae</i> by Mr. I. Millar, Job number 1998/137. Material collected from a Nelspruit laboratory culture on butternuts and lemon 14 January 1998, original collection from citrus (J. Fourie, Tshipise, November 1997).	<i>D. elisabethae</i>	<i>D. elisabethae</i> (Ac167, Job number 1998/137)

* Sub-samples A & E = South Korean NPQS reference collection (except for 5E), sub-samples C & G = SA DPHQ reference collection, sub-samples D & H destined for morphological ID by Mr. I. Millar, sub-samples B & F and samples 5E, 8 to 16 destined for PCR ID by Ms. A. Severn-Ellis.

Sample number 17 was collected by Dr. Vaughan Hattingh in Somerset West on 09 February 2003 on the stems of the ornamental plant *Agapanthus sp.* The mealy bug species was unknown, but was considered by the collector to be different from the seven species associated with citrus in SA. The sample was split into sub-samples 17 A (South Korean NPQS reference collection), 17 C (SA DPHQ reference collection), 17 D (assigned for morphological ID) and 17 B (assigned for PCR ID, PCR ID number PCR22).

Table 2.4.2. Mealybug samples collected at Stellenbosch University on 14 February 2003.

Sample number	Sub-samples	PCR sample number	Sample details	Origin of culture or collection	Tentative sp ID assigned during collection	ID assigned by morphological inspection
18	A, B, C, D	PCR23	Approximately 5 x adult specimens in each sub-sample	Field collection from citrus orchards (lemons, Rhodes Farms, Franschoek, W. Wakgari, 26 January 2003) held in laboratory (Stellenbosch) until 14 February 2003.	<i>P. calceolariae</i>	<i>P. calceolariae</i>
18	E		Approximately 4 x I/II instar specimens	As for 18 A, B, C, D.	<i>P. calceolariae</i>	Same as samples 18 A, B, C & D (<i>P. calceolariae</i>)
19	A, B, C, D	PCR25	Approximately 4 x III/IV instar specimens in each sub-sample	Stellenbosch laboratory culture on sprouted potatoes. Originally collected from citrus trees, Rhodes Farms, Franschoek, by W. Wakgari, March 2002.	<i>P. calceolariae</i>	<i>P. calceolariae</i>
20	A		Approximately 4 x adult specimens	Field sample from branches of citrus trees at Zebediela Estates, collected by F. Honniball, February 2003, maintained in laboratory in Stellenbosch on branches as originally collected.	<i>N. viridis</i>	<i>N. viridis</i>
20	B	PCR26	Approximately 4 x egg sacs	As for sample 20 A.	<i>N. viridis</i>	Same as sample 20A (<i>N. viridis</i>)
21	-	PCR27	1 x adult specimen	Stellenbosch laboratory culture on sprouted potatoes. Established with material from Nelspruit, origin uncertain. Only one live specimen was found in the sample.	<i>F. virgata</i>	-
22	A, B, C, D		Approximately 5 x adult / sub-adult specimens per sub-sample	Stellenbosch laboratory culture on citrus seedlings. Origin: a combination of material forms the Nelspruit culture and field collections on citrus in Citrusdal, W. Wakgari, April 2001.	<i>P. burnerae</i>	<i>P. burnerae</i>
23	A, B, C, D		Approximately 5 x II/III instar specimens per sub-sample	Stellenbosch culture on citrus seedlings. Origin: citrus (mandarins) in Franschoek, W. Wakgari, March 2002.	<i>P. longispinus</i>	<i>P. longispinus?</i> (ID provisional due to immature life stage)
24	-		Approximately 5 x adult <i>P. citri</i> specimens + 2 x II/III instar <i>P.</i>	<i>P. citri</i> = Stellenbosch culture on lemons and butternuts. <i>P. burnerae</i> as in sample 22.	<i>P. citri</i> + <i>P. burnerae</i>	-

			<i>burnerae</i> (same as sample 22)			
25	-		Approximately 5 x adult <i>P. citri</i> specimens + 2 x II/III instar <i>P. longispinus</i> (same as sample 23)	<i>P. citri</i> as in sample 24 and <i>P. longispinus</i> as for sample 23.	<i>P. citri</i> + <i>P. longispinus</i>	-
26	-		Approx 5 x adult <i>P. citri</i> specimens + 1 x II/III instar <i>P. calceolariae</i> (same as sample 19)	<i>P. citri</i> as in sample 24 and <i>P. calceolariae</i> as for sample 19	<i>P. citris</i> + <i>P. calceolariae</i>	-
27	-		Approx 5 x adult <i>P. citri</i> specimens + 1 x adult <i>N. viridis</i> (same as sample 20)	<i>P. citri</i> as in sample 24 and <i>N. viridis</i> as for sample 20.	<i>P. citri</i> + <i>N. viridis</i>	-

* Sub-samples 18a, 19A, 22A & 23A = South Korean NPQS reference collection; sub-samples 18C, 19C, 22C & 23C = SA DPHQ reference collection; sub-samples 18D, 19D, 20A, 22D & 23D destined for morphological ID by Mr. I. Millar; sub-samples 18B, 18E, 19B, 20B, 21, 22B, 23B, 24 to 27 destined for PCR ID by Ms. A. Severn-Ellis.

Table 2.4.3. Mealy bug samples collected at Citrus Research International (CRI) Nelspruit on 24 February 2003.

Sample number	Sub-samples	PCR number	sample	Sample details	Origin of culture or collection	Tentative sp ID assigned during collection	ID assigned by morphological inspection
28 (replaces sample 12)	-	PCR28	replaces PCR17	Approximately 15 x I/II instar <i>P. citri</i> specimens (same as sample 6) + 3 x II/III/IV instar <i>F. virgata</i> (Ac201 = sample 31)	<i>P. citri</i> = sample 6, <i>F. virgata</i> = sample 31.	<i>P. citri</i> + <i>F. virgata</i>	Same as samples 6 & 31 (<i>P. citri</i> & <i>F. virgata</i>)
29 (replaces sample 15)	-	PCR29	replaces PCR20	Approximately 15 x I/II instar <i>P. citri</i> specimens (same as sample 6) + 1 x II/III/IV instar <i>D. elisabethae</i> (Ac167 = sample 16)	<i>P. citri</i> = sample 6, <i>D. elisabethae</i> = sample 16.	<i>P. citri</i> + <i>D. elisabethae</i>	Same as samples 6 & 16 (<i>P. citri</i> & <i>D. elisabethae</i>)
30 (replaces sample 13)	-	PCR30	replaces PCR18	Approximately 15 x I/II instar <i>P. citri</i> specimens (same as sample 6) + 3 x II/III instar <i>P. burnerae</i> (same as sample 4).	<i>P. citri</i> = sample 6, <i>P. burnerae</i> = sample 4.	<i>P. citri</i> + <i>P. burnerae</i>	Same as samples 6 & 4 (<i>P. citri</i> & <i>P. burnerae</i>)
31 (replaces sample 3)	A			5 x adult / sub-adult specimens	Nelspruit reference material preserved in 70% ethanol in icebox, Nelspruit accession number Ac201, collected on citrus, Tshipise by S. Kamburov August 1998, maintained on potatoes and lemons in Nelspruit laboratory until 26 October 1998.	<i>F. virgata</i>	<i>F. virgata</i>
31 (replaces sample 3)	B	PCR31B	replaces PCR4	Approximately 3 x adult / sub-adult specimens	As in sample 31 A.	<i>F. virgata</i>	Same as sample 31A (<i>F. virgata</i>)

* Samples 28, 29, 30, 31B assigned for PCR ID by Ms. A. Severn-Ellis; sample 31A assigned for morphological ID by Mr. I. Millar.

Morphological identification

Morphological identifications were done by Mr. I. Millar, an internationally recognised Pseudococcidae systematics specialist from the National Collection of Insects, Plant Protection Research Institute, Agricultural Research Council Pretoria, on 19 and 20 February 2003. Both South Korean NPQS and SA DPHQ officials witnessed the procedure.

Molecular identifications

Molecular identifications were done by Ms. A. Severn-Ellis, a biotechnologist from the Institute of Tropical and Subtropical Crops, Agricultural Research Council, Nelspruit on 17 and 18 February 2003 (witnessed by the South Korean NPQS and SA DPHQ officials) and 24, 25 and 26 February 2003 (witnessed by the South Korean NPQS official). The molecular identifications were conducted in accordance with the PCR technique described in Addendum A, with amendments to PCR conditions as indicated in the Results section of this report, where relevant.

Results

The Morphological identifications by Mr. I. Millar confirmed the accuracy of the provisional identifications assigned by Dr. V. Hattingh during collection of the samples, with one exception, namely sample 3 that was identified by Mr. Millar as *F. malvastra* and not *F. virgata* as assumed during collection of the sample.

Microscopic inspection of morphological characteristics by Mr. Millar confirmed that sample 17 was not one of the seven species associated with citrus. Molecular diagnosis of this sample number 17 (PCR sample number PCR22) produced no positive matches with any of 7 citrus species.

Due to time and equipment constraints it was not feasible to conduct PCR diagnoses on all the samples collected. Consequently the South Korean NPQS official selected a number of representative samples for molecular identification using the PCR technique. Three series of PCR testing were conducted. The results have been collated on a species basis and are reported below.

Nipaecoccus viridis primer

Series 1: Samples that should have been positive (PCR13, PCR15, PCR26) produced distinct banding and were diagnosed as positive relative to the positive control (plasmid P4) (Fig. 2.4.1). There was no non-specific banding and there were no false positives.

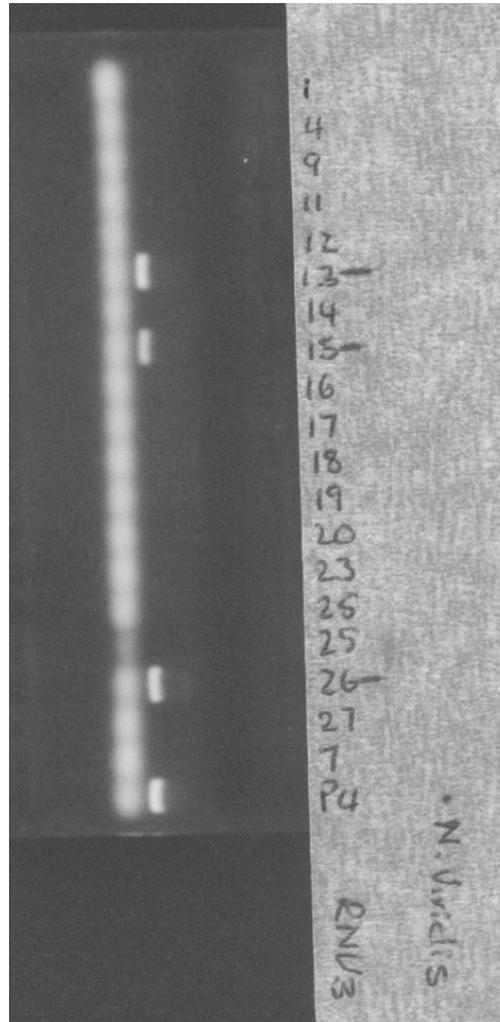


Figure 2.4.1. Series 1 PCR gel using *N. viridis* primer under original PCR conditions. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2).

It was concluded from Series 1 results that this primer and the PCR conditions as set out in the original PCR treatment procedure (Addendum A) are adequate for use in a diagnostic procedure.

Pseudococcus longispinus primer

Series 1: All samples that should have tested positive (PCR9, PCR12, PCR16) were clearly diagnosed as positive relative to the positive controls (Plasmid P2 and a *P. longispinus* DNA extraction D2) and there were no false positive results (Fig. 2.4.2). There was non-specific banding, but this did not detract from the ability to accurately make positive or negative diagnoses. In an attempt to reduce the extent of this non-specific banding, the *P. longispinus* primer was included in a second series of testing and the annealing temperature was increased from 65°C to 66°C.

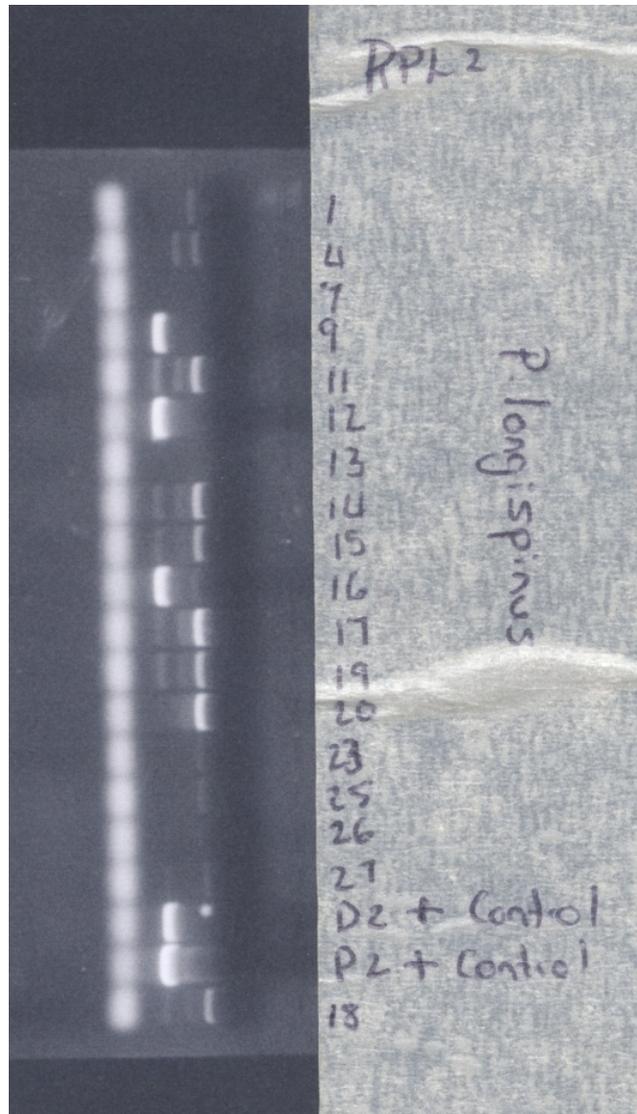


Figure 2.4.2. Series 1 PCR gel using *P. longispinus* primer under original PCR conditions. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2).

Series 2: Increasing the annealing temperature to 66°C, removed the non-specific banding, and all the positive samples remained clearly positive without any false positives (Fig. 2.4.3).

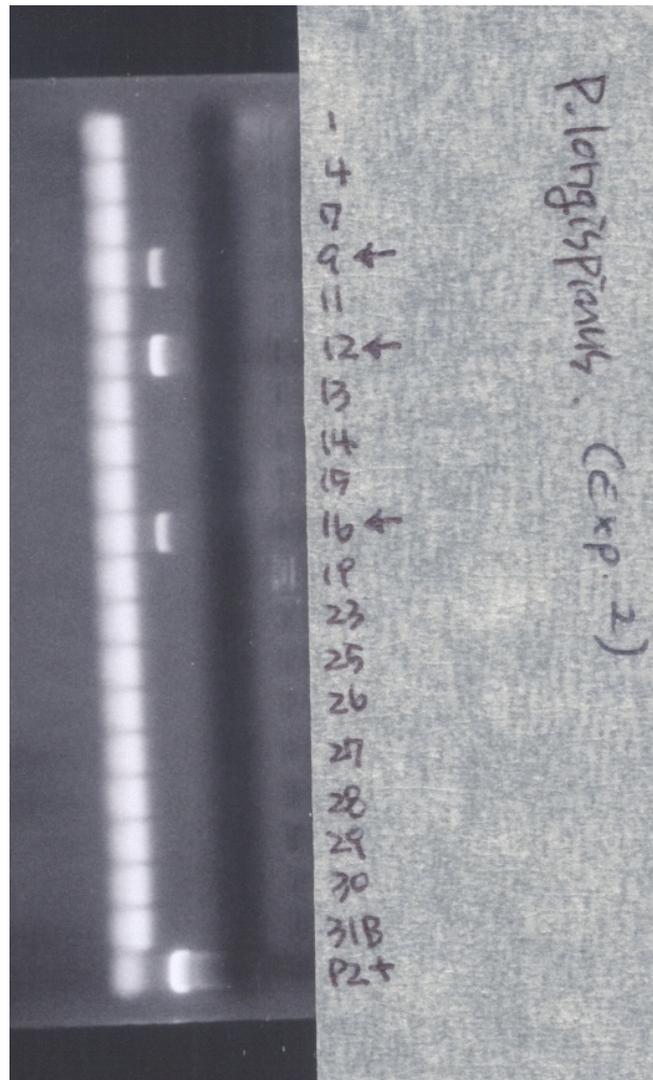


Figure 2.4.3. Series 2 PCR gel using *P. longispinus* primer with annealing temperature increased to 66°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 & 2.4.3)

It was concluded from Series 2 results that the PCR conditions for this primer should be amended by increasing the annealing temperature from 65°C as set out in the original testing procedure (Addendum A) to 66°C and that with this amendment the primer would be adequate for use in a diagnostic procedure.

Pseudococcus calceolariae primer

Series 1: All samples that should have tested positive (PCR19, PCR23, PCR25) were clearly diagnosed as being positive relative to the positive controls (Plasmid P7 and an extraction of *P. calceolariae* DNA D7) (Fig. 2.4.4). There were no false positive results. There was some faint non-specific banding, but this did not detract from the ability to accurately make positive or negative diagnoses. In an attempt to reduce this non-specific banding, the *P. calceolariae* primer was included in a second series of testing and the annealing temperature was increased from 62°C to 64°C.

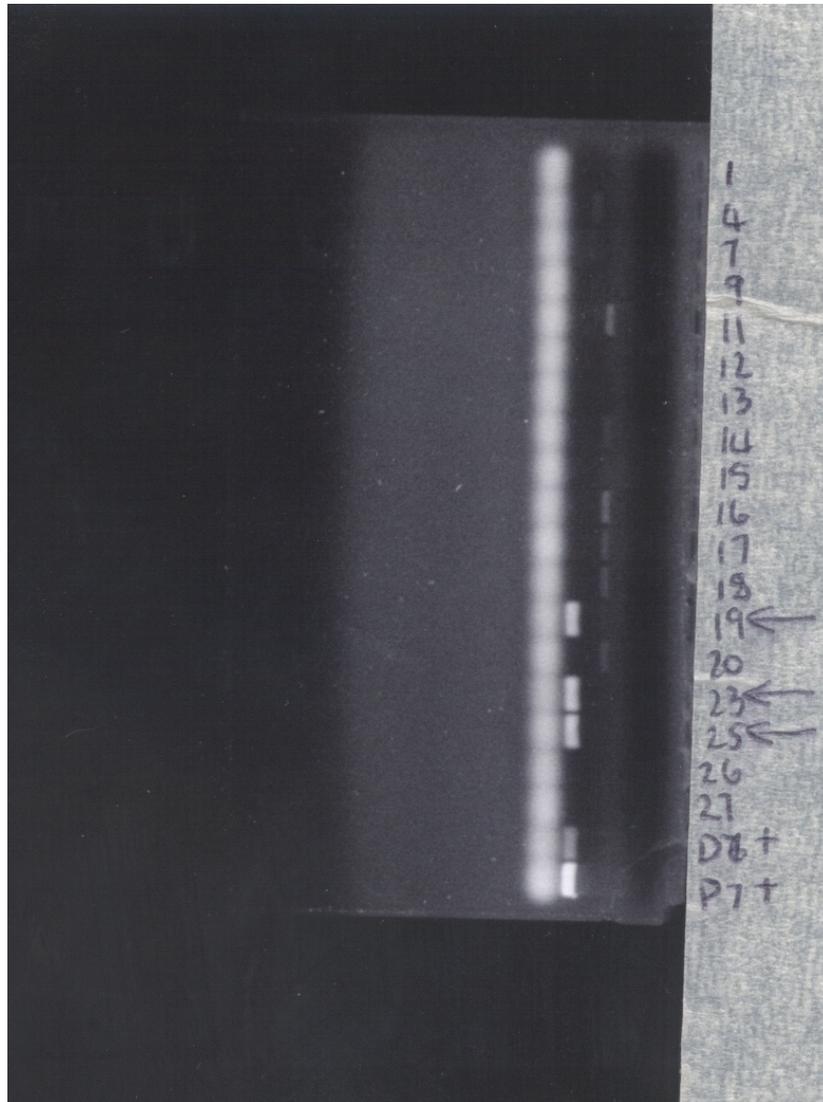


Figure 2.4.4. Series 1 PCR gel using *P. calceolariae* primer under original PCR conditions. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2)

Series 2: Increasing the annealing temperature to 64°C, did not remove the non-specific banding, and the positive samples produced less intensive banding (Fig. 2.4.5).

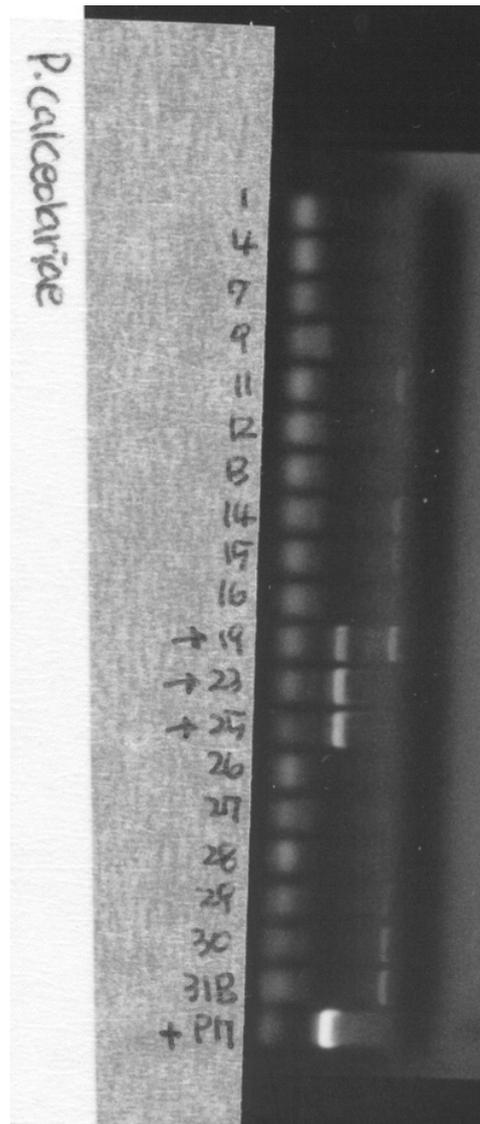


Figure 2.4.5. Series 2 PCR gel using *P. calceolariae* primer with annealing temperature increased to 64°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 & 2.4.3).

It was concluded that the original PCR conditions for this primer, as set out in Addendum A should be retained. The faint non-specific banding did not detract from the accuracy of positive diagnoses and did not give rise to any false positives. Together with the absence of any false negatives, this indicated that the primer would be adequate for inclusion in a diagnostic procedure.

Ferrisia virgata primer

Series 1: The positive controls (Plasmid P5 and a *F. virgata* DNA extraction D5) produced clear bands (Fig. 2.4.6). Sample PCR27 (Stellenbosch culture), tested strongly positive for *F. virgata*. Sample PCR4 (Nelspruit culture) gave a faint positive banding whereas sample PCR17 (a mixture of *P. citri* and *F. virgata* from the Nelspruit culture) tested negative for *F. virgata*. There was also a fair amount of non-specific banding.

The morphological identifications conducted by I Millar indicated that the Nelspruit culture (PCR4 & PCR17) that was provisionally identified as *F. virgata* during collection of the sample, was *F. malvastra*. This culture had been established from field collections on an ornamental plant, not citrus, and the *Ferrisia* sp. resembling *F. virgata* had not been positively identified prior to collection of the sample for PCR testing. The faint positive banding of sample PCR4 indicated that there was a weak cross reaction between the *F. virgata* primer and *F. malvastra* DNA, but this weak cross reaction was not evident in a dilution sample (PCR17).

In an attempt to reduce the extent of non-specific banding, the *F. virgata* primer was included in a second series of testing, and the annealing temperature increased from 65°C to 66°C. The sample PCR17 was replaced with PCR28 and PCR31B was added (Tables 2.4.1 & 2.4.3).

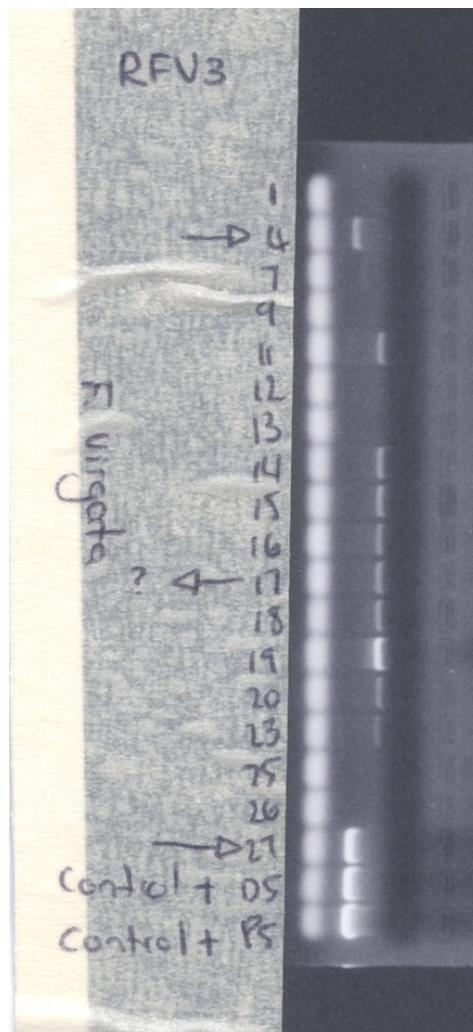


Figure 2.4.6. Series 1 PCR gel using *F. virgata* primer under original PCR conditions. Numbers on the margins of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2).

Series 2: Increasing the annealing temperature to 66°C reduced the intensity of non-specific banding, but a faint false positive band was evident in sample PCR30 (Fig. 2.4.7). The faint cross-reaction with *F. malvastra* (sample PCR4) was still evident. The positive sample PCR27 and replacement positive samples PCR28 and PCR31B were all strongly positive. In a further attempt to reduce the non-specific banding, the *F. virgata* primer was included in third series of testing where the annealing temperature was further increased to 67°C.

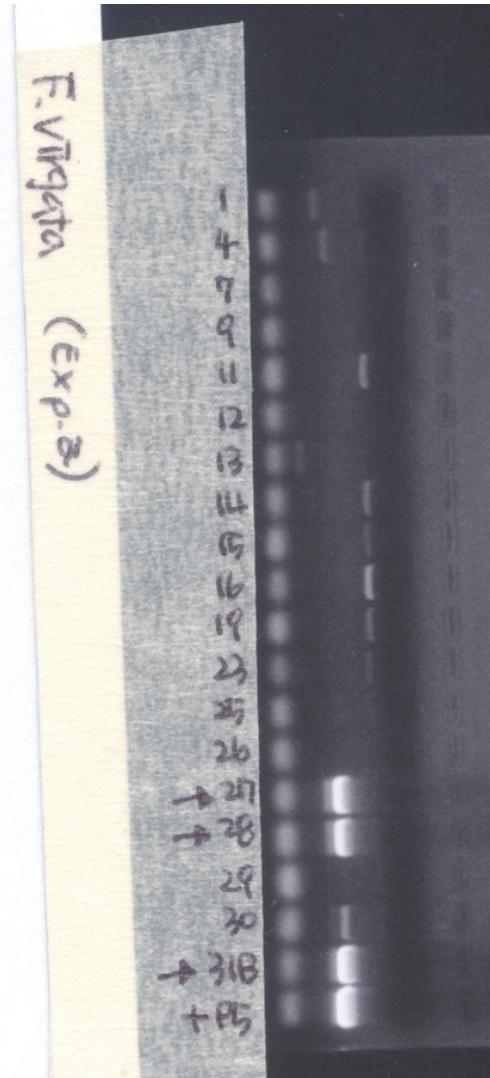


Figure 2.4.7. Series 2 PCR gel using *F. virgata* primer with annealing temperature increased to 66°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 and 2.4.3).

Series 3: Increasing the annealing temperature to 67°C eliminated the non-specific banding (Fig. 2.4.8). All positive test samples showed strongly positive banding and there were no false positives. The faint cross-reaction with *F. malvastra* was also no longer evident.

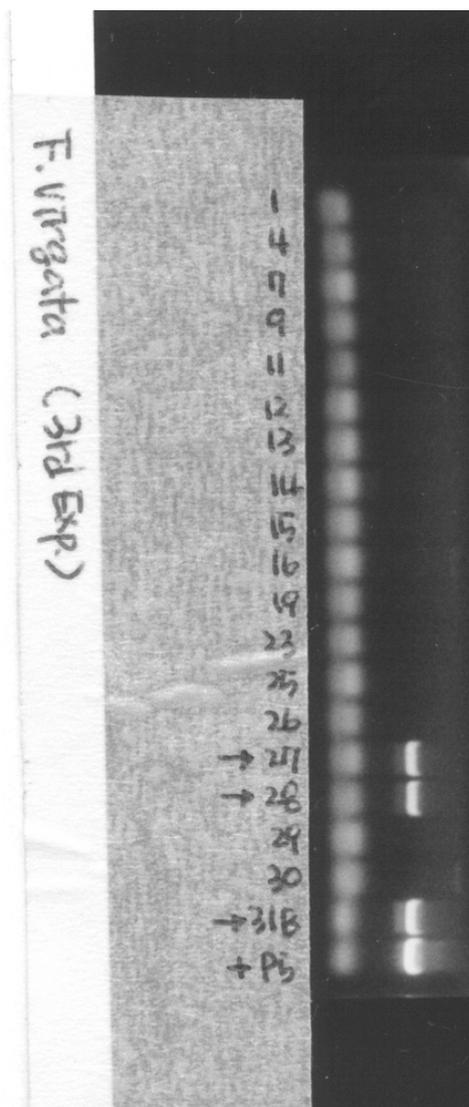


Figure 2.4.8. Series 3 PCR gel using *F. virgata* primer with annealing temperature increased to 67°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 and 2.4.3).

It was concluded that the annealing temperature should be increased to 67°C, and under these conditions the *F. virgata* primer would be adequate for inclusion in a diagnostic procedure

Paracoccus burnerae primer

Series 1: The results of the first series of testing were confusing. Firstly, the plasmid used as a positive control (P1) did not accurately match the other positive control (*P. burnerae* DNA extraction D1) (Fig. 2.4.9). Secondly, positive test sample number PCR18 was not diagnosed as positive, whereas positive test sample PCR7 did show strongly positive banding. Non-specific banding was also evident.

The absence of positive banding with sample PCR18 (a dilution sample) could have been due to loss of the *P. burnerae* individuals from the sample bottle prior to DNA extraction. This may have occurred when excess ethanol was removed from the sample under vacuum. On removal of the samples from the vacuum chamber it was noticed that the ethanol had boiled and several mealy bug specimens had been expelled from the sample bottles.

Consequently it was decided to include the *P. burnerae* primer in a second series of testing with a replacement plasmid as positive control (P1), and a new mixed species sample (PCR30) was collected to

replace sample PCR18. In the second series of testing the annealing temperature was increased from 70°C to 70.5°C in an attempt to reduce the non-specific banding.

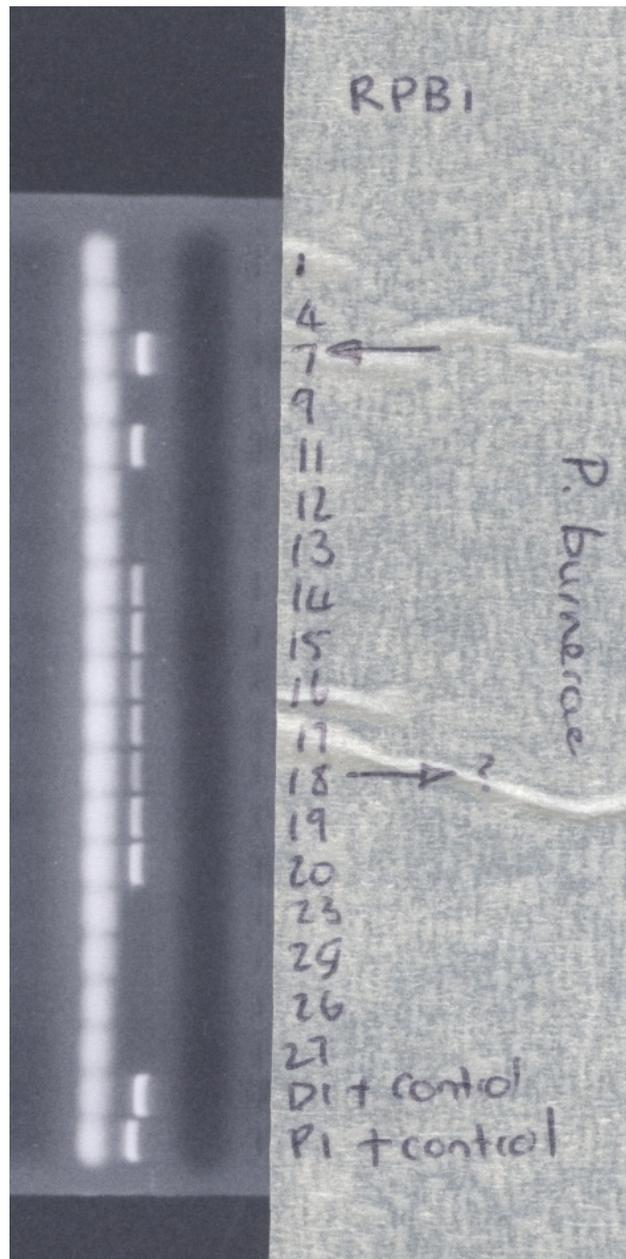


Figure 2.4.9. Series 1 PCR gel using *P. burnerae* primer under original PCR conditions. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2)

Series 2: Increasing the annealing temperature to 70.5°C eliminated the non-specific banding (Fig. 2.4.10). However, the positive test sample PCR30 did not show a positive band, and the intensity of the banding with the positive test sample PCR7 and the positive control (P1) was reduced. It was consequently decided to repeat the test in a third series, using the original annealing temperature of 70°C.

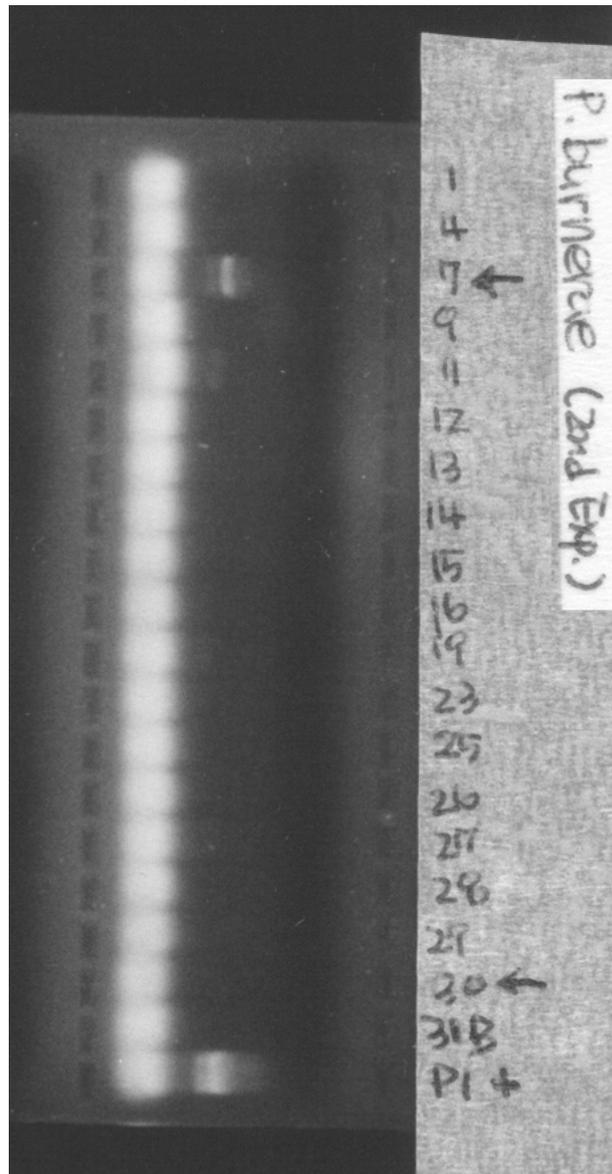


Figure 2.4.10. Series 2 PCR gel using *P. burnerae* primer with annealing temperature increased to 70.5°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 and 2.4.3).

Series 3: The positive test samples (PCR7 & PCR30) produced strong positive banding and the positive control (P1) also produced strong banding (Fig. 2.4.11). Although, fainter non-specific banding was present, this did not interfere with the accuracy of both appropriate positive and negative diagnoses.

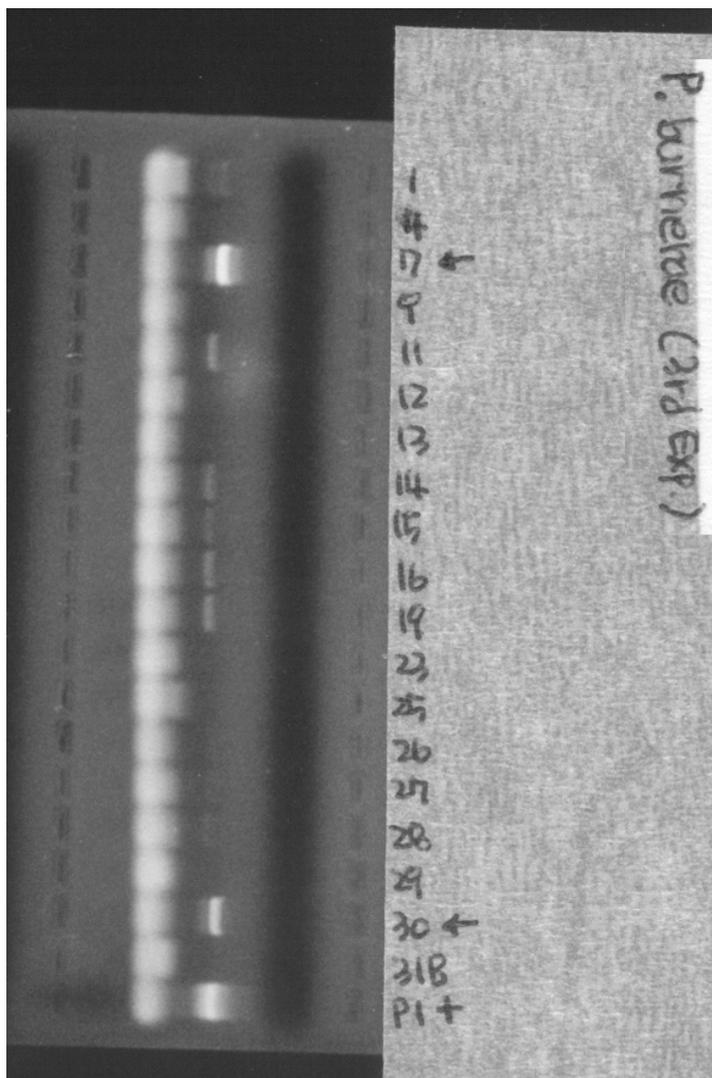


Figure 2.4.11. Series 3 PCR gel using *P. burnetiae* primer with the original annealing temperature of 70°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 and 2.4.3).

It was concluded that the *P. burnetiae* primer should be used under the original conditions described in Addendum A. The non-specific banding did not preclude accurate diagnoses and therefore the primer was considered to be adequate for use in the proposed diagnostic procedure.

Delottococcus elisabethae primer

Series 1: The positive controls (plasmid P6 and a *D. elisabethae* DNA extraction D6) showed strong banding and the positive test sample PCR1 showed strong positive result (Fig. 2.4.12). The positive test sample PCR20 did not show positive banding.

The absence of positive banding with sample PCR20 (a dilution sample) could have been due to loss of the *D. elisabethae* specimen from the sample bottle prior to DNA extraction. This may have occurred when excess ethanol was removed from the sample under vacuum. On removal of the samples from the vacuum chamber it was noticed that the ethanol had boiled and several mealy bug specimens had been expelled from the sample bottles.

Consequently it was decided to include the *D. elisabethae* primer in a second series of testing with sample PCR20 being replaced with a new mixed species sample PCR29.

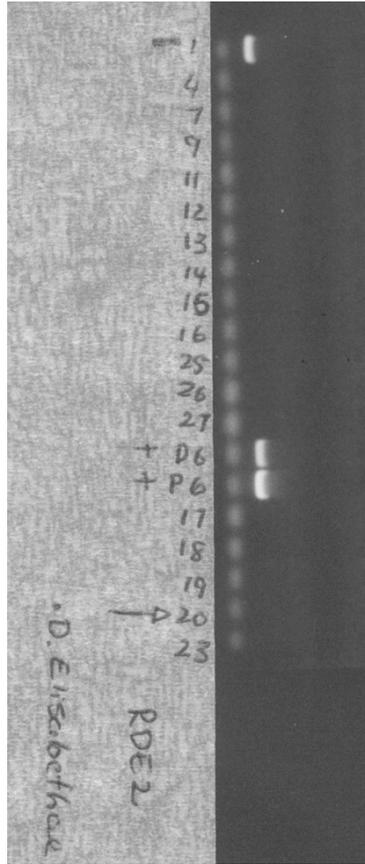


Figure 2.4.12. Series 1 PCR gel using *D. elisabethae* primer under original PCR conditions. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2)

Series 2: All positive samples (PCR1 & PCR29) showed clear intense positive banding (Fig. 2.4.13). Some faint false positive banding was evident. Where such banding may be encountered during the execution of a diagnostic procedure the test would be repeated to seek clarification. The gel from series 1 had none of the weak false positives found in series 2. The occurrence of this additional banding in series 2 may be attributable to primer deterioration.

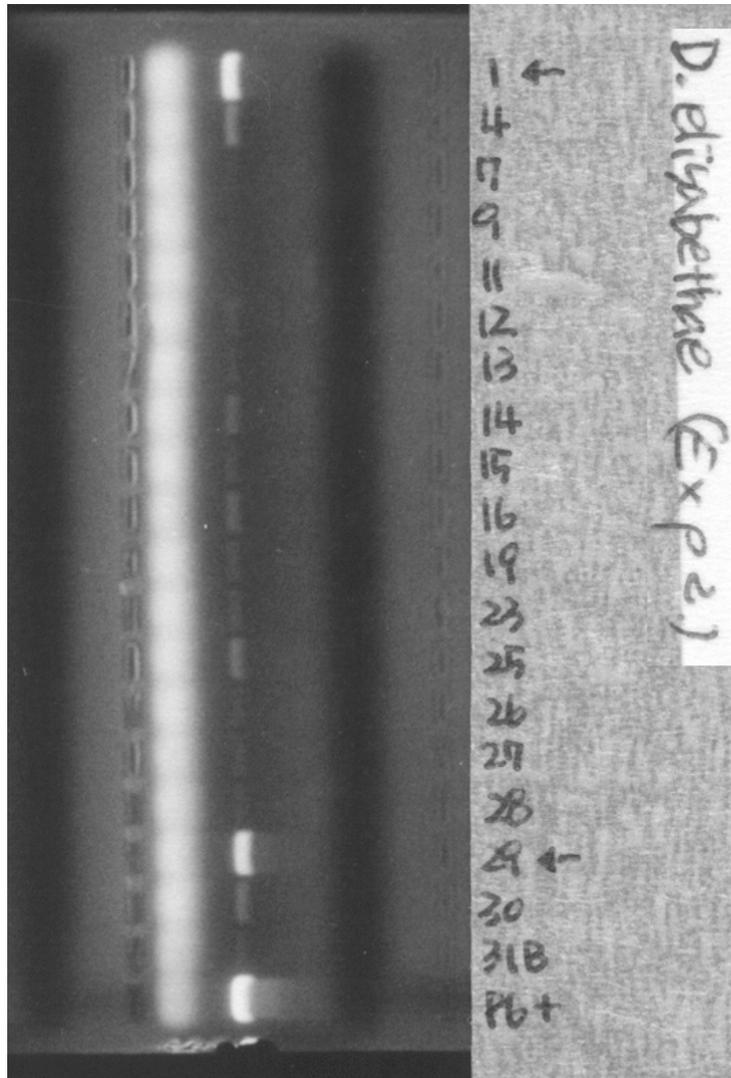


Figure 2.4.13. Series 2 PCR gel using *D. elisabethae* primer. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 and 2.4.3).

On the basis of the clarity of the series 1 gel, and the subsequent confirmation in series 2 and 3 that the *D. elisabethae* specimen had been lost during the processing of sample PCR20, the *D. elisabethae* primer was considered adequate for use in the proposed diagnostic procedure.

Planococcus citri primer

Series 1: The results of series 1 testing were clear. All positive test samples (PCR11, PCR14 to PCR20) showed strong positive banding that matched the positive controls (primer P3 and a *P. citri* DNA extraction D3) (Fig. 2.4.14). There was some faint non-specific banding that did not confuse the diagnostic result. In an attempt to eliminate this non-specific banding the *P. citri* primer was included in a second series of testing with the annealing temperature increased from 65°C to 65.5°C.

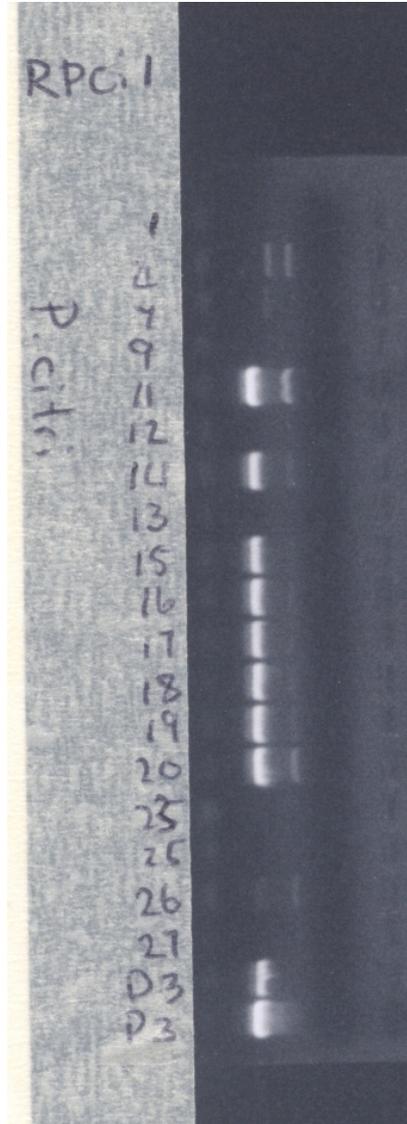


Figure 2.4.14. Series 1 PCR gel using *P. citri* primer under original PCR conditions. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1 and 2.4.2)

Series 2: Increasing the annealing temperature to 65.5°C did not eliminate the non-specific banding (Fig. 2.4.15). The replacement positive test samples (PCR28, PCR29 & PCR30) were all clearly diagnosed as positives.

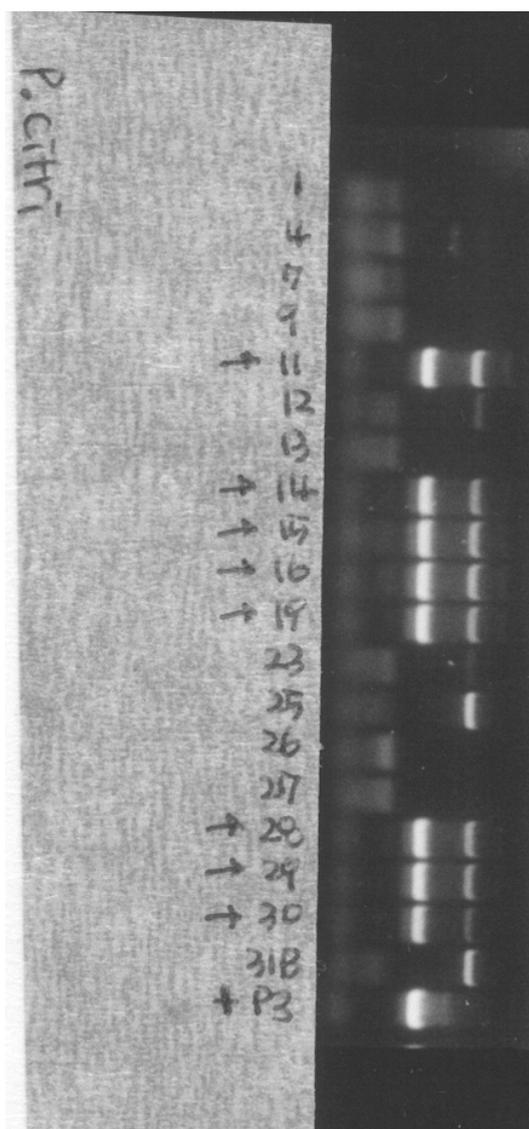


Figure 2.4.15. Series 2 PCR gel using *P. citri* primer with annealing temperature increased to 65.5°C. Numbers on the margin of the gel correspond to PCR sample numbers (Tables 2.4.1, 2.4.2 and 2.4.3).

It was concluded that the *P. citri* primer should be used under the original conditions described in Addendum A. The non-specific banding did not preclude accurate diagnoses and therefore the primer was considered adequate for use in the proposed diagnostic procedure.

Conclusion

In considering whether a PCR ID technique is suitable for adoption in phytosanitary inspections, it is essential to ensure that the phytosanitary security of the importing country is not jeopardised. In the case of mealy bugs occurring on citrus exported from South Africa to South Korea, where *P. citri* is non-actionable and the other mealy bug species associated with citrus are actionable, it is important that the technique should not give false positives with the *P. citri* primer and should also not give false negatives with the other primers. It has been demonstrated that, barring human error during the processing of the samples, the technique meets the above requirements.

Non-specific banding was evident with the use of some of the primers. The reason for the occurrence of this banding was unclear. Such banding had not been evident in testing conducted prior to this joint trial as reflected in the workbook of the responsible biotechnologist, A. Severn-Ellis. The only change brought about between such testing and the joint trial was that the stock of primers had been replaced with a newly

manufactured batch. Whether this could explain the sudden appearance of such non-specific banding was unclear.

The presence of non-specific banding was eliminated with some of the primers by modifying the annealing temperatures. Nonetheless, the non-specific banding did not increase the risk of returning either a false positive with the *P. citri* primer or a false negative with any of the other primers. As such, the presence of the type of non-specific banding evident in these trials, would not compromise the phytosanitary security of the importing country, should this technique be used as a diagnostic tool in the export inspection process.

The technique provides a means of significantly reducing unnecessary rejection of consignments of citrus presented for export from South Africa to South Korea due to the presence of previously unidentifiable immature mealy bugs. Adoption of this diagnostic technique into the export inspection process would significantly enhance the value of this export programme, without exposing the importing country to an increased exposure to phytosanitary risk.

ADDENDUM A: MOLECULAR IDENTIFICATION PROCEDURE FOR MEALYBUGS OCCURRING ON SOUTH AFRICAN CITRUS EXPORTS

Developers: A.A. Severn-Ellis¹, V. Hattingh² and M. Luttig¹ (SA patent registered)

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Summary

Molecular identification techniques were established to reliably distinguish between seven mealy bug species which may be found on citrus in southern Africa namely: *Planococcus citri*, *Ferrisia virgata*, *Paracoccus burnerae*, *Pseudococcus longispinus*, *Nipaecoccus viridis*, *Delottococcus elisabethae* and *Pseudococcus calceolariae*. *Nipaecoccus viridis* was included although this species is primarily associated with wood and very young fruitlets and not with ripe fruit. Species-specific primers were designed to permit the identification of the mealy bug species at different stages of their life cycles.

Testing procedure

Insect material

Specimens collected during the export inspection process should be placed in small sample vials filled with 70% ethanol. All mealy bug specimens collected from a particular consignment can be combined into a single sample vial.

Preparation of DNA for PCR

On receipt of the sample by the testing laboratory, the specimens may be separated depending on the number and sizes of individuals in the sample. Samples consisting of individuals with both large (adult or sub-adult) and small (I, II or III instar) individuals will be split so that small insects are separated from the larger specimens. Samples consisting of large numbers of individuals will be sub-divided into sub-samples that each contain no more than 10 medium to large individuals. Samples consisting of larger quantities of small individuals can be processed without sub-dividing.

The total nucleic acid extraction procedure was modified from Nagaraja & Nagaraju, 1995 (Nagaraja G.M. and Nagaraju J., 1995. Genomic fingerprinting of the silkworm, *Bombyx mori* using random arbitrary primers. Electrophoresis 16:1633-1638). Mealybugs are homogenised in 5, 10, 20, 30, 50 and 100 µl of nucleic lysis buffer (LB) depending on the size and number of insects. The lysis buffer contained 100 mM Tris-HCl, pH 8.0, 50 mM NaCl, 50 mM EDTA, 1% SDS, and 5 µl of 100 µg/ml Proteinase K. The homogenised insects are then incubated at 37°C for 30 min. The DNA is extracted once with an equal volume of phenol: chloroform: iso-amylalcohol (24:24:1). If the supernatant still contains impurities a second extraction is carried out with chloroform: iso-amylalcohol (24:1). The supernatant DNA is then precipitated with 100% ethanol, washed with 70% ethanol, and re-suspended in 20 - 100 µl TE buffer. Two µl RNase are added and incubated at 37°C for 30 min. The extracted DNA is visualised on a 1% agarose gel and the concentration/ optical density (OD) determined at 260 nm.

In the case of processing single first or second instar insects lysis in 20 µl is suggested, while the extraction

with an equal volume of phenol: chloroform: iso-amylalcohol (24:24:1) can be omitted and the DNA precipitated with ethanol from the lysis buffer supernatant. It is however important to add RNase to the suspended DNA.

For larger insects and samples containing more than one insect, it is suggested that 50 to 200 µl lysis buffer is used and the DNA is extracted with phenol: chloroform: iso-amylalcohol (24:24:1). If the supernatant cannot be removed without impurities, a second extraction with chloroform: iso-amylalcohol (24:1) should be carried out.

Species-specific plasmids have been cloned to serve as positive controls.

Primers

A reverse or 3' 18 mer species specific, reverse primer has been designed for each of the 7 mealy bug species, while a single common 18 mer forward or 5' primer has been selected. The selected oligonucleotides are synthesised by MWG Biotech or Integrated DNA Technologies Inc.

PCR and electrophoresis

PCR amplifications are conducted in 25 µl reaction volumes containing 20 ng of total DNA of sample insects, 2 µl of 10 µM of the forward primer (FW1) and 2 µl of 10 µM species specific reverse primer. Various amplification conditions apply to the various primers.

The amplification parameters for the species specific primers for *P. burnerae* (RPB1), *N. viridis* (RNV3) and *D. elisabethae* (RDE2) are 94°C for 3 min for one cycle for initial denaturation, followed by 35 cycles of denaturing at 94°C for 1 min., annealing at 70°C for 20 seconds and extension at 72°C for 45 seconds and one final extension cycle at 72°C for 2 min. Amplification conditions for the species specific primers for *P. longispinus* (RPL2), *P. citri* (RPCi 1) and *F. virgata* (RFV3) are initial denaturing at one cycle at 94°C for 3 min, followed by 35 cycles of denaturing at 94°C for 1 min., annealing at 65 °C for 45 seconds and extension at 72°C for 45 seconds and one final extension cycle at 72°C for 2 min. For the species specific primer of *P. calceolariae* (RPCA 1), amplification conditions are an initial denaturing at 94°C for 3 min, followed by 35 cycles of denaturing at 94°C for 1 min., annealing at 62°C for 30 seconds and extension at 72°C for 45 seconds and one final extension cycle at 72°C for 2 min. The amplification products are visualised on a 1.2 % agarose gel stained with ethidium bromide.

* Annealing temperatures have subsequently been amended as follows: *P. longispinus* = 66°C, *F. virgata* = 67°C.

2.5 Survey of potential phytosanitary pests and diseases in Mozambique and Zimbabwe 25 July – 7 August 2003

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Introduction

The southern African citrus industry has been threatened by some serious citrus diseases over the past century. One of these threats was the introduction of citrus canker into South Africa in 1909. The eradication thereof is an achievement still mentioned in the international media. A second threat was citrus greening disease which destroyed four of the eleven million citrus trees in South Africa during the 1970s. Once again the authorities and citrus research infrastructure within South Africa had the capacity to address the problem and to develop strategies to cope with this disease.

A disease present in parts of southern Africa that could pose a threat to the South African citrus industry is *Pseudocercospora angolensis*, formerly known as *Cercospora angolensis*, *Phaeoramularia angolensis* and *Phaeosariopsis angolensis*. During the 1990s, researchers working for Outspan International cooperated with the South African and Zimbabwean authorities, as well as the Zimbabwean citrus industry, to survey Zimbabwe and attempt to eradicate this disease. The disease was never eradicated but the districts in which the disease could be found were reduced to Matepatepa and Bindura.

During the late 1990s, visitors to Inhambane reported lesions similar to those of *Pseudocercospora* on mandarin trees in that area. A survey done in 2002 proved that these were caused by *Alternaria*. There was, however, the perception that *Pseudocercospora* could move from the northern part of Mozambique to the south and could then pose a threat to the Mpumalanga Lowveld and Swaziland citrus industries. In the past, Outspan International was very active in both the south of Mozambique and the Beira corridor. This involvement ceased a few years ago and our information on the citrus pest and disease status in Mozambique came to an end. Although there remains contact between the South African authorities and their Mozambiquean counterparts, the CGA was concerned about the actual status of any threats posed by the citrus in Mozambique to the rest of southern Africa.

It is known that some of the citrus farms in Zimbabwe were taken over by “war veterans” and that the trees on these farms were under no chemical programmes to control *Pseudocercospora*. It was also rumoured that the disease had spread to the Chegutu area which was free of the disease during previous surveys. If this was correct, this would then be the southernmost point of the disease’s reported presence. The presence of the disease in this area and in areas where the disease was reduced to undetectable levels during the 1990s, had to be verified.

Apart from the threat of new diseases, there is also the threat of new insect or mite pests and a new whitefly pest is known to occur on citrus in Kenya. Inspection of citrus in different parts of Mozambique would show whether any unusual pests were present that may threaten the citrus industry in the rest of southern Africa.

Adra & Africare

There are several non-governmental organisations active in Mozambique. Many of these organisations are religion based. ADRA for example has connections with the Adventist church in Brazil. These organisations are involved in teaching the local population how to propagate fruit trees in order to establish a more healthy diet amongst the locals. During a visit to CRI in Nelspruit by representatives from these NGOs, contacts were established between them and the Citrus Foundation Block. ADRA contacted the CGA and requested help in establishing citrus nurseries at Homoine (Inhambane Province), Nicoadala and Mocuba. (Zambezi Province). One of the most important aims of this visit was to convince ADRA not to import any citrus material from Brazil as there are several citrus diseases in Brazil which are not present in South Africa e.g., Citrus canker, Citrus variegated chlorosis (which is seed transmissible), Leprosis virus, Rubilose and *Colletotrichum* causing post bloom fruit drop.

ADRA also requested inputs on pest control and other orchard practices.

CGA

In the past Mozambique was represented by Swaziland within the citrus industry. Swaziland, however, decided that they would not be representing Mozambique in future. We would therefore try to establish links between the more prominent citrus producers in Mozambique and the CGA and, if justified, the CRI would set up a citrus studygroup in that area.

Swaziland

Furthermore, the Swaziland citrus industry requested CRI to investigate the orchards in southern Mozambique to ensure that there was no phytosanitary risk in sending fruit to Swazi-Can for processing.

Programme

Day	Date	Contact	Purpose	Overnight
Fr	25/7	Alex Negroa (Citrum) 092581775009(W) 0925882318555(C) Jaycey Strauss (Capespan) 0925882309639(C)	Travel: Nsp-Matola- Xai-Xai. Visit Citrum and determine the status of citrus production between the Swazi border and Xai-Xai. Phytosanitary inspections with Gerd Hoppner from IYSIS.	Xai-Xai
Sa	26/7	Dries van Wyk 082 443 7524(C)	Travel: Xai-Xai- Inhambane. Inspect citrus trees <i>en route</i> for pests and diseases.	Paindane

So	27/7	Dries van Wyk	Inhambane (Day off. Visit Tofo, Guinjata, Painsane).	Painsane
Mo	28/7	Josef Pudivitr, ADRA	Visit ADRA research farm near Maxixe and nursery. Visit citrus at Homoine Agric College. Josef Pudivitr 0925882484199	Painsane
Tu	29/7	Vilanculos	Travel: Inhambane – Vilanculos Visit orchards in Morrumbene district <i>en route</i> . Overnight: Beach Lodge. Ed Brits. 021 7060517. beachlodge@vilanculos.co.za Josef Pudivitr 02382140 Casa Josef e Tina on beach near Quiosque Tropical.	Vilanculos
We	30/7	AFRICARE (Chimoio)	Travel: Vilanculos-Chimoio. Inspect citrus trees <i>en route</i> for pests and diseases. Overnight: Castelo Branco Hotel 082 438601 (Mr Erasmo)	Chimoio
Th	31/7	AFRICARE (Chimoio)	Discussions on citrus developments in Manica Province with Africare . Manuel Ginga 082501969. chimoio@teledata.mz Orchard inspections at Citrinos de Chimoio. (Joao Ferreira dos Santos 5 km from Chimoio on Beira road.) Overnight: Gorongosa National Park.	Gorongosa
Fr	1/8	Quelimane	Travel: Gorongosa-Quelimane. Overnight: Hotel Chuabo	Quelimane
Sa	2/8	Quelimane	Day off.. Site seeing. Overnight: Hotel Chuabo	Quelimane
So	3/8	Nicoadala/ Mocuba	Meeting with ADRA to discuss citrus developments in Zambezi Province. Visit orchards and nursery at Nicoadala and Mocuba. Josef Pudivitr 092584810450(W) 0925882484199(C)	Gorongosa
Mo	4/8	Gorongosa –Harare.	Travel from Gorongosa to Harare	Harare
Tu	5/8	Harare-Bindura-Mvurwi-Chegutu	Visit orchards including farms occupied by war-veterans in Bindura -Frank Reed 011-801063(C), and Mvurwi - Chris Maggs 011-419624(C) 077 2759(W) and inspect for <i>Pseudocercospora angolensis</i> .	Chegutu
We	6/8	Chegutu –Louis Trichardt	Orchard inspections for <i>P. angolensis</i> Richard Etheredge 091-230014(C)	Louis Trichardt
Th	7/8	Louis-Trichardt-Nelspruit.	Travelling back to Nelspruit, Uitenhage and Groblersdal, respectively.	

Investigation

Mozambique

Area between Maputo and Swaziland

The citrus export industry in Mozambique has collapsed. The only farm that is still exporting citrus is the farm Citrum, the former Lonro and then Lomaco. This farm is situated between Matola and Boane and is farmed by a group of which Alex Nigrau is part. Citrum exported 25 000 cartons of grapefruit this year and hope to export 150 000 in the years to come. This farm as well as the old Citrinos de Maputo which is now also part of Citrum are both in poor condition and will take time and money to recover. The Star Ruby grapefruit are showing Tristeza problems and as these trees are all about twelve years old and preimmunised with the Nartia cross protection strains, their productive life span cannot be much longer. However, the quality of the red grapefruit is good and the recommendation would be to revitalise the farm through a replant programme to increase the number of export cartons. Citrus black spot (CBS) and melanose were commonly found on

the Valencias. Citrum, who is exporting through Capespan, do not intend to become part of CGA at this stage.

Area between Maputo and Morrumbene

No commercial citrus is left in this region but there are several neglected old orchards, mainly mandarins. A large number of mandarins are produced by subsistence farmers from a few trees in each backyard. *Alternaria* is common on the rough lemon trees as is scab (*Elsinoë fawcetti*). CBS is also present and greening-like symptoms were observed at Morrumbene. It seems to be cooler at Morrumbene than south of Maputo where the grapefruit does well. However, Dr. Fanie van Vuuren from the ITSC did PCR tests on the leaves and they proved to be negative for *Liberibacter*.

At Homoine in the Inhambane province ADRA has a nursery, mainly for cashew nuts but also producing a few thousand citrus trees per year. The cultivars propagated are mandarins and Valencias. CRI gave recommendations and also presented them with "Guidelines for the production of container grown citrus nursery trees in South Africa." The scale insect *Morganella longispina* was found on a few citrus fruit here but is not considered significant.



ADRA citrus nursery between Maxixe and Homoine

Area between Morrungulu and Inchope (Beira corridor)

With the exception of a few trees at Vilanculos and a few backyard trees along the road, there is a five hundred kilometre barrier between Morrungulu and Inchope which is citrus free. The chances of any airborne disease being spread from the north of Mozambique to the south is therefore slim. The way in which it will spread is through the movement of planting material which is at this stage unlikely.

Area between Mutare (Machipanga) and Inchope (Beira corridor)

All the commercial citrus plantings from Machipanga on the Zimbabwean border at Mutare to Inchope, with the exception of less than ten hectares, have been neglected. All the effort put into Citrinos de Chimoio and the other citrus estates around Chimoio has been wasted. In some cases the trees are still there but the fruit are small because of no irrigation and riddled with CBS. Some orchards were removed to plant tobacco. The area has a large number of mandarin trees belonging to subsistence farmers along the road between Machipanga and Inchope. Though we did not visit the area we were told that it is the same on the road between Inchope and Beira. No signs of *Pseudocercospora* could be found. Africare is involved in this area with agriculture.



Lemon plantings at Chimoio

Area between the Beira corridor over the Zambezi river up to Nicaudala

Once you are a few kilometres from Inchope on the road to Nicaudala there are no citrus trees with the exception of a few backyard trees in Gorongosa and Caia towns. This is a stretch of more than four hundred kilometres, which once again reduces the chance of citrus diseases moving from the north to the south other than through planting material which is unlikely at this stage.

Area between Quelimane, Nicaudala and Mocuba

Once again mandarin trees were abundant with many subsistence farmers having a few backyard trees each. At Nicaudala there is a government farm which also has Bahianina navels. Rust mite and red scale (largely due to pugnacious ant activity) are a problem and many of the branches had *Phytophthora* lesions carried into the trees by the pickers' shoes. *Alternaria* and scab could once again be found on rough lemon trees. CBS was common but no signs of *Pseudocercospora* were found.

ADRA has a nursery at Mocuba which includes citrus. They have budded several different varieties which they received from the Citrus Foundation block. Once again recommendations were made, Guidelines were presented to them and the importance of avoiding planting material from Brazil was emphasised. Joseph Pudivitr from ADRA has until now been involved in Mocuba with ADRA but may be moving South to Homoine.



ADRA citrus nursery at Mocuba (cashew trees in background)

Zimbabwe

The Bindura, Mvurwi and Chegutu areas were visited. Forty percent of the commercial citrus farms in Zimbabwe have been occupied by the so called war veterans. The areas in the north were more affected

than those in the Beitbridge area. In most cases these farms were not kept on any spray programme. However, as the farms were taken over in a good condition the export crop of Zimbabwe only declined from 3 million to 2,5 million export cartons. Diseases such as CBS were easily found in the orchards taken over from the commercial farmers and these can be expected to be much more of a problem on these farms in the years to come.

Pseudocercospora was found in the Bindura area where it used to be many years ago. Unfortunately, *Pseudocercospora*-like symptoms have reappeared in all three of the orchards visited in the Mvurwi area on farms occupied by war veterans. The incidences are low but the disease will have to be controlled. Chris Maggs has a good relationship with these new farmers and we rely on him to convince these growers to do CBS and *Pseudocercospora* sprays during this coming season.



Pseudocercospora angolensis on fruit and leaves

Citrus farms were also visited in the Chegutu area and rumours of *Pseudocercospora* spreading to this area were investigated. No *Pseudocercospora* was found and it is believed that either leafhopper damage or severe grey mite infestations and sunburn lesions on the concentric ring blotches may have been mistaken for *Pseudocercospora*.



Concentric ring blotch caused by citrus grey mite

Summary and conclusions

Mozambique

- With the exception of Citrum there is no commercial citrus export industry left in Mozambique.
- It is unlikely that any citrus industry will be established in the near future because of a lack of expertise and infrastructure. This is in spite of the fact that excellent grapefruit and Valencias can be produced in the south.
- No citrus diseases other than those found in South Africa and Swaziland could be found. Although *Pseudocercospora angolensis* or *Cercospora angolensis* as it was known previously, was reported in old literature, no symptoms could be found between the Swaziland border (27° South) and as far north as Mocuba (17° South). No symptoms were seen on the leaves or fruit inspected on trees nor on any of the thousands of fruit sold by roadside traders.
- Only one unusual insect pest was found on citrus and that was a scale insect. It has been sent off for identification. Although some unusual mites were found on foliage, none of them appeared to be pests.
- At this stage, most of the citrus produced is Empress mandarins and this is grown by subsistence farmers. Trees produced for these subsistence farmers currently are Empress mandarins, old clone Valencias and Bahianina navels.
- ADRA, who is involved in producing citrus in nurseries at Homoine, Nicaudala and Mocuba, requested recommendations for other citrus cultivars to extend their season. At this stage it is recommended that the subsistence farmers should stick to fruit with seed to ensure good fruit set. It is also recommended that growers should avoid citrus susceptible to *Alternaria* e.g. Novas and Minneola tangelos. To extend the Bahianina season, Dr Graham Barry recommended the Autumn gold and Royal late. To extend the mandarin season he recommended the Daisy (not Dancy) which is earlier. The Tambor is recommended to follow the Empress and then the Turkey and the Valencia Late. (The latter they already produce.)
- The movement of citrus diseases from the Beira corridor to the south of Mozambique is unlikely, except through the movement of planting material. This is due to a 500 km citrus free zone south of the Beira corridor. To the north of the Beira corridor there is also an area of almost 400 km which is free of citrus, allowing a further buffer to the north.
- Both ADRA and Africare were encouraged to use only the Citrus Foundation Block as seed and budwood source. No material should be imported from any other country especially Brazil, (with whom ADRA has links), without going through plant quarantine. This also applies to seed as it has just become known that Citrus Variegated Chlorosis (CVC) can also spread by means of seed. Fortunately, ADRA is advised on agriculture by Josef Pudevitr who has an understanding of the dangers of importing citrus material from Brazil. The CRI should maintain close contact with ADRA to ensure that this message is continuously emphasised.

Zimbabwe

- The citrus industry was found to still be in a surprisingly good condition. In spite of 40% of the farms being taken over by war veterans the exports will only drop from 3 million to 2,5 million cartons this year. This is because the farms occupied by the war vets were in a good condition when they did so and some obtained packouts as high as 60%. This will not continue if they do not get their spray programmes, irrigation and fertilization right this coming season.
- *Pseudocercospora* was found at Bindura as expected. There was unfortunately also a low incidence on some of the farms occupied by the war vets in the Mvurwi area. This was an area where *Pseudocercospora* was once reduced to undetectable levels. Farms inspected in the Chegutu area were still free of the disease. Once the political situation in Zimbabwe stabilises there should again be a drive to eradicate the disease.

Future preparedness with regard to biosecurity

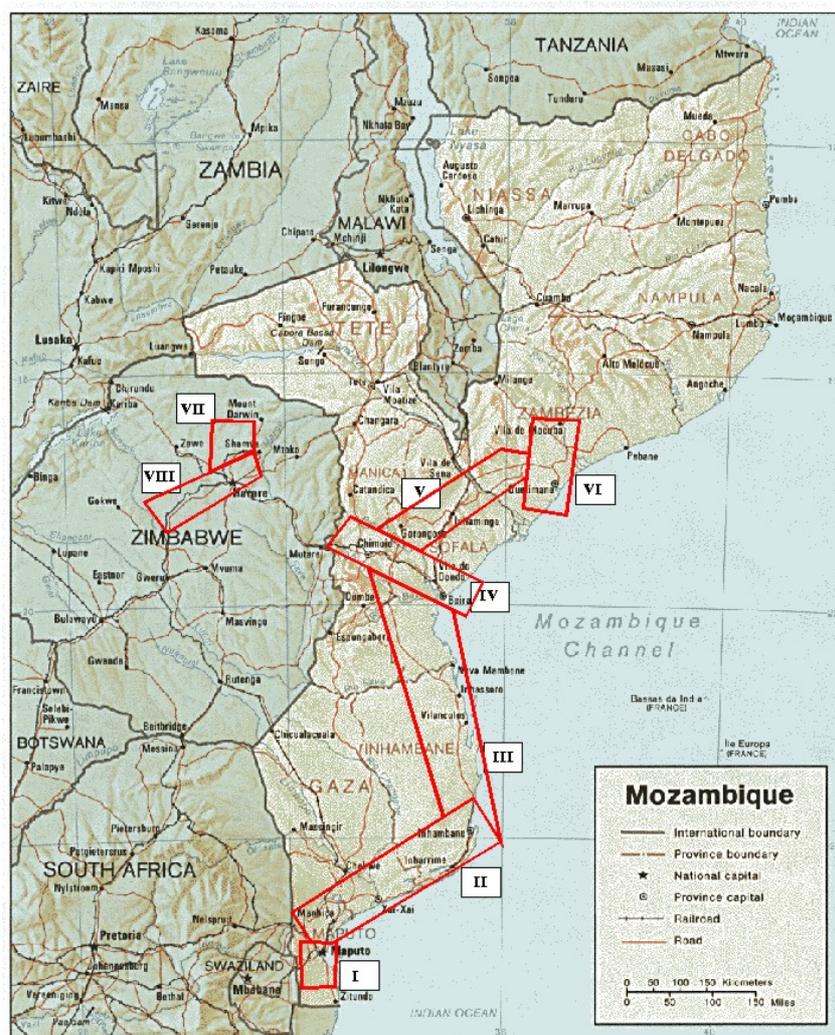
This survey indicated that other than *Pseudocercospora* there are no current plant pathological dangers to the South African citrus industry from its neighbouring countries to the east and the north. (Mozambique and Zimbabwe) No threats are expected from Namibia or Botswana. This can however change because of natural or intentional introduction of plant pathogens. For this reason it is important that the citrus industry should follow the guidelines as were formulated by the American Phytopathological Society in March 2003 during a meeting held on Crop Biosecurity.

From a southern African citrus perspective this would mean that:

- CRI develop a rating system for citrus pest and disease threat levels.
- CGA/CRI ensure that the current capacity to diagnose citrus pests and diseases are maintained and improved. (CRI, Universities, ARC and private.)
- CRI increase the education and awareness of first line responders (researchers, technicians, current grower generation both commercial and upcoming) so that they know what to look for and who to contact and to report potential threats. This was done during this visit to Mozambique, through the publication of the article "Importance of adhering to the Citrus Improvement Programme Procedures" in the Febr/March 2003 issue of the SA Fruit Journal and will be done at the Letsitele Symposium in September 2003 during the talk "Eksotiese sitrussiektes en die potensiele gevaar wat dit vir die suider Afrikaanse sitrusbedryf inhou".
- CGA ensure that CRI maintain a worldwide network with specialists to be able to share knowledge and resources to be able to deal with any potential threat should it arise. This would mean attending symposia such as the ICC and the IOCV both to be held in 2004.

Acknowledgements

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- To Dries van Wyk for accommodation at Paindane.
- To Mr and Mrs Etheredge for accommodation at Chegutu.
- To Henry's Electrical in Nelspruit for camping and survival equipment.



Map of Mozambique and Zimbabwe showing the areas (I-VIII) visited.

3 **PROGRAMME: INTEGRATED PEST MANAGEMENT**

3.1 **PROGRAMME SUMMARY**

By Tim G. Grout (Research and Technical Manager)

Due to their present, and increasing significance as phytosanitary pests, most resources in the IPM programme were focused on false codling moth (FCM) and fruit flies. Excellent progress was made in developing the groundwork for use of the Sterile Insect Technique to control FCM in the future. Further strides were also taken in preparing for the commercialisation of the FCM granulovirus. Labour-intensive research on the cold sterilisation of Medfly in Clementines had to be repeated to address queries received by the Japanese. The use of area-wide management strategies for the control of fruit flies may be a valuable risk mitigation tool in the future and use of the M3 for this purpose appears feasible after some large-scale trials. Mealybugs are also phytosanitary pests for certain markets and descriptions to simplify identification of six of the seven species found on citrus should now facilitate marketing decisions. With the combined pressure of EUREPGAP requirements and tighter restrictions on permitted chemical residues, there is a need for less disruptive thripicides as repercussion pests are more difficult to control. Although populations of citrus thrips were once again low, more results were achieved than in the previous season. Other than Tracer plus oil, no suitable alternative to abamectin plus oil was found with similar efficacy and IPM compatibility. Work on a database of non-target effects on five key natural enemies has now been brought to a close as most toxic chemicals have been evaluated against at least one natural enemy and new products will be evaluated as contract research. Various other aspects essential for efficient running of IPM citrus orchards for export markets, were also addressed. It is probable that future research in this programme will be increasingly directed towards market access issues.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsing & Tegnieuse Bestuurder: CRI)

As gevolg van hulle huidige en toenemende betekenis as fitosanitêre plae, was meeste hulpbronne in die IPB program gerig op vals kodlingmot (VKM) en vrugtevlieë. Uitstekende vordering is gemaak met die ontwikkeling van voorbereidingswerk vir die gebruik van die Steriele Insektegniek om VKM in die toekoms te beheer. Verdere stappe is ook geneem met die kommersialisering van die VKM granulovirus. Arbeidsintensiewe navorsing oor die koue-sterilisering van Medvlieg in Clementines moes herhaal word om vroeë wat deur die Japanese gestel is, te beantwoord. Die toepassing van gebiedsweye bestuurstrategieë vir die beheer van vrugtevlieë mag 'n nuttige wyse van risiko-vermindering wees in die toekoms, en gebruik van die M3 vir hierdie doel blyk doenlik te wees ná 'n aantal uitgebreide proewe. Wolluise is ook fitosanitêre plae vir sekere markte en beskrywings om die identifisering van ses van die sewe spesies wat op sitrus aangetref word te vergemaklik, behoort nou markbeslissings te vergemaklik. As gevolg van die gesamentlike druk van EUREGAP-vereistes en strenger beperkings op toegelate chemiese residu's, bestaan daar 'n behoefte aan minder-ontwrigtende blaaspootjiedoders aangesien reperfussieplae moeiliker is om te beheer. Alhoewel blaaspootjiepopulasies weereens laag was, is meer resultate verkry as in die vorige seisoen. Behalwe vir Tracer plus olie, is geen geskikte alternatief gevind vir abamektien plus olie met vergelykbare doeltreffendheid en IPB verenigbaarheid nie. Werk aan 'n databasis vir nie-teikeneffekte van vyf natuurlike vyande van sleutelbelang het nou tot 'n einde gekom aangesien meeste toksiese chemiese middels op ten minste een natuurlike vyand getoets is en nuwe produkte by wyse van kontrakanavorsing getoets sal word. Verskeie ander aspekte noodsaaklik vir die doeltreffende werking van IPB sitrusboorde vir uitvoermarkte, is ook aangespreek. Toekomstige navorsing in hierdie program sal waarskynlik toenemend gerig wees op aspekte van marktoegang.

3.2 **PROJECT: BIOCONTROL DISRUPTION**

Project Co-ordinator: Tim G. Grout (CRI)

3.2.1 **Project summary**

With a reduction in the number of pesticides available for use on export citrus, the conservation of natural enemies is becoming more important. Research on biocontrol disruption therefore continues even though the focus may shift from year to year. In 2003, another 37 pesticide dosage-natural enemy combinations were tested in bioassays as the last contribution to a database of non-target effects on five key natural enemies (3.2.2). Only contract bioassays on the key natural enemies will continue in the future and the results will be added to the database if the products are registered for use on citrus. The non-target effect database will be available at www.citrusres.com. Two improved versions of old ant barriers were tested in 2003. One of these was Ant-Off gel, a less phytotoxic formulation of Tree gum, and the other was a less expensive version of the fibrous Protector or Ant cap barrier. The Ant-Off gel was only effective for one week against pugnacious ant and the tape used on the Protector only lasted three weeks, even though the barrier

itself was effective for longer (3.2.3). Further research on ants will only be conducted promising new products become available.

Projekopsomming

Die bewaring van natuurlike vyande word al hoe belangriker namate die aantal plaagdoders beskikbaar vir gebruik op uitvoersitrus afneem. Navorsing oor biobeheer-ontwrigting word daarom voortgesit, alhoewel die fokus van jaar tot jaar kan verskil. In 2003 is 'n verdere 37 plaagdoderdosis-natuurlike vyand kombinasies getoets in bioessaiërings as finale bydrae tot 'n databasis van nie-teikeneffekte op die vyf natuurlike vyande van sleutelbelang (3.2.2). Slegs kontrak-bioessaiërings op hierdie natuurlike vyande sal in die toekoms voortgaan en die resultate sal tot die databasis gevoeg word indien die produkte vir gebruik op sitrus geregistreer word. Die nie-teikeneffek databasis sal beskikbaar wees by www.citrusres.com. Twee verbeterde weergawes van ou mierversperrings is in 2003 getoets. Een hiervan was "Ant-Off gel", 'n minder-fitotoksiese formulasie van "Tree gum", terwyl die ander 'n goedkoper weergawe is van die veselrige "Protector" of "Ant cap" versperring. "Ant-Off gel" was net vir een week doeltreffend teen malmiere terwyl die band in die "Protector" slegs drie weke gehou het, alhoewel die versperring self langer doeltreffend gebly het (3.2.3). Verdere navorsing op miere sal bepaal word deur die beskikbaarheid van belowende nuwe middels.

3.2.2 Develop a database of non-target effects of pesticides on beneficial arthropods

Experiment 275 by Tim G Grout, Kim Stoltz and Bruce Tate (CRI)

Opsomming

Meeste van die plaagdoders wat algemeen gebruik word op sitrus in suidelike Afrika is nou getoets teen ten minste een van die vyf natuurlike vyande van sleutelbelang. Resultate is gekombineer om 'n databasis te vorm waartoe gegewens van kontraknavorsing gevoeg word namate produkte op sitrus geregistreer word. Hierdie databasis sal bygewerk bly op die CRI webwerf (www.citrusres.com). In 2003 is 37 kombinasies van plaagdoderdosis en sleutelbelangrike natuurlike vyande in bioessaiërings op Nelspruit getoets. Hierdie gegewens is ingesluit in die databasis wat in sy geheel hier gegee word. Dit beëindig navorsing op geregistreerde produkte sover dit die vyf belangrikste natuurlike vyande aanbetref. Bioessaiërings word beplan met 'n aantal plaagdoders om te bepaal of die gevoeligheid van *Androlaelaps* en *Franklinothrips megalops* noemenswaardig verskil van dié van bogenoemde natuurlike vyande.

Introduction

In order to reduce disruption to established natural enemy complexes in citrus orchards, the non-target effects of pesticides registered for use on citrus in South Africa must be known. Contract research is conducted on new pesticides to ensure that their effect on natural enemies is displayed on the label, but in order to compare these results with products that are currently being used or have been used in the past, bioassays have been conducted for a number of years to compile a non-target effect database. Although many possible combinations of pesticide dosage and natural enemy have not been tested, the IPM research committee felt that the most commonly-used products had been evaluated and that the research should end after the 2003 results.

Materials and methods

All techniques used are those that have been previously developed and described in annual reports and by Hattingh et al. (2000). The only change to the method was that *Chilocorus nigritus* adults were sourced from insectaries in order to reduce the costs and labour involved in rearing large numbers. The products evaluated were those for which results had not yet been obtained and which were in current use. The mealybug parasitoid *Coccidoxenoides peregrinus* has had its name changed to *Coccidoxenoides perminutus*. Twelve natural enemy-product combinations were tested against various formulations of CRI's new granulovirus for false codling moth.

Results and discussion

Thirty-nine new natural enemy-product combinations were tested during 2003. Rather than present individual results, the new results have been included in the database and the whole database presented as Table 3.2.2.1. This includes results up to March 2004 and concludes the research on these natural enemies. The database can be downloaded from the website www.citrusres.com by registered members and will be updated with results from contract research once products are registered. Contract research results for products that have now been registered on citrus have been included in Table 3.2.2.1.

Some of the more interesting results from the recent bioassays were the 20 ml rate of abamectin being more detrimental to *Chilocorus* and *Aphytis* than the 10 ml rate, and Meothrin at 60 ml without Elsan being more detrimental than the combination with Elsan for all parasitoids except *Aphytis*. The bioassays of the granulovirus with or without Agral 90 or molasses showed little differences except for *Trichogrammatoidea* which appeared to be susceptible to molasses.

Conclusion

Thirty-nine more gaps in the database were filled and most products used on citrus farms have now been tested against at least one natural enemy. This concludes work on this database. As new products are registered their data will be added to this database on www.citrusres.com.

Future research

Further research on this database will not be conducted but contract research on new products will continue and research on some other natural enemies will start in 2004.

Reference cited

Hattingh, V., A.B. Ware & T.G. Grout. 2000. The development of a non-target evaluation system for southern African citrus. Proc. Intl. Soc. Citricult. IX Congr. 795-797.

Table 3.2.2.1. Database of non-target impact ratings (similar to percentage mortality but taking persistence into account) against five key natural enemies in citrus. (Categories: 0-25 Harmless; 26-50 Slightly Harmful; 51-75 Harmful; 76-100 Very Harmful)

Active Ingredient	Trade Name	Dosage/hl	Euseius	Chil.nigrinus	Aphytis	Coc.perm.	Trich. crypt
abamectin 18 EC	Agri-mec (unregistered without oil)	10 ml		2.3	0.0		
abamectin 18 EC + Sunspray 7E	Agri-mec + Oil	10 ml + 300 ml	16.0	0.6	8.0	1.0	28.1
abamectin 18 EC + Sunspray 7E	Agri-mec + Oil	20 ml + 300 ml		23.5	25.8	1.3	26.8
acetamiprid 200 SP	Mospilan	50 g		94.8	98.0	63.9	71.8
aldicarb 150 G.R.	Temik	30 g/m ² (soil treatment)	19.0				
amitraz 200 EC	Mitac	150 ml		1.7	30.0	0.0	29.6
amitraz 200 EC	Mitac	200 ml	6.0				
azadirachtin 4.5 %	Neemix	50 ml			21.0		
azoxystrobin 250 EC	Ortiva	20 ml			3.0		
azoxystrobin 250 EC	Ortiva + Dithane + Oil	20 ml + 200 g + 500 ml		1.1	0.0	26.0	22.9
azoxystrobin 250 EC	Ortiva + Mancozeb + Oil	30 ml + 150 g + 0.5 % Oil					24.7
B. thuringiensis var. kurstaki 16000 IU/mg	Dipel	25 g	23.0				
B. thuringiensis 32000 IU/mg	Dipel	12.5 g		18.2 *			21.9
benomyl 500 WP + mancozeb 800 WP + Citrex oil	Benlate+Dithane+Oil	50 g + 200 g + 500 ml	24.0	35.0	0.0	16.2	35.9
benomyl 500 WP + Oil	Benlate + Oil	50 g + 500 ml			0.0		
bromopropylate 500 EC	Acarol	75 ml	11.0	1.1	34.0	2.1	30.3
buprofezin 500 WP + Citrex	Applaud + Oil + Agral 90	30 g + 250 ml + 10 ml			2.0		
buprofezin 500 WP + Sunspray 7E	Applaud + Oil	30 g + 0.25 %	36.0	100.0			
cadusafos 100 GR	Rugby	20 g/m ² (soil)	2.0				
carbendazim 500 SC	Bavistin	57 g			0.0		
carbendazim 500 SC + mancozeb 800 WP	Bavistin + Dithane + Oil	57 g + 200 g + 500 ml			0.0		
chinomethionat 250 WP	Morestan	75 g	10.0				
chlorfenapyr 360 SC	Hunter	30 ml	68.0	27.2	74.0	70.0	71.6
chlorobenzilate EC	Akar (withdrawn)	25 g					3.0
chlorpyrifos 480 EC	Dursban	40 ml	21.0				28.1
chlorpyrifos 480 EC	Dursban	50 ml		23.4		13.8	54.3
chlorpyrifos 480 EC	Dursban	100 ml	58.0	29.7		45.4	
copper oxychloride 850 WP	Copper oxychloride	200 g	16.0	11.9	1.0	3.4	3.1
cypermethrin 200 EC	Cypermethrin	20 ml	98.0	99.0	89.0	49.2	
cypermethrin 200 EC	Ripcord	5 ml					52.0

Active Ingredient	Trade Name	Dosage/hl	Euseius	Chil.nigrinus	Aphytis	Coc.perm.	Trich. crypt
permethrin-high cis 25EC + profenofos 400 EC	Trip	100 ml	100.0				
cyprodinil 500 WG	Chorus	80 g			0.0		
dicofol 185 WP	Kelthane	200 g	10.0	20.8			20.0
dimethoate 400 EC	Rogor	40 ml	26.0				
diofenolan 500 EC	Aware	40 ml	37.0		5.0		
endosulfan 475 SC	Thioflo	100 ml	64.0	20.6	42.6	53.0	26.8
endosulfan 475 SC	Thioflo	300 ml			39.0		
endosulfan 475 WP	Thiodan	285 g		69.2	42.0	53.5	28.0
fenamiphos 100 GR	Nemacur	40 g/m ² (soil treatment)	4.0				
fenazaquin 200SC	Pride	25 ml	41.6				
fenbutatin-oxide 550 SC	Torque	30 ml		0.0	0.0		
fenbutatin-oxide 550 SC	Torque	55 ml	20.0	27.5	31.8	53.5	27.5
fenpropathrin 200 EC	Meothrin	30 ml				100.0	
fenpropathrin 200 EC	Meothrin	60 ml		77.3	61.0	98.6	91.5
fenpropathrin 200 EC + phenthoate 500 EC	Meothrin + Elsan	30 ml + 100 ml	100.0				
fenpropathrin 200 EC + phenthoate 500 EC	Meothrin + Elsan	60 ml + 100 ml		54.6	76.0	70.0	84.9
fipronil 200 SC	Regent (shade)	10 ml			93.0		
fipronil 200 SC	Regent (sun)	10 ml	86.0	60.1	57.0	59.0	
formetanate 500 SP	Dicarzol	75 g	100.0	30.7 *		99.1	
formetanate 500 SP + sugar	Dicarzol bait	25 g + 200 g	98.0	2.4	52.0		
formetanate 500 SP + sugar	Dicarzol bait + rain	25 g + 200 g + 3 mm			41.0		
fosetyl-AI 440 WP	Aliette	250 g	21.0				
imidacloprid 200 SL	Confidor	15 ml/stem	3.0				
iprodione 255 SC + mancozeb 800 WP	Rovral + Mancozeb	200 ml + 200 g		2.7		0.0	24.8
isofenphos 500 EC	Oftanol	100 ml	39.0				
kresoxium-methyl 500 WG + mancozeb 800 WP	Stroby + Dithane + Oil	20 g + 200 g + 500 ml			0.0		
mancozeb 800 WP	Dithane	200 g	35.0	0.0	0.0	0.0	6.4
mercaptotion 250 WP	Malathion (unregistered discrete-droplet (bait-type) application)	300 g			47.0		
mercaptotion 250 WP	Malathion + rain	300 g + 3 mm			34.0		
mercaptotion 250 WP + Buminal	Malathion bait	300 g + 400 ml			39.0	32.0	
mercaptotion 250 WP + protein hydrolysate 580 EC	Malathion + Buminal	300 g + 400 ml			39.0	32.0	

Active Ingredient	Trade Name	Dosage/hl	Euseius	Chil.nigrinus	Aphytis	Coc.perm.	Trich. crypt
mercaptothion 250 WP + protein hydrolysate 580 EC	Malathion + Buminal + rain	300 g + 400 ml + 3 mm			28.0		
mercaptothion 250 WP + sugar	Malathion bait	300 g + 200 g			48.0		
mercaptothion 250 WP + sugar	Malathion bait + rain	300 g + 200 g + 3 mm			34.0		
methamidophos 500 AL	Citrimet	stem treatment	9.0				
methidathion 420 EC	Ultracide	100 ml	70.0	47.1	75.0	55.0	
methidathion 420 EC	Ultracide	150 ml					42.3
methiocarb 800 WP	Mesuroi (unregisterd film-wetting spray)	10 g		78.8		100.0	
methiocarb 800 WP	Mesuroi (unregistered discrete-droplet (bait-rype) application)	20 g			30.0		
methiocarb 800 WP	Mesuroi (unregistered film-wetting sprary)	20 g			56.0		
methiocarb 800 WP + sugar	Mesuroi bait	10 g + 200 g	100.0	17.1	46.0		
methiocarb 800 WP + sugar	Mesuroi bait (unregistered conc)	20 g + 200 g			44.0		
methiocarb 800 WP + sugar	Mesuroi bait + rain	10 g + 200 g + 3 mm			31.0		
methomyl 200 SL	Lannate	90 ml	59.0				
methomyl 900 SP	Lannate	20 g			54.3	53.5	
methomyl 900 SP	Lannate	25 g		42.0			
methomyl 900 SP	Lannate	100 g	53.0	80.0	97.0	100.0	98.0
methyl-parathion 240 SC (encapsulated)	Penncap	200 ml			100.0		100.0
mevinphos 150 EC	Phosdrin	100 ml	33.0	18.8		10.1	29.0
mevinphos 500 SL	Phosdrin	30 ml		6.0	15.0		21.3
mineral oil (heavy)	Bac oil	1000 ml		1.8	5.0	39.0	1.8
mineral oil (medium)	Orchex	1.25 l		24.2	38.0	10.5	58.0
mineral oil (medium-heavy)	Citrex oil	1.25 l	32.0				
monocrotophos 400 SL	Azodrin	50 ml	40.0				
omethoate 800 SL + oil	Folimat + Oil	50 ml + 1 l	58.0				
parathion 250 WP	Parathion	300 g		62.5	90.0		
parathion 500 EC	Parathion	125 ml	59.0				
phenthoate 500 EC	Elsan	200 ml	60.0				
phosphorous acid 980 SP + potassium carbonate	Phosphorous acid + Potassium carbonate	206 g + 206 g	40.0				
potassium phosphonate 200 SL	Phytex	500 ml			0.0		

Active Ingredient	Trade Name	Dosage/hl	Euseius	Chil.nigrinus	Aphytis	Coc.perm.	Trich. crypt
potassium phosphonate 200 SL	Phytex	1000 ml		1.0 *			12.5
procymidone 200 SC	Sumisclex	200 ml	34.0				
profenofos 500 EC	Curacron	75 ml		25.1	39.0	36.0	
profenofos 500 EC	Curacron	100 ml	61.0		60.9	22.7	
profenofos 500 EC	Selecron	50 ml	100.0				
propargite 300 WP	Omite	200 g		1.0	24.4	35.2	40.1
protein hydrolysate 580 EC	Buminal	400 ml			3.0		
protein hydrolysate 580 EC	Buminal + rain	400 ml + 3 mm			4.0		
prothiofos 960 EC	Tokuthion	50 ml	65.0	12.8	32.0	7.5	
pyriproxyfen 100 EC + Cipron	Nemesis + oil	30 ml + 0.3 %	13.0	100.0	3.0	29.3	
spinosad 480 SC	Tracer	12.5 ml + 0.3 % Orhex (mineral oil)	34.9	25.9	56.5	99.1	41.1
spirodiclofen 240 SC	Envidor	10 ml	9.1	39.2	13.0	52.9	21.2
spirodiclofen 240 SC	Envidor	15 ml	9.1	32.0	13.3	38.0	15.4
tartar emetic 995 SP	Tartox	200 g			22.0		
tartar emetic 995 SP + sugar	Tartox bait	400 g + 400 g	19.0				
tartar emetic 995 SP + sugar	Tartox bait	200 g + 200 g	9.0	0.4	50.0		
tartar emetic 995 SP + sugar	Tartox bait + rain	200 g + 200 g + 3 mm rain			36.0		
tau-fluvalinate 240 EC	Klartan	30 ml	98.0	97.0	67.1	52.6	82.3
tebuconazole 250 EC	Folicur	80 ml		0.0			
tebuconazole 250 EC	Horizon	80 ml			1.0		
teflubenzuron 150 SC	Nomolt	40 ml	45.0		2.0		
temephos 500 EC	Abate	40 ml	15.0				
tetradifon 81 EC	Tedion	200 ml	0.0	5.9	0.0	1.5	11.8
thiacloprid 480 SC	Calypso	30 ml	24.3	94.2	16.9	94.8	43.9
triazophos 400 EC	Hostathion	90 ml	20.0				
trichlorfon 950 SP + protein hydrolysate 580 EC	Dipterex + Buminal	50 g + 400 ml			24.0		
trichlorfon 950 SP + protein hydrolysate 580 EC	Dipterex + Buminal + rain	50 g + 400 ml + 3 mm			16.0		
trichlorfon 950 SP + sugar	Dipterex bait	50 g + 8 kg sugar			24.0		
triflumuron 480 SC	Alsystin	20 ml	6.0	100.0	2.0	0.0	2.6
* Impact on <i>C.nigrinus</i> adults survival only							
IGR tests completed							

3.2.3 Efficacy and safety of new ant-band deterrents Experiment 731 by Tim G Grout and Kim Stoltz (CRI)

Opsomming

Die ideale benadering tot mierbeheer is om hulle uit bome te hou sonder om populasies op die boordvloer te verminder. Twee nuwe weergawes van ou mierversperrings is evalueer. "Ant-Off gel" is 'n minder-fitotoksiese formule as die ou "Tree gum", maar het vir minder as twee weke gewerk teen malmiere. Die nuwe weergawe van die "Protector cap" het vir drie weke goed gewerk totdat die kleefband waarmee die veselmateriaal aan die boom in posisie gehou word nie verder wou kleef nie. Laasgenoemde versperring kan doetreffend wees indien dit voorsien word met 'n ander tipe band. 'n Alternatiewe formulاسie vir "Ant-Off gel" is onwaarskynlik in die nabye toekoms a.g.v. die groot hoeveelhede wat vervaardig sal moet word. Geen tekens van fitotoksiteit is waargeneem vir 'n jaar na aanwending van "Ant-Bar", "Tree gum" of "Ant-Off gel" aan Empress mandaryn, Star Ruby pomelo of Olinda Valencia nie. Verdere navorsing oor miere word nie beplan nie tot tyd en wyl alternatiewe produkte beskikbaar word.

Introduction

Recent research in the Eastern Cape (Bownes et al. 2001) once again showed the benefit of ants on the orchard floor yet the sticky ant bands are not very user friendly and not compatible with windy conditions. The Lithium-based grease sold as Tree Gum previously has now been modified and all additives removed to reduce phytotoxicity. As this product is easier to handle than polybutene and is not bridged as easily with orchard debris in windy conditions, it is worthwhile evaluating to determine whether it is as phytotoxic as the last product and whether it is effective against both the pugnacious ant and the brown house ant. The new product is called Antoff Gel. In addition, the Protector or Ant Cap is again available at a competitive price so this was included in the evaluation.

Materials and methods

Evaluation of ant barriers was initiated on 3 March 2003. The trial comprised two parts: efficacy evaluation against the pugnacious ant *Anoplolepis custodiens* and phytotoxicity on potted plants. The efficacy evaluations included four products: Antbar – the standard polybutene ant barrier, Tree Gum – the phytotoxic Lithium-based grease, Ant-Off gel – the new Lithium-based grease and the Protector Cap sprayed underneath with Fastac.

A site was used outside Nelspruit where several large pugnacious ant nest mounds were within a few metres of each other. To determine efficacy, dowel rods (50 cm long) were used to each support a small petridish containing a food item. Ten such bait stations were used per barrier type and for a control that had food items without barriers. The rods were randomly placed in the soil near the nests and the gums or polybutene applied directly to the middle of the rods in a band about 3 cm high. The Protector caps were sprayed with Fastac (250 ml/l) and allowed to dry before they were folded around each rod, stapled where they overlapped and taped at the top with the red adhesive supplied for the purpose. The food placed in the petridishes was a combination of Peck's Anchovette fish paste and shredded Enterprise viennas. Observations were made once a week on the amount of food removed from the petridishes and the presence of ants above the barriers.

To check for phytotoxicity, three different scions were used. Large potted plants with stems of about 20 mm diameter were used for this purpose. The scion/rootstock combinations were: Empress mandarin on Swingle rootstock, Star ruby grapefruit on Swingle and Olinda Valencia on Volckameriana. Twenty trees each of the mandarins and grapefruit were used with Tree gum, Ant-Off gel and Ant-bar being applied directly to five of each type and five trees kept as a control. Thirty-five Valencia trees were used. Twenty of these were treated as above but the remaining 15 were wounded with a stapler and the various barrier products applied on top of the wound. In all cases the barriers were applied on the scion in a band 20 mm high and 3-5 mm thick on 4 January 2003. Trees were inspected from this date until 12 March on a weekly basis for phytotoxicity. Trees were kept outside so that they could be exposed to all natural environmental conditions.

Results and discussion

After the first week of the efficacy trial all four products worked effectively and prevented all ants from reaching the food sources. After the second week, ants were reaching the food source past Ant-Off Gel and Tree Gum and some ants even got over the Protector but after collecting some of these ants it was found that they died within 20 minutes. After three weeks the red tape on the Protectors started to peel off allowing

the polyester fibres to move away from the wood and providing gaps that the ants could get through. With more effective tape, the Protector caps would have lasted much longer. After five weeks one of the 10 Ant-Bar barriers had a bridge across it formed from dead ants while the others were still effective. Exact figures for the amount of food removed cannot be provided because after the second week a dog (and later some children) found the bait stations and removed the food. However, it was clear that the two new products were not as effective as Ant-Bar. With different adhesive tape the Protector cap could be almost as effective as Ant-Bar.

In the phytotoxic evaluations there were no signs of phytotoxicity at all within the period that observations were made. When the Tree gum and Ant-Off gel were first applied, the solvent (oil?) spread above and below the band. This solvent repelled the ants at first but this effect did not last much longer than a week. Once the gels dried out, the ants could walk over them. A similar drying out of the barriers was found when they were applied directly to the dowel rods in the efficacy trials. Perhaps if they were applied to plastic wrapped around the tree they would not dry as rapidly as the solvent would not be absorbed. Ant-Bar applied directly to the young trees did not appear to cause the phytotoxic problems that have sometimes been attributed to it.

The supplier of Ant-Off gel could not provide a different formulation as the minimum batch size is more than a ton. Further research with this product was therefore not possible. A suitable alternative to the red tape supplied with the Protector caps has also not yet been found so further research with that barrier was also not possible.

Conclusion

The new ant deterrent, Ant-Off gel was only effective for less than two weeks. However, it did not appear to be phytotoxic. The Protector cap was very effective while the adhesive tape holding the top of the cone together lasted (approximately three weeks). An alternative tape is required but has not yet been supplied.

Future research

As no alternative products have been supplied to evaluate, no further research is planned at present.

Reference cited

Bownes, A., Villet, M. & Moores, S.D. 2001. The beneficial effect of ants as predators in citrus orchards. pp. 12-20. In: CRI Group annual research report, Nelspruit.

3.3 PROJECT: COSMETIC PESTS

Project Co-ordinator: Tim G. Grout (CRI)

3.3.1 Project summary

The status of citrus thrips as a pest of citrus in southern Africa seems to have declined in the last few years in the northern production regions. The reason for this is not obvious but may be due to climatic changes, the use of less disruptive spray programmes or a combination of these. Nevertheless, citrus thrips remains an important pest because of its ability to render fruit unsuitable for export in the period of a week or two. Four years of research on the augmentation of predatory mites in the soil below citrus trees was brought to a close in 2003 with negative results (3.3.2). Despite populations of citrus thrips being low at most sites, mass releases of soil mites seldom reduced economic thrips damage. Perhaps the percentage of citrus thrips that drop to the ground to pupate in the soil is smaller than expected. Another aspect of citrus thrips biology that is not yet known is the lower developmental threshold. Research to determine this temperature, and possibly an estimate of the upper developmental threshold, is being conducted at the University of Pretoria. However, progress has been slow due to difficulties with incubators and rearing techniques (3.3.3). The search for alternative thripicides that would be suitable in IPM or organic production continued but was frustrated by low numbers of citrus thrips at two of the three sites (3.3.4). Erador was less efficacious than abamectin and other unregistered soft options were no better than tartar emetic plus sugar. The fact that citrus thrips is not an economic pest on citrus along the Orange River may be partly due to the winter climate. The predatory mite complex in the trees is better than in most production regions although the numbers of predatory mites are not high (3.3.5). The predatory thrips *Franklinothrips megalops* was found at half the sites and may be contributing to the suppression of citrus thrips. Unlike the phytoseiid mite complex in citrus trees in southern Africa that varies considerably in different areas, tydeid mites on citrus seem to be largely limited to *Pronematus ubiquitus* and *Tydeus munsteri* (3.3.6). A key will shortly be drawn up to separate the species recovered in the region. Another complex of species on citrus of which little is known

is that of the lemon moth complex comprising four different species, one of which has been recorded from Nadorcotts. Research has shown that Alsystin and mevinphos offer some control options but the timing of applications requires further attention (3.3.7).

Projekopsomming

Dit wil voorkom of die status van sitrusblaaspootjie die afgelope jare afgeneem het as plaag van sitrus in die noordelike produksie-gebiede in suidelike Afrika. Die rede hiervoor is nie voor die hand liggend nie, maar kan te wyte wees aan klimaatsveranderinge, die gebruik van minder-ontwrigtende spuitprogramme, of beide. Sitrusblaaspootjie bly nietemin 'n belangrike plaag a.g.v die vermoë van die insek om vrugte binne die bestek van 'n week of twee ongeskik te maak vir uitvoer. Vier jaar van navorsing gemik op die vermeerdering van roofmyte in grond onder sitrusbome is in 2003 beëindig met negatiewe resultate (3.3.2). Ondanks lae populasies van sitrusblaaspootjie op meeste plekke, het massa-vrylating van grondmyte selde ekonomiese blaaspootjieskade verminder. Moontlik is die persentasie blaaspootjies wat afval om in die grond te verpop kleiner as wat vermoed word. 'n Verdere aspek van die biologie van sitrusblaaspootjie wat tans onbekend is, is die onderste temperatuurdrempel vir ontwikkeling. Navorsing om hierdie temperatuur te bepaal, sowel as 'n moontlike beraming van van die boonste drempel, word tans by die Universiteit van Pretoria onderneem. Vordering is egter vertraag a.g.v. probleme met broeikaste en teeltgnieke (3.3.3). Die soektog na alternatiewe blaaspootjiemiddels geskik vir IPB of organiese produksie het voortgegaan maar is gekortwiek deur die lae getalle sitrusblaaspootjie by twee van die drie lokaliteite (3.3.4). Erador was minder doeltreffend as abamektien, terwyl ander ongeregistreerde sagte opsies nie beter was as braakwynsteen plus suiker nie. Die feit dat sitrusblaaspootjie nie 'n ekonomiese plaag is op sitrus langs die Oranjerivier nie, kan gedeeltelik toegeskryf word aan die witerklimaat. Die roofmytkompleks in bome aldaar blyk beter te wees as in meeste ander produksiegebiede, alhoewel die getalle nie hoog is nie (3.3.5). Die roofblaaspootjie, *Franklinothrips megalops*, is by helfte van die lokaliteite aangetref en kan bydra tot die onderdrukking van sitrusblaaspootjie. Anders as die fitoseïd mytkompleks in sitrusbome in suidelike Afrika, wat aansienlik van gebied tot gebied wissel, lyk dit of tydeïdmyte wat op sitrus voorkom hoofsaaklik beperk is tot *Pronematus ubiquitous* en *Tydeus munsteri* (3.3.6). 'n Sleutel om te onderskei tussen spesies in die gebied sal binnekort opgestel word. Nog 'n kompleks van spesies op sitrus waarvoor min bekend is, is die suurleroemot-kompleks wat vier spesies bevat, een waarvan aangemeld is vanaf Nadorcott. Navorsing het getoon dat Alsystin en mevinfos sekere beheeropsies bied, maar die skedulering van toedieningstye verg verdere ondersoek (3.3.7).

3.3.2 Mass releases of soil predators to control winter populations of citrus thrips, *Scirtothrips aurantii*

Experiment 527 by Tim G Grout and Peter R Stephen (CRI)

Opsomming

Die roofmyt, *Androlaelaps* sp., is vir die vierde en finale jaar onder sitrusbome vrygelaat met die doel om sitrusblaaspootjies in die grond te dood. Die bekende blaaspootjie-predator, *Neoseiulus barkeri*, is ook vir die eerste keer onder bome vrygelaat. Die proewe is by twee lokaliteite in Mpumalanga gedoen. Een van die lokaliteite is die vorige jaar aangewend vir proefdoeleindes, wat dennebasmolm as 'n behandeling ingesluit het, terwyl die ander lokaliteit nuut was. Getalle blaaspootjies by die lokaliteit wat met dennebas gedek is was te laag om enige beraming van besmetting te regverdig, maar die omvang van skade kon bepaal word. Blaaspootjiesgetalle was net effens hoër by die ander lokaliteit. 'n Geringe vermindering in totale blaaspootjieskade is waargeneem na vrylatings van *N. barkeri* by een lokaliteit, maar dit was nie weerspieël in die hoeveelheid ernstige skade aldaar nie, nóg op enige wyse by die ander lokaliteit. Geen betekenisvolle voordele kon toegeskryf word aan *Androlaelaps*-vrylatings by beide lokaliteite nie, alhoewel hoër getalle van hierdie spesie herwin is uit grond na vrylatings plaasgevind het. Sitrusblaaspootjiepopulasies in Mpumalanga het deurgaans relatief laag gebly tydens die vier jaar van navorsing. Onder hierdie toestande het vrylatings van *Androlaelaps* myte net by een geleentheid geringe voordele tot gevolg gehad. Inaggenome die moontlikheid dat sommige blaaspootjies in die boom verpop en dat die getalle natuurlike vyande oor die jare toegeneem het met minder-ontwrigtende spuitprogramme, blyk bykomende vrylating van roofmyte onder sitrusbome nie geregverdig te wees nie. Verdere navorsing oor massa-vrylatings van grondpredatore onder sitrusbome word nie beoog nie, maar 'n ondersoek sal onderneem word na die nie-teiken effekte van plaagdoders op *Androlaelaps*.

Introduction

Mass releases of the laelapid predatory mite *Androlaelaps* sp. have now been made for three years at different sites in Mpumalanga. In every case, populations of citrus thrips have remained low in the untreated controls so that the impact of the releases could not be determined with respect to infestation of fruit. It was decided that a fourth and final attempt would be made in 2003 and that in addition, releases of the known thrips predator *Neoseiulus barkeri* would be made as a

Materials and methods

Cultures of the mite *Tyrophagus putrescentiae* were maintained on ground, dry cat food as prey items for both predatory mites. *Androlaelaps* was reared as before with a substrate of sterilised compost and soil on a slightly damp ceramic tile. *Neoseiulus barkeri* was reared in a similar way but a more porous tile was used that kept the substrate more damp. The *N. barkeri* culture was plagued with contamination by ascid mites of the genus *Blattisocius* that could obviously out-compete *N. barkeri* on the same diet and substrate. These mites seemed to be able to get through the seal on the plastic rearing boxes and numerous cultures had to be destroyed. However, despite these setbacks, sufficient mites were reared for planned releases to be made.

Two sites in eastern Mpumalanga were used for the releases. A Midnight orchard at Friedenheim Estates had been used the previous year when bark mulch had been placed under some trees in order to try to improve the environment for existing predatory mites in the soil. The mulch-treated blocks were retained as a treatment in the trial in addition to releases of both mites and an untreated control. In all cases, treatment blocks were at least nine trees long and five rows wide. At a Eureka lemon orchard at Bakgat in the Schoemanskloof Valley, releases of both mites were made in comparison with an untreated control. At both sites, a nested block design was used with each half of the site being divided into the required numbers of blocks. Treatments were assigned randomly to the blocks.

Releases of mites were made under all trees in each block. Releases of 90 *Neoseiulus* per tree were made on 6 August 2003 at Friedenheim and releases of 200 *Androlaelaps* per tree under the central three rows and 100 per outside row tree were made on 7 August. Further releases were made on 18 September of 86 *Neoseiulus* per tree and 144 *Androlaelaps* per tree. At Bakgat, releases of 150 *Androlaelaps* per tree and 150 *Neoseiulus* per tree were made on 4 September and further releases of 151 per tree and 123 per tree, respectively, were made on 16 October. When making the releases, a small heap of substrate containing the estimated number of predatory mites as well as *Tyrophagus* was placed about 30 cm from each tree trunk.

Four yellow sticky card traps were hung in each replicate block to monitor adult thrips populations. The traps were changed at approximately two-week intervals and catches were converted to numbers per week. At Bakgat, traps were hung from 4 September to 3 December while at Friedenheim, traps were hung from 7 August to 14 November. An evaluation of fruit infestation by larval and adult thrips was conducted at Bakgat using eight of the centre trees per replicate and 20 fruit per tree. Only fruit less than 40 mm in length were inspected for this purpose. At the same time, fruit with recognisable thrips scars were recorded. Numbers of thrips at Friedenheim did not justify an evaluation of fruit infestation but an evaluation of scarring was conducted on 5 March 2004 using 20 fruit on each of eight trees per replicate. Scars were recorded as slight or export cull. All data were analysed by ANOVA after appropriate transformations were applied. Where F tests were significant at $P=0.05$, means were compared further using Student-Newman-Keuls test.

To determine whether released mites were contributing to the fauna in the soil below the trees, soil samples were collected on 19 November at Bakgat, 34 days after the second mass releases. A small spadeful of soil, 15 mm deep was taken from below eight trees in the middle of each block. The average weight of the soil sample per block was 2.274 kg and the average volume was 2.787 litres. The soil samples were each placed on Tullgren funnels for four days to extract arthropods into containers of ethanol and glycol. One sample was produced for each replicate and all mites resembling phytoseiids or laelapids were mounted on microscope slides for identification. The numbers of *Androlaelaps* sp. and *Neoseiulus barkeri* individuals recovered were then recorded.

Results and discussion

Releases of both mites could only be evaluated from a thrips infestation basis at Bakgat. There appeared to be no impact on fruit scarring caused by thrips (Table 3.3.2.1). There was a very slight suppression of larval infestation by *Androlaelaps* but this was not significant ($P>0.05$). The scarring evaluation at Friedenheim showed no significant differences in severe scarring between release blocks and the control. However, the total scarring was significantly lower in the treatment with the mulch and the *N. barkeri* releases than in the control (Table 3.3.2.2).

Table 3.3.2.1. Evaluation of thrips infestation and scarring after releasing mites on the soil at Bakgat (no significant differences)

Treatments	Larval infestation of fruit on 3 Dec 2003 (%) ¹	Adult infestation of fruit on 3 Dec 2003 (%) ²	Fruit scarred by thrips (%) ³
Untreated control	44.1	5.9	71.9
<i>Androlaelaps</i> sp. released below trees on 4 Sep & 16 Oct.	37.5	7.5	67.8
<i>N. barkeri</i> released below trees on 4 Sep & 16 Oct.	47.5	6.6	69.7

¹ F=2.35, P=0.11, DF=2. ² F=0.42, P=0.66, DF=2. ³ F=0.49, P=0.62, DF=2.

Table 3.3.2.2. Evaluation on 5 March 2004 of thrips scarring after releasing mites on the soil at Friedenheim

Treatments	Fruit with any thrips scar (%)	Fruit with scarring causing export cull (%)
Untreated control	13.4 a	3.4 a
<i>Androlaelaps</i> sp. released below trees on 7 Aug & 18 Sep.	15.3 a	3.4 a
<i>N. barkeri</i> released below trees on 6 Aug & 18 Sep.	7.2 b	1.3 a
Bark mulch under trees for 1 yr.	6.3 b	0.6 a

Means in the same column followed by the same letter are not significantly different (P>0.05) (SNK test)

There were no consistent differences between treatments and controls in the yellow trap catches of citrus thrips at Bakgat (Fig. 3.3.2.1). At Friedenheim, numbers of thrips on traps in the *N. barkeri* release blocks remained the lowest and this supported the lower amount of total thrips scarring in this treatment (Fig. 3.3.2.2). However, the reason for the flight peak in the mulch treatment is not known.

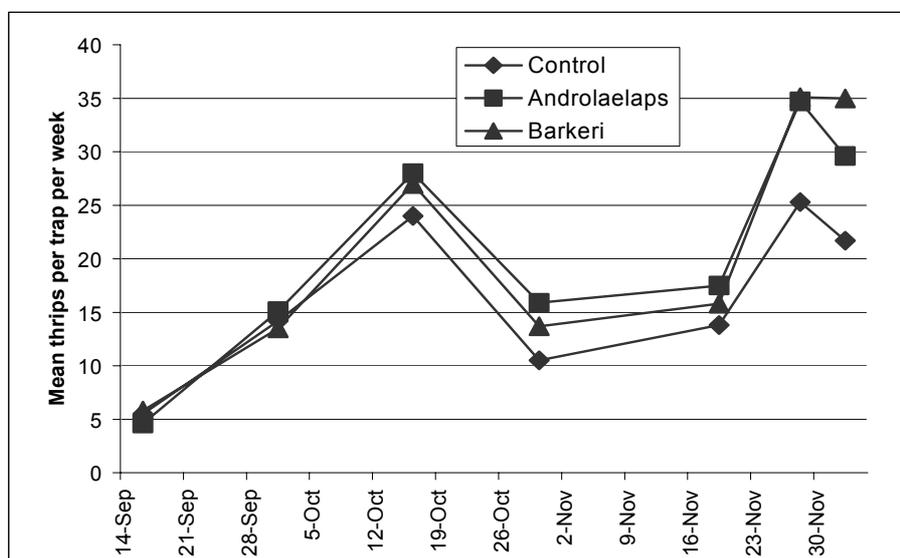


Figure 3.3.2.1. Numbers of citrus thrips caught on yellow traps at Bakgat, Schoemanskloof

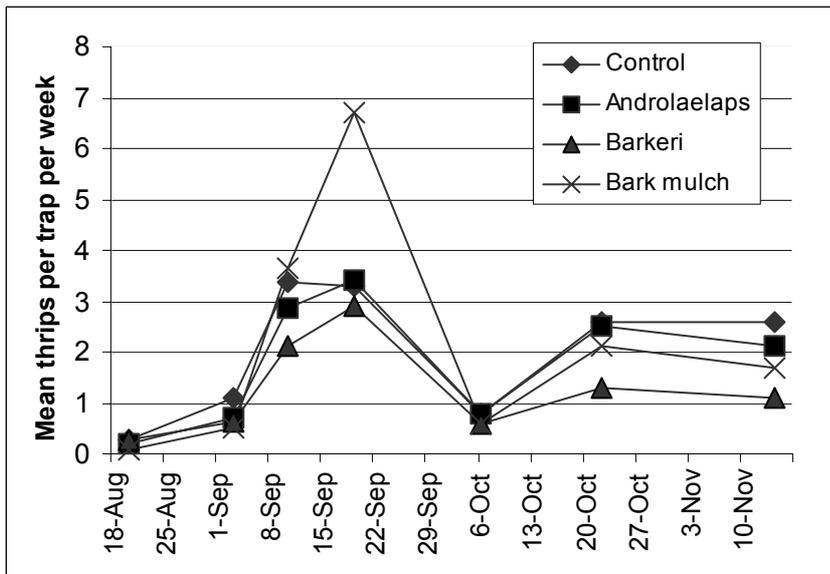


Figure 3.3.2.2. Numbers of citrus thrips caught on yellow traps at Friedenheim, Karino

From the recovery of mites under the trees at Bakgat it appeared that the natural numbers of *N. barkeri* in all treatments were similar to the numbers recovered after releasing *N. barkeri* (Table 3.3.2.3). The released mites may have dispersed both in the horizontal plane and vertically into weeds and other ground cover as unlike *Androlaelaps*, *N. barkeri* does occur on plants (see records on citrus in 3.3.5). It was clear that the releases of this species did not augment the natural population present in the soil. No *Androlaelaps* were found in the non-release control and only one specimen was found in an *N. barkeri* release block, whereas the total recovered from *Androlaelaps* release blocks was 44. The specimen found in the *N. barkeri* block may have moved from the *Androlaelaps* block as the “A” replicates for these two treatments were adjacent. Clearly the releases of *Androlaelaps* did therefore contribute to the population in the soil under the tree.

Table 3.3.2.3. Recovery of mites from approximately 2.8 l soil per replicate from under eight citrus trees 34 days after releases were made

Treatment	No. of mites in Replicate A	No. of mites in Replicate B	Total numbers of mites recovered
No release	– <i>N. barkeri</i> 5	– <i>N. barkeri</i> 1	– <i>N. barkeri</i> 6
<i>Androlaelaps</i> sp. release	<i>Androlaelaps</i> sp. 26 <i>N. barkeri</i> 1	<i>Androlaelaps</i> sp. 18 <i>N. barkeri</i> 3	<i>Androlaelaps</i> sp. 44 <i>N. barkeri</i> 4
<i>Neoseiulus barkeri</i> release	<i>Androlaelaps</i> sp. 1 <i>N. barkeri</i> 4	– <i>N. barkeri</i> 5	<i>Androlaelaps</i> sp. 1 <i>N. barkeri</i> 9

Conclusion

After four years of releasing *Androlaelaps* in orchards with low to moderate thrips populations it could not be confirmed that these releases were contributing to the biological control of citrus thrips. Releases of *N. barkeri* did not appear to augment the orchard floor population and only slightly reduced thrips damage at one site. Perhaps the complex of natural enemies in the soil is so rich that the releases did not contribute much, or a significant percentage of thrips larvae do not drop to the ground to pupate and are therefore not exposed to these predators. Over the years that this research was conducted the status of citrus thrips as a pest seems to have diminished. Perhaps this is due to the increased survival of predators such as those that were being mass reared, due to growers using less disruptive spray programmes.

Future research

No future research is planned with mass releases of predatory mites. However, as *Androlaelaps* may be an important natural enemy in the soil in certain locations, its susceptibility to a range of pesticides will be determined and compared to the phytoseiid predatory mites found in the trees.

3.3.3 Determining the developmental thresholds for citrus thrips

Experiment 697 by Khakhathi David Matshaya (UP) and Tim Grout (CRI)

Opsomming

Die doelwit van die studie is om ondersoek in te stel na die effek van temperatuur op die ontwikkeling en oorlewing van *Scirtothrips aurantii* ten einde onderste en boonste waardes daar te stel vir die ontwikkeling op sitrus. Verskillende metodes vir die teel van *S. aurantii* is getoets. Drie loodsproeue, om broeikaste te toets en die metode gebruik vir die waarneming van die verskillende ontwikkelingsstadia in spesiale houers te verfyn is uitgevoer. 'n Proef by vier temperature (18, 25, 30 en 35 °C) met vyf herhalings elk word tans onderneem. Resultate sal in die volgende jaarverslag verskyn.

Introduction

Although *Scirtothrips aurantii* has been a citrus pest in southern Africa for more than 70 years, the developmental thresholds of this species have not been determined. As a result, apart from the influence of rain and available growth flush on populations, its phenology is not well understood. Developmental thresholds for *S. citri* and *S. dorsalis* have been determined and can be used in degree-day models to fine-tune early season insecticide applications. Knowing the developmental threshold of *S. aurantii* may thus improve IPM of citrus thrips in South Africa. By rearing citrus thrips at different constant temperatures, the lower developmental threshold can be determined and an estimate for an upper developmental threshold can be made. The number of degree-days required for a generation can then be determined and a basic degree-day phenology model developed. As a next step, field data from citrus growers using yellow traps and scouting can be compared with generation times based on the daily maximum and minimum temperatures, and the ability to predict emergence of thrips larvae soon after petal fall based on earlier adult flights can be tested.

Materials and methods

Plants

Citrus seedlings ('Rough Lemon'), for the establishment and maintenance of a culture of *S. aurantii* were obtained from Casmar Nursery (Mooiwooi, North-West Province). Citrus seedlings for experiments ('Rough Lemon') were grown from seeds in a soil mixture consisting of one part of sandy soil and three parts of pine bark. Plants were fertilised every 14 days with liquid nitrogen fertilizer (Wonder 3:2:1 (22) Supranure Plus) and grown in insect-free gauze cages at approximately 25°C under natural humidity and 16L:8D photoperiod.

Insects

A culture of *Scirtothrips aurantii* was established with specimens collected from infested citrus trees at Groblersdal (Mpumalanga) and Nelspruit (Mpumalanga) in 2003. The culture was established on insect-free citrus seedlings kept in the laboratory at 25°C, natural humidity and 16L:8D photoperiod using a combination of cool white (Osram) and plant (Osram 'Fluora') fluorescent lights. Seedlings infested with *S. aurantii* were placed in gauze cages (460 x 460 x 920 mm) with a glass panel on top or openly on trays (450 x 520 mm). The seedlings were watered every three days and fertilized every 14 days with liquid nitrogen fertilizer (Wonder 3:2:1(22) Supranure Plus) to encourage flushing. When leaves of seedlings started to become unsuitable for feeding and oviposition in one tray, the plants were pruned with pruning scissors and then fertilized to encourage flushing. When seedlings started flushing, thrips from seedlings in neighbouring trays where leaves had hardened off, migrated to the tray with new flushes. Plants were also sprayed regularly with water to keep red spider mites (*Tetranychus* sp. (Acari: Tetranychidae)) under control.

Experimental design

A rearing system for citrus thrips that was recently developed by Peter Stephen (CRI) based on a citrus seedling placed within two plastic honey jars, was used. One honey jar was filled with soil and had holes in the bottom for absorbing water and nutrients. The plastic lid was tightly fastened on this bottle and another lid glued back-to-back on this lid so that an inverted bottle could be screwed onto the second lid. The stem of the seedling passed through a hole drilled in the centre of both lids and this was tightly sealed with wax (Sasol, Micro 3971). Citrus seedlings pruned back to 5-6 cm height or 3-week-old citrus seedlings were transplanted into the jars. Adult *S. aurantii* were placed on the foliage in the upper jar and the jar removed for close inspection of non-alate stages under the microscope.

The development time of *S. aurantii* was examined in incubators at approximately 18, 25, 30 and 35°C and 16L:8D photoperiod. Minimum and maximum temperatures, as well as the current temperature in the incubators were recorded daily. The minimum and maximum temperatures were influenced by the

lights, which emitted considerable heat in the incubators after being switched on, with the result that temperatures were temporarily higher after lights came on in the morning and lower after lights switched off in the evening until the temperature in the incubators adjusted again. Therefore, the current temperature in the incubators was checked during different times of the day. Further experiments will be conducted at two additional temperatures in order to obtain more reliable estimates of the developmental thresholds.

Adult *S. aurantii* were transferred from the culture to the seedlings in the honey jars using either a fine paint brush or small leaf cuttings with four adults (3 females, 1 male). The leaf cuttings were placed on the seedlings in the honey jars. When the leaf cuttings desiccated, the thrips moved from the cuttings to the plant. The leaf cuttings were removed after 6 hours. Honey jars containing citrus seedlings with thrips were then placed in one of the four incubators. Adult *S. aurantii* were left inside the honey jars for a period of two days to allow the females to oviposit. After two days, adults were removed with a fine paintbrush. Daily records were made of the developmental stages observed in the jars.

Preliminary results

Establishment of an *S. aurantii* culture in gauze cages proved difficult and better results were obtained in rearing the thrips on seedlings placed openly in trays. A possible reason for the problems experienced with the gauze cages could be inadequate lighting, which could have been responsible for the slower growth of plants, i.e. fewer new leaf flushes, compared to seedlings kept openly in trays. Three pilot trials have been conducted to test the upgraded incubators and adjust the method. During the first trial, problems were experienced with the temperature control of the incubators after installation of additional lights, which were subsequently solved by installing extractor fans. During the first trial it was observed that the age of the seedlings used is important. When using older seedlings, which were pruned back from a height of 40 cm to 5-6 cm, it was found that adults and larvae were difficult to observe on the relatively old stems. The second and third pilot trials with three replicates for each temperature were done using seedlings grown from seeds with 3-4 leaves (3 weeks after germination). These trials served to learn to differentiate between the larval stages and to fine-tune observation methods, i.e., avoiding escape of specimens when opening jars for observation. A trial with five replicates for each temperature (18, 25, 30 and 35°C) is currently underway. The results on the development of *S. aurantii* at the four temperatures are expected to be available by April 2004.

Conclusion

Most time has been spent developing techniques and results will only be available for the 2004 annual report.

3.3.4 OP alternatives for citrus thrips, compatible with bio-intensive IPM or organic production

Experiment 713 by Tim Grout, Peter Stephen and Bruce Tate (CRI)

Opsomming

Alternatiewe vir organofosfate en abamektien word dringend benodig vir die beheer van myte. Proewe is uitgevoer by drie wydliggende lokaliteite in Mpumalanga, maar blaaspootjiebesmettings was deurgaans laag by twee hiervan. Resultate het getoon dat Erador (voorheen OrganoZ) minder doeltreffend was as abamektien (20 ml/ha) plus olie. Geen van die nuwe produkte wat getoets is was so doeltreffend as abamektien nie, behalwe Tracer plus olie wat alreeds geregistreer is. Verskeie produkte het sitrusblaaspootjie onderdruk tot 'n mate swakker as braakwynsteen maar effens beter as swael. In meeste gevalle sal dit onprakties wees om sulke produkte te gebruik behalwe waar blaaspootjiepopulasies baie laag is. Van hierdie produkte kan die kaolienformulasie, Pyrofil, as 'n enkel toediening gebruik word laat in die somer, alhoewel verdere evaluasie aangewese is. Mangocote het 'n soortgelyke onderdrukking tot gevolg gehad. Dit is moeilik om op hierdie stadium enige ander afleiding te maak a.g.v. die lae besmettingsvlakke. Die soektog na alternatiewe blaaspootjiedoders duur voort.

Introduction

The need for alternatives to OPs was given a high priority by citrus growers and there is growing interest in organic production for which thrips management is a challenge. Erador (previously OrganoZ), has been approved for use in organic production and was recently registered on citrus in South Africa. Tracer (spinosad) is already registered on citrus in South Africa and may soon be approved for use in organic production. Other botanical products such as rotenone are becoming more available and may be effective against citrus thrips. Registered products such as Hunter could be evaluated at lower dosages and volumes

with sugar to see whether they could be used as effective thripicides without causing the repercussions they have become known for. For these reasons, various products were included in evaluations against citrus thrips at a number of locations in Mpumalanga in 2003.

Materials and methods

Due to recent difficulties in finding sites with high population densities of citrus thrips, trials were spread over three sites between Burgersfort and Hectorspruit in Mpumalanga. No citrus thrips developed at the site at Hectorspruit so this was eventually scrapped and a site used near Komatipoort where no pesticides had been sprayed after petal fall. These summer sprays at the latter site therefore fulfilled the request by the IPM research committee of applying some treatments later in summer when thrips numbers were lower.

The site near Burgersfort was an orchard of mature Palmer navels (no. 5) on Johan Vriis' farm. An orchard of young Midnights was used on the farm Bushrock near Karino, northeast of Nelspruit. The third site was initially at TSB Hectorspruit but when that had no thrips it was moved to a block of young Valencias (W12) at Die Brug near the Crocodile River, north of Komatipoort. The layout of the trials at each of the three sites was similar. Blocks of trees, either three rows wide and 10 trees long, or four rows wide and seven trees long, were used for each treatment. Eight trees from the centre row or rows were used as data trees. Each site was divided in two and a replicate block of each treatment was randomly assigned in each half. Treatment and evaluation dates are provided in the data tables. Treatments were all applied when the temperature was below 30°C. Yellow sticky traps were hung in the control blocks to give an indication of when evaluations or reapplications were required.

Evaluations involved inspecting 20 outside fruit on each data tree (25 at Johan Vriis) and recording whether they were infested with either immature or adult citrus thrips. With the second evaluation at Johan Vriis (23 October 2003), slight scarring by thrips was recorded at the same time. After this evaluation, the whole trial site was sprayed out by the grower with abamectin (20 ml/hl) plus oil (0.3%) and this was followed by an evaluation of thrips infestation on fruit on 29 October. At the Bushrock site, numbers of thrips were never adequate in the controls to justify an evaluation of infestation. However, the treatments were applied twice and finally evaluated on scarring to determine whether any treatment effects were noticeable. Twenty fruit per data tree were recorded as being unscarred, slightly scarred or scarred sufficiently to be culled from export, if the fruit were full size. Scarring could not be evaluated at Die Brug because the trial was only initiated in December and earlier damage was already present. Only one infestation evaluation was conducted because thrips numbers declined rapidly in the whole site after treatments were applied. All data were transformed to arc sine before an ANOVA was conducted. Where F tests were significant, means were compared further using Student-Newman-Keuls test at $P=0.05$.

At the Burgersfort site an evaluation of red scale and mealybug infestation of fruit was conducted on 31 March 2004 to determine whether any of the treatments caused a repercussion. Only selected treatments were evaluated and some trees in an adjacent block managed by the grower. Twenty-five fruit per tree were rated as being clean, infested with one to 10 red scale or more than 10 red scale. The same fruit were also rated for the presence of live mealybug. Data were transformed and statistically analysed as for citrus thrips.

Results and discussion

With the exception of Johan Vriis' farm near Burgersfort, populations of citrus thrips were disappointingly low. At Johan Vriis, the opposite was true and numbers were too high for several of the weak treatments used. In the first evaluation (Table 3.3.4.1), larval infestation exceeded the treatment threshold of 2% in Erador, Expellar, Pyrofil and Sulphur treatments. However, these treatments were all suppressing citrus thrips relative to the control infestation. After this evaluation, the Expellar, Pyrofil and Sulphur blocks were sprayed out with Hunter (30 ml/hl) or Regent (10 ml/hl) and replicate A of the control was also sprayed out with Regent to limit losses for the grower.

The evaluation of the remaining blocks after the second round of sprays showed that the double treatment of Tracer plus oil was similar in efficacy to a double treatment of abamectin (20 ml/hl) plus oil (Table 3.3.4.2). Both double treatments had some thrips scarring but this would not have been sufficient to cause export cull. The double Erador treatment had much more damage than the latter treatments and was significantly inferior ($P<0.05$). Fruit scarring was similar to that in the untreated control and thrips infestation was slightly higher than in the control, although this was not tested statistically due to only one replicate being evaluated in the control. The Erador treatment following an abamectin plus oil treatment (no. 7) was showing slightly inferior control to the double abamectin plus oil treatment (no. 8) but significantly better control than the double Erador treatment (Table 3.3.4.2). After the grower sprayed the orchard with abamectin plus oil, thrips infestation was still not quite under control in treatment 2 that had received two Eradors followed by the

abamectin (Table 3.3.4.3). There were no significant differences between treatments 3, 7 and 8.

Table 3.3.4.1. Infestation evaluation nine days after the first treatments were applied at J. Vriis

Trt. no.	Treatments applied on 23 Sep 2003 using 20 ℓ/tree	Larval infestation of fruit on 2 Oct 2003 (%)	Adult infestation of fruit on 2 Oct 2003 (%)
1	Untreated control	16.5 a	14.5 a
2	Erador (pyrethrum 5.44 + azadirachtin 0.003 g/l EC) 75 ml/hl	2.5 cd	8.0 ab
3	Tracer (spinosad 480 SC) 15 ml/hl + Orchex 0.3%	0.3 d	1.3 c
4	Expellar (rotenone 100 EC) 500 ml/hl	5.8 bc	11.5 ab
5	Pyrofil (kaolin WP) 2.5 kg/hl	5.0 bc	7.5 ab
6	Sulphur (800 WP) 300 g/hl	9.3 b	8.8 ab
7	Biomectin (abamectin 18 EC) 20 ml/hl + Orchex 0.3%	0.0 d	1.0 c
8	Biomectin (abamectin 18 EC) 20 ml/hl + Orchex 0.3%	0.0 d	4.0bc

Means in the same column followed by the same letter are not significantly different ($P>0.05$) (SNK)

Table 3.3.4.2. Infestation and scar evaluation 16 days after the second round of treatments were applied at J. Vriis

Trt. no.	Treatments reapplied on 7 Oct 2003 using 20 ℓ/tree	Larval infestation of fruit on 23 Oct 2003 (%)	Adult infestation of fruit on 23 Oct 2003 (%)	Fruit scarred by thrips (%)
1	Untreated control (replicate B only)	23.0	11.5	92.5
2	Erador (pyrethrum 5.44 + azadirachtin 0.003 g/l EC) 75 ml/hl	36.0 a	30.0 a	86.0 a
3	Tracer (spinosad 480 SC) 15 ml/hl + Orchex 0.3%	1.0 b	2.8 b	6.5 b
7	Erador (pyrethrum + neem 500 EC) 75 ml/hl	2.5 b	5.8 b	11.5 b
8	Biomectin (abamectin 18 EC) 20 ml/hl + Orchex 0.3%	0.3 b	3.0 b	5.8 b

Means in the same column followed by the same letter are not significantly different ($P>0.05$) (SNK)

Table 3.3.4.3. Citrus thrips infestation evaluation 5 days after all treatments were commercially sprayed with abamectin plus oil on 24 October 2003 at J. Vriis

Trt. no.	Larval infestation of fruit on 29 Oct 2003 (%)	Adult infestation of fruit on 29 Oct 2003 (%)
1*	-	-
2	5.0 a	14.3 a
3	0.0 b	1.0 b
7	0.3 b	1.3 b
8	0.3 b	1.0 b

* Insufficient fruit as most trees had earlier been sprayed with Regent to limit losses

Means in the same column followed by the same letter are not significantly different ($P>0.05$) (SNK)

The scarring evaluation at Bushrock (Table 3.3.4.4) provided little conclusive data. One of the tartar emetic baits was significantly more efficacious than one of the Erador treatments and the Hunter bait was not as effective as the Dicarzol bait, but few other conclusions could be drawn. The yellow traps in the control at Bushrock showed that the thrips numbers were high until just after petal fall when the treatments were applied in the other blocks (data not shown). The numbers remained low for the rest of the trial.

Table 3.3.4.4. Scarring by citrus thrips at Bushrock evaluated on 26 Feb 2004 (thrips numbers were never adequate to evaluate infestation)

Treatments applied on 25 Sep and reapplied 11 Nov 2003 at 3.5 ℓ/tree for outside cover and 1.1 ℓ/tree for baits	Slight scarring (%)	Export cull scars (% (no signif. diffs.)*
Untreated control	20.0 abc	1.9
Erador (pyrethrum 5.44 + azadirachtin 0.003 g/ℓ EC) 50 ml/hl	20.3 abc	1.6
Erador (pyrethrum 5.44 + azadirachtin 0.003 g/ℓ EC) 75 ml/hl	13.1 abc	1.6
Erador (pyrethrum 5.44 + azadirachtin 0.003 g/ℓ EC) 100 ml/hl	23.4 a	2.5
Tartar emetic (995 SP) 200 g + white sugar 200 g/hl	8.4 bc	0.9
Tartar emetic (995 SP) 200 g + white sugar 400 g/hl	12.2 abc	1.3
Tartar emetic (995 SP) 200 g + brown sugar 200 g/hl	14.1 abc	0.3
Hunter (chlorfenapyr 360 SC) 10 ml + white sugar 200 g/hl	21.6 ab	2.5
Dicarzol (formetanate 500 SP) 25 g + white sugar 200 g/hl	8.4 c	0.6
Biomectin (abamectin 18 EC) 20 ml/hl + Orchex 0.3%	16.3 abc	1.9
Exterminator (pyrethrum 20 g/ℓ + plant oils EC) 250 ml/hl	15.3 abc	1.6

Means in the same column followed by the same letter are not significantly different ($P>0.05$) (SNK)

* $F=0.68$, $P=0.74$, $DF=10$.

Although thrips infestation at the time of spraying at Die Brug was good, numbers crashed soon after and were disappointingly low when treatments were evaluated 13 days after application. All treatments did suppress thrips populations relative to the control but few differences were apparent between treatments (Table 3.3.4.5). Larval infestations of 2% or more were found in the Erador, Expellar and Fulvic acid treatments. Pyrofil and Mangocote had some of the lowest infestation levels, particularly of the adults, so there may be opportunity for a single application of kaolin late in summer to stop late thrips. Mangocote and Expellar were found to cause excessive foaming in the spray tank.

Table 3.3.4.5. Treatments and citrus thrips infestation at Die Brug

Treatments applied on 2 & 3 Dec 2003 using 3.5 ℓ/tree for baits and 7.5 ℓ/tree for outside cover	% Larval infestation of fruit on 15 Dec 2003	% Adult infestation of fruit on 15 Dec 2003
Untreated control	12.8 a	14.5 a
Erador (pyrethrum 5.44 + azadirachtin 0.003 g/ℓ EC) 75 ml/hl	2.0 b	4.8 bc
Tracer (spinosad 480 SC) 15 ml/hl + Orchex 0.3%	0.0 b	1.0 bc
Dursban (chlorpyrifos 64 WG) 64 g/hl	0.3 b	2.5 bc
Expellar (rotenone 100 EC) 500 ml/hl	3.5 b	5.5 bc
Biomectin (abamectin 18 EC) 20 ml/hl + Orchex 0.3%	0.3 b	5.0 bc
Sulphur (800 WP) 300 g/hl	1.0 b	4.0 bc
Pyrofil (kaolin WP) 2.5 kg/hl	0.3 b	0.5 bc
Mangocote (copper oxychloride 100 g/kg and kaolin WP) 3 kg/hl	0.3 b	0.3 c
Tartox (995 SP) 200 g + white sugar 200 g/hl	0.0 b	6.0 b
Tartox (995 SP) 200 g + brown sugar 200 g/hl	0.5 b	6.0 bc
Fulvic acid 2%	2.5 b	1.5 bc
Hunter (chlorfenapyr 360 SC) 10 ml + sugar 200 g/hl	1.3 b	5.8 bc
Hunter (chlorfenapyr 360 SC) 15 ml + sugar 200 g/hl	0.5 b	5.3 bc
Dicarzol (formetanate 500 SP) 25 g + sugar 200 g/hl	0.0 b	1.3 bc

Means in the same column followed by the same letter are not significantly different ($P>0.05$) (SNK)

The results of the evaluations of red scale and mealybug infestations at Burgersfort are presented in Table 3.3.4.6. The infestation in a few trees in an adjacent orchard on the grower's spray programme could not be included in the analysis but gave an indication of the degree of control of red scale and mealybug where a profenofos 100 ml/hl spray was applied in September, followed by two Klartan sprays, an abamectin plus oil and Applaud plus oil in January. The mealybug infestation was low in both orchards and there was no indication that the double Erador treatment or double Tracer plus oil treatment caused a mealybug repercussion. However, mealybug infestation in trees sprayed with Regent was the highest. The red

scale infestation in the trial block was generally high. However, treatments 3 and 8 both received three applications of 0.3% oil that significantly suppressed the scale. As the double Erador treatment (trt. 2) received only one spray that contained 0.3% oil, the higher levels of red scale cannot be attributed to an Erador repercussion. Treatment 1 also received only 0.3% oil but had significantly more red scale than treatment 2. This was either due to some degree of control given by the Erador in treatment 2 or a detrimental effect of Regent on red scale natural enemies in treatment 1.

Table 3.3.4.6. Fruit infestation by red scale and mealybug on 31 March 2004 at J. Vriis

Trt. no.	Insecticides applied to fruit in spring and summer	Fruit with red scale (%)	Fruit with >10 red scale (%)	Fruit with live mealybug (%)
1*	Nothing; Regent; abamectin plus oil	87.5 a	64.5 a	4.0 a
2	Erador; Erador; abamectin plus oil	64.0 b	36.3 b	1.8 ab
3	Tracer plus oil; Tracer plus oil; abamectin plus oil	45.3 c	20.5 c	0.3 b
8	Biomectin plus oil; Biomectin plus oil; abamectin plus oil	43.5 c	20.8 c	2.8 ab
Grower	Grower programme with Klartan & Applaud	28.0	10.3	1.5

* Insufficient fruit on data trees not sprayed with Regent so Regent-sprayed border trees used
Means in the same column followed by the same letter are not significantly different ($P>0.05$) (SNK)

Conclusions

The efficacy of Erador was generally poor and worse than abamectin 20 ml/hl plus oil which gave similar control to Tracer plus oil. The inexpensive kaolin formulation Pyrofil gave some thrips suppression or repulsion that was at least as good as wettable sulphur and it may be useful as a single, late summer spray if fruit can be cleaned with water jets in the packhouse. Mangocote had a similar effect but contains copper. Other products such as fulvic acid, Expellar and Exterminator were not very promising but trials with higher thrips infestations are required to confirm this. Attempts to use Hunter as a bait suggest that the dosage is too low at 10 ml/hl. The debate about white or brown sugar with tartar emetic could not be resolved. There was no evidence of Erador or Tracer causing repercussions of red scale or mealybug.

Future research

Alternatives to organophosphates and abamectin for thrips control are urgently required so further research will be conducted with this objective.

3.3.5 Natural enemies of citrus thrips in the Orange River area Experiment 714 by Tim Grout and Peter Stephen (CRI)

Opsomming

Sitruskwekers in die omgewing van Kakamas en Upington aan die Oranjerivier het versoek dat moontlike natuurlike vyande geïdentifiseer word aangesien sitrusblaaspootjie nie 'n probleem is in die omgewing nie. Agt plase is in Mei en Desember 2003 besoek. Grondmonsters is in Mei by vier van die plase geneem en natuurlike vyande is in Mei en Desember vanaf loof versamel op al agt die plase. Geen sitrusblaaspootjie was op enige van die twee datums teenwoordig nie, alhoewel 'n mate van skade by 'n paar plekke waargeneem is op laat-somer uitloopsels. Dit is moontlik dat die klimaat gedurende die winter nie geskik is vir oorlewing van sitrusblaaspootjiepopulasies nie. Lae getalle spinnekoppe is aangetref in Mei, te wyl verskeie grondmyte teenwoordig was in die grondmonsters. Etlike verskillende roofmyte het in die loof voorgekom, maar hulle getalle was laag. Die belangrikste ontdekking was die roofblaaspootjie, *Franklinothrips megalops*, wat heelwaarskynlik parasiteer op sitrusblaaspootjie. Toekomstige navorsing sal die teel van hierdie predatoor insluit, sowel as 'n ondersoek na die gevoeligheid daarvan vir plaagdoders.

Introduction

Citrus growers in the vicinity of Kakamas and Upington in the Northern Cape requested research on natural enemies of citrus thrips, *Scirtothrips aurantii*, because unlike most citrus production regions in the country, this insect is not a pest in their area. The reason for this is not known but in case it is due to a well-established natural enemy complex they wanted to know more about the latter before pesticides disturbed it.

Two surveys were therefore planned by the first author to investigate what natural enemies were present that may be preying on citrus thrips.

Materials and methods

Two visits were made to the area in May and December 1983. Eight citrus farms were visited from 20 km north of Upington to a few kilometres south of the Augrabies Falls (Blouputz). At the first visit between 13 and 15 May, sampling was more intensive than between 9 and 10 December. Soil samples were taken from under the trees at four of the eight sites. At each site, soil samples were collected in two zones, each zone's sample comprised five sub-samples taken under every third tree to a depth of about 1 cm and a surface area of 729 cm². An insect net was used to sweep along both sides of the trees for 20 m in each zone. Foliage and branches were beaten at 10 spots on each side of the row in each zone. The branches were beaten five times per spot over a blue plastic disk (28.5 cm diameter) using a length of irrigation tubing (25 mm diameter). Arthropods collected in beating and sweep samples were placed in 70% ethanol in the orchard. Soil samples were transported back to the laboratory in Nelspruit where they were placed on Tullgren funnels over a mixture of alcohol and glycol for five days to catch any emerging predators. In December, only beating samples were taken from each site, as this sampling method had been the most successful in May. Observations were made on both occasions for any sign of damage by citrus thrips on new growth or fruit.

Results and discussion

In May 2003, there was some typical citrus thrips damage on the summer flush at a few of the sites but there were no citrus thrips present at the time, nor any earlier damage on the fruit. In December there were plenty of semi-expanded new leaves available but again there were no citrus thrips and no sign of damage to leaves or fruit. The complete absence of citrus thrips in spring and early summer suggests that it is not overwintering in the orchards and takes a long time to recolonise the orchard. This may be due to unsuitable climate in winter as is found in parts of the Western Cape. Although the orchard near Upington had virtually no alternative host plants for citrus thrips in the vicinity, most of the other orchards near the Orange River had *Rhus* spp. or *Ricinus communis* nearby that citrus thrips could survive on if there was new growth. Perhaps the winter conditions are such that none of the plants have suitable foliage to sustain populations of citrus thrips. When numbers do slowly start increasing, the natural enemy complex may effectively suppress them.

The identification of predators in the soil samples is not yet complete and some of the predators on the foliage have not been identified to species (Table 3.3.5.1). The December samples are still being processed. There appears to be quite a lot of variation in the predatory mites in the tree although *Typhlodromus lootsi* was present at most sites and this genus is known to be a more specialised predator than *Euseius addoensis* that occurred at several sites (McMurtry & Croft 1997). The predatory mite *Neoseiulus barkeri* was collected from the foliage at one site and from citrus in a home garden at Kuruman. This is the first time that this predator has been found in the citrus tree, as it is more common in the soil below the tree. However, the low incidence of this natural enemy suggests that it is not playing an important role in preying on citrus thrips. Low numbers of coniopterygids (dusty wings) were found at several sites. Although they are known to prey on mites it is not known whether they will prey on citrus thrips. A few spiders were also found in May (Table 3.3.5.1) but these were not evident in December.

The most interesting discovery was that at four of the eight farms visited, a large predatory thrips resembling a parasitic wasp was collected from citrus foliage. This predator is *Franklinothrips megalops* belonging to the family Aeolothripidae and according to M. Stiller at the Biosystematics Division of the Plant Protection Research Institute, it is widespread in Africa with records from various indigenous trees, but not citrus. *F. megalops* has been used for biocontrol in glasshouses in Belgium, France, Netherlands and Spain since 1992 (Anonymous 2002) and is a known predator of *Heliothrips haemorrhoidalis* in Israel (Wysoki 1999). A related species, *F. orizabensis* has been shown to be an important predator of the Avocado thrips *Scirtothrips perseae* in California and a rearing technique has been developed for augmentative releases (Hoddle *et al.* 2001).

It is possible that *F. megalops* may be preying on citrus thrips in the Orange River Valley and further investigation of this predator will be conducted to verify this and determine how susceptible the predator is to sprays normally applied in IPM orchards. Due to its resemblance to a parasitic wasp, the adult predator may not be readily recognised but the larvae are very striking with swollen abdomens and bright red transverse markings (see photographs in Grout 2003). Identification of remaining predators will continue.

Table 3.3.5.1. Possible thrips predators in the Orange River valley collected in May 2003

Location	Foliage 13-15/5/03
Kuruman, home garden citrus	Coniopterygid adult (1) Mirid adult (1): pale green Mites: Euseius tutsi (12) Typhlodromus lootsi (6) Neoseiulus barkeri (1) Tydeus munsteri (5)
NE of Upington, Iconos farm, Delta Valencias 3-4 m high, only 2 fruit fly baits (trichlorfon) sprayed 28°23,58'S 21°24.21'E	Coniopterygid adults 2 spp. (1) Coniopterygid larvae (4) Mites: Typhlodromus griekwensis (4) Ascidae G (1) Cunaxidae (1) Cheyletidae B (4) Pronematus ubiquitous (7) Spiders: Clubionidae (1)
E of Kakamas, Kromhout Boerdery, Star Ruby grapefruit 15 years old, RS and <i>Aphytis</i> present & green leafhoppers 28°47.19'S 20°39.17E	Coniopterygid larvae (8) Franklinothrips megalops (adult and larva) Mites: Typhlodromus lootsi (7) Neoseiulus barkeri (1) Pronematus ubiquitous (52) Spiders: Miturgidae (1)
10 km NE of Kakamas, Zwartbosberg farm, 7 yr old Eureka lemons, Organic. Earlier had grey mite. 28°45.23'S 20°42.25'E	Franklinothrips megalops (adult [1] & larvae [4]) Other Thripidae (1) Mites: Typhlodromus lootsi (13) Pronematus ubiquitous (31)
SE of Augrabies Falls NP, Augrabies farm, Delta Valencias 15 yrs old with complete flood irrigation. White fly and fruit flies present. 28°39.9'S 20°27.5'E	Franklinothrips megalops (adult [1] & larvae [2]) Mites: Trombidiidae? (1) Euseius addoensis (72) Tydeidae (21): Pronematus ubiquitous Metatriophtydeus sp. Homeopronematus sp. Tydeus sp. Lorryia sp. Spiders: Miturgidae (2) Clubionidae (2) Lycosidae (1)
NW of Augrabies Falls NP, Zeekoeisteeck, Star Rubies 8 yrs old, RS and <i>Aphytis</i> , summer flush with thrips damage, microsprinklers. 28°27.9'S 20°05.4'E	Coniopterygid larva (1) Mites: Typhlodromus lootsi (10) Euseius addoensis (7) Cheyletidae F (2) Tydeidae (34): Pronematus ubiquitous Metatriophtydeus sp. Spiders: Araneidae, Cyrtophorinae (1) Araneidae (1)

NW of Kakamas, Rooiduin farm, Delta Valencias & Eureka lemons 3 yrs old, thrips damage on summer flush. 28°42.11'S 20°28.02'E	Thysanoptera adults (2) Mites: Typhlodromus lootsi (7) Euseius addoensis (1) Pronematus ubiquitous (28) Spiders: Salticidae (1) Thomisidae (1)
Nr. Kakamas, JH Retief Boord, Eureka lemons 5 yrs old, no sign of thrips damage on leaves. Alt. 680 m. 28°45.4'S 20°31.9'E	Coniopterygid larvae (7) Mites: Typhlodromus lootsi (9) Ascidae/Laelapidae G (1) Bdellidae (1) Eupalopsellus sellnicki (1) Pronematus ubiquitous (9) Spiders: Salticidae (1)
NE of Kakamas, Warmzand farm, 8 yr old navels, some thrips damage on summer flush, RS, orange dog and fruit fly present. Alt. 735 m. 28°44.4'S 20°48.7'E	Franklinothrips megalops (larva [1]) Mites: Typhlodromus lootsi (1) Eupalopsellus sellnicki (18) Pronematus ubiquitous (100) Spiders: Salticidae (1)

Conclusion

Although the natural enemy complex is probably the most undisturbed and therefore effective of any citrus production area in southern Africa, the winter climate and lack of palatable host plant material may be largely responsible for the absence of citrus thrips in spring and early summer. Once citrus thrips does move into citrus orchards a predatory thrips and several different species of predatory mites probably suppress populations sufficiently to prevent damage to fruit.

Future research

The predatory thrips *Franklinothrips megalops* will be reared and screened for non-target effects of pesticides that may be used in IPM orchards as this predator is not known from citrus in other parts of southern Africa and may be very susceptible to certain treatments.

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3.3.6 Identification of tydeid predatory mites on citrus

Experiment 696 by Tim G Grout (CRI) and Eddie Ueckermann (ARC-PPRI)

Opsomming

Tydeïdmyte is vir 'n tweede jaar versamel vanaf sitrus op verskillende plekke in suidelike Afrika deur loof en takke te klop. *Pronematus ubiquitous* (McGregor) is algeheel die algemeenste aangetref, gevolg deur *Tydeus munsteri* Meyer & Ryke. Dit wil voorkom of sommige spesies beperk is tot sekere klimaatsgebiede, bv. *Paraprotonematus geminus* Meyer & Rodriques wat slegs gevind is in die kusstreke van Mosambiek en *Metatriophtydeus* sp. wat net in die droë benede-Oranjeriviervallei aangetref is. 'n Sleutel om te onderskei tussen die tydeïdmyte wat gevind is word deur die tweede outeur opgestel om hulle identifisering te vergemaklik en verdere navorsing aan te moedig oor hulle rol in die sitrussekosisteem in suidelike Afrika. Die sleutel sal

gepubliseer word met sketse van die meer ongewone spesies. Geen verdere navorsing word tans beoog nie.

Introduction

Tydeids have been recorded on citrus foliage in many parts of the world and certain species are widespread. One such species is *Tydeus californicus* Banks that has been recorded from citrus in California (McGregor, 1956), Israel (Gerson, 1968), Egypt (Rasmy et al., 1972), Spain (Garcia-Mari et al., 1985) and Portugal (Carmona, 1970). *Pronematus ubiuitus* (McGregor) is also widespread with records on citrus from California (McGregor, 1956), Spain (Garcia-Marí et al., 1985), Italy (Vacante and Nucifora, 1984-5) and Egypt (Rasmy et al., 1972). These species probably serve as prey for various predatory mites on citrus as has been found in other crop systems (Flaherty and Hoy, 1971; Calis et al., 1988). Some tydeids are predators themselves of phytophagous mites on citrus (Muma, 1965; Rasmy, 1969). Only one tydeid species has been considered a pest of citrus and that is *Lorryia formosa* Cooreman (Flechtmann, 1973; Garcia-Marí et al., 1985). This species is also widespread and is common on citrus in Florida (Aguilar and Childers, 2000), north-eastern Mexico (Badii et al. 2001) and Spain (Garcia-Mari, 1985). This may also have been the species of *Lorryia* referred to by Smirnof (1957) as a citrus pest in Morocco. In Mexico, *L. formosa* was found to be more suited to orange leaves than grapefruit leaves and was considered only a casual visitor on the latter *Citrus* species (Badii et al., 2001). This mite is also known to feed on sooty mould or honey-dew (Smirnof, 1957; Mendel and Gerson, 1982) and this may apply to other tydeids too.

The phytoseiid mite fauna on citrus foliage in southern Africa has been fairly well studied (Grout, 1994; Grout, 2001) and certain species are known to contribute to the biological control of citrus pests (Keetch 1972, Grout and Richards, 1992). However, little is known about tydeid mites on citrus in southern Africa, even though they are encountered as frequently as phytoseiids. Meyer (1998) considers *T. munsteri* Meyer & Ryke to be a predator of *Eutetranychus orientalis* (Klein) in South Africa and the first author has often collected *P. ubiuitus* in association with citrus grey mite *Calacarus citrifolii* Keifer. This study was undertaken to facilitate the identification of the species that are present before establishing what contribution they may be making towards the biological control of citrus pests, or whether any of the species are phytophagous.

Materials and methods

Most of the tydeid mites were collected from citrus foliage and branches during 2002 and 2003. Some specimens were collected on earlier occasions or by colleagues working in outlying areas (thanks to H. Hofmeyr, P.R. Stephen and W. Kirkman for collecting some of the tydeids). In all cases, mites were collected by beating branches with a length of black polyethylene irrigation tubing (25 mm diameter) over a dark blue plastic board. Usually, four or five trees were beaten for a period of about five minutes. No attempt was made to compare population densities but only to acquire enough tydeids to serve as a representative sample. The tydeids were transferred from the board to 75% ethyl alcohol by using a very fine paintbrush. Temporary mounts were made using Hoyer's solution (Krantz, 1978). The second author identified all the mites.

Results and discussion

Only two samples were obtained from the Western and Eastern Cape provinces so it is likely that other species may occur on citrus there. The records (Table 1) show that *Pronematus ubiuitus* is the most widespread tydeid mite on citrus foliage in southern Africa and that *Tydeus munsteri* is the next most likely to be found. *Parapronematus geminus* Meyer & Rodriques was well distributed amongst the low altitude localities of Mozambique with quasi-coastal conditions. *Metatriophtydeus* sp. was only found in the arid climate of the lower Orange River Valley. The remaining species were each only found at a few localities, but in some cases these localities were thousands of kilometres apart. This was the case for *T. grabouwi* and *T. meyeriae*. It is therefore likely that the latter two species will occasionally be found on citrus anywhere in the region. None of the tydeids appeared to be causing damage to citrus so are beneficial, either directly as predators or scavengers of honeydew and sooty mould, or indirectly as alternative prey items for phytoseiid mites. Once the key becomes available it will be easier to make predator-prey associations between tydeid mites and phytophagous pests that they may be preying on.

Table 3.3.6.1. Distribution of tydeid species collected from citrus foliage and branches in southern Africa

Genus	Species	Locality	SA province or country
Homeopronematus	sp.	Kakamas	Northern Cape
Lorryia	sp.	Kakamas	Northern Cape
Metatriophtydeus	sp.	Blouputz	Northern Cape
		Kakamas	Northern Cape
Paralorryia	monticola	Brits	North-West
	sp.	Malelane	Mpumalanga
Parapronematus	geminus	Boane	Mozambique
		Mapinhane	Mozambique
		Maxixe	Mozambique
		Nicuadaala	Mozambique
		Zandamela	Mozambique
Perafrotydeus	sp.	Letsitele	Limpopo
		Tzaneen	Limpopo
Pronematus	ubiquitus	Melmoth	KwaZulu-Natal
		Nkwaleni	KwaZulu-Natal
		Bela-Bela	Limpopo
		Hoedspruit	Limpopo
		Letsitele	Limpopo
		Mokopane	Limpopo
		Naboomspruit	Limpopo
		Burgersfort	Mpumalanga
		Groblerdsdal	Mpumalanga
		Hazyview	Mpumalanga
		Komatipoort	Mpumalanga
		Malelane	Mpumalanga
		Nelspruit	Mpumalanga
		Blouputz	Northern Cape
		Kakamas	Northern Cape
		Uppington	Northern Cape
		Brits	North-West
		Mapinhane	Mozambique
		Bindura	Zimbabwe
		Chegutu	Zimbabwe
		Chiredzi	Zimbabwe
		Harare	Zimbabwe
		Mazowe	Zimbabwe
		Mutare	Zimbabwe
		Mvurwi	Zimbabwe
		sp.	Near Homoine
	Triophtydeus	myacanthus	Nkwaleni
Tydeus	grabouwi	Citrusdal	Western Cape
		Mvurwi	Zimbabwe
	meyerae	Hectorspruit	Mpumalanga
		Bindura	Zimbabwe
	munsteri	Addo	Eastern Cape
		Nkwaleni	KwaZulu-Natal
		Tshipise	Limpopo
		Burgersfort	Mpumalanga
		Malelane	Mpumalanga
		Nelspruit	Mpumalanga
		Kuruman	Northern Cape
		Tshaneni	Swaziland
	Chegutu	Zimbabwe	
	spathatus	Nicuadaala	Mozambique
sp.	Nelspruit	Mpumalanga	
	Komatipoort	Mpumalanga	

Genus	Species	Locality	SA province or country
		Kakamas	Northern Cape

Conclusion

Pronematus ubiquitus and *Tydeus munsteri* are the most widespread of the tydeids on citrus. Both have been associated with phytophagous mites and may therefore be predators. A key to distinguish between the tydeids mentioned here is under construction and will assist in identifying future predator-prey associations.

Future research

Further specific research on tydeid mites is not planned.

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3.3.7 The status and control of new moth pests on lemons

Experiment 715 by Sean D. Moore, Garth I. Richards and Wayne Kirkman (CRI)

Opsomming

Die doel van hierdie eksperiment was om te bevestig watter motspesies gom- en nekrosekolle op suurlemoene in die Oos- en Wes-Kaap veroorsaak het. Die chemiese bestryding van die motte is ook ondersoek.

Cryptoblabes gnidiella-feromoonlokvalle is oor 'n tydperk van 6 weke van Oktober tot Desember in 3 suurlemoenboorde in die OosKaap gehang. Eiers of vrugte en larwes en papies op blom- en vrugtrosse was opsigtelik in elke boord. Geen motte is in enige van die lokvalle gevang nie. Larwes en papies is van suurlemoenboorde in die Oos-Kaap en van Nadorcott in die Wes-Kaap versamel. Alle motte wat ontwikkel het is as die sitrusblommot, *Prays citri*, geïdentifiseer. Dertig persent van die larwes wat in die Wes-Kaap versamel is, is deur 'n *Apanteles* sp. geparasiteer. Tot op hede is 4 motspesies óf definitief óf tentatief geassosieer met die skade op suurlemoene wat beskryf is. Hulle is *C. gnidiella*, *P. citri*, *Lobesia stericta* en *Pyroderces tripola*. Dit sal daarom op dié stadium meer akuraat wees om na die suurlemoenmot-kompleks, eerder as die suurlemoenboordermot, te verwys.

In 'n chemiese bestrydingsproef het alle behandelings 'n afname in eiergetalle en vrugskade veroorsaak. Die afname in eiergetalle is net vir mevinphos en Alsystin betekenisvol. Daar word vermoed dat behandelings 1-2 weke vroeër, gemik teen die larwes op die blomme, toegedien behoort te word. Daar is bepaal dat eierlegging op vrugte en die daaopvolgende vrugskade, deur die tweede generasie motte op die boom veroorsaak word.

As gevolg van die onsekerheid wat bestaan oor watter motspesie die belangrikste is, die akkurate monitering van hierdie motte, asook die behoefte vir 'n verdere chemiese proef, word dit aanbeveel dat verdere werk uitgevoer word.

Introduction

During the 2000/01 season conspicuous brown scabs, not dissimilar to leafhopper damage, were noticed on lemons in the Eastern and Western Cape Provinces. During the 2001/02 season this damage reoccurred and was associated with the honeydew moth, *Cryptoblabes gnidiella*, and possibly with two other lepidopteran species, *Lobesia stericta* and *Pyroderces tripola* (Moore, 2003). This is the first report of these species on citrus in South Africa, however, *C. gnidiella* is well known as a citrus pest in other parts of the world (Anshelevich *et al.*, 1993; Avidov & Gothlif, 1960). Although the damage in the Western Cape was associated with a lepidopteran, it was not possible to identify the species. What is peculiar is that elsewhere in the world *C. gnidiella* is associated with honeydew and therefore the pest species which excrete honeydew (Wysoki *et al.*, 1975; Wysoki, 1989; Swirski *et al.*, 1980). In none of the orchards in which *C. gnidiella* nor its presumed damage were recorded, was any conspicuous level of honeydew-producing insects present. The identification of *C. gnidiella* and the other moth species, and their association with damage on lemons must therefore be confirmed in both the Eastern and Western Cape Provinces. Means of controlling these moths must also be investigated. Another important factor justifying this research is the possible ability of *C. gnidiella* (and the other lepidopteran species identified) to act as a vector of *Botrytis* (Fermaud & LeMenn, 1992).

Materials and methods

Monitoring and surveying

During spring of the 2003/04 season, three lemon orchards in Sundays River Valley (SRV) with signs of lepidopteran presence were identified. Lepidopteran presence was defined as the occurrence of larvae, pupae or eggs on fruit or blossoms. On 29 October 2003 one bucket (IPS) trap was hung in each of the three orchards. A *C. gnidiella* pheromone dispenser was inserted into the lid of each trap. Dispensers were obtained from Yogeve in Israel. Traps were erected in the third tree of the third row on the southern side of each orchard. As the prevailing wind direction was south-easterly or south-westerly, the pheromone could be carried by the wind into the orchard, and detected by any male moths in the orchard. Traps were checked weekly on the same day for a period of 6 weeks (i.e. from 29 October to 10 December 2003). Pheromone dispensers were replaced every three weeks. Simultaneously, inspections of fruit and blossoms were conducted for the presence of lepidopteran larvae and eggs. Samples of larvae and eggs were collected. These were reared to adulthood, on artificial diet (Moore & Richards, 2000) and identified. Contact was made with technical personnel operating ⁷³ in other citrus-producing areas of the country,

particularly the Western Cape where problems with lemon moths had previously been reported. These persons were requested to be on the lookout for any life stages of Lepidoptera on lemons. Samples of such were collected and sent to CRI, PE for identification.

Chemical control

A chemical control trial was conducted in a 4-year old lemon orchard (orchard 16, Woodridge Farm, SRV) in which there was a conspicuous level of infestation. Two replicates of each of 6 treatments (Mevinphos, Agrimec, Alsystin, 2 oil (BP medium) concentrations and DiPel) were applied to randomly laid out blocks of 5 trees each. Untreated trees were retained as control blocks. Sprays were applied on 24 November 2003 with hand held spray guns at 7 ℓ/tree. The efficacy of the treatments was evaluated on 10 December 2003. Ten fruit of similar size, were otherwise randomly picked from the 3 centre trees in each block. Fruit were taken back to the laboratory and microscopically inspected for eggs and blemishes (gumming spots). Mean numbers of eggs and blemishes per fruit were statistically compared between treatments. This was done by conducting an ANOVA and comparing means with a Bonferroni LSD multiple range test (Statistical Graphics Corporation, 1996).

Results and discussion

Monitoring and surveying

No moths were caught in any of the traps during the 6-week monitoring period (i.e. from 29 October to 10 December 2003), despite the conspicuous presence of eggs on fruit and larvae and pupae on blossom and fruitlet clusters. The identification of the moths observed was therefore brought into question. Samples of larvae and pupae, collected from orchard 16 at Woodridge Farm and from a 3-year old orchard of Nadorcotts in the Western Cape, were reared to adulthood and identified by Dr. Martin Krüger of the Transvaal Museum. All specimens were identified as the citrus flower moth, *Prays citri*.

This was a first record of the lemon borer type damage on a cultivar other than lemons. However, Nadorcotts are a new cultivar in South Africa and there is therefore still much to be learned about them. *P. citri* is known as a pest of blossoms on lemons and limes in South Africa, however, has previously been found on certain midseason varieties such as tomangoes and Valencias (Annecke & Moran, 1982). Kamburov (1987) reported that *P. citri* larvae might occasionally attack young fruit. However, the type of damage observed here (i.e. eggs deposited onto fruit and emerging larvae causing gumming and necrosis) has not previously been associated with *P. citri*. Ebeling (1959) refers to early records of *P. citri* larvae feeding on the rind of fruit in India and the Philippines, but no details are given.

Of the larvae, presumably all *P. citri*, which were collected from the Nadorcott orchard in the Western Cape, 30% were parasitised by *Apanteles* sp. Considering that the larvae were collected early in the season (i.e. 17 October 2003), this is considered to be a very impressive level of parasitism. As the orchard was sprayed with Ultracide shortly thereafter (for mealybug control) it was not possible to observe the impact of the parasitoids on the moth population. No parasitism was found in the Eastern Cape. Translocation of these parasitoids to the Eastern Cape can be considered, particularly as the problem appears to be greater in the Eastern Cape than in the Western Cape.

To date, four different moth species have been definitely or tentatively associated with the gumming and necrotic blotch damage to lemons. These are *Cryptoblabes gnidiella*, *Lobesia stericta*, *Pyroderces tripola* and *Prays citri*. In this regard, it might therefore be more accurate and less confusing to refer to the lemon moth complex, rather than to the lemon borer moth. In future *P. citri* pheromone traps should be erected in orchards in an attempt to examine the association of this moth with damage to lemon fruit. Further collections of eggs on fruit should be conducted in order to confirm which species of moth/s are ovipositing in these.

Chemical control

All treatments resulted in some reduction in numbers of eggs per fruit (Table 3.3.7.1). The reduction was only significant for mevinphos and Alsystin. Not surprisingly, Alsystin was the most effective treatment in reducing egg numbers, as it was the only specific ovicide used. All treatments significantly reduced damage to the fruit, but there was no significant difference between treatments (Table 3.3.7.1). Surprisingly, less damage was recorded on fruit sprayed with 0.3% oil than with 0.8% oil, although once again, this was not significant.

Probably most valuable was not the data recorded on the efficacy of treatments but what was observed on the phenology of the moth. It is believed that applications should have been conducted 1-2 weeks earlier, being targeted against the larvae on the blossoms. It has been ascertained that the egg laying on fruit and consequent damage to this fruit is caused by the second generation of moths on the trees. The first generation, whose larvae attack the blossom, should therefore be controlled in order to prevent the occurrence of a second generation. If this is done, it is very likely that a product as soft as DiPel could provide adequate control. It appears that the moths associated with the described damage on lemons are very sensitive to sprays, as larvae and fruit damage have been observed almost exclusively in unsprayed lemon orchards (i.e. orchards treated with Confidor). A similar chemical control trial should be conducted, however, targeting sprays at the first occurrence of larvae on blossoms.

Table 3.3.7.1 Lepidopteran eggs and damage on lemon fruit (10 December 2003) subjected to different treatments (29 October 2003).

Treatment	Concentration per 100 ℓ water	Eggs/fruit*	Blemish spots/fruit*
Untreated control	-	8.62a	1.30a
Mevinphos	65 ml	5.28bc	0.80b
Agrimec BP medium oil	20 ml 300 ml	6.48ab	0.48b
Alsystin	10 ml	3.53c	0.52b
BP medium oil	300 ml	5.48abc	0.48b
BP medium oil	800 ml	5.48abc	0.78b
DiPel	12.5 g	6.72ab	0.78b

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

Conclusion

No moths were caught in any *C. gnidiella* pheromone traps erected in 3 lemon orchards in the Eastern Cape over a 6 week period spanning October to December, despite the conspicuous presence of eggs on fruit and larvae and pupae on blossom and fruitlet clusters. Samples of larvae and pupae, collected from lemons in the Eastern Cape and Nadorcotts in the Western Cape, were identified as the citrus flower moth, *Prays citri*. Thirty percent of the larvae collected from the Western Cape were parasitised by *Apanteles* sp.

In a chemical control trial, all treatments resulted in some reduction in numbers of eggs per fruit and damage to fruit. The reduction in egg number was only significant for mevinphos and Alsystin. It is believed that applications should have been conducted 1-2 weeks earlier, being targeted against the larvae on the blossoms. It has been ascertained that the egg laying on fruit and consequent damage to this fruit is caused by the second generation of moths on the trees.

Future research

Further work on this experiment was not planned for the following season. However, due to the uncertainty that still remains on which moth species are the most important, the accurate monitoring of these moths, and the need for another chemical control trial, it is recommended that further work be conducted.

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3.4 **PROJEK: VALSKODLINGMOT** Projekkoördineerder: Hendrik Hofmeyr (CRI)

3.4.1 **Projekopsomming**

Navorsing is die afgelope jaar voortgesit om 'n manier te vind waardeur valskodlingmot (VKM) hokgeslaan kan word. Die vordering wat gemaak is, is egter nie van so 'n aard dat sitrusprodusente onmiddellik baat daarby sal vind nie. Desnieteenstaande is dit nog steeds 'n ligpunt dat alle beskikbare tegnieke vir VKM-bestryding nog nie volledig ondersoek is nie en die hoop vir 'n oplossing wat vir beide produsente en klante kommersieel aanvaarbaar is, mag nie verloor word nie.

Ontwikkeling van die CrleGV-virus word voortgesit om die produk se produksie en gebruik te verfyn, terwyl daar terselfdertyd aan 'n aansoek om registrasie van die produk gewerk word (3.4.2; 3.4.16). Goeie resultate is die derde agtereenvolgende jaar met die paringsontwrigtingprodukt, Isomate, verkry (3.4.5). Die maatskappy sal waarskynlik aan die einde van die 2004-seisoen besluit of die produk geregistreer moet word.

Navorsing met die Steriele Insekloslatingstegniek vorder nog steeds uitstekend. 'n Hokproef is uitgevoer waarin vroeëre laboratoriumresultate bevestig is (3.4.8). Die potensiële nut van die tegniek, selfs onder die hewige besmettingstoestande wat kunsmatig in die hokke geskep is, het duidelik geblyk. Vordering word gemaak met die verbetering van massateelt tegnieke vir VKM, wat noodsaaklik is indien miljoene insekte geteel moet word vir gebruik in 'n SIL-program (3.4.9). Beide apparaat en voedingsmedium is verbeter en die navorsing word voortgesit.

Navorsing op die biologiese beheer van VKM het weer eens aandag geniet. Bevindings dui daarop dat dit die moeite werd kan wees om die lewensvatbaarheid van 'n kunsmatige teel en loslaatprogram van 'n sekere larweparasitoïed te ondersoek (3.4.10).

'n Ondersoek het getoon dat VKM sekere nawellemoenvariëteite soos Lina, Chislett, Tulegold, Autumn Gold en Cara-Cara verkies (3.4.11). Dit skep die vraag of produsente nie die aanplant van sulke vatbare variëteite moet vermy nie.

Daar is in die verlede vermoed dat VKM maklik van boord tot boord of plaas tot plaas beweeg om besmettings oor te dra. 'n DNS-studie dui daarop dat dit nie so is nie en dat VKM redelik gebiedsgebonde is (3.4.14; 3.4.15). Die genetiese variasie in en tussen motbevolkings is ook relatief groot en toon dat die insek in staat sal wees om onder andere weerstand teen insekdoders te verwek en goed by wisselende omgewingstoestande aan te pas.

Dit is nog steeds 'n risiko om die bestaande kouedisinfestasieprotokol vir VKM in verpakte vrugte toe te pas as gevolg van die potensiaal vir skilskade. 'n Alternatiewe tegniek is daarom noodsaaklik. Die behandeling van VKM-larwes met gammabestraling is in 'n voorlopige proef getoets en baie goeie resultate is behaal wat verdere navorsing met dié tegniek nodig maak (3.4.12).

Project summary

During the past year research was continued into ways to combat false codling moth (FCM). The progress was not such that producers would be benefited immediately. However, research on all possible means of controlling FCM have not been finalized and there is hope for a solution that would be acceptable to producers and clients alike.

Development of the CrleGV virus was continued to refine the product's manufacture and usage, while an application for its registration is being finalized (3.4.2; 3.4.16). Good results with the mating disruptor, Isomate, were obtained for the third year running (3.4.5). The parent company will probably decide whether the product should be registered for use on citrus at the end of the 2004 season.

Research into the Sterile Insect Technique (SIT) is still progressing apace. A cage experiment was conducted to confirm earlier laboratory results (3.4.8). Even under the extremely severe infestation conditions created artificially the potential value of the technique was clearly demonstrated. Progress was made with the improvement of mass rearing techniques essential for use in a Sterile Insect Release (SIR) program (3.4.9). Rearing apparatus and artificial rearing medium were improved and the research is continuing.

Attention was given to the biological control of FCM. Results show that it would be valuable to investigate the viability for the artificial rearing and release of a certain larval parasitoid (3.4.10).

An investigation showed that FCM prefers certain navel orange varieties such as Lina, Chislett, Tulegold, Autumn Gold and Cara-Cara (3.4.11). It is questionable whether producers should continue planting these varieties.

In the past it was suspected that FCM are able to easily move from orchard to orchard or farm to farm to spread an infestation. A DNA study proved that this is not so and that FCM are quite territorially orientated (3.4.14; 3.4.15). The genetic variation in and between different populations are also relatively large and demonstrate that the insect will be able to, *inter alia*, develop resistance to insecticides and to adapt quite well to varying environmental conditions.

It is still risky to apply the current disinfestation protocol for FCM in packed fruit due to the potential for cold damage to the rind. The development of an alternative technique is therefore essential. The treatment of FCM larvae with gamma radiation was evaluated in a preliminary experiment (3.4.12). Very promising results were obtained and the continuation of this investigation is essential.

3.4.2 Die doeltreffendheid van 'n granulovirus vir die bestryding van valskodlingmot

Proef 169 deur Sean D. Moore, Garth I. Richards, Wayne Kirkman en Peter Stephen (CRI)

'n Granulovirus (GV) is 'n paar jaar gelede in VKM-larwes afkomstig van Goedehoop Sitruskoöperasie se insektarium op Citrusdal ontdek. Die virus is deur DNS-ontleding as 'n nuwe isolaat van *Cryptophlebia leucotreta* GV (CrleGV-SA) geïdentifiseer. Die virus is tot nou toe deeglik in laboratorium- en boordproewe getoets. Daar is bewys dat dit groot potensiaal as 'n biologiesebeheeragent van valskodlingmot inhou.

In proewe wat in 2003 afgehandel is, is die produksie van CrleGV uit larwes saam met die larfdieët net so vrugbaar, indien nie meer vrugbaar nie, as die produksie van CrleGV uit besmette larwes alleen. Die twee metodes het gemiddeld 1.105×10^{13} GPs en 4.019×10^{13} GPs onderskeidelik, per bak (as 600 larwes per bak gebruik is) geproduseer. As gevolg van die arbeidsbesparing met die oes van larwes saam met hulle dieët, lyk hierdie metode baie aanloklik.

As CrleGV teen 10°C gehou is, is geen afname in patogenie gemeet vir 3 verskillende formulasies na 12 weke nie. As CrleGV teen 27°C gehou is, het dit gelyk asof die virus in 'n vloeibare suspensie na 12 weke begin afbreek het.

Voorlopige biotoetse het aangedui dat mengsels van CrleGV saam met Agrimec en olie, of saam met Dithane, Benlate en olie, geen nadelige effek op die virus het. Abamectin kan dalk selfs 'n onderdrukkende invloed op VKM-larwes hê.

Vier boordproewe met CrleGV het almal goeie resultate teen VKM op nawellemoene gewys. In een proef is VKM-besmetting met gemiddeld 80% verminder vir die eerste 10 weke na toediening en met gemiddeld 70% vir 16 weke na toediening. Dit het gelyk asof molasse die werking van CrleGV verbeter.

Navorsing op CrleGV sal gedurende 2004 voortgesit word. Die rակlewe van verskeie formulasies sal vir 'n tydperk van tot 18 maande ondersoek word. Boordproewe sal op ander kultivars as slegs nawellemoene gedoen word. 'n Aansoek vir die registrasie van CrleGV sal ingedien word.

Introduction

Chemical control of FCM is fraught with problems. Simultaneously, the justification for adopting an IPM approach in the citrus industry is increasing. Consequently, an effective and IPM compatible means of controlling FCM has been sought. A few years ago a granulovirus (GV) was identified from FCM larvae from the Goedehoop Citrus Co-op insectary at Citrusdal. Through restriction enzyme analyses the virus was identified as a novel isolate of *Cryptophlebia leucotreta* GV (CrleGV). Bioassays against neonate larvae, confirming its potential as a biocontrol agent, have led to fairly extensive field trials since 2000. These trials, in which FCM infestation was reduced by over 60%, confirmed the concentration and coverage required to achieve the best results. During 2003 further work was conducted with various formulation additives, in an attempt to improve the efficacy of the virus and reduce the concentration required to a more cost effective level. This, and further work conducted on the production, formulation and storage of the virus is described in this report.

Materials and methods

Virus production

CrleGV was mass produced in 220 ml glass pie dishes. The artificial diet (94 g dry ingredients with 100 ml distilled water) was prepared in the same manner as for rearing of FCM larvae. The only difference between the diet used for virus production and the diet used for host rearing was that 33 % higher concentrations of the anti-microbial agents (nipagin and sorbic acid) were used in the virus production diet. Once cooled, the surface of the diet was inoculated with a total of 1.732×10^{11} OBs, suspended in 14 ml of distilled water, using a 1 l plastic spray bottle. The inoculated diet was placed in a laminar flow cabinet for ± 20 minutes, to dry. A total of 600 fifth instar larvae were placed on the diet surface. The pie dish was covered with a double layer of "Gladwrap" (plastic stretch film) or a 2 mm thick sheet of glass to prevent larvae from escaping. Symptomatic infection of larvae usually began 5-7 days after introduction onto inoculated diet. Larvae were collected each day, recorded, and stored at -40°C . All possible harvesting of infected larvae was completed by 10 days after introduction of larvae onto the diet.

Periodically, outbreaks of virus were observed in the FCM larval culture. When this happened, rearing jars with symptomatically infected larvae were transferred to the virus production room. Infected larvae were removed and stored with the harvested larvae from deliberate virus production, at -40°C . Infected larvae were harvested from the jars each day until no new infection appeared.

At a later stage, larvae were defrosted and virus liberated by crushing with a mortar and pestle. The homogenate was then diluted with water and filtered through a double layer of muslin cloth, to produce a crude viral suspension. Enumeration of the filtered unpurified virus was conducted using a 0.02 mm depth Thoma bacterial counting chamber at 400X magnification under dark field light microscopy. If virus was purified by rate-zonal centrifugation, it became possible to estimate virus concentration with the use of a spectrophotometer.

Concentration of virus per larva and per mass of larvae was determined for different batches. This enabled estimation of virus production parameters.

A method of producing and harvesting virus, which was less labour intensive, was investigated. Two pie dishes of diet were prepared and inoculated with virus, as described above. 600 fifth instar larvae were placed onto each dish as above, and dishes were closed and sealed. After 9 days, larvae and diet were harvested and homogenised in a domestic food processor. Plain distilled water was added to one of the samples, whereas 1% sodium dodecyl sulphate (SDS), in distilled water, was added to the other. This was in order to determine whether SDS improved the recovery of virus from the homogenate. The homogenate was then filtered through a double layer of muslin cloth. The diet particles in the suspension made it impossible to enumerate the virus concentration with a bacterial counting chamber. Therefore, in order to estimate the virus concentration, a 7-treatment 5-fold dilution series bioassay was conducted with neonate FCM larvae on artificial diet in 25-cell trays. One bioassay was conducted for the homogenate from each of the 2 dishes. The concentration of virus in each homogenate was determined by inserting empirical probits for mortality recorded, into a regression analysis forecast model (Statistical Graphics Corporation, 1996), which had been programmed with log doses and empirical probits from dose mortality bioassays with neonate FCM larvae (Moore, 2002).

Impact of freeze-drying on the virus

Due to certain potential problems with virus in liquid suspension, freeze-drying of CrleGV was investigated. The first thing to determine was whether freeze-drying affected pathogenicity. An aliquot (1 ml) of suspended CrleGV at a concentration of 7.106×10^{10} OBs/ml was freeze-dried and resuspended to again make up a total of 1 ml. This was bioassayed against an identical suspension, which had not been freeze-dried. The bioassay was an 8-treatment 5-fold dilution series bioassay with neonate FCM larvae on artificial diet in 25-cell trays. Bioassays were evaluated after 7 days. Larvae were recorded as alive or dead. Dose-response curves were calculated using PROBAN (Van Ark, 1995), a computer 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, LC_{50} and LC_{90} (concentrations required to kill 50% and 90 % of larvae in a sample, respectively) were calculated for each assay.

Virus shelf-life

A total of 33 aliquots of CrleGV were made. Each was 0.25 ml (250 µl), with a concentration of 2.591×10^{11} OBs/ml. Eleven of these were freeze-dried; 11 were spin-dried (centrifuged at 10000 rpm for 5 minutes); and the remaining 11 were left in liquid suspension. One aliquot of each of the 3 treatments was frozen at -40°C. The remaining vials were individually wrapped in aluminium foil to protect them against any possible UV-inactivation. Five vials of each treatment were placed in a room at 27°C and the other 5 of each treatment were placed in a refrigerator at 10°C. One vial of each treatment, from each temperature, was removed and frozen at -40°C at the following intervals after trial initiation: 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks. After 12 weeks, all samples were bioassayed (dose-response) against neonate FCM larvae on artificial diet.

Compatibility with other products

Compatibility of the virus with certain other plant protection products is important. The products deemed most likely to be applied at the same timing as CrleGV are abamectin with oil, and dithane and benlate with oil. A detached fruit (out of season Valencia oranges) bioassay was conducted. Oranges were treated with one of 4 different treatments (Table 3.4.2.1). Twenty five fruit were dipped into beakers containing suspensions/solutions of each treatment. After dipping, fruit were placed onto a wire mesh rack until dried. Thereafter, four FCM neonate larvae were placed onto each fruit using a size 000 paintbrush. Fruit were then kept at 27°C for 14 days. Thereafter they were evaluated for decay and penetration marks (suspected to be caused by FCM larvae). They were then dissected and FCM infestation (number and instar of larvae) recorded.

Table 3.4.2.1 Detached fruit (Valencia orange) bioassay treatments, testing compatibility of CrleGV (at a concentration of 9×10^6 OBs/ml) with certain plant protection products.

Treatment No.	Treatment
1	Distilled water
2	CrleGV + molasses (0.5%)
3	CrleGV + molasses (0.5%) + Agrimec (20 ml)* + oil (0.3%)
4	CrleGV + molasses (0.5%) + dithane (200 g)* + benlate (50 g)* + oil (0.3%)

* Dosages are per 100 l water.

Field trials

During the 2002/03 season, five trials with CrleGV were applied to test its efficacy against FCM on citrus. One of the trials was conducted by Hendrik Hofmeyr in the Western Cape, and is described in a separate section within this report (Section 3.4.3).

The first trial was applied at Carden Farm in the Sundays River Valley. The orchard used, consisted of 10 year old Palmer navel orange trees on rough lemon rootstock. The orchard was planted at a density of 555 trees per hectare. The trial was laid out in a semi-commercial block format, with an average of 82 trees per block, and two blocks (replicates) per treatment. Two treatments were applied (Table 3.4.2.2). A replicated untreated control was retained. Treatments were applied using a mist-blower with an oscillating tower, and were applied after 17h30 on 4 December 2002. An average of 18.55 l of spray mix was applied per tree.

Table 3.4.2.2. Treatments applied on 4 December 2002 for the control of FCM on Palmer navel orange trees at Carden Farm.

Treatment number	Product	Concentration in water	Rate per ha (for CrleGV)	Additive	Concentration per 100 l water
1	Untreated	-	-	-	-
2	Alsystin	10 ml/100 l	-	-	-
3	CrleGV	1.326×10^8 OBs/ml	1.435×10^{15} OBs	Molasses plus Agral 90	500 ml plus 18 ml

The second trial of the 2002/03 season was applied at Schoeman Landgoed in Mpumalanga Province. The trial was conducted in an orchard of mature Robyn navel orange trees, spaced at 340 trees per

hectare, on 16 January 2003. Three treatments were applied: Alsystin and two concentrations of CrleGV (Table 3.4.2.3). An untreated control was retained. The trial was laid out in single-tree replicates in a randomised block design, ensuring that there were buffer trees between treatments in order to minimise any influence of spray drift. Ten replicates of each treatment were used. An average of 35 ℓ of spray mix was applied per tree, as a high pressure spray, using hand-held spray guns. Spraying of CrleGV treatments was initiated at 16h40.

Table 3.4.2.3. Treatments applied on 16 January 2003 for the control of FCM on Robyn navel orange trees at Schoeman Landgoed.

Treatment number	Product	Concentration in water	Rate per ha (for CrleGV)	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-	-
2	Alsystin	10 ml/100 ℓ	-	-	-
3	CrleGV	9.948 x 10 ⁶ OBs/ml	1.6 x 10 ¹⁴ OBs/ha	Molasses plus Agral 90	500 ml plus 18 ml
4	CrleGV	4.974 x 10 ⁷ OBs/ml	8.0 x 10 ¹⁴ OBs/ha	Molasses plus Agral 90	500 ml plus 18 ml

The third trial was applied on an orchard of 20 year old Palmer navel orange trees, spaced at 417 trees per hectare, on the Farm, Dirkie Uys, in the Sundays River Valley. The trial was laid out in single-tree replicates in a randomised block design, with 10 replicates of each treatment. Six treatments (Table 3.4.2.4) were applied – treatments 2 and 3 (Table 3.4.2.5) on 18 February 2003 and treatments 4, 5, 6 and 7 (Table 3.4.2.4) on 20 February. An untreated control was retained. Meothrin was included as a standard registered treatment for FCM control. CrleGV application was initiated at 15h30. An average of 30.2 ℓ of spray mix was applied per tree for all treatments, as a high pressure spray, using hand-held spray guns. After treatments applied on 18 February had dried, 25 mm of rainfall was recorded in the Kirkwood area (in which Dirkie Uys Farm is included). The following evening (19 February), 30 mm of rainfall was recorded.

Table 3.4.2.4. Treatments applied on 18 February 2003 for the control of FCM on Palmer navel orange trees at Dirkie Uys Farm.

Treatment number	Product	Concentration in water	Rate per ha (for CrleGV)	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-	-
2	Meothrin	30 ml/100 ℓ	-	-	-
3	CrleGV	6.003 x 10 ⁷ OBs/ml	7.560 x 10 ¹⁴ OBs	Molasses	500 ml
4	CrleGV	3.001 x 10 ⁷ OBs/ml	3.780 x 10 ¹⁴ OBs	Molasses	500 ml
5	CrleGV	3.001 x 10 ⁷ OBs/ml	3.780 x 10 ¹⁴ OBs	Molasses plus Agral 90	500 ml plus 18 ml
6	CrleGV	3.001 x 10 ⁶ OBs/ml	3.780 x 10 ¹³ OBs	Molasses plus Agral 90	500 ml plus 18 ml
7	CrleGV	3.001 x 10 ⁷ OBs/ml	3.780 x 10 ¹⁴ OBs	Skimmed milk powder plus Agral 90	1 kg plus 18 ml

The fourth trial of the season was applied on Vergenoeg Farm in the Gamtoos River Valley. An orchard of 17 year old Robyn navel orange trees, spaced at 555 trees per hectare was used. The trial was laid out in single-tree replicates in a randomised block design, ensuring that there were buffer trees between treatments in order to minimise any influence of spray drift. Ten replicates of each treatment were used. Four treatments were sprayed on 24 April 2003 (Table 3.4.2.5). Untreated control trees were included in the trial. CrleGV treatments were applied after 15h15. An average of 19.37 ℓ of spray mix was applied per tree for all treatments, as a high pressure spray using hand-held spray guns. Evaluation of fruit drop was only initiated four weeks after application of treatments.

Table 3.4.2.5. Treatments applied on 24 April 2003 for the control of FCM on Robyn navel orange trees at Vergenoeg Farm.

Treatment number	Product	Concentration in water	Rate per ha (for CrleGV)	Additive	Concentration per 100 ℓ water
1	Untreated	-	-	-	-
2	Meothrin	30 ml/100 ℓ	-	-	-
3	CrleGV	9.017 x 10 ⁷ OBs/ml	9.694 x 10 ¹⁴ OBs	Molasses plus Agral 90	500 ml plus 18 ml
4	CrleGV	9.017 x 10 ⁶ OBs/ml	9.694 x 10 ¹³ OBs	Molasses plus Agral 90	500 ml plus 18 ml
5	CrleGV	9.017 x 10 ⁵ OBs/ml	9.694 x 10 ¹² OBs	Molasses plus Agral 90	500 ml plus 18 ml

Results and discussion

Virus production

There was great variation in the virus production per larva and per gram of larva (Table 3.4.2.6). It was not possible to associate this variation with any differences in the virus production parameters and techniques, as larvae were harvested over a period of time and pooled. Virus was extracted from each batch of pooled larvae. Larvae within a batch are likely to have been subjected to different regimes (e.g. density of larvae and exact stage at which harvested).

The productivity ratio (PR), defined as the difference between the size of the infecting inoculum and the per-larval OB yield, is calculated as:

$$PR = \text{No. OBs yielded/larva} \div \text{No. infecting OBs (OBs/mm}^2 \text{ for surface inoculation)}$$

If the mean number of OBs per larva is 3.070×10^{10} (Table 3.4.2.6) and the number of infecting OBs per mm² is 9.135×10^6 (OBs introduced onto diet surface \div surface area), then $PR = 3.361 \times 10^3$. Productivity was about one third of what was previously recorded (Moore, 2002). However, it is believed that the estimation recorded here should be considered more realistic than previously recorded, as projections should preferably be conservative.

Table 3.4.2.6. Yield of CrleGV by *in vivo* production in fifth instar *C. leucotreta* larvae and harvesting of larvae individually.

Number of CrleGV infected larval cadavers	Total Mass	Mass per larva (g)	Total OBs	Mean OBs per larva (larval equivalent) \pm SE	Mean OBs per gram \pm SE
6161	384.54 g	0.062	1.110×10^{14}	1.802×10^{10}	2.886×10^{11}
7203	415.97 g	0.058	3.340×10^{13}	4.637×10^9	8.029×10^{10}
10484	602.38 g	0.057	8.059×10^{14}	7.687×10^{10}	1.338×10^{12}
7613	366.53 g	0.048	1.771×10^{14}	2.327×10^{10}	4.833×10^{11}
Mean				3.070×10^{10} $\pm 1.588 \times 10^{10}$	5.475×10^{11} $\pm 2.760 \times 10^{11}$

OB production per final mg of body weight for GVs in Lepidoptera seems to be about 2×10^7 (Entwistle & Evans, 1985). The mean production of 5.475×10^8 OBs per mg of final body weight (Table 3.4.2.6) achieved with CrleGV in fifth instar FCM larvae was therefore impressive.

If 600 larvae are placed onto a dish of virus inoculated diet, and 60% of larvae are recovered infected with virus (Moore, 2002), then virus production per dish₁ would average 1.105×10^{13} OBs.

Previous trials indicated that there was very little difference in virus yield when virus with diet was harvested 8, 9 or 10 days after introduction of healthy larvae (Moore, 2002). However, production of virus by this method was also observed to be substantially lower than when larvae were harvested without the diet. As the labour saving in harvesting diet with larvae would be so immense, this trial was repeated.

Table 3.4.2.7 Mortality of neonate *C. leucotreta* larvae in five-fold series dilution dose-response bioassays with two samples of virus harvested with diet. Twenty five individuals were tested per treatment – 7 treatments per bioassay.

Dilution rates of CrleGV	Sample 1 (no SDS)			Sample 2 (1% SDS)		
	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit
Distilled water control	20	-	-	20	-	-
x/625	36	20.0	4.158	32	15.0	3.964
x/125	40	25.0	4.325	44	30.0	4.476
x/25	68	60.0	5.253	72	65.0	5.385
x/5	84	80.0	5.842	84	80.0	5.842
x	96	95.0	6.645	92	90.0	6.282

Virus harvested with diet cannot be enumerated by light microscopy unless the homogenate is purified (after filtration) by rate zonal centrifugation. As the equipment and consumables necessary for this process were not available at the time this trial was conducted, the virus was enumerated by conducting surface inoculation dose-response bioassays with neonate larvae on artificial diet instead. In order to estimate what dilution rates to use, the concentration of virus in the homogenates was surmised. This speculation proved sufficiently accurate to obtain mortality trends (Table 3.4.2.7) from which virus concentrations could be deduced.

Table 3.4.2.8 Virus concentrations estimated for two samples of CrleGV harvested with diet. Estimates were conducted by inserting empirical probits for mortality recorded, into a regression analysis forecast model.

Sample 1 (no SDS)		Sample 2 (1% SDS)	
Empirical probit	Estimated concentration of CrleGV (OBs/ml)	Empirical probit	Estimated concentration of CrleGV (OBs/ml)
4.158	4.518×10^2	3.964	2.805×10^2
4.325	6.792×10^2	4.476	9.840×10^2
5.253	6.577×10^3	5.385	9.099×10^3
5.842	2.780×10^4	5.842	2.780×10^4
6.645	1.986×10^5	6.282	8.166×10^4

By converting mortality (Table 3.4.2.8) to empirical probits and inserting these into a regression analysis forecast model, quantity of OBs in sample 1 and 2 were estimated at 5.696×10^{13} OBs and 2.342×10^{13} OBs, respectively. These are the numbers of OBs harvested per dish of 600 larvae. This compares very favourably with virus productivity when larvae are harvested individually. The addition of SDS to the homogenate provided no apparent benefit. It has subsequently been ascertained that SDS can even be harmful to baculoviruses (Lua *et al.*, 2003).

Impact of freeze-drying on the virus

The need for virus formulation encompasses two main areas: shelf and tank (finished spray) formulations (Hunter-Fujita *et al.*, 1998). Shelf life of a baculovirus product can be retained for a reasonable length of time by freezing, or by developing a formulation that confers stability under a reasonable range of shelf storage conditions (Hunter-Fujita *et al.*, 1998). As it has been shown that wet virus breaks down more rapidly than dry virus (David, 1969) and as bacterial contamination can increase in a wet virus preparation, freeze-drying of CrleGV was investigated.

Table 3.4.2.9. Mortality of neonate *C. leucotreta* larvae in dose response bioassays with 6 concentrations of non-freeze-dried CrleGV, replicated twice. Twenty five individuals were tested per treatment per replicate.

Treatment (CrleGV in OBs/ml)	Replicate 1			Replicate 2		
	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit
Distilled water control	20.0	-	-	19.0	-	-
1.219×10^2	24.0	5.0	3.355	28.0	11.11	3.779
6.093×10^2	40.0	25.0	4.326	32.0	16.05	4.008
3.046×10^3	56.0	45.0	4.874	64.0	55.56	5.140
1.523×10^4	92.0	90.0	6.282	76.0	70.37	5.535
7.616×10^4	100	100	-	100	-	-
3.808×10^5	100	100	-	100	-	-

Table 3.4.2.10. Mortality of neonate *C. leucotreta* larvae in dose response bioassays with 6 concentrations of freeze-dried CrleGV, replicated twice. Twenty five individuals were tested per treatment per replicate.

Treatment (CrleGV in OBs/ml)	Replicate 1			Replicate 2		
	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit
Distilled water control	20.0	-	-	19.0	-	-
1.219×10^2	36.0	20.0	4.158	20.0	1.23	2.752
6.093×10^2	36.0	20.0	4.158	40.0	25.93	4.355
3.046×10^3	60.0	50.0	5.000	96.0	90.12	6.288
1.523×10^4	84.0	80.0	5.842	96.0	95.06	6.651
7.616×10^4	100.0	100.0	-	100.0	100.00	-
3.808×10^5	100.0	100.0	-	100.0	100.00	-

From dose response bioassays with non-freeze-dried virus (Table 3.4.2.9), mean LC_{50} and LC_{90} were estimated at 2.993×10^3 OBs/ml and 4.884×10^4 OBs/ml, respectively. From dose response bioassays with freeze-dried virus (Table 3.4.2.10), mean LC_{50} and LC_{90} were estimated at 1.831×10^3 OBs/ml and 3.244×10^4 OBs/ml, respectively. It is therefore clear that the freeze-drying process in no way affected the infectivity of CrleGV. The lower LC values recorded for the freeze-dried virus cannot be considered as being significant, as it is unperceivable that freeze-drying could enhance pathogenicity of a virus in any way. The LC values estimated here are lower than those previously determined for CrleGV (Moore, 2002).

Virus shelf-life

Thus far bioassays have only been replicated once for each duration of storage at each temperature. Another two replicates should be conducted with each sample. Nevertheless, there was no indication of any reduction in pathogenicity of CrleGV, stored at 10°C for up to 3 months. This was determined through bioassay of CrleGV samples against neonate FCM larvae at various time intervals. No meaningful increase in either LC_{50} (Fig. 3.4.2.1) or LC_{90} (Fig. 3.4.2.2) values over this time was recorded. Apparent increases in values such as the LC_{90} of spin-dried CrleGV after 4 weeks at 10°C, must be nothing more than a slight experimental aberration, as the LC_{90} was down again at 8 and 12 weeks of storage. Such aberrations should be diminished by repetition of the bioassays.

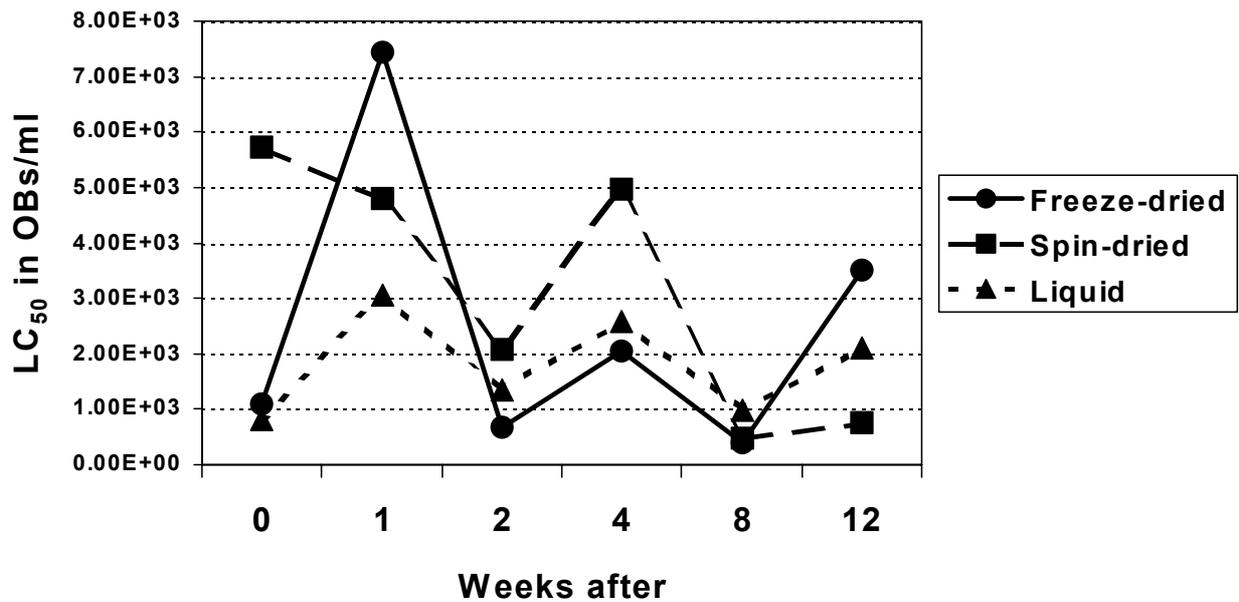


Fig. 3.4.2.1 LC₅₀ of freeze-dried, spin-dried and liquid suspension virus after various intervals at 10°C against neonate FCM larvae.

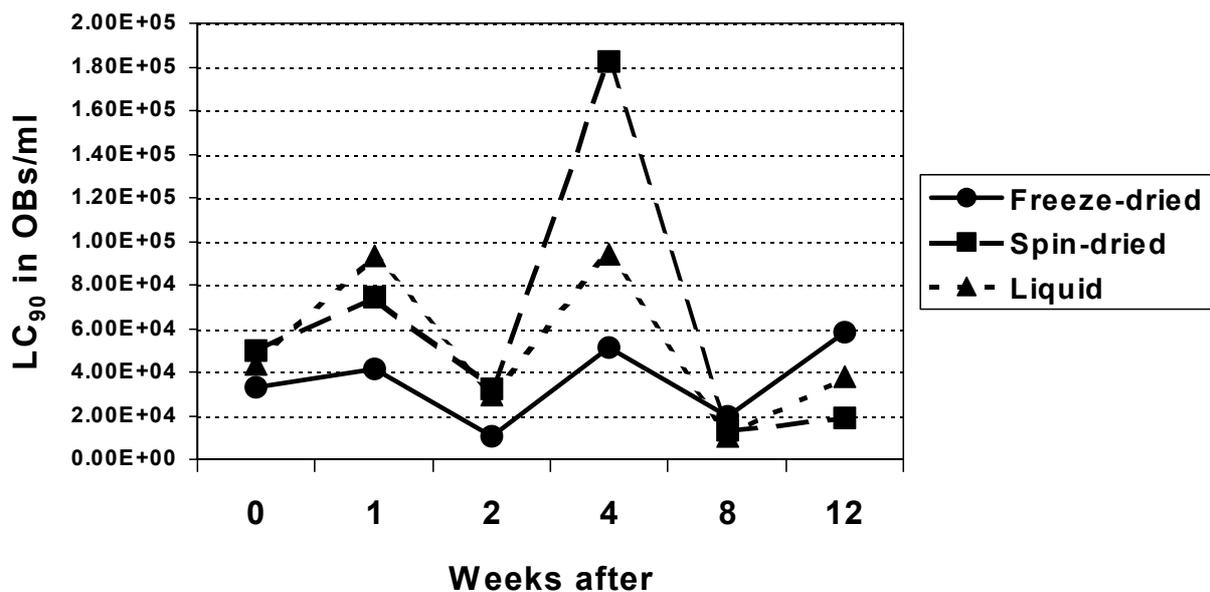


Fig. 3.4.2.2 LC₉₀ of freeze-dried, spin-dried and liquid suspension virus after various intervals at 10°C against neonate FCM larvae.

One bioassay replicate was conducted with samples of CrleGV kept at 27°C at each time interval, except at 1 week of storage. This bioassay, as well as a further two replicates with all of the samples, must still be conducted. However, it is apparent from the bioassays already conducted that the LC₅₀ (Fig. 3.4.2.3) and LC₉₀ (Fig. 3.4.2.4) of CrleGV in liquid suspension were substantially higher after 12 weeks at 27°C. After 8 weeks at that temperature, there was no noteworthy change in dose-response of neonate larvae. This indicates that CrleGV in liquid suspension has begun to lose pathogenicity by 12 weeks at 27°C. There were some indications that the freeze-dried virus might also have been affected by this stage. However, this was by no means conclusive. These results must all be confirmed by replicating the bioassays.

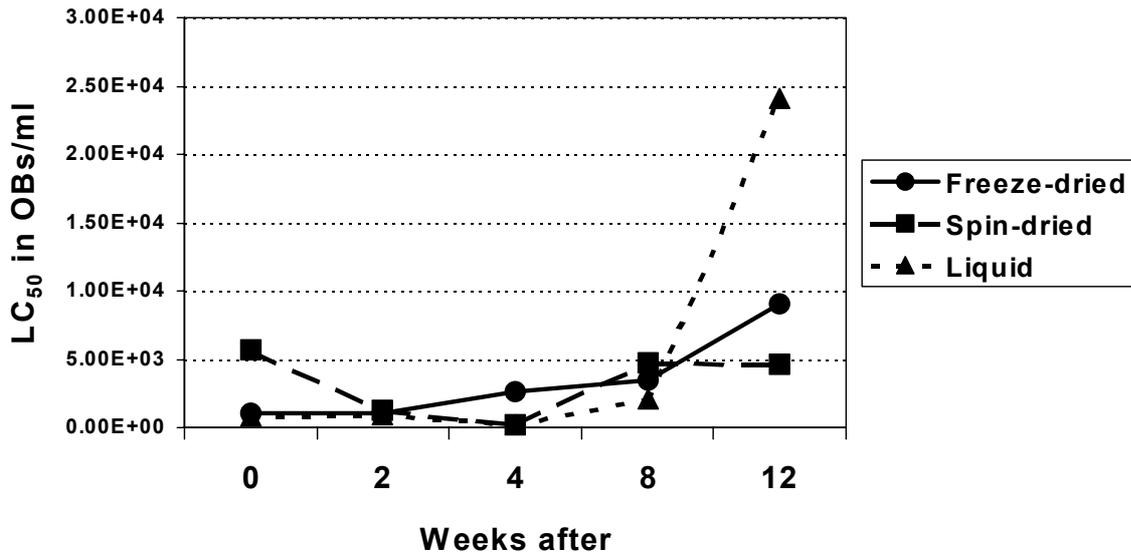


Fig. 3.4.2.3 LC₅₀ of freeze-dried, spin-dried and liquid suspension virus after various intervals at 27°C against neonate FCM larvae.

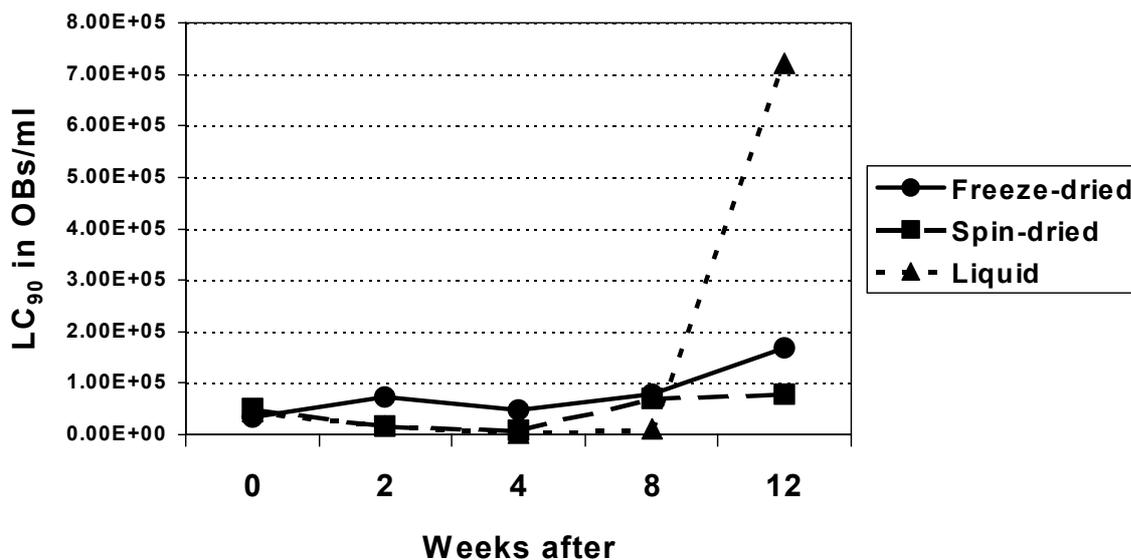


Fig. 3.4.2.4 LC₉₀ of freeze-dried, spin-dried and liquid suspension virus after various intervals at 27°C against neonate FCM larvae.

Results obtained in this trial confirmed that virus in suspension breaks down more rapidly than dry virus (David, 1969). However, it is still questionable as to whether CrleGV should be produced as a dry formulation for commercial use. Freeze-drying (not the only method of drying) is a very expensive and time consuming process, and liquid formulations are more user-friendly. Further trials are required to test the shelf-life of the different formulations at a cool storage temperature over an extended period (i.e. 12 - 18 months).

A shelf-life of at least 12 weeks can be proclaimed for a liquid suspension formulation. Trials currently being conducted will indicate how much longer the shelf-life is than this. Various ingredients have been added to the liquid suspension in these trials, in order to try and extend the shelf-life.

Compatibility with other products

It is envisaged that under commercial conditions, the first treatment of CrleGV will be applied somewhere between late November and early January in a number of citrus producing areas of South Africa. This is

because it has been confirmed that the first major peak in FCM levels occurs around this time, at least in the Eastern Cape (Moore & Fourie, 1999; Moore & Richards, 2000; 2001; 2002; SRCC, unpublished data; PSB, unpublished data) and Mpumalanga (Language, 2002). It is very possible that growers will need to control other pests around this time – one of the most important being citrus thrips. The treatment of choice is likely to be abamectin with oil. In some areas it might also be necessary to spray for the control of blackspot. Mancozeb and benomyl with oil is likely to be the most popular treatment.

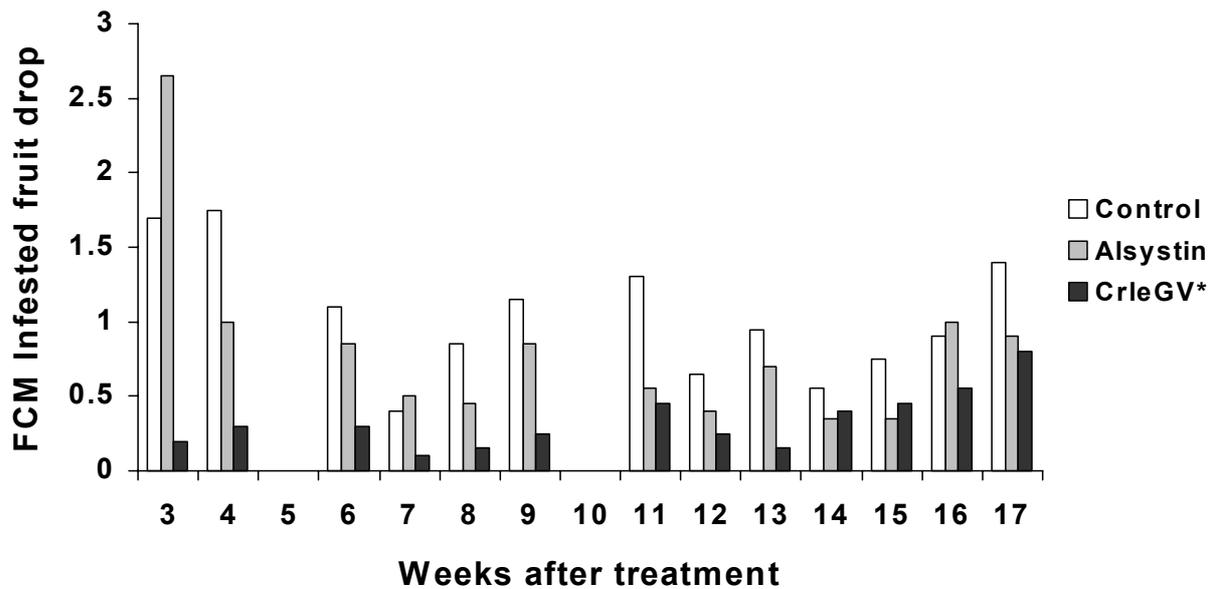
Table 3.4.2.11. Damage to and infestation of fruit (Valencia oranges) treated with CrleGV (9×10^6 OBs/ml) and certain other plant protection products to test compatibility. (Four neonate larvae were placed onto each fruit, and 25 fruit were used per treatment).

Treat. No.	Treatment	Number of fruit decaying	Number of fruit with penetration marks	Total number of penetration marks	Number of fruit infested	Total number of larvae	Average larval instar
1	Distilled water	1	20	31	16	20	3.75
2	CrleGV + molasses (0.5%)	1	16	25	5	7	3.14
3	CrleGV + molasses (0.5%) + Agrimec (20 ml)* + oil (0.3%)	0	5	8	0	0	-
4	CrleGV + molasses (0.5%) + Dithane (200 g)* + Benlate (50 g)* + oil (0.3%)	0	8	9	4	5	3.6

The detached fruit bioassay gave no indication that either the thrips treatment or the black spot treatment had any detrimental effect on the efficacy of the CrleGV. Infestation was similarly low in fruit treated with the virus and molasses alone and in fruit treated with Dithane, Benlate and oil, in addition to these (Table 3.4.2.11). There was even an indication that abamectin might have a controlling effect on the neonate FCM larvae (Table 3.4.2.11). Further detached fruit bioassays will be conducted to confirm these results. The chemical treatments will also be included in these bioassays without CrleGV.

Field trials

As a result of the conclusions drawn from the two trials conducted during the previous season, CrleGV was applied as a full cover spray at an estimated 10^{15} OBs/ha. Ultimately 1.435×10^{15} OBs/ha were applied, with 0.5% molasses. Results surpassed those previously achieved, not only with CrleGV but with any insect virus on which field data was found in available literature. Mean reduction in FCM infestation by CrleGV was around 80% for the 10 weeks post-application, and around 70% for 16 weeks post-application (Fig. 3.4.2.5). CrleGV appeared to have broken down completely by 19 weeks after application. These excellent results could be ascribed to the addition of molasses or to the block effect (all previous trials had been applied to single tree replicates), or a combination of both. Once again, Alsystin had very little effect on FCM (Fig. 3.4.2.5).



* approx. 10^{15} OBs/ha

Fig. 3.4.2.5. Weekly fruit (Palmer navel oranges) drop for treatments applied for control of FCM at Carden Farm, Eastern Cape Province, on 4 December 2002. (CrleGV = 1.326×10^8 OBs/ml).

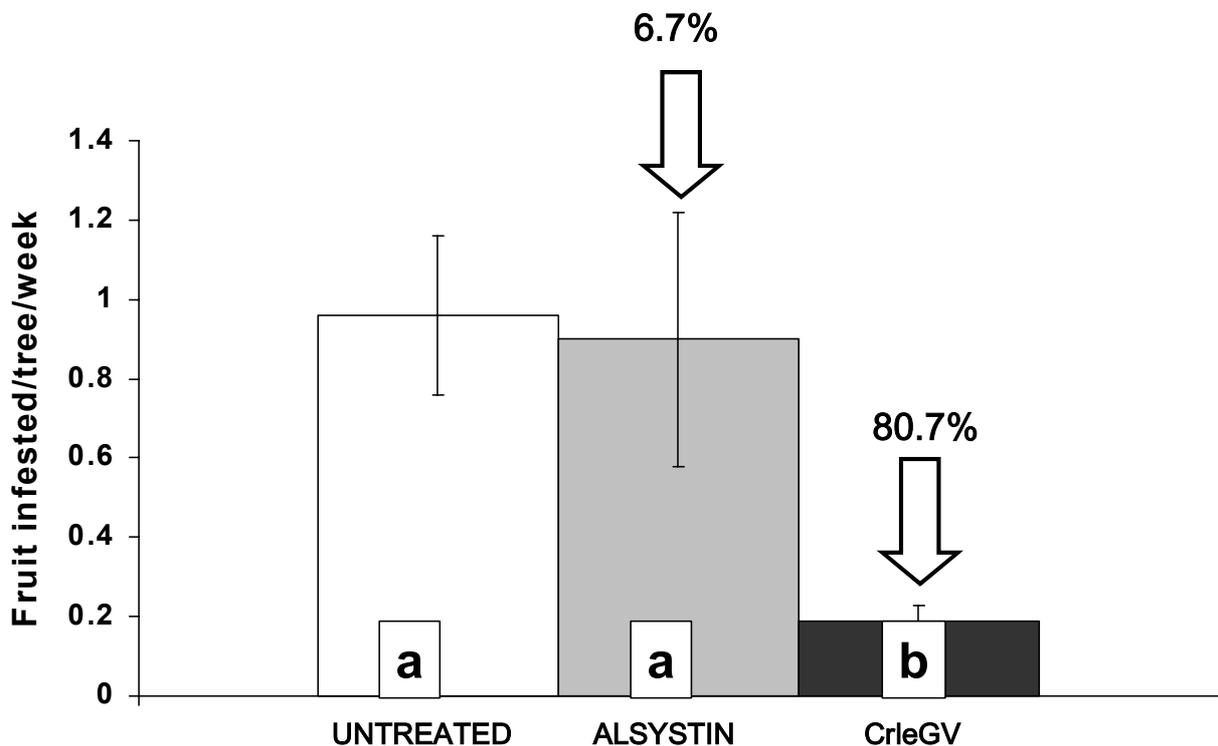
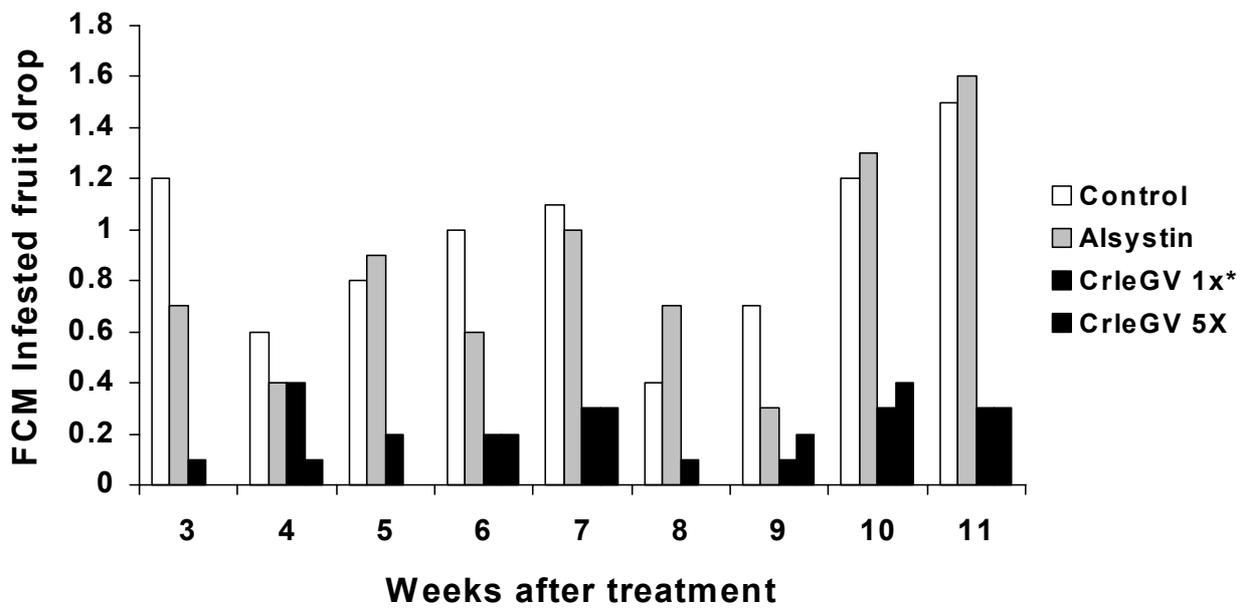


Fig. 3.4.2.6. Mean fruit (Robyn navel oranges) drop per tree per week for a period of 3 - 10 weeks after treatments applied for control of FCM at Carden Farm, Eastern Cape Province, on 4 December 2002 (including standard error bars). Columns with the same letter are not significantly different ($P > 0.05$; Bonferroni LSD multiple range test). Arrows indicate percentage reduction in FCM larval infestation of fruit, relative to untreated control trees.

In the Carden Farm trial, mean reduction in FCM infestation over an eight week period (weeks 3 - 10 post-application) from CrleGV was 80.7% (Fig. 3.4.2.6). This was against an FCM pressure which could be considered to be moderate i.e. a loss of an average of 0.87 just under one FCM infested fruit per tree per

week. Alsystem succeeded in only reducing FCM infestation by 6.7% over the same time period (Fig. 3.4.2.6).

As a result of the outstanding results achieved with CrleGV at Carden Farm in the Eastern Cape (Figs. 3.4.2.5 & 3.4.2.6), CrleGV was applied at two lower concentrations (approximately one half and one tenth of the per hectare rate used at Carden Farm) in a subsequent trial in Mpumalanga. Despite this reduction in concentration and the use of single tree replicates, as opposed to block treatments, reduction in FCM infestation was again vivid (Fig. 3.4.2.7). Yet again, Alsystem did not appear to have much effect on FCM. Although CrleGV was still working well at 11 weeks after application (Fig. 3.4.2.7) (and could have continued to have an effect for a number of weeks) monitoring of the trial was terminated.



* approx. 10^{14} OBs/ha

Fig. 3.4.2.7. Weekly fruit (Palmer navel oranges) drop for treatments applied for control of FCM at Schoeman Landgoed, Mpumalanga Province, on 16 January 2003. (CrleGV 1X = 9.948×10^6 OBs/ml; CrleGV 5X = 4.974×10^7 OBs/ml).

Over the entire nine week period of monitoring, i.e. week 3-11 after application, Alsystem reduced FCM infestation by only 11.8% (Fig. 3.4.2.8). The two CrleGV treatments (9.948×10^6 OBs/ml and 4.974×10^7 OBs/ml) reduced infestation by 76.5% and 82.3%, respectively, over this period (Fig. 3.4.2.8).

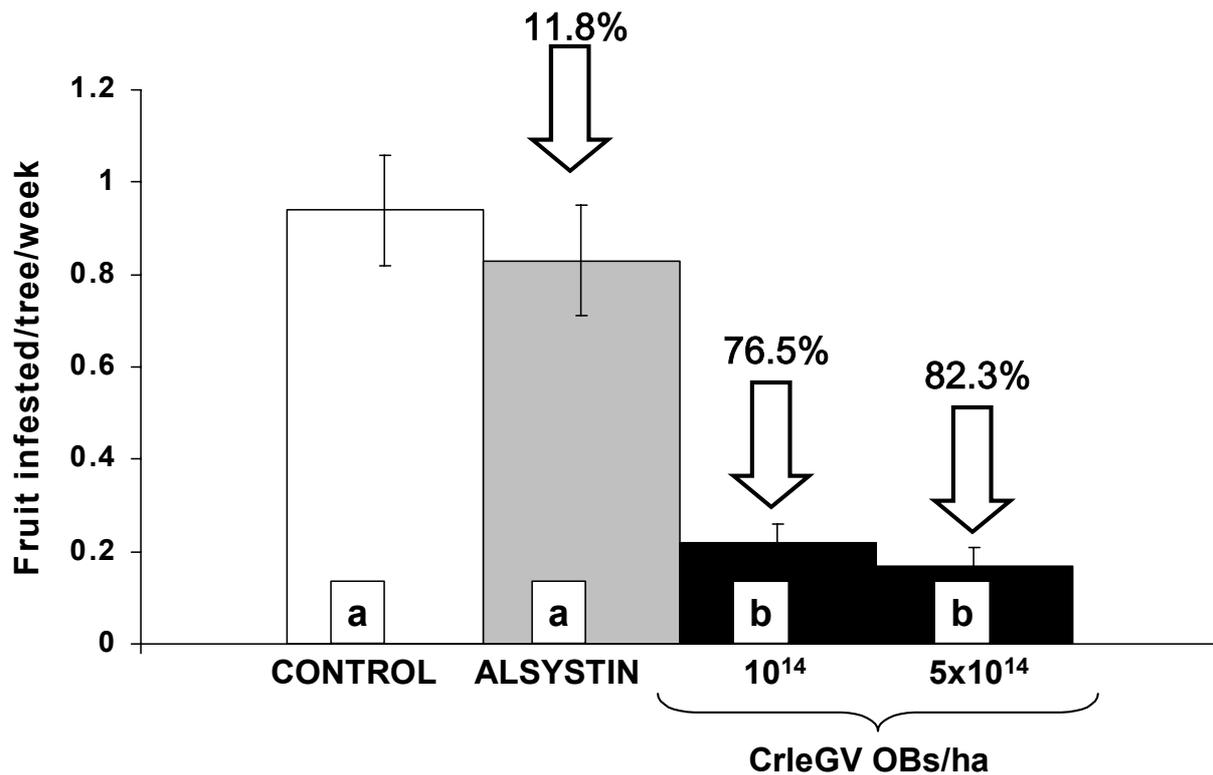


Fig. 3.4.2.8. Mean fruit (Palmer navel oranges) drop per tree per week for a period of 3 - 11 weeks after treatments applied for control of FCM at Schoeman Landgoed, Mpumalanga Province, on 16 January 2003 (including standard error bars). Columns with the same letter are not significantly different ($P > 0.05$; Bonferroni LSD multiple range test). Arrows indicate percentage reduction in FCM larval infestation of fruit, relative to untreated control trees.

The trial at Dirkie Uys Farm was only monitored for a total of four weeks, as infestation was disappointingly low (an average of 0.125 FCM infested fruit per tree per week). It was felt that with such a low level of infestation, conclusive results could not be achieved. Results obtained were nevertheless interesting. Four of the CrleGV treatments reduced FCM infestation. The only one not doing so, being the one applied with milk powder. FCM infestation was 80% lower where 3.001×10^7 OBs/ml with molasses (0.5%) and Agral 90 were applied (Fig. 3.4.2.9). The same concentration with molasses, but without the Agral 90, reduced infestation by 60% (Fig. 3.4.2.9). Surprisingly, FCM infestation was higher where Meothrin had been applied than in the untreated control (Fig. 3.4.2.9).

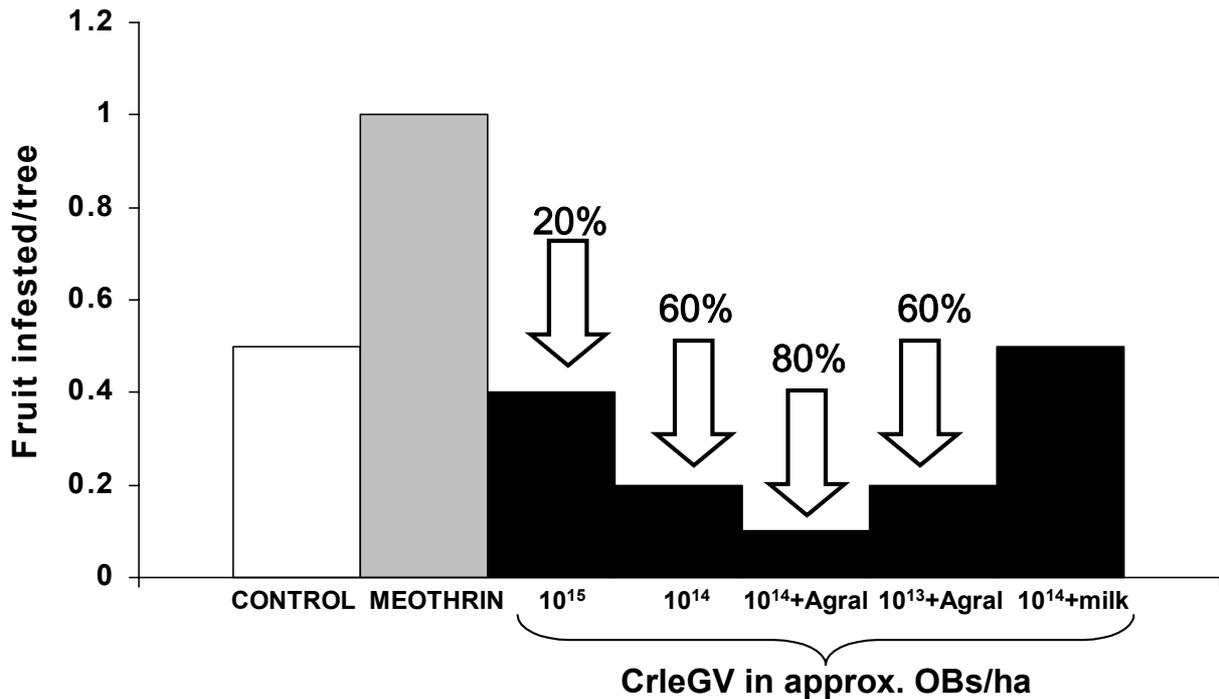


Fig. 3.4.2.9. Mean fruit (Palmer navel oranges) drop per tree per week for a period of 3 - 6 weeks after treatments applied for control of FCM at the farm, Dirkie Uys, Eastern Cape Province, on 18 and 20 February 2003. Arrows indicate percentage reduction in FCM larval infestation of fruit, relative to untreated control trees.

The trial at Vergenoeg Boerdery was monitored for six weeks. Although the trial was conducted on Robyn navel oranges, which would have been harvested in July, monitoring ceased on 19 May, which is around the time when harvesting of the majority of navel orange plantings in the Eastern Cape begins. This is interesting, as this trial gives an indication of the capacity of CrleGV to reduce post-harvest problems related to FCM infestation. During the last three weeks of monitoring, no FCM infested fruit were recorded in the 9.017×10^6 OBs/ml CrleGV treatment (Fig. 3.4.2.10).

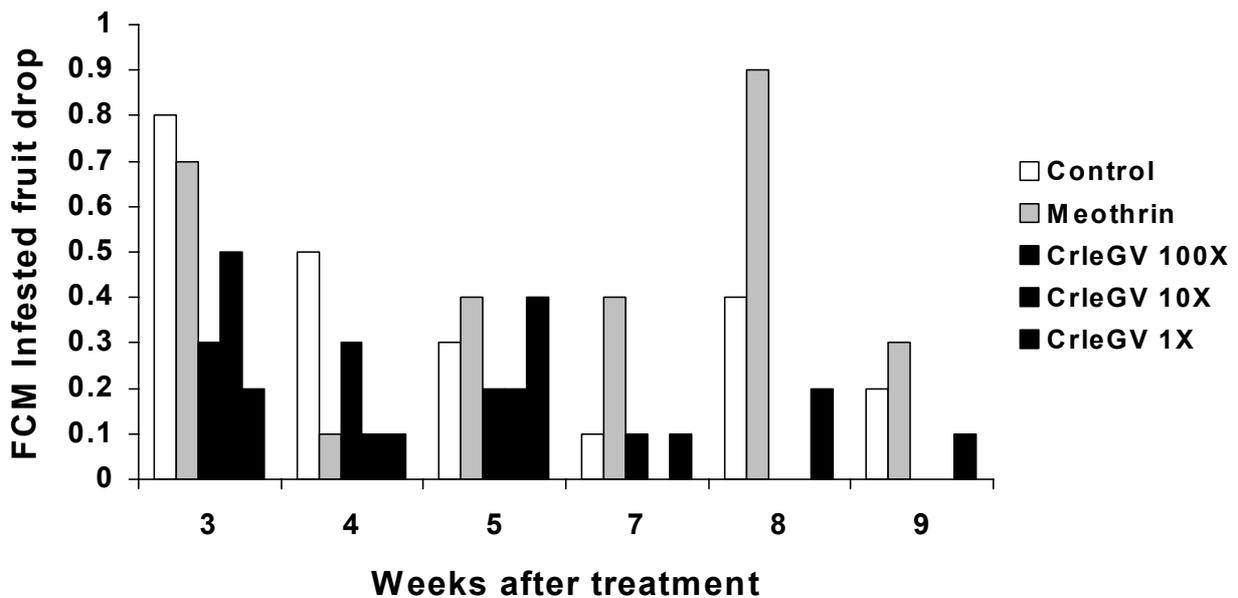


Fig. 3.4.2.10. Weekly fruit (Robyn navel oranges) drop for treatments applied for control of FCM at Vergenoeg Boerdery, Eastern Cape Province, on 24 April 2003. (CrleGV: 1X = 9.017×10^5 OBs/ml, 10X = 9.017×10^6 OBs/ml, and 100X = 9.017×10^7 OBs/ml).

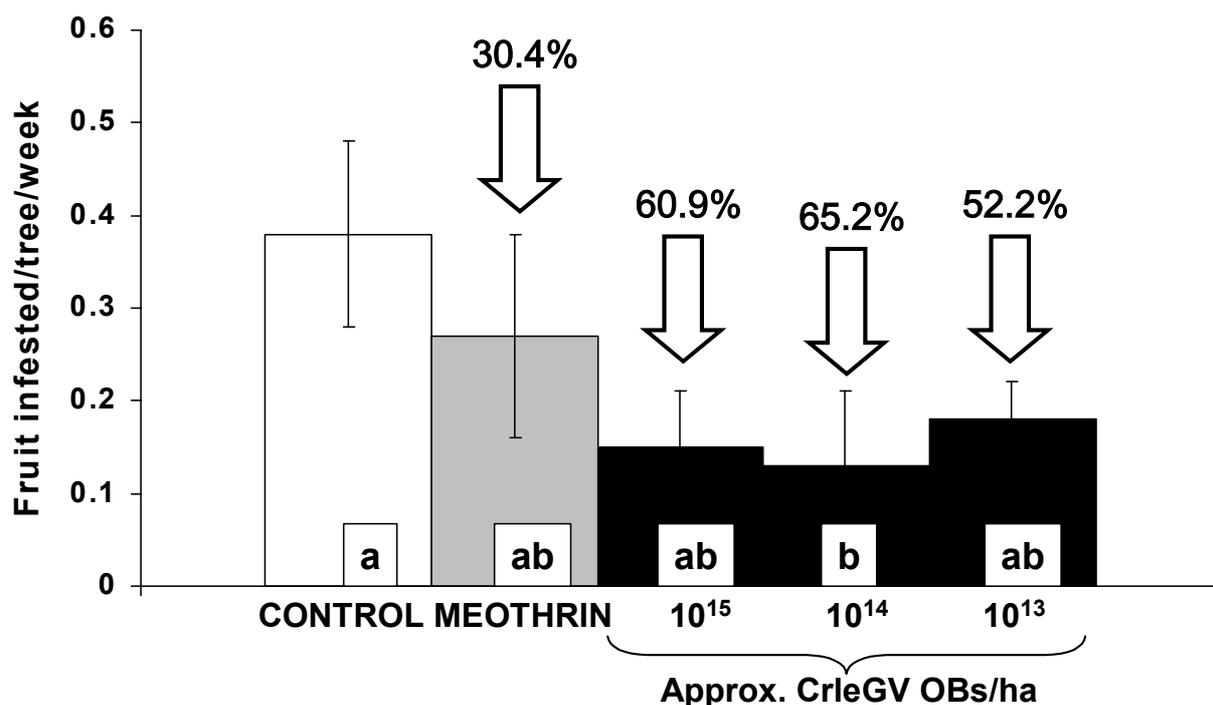


Fig. 3.4.2.11. Mean (and SE) fruit (Robyn navel oranges) drop per tree per week for a period of 3 - 9 weeks after treatments applied for control of FCM at Vergenoeg Boerdery, Eastern Cape Province, on 24 April 2003 (including standard error bars). Bars with the same letter are not significantly different ($P > 0.05$; Bonferroni LSD multiple range test). Arrows indicate percentage reduction in FCM larval infestation of fruit, relative to untreated control trees.

Over the six week monitoring period in the Vergenoeg trial, the best CrleGV treatment reduced FCM infestation by an average of 65% (Fig. 3.4.2.11). The most concentrated CrleGV treatment was not more effective than the second most concentrated treatment (Fig. 3.4.2.11). However, the weakest treatment was notably less effective (Fig. 3.4.2.11), although not significantly so. This, once again, was an indicator of the CrleGV concentration which would be suitable for registration.

As was the case in the previous trial in which Meothrin was included as the chemical standard (Fig. 3.4.2.9), this product was once again the least effective treatment (Fig. 3.4.2.11).

Conclusions

Harvesting of CrleGV with diet was found to be at least as productive, if not more productive, than harvesting of larvae individually. The two methods of harvesting produced averages of 1.105×10^{13} OBs per dish and 4.019×10^{13} OBs per dish, respectively (if 600 larvae were placed onto a dish). Due to the labour saving of harvesting larvae with diet, this method appears very attractive.

If stored at 10°C, no loss of pathogenicity was recorded for three different formulations of CrleGV for 12 weeks. However, if stored at 27°C, CrleGV in liquid suspension appeared to be breaking down by 12 weeks in storage.

Initial bioassays indicated that tank mixes of CrleGV with Agrimec and oil or with Dithane, Benlate and oil, would have no detrimental effect on the virus. Abamectin might even have a controlling effect on FCM larvae.

Four field trials with CrleGV showed consistently good results against FCM on navel oranges. In one trial, FCM infestation was reduced by an average of around 80% up to 10 weeks after application and an average of around 70% up to 16 weeks after application. Molasses seemed to improve the efficacy of CrleGV.

Future research

This trial is ongoing. The shelf-life of various formulations will be examined for up to 18 months. Field trials will be conducted on cultivars other than navels. An application will be submitted for the registration of CrleGV.

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3.4.3 Evaluasie van die granulovirus CrleGV teen valskodlingmot op nawellemoene Proef 169 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

The efficacy of the entomopathogenic virus, CrleGV, was evaluated in an orchard experiment against false codling moth. It suppressed crop loss due to FCM to below the economic threshold. However, the ability of CrleGV to suppress severe FCM infestations is still uncertain and needs to be investigated.

Inleiding

Die proef onder bespreking is deel van 'n reeks wat onder leiding van Sean Moore uitgevoer word om die doeltreffendheid van die CRI-granulovirus CrleGV in verskillende sitrusproduserende gebiede van die land vas te stel.

Materiale en metodes

Uitleg: Die proef is terselfdertyd as proef 733 (Afd. 3.4.13) op die plaas Rivierplaas uitgevoer en die kontrolebome en standaard Alsystem-behandeling was daarom gemeenskaplik. Die tegniek word volledigheidsonthalwe herhaal. Agtienjaarou Palmer-nawelbome is gebruik. 'n Ewekansige blokontwerp met 10 herhalings per behandeling is gebruik. Elke herhaling het uit 'n enkele boom bestaan wat ewekansig in elk van 10 blokke toegeken is. Geen skutbome tussen behandelings is gebruik nie.

Toediening van insekdoders: Alle spuitbehandelings is een keer op die oggend van 5 Maart 2003, as hoë druk, matige dekbespuitings met verstelbare handspuiter, gekoppel aan 'n 2 000 l spuitenk, toegedien. Die lower en vrugte binne en buite elke boom is deeglik tot op die punt van afloop natgespuit. 35 liter spuitmengsel is gemiddeld per boom toegedien. Geen reën het binne 72 uur na toediening geval nie.

Evaluasie: Doeltreffendheid van die verskillende behandelings is geëvalueer deur alle afvalvrugte onder die databome vyf weke lank vanaf vier weke na behandeling tot oestyd bymekaar te maak. Die vrugte is oopgesny en vir simptome van VKM-besmetting ondersoek. 'n Vrug met 'n lewendige of dooie larwe, of larwale uitwerpsels, is as besmet beskou.

Doeltreffendheid: Die vrugval in 'n betrokke behandeling is met die onbehandelde kontrole vergelyk. Daarbenewens moes die totale vrugval in 'n behandeling ook minder as die ekonomiese

drempelwaarde (minder as gemiddeld een besmette vrug per boom per week) wees.

Behandelings: Die konsentrasie van die geformuleerde virusprodukt was $7,106 \times 10^{10}$ GP's/ml. 0,5% molasse is by al drie CrleGV-behandelings gevoeg om die UV-weerstand van die virusformulasie te probeer verhoog.

Die handelings en resultate word in Tabel 3.4.3.1 verstrekk.

Resultate en bespreking

Vrugval weens VKM-besmetting in die kontrole het die ekonomiese drempelwaarde deurgaans gedurende die vyf weke verloop van die proef oorskry (Fig. 3.4.3.1). Die besmetting was egter matig en ernstige oesverliese is nie gelyk nie. Die gemiddelde vrugval in al vier spuitbehandelings was daarenteen beduidend laer as in die kontrole en onder die drempelwaarde (Tabel 3.4.3.1). Vrugval in die CrleGV-behandelings het nie van mekaar of van die Alsystin-behandeling verskil nie.

Tabel 3.4.3.1. Gemiddelde vrugval per behandeling weens valskodlingmot op nawelbome in Citrusdal

Insekdoder	Dosis per hℓ water	Gemiddelde aantal besmette vrugte per boom per week*
Onbehandelde kontrole	-	1,9 a
Alsystin	10 ml	0,8 b
CrleGV + molasse	2 ml + 500 ml	0,9 b
CrleGV + molasse	7 ml + 500 ml	0,8 b
CrleGV + molasse	16 ml + 500 ml	0,9 b

*Variansie-ontleding is uitgevoer op die data en die behandelinggemiddeldes is met Student se t-KBV vergelyk (5%). Data gevolg deur dieselfde letter verskil nie betekenisvol van mekaar nie.

Indien die vrugvalpatroon in Fig. 3.4.3.1 beskou word, is dit duidelik dat CrleGV waarskynlik nie, te oordeel aan dié een proef, besonder goed teen 'n hewige VKM-besmetting sal vaar nie. Alhoewel vrugval in die spuitbehandelings altyd laer as in die kontrole was, het dit dieselfde patroon gevolg en die drempelwaarde inderdaad oorskry toe die besmetting, relatief gesproke, die hewigste was. Dit beteken dat die onderdrukking deur CrleGV moontlik heelwat swakker sou gewees het indien die besmetting hewiger was.

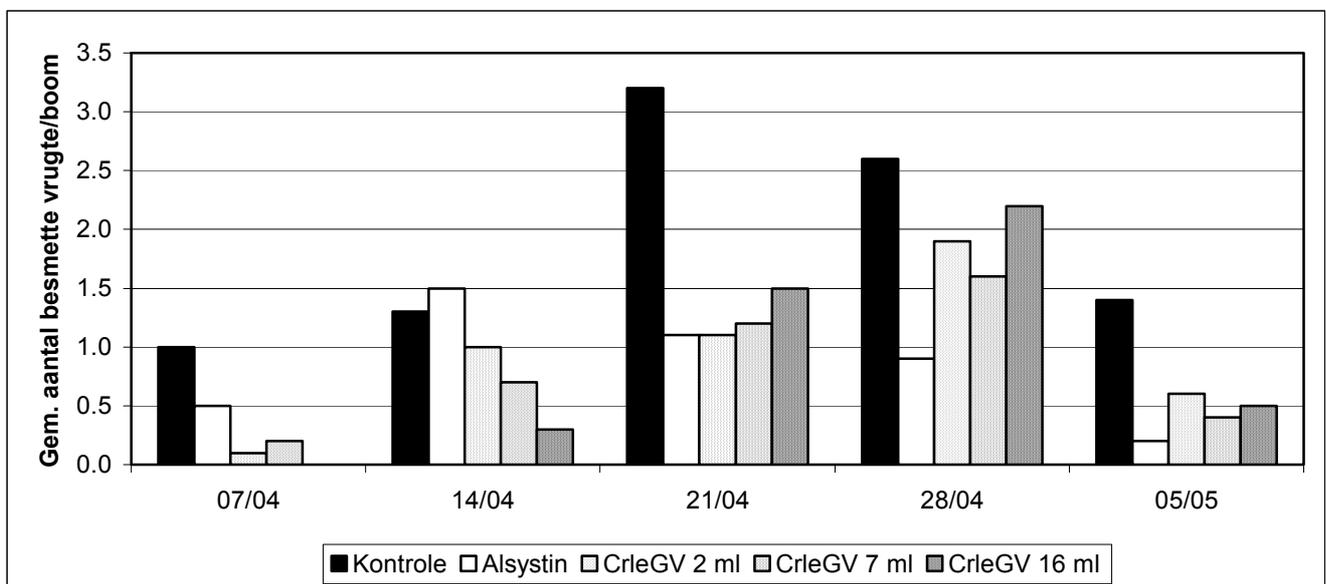


Fig. 3.4.3.1. Vrugvalpatroon weens valskodlingmotbesmetting in 'n boordproef met CrleGV op nawelbome in Citrusdal gedurende 2003.

Insekbstryding met "tradisioneel" geformuleerde virusprodukte word gewoonlik deur die uitklop van die betrokke plaag kort na toediening gekenmerk. Daar word aangevoer dat die virusse baie vatbaar vir UV-bestraling is en dus gou afbreek. Sulke produkte het derhalwe geen nablywende werking van betekenis nie. Daarenteen lyk dit egter asof CrleGV-residu's wel nablywend aktief is. Dit is opsigself belowend en, indien UV-bestraling 'n rol speel, kan die beskerming van die produk met 'n UV-beskerende bymiddel moontlik 'n groot bedrae tot die algemene doeltreffendheid van CrleGV maak.

Gevolgtrekking

Die ontwikkeling van CrleGV vir die bestryding van VKM, in kombinasie met verskillende UV-beskerende bymiddels, behoort voortgesit te word.

Literatuur

Introduction to the analysis of quantal response. Agricultural Research Council Agrimetrics Institute: Pretoria.

3.4.4 Ontwikkeling van die Lorelei II-feromoonvrysteller vir kommersiële gebruik in lokvalle Proef 573 deur Hendrik en Marsheille Hofmeyr

Summary

Experiments were conducted with a new pheromone dispenser to replace the Lorelei dispenser currently being used by the citrus industry on a commercial scale to monitor false codling moth. The new dispenser body is adequate and is a big improvement on the older type with regard to cost and ease of manufacture. The dispenser septum, however, proved to be difficult to activate and little progress was made. The investigations continues.

Inleiding

Die Lorelei-feromoonvrysteller is die eerste konstante tempo vrysteller, met 'n lang nabywende werking, wat wêreldwyd vir gebruik in lokvalle geskikbaar is. Dit word alreeds sewe jaar lank deur die navorsingsdepartement (huidiglik CRI) vervaardig en aan produsente in suider-Afrika beskikbaar gestel. Dit is egter moeilik en tydrowend om te vervaardig.

Scentry (VSA) het etlike seisoene gelede 'n nuwe feromoonvrysteller vir ontwikkeling beskikbaar gestel. Die vrysteller is veel makliker en goedkoper om te vervaardig as die Lorelei-vrysteller. Geen vordering kon egter met die toetsing daarvan gemaak word nie omdat die vervaardigers nie sekere tegniese probleme kon oplos nie. Daar is dus besluit om 'n soortgelyke vrysteller in samewerking met Quest Ontwikkelings te ontwerp.

'n Matrys vir die vervaardiging van die vrystellers is vervaardig en getoets. Prototipe vrystellers is vervolgens deur Quest vervaardig. Die vrysteller bestaan uit 'n ondeurlaatbare plastiekferomoonhouer en 'n deurlaatbare septum, wat vir die vrystelling van die feromoon verantwoordelik is. Die septum moet feromoon teen 'n voorafbepaalde tempo kan vrystel. Die deurlaatbaarheid van die septum word deur die materiaal waarvan dit vervaardig is, asook die grootte van die oppervlakte daarvan wat aan die lug blootgestel is, bepaal. Ondersoeke om die regte tipe septum te vind, is uitgevoer.

Materiale en metodes

Die vrystellingstempo van die Lorelei-vrysteller wissel gewoonlik van 1,0 tot 2,5 mg per week. Daar is gepoog om 'n septum te ontwikkel wat nagenoeg dieselfde vrystellingstempo het. Verskillende septa is óf deur Quest Ontwikkelings verskaf óf is uit eie voorraad in drie proewe gebruik. Drie vrystellers is per behandeling gebruik. Elke vrysteller is met 50 mg feromoon gevul en in die laboratorium aan 'n pasgemaakte rak opgehang. Maksimum temperature in die vertrek het gedurende die verloop van die verskillende proewe van 20 tot 33°C gewissel.

Die vrystellers is weekliks met behulp van 'n analitiese weegskaal geweeg en die gemiddelde massaverlies per vrysteller is bereken. Die resultate word in Fig'e. 3.4.4.1-3 verstrekk.

Resultate en bespreking

In proef 1 is twee verskillende feromoonvrystellers, asook twee verskillende septa, vergelyk. Die vrystellers was van poliëtileen- of polipropileenplastiek vervaardig en is spesiaal behandel om hulle ondeurlaatbaar te maak. Die tipe plastiek het geen invloed op die vrystellingstempo gehad nie (Fig. 3.4.4.1). Geeneen van die septa kon die vereiste vrystellingstempo handhaaf nie. Die Lorelei-vrystellers het feromoon aanvanklik heelwat vinniger as die verlangde tempo vrygestel. Die tempo het egter vinnig afgeneem en by nagenoeg 3 mg per week gestabiliseer. Die septa in proef 1 se vrystellingstempo was aanvanklik hoog, maar het in die tweede week tot 1 mg afgeneem en daarna voortdurend verder verminder. In proewe 2 (Fig. 3.4.4.2) en 3 (Fig. 3.4.4.3) was die vrystellingstempo deurgaans ongeveer 0,5 mg per week, wat baie minder as die verlangde tempo is.

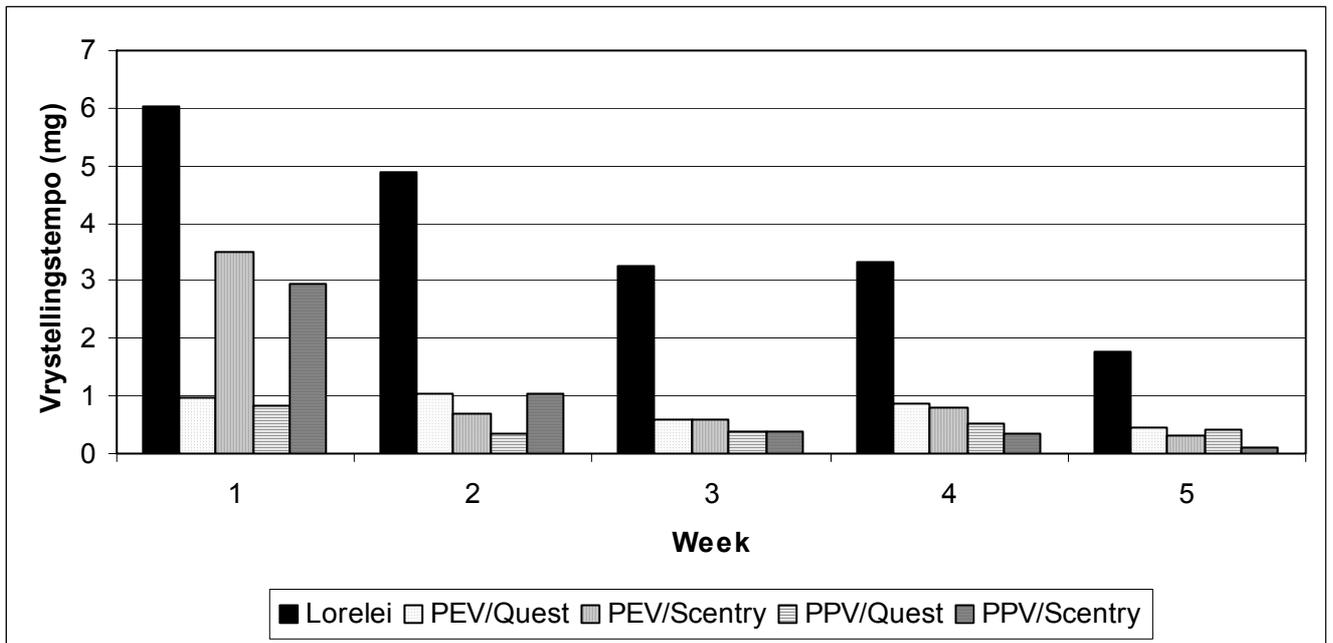


Fig. 3.4.4.1. Vrystellingstempo van twee verskillende septa. 'n Rubberseptum van Scentry en 'n plastiekseptum van Quest is vergelyk (PEV = poliëtileenvrysteller; PPV = polipropileenvrysteller).

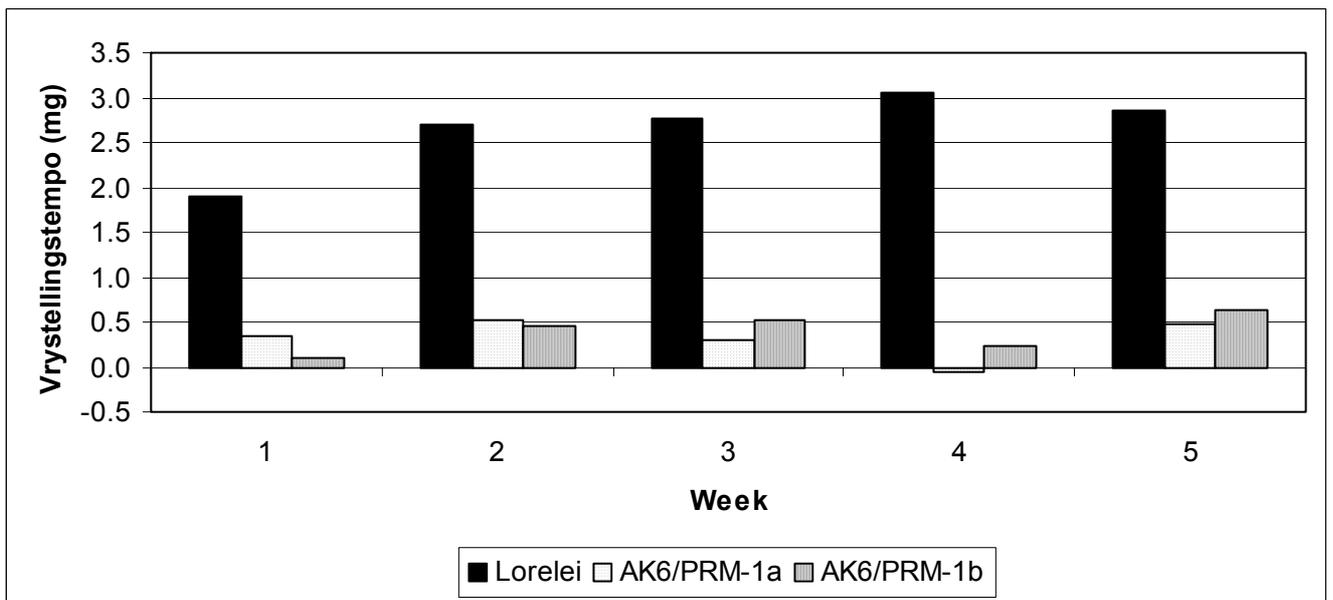


Fig. 3.4.4.2. Vrystellingstempo van twee verskillende septa uit poliëtileenvrystellers. Twee plastiekseptu van verskillende samestelling is vergelyk.

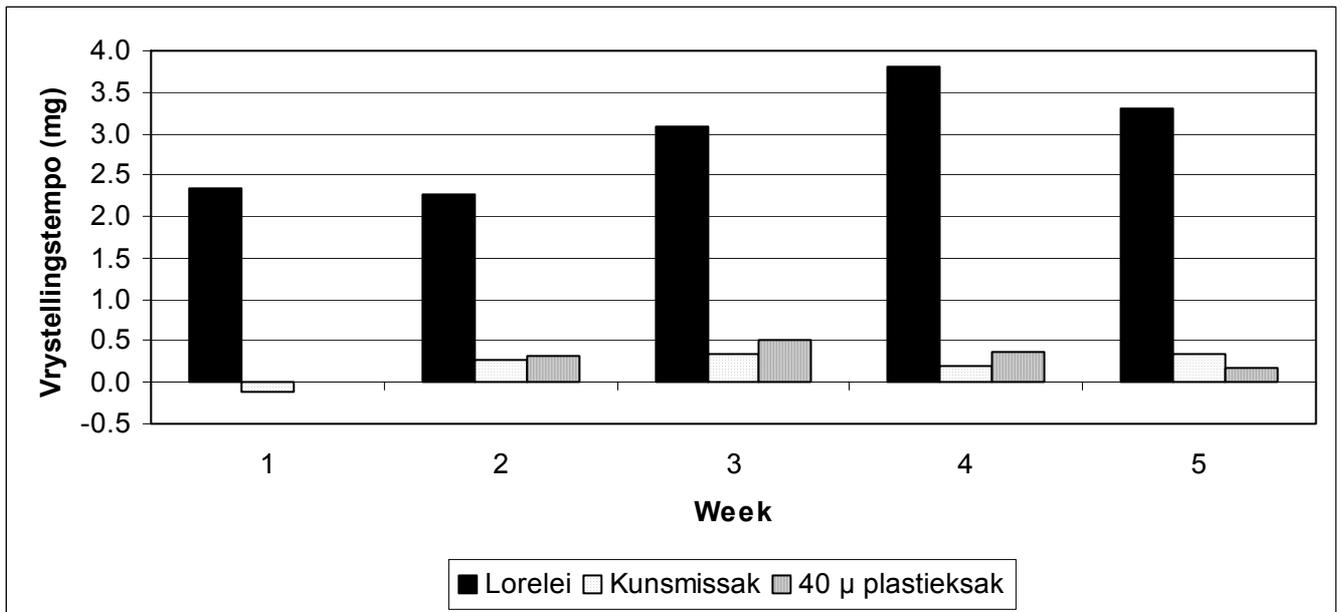


Fig. 3.4.4.3. Vrystellingstempo van twee verskillende septa uit poliëtienvrystellers. Twee septa van poliëtielenplastiek is vergelyk.

Geeneen van die getoetste septa kon feromoon teen die verlangde tempo van 1-2,5 mg per week vrystel nie. Selfs die dunste poliëtielenplastiek wat in die vrysteller gebruik kon word sonder om ander tegniese probleme te skep, het misluk. Omdat die plastiek dun genoeg is om feromoon deur te laat, moet die oorsaak van die probleem dus aan 'n ander faktor te wyte wees. Dit kan moontlik aan die blootstellingsoppervlakte wat vir feromoonvrystelling verantwoordelik is, toegeskryf word. In vergelyking met die totale oppervlakte van die Lorelei-vrysteller se poliëtielenpypie (wat die feromoon vrystel) is die Lorelei II se septum-oppervlakte baie kleiner. Dit is dus waarskynlik dié eienskap wat die probleem veroorsaak.

Gevolgtrekking

Dit is van belang om 'n verbeterde feromoonvrysteller te vervaardig. Die soektog na 'n geskikte septum sal daarom voortgesit word.

3.4.5 Evaluasie van die paringsontwrigter Isomate vir die kommersiële bestryding van valskodlingmot op nawelbome

Proef 636 deur Hendrik en Marsheille Hofmeyr

Summary

The mating disruption product, Isomate, was evaluated against false codling moth for the third successive year. A double application of Isomate in November and February suppressed FCM commercially to below the economic threshold. Infestations in the experimental site were light to moderate and the ability of Isomate to suppress severe FCM infestations is still unknown.

Inleiding

Drie proewe is gedurende 2001 en 2002 in Citrusdal uitgevoer om die doeltreffendheid van Isomate vir die paringsontwrigting van VKM op sitrus te ondersoek. Die VKM-besmetting in die 2001-proef, asook 'n daaropvolgende proef in dieselfde perseel gedurende 2002 uitgevoer, was lig en min bruikbare inligting is ingewin. Data van 'n tweede proef in 2002 in 'n ander perseel was wel bruikbaar en het gewys dat 'n dubbele toediening van Isomate in staat was om oesskade weens VKM tot minder as die ekonomiese drempelwaarde (minder as een vrug per boom week gemiddeld) te beperk. Resultate van 'n proef wat terselfdertyd in aangrensende boorde met die lok&vrekprodukt, Last Call, uitgevoer was, was minderwaardig.

'n Kontrakproef is gedurende 2003 uitgevoer om die ondersoek na die potensiaal van Isomate om kommersieel skadelike besmettings van VKM hok te slaan, verder te ondersoek.

Materiale en metodes

Perseel en uitleg: Dieselfde perseel as die suksesvolle 2002 Isomate-proef op die plaas Hexrivier Landgoed, Citrusdal, is weer gebruik. Die perseel het uit 12 boorde met volwasse lemoenbome bestaan wat in 'n 6 x 2 patroon gerangskik was. Die eerste 9 boorde (4 x 2 + 1) was Washington-nawelbome, terwyl die origes Valencias was (Fig. 3.4.5.1).

Die proefuitleg was dieselfde as die vorige seisoen (bespreek in die CRI Jaarverslag vir 2002), behalwe dat Isomate ook in die boorde wat voorheen met Last Call behandel was, toegedien is (boorde met datapunte S1 tot S5 = Blok A). Daarby is Isomate weer op dieselfde bome as voorheen toegedien (boorde met datapunte S7 tot S12 = Blok B). Blokke A en B is van mekaar geskei deur twee boorde onbehandelde kontrolebome (boorde met datapunte K1 tot K4). Een datapunt is ook in elk van die laaste twee Valenciaboorde geplaas (K5 en K6).

S1 N	S3 N	S4 N	K2 N	K3 N	S7 N	S8 N	S10 N	S11 N	K5 V
S2 N	S5 N	S5 N	K1 N	K4 N	S6 N	S9 N	S12 V	S12 V	K6 V

Fig. 3.4.5.1. Diagrammatiese voorstelling van die proefperseel te Hexrivier Landgoed, Citrusdal (S = Isomate, K = Kontrole; 1, 2, 3, ens. = Datapunte; N = Nawelboord, V = Valenciaboord).

Meer besonderhede word in Tabel 3.4.5.1 verstrek.

Tabel 3.4.5.1. Besonderhede van die proef met Isomate by Hexrivier Landgoed, Citrusdal gedurende 2003.

Eienskap	Perseel
Bome per ha (plantafstand)	444 (6,6 m x 3,4 m)
Oppervlakte van behandelde gebied (ha)	Blok A: 9,52 ha Blok B: 10,81 ha
Lokvaltellings begin	24 Oktober 2002
Eerste Isomate-toediening	13 November 2002
Vrugvalopname begin	18 Desember 2002
Tweede Isomate-toediening	5 Februarie 2003

Tegniek

Toediening van feromoonvrystellers: Isomate is twee keer toegedien. Die eerste keer is een vrysteller aan elke boom opgehang, dus 'n minimum van 444 vrystellers per ha. 'n Tweede vrysteller is aan alle buitenste bome in elk van die 8 behandelde boorde opgehang. Die tweede keer, 12 weke later, is een vrysteller aan elke tweede boom gehang; die aantal vrystellers per ha is dus gehalveer. Die buitenste bome het nie weer bykomende vrystellers gekry het nie.

Die vrystellers is met spesiale toedieners in elke boom aan 'n geskikte tak in die boomtop gehang sodat dit in gedeeltelike skaduwee gehang het, maar nie só diep in die boom in dat vrystelling van die feromondamp deur te min lugbeweging verhinder sou word nie.

Evaluasie van doeltreffendheid: 'n Aantal datapunte, elk bestaande uit 'n VKM-lokval en vyf databome, is gebruik om behandelingsdoeltreffendheid te bepaal.

- **Motaktiwiteit:** Motaktiwiteit is met behulp van standaard Lorelei® VKM-lokvalle ondersoek. Lokvalle is in die kontrole- en behandelde boorde versprei, naamlik 6 en 12 lokvalle onderskeidelik. Lokvaltellings is een keer week vanaf drie weke voor toediening van die vrystellers, tot oestyd in Mei uitgevoer.
- **Vrugval:** Vyf aangrensende bome met 'n verteenwoordigende oes is by elke lokval gebruik.

Vrugvalopnames is nie in die 3 Valenciaboorde uitgevoer nie, alhoewel motaktiwiteit met lokvalle nagegaan was (Fig. 3.4.5.1; datapersele S12, K5 en K6).

Oesverliese as gevolg van VKM-besmetting is geëvalueer deur alle afvalvrugte onder die databome een keer per week vanaf vier weke na toediening van die vrystellers tot oestyd bymekaar te maak. Die vrugte is oopgesny en vir simptome van besmetting ondersoek. 'n Vrug met 'n lewendige of dooie larwe, of larwe-uitwerpsels, is as VKM-besmet beskou. Vrugvaltellings het vier weke na toediening van die vrystellers begin en tot oestyd geduur.

- **Feromoonvrystellingstempo:** Die vrystellingstempo uit die Isomate-vrystellers is op twee maniere bepaal. Eerstens is vyf Isomate vrystellers op kophoogte in 'n boom aan 'n spesiale rak opgehang. Die rak is een keer per maand verwyder en die vrystellers is in die laboratorium individueel op 'n analitiese weegskaal geweeg om die massaverlies weens feromoonvrystelling te bepaal. Tweedens is bondeltjies vrystellers aan die begin van die proef in die boonste derde van etlike bome in die proefperseel opgehang. Elke bondeltjie het uit vyf vrystellers bestaan. Een bondeltjie is elke week tot oestyd verwyder, in aluminiumfoelie toegedraai en in 'n yskas by 2°C bewaar. Die vrystellers is met afsluiting van die proef aan die verskaffer vir ontleding oorhandig.

Resultate en bespreking

Lokvalvangste: Motvangste in die kontroles en die boorde wat nog behandel sou word, was voor die toediening van Isomate baie dieselfde (Fig. 3.4.5.2). Motgetalle in die kontrolelokvalle het skerp afgeneem na toediening van die Isomate in die aangrensende boorde, maar het telkens 'n paar weke later toegeneem. Die kontroleboorde was aan twee kante deur Isomate-behandelde boorde begrens, met bosse aan die ander kante. Dit kom voor asof die mannetjies in die kontroleboorde deur die Isomate in die aangrensende boorde beïnvloed was. Dit is onbekend of die afname in vangste te wyte is aan die mannetjies wat uit die kontroleboorde weggelok is en of hulle deels deur die Isomate onderdruk was.

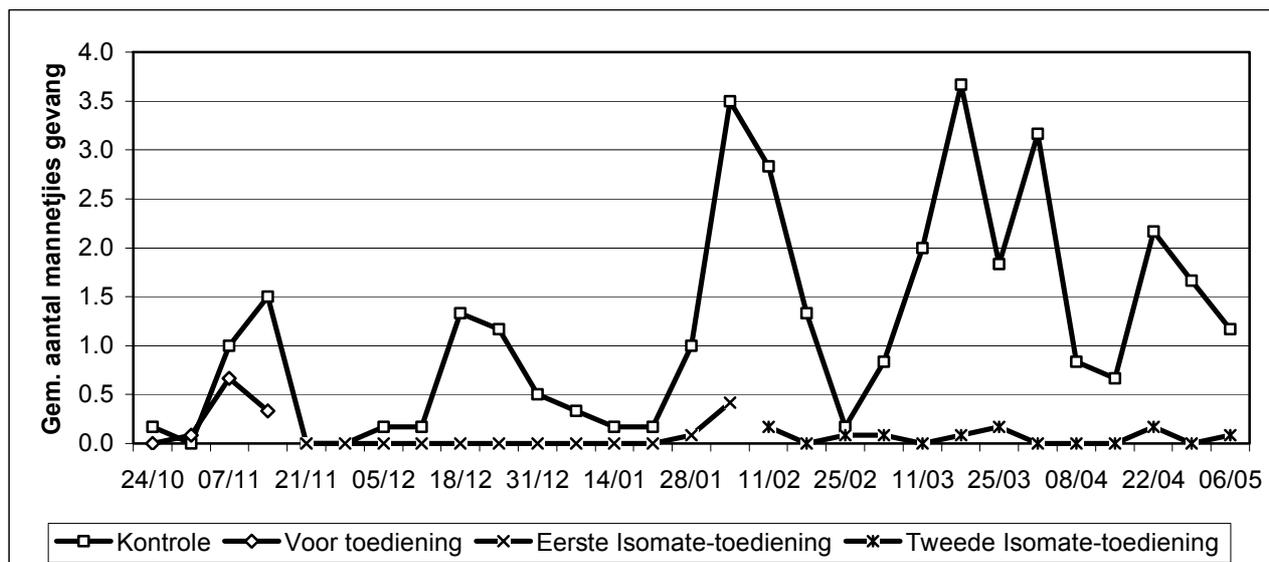


Fig. 3.4.5.2. Gemiddelde aantal valskodlingmotmannetjies in Isomate-behandelde boorde gevang by Hexrivier-landgoed, Citrusdal, in 2003. Isomate is op 13 November 2002 en 5 Februarie 2003 toegedien.

Die Isomate-behandelings het lokvalvangste gedurende die verloop van die hele proef tot 'n gemiddeld van 0,06 mannetjies per lokval per week (16 mannetjies is in 12 lokvalle in 21 weke gevang) verminder, in vergelyking met 1,5 mannetjies in die kontroleboorde (185 mannetjies in 6 lokvalle in 21 weke). Dit is baie klein getalle en, veral in die onbehandelde kontroleboorde, ver onder die lokvaldrempelwaarde vir ekonomiese skade. Die grootste vangs in die kontrolelokvalle was trouens 10 mannetjies in een lokval in een week – die ander kontrolelokvalle het elk deurentyd minder as 10 mannetjies per week gevang. Motvangste in die Isomate-behandelde boorde het effens toegeneem kort voordat Isomate vir die tweede keer toegedien is. Dit was waarskynlik aan feromoonuitputting in die eerste reeks vrystellers te wyte, wat aangehelp is deur groter motaktiwiteit in die proefperseel, soos blyk uit die skerp toename in motvangste in die kontrolelokvalle.

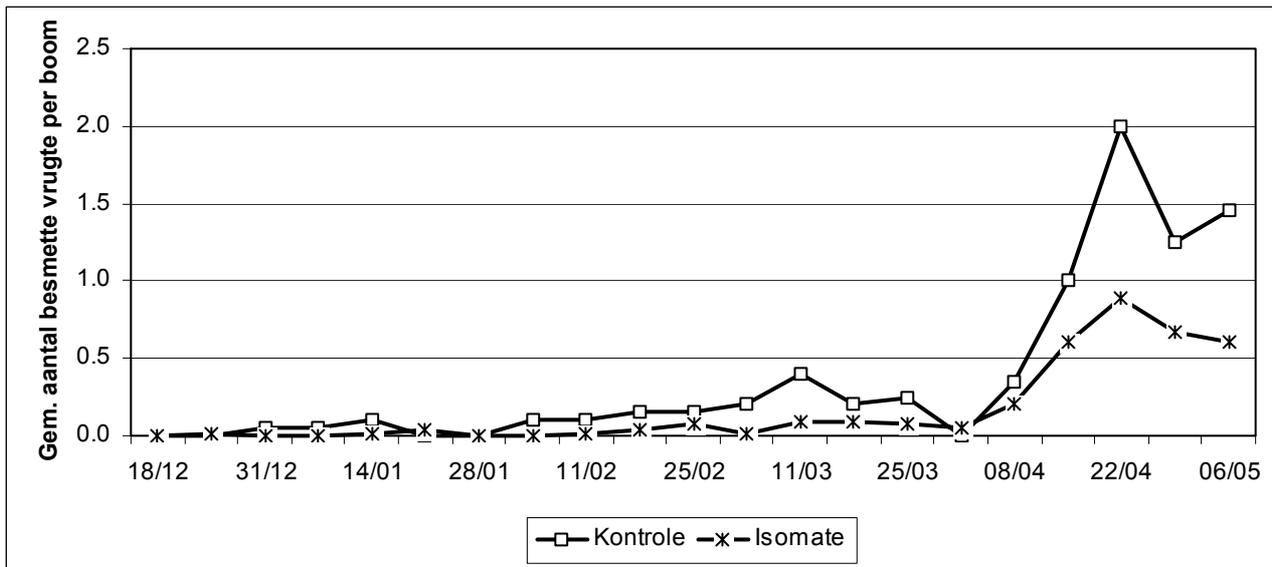


Fig. 3.4.5.3. Gemiddelde aantal vrugte deur valskodlingmot besmet in Isomate-behandelde boorde by Hexrivier Landgoed, Citrusdal, in 2003. Isomate is op 13 November 2002 en 5 Februarie 2003 toegedien.

Ten spyte van die lae vangste stem die lokval- en vrugvalpatroon in die kontroleboorde redelik ooreen in dié opsig dat die vrugval wat onderskeidelik op 11 Maart en 22 April 'n hoogtepunt bereik het (Fig. 3.4.5.3) deur groter motaktiwiteit nagenoeg vier weke vroeër voorafgegaan is.

Vrugval: Vrugval weens VKM-besmetting was baie lig gedurende die hele seisoen - soos deur die lokvalvangste voorspel is. Die ekonomiese vrugvaldrempelwaarde (gemiddeld 1 vrug per boom per week) is slegs kort voor oestyd in die kontroleboorde oorskry, terwyl dit in die Isomate-behandelde boorde tot onder die drempelwaarde beperk is.

Feromoonvrystellingstempo: Weeklikse meting van die vrystellingstempo was weens die afstand van die proefboorde nie moontlik nie. Dit bemoelijk enige gevolgtrekkings oor die vrystellingstempo van die Isomate-vrystellers wat gehandhaaf moet word om bevredigende motonderdrukking te verkry. 'n Paar meer mannetjies is in die tweede helfte van die proef gevang as in die eerste helfte. Alhoewel die relatiewe verskil baie klein is, kan dit egter daarop dui dat die halvering van die aantal Isomate-vrystellers wat die tweede keer toegedien is, moontlik met hewiger VKM-besmettings te min vir bevredigende motonderdrukking kan wees, al word daar nog feromoon deur die eerste reeks vrystellers afgeskei (Fig. 3.4.5.4). Daar moet onthou word dat feromoonafskeding deur die vrystellers daardie tyd van die jaar sal afneem as gevolg van die naderende winter en gevolglike laer temperature. Dit kan dus wys wees om nie die aantal vrystellers wat toegedien moet word, te halveer nie, maar die volle getal te gebruik – veral indien maksimum paringsontwrigting in die paar maande kort voor oestyd verlang word.

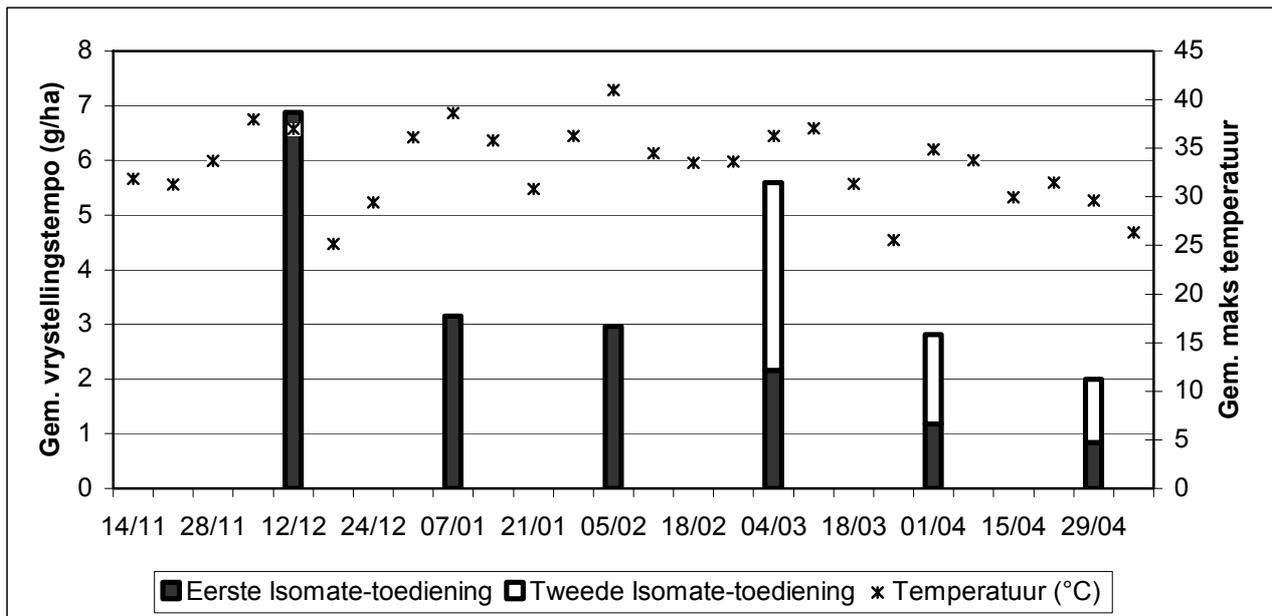


Fig. 3.4.5.4. Vrystellingstempo van Isomate-feromoonvrystellers twee keer toegedien by Hexrivier Landgoed, Citrusdal, in 2003. Feromoonvrystelling is een keer per maand gemeet; die data verteenwoordig die berekende gemiddelde vrystellingstempo per week per ha.

In die geval van die geregistreerde paringsontwrigter Quant, is voorheen aanvaar dat 'n minimum van 4-6 g feromoon per week per ha vrygestel moet word om VKM bevredigend te onderdruk. Soos in die 2002 Isomate-proewe het lokvalvangste egter nie toegeneem toe die Isomate-vrystellingstempo onder dié vlak gedaal het nie. Dit kan die voordeel van 'n produk wees waarvan die feromoon nader verwant aan die natuurlike VKM-feromoon is, naamlik 'n oormaat (E)-8-dodekanielasetaat, in verhouding met (Z)-8-dodekanielasetaat, in plaas van soos by Quant, wat meer (Z)-8-dodekanielasetaat as (E)-8-dodekanielasetaat bevat.

Die resultate was baie dieselfde as dié wat in dieselfde perseel in 2002 ingesamel was. Al vier proewe wat in drie jaar se tyd in Citrusdal uitgevoer was, het onder besmettings gebuk gegaan wat lig gedurende die seisoen was, maar kort voor oestyd tot matig vermeerder het. Dié neiging het tipies vir VKM in die Wes-Kaap gedurende die afgelope vier tot vyf jaar geword. Dit is nog steeds onseker of dit 'n natuurlike verskynsel is, of die gevolg van beter boordsanitasie deur sitrusprodusente sedert 2000. Die eierparasitoïed *Trichogrammatoidea cryptophlebiae* was ook aktiewer as gewoonlik, wat ook 'n voordelige uitwerking kon gehad het. Die uiteinde van die ondersoek is dat Isomate nog steeds nie onder hewige besmettingstoestande getoets kon word nie en die doeltreffendheid daarvan onder dié toestande nog steeds onbekend is. Die huidige besmettingspatroon blyk egter ten minste vir die kort termyn gevestig te wees en Isomate sal dus onder sulke omstandighede gebruik word. Geen resultate is ingesamel wat daarop dui dat Isomate nie in staat sal wees om kommersieel skadelike VKM-besmettings te onderdruk nie.

Gevolgtrekking

Geen verder navorsing word beoog tensy dit deur die verskaffers verlang word nie. Laasgenoemde beoog om 'n finale besluit oor die moontlik registrasie van Isomate te neem na afloop van 'n verdere seisoen se toetsing teen VKM in ander gebiede.

3.4.6 Development of semiochemical odorants for the attraction and repellence of false codling moth in citrus

Proef 648 deur Christo Smit (Desense Pest Control)

Nota deur die Projekkoördineerder

'n Deel van die navorsing wat vroeg in die huidige verslagtydperk uitgevoer was, is saam met die 2002-navorsing in die Jaarverslag vir 2002 vervat. Dit is gedoen om onnodig fragmentasie van die inligting te voorkom. Die betrokke navorsing het op daardie tydstip tot op 'n stadium gevorder dat dit duidelik was dat fasiliteite en toerusting, wat onder onbeheerbare omgewingstoestande gebruik moes word, ontoereikend geword. Proewe kon nie onder dieselfde toestande uitgevoer word nie en herhaalbare resultate kon dus nie verkry word nie. Dit het daarom noodsaaklik geword om 'n temperatuurbeheerde vertrek, toegerus met

olfaktometers, te bou. Dié infrastruktuur is gedurende 2003 geskep. Daarbenewens is 'n groot aantal verbindings in potensieel belangrike chemiese groepe van oorsee vir evaluasie ingevoer. Die navorsing is gedurende die einde van 2003 hervat en die resultate sal in die Jaarverslag vir 2004 ingesluit word.

Note by the Project Co-ordinator

A part of the research conducted early on during the current report period, word incorporated into the Annual Report for 2002. This was done to avoid unnecessary fragmentation of the information. The research had also developed to such an extent that it was clear that the facilities and equipment, which were used under uncontrollable ambient conditions, were insufficient. Experiments could not be repeated under similar conditions and it was therefore very difficult to achieve consistent, repeatable results. It was decided to build a temperature controlled room, fitted with olfactometers. This infrastructure was created during 2003. A large number of compounds in potentially important chemical groups were also imported for evaluation. The research was resumed at the end of 2003 and the results will be included in the annual Report for 2004.

3.4.7 Evaluasie van Elektrostatische Poeiertegnologie vir die bestryding van valskodlingmot Proef 660 deur Hendrik en Marsheille Hofmeyr (CRI)

Opsomming

Swak resultate is in navorsing deur 'n plaaslike agent vir die elektrostatische poeiertegnologie, verkry. Soortgelyke swak resultate is ook voorheen verkry in navorsing deur Hofmeyr en Hofmeyr (Jaarverslag vir 1998). Weens beperkte tyd is daar besluit om dié navorsing op te skort.

Summary

Poor results were obtained during 2003 in initial research by a local agent for electrostatic powder technology. This finding was preceded by equally poor results in research by Hofmeyr and Hofmeyr (Annual Report for 1998). Due to time constraints it was therefore decided to postpone research for the time being.

3.4.8A Bestryding van valskodlingmot met steriele insekloslatings: A. Eierlegging deur bevrugte en onbevrugte valskodlingmotwyfies Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

The egg-laying capabilities of virgin and mated FCM female moths were investigated in laboratory experiments. Virgin females deposited up to 300 sterile eggs during an average life-span of 9 days. Approximately 23% of the females did not lay any eggs. Mated females laid up to 600 viable eggs over 5 days. The results will be used to enhance decision making during the planning stages of SIT experiments.

Inleiding

Dit is wenslik om te weet hoeveel eiers deur VKM-wyfies gelê kan word. Navorsing is byvoorbeeld uitgevoer om vas te stel hoe geskik VKM-eiers wat deur gammabestraalde VKM gelê word, vir die eierparasitoïed *Trichogrammatoidea cryptophlebiae*, is (Bloem *et al.*, 2004). In dié proewe was dit nodig om by benadering te weet hoeveel eiers deur 'n bevrugte wyfiemot gelê kan word sodat die aantal eierparasitoïede wat in gedwonge/ongedwonge keuseproewe gebruik moes word, bereken kon word. Dié proewe het laasgenoemde ondersoeke voorafgegaan. Dit is eweneens belangrik om te weet of en indien wel hoeveel, eiers deur onbevrugte wyfies gelê kan word. Dié faktor moet in berekening gebring word wanneer die invloed van gammabestraling byvoorbeeld op die eierlêvermoë van die wyfies ondersoek word.

Materiale en metodes

Twee proewe is in die laboratorium met insektariumgeteelde VKM uitgevoer. In kommersiële VKM-insektaria word VKM in omgekeerde huishoudelike meelsiwwe op waspapier geplaas sodat eierlegging op die papier kan plaasvind. 'n Soortgelyke tegniek is in dié proewe gevolg, behalwe dat koepelvormige, bodemlose hokkies van vleklosestaalgaas, 50 mm in deursnee, op waspapier gebruik is. In proef A1 is 'n maagdelike wyfie in elk van 30 hokkies geplaas. Die waspapier is ses dae later met nuwes vervang en toe ongesteurd gelaat totdat alle motte gevrek het. Nat watterpluisies op elke hokkie het as waterbron vir die wyfies gedien. In proef A2 is een paar VKM twee dae lank onder elk van 10 hokkies geplaas, waarna die waspapier vervang is en vir 'n verdere drie dae ongesteurd gelaat is. Alle eiers op die waspapiervelle is na afloop van die proewe getel. Die wyfies is gedissekteer en vir die₁₀₁aanwesigheid van spermatofore in die *bursa*

copulatrix ondersoek om te bevestig dat paring plaasgevind het. Die proewe is by 26°C uitgevoer.

Resultate en bespreking

Proef A1 (Onbevrugte VKM-wyfies): Onbevrugte VKM-wyfies kan eiers lê (Fig. 3.4.8.1). Die wyfies het gemiddeld 9 dae lank gelewe, alhoewel enkele tot 20 dae oud geword het. Van die 30 wyfies wat ondersoek is, het 7 (23.3%) geen eiers gelê nie, 17 (56,7%) het tot 50 eiers gelê en 6 (20%) het meer as 50 eiers (maks. = 292) gelê. Heelwat meer eiers is gedurende die tweede deel van die proef gelê as gedurende die eerste ses aande. Nie een eier het uitgebroei nie.

Die resultaat beteken dat die verskynsel van onbevrugte wyfies wat steriele eiers lê sonder dat gammabestraling betrokke is, by SIT-navorsing in aanmerking geneem moet word.

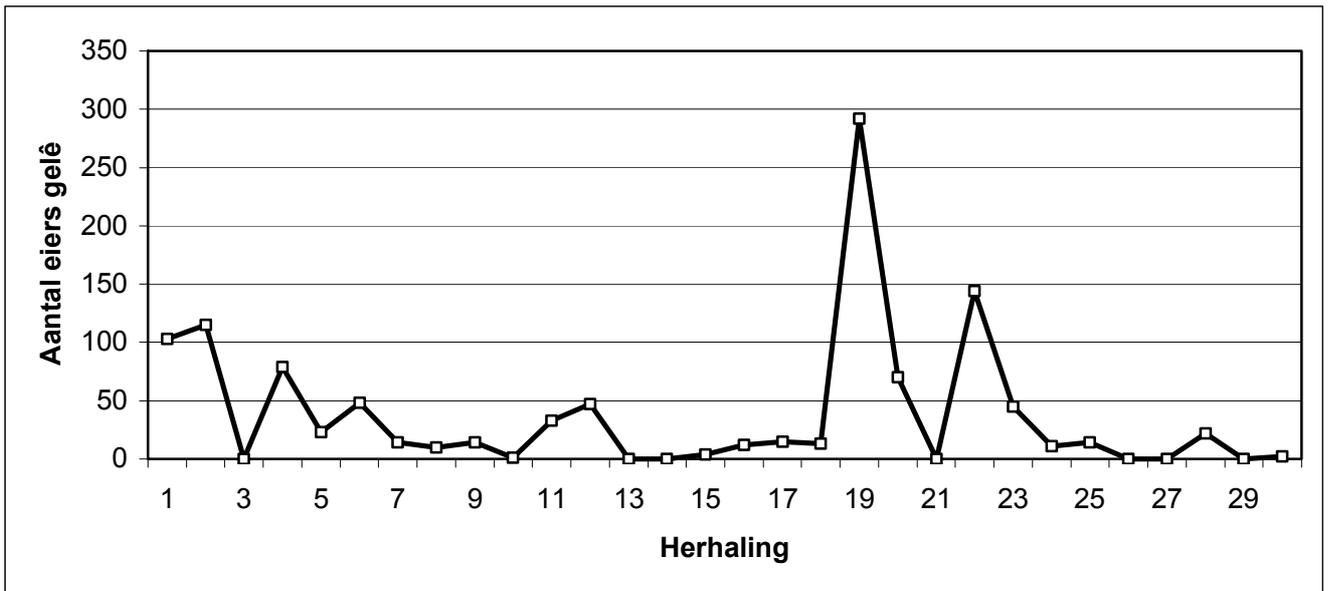


Fig. 3.4.8.1. Aantal eiers wat onder laboratoriumtoestande deur onbevrugte valskodlingmotwyfies gelê kan word.

Proef A2 (Bevrugte VKM-wyfies): Spermatofore is in al die wyfies gevind, wat daarop dui dat hulle gepaar het. Nege van die 10 bevrugte wyfies het meer as 400 eiers in 5 aande gelê (die meeste het meer as 500 eiers gelê) (Fig. 3.4.8.2), terwyl 1 wyfie slegs 84 eiers gelê het. Die totale getal eiers sou dus heelwat meer gewees het indien hulle onbepaalde tyd gegee is (in Proef E hierna het sommige wyfies meer as 1 000 eiers tydens hulle lewensduur van 10-14 dae gelê). Die meeste van die eiers is gedurende aande 3 tot 5 gelê.



Fig. 3.4.8.2. Aantal eiers wat onder laboratoriumtoestande deur bevrugte valskodlingmotwyfies gelê kan word.

Bogenoemde ondersoek kan heelwat uitgebrei word om die eierlegging van wyfies onder verskillende omstandighede te ondersoek. Die inligting wat ingewin is, is egter tydelik voldoende om ingeligte besluite te neem en sal deur waarnemings in ander proewe gerugsteun word.

3.4.8B Bestryding van valskodlingmot met steriele insekloslatings: B. Invloed van bestraling op onvolwasse valskodlingmotpapiës

Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

Three day old pupae were irradiated to study the potentially detrimental influence of 100 Gy and 250 Gy of gamma radiation on their sustained development. The rate of development was not affected, but the number of eclosed moths was severely reduced. The importance of only irradiating pharate pupae if exploited in a Sterile Insect Release Programme, is emphasized.

Inleiding

Die resultate van twee ondersoeke waarin die bestralingsbiologie en oorgeërfde steriliteit van VKM-papiës en motte ondersoek is, is breedvoerig in die CRI-jaarverslag vir 2002 bespreek. Etlke proewe is uitgevoer om meer inligting oor verskeie aspekte te kry wat belangrik sal word namate SIT-navorsing vorder en die tyd aanbreek wanneer die tegniek in 'n loodsprojek toegepas moet word. Die proef onder bespreking het as 'n voorloper gedien om die bestralingstegniek vir VKM-papiës te ondersoek met die oog op die uitgebreide ondersoek wat sou volg. Dit is nie in die vorige jaarverslag bespreek nie, maar verdien vermelding aangesien die resultate later van tyd van belang kan wees.

Lepidoptera soos Kodlingmot of VKM kan vir SIL-doeleindes in die papie- of motstadium bestraal word. Motte is minder sensitief vir bestraling as papies, wat voordelig is. Mottemoet egter verkoel vervoer en bestraal word om te veel beweging en aktiwiteit te voorkom. Dit kan logistiese probleme skep. In dié opsig is dit makliker om papies te bestraal, wat uiteraard onaktief is en nie verkoel hoef te word nie. Omdat hulle nog heelwat fisiese ontwikkeling moet ondergaan, word dié stadium egter gewoonlik makliker deur gammabestraling benadeel. Die invloed van bestraling is in hierdie proef ondersoek.

Materiale en metodes

Driehonderd en dertig 3-daeoue VKM-papiës is in elk van drie behandelings gebruik. Die papies is individueel in 5 ml pilbotteltjies geplaas en met sponsproppe toegemaak. Die papies van twee behandelings het bestralingsdosisse van onderskeidelik 100 Gy en 250 Gy ontvang. Die papies is by 27°C gehou totdat alle lewendiges tot motte ontwikkel het.

Resultate en bespreking

Die 250 Gy bestralingsbehandeling het 'n uiters nadelige uitwerking op die relatiewe jong papies gehad en die meeste het gevrek (Tabel 3.4.8.1). Baie meer papies het die 100 Gy behandeling oorleef om in motte te ontwikkel.

Tabel 3.4.8.1. Invloed van gammabestraling op drie-dae oue valskodlingmotpapies

	Bestralingsdosis		
	0 Gy (kontrole)	100 Gy	250 Gy
Aantal papies uit 330 wat in motte ontwikkel het	266	235	19
Persentasie	79.9	70.6	5.7

Gammabestraling vertraag dikwels die ontwikkeling van onvolwasse insekte. In dié proef is die ontpoping (gedaanteverwisseling van papies tot motte) van bestraalde papies nie beduidend vertraag nie (Fig. 3.4.8.3). 'n Bestralingsdosis van 100 Gy het relatief min invloed op die papies se ontwikkelingstempo en hul oorlewing gehad. Die mortaliteit van papies wat die 250 Gy dosis ontvang het, was egter so hoog dat die bestralingsinvloed op hul ontwikkelingstempo nie betroubaar vasgestel kon word nie.

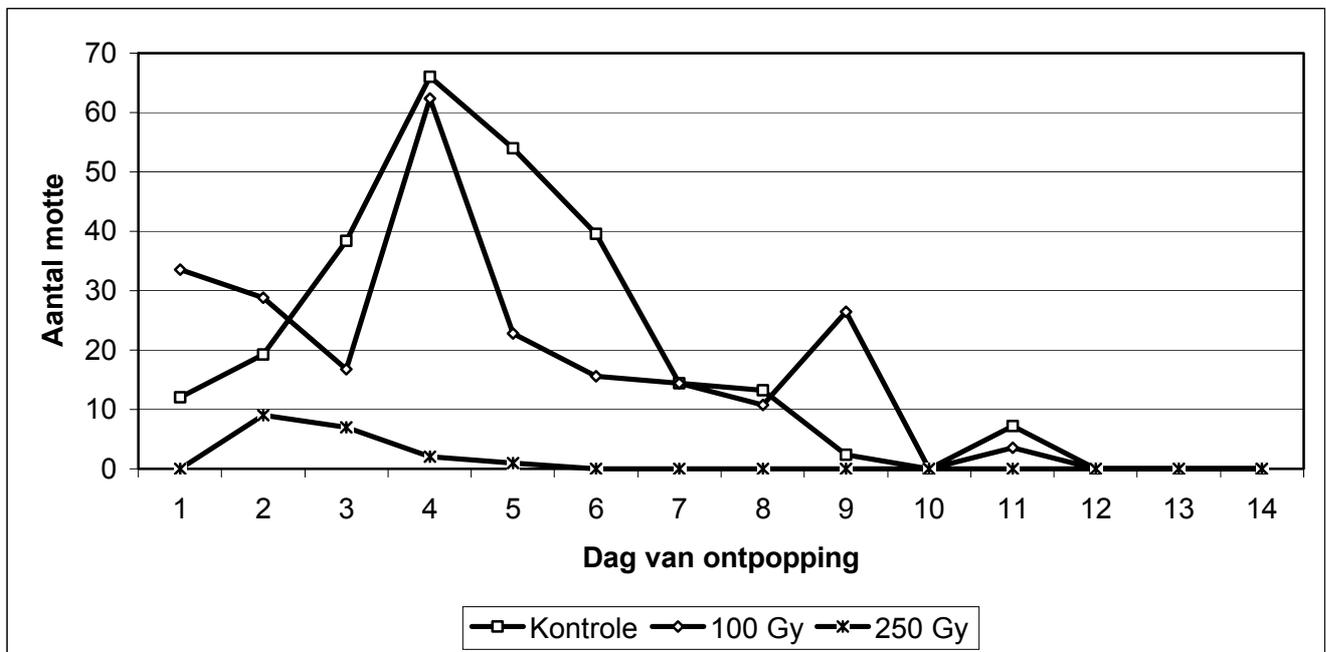


Fig. 3.4.8.3. Ontwikkelingstempo van gammabestraalde drie-dae oue valskodlingmotpapies.

In 'n SIL-program (steriele insekloslaatprogram) kan VKM óf in die papiestadium óf as motte bestraal word. Hantering van die insekte kan baie vergemaklik word deur papies in plaas van motte te bestraal. Dit is egter noodsaaklik dat volwasse papies bestraal word (papies wat so ver ontwikkel is dat die motte alreeds in die papiedop sigbaar is en dus binne nagenoeg 24 tot 36 uur sal ontpop). In vroeëre navorsing met sulke papies het die bestralingsdosisse wat gebruik was, nie 'n noemenswaardige invloed op motontpoping gehad nie (Bloem *et al.*, 2004). Dié vereiste kan opsigself 'n probleem skep aangesien dit tans met huidige teeltgnieke nagenoeg onmoontlik is om alle papies tegelykertyd in dieselfde stadium van ontwikkeling te kry. Hoe meer hul ontwikkeling verskil, hoe groter sal die verlies aan papies wees omdat 'n beduidende persentasie óf te vroeg óf te laat bestraal sal moet word. In hierdie proef blyk dit duidelik dat relatiewe jong papies maklik deur bestraling banadeel word. Dit sal daarom spesiaal in ag geneem moet word indien papiebestraling ooit in 'n SIL-program gebruik word.

As alternatief vir papiebestraling kan motte wat nagenoeg 24-uur oud is, bestraal word (sodat die motte 'n mate van rypheid kan ontwikkel wat die skadelike invloed van bestraling ietwat sal verminder). Die nadeel van motbestraling is egter dat die motte onder spesiale toestande van lig en temperatuur gehou, hanteer, vervoer en bestraal moet word, wat 'n logistiese nagmerrie kan wees wanneer tienduisende motte betrokke is.

In beide gevalle moet gespesialiseerde tegnieke en apparaat ontwikkel word om die verlangde aantal insekte bymekaar te maak. Die sukses waarmee dit gedoen word en die doeltreffendheid van die tegnieke, sal ook die finale keuse van welke ontwikkelingstadium gebruik word, beïnvloed.

3.4.8C Bestryding van valskodlingmot met steriele insekloslatings: C. Invloed van gammabestraling op die ontwikkelingstempo van bestraalde motte se nageslag Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

The effect of gamma radiation on the progeny (F1) of irradiated false codling moth was investigated. It was established that larval and pupal survival of the F1 generation was reduced. The development of larvae and pupae was progressively retarded with increasing doses of radiation from 0 Gy to 250 Gy. The sex ratio of the F1 generation also changed progressively in favour of males.

Inleiding

Die invloed van gammabestraling op die vrugbaarheid ("fertility") en geilheid ("fecundity") van VKM is in die Jaarverslag vir 2002 (Afd. 3.4.11) bespreek. Sekere bevindinge is egter nie vermeld nie, naamlik die invloed van gammabestraling op sekere aspekte van die bestraalde motte se F1-nageslag. Alhoewel dié waarnemings nie direk met die praktiese toepassing van die resultate verband hou nie, verdien dit tog vermelding, aangesien dit aspekte is wat 'n beter insig in die volle uitwerking van gammabestraling op VKM gee.

Materiale en metodes

VKM-mannetjies is met dosisse gammabestraling wat van 100 tot 250 Gy gewissel het, behandel en daarna met onbestraalde wyfies gepaar. Vyf paar motte is in elk van drie hokkies geplaas en toegelaat om 4 aande lank eiers te lê. Die eiers is daarna in teelflesse ingeënt sodat die larwes kon ontwikkel. Die teelflesse is daaglik ondersoek en die watterproppe is met riefelkartondoppe vervang toe die eerste larwes begin pupleer het. Die aantal kokonne is daaglik getel. Die papies is daarna uit die kokonne verwyder en individueel in 5 ml glas pilbotteltjies geplaas. Motontopping is daaglik ondersoek.

Resultate en bespreking

Twee aspekte het veroorsaak dat die tempo waarteen die F1-larwes verpop het (gedaanteverwisseling van larwes tot papies), nie akkuraat bepaal kon word nie:

(i) Die larwes het kokonne in die kartonproppe gespin en die presiese tydstip waarop elkeen verpop het, kon dus nie waargeneem word nie. Die verpopingstempo is dus inderwaarheid beoordeel aan die tempo waarteen die larwes in die verskillende behandelings kokonne gespin het en daar word aangeneem dat alle larwes daarna teen 'n vaste tempo verpop het.

(ii) Meer as twee larwes het somtyds in elke riefel van die kartonproppe verpop. Die twee buitenstes kokonne was uiteraard aan weerskante van die kartonstrook in die riefel sigbaar, maar enige larwe wat tussen-in pupleer het, was onsigbaar.

Ten spyte van bogenoemde probleme word daar aanvaar dat die resultate waarskynlik nie wesentlik beïnvloed is nie.

Die tempo waarteen kokonne deur larwes gevorm is waarvan die ouers met 100 Gy en 150 Gy behandel was, het min van die kontrole (0 Gy) verskil (Fig. 3.4.8.4). Die larwes waarvan die ouers die 200 Gy en 250 Gy dosisse ontvang het, se tempo was egter vertraag en 'n groter persentasie kokonne is ietwat later as in die ander behandelings gevorm.

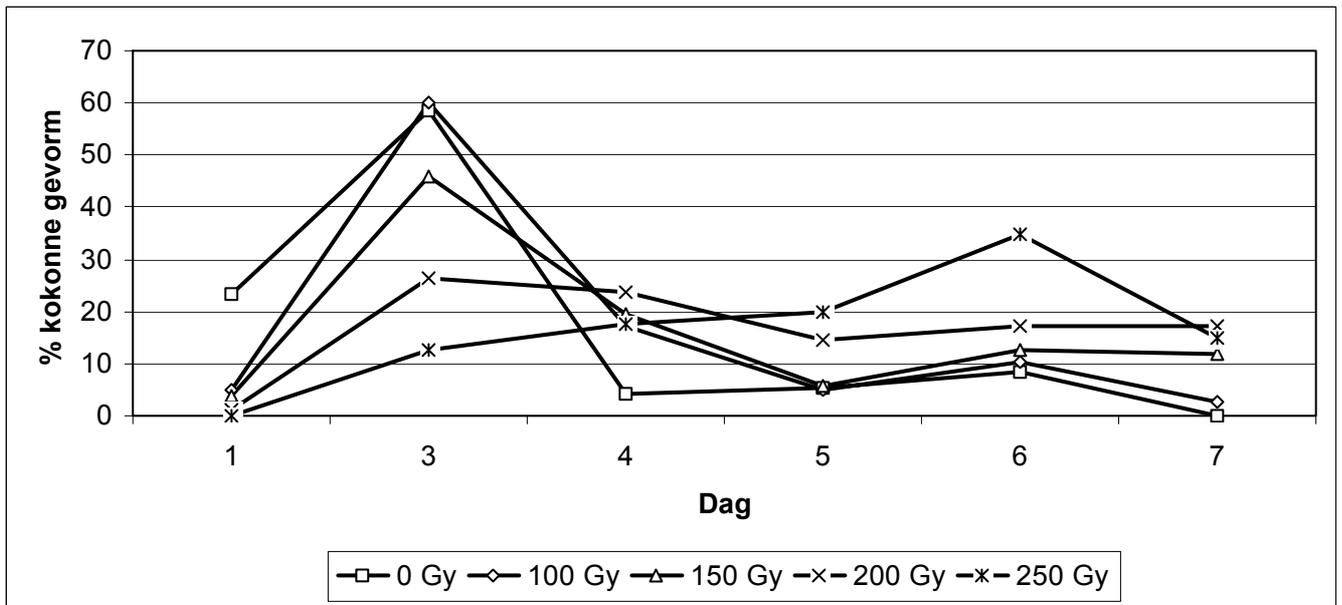


Fig. 3.4.8.4. Tempo waarteen kokonne deur gammabestraalde valskodlingmotlarwes gevorm word.

Die aantal larwes wat in papier ontwikkel het en die ontpoppingstempo van die papier kon akkuraat bepaal word. Bestraling van die ouers het die lewensvatbaarheid van hul nageslag aangetas en 'n al hoe groter persentasie larwes en papier het met toenemende bestralingsdosis gevrek (Tabel 3.4.8.2).

Tabel 3.4.8.2. Invloed van bestralingsdosis op die oorlewing en ontwikkelingstempo van die F1-geslag van bestraalde valskodlingmotte

Bestralingsdosis (Gy)	Aantal papier wat gevorm is	% larve-papiermortaliteit	Aantal motte
0	314	1,6	309
100	233	5,2	221
150	125	9,6	113
200	141	12,1	124
250	78	21,8	61

Dit was opsigtelik dat die papier al hoe langer geneem het om te ontpop hoe hoër die bestralingsdosis was (Fig. 3.4.8.5). Die vinnigste ontpoppingstempo van elke behandeling was een tot twee dae later met elke 50 Gy verhoging in dosis.

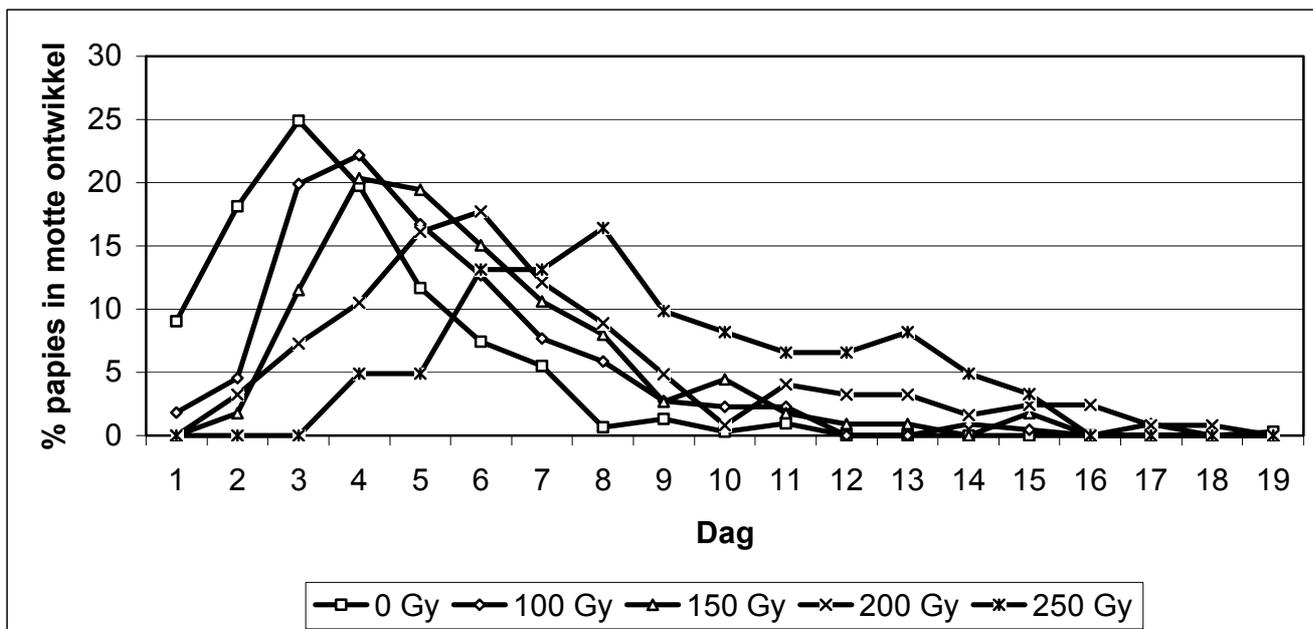


Fig. 3.4.8.5. Ontpoppingstempo van die nageslag (F1)-papies van gammabestraalde valskodlingmot.

'n Verskynsel wat uiters voordelig kan wees wanneer VKM in 'n SIT-program bestry word, is in dié proef waargeneem. Dit is naamlik dat die geslagsverhouding van die F1-generasie verander wanneer die ouerlike motte bestraal word (Fig. 3.4.8.6). Al hoe meer mannetjies ontwikkel soos die bestralingsdosis toeneem. Die mannetjies is die belangrikste oordraer van steriliteit aangesien hulle meer dikwels as die wyfies paar. Hoe meer F1-mannetjies daar dus is, hoe beter. Die verskynsel kan egter nie ten volle benut word nie, aangesien die lewensvatbaarheid van die motte toenemend aangetas word. Dit lyk tans asof óf 150 Gy óf 200 Gy vir die kommersiële bestraling van VKM gebruik sal word. Volgens die data lyk dit asof nagenoeg 80% van die F1-generasie uit mannetjies sal bestaan, wat heelwat gunstiger is as die gemiddelde 1:1 geslagsverhouding wat by onbestraalde motte in die insektarium en in die natuur aangetref word.

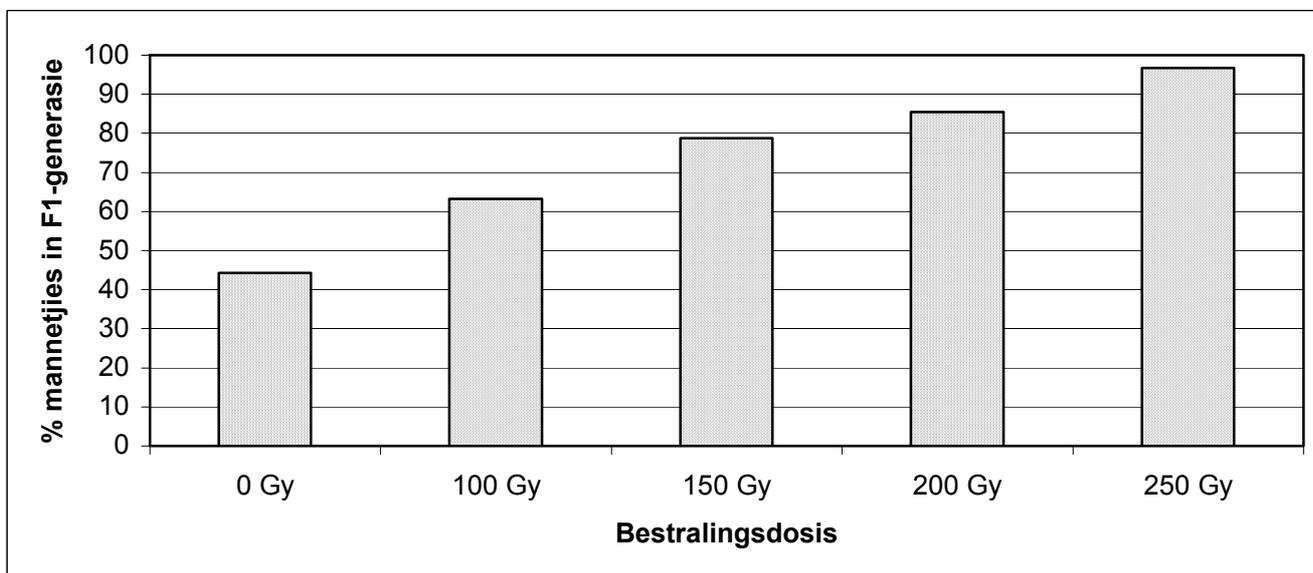


Fig. 3.4.8.6. Verandering in die geslagsverhouding van die nageslag (F1) van gammabestraalde valskodlingmot.

Die data ten opsigte van die papies stem grotendeels met die resultate van die vorige proef (Proef B) in dié reeks ooreen, d.w.s. dat bestraling die oorlewing en ontwikkeling van die nageslag van bestraalde VKM aantass.

Die afname in lewensvatbaarheid van die nageslag van bestraalde motte is ongewens aangesien daar op F1-steriliteit staatgemaak word sodat SIT doeltreffend kan werk. Dit beteken dat die F1-nageslag nie net steriel moet wees nie, maar dat hulle nog steeds lewensvatbaar genoeg moet wees om met wilde motte te

kan meeding om die oordrag van steriliteit ten beste te benut. Die bestralingsdosis wat uiteindelik in 'n SIT-program gebruik word, sal daarom versigtig gekies moet word.

3.4.8D Bestryding van valskodlingmot met steriele insekloslatings: D. Inwendige kleuring van valskodlingmot vir hervangsproeue

Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

It is necessary to dye moths internally or externally for use in mark and release experiments, as well as to monitor the efficacy of sterile insect releases in a SIT programme. Various dyes were investigated, but only Calco Oil Red[®] proved to be useful as an internal dye. External DayGlo[®] dyes will be investigated in future experiments.

Inleiding

Dit is om 'n hele paar redes nodig dat veral mannetjiemotte gekleur word. Eerstens moet die bewegingsdinamika van die motte bestudeer word om onder andere te bepaal (i) of mannetjiemotte se mededingendheid onder boordtoestande deur bestraling aangetas word, (ii) hoe ver 'n losgelate mannetjiemot kan of wil vlieg om by 'n wyfie (of lokval) te kom, (iii) hoe lank dit neem om so 'n afstand af te lê, (iv) hoe lank na loslating mannetjies in die loslaatgebied sal verwy, (v) met hoeveel sukses losgelate mannetjies weer gevang kan word (vir die kalibrasie van verskillende tipes lokvalle) en (vi) om tussen wilde en losgelate mannetjies te onderskei en sodoende die sukses van SI-loslatings te bepaal.

Motte kan op twee maniere gemerk word:

(i) Mannetjies kan uitwendig met 'n gekleurde poeier gemerk word. Dié poeiers ("DayGlo" reeks) is fluoriserend en vertoon duidelik wanneer 'n bepoeierde mot in die donker met 'n ultravioletlamp (UV-lamp) belig word. Die voordeel van dié tegniek is dat dit vinnig en sonder spesiale voorbereiding gedoen kan word. Daar is ook verskeie kleure poeiers beskikbaar, wat beteken dat meer as een behandeling tegelykertyd met mekaar vergelyk kan word. Die nadeel is dat die poeier met oorleg gebruik moet word, aangesien motte met te veel poeier op hul liggame en antennae skynbaar geïnaktiveer word en nie op 'n lokstimulus reageer nie.

(ii) 'n Spesiale, onskadelike kleurstof word saam met die voedingsmedium in die teelflesse gemeng. Die larwes vreet die kleurstof saam met die voedingsmedium en die gevolg is dat die motte inwendig gekleur word. Wanneer so 'n mot stukkend gedruk word, kan die kleurstof waargeneem word. Die nadeel is dat daar net een bekende kleurstof is wat die gewenste eienskappe het. Slegs een behandeling kan daarom op 'n slag gebruik word wat vergelyking met ander behandelings bemoeilik.

Verskeie proewe is uitgevoer om inwendige kleurstowwe te ondersoek.

Materiale en metodes

Proef 1: 'n Plaaslik verkrygbare voedselkleurstof, Sicovit Azorubine 85E122 (# 01059101) is getoets. 0,035 g van die wateroplosbare, rooi kleurstof is by 70 g meliemeel (die standaard hoeveelheid wat per teelfles gebruik word) in elk van 10 teelflesse gevoeg. Sewentig ml water is bygevoeg, die mengsel is goed geroer en daarna op die voorgeskrewe wyse met VKM-eiers ingeënt. Tien teelflesse met ongekleurde voedingsmedium is as kontrole gebruik. Die 20 ingeënte flesse is by 26°C gehou totdat die larwes tot motte ontwikkel het. Verskeie motte uit elke teelfles is vervolgens papgedruk en ondersoek om vas te stel of hulle inwendig gekleur was.

Proef 2: Twee kleurstowwe, Calco Oil Blue en Calco Oil Red, is met 'n ongekleurde kontrole vergelyk. 0,01 g en 0,02 g van die 2 kleurstowwe is soos hierbo met meliemeel in elk van 5 teelflesse gemeng. 0,3 ml Canola-olie is by elke fles gevoeg om die meng van die wateronoplosbare poeiers moontlik te maak. Die flesse is soos voorheen met VKM-eiers ingeënt wat toegelaat is om tot motte te ontwikkel.

Proef 3: Dieselfde tegniek as in Proef 1 is gevolg en 10 teelflesse is per behandeling gebruik ('n kontrole-behandeling is ingesluit). Slegs een kleurstof, naamlik 0,01 g Calco Oil Red plus 0,3 ml Canola-olie, is getoets.

Die motte wat in dié proef herwin is, is in vier opeenvolgende merk-en-loslaatproewe gebruik. Die tegniek in al die proewe het ooreengestem. Vyftig tot 100 mannetjies is met sonsondergang op drie aangrensende bome in die verskillende proewe in 'n nawelboord 30 m windaf van 'n enkele VKM-lokval losgelaat. Die

lokvalle was met sintetiese feromoon toegerus. Motte is slegs een keer in elke proef losgelaat en lokvaltellings is vier dae lank uitgevoer. Gevangde motte is op 'n wit karton pagedruk en vir tekens van rooi kleurstof ondersoek.

Resultate en bespreking

Proef 1: Die Sicovit Azorubine voedselkleurstof was op die oog af onskadelik vir die larwes. Die motte is egter nie inwendig gekleur nie en daar word vermoed dat die kleurstof ten volle metabolies afgebreek is.

Proef 2: Die Calco Oil Blue kleurstof was ooglopend giftig en baie min larwes het daarin geslaag om in motte te ontwikkel. Die blou kleurstof was baie onduidelik sigbaar toe die enkele oorlewende motte op 'n wit agtergrond pagedruk is.

Larwes in die Calco Oil Red teelflesse het normaal ontwikkel. Daar was geen ooglopende verskil in die aantal geproduseerde motte tussen die kontrole en die 0,01 g en 0,02 g behandelings nie. Daar was ook geen opsigtelike verskil in die graad van inwendige kleuring tussen die twee dosisse kleurstof nie. Duidelike rooi vlekke is gevorm toe motte van beide behandelings op wit karton pagedruk is.

Proef 3: Die motte was inwendig goed deur die Calco Oil Red gekleur en daar was geen ooglopende verskille tussen die kontrole en kleurstofbehandeling ten opsigte van die aantal motte wat per teelfles geproduseer is nie.

Twintig tot 50% van die aantal gemerkte, losgelate mannetjies is weer in die verskillende proewe gevang. Dié resultaat wys dat die metode van merking die mannetjies nie so erg aantast dat hulle nie op 'n feromoonstimulus kan reageer nie. Dit is onbekend of die hervangspersentasie deur die inwendige kleurstof benadeel is. In toekomstige proewe sal sulke gemerkte mannetjies met motte wat uitwendig met fluoriserende poeier gemerk is, vergelyk word.

Gevolgtrekking

Calco Oil Red sal waarskynlik in die toekoms vir die inwendige kleuring van motte gebruik moet word wanneer laasgenoemde op groot skaal vir SI-loslatings geteel word. Bogenoemde ondersoek sal daarom voortgesit word om te bevestig dat (i) die larwes se lewensvatbaarheid (vermoë om uiteindelik in motte te ontwikkel) en (ii) die motte se mededingendheid wanneer hulle in 'n boord losgelaat word, nie benadeel word nie.

Die fluoriserende Day-Glo poeiers sal in merk-en-loslaat boordproewe geëvalueer word wanneer aspekte (i) tot (v) waarna in die Inleiding verwys is, ondersoek word.

3.4.8E Bestryding van valskodlingmot met steriele insekloslatings: E. Evaluasie van F1-steriliteit in hokproewe op nawelbome

Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI), Stephanie Bloem (USDA/APHIS en Konsultant: IAEA), Jim Carpenter (USDA-ARS) en Frikkie Calitz (LNR Biometrie-eenheid, Stellenbosch)

Summary

Navel orange trees were individually enclosed in 125 μm gauge nylon mesh cages. FCM treated with 150 Gy and 200 Gy gamma radiation, were released into the cages with unirradiated moths in ratios of 5:1 and 10:1 respectively. Although the experiment was not designed to reduce crop losses there was a large reduction in the number of larval induced injuries caused by the F1-progeny of irradiated moths, with a commensurate reduction in fruit drop of up to 39%. Earlier laboratory results indicating a shift in the sex ratio of F1-moths reared from irradiated parents, to male dominated progeny, were confirmed.

Due to inherited sterility the numbers of eggs deposited by the F1-generation were reduced, while up to 69,0% of all matings involving, *inter alia*, normal females mated to F1-males, were totally sterile and resulted in 100% egg mortality. F1-females were not as effective in transmitting sterility when mated to normal male moths. However, up to 51% of all eggs produced from matings by, *inter alia*, apparently partially sterile F1-females and normal males, remained undeveloped.

The results establish a firm foundation for the continuation of SIT research and lend support for the application of SIT for the control of FCM in a future pilot study.

Inleiding

In die Jaarverslag vir 2002 is verslag gelewer oor navorsing op die bestralingsbiologie en oorgeërfde F1-steriliteit van VKM. In daardie navorsing is die invloed van verskillende bestralingsdosisse op die vrugbaarheid en geilheid van VKM bestudeer. 'n Uitgebreide proef is ook uitgevoer om vas te stel of steriele eiers wat deur die nageslag (F1-generasie) gelê word, deur die eierparasitoïed *Trichogrammatoidea cryptophlebiae* geparasiteer kan, of sal, word. Alle inligting wat ingewin was, is uiters belowend en wys onder andere dat (i) die beginsel van F1-steriliteit direk van toepassing op VKM is, (ii) dat sterilisasietegnieke geskik vir toepassing op VKM is en (iii) dat steriele VKM-eiers wel deur *T. cryptophlebiae* geparasiteer kan en sal word. Die volgende stap in die logiese ontplooiing van die SIT-navorsing is om die inligting wat tot dusver ingewin is, op 'n praktieser wyse te bevestig. Dié inligting sal van groot nut wees wanneer SIT onder kommersiële toestande in 'n loodsprojek getoets word.

Dit is nodig om baie meer gesteriliseerde motte in 'n SIL-program los te laat as wat daar wilde motte in die omgewing is. 'n Loslaatverhouding (LLV) van 10:1 word byvoorbeeld internasionaal vir die SIT-bestryding van Kodlingmot gebruik. Die doel van die proef was om inligting onder natuurliker toestande (as in die laboratorium) oor 'n geskikte LLV van bestraalde tot onbestraalde VKM, asook 'n geskikte bestralingsdosis, in te win. Dit is gedoen deur VKM (P1) te bestraal, hulle toe te laat om vrugte te besmet en die oorgeërfde uitwerking op die F1-nageslag se eiers te ondersoek.

Materiale en metodes

'n Statisties volledige ewekansige blokontwerp proef is uitgevoer met 5 behandelings wat ewekansig in elk van 3 statistiese blokke (herhalings) toegeken is. Die behandelingsontwerp was 'n 2 x 2 faktoriaal plus 'n onbestraalde kontrole. Die faktore was twee loslaatverhoudings (5:1 en 10:1) en twee bestralingsdosisse (150 Gy en 200 Gy) (Tabel 3.4.8.3).

Tabel 3.4.8.3. Bestralingsdosis en loslaatverhouding wat in die hokproef gebruik is.

Aantal bestraalde VKM (pare)	Aantal onbestraalde VKM (pare)	Loslaatverhouding bestraal: onbestraal	Bestralingsdosis (Gy)
0 (kontrole)	10	0:1	0
50	10	5:1	150
100	10	10:1	150
50	10	5:1	200
100	10	10:1	200

Die proef is in 'n boord met 7-jaaroue, vrugdraende Lina-nawelbome uitgevoer. 'n Raamwerk van houtpale en ysterbalke is oor 15 aangrensende bome opgerig (Fig. 3.4.8.7). Vyftien hokke van nylongaasmateriaal, met 'n maasgrootte van 125 μ , is vervaardig. Vooraf toetsing het getoon dat *T. cryptophlebiae* nie deur dié maasgrootte kan dring nie. 'n Hok, wat aan die raamwerk vasgemaak was, is oor elke boom gehang en die onderrande is 300 mm diep in die grond begrawe. 'n Ritssluiters, 1,2 m lank, wat in een sy van die hok ingesit is, is gebruik om die hok binne te gaan. Een boom in 'n hok is as 'n eksperimentele eenheid beskou.



Fig. 3.4.8.7. 'n Nawelboom met oes in 'n nylongaashok met ritssluiting.

Die bome is op 2 April 2003, nagenoeg 11 weke voor oestyd, ingehok. Die bome is vier weke lank elke dag ondersoek en alle afvalvrugte is verwyder. Dié vrugte is oopgesny om te verseker dat geen VKM-larwes uit besmette vrugte ontsnap het nie. Met die uitsondering van 50 ewekansig verspreide vrugte in elke databoom, is alle ander vrugte daarna gepluk en verwyder.

Mannetjie- en wyfiemotte is op 2 Mei 2003 aan teenoorstaande kante van elke boom losgelaat. Die hokke is vervolgens verseël en slegs weer oopgemaak om afgevalde vrugte te verwyder. Dié vrugte is in die laboratorium ondersoek, alle VKM-vreetmerke getel en daarna individueel in 500 ml plastiekbakkies met gaasdeksels geplaas (Fig. 3.4.8.8). Alle vrugte wat nog nie afgeval het nie, is op 24 Junie 2003 gepluk, die vreetmerke getel en ook in bakkies geplaas. Tien tot 15 stukkies plastiekkoele drankstrooitjies, 20 mm lank, is in elke bakkie geplaas sodat F1-larwes wat die besmette vrugte verlaat het, plek sou hê om in te puepeer. Vrugte wat tekens van bederf getoon het, is oopgesny en alle larwes is daaruit verwyder. Die larwes is in teelflesse met voedingsmedium oorgeplant (een fles per vrug) om hul lewensiklus te voltooi. Die teelflesse is daagliks ondersoek, alle F1-papies is verwyder en individueel in 5 ml glas pilbotteltjies met sponsproppe oorgeplaas. Alle vrugte en papier is by 26°C in die laboratorium gehou.

Elke F1-mot wat ontpop het, se geslag is bepaal en met 'n "metgesel"-mot (M; motte waarvan die ouers nie bestraal was nie) van die teenoorgestelde geslag gepaar. Laasgenoemde motte was van papier afkomstig wat met gereelde tussenposes by Ceder Biocontrol-insektarium in Citrusdal gekry is om as metgeselle vir die proefmotte te dien. Elke paar motte is vir paring en eierlegging in 'n 90 ml plastiekbakkie geplaas. Elke bakkie se deksel is van 'n watterprop voorsien wat nat gehou is totdat die wyfie gemiddeld 14 dae later in die hokkie gevrek het. Dooie wyfies is verwyder en gedissekteer om vas te stel of hulle gepaar het. Eierlegging het teen die wande, bodems en deksels van die bakkies plaasgevind. Die bakkies is gebêre totdat dit seker was dat alle lewensvatbare eiers uitgebroei het. Die bakkies is daarna in twee geknip en alle eiers is onder die mikroskoop ondersoek en getel om die persentasie dooie eiers te bepaal.



Fig. 3.4.8.8. Afgevalde vrugte een-een in 500 ml plastiekbakkies geplaas.

Resultate en bespreking

Die meeste evaluasies was binomiaal van aard (beskadig of onbeskadig, dood of uitgebroei) en kon as persentasies uitgedruk word. Die veranderlikes van belang is statisties met behulp van die SAS statistiese pakket (weergawe 8.2) verwerk (SAS, 1999). Variansie-analises met gepaste effekte en wisselwerkings is op die data uitgevoer en residuele afwykings is vir nie-normaliteit getoets (Shapiro en Wilk, 1965). Daar was nie genoegsame getuienes ($P > 0.05$) teen normaliteit nie; transformasie was daarom nie nodig nie en daar is met die vertolking van die resultate voortgegaan. Student se t-KBV (kleinste betekenisvolle verskil) is by 'n 5% betekenispeil bereken om gemiddeldes te vergelyk (Snedecor, 1967).

(1) P1-steriliteit en die beskerming van vrugte teen VKM-besmetting: Vir navorsingsdoeleindes word tans aanvaar dat daar op enige enkele aand gemiddeld 50 paar wilde VKM per ha in 'n boord aanwesig is. Dit beteken dat daar gemiddeld een wyfie vir elke 10 bome (500 bome/ha) is. Daar is egter baie meer motte, onderskeidelik 10 (kontrole), 60 (5:1 LLV) en 110 (10:1 LLV) wyfies per boom in die verskillende behandelings losgelaat. Alhoewel dit baie wissel, kan 'n enkele wyfie onder ideale toestande meer as 1 000 eiers lê in haar lewensduur van ongeveer 7 tot 14 dae lank. Indien daar argumentsonthalwe aanvaar word dat 500 eiers deur 'n enkele wyfie gelê word (raadpleeg Proef A2 hierbo), sou al 50 vrugte op 'n databoom potensieel maklik oor en oor deur een individu besmet kon word. Daarbenewens moet in gedagte gehou word dat 'n sekere persentasie vrugte deur die bestraalde ouerlike motte se F1-larwes beskadig kan word, aangesien die betrokke bestralingsdosis nie groot genoeg is om alle P1-mannetjies ten volle te steriliseer nie (Bloem *et al.*, 2004). **Die proef was dus nie daarop gemik om die doeltreffendheid van SIL om oesskade te verhoed, te ondersoek nie.** Daar was desnieteenstaande opvallende, statisties beduidende verskille tussen die behandelings ten opsigte van die aantal vreetmerke wat deur die F1-larwes op die vrugte aangerig was (Fig. 3.4.8.9). Alle letsels wat deur die larwes veroorsaak was, is in aanmerking geneem. Omdat die vrugte gehou is sodat die larwes daarin kon ontwikkel, kon geen onderskeid tussen skilletfels sonder of met larwes daarin, getref word nie.

Die larwes wat vir die vreetmerke verantwoordelik was, was die gevolg van P1-parings (O = onbestraal, B = bestraal) wat totaal vrugbaar ($O_{\text{♀}} \times O_{\text{♂}}$) of gedeeltelike steriel ($O_{\text{♀}} \times B_{\text{♂}}$) was. Dié verskynsel bevestig navorsing wat vantevore uitgevoer was (Bloem *et al.*, 2004). Die data is bereken deur die aantal vreetmerke op al 50 vrugte per hok met die totale aantal bestraalde en onbestraalde mannetjies wat in die hok losgelaat was, te vergelyk (volgens vroeëre navorsing was die bestraalde wyfies heeltemal steriel en kon geen nageslag produseer nie). In terme van die aantal letsels wat veroorsaak is, het die 150 Gy/10:1-kombinasie

van behandelings net so goed soos die 200 Gy/10:1-kombinasie gevaar. Die 150 Gy/5:1-kombinasie was betekenisvol swakker.

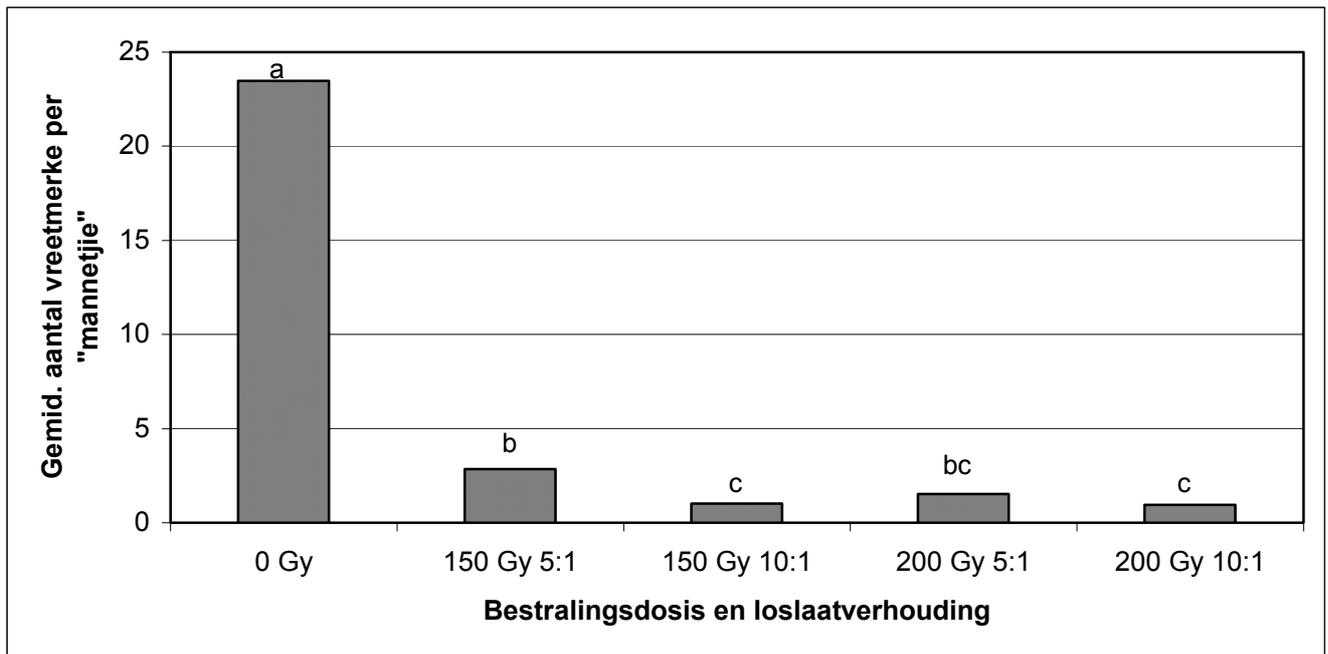


Fig. 3.4.8.9. Vrugskaede deur die F1-larwes van gammabestraalde valskodlingmot aangerig. Die aantal vreetmerke wat op 50 vrugte aangerig is, is met die aantal mannetjies wat per hok losgelaat was, vergelyk. Kolomme met dieselfde letter verskil nie betekenisvol van mekaar nie (t-KBV = 1.419).

Dié vermindering in vreetmerke het tot gevolg gehad dat minder vrugte beskadig is (Fig. 3.4.8.10). Dié oënskynlik duidelike verskille tussen die behandelings is egter nie statisties betekenisvol nie. Daar was uiteenlopende paringskombinasies wat lukraak in een hok kon plaasvind, naamlik $O_{\text{♀}} \times O_{\text{♂}}$, $O_{\text{♀}} \times B_{\text{♂}}$, $B_{\text{♀}} \times O_{\text{♂}}$ en $B_{\text{♀}} \times B_{\text{♂}}$, wat van heeltemal vrugbaar tot heeltemal steriel verskil. Indien die potensiaal van een onbestraalde wyfie om 50 vrugte maklik opsigself te besmet, in gedagte gehou word, kon slegs een of twee bykomende vrugbare parings genoeg variasie tussen herhalings in 'n behandeling veroorsaak het onbetekenisvolle verskille te verhoed. Ondanks die afwesigheid van statistiese verskille, is die neiging dieselfde as by die vreetmerke, naamlik dat die 150 Gy/10:1-kombinasie goed met die 200 Gy-bestralingsdosis vergelyk.

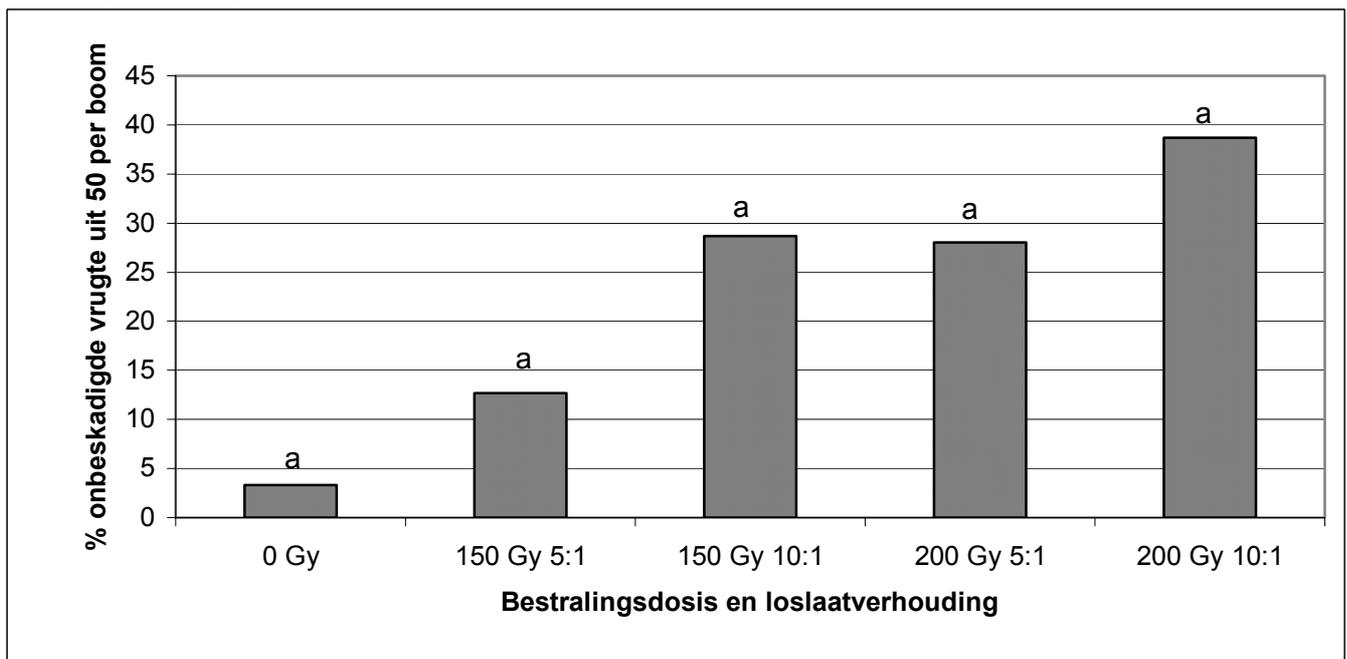


Fig. 3.4.8.10. Afname in oesskade as gevolg van die bestraling van valskodlingmot. Kolomme met dieselfde letter verskil nie betekenisvol van mekaar nie (t-KBV=45.65). Geen verskille is statisties beduidend nie.

(2) **Oorgeërfde F1-steriliteit:** Bestraling van die P1-ouers het die F1-generasie se voortplanting grotendeels ontwig. Die volgende is waargeneem:

- **Afname in eierlegging:** Baie minder eiers is gelê deur parings tussen F1-mannetjies en -wyfies met metgeselmotte ($M_{\text{♀}} \times F1_{\text{♂}}$, $F1_{\text{♀}} \times M_{\text{♂}}$) (Fig. 3.4.8.11). Soos hierbo, neig die 150 Gy/10:1 behandeling weer eens om baie dieselfde as die 200 Gy-bestralsingsdosis te presteer. Die patroon is deurlopend en word nie deur die tipe paring ($M_{\text{♀}} \times F1_{\text{♂}}$ of $F1_{\text{♀}} \times M_{\text{♂}}$) beïnvloed nie. Die grootste afname is deur die 200 Gy-bestralsingsdosis en die 10:1 LLV veroorsaak. In die geval van die 150 Gy/10:1-behandeling het die groter aantal motte wat losgelaat was, vir die ietwat swakker werking van die laer bestralsingsdosis vergoed. Die 150 Gy/5:1-behandeling het die swakste presteer.

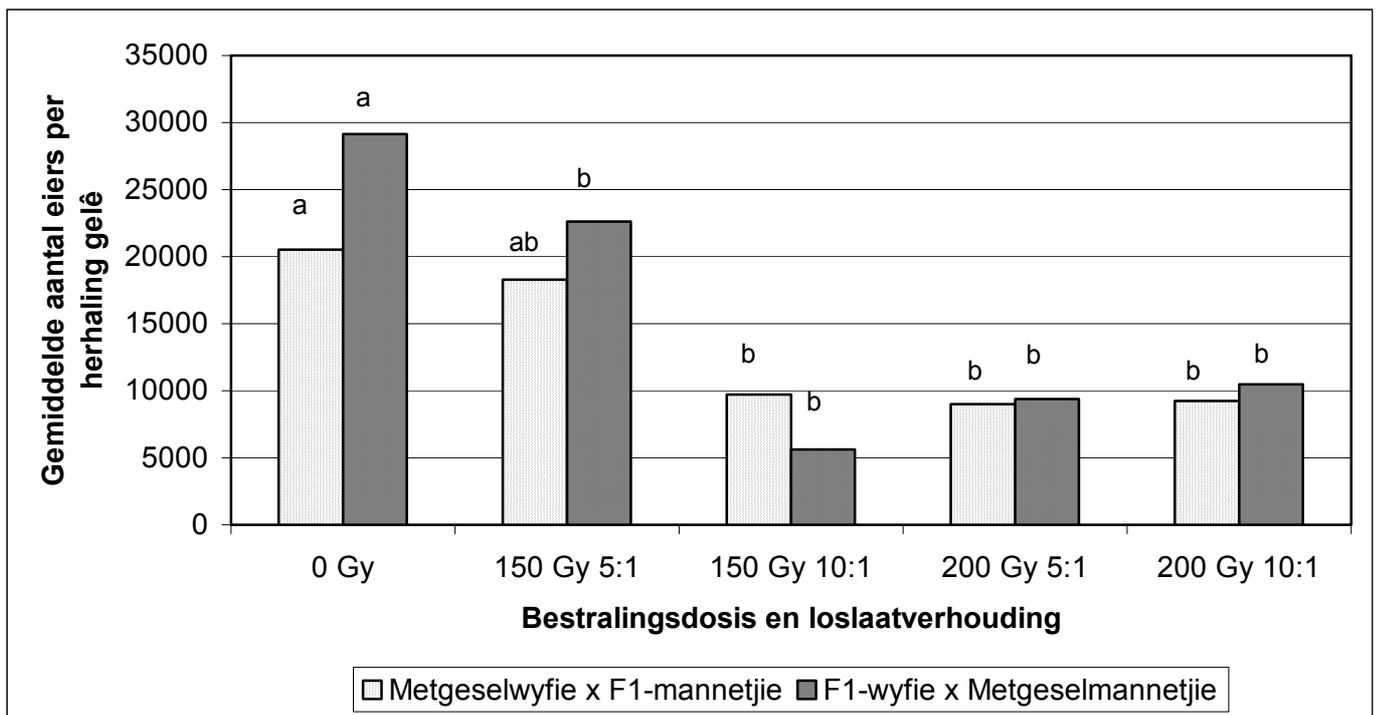


Fig. 3.4.8.11. Afname in eierlegging van parings tussen F1-mannetjies en -wyfies met afstammeling van onbestraalde motte (M = metgeselmot; F1 = nageslag van bestraalde ouerlike motte). Kolomme met dieselfde letter verskil nie betekenisvol van mekaar nie (t-KBV = 10774.6). Slegs behandelings in 'n betrokke kruising moet met mekaar vergelyk word en nie behandelings in verskillende kruisings nie.

- **Groter eiersteriliteit:** Baie meer eiers wat deur die F1 x metgeselkruisings gelê was, was steriel as in die kontrole (0 Gy). Daar was nie net 'n toename in die aantal volkome steriele wyfies en mannetjies nie (parings waarvan geen eiers uitgebroei het nie), maar daar was ook 'n toename in die aantal motte wat gedeeltelik gesteriliseer was en groot getalle steriele eiers gelê het (Tabel 3.4.8.4). Volkome steriliteit is beter deur die F1-mannetjies ($M_{\text{♀}} \times F1_{\text{♂}}$) as deur die F1-wyfies ($F1_{\text{♀}} \times M_{\text{♂}}$) oorgedra. As die totale aantal dooie eiers beskou word (hokkies waarin alle eiers, of slegs 'n persentasie daarvan, dood was), blyk dit dat daar persentasiegewys meer deels-steriele wyfies in die $F1_{\text{♀}} \times M_{\text{♂}}$ -kruisings as in die $M_{\text{♀}} \times F1_{\text{♂}}$ -kruisings was. Dit kan gesien word aan die relatiewe klein toename in die totale persentasie dooie eiers in vergelyking met die aantal hokkies waarin alle eiers dood was in die $M_{\text{♀}} \times F1_{\text{♂}}$ -kruisings, in teenstelling met die redelik groot toenames van die een na die ander faktor in die $F1_{\text{♀}} \times M_{\text{♂}}$ -kruisings (Tabel 3.4.8.4).

Tabel 3.4.8.4. Steriliteit van eiers deur die F1-generasie van gammabestraalde valskodlingmot gelê.

Behandeling	Gemiddelde % hokkies waarin geen eiers uitgebroei het nie		Gemiddelde % dooie eiers	
	M♀ x F1♂	F1♀ x M♂	M♀ x F1♂	F1♀ x M♂
0 Gy	2.40 a	1.32 a	11.17 a	5.76 a
150 Gy 5:1	43.52 b	3.79 a	48.39 b	29.01 ab
150 Gy 10:1	53.03 b	8.33 a	64.15 b	49.27 b
200 Gy 5:1	42.58 b	15.26 a	42.86 b	24.19 ab
200 Gy 10:1	61.60 b	10.86 a	57.90 b	21.08 ab
t-KBV(P = 0.05)	36.776		31.089	

M = Metgeselmotte. Syfers in een kolom gevolg deur dieselfde letter verskil nie betekenisvol van mekaar nie.

Die M♀ x F1♂ kruisings het in tot 61% van die hokkies volkome steriliteit van F1-eiers veroorsaak, terwyl dit in die omgekeerde F1♀ x M♂-kruisings veel laer was (tot slegs 15%; geen onderlinge statistiese verskille nie). In terme van die totale persentasie dooie eiers in die twee kruisings, het die M♀ x F1♂-kruisings se resultaat nie veel verander nie, maar die F1♀ x M♂-kruisings het veel beter gevaar (bv. 8% tot 49%).

Alhoewel die verskille tussen behandelings (0 Gy uitgesluit) nie deurgaans groot genoeg is om betekenisvol te wees nie, het die 150 Gy bestralingsdosis teen 'n 10:1 LLV goed presteer. Dit was mededingend met die 200 Gy/10:1 kombinasie in terme van beide die aantal parings wat heeltemal steriel was én die totale aantal steriele eiers wat gelê was.

- **Toename in mannetjiemotte:** Soos in proef C hierbo (Afd. 3.4.8C) het die grootte en geslagsverhouding van die F1-nageslag ook in dié proef verander nadat die ouerlike motte bestraal is. 'n Dosisreaksie is nie so duidelik soos in Proef C te sien nie, wat waarskynlik te wyte is aan die mengsel van bestraalde en onbestraalde P1-ouers in die hokke, wat lukrake parings tot gevolg gehad het. Daar was desnieteenstaande 'n betekenisvolle verskuiwing in die geslagsverhouding van die nageslag en baie meer F1-mannetjies het in die bestralingsbehandelings ontwikkel (Fig. 3.4.8.12). Dié verskynsel is nie tot VKM beperk nie – gammabestraling het dieselfde uitwerking op die Kodlingmot *Cydia pomonella* (Bloem *et al.*, 1999). Die toename in mannetjiegetalle is nie so groot soos in proef C nie en kan weer eens aan die lukrake aard van die paringskombinasies wat moontlik was, toegeskryf word. Beide bestralingsdosisse het teen 'n LLV van 10:1 die beste presteer.

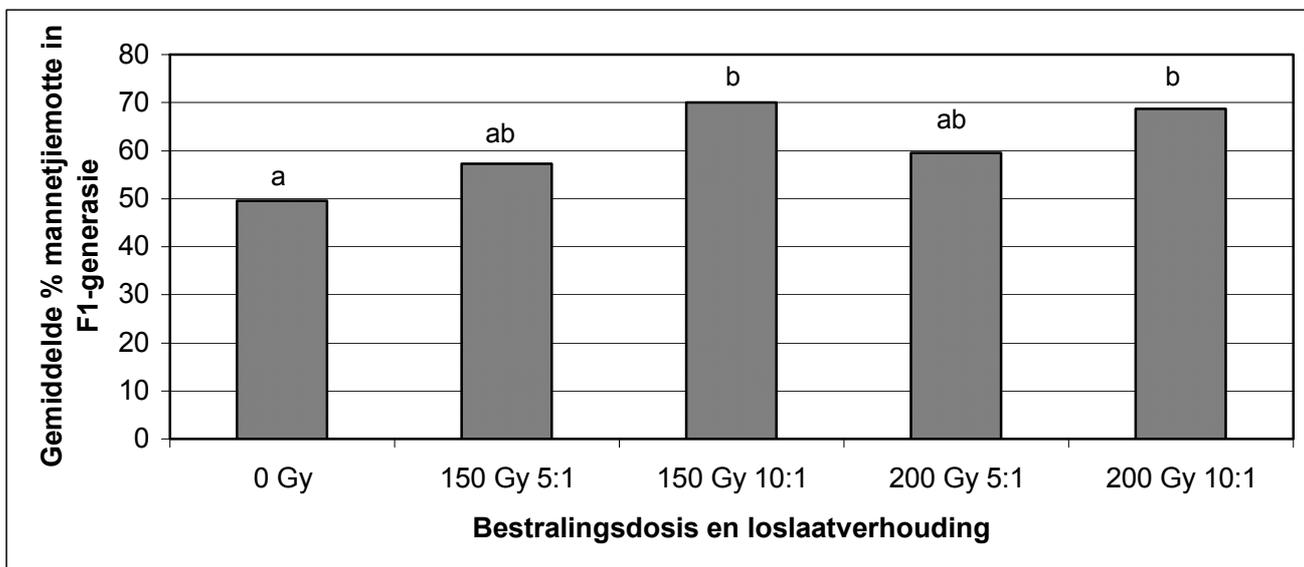


Fig. 3.4.8.12. Verandering in die geslagverhouding van die F1-generasie wanneer die ouerlike (P1) motte bestraal word. Kolomme met dieselfde letter verskil nie betekenisvol van mekaar nie (t-KBV = 16.164).

Dieselfde patroon kan deurgaans in die resultate van die verskillende faktore wat bestudeer is, opgemerk word. Die 150 Gy/5:1-kombinasie is beslis die swakte behandeling, terwyl die 150 Gy en 200 Gy bestralingsdosisse, beide teen 'n 10:1 LLV, die beste presteer het. Die 200 Gy/5:1-kombinasie was deurentyd iewers in die middel.

Hoe laer die bestralingsdosis, hoe voordeliger is dit vir die bestraalde mannetjies in terme van die

behoud van hul lewenskrag en mededingendheid. Met 'n 5:1 LLV het die 200 Gy-dosis telkens beter as die 150 Gy-dosis gevaar. Dié verskil is nie by die 10:1 LLV sigbaar nie en dui daarop dat die groter LLV vir die kleiner doeltreffendheid van die 150 Gy-dosis vergoed het. Daar sal dus besluit moet word welke faktor in die praktyk die belangrikste is. Enersyds sal 'n bestralingsdosis van 200 Gy minder vrugbare P1-mannetjies tot gevolg hê (Bloem *et al.*, 2004), met gevolglike ligter oesskade deur die F1-generasie. Andersyds hang die doeltreffendheid van die SIT-benadering egter van goeie F1-steriliteit af, wat meebring dat mannetjies wat fisies in alle opsigte op gelyke voet met die wildes kan meeding, noodsaaklik is. In dié opsig kan die toepassing van 'n 200 Gy-dosis nadelig wees. Die vlieg- en paringsvermoë van bestraalde motte onder boordtoestande sal in 'n volgende reeks proewe bestudeer word, wat moontlik inligting sal verskaf wat die keuse sal vergemaklik.

Gevolgtrekking

Goeie resultate is met behulp van die hokproef verkry, wat laboratoriumresultate bevestig en die toepassing van SI-loslatings in die naby toekoms 'n groter werklikheid maak. Die SIT-navorsing word voortgesit.

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3.4.9 Development of a technique for mass rearing of FCM for SIT purposes

Experiment 689 by Sean D. Moore, Garth I. Richards (CRI) and Nolitha Mlanjeni (Rhodes University)

Opsomming

'n Belangrike voorwaarde om sukses met die steriele insektegniek (SIT) te behaal is die ontwikkeling van 'n stelsel vir die massateel van baie groot getalle insekte. Die getalle VKM wat met huidige stelsels vir parasitoïedproduksie geteel kan word, is ontoereikend.

'n Nuwe eierlêhok van vleklosestaalgaas was 'n opmerklige verbetering op die hok wat voorheen gebruik is. Die nuwe hokke is makliker om uitmekaar te haal en skoon te maak, is sterker en sal dus langer hou. Die hok is ook aangepas om omgewingskontaminasie met motskubbe te verminder.

Van alle sojaboontjiemengsels wat getoets is, was sojameelblom en soja-oliekoek die geskikste basiese bestanddele vir 'n VKM-larwedieët. Groter getalle papies het oor die algemeen op hierdie dieët ontwikkel as op dieëte wat op ander soja-mengsels gebaseer was.

Die dieët wat by die SIL (Steriele Insekloslating)-fasiliteit in Kanada vir die massateling van Kodlingmot gebruik word, is blykbaar ook vir VKM geskik. Mieliemeel, sojameelblom en hawermeel, as basiese bestanddele, is egter blykbaar meer geskik vir VKM-teling as canolameel (die basiese bestanddeel in die Kanadese Kodlingmot-dieët). Saagsels, wat gebruik word om die dieët te bind en ook as 'n vulstof dien, het ontwikkeling van larwes verhoed wanneer die dieët met 'n outoklaaf gestoomsteriliseer, in plaas van gekook, is.

Opperrolde stroke riffelkarton skep 'n doeltreffende oppervlak vir verpoping (papievorming deur larwes).

Navorsing om die dieët, die voorbereiding daarvan en die houers wat gebruik word, te verbeter, word voortgesit.

Introduction

Researchers in the southern African citrus industry, the deciduous fruit industry, the USDA (United States Department of Agriculture) and the IAEA (International Atomic Energy Agency) are collaborating on the investigation and implementation of the sterile insect technique (SIT) for control of false codling moth (FCM), codling moth and fruit flies in the Western Cape. SIT has a history of success in the suppression and even elimination of insect pests from various parts of the world. It is important that this system also be tested against FCM on citrus in southern Africa, starting in the West Cape. The IAEA has agreed to sponsor this project by funding expert advice, overseas visits and the purchase of capital equipment. This funding has been pledged until 2005. An important prerequisite to achieving success with SIT is to develop a system for mass rearing extraordinarily large numbers of insects. The numbers of FCM that can be produced with currently known systems, used for parasitoid production, are inadequate. Improvements in FCM mass rearing techniques have already been made (see exp. 402; Moore & Richards, 2000 & 2001). However, these changes were not made specifically with SIT in mind. Improvements geared towards SIT specifically, were only initiated more recently (Moore *et al.*, 2002). Changes are aimed at increasing production while keeping labour inputs and costs as low as possible.

Materials and methods

Oviposition improvement

The standard oviposition cages (Moore & Richards, 2001; Moore *et al.*, 2002) consisted of a galvanised metal rod framework, covered with an organdy net sock. A new oviposition cage was designed, constructed and tested. This cage was constructed from stainless steel mesh and followed the same principle as the previously used cage. A roll of wax paper (the oviposition substrate) was fed through a slit on one side of the cage and out of a slit on the other side. The wax paper was positioned vertically, facing the natural light coming through a window in the room. Every 24 h the wax paper was moved on one length and severed. The eggs could then be used for further production of FCM. One difference between this and the existing cages was that a sheet of Perspex was hooked underneath the new cage in order to capture any moth scales falling through the cage.

As this cage was exactly the same size and followed exactly the same principle as the previous cage, no trials were conducted to measure and compare egg production. However, observations were made in key areas where the new cage was deemed to offer improvements over the old cage. These included aspects such as oviposition on the cage itself, durability of the cage, ease of cleaning, moth escape, and capture of moth scales.

Diet improvement

As earlier research indicated that soya flour might be a more favourable base ingredient than maize meal in the FCM larval diet, different types of soya preparations were tested in diets (Table 3.4.9.1). All ingredients, other than the base ingredient (i.e. maize meal or soya) remained the same. These were wheat germ, yeast, milk powder (or casein), nipagin and sorbic acid (Moore & Richards, 2000; Moore, 2002). Diets in jars were replicated twice whereas diets in plastic tubs were replicated only once. Once cooled, 160 FCM eggs were placed into each jar, and 320 eggs into each plastic tub. Jars were sealed with cotton wool stoppers and tubs were sealed with foam rubber sponges. The following information was recorded: Date at the start of pupation (therefore duration of larval development), number of pupae per container, level of contamination (fungal, viral or bacterial) in the container and any other important observations.

Table 3.4.9.1. Base ingredients, quantities and containers used in diet comparison trials with FCM larvae.

Treat. No.	Base ingredient	Description	Container used*	Total mass of dry ingredients	Volume of water added	Autoclaved/uncooked**
1	Maize meal	Impala Maize Meal	Jars	50 g	50 ml	Autoclaved
2	Soya flour	Factory milled (oil free)	Jars	50 g	85 ml	Autoclaved
3	Soya meal	Beans - factory ground	Jars	50 g	50 ml	Autoclaved
4	Soya cake oil	First pressed to remove some oil and then ground	Jars	50 g	85 ml	Autoclaved

5	Full fat soya	First ground, then heated to 160°C to remove some oil	Jars	50 g	40 ml	Autoclaved
6	Ground soya beans	Beans: self-ground	Jars	50 g	50 ml	Autoclaved
7	Soya flour	See above	Jars	50 g	85 ml	Uncooked
8	Soya meal	See above	Jars	50 g	50 ml	Uncooked
9	Soya oil cake	See above	Jars	50 g	85 ml	Uncooked
10	Full fat soya	See above	Jars	50 g	40 ml	Uncooked
11	Ground soya beans	See above	Jars	50 g	50 ml	Uncooked
12	Soya flour	See above	Plastic tubs	100 g	170 ml	Cooked
13	Soya meal	See above	Plastic tubs	100 g	100 ml	Cooked
14	Soya oil cake	See above	Plastic tubs	100 g	170 ml	Cooked
15	Full fat soya	See above	Plastic tubs	100 g	80 ml	Cooked
16	Ground soya beans	See above	Plastic tubs	100 g	100 ml	Cooked

*Jars had a capacity of 350 ml; plastic tubs had a capacity of 1 l.

**Hot water was added to dry ingredients for "uncooked" diet.

A second diet trial was conducted to further determine the suitability of a soya oil cake based diet. The recipe used was identical to that described above. Diet was made in plastic tubs (1 l capacity), and 142 g of dry ingredients with 255 ml hot boiled distilled water was used in each (5 replicates). After cooling, 500 surface sterilised eggs were placed into each container. Each container was fitted with a corrugated cardboard lid. The intention was that this should act as a suitable pupation substrate. Dishes were kept in a room at 27°C and 25% humidity. The room was dehumidified to reduce fungal contamination on the diet. Numbers of pupae developing in each container were counted.

A third diet trial was conducted, also with soya oil cake. Ten different diets were prepared (Table 3.4.9.2). Some of the diets were autoclaved and others were cooked. Larger quantities of diet than usual (50 g dry ingredients plus 50 ml water) were also tested in the jars. In two of the diets, paper pulp noodles were added as a bulking and bonding agent. After cooling, surface sterilised FCM eggs were placed onto the surface of each diet. Numbers of pupae developing in each container were counted, and observations on the state of the diet and contamination were recorded.

Table 3.4.9.2. Basic ingredients, quantities and containers used in diet comparison trials with FCM larvae.

Treat. No.	Base ingredient	Container used*	Total mass of dry ingredients	Volume of water added	Autoclaved/cooked*	Eggs per container
			OR Total mass in container (if diet was boiled)			
1	Maize meal	Jars	50 g	50 ml	Autoclaved	200
2	Soya oil cake	Jars	50 g	85 ml	Autoclaved	200
3	Maize meal	Jars	100 g	50 ml	Autoclaved	400
4	Soya oil cake	Jars	100 g	85 ml	Autoclaved	400
5	Soya oil cake	Jars	175 g		Cooked	200
6	Soya oil cake	Jars	350 g		Cooked	400
7	Soya oil cake	1 l plastic tub	350 g		Cooked	400
8	Soya oil cake	2 l plastic tub	700 g		Cooked	800
9	Soya oil cake + paper pulp noodles	1 l plastic tub	350 g		Cooked	400

10	Soya oil cake + paper pulp noodles	2 l plastic tub	700 g	Cooked	800
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*Cooked at 90°C for 10 min; decanted; cooled overnight in laminar flow cabinet.

A fourth diet trial was conducted to examine the diet and method of rearing codling moth larvae, used at the Sterile Insect Release (SIR) facility in British Columbia, Canada. The ingredients are listed in Table 3.4.9.3. The paper pulp was soaked in water for 2 h. The first fraction (Table 3.4.9.3) was then placed into a pot and brought to boiling point. Fraction 2 (Table 3.4.9.3) was then added and again brought to boiling. The diet was constantly mixed while it cooked for 7 min. It was then cooled to 65°C, and the last fraction (Table 3.4.9.3) added. The diet was then mixed thoroughly and dispensed in equal portions into two stainless steel trays. The diet was cooled overnight in a laminar flow cabinet. An estimated 3000 eggs were then placed onto the surface of the diet in each container, and containers were covered with flat glass lids. They were kept at 27°C and 50% RH. Regular observations were made on development, survival and the condition of the diet.

Table 3.4.9.3. Diet used for rearing codling moth at the SIR facility in Canada.

Fraction	Ingredient	Quantity
1	Water	7.33 l
	Paper pulp	75 g
	Saw dust	625 g
2	Canola meal	780 g
	Whole wheat flour	400 g
	Sugar	156 g
	Wheat germ	52 g
	Gluten	30 g
	Fumaric acid	47 g
	Choline choride	12 g
	Salt mixture	32 g
	Canola oil	15 ml
3	Nipagin	9 g
	Vitamin mixture	33 g
	Formaldehyde solution (24 ml of 37% in 600 ml water)	3 ml

Before the fourth trial was repeated, it was decided to determine whether canola meal was the most suitable base ingredient or if there was something else more favourable for the larvae. This was done in 350 ml capacity jars. Maize meal, soya flour, canola meal, whole wheat flour, oat meal and oat bran were compared. The recipe used was as described for the first trial under this section. The diets were autoclaved. Each diet was replicated 4 times, and 250 eggs were placed into each jar. A volume of 50 ml of distilled water was added to each jar, except the soya meal and canola meal based diets, to which 85 ml and 65 ml were added, respectively. Once larvae had pupated, pupae were placed into larger jars so that moths could eclose freely. Moths were then counted and sexed.

A sixth diet trial was conducted in order to compare paper pulp and sawdust as bulking and bonding agents (Table 3.4.9.4). Diets were all based on the standard aseptic maize meal diet ((Moore & Richards, 2000; Moore, 2002) described for the first trial under this subheading. Each diet was replicated 5 times. Diets were autoclaved. After cooling, 250 eggs were placed into each container. Numbers of pupae developing in each container were counted.

Table 3.4.9.4. Recipes of diets used in trial to compare paper pulp and sawdust and bulking and bonding agents.

Treatment no.	Diet	Mass of paper pulp/sawdust	Additional nipagin and sorbic acid	Volume of distilled water/jar
1	Standard	-	-	50 ml
2	Standard + paper pulp	1.7 g/jar (3.3% of dry ingredients)	0.01 g nipagin 0.01 g sorbic acid	50 ml
3	Standard + sawdust	18.3 g/jar (28% of dry ingredients)	0.08 g nipagin 0.04 g sorbic acid	85 ml
4	Standard + paper pulp + sawdust	As above	0.1 g nipagin 0.05 g sorbic acid	85 ml

Pupation substrate

Either pupae or adults (moths) can be irradiated for SIT. If pupae are to be irradiated, then a means of collecting the pupae in such a way so as to enumerate and sex them without damaging them, must be developed. Hendrik Hofmeyr developed a lid which could enable this. The lid consists of a double layer of PVC piping (63 mm outer diameter; 53 mm inner diameter; approximately 23 mm depth) with a fine stainless steel gauze mesh heat-impressed to close one end. Corrugated cardboard (approximately 35 mm wide) is rolled up, fairly tightly, and fitted into the piping. This is then inserted into a jar of diet, inoculated with FCM eggs. Final instar larvae could climb up into the corrugations of the cardboard to pupate. Pupae could then be removed and counted. The use of these lids was tested in three trials. In each trial, they were used in 10 jars of diet, each inoculated with 200 eggs. Numbers of pupae collected per jar were compared with 10 jars in which the standard cotton wool stoppers were used.

Results and discussion

Oviposition improvement

The new stainless steel cages proved to be a substantial improvement on the previously used cages, as summarised in Table 3.4.9.5.

Table 3.4.9.5. Comparison between old (organdy net) and new (stainless steel mesh) oviposition cages.

Aspect	Old cage	New cage
Egg production	Very good (Moore <i>et al.</i> , 2002)	No reason to suspect that production is not as good as with the old cage. One problem is that moths seem to be able to oviposit on the outer side of the wax paper more than is observed with the old cage. This makes enumeration of eggs more difficult. This might be remedied by constructing a guide in the cage to eliminate the gap between the paper and the side of the cage.
Oviposition on cage	Wide bars in metal framework provide an alternative oviposition substrate. There is therefore some loss of eggs.	Mesh is too thin to act as an oviposition substrate. There is therefore a reduced loss of eggs relative to the old cage.
Escape of moths	Netting tends to tear, which allows moths to escape.	Initially there was some escaping of moths. This has been remedied by straightening cage edges and reducing width of wax paper slits.
Cage cleaning	When removing and cleaning cages there are always some moths escaping.	Due to the rigidity of the cage, it is possible to prevent moths from escaping when the cage is removed. Cleaning is also easier than is the case with the soft net socks.
Durability	Do not last long. Tend to tear.	Should last for several years without tearing, breaking or rusting.
Moth scales	Tend to escape into the environment of the rearing room, particularly when nets are removed.	Scales fall through the bottom of the cage and are captured by a Perspex sheet. The sheet can be smeared with oil to improve adherence of scales.

Diet improvement

Unfortunately, a level of virus contamination was prevalent throughout most of the treatments in the first diet trial. Considering both duration of development (of larvae from egg to pupation) and production of pupae, soya flour, soya meal and soya oil cake appeared to be the most promising of the diets autoclaved in jars (Table 3.4.9.6). There were not great differences between the numbers of pupae from the jars of uncooked diet. However, the duration of larval development was shorter for the same three diets than for the others (Table 3.4.9.6). In the plastic tubs, there was little or no development from all diets except the soya flour and soya oil cake (Table 3.4.9.6). As the soya flour was a very expensive commercial brand, and as the soya oil cake could be obtained from the mill at a reasonable price, it was decided that further trials with soya should be conducted with this preparation.

Table 3.4.9.6. Development of FCM larvae on different soya based artificial diets, relative to the standard maize meal based diet.

Treat. No.	Base ingredient	Details	Duration of larval development (days)	Number of pupae/ container	Contamination and other observations
1	Maize meal	Jars, autoclaved	15	55.0	Very low level of virus
2	Soya flour		15	69.5	Low level of virus
3	Soya meal		15	83.0	Very low level of virus
4	Soya oil cake		14	74.5	Very low level of virus
5	Full fat soya		18	39.0	Low level of virus
6	Ground soya beans		17	41.0	Low level of virus
7	Soya flour	Jars, uncooked	14	44.0	Low level of virus
8	Soya meal		15	37.5	Low level of virus
9	Soya oil cake		15	44.0	Low level of virus
10	Full fat soya		17	34.5	Very low level of virus
11	Ground soya beans		17	49.5	Low level of virus
12	Soya flour	Plastic tubs, uncooked	16	105	None
13	Soya meal		-	0	None
14	Soya oil cake		16	77	Low levels of virus and fungus. Diet had desiccated and shrunk to the point of being detrimental to larvae (due to foam rubber sponge being too loose).
15	Full fat soya		-	0	None.
16	Ground soya beans		17	3	None

Mean number of pupae recorded per container in the second trial (i.e. 5 plastic tubs of soya oil cake based diet) was 44.0 ± 2.1 (mean \pm SE). Some viral infection of larvae was observed in each container. Very little pupation took place between the cardboard corrugations. Most of the larvae pupated within the diet, as the diet was sufficiently desiccated to be a suitable substrate for pupation. However, the diet appeared a bit too desiccated and might therefore have caused some mortality. If this trial is repeated, the humidity in the room should be increased to slow down desiccation of the diet. The addition of binding and bonding agents (e.g. paper pulp, wood chips) to the diet to assist the diet in retaining structure, should also be considered.

Unfortunately, there was once again an unacceptable level of contamination of larvae and diet in the third diet trial (Table 3.4.9.7). The overall high level of bacterial contamination might have been due to moisture levels being too high. Interestingly, no bacterial contamination was observed in the maize meal based diets. Bacterial contamination was worst in the diets to which paper pulp was added. This probably occurred, as the quantities of anti-microbial agents (i.e. nipagin and sorbic acid) in the diets were not sufficient for the increased total volumes, due to the addition of paper pulp.

Table 3.4.9.7. Development of FCM larvae on different soya oil cake based artificial diets, relative to the standard maize meal based diet.

Treat. No.	Base ingredient	Details	Number of pupae/ container	Virus infected larval cadavers per container	Contamination and other observations
1	Maize meal 1X	Jars, autoclaved	24.5	-	-
2	Soya oil cake 1X		49.0	1.0	Some bacterial and fungal contamination.
3	Maize meal 2X		53.0	11.5	-
4	Soya oil cake 2X		42.5	1.0	Severe bacterial contamination.

5	Soya oil cake	Jars, cooked	45.0	7.0	Diet too moist – appears to be some bacterial contamination.
6	Soya oil cake		92.5	14.0	Diet too moist – no larval penetration into bottom half of diet.
7	Soya oil cake	1 ℓ plastic tub	7	202	-
8	Soya oil cake	2 ℓ plastic tub	19	284	-
9	Soya oil cake + paper pulp noodles	1 ℓ plastic tub	-	-	Severe bacterial infection – no larval development. Probably not sufficient antimicrobial agents (i.e. nipagin & sorbic acid) for the increased total volume due to the addition of paper pulp.
10	Soya oil cake + paper pulp noodles	2 ℓ plastic tub	-	-	

In the fourth diet trial, both trays of Canadian diet looked very good. Diets were well bulked and bonded by the addition of paper pulp and sawdust. As a result, very little shrinking and no cracking of diet took place. There was also no contamination of diet. Unfortunately, a large number of neonate larvae were lost by drowning in moisture which accumulated on the ceiling of the glass lids. Consequently, not many larvae developed in the diet. However, those which developed appeared healthy, and unlike previous trials, there was no sign of viral infection. The Canadian diet therefore appeared very promising. Before testing this diet again, different base ingredients were tested as possible replacements for the canola meal. Simplification of the diet was imperative, as the diet was extremely complex (including certain ingredients which are not available in South Africa) and therefore also expensive.

The fifth diet trial was conducted to determine whether canola meal was the most suitable base ingredient for the Canadian diet, or whether there was something better. Higher numbers of moths were produced on the maize meal, soya flour and oat bran based diets than on the canola meal based diet (Table 3.4.9.8). These differences were not statistically significant. However, this might have been due to the small sample size i.e. only 4 jars per treatment. Rate of development (from egg to pupa) was also more rapid on all diets (except oat meal) than on the canola meal based diet (Table 3.4.9.8). Although rate of development in an ongoing production cycle is immaterial, this could be indicative of the nutritional suitability of the diet. Although diets were autoclaved in this trial, and the Canadian diet is boiled, maize meal, soya flour and oat bran (or possibly a combination of more than one of these) should be tested as a replacement for canola meal in the Canadian diet.

Table 3.4.9.8. Mean number of moths developing per jar per diet, comparing autoclaved diets with different base ingredients.

Treatment no.	Base ingredient	Duration of larval development (to pupation) (Days)	Mean (\pm SE) moths per jar	Females (%)
1	Maize meal	14	78.0b \pm 30.9	55
2	Soya flour	13	65.5b \pm 26.4	55
3	Canola meal	15	46.5ab \pm 8.1	51
4	Whole wheat flour	14	44.5ab \pm 13.3	44
5	Oat meal	15	15.0a \pm 8.1	52
6	Oat bran	13	59.0ab \pm 23.2	44

The addition of sawdust to the Canadian diet (which is boiled, but not autoclaved) appeared to cause no problems for larval development. However, when the diet containing sawdust was autoclaved, no larval development occurred (Table 3.4.9.9). The extreme heat of autoclaving might have somehow caused the development or release of toxins from the sawdust. Larval development on the diet containing paper pulp was lower than that on the standard diet (Table 3.4.9.9). However, this was not significant.

Table 3.4.9.9. Recipes of diets used in a trial to compare paper pulp and sawdust and bulking and bonding agents.

Treatment no.	Diet	Mean (\pm SE) pupae per jar
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1	Standard	41.25a ± 7.65
2	Standard + paper pulp	29.00a ± 5.79
3	Standard + sawdust	0b
4	Standard + paper pulp + sawdust	0b

Pupation substrate

In the first trial there was an unacceptably high level of viral contamination in the jars using the PVC lids. The trial was therefore repeated. In this trial, some escape of larvae, particularly 1st and 2nd instar, was observed. Larval infestation of diet was therefore very low and the trial was terminated. Previously, the PVC lids with cardboard had been used for collecting pupae, by initially inserting cotton wool stoppers into the jars. Only shortly before pupation began, were these replaced with the PVC-cardboard lids. However, this was considered to be far too laborious to be used on a large scale. A new, but similar lid should therefore be designed, replacing the gauze mesh with a denser material, through which larvae cannot pass, and preferably also sufficiently dense to inhibit the introduction of microbial contaminants. It was nevertheless determined, in a non-comparative usage of the PVC-cardboard lids, that mean number of pupae recovered per cardboard roll (from 13 jars) was 53.5 ± 8.9 (SE).

Conclusion

A new stainless steel gauze oviposition cage proved to be a substantial improvement on the previously used cages. Cages were easier to remove and clean, and were more durable and longer lasting. An adaptation to reduce the volume of moth scales escaping into the air, was also introduced.

Soya flour and soya oil cake were identified as the most suitable preparations of soya as base ingredients for the FCM larval diet. Numbers of pupae developing on these diets was generally higher than on diets based on other soya preparations.

The diet used for rearing codling moth at the SIR facility in Canada appeared to be suitable for FCM too. However, in another trial, maize meal, soya flour and oat bran appeared to be more suitable base ingredients for FCM than was canola meal (the base ingredient of the Canadian diet). Sawdust, which is used as a bulking and bonding agent in the diet, prevented larval development when the diet was autoclaved rather than boiled.

Rolls of corrugated cardboard appear to provide an attractive and effective pupation substrate for FCM larvae.

Future research

Research to improve the diet, its preparation and the rearing containers used, will be continued.

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3.4.10 Understanding and improving biological control of false codling moth larvae Experiment 690 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

'n Voorlopige opname van parasitisme van VKM-larwes is maandeliks gedurende die 2001/02-seisoen in die Oos-Kaap, Wes-Kaap en Mpumalanga gedoen. Hierdie opname het gewys dat larwe-parasitoïede dalk doeltreffender is as wat voorheen vermoed is en 'n verdere (weeklikse) opname is gedurende Desember 2003 in die Oos-Kaap uitgevoer. Soos in die 2001/02-seisoen, was *Agathis bishopi* weer eens die enigste

parasitoïed wat in die streek gekry is. In Desember is parasitisme van tot 40% opgemerk, met 'n gemiddeld van 23%. Hierdie opname is nog aan die gang. In 'n moontlike uitbreiding van die studie sal gepoog word om die dominante larwe-parasitoïed in die laboratorium te teel en om aanvullings- en hervestigingsproewe uit te voer.

Introduction

Much emphasis has been placed on studying and exploiting the egg parasitoid of FCM, *Trichogrammatoidea cryptophlebiae*. The next step in advancing the biological control of FCM is to examine the potential for improvement of control of the larval stage. A total of nine larval or egg-larval parasitoids have been identified from FCM on citrus in southern Africa. Six of these species occur in South Africa. Larval parasitoids of FCM have been discussed by Ulyett (1939), CIBC (1984) and Prinsloo (1984). They speculated that, perhaps due to the inaccessibility of the host, they do not seem to be important mortality factors. Ulyett (1939) found that many of the larval parasitoids were poorly distributed and suggested the exchange of parasitoids between the different provinces of South Africa. The distribution, seasonal occurrence and effectiveness of these parasitoids is, however, not sufficiently clear. Knowledge of the natural enemies of a pest species and the control they exert is important when considering commercial control measures. Such a survey may lead to the translocation of one or more species of parasitoid from one area to another or a parasitoid augmentation programme.

A preliminary survey on FCM larval parasitoids was conducted in the Eastern Cape, Western Cape and Mpumalanga from December 2001 to May 2002 (Sishuba *et al.*, 2002; Sishuba, 2003). Two parasitoids were reared from FCM larvae in this study: *Agathis bishopi* and *Apophua leucotretae*. *Agathis bishopi* was the more abundant of the two and appeared to be a valuable parasitoid of FCM on citrus, but was only found in the Eastern Cape Province. *Agathis bishopi* and *T. cryptophlebiae* seemed to compliment each other. *Agathis bishopi* exhibited high parasitism rates early in the season, at a time when *T. cryptophlebiae* was either absent or at very low levels. Egg parasitism increased in the latter part of the season when the larval parasitoid was at low levels. It is interesting, therefore, to speculate on the effect of releasing large numbers of the larval parasitoid in the latter part of the season and the egg parasitoid in the early part of the season, when wild populations of the parasitoids are often low. Because these surveys were only conducted monthly, during 2003 weekly surveys of larval parasitoids were conducted in an Eastern Cape navel orange orchard with consistently high levels of FCM activity. No surveys were conducted during the 2002/03 season as this work was scheduled to be conducted by a post-graduate. Despite advertising, no student was forthcoming.

Materials and methods

An orchard of navel oranges in the Sundays River Valley (orchard 1, Carden Farm) with a reputation for FCM problems was selected for the trial. Ten trees were marked and fruit underneath the trees were removed on 26 November 2003. Weekly, from 3 December, fruit that had dropped from the 10 trees was collected and taken back to the laboratory, where they were dissected and inspected to determine the cause of drop. FCM infestation was recorded. Care was taken when dissecting fruit, to not damage any live FCM larvae. Larvae were removed from fruit and transferred to glass vials containing plugs of the artificial diet, used for rearing FCM (Moore, 2002; Moore & Richards, 2001). A tightly fitting cotton wool plug was inserted into the opening of each glass vial. The life stage of the larva in each vial was recorded on the vial. These were monitored daily for parasitoid emergence. Parasitoids were identified and the life stage from which the parasitoid emerged was recorded.

Results and discussion

During the first 3 weeks that the trial was conducted, FCM infestation was high (peaking at 3.7 infested fruit per tree per week on the third week) (Table 3.4.10.1). Infestation declined during the following 2 weeks. Consequently the number of larvae collected also declined. Over 5 weeks, during the month of December (2003), a total of 113 larvae were collected (Table 3.4.10.1), of which most were 2nd and 3rd instars.

Table 3.4.10.1. FCM infestation of navel oranges collected from four study sites.

Collection date	Number of fruit collected from 10 data trees	Number of fruit infested	Number of each larval instar					Total number of larvae collected
			1 st instar	2 nd instar	3 rd instar	4 th instar	5 th instar	
03/12	295	32	0	1	8	2	1	12

10/12	220	22	0	7	5	0	0	12
17/12	127	37	1	2	5	2	3	13
23/12	94	12	0	1	2	1	0	4
30/12	49	10	1	1	0	1	1	4
Total	785	113	2	12	20	6	5	45

In total, over December 2003, 23.1% of larvae (excluding larvae which died inexplicably) were parasitised (Table 3.4.10.2). All parasitoids were identified as *Agathis bishopi*. This was also the only species of larval parasitoid found attacking FCM in the Eastern Cape during the 2001/02 season (Sishuba, 2003; Sishuba *et al.*, 2002). The highest level of parasitism (40%) was recorded on 10 December (Table 3.4.10.2). This coincided with findings two seasons ago, when the highest level of parasitism (37%) was recorded on 11 December (Sishuba, 2003; Sishuba *et al.*, 2002).

Table 3.4.10.2. Parasitism of the different FCM larval instars and parasitoid sex ratios.

Collection date	% Larvae parasitised (excluding larvae which died)	Number of each larval instar parasitised				
		1st	2nd	3rd	4th	5th
03/12	28.6	0	0	2	0	0
10/12	40.0	0	1	1	0	0
17/12	12.5	1	0	0	0	0
23/12	33.3	0	0	0	1	0
30/12	0	0	0	0	0	0
Total	-	1	1	3	1	0
% larvae parasitised	23.1	50.0	8.3	15.0	16.7	0

Conclusion

During the second season that a survey of larval parasitoids of FCM was conducted in the Eastern Cape, *Agathis bishopi* was once again the only parasitoid found. Over the month of December (2002), parasitism peaked at 40% and averaged 23%.

Future research

This survey described in this experiment is still ongoing. A possible follow on from this will be to laboratory rear the dominant parasitoid in order to conduct augmentation and translocation trials.

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3.4.11 Ovipositional preferences and relative susceptibility amongst navel orange varieties by FCM Experiment 705 by Sean D. Moore and Garth I. Richards (CRI)

Opsomming

Die eierleggingsvoorkeure van VKM vir 'n reeks van kommersiële en eksperimentele nawelmoenvariëteite is in die laboratorium getoets. Die vatbaarheid van hierdie variëteite vir VKM is in beide laboratorium- en boordproewe ondersoek.

In eierleggingsvoorkeurproewe is bepaal dat vrugkleur die dominante aantrekkingsfaktor vir die motte is. Nawelendgrootte beïnvloed motte blykbaar op 'n manier motte om eiers op vrugte te lê, al is die vrugte nog relatief groen. Dit was veral met Tulegold die geval gewees. In twee vrugvatbaarheidsproewe wat in die laboratorium uitgevoer is, is Chislett en Newhall min deur VKM besmet. Tulegold en Lina was onder die vier mees besmette variëteite in albei proewe. In boordproewe is die hoogste vlak van besmetting in Lina, Chislett, Tulegold, Autumn Gold en Cara-Cara gekry. Die laagste vlakke van besmetting was in Cambria, Rustenburg, Newhall en Bahiahnina.

Oor die algemeen lyk dit asof Tulegold die vatbaarste nawelmoenvariëteit vir VKM-besmetting is. Dit word deur Lina gevolg. Cambria, Rustenburg en Newhall is blykbaar die minste gevoelig van die variëteite wat getoets is. Hierdie inligting kan vir produsente van belang wees in hul keuse van nawelmoenvariëteite.

Geen verdere werk word beplan nie.

Introduction

False codling moth is potentially the most destructive member of the southern African citrus pest complex. It is also extremely difficult to control with conventional methods. Consequently it is regarded as the number one insect pest in the industry. Experience in the Western Cape has shown certain navel orange types to be almost as susceptible to infestation as are some soft citrus varieties (Hendrik Hofmeyr, personal communication). In the Eastern Cape, navel oranges appear to be the most susceptible cultivar to FCM attack. Because navel oranges are one of the most widely planted of all cultivars in southern Africa, particularly in the southern regions, it is imperative to consider susceptibility to FCM when selecting which variety to plant. More than a decade ago a preliminary ovipositional preference study was conducted with five different selections (some of them rather obscure), showing significant differences (Newton, 1990). In this experiment we propose to compare the FCM ovipositional preference to – and susceptibility of – as many different commercially popular and experimental navel orange varieties as possible.

Materials and methods

On 9 May 2002, 50 fruit were picked of each of 14 different navel orange varieties (Table 3.4.11.1), grown at the Citrus Foundation Block (CFB) in the Eastern Cape. The fruit diameter, navel diameter and colour rating (Anonymous, 1995) of each of 12 fruit were measured and mean values calculated (Table 3.4.11.1).

Table 3.4.11.1. Navel orange varieties used in laboratory trials to compare FCM ovipositional preference and host suitability (2002).

Variety	Abbreviation	Mean fruit diameter	Mean colour plate standard	Mean navel diameter
Palmer	PN	74.30	6.95	3.45
Washington	WN	70.20	6.25	3.60
McLean	MN	72.20	5.50	1.27
Lina	LN	70.70	5.25	3.62
Bahianihna	BN	74.15	5.95	0.45
Rustenburg	RUN	69.05	7.20	0.95
Tulegold	TN	74.95	5.30	3.96
Newhall	NEN	75.25	4.85	6.22
Cara Cara	CCN	72.65	6.25	4.24
Cambria	NCA	67.55	7.80	2.20
Chislett	CSN	69.25	7.40	4.10
Powell Summer	PSN	71.70	7.35	4.21
Dream	NDR	74.50	6.60	4.86
Autumn Gold	AGN	69.10	7.00	3.26

On 3 June 2003, another 42 fruit of the same 14 navel orange varieties (Table 3.4.11.2) were picked at the CFB, and the same measurements made, as during the previous season.

Table 3.4.11.2. Navel orange varieties used in laboratory trials to compare FCM ovipositional preference and host suitability (2003).

Variety	Abbreviation	Mean fruit diameter	Mean colour plate standard	Mean navel diameter
Palmer	PN	73.81	5.25	7.90
Washington	WN	66.53	4.20	4.82
McLean	MN	68.87	5.80	3.76
Lina	LN	71.06	4.95	5.83
Bahianihna	BN	72.51	6.10	3.13
Rustenburg	RUN	63.26	7.05	3.02
Tulegold	TN	81.76	4.20	13.80
Newhall	NEN	72.69	4.70	5.03
Cara Cara	CCN	70.87	4.75	8.92
Cambria	NCA	67.55	6.90	2.72
Chislett	CSN	73.68	6.65	7.63
Powell Summer	PSN	75.66	5.90	6.39
Dream	NDR	74.49	5.20	7.17
Autumn Gold	AGN	71.15	6.85	7.67

Ovipositional preference

Six fruit from each variety were mixed-up and randomly placed into a large organdy net-covered moth cage (1 m x 330 mm x 325 mm). Approximately 100 moths which had eclosed within the previous 24 h were released into the cage. Approximately hourly, random selections of fruit were inspected for FCM eggs. After several hours, once inspection revealed that eggs had been oviposited on most of the fruit, all fruit were removed from the cage. The remaining 6 fruit of each variety were then placed randomly into the cage and fruit were later removed, as previously explained. Numbers of eggs laid on each fruit were counted. Fruit with eggs were retained in order to determine penetration and development of hatching larvae. This could possibly serve as an additional test of host suitability. Mean values for each variety were calculated. This trial was conducted in May 2002 and repeated in June 2003.

Host susceptibility

During May 2002, varietal suitability to FCM was tested by placing two neonate larvae (using a size 000 paintbrush) onto each of 38 fruit of each variety. Varieties were kept separately at 27°C and approximately 60% RH. Two weeks after surface inoculation with larvae, fruit were inspected both externally (for signs of attempted penetration) and internally (for successful penetration). Mean values for each variety were calculated. This trial was repeated during June 2003 with 30 fruit per variety.

Field infestation

For an 8-week period from 27 February to 10 April 2003, 5 trees of each of 14 different navel orange varieties were monitored for FCM infestation. Each fortnight, fruit which had dropped under each tree was collected and evaluated for cause of drop. In particular, FCM infestation (larvae or frass) was recorded. Mean number of infested fruit per tree per variety was determined for the 8-week evaluation period.

Statistical analysis

Mean values for egg numbers, penetration marks and larval infestation, for each variety, were compared by using Bonferroni LSD multiple range tests, after analysis by ANOVA. Regression analyses were conducted to determine whether there were correlations between eggs per fruit (attractiveness of fruit to moths) or larval infestation, and fruit size, fruit colour and navel size.

Results and discussion

Ovipositional preference

A regression analysis revealed that there was no statistical relationship between egg numbers and fruit size or navel size. However, the relationship between eggs per fruit and fruit colour could be considered significant ($r^2 = 24.3\%$; $P < 0.07$). In support of the attractiveness of colour to FCM as an oviposition substrate, the only 5 varieties which had a mean colour plate rating (Anonymous, 1995) of over 7 (Table 3.4.11.1) were amongst the 6 varieties with the most 127 eggs per fruit (Table 3.4.11.3). The other variety

(Dream) amongst the top 6 had a mean colour plate standard of 6.6 (Table 3.4.11.1), being the seventh most “coloured up” of all varieties.

Table 3.4.11.3. Mean numbers of FCM eggs, penetration marks and larvae per fruit for each of 14 different navel orange varieties (2002).

Variety	Fruit with eggs (%)	Mean eggs/fruit*	Mean penetration marks/fruit*	Mean number of larvae/fruit*
Palmer	50.0	4.91a	3.25ab	0a
Cara Cara	58.3	5.25a	1.33a	0.17ab
Washington	83.3	6.50ab	1.42a	0.17ab
McLean	75.0	6.83ab	3.25ab	0.83ab
Tulegold	50.0	8.67ab	1.25a	0.08ab
Bahianihna	83.3	9.08abc	1.58a	0.58ab
Newhall	83.3	9.08abc	2.41ab	0.91ab
Lina	83.3	10.08abc	1.58a	1.25b
Chislett	75.0	10.75abc	2.00ab	0.33ab
Autumn Gold	91.6	11.41abc	3.17ab	0.17ab
Dream	83.3	11.58abc	3.58ab	0.91ab
Rustenburg	91.6	12.17abc	5.58b	0.58ab
Cambria	83.3	14.17bc	5.83b	0.25ab
Powell Summer	81.8	20.45c	3.90ab	0.72ab

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

No correlation could be established between the number of eggs per fruit and fruit colour, fruit size or navel size, with data from the 2003 trial. However, there was still substantial evidence to indicate the attractiveness of colour to moths. The lowest numbers of eggs per fruit were counted for Cara Cara, Washington and Newhall. Apart from Tulegold, these varieties had the greenest fruit, all with a mean colour plate rating (Anonymous, 1995) of well below 5 (Table 3.4.11.2). The highest numbers of eggs per fruit were counted for Autumn Gold, Chislett, Cambria, Palmer and Tulegold (Table 3.4.11.4). The first of these 3 listed were amongst the 4 varieties with the highest mean colour plate ratings (Table 3.4.11.2). The obvious exception to this trend of relating egg numbers to colour, was Tulegold. Tulegold fruit were the greenest (Table 3.4.11.2) and yet they had the highest mean number of eggs. This might be related to the fact that the mean diameter of the navels of the Tulegold fruit was far larger than that of any other fruit (Table 3.4.1.2). Bahianihna are reputed to be less affected by FCM than most other navel orange varieties (Newton, 1990). In a detached fruit bioassay to test the FCM granulovirus, Rustenburg navels were observed to be relatively unsusceptible to FCM. Both of these cultivars have very small navel ends (Tables 3.4.11.1 & 3.4.11.2).

Table 3.4.11.4. Mean numbers of FCM eggs, penetration marks and larvae per fruit for each of 14 different navel orange varieties (2003).

Variety	Fruit with eggs (%)	Mean eggs/fruit*	Mean penetration marks/fruit*	Mean number of larvae/fruit*
Cara Cara	75.0	2.33a	0.25a	0.25ab
Washington	33.3	2.42a	0.42abc	0.33abc
Newhall	66.7	2.58a	0.33ab	0.08a
Bahianihna	66.7	3.08a	0.75abc	0.17a
Dream	75.0	3.33a	0.50abc	0.25ab
Powell Summer	50.0	4.17a	0.58abc	0.33abc
Rustenburg	83.3	4.33a	0.92bc	0.42abc
McLean	75.0	4.75a	0.67abc	0.33abc
Lina	58.3	5.00a	0.75abc	0.50abc
Autumn Gold	75.0	5.92a	0.75abc	0.50abc
Chislett	91.7	6.42a	1.00c	0.75c
Palmer	75.0	6.50a	0.42abc	0.08ac
Cambria	58.3	6.58a	1.00c	0.67bc
Tulegold	66.7	7.25a	0.92bc	0.42abc

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

Host susceptibility

In these trials, attractiveness of the fruit to FCM was not an issue. Only susceptibility of fruit to FCM infestation was measured. In the 2002 trial, only Newhall, Cambria and Chislett had no infestation (Table 3.4.11.5). Tulegold, McLean, Autumn Gold and Lina were the most heavily infested. No correlation could be established between levels of infestation and fruit colour, fruit size and navel diameter.

Table 3.4.11.5. FCM induced-damage to, and FCM infestation of, 14 different navel orange varieties (2002).

Variety	Fruit decaying (%)	Mean penetration marks/fruit*	Fruit infested (%)	Mean number of larvae/fruit*	Mean larval instar
Cambria	7.9	0a	0.0	0a	-
Chislett	18.4	0a	0.0	0a	-
Newhall	36.8	0.026a	0.0	0a	-
Cara Cara	42.1	0.053a	2.6	0.026a	2.0
Dream	39.5	0.158a	2.6	0.026a	2.0
Palmer	21.0	0.026a	2.6	0.026a	3.0
Washington	28.9	0.053a	2.6	0.026a	3.0
Bahianihna	42.1	0.132a	2.6	0.026a	3.0
Rustenburg	18.4	0.037a	5.3	0.053a	2.5
Powell Summer	7.9	0.079a	5.3	0.053a	3.0
Lina	42.1	0.079a	7.9	0.079ab	2.0
Autumn Gold	15.8	0.105a	7.9	0.079ab	4.0
McLean	36.8	0.132a	10.5	0.105ab	2.25
Tulegold	36.8	0.316	23.7	0.237b	3.0

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

In the 2003 trial, Chislett, Rustenburg, Palmer and Newhall, were the varieties with the lowest levels of FCM infestation (Table 3.4.11.6). Washington, Tulegold, Bahianhina and Lina were the most heavily infested varieties. Once again, no correlation could be found between infestation of fruit and fruit measurements listed (Table 3.4.11.2).

Table 3.4.11.6. FCM induced-damage to, and FCM infestation of, 14 different navel orange varieties (2003).

Variety	Fruit decaying (%)	Mean penetration marks/fruit*	Fruit infested (%)	Mean number of larvae/fruit*
Chislett	50.0	0.60ab	20.0	0.23a
Rustenburg	36.7	0.40a	26.67	0.27ab
Palmer	50.0	0.60ab	33.33	0.37ab
Newhall	20.0	0.57ab	36.67	0.40ab
Powell Summer	23.3	0.63abc	40.0	0.40ab
Cara Cara	53.3	0.63ab	36.67	0.43ab
McLean	50.0	0.67abc	40.00	0.43ab
Autumn Gold	46.7	0.53ab	33.33	0.47ab
Cambria	30.0	0.67abc	46.67	0.53abc
Dream	30.0	0.57ab	43.33	0.53abc
Lina	43.3	0.73abc	53.33	0.53abc
Bahianihna	16.7	0.70abc	46.67	0.60abc
Tulegold	58.8	0.94bc	35.29	0.65bc
Washington	30.0	1.00c	60.00	0.87c

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

In both of the fruit susceptibility trials, Chislett and Newhall had little or no infestation. Tulegold and Lina were amongst the four most heavily infested varieties in both trials. Due to the variability in results it was important that orchard inspections of levels of FCM infestation in the different varieties were also conducted.

Field infestation

Over the 8-week period that the trial was monitored, the highest mean level of infestation per tree per week was found for Lina, Chislett, Tulegold, Autumn Gold and Cara-Cara (Table 3.4.11.7). The results with Lina, Tulegold and Autumn Gold confirmed those obtained in the fruit susceptibility laboratory trials. However, Cara-Cara was not heavily infested in the laboratory trials and Chislett was the most lightly infested variety in both laboratory trials. The level of infestation recorded for these two varieties in the field is therefore a little divergent.

The lowest levels of infestation were recorded for Cambria, Rustenburg, Newhall and Bahiannina (Table 3.4.11.7). All but the last mentioned of these four varieties were also of the most lightly infested fruit in at least one (if not both) of the laboratory trials. Bahiannina was on average, relatively moderately infested in the two laboratory trials (Tables 3.4.11.x & 3.4.11.x). However, this variety is regarded to be one of the least susceptible to FCM (Newton, 1990).

Table 3.4.11.7. FCM infestation of 14 different navel orange varieties at the Citrus Foundation Block during 2003.

Variety	Fruit infested/tree/fortnight				Total fruit infested/tree
	27 Feb	13 March	27 March	10 April	
Lina	0.80	0.20	3.20	0.40	4.6
Chislett	2.60	0	0.60	0.20	3.4
Tule Gold	2.25	0	0.75	0	3.0
Autumn Gold	1.00	1.00	0.33	0.67	3.0
Cara-Cara	1.80	0.40	0.80	0	3.0
McClellan	0.40	0	1.80	0	2.2
Palmer	0.40	0	0.80	0.60	1.8
Washington	0	0.20	0.75	0.20	1.15
Dream	0.50	0	0.50	0	1.0
Powell Summer	0.80	0	0.20	0	1.0
Bahiannina	0.40	0	0.20	0.20	0.8
Newhall	0	0	0.80	0	0.8
Rustenburg	0	0	0.40	0	0.4
Cambria	0	0	0	0	0

Conclusion

In oviposition preference trials conducted in the laboratory, it was determined that fruit colour was the predominant factor attracting moths to oviposit on the fruit. However, it appeared that a large navel size could somehow influence moths to preferentially oviposit on fruit, even if the fruit was comparatively green. This was observed to be the case with Tulegold. In both of the fruit susceptibility trials conducted in the laboratory, Chislett and Newhall had little or no infestation. Tulegold and Lina were amongst the four most heavily infested varieties in both trials. In field trials, the highest level of infestation was found in Lina, Chislett, Tulegold, Autumn Gold and Cara-Cara. The lowest levels of infestation were recorded for Cambria, Rustenburg, Newhall and Bahiannina.

Overall, it therefore appears that Tulegold is the most susceptible navel orange variety to FCM attack, of those tested. This is closely followed by Lina. Cambria, Rustenburg and Newhall appear to be the least susceptible of the varieties tested. This information can assist growers in selecting which navel orange varieties to plant.

Future research

No further work is planned on this experiment.

References cited

- Anonymous. 1995. *Colour prints for blemish standards*. Outspan International, South Africa.
Newton, P.J. 1990. Ovipositional preferences amongst navel sweet orange types by the false codling moth, *Cryptophlebia leucotreta*. *Annals of Applied Biology* 116: 143-150.

3.4.12 **Behandeling van valskodlingmotlarwes met gammabestraling as metode vir die disinfestasië van verpakte sitrusvrugte**

Proef 719 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

Various methods such as treatment with ozone, controlled atmosphere, fumigation and cold were previously investigated for the disinfestation of citrus fruit for false codling moth. Due to a variety of reasons none of these methods were suitable. An introductory experiment was therefore conducted to investigate the sensitivity of three different age groups of false codling moth larvae to gamma radiation.

100, 200 and 300 Gy of radiation were evaluated against 3rd to 5th instar FCM larvae in culture jars. A large percentage of all ages of irradiated larvae died before pupation. Only 3% to 5% of the remaining pupae in the 100 Gy treatment emerged as moths. No pupae in the 200 Gy and 300 Gy treatments eclosed as adults.

Gamma radiation therefore seems a likely candidate for the disinfestation of fruit for FCM and should be further investigated.

Inleiding

Dit is om 'n verskeidenheid van redes onprakties, indien nie onmoontlik nie, om VKM so goed in die boord te bestry dat dit geen fitosanitiëre bedreiging is wanneer die vrugte uitgevoer word nie. Die aard van VKM bemoeilik bestryding om 'n verskeidenheid van redes. Daarbenewens verhoed etlike veranderlike faktore, soos spuitdoeltreffendheid, verkeerde bestrydingsprogramme, wisselende weerstand teen insekdoders, ens., dat geregistreerde insekdoders doeltreffend op alle vrugte in 'n boord toegedien kan word. Dit geld vir alle behandelings soos lowerbespuiting met insekdoders en selfs ook ongewone metodes soos paringsontwrigting.

Die enigste uitweg is daarom om ge-oeste vrugte voor of na verpakking te behandel en die larwes in die vrugte te dood. Alle náverpakkingstegnieke wat voorheen ondersoek is om VKM te dood, was egter óf ondoeltreffend, óf was nadelig vir vrugkwaliteit. Tegnieke soos osoon en beheerde atmosfeer was ondoeltreffend, terwyl berokking met metielbromied onder andere die raklewe van verpakte vrugte beduidend verkort het. Subzero-verkoeling word alreeds etlike jare lank met sukses gebruik om VKM-larwes in lemoene te dood. Dié temperatuur is egter potensieel skadelik aangesien die skil van verpakte vrugte beskadig kan word.

Gammabestraling met 'n kobaltbron word alreeds internasionaal gebruik om Mediterreense vrugtevlieglarwes in vrugte te dood. Navorsing is ook alreeds op verwante spesies, *Cryptophlebia batrochopa* en *C. illepidata*, in Hawaii uitgevoer (Follet en Lower, 2000). Dié tegniek skep die moontlikheid dat vrugte op vervoerbande in 'n pakhuis vóór verpakking deur middel van 'n liniëre versnellerbron, of ná verpakking met 'n kobaltbron bestraal sal kan word.

'n Inleidende proef is uitgevoer om vas te stel of water invloed gammabestraling op VKM-larwes van verskillende ouderdomme het.

Materiale en metodes

Teelflesse met onderskeidelik 5 mm, 10 mm en 15 mm lange larwes is gebruik. Die ouderdomme van die onderskeie groottes is vasgestel deur 100 larwes ewekansig uit verteenwoordigende teelflesse te verwyder en kopkapsulemetings uit te voer. Sodoende is vasgestel dat die larwe-groottes met derde, vierde en vyfde (laaste) instar VKM-larwes ooreenstem. Drie teelflesse per larwe-ouderdom is vervolgens met 100, 200 en 300 Gy bestraling in 'n panoramiese gammabestralingsbron te Infruitec, Stellenbosch, behandel. Die teelflesse is na bestraling by 26°C gehou sodat die larwes kon ontwikkel.

Die teelflesse word normaalweg met gevormde watterbolle toegeprop. Wanneer volwasse vyfde instar larwes gereed is om te verpop, spin hulle kokonne in dié proppe. Dit is uiters arbeidsintensief om honderde sulke kokonne oop te knip om die papies te verwyder. Gereelde waarnemings is derhalwe uitgevoer en die watterproppe is met riffelkartonproppe vervang toe die eerste larwes in die kontrole-behandeling (0 Gy) begin verpop het. Papies word veel makliker uit dié riffelkartonstroke verwyder.

Om waarneming moontlik te maak is alle papies uit die riffelkarton verwyder en individueel in 5 ml pilbottels met sponsproppe geplaas totdat ontpopping₁₃₁ afgehandel was. Alle papies en motte is getel.

Resultate en bespreking

Kopkapsulemetings het gewys dat daar larwes van hoogstens twee verskillende ouderdomme in die teelflesse was. 75% tot 95% van die larwes in die drie groepe teelflesse was egter van dieselfde ouderdom (Fig. 3.4.12.1). Eiers wat in die teelflesse geplaas word, kan tot 24 uur in ouderdom van mekaar verskil. Dít, asook die wisselende tempo waarteen die insekte ontwikkel, is waarskynlik vir die verskille verantwoordelik.

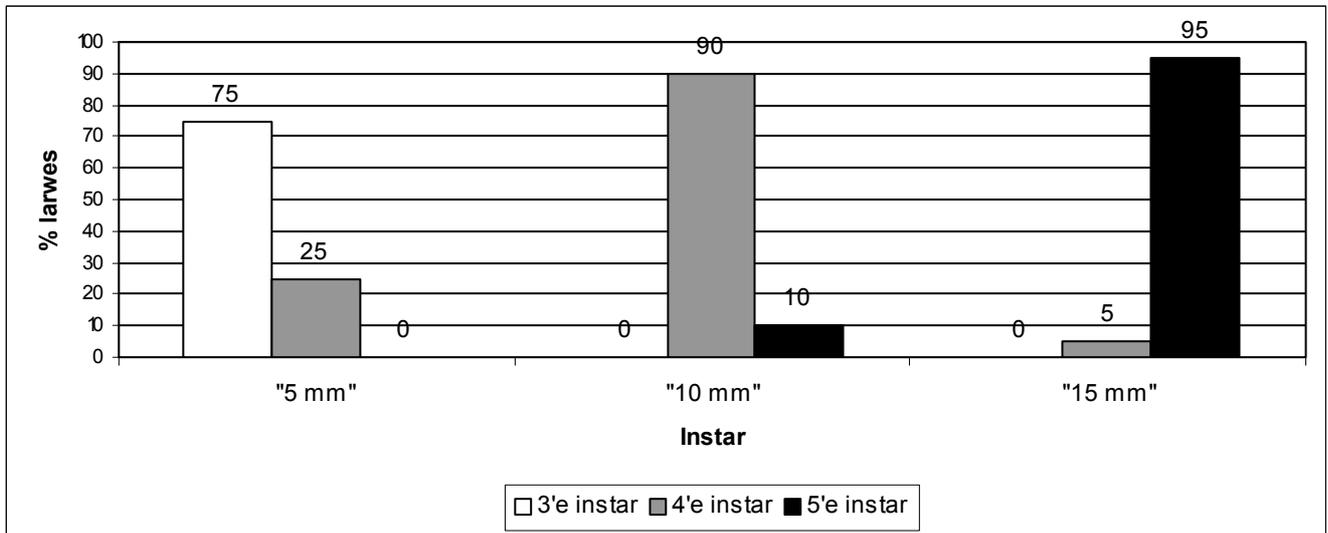


Fig. 3.4.12.1. Ouderdomme van valskodlingmotlarwes in die teelflesse wat met gammabestraling behandel is.

Die bestraalde larwes se ontwikkeling is ernstig benadeel. Daar kan van die relatief geringe aantal larwes wat in staat was om tot papies te ontwikkel, afgelei word dat heelwat van hulle deur die bestraling gedood is (Tabel 3.4.12.1). Daar kan nie vasgestel word hoe gou na bestraling dit was nie, aangesien hulle in die kultuurmedium in die teelflesse was en nie ondersoek of getel kon word nie.

Tabel 3.4.12.1. Aantal motte wat ontwikkel het nadat valskodlingmotlarwes van verskillende ouderdomme met gammabestraling behandel is.

Ontwikkelings stadium van bestraalde larwes (instar)	Bestralingsdosis (Gy)	Aantal papies wat uit bestraalde larwes ontwikkel het	Aantal motte wat uit oorlewende papies ontwikkel het
3'de	0	671	611
	100	293	20
	200	0	-
	300	0	-
4'de	0	802	728
	100	473	39
	200	2	0
	300	0	-
5'de	0	799	746
	100	254	36
	200	19	0
	300	7	0

Soos verwag kan word, is al drie ouderdomme larwes die minste deur die laagste bestralingsdosis aangetas. 'n Groter persentasie 4'de instar larwes het die 100 Gy bestraling oorleef (Fig. 3.4.12.2); dit is tans onbekend of dit 'n eksperimentele fout is en of daardie betrokke ouderdom larwes meer bestralingsbestand is. Laasgenoemde is hoogs onwaarskynlik aangesien die bestralingsweerstand van verskillende ontwikkelingsstadia by insekte gewoonlik toeneem van eier tot volwassene. In enige betrokke ontwikkelings stadium is die jongste individue gewoonlik ook minder bestand teen bestraling as die oueres. Dié verskynsel is in die proef waargeneem waar enkele 4'de en 5'de instar larwes wat onderskeidelik met 200 Gy en 300 Gy bestraal was, oorleef het en in papies ontwikkel het (Tabel 3.4.12.1).

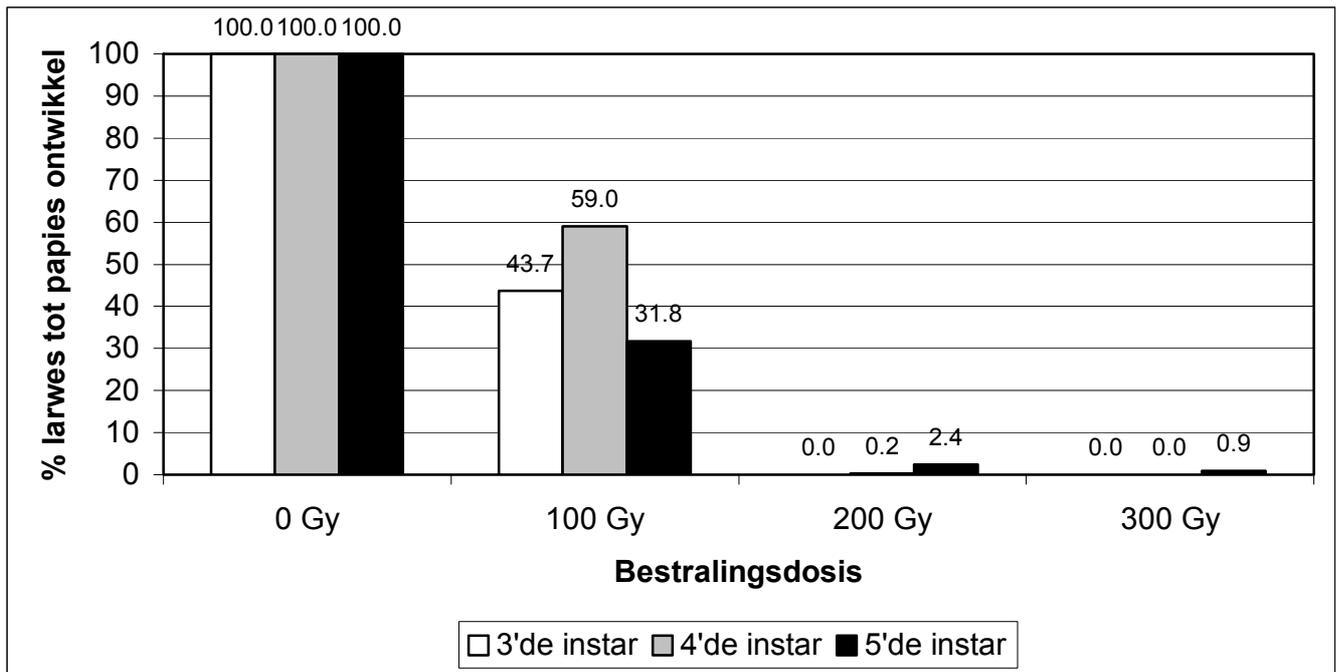


Fig. 3.4.12.2. Persentasie valskodlingmottlarwes wat verskillende bestralingsdosisse oorleef het en in staat was om in papies te ontwikkel.

Baie larwes van al drie ouderdomme wat bestraal is, het in die papiestadium gevrek (Tabel 3.4.12.1). Slegs 3% tot 5% van die papies in die 100 Gy behandeling het in motte ontwikkel (Fig. 3.4.12.3). Dié motte was deurgaans erg misvorm en die meeste kon nie vlieg nie. Alhoewel dit nie in dié proef ondersoek is nie, sou hulle hoogs waarskynlik almal steriel gewees het.

Daar is 'n duidelike toename in die weerstand van larwes soos hulle ouer word. Desnieteenstaande was geen 3'de tot 5'de (finale) instar larwe wat minstens 200 Gy ontvang het, in staat om tot 'n mot te ontwikkel nie. Daar kan dus aanvaar word dat alle 1'ste en 2'de instar larwes nog sensitiewer vir dié dosis sal wees en geen ondersoek benodig nie.

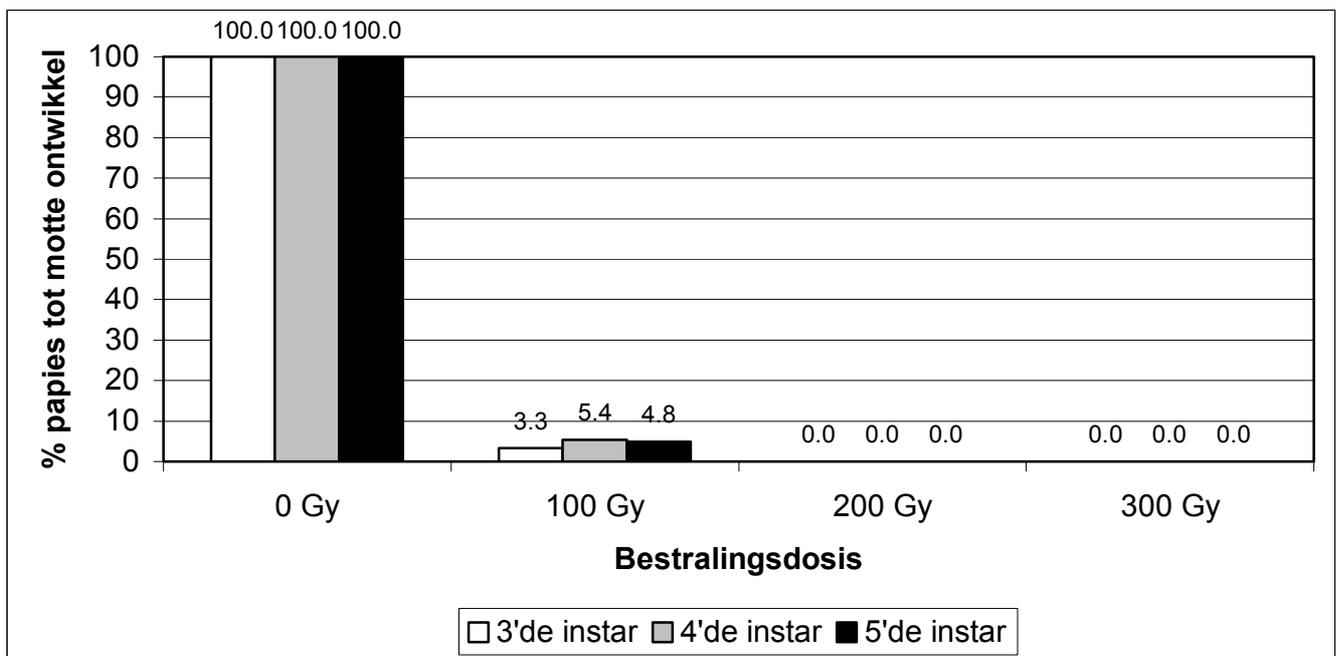


Fig. 3.4.12.3. Persentasie valskodlingmotpapies wat daarin geslaag het om in motte te ontwikkel.

Uit 'n kommersiële oogpunt sal dit uiteraard wenslik wees om 'n behandeling te vestig wat alle larwes in bestraalde vrugte sal dood sodat inspekteurs nie oor die doeltreffendheid van die behandeling hoef te twyfel

nie. Daar is nog nie vasgestel watter bestralingsdosis alle ouderdomme larwes onmiddellik, of slegs binne enkele ure na bestraling, sal dood nie, maar dit sal beslis heelwat hoër as 300 Gy wees. So 'n hoë dosis kan vrugkwaliteit benadeel. Dit is tans onbekend watter vlak van doeltreffendheid deur byvoorbeeld die VSA aanvaar sal word. Daar is tekens dat doeltreffendheidsdata aanvaar sal word wat nie noodwendig op larwedoding gebaseer is nie. Oortuigende bewys sal egter gelewer moet word dat die behandeling tot gevolg sal hê dat enige oorlewende larwes in die daaropvolgende papie- of motstadium sal vrek. Alhoewel dit seker, maar onbewese, is dat die motte wat uit bestraalde larwes ontwikkel, steriel sal wees, sal dié tipe inligting waarskynlik nie aanvaarbaar wees nie.

Gevolgtrekking

Die data wat ingewin is is uiters belowend en sal in verdere soortgelyke proewe met larwes in teelflesse en in lermoene opgevolg word. Dit sal raadsaam wees om die USDA se mening oor die voorgenome proefprotokol te verkry voordat die navorsing begin.

Literatuurverwysing

Follet, P. A., and R. A. Lower, 2000. Irradiation to Ensure Quarantine Security for *Cryptophlebia* spp. (Lepidoptera: Tortricidae) in Sapindaceous Fruits from Hawaii. *J. Econ. Entomol.* 93(6): 1848-1854.

3.4.13 Evaluasie van Triflumuron 480 SC vir die bestryding van valskodlingmot Proef 733 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

An alternative triflumuron product, Triflumuron 480 SC, was evaluated against false codling moth in an orchard experiment. With and without the additive Booster Oil, Triflumuron performed as well as the registered product, Alsystin. The addition of Booster Oil did not enhance the effectiveness of Triflumuron or Alsystin to a significant degree.

Inleiding

Relatief teleurstellende resultate is gedurende 2002 in laboratoriumproewe verkry met Triflumuron, 'n alternatiewe produk vir Alsystin, wat ook die aktiewe bestanddeel triflumuron bevat. Formulasieprobleme is vermoedelik met die produk ondervind, wat 'n dik, moeilik-oplosbare neerslag in die bottel tot gevolg gehad het. Dié neerslag het waarskynlik grotendeels uit die aktiewe bestanddeel bestaan en kon vir die swak resultaat verantwoordelik gewees het. Die verspreiders het gevolglik besluit om die produk te laat herevalueer. 'n Nuwe voorraad insekdoder is verskaf en 'n boordproef is vervolgens onder kontrak uitgevoer.

Materiale en metodes

Uitleg: Die proef is op die plaas Rivierplaas naby Citrusdal op 18-jaarou Palmer-nawelbome uitgevoer. 'n Ewekansige blokontwerp met 10 herhalings per behandeling is gebruik. Elke herhaling het uit 'n enkele boom bestaan wat ewekansig in elk van 10 blokke toegeken is. Geen skutbome tussen behandelings is gebruik nie.

Toediening van insekdoders: Alle spuitbehandelings is een keer op 5 Maart 2003, as hoë druk, matige dekbespuitings met verstelbare handspuite, gekoppel aan 'n 2 000 l spuitenk, toegedien. Die lower en vrugte binne en buite elke boom is deeglik tot op die punt van afloop natgespuit. 35 liter spuitmengsel is gemiddeld per boom toegedien. Geen reën het binne 72 uur na toediening geval nie.

Evaluasie: Doeltreffendheid van die verskillende behandelings is geëvalueer deur alle afvalvrugte onder die databome vyf weke lank vanaf vier weke na behandeling tot oestyd bymekaar te maak. Die vrugte is oopgesny en vir simptome van VKM-besmetting ondersoek. 'n Vrug met 'n lewendige of dooie larwe, of larwale uitwerpsels, is as besmet beskou.

Doeltreffendheid: Die vrugval in 'n betrokke behandeling is met 'n onbehandelde kontrole vergelyk. Daarbenewens moes die totale vrugval in 'n behandeling ook minder as die ekonomiese drempelwaarde (minder as gemiddeld een besmette vrug per boom per week) wees.

Behandelings: Triflumuron en Alsystin is beide teen 10 ml produk per hl water getoets. Beide produkte is ook op versoek getoets in mengsels met 'n bymiddel van die verspreiders, "Booster Oil".

Die behandelings en resultate word in Tabel 3.4.13.1 verstrek.

Resultate en bespreking

Die VKM-besmetting in die kontrole van die proefperseel het tot week 3 toegeneem, waarna dit afgeneem het (Fig. 3.4.13.1). Vrugval in die spuitbehandelings het dieselfde neiging getoon. Die gemiddelde besmetting op die onbehandelde kontrolebome het die ekonomiese drempelwaarde oorskry (Tabel 3.4.13.1). Die skade in al die insekdoder-behandelings was minder as die drempelwaarde en kan onder die betrokke besmettingstoestande wat geheers het, as doeltreffend beskou word. Daar was geen betekenisvolle verskille tussen die verskillende spuitbehandelings nie.

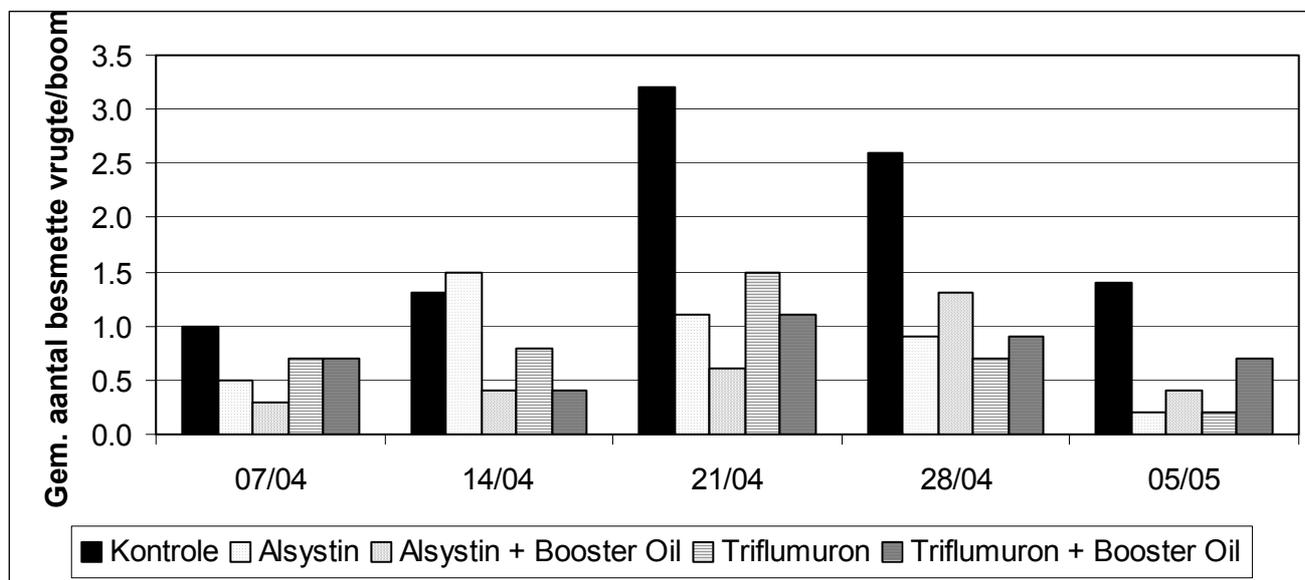


Fig. 3.4.13.1. Vrugvalpatroon weens valskodlingmotbesmetting in 'n boordproef met Triflumuron op nawelbome in Citrusdal gedurende 2003.

Die besmetting was oor die algemeen matig (gemiddelde vrugval van 1,9 in die kontrole) en Triflumuron se doeltreffendheid wanneer dit hewiger besmettings moet onderdruk, is dus onbekend. Daar kan waarskynlik aanvaar word dat dit onder alle omstandighede nie swakker as Alsystin sal vaar nie.

Tabel 3.4.13.1. Gemiddelde vrugval per behandeling weens valskodlingmot op nawelbome in Citrusdal.

Insekdoder	Dosis per h ℓ water	Gemiddelde aantal besmette vrugte per boom per week*
Onbehandelde kontrole	-	1,9 a
Alsystin	10 m ℓ	0,8 b
Alsystin + "Booster Oil"	10 ml + 200 m ℓ	0,6 b
Triflumuron	10 m ℓ	0,8 b
Triflumuron + "Booster Oil"	10 ml + 200 m ℓ	0,8 b

*Variansie-ontleding is op die data uitgevoer en die behandelinggemiddeldes is met Student se t-KBV vergelyk (5%). Data gevolg deur dieselfde letter verskil nie betekenisvol van mekaar nie.

In 'n 2002-proef het Booster Oil die swak-geformuleerde(?) Triflumuron se werking verbeter. In die 2003-proef was die resultate van Alsystin en Triflumuron saam met die bymiddel gedurende die eerste drie weke na behandeling skynbaar beter as daarsonder. Daarna het die mengsels met die Booster Oil swakker as daarsonder presteer (Fig. 3.4.13.1). Daar is egter nie genoeg getuieis om dié waarnemings statisties betekenisvol te bewys nie ($p = 0,14$). Dit is moontlik dat die vorige, swakker Triflumuron-formulasie se werking inderdaad deur die bymiddel versterk is, maar onnodig is in die geval van 'n behoorlik-geformuleerde produk. Alhoewel die bymiddel daarteenoor Alsystin se werking in dié proef skynbaar verbeter het, het verskeie bymiddels wat al in die verlede saam met die produk getoets was, geen verbetering bewerkstellig nie en dié resultaat is waarskynlik toevallig.

Gevolgtrekking

VKM het alreeds landswyd 'n wisselende graad van weerstand teen die bensoïelureum-insekdoders ontwikkel. Die resultate van die proef dui daarop dat Alsystin en Triflumuron nog steeds 'n verskil kan maak, alhoewel die werking daarvan, veral in gevalle van hewige VKM-besmettings, moontlik nie watwonders sal wees nie. Op plase of in gebiede waar weerstand nog nie 'n beduidende rol speel nie, kan die produkte egter nog gebruik word. Indien Triflumuron kostegewys met Alsystin kan meeding, kan dit alternatiewelik vir VKM-bestryding gebruik word.

Verdere toetsing van die produk word nie beoog nie.

3.4.14 The spatial and temporal population dynamics of the false codling moth and its biocontrol agents relative to an agricultural landscape mosaic

Experiment US/MS by Michael Schmeisser (University of Stellenbosch)

Note from the Project Co-ordinator

This project was funded by CRI as a Ph.D. study. Unfortunately, unforeseen circumstances necessitated the incumbent to terminate his research prematurely. This was after only a few months of work and before many of the envisaged areas of investigation could be initiated. However, a start was made with some preliminary work and this is reported on briefly.

Opsomming

Die rede vir die studie word bespreek. Waarnemings dui daarop dat 'n alternatiewe gasheerplant soos akkerbome nie deur VKM besmet word indien hulle nie aan sitrusboorde grens nie.

Opnames dui daarop dat die meeste VKM in sitrusboorde gevang word en selde in groot getalle daar buite. Geen VKM-papies is onder sitrusbome gevind nie. Selfs waar daar larwes op die grond losgelaat is, kon die papies nie later opgespoor word nie.

Introduction

The citrus industry is a multi-million Rand export business in South Africa, being the third largest exporter of fresh citrus in the world after Spain and the United States of America. The income from export exceeds 92% of the total citrus industry revenue. With increasing pressure by international markets for the production of pest free, high quality fruit, while limiting the use of pesticides, the citrus industry is faced with a difficult challenge to overcome the large insect pest complex known to attack citrus, including the major indigenous pest, the false codling moth.

Chemical control of FCM has proved to be inefficient, as it is expensive, leads to pesticide resistance and unacceptable chemical residues in export fruit. This is complicated further by FCM being capable of laying eggs throughout the entire fruiting season and that the larvae, which bore into the fruit, are difficult-to-reach targets for chemical control. Biological and sanitary control measures have only achieved limited results, as the success of control appears to be unpredictable, varying from orchard to orchard and from season to season.

Although the population dynamics of FCM and that of its egg parasitoids have been studied within the citrus orchard, very little attention has been given to population studies outside the orchard system. FCM is known to feed on a vast range of alternate host plants besides citrus, allowing it to be active and pose a threat throughout the year. The succession and composition of alternate cultivated and wild host plants may have an important influence on FCM population fluctuations within the citrus orchard. It has long been suggested by Ulyett & Bishop (1938), that initial FCM infestations in citrus orchards stem from alternate host plants in the vicinity of citrus, and that these could significantly increase population levels throughout the year.

Hence there are some pertinent questions that require more detailed investigations. What population numbers are sustained outside the citrus orchards and how do these change throughout the season? How capable is FCM of utilising a succession of alternate hosts and are there specific host preferences? What is the capability of FCM to migrate back into citrus orchards and cause a re-infestation early in the season? Furthermore, does FCM migrate in and out of citrus orchards during the season? If so, this could explain, in part, the lack of the biological control success. Similar questions arise when considering the ecological behaviour of the egg or larval parasitoids. What are the population dynamics of the various parasitoids in cultivated versus non-cultivated areas and to what¹³⁶ extent are they suppressed? Answers to these

questions could highlight the need for an alternate approach to the current control methods e.g. that control measures in the orchard alone are insufficient for the successful control of FCM and that a mass release of parasitoids in the regions around orchard containing alternate hosts may be required.

This study is on the behavioural ecology of FCM and its control agents in terms of the spatial distribution and population dynamics within and outside the citrus orchard system.

Materials and method

After accepting the project at the end of April 2003, the first months were largely comprised of reviewing literature on FCM, including *inter alia*, identification of the pest from the larva, pupa and adult stages, and its parasitoids. A comprehensive project proposal was designed at the same time.

The farm Floreat, near Citrusdal, was identified as an experimental site. During the initial scouting of the farm, GPS points of some of the orchards and alternate host plant patches were taken to allow the creation of maps with more accurate zoning of hosts and broader vegetation types.

• Inspection of oak trees as an alternative host for FCM

Acorns were collected on 3 occasions from the soil beneath several oak trees 10-20 m away from three citrus orchards. The nuts were cracked open and inspected. Similar inspections were conducted beneath oak trees situated **at least 300 m** away from the citrus orchards.

Fifteen pink, presumably final instar larvae, were collected from acorns and released on the ground underneath an oak tree. Once a larva had settled under leaf litter or piece of bark and became quiescent for at least 5 minutes, the location was demarcated and inspected for pupae two weeks later.

• Mounting of pheromone traps

With the aid of GPS 19 delta traps were distributed in and outside the orchard system at Floreat. The traps were mounted on wooden poles at a height of 2 m and were supplied with Lorelei pheromone dispensers. All traps were a minimum of 300 m apart to prevent pheromone interference. The traps were monitored every two weeks from September to December 2003. All trapped FCM males were counted and removed during each of the seven counts that were made.

• Search for pupation sites

A grid system was used to search for FCM pupation sites within citrus orchards, as well as oak tree patches. A grid typically consisted of a demarcated square beneath a tree from the trunk outwards. Each grid was 3 m x 3 m, and subdivided into smaller squares of 0,5 m x 0,5 m. Two such grids were used per orchard in each of three orchards. This formal approach was changed after three observations to an informal system where the soil beneath and around 5 to 10 trees in an orchard was inspected, but not in a grid system. During the formal inspections, the soil was sifted and the litter remaining in the sieve was inspected for FCM pupae and pupal cases. During the informal inspections, the soil was disturbed superficially and vegetative litter was scrutinized closely. Sampling was conducted on all inspection dates from April to October.

Results

• **Oak trees:** The inspected acorns showed relatively high infestations of larvae. Eleven, 8 and 9 out of 30 nuts cracked open on each of three occasions contained pink, presumably adult, larvae. It was noted that most nuts had a single large larva in the centre of the nut. However, up to 3 larvae were encountered in some nuts and it appeared that the central larva was the most developed. The others tend to feed only between the husk of the shell and the nut. As this method involved cracking the acorns, exposing the larvae, and thus preventing further feeding, the moths were not reared. Hence it is uncertain whether the larvae were indeed FCM¹.

No similar larvae were found in acorns collected from the soil beneath oak trees away from citrus orchards.

¹ JHH: Similar observations were conducted in Citrusdal in 1981-82. No larvae from other species similar in appearance to FCM larvae were found in acorns. Although still not conclusive, what the author recovered above were probably FCM larvae.

No cocoons, pupae or pupal cases were recovered at the demarcated locations where larvae were released.

- **Traps:** More data needs to be collected. However, according to the data accumulated, most male activity occurred within or close by citrus orchards (Table 3.4.14.1). Highest catches were in navel orchards or in a mixture of navel and Valencia orange trees, as well as in oak trees close by orchards. Traps located well away from orchards in natural bush, generally attracted the least males.

Table 3.4.14.1. Total number of false codling moth males trapped from 18 September to 11 December 2003. The results are not arranged chronologically, but according to numbers of males trapped.

Trap	Location	Total number of males trapped
12	Road down between two orchards 11 & 12, deep in the fynbos (about 200-250 m from nearest orchard)	0
8	First trap up in kloof facing the Citrusdal Valley enclosed with mountains on both sides	1
9	Second trap up in kloof facing the Citrusdal Valley enclosed with mountains on both sides	1
14	At the office, mixture of plants - wild olive, Sour plum etc.	1
7	Placed in Fynbos - estimated 200m from orchards	4
16	Olive tree high up near mountain (about 250 m from nearest orchard)	4
3	Eucalyptus trees & open grass field, near rivulet with reeds, nearest oaks about 60 m; closest orchard about 150 m	7
15	Near gate, between orchards 11-12, at blue gum tree on main road leading to farm : windbreak (about 10 m from orchard)	7
17	Orchard 13 (Valencia: 284 trees: Navel: 515 trees) Between row 3-4 from top near gate	7
5	Near orchard 01 (mostly navels: 414 trees : Valencia 87 trees), surroundings: fynbos, reeds, brambles	8
2	Open grass field, near orchard 06 (Valencia & Navel mix) (about 100 m from nearest orchard)	11
4	In orchard 06 (Valencia: 62 trees : Navel 32 trees), row 3-4	12
18	Orchard 14 (Valencia: 170 trees : Navels 10 Trees), near farmhouse, 3 rows from bottom	12
19	Gate on main road near the last orchard 12 (in the fynbos and about 100-150 m from nearest oak tree)	13
1	Placed at periphery of oak trees - near Valencia orchard 07 (about 15 m from orchard)	20
6	Place at periphery of single big oak, about 10 m from orchard 02 (navels:181 trees) with windbreak in between	35
10	Within Orchard 08 (Navels: Palmers 706 trees) - straight through gate	42
13	Orchard 12, Valencia: Midnight: 1470 trees , row 5-6 from bottom	46
11	Between Orchards 9-11 Navel: Robyn: 800 and 695 trees	59

- **Pupation sites:** Not a single pupa was found under the citrus or oak trees in any of the formal and informal inspections. Two pupae were discovered within empty acorns shells, but when reared were identified as members of the family Phyctinidae.

Conclusion

All aspects investigated above need to be looked at more closely and the scope need to be enlarged. The aspects covered by the study are of great importance to facilitate a better understanding of FCM. In doing so, costly mistakes can perhaps be prevented when techniques such as SIT and mating disruption (where the aim is to interfere with the insect's behaviour, rather than killing it with insecticides) are applied. If another candidate can be found, the study will be continued.

3.4.15 Genetic differentiation in some South African populations of the false codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), inferred from molecular markers

Experiment US/AT by Alicia Timm, Henk Geertsema & L Warnich* (Department of Entomology and Nematology; *Department of Genetics: Univ. of Stellenbosch)

Opsomming

Die patroon van genetiese verskeidenheid in valskodlingmot is in 20 populasies van sitrus, akkers en avokado's van die Wes-Kaap, Mpumalanga en Limpopo bestudeer. Populasies is ontleed deur die gebruik van versterkte fragment lengte polimorfismes (VFLM). Ontleding van 402 VFLM loci het aangetoon dat genetiese variasie besonder hoog was, met 98.3% van die loci polimorfies. Gegewens dui aan dat die VKM 'n redelike sterk geografiese substruktuur vertoon. Bevolkings van Limpopo en Mpumalanga was heterogeen ($G_{st} = 0.0533$, $N_{m_{est}} = 4.27$). Daarenteen het bevolkings van die Wes-Kaap 'n hoë graad van inteelt vertoon en verdere bevolkingsstruktuur- ($G_{st} = 0.28$, $N_{m_{est}} = 0.63$) en gepaarde hoofkoördinaat-ontledings (GHKO) kon gebruik word om tussen motte van verskillende bevolkings te onderskei. Ontleding van molekulêre variasie het getoon dat 5% van die variasie aan verskille tussen populasies, met 'n verdere 3% as gevolg van gasheerverskille, toegeskryf kon word. Populasies van motte afkomstig van sitrus en akkers was nader verwant as bevolkings vanaf avokado's. Dit wil voorkom asof mannetjiemotte meer geredelik versprei as die wyfies, wat bydra tot die genetiese diversiteit van bevolkings oor 'n relatief wye gebied. Die resultate tot dusver verkry, kan moontlik die sukses van die plaag in baie omgewings verklaar en het belangrike implikasies vir die bestryding van VKM.

Introduction

The False Codling Moth (FCM) is one of the most catholic pests of fruit, with a host range of at least 35 reported hosts and feeding on crops as diverse as citrus, pome fruit, stone fruit, litchis, macadamias and non-cultivated hosts such as acorns, Port Jackson galls and *Eucalyptus*. False codling moth is indigenous to Sub-Saharan Africa, and is found throughout South Africa. It is one of the most economically important pests on citrus and is also important on litchis and macadamias. Recent conventional control methods aimed at this moth have not always been successful and it may well be that different populations of FCM in South Africa show different degrees of tolerance to such treatments (Newton, 1998). One of the more recent environmental-friendly control strategies makes use of the Sterile Insect Technique (SIT) and it is imperative that the various population structures be assessed to determine differences in genetic make-up and to specifically target certain populations to ensure the successful application of this control strategy.

The need to understand the nature of genetic variation between and within pest populations has been demonstrated many times, for many different taxa. Applied entomologists are starting to realize that knowledge of the genetic variation of an insect pest population can aid in the understanding of its biology and ultimate control in an integrated pest management system (Loxdale & Lushai, 1998). Genetic variability is the basis of any evolutionary process since it provides the raw genetic material for individuals to adapt to a given situation. The existence of variation in natural populations therefore determines its success under changing conditions (Armstrong & Wratten, 1996). It is generally assumed that species exhibiting high levels of genetic variation are more persistent, as they have the ability to respond to both biotic and abiotic changes. In populations with high levels of genetic variation biotype development is common and can allow adaptation to a variety of changes in the environment (Hales *et al.*, 1997). However, the survival of a species with low genetic variability is threatened by these changes (Maynard Smith, 1978, Blackman, 1979). Therefore, it is clear that knowledge of the genetic structure of a pest population can provide invaluable information on which to construct and modify pest management and forecasting systems.

In recent years, the advent of molecular techniques, especially in the past decade, has provided unique insight into the biology, ecology and control of insect pests in general. In particular, the use of multilocus techniques such as random amplification of polymorphic DNA (RAPD) (Williams, 1990, Welsh & McClelland, 1990), microsatellites (Cregan & Quigley, 1997) and amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995) have been found to provide a high level of resolution for characterizing the genetic structure.

The primary objective of the present study was to determine the genetic structure of FCM populations with regard to the amount and distribution of genetic variation and the relationships between different populations, including on different hosts, in South Africa. Furthermore, it was aimed to use this information to provide practical insight into FCM dispersal and control.

Material and methods

A total of 200 FCM samples were analyzed. These were collected from four regions in the West-Cape (Citrusdal, Paarl, Stellenbosch and Retreat), from Nelspruit in Mpumalanga Province and Magoebaskloof in the Limpopo Province. Also included were individuals obtained from a laboratory colony maintained at Citrusdal, started approximately 10 years ago with material, not supplemented with wild material in the last five years, from Citrusdal and Tzaneen (as part of a biological control program). Five individuals per sampling site were collected where possible, with the exception of the laboratory colony from which ten individuals were used.

FCM moths were collected from infested fruit from trees or from the ground, or from sticky traps in the field. Fruit was placed in emergence boxes provided with sand to facilitate pupation of larvae emerging from fruit. Pupae were removed and placed in a container to emerge. Live moths caught in pheromone traps were removed from the sticky bases by using paraffin.

DNA EXTRACTION

Extraction tissue: The minimum amount of tissue necessary to perform AFLP analysis reproducibly was by using the head and legs of moths. The remainder of the specimen was stored in 100% ethanol as voucher material. Where only larvae could be obtained, a portion of the thorax was used, leaving the head and abdomen free for storage as voucher material. A modified procedure of Reineke *et al.* (1998) was used. Insect tissue was macerated in 500 μ l lysis buffer containing 0.1 M Tris (pH 8), 10 mM EDTA, 2% SDS (w/v) and 0.2 mg/ml proteinase K. After incubation at 58°C for three hours, 1/10 volume CTAB and 140 μ l of a 5 mM NaCl solution were added. Incubation was continued for 30 minutes at 65°C. DNA was extracted once with chloroform/isoamyl alcohol (24:1 v/v) and polysaccharides removed by the addition of 225 μ l of a 5 M NH₄Ac solution. DNA was precipitated using 0.25 volume 30% (v/v) PEG. After centrifugation for 20 minutes at 4°C, the precipitate was washed three times with 70% ethanol and dried. Finally, the precipitate was suspended in 20 μ l TE buffer (10 mM Tris HCl, 0.1 mM Na₂EDTA, pH 8.0) and RNA removed by the addition of 1 μ l Rnase followed by incubation at 37°C for two hours. DNA concentration and quality was estimated by comparison with standard λ DNA concentrations (Promega) on 0.8% (w/v) agarose gels (Seakem).

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

Restriction-ligation: At least 200 ng genomic DNA from each insect was digested in a total volume of 40 μ l with five units each of the restriction enzymes *MseI* (New England Biolabs) and *EcoRI* (Promega), One-Phor-All buffer (Pharmacia) and 0.1 μ g/ μ l BSA for three hours at 37°C. An adaptor ligation mixture of 10 μ l containing 5 pmoles of *MluI* adaptor, 50 pmoles of *MseI* adaptor, 1 unit of T4 DNA ligase (Promega), 1 mM ATP (Roche) in One-Phor-All reaction buffer was added to each of the digestion reactions and incubated overnight at 37°C. Reactions were diluted 1:10 in 1 x TE buffer and stored at -20°C prior to pre-selective amplification.

Pre-selective amplification: Thirteen μ l of the template resulting from restriction enzyme digestion and adaptor ligation were added to a reaction consisting of 75 ng of each *EcoRI* and *MseI* preselective primer 200 μ M of all four dNTPs, 1 unit of *Taq* polymerase (Promega) and 1.5 mM MgCl₂ in a final volume of 50 μ l. Amplification was performed in the GeneAmp PCR Instrument System 9600 DNA thermocycler (Applied Biosystems). The following cycle program was used: 5 minutes at 72°C followed by 30 cycles with the cycle profile 30 seconds at 94°C, 1 minute at 56°C and 1 minute at 72°C. The reaction was completed by a final extension period of 72°C for 5 minutes and stored at 4°C. Successful amplification was determined by agarose gel electrophoresis of 10 μ l of the preamplification product. The remaining 40 μ l of the amplification product was diluted tenfold in 1 x TE buffer and stored at -20°C.

Primer labeling: *EcoRI* selective primers were end-labelled prior to amplification. This was performed using 5 ng *EcoRI* selective primer, 0.25 μ Ci [γ -33P] ATP (Easytides, NEN) and 0.05 units T4 polynucleotide kinase (Promega) in One-Phor-All buffer in a total volume of 0.5 μ l per reaction. The reaction was incubated for three hours at 37°C and the enzyme inactivated by incubation at 65°C for 10 minutes.

Selective amplification: Selective amplification was carried out in a standard PCR reaction using 2.5 μ l diluted pre-amplified DNA, 200 μ M of each dNTP, 0.025 units *Taq* (Promega), 1.5 mM MgCl₂, 15 ng *MseI* primer and 0.5 μ l of the labelling reaction in a final volume of 10 μ l. The cycle profile was as follows: 30 seconds at 94°C, 30 seconds at 65°C, and 1 minute at 72°C. The annealing temperature was reduced by 0.7°C for the next 12 cycles and continued at 56°C for 24 cycles.

Gel electrophoresis: Amplification products were added to 3.5 µl formamide dye (98% formamide, 10 mM EDTA and 0.05% each of bromophenol blue and xylene cyanol FF) and denatured at 95°C for 5 minutes. Four µl of the mixture was loaded onto a 6% (w/v) denaturing polyacrylamide gel (40% acrylamide stock solution (Promega), 6M urea, 1 x TBE) and electrophoresed in 1 X TBE buffer (100 mM Tris, 100 mM boric acid, 10 mM EDTA, pH 8.3) at 60 W for 3 hours. Gels were dried on blotting paper and exposed to Kodak Biomax X-ray films for up to ten days.

Data scoring: AFLP markers were scored dominantly. Only two characters were distinguished viz. band absence and band presence. Band presence was assumed to represent either a dominant homozygote (AA) or heterozygote (Aa) whereas band absence denoted a recessive homozygote (aa). Fragments of the same size were assumed to represent homologous DNA sequences. Only bands that could be scored clearly and unambiguously were considered for analysis. Band intensity was disregarded.

DATA ANALYSIS

Amount of genetic diversity: The percentage of polymorphic bands was used to measure the amount of diversity present in a population. A polymorphic band was defined as a band either present or absent in at least one individual.

Distribution of genetic diversity: The distribution of diversity was analyzed using analysis of molecular variance (AMOVA) performed in GenAEx (Peakall & Smouse, 2001). The genetic distance matrix was used to perform a hierarchical analysis of molecular variance (AMOVA). The AMOVA procedure as calculated in GenAEx followed the methods of Excoffier *et al.* (1992) and Huff *et al.* (1993). Variation was summarized as the percentage of the total variance present. Statistical significance was tested by random permutation, with the number of permutations set to 999. Due to the size of the data set, only polymorphic bands were used in this analysis. AMOVA analysis was performed using individuals from different geographic areas as well as from different hosts.

Relationships between populations: Two types of cluster analysis were performed. The first, based on Gower's similarity coefficient, was principal coordinate analysis (PCOA) conducted in Multivariate Statistical Program (MVSP) (Kovach, 1999). F-statistics and measures of the estimated number of absolute migrants ($N_{m_{est}}$) were calculated using this software Popgen32 (Aspi, J., 1995).

Results

Level of genetic variation: AFLP analysis of 123 *C. leucotreta* individuals from three provinces in South Africa provided a total of 402 fragments using five primer pairs. Genetic variation was extremely high. Of the 402 fragments analysed, a total of 395 (98.3%) were polymorphic in the entire population. There was, however, no statistical difference between the level of variation within the seven populations (Table 3.4.15.1).

Table 3.4.15.1. Percentage polymorphic bands detected by AFLP for *C. leucotreta* populations from South Africa

Origin of population	Number of fragments	% Polymorphic fragments
Lab colony	214	81.78
Citrusdal	345	95.9
Stellenbosch	334	98.0
Retreat	247	83.8
Paarl	212	81.6
Nelspruit	326	92.94
Magoebaskloof	178	74.7
Total	402	98.3

($\chi^2 = 5.21$, $P > 0.05$)

Distribution of molecular variance: The results of analysis of molecular variance (AMOVA) for the total AFLP data set showed that most of the variation could be found within populations. Five percent of the variation could be attributed to geographic variation with a further three percent a result of host variation. The remaining 92% could be attributed to differences within populations.

Relationships between geographical populations: The results of principal co-ordinate analysis for the South African population are shown in Fig 3.4.15.1. The Citrusdal laboratory colony formed a cluster distinct from the other populations. The Citrusdal and Stellenbosch populations also showed differences to the remaining populations. The absolute number of estimated migrants ($N_{m_{est}}$) exchanged between all populations was calculated as 0.678 ($F_{st} = 0.269$). Pairwise PCOA could be used to distinguish Citrusdal, Stellenbosch and Nelspruit populations. PCOA analysis comparing Citrusdal and Nelspruit populations is shown in Fig 3.4.15.2.

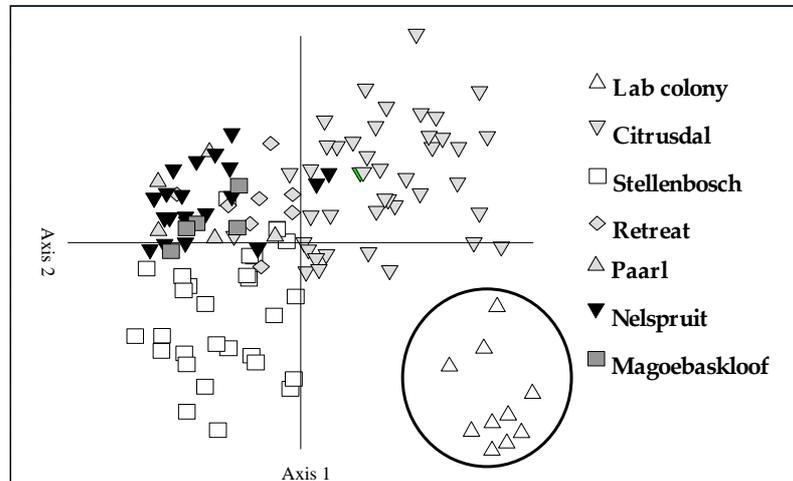


Fig. 3.4.15.1. PCOA of *Cryptophlebia leucotreta* populations from South Africa

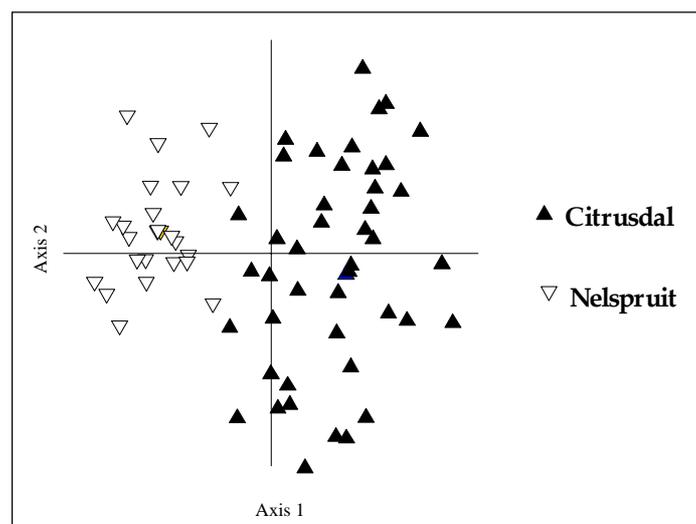


Fig. 3.4.15.2. PCOA comparing Citrusdal and Nelspruit populations of *C. leucotreta*

Relationships within geographical populations: In addition to distinguishing between moths from different regions, PCOA could also be used to distinguish moths collected from different farms within the same region and, in some cases, from different orchards within the same farm. An example of the latter is shown in Fig 3.4.15.3. Gene flow between populations within the same region differed between regions. For example, Nelspruit populations interchanged more genetic material than Citrusdal populations. The estimated number of absolute migrants ($N_{m_{est}}$) exchanged between populations within the same region is shown in Table 3.4.15.2.

Table 3.4.15.2. Gene flow (G_{st}) and the absolute number of migrants (Nm_{est}) exchanged within seven *C. leucotreta* populations in South Africa

Origin of population	G_{st}	Nm_{est}
Lab colony	_*	_*
Citrusdal	0.2075	0.85
Stellenbosch	0.1581	1.33
Retreat	0.1293	1.68
Paarl	0.511	0.239
Nelspruit	0.0553	4.27
Magoebaskloof	_*	_*

* Not calculated as only one population was sampled.

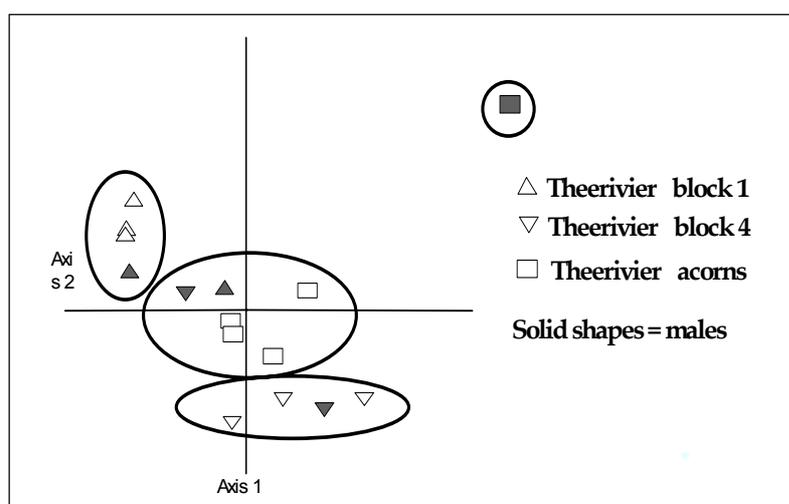


Fig. 3.4.15.3. PCOA of *C. leucotreta* individuals collected from Theerivier farm in Citrusdal.

Relationships between host populations: Moths collected from different hosts showed no distinct clusters. However, UPGMA analysis showed that moths collected from citrus and acorns were more closely related than those collected from avocados (data not shown).

Differential dispersal of sexes: In some cases male individuals seemingly clustered into the “wrong” group. An example of this can be seen in Fig. 3.4.15.3.

Discussion

Level of genetic variation: AFLP analysis using five primer pairs showed that a high level of genetic variation is present in *C. leucotreta* populations in South Africa. A total of 98.3% of the AFLP fragments were polymorphic. This value is amongst the highest of that recorded for insects and even more than the reported high values found in similar studies for the gypsy moth *Lymantria dispar* where 89% of the fragments were polymorphic (Reineke *et al.*, 1999) and the winter pine processionary moth (*Thaumetopoea pityocampa – wilkinsoni* complex) where 84% of the fragments were polymorphic (Salvato *et al.*, 2002). Furthermore, variation is high (greater than 70% polymorphic AFLP fragments) in all *C. leucotreta* populations, including the laboratory colony. This is unexpected as inbreeding in an isolated population usually leads to a decrease in genetic variation. This may indicate that *C. leucotreta* has a mechanism for maintaining increased levels of genetic variation.

The high level of genetic variation that was found has important practical implications for control. It is generally assumed that species exhibiting high levels of genetic variation are more persistent, as they have the ability to respond to both biotic and abiotic changes. In populations with high levels of genetic variation biotype development is common and can allow adaptation to a variety of changes in the environment (Hales *et al.*, 1997). This may explain the rapid emergence of *C. leucotreta* biotypes resistant to insecticides (Hofmeyr & Pringle, 1998) and provides an explanation for the problems associated with control of *C. leucotreta* populations.

Distribution of genetic diversity: Results showed that whereas 5% of the variation could be attributed to differences between geographically distinct populations, only 3% was attributed to differences between populations collected from different hosts. The latter value is likely inflated since host populations from different geographic locations were analyzed. Geographic isolation plays a far greater role than host differentiation in influencing patterns of genetic differences in *C. leucotreta* populations in South Africa. In practice, this indicates that moth populations from different hosts within the same geographical area are intermixed to such an extent that they can be treated as one population.

Relationships between populations: PCOA analysis of South African *C. leucotreta* populations showed that the laboratory colony formed a cluster separate to the field populations (Fig. 3.4.15.1). Furthermore, three groups could be distinguished in the field populations. The first consisted of individuals from Citrusdal, the second of individuals from Stellenbosch and the third from the remaining individuals from the Western Cape as well as from Mpumalanga and Magoebaskloof. These clusters are not very distinct, but became so with pairwise comparisons (Fig. 3.4.15.2). PCOA analysis allowed clear discrimination between individuals from Citrusdal, Stellenbosch and Nelspruit.

The Stellenbosch population most probably originated from Nelspruit (Riedl *et al.*, 1998) during the thirties. It seems likely that this population became isolated from its founder population and became genetically differentiated. The origin of the Citrusdal population is not known.

The estimated number of absolute migrants ($N_{m_{est}}$) exchanged between populations was calculated as 0.678. As a benchmark, values of $N_{m_{est}} < 1$ are typical of "low gene flow" species, which will become differentiated in the absence of counteracting factors. If $N_{m_{est}}$ is >1 there will be sufficient gene flow to negate the effects of genetic drift. Therefore, one can expect that *C. leucotreta* populations in South Africa will become even more distinct over time.

Genetically distinct populations have important implications for control. For example, different populations may differ in terms of their biology and/or ecology and may therefore also require different control practices. This should be taken into account when evaluating future control practices, for example when considering sterile insect techniques. Such experiments require evaluation using a sample population of *C. leucotreta* representative of the total genetic diversity of FCM in South Africa. If such a genetically representative sample is not used, geographic differences or narrow genetic variability within the pest population will give false results during such trials which, in turn, could lead to poor control in the field (Shufran *et al.*, 2000). To avoid this, control practices should be exposed to as much of the diversity of the pest populations as possible to obtain meaningful results. This study confirmed that the genetic structure of *C. leucotreta* is such that all three populations from, for example, Citrusdal, Stellenbosch and Nelspruit should be treated as an entity in order to make general recommendations for control in South Africa.

Relationships within populations: The number of migrants exchanged within FCM populations differed between regions. In Stellenbosch, Retreat and Nelspruit populations, $N_{m_{est}}$ values are greater than one, indicating that the probable development of distinct populations in these areas is unlikely. However, $N_{m_{est}}$ values for Citrusdal and Paarl are less than one. In these populations, genetically distinct populations were found. In Citrusdal, moths from different farms as well as from different orchards within the same farm (Fig 3.4.15.3) could be distinguished. This may indicate that moths disperse over wide areas in some regions, while being limited to areas as small as a farm in other instances. This may be influenced by environmental factors including host availability. Insight into pest dispersal can be valuable for designing a pest control system, especially when considering SIT. For example, populations of FCM from Nelspruit and Citrusdal are genetically distinct and exhibit differential dispersal behaviour which could affect the success of an SIT program.

Relationships between host populations: Moths collected from three different hosts, *viz.* citrus, avocados and acorns, showed no distinct clustering. This implies that moths readily interchange between hosts and that there has been no host-biotype formation. However, FCM populations from additional hosts, both cultivated and from the wild, should be investigated as well.

Differential gender dispersal: In some cases males clustered seemingly into the "wrong" group. From these analyses, it became apparent that males appear to disperse more readily than females. Males therefore contribute to the genetic diversity of populations over a relatively wide range, reducing the risks of inbreeding and genetic loss associated with bottlenecks occurring in isolated populations. The implication is that special efforts should be developed to reduce females in a population. The effect of males on population dispersal is presently being quantified using mitochondrial DNA, which is maternally inherited, and therefore able to measure the effect of dispersal in a population devoid of males.

Implications for dispersal and control: From these studies, it is clear that FCM is fairly homogenous in the Mpumalanga and Limpopo Provinces, but not so in the Western Cape. The heterogeneity in the Western Cape may point to two separate introductions into the Western Cape. Conventional control of FCM does not always meet the expected results (Hofmeyr & Pringle, 1998) and are indicative that populations within or between different locations exhibit different characteristics. With the introduction of newer control strategies, this important aspect should be kept in mind.

Conclusions

The level of variation in FCM populations in South Africa is extremely high. The distribution of this variation was such that most of the variation was present within populations, with geographical distribution having a greater effect on the pattern of variation than host differentiation. Significant differences, to a lesser or greater degree, were found between different populations and each population may therefore exhibit variation in responses to, for example, SIT. Further studies are being continued.

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3.4.16 Evaluation of a genetically modified pathogen for control of false codling moth

Experiment 569 by Sean D. Moore, Garth I. Richards & Wayne Kirkman (CRI)

Opsomming

'n Virus wat deur Horticulture Research International (HRI) in die VK geneties verander is, word teen VKM getoets om te bepaal of dit vinniger as die wilde-tipe virus (CrleGV) doodmaak. In oppervlakbesmetting dosisreaksie biotoetse is die LC_{50} en LC_{90} (konsentrasies wat onderskeidelik 50% en 90% van individue in 'n monster van 'n bevolking sal doodmaak) van pasuitgebroeide larwes as 2.257×10^8 OBs/ml en 1.854×10^{10} OBs/ml, geskat. Dit is heelwat hoër as wat voorheen geskat is en heelwat hoër as dieselfde waardes vir CrleGV. Dit sal waarskynlik verhoed dat die geneties-veranderde virus kommersieel gebruik sal kan word, want teen die konsentrasies wat deur die biotoetse aangedui word sal dit te duur wees. Die LT_{50} en LT_{90} (tye wat dit neem om onderskeidelik 50% en 90% van individue in 'n monster van 'n bevolking dood te maak) van die geneties-veranderde virus teen pasuitgebroeide VKM-larwes is as 3 dae 16 h 30 min en 8 dae 8 h 20 min onderskeidelik, geskat. Die LT_{50} is korter as die van CrleGV maar die LT_{90} is langer. Dit wil daarom voorkom asof die geneties-veranderde virus min of geen voordeel bo CrleGV sal bied nie. Biotoetse op vrugte sal binnekort uitgevoer word, waarna enige verdere navorsing onwaarskynlik met die virus gedoen sal word nie.

Introduction

A granulovirus (CrleGV) is currently being investigated for control of FCM (Section 3.4.2). A typical drawback with wild type baculoviruses is their slow speed of kill. This can be remedied through genetic modification of the pathogen. A heterologous virus has been genetically modified by Horticulture Research International (HRI) in the UK. Coincidentally, this virus has been found to be highly effective against FCM in laboratory bioassays. This potential was further tested by CRI during 2002 by conducting dose-response bioassays with neonate larvae. During 2003, these were repeated and time-response bioassays were conducted. The results of these are described here.

Materials and methods

Dose-response bioassays with neonate larvae

Surface dose bioassays were conducted in 25 cell bioassay trays. Each cell was filled with a layer of 5 - 10 mm of diet with agar. Five five-fold dilutions of purified GM-virus in sterile distilled water and a sterile distilled water control were used. Twenty-five larvae were treated per dose, using 25 cell bioassay trays. A volume of 50 μ l of each virus dilution and of the control was pipetted onto the centre of the diet surface in each cell using an autopipette. The fluid was spread evenly over the diet surface by tilting and rotating the tray slowly. Inoculated bioassay trays were left within the laminar flow cabinet for ± 30 minutes, until the diet had dried adequately. One neonate larva was then placed into each cell. All larvae were from the same batch of eggs, having all hatched on the morning that the assay was initiated. Trays were sealed with multiple layers of paper towelling, followed by a layer of thick transparent plastic and the tray lid. These were firmly held in place with the aid of four Bulldog clips – one on each side of the tray. Trays were then marked and kept at 27°C.

After 7 days, trays were opened and inspected. Larvae were recorded as alive or dead. The dose-response curve was calculated using PROBAN (Van Ark, 1995), a computer 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, LC_{50} and LC_{90} (concentrations required to kill 50% and 90% of larvae₁₄₆ in a sample) were calculated.

Time-response bioassays with neonate larvae

Surface dose bioassays were conducted in 30 ml plastic pots. Twenty round plugs were cut from a pie dish of diet. Ten plugs were dipped into sterile distilled water and the other 10 plugs were dipped into a LC₉₀ (calculated from the dose-response bioassays) solution of GM virus. Plugs were placed on a thin wire mesh drying rack, in a laminar flow cabinet until sufficiently dry (± 30 min). They were then inserted into the pots. Five neonate larvae were then placed into each pot, onto the diet surface. This was done with the aid of a 000 paint brush, first placing larvae onto the control diet and then onto the virus treated diet. Pots were sealed with filter paper and plastic lids, marked and kept at 27°C. There were therefore 50 larvae per treatment.

After 8 hours, pots were opened individually, starting with the control pots, and inspected for any dead larvae. Thereafter, pots were checked every 8 h (three times a day): at 07h00, 15h00 and 23h00. When dead larvae were observed, they were immediately and gently (so as not to rupture them) removed from the pots, using a 000 paint brush. Inspections were continued at the stated time intervals until virus induced mortality appeared to have ceased i.e. when no mortality was observed during 48 consecutive hours. Diet was then dissected so that surviving larvae could be recorded. The time-response relationship was determined using PROBAN (Van Ark, 1995). Strictly speaking, a logit analysis rather than a probit analysis should be conducted (Jones, 2000). However, no computer package which could conduct a logit analysis was available. In reality, time-response relationships are often estimated using probit analysis, which will not give results much different from a logit analysis (Jones, 2000). From this, LT₅₀ (time to kill 50 % of larvae in a sample) and LT₉₀ (time to kill 90 % of larvae in a sample) were calculated

Results and discussion

Dose-response bioassays with neonate larvae

The regression (probit) line fitted to the corrected data in Table 3.4.16.1 had the equations $y = 1.1057 + 0.4471x$ (SE of slope = 0.1566). Mean LC₅₀ and LC₉₀ from the 2 replicates were estimated to be 2.257×10^8 OBs/ml and 1.854×10^{10} OBs/ml respectively. Results from the 2 bioassays were similar and therefore supported one another. These values were substantially higher than those obtained in similar bioassays during the previous year (Moore & Richards, 2002), but should be considered as being more accurate. The estimated LC₅₀ and LC₉₀ values are disappointingly high. The LC₅₀ and LC₉₀ values estimated for the wild-type homologous FCM virus (CrleGV) were 4.095×10^3 and 1.185×10^5 OBs/ml, respectively (Moore, 2002), meaning that the GM-virus is not as infectious. This would most likely preclude the possible commercial use of the GM virus, as it would be too expensive to be used at the high rates implied by the bioassay results.

Table 3.4.16.1 Mortality of neonate FCM larvae in dose response bioassays with five concentrations of GM-virus. Twenty-five individuals were tested per treatment.

Treatment (CrleGV in OBs/ml)	Replicate 1			Replicate 2		
	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit	Larval mortality (%)	Mortality corrected for control mortality (%)	Empirical probit
Distilled water control	20.0	-	-	20.0	-	-
1.90×10^6	36.0	20.0	4.158	28.0	10.0	3.718
9.50×10^6	48.0	35.0	4.615	24.0	5.0	3.355
4.75×10^7	48.0	35.0	4.615	40.0	25.0	4.326
2.38×10^8	56.0	45.0	4.874	48.0	35.0	4.615
1.19×10^9	84.0	80.0	5.842	80.0	75.0	5.674

Time-response bioassays with neonate larvae

According to Finney (1971), subjects should ideally be tested only once each, in order to use these statistical techniques. This is in order to make the independent variables (time) completely independent of one another. However, in practice this would not only be extremely wasteful of larvae but would be totally impractical. Instead of using 100 larvae (per replicate: treated and untreated), around 2 300 larvae would be required.

Once the bioassays had been completed (i.e. no mortality had been recorded for 48 hours), diet was dissected to search for any survivors or cadavers. None could be found. As a total of only 31 dead larvae were recorded during the trial, it must be assumed that the remaining 19 larvae must either have escaped or more likely have died and disintegrated within the diet. These “non-responding” larvae were therefore not considered in the calculation of the time-response curve. This is known as artificial truncation of the data (Bliss, 1935), as truncation is not inherent in the process under investigation but is due to the method of experimentation and presumably could be eliminated by a change in technique. The total number of individuals considered exposed in the bioassay was therefore reduced from 50 to 31.

The LT_{50} and LT_{90} , estimated from the recorded data (Table 3.4.16.2), was 3 days 16 h 30 min and 8 days 8 h 20 min, respectively. The LT_{50} and LT_{90} of CrleGV were estimated at 4 days 22 h and 7 days 8 h, respectively (Moore, 2002). Therefore, relative to CrleGV, the GM virus induces mortality sooner (hence the shorter LT_{50}). The first virus-induced mortality was recorded after 8 h (Table 3.4.3.2), compared to bioassays with CrleGV in which initial virus-induced mortality was recorded after 40 h (Moore, 2002). However, the LT_{90} of the GM virus is longer. This is disappointing, as the professed benefit of genetic modification is improvement of the speed of kill. It appears that the GM virus will offer little, if any, advantage over CrleGV.

Table 3.4.16.2 Mortality and response of neonate *C. leucotreta* larvae in time-response bioassays with the LC_{90} concentration of a GM virus.

Time after treatment		Cumulative larval mortality (corrected for non-virus induced control mortality) (%)	Larvae not penetrating the diet (%)	
Days	Hours		Control	Treatment
0	8	3.23	16.13	0.00
	16	3.23	0	0.00
1	0	6.45	0	3.23
	8	6.45	0	3.23
	16	9.68	0	9.68
2	0	12.90	0	12.90
	8	16.13	0	12.90
	16	19.35	0	16.13
3	0	22.58	0	16.13
	8	25.81	0	9.68
	16	29.03	3.23	3.23
4	0	45.16	0	9.68
	8	61.29	0	9.68
	16	70.97	0	9.68
5	0	80.65	0	9.68
	8	93.55	0	6.45
	16	96.77	0	3.23
6	0	100.00	0	0.00

It must be noted that as is the case with CrleGV (Moore, 2002), mortality was not the only benefit provided by infection. There was also a conspicuous difference in behaviour between larvae on inoculated diet and those on untreated diet. From 24 h post-infection larvae were recorded on the surface of the treated diet, or even on the inside surfaces of the containers (Table 3.4.16.2). This was rarely the case for larvae on the untreated diet. This was either a response to viral infection, preceding death, or a deterrent effect of the virus to penetration by the larvae into the diet, or a combination of the two.

Conclusion

LC_{50} and LC_{90} of the GM-virus against neonate FCM larvae were estimated to be 2.257×10^8 OBs/ml and 1.854×10^{10} OBs/ml respectively. This is substantially higher than that of CrleGV. LT_{50} and LT_{90} of the GM-virus against neonate FCM larvae were estimated at 3 days 16 h 30 min and 8 days 8 h 20 min, respectively. The LT_{50} is shorter than that of CrleGV. However, the LT_{90} is longer. It therefore appears that the GM-virus will offer little, if any, benefit over CrleGV. It will also be more expensive to produce and will probably have to be applied at a higher concentration than CrleGV, further raising the cost.

Future research

Detached fruit bioassays will be conducted shortly. Thereafter it is likely that no further work on the GM virus will be conducted, as results obtained here already indicate that its use will not be commercially viable.

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3.5 PROJECT: FRUIT FLY

Project Co-ordinator: Tony Ware (CRI)

Project summary

Fruit flies continue to receive a large amount of attention from the southern African fruit industries. Research has been undertaken on a broad front, both in orchards and in the laboratory, in order to open new markets and maintain the traditional ones. Rearing a good quality fruit fly (3.5.2) plays the pivotal role in market access research. The insects were used for research on mitigating treatments to ensure disinfestation of Mediterranean fruit fly-infested Clementine mandarins (3.5.4) and Barlinka grapes (3.5.5). A 14-day exposure at -0.5°C ($\pm 0.5^{\circ}\text{C}$) on Mediterranean fruit fly-infested Clementines and a 16-day exposure at 0.5 ($\pm 0.5^{\circ}\text{C}$) of Mediterranean fruit fly-infested Barlinka grapes prove to be effective and satisfy Probit 9 requirements. Other forms of mitigating treatments were investigated and included subjecting insects to hypobaric (low) pressure or methyl bromide fumigation (3.5.6). Neither was particularly successful.

Other research aspects investigated were the monitoring and control of fruit fly in the orchard. This was considered an important research component after the interception of Natal fruit fly in a southern African citrus consignment in Spain. Two surveys were conducted (3.5.3.1 and 3.5.3.2) and it was demonstrated that towns can harbour large numbers of flies and that Natal fruit fly populations were low in both the Kakamas Orange River and Vaalharts irrigation scheme areas. The use of the M3 bait station for area-wide control of fruit flies was investigated in Clementine orchards in the Eastern Cape province (3.5.3.3 and 3.5.3.4), grapefruit in the Onderberg (3.5.3.5), mangoes and litchis in the Onderberg (3.5.3.6 and 3.5.3.7) and grapes in Kakamas (3.5.3.8). Control was satisfactory in all but one site (3.5.3.4) where excessive fruit damage by birds is believed to have resulted in fruit rot that competed with M3 bait station as a protein food source. Spinosad (GF120 NF) was tested in the field (3.5.8) and in cages (3.5.9) and was found to be comparable in efficacy to the traditional protein hydrolysate/mercaptothion treatments. An M3 organic bait station prototype was developed and tested (3.5.10) and was shown to have potential for this type of farming operation. The option of using Capilure and Questlure in the same trap was investigated (3.5.11). In general the combination attracted fewer flies except for Natal fruit fly in which the mixture appeared to be better. Research was also conducted on an alternative fungicide for the M3 bait station that was longer lasting than the currently used product (3.5.11). A survey for exotic fruit fly in the region was conducted and, although identification still has to be completed, no exotic fruit flies were recorded (3.5.12).

Future research will focus primarily on market access issues. Research is to be conducted on lemons, Clementines, grapefruit and oranges using a mitigating disinfestation treatment of 0.5°C . Disinfestation research will also be conducted on behalf of litchi and persimmon industries. Alternatives to organophosphates for fruit fly control will be investigated while the monitoring for Natal fruit fly will also receive attention.

Projekopsomming

Vrugtevlieë ontvang nog steeds heelwat aandag van die Suider-Afrikaanse vrugtebedryf. Navorsing is oor 'n wye gebied onderneem, beide in die boord en in die laboratorium, met die doel om nuwe markte te open en bestaandes te behou. Teling van goeie gehalte vrugtevlieë (3.5.2) speel 'n sleutelrol in navorsing gemik op toegang tot markte. Die insekte is gebruik in navorsing oor verliggende behandelings vir die ontsmetting van Mediterreense vrugtevlieg-besmette clementine mandaryn (3.5.4) en Barlinka druiwe (3.5.5). Blootstelling van besmette Clementines en Barlinka druiwe aan onderskeidelik $-0.5\text{ }^{\circ}\text{C}$ ($\pm 0.5\text{ }^{\circ}\text{C}$) vir 14 dae en $0.5\text{ }^{\circ}\text{C}$ ($\pm 0.5\text{ }^{\circ}\text{C}$) vir 16 dae, was suksesvol en het voldoen aan Probit 9 vereistes. Verdere vorms van verliggende behandelings wat ondersoek is het die blootstelling van insekte aan hipobariese (lae) druk of metielbromiedberoking ingesluit (3.5.6). Nie een hiervan was besonder suksesvol nie.

Ander navorsingsaspekte wat ondersoek is was onder andere die monitering en beheer van vrugtevlieg in die boord. Dit was geag as belangrik ná die onderskepping van 'n Suider-Afrikaanse besending sitrus in Spanje. Twee opnames is onderneem (3.5.3.1 en 3.5.3.2) wat aan die lig gebring het dat stede en dorpe groot getalle van die vlieë kan huisves en dat populasies van Natalse vrugtevlieg laag was in beide die Kakamas Oranjerivier en Vaalharts besproeiingsgebiede. Gebruik van die M3 lokaasstasie vir streekswyse beheer van vrugtevlieë is ondersoek in Clementine boorde in Oos-Kaap Provinsie (3.5.3.3 en 3.5.3.4), asook pomelo (3.5.3.5), mango en lietsjie (3.5.3.6 en 3.5.3.7) in die Onderberg en druiwe in Kakamas (3.5.3.8). Beheer was bevredigend behalwe in een geval (3.5.3.4) waar uitermatige skade deur voëls klaarblyklik gelei het tot die verrotting van vrugte om sodoende 'n substraat te skep wat met die M3 lokaasstasie gekompeteer het as proteïen-voedselbron. Spinosad (GF 120 NF) is in die veld (3.5.8) en in hokke (3.5.9) getoets en die doeltreffendheid daarvan was vergelykbaar met dié van tradisionele hidrolisaat/merkaptotien behandelings. 'n M3 organiese lokaasstasie is ontwikkel en getoets (3.5.10) en blyk belofte in te hou vir hierdie tipe boerdery-onderneming. Die opsie om Capilure en Questlure in dieselfde lokval te gebruik is ondersoek (3.5.11). Oor die algemeen het die kombinasie minder vlieë gelok, behalwe vir Natalse vrugtevlieg, waar die mengsel oënskynlik beter gewerk het. Navorsing is ook onderneem om 'n swamdoder met langer werking as die huidige te vind vir die M3 lokaasstasie (3.5.11). 'n Opname van eksotiese vrugtevlieë in die gebied (3.5.12) het geen vreemde spesies opgelewer nie, alhoewel identifisering van die vlieë nog nie afgehandel is nie.

Toekomstige navorsing sal hoofsaaklik gerig wees op aspekte van toepassing op toegang tot markte. Ondersoek sal ingestel word na die verliggende effek wat blootstelling aan $0.5\text{ }^{\circ}\text{C}$ het by suurlemoene, Clementines, pomelos en lemoene. Navorsing oor ontsmetting sal ook namens die lietsjie en dadelpruim bedrywe onderneem word. Organofosfaat-alternatiewe vir die beheer van vrugtevlieë sal ondersoek word, terwyl monitering van Natalse vrugtevlieg ook aandag sal geniet.

3.5.2 Fruit fly Rearing

Experiment 407 by Tony Ware, John-Henry Daneel and Rooikie Beck (CRI)

Opsomming

Beskikbaarheid van goeie biologiese materiaal is noodsaaklik vir die verkryging van herhaalbare resultate. Die doel van hierdie navorsingsprojek was om vrugtevlieë van goeie gehalte te teel in getalle voldoende vir gebruik in ander projekte. In dié verband is aandag gegee aan die ontwikkeling van diëte en tegnieke vir die teling van die drie ekonomies-belangrike spesies in suidelike Afrika, nl. *Ceratitis capitata*, *C. rosa* en *C. cosyra*.

Introduction

It is essential that one use good biological material if one is to produce reproducible results. The object of this research project was to produce fruit fly of good quality and in the required volumes to support other research projects. To this end some time and effort has gone into developing diets and techniques to rear the three economically important southern African species namely *Ceratitis capitata*, *C. rosa* and *C. cosyra*.

Materials and methods

A single diet that is able to support the rearing of the three species has been developed. Other than that, techniques have not been altered to those previously reported.

Results and discussion

The three species have been reared in sufficient quantities with the required quality to meet research demands. Mediterranean fruit fly was supplied for the cold disinfestation treatment of Clementines (experiment 709) and Barlinka grapes (DFPT contract) and avocado (SAAGA contract). Natal fruit fly was used for avocado disinfestation research (SAAGA contract) and for GF 120 cage tests (Dow AgroScience contract). Marula fruit fly was also used in avocado disinfestation research and for the GF 120 cage tests. All three species were used in research that investigated methyl bromide and hypobaric disinfestation of fruit fly-infested material.

Conclusion

The project continues to satisfy local fruit fly research needs.

Future research

The generating of suitable data comparing these diets with traditional diets is needed. A method to collect Natal fruit fly eggs in large quantities requires attention. This is essential if the mass rearing of that species is to be undertaken for SIT or other purposes.

3.5.3 Area-Wide Control of Fruit Fly Experiment 694 By Tony Ware (CRI)

Opsomming

Die belang van vrugtevlieë is nie soseer die skade wat hulle aanrig nie, maar eerder die fitosanitêre implikasies meegebring deur die teenwoordigheid van 'n enkele individu in 'n besending. Om die moontlikheid van sulke onderskeppings deur die owerheid van 'n invoerende land te beperk, moet kwekers die plaag in hulle boorde beheer. Tradisioneel is beheer toegepas deur die insekte te lok met proteïenhidrolisaat en 'n organofosfaate soos merkaptotion. Die M3 lokaasstasie bied 'n IPB-verenigbare alternatief vir hierdie tradisionele metodes. Dit is "veilig" vir natuurlike vyande en die omgewing en kans is gering dat vrugte met 'n plaagdoder besoedel sal word. Die langdurende werking daarvan (ongeveer 16 weke) maak dit geskik vir die daarstelling en handhawing van vrugtevlieg-vrye gebiede (of ten minste gebiede met 'n lae voorkoms).

Twee vrugtevliegopnames is gedoen, een in Vaalharts en die ander in Kakamas. Mediterreense vrugtevlieg was die oorheersende spesie in beide gebiede. Slegs een Natalse vrugtevlieg is in Kakamas gevang, wat daarop dui dat die spesie nog nie in die gebied gevestig het nie, of nie daartoe in staat is nie. Populasies van Natalse vrugtevlieg in Vaalharts was laag en beperk tot 'n kort tyd van die jaar. Die M3 lokaasstasie het vrugtevlieë doeltreffend beheer op Clementine (Patensie - Oos-Kaap Provinsie), asook Marsh pomelo, lietsjie en mango (Onderberg - Mpumalanga). Een geval waar die behandeling gefaal het om vrugtevlieg te beheer was in 'n Clementine boord in Patensie waar muisvoëls aansienlike skade aan die vrugte aangerig het. Die gevolglike verrotting van vrugte deur bakterieë en swamme het 'n alternatiewe proteïenbron geskep wat doeltreffend met die M3 lokaasstasie meegeeding het as voedselbron. Die betrokke kweker kon die vorige seisoen nie vrugtevlieë beheer met tradisionele lokaasmetodes nie.

Alhoewel die M3 lokaasstasie doeltreffend blyk te wees teen sterktes laer (250/ha) as waarteen dit geregistreer is (300-400/ha), dui die mislukking daarvan by die boord in Patensie daarop dat versigtigheid aan die dag gelê moet word met die toepassing van so 'n strategie. Indien die kostekwessie aangespreek kan word het die M3 lokaasstasie potensiaal om gebruik te word in streekswye vrugtevlieg-beheerprogramme.

Summary

The importance attached to fruit fly is not so much the damage it does, but rather in the phytosanitary implications of finding a single individual in a consignment. In order to minimise the chances of such interceptions by authorities from an importing country, the grower must control the pest in the orchard. These control measures are traditionally done through baiting using protein hydrolysate and an organophosphate such as mercaptotion. The M3 bait station offers an IPM compatible alternative to these traditional methods. It is "safe" to natural enemies and the environment and there is little chance that the fruit will be contaminated with pesticide. Its long lasting activity (approximately 16 weeks) makes it a candidate for use in creating and maintaining fruit fly free areas (or at least areas of low prevalence).

Two fruit fly surveys, one in the Vaalharts and the other in Kakamas, were done. Mediterranean fruit fly was the predominant species in both areas. Only one Natal fruit fly was caught in Kakamas indicating that the species may not have (or cannot) established in the region. The Vaalharts Natal fruit fly population was low and confined to a short period of the year. The M3 bait station was effective in controlling fruit fly in Clementine (Patensie - Eastern Cape Province), Marsh grapefruit, litchis and mangoes (Onderberg – Mpumalanga). The one occasion where the treatment failed to control fruit fly was in a Clementine orchard in Patensie (Eastern Cape Province) where mousebirds had caused considerable damage to the fruit. The consequent rotting of the fruit by bacteria and fungi resulted in an alternate protein source that effectively competed with the M3 bait station as a food source. The grower had been unable to control fruit flies using traditional baiting methods in the previous season.

While the M3 bait station can be effective when used at lower rates (250/ha) than at which it is registered (300-400/ha), the breakdown in its effectiveness at the one Patensie site indicates that care should be taken when adopting this strategy. Should the cost issue be addressed then the M3 bait station has the potential to be used in fruit fly area-wide control programmes.

General Introduction

There are more than 4000 species of fruit fly (Tephritidae) throughout the world but only approximately 100 of these are of economic importance (White and Elson-Harris, 1992). In southern Africa there are three species of agricultural importance. These are the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]), the Natal fruit fly (*Ceratitis rosa* Karsch) and the marula fruit fly (*Ceratitis cosyra* [Walker]).

The Mediterranean fruit fly (Medfly) has a wide host range and is considered to be the most serious pest of the three species. It occurs in most of the fruit growing regions of the world with the notable exceptions of East Asia and Japan, New Zealand and parts of Australia. Although the United States of America is considered to be free of this particular fruit fly pest there have been periodic outbreaks in California and Florida (Dowell *et al.*, 2000). All these countries consider Medfly as a phytosanitary pest and all fruit imported from southern Africa must carry the guarantee that it does not harbour the pest. This often means that the fruit must undergo a post-harvest mitigating treatment such as cold sterilization or methyl bromide fumigation before being allowed entry. These treatments are often expensive and can impact negatively on fruit quality, often limiting the period for which the fruit can be stored. Countries already harbouring Medfly do not restrict the movement of produce but the inability to control the pest may lead to high wastage and loss of market confidence in the product.

Both marula and Natal fruit fly have a more restricted distribution than Medfly and only occur in sub-Saharan Africa although the latter also occurs on the island of Reunion (White and Elson-Harris, 1992). The host range of Natal fruit fly is similar to Medfly and it is viewed as a phytosanitary pest. It is more prevalent in the more humid parts of the country and is rare or absent in the desert or semi-desert areas. In contrast, marula fruit fly has both a limited host and distribution range and is not considered a major pest on most southern African fruit crops with the possible exceptions of mangoes and guavas.

The traditional method of controlling fruit flies is through the use of bait sprays. In South Africa these are generally a mixture of the bait (protein hydrolysate – Hym lure, Buminal or Nasiman) plus a toxicant (Dipterex SP, Malathion EC or Malathion WP) (Nel *et al.*, 2002). The diluted mixtures are applied using a tractor-mounted applicator or a knapsack sprayer. The spray equipment is modified to ensure that the droplets applied are coarse. Because the baits are most effective on the day of application and rapidly lose efficacy thereafter, they should be applied in the morning. The baits are rendered ineffective by rain.

While organophosphate baits are the mainstay of the fruit fly baiting programme, concerns about the continual use of this group of pesticides has been expressed. There is strong market pressure to phase out these chemicals and many have been earmarked for withdrawal. The situation is further compounded by EUREPGAP legislation in that growers are required to minimise pesticide usage because of human safety and environmental concerns. Discerning markets have already indicated their intention to source produce that has not been treated with Malathion.

A less traditional approach is the use of the sterile insect technique (SIT) to control fruit flies. This involves the irradiation of large numbers of male Medfly, rendering them sterile, that are then released and compete for the favours of the wild females. The females that are inseminated with sperm from these sterile males are unable to produce eggs and theoretically the population crashes. However, to be effective there must be an overwhelming number of competitive sterile males which means that chemical control is a pre-release prerequisite to lower the insipient population levels. A pilot project is currently being undertaken in the Hex River Valley (Barnes and Eyles, 2002) but suffers various drawbacks including no central

government funding. The presence of a second species of fruit fly, the Natal fruit fly, is problematic in that no sterile insect technique has been developed for this species. Indigenous trees and backyard fruit trees that provide a continual source of flies have been placing pressure on the SIT programme. Furthermore, there is little hope that the government will provide a legal framework that will prevent the transport of fruit into the area as this would impact on the hawker trade and infringe on the constitutional rights of these individuals.

The M3 bait station (Figure 3.5.3.1) was developed in order to address some of these concerns. The following research was undertaken in order to demonstrate the effectiveness of this “attract and kill” technology” and to assess its future role in providing area-wide control.

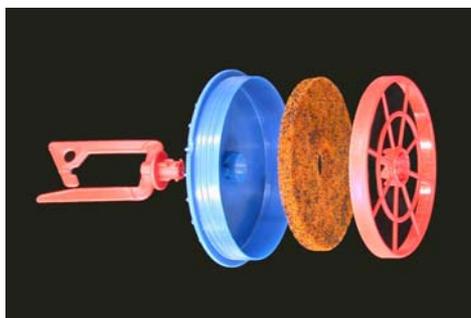


Figure 3.5.3.1. Exploded view of the M3 bait station

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3.5.3.1 Kakamas survey

Experiment by Tony Ware (CRI), John-Henry Daneel (CRI) and Francois Reyneke (Kromhout Boerdery)

Materials and methods

Twelve Sensus traps containing Capilure were placed at various locations in and around Kakamas town in the Northern Cape Province (Figure 3.5.3.1.1.). Trap 1 was placed in a peach tree on the outskirts of town bordering the vineyards. Trap 2 was placed in a nursery in an unidentified non-fruiting tree. Trap 3 was placed in a non-fruiting tree near the AGS church. A few fruiting trees were in the vicinity. Trap 4 was placed in a hedge near a taxi rank while trap 5 was positioned in an orange tree surrounded by a vegetable garden. Trap 6 was placed in a citrus tree and trap 7 in a mango tree, trap 8 and 9 were hung in a peach tree and a guava tree respectively. Trap 10 was placed in a vine of black grapes while trap 11 was hung in a small block of grapes that were used for raisin production. The last trap was hung in a quince tree that was surrounded by numerous other fruiting trees.

The traps were emptied weekly over a 12 month period beginning in January 2002. All the flies collected were sent to the laboratory in Nelspruit where they were counted and identified to species. The attractant lure and dichlorvos were changed every six weeks. No attempt was made to control the flies in the town.

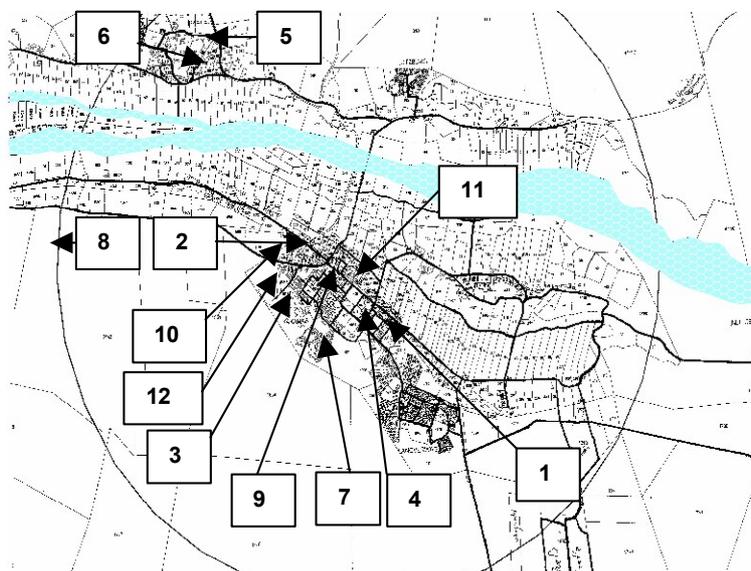


Figure 3.5.3.1.1. Positions of Sensus traps charged with Capilure in Kakamas town. Trap 8 was placed at Marchand some 14 km from Kakamas. The circle represents a radius of 5 km.

Results and discussion

A total of 39089 Mediterranean fruit flies were trapped over the 12-month sampling period. A single Natal fruit fly was trapped during this time indicating the relative scarcity of this species in the area. Based on these results it is assumed that Natal fruit fly does not permanently inhabit the area and is probably introduced in fruit imported for local consumption. This observation is of particular importance in demonstrating that the area has low Natal fruit fly populations and could be earmarked for European export should those authorities insist on post-harvest mitigating treatments against Natal fruit fly.

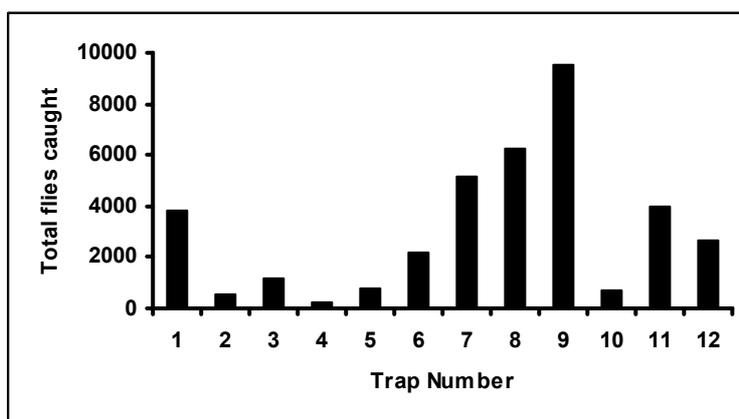


Figure 3.5.3.1.2. Total number Mediterranean fruit fly caught at various sites (see Figure 3.5.3.1.1) in Kakamas.

Exceptionally high numbers were trapped in the Sensus trap (trap 9) positioned on the guava tree (Figure 3.5.3.1.2). In general those traps placed in fruiting trees caught more flies. The traps placed in the nursery (trap 2), in the church grounds (trap 3), near a taxi₁₅₄ rank (trap 4), in a citrus tree (trap 5) and near the

black grapes (trap 11) caught few flies. These results confirm the recommendations by the USDA (2001) and IAEA (2003) of the importance of placing the traps in host trees when monitoring fruit fly.

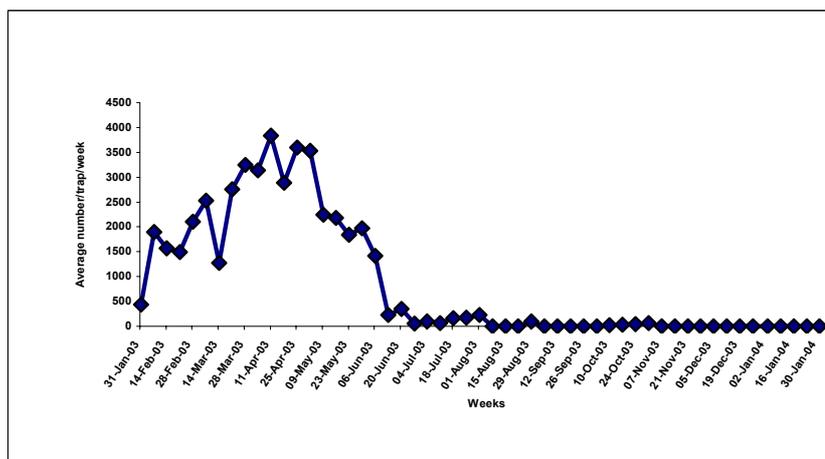


Figure 3.5.3.1.3. Weekly trap counts of fruit fly caught in Sensus traps with Capilure in Kakamas town.

Most of the flies were caught in the summer and autumn months and the numbers tapered off in the second half of the year (Figure 3.5.3.1.3). This implies that few suitable hosts were available for infestation after June.

Conclusion

Kakamas and, by extrapolation, the Northern Cape Orange River area only have one species of fruit fly permanently inhabiting the areas. Most of the flies are present during the first half of the year and any fruit ripening during this time would be under threat. Citrus that matures later in the year should escape the attention of fruit fly.

References cited

International Atomic Energy Agency (2003). *Trapping Guidelines for Area-wide Fruit Fly Programmes*. Vienna, Austria. pp46.
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3.5.3.2 Vaalharts fruit fly survey

Experiment by Tony Ware, John-Henry Daneel (CRI) and Johanna Mathewson (Saamfarm)

Materials and methods

Two Sensus fruit fly traps containing either Capilure or Questlure attractants were placed at each of 24 locations throughout the Vaalharts irrigation area. There were approximately 50 km between the two outermost traps. A description of the individual locations was Area 1 - Harts Valley: Trap location 1. Saamfarm in an unsprayed backyard orchard. Trap location 2. Saamfarm in beefwood windbreak adjacent to veld. Trap location 3. Hammies in a garden near to citrus. Trap location 4. Pieter van Wyk in an unsprayed citrus orchard. Trap location 5. Verweg citrus in beefwood adjacent to citrus. Trap location 6. Lourens de Jager in beefwoods between citrus and grapes. Area 2 - Jan Kempdorp: Trap location 7. Chempool Jan Kempdorp in garden. Trap location 8. Wessel Hamman in town. Flies controlled using M3 bait stations. Trap location 9. Agricultural School in deciduous fruit trees. Trap location 10. Braam van Wyk near citrus and peaches. Trap location 11. Wessel Saunders in louquat tree near citrus. Trap location 12. Josias Delpont in deciduous trees. Trap location 24. Pieter Burger in abandoned citrus orchard. Area 3 - Hartswater: Trap location 13. T.C. Meyer in backyard near citrus. Trap location 14. Hansie Lategan in beefwoods in citrus orchard. Trap location 15. Naas Sauerma in town backyard. Trap location 16. Vaalharts citrus nursery in town. Trap location 23. Wynhuis in garden trees. Area 4 – Magogong: Trap location 17. Willie de Bruin near citrus. Trap location 18. Louisa Lubbe near citrus. Trap location 19. Johan Smit between citrus and deciduous orchards. Trap location 20. Marius de Bruin between citrus and deciduous orchards. Trap location 21. Willem de Bruin in backyard. Trap location 22. Frans Venter Nursery. The lure and attractants were changed approximately every six weeks. All flies were collected every

fortnight and identified to species and sexed. Fruit fly trapping was initiated on 18 April 2003 and terminated 44 weeks later.

Results and discussion

Most of the flies that were trapped were caught in the first 8 weeks and in the last 4 weeks of the trial (Figure 3.5.3.2.1). The majority of the flies caught were *C. capitata*. (95.7%) with the rest being *C. rosa* (Table 3.5.3.2.1). The winter period proved to be quiet with few flies being recorded corresponding to the time of year when there are few hosts.

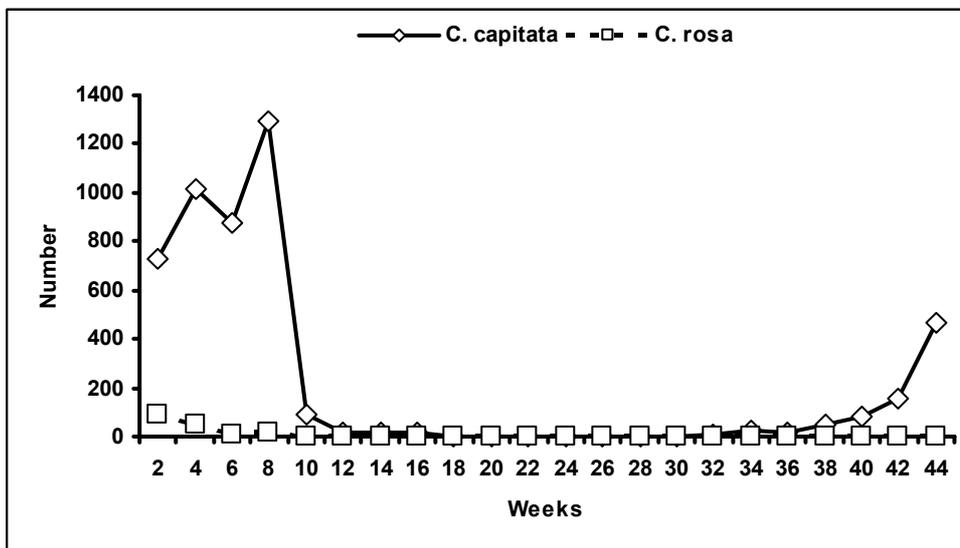


Figure 3.5.3.2.1. Fruit fly trap catches in the Vaalharts irrigation area

A total of 5634 fruit flies were trapped throughout the area, 86% were *C. capitata* trapped in the Sensus traps containing Capilure. In this trial site Capilure proved to be more attractive to *C. rosa* than Questlure (see other results in this report). The ratio of fruit fly catches between the Capilure and Questlure was almost 10:1.

Table 3.5.3.2.1. Fruit flies caught in the Vaalharts irrigation area in 2003/4

	<i>C. capitata</i> females	<i>C. capitata</i> males	<i>C. rosa</i> females	<i>C. rosa</i> males	Total
Capilure	17	4867	0	167	5051
Questlure	407	101	54	21	583
Total	424	4968	54	188	5634

Nearly 80% of the flies caught were trapped in the Harts Valley and Jan Kempdorp areas (Table 3.5.3.2.2). This may be a result of the presence of untreated host trees in these areas but also may reflect the efficiency of fruit fly baiting in the other two areas. In general the number of fruit fly trapped was low with only 2.7 flies /trap/week being recorded. The low number of *C. rosa* could allow for the creation of a Natal fruit fly area of low prevalence. This may be important if Europe decides to impose mitigating treatments for citrus originating from areas that have this species.

Table 3.5.3.2.2. Fruit fly catches by area in the Vaalharts irrigation area.

Area	Capilure	Questlure	Total
Harts Valley	1960	477	2437
Jan Kempdorp	1991	61	2052
Hartswater	224	22	246
Magogong	876	23	899
Total	5051	583	5634

Conclusions

1. The Vaalharts area appears to be a region that has a naturally low fruit fly population.
2. The fruit fly populations drop to negligible levels during winter.
3. Although *C. rosa* occurs in the area it appears to be seasonal and may never occur in large numbers.

Acknowledgements

We would like to extend our thanks to all the collaborators and to Saamfarm for their support.

3.5.3.3 Patensie - Nuwelande – Clementines

Experiment by Tony Ware and Garth Richards (CRI)

Materials and methods

The site used was a four ha site situated in the Patensie Valley of the Eastern Cape Province of South Africa and was approximately 400 m from the edge of the town. The bait stations were placed in the trees approximately four weeks (11 December 2001) before colour break at a rate of 350 stations per ha. The fruit fly population was monitored using Sensus traps baited with either Capilure or Questlure. The traps were placed as illustrated in Figure 3.5.3.3.1. The traps surrounding the orchard were approximately 10 m from the nearest Clementine tree. Those traps designated to be on the border were placed in the second tree from the edge of the orchard. Two traps, one containing Capilure and one with Questlure, were placed in the centre of the orchard. The traps were monitored weekly and all fruit flies caught were sexed and identified to species. At harvest the orchard was inspected for fruit fly damage.

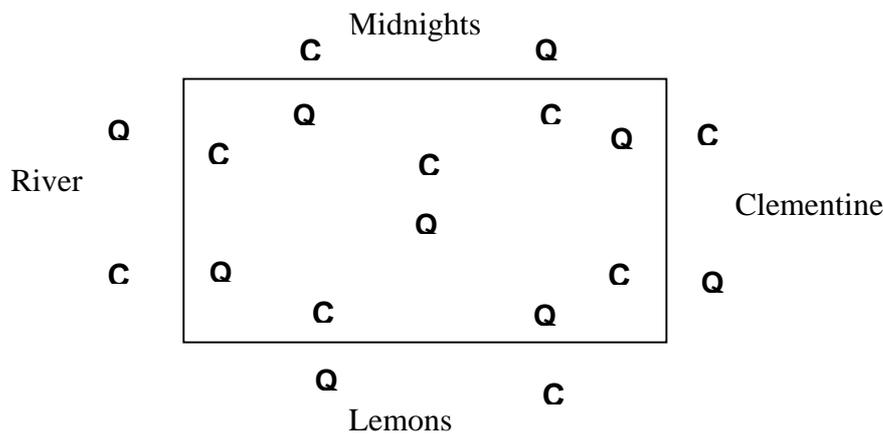
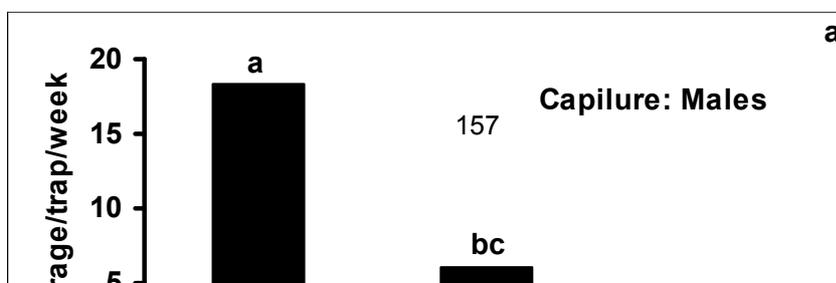


Figure 3.5.3.3.1. Positions of Sensus traps containing Capilure and Questlure attractant in and around a 4 ha Clementine orchard situated in the Patensie area of the Eastern Cape Province.

Results and discussion

Only Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]) were caught in the traps. Most flies were caught in the traps placed on the outside the orchard, fewer flies on the border and low numbers in the centre of the orchard using Capilure (Figure 3.5.3.3.2). Although the number of flies caught in the Sensus traps containing Questlure were lower than those charged with Capilure, the pattern of trap catches was similar to the Capilure traps. There was no statistical difference between the number of fruit fly caught in the traps placed on the border and those placed in the centre of the orchard for either lure.



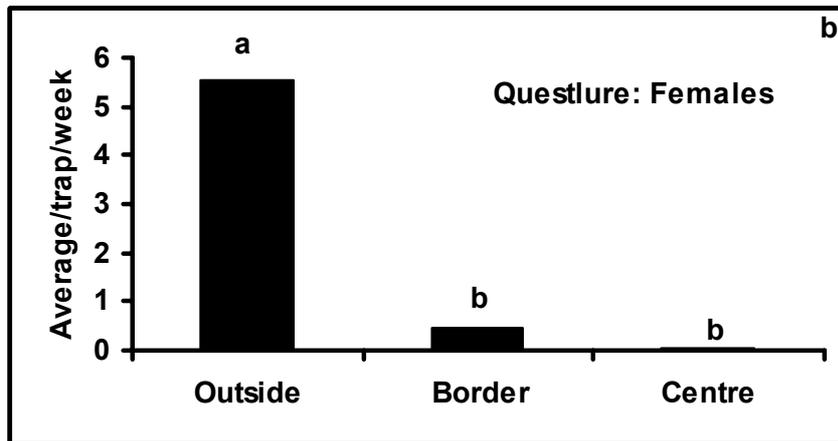


Figure 3.5.3.3.2. Mediterranean fruit fly catches in Sensus traps placed outside, in the border trees and in the centre of the orchard. Similar letters indicate no statistical difference between the number of fruit fly caught (ANOVA with the means compared using Fisher's LSD test ($P < 0.05$)). a. Capilure b. Questlure.

Temporal catches over the trial period for the Capilure and Questlure baited fruit fly traps are presented in Figure 3.5.3.3.3. The effect of the bait stations and routine fruit fly baiting in the surrounding orchards is reflected in the decrease in the number of fruit fly trapped from week four in the traps positioned on the outside of the orchard (Figure 3.5.3.3.3A). No doubt the proximity of the untreated town fruit trees resulted in continuous fruit fly pressure. Although the scale of the two y-axes are different (indicating that for Mediterranean fruit fly that Capilure is the more efficient trap), the relative pattern of fruit fly catches were similar. If one considers the treatment threshold to be 7 flies/trap/week using Capilure and 2 flies/trap/week using Questlure, one would have applied fruit fly baits on 15 occasions with respect to the Capilure trap catches and 14 occasions if one was reacting to the Questlure numbers.

Small numbers of flies were trapped in the Sensus traps placed on the border of the orchard (Figure 3.5.3.3.3B). At no stage did the numbers exceed the treatment thresholds. A similar result was obtained from the traps placed in the centre of the orchard with the exception of the first week of trapping when the treatment threshold was exceeded in the Capilure baited traps.

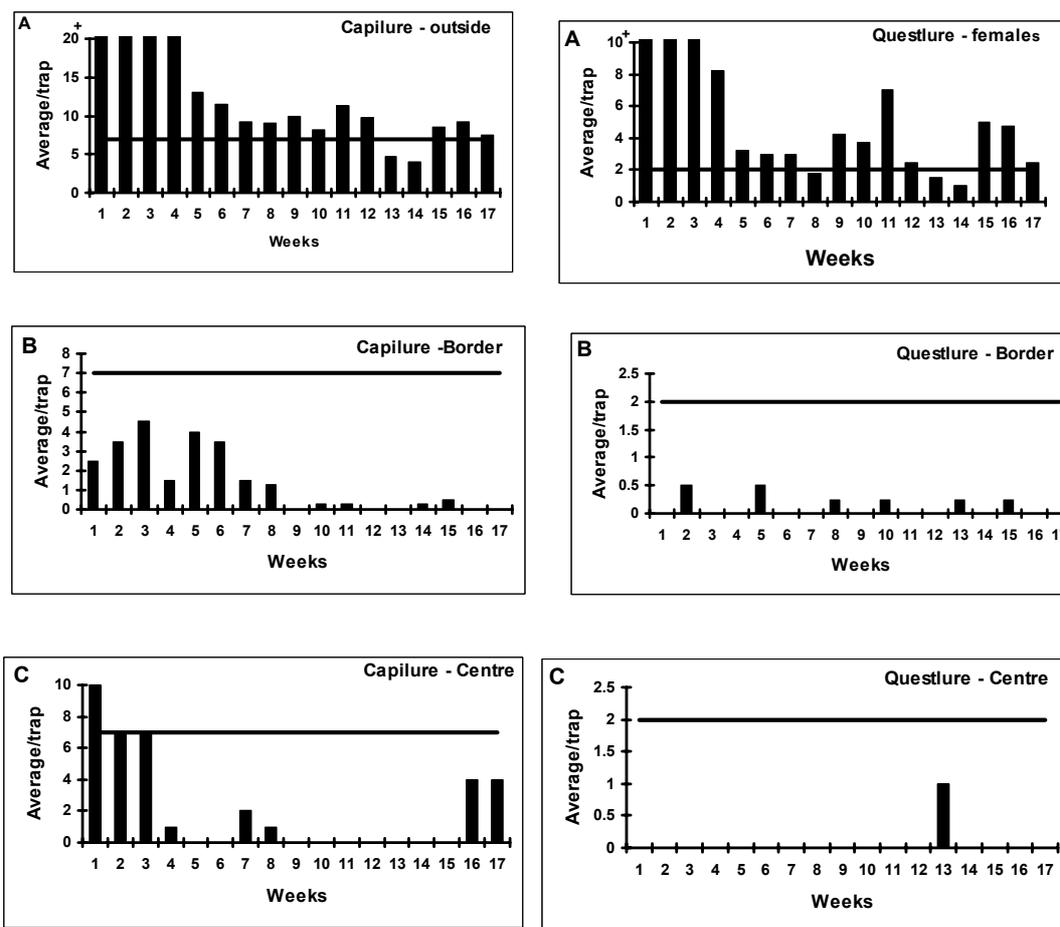


Figure 3.5.3.3.3. Mediterranean fruit fly (*Ceratitis capitata*) trapped in Sensus traps containing Capilure or Questlure positioned outside (A), on trees near the border (B) and in the centre of a Clementine orchard (C) that was treated with M3 bait stations.

These results indicate that baiting does **not** selectively remove females. If this were the case then there would be a build up of male numbers in the orchard, a result not indicated by the data.

A large number of fruit were damaged by false codling moth but only two fruit were considered to have been subject to fruit fly attack. These data indicated that the M3 bait stations were an effective fruit fly control measure.

Conclusions

- The M3 bait station was an effective fruit fly treatment.
- The two attractants, albeit relatively different, produced similar results in relationship to their individual treatment threshold values.
- Baiting did not selectively remove females.

3.5.3.4 Patensie – Stuk van Acht – Clementines

Experiment by Tony Ware, Garth Richards (CRI) and Michael van Rensburg (Patensie Co-op.)

Materials and methods

The lures, and the positioning thereof, were similar to the Nuwelande trial. The trial site differed from the Nuwelande site in that the orchard was situated a number of kilometres from the town between the road and the river. The orchard was more elongated, consisting of only 15 rows of trees. Approximately 320 M3 bait stations were used to treat every hectare.

Results and discussion

In this experiment the border traps attracted the largest number of flies regardless of the attractant used. The low number of flies caught in the traps placed outside the orchard indicated that the fly populations emanated from within the orchard, in particular on the orchard edges (Figure 3.5.3.4.1).

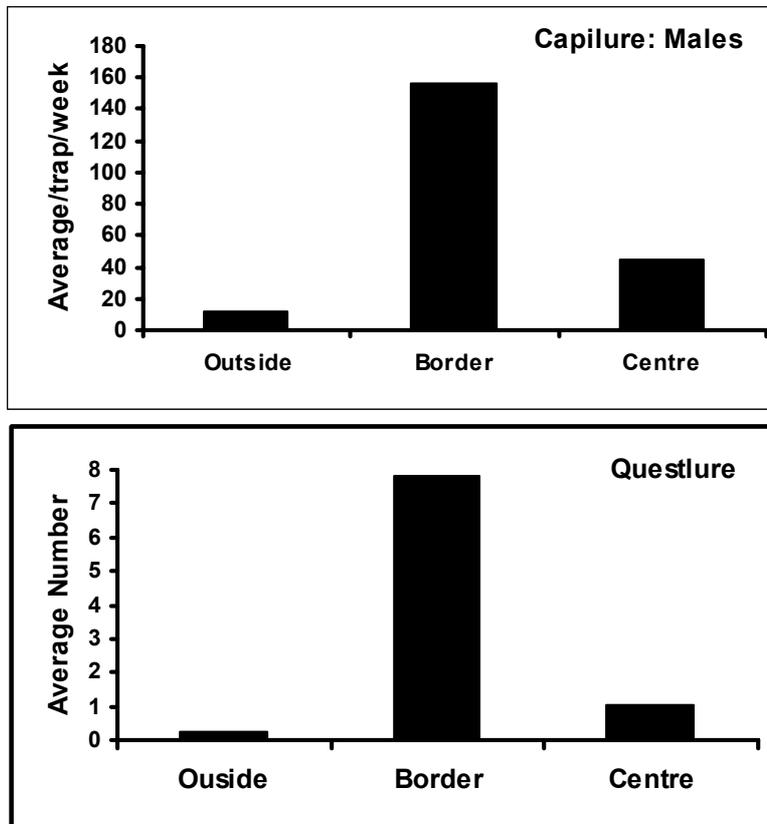
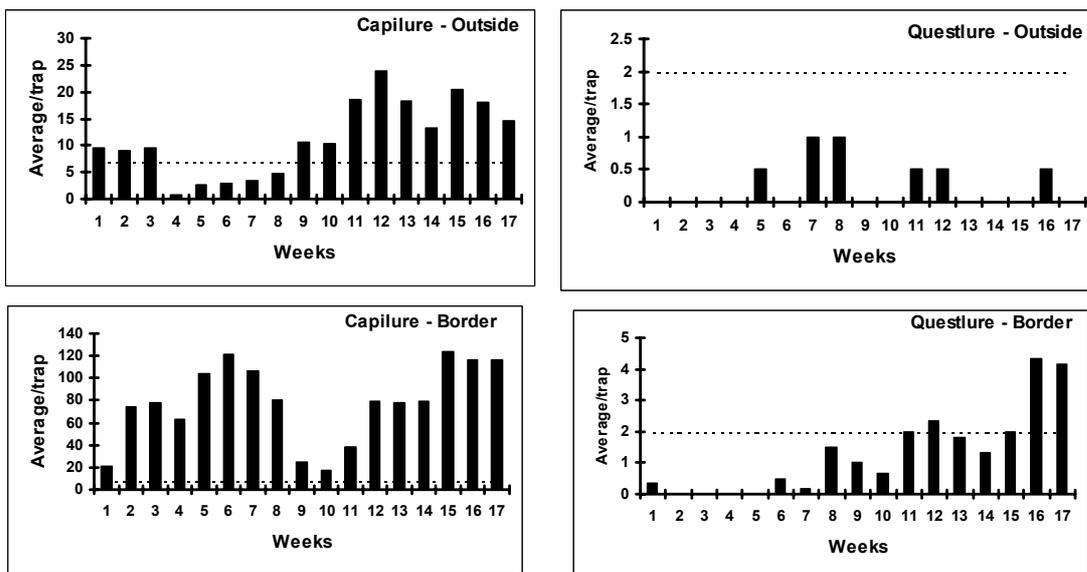


Figure 3.5.3.4.1. Mediterranean fruit fly catches in Capilure and Questlure baited Sensus traps baited placed outside, in the border trees and in the centre of the orchard.

The temporal fruit fly trap catches differed from the previous trial in that the two attractants did not mirror each other in respect of their respective treatment threshold values (Figure 3.5.3.4.2). Trap catches using Capilure indicated that there were excessive numbers of flies throughout the trial period while Questlure traps only indicated that the last few weeks were problematic.



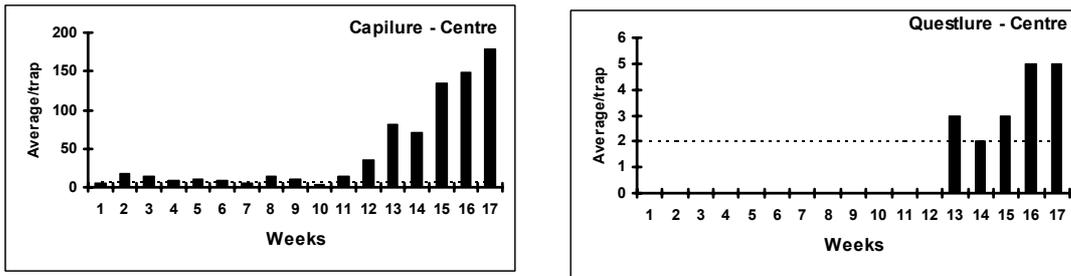


Figure 3.5.3.4.2. Mediterranean fruit fly (*Ceratitis capitata*) trapped in Sensus traps containing Capilure or Questlure positioned outside, on trees near the border and in the centre of a Clementine orchard that was treated with M3 bait stations.

In order to explain this pattern of fruit fly catches the orchard was inspected a week before the proposed harvest date. The number of dropped fruit/tree was high on the side of the orchard nearest the road and decreased towards the centre of the orchard (Figure 3.5.3.4.3). A large number of fruit fly-infested fruit was sampled (Figure 3.5.3.4.4) and was highest in the first four rows of the orchard. The percentage infestation tapered off as one transversed across the orchard and the immediate conclusion is that fruit fly damage was responsible for the fruit drop. However, closer examination demonstrated that large numbers of fruit in the trees in rows 1 to 4 had been damaged by mousebirds (*Colius* sp.). It is surmised that this fruit was infected by fungi and bacteria, the natural sources of protein for fruit fly, and were in direct competition with the bait station. In these circumstances the flies were not attracted to the station as there was an abundance of protein in the vicinity and were therefore not controlled. However, the birds did not damaged the fruit on the side of the orchard nearest the river and in this situation the bait stations appeared to be effective. The orchard has a history of fruit fly that the grower has not been able to control using more traditional chemical control measures. Mousebirds have always been responsible for damage in this orchard.

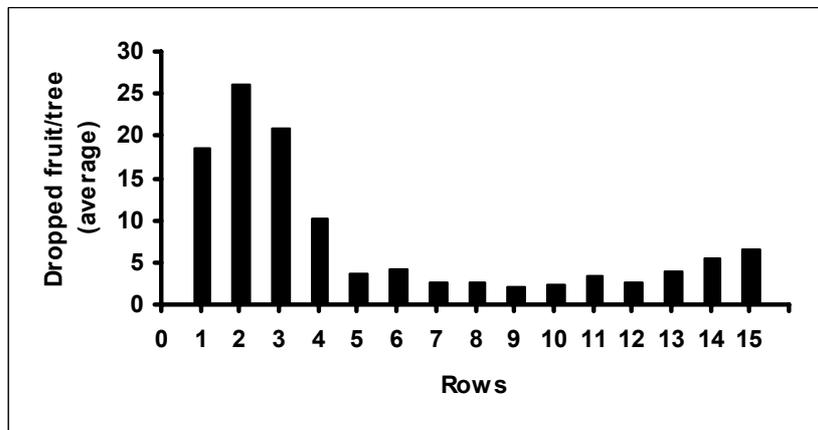


Figure 3.5.3.4.3. The average number of fruit per tree that had dropped across the 15 rows of the Stuk van Acht Clementine orchard.

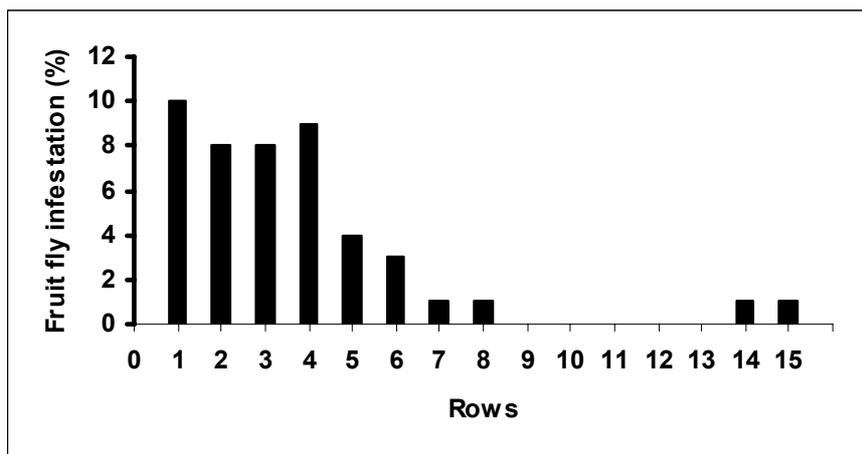


Figure 3.5.3.4.4. The percentage of fruit infested with fruit fly across the Clementine orchard.

Conclusions

- a. If excessive numbers of fruit flies are trapped within an M3 bait station treated orchard, then reasons for this increase should be determined.
- b. Excessive damage to fruit, whether caused by animal or environmental conditions such as hail, may render fruit fly control ineffective.

3.5.3.5 Onderberg – Neos Estates – Marsh grapefruit

By Tony Ware, John-Henry Daneel and Bruce Tate (CRI)

Introduction

This experiment was undertaken in order to determine whether the number of M3 bait stations normally used to control fruit fly (registered rate 300/ha) could be reduced if large areas were treated. The rationale was that fruit fly threat occurs from outside the orchard and that if large numbers of traps were applied to the borders of the orchard then fewer traps could be used in the centre (insurance).

Materials and methods

2720 M3 bait stations were placed in the 10ha Marsh grapefruit orchard on Neos Estates in the Onderberg area on 12 February 2002. A single bait station was hung in each tree in the outside three rows and/or the first and last three trees in each row. The bait station density was then lowered to one station/two trees for the next six trees, thereafter bait station density was maintained at one station/three trees. Sensus traps containing either Capilure or Questlure were placed at various positions in and around the orchard (Figure 3.5.3.5.1). Two McPhail traps containing Biolure (Consep Inc.) were placed in the centre of the orchard. The lures and dichlorvos were changed every 6-8 weeks. The traps were emptied weekly and all insects caught were taken to the laboratory. Fruit flies were sexed and identified to species. All other insects were assigned to their insect order.

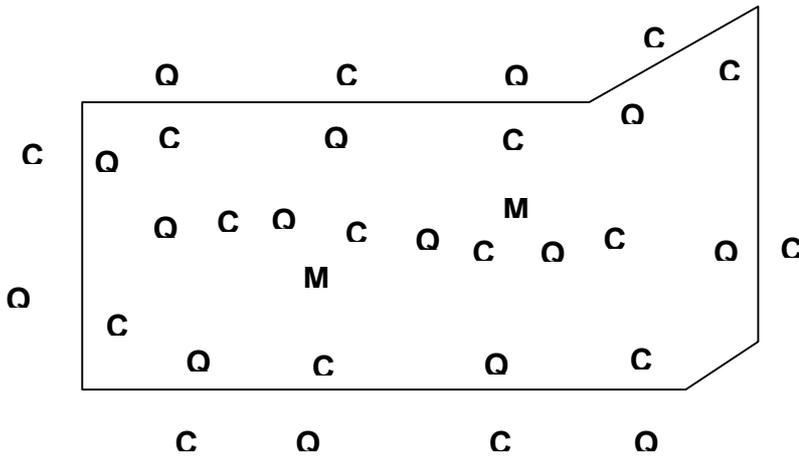


Figure 3.5.3.5.1. Positions of Sensus traps containing either Capilure (C) or Questlure (Q) and Biolure (M) in McPhail traps in a 10 ha Marsh grapefruit orchard situated at Neos Estates in the Onderberg area of Mpumalanga.

Results and discussion

At this site all three species of economically important fruit fly were present. Mediterranean fruit fly was the most abundant, followed by Natal fruit fly (Figure 3.5.3.5.2). The outside traps caught significantly more flies than either the border traps or those placed in the centre of the orchard.

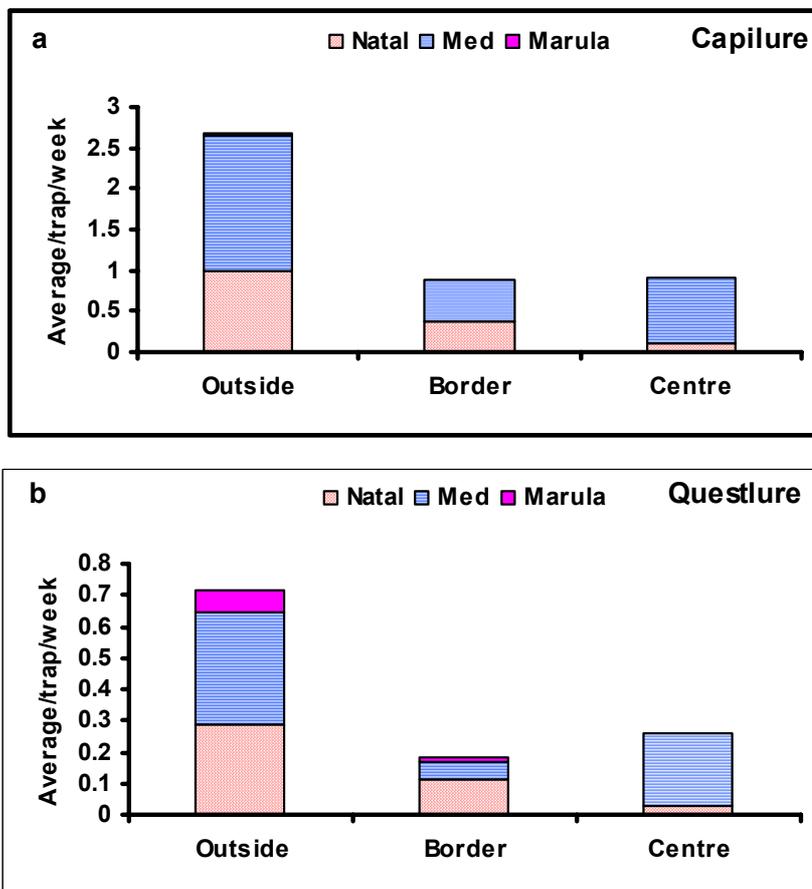


Figure 3.5.3.5.2. Fruit fly caught in Sensus traps using Capilure (a) or Questlure (b).

Trap returns were highest over the first few weeks of the trial (Figure 3.5.3.5.3). Because there were so few flies caught at this trial site no differences were recorded between the traps placed on the outside of the orchard and those placed on either the border or the centre. No fruit fly-infested fruit were found when the orchard was inspected at harvest.

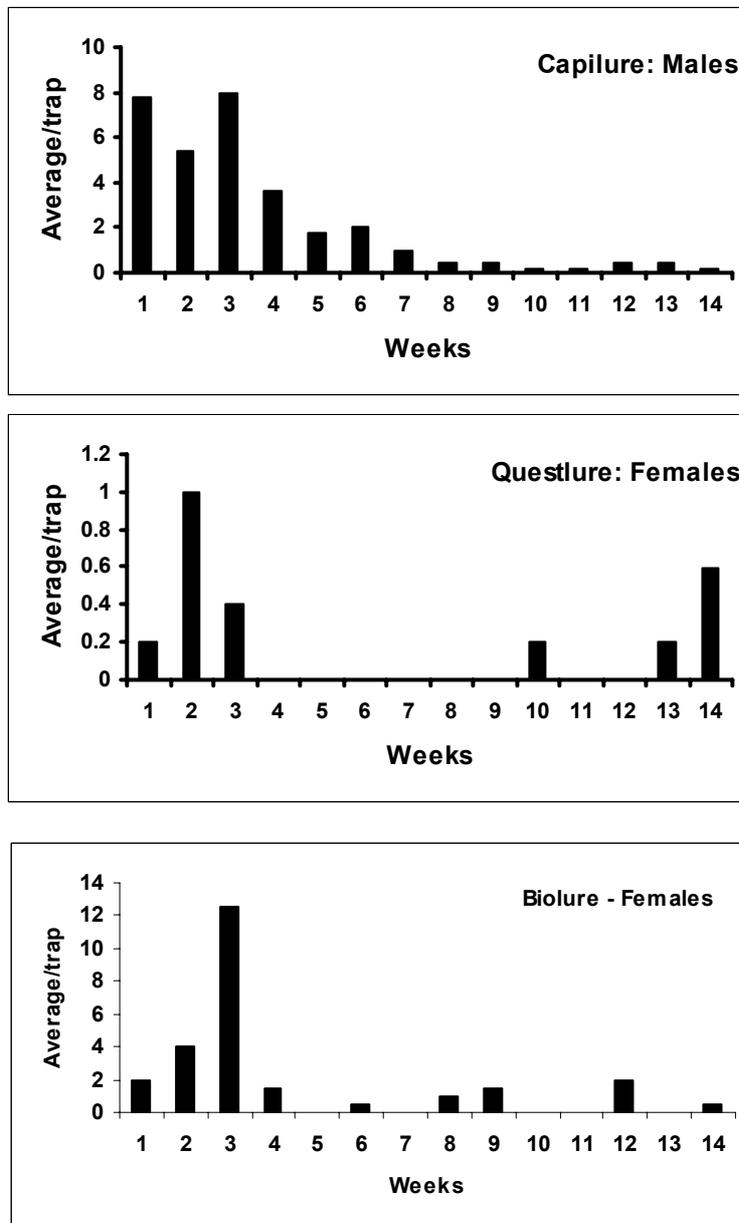


Figure 3.5.3.5.3. Fruit flies (regardless of species) caught in Capilure-charged (top graph) and Questlure-charged (centre graph) Sensus traps and Biolure-charged McPhail trap over the trial period.

Both Questlure and Biolure are reputed to be attractive to female fruit flies. In all cases the McPhail trap/Biolure outperformed the Sensus trap/Questlure combinations (Table 3.5.3.5.1). The large numbers of female *C. rosa* caught in the Biolure/McPhail may be an important result as this species is of phytosanitary significance and one may wish to use the “best” attractants in order to determine whether the species represents a threat to the industry.

Table 3.5.3.5.1. Comparison between fruit fly attractants and traps

		Ratio Quest : McPhail
<i>C. capitata</i>	Male	1: 0.86
	Female	1:2.77
<i>C. rosa</i>	Male	1:2.5
	Female	1.:11.5

The non-target insects caught are presented in Table 3.5.3.5.2. In general the traps were specific for fruit fly, although various Diptera species were attracted in reasonably numbers to the McPhail traps. None of the traps are likely to cause any pest repercussions.

Table 3.5.3.5.2. Non-target insects caught in the Sensus traps containing Capilure or Questlure and McPhail traps containing Biolure placed in and around a Marsh grapefruit orchard in the Onderberg area of Mpumalanga Province.

Insect group	Questlure	Capilure	McPhail
Hymenoptera (Formicidae)	1.64	0.15	0
Hymenoptera (Parasitica)	0.7	1.5	1.5
Hymenoptera (Apidae)	0.9	0.6	0.5
Diptera	4.8	2.2	114.2
Lepidoptera	1	0.5	0.5
Neuroptera	0	0	0.5
Odonata	0	0.07	1.0
Hemiptera	2.4	0	0.5
Arachnidae	0.9	0.9	0

Conclusions

- It appears that M3 bait station numbers can be reduced if one protects the orchard by placing a “barrier” around the block of trees and reducing the number in the centre.
- The Biolure may be a more effective attractant for *C. rosa* and this aspect needs to be investigated.
- None of the traps are likely to cause pest repercussion outbreaks by removing natural enemies.

3.5.3.6 Neos Estates – Mangos “ Tommy Atkins” – Onderberg

By Tony Ware (CRI), John-Henry Daneel (CRI) and Ian Johnson (Neos Estates)

Introduction

Mango is recorded as a host of marula fruit fly (*Ceratitis cosyra*), Mediterranean fruit fly (*C. capitata*) and Natal fruit fly (*C. rosa*) (White and Elson-Harris 1992) This experiment was undertaken in order to test the efficacy of the M3 bait station against these species.

Materials and methods

A five ha orchard situated on Neos Estates was treated with M3 bait stations on 7 November 2002. Each tree received one bait station placed on the southern aspect of the plant (shady side). Fruit fly populations were monitored using Sensus traps containing either Capilure or Questlure. Four traps of each were placed in and immediately adjacent to the orchard. Trapped flies were removed weekly and taken to the laboratory where they were identified and sexed. The trial was terminated at harvest at the end of December. Fruit was examined for fruit fly damage at this juncture.

Results and discussion

Very few flies were trapped over the nine week observation period and are reported in Table 3.5.3.6.1. No fruit fly-damaged fruit was found at harvest.

Table 3.5.3.6.1. The total number of fruit flies caught in Sensus traps placed in and around a mango orchard situated at Neos Estates in the Onderberg area of Mpumalanga.

Species	Sex	Questlure	Capilure
<i>C. capitata</i>	Male	0	12
<i>C. capitata</i>	Female	0	0
<i>C. rosa</i>	Male	0	12
<i>C. rosa</i>	Female	0	0
<i>C. cosyra</i>	Male	2	0
<i>C. cosyra</i>	Female	2	0

Conclusion

M3 bait stations were effective at the low fruit fly pressures experienced at this trial site.

Future research

No further research on mangos is planned.

Literature cited

White, I.M. and Elson-Harris, M.M. 1992. *Fruit flies of Economic Significance: Their Identification and Bionomics*. CAB International Wallingford.

3.5.3.7 Laughing Waters – Litchis – Onderberg

By Tony Ware and John-Henry Daneel (CRI)

Introduction

This experiment was essentially a demonstration trial for the litchi industry.

Materials and Methods

The layout was similar to the Neos mango trial reported above with the exception that 6 Sensus traps, each containing one Questlure or Capilure capsule were used.

Results and Discussion

The fruit fly at this site was low with only 67 flies being caught over the 9 week trapping period. The relative proportion of flies trapped is recorded in Table 3.5.3.7.1.

Table 3.5.3.7.1. Fruit fly trapped in Sensus traps containing Questlure or Capilure on the farm Laughing Waters in the Onderberg area of Mpumalanga.

Species	Sex	Questlure	Capilure
<i>C. capitata</i>	Male	3	15
	Female	4	1
<i>C. rosa</i>	Male	15	9
	Female	11	0
<i>C. cosyra</i>	Male	6	0
<i>C. cosyra</i>	Female	2	0
Total		42	25

These results indicate that Questlure might be a superior attractant for Natal fruit fly while Capilure appears better at attracting Mediterranean fruit fly. These results need to be confirmed. These data confirm that marula fruit fly is not attracted to Capilure but is attracted to Questlure.

No fruit fly-infested fruit was found at harvest.

Conclusion

The M3 bait station appeared to control fruit fly in litchis.

Future research

No further research on litchis is planned.

3.5.3.8 Kakamas – grapes

Experiment by Tony Ware, John-Henry Daneel (CRI) and Francois Reyneke (Kromhout Boerdery)

Introduction

This fruit fly work was initiated by Kromhout Boerdery and aimed to test the M3 bait station as an area-wide application.

Materials and methods

Three vineyards of approximately 40 ha each were treated with M3 bait stations on 2 October 2002. Fruit fly populations were monitored using Sensus traps containing either Questlure or Capilure. A proportion of the traps were placed outside the vineyard, some within 10 m of the edge of the vineyard and some were placed near the centre. The traps were serviced weekly and all flies trapped were removed, counted, identified and sexed. The final readings were made on the 28 May 2003. Harvesting took place in January 2003. The M3 bait stations were not removed from the vineyards even though they could not be considered to be highly effective after 16 weeks.

Results and discussion

No fruit flies were caught in the Capilure or Sensus traps until after the grapes were harvested at about 10 weeks after monitoring had started (Figure 3.5.3.8.1). Fruit flies were only noted in the treated vineyards from week 26. It is assumed that these flies were targeting the “na trossies”. These are grapes left on the vine after harvest and gradually loose moisture becoming raisin-like. Grapes at harvest are not a good fruit fly host as the eggs and larvae tend to drown in the juice. In contrast to the treated vineyards large numbers of flies were trapped in Kakamas town (see report on survey above) indicating that there were large numbers of flies in the vicinity. The small numbers caught in the vineyard could be attributed to the M3 bait stations or to the fact that the flies were not attracted to the vineyards. If the former was the case then it indicates that the bait station’s effective life in the environment of Kakamas is some 26 weeks, well in excess of the manufacturer’s claim of 16 weeks.

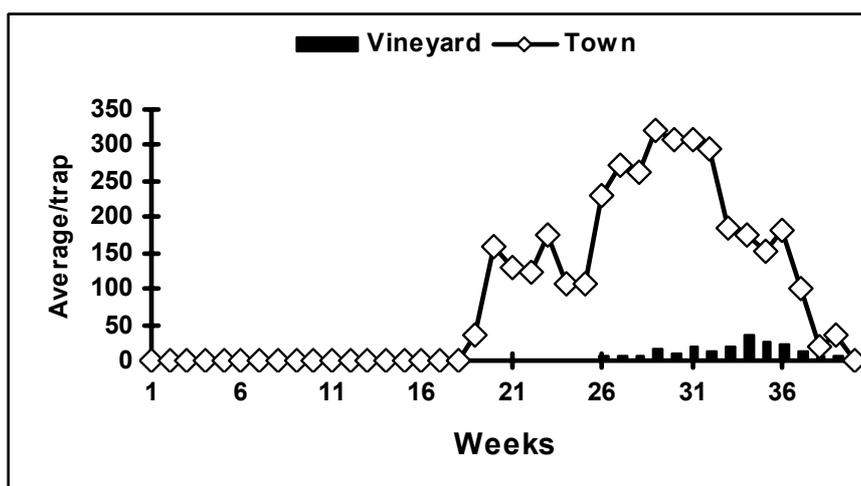


Figure 3.5.3.8.1. Fruit flies caught in Sensus traps containing Capilure placed in Kakamas town and in M3 bait station treated vineyards approximately 5 km from the town. Harvesting of the vineyards was done at week 12.

A total of 7074 flies were caught using the traps containing Capilure against 563 flies in the traps containing Questlure, a ratio of 12.6:1. The low numbers of fruit fly caught in Questlure traps has been a concern to many growers but this is merely an indication that the ¹⁶⁷ trap is some ten times less sensitive for *C.*

capitata and the threshold treatment values pertaining to the two traps reflect this difference in sensitivity. Accumulative trap catches indicate that fruit flies were noted to occur from outside the vineyard about three weeks before they were recorded from within the vineyard (Figure 3.5.3.8.2). This period reflects the approximate fruit fly generation period. Thereafter there was a steady increase in the fruit fly populations along the border of the vineyards. However, the fruit fly counts remained low in the centre of the vineyards, another indication that the effective period of the bait stations under these environmental conditions may be far longer than that claimed by the manufacturer.

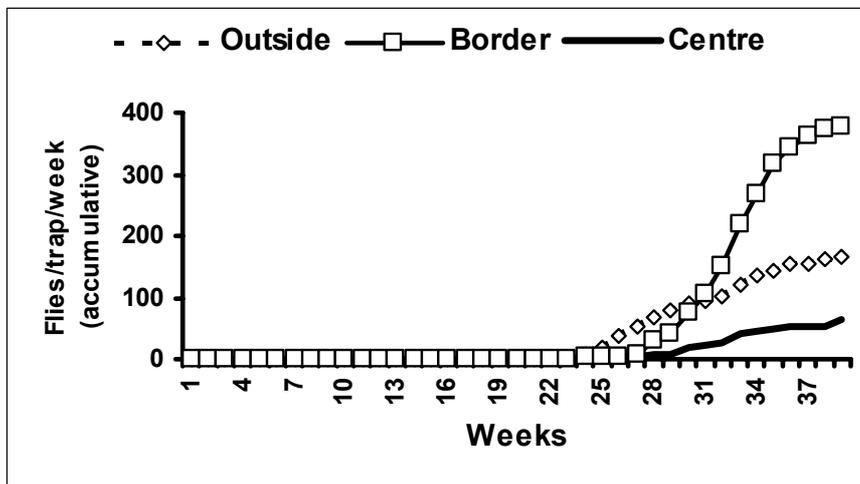


Figure 3.5.3.8.2. Accumulative fruit fly trap catches made in Sensus traps containing Capilure positioned in and around vineyards on the farm Kromhout near Kakamas.

A similar pattern of fruit fly trap catches to that made in the M3 bait station-treated vineyards were made in untreated vineyards (Figure 3.5.3.8.3). This result indicated that fruit flies were not present in the vineyards until after harvest. This implies that it is unnecessary to treat the grapes as they appear not to be susceptible to fruit fly attack until after harvest.

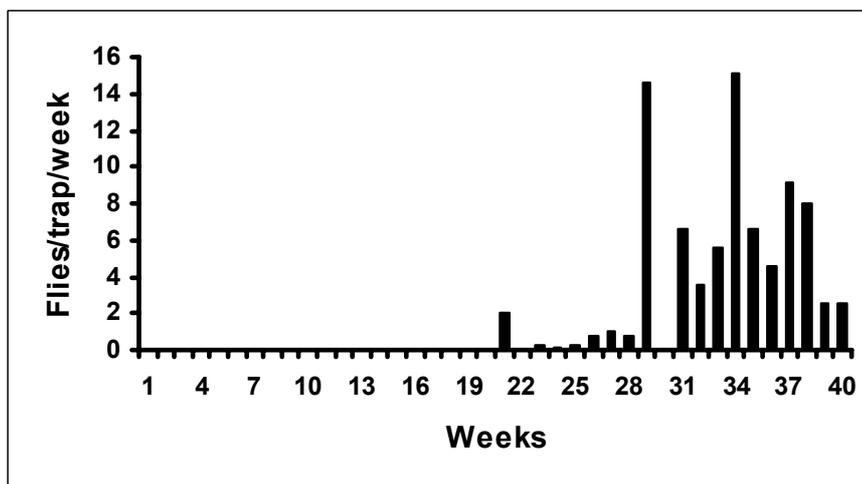


Figure 3.5.3.8.3. Fruit fly caught in Sensus traps containing Capilure placed in untreated vineyards on the farm Kromhout near Kakamas.

Conclusions

1. Fruit fly is not a threat to grapes until after harvest when the condition of the fruit through the loss of water allows the pest to develop.
2. The presence of “na trossies” will allow a build up of fruit fly populations that is a threat to citrus production.

Future research

No further research is planned.

3.5.4 Cold disinfestation treatment for Medfly-infested Clementines

Experiment 709 by Tony Ware and John-Henry Daneel (CRI)

Opsomming

'n Fout het ingesluit met die oorsetting van data van die groot Mediterreense vrugtevlieg-besmette Clementine proef vanaf tabel- na grafiese formaat. As gevolg hiervan is die proef herhaal. 'n Groot getal (51 057) derde-instar vrugtevlieë is vir 14 dae blootgestel aan -0.5°C ($\pm 0.5^{\circ}\text{C}$). Daar was geen oorlewendes nie, wat die tabelwaardes van die vorige proef bevestig.

Introduction

Previous research submitted to Japan contained conflicting data in that the graphic representation of the thermoprobe readings did not accurately reflect the original tabular data. Good scientific protocol required the experiment be repeated in order to confirm which one of the data sets was accurate. An independent assessor (Dr Piet Joubert - Head of the Entomology Department of the Institute for Tropical and Subtropical Crops [Agricultural Research Council]) was appointed to verify the tabular temperature data.

Materials and Methods

These were as previously described. Calibration of the thermoprobes was done immediately prior to initiating a treatment and is reported in Table 3.5.4.1. The treatment was deemed to have begun once the mean of the thermoprobe reading had dropped below 0°C .

Table 3.5.4.1. Reading of thermoprobes placed on melting ice. These readings were used to correct logged temperatures.

Thermoprobe number	Replicate			
	1	2	3	4
1	0.10	0.10	0.01	0.08
2	-0.01	-0.01	0.03	0.09
3	-0.06	-0.08	0.05	0.06
4	-0.17	-0.20	0.06	0.00
5	0.19	-0.04	0.76	0.88
6	0.07	-0.17	0.76	0.88
7	-0.01	-0.19	0.73	0.78
8	-0.04	-0.21	0.68	0.73
9	-0.13	-0.29	0.55	0.52
10	-0.29	-0.33	0.48	0.25
11	-0.31	-0.35	0.42	0.40
12	-0.32	-0.29	0.30	0.25
13	0.15	-0.04	0.80	0.88
14	0.10	-0.02	0.63	0.73
15	0.09	0.02	0.52	0.51
16	0.09	0.10	0.42	0.42

Results and discussion

The positions of the fruit cartons with their associated thermoprobes within the cold chamber are illustrated in Figures 3.5.4.1 to 3.5.4.4. The average thermoprobe reading for each replicate is presented in Figures 3.5.4.5 - 3.5.4.8. The individual tabular and graphic thermoprobe data are presented in Appendices A-D.

The larval survival in both the untreated control and cold treated fruit is reported in Table 3.5.4.2. Replicate 3 was aborted and no assessment of survival in the treated group was made as the fruit was subjected to cold treatment for 15 days and not 14 days (Figure 3.5.4.7). In Replicate 4 two probes (3 and 7 positioned in the bottom tier of fruit cartons at the back of the room (Figure 3.5.4.3)) registered temperatures below the -1°C threshold and, although these fruit were assessed, they were not included in the final tally. In total 5561 fruit were treated and it was estimated that 51067 third instar larvae were subjected to the cold treatment.

There were no survivors.

Table 3.5.4.2. The survival of third instar Mediterranean fruit fly in Clementines after 14 days cold treatment at $-0.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The control fruit was not subjected to cold treatment.

Replicate (date)	Control			Treated			
	Number of fruit treated	Number of live larvae	Mean number of live larvae/fruit	Number of treated fruit	Estimated number of larvae treated	Number of larvae surviving treatment	Mortality (%)
1	500	6090	12.2	2008	24457	0	100
2	500	2750	6.3	1864	11650	0	100
3 ¹	540	3919	7.3	-	-	-	-
4 ²	616	5456	8.9	1939 (1689)	17174 (14960)	0	100
Total	1616	14296	8.9	5561	51067	0	100

¹In cold room for extra 24 hours – not assessed

²Probes 3 and 7 registered temperatures below -1°C . Figures in parentheses indicated numbers assessed that do not include cartons in which these probes were placed.

This experiment demonstrated the effectiveness of cold treatment disinfestations ($0.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 14 days) of Mediterranean fruit fly-infested Clementines. Furthermore, the results confirm that the tabular results as previously presented (2000) were correct.

Conclusion

The tabular data set was confirmed to be the correct data set.

Future research

Research determining the disinfestation potential of $+0.5^{\circ}\text{C}$ is destined to begin in 2004.

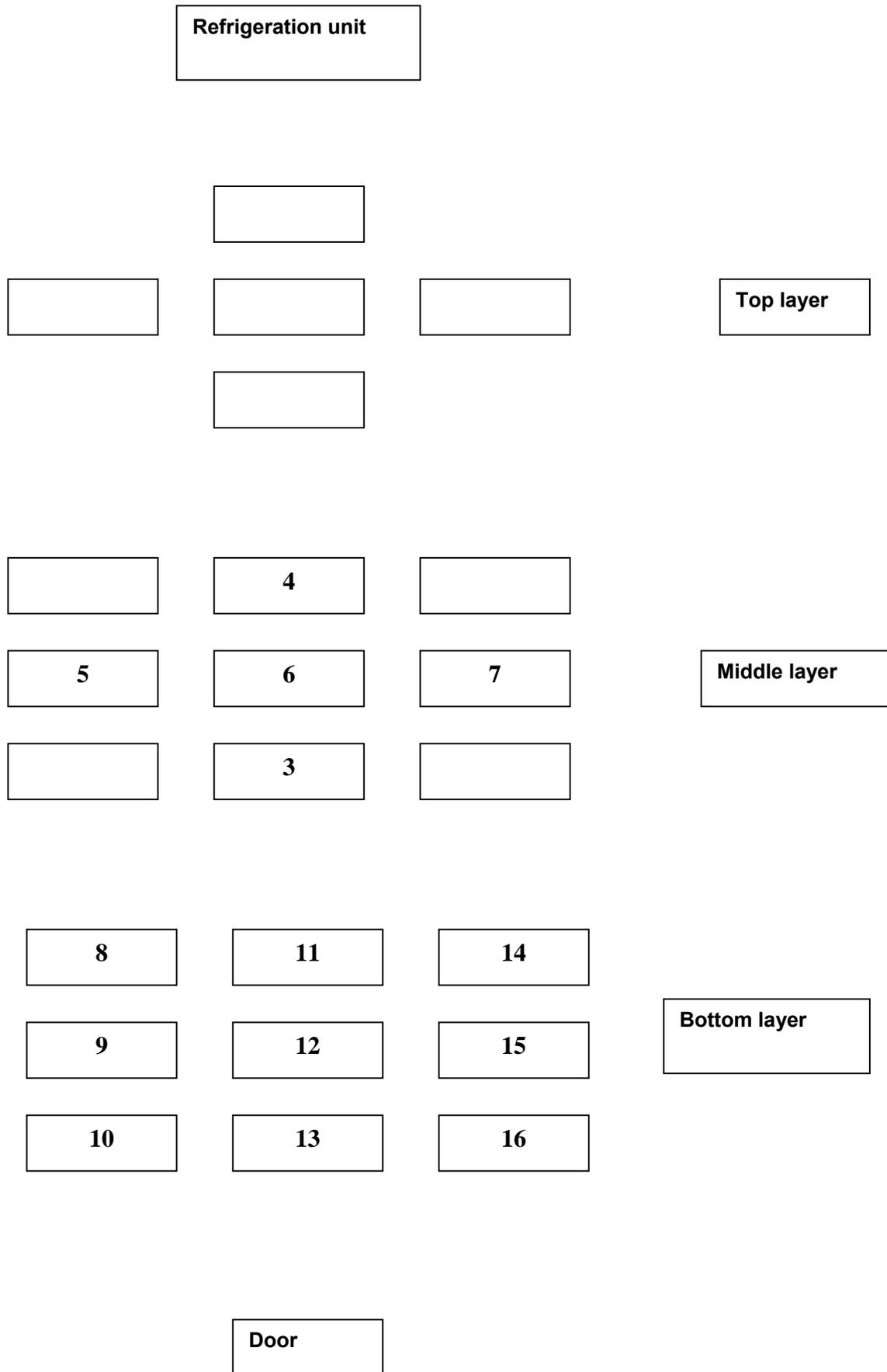


Figure 3.5.4.1. Floor plan – Replicate 1 (6 June 2002). Numbers represent cartons containing thermoprobes. Unmarked cartons contained experiment fruit but no thermoprobes.

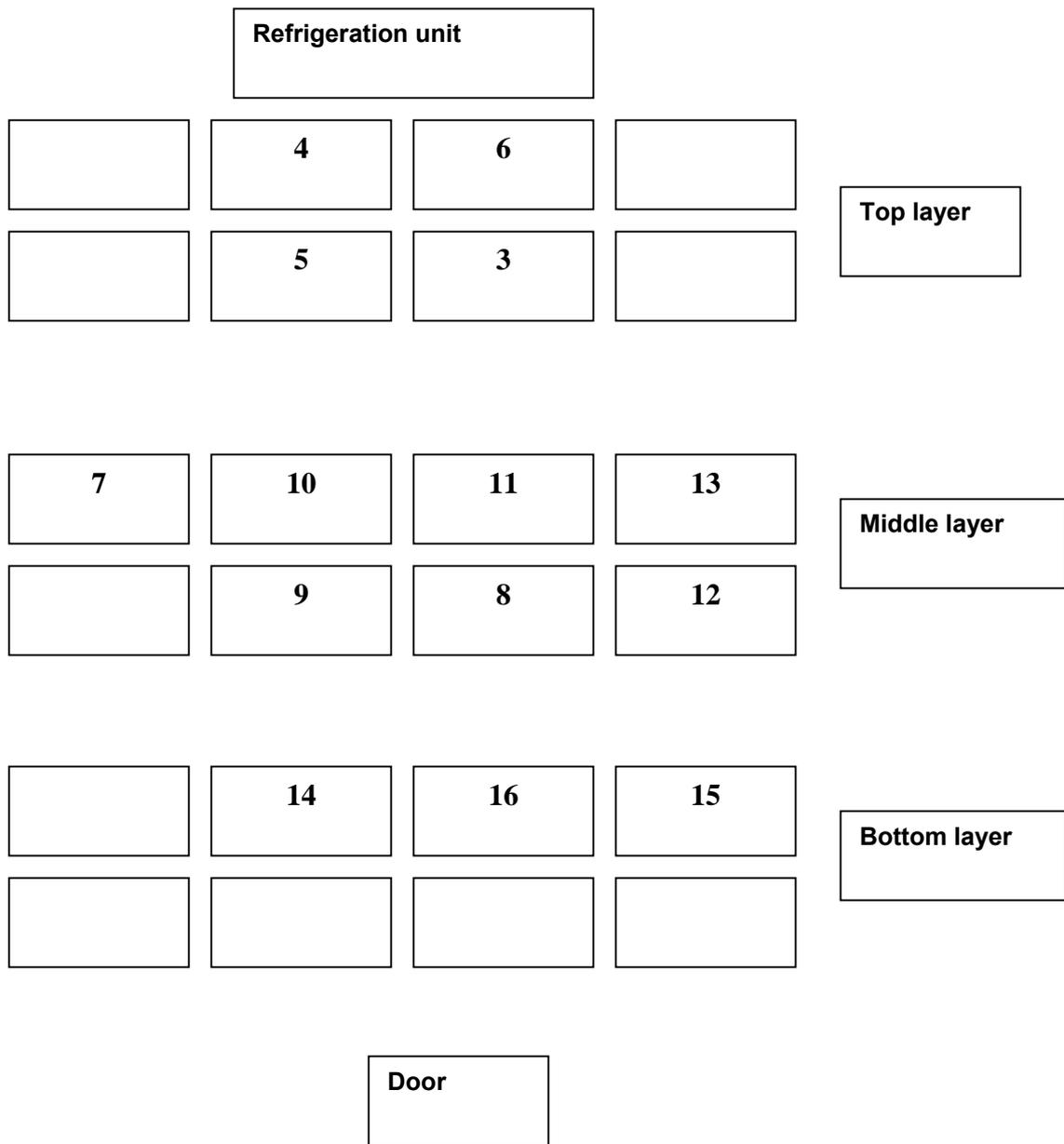


Figure 3.5.4.2. Floor plan – Replicate 2 (12 August 2002). Numbers represent cartons containing thermoprobes. Unmarked cartons contained experiment fruit but no thermoprobes.

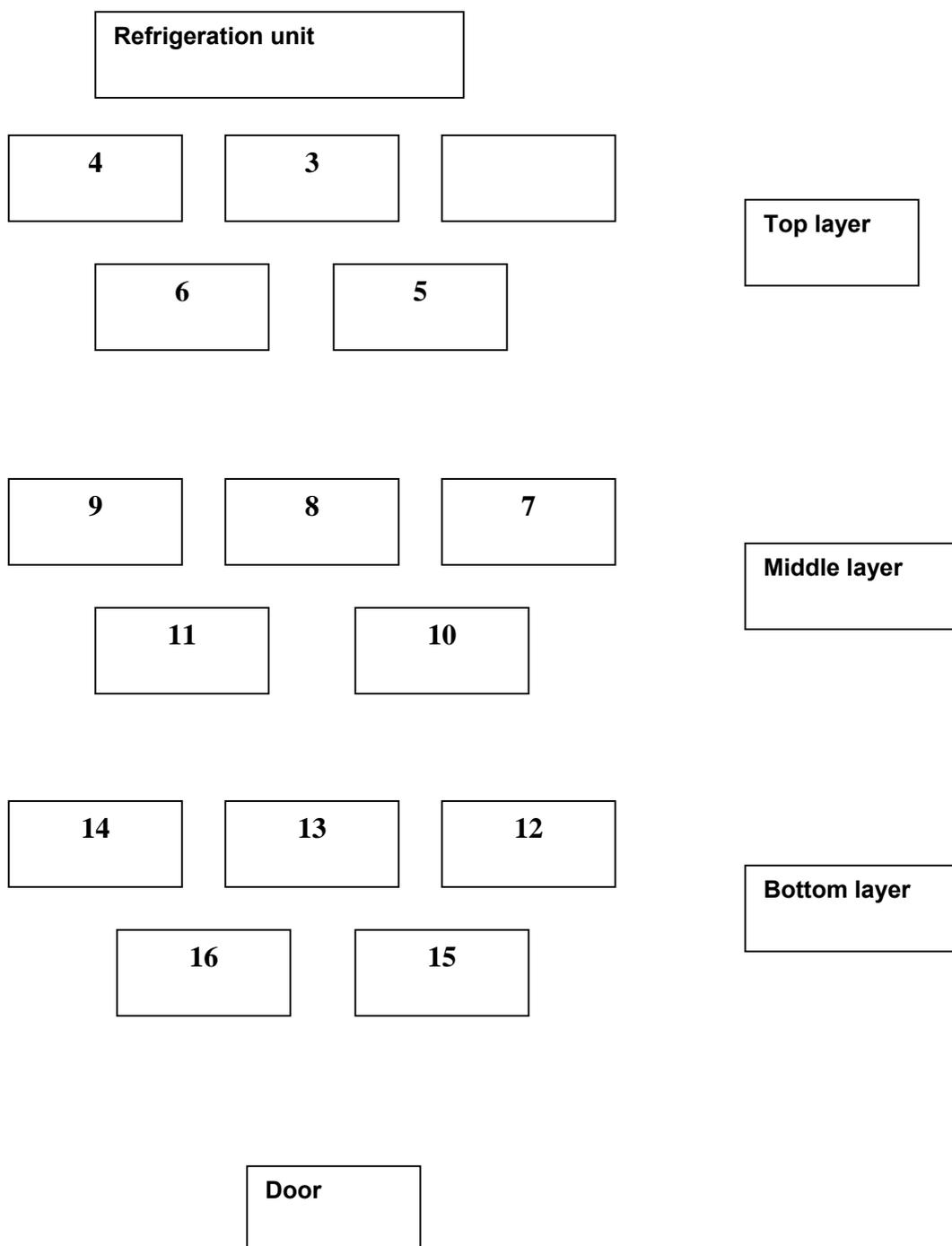


Figure 3.5.4.3. Floor plan – Replicate 3 (13 September 2002). Numbers represent cartons containing thermoprobes. Unmarked cartons contained experiment fruit but no thermoprobes.

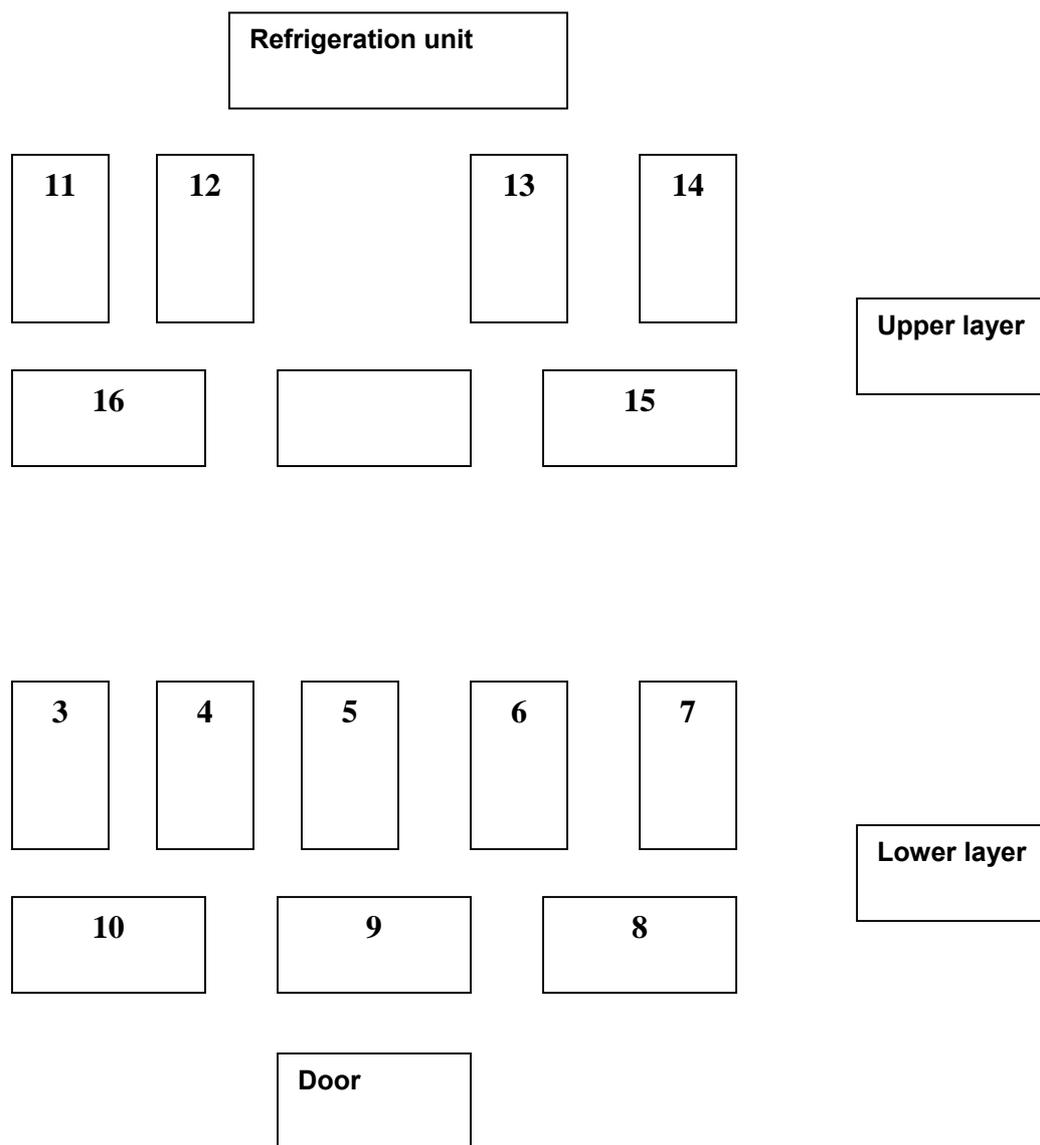


Figure 3.5.4.4. Floor plan – Replicate 4 (18 October 2002). Numbers represent cartons containing thermoprobes. Unmarked cartons contained experiment fruit but no thermoprobes.

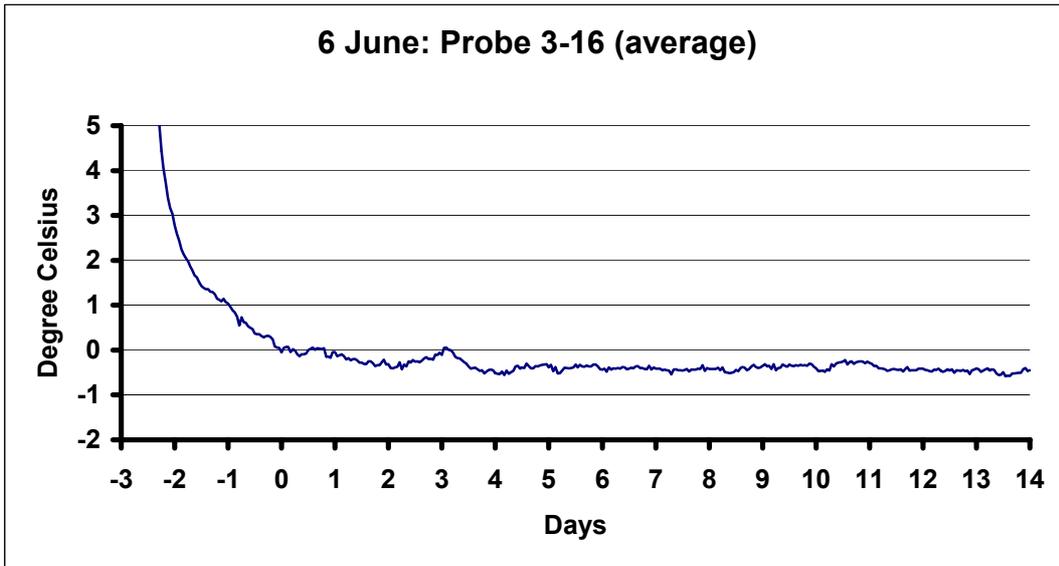


Figure 3.5.4.5. Average of temperature readings of thermoprobes placed in Clementine fruit on 6 June 2002.

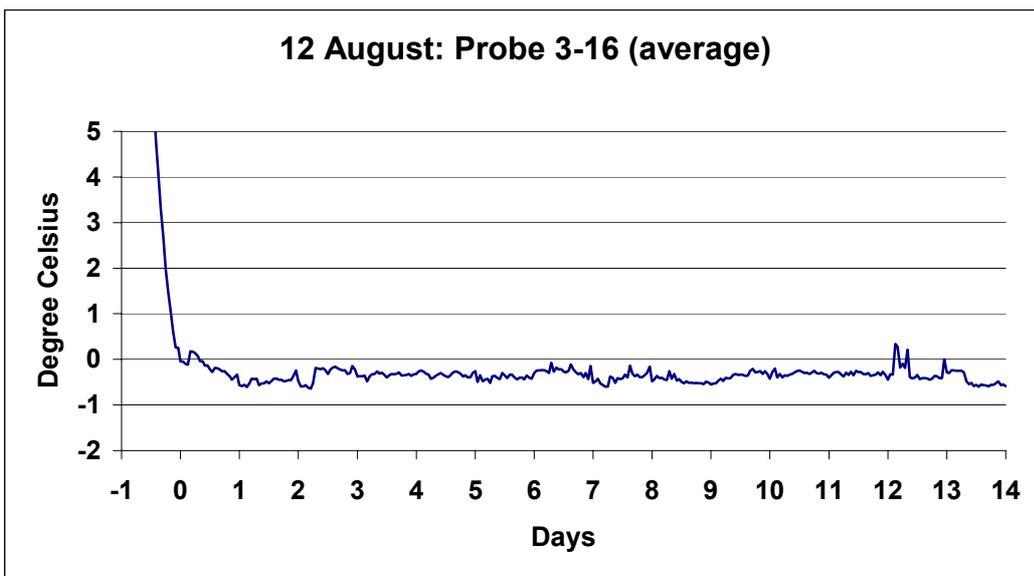


Figure 3.5.4.6. Average of temperature readings of thermoprobes placed in Clementine fruit on 12 August 2002.

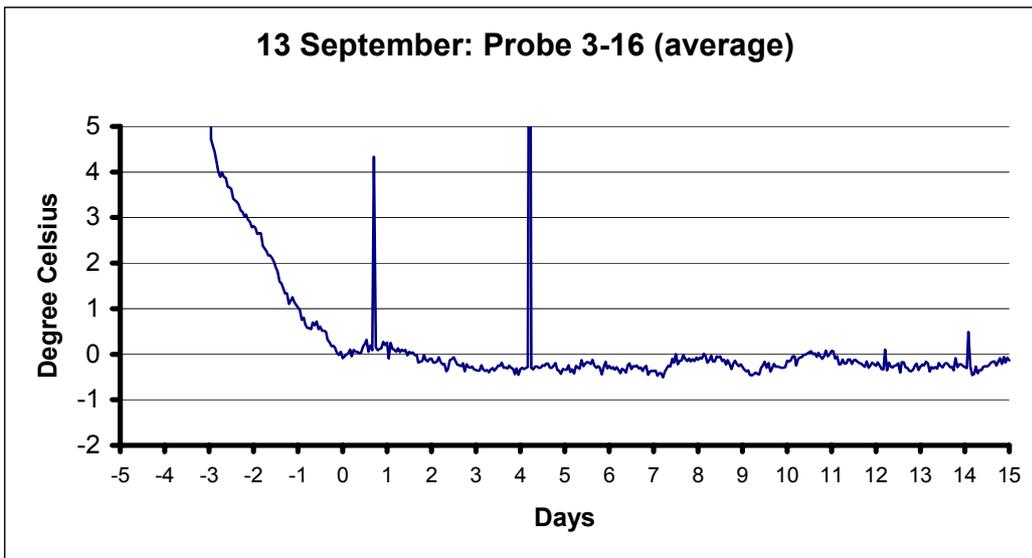


Figure 3.5.4.7. Average of temperature readings of thermoprobes placed in Clementine fruit on 13 September 2002.

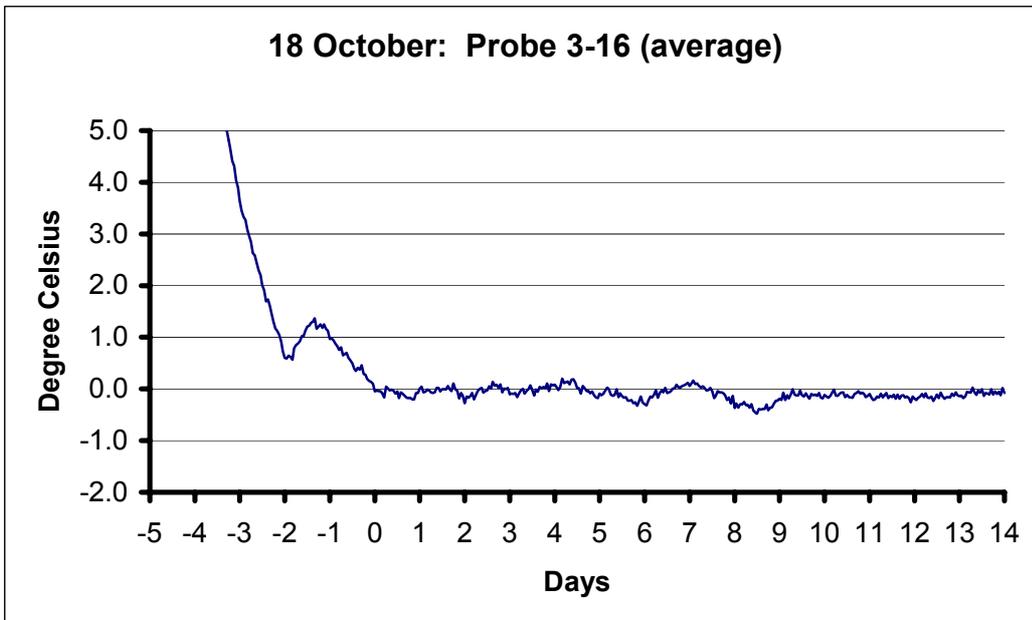


Figure 3.5.4.8. Average of temperature readings of thermoprobes placed in Clementine fruit on 18 October 2002.

3.5.5 Cold disinfestation of Medfly-infested Barlinka grapes

Contract for DFPT by Tony Ware, Peter Stephen, Bruce Tate and John-Henry Daneel (CRI)

Opsomming

CRI is deur die sagtevrugte Produsente Trust gekontrakteer om 'n koue-ontsmettingsproef uit te voer met Barlinka druive. Mediterreense vrugtevlieë (84 728 derde-instar) is vir 16 dae blootgestel aan 0.5 °C (± 0.5 °C). Geen vlieë het oorleef nie, wat daarop dui dat die behandeling doeltreffend was. Dit bied dus aan die Japannese die versekering dat sodanig-behandelde druive nie 'n bedreiging inhou sover dit die toevallige invoer van Mediterreense vrugtevlieë aanbetref nie.

Introduction

Previous research (Conlong, 1999) had shown that Mediterranean fruit fly (*Ceratitidis capitata* [Wiedemann]) mature larvae were able to withstand cold treatment of 0.6 +/- 0.5°C for 14 days. The Deciduous Fruit

Producers' Trust commissioned Citrus Research International (Pty) Ltd to repeat phase 4 (large disinfestation trial) of the research but this time increasing the number of days the fruit fly-infested grapes would be subjected to the cold treatment, to 16 days.

Materials and methods

Barlinka grapes were sourced from the Hex River Valley in the Western Cape Province of South Africa and maintained at approximately 0.6°C until required. A day before the grapes were inoculated with Mediterranean fruit fly, they were removed from the cold chamber and allowed to warm to room temperature (approximately 23°C). Immediately before the grapes were infested, they were dipped in fungicide (Imazalil; 66 g/hl) to prevent excessive decay. A hole was made in the stalk end using a cork borer (8 mm internal diameter). Aliquots of approximately 50 fruit fly eggs in water, not more than 24 hours old, were placed into the hole using an automatic pipette. Absorbent cotton wool was then used to seal the hole. The fruit was placed upside down (i.e. stalk end at the bottom) to ensure that excess grape juice drained into the cotton wool and the fruit fly eggs did not drown. The fruit was then placed into a screened cage (to prevent oviposition by vinegar flies (*Drosophilidae*)) at 26°C for 10 days to guarantee development to third instar. Approximately 25% of the fruit were then assessed for survivors (control fruit) while the balance was packed into grape cartons and placed among untreated grapes in the cold chamber. The cold room temperature was monitored using a Grant Squirrel data logger attached to T type thermoprobes. The thermoprobes had been calibrated on melting ice immediately prior to each trial. The calibration factor used for the probes is given in Table 3.5.5.1. The probes were placed into untreated grapes and at least one probe was placed into each carton containing the fruit fly-infested grapes (Figures 3.5.5.1-3.5.5.4). The position of cartons and their associated thermoprobes are recorded in Figures 3.5.5.1-3.5.5.4. The monitoring of the thermoprobes was initiated immediately after the fruit was placed in the cold chamber and was done hourly. The target temperature and range was 0.6°C +/- 0.5°C. Treatment was deemed to have commenced once the average of the thermoprobe reading had reached 1°C and is recorded as Day 0. After 16 days cold treatment the grapes were removed from the cold chamber and placed at 26°C for 48 hours. The fruit was then dissected to determine the survival rate, if any.

Table 3.5.5.1. Calibration factors used for each thermoprobe.

Thermoprobe number	Replicate					
	1	2	3	4	5	6
1	0.32	0.32	0.05	0.05	0.05	0.07
2	0.31	0.31	0.10	0.08	0.08	0.09
3	0.29	0.29	0.25	0	0.11	0.17
4	0.20	0.42	0.32	0	0.15	0.26
5	1.22	0.92	0.63	0.6	0.76	0.88
6	1.18	1.18	0.75	0.6	0.83	0.92
7	1.07	1.07	0.68	0.6	0.76	0.87
8	0.98	0.98	0.73	0.7	0.75	0.81
9	0.82	0.51	0.66	0.5	0.65	0.64
10	0.64	0.64	0.66	0.4	0.61	0.66
11	0.53	0.53	0.62	0.4	0.56	0.58
12	0.36	0.36	0.53	0.3	0.45	0.47
13	1.14	0.84	0.70	0.5	0.85	0.85
14	0.99	0.79	0.57	0.6	0.66	0.69
15	0.86	0.63	0.50	0.5	0.54	0.57
16	Not used	Not used	0.41	0.4	0.45	0.46

Results and discussion

The survival results of the six replicates of the large-scale disinfestation trial are shown in Table 3.5.5.2. The average thermoprobe readings for all the replicates are presented in Figures 3.5.5.5-3.5.5.10. The first two of these replicates were aborted as the thermoprobes showed that the temperature was below that of the minimum temperature prescribed for the trial (Figures 3.5.5.5 and 3.5.5.6). The first of these was due to an irregularity with respect to the cold chamber controller. This was replaced before the second replicate was initiated. The second replicate was also aborted but this was due to the grapes being cooled to below the target temperature range at the start of the trial. Two cartons of the third replicate were not assessed as the

thermoprobes indicated that the temperature had dropped below the prescribed temperature for an excessive period in these cartons (not reported). The two cartons in question were those that contained probe 3 and probes 15 and 16 (Figure 3.5.5.1). These cartons had been placed on the right hand side of the cold chamber near the floor indicating that this part of the room was colder than the rest. This resulted in less than the required 2000 fruit being assessed. In the fourth replicate a number of the thermoprobes again dropped below the required temperature threshold. These were in cartons containing probes 4,5,16, 14 and 15, 6 and 7 (Figure 3.5.5.2). As for the previous replicate the right hand side of the chamber was coldest. These cartons were not assessed and this resulted in less than 2000 fruit being assessed. However, together replicates 3 and 4 met the fruit number requirement. The high temperatures recorded on day 14 were a result of a malfunction in the unit controlling the defrosting cycles. Replicates 5 and 6 generally went smoothly. Some of the thermoprobe readings were above the higher specification but the lack of survival in these fruit demonstrated that the temperature of 0.6°C for 16 days is conservative and will provide the necessary security that grapes infested with Mediterranean fruit fly will not be accidentally imported.

Table 3.5.5.2. The survival of third instar Mediterranean fruit fly in Barlinka grapes after 16 days cold sterilization at 0.6 +/- 0.5°C.

Replicate (date)	Control			Treated			
	Number of fruit	Number of live larvae	Mean live larvae/fruit	Number of fruit	Estimated number of larvae treated	Number of survivors	Mortality (%)
1 (3 Apr)	258	407	1.6	2035	3460	- ¹	-
2 (24 Apr)	474	2918	6.1	2370	14457	- ¹	-
3 (2 May)	560	6964	12.4	1520 ²	20588	0	100
4 (13 Jun)	560	6467	11.5	1160 ²	13340	0	100
5 (5 Jul)	560	3839	6.3	2000	12600	0	100
6 (8 Aug)	640	12237	19.1	2000	38200	0	100
Total ³	2320	29507	12.7	6680	84728	0	100

¹ Run cancelled – all probes out of target range temperature

² Grape cartons that contained thermoprobes below the minimum target range temperature were not assessed – 2000 fruit originally inoculated

³ Replicates 3-6 only

References cited

Conlong, D.E. 1999. Mediterranean fruit fly (*Ceratitis capitata*) rearing and cold sterilization procedures in grape varieties Barlinka table grapes. Report submitted to Japan.

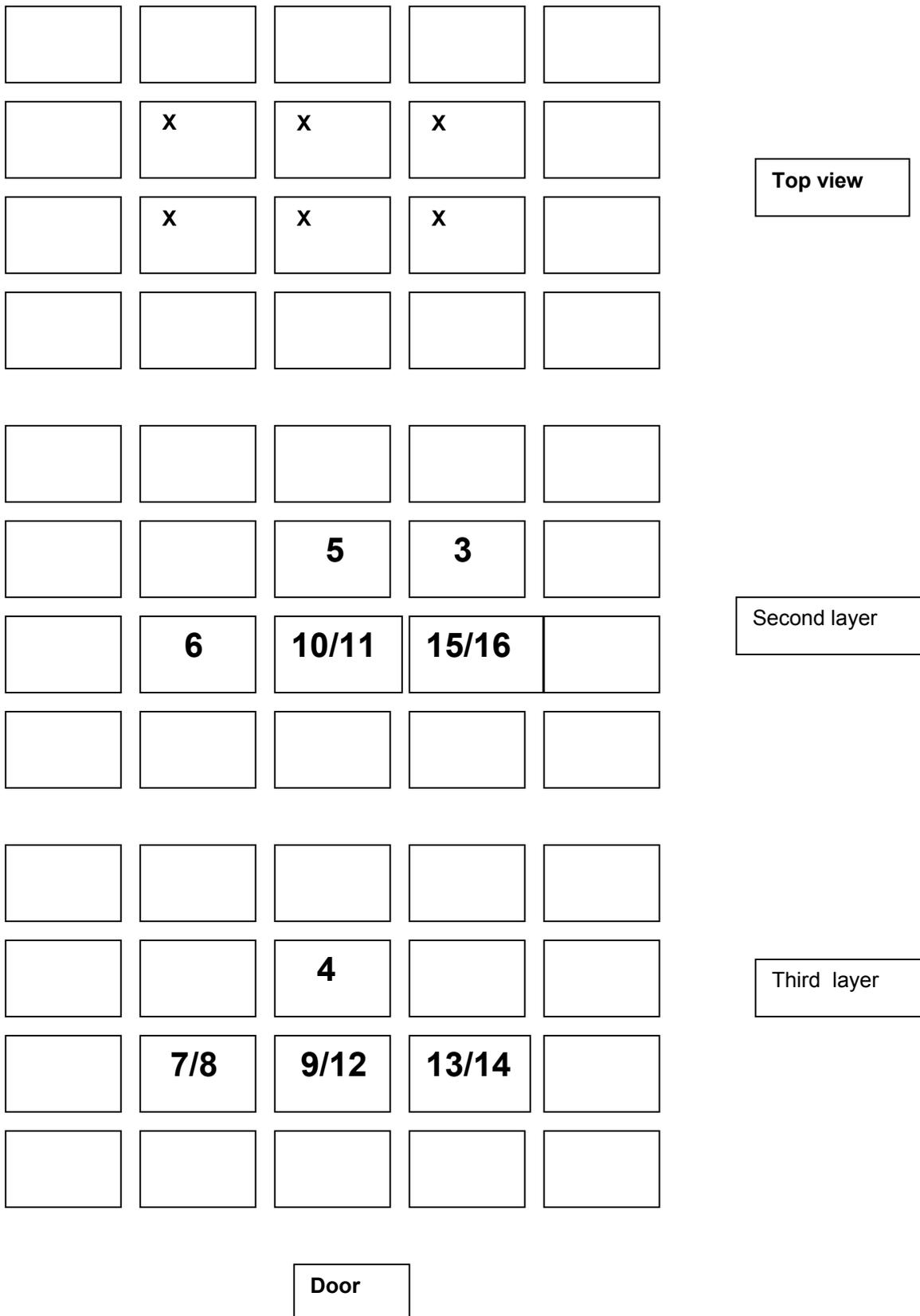


Figure 3.5.5.1. Floor plan - Replicate 3 (22 May 2002) Cartons 4 high. X indicates position of experimental cartons. First and fourth layer did not contain experimental fruit. Position of thermoprobes indicated by numbers and all unmarked cartons did not contain experimental fruit.

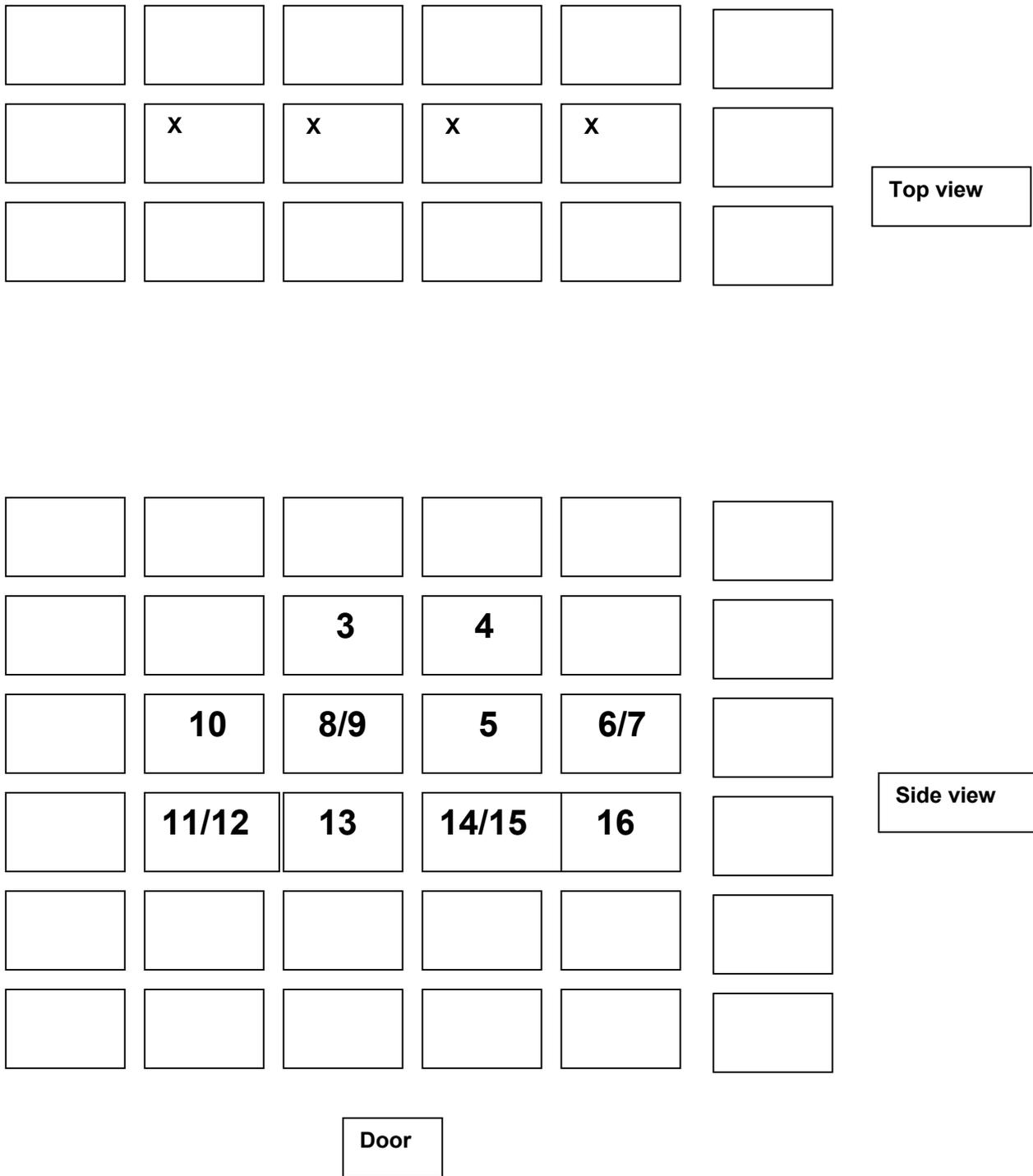
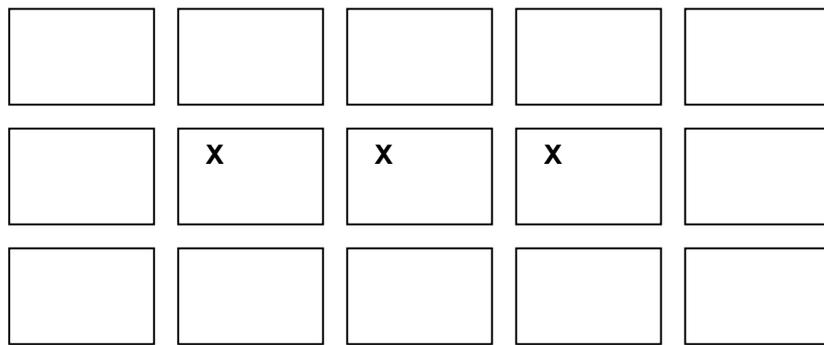


Figure 3.5.5.2. Floor plan - Replicate 4 (25 June 2002). X indicates position of experimental cartons. Position of thermoprobes indicated by numbers and all unmarked cartons did not contain experimental fruit.



Top view



Side view

Door

Figure 3.5.5.3. Replicate 5 (13 July 2002). X indicates position of experimental cartons. Position of thermoprobes indicated by numbers and all unmarked cartons did not contain experimental fruit.

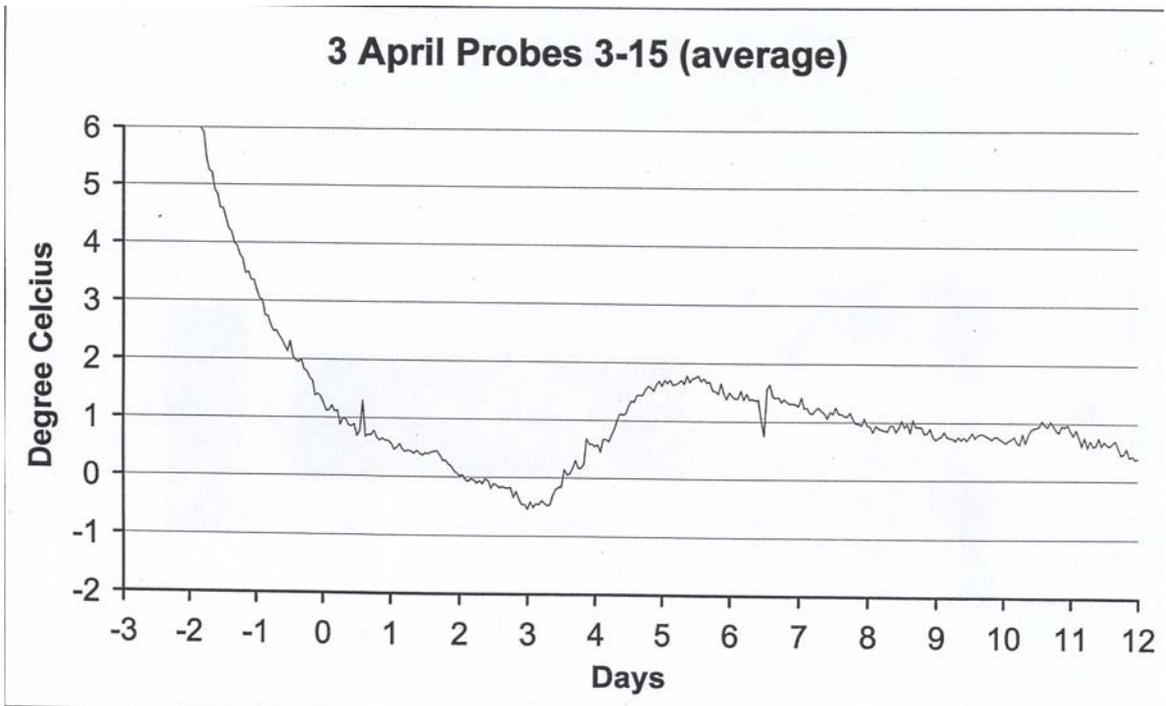


Figure 3.5.5.5. Average of the temperature readings from thermoprobes in fruit – replicate 1 (3 April).

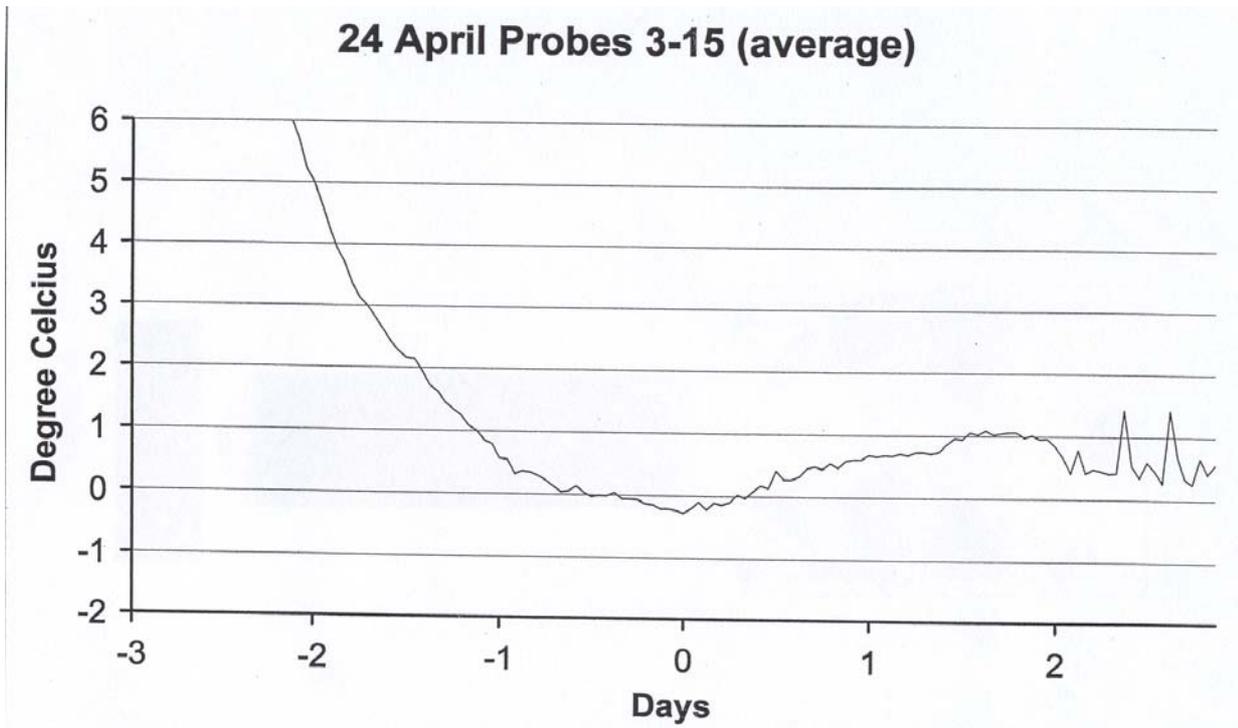


Figure 3.5.5.6. Average of the temperature readings from thermoprobes in fruit – replicate 2 (24 April).

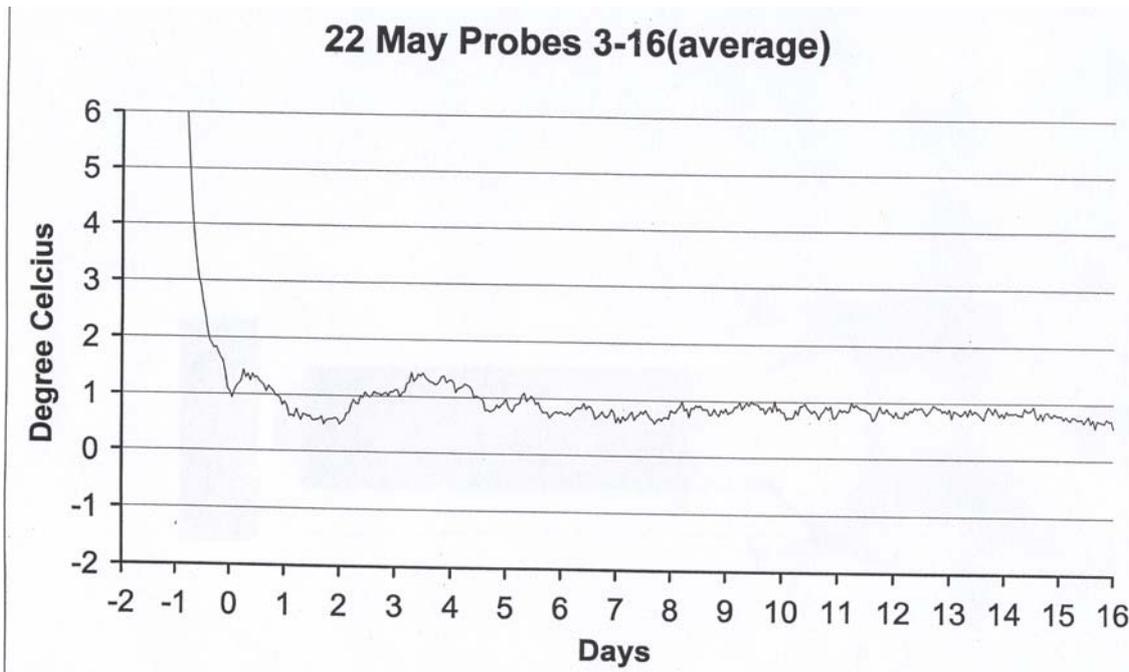


Figure 3.5.5.7. Average of the temperature readings from thermoprobes in fruit – replicate 3 (22 May).

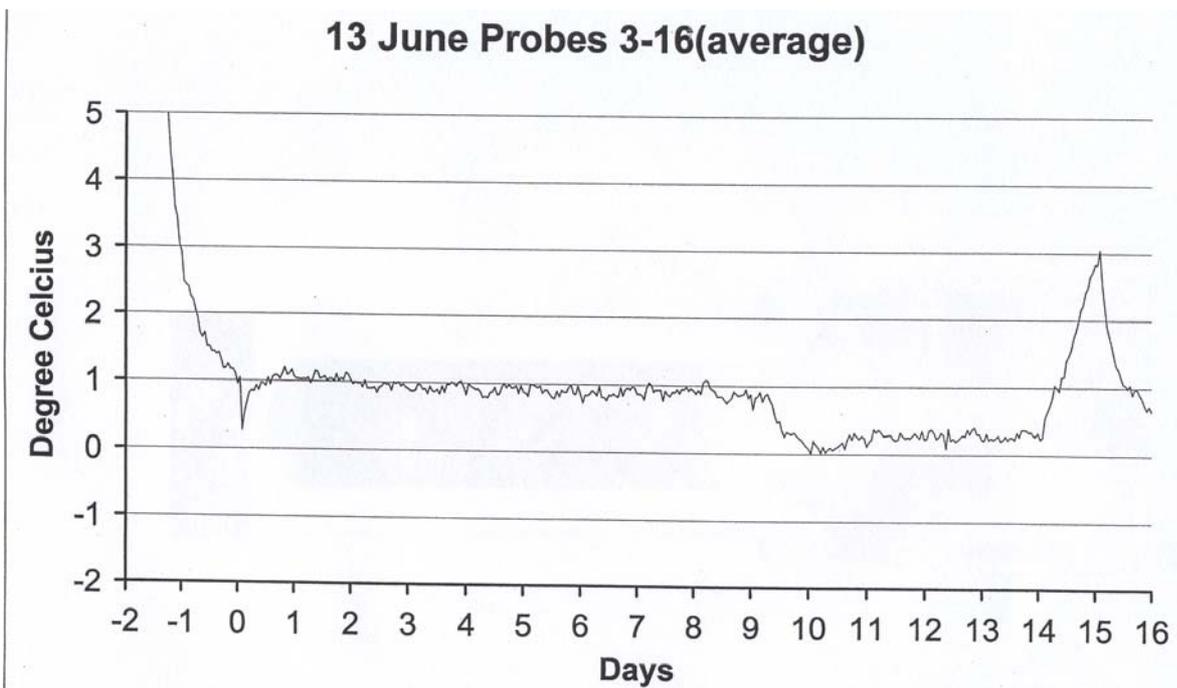


Figure 3.5.5.8. Average of the temperature readings from thermoprobes in fruit – replicate 4 (13 June).

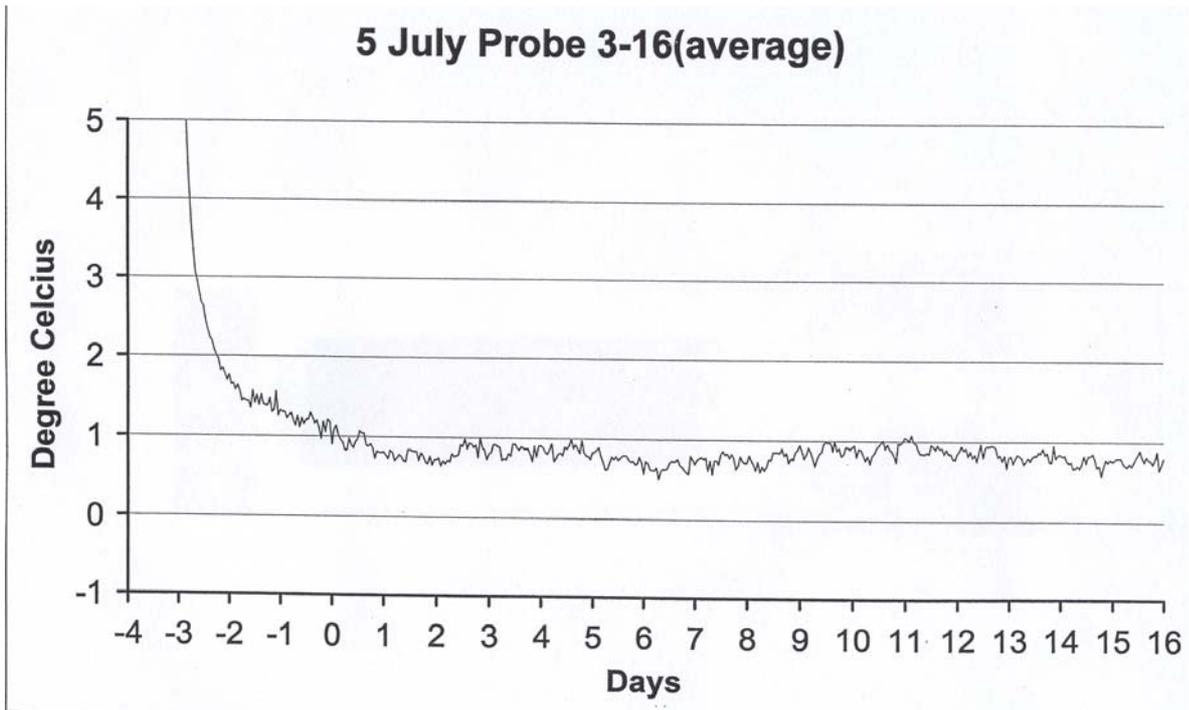


Figure 3.5.5.9. Average of the temperature readings from thermoprobes in fruit – replicate 5 (5 July).

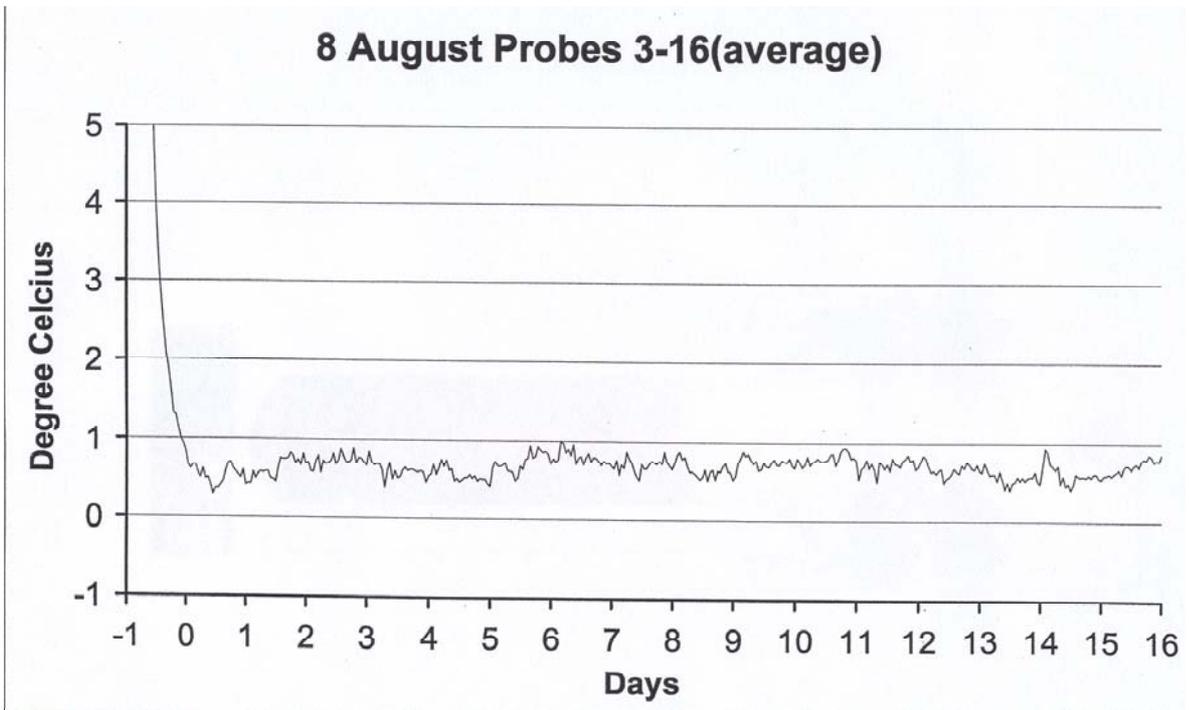


Figure 3.5.5.10. Average of the temperature readings from thermoprobes in fruit – replicate 6 (8 August).

Conclusion

A temperature of 0.6°C ($\pm 0.5^\circ\text{C}$) for 16 days was an effective disinfestation treatment for Mediterranean fruit fly-infested Barlinka grapes.

Future research

There may be a request that the research be extended to cultivars other than Barlinka grapes.

3.5.6 Disinfestation treatments for citrus pests of phytosanitary significance Experiment 695 by Bruce Tate and Tony Ware (CRI)

Opsomming

Fitosanitiere plaë is van die belangrikste hindernisse in die uitvoer van vrugte. Mediterreense (*Ceratitis capitata*), Natalse (*Ceratitis rosa*) en maroela (*Ceratitis cosyra*) vrugtevlieë en vals kodlingmot is vier van die insekte wat 'n bedreiging inhou vir die teiken-uitvoermark, die Verenigde State van Amerika. Eiers en verskeie larvale ontwikkelingsstadiums is blootgestel aan 4.5 of 3.0 °C vir onderskeidelik 7, 14 en 21 dae (3.5.6.1). 'n Klein aantal het die hoër temperatuur vir meer as 14 dae oorleef, maar geen oorlewing het is oor hierdie tydperk waargeneem by die laer temperatuur nie. Geeneen van die vrugtevlieë geïnkuleer in Clementine mandaryn het blootstelling aan 4.5 °C by 26 mBar oorleef nie, wat daarop dui dat hipobariese druk 'n sinergistiese invloed het. In 'n eksperiment met insekte geteel by 10.0 °C in 'n medium by 25 mBar en 2.0 °C by 15 mBar het sommige nogtans die blootstelling oorleef. Volgens hierdie eksperimente wil dit voorkom of hipobariese druk nie enige voordeel inhou bo tradisionele kouebehandeling nie. Kouebehandeling by 3.0 °C of laer vir 'n tydperk langer as 14 dae mag voldoende ontsmetting bied. Vrugkwaliteit is nie nadelig beïnvloed tydens hipobariese en koelopberging nie. VKM larwes in avokado het blootstelling aan metielbromied oorleef (3.5.6.2.).

Introduction

The avocado industry is currently investigating the possibility of exporting produce to the United States of America. However, this effort is being hampered by the presence of some pest species. To realise this opportunity the industry must treat the produce in such a manner in order to provide the importing country with the security that the threat of accidental importation of these phytosanitary pests is alleviated. Traditional disinfestation programmes use cold treatment procedures. However, many of these treatments result in fruit condition deterioration and the loss of shelf life. Some results from the USA have indicated that fruit condition can be maintained for long periods using low pressure. Furthermore reports that a combination of low pressure and temperature may be used in disinfestation treatments were received. Such treatments require sophisticated equipment that was available in Port Elizabeth. Arrangements were made to transport a container-sized hypobaric chamber to Nelspruit where research into its disinfestation potential could be examined. The research undertaken and reported in this paper examines the effects of different temperature and pressure regimes on the survival of four important phytosanitary pest species.

3.5.6.1 Hypobaric and low temperature

Materials and methods

Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]), Natal fruit fly (*Ceratitis rosa* Karsch) and marula fruit fly (*Ceratitis cosyra* Walker) reared at the Nelspruit facility were used in the experiments. False codling moth (*Cryptophlebia leucotreta* (Meyrick)) was obtained from the Citrusdal insectary. In the first experiment the insects were reared to the desired developmental stage in media and subjected to temperatures of 4.5°C and 3.0°C for 7, 14 and 21 days. The surviving larvae were then allowed to pupate and the number pupating was noted. In the second experiment Clementine fruit was inoculated with approximately 30 eggs using the method described by Ware (1999). Fruit with eggs were treated in a cold room at normal atmospheric pressure at 1013mBar at 4.5°C for 14 days. The fruit was then removed and placed at 26°C where the surviving insects were allowed to pupate. The number of pupae was then determined. Treatments at 26mBar were similarly undertaken.

In the third experiment, fruit fly eggs were placed on media. Some of the Petri dishes with eggs were treated while others were allowed to develop into mature larvae (third instar) before being subjected to treatment. False codling moth larvae were allowed to develop on media in Petri dishes and once developed into third and fifth instars, were treated. The treatment parameters were 10.0°C at 25mBar or 2.0°C at 15mBar, each for 14 days. Once treatment had been terminated the Petri dishes were stored at 26°C and the surviving insects allowed to pupate. The number of insects reaching this stage of development was then determined. The control insects were allowed to develop at 26°C at normal temperature.

Avocados were subjected to various temperatures and pressures and were examined for weight loss and fruit quality.

Results and discussion

None of the life stages of any of the species tested survived the 3°C for more than 14 days (Table 3.5.6.1.1). In contrast third instar *C. capitata* and third instar *C. cosyra* tolerated 21 days treatment at 4.5°C. Egg and first instar *C. leucotreta* survived 14 days but not 21 days at 4.5°C (Table 3.5.6.1.1). Based on these results it would appear that the 3°C treatment may have potential as a disinfestation treatment for all the species tested and that the 4.5°C would not provide the necessary security. Further research using the 3°C treatment or less may be warranted.

Table 3.5.6.1.1. Mortality (%) of *C. capitata*, *C. rosa*, and *C. cosyra* fruit fly species and *C. leucotreta* (false codling moth) after cold treatment of 4.5°C (treatment 1) and 3.0°C (treatment 2), both at normal atmospheric pressure.

Days	Egg	First instar	Second instar	Third instar	Fifth instar
Treatment 1					
<i>C. capitata</i>					
7	99.3	100	95.6	87.0	-
14	100	100	-	100	-
21	100	100	100	99.8	-
<i>C. rosa</i>					
7	100	100	100	-	-
14	100	100	100	-	-
21	100	100	-	-	-
<i>C. cosyra</i>					
7	97.0	97.0	89.1	100	-
14	100	100	100	99.8	-
21	100	100	100	99.8	-
<i>C. leucotreta</i>					
7	99.0	94.3	99.2	99.8	-
14	99.7	99.7	100	100	-
21	100	100	100	100	-
Treatment 2					
<i>C. capitata</i>					
7	53.6	98.8	100	98.6	-
14	100	100	100	100	-
21	100	100	100	100	-
<i>C. rosa</i>					
7	97.2	100	100	100	-
14	100	100	100	100	-
21	100	100	-	100	-
<i>C. cosyra</i>					
7	95.4	89.6	86.6	97.8	-
14	100	100	100	100	-
21	100	100	100	100	-
<i>C. leucotreta</i>					
7	-	71.5	-	31.2	47.7
14	-	100	-	100	100
21	-	100	-	100	100

C. rosa larvae tolerated 4.5°C in Clementines thereby confirming the ability of fruit flies to survive 14 days at this temperature (Table 3.5.6.1.2). Under reduced atmosphere no eggs or larvae survived, indicating that the reduced pressure may have a synergistic effect.

Table 3.5.6.1.2. The number of fruit flies alive after cold treatment of 4.5°C for 14 days at normal atmospheric pressure or 26mBar

Treatment	Life stage	<i>Ceratitidis capitata</i>	<i>Ceratitidis rosa</i>	<i>Ceratitidis cosyra</i>
Normal atmosphere	Eggs	0 (n=20)	0 (n=20)	0 (n=20)
Normal atmosphere	Larvae	0 (n=20)	2 (n=20)	0 (n=20)
26 mBar	Eggs	0 (n=21)	0 (n=15)	0 (n=16)
26 mBar	Larvae	0 (n=14)	0 (n=15)	0 (n=14)

Based on the above results and the advice of Dr Stanley Berg (United States) the next experiment was conducted with an elevated temperature. This was supposed to induce more rapid metabolism of the insect resulting in a build up of lactic acid and that would result in death in the absence of enough oxygen. However, third instar *C. capitata*, third instar *C. cosyra* and fifth instar *C. leucotreta* tolerated the conditions. In reaction to these results (Table 3.5.6.1.3) the experiment was repeated at a reduced temperature and pressure. Under these conditions no *C. capitata* or *C. rosa* survived, although small numbers of egg and second instar *C. cosyra* tolerated the treatment. By increasing the treatment period to 16 days an acceptable level of disinfestation may be achieved but this could probably also be achieved without the expense of hypobaric pressure.

Table 3.5.6.1.3. The average number of flies per replicate surviving 14 days at 10.0°C and 25mBar (treatment 1) or 14 days at 2.0°C at 15mBar (treatment 2).

Treatment	Species	Life stage	Control	Test	Survival (%)
1	<i>Ceratitidis capitata</i>	Egg	47.7	0	0
		Third instar	44.6	26.0	58
	<i>Ceratitidis rosa</i>	Egg	0.3	0	0
		Third instar	2.4	0	0
	<i>Ceratitidis cosyra</i>	Egg	18.6	0	0
		Third instar	28.0	8.0	29
	<i>Cryptophlebia leucotreta</i>	Third instar	7.0	0	0
		Fifth instar	7.3	0.6	21
2	<i>Ceratitidis capitata</i>	Egg	7.5	0	0
		First instar	39.0	0	0
		Second instar	42.8	0	0
		Third instar	32.2	0	0
	<i>Ceratitidis rosa</i>	Egg	2.7	0	0
		First instar	2.8	0	0
		Second instar	8.3	0	0
		Third instar	28.2	0	0
	<i>Ceratitidis cosyra</i>	Egg	?	0.2	?
		First instar	28.8	0	0
		Second instar	16.2	0.2	1
		Third instar	14.8	0	0

The avocados stood up well to low temperature and low pressure treatments (Table 3.5.6.1.4).

Table 3.5.6.1.4. Fruit quality assessments.

Treatment	Result
Fuerte: 5 days at 10°C followed by 14 days at 12.5°C at 52 mBar and 88% RH. Foil covered and naked.	Less than 10% weight loss in both groups. Fruit quality fine
Hass: 3 days at 7°C followed by 14 days at 10°C at 24 mBar followed by 8 days at 23°C. Some fruit deliberately damaged by piercing.	Fruit fine. No fungal growth on damaged fruit.
Hass: 14 days at 2°C at 15 mBar followed by 7 days at 23°C. Control fruit at 7°C. Foil covered and naked.	Hypobaric-treated fruit approximately 5% more weight loss. No difference in weight loss of fruit in foil and those not covered. Quality of 90% fruit good

Powell (2003) noted that the duration of the cold treatment needed for disinfestation may be more important than the nominal storage temperature and further research should be based on cold treatment by extending the treatment period. These results indicate that while low pressure may be synergistic it was not markedly so and that little benefit would be achieved in using this expensive apparatus.

3.5.6.2 Methyl bromide

Materials and methods

Initial testing demonstrated that mature FCM larvae were the most tolerant life stage to methyl bromide treatment. Twenty-one holes were made in each avocado using a cork borer (12 mm diameter). The holes terminated at the pip. Larvae of the selected age (10-12 day-old) were removed from artificial media and 5 were placed into each hole. The holes were then sealed with plugs from another avocado. These plugs were created using a larger cork borer (14 mm diameter) and excess pulp was removed. Two methyl bromide regimes were used. The first treatment of 32 gm⁻³ methyl bromide (MBr) was bled into the container and after 30 minutes the concentration was reduced to 26 gm⁻³ by purging with air. The concentration was again lowered after 2 hours to 16 gm⁻³ and was maintained at that level for the remainder of the four-hour experiment. In the second of the fumigation trials the initial concentration was 32 gm⁻³ and after 15 minutes this was reduced to 29 gm⁻³, after 30 minutes adjusted to 26 gm⁻³, after 75 minutes to 21 gm⁻³, after 2 hours to 16 gm⁻³, after three hours to 15 gm⁻³ where it was maintained for the remainder of the experiment. The air temperature was 26°C in both cases. Controls were infested fruit maintained at 26°C without undergoing fumigation. The number of survivors was determined 48 hours after treatment.

Results and discussion

Control survival was 97.5% (2.5% natural mortality; n=420). In the first replicate there was 97.1% mortality (n=1680) and in the second 94.6% mortality (n=2100). Many of the MBr-treated survivors were sluggish and probably would not have pupated.

Conclusion

Methyl bromide did not disinfest FCM-infested avocado fruit.

Future research

SAAGA has requested that no further research be conducted on FCM at this stage but that the fruit fly research continue.

References cited

- Powell, M.P. 2003. Modeling the response of the Mediterranean fruit fly (Diptera: Tephritidae) to cold treatment. *Journal of Economic Entomology* 96: 300-310.
- Ware, A. B. 1999. Cold sterilization of Mediterranean fruit-fly-infested mandarins. *Outspan Citrus Centre Annual Research Report* 72-85.

3.5.7 **Marula fruit fly. Is it a threat to the citrus industry?**
Experiment 725 by Tony Ware and John-Henry Daneel (CRI)

Opsomming

Met die tegniek wat gebruik is kon nie bewys word dat maroela vrugtevlieg 'n bedreiging inhou vir die sitrusbedryf nie. Alhoewel groot getalle van die insek teenwoordig was in die boord het hulle net beduidend voorgekom wanneer Satsuma op die punt was om gepluk te word. Populasies het afgeneem namate later-ontwikkelende kultivars oesgereedheid bereik het. Maroela vrugtevlieg is slegs geteel van Satsuma vrugte wat op die grond versamel is. Hierdie resultate moet nog bevestig word. Indien maroela vrugtevlieg 'n plaag van sitrus blyk te wees, alhoewel in lae getalle, word dit 'n fitosanitêre bedreiging wat navorsing oor verliggende naesbehandelings regverdig.

Introduction

Marula fruit fly (*Ceratitis cosyra* Walker) is common in areas where marula trees grow. They have been recorded from mango (from whence they have obtained their alternate common name of mango fruit fly) and guava (White and Elson-Harris, 1992). Citrus is considered as a host in Zimbabwe. However, in a laboratory experiment grapefruit and Delta Valencia oranges appear to be unsuitable hosts (Grout, 1999). These trials examine the suitability of citrus cultivars in the field.

Materials and methods

Sensus traps containing Questlure were hung in an untreated mixed variety orchard situated at the Lowveld Agricultural College near Nelspruit. The traps were examined weekly over twenty weeks and all flies trapped were taken to the laboratory where they were identified to species and sexed. Fallen fruit was collected and kept on sand. Any emerging flies were collected and identified. Traps were first placed on the 13 February 2003.

Results and discussion

Over the period of observation from colour break to harvest a large number of *C. capitata* and *C. cosyra* were trapped in the orchard (Table 3.5.7.1). Less than 10% of the flies caught were *C. rosa* while 13 *C. pedestris* were caught.

Table 3.5.7.1. Weekly trap catches of fruit flies caught in Sensus traps containing Questlure over 20 weeks from 20 February 2003 in a mixed variety orchard near Nelspruit.

Week	<i>C. capitata</i>		<i>C. rosa</i>		<i>C. cosyra</i>		<i>C. pedestris</i>	
	Male	Female	Male	Female	Male	Female	Male	Female
1	0	1	0	4	6	16	0	0
2	0	2	3	5	34	45	0	0
3	2	3	9	12	27	65	1	0
4	7	12	13	19	34	104	0	2
5	21	29	8	15	32	147	0	1
6	22	33	14	15	40	127	0	1
7	14	47	8	26	49	147	1	3
8	11	26	2	10	46	119	3	0
9	12	36	3	7	17	71	0	0
10	21	80	9	23	41	78	0	0
11	35	108	9	17	25	48	0	0
12	52	208	19	35	31	49	0	0
13	51	255	15	38	21	67	0	0
14	35	209	9	25	19	35	0	0
15	74	376	16	22	8	41	0	0
16	31	218	6	10	4	12	0	0
17	10	79	2	11	2	8	0	0
18	46	273	2	14	7	19	0	0
19	15	100	2	7	1	9	0	0
20	5	196	1	2	2	0	0	0
Total	464	2291	150	317	446	1207	5	8
%	9.5	46.9	3.1	6.5	9.1	24.7	0.1	0.2

There appears to be seasonal distribution of fruit fly species (Figure 3.5.7.1). Early in the season there is a predominance of *C. cosyra* with this population tapering off as the season progressed. This result is in agreement with Grout and Stephen (1999). *C. rosa* appeared to have two population peaks. It is unclear why this occurred but environmental conditions may have been responsible. There were few *C. capitata* early in the season. These populations increased as the weather got colder.

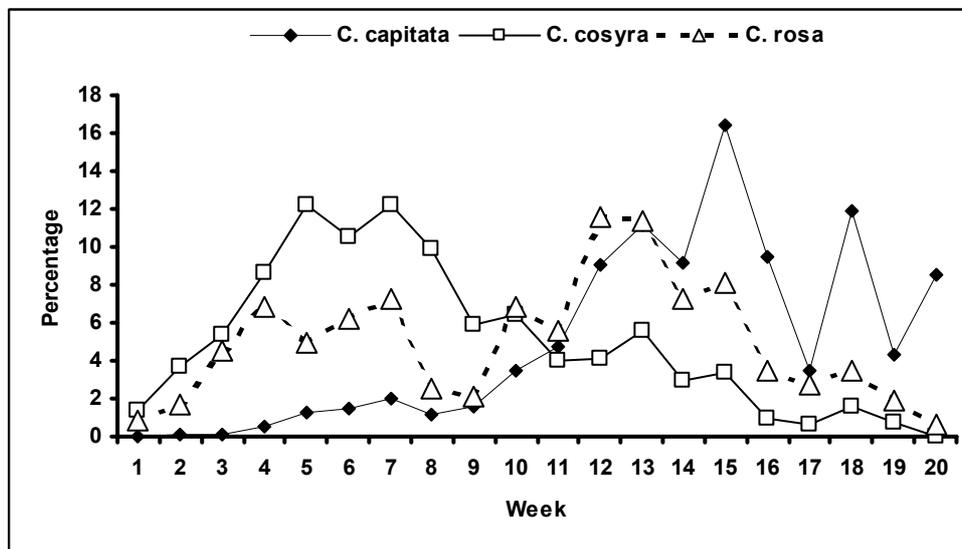


Figure 3.5.7.1. Temporal *C. capitata*, *C. rosa* and *C. cosyra* female populations expressed as a weekly percentage of their individual total number in a mixed variety orchard near Nelspruit.

Fallen fruit was collected on three occasions from Satsumas (harvesting began during week 8 on this cultivar). Two of these collections did not yield any fruit fly but the third batch produced 3 male and 1 female *C. capitata*, 2 male and 1 female *C. rosa* and 1 male *C. cosyra*. This observation with respect to marula fruit fly was also noted by Grout (1999) who found, in the laboratory, that low numbers of marula fruit flies emerged from Clementines exposed to high numbers of marula fruit flies, but no marula fruit flies emerged from grapefruit or Delta Valencia oranges exposed in the same way. Although fruit was collected from the other cultivars (Empress, Ellendale and Clementine) no fruit fly emerged.

This result confirmed the result of Grout (1999) that *C. cosyra* was able to utilise Satsumas as a host. However, the fruit was gathered from the ground and it is uncertain whether *C. cosyra* oviposits while the fruit is on the tree. This species of fruit fly appears to only lay its eggs on fruit that has fallen. Further research is needed to clarify the position.

Conclusion

Although high numbers of *C. cosyra* and *C. capitata* were present in the orchard, few progeny were raised from the fruit collected. It is still uncertain whether marula fruit fly poses a threat to citrus and further research is needed.

Future research

Further research into the natural behaviour and preferences of marula fruit fly needs to be undertaken.

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3.5.8 Spinosad GF 120 cage tests

Contract research for Dow AgroScience by John-Henry Daneel, Peter Stephen and Tony Ware (CRI)

Opsomming

Spinosad is 'n nuwe geslag plaagdoders wat vervaardig word deur fermentasie met die grondbakterie, *Saccharopolyspora spinosa*, en geformuleer is as 'n vrugtevlieglokaas (GF 120 NF). As deel van die registrasieproses is die doetreffendheid daarvan in hokke getoets teen Natalse en maroela vrugtevlieë. Die laagste dosis waarteen dit getoets is (750 ml/10 l water) blyk net so doetreffend te wees as as die hoër konsentrasies. Die produk het bewys dat dit net so effektief is as die tradisionele hidrolisaat/merkaptotien mengsel.

Introduction

Fruit flies are important pests of the fruit industry. The fruit is damaged by the feeding activity of the larvae and blind stings (aborted oviposition) can lead to post harvest losses through fungal infection. Furthermore, the presence of fruit fly is a phytosanitary concern and many lucrative markets are in countries that demand that the exporter guarantee that the produce is fruit fly free. Current control methods applied to citrus in South Africa include pre-harvest spraying with a protein hydrolysate mixed with either of the organophosphates mercaptothion or trichlorfon (Nel *et al.*, 2002) and/or post-harvest cold treatment disinfestations.

There is worldwide pressure to phase out the use of organophosphates and the currently used pesticides used to control fruit fly are perceived to have a limited life. It is therefore essential that alternatives be investigated so that the industry is not left high and dry should these organophosphates be withdrawn from the market. One of the promising candidates is Spinosad, a new generation pesticide to a new class of insect control products called the Naturalytes, has been developed by Dow AgroSciences and the USDA-ARS (Dow AgroSciences, undated). Spinosad is produced by fermentation of a soil bacterium *Saccharopolyspora spinosa*. It has a low acute mammalian toxicity and, being a “naturally-occurring” product, is considered organic in nature (although the formulated product may not be). A bait formulation (GF 120 NF) for fruit fly control has been developed.

It is essential that the product be shown to be effective against the three southern African agriculturally important fruit fly species. The research on Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]) has shown that the product is effective (Van der Merwe and Allsopp, undated). This paper reports on research determining the effectiveness of the product using outdoor cage tests and Marula fruit fly (*Ceratitis cosyra* [Walker]) and Natal fruit fly (*Ceratitis rosa* Karsch).

Materials and methods

The research was conducted at the Citrus Research International (Pty) Ltd premises situated in Nelspruit in the Mpumalanga Province of South Africa. The experiments were done in metal-frame cages (dimensions 1100X600X1800mm – lengthXbreadthXheight) enclosed in insect netting placed under a lean-to in order to protect the flies from rain and direct sunlight.

Three potted rough lemon trees that had never been exposed to pesticide treatments were placed in each cage. The trees were suspended above the cage floor in order to facilitate the collection of dead flies. Only the centre tree in each cage was treated (Tables 3.5.8.1 and 3.5.8.2). All applications were made using a hand-spray apparatus at low pressure to ensure the deposit of coarse droplets (Figure 3.5.8.1). The treated trees were allowed to dry (\pm 3 hours) before being placed into the cages. The position of the cages was changed for every replicate. The daily maximum and minimum ambient temperature was recorded.

The Natal and marula fruit flies used were obtained from laboratory-reared colonies that were established four years previously and that had been supplemented annually with “wild” flies. Approximately 50 male and 50 female flies, aged five days, were placed in the cages. These flies were fed *ad lib* with sugar and water before and during the experiment (no protein was supplied). A mortality estimate was done by counting and then removing all dead flies from the cage floor three hours after their release. Estimates were repeated at 24 hours intervals thereafter. The experiment was terminated after 72 hours when all living flies were counted. The marula fruit fly trial was performed five times (11 November 2002, 22 November 2002, 10 December 2002, 14 January 2003, 4 February 2003) and the Natal fruit fly trial four times (23 June 2003, 14 July 2003, 18 August 2003, 26 August 2003),

Statistical analysis was done on the mortality estimates using Polo-PC (LeOra, 1987). The program calculates the lethal time that 50% of the population (LT_{50}) dies using Chi-squared goodness of fit test on probits obtained from mortalities and least square linear regressions of log (time) on probit (mortality). The fiducial limits (the probability that the true value lies between the calculated high and low limits) are estimated using $g(0.9)$ (index of significance for potency estimation at 90% confidence level). Where the data set has too much variability the fiducial limits could not be estimated. Analysis of LT_{50} and mortality data was done using ANOVA followed by a comparison of means using LSD ($P < 0.05$).

Table 3.5.8.1. Treatments and dose rates for evaluating Spinosad GF 120 fruit fly bait using marula fruit flies

Treatment no.	Treatment	Dose rate (10mℓ/tree)
1	Untreated control	Water
2	Hymlure + mercaptothion	700mℓ mercaptothion + 2000ℓ Hymlure/100ℓ
3	Spinosad	750mℓ/10ℓ water/ha
4	Spinosad	1ℓ / 10ℓ water/ha
5	Spinosad	1.5ℓ / 10ℓ water/ha
6	Spinosad	1ℓ / 3ℓ water/ha

Table 3.5.8.2. Treatments and dose rates for evaluating Spinosad GF 120 NF fruit fly bait using Natal fruit flies

Treatment no.	Treatment	Dose rate (10mℓ/tree)
1	Untreated control	Water
2	Hymlure + mercaptothion	700mℓ mercaptothion + 2000ℓ Hymlure/100ℓ
3	Spinosad	750mℓ/10ℓ water/ha
4	Spinosad	1ℓ / 10ℓ water/ha
5	Spinosad	1.5ℓ / 10ℓ water/ha
6	Spinosad	1ℓ / 3ℓ water/ha
7	Spinosad GF 120 (old)	1ℓ / 10ℓ water/ha

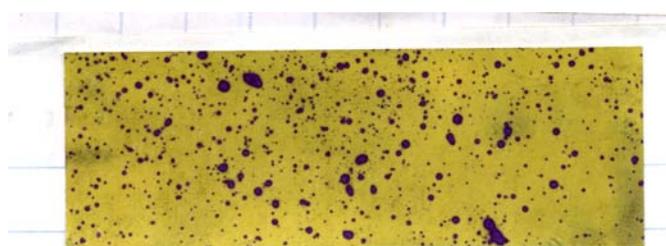


Figure 3.5.8.1. Spray deposits on trees as indicated using water sensitive indicator paper (Ciba-Geigy).

Results and discussion

The percentage fruit fly mortality over 72 hours exposure is reported in Table 3.5.8.3 and 3.5.8.4. All treatments had almost double the mortality of the control. There was no statistical difference between the traditional treatment (protein + mercaptothion – treatment 2) and any of the Spinosad GF 120 treatments (treatment 3,4,5 and 6). Furthermore, there was no concentration related efficacy differences between the Spinosad treatments (treatments 3, 4, 5 & 6). This result implies that the lowest rate tested would suffice.

Table 3.5.8.3. Percentage marula fruit fly mortality₁₉₃(both male and female) over 72 hours exposure to

various concentrations of Spinosad GF 120 and the traditional Hymlure + mercaptothion-treated rough lemon (potted) in outdoor cages.

Replicate	Treatment					
	1	2	3	4	5	6
1	39	85	98**	95	93	91
2	54	86	97**	97	98	98
3	50	89	88	94	92	88
4	47	90	99***	63	59	97
5	35	87	95	93	95	94
Mean*	45 ^a	87 ^b	95 ^b	88 ^b	87 ^b	94 ^b

* Same letters indicate no statistical difference (ANOVA followed by Fisher's LSD – P<0.05)

** Hole in cage – many escapees

*** Ants in cage – removed some cadaver

Table 3.5.8.4. Percentage Natal fruit fly mortality (both male and female) over 72 hours exposure to various concentrations of Spinosad GF 120 NF and the traditional Hymlure + mercaptothion-treated rough lemon (potted) in outdoor cages.

Replicate	Treatment						
	1	2	3	4	5	6	7
1	56	81	79	90	81	78	80
2	38	84	94	90	85	86	90
3	93	99	100	95	98	92	98
4	88	99	94	99	94**	90	95
Mean*	69 ^a	91 ^a	92 ^a	94 ^a	90 ^a	87 ^a	91 ^a

* Same letters indicate no statistical difference (ANOVA followed by Fisher's LSD – P<0.05)

** Hole in cage – many escapees

Table 3.5.8.5 and 3.5.8.6 indicates the LT₅₀ (hours). There is a large variation in the results, some of which may be explained by differences in ambient temperature (Table 3.5.8.7 and 3.5.8.8) between replicates. In general fly activity will be less when temperatures are low. This would reflect in a less feeding activity that, will in turn, be reflected in a higher LT₅₀. However, even with the high variation in results all treatments had lower LT₅₀ than the untreated controls (Table 3.5.8.5 and 3.5.8.6). The loss of flies through ant activity and escape in treatments did not appear to affect the results. There was no apparent difference between the conventional mercaptothion treatment and the Spinosad treatments. Furthermore there was no rate related differences between the different Spinosad concentrations or between formulations.

Table 3.5.8.5. The LT₅₀ (fiducial limits) of the marula fruit flies (both sexes) subjected to various concentrations of Spinosad GF 120 and the traditional hymlure/mercaptothion treatment.

Rep.	Treatment					
	1	2	3	4	5	6
1	112.1 (83.5-198.5)	33.2 (-)	24.3** (-)	32.1 (29.1-34.8)	36.1 (26.3-45.5)	33.6 (-)
2	78.9 (47.5 – 438.6)	28.9 (-)	20.6** (5.7-35.2)	38.9 (34.2-44.1)	14.7 (9.2-20.1)	12.9 (-)
3	84.1 (72.3-106.7)	38.2 (25.1-57.8)	41.1 (30.9-54.8)	29.0 (22.3-35.4)	24.6 (13.0-32.8)	36.7 (32.0-41.0)
4	48.9 (-)	30.1 (15.5-60.7)	13.8*** (10.0-17.9)	23.5 (19.9-26.9)	18.6 (5.5-35.0)	13.5 (11.2-15.8)
5	149.0 (100.2- 308.1)	10.6 (8.4-13.0)	15.3 (9.8-20.9)	12.1 (8.0-16.1)	11.4 (4.7-19.5)	11.0 (9.8-14.0)
Mean*	94.6 ^a	28.2 ^b	23.0 ^b	27.1 ^b	21.1 ^b	21.7 ^b

* Same letters indicate no statistical difference (ANOVA followed by Fisher's LSD – P<0.05)

** Hole in cage – many escapees

*** Ants in cage – removal of cadaver

Table 3.5.8.6. The LT₅₀ (fiducial limits) of the Natal fruit flies (both sexes) subjected to various concentrations of Spinosad GF 120 NF and the traditional hylmlure/mercaptotion treatment.

Rep.	Treatment						
	1	2	3	4	5	6	7
1	74.3 (43.3 – 382.3)	17.0 (10.0 – 26.3)	36.2 (31.5 – 41.2)	23.8 (12.7 – 33.6)	25.0 (14.8 – 36.4)	24.8 (9.8 – 43.6)	30.6 (26.2 – 34.9)
2	102.5 (81 – 160)	10.6 (5.3 – 17.0)	26.1 (22.4 – 29.8)	18.9 (8.4 – 28.5)	27.7 (23.7 – 31.8)	31.3 (27.2 – 35.2)	19.1 (13.0 – 27.3)
3	26.6 (16.0 – 37.8)	6.2 (5.0 – 7.5)	13.7 (6.0 – 21.7)	20.3 (16.9 – 23.3)	8.5* (7.0 – 10.0)	10.6 (6.5 – 15.2)	17.8 (14.9 – 20.6)
4**	-	13.5	30.9	25.2	22.7	26.8	37.1
Mean	67.8	11.8	26.7	22.2	21.0	23.4	26.1

* Hole in cage – many escapees

** No fiducial limits or statistical analysis was performed because of the variability of the data

Table 3.5.8.7. The ambient temperature (°C) range experienced during the marula fruit fly experiments.

Replicate	Temperature °C			
	Application	0-24 hrs	24-48 hrs	48-72 hrs
1	24	16-26	15-31	18-31
2	27	30-35	19-31	12-32
3	25	19-28	20-31	20-31
4	30	20-30	20-34	20-27
5	36	23-37	22-37	21-32

Table 3.5.8.8. The ambient temperature (°C) range experienced during the Natal fruit fly experiments.

Replicate	Temperature °C			
	Application	0-24 hrs	24-48 hrs	48-72 hrs
1	27	19-22	17-24	14-25
2	26	16-28	16-30	18-30
3	28	26-30	20-33	22-28
4	26	24-27	9-26	18-32

Conclusions

1. Spinosad GF 120 appears to be as efficacious against Marula fruit fly as the currently registered Hylmlure/mercaptotion treatment.
2. Spinosad GF 120 NF appears to be as efficacious against Natal fruit fly as the currently registered Hylmlure/mercaptotion treatment.
3. Temperature plays a role in the effectiveness of the products.
4. Under the conditions these experiments were performed, the lowest Spinosad rate was found to be as efficacious as the higher rates.

References cited

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3.5.9 Spinosad GF 120 field trial

Contract for Dow AgroSciences by John-Henry Daneel, Peter Stephen and Tony Ware (CRI)

Opsomming

Spinosad is 'n nuwe geslag plaagdoder wat vervaardig word deur fermentasie met die grondbakterie, *Saccharopolyspora spinosa*, en geformuleer is as 'n vrugtevlieglokaas (GF 120 NF). As deel van die registrasieproses is die doeltreffendheid daarvan in die veld getoets. Die produk was net so effektief as die tradisionele proteïenhidrolisaat/merkaptotien mengsel. Wanneer volgens kleur gepluk word is dit belangrik dat die lokvalle gedurende die tydperk in werking bly aangesien die vrugte vatbaar is en vrugtevlieggpopulasies neig om vinnig op te bou. Daar was ook 'n verskil in die tydperk waartydens die twee oorheersende spesies teenwoordig was. Natalse vrugtevlieg het vroeg in die seisoen voorgekom en Mediterreense vrugtevlieg eers later namate die weer koeler geword het.

Introduction

Fruit flies not only pose a direct threat to citrus through the laying of eggs and subsequent damage done by the developing larvae, but they are also considered a phytosanitary pest. Interceptions of fruit fly in Spanish consignments of soft citrus effectively closed that lucrative market to Spanish citrus. This resulted in severe financial hardship for growers and highlights the importance of this pest. Traditionally the pest has been controlled commercially using a bait mixture of protein hydrolysate plus the toxicant Malathion (mercaptotion) or Dipterex (trichlorfon). However, there is worldwide pressure to phase out the use of organo-phosphates thus limiting the life of these products. Currently the southern African fruit growing industry does not have a registered alternative bait spray.

Spinosad is a pesticide of low mammalian toxicity produced by bacteria and, as such, is considered "organic". Dow AgroSciences has formulated Spinosad into a bait and is currently registering the product for fruit fly control in South Africa. The results reported are a field evaluation of the bait and form part of the registration process.

Materials and methods

Nova mandarins grown on the farm Bakgat in the Schoemanskloof Valley near Nelspruit in the Mpumalanga province of South Africa were used in the trial. An orchard (4 years old) consisting of 800 trees was treated weekly with a bait made up of 6000 ml protein hydrolysate (Hym lure) plus 175 ml mercaptotion (malathion) in 100 l water. A further 800 trees (8 years old) immediately adjoining the above orchard (Figure 3.5.9.1) were treated with M3 bait stations (one per tree). The M3 bait stations were hung a week before spraying commenced and a week after the monitoring "Sensus" traps were commissioned. Two adjoining orchards of 800 trees each (one 8 years and the other 4 years of age) were treated weekly with Spinosad GF 120 (1 l in 9 l of water). GF120 spray mixtures were applied using a "Mantis" spray machine designed to deliver 10 ml/tree in coarse droplets. Spraying was terminated after 7 applications the week before harvesting commenced although the M3 bait stations were not removed.

The attractants used in the monitoring traps were Capilure (a trimedlure formulation commonly used to monitor Mediterranean fruit fly, *Ceratitidis capitata* [Wiedemann]) or Questlure (used to monitor females of all three economically important species, *C. capitata*, *C. rosa* Karsch (Natal fruit fly) and *C. cosyra* (Walker) (Marula fruit fly). The traps were positioned as illustrated in Figure 3.5.9.1 on 28 February 2003 a week before treatment. Those traps placed outside the orchards were considered as untreated controls.

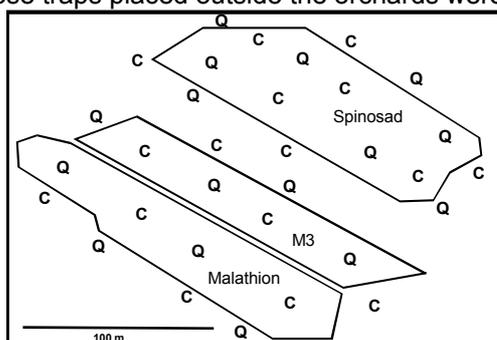


Figure 3.5.9.1. Sensus trap positions (C = Capilure and Q = Questlure) and orchard treatments

Fifty fruit were collected each week from each orchard floor and placed on sand so that flies could emerge. All emergent flies were identified and sexed.

Results and discussion

The effect of the treatments on the fruit fly populations is reflected in Table 3.5.9.1. The variation in Capilure fly catches before treatments were applied was high with an average of 13.8 flies per trap caught in the Spinosad treated block and only an average of one fly per trap in the malathion-treated orchard. Trap catches with Questlure showed an even higher variation in pre-treatment trap counts in the four areas although this time the M3 treated block of trees caught the most flies. This was more than ten times the number of flies trapped in the adjoining malathion-treated block. These results imply that the fly pressure was not consistent throughout the area with the result that the catches from the different treatments could not be compared statistically. There was a 92.4% decrease in Capilure and 93.3% in the Questlure fly numbers over the whole experimental area after the first treatment.

The current recommended treatment threshold level for fruit flies using Capilure is five (previously 7), or more, flies per trap per week. Fruit fly catches in the M3 and malathion treated blocks were below the threshold value when the trial was initiated. Only on one occasion during the treatment period did the number trapped climb to above the threshold level (M3- week 1). In the Spinosad-treated orchard this level was exceeded before treatments were initiated. In the week (7) after spraying had stopped the trap catches again exceeded the treatment level.

The recommended treatment threshold level for Questlure (two females/trap/week) was exceeded on all but two occasions (week 3 and 4) in the untreated control traps. Once spraying had ceased the fly numbers increased (week 8 and 9) although in the case of the M3 treated block this increase was contained to below the threshold value in week 8.

The discrepancy between Capilure and Questlure fruit fly catches is probably due to differences in the attractiveness of the lures to the different species. Capilure attracted 346 male Mediterranean fruit flies but no females. In contrast, Questlure traps produced 35 males and 91 females. In contrast, the trap catch ratio for Natal fruit fly between Capilure and Questlure traps was 1:2.8 with 208 males and 18 females being caught using Capilure, and 256 males and 578 females using Questlure. Marula fruit fly catches were comparatively low with a single male being caught in the Capilure traps against 16 males and 41 females in the Questlure traps. Three *C. pedestris* (Bezzi) females and a single male were caught in the Questlure traps.

No fruit flies emerged from the fruit gathered and no fruit was rejected in the packhouse because of damage that could be attributed to fruit flies. Based on these results it would appear that there was no difference in the efficacy of Spinosad GF 120 and the two registered treatments with which it was compared. The increase in the number of flies caught after spraying was stopped, indicates that fruit fly baiting should continue during harvesting.

Table 3.5.9.1. Average number of fruit flies per trap in Sensus traps charged with Capilure or Questlure. Week in which treatment was initiated is designated 0. The last spray was applied at week 6 and the first fruit were harvested in week 7. Those traps placed outside the orchards were designated untreated.

CAPILURE											
Treatment	Total no. of traps	Weeks									
		-1	0	1	2	3	4	5	6	7	8
Untreated	4	8.0	0.3	0.1	0.8	0.8	0.5	0.9	3.5	9.4	17.8
Spinosad GF120	4	13.8	1.0	1.8	4.8	0.5	3.8	3.5	8.3	36.8	26.0
M3	2	3.0	1.0	5.5	2.5	3.5	1.0	3.0	2.0	8.5	4.4
Malathion	2	1.0	0	0.5	0	1.0	1.5	0.5	2.0	10.5	11.0
QUESTLURE											
Untreated	4	21.3	3.4	3.0	5.5	0.9	1.5	6.3	9.3	9.6	3.5
Spinosad GF120	4	16.8	1.5	1.1	2.1	0.5	1.8	1.4	4.0	5.8	31.0
M3	2	74.0	0.5	0	0.5	0.5	0	1.0	0	0.5	4.0
Malathion	2	7.0	0	0	0	0.5	0.5	0	2.0	5.5	11.0

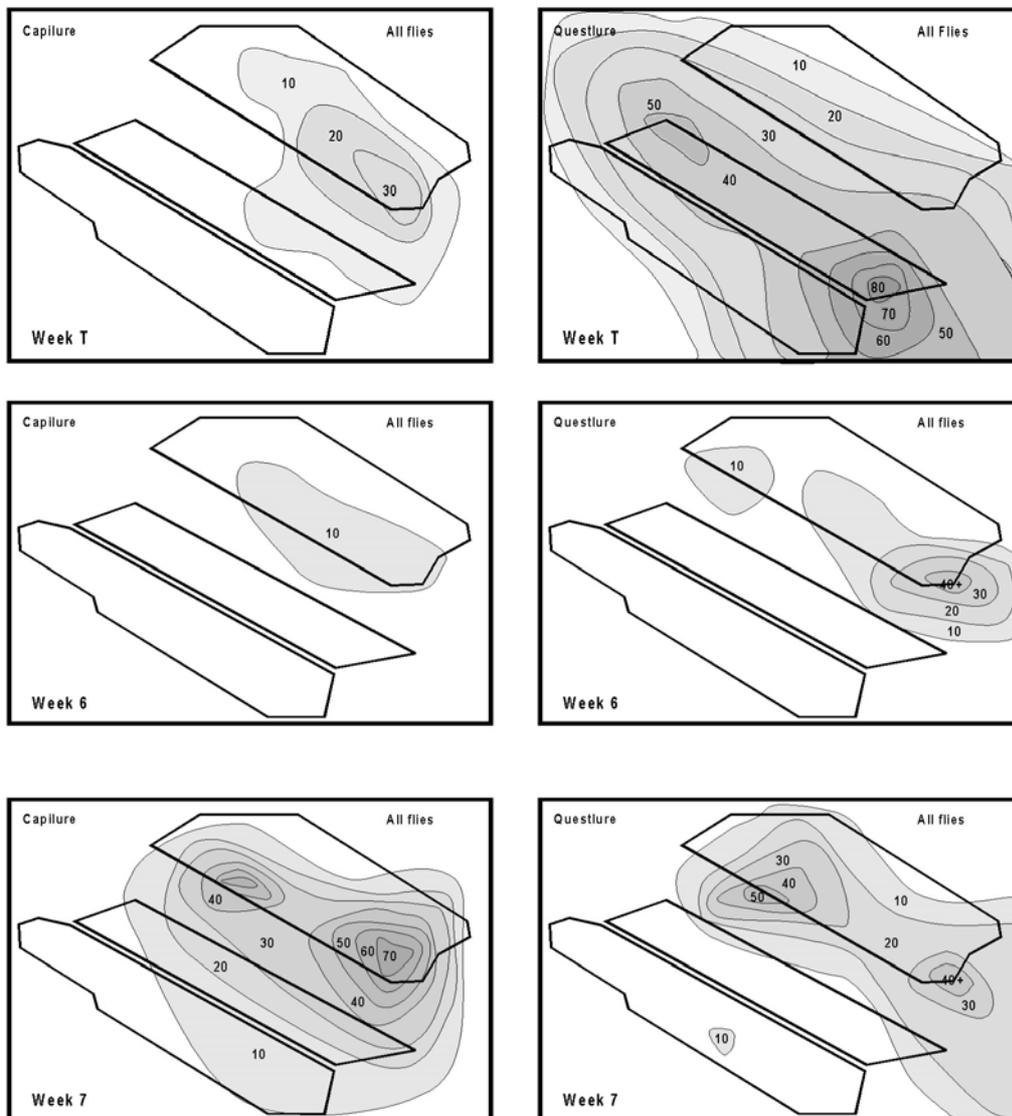
Conclusion

Spinosad GF 120 gave effective fruit fly control at 1 ℓ per 9 ℓ water per hectare per week.

Additional data analysis

The large number of traps used in the trial provided additional information on the response of the different species to the different attractants. The data from each trap was plotted and fly density was determined by determining the distance between two neighbouring points and then calculating the fly numbers between the points based on a linear relationship. The joining of these points resulted in area density charts being drawn.

Figure 3.5.9.2 shows the fly distribution of fruit flies regardless of species and sex. Before treatment the flies caught with Capilure indicated that they were concentrated in the Spinosad GF120 treated orchard (Week T-1). In contrast Questlure trap catches demonstrated high number of flies in all the orchards with a “hot” spot in the M3 bait station treated orchard. After 7 treatments (week 6) few flies were trapped with Capilure attractant in the Spinosad GF 120 treated orchard. Data from Questlure traps indicated the presence of a source of fruit fly on the boundary of the Spinosad GF 120 orchard, a guava tree with ripe fruit. It was somewhat surprising that the Capilure traps did not reflect this threat. By week 8 and 9 the Capilure traps indicated that the flies were widely distributed over the experimental area, a result not borne out by the Questlure trap fruit fly distribution determination. The question then arose of whether these different patterns could be explained by the different attractants being favoured by any particular species.



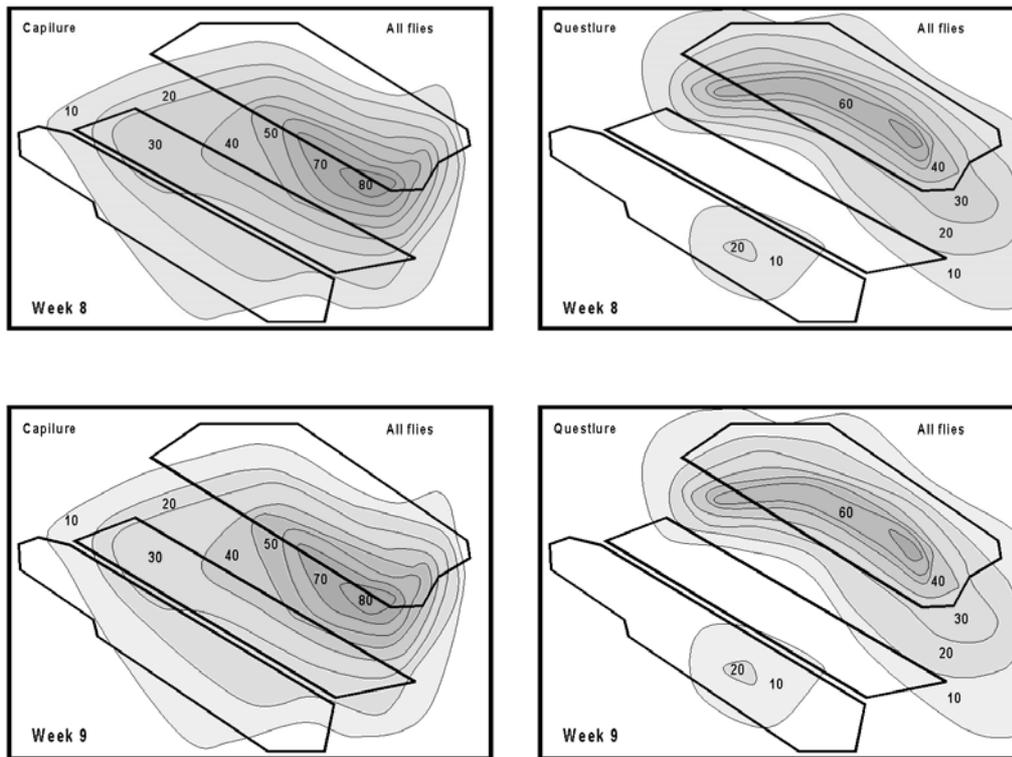
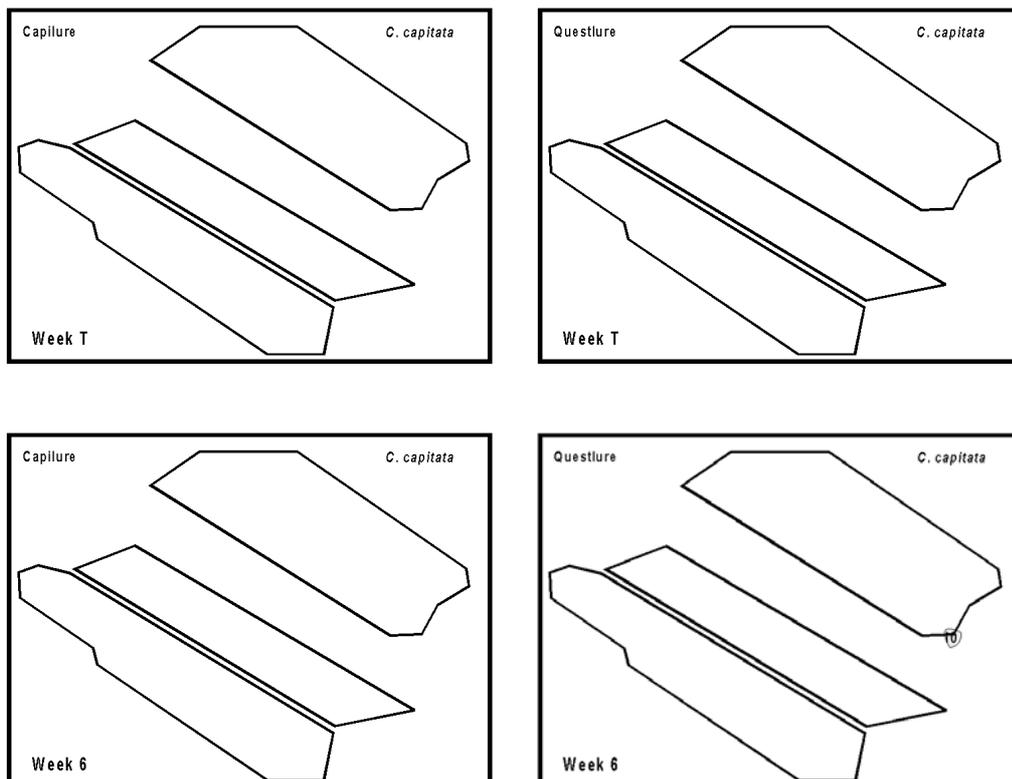


Figure 3.5.9.2. Density distribution of fruit flies caught in Sensus traps baited with either Capilure or Questlure attractants. Unshaded areas indicate areas in which less than 10 flies were caught.

The distribution of *C. capitata* as reflected by Capilure and Questlure trap catches is shown in Figure 3.5.9.3. Few specimens were caught before spraying was terminated (T+6). Thereafter there was a weekly increase in the numbers caught although the number caught, in the Questlure traps was consistently lower. These results implied that Capilure was the more effective trap for this species.



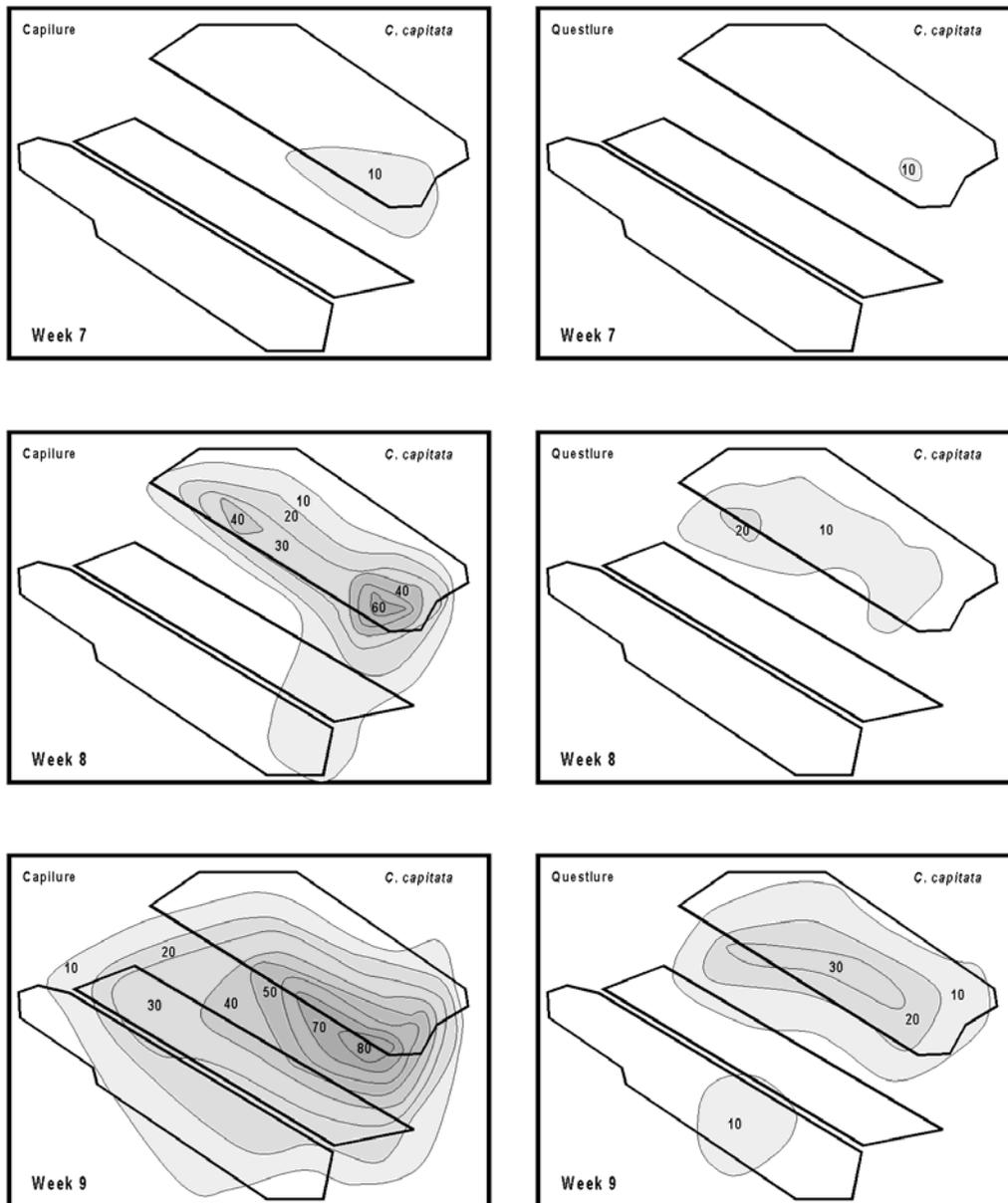
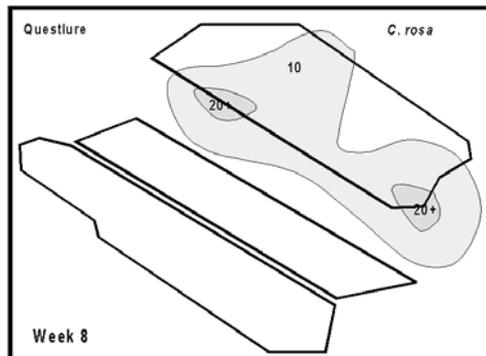
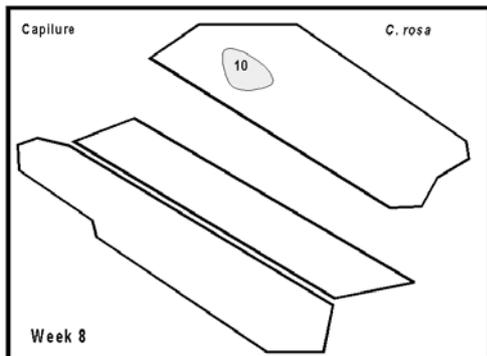
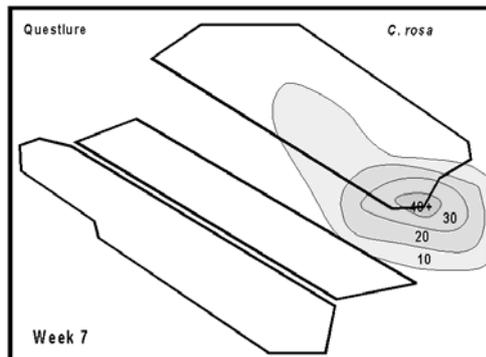
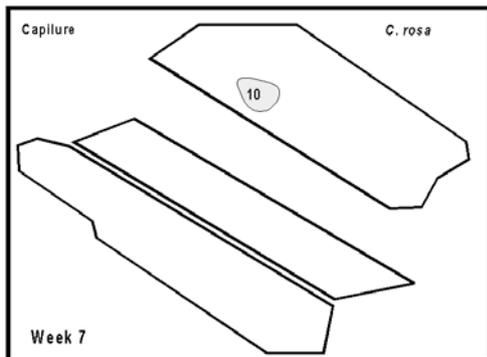
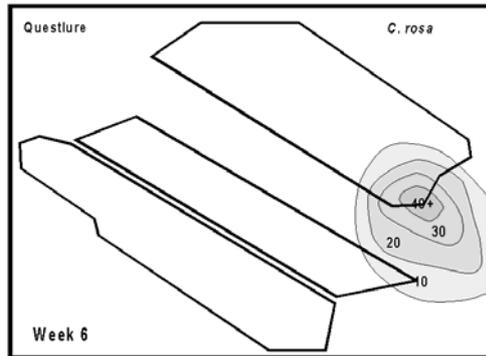
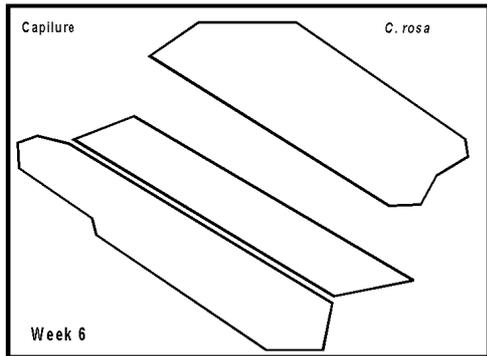
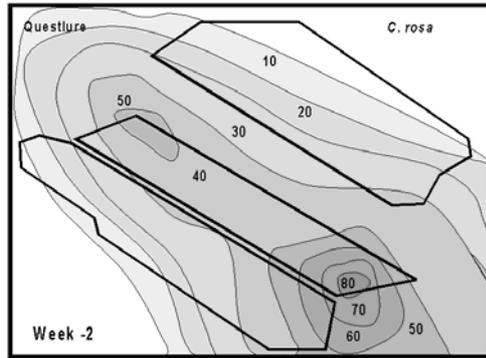
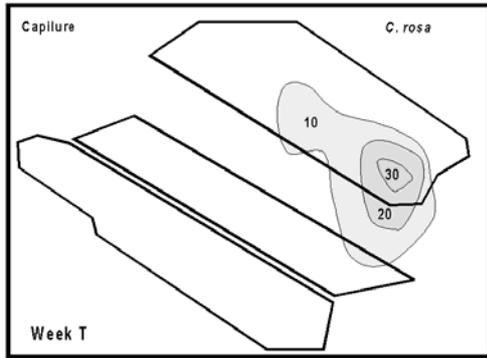


Figure 3.5.9.3. Density distribution of *C. capitata* fruit flies caught in Sensus traps baited with either Capilure or Questlure attractants. Unshaded areas indicate areas in which less than 10 flies were caught.

The *C. rosa* fruit fly trap catches are illustrated in Figure 3.5.9.4. In contrast with *C. capitata*, the most catches of this species were made prior to treatment regardless of the attractant used. However, Questlure outperformed Capilure indicating that the former was the superior attractant for this species. *C. rosa* was the predominant species from the guava tree (T6 and T7). The lack of fruit fly caught in the Capilure trap did not reflect the seriousness of *C. rosa* numbers in those orchards.



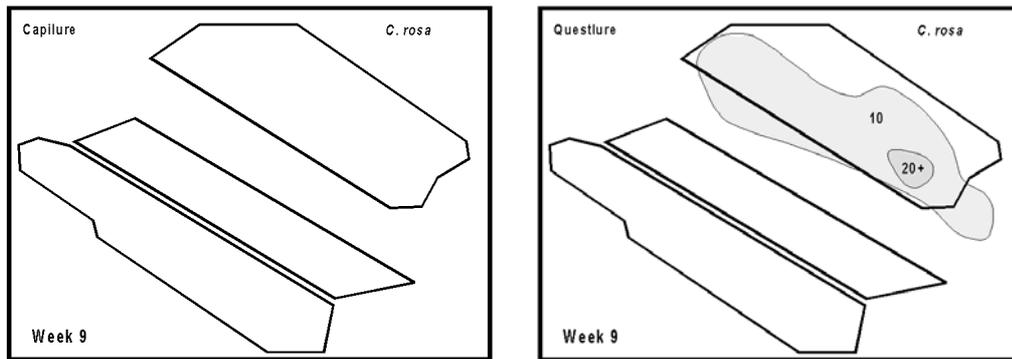


Figure 3.5.9.4. Density distribution of *C. rosa* fruit flies caught in Sensus traps baited with either Capilure or Questlure attractants. Unshaded areas indicate areas in which less than 10 flies were caught.

The status of *C. cosyra* as a pest of citrus is still under scrutiny. It has been established that Capilure does not attract *C. cosyra* (White and Elson-Harris, 1992). Trap catches in these orchards have confirmed the unsuitability of Capilure as a monitoring tool for this species. On the other hand Questlure traps attracted a number of specimens (Figure 3.5.9.5). As in the case of *C. capitata*, no *C. cosyra* were caught in the beginning of the trial while the populations appeared to increase later in the season (probably a direct result of flies emerging from the guava tree).

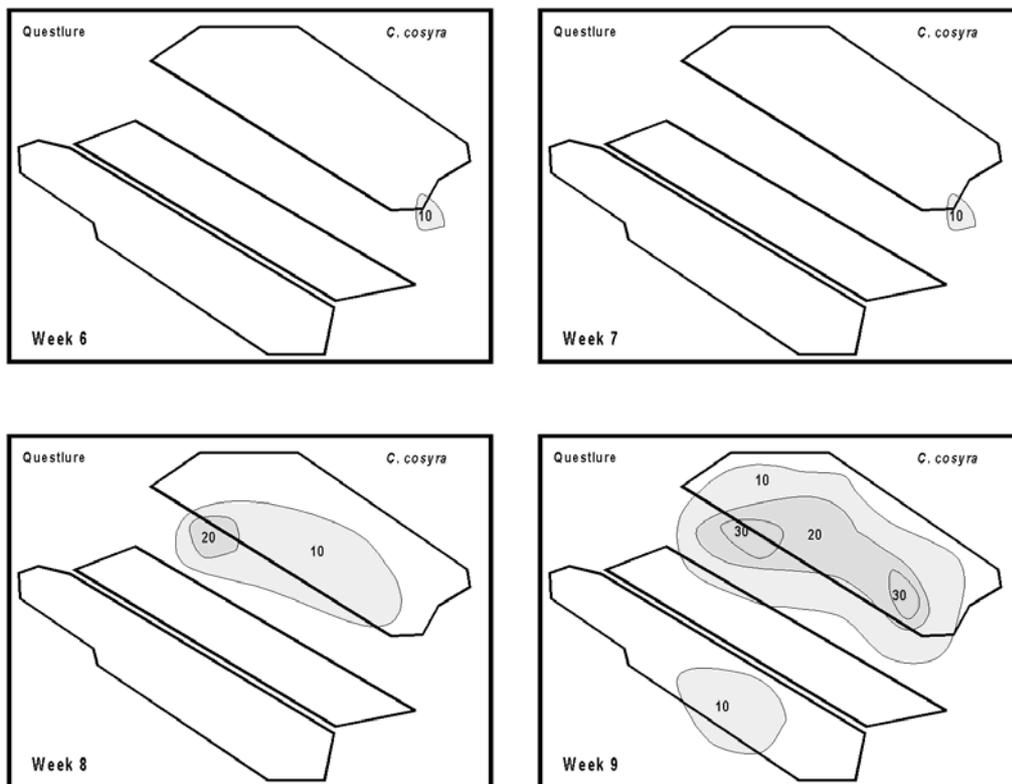


Figure 3.5.9.5. Density distribution of *C. cosyra* fruit flies caught in Sensus traps baited with either Capilure or Questlure attractants. Unshaded areas indicate areas in which less than 10 flies were caught.

Recent research has demonstrated that male and female *C. capitata* can exhibit different spatial dispersion patterns (Papadopoulos *et al.*, 2003). An examination of the Questlure data (T+9) (Figure 3.5.9.6) did not demonstrate any spatial differences in the two sexes. Long term data gathered after moving the position of Sensus/Questlure traps weekly led to the establishment of a females:males of 70:30 for *C. capitata*, *C. rosa* and *C. cosyra* (Ware, unpublished).

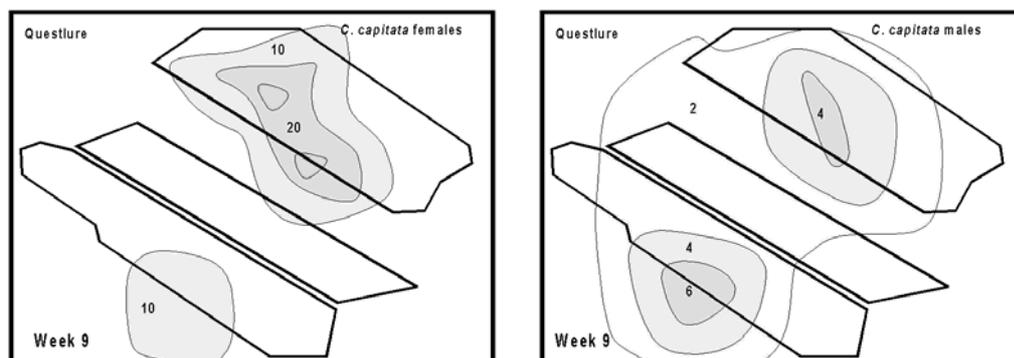


Figure 3.5.9.6. Density distribution of *C. capitata* fruit flies caught in Sensus traps baited with Questlure attractant. Unshaded areas indicate areas in which less than 10 female flies or less than 2 male flies were caught.

However, *C. rosa* males were found to concentrate at the edge of the orchards with the females distributed between (Figure 3.5.9.7). The reason for this behaviour is unknown but these results do help to explain some of the variability in trap catches within small areas.

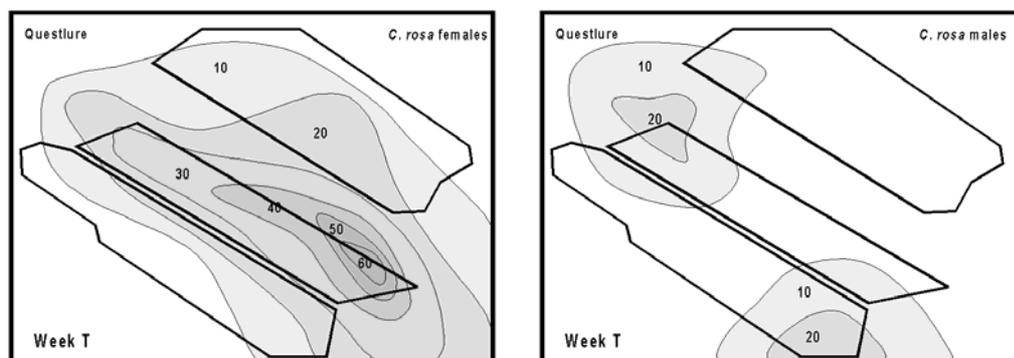


Figure 3.5.9.7. Density distribution of *C. rosa* fruit flies caught in Sensus traps baited with Questlure attractant. Unshaded areas indicate area in which less than 10 flies were caught.

These data confirm the patchy distribution of flies within any particular area over any particular time period (Bateman, 1972) and generally indicate that flies arise from or congregate in “hot” spots (Dimou *et al.*, 2003). There is a definite indication that *C. rosa* is more prevalent early in the season while *C. capitata* was all but absent. This pattern changed over the eight week observation period when the numbers of *C. capitata* increased, displacing the *C. rosa* at the end of the observation period. It is unlikely that this is competition between species but it may be related to environmental conditions. *C. rosa* is thought to prefer more humid conditions.

References cited

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- Dimou, I., Koutsikopoulos, C., Econompoulos, A. and Lykakis, J. 2003. The distribution of olive fruit fly captures with McPhail traps within an olive orchard. *Phytoparasitica* 3; 124-131.
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3.5.10 Attractant combinations for Sensus trap fruit fly monitoring

Experiment by Tony Ware and John-Henry Daneel (CRI)

Opsomming

Questlure is 'n swak lokmiddel vir vrugtevlieë maar het die voordeel bo Capilure dat dit wyfies en maroela vrugtevlieg lok. Die vraag het dus ontstaan of die twee lokmiddels gekombineer kan word in een wat algemeen

aanvaarbaar is. Resultate het getoon was dat 'n kombinasie van die twee middels antagonisties was teen Mediterreense vrugtevliegwyfies, maar 'n doeltreffende lokmiddel mag wees vir Natalse vrugtevliegmannetjies. Die mengsel was ondoeltreffend teen maroela vrugtevlieg.

Introduction

The knowledge of fruit fly populations is essential if they are to be controlled effectively. Traditionally, Capilure has been used to monitor fruit fly and, based on the number of flies trapped, decide when to initiate control measures, usually through baiting. Unfortunately, Capilure does not attract female Mediterranean and Natal fruit fly nor does it attract marula fruit fly. Recently a female lure (Questlure) has been developed that also attracts marula fruit fly. However, this lure attracts fewer flies and growers have expressed concern that it does not work. In an attempt to improve the number of flies caught and produce a trap that could be used universally, it was decided to combine Capilure and Questlure in the same trap. This paper reports on the results of this experiment.

Materials and Methods

Six Sensus traps containing Capilure and a further six traps containing Questlure were placed in an untreated mixed variety orchard at the Lowveld Agricultural College near Nelspruit. Another six traps containing both attractants were employed. The traps were made by dividing the capsule containing the attractant in half and placing one half of each into the traps. These traps were designated as the combined traps. Two traps containing a mixture (liquids mixed in equal quantities and placed on capsule felt) of Capilure and Questlure and two McPhail traps containing Questlure were also used.

The trap positions were rotated and the traps emptied weekly. All flies caught were taken to the laboratory where they were sexed and identified to species level. The trial ran over 5 weeks beginning 24 July 2003.

Results and discussion

A total of 4599 flies were caught over the trial period. The relative proportions of the species trapped were *C. capitata* (85.0%), *C. rosa* (9.3%), *C. cosyra* (4.6%) and *C. pedestris* (1.1%). The number of flies trapped using Capilure and Questlure in Sensus traps was similar. The other traps attracted fewer flies on average (Figure 3.5.10.1).

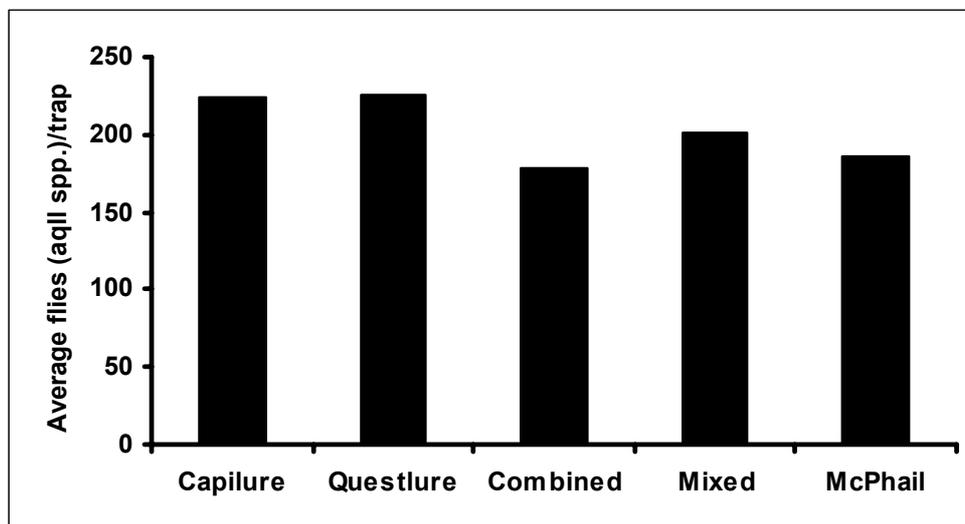


Figure 3.5.10.1. The average number of fruit flies of all species trapped in Sensus traps containing Capilure or Questlure or a combination (see text) or mixture of these two attractants. The McPhail traps containing Questlure.

Capilure attracted the highest number of male *C. capitata* over the trial period (Figure 3.5.10.2). It appears that when this attractant is mixed with Questlure it becomes less attractive. However, this result may be because half the volume of each attractant was used in the combined and mixed formulations. The type of trap did not make a difference to the number of male flies caught.

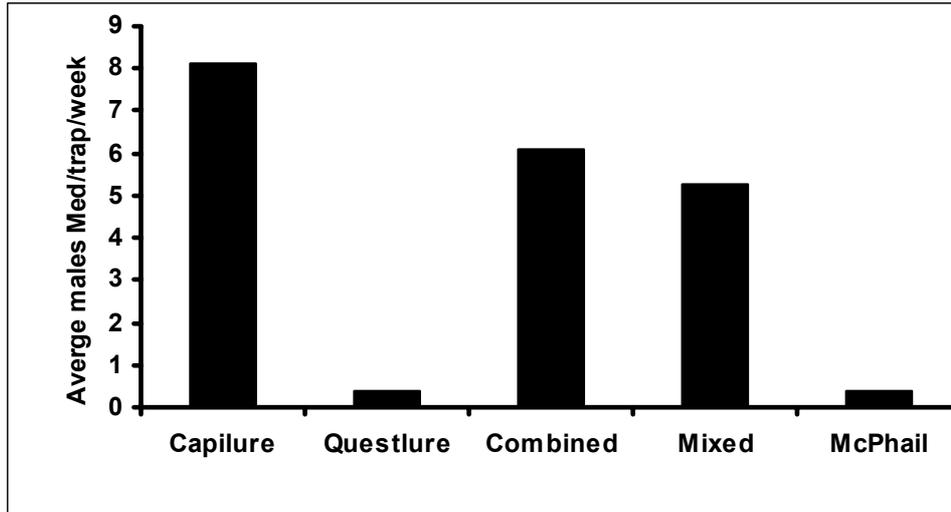


Figure 3.5.10.2. The average number of *C. capitata* males trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

The average number of female *C. capitata* trapped was highest in the two traps containing only Questlure (Figure 3.5.10.3). Capilure did not attract female *C. capitata*. However, when this attractant was mixed with Capilure it apparently either repelled the females or in some way masked the attractiveness of the lure. A similar result was obtained by Tóth *et al.* (2004) using trimedlure and Three-component lure.

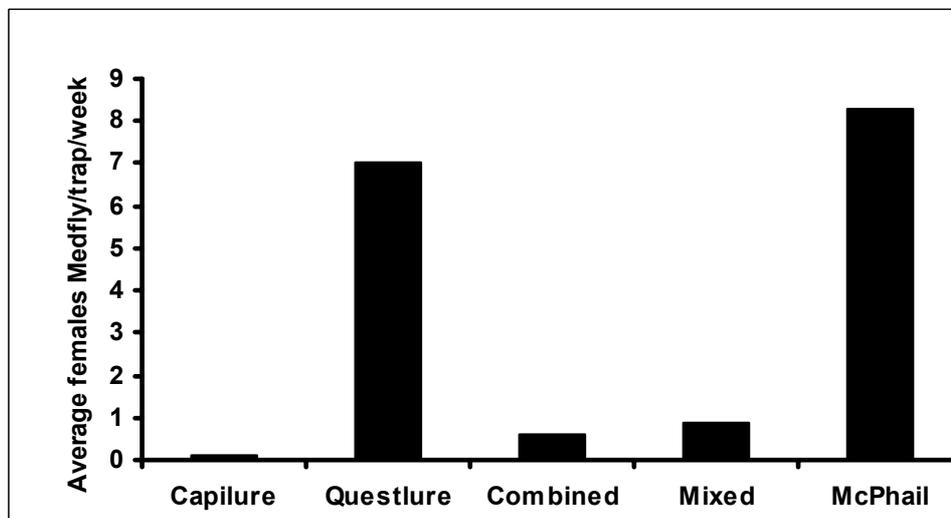


Figure 3.5.10.3. The average number of *C. capitata* females trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

When sex of *C. capitata* is taken out of the equation, there is apparently little to choose between any of the trap/attractant combinations tested (Figure 3.5.10.4) - less than one fly/trap/week was the difference.

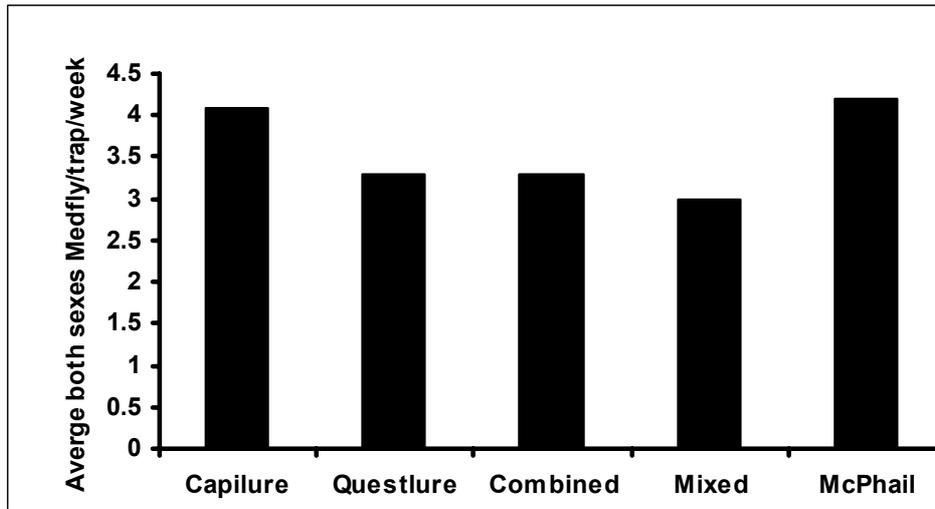


Figure 3.5.10.4. The average number of *C. capitata* of both sexes trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

In contrast with *C. capitata*, relatively few *C. rosa* males were attracted to the traps containing Capilure as compared with traps containing Questlure (Figure 3.5.10.5). In this case the addition of Capilure to Questlure appeared to have a synergistic effect with the mixture being most effective. In the case of Natal fruit fly the McPhail trap appears to be slightly more effective.

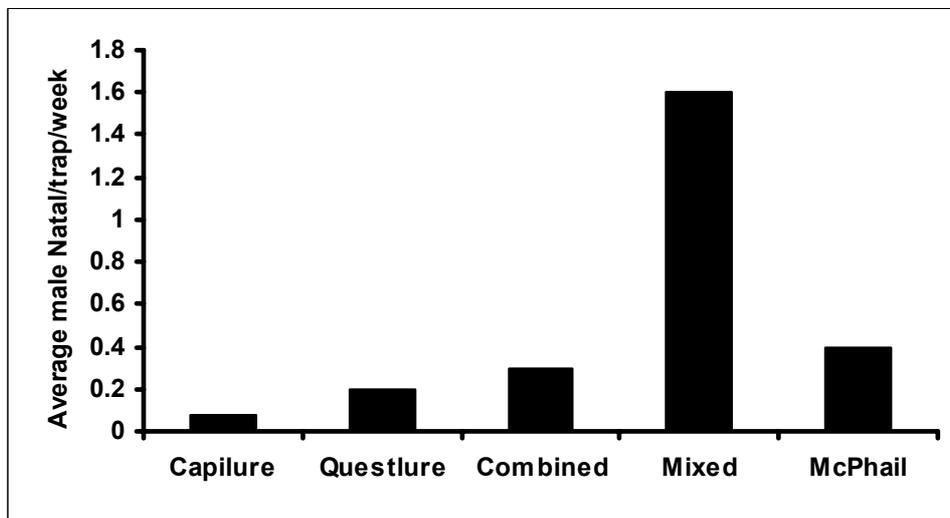


Figure 3.5.10.5. The average number of *C. rosa* males trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

In the case of *C. rosa* females, the addition of Capilure appears to be antagonistic (Figure 3.5.10.6). As with male *C. rosa*, female *C. rosa* appear to be attracted in greater numbers to McPhail traps than Sensus traps.

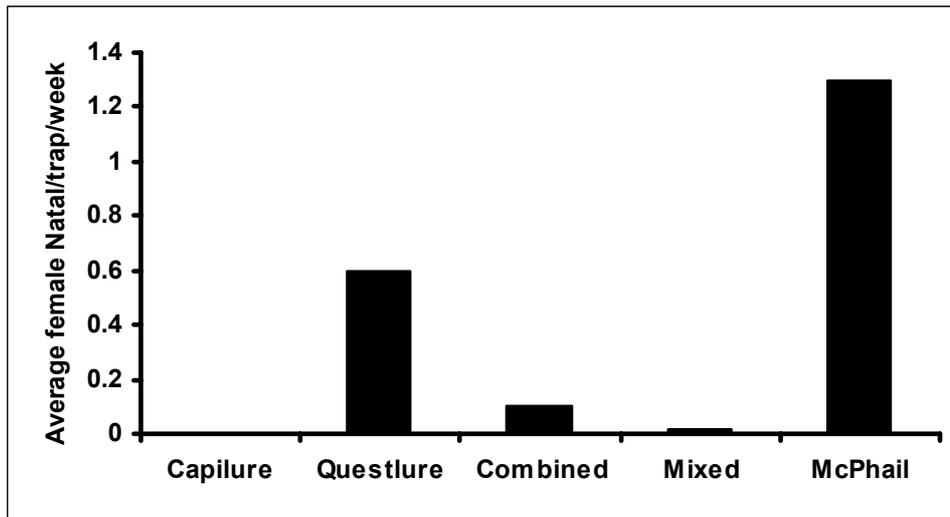


Figure 3.5.10.6. The average number of *C. rosa* females trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

C. rosa found the Sensus trap with both Capilure and Questlure less attractive than the individual components (Figure 3.5.10.7). However, if the components were mixed then the results indicate that it is twice as effective. The McPhail trap again proved to be effective.

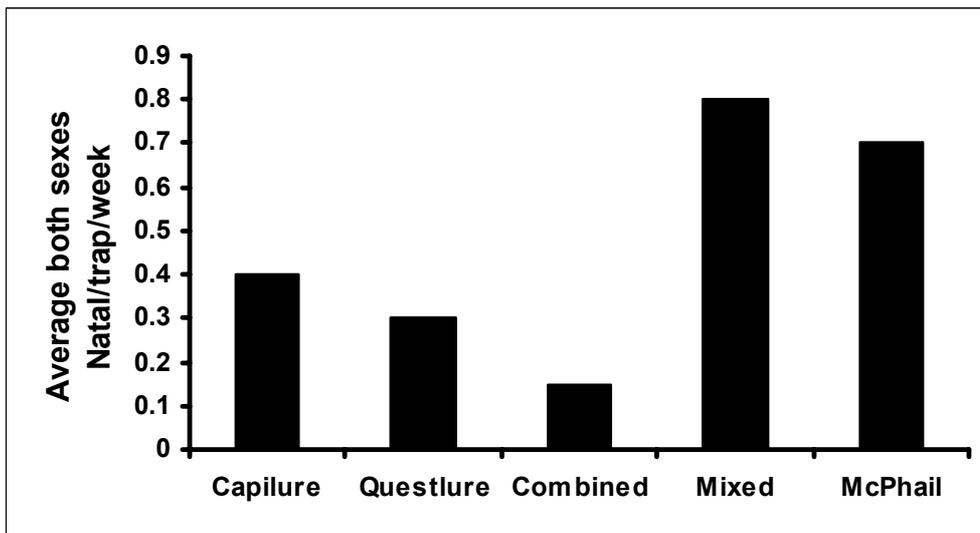


Figure 3.5.10.7. The average number of *C. rosa* (both sexes) trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

Male *C. cosyra* are not attracted to Capilure and may even be repelled by this parapheromone (Figure 3.5.10.8). Questlure was found to be an effective attractant. Questlure placed in a McPhail trap appeared to be the most effective combination for the males of this species.

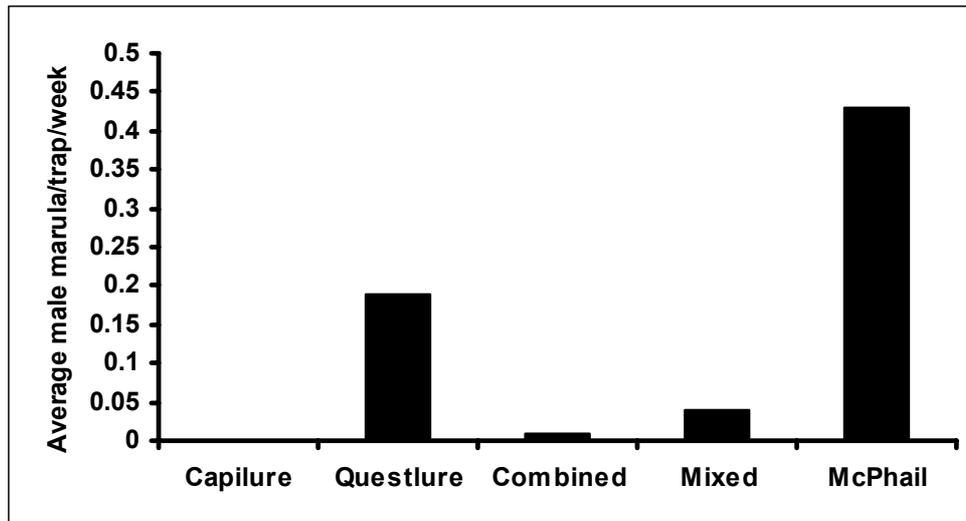


Figure 3.5.10.8. The average number of *C. cosyra* males trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

The pattern of trap catches for *C. cosyra* females (Figure 3.5.10.9) mimics that of the males (Figure 3.5.10.8) but at a higher level. The premise that Questlure is predominantly a female fruit fly attractant is again borne out in these results.

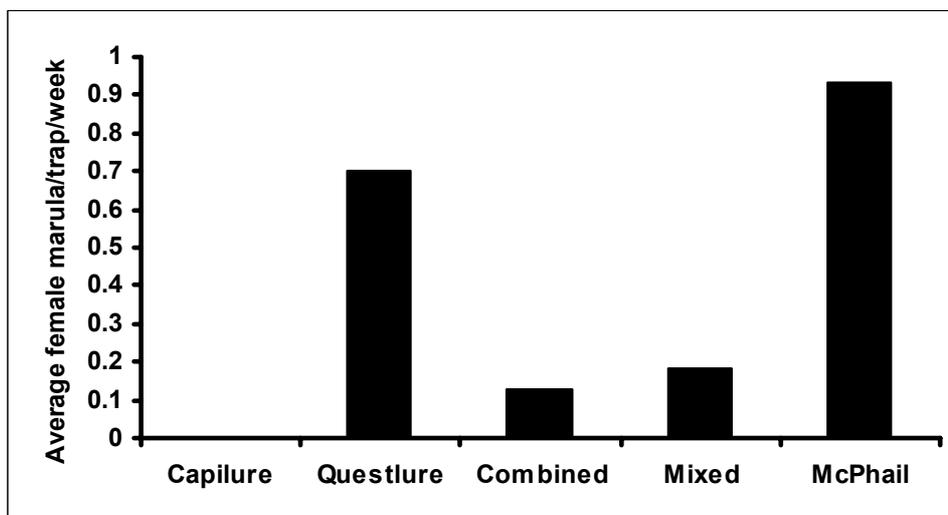


Figure 3.5.10.9. The average number of *C. cosyra* females trapped per week in Sensus traps containing Capilure, Questlure, a combination of these two attractants or a mixture of these attractants or McPhail traps containing Questlure.

Conclusions

1. Capilure is an efficient attractant for male *C. capitata*.
2. The addition of Capilure to Questlure in Sensus traps appears to be antagonistic to female *C. capitata*.
3. Questlure appears to be more efficient than Capilure as a male *C. rosa* attractant.
4. A mixture of Questlure and Capilure needs to be investigated further.
5. *C. rosa* females apparently find Capilure repellent.
6. Capilure does not attract *C. cosyra* and may even be repellent.
7. The McPhail trap appears to be more efficient than the Sensus trap. Perhaps it is the yellow colour although previous research has indicated that colour does not play a

significant attractive role (Ware & Joubert, 2001).

Future research

1. Investigate whether Capilure/Questlure mixture can be used as a *C. rosa* attractant
2. Investigate the reason why the McPhail trap is more efficient than the Sensus trap. Also investigate using the Tephri trap.

Reference cited

- Tóth, M., Nobili, P., Tabilio, R and Ujváry, I. 2004. Interference between male-targeted and female-targeted lures of the Mediterranean fruit fly *Ceratitis capitata* (Dipt., Tephritidae) in Italy. *Journal of Applied Entomology* 128; in press.
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3.5.11 Organic M3 bait station

Experiment by Tony Ware and John-Henry Daneel (CRI)

Opsomming

Die steeds-toenemende neiging om organies te boer skep 'n vraag na 'n organies-aanvaarbare metode van vrugtevliegbeheer. Met dié doel voor oë is die plaag- en swamdoderkomponente verwyder uit die M3 lokaasstasie en 'n kleefband aangebring om alle vrugtevlieë wat na die stasie gelok word te dood. Die organiese lokval het net effens minder vlieë gevang as die vervaardigde produk en het potensiaal om verder ontwikkel te word.

Introduction

There is a general trend towards organic farming and fruit produced through this method of farming have a ready niche market both in the overseas and local markets. Generally this type of farming is considered environmentally friendly and is considered the ultimate IPM tool. However, the production of good quality fruit using such organically accredited methods is a challenge as many remedies used in traditional farming practices are not permitted. The M3 bait station, although a good example of an IPM compatible product, is not permitted because of the pesticide and fungicide it contains and even transport through an organically accredited orchard is not allowed. This experiment was designed to see whether the M3 bait station could be modified in such a way as to be acceptable to the organic farmers.

Materials and methods

The M3 bait stations were modified. The fungicide and pesticide were removed from the formulation leaving only the attractive component. These consisted of protein hydrolysate and some plant extracts. In order to trap insects a sticky trap similar to that described by Georgala and Stephen (1987) was attached to each trap (Figure 3.5.11.1). A plastic pipe measuring approximately 30 cm and a diameter of 4 cm was attached to the base of the M3 bait station. The pipe was then covered with GLADWRAP and painted with FlyTack. The Gladwrap was replaced weekly. The old Gladwrap was taken to the laboratory where all fruit flies trapped were sexed and identified to species level. Sometimes the insects had to be removed from the sticky surface using a solvent in order to be accurately identified.

Six organic M3 bait stations and six control traditional M3 bait stations (also with sticky stick traps attached) were then hung in an untreated mixed variety orchard situated at the Lowveld Agricultural College near Nelspruit in the Mpumalanga Province of South Africa. The traps were rotated weekly with an organic bait station being replaced by a traditional bait station and *visa versa*. The trial was initiated on 27 February 2003 and was terminated 13 weeks later.

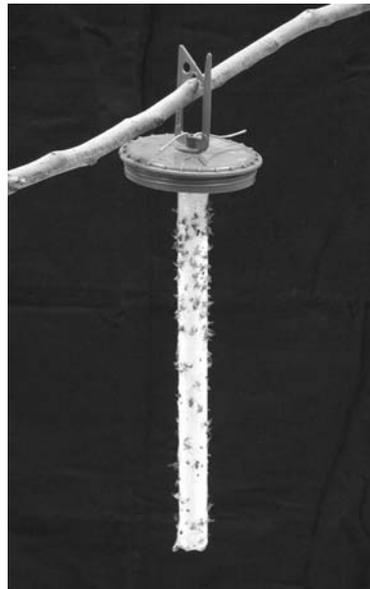


Figure 3.5.11.1. Modified M3 bait station

Results and discussion

Small numbers of Natal fruit fly were trapped over the trial period (Figure 3.5.11.2) and large numbers of Mediterranean fruit fly and marula fruit fly. The few *Dacus* sp. that were caught are described as “other”. These were not sexually differentiated. In all cases the traditional traps caught more flies of each sex than the organic trap. However, because of the large differences in the trap catches none of these differences could be considered to be statistically significant (statistical testing was not considered necessary).

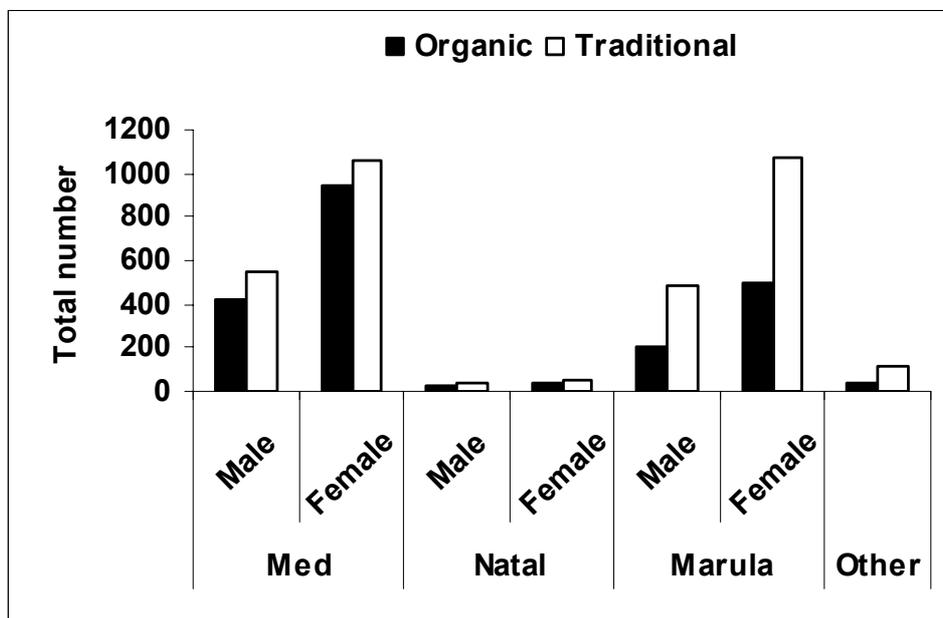


Figure 3.5.11.2. Fruit flies trapped on sticky stick traps using traditional M3 bait station and an organic M3 bait station (fungicide and pesticide removed)

Conclusion

There is potential to develop and market an organic M3 bait station. The cost of such a registration and return on investment would probably not warrant such input. The removal of the fungicide would probably result in loss of attraction because of consequent fungal growth and the attractive component (sponge) would have to be replaced regularly.

The adhesive would have to be replaced regularly as dust and dirt would render it ineffective.

Future research

No further research is planned.

Reference cited

Georgala, M.B. and Stephen, P.R. 1987. South African Co-op Citrus Exchange research report for the years 1985-1987.

3.5.12 M3 Product development

Opsomming

Dit is onvermydelik dat enige produk wat bekend gestel word, verdere verfyning, ontwikkeling en tegniese ondersteuning sal verg. Die verslag hieronder sluit 'n ondersoek in na produkklagtes en het aanleiding gegee tot navorsing oor die vervanging van die swamdoder in die M3 lokaasstasie. Kaptafol was 'n geskikte alternatief vir Folpan, maar koperoksichloried, wat organies meer aanvaarbaar is, het nie swamgroei onderdruk nie. Twee produkklagtes is ondersoek. Een het gehandel oor swamgroei en gelei tot die navorsing hierbo. Die tweede klagte dat die M3 lokaasstasie bye lok en dood, kon nie bevestig word nie en is verwerp.

Summary

It is inevitable that any product launched will require refining, further development and technical support. The report includes investigation of product complaints and led to research into a replacement fungicide in the M3 bait station. Captafol was found to be a suitable replacement for Folpan but copper oxychloride, a more organically acceptable alternative, did not prevent fungal growth. Two product complaints were investigated. The first one concerned fungal growth and this led to the research described above. The second complaint that the M3 bait station attracted and killed bees was unsubstantiated and was rejected.

3.5.13 Replacement fungicide for Folpan in the M3 bait station

Experiment by Tony Ware, Laura Huisman and John-Henry Daneel (CRI)

Opsomming

Boordtoestande het getoon dat die swamdoder wat tans gebruik word in die M3 voortydig afbreek. Kaptafol en koperoksichloried is getoets as plaasvervangers. Kaptafol het 'n langer nawerking gehad as beide Folpan en koperoksichloried. Daar word aanbeveel dat Kaptafol Folpan in die toekoms moet vervang.

Introduction

Folpan is currently used in the M3 bait station to prevent the growth of fungi that occurs under some environmental conditions. The growth inactivates the bait station by utilizing the protein hydrolysate. The bait station is designed to last 4 months but it is suspected that the fungicide is not effective for this length of time and a more long lasting candidate was desirable. Captafol was identified as a suitable candidate but it is necessary to demonstrate that its addition to the bait did not repel fruit flies and was able to prevent fungal growth over a protracted period. This research was designed to answer these questions.

Materials and methods

1. Does Captafol repel fruit flies?
M3 bait stations were provided by Quest Developments CC with either Folpan or Captafol. The bait stations were modified by the addition of sticky traps (see Organic Bait stations section 3.5.11) and three traps of each formulation were hung out in an untreated mixed variety orchard situated at the Lowveld Agricultural College near Nelspruit. The traps were left out for one week in June 2003 and then brought back to the laboratory where all the flies trapped were identified and sexed.
2. How effective is Captafol at preventing *Cladosporium* growth?
Ninety blank M3 bait stations were treated as follows: 15 using copper oxychloride (2 g/l); 15 using copper oxychloride (4 g/l); 15 using Captafol (5 ml/l); 15 using Captafol (10 ml/l); 15 using Folpan (2 ml/l) and 15 using Folpan (4 ml/l). Five bait stations were placed at 24, 30 and 40°C. A single bait

station was removed after 2, 4, 8, 12 and 16 weeks. A disc was cut from each bait station and placed on potato agar in a petridish inoculated with *Cladosporium*. The zone on inhibition was measured at convenient intervals.

Results and discussion

1. There was no obvious repellent effect using Captafol (Table 3.5.13.1). This finding resulted in the testing of the chemical against the fungus.

Table 3.5.13.1. Number of fruit flies caught in modified M3 bait station using Folpan or Captafol as the fungicide.

Species	Sex	Folpan	Captafol
C. capitata	Male	23	23
	Female	53	92
C. rosa	Male	3	0
	Female	1	1

2. The copper treatment did not inhibit the growth of *Cladosporium*. The results obtained using Folpan were ambiguous in that the higher dosage produced more negative results than the lower dosage (Table 3.5.13.2). Captafol (10 ml/l) appeared to be the best treatment and apparently can withstand high temperatures for extended periods.

Table 3.5.13.2. Zones of inhibited growth after 40 days on *Cladosporium*-inoculated potato agar (X = no growth).

Temperature (°C)	Time (weeks)	Captafol (5 ml/l)	Captafol (10ml/l)	Folpan (2 ml/l)	Folpan (4 ml/l)
24	2	√	√	X	√
	4	√	√	√	√
	6	X	√	√	√
	8	√	√	√	X
	12	√	√	√	√
	16	√	√	√	√
30	2	√	√	√	X
	4	√	√	√	√
	6	√	√	√	X
	8	√	√	√	X
	12	√	√	√	X
	16	√	√	√	X
40	2	√	√	√	X
	4	√	√	√	X
	6	√	√	X	X
	8	√	√	√	X
	12	X	√	X	X
	16	√	√	√	X

Conclusions and recommendations

1. Captafol does not repel fruit flies.
2. Captafol can withstand high temperatures (40°C) for extended periods without loss of efficacy.
3. Captafol should be considered as a replacement for Folpan in the M3 bait station.

3.5.14 Maputo Corridor Survey of Fruit Flies

Experiment 727 by Tony Ware and John-Henry Daneel (CRI)

Opsomming

'n Opname is gemaak van vrugtevlieë in die gebiede Nelspruit, Malelane, Komatipoort, Swaziland en Maputo met behulp van Sensus lokvalle bevattende metiel-eugenol, Cuelure en Questlure. Identifisering van die vlieë

word tans onderneem en gedetailleerde resultate sal in die volgende jaarverslag voorgelê word. John-Henry Daneel het 'n kursus bygewoon oor die identifisering van Tephritidae wat aangebied is deur Mervyn Mansell van die Nasionale Versameling van Insekte.

Summary

Fruit fly in the Nelspruit, Malelane, Komatipoort, Swaziland and Maputo areas have been surveyed using Sensus traps containing methyl eugenol, Cuelure and Questlure. Identification of the flies is currently being undertaken and will be detailed in the next annual report. John-Henry Daneel attended a course on the identification of Tephritidae conducted by Mervyn Mansell of the National Collection of Insects.

3.6 PROJECT: MEALYBUG AND OTHER PHYTOSANITARY PESTS

Project Co-ordinator: Sean Moore (CRI)

3.6.1 Project summary

Five experiments were conducted under this project during 2003. In the first, an identification guide consisting of descriptions and illustrations of 6 of the 7 mealybug species occurring on citrus, was completed (3.6.2). This report is a summarised version of the full document. The full document, which has been submitted for publication, should make it possible to identify any stage of any mealybug species found on export fruit. In the second experiment, the suitability of 4 mealybug species as hosts for the parasitoid, *Coccidoxenoides perminutus (peregrinus)* was tested (3.6.3). *Planococcus citri* was found to be the favoured host. In the third experiment a survey conducted in Eastern Cape citrus orchards revealed that *Paracoccus burnerae* is the dominant mealybug species in 79% of orchards (3.6.4). *Leptomastix* sp. was found to be the dominant parasitoid species attacking *P. burnerae*. No work was conducted on the fourth experiment, which was to investigate the grain chinch bug, *Macchiademus diplopterus*, as a phytosanitary pest (3.6.5). This was because no insects were available for the study. Work on the fifth experiment did also not progress very far. In this experiment the disinfestation of mealybug infested packed citrus fruits by gamma irradiation was to be investigated (3.6.6). Attempts to rear *P. burnerae* for the study were unsuccessful. This work will be conducted during the following season.

Projekopsomming

Vyf eksperimente is onder hierdie projek gedurende 2003 uitgevoer. In die eerste een is 'n identifikasiegids met beskrywings en tekeninge van 6 van die 7 witluis spesies wat op sitrus voorkom, voltooi (3.6.2). Die verslag is 'n opsomming van die volle opskrif, wat vir publikasie ingedien is. Die gids sal die identifikasie van enige stadium van enige witluis spesie wat op uitvoer vrugte gevind is, moontlik maak. In die tweede eksperiment is die geskiktheid van 4 witluis spesies as gashere vir die parasiet, *Coccidoxenoides perminutus (peregrinus)* getoets (3.6.3). Dit is gevind dat *Planococcus citri* die voorkeur gasheer is. In die derede eksperiment het 'n opname wat in die Oos Kaap uitgevoer is gewys dat *Paracoccus burnerae* die dominante witluis spesie in 79% van boorde is (3.6.4). *Leptomastix* sp. is die dominante parasiet spesie op *P. burnerae*. Geen werk is op die vierde eksperiment uitgevoer nie. In hierdie eksperiment sou die graanstinkbesie, *Macchiademus diplopterus*, as 'n fitosanitêre plaag bestudeer word (3.6.5). Werk is gestaak omdat geen insekte vir die studie beskikbaar is nie. Die vyfde eksperiment is ook nie suksesvol uitgevoer nie. In hierdie eksperiment sou die disinfestasië van verpakte witluisbesmette sitrusvrugte met gammabestraling ondersoek word (3.6.6). Pogings om *P. burnerae* vir die studie te teel is onsuksesvol. Hierdie werk sal gedurende die komende seisoen voortgesit word.

3.6.2 Descriptions of the adults and immature females of six South African mealybug species (Hemiptera: Pseudococcidae) found on citrus

Experiment USE1-02 by Waktola M. Wakgari and Jan H. Giliomee (University of Stellenbosch)

Opsomming

Omdat sekere witluis spesies as fitosanitêre plaë beskou word, kan hulle teenwoordigheid op vrugte of die teenwoordigheid van witluis wat nie geïdentifiseer kan word nie, na afkeuring van vrugte vir uitvoer lei. Dit is belangrik dat dit moontlik moet wees om al die stadia van enige witluis wat op uitvoer vrugte gevind word te kan identifiseer. Ons het nou die beskrywings en tekeninge van 6 van die 7 witluissoorte wat al op sitrus gevind is, voltooi. Hierdie lang dokument is vir publikasie ingedien en as 'n verslag gee ons hier net die sleutels wat deel vorm van die manuskrip. Geen verdere werk word op hierdie eksperiment beplan nie.

Introduction

Mealybugs are important pests on export fruit. Some species are endemic (found only in our region) and importing countries reject consignments of fruit carrying these species. Since it has not been possible to identify immature mealybugs, even of the cosmopolitan and therefore "acceptable" species, their presence has led to the rejection of fruit. The object of this project was to describe and illustrate all stages of all species found on citrus.

Results

We were able to obtain and breed six of the seven species of mealybugs that have been recorded from citrus, i.e. the citrus mealybug *Planococcus citri*, the citrophilous mealybug *Pseudococcus calceolariae*, the oleander mealybug *Paracoccus burnerae*, the striped mealybug *Ferrisia virgata*, the spherical mealybug *Nipaecoccus viridis* and the longtailed mealybug *Pseudococcus longispinus*. We have been unable to find one species which rarely occurs on citrus, namely *Delottococcus elizabethae*.

All the stages of most of the species can now be separated using the keys below, together with the descriptions and illustrations. The descriptions and illustrations appear in the manuscript we have submitted for publication.

Key to immature instars and adult females of six mealybug species

1. Antennae 6-segmented 2
- Antennae 7-segmented 7
- 2 (1). Anal ring $\leq 32\mu\text{m}$ wide; a pair of conical or lanceolate setae only on anal lobe cerarius..... 3
- Anal ring $38\text{--}43\mu\text{m}$ wide; a pair of conical or flagellate setae on at least three posterior abdominal cerarii..... 8
- 3 (2). Anal lobe bar present 4
- Anal lobe bar absent..... 6
- 4 (3). Discoidal pores present first-instar nymph *Ferrisia virgata* and *Paracoccus burnerae*
- Discoidal pores absent 5
- 5 (4). Both dorsal and ventral setae flagellate; circulus $\leq 35\mu\text{m}$ wide and $\leq 25\mu\text{m}$ long; 2-3 sub-apical setae present near each apical seta first-instar nymph *Planococcus citri*
- Dorsal setae conical; ventral setae flagellate; circulus $\geq 55\mu\text{m}$ wide and $\geq 40\mu\text{m}$ long; 1-2 sub-apical setae near each apical seta first-instar nymph *Nipaecoccus viridis*
- 6(3). Only one sub-apical seta present next to each apical seta; circulus about $63\mu\text{m}$ wide and $50\mu\text{m}$ long first-instar nymph *Pseudococcus calceolariae*
- Up to three sub-apical setae present next to each apical seta; circulus about $58\mu\text{m}$ wide and $37\mu\text{m}$ long first-instar nymph *Pseudococcus longispinus*
- 7 (1). Anal lobe cerarius not sclerotized 14
- Anal lobe cerarius sclerotized 17
- 8(2). Dorsal oral-collar tubular ducts present..... 9
- Dorsal oral-collar tubular ducts absent 10
- 9 (8). Anal lobe bar present second-instar female *Paracoccus burnerae*
- Anal lobe bar absent second-instar female *Pseudococcus calceolariae*
- 10 (8). Both dorsal and ventral setae flagellate or lanceolate; stout conical setae only on anal lobe cerarius 11
- Dorsal setae stout conical; ventral setae flagellate or lanceolate; setae on three posterior abdominal cerarii stout or flagellate 13
- 11 (10). About 28 dorsal slender tubules with 2-3 flagellate setae arising from the sclerotized orifice or rim encircling base of each tubule 12
- Dorsal slender tubules absent..... second-instar female *Planococcus citri*
- 12 (11). Only 1 auxiliary seta on anal lobe cerarius; no oral-collar tubular ducts on both body surfaces second-instar female *Ferrisia virgata*
- Six-8 auxiliary setae on anal lobe cerarius; many oral-collar tubular ducts at least on ventral sub-margin and margin..... 21
- 13 (10). Antennae $\leq 180\mu\text{m}$ long; ≤ 9 pairs of cerarii discernible; no auxiliary setae present on any cerarii second-instar female *Nipaecoccus viridis*
- Antennae $\geq 210\mu\text{m}$ long; ≥ 15 pairs of cerarii discernible; 2-3 auxiliary setae present on anal lobe cerarius..... 19
- 14 (7). A few oral-rim tubular ducts present on margin₂₁₄ and sub-margin of abdominal dorsum

.....	15
--- Oral-rim tubular ducts absent	16
15 (14) A few oral-collar tubular ducts present on both body surfacessecond-instar female <i>Pseudococcus longispinus</i>	
--- A few oral-collar tubular ducts present only on venter	18
16 (14) About 2-3 oral-collar tubular ducts present anterior to clypeolabral shield; 18 pairs of cerarii present third-instar female <i>Planococcus citri</i>	
--- Oral-collar tubular ducts absent; 17 pairs of cerarii present	
..... third-instar female <i>Paracoccus burnerae</i>	
17 (7). Oral-rim tubular ducts on marginal and sub-marginal of dorsum as well as on abdominal venter third-instar female <i>Pseudococcus longispinus</i>	
--- Oral-rim tubular ducts absent on either body surface	
..... third-instar female <i>Pseudococcus calceolariae</i>	
18 (15). Auxiliary setae present only on anal lobe, frontal and ocular cerarii; dorsal slender tubules absent	20
--- Auxiliary setae present only on anal lobe cerarius; many dorsal slender tubules present, each with 2- 4 flagellate setae surrounding it at its orifice	third-instar female <i>Ferrisia virgata</i>
19 (13). Anal lobe cerarius not sclerotized; discoidal pores absent on both body surfaces; multilocular disc pores absent; translucent pores absent on hind legs; anal ring about 55µm wide	third-instar female <i>Nipaeococcus viridis</i>
--- Anal lobe cerarius sclerotized; a few discoidal pores on anterior dorsal and ventral sub-margin; many multilocular disc pores on posterior abdominal venter and mid-thoracic area; translucent pores on hind leg coxa and tibia; anal ring about 106µm wide	adult female <i>Nipaeococcus viridis</i>
20 (18). Eighteen pairs of cerarii present; non-sclerotized; oral-rim tubular ducts absent; anal ring about 80µm wide	adult female <i>Planococcus citri</i>
--- Seventeen pairs of cerarii present; at least anal lobe cerarius sclerotized; oral-rim tubular ducts present on dorsum; anal ring about 74µm wide	adult female <i>Paracoccus burnerae</i>
21 (12). As many as 130 dorsal slender tubules present with 2-5 flagellate setae surrounding from orifice of each tubule; only a pair of anal lobe cerarius discernible, each with 6-8 auxiliary setae; anal ring about 132µm wide	adult female <i>Ferrisia virgata</i>
--- No dorsal slender tubules present; 17 pairs of cerarii, each with 4-8 auxiliary setae; anal ring < 132µm wide	22
22 (21). Only anal lobe cerarius sclerotized; a single oral-rim tubular duct next to most cerarii; 4-5 cisanal setae, each about 66µm long; apical setae about 211µm	adult female <i>Pseudococcus calceolariae</i>
--- Anal lobe and penultimate cerarii sclerotized; 2-3 oral-rim tubular ducts next to each cerarius; 4-8 cisanal setae, each about 50µm long; apical setae about 134µm long ..	adult female <i>Pseudococcus longispinus</i>

Conclusion

The project on describing and illustrating all stages of the mealybugs found on citrus has been completed and once the manuscript based on this work has been published in a scientific journal, importing countries would no longer be able to justify rejection of our citrus fruit on the grounds that it contains unknown and therefore potentially dangerous mealybug species. The only exception would be if specimens are found that do not fit the descriptions, but that is very unlikely.

Future research

Beyond publication of this work in a scientific journal, no further research is planned.

3.6.3 Evaluation of the host range of *Coccidoxenoides peregrinus* and the species composition of parasitoids associated with some mealybug species occurring in the Western Cape

Experiment USE2-02 by Waktola M. Wakgari and Jan H. Giliomee (Stellenbosch University)

Opsomming

Die doel van hierdie eksperiment was om te bepaal of *Coccidoxenoides peregrinus* spesifiek vir *Planococcus* spp. is of as dit ook teen ander ekonomies belangrike witluis spesies doeltreffend kon wees. Die gasheerreeks van die witluisparasitoïd is in die laboratorium ondersoek. Die parasitoïd het 'n betekenisvolle ($P < 0.001$) voorkeur vir 'n mengsel van individue bestaande uit eerste en tweede instars van *Planococcus citri* getoon bo *Planococcus burnerae*, *Pseudococcus calceolariae* en *Pseudococcus longispinus* in beide

keuse en nie-keuse toetse. Dit dui daarop dat *Planococcus citri* waarskynlik die natuurlike (voorkeur) gasheer van *C. peregrinus* is en slegs suksesvol vir die doeltreffende beheer van hierdie spesie in Suid-Afrikaanse sitrusboorde aangewend kan word. Geen verdere werk word op hierdie eksperiment beplan nie.

Introduction

Coccidoxenoides peregrinus (Timberlake) is an important commercially produced endo-parasitoid used in South Africa for the augmentative biocontrol of grapevine and citrus mealybugs. However, no information is currently available on whether this parasitoid is specific to *Planococcus* spp. or if it could also be effective against other economically important mealybug pests. Thus the host range of *C. peregrinus* requires assessment in order to establish if *C. peregrinus* could be used in orchards with a composite population of different mealybug species.

Materials and Methods

The parasitoid

A starter culture of *C. peregrinus* was obtained from ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa, where it has been under commercial production for augmentative biocontrol of grapevine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). It was subsequently reared in the laboratory using the methods described by Hattingh & Tate (1996).

The mealybug hosts

Four mealybug species that are important pests of citrus in South Africa were used in the experiments. These are the oleander mealybug (*Paracoccus burnerae*), the citrus mealybug (*Planococcus citri*), the citrophilous mealybug (*Pseudococcus calceolariae*) and the longtailed mealybug (*Pseudococcus longispinus*). The founder culture of *P. burnerae* was obtained from Nelspruit, Mpumalanga, collected by B. Tate [ex *Citrus limon* (L.)], and subsequently reared on seedlings of *C. limon* in the laboratory at the University of Stellenbosch. The latter three species were collected on *C. limon* and *C. reticulata* at Franschhoek and reared in the laboratory on sprouted potatoes.

No-choice test

For each of the four mealybug species, 10 similar-sized fresh lemon fruits (*C. limon*) were used as the host substrate. Each fruit was infested separately with 100 mixed individuals of first and second-instars of each mealybug species. The infested fruits were placed in Perspex boxes (24cm x 24cm x 17cm) and maintained in an incubator at 25°C for 48 hr to ensure that the mealybugs were settled. Mixed sexes of one-day-old *C. peregrinus* were then released into the boxes at the ratio of 1 parasitoid to 5 mealybugs (i.e. 200 parasitoids to 1000 mealybugs per box per species). The parasitoids were allowed to parasitize the mealybug host for 48 hr after which they were removed from the boxes. A cotton wool pad soaked in a diluted sugar solution was supplied in plastic petri dishes as food for the parasitoids during the 48 hr exposure period. The infested mealybugs in Perspex boxes were maintained at ambient temperature and humidity for three weeks after which 20-50 mealybugs per fruit per species were dissected under a stereomicroscope to check for parasitism. Whenever mummies were evident, dissection was not necessary.

Choice test

Six lemon fruits of comparable sizes were each separately infested with 75 mixed individuals of first and second-instars of each of the four mealybug hosts in Perspex boxes and maintained in an incubator at 25°C for 48 h. The fruits infested with each species were pooled into a large Perspex box (40cm x 30cm x 20cm) and arranged in a completely randomized design. Mixed sexes of one-day-old *C. peregrinus* were released into the box at the ratio of 1 parasitoid to 5 mealybugs (i.e. 360 parasitoids to 1800 mealybugs). The parasitoids were left in the box for 48 h during which time they were provided with a diluted sugar solution on cotton wool pads as food. The Perspex box was maintained at ambient temperature and humidity for three weeks after which 10-40 mealybugs per fruit per species were dissected under a stereomicroscope to check for parasitism.

Data analyses

The percentage parasitism of the four mealybug species presented to *C. peregrinus* in both choice and no-choice experiments was compared using a one way analysis of variance. Differences in toxicity of insecticides against *C. peregrinus* were analysed using ANOVA and contrasted by Tukey's honestly significant difference (HSD) test when significant F-values for these treatments were recorded (Zar, 1996).

Results and discussion

Coccidoxenoides peregrinus showed a significant preference ($P < 0.001$) for a composite population of first and second instar *P. citri* than for *P. burnerae*, *P. calceolariae* and *P. longispinus* in both choice and no-choice experiments (Table 3.6.3.1). Although the rate of parasitism of *P. citri* was less in the choice test than in the no-choice test, it was nevertheless significantly higher than the rate of parasitism of the other three mealybug species exposed to *C. peregrinus*.

Table 3.6.3.1. Mean percentage parasitism \pm SE of four mealybug species exposed to *Coccidoxenoides peregrinus* in choice and no-choice trials. N = Total number of mealybug dissected per 6 fruits (choice test) and 10 fruits (no-choice test).

Mealybug species	% Parasitism in choice test	N	% Parasitism in no-choice test	N
<i>P. burnerae</i>	13.3 ^a \pm 4.9	95	22.6 ^a \pm 6.8	200
<i>P. citri</i>	56.8 ^b \pm 10.6	80	86.7 ^b \pm 2.1	230
<i>P. calceolariae</i>	11.7 ^a \pm 1.8	190	11.4 ^a \pm 1.6	335
<i>P. longispinus</i>	8.3 ^a \pm 4.0	80	16.5 ^a \pm 2.9	220

Mean values within a column followed by unlike letter are significantly different ($P < 0.001$; One-way ANOVA; mean percentage parasitism contrasted by Tukey's honestly significant difference (HSD) test.)

Conclusions

Results of a laboratory experiment on the host range of *C. peregrinus* showed that *C. peregrinus* tends to be more specific to *Planococcus citri* (and possibly *Planococcus* spp. in general) than other mealybug species that are also important economic pests in citrus orchards. In a laboratory evaluation of the efficacy of *C. peregrinus* against *P. citri* and *P. burnerae*, Hattingh & Tate (1997) also demonstrated that *C. peregrinus* was not effective against the latter. However, a broad field trial is warranted to further verify the effect of *C. peregrinus* against the four mealybug species used in the current laboratory assay.

Future research

No further work is planned on this experiment.

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3.6.4 Investigating biocontrol agents of mealybug species other than citrus mealybug Experiment 692 by Sean D. Moore and Wayne Kirkman

Opsomming

Die dominansie en daarom plaagstatus van oleander witluis, *Paracoccus burnerae*, is in die Gamtoos Rivier Vallei (GRV) en Kat Rivier Vallei (KRV) in die Oos Kaap bepaal. 'n Opname word huidiglik in die Sondags Rivier Vallei (SRV) gedoen. As gevolg van die relatiewe oneffektiwiteit van *Coccidoxenoides peregrinus* teen *P. burnerae*, word 'n opname van sy parasiete huidiglik uitgevoer.

In Januarie 2003 is 68.6% van witluis individue wat van sitrus boorde in GRV versamel is as *P. burnerae* geïdentifiseer. Net 19.0% was *P. citri* en 12.4% was *P. longispinus*. *P. burnerae* was dominant in 78.6% van boorde wat ondersoek is. Dit wil voorkom of daar 'n dramatiese verandering in die demografie van

witluis spesies in sitrus boorde in die Oos Kaap is. Opnames wat van 1995 tot 1998 gedoen is het gewys dat *P. citri* die dominante spesie was.

Leptomastix sp. is die enigste parasiet spesie wat van *P. burnerae* in SRV gekry is en is die dominante spesie van *P. burnerae* in GRV. Een monster van *C. perminutus* is van *P. burnerae* in SRV versamel.

Hierdie eksperiment word voortgesit. Nadat die dominante parasiet spesies van *P. burnerae* geïdentifiseer is, sal die moontlike gebruik van hierdie spesies vir vrylating ondersoek word. Opnames van witluis spesies sal in die Oos Kaap voltooi word, en moontlik ook in ander dele van die land. As dit gevind word dat ander witluis spesies soos *P. longispinus* belangrik genoeg is sal biologiese beheer van hierdie spesies ook ondersoek word.

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*). It has, however, been shown that the oleander mealybug, *Paracoccus burnerae*, is approximately 100 times less suitable a host for *C. peregrinus* 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 and Korea (and potentially many others) as being a phytosanitary pest. Important and effective natural enemies of *P. burnerae*, and other important mealybug species, should be identified. Ultimately, the objective should be to establish an augmentation technique with natural enemies effective against these "other" species of mealybug. Recently, Wakgari & Gilliom (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*. However, this has not been done for *P. burnerae*. It is also imperative that important aspects of this biocontrol work be conducted in the Eastern Cape and northern citrus production areas.

Materials and methods

From 22-29 January a survey of mealybug species was conducted in 14 orchards in the Gamtoos River Valley. Samples of 20-50 fruit which appeared to be infested with mealybug were collected from orchards. Fruit were microscopically inspected and mealybug species (where possible) and life stages were identified, and numbers of mealybug counted.

A starter culture of *P. burnerae* was obtained from Bruce Tate (CRI, Nelspruit). Their identification was confirmed by Dr. Ian Millar of the Biosystematics Unit of the Plant Protection Research Institute (PPRI). The mealybugs were transferred onto Clementine Mandarin seedlings, which were kept under Growlux (UV-B) lights in the laboratory. Trees were watered and fertilised regularly. Small trifoliolate seedlings (approximately 20 cm from soil to tip) were obtained from the Citrus Foundation Block. Mealybug infested leaves from the Clementines were removed and placed onto the trifoliolate seedlings to facilitate movement of the mealybug onto the trifoliolate seedlings.

Two orange orchards, in which there were conspicuous levels of mealybug infestation, were selected. One of these was in the Gamtoos River Valley (GRV) (Tierhok Farm, orchard 4, Delta Valencias) and the other in the Sundays River Valley (SRV) (Rosedale Farm, orchard 3, Washington navels). Two gauze cages were assembled and each was fitted onto a platform on the top of a pole. These were inserted into the ground in each orchard, so that the cage was about 1.5 m above the ground, and the cage was not in contact with any of the trees in the orchard. Twenty mealybug-infested trifoliolate seedlings were placed into each cage (approximately 1 week after mealybug infested Clementine leaves had been placed onto the seedlings). Tangletrap glue was smeared around each pole so that ants and other wingless predators could not access the cages. Seedlings were retrieved from each orchard after 1 week. Numbers and life-stages of mealybug were estimated and seedlings were placed into emergence boxes. Emerging parasitoids were collected, placed into 70% ethanol, identified and counted. This survey was conducted monthly in each orchard. However, only the results obtained during 2003 are presented here.

Results and discussion

In total, 14 orchards in GRV were evaluated for mealybug during January 2003. In total 46.1% of mealybug individuals observed could be identified to species (Table 3.6.4.1), with acceptable certainty. These were all adults and sub-adults (3rd instar larvae). Crawlers (1st instar) and second instar larvae were not included, even if it was possible to identify them. Of the individuals identified, 68.6% were *P. burnerae*, 19.0% were *P. citri* and 12.4% were *P. longispinus* (Table 3.6.4.1). During collection of fruit two other species were

observed in orchards, namely striped mealybug, *Ferissia virgata*, and karoo-thorn mealybug, *Nipaecoccus viridis*. However, neither of these species appeared on the fruit samples that were collected and inspected microscopically. Probably most important, was the fact that *P. burnerae* was dominant in 78.6% of orchards inspected (Table 3.6.4.1). Random surveys conducted in the Kat River Valley (KRV) during 2003 revealed that *P. burnerae* was the dominant or only species in all orchards inspected (Bruce Tate & Freyni Killer, personal communication). Similar surveys will soon be conducted in SRV. From 1995 to 1998, trials with augmentation of *C. peregrinus* for control of mealybug, were conducted extensively in orchards in the Eastern Cape (SRV, GRV and KRV) (Hattingh *et al.*, 1997 & 1998). *P. citri* was observed to be the dominant species in all but one of the orchards inspected over this period. There therefore appears to have been a dramatic change in the demographics of mealybug species in citrus orchards in the Eastern Cape.

Table 3.6.4.1. Mealybug species complex in Gamtoos River Valley orchards (22-29 January 2003).

Farm	Orchard no.	Cultivar	Identifiable mealybug (% of total observed)	% of identified mealybug		
				<i>P. citri</i>	<i>P. burnerae</i>	<i>P. longispinus</i>
Tierhok	1	Navels	34.5	0	100	1
Vergenoeg	2	Navels	57.5	30.4	21.7	47.9
	8	Midknights	35.9	28.6	57.1	14.3
	9a	Midknights	18.8	0	0	100
	P1	Nardacotts	83.9	46.2	53.8	0
Stuk-Van-Acht	2	Midknights	49.1	3.8	92.4	3.8
	5	Navels	86.4	0	100	0
	8	Navels	57.1	12.5	87.5	0
	10b	Midknights	16.0	0	100	0
	10c	Midknights	44.4	0	100	0
	103	Navels	50.0	0	100	0
	106	Novas	41.2	28.6	71.4	0
	107	Minneolas	14.6	100	0	0
	109	Navels	56.5	15.4	76.9	7.7
Mean			46.1	19.0	68.6	12.4
Percentage of orchards in which each species was dominant				7.1	78.6	14.3

Leptomastix sp. was the only species of parasitoid found parasitising oleander mealybug in Sundays River Valley, and the dominant species attacking *P. burnerae* in GRV. Samples of this species have been sent to the Biosystematics Unit of the PPRI, for identification. Results are still outstanding. It is most likely that the species is *L. dactylopii* (Prinsloo, 1984). One specimen of *Coccidoxenoides perminutus* was collected from SRV (Table 3.6.4.2). The identification of this species will also be confirmed. *C. perminutus* is known to be far less effective against *P. burnerae* than against *P. citri* (Hattingh & Tate, 1997). However, this does not imply that *P. burnerae* is not attacked by *C. perminutus*. If the numbers of mealybug that were counted before placement into the emergence boxes, are accurate, then 7.6% mealybug was parasitised in the GRV orchard and 8.2% was parasitised in the SRV orchard. The numbers of mealybug counted might have been an underestimation of the actual numbers present, as individuals could have been hidden on the seedlings.

Table 3.6.4.2 Mealybug placed into emergence boxes (26 November 2003) and parasitoids and mealybug males collected from emergence boxes (18 December 2003).

			Gamtoos River Valley	Sundays River Valley
Mealybug life-stages counted before placement in emergence box	Egg sacs		77	124
	Crawlers (1 st & 2 nd instars)		32	16
	Sub-adults (3 rd instar)		9	0
	Adults		27	19
Collected from emergence box	Mealybug	Males	52	17
	Parasitoids	<i>Leptomastix</i> sp.	8	13
		<i>Coccidoxenoides perminutus</i>	1	0

Conclusion

In January 2003, 68.6% of mealybug individuals collected from citrus orchards in GRV were identified as *P. burnerae*, 19.0% were *P. citri* and 12.4% were *P. longispinus*. *P. burnerae* was dominant in 78.6% of orchards inspected. There appears to have been a dramatic change in the demographics of mealybug species in citrus orchards in the Eastern Cape.

Leptomastix sp. was the only species of parasitoid found attacking oleander mealybug in SRV, and the dominant species attacking *P. burnerae* in GRV. One specimen of *C. perminutus* was collected from SRV.

Future research

This experiment is ongoing. Once the dominant parasitoid species, attacking *P. burnerae*, are identified, the possible use of these species for augmentative biocontrol will be investigated. Surveys of mealybug will be completed in the Eastern Cape, and possibly elsewhere in South Africa. If other species of mealybug, such as *P. longispinus*, are found to be sufficiently important, biocontrol of these species will also be investigated. Some work on parasitism of this species has already been conducted (Wakgari & Gilliomee, 2002).

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3.6.5 Proef 717: Navorsing op die graanstinkbesie, *Macchiademus diplopterus*, as 'n fitosanitêre plaag op sitrus

Opsomming

Die graanstinkbesie *M. diplopterus* is 'n plaag wat oorsomer en slegs vir 'n relatiewe kort tydjie in die winter tot vroeë somer aktief is. As sodanig moet daar ook, soos in die geval van witluise (Afd. 3.6.6), op insektariumgeteelde insekte vir navorsingsdoeleindes staatgemaak word. Daar bestaan egter nog nie 'n tegniek om *M. diplopterus* kunsmatig te teel nie. Die ontwikkeling van so-iets verg baie tyd en aandag en die navorsing is daarom opsy geskuif. Geen werk word op hierdie eksperiment in die nabye toekoms beplan nie.

Summary

The grain chinch bug, *M. diplopterus*, is a pest that oversummers and is mainly active for a relatively short time during winter to early summer. As in the case of mealybugs (Section 3.6.6), research therefore depends on the availability of insectary-reared insects. A technique to artificially rear *M. diplopterus* does not exist. To develop such techniques is time consuming and research was abandoned for the time being. No work is planned on this experiment in the immediate future.

3.6.6 Die ontwikkeling van gammabestraling vir die disinfestasië van verpakte witluisbesmette sitrusvrugte

Proef 718 deur Hendrik en Marsheille Hofmeyr

Summary

Many different techniques for the disinfestation of exported citrus fruit have been evaluated without success. Techniques such as ozone treatment, controlled atmosphere and fumigation were all either ineffective, or were detrimental for fruit quality. Cold disinfestation of fruit for FCM is being used commercially, but the

subzero temperature used is variably injurious to the rind and also do not kill all mealybugs. Gamma irradiation of fruit, whether by linear accelerator or a cobalt source, is a potential solution for the problem.

Attempts to rear the mealybug *Paracoccus burnerae*, in Citrusdal for experimental purposes, were unsuccessful. This was due to inadequate resources and time constraints imposed by an extensive sterile insect release research programme for false codling moth. The need for mealybug disinfection has been reduced to some extent by the development of the PCR technique for the simplified identification of mealybug species.

Work on this experiment is planned for the coming season.

Bespreking

Dit is om 'n verskeidenheid van redes onprakties, indien nie onmoontlik nie, om verskeie plae op lemoene so goed in die boord te bestry dat hulle geen fitosanitêre bedreiging is wanneer die vrugte uitgevoer word nie. Dit is omdat baie veranderlike faktore, soos spuitdoeltreffendheid, skuilplekke op die boom, verkeerde bestrydingsprogramme, wisselende weerstand teen insekdoders, ens., verhoed dat 'n betrokke behandeling presies ewe doeltreffend op alle vrugte in 'n boord toegedien kan word. Dit geld vir alle behandelings soos byvoorbeeld lowerbespuiting met insekdoders, grondbehandeling met sistemiese insekdoders en selfs ook ongewoner metodes soos paringsontwrigting.

Die enigste uitweg is daarom om ge-oeste vrugte voor of na verpakking te behandel en enige plaagindividue op of in die vrugte te dood. Alle náverpakkingstegnieke wat voorheen ondersoek is om plaagindividue wat van fitosanitêre belang is, te dood, was egter óf ondoeltreffend, óf was nadelig vir vrugkwaliteit. Tegnieke soos osoon behandeling en beheerde atmosfeer was ondoeltreffend, terwyl berokking met metielbromied onder andere die raklewe van verpakte vrugte beduidend verkort het. Subzero-verkoeling word alreeds etlike jare lank met sukses gebruik om VKM-larwes in lemoene te dood. Dié temperatuur is egter potensieel skadelik vir vrugte aangesien die skil van verpakte vrugte beskadig kan word. Dit is ook nie in staat om 'n plaag soos die oleanderwitluis, *Paracoccus burnerae*, hok te slaan nie.

Gammabestraling met 'n kobaltbron word alreeds internasionaal gebruik om Mediterreense vrugtevlieglarwes in vrugte te dood. Voorlopige gegewens dui daarop dat selfs VKM-larwes waarskynlik doeltreffend in lemoene bestry sal kan word. Daar is inligting wat daarop dui dat baie skadelike insekte op dié wyse uitgekakel sal kan word. Dié tegniek skep die moontlikheid dat vrugte op vervoerbande in 'n pakhuis vóór verpakking deur middel van 'n liniêre versnellerbron, of ná verpakking met 'n kobaltbron bestraal sal kan word.

Toetsing van die tegniek is afhanklik van 'n groot genoeg, standhoudende insektariumbevolking wat in proewe gebruik kan word. Aanvanklike pogings om *P. burnerae* in die CRI-laboratorium te Citrusdal te teel, het om twee redes misluk. Daar was (en is) enersyds nie fasiliteite wat vir die teel van sulke insekte geskik is nie, terwyl navorsing op die Steriele Insektoslatings-tegniek vir VKM die meeste beskikbare tyd in beslag geneem het. Sedert die aanvanklike pogings, het die PCR-tegniek vir die identifikasie van etlike witluisspesies op sitrusvrugte beskikbaar geraak. Dit het die onmiddellike dringendheid vir 'n disinfestasietegniek vir witluis ietwat verlig en daar is besluit om eerder aandag aan die ontwikkeling van die tegniek vir VKM en Natalse vrugtevlieg te gee.

3.7 PROJECT: PRODUCTION PESTS

Project Co-ordinator: Tony Ware (CRI)

3.7.1 Project summary

Little research was conducted within this project. Attempts at rearing unidentified armoured scale on butternuts in an attempt to establish an *A. africanus* colony were unsuccessful (Experiment 611). Research into psylla control was placed on hold because no suitable field populations were available (Experiment 586). Soft scale biology was investigated (experiment 691). A fungus, *Verticillium lecanii*, a parasitoid, *Coccophagus pulvinariae*, and a beetle, *Cryptolaemus montrouzieri*, were efficient natural enemies. The pest could be controlled chemically with Mitac and oil showing good results. All research in this project will receive more attention in the following year.

Projekopsomming

Min navorsing is in hierdie projek onderneem. Pogings om ongeïdentifiseerde harde dopluis op grysneut te kweek met die doel om 'n *A. africanus* kolonie te vestig, was onsuksesvol (eksperiment 611). Die

biologie van sagtedopluis was geondersoek. 'n Swam, *Verticillium lecanii*, 'n parasite, *Coccophagus pulvinariae*, en 'n kewer, *Cryptolaemus montrouzieri*, was vasgestelde natuurlike vyande. Die pes kan chemies beheer word met Mitac en olie. Al die fasette van hierdie projek sal meer aandag geniet in die komende jaar.

3.7.2 Investigating control options for soft green scale

Experiment 691 by Xolani C. Mavi (Port Elizabeth Technikon), Sean D. Moore, Garth I. Richards and Wayne Kirkman (CRI)

Opsomming

Hierdie studie is voortgesit as gevolg van die verhooging in plaagstatus van sagtegroendopluis in die Oos Kaap oor die laaste paar jaar. Die tydperk tussen 'n kruiper beweging piek en 'n piek in getal volwassenes is as 1281.5 dag grade gemeet. Hierdie ontwikkeling sal tussen 53 tot 91 dae duur afhangend van temperatuur. Die lewensiklus van sagtegroendopluis van eier tot eier sal heel waarskynlik 'n hele paar dae langer duur. Daarom is daar moontlik 3 generasies per jaar op sitrus in Suid Afrika. Die enigste natuurlike vyande wat gekry is wat sagtegroendopluis aanval is die swam *Verticillium lecanii* en die parasiet *Coccophagus pulvinariae*. In 'n glashuis het die laasgenoemde 77% van tweede instars en 74% van volwassenes gearasiteer. 'n Vrylating van hoë getalle van *Cryptolaemus montrouzieri* kewers het in vergelyking met 'n onbehandelde kontrole, 'n 53% afname in sagtegroendopluis besmetting veroorsaak. Dit kon nie bepaal word of *Chilocorus cacti* kewers enige impak op sagtegroendopluis gehad het nie. In 'n chemiese beheer proef het alle behandelings 'n betekenisvolle vermindering in besmetting veroorsaak. As gevolg van hulle "IPM" verenigbaarheid is Mitac en olie in 'n tweede proef getoets, met baie goeie resultate. Behalwe die voltoeing van biotoetse met *V. lecanii* teen sagtegroendopluis, word geen verdere navorsings werk beplan nie.

Introduction

The pest status of soft green scale in the Eastern Cape was deemed to have increased in the few years leading up to the initiation of this study. So much so that it was regarded by many growers in the region as a research priority. Knowledge of soft green scale is incomplete. In this study, it was considered important to firstly determine the life cycle of soft green scale, and the status of its natural enemies. Subsequent to this, various control options (both biological and chemical) were investigated. This included augmentative and new association releases of natural enemies and the study of new chemical control options.

Materials and methods

Life-cycle and population dynamics

Two trials were conducted to determine the life-cycle and population dynamics of soft green scale on citrus. The first trial was conducted at the Citrus Foundation Block (CFB), in a small orchard of Minneola x Trifoliolate (MxT) rootstock trees, which was heavily infested with soft green scale. The orchard consisted of 8 rows of 10 trees each. The 2 outer rows and the outermost trees in each row were considered as buffer trees in the trial and were not used for data collection. Forty-eight trees were therefore used for the trial. The fifth and sixth trees in each row were trimmed so that they did not touch one another. In the first row, the first 4 trees were ant-banded. In the following row the second 4 trees were ant-banded, and so on for the remaining rows. This provided an opportunity to simultaneously determine the impact of ants on the scale (through their effect on natural enemies). In May 2002 evaluation of the trial was initiated, and monitoring was conducted once a fortnight and continued until October 2002. On each tree, a cluster of leaves was randomly selected on each of the northern, southern, eastern and western aspects. On the furthestmost 5 leaves (and adjoining stems), numbers of soft green scale crawlers (1st instar larvae), sub-adults (2nd instar) and adults, were recorded.

The second trial was conducted in a temperature controlled (minimum: 19°C; maximum: 28°C; mean: 23.3°C) glasshouse at PE Technikon. Twelve citrus trees were obtained from the CFB and placed into the glasshouse. Soft green scale infested twigs and leaves were collected from a farm in Sundays River Valley (SRV), and placed on the trees in the glasshouse until conspicuous numbers of crawlers had move onto and settled on the trees. Trees were separated from one another to prevent movement of scale from one tree to another. Five inspection points (consisting of 5 leaves each) were selected and marked on each of the 12 trees. Numbers of soft green scale of each of the 3 life stages, were recorded on the same inspection points each fortnight.

Natural enemies

During the field trial conducted at the CFB, soft green scale-infested leaves were collected each fortnight from non-data trees. After counting the numbers of each life stage of soft green scale, leaves were placed into emergence boxes. Emerging parasitoids were recorded for identification.

Any predators or pathogens observed attacking soft green scale in any of the orchards in which field trials were conducted, were collected for identification. Scale on the trees in the glasshouse life-cycle trial appeared to be parasitised during late August 2003. Five scale infested leaves were collected from each of the 10 trees and placed into emergence boxes, after recording the numbers and life stages of the scale on the leaves. Consequently, percentage parasitism could be recorded for each life stage and parasitoid species could be identified.

Biological control

An orchard which was well infested with soft green scale was selected for a *Cryptolaemus montrouzieri* release trial. Ten heavily infested trees were selected and marked on each side of the orchard. Soft green scale infestation was recorded in each of the trees by randomly selecting 10 inspection points (cluster of 5 leaves) around the canopy of each tree. On 10 October 2002, 50 *C. montrouzieri* adults were released into each of the 10 trees on one side of the orchard. Seven days later (on 17 October) and again 2 weeks later (on 7 November), soft green scale infestation on the 20 data trees was recorded to determine whether the beetles had caused any reduction in infestation, and to quantify such a reduction.

In a second biological control trial, two navel orange trees which were heavily infested with soft green scale were identified on a private residential property in SRV. An organdy net covered cage was designed and erected over one of the trees, in order to eliminate any predators. Before this was done, soft green scale infestation was evaluated on each of the trees (on 10 inspection points of 5 leaves on each tree) and trees were inspected to ensure that predators were absent. Three hundred *Chilocorus cacti* adults were then released into the cage on 15 July 2003. On 2 September 2003 soft green scale infestation was re-evaluated on each tree.

Chemical control

An orchard of navel orange trees, which was well infested with soft green scale, was selected for this trial. The trial was laid out in a single tree random block design, with 10 replicates per treatment. Seven treatments (Table 3.7.2.1) were applied as full cover sprays with hand-held spray guns on 10 October 2002. An eighth treatment (Mospilan) was applied to the trunk of the trees with a paint brush, and an untreated control was retained. Four weeks later (on 7 November), infestation on all of the trees was evaluated. This was done as described for the *C. montrouzieri* release trial. Mean values for each treatment were calculated and data was subjected to an ANOVA. An LSD multiple range test revealed any statistical differences in infestation between treatments.

Table 3.7.2.1 Treatments applied on 9 October 2002 for the control of soft green scale on navel orange trees on Pennyholme Farm, SRV.

Treatment number	Product	Active ingredient	Concentration per 100 l water
1	Ultracide BP Medium	Methidathion Oil	150 ml 500 ml
2	Lannate BP Medium	Methomyl Oil	20 g 500 ml
3	Proton Agral 90	Profenofos Nonylphenoxy- polyethoxyethanol	100 ml 18 ml
4	Mitac Agral 90	Amitraz Nonylphenoxy- polyethoxyethanol	150 ml 18 ml
5	Applaud BP Medium	Buprofezin Oil	30 g 500 ml
6	Nemesis BP Medium	Pyriproxyfen Oil	30 ml 500 ml
7	BP Medium	Oil	800 ml
8	Mospilan	Acetamiprid	

A second trial was applied against a moderate level of soft green scale infestation in a navel orange orchard on Rawdon Farm in SRV. The trial was again laid out in a single tree random block design, this time with 12 replicates per treatment. Six treatments (Table 3.7.2.2) were applied as full cover sprays with hand-held spray guns on 12 August 2003. Benomyl was included with each of the treatments, as the entomopathogenic fungus, *V. lecanii* was observed to be attacking the scale at this site. Being a fungicide, it was hoped that benomyl would kill the *V. lecanii*, so that the impact of the insecticides alone on the scale could be measured. In order to measure the effect of the *V. lecanii* on the scale, benomyl was also sprayed on its own. Three weeks later (on 2 September) infestation on each tree was evaluated. Mean values for each treatment were calculated and data was subjected to an ANOVA. A Bonferroni LSD multiple range test revealed any statistical differences in infestation between treatments.

Table 3.7.2.2 Treatments applied on 12 August 2002 for the control of soft green scale on navel orange trees on Rawdon Farm, SRV.

Treatment number	Product	Active ingredient	Concentration per 100 ℓ water
1	Ultracide Agral 90 Benlate	Methidathion Nonylphenoxy- polyethoxyethanol Benomyl	150 ml 18 ml 50 g
2	Mitac Agral 90 Benlate	Amitraz Nonylphenoxy- polyethoxyethanol Benomyl	150 ml 18 ml 50 g
3	Mitac BP Medium Benlate	Amitraz Oil Benomyl	150 ml 500 ml 50 g
4	BP Medium Benlate	Oil Benomyl	800 ml 50 g
5	BP Medium Benlate	Oil Benomyl	1000 ml 50 g
6	Benlate	Benomyl	50 g

Results and discussion

Life-cycle and population dynamics

Soft green scale infestation was monitored on MxT rootstock trees fortnightly from May to October 2002. During this period, levels of all three life-stages monitored, declined steadily (Fig. 3.7.2.1). There was no clear reason why this occurred. Consequently, it was not possible to determine the duration of the life-cycle or the relative proportions of the different life stages (with sufficient confidence), or to record any other trends pertaining to the population dynamics of the scale.

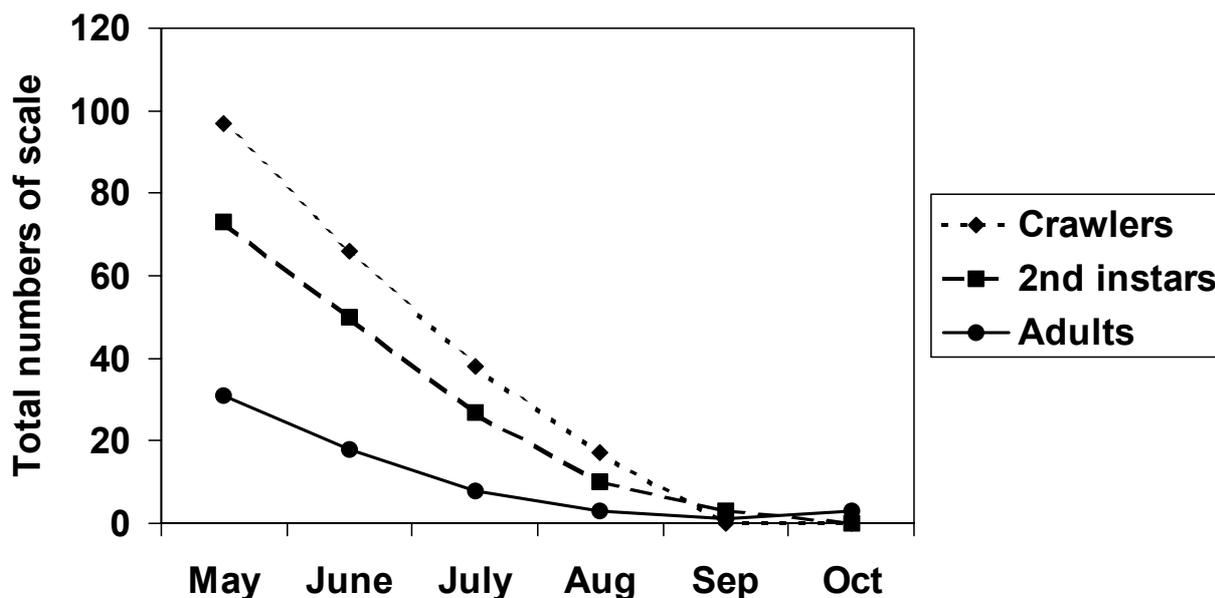


Figure 3.7.2.1 Total numbers of soft green scale crawlers, second instars and adults counted on unbanded data trees from May to October 2002.

Despite the obvious presence of large numbers of *Anoplolepis custodiens* ants in the MxT orchard, and a conspicuous level of activity in trees, there was no meaningful difference in levels of soft green scale infestation between ant-banded and unbanded trees (Fig. 3.7.2.2). This might have been because soft green scale numbers were declining (inexplicably) in both treatments in any case, or because there was no natural enemy activity for the ants to disrupt (see next sub-section on Natural Enemies).

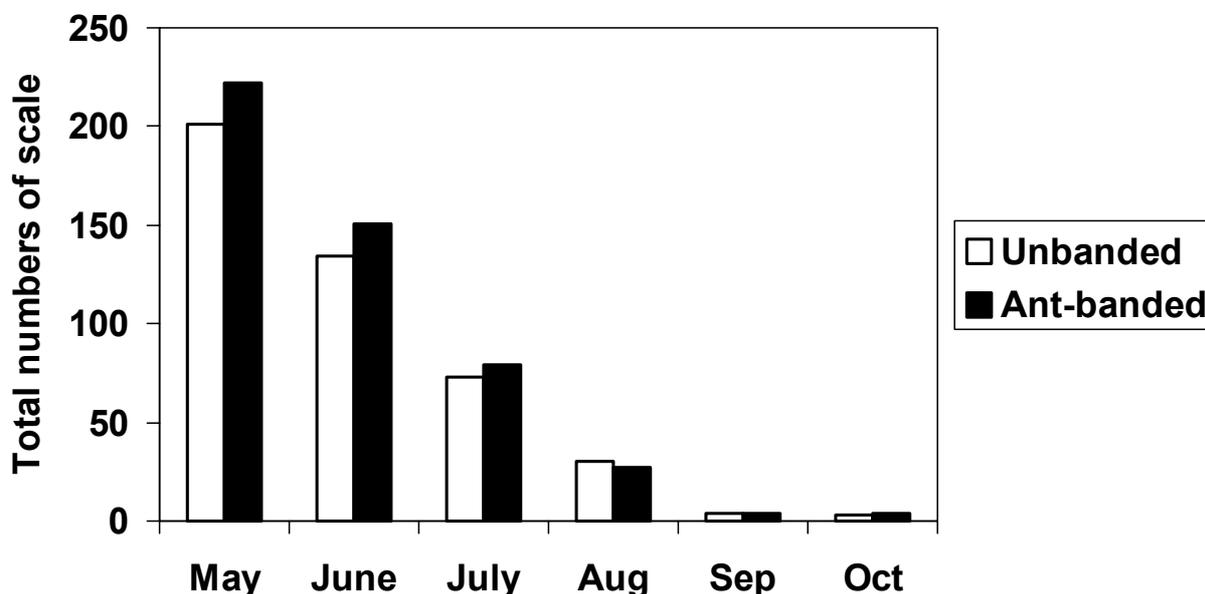


Figure 3.7.2.2 Total numbers of soft green scale crawlers, second instars and adults counted on unbanded and ant-banded data trees from May to October 2002.

In the trial conducted in the glasshouse, soft green scale also began to decline rapidly during late August. The cause of this was determined to be the inadvertent appearance of parasitism (see next sub-section on Natural Enemies). However, before this occurred, a peak in crawler infestation was recorded on 25 May (Fig. 3.7.2.3). This was followed by a peak in adult numbers on 19 July. This is a gap of 55 days. At an average temperature of 23.3°C, this would be a period of 1281.5 day degrees. In mid-summer the average daily temperature in SRV can be around 24°C (data₂₂₅ obtained from South African Weather Services for

Addo in January 2001). In mid-winter the average daily temperature in the same region can be around 14°C (data obtained from South African Weather Services for Addo in August 2001). This period from crawler to adult is therefore speculated to take from 53 to 91 days depending on the time of the year and the temperature. The life-cycle of soft green scale from egg to egg can be considered to be several days longer than this, as one needs to consider pre-oviposition and incubation periods. The latter is apparently of longer duration than that of *Coccus hesperidum*, soft brown scale (Annecke, 1998b). There are therefore probably around 3 generations per year on citrus in South Africa, as is the case with the soft brown scale (Annecke, 1998a). The coffee green scale, *Coccus viridis*, undergoes 3-4 generations per year on citrus in Australia (Smith *et al.*, 1997).

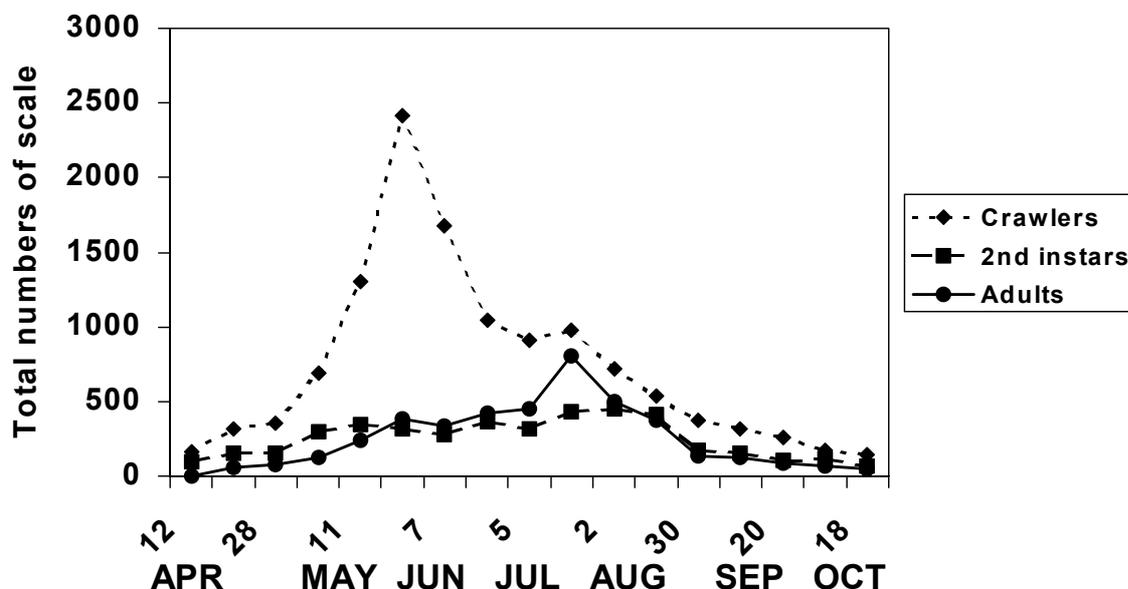


Figure 3.7.2.3 Total numbers of soft green scale crawlers, second instars and adults counted on citrus seedlings in a glass house from April to August 2003.

Natural enemies

No parasitoids were obtained from the soft green scale, collected from the CFB, which was placed into emergence boxes. No predators were observed feeding on the scale in orchards either. The fungus *Verticillium lecanii* was found attacking soft green scale at two sites in SRV: navel orange orchards at Pennyhome and Rawdon Farms.

During late August a high percentage of soft green scale on the potted seedlings in the hot house appeared to be parasitised i.e. they became brown or black and appeared to die. No emergence holes could be found in the scale coverings. Of the scale which was placed into the emergence boxes, no crawlers were parasitised, 76.99% of second instars were parasitised, and 73.70% of adults were parasitised (Fig. 3.7.2.4). All parasitoids were identified as *Coccophagus pulvinariae*, a known parasitoid of soft green scale on citrus in South Africa (Prinsloo, 1984). Four other species of parasitoid have been recorded attacking soft green scale. These are *C. nubes*, *C. basalis*, *C. rusti* and *Metaphycus helvolus*. It is highly unlikely that any of the parasitoid species are as prolific or effective in the field as *C. pulvinariae* was in the protected glasshouse environment.

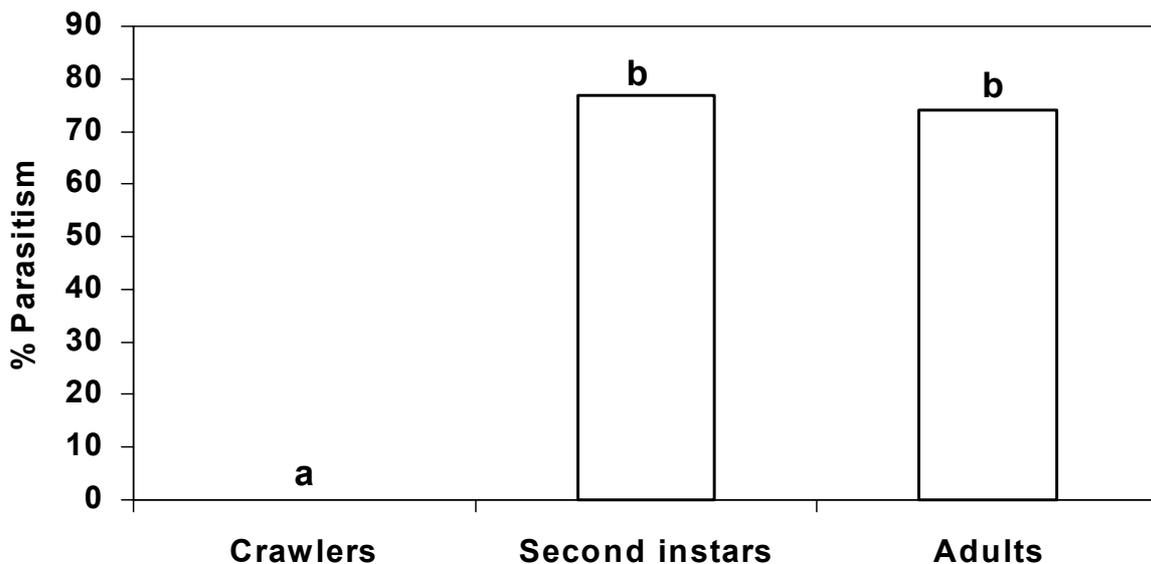


Fig. 3.7.2.4 Percentage of different soft green scale life stages parasitised by *Coccophagus pulvinariae* on citrus trees in a glass house during October 2003. (Bars with the same letters are not significantly different; $P < 0.05$; LSD multiple range test).

Biological control

In the trial in which *C. montrouzieri* beetles were released for control of soft green scale, scale infestation had declined 7 days after release (Fig. 3.7.2.5). However, this was also so for non-release trees. At this stage *C. montrouzieri* did not yet appear to have had any effect on the level of infestation. Infestation in the release trees had declined by 30%. However, infestation in the non-release trees had declined by 40%. It is not clear what caused this decline. *C. montrouzieri* beetles were conspicuous in the release trees at this time, but not in the control trees. From the evaluation conducted 7 days after release to the second evaluation conducted 21 days after release, infestation in control trees had declined by 27%, whereas infestation in release trees had declined by 57% (Fig. 3.7.2.5). This shows a 53% greater reduction in infestation where the beetles were released. However, at 21 days after release no *C. montrouzieri* could be found at the release site. It is speculated that they had dispersed due to the declining density of scale. A further two weeks later scale infestation had declined to an almost inconspicuous level. It was therefore not possible to conduct another evaluation.

Even though the reduction in infestation presumably caused by *C. montrouzieri* was noteworthy, it is not yet clear whether this predator is a suitable release candidate for the control of soft green scale. The numbers released per tree were extremely high, and would be far too costly to consider commercially. It must therefore be determined whether far lower numbers would be effective, and whether a commercially acceptable level of soft green scale control (probably greater than 53% reduction in infestation) could be achieved.

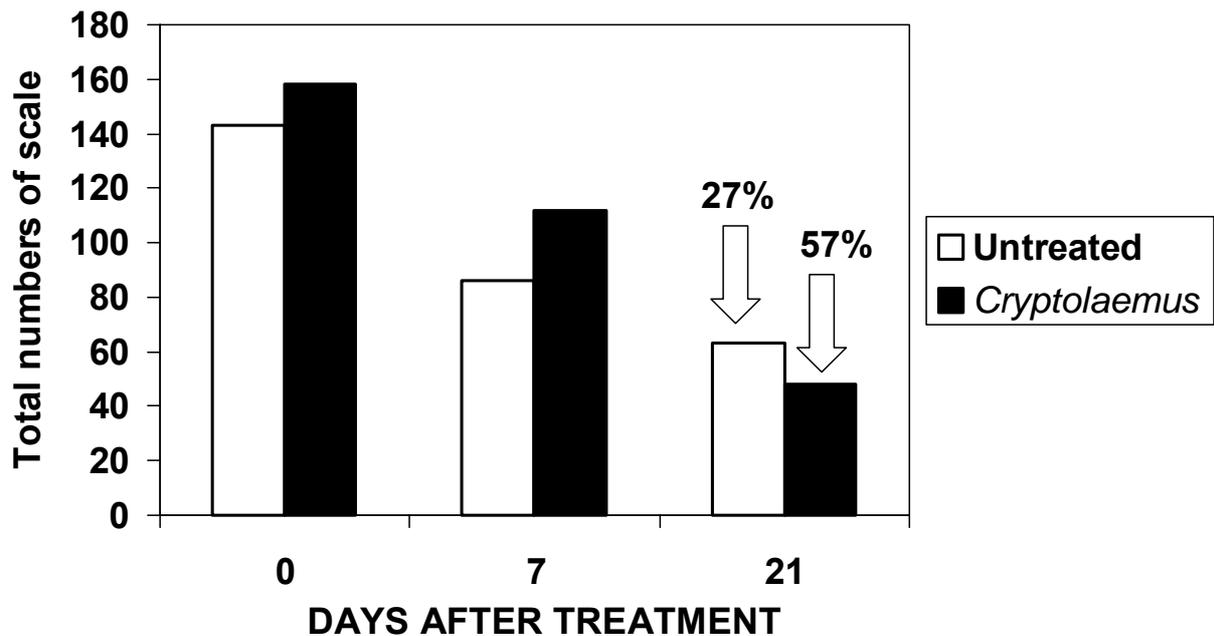


Figure 3.7.2.5 Total numbers of soft green scale on control trees and trees on which *Cryptolaemus montrouzieri* was released. (Arrows indicate reduction in infestation since previous evaluation (7 DAT)).

Very little was learned from the *C. cacti* trial. There were several problems with the trial. Only one release tree and one control tree were used. The level of infestation in the release tree was notably higher than in the control tree (Fig. 3.7.2.6). Also, a high level of *Pheidole megacephala* ant activity was noted in the trees when the trial was evaluated. This could have been disruptive to the beetles. When the trial was evaluated, infestation in both trees had declined markedly (Fig. 3.7.2.6). However, it is not possible to say whether or to what extent *C. cacti* contributed to the decline on the release trees. *C. cacti* does not occur naturally in the Eastern Cape. Releases have previously been conducted against bamboo scale on the CFB (in the Eastern Cape) (Hattingh & Tate, 1997) and against red scale in Sunday River Valley (Moore, unpublished data). Beetles overwintered at the CFB, but no recoveries were made from release orchards in SRV. It is therefore not certain whether *C. cacti* would survive from one season to the next, even if found to be effective as biocontrol agent, released inundatively.

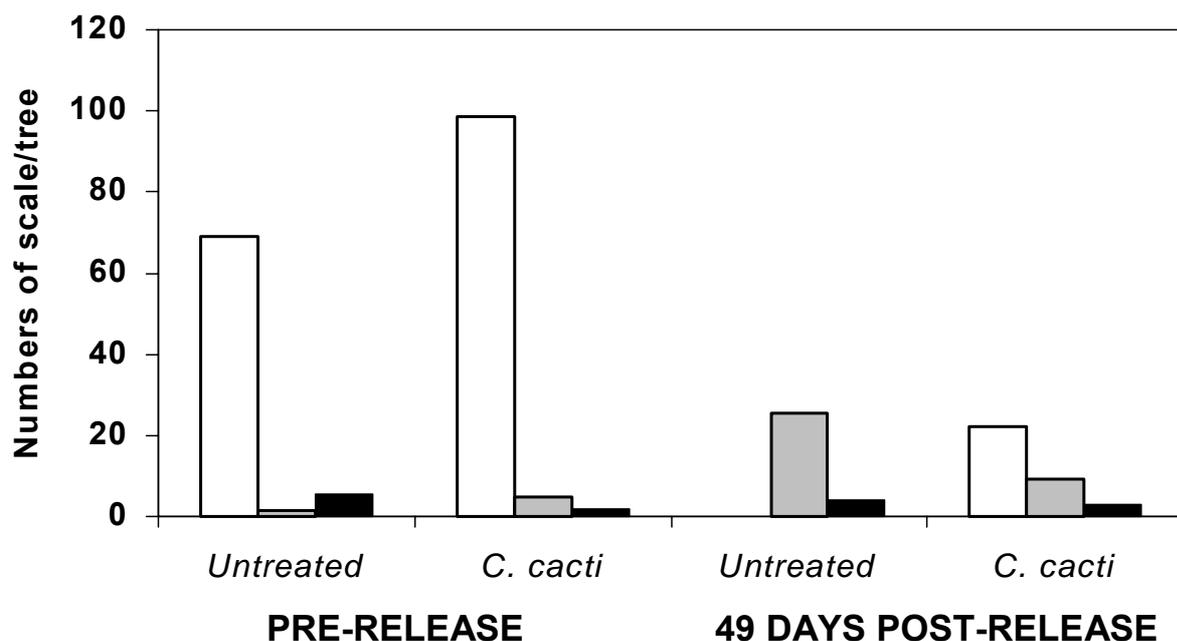


Fig. 3.7.2.6 Soft green scale infestation on the control tree and on the caged tree on which *Chilocorus cacti* beetles were released. White columns = crawlers; grey columns = second instars; black columns = adults.

Chemical control

In the first chemical control trial, all treatments significantly reduced soft green scale infestation, relative to the control (Fig. 3.7.2.7). The most effective treatments were the registered options: Ultracide and Lannate. However, within an IPM programme, the use of either of these products might be undesirable and detrimental, depending on the time of year that they are applied. As both oil and Mitac can be considered as being substantially more IPM compatible (Grout *et al.*, 2002), these two treatments were tested again in the next trial.

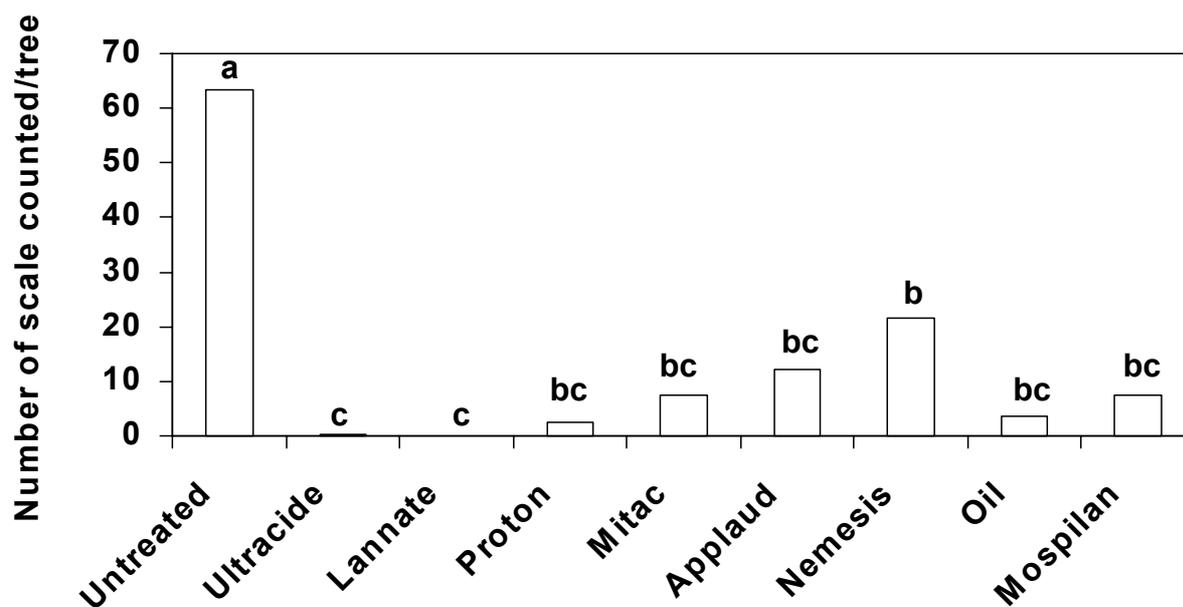


Fig. 3.7.2.7 Soft green scale infestation (7 November 2002) in navel orange trees (on Pennyholme Farm) treated with various pesticides (10 October 2002). (Columns with the same letters are not significantly different; $P < 0.05$; LSD multiple range test).

In the second chemical control trial conducted at Rawdon Farm, all of the treatments worked extremely well i.e. as well as the registered standard treatment (Ultracide). Oil at 1% was not more effective than at 0.8% (Fig. 3.7.2.8). There was no difference in control with Mitac, regardless of whether it was applied with oil or with a wetter (Fig. 3.7.2.8). As infestation in the untreated control and on the benomyl treated trees was not significantly different (Fig. 3.7.2.8), it appeared that *V. lecanii* had no controlling effect on the scale. Infestation was actually slightly lower where benomyl had been applied, indicating that this had some suppressive effect on the scale, particularly on the crawlers (not indicated in Fig. 3.7.2.8). Despite these results, it is still believed that *V. lecanii* could control soft green scale. It is possible that by the time the sprays were applied, the fungus had already had its full effect, and that due to the declining density of scale, the effect of the fungus had also declined.

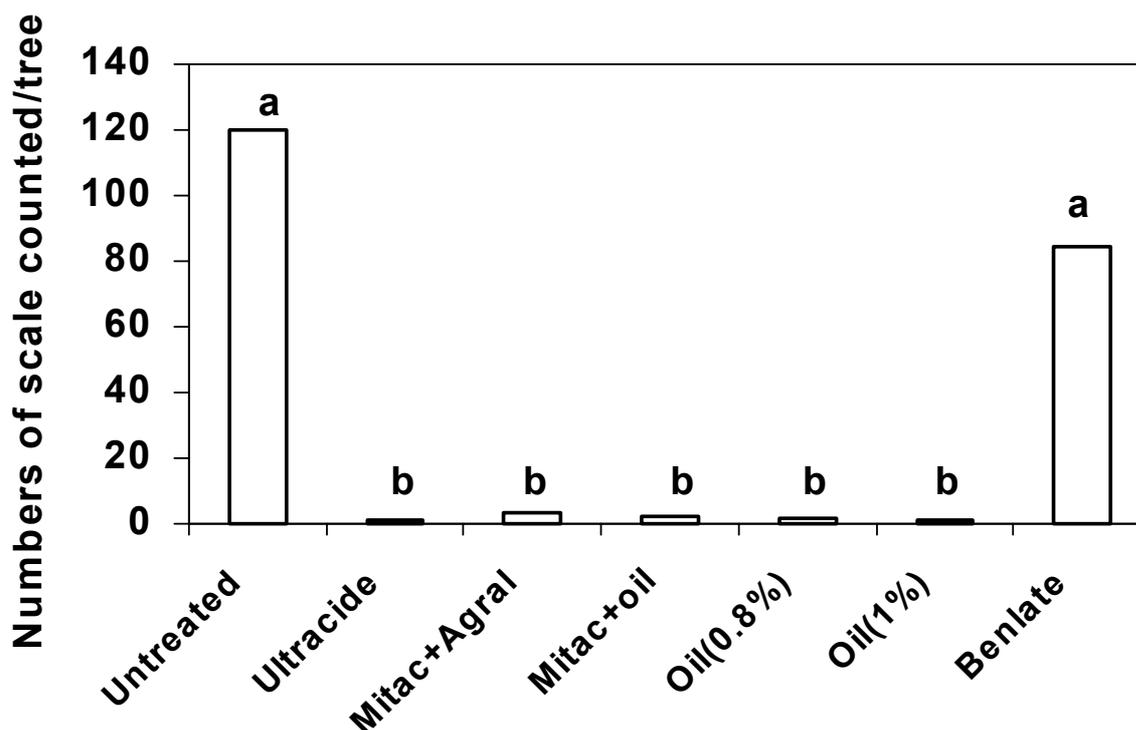


Fig. 3.7.2.8 Soft green scale infestation (2 September 2003) in navel orange trees (on Rawdon Farm) treated with various pesticides (12 August 2003). (Columns with the same letters are not significantly different; $P < 0.05$; LSD multiple range test).

Conclusion

A period of 1281.5 day degrees was recorded between a peak in crawler movement and a peak in adult numbers. This period is therefore speculated to be from 53 to 91 days depending on temperature. The life-cycle of soft green scale from egg to egg can be considered to be several days longer than this. There are therefore probably around 3 generations per year on citrus in South Africa.

The only natural enemies recorded attacking soft green scale were *Verticillium lecanii* and *Coccophagus pulvinariae*. In a glasshouse, the latter was found to parasitise 77% of second instars and 74% of adults. High density releases of *Cryptolaemus montrouzieri* beetles caused a 53% reduction in soft green scale infestation relative to the control. It could not be demonstrated that *Chilocorus cacti* had any impact on soft green scale.

In a chemical control trial, all treatments caused a significant reduction in infestation. Due to their IPM status, Mitac and oil were tested in a second trial, with very good results.

Future research

No future work has been planned on this experiment. However, Xolani Mavi is expected to complete a laboratory assay with *V. lecanii* against soft green scale.

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4 PROGRAMME: DISEASE MANAGEMENT

4.1 PROGRAMME SUMMARY

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

For a number of years the focus in the Disease Management programme has been on providing assurances to the European Union that citrus black spot-infested fruit from southern Africa is not a threat to their citrus industry. Some of these objectives were met in 2003 and focus can now increasingly shift towards more efficient management of this disease. Unfortunately, little progress was made with field trials in 2003 due to unsuitable weather. As our citrus industry continues to grow and profit margins shrink, it becomes increasingly important to have trees with a long, highly productive life, producing fruit of good size and quality. The progress achieved in obtaining better virus strains for immunisation against Citrus Tristeza Virus and the biological control of the citrus nematode, will help to meet these requirements. The value to the citrus industry of applied plant pathologists was once again demonstrated in the provision of guidelines for the continued post-harvest use of 2,4-D and identifying the cause of unusual symptoms on citrus in different parts of the production region.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsing & Tegnieuse Bestuurder: CRI)

Die Siektebeheerprogram het vir 'n aantal jare daarop gefokus om versekering te gee aan die Europese Unie dat sitruswartvlek-besmette vrugte van Suider-Afrika nie 'n bedreiging inhou vir die sitrusbedryf aldaar nie. Sommige van die doelwitte is in 2003 bereik en klem kan nou toenemend verskuif na doeltreffende bestuur van hierdie siekte. Ongelukkig is min vordering in 2003 gemaak met veldproewe as gevolg van ongunstige weer. Namate die plaaslike sitrusbedryf voortgaan om te groei en winsmarges daal, word dit toenemend belangrik om te beskik oor bome met 'n lang, hoogs-produktiewe lewensduur wat vrugte van goeie grootte en kwaliteit produseer. Vordering gemaak met die verkryging van beter virusstamme vir immunisering teen Citrus Tristeza virus en die biologiese beheer van sitrusaalwurm, sal bydra om hierdie vereistes te verwesenlik. Die waarde van toegepaste plantpatoloë vir die sitrusbedryf is weereens bevestig met die daarstelling van riglyne vir die voortgesette na-oesgebruik van 2,4-D en identifisering van ongewone simptome op sitrus in verskillende streke van die produksiegebied.

4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project Co-ordinator: S.P. van Vuuren (ARC-ITSC)

4.2.1 Projekopsomming

Vordering in *Citrus tristeza virus* (STV) navorsing help om die siekte beter te verstaan en daardeur word die toepassing van beheermaatreëls bevorder. Aangesien STV endemies is in suider Afrika as gevolg van plantluis vektore, is ligte ras kruisbeskerming die enigste beheermaatreël. Die langtermyn waardebeoordeling van nuwe verbeterde kruisbeskermings-isolate is 'n beperking wat optimale beheermaatreëls vertraag om maksimum produksie te verkry. Pomelos vorm 'n belangrike deel van die suider Afrikaanse sitrus bedryf, maar die produksie en vruggrootte word grootliks beïnvloed deur STV. Die soeke na beter kruisbeskermings-isolate is daarom 'n voortdurende aksie. 'n Groot aantal nuwe isolate wat versamel is in verskillende produksiegebiede is in die glashuis geëvalueer. Eenhonderd en sewe bome is geïdentifiseer in die verskillende pomelo produksie gebiede. Enthout is van hierdie bome gesny en in die glashuis geïnkuleer op virusvrye Meksikaanse lemmetjie (sensitiwe indikator kultivar vir STV). Indeksering gedurende 2002 het slegs 19 isolate van die oorspronklike 107 geïdentifiseer wat potensiaal het om as kruisbeskermers gebruik te word. 'n Verdere biologiese indeksering is gedoen om die 19 isolate se virulensie te bepaal asook hoe hulle vergelyk met die huidige GFMS 12 en GFMS 35 STV isolate. Nartia sub-isolate van die ITSG en Beltsville is ook ingesluit in hierdie evaluering. Die boord isolate is ook geïndekseer op virusvrye Etrog sitroen om te bepaal of die isolate vry is van sitrus viroïde. Daar is gevind dat vyf van die isolate met viroïde besmet is, agt het groei gestrem en strawwe stamgleuf veroorsaak terwyl twee lae virus titer getoon het. Dus, van die 107 isolate is daar vier wat uiteindelik in die boord ge-evalueer gaan word (afdeling 4.2.2). Biologiese indeksering van die 21 beste bome in 'n proef aanplanting te New Venture het getoon dat slegs twee bome met ligte virus isolate besmet is. Die twee isolate is geïnkorporeer by die ander boord isolate waar 'n vergelykende toets gedoen is in afdeling 4.2.2. Een isolaat sal verder in boordproewe ge-evalueer word. Die huidige boordproef is vir die tweede agter-eenvolgende jaar deur die boer ge-oes sonder dat data geneem is en aangesien daar geen behoorlike kontrole behandeling is om te vergelyk nie, word die proef gestaak (afdeling 4.2.3). Uiteindelik is die Beltsville isolate in die boord. Star Ruby en Marsh pomelo boompies is met die beste sub-isolate geïnkuleer en ELISA is gedoen om preïmmunisasie te bevestig. ELISA het egter getoon dat twee van die sub-isolate swak vermeerder en beweeg en daarom word hulle

uitgesluit van verdere evaluasie. Die Star Ruby bome is in Swaziland by Tambuti Estates geplant en die Marsh bome by Riversbend in die Nkwaleni Vallei (afdeling 4.2.4). In die proef waar verskillende STV isolate as kruisbeskermings-agente vir Star Ruby geëvalueer word, het bome met isolate GFMS 35 en GFMS 78 die beste produksie oor 'n periode van vyf jaar gelewer. Die verskil in die kumulatiewe produksie van bome met die twee isolate was 0,5 kg. Bome met hierdie twee isolate het betekenisvol beter geproduseer as bome wat virusvry geplant is, die wat met GFMS 12 (Nartia) en GFMS 67 gepreïmmuniseer is en die met die twee strawwe isolate. Berekening van die oeswaarde (vruggrootheid en mark pryse) wys dat die oeswaarde van bome met GFMS 78 1% beter is as die met GFMS 35. Gedurende die vorige seisoen (2002) was bome met GFMS 35 5% beter as die met GFMS 78. Bome met GFMS 12a het die derde beste oeswaarde gehad en was 12% laer as die van GFMS 78. Isolaat GFMS 12a is van die oorspronklike goeie Star Ruby moederboom by die Sitrus Grondvesblok versamel. Die meerderheid van die Star Ruby bome in die bedryf wat tans in produksie is se oorsprong is vanaf die moederboom. Volgens die status van die proef moet dit gesluit word. Die resultate toon dat isolate GFMS 35 (kruisbeskermings-agent tans vir Star Ruby gebruik word) en GFMS 78 uitstaande kruisbeskermers vir Star Ruby pomelo is in vergelyking met die ander isolate wat ge-evalueer is. Nietemin, dit sal voordelig wees dat die proef voortgesit word om te bepaal tot watter mate boom leeftyd en ekonomiese produksie verleng word (afdeling 4.2.5). Met die evaluasie van STV isolate vir Star Ruby pomelo in twee lokaliteite, het resultate van die Nelspruit lokaliteit bevestig dat GFMS 12 (Nartia) nie 'n geskikte beskermer vir Star Ruby is nie. Jonger bome op Malelane bevestig nie die resultaat op hierdie stadium nie. Op Nelspruit, met sy koeler klimaat, is die invloed van die STV isolate op groei en stamgleuf ontwikkeling meer drasties. Op Malelane verskil stamgleuf ontwikkeling op bome wat met ligte isolate geïnkuleer is nie van die wat met die strawwe isolaat geïnkuleer is nie wat 'n aanduiding is van hoe hoë temperatuur die virus ondedruk (afdeling 4.2.6). Vyf-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red, het baie eenvormig gereageer met vier STV isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Daar is aanduidings van interaksies tussen sommige pomelo seleksies en sekere STV isolate. Die kruisbeskermings-eienskappe van elk van die vier isolate vir die verskillende pomelo seleksies sal met tyd bekend word (afdeling 4.2.7). Sewe sub-isolate van GFMS 12 (Nartia), vier enkel plantluis oordragings en drie gasheer skeidings, word ge-evalueer vir hul kruisbeskermings-eienskappe (virulensie, vermenigvuldiging, beweeglikheid) in vyf pomelo seleksies (Marsh, Star Ruby, Flame, Rio Red, Henderson) en vergelyk met die twee isolate wat tans vir pomelos in die bedryf gebruik word. Twee sub-isolate wat deur plantluis skeiding verkry is het goeie eienskappe vir kruisbeskerming getoon en was beter as beide die huidige kruisbeskermings-isolate. Sub-isolate 12/7 en 12/9 was hoogs oordraagbaar, vertoon eenvoudige enkel-string konformasie polimorfisme (SSCP) profile (n aanduiding van 'n enkel ligte ras) en het geen negatiewe effek op groei van al die pomelo seleksies gehad nie. Evaluasies van hierdie twee sub-isolate word voortgesit, afsonderlik en in kombinasie (afdeling 4.2.8).

Studies om die effek van STV op tolerante cultivars te bepaal en om geskikte isolate vir kruisbeskerming te identifiseer, gaan voort. 'n Proef is op Addo gevestig om die invloed van STV isolate op Clementine te bepaal en om geskikte ligte isolate te identifiseer wat in 'n kruisbeskermingsprogram gebruik kan word. Die Marisol bome, op verskillende onderstamme, is nou sewe jaar oud en is met verskillende STV isolate gepreïmmuniseer voordat hulle in die boord geplant is. Die effek van die verskillende STV isolate op vruggehalte (grootte) word uitgedruk in hul markwaarde. Die berekening van die produksiewaarde, gebaseer op produksie, vruggrootheid en markpryse, toon aan dat bome wat met GFMS 12 (Nartia) ge-preïmmuniseer was, 'n 12% hoër inkomste gelewer het as bome wat met LMS 6 ge-preïmmuniseer was. Die verskil is 8% minder as in 2002 en dit is moontlik dat die huidige voordeel van bome met GFMS 12 oor die lang termyn verder sal afneem. Nietemin, die verskil tussen bome met die twee isolate is nie betekenisvol nie en regverdig nie 'n verandering van die preïmmuniserings-isolaat nie. Die doel van die proef is bereik en word gesluit (afdeling 4.2.9). Die effek van verskillende STV isolate word op drie Valencia bostamme (McClellan, McClellan Saadloos en Delta) ge-evalueer. Die drie-jaar oue bome het vir die eerste keer vrugte geproduseer. Van die bostamme, het McClellan saadloos betekenisvol beter geproduseer as McClellan en Delta bome. Bome wat gepreïmmuniseer is met LMS 6 was nie die grootste nie maar het die beste geproduseer en daarom ook die hoogste produksie doeltreffendheid gehad (afdeling 4.2.10). Verskillende STV isolate word in Palmer nawel op verskillende kommersiële onderstamme (Growweskil suurlemoen, Troyer citrange, Swingle citrumelo, C35 citrange) in die Oos Kaap geëvalueer. Die vier-jaar oue bome met LMS 6 en SM 45 isolate was betekenisvol groter as bome wat met isolate SM 36 en SM 41 gepreïmmuniseer is asook die met die bekende strawwe isolaat. Die onderstamme het tans geen effek op boom grootte nie (afdeling 4.2.11). Indeksresultate gedurende 2002 het getoon dat die STV isolate vanaf Shamouti bome sonder entverbindings-abnormaliteit nie noodwendig lig is nie. Dit dui aan dat daar nie 'n korrelasie is tussen die STV strafheid en entverbindings-abnormaliteit by Shamouti bome nie. Turkey Valencia het stamgleuf simptome ontwikkel na die inokulasie van sekere STV isolate en daarom wil dit voorkom asof dit meer gevoelig is vir STV as wat aangeneem word. 'n Ondersoek is geloods na die teenwoordigheid van stamgleuf in Turkey Valencia in boord bome in verskillende lokaliteite. Bome op verskeie onderstamme by die Sitrus Grondvesblok (ou oopblok bome), Addo Navorsingstasie en Crocodile Valley Landgoed is ondersoek vir die

teenwoordigheid van stamgleuf. Stamgleuf is gevind in bome in al die lokaliteite ongeag die onderstam. Aangesien Turkey Valencia 'n vroeë Valencia is, vorm dit 'n belangrike deel van sitrus produksie in die bedryf en daarom bly dit 'n hoë prioriteit om 'n geskikte STV isolaat te vind om Turkey Valencia te preïmmuniseer (afdeling 4.2.12). Die biologiese indeksering van sitrus bontblaar virus was onsuksesvol. Daar is geen bewyse van die teenwoordigheid van die virus in Suid Afrika nie. Volgens navorsers in Spanje is die virus nie maklik na indikator plante oordraagbaar nie, moontlik as gevolg van 'n lae virus titer (afdeling 4.2.13).

Die inokulasie van sitrusskroei in Delta Valencia bome op verskillende onderstamme induseer 'n afname in boom grootte en oesopbrengs in vergelyking met ongeïnokuleerde bome. Serologiese analises van die 12-kd proteïen wat slegs in sitrusskroei besmette bome voorkom, is gebruik om bome te identifiseer wat met sitrusskroei besmet is. Uitslae bevestig die visuele simptome in die boord maar identifiseer ook die siekte in 'n vroeë stadium wanneer geen simptome nog waargeneem kan word nie. Onderstamme soos C35 citrange, Empress mandaryn en Carrizo citrange is die meeste ge-afekteer deur sitrusskroei. Bome op M&T en Swingle onderstamme, wat in sitrusskroei gebiede gebruik word om dooie bome te vervang, se boomgrootte is met 16 en 25 % repektiewelik, verminder in die proef. Onderstam X639 toon die meeste verdraagsaamheid (afdeling 4.2.14).

Die volgende projekte is gesluit na die bedanking van mnr. Michael Luttig (LNR-ITSG): 1) Serologiese opsporing van entoordraagbare siektes van sitrus; 2) Onderskeiding van *Sitrus tristeza virus* isolate gebaseer op die 5'-terminale area van die genoom; 3) Die bepaling van beweging en verspreiding van ligte *Sitrus tristeza virus* isolate in gashere, 'n eienskap wat belangrik is vir doeltreffende kruisbeskerming; 4) Die teenwoordigheid van *Liberibacter*, die veroorsakende organisme van Huanglongbing (vergroening), in boord sitrus bladvlooi; 5) Skeiding van GXI *Sitrus tristeza virus* rasse, karakterisering en evaluasie vir kruisbeskerming teen Huanglongbing en *Sitrus tristeza virus*.

Project summary

Progress in *Citrus tristeza virus* (CTV) research enables a better understanding of the disease and therefore assists with the application of control measures. Since CTV is endemic in southern Africa, the only control measure for the disease is mild strain cross-protection. The long-term assessment of new better cross-protecting isolates is a restriction that delays optimal control measures to obtain maximum production. Grapefruit forms an important part of the southern African citrus industry, however, production and fruit size are greatly affected by CTV. The search for better cross-protecting isolates is therefore a continuous process. A large number of new isolates that were collected in different grapefruit production areas were evaluated in the glasshouse. Indexing during 2002 showed that only 19 of 107 isolates have potential as cross-protectors. Additional indexing was done to compare the isolates with the present pre-immunizing isolates. Sub-isolates of GFMS 12 (Nartia) from the ITSC and Beltsville were included for the evaluation. The field isolates were also indexed for the presence of citrus viroids on virus-free Etrog citron. It was found that five isolates were contaminated with viroids, eight reduced growth and induced severe stem pitting, while two had low virus titre. Therefore, of the 107 isolates, only four will be evaluated in the field for their cross-protecting abilities (section 4.2.2). Biological indexing of the 21 best trees of the trial planting at New Venture revealed that only two trees contain mild isolates. These isolates are incorporated with other isolates for comparative tests in section 4.2.2. The present field trial was harvested for the second year by the farmer without taking records and because no proper controls were included in the lay-out of the trial, it will be terminated (4.2.3). Finally the Beltsville sub-isolates are in the orchard. Star Ruby and Marsh grapefruit trees were inoculated with the best sub-isolates and pre-immunization was confirmed by ELISA before they were planted in the field. The Star Ruby trees were planted at Tambuti Estates in Swaziland and the Marsh trees at Riversbend in the Nkwaleni Valley (4.2.4). In the trial where different new CTV isolates are evaluated as cross-protectors for Star Ruby grapefruit, trees pre-immunized with GFMS 35 (the present cross-protecting isolate for red grapefruit) and GFMS 78 gave the best production over a five-year period. The difference in production between the trees of these two isolates was 0.5 kg. These trees produced significantly better than trees that were planted virus-free, trees with mild isolates GFMS 12, GFMS 67 and those with the two severe isolates. Calculating the crop value (fruit size and market prices), the crop value of trees with GFMS 78 was 1% better than that of trees with GFMS 35. During the previous season, trees with GFMS 35 were 5% better than those with GFMS 78. Trees with GFMS 12a were third best and were 12% lower than the best. The latter isolate was collected from the original good parent tree at the Citrus Foundation Block. According to the status of this trial it should be terminated. At this stage the results have shown that CTV isolate GFMS 35, which is the present pre-immunizing isolate for red grapefruit, together with isolate GFMS 78, are superior to the other isolates under test. However, it will be beneficial to see if the superiority will be maintained and to what extent tree life and economic production is increased (4.2.5). With the evaluation of CTV isolates at two localities, results at Nelspruit confirm that GFMS 12 is not suitable as a cross-protector for Star Ruby grapefruit. However, the results of younger trees at Malelane do not compliment the Nelspruit results at this stage. At Nelspruit, with its cooler climate, the effect of the CTV

isolates on growth and stem pitting development is more drastic. At Malelane there is no difference in stem pitting development in trees with the mild isolates and the severe isolate, indicating a suppression of the virus by high temperature (4.2.6). Five-year-old trees of seven red grapefruit selections (Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben, Oran Red) reacted very similarly to four mild CTV isolates (GFMS 12, GFMS 35, GFMS 67, GFMS 73). There are indications of interactions between some isolates and grapefruit selections *viz.* canopy volumes of Rio Red trees with GFMS 12, and Star Ruby, Rio Red and Ruben trees with GFMS 73 were significantly reduced. There was no difference in the occurrence of stem pitting between selections and isolates. The cross-protection abilities of each of the isolates in the different selections will be revealed over time (4.2.7). Seven sub-isolates of GFMS 12 (Nartia), four single aphid transmitted and three host separated, are evaluated for their cross-protecting traits in five grapefruit selections (Marsh, Star Ruby, Flame, Rio Red, Henderson) and compared to the two cross-protecting isolates currently being used in the industry. Two sub-isolates showed good traits as cross-protectors and were better than both the isolates in the industry. Sub-isolates GFMS 12/7 and GFMS 12/9 were highly transmissible, display simple bands (indication of single strains by SSCP analysis) and had no effect on growth of all the grapefruit selections. Therefore, evaluations of these two subs, singly and in combination will be continued in the glasshouse and in the field (4.2.8).

Studies to determine the effect of CTV on tolerant cultivars and to identify suitable isolates for cross-protection are continuing. A trial was initiated to establish the effects of CTV on Clementine and to identify isolates that can be used beneficially as a pre-immunizing agent. The effect of different CTV isolates on fruit quality (size) is expressed in their market value. It is essential that trees pre-immunized with a mild isolate should produce a high yield of good quality. This will ensure the highest income to the producer. The projection of the crop value, based on yield, fruit size and market prices, shows that 7-year-old Clementine trees pre-immunized with GFMS 12 had a 12% better income than those pre-immunized with LMS 6. The difference is 8% less than 2002 and it is possible that the present benefit of GFMS 12 will further decrease in the long term. However, the difference between these two isolates is not significant and LMS 6 can remain as the pre-immunizing isolate for Clementine. The objective of the trial has been achieved and it will be terminated (4.2.9).

The effects of CTV on three Valencia scions (McClellan, McClellan Seedless and Delta Valencia) were evaluated at Malelane. The three-year-old trees bore fruit for the first time this year. Of the scions, McClellan Seedless Valencia yielded significantly better than McClellan and Delta Valencia trees. Trees with isolate LMS 6 were not the largest but yielded best and therefore had the highest yield efficiency (4.2.10). Results on the evaluation of different CTV isolates in Palmer navel on four commercial rootstocks (Rough lemon, Troyer citrange, Swingle citrumelo, C35 citrange) for the Eastern Cape showed that trees with isolates, LMS 6 and SM 45 were significantly larger and did not differ from trees that were planted virus-free. Overall the rootstocks did not affect tree size (4.2.11). Turkey Valencia developed stem pitting after the inoculation with mild CTV isolates. An investigation was made of the occurrence of stem pitting on field trees at different locations. Trees on several rootstocks at the Citrus Foundation Block (old open block trees), Addo Research Station and Crocodile Valley Citrus Co. were examined for the presence of stem pitting. It was found in all the trees regardless of the rootstock. The finding indicates that Turkey Valencia is more sensitive to CTV than other Valencia cultivars. Since Turkey Valencia is an early cultivar, it forms an important part of citrus production, and therefore the identification of a suitable CTV isolate for cross-protection remains a high priority (4.2.12). The biological indexing for citrus leaf blotch virus was unsuccessful. There is no evidence that this virus is present in South Africa. According to researchers in Spain, the virus is not easily transmitted to host plants, possibly because of a low titre (4.2.13).

The inoculation of citrus Blight (CB) to Delta Valencia trees on different rootstocks induced a decrease in tree size and production in comparison with un-inoculated trees. Serological analysis of the 12-kd protein, that only occurs in CB-affected trees, was used to identify infected trees. Results confirmed the visual symptoms in the orchard but also identified early infections where no symptoms could be observed. Rootstocks such as C 35 citrange, Empress mandarin and Carrizo citrange were the most affected. The sizes of trees on MxT and Swingle citrumelo rootstocks were reduced by 16 and 25% respectively. These two rootstocks are used to replace trees in CB areas. Rootstock X639 showed the most tolerance (4.2.14).

The following experiments were terminated with the resignation of Mr. Michael Luttig (ARC-ITSC): 1) Serological detection of citrus graft transmissible diseases; 2) *Citrus tristeza virus* differentiation based on 5'-terminal genomic region; 3) Determination of the movement and distribution of mild CTV isolates in hosts, a trait important for effective cross-protection; 4) Presence of *Liberibacter*, the causal agent of Huanglongbing, in field psylla; 5) Separation of GXI strains, characterization and evaluation for cross-protection against Huanglongbing and *Citrus tristeza virus*.

4.2.2 Glasshouse evaluation of *Citrus tristeza virus* isolates for cross-protection of grapefruit and sweet oranges

Experiment 49 by JHJ Breytenbach (CRI)

Opsomming

Een van die belangrikste eienskappe in 'n suksesvolle kruisbeskermingsprogram om *Sitrus tristeza virus* (STV) te beheer is om gedurig op soek te wees na 'n beter beskermings-isolaat. As gevolg van die feit dat die Nartia STV isolaat (GFMS 12), wat huidiglik gebruik word om wit pomelo enthout te beskerm, besmet is met 'n strawwe STV ras, is dit noodsaaklik om te soek na beter isolate in boorde vanaf ou bome wat steeds 'n goeie produksie lewer van goeie gehalte uitvoerbare vrugte en min stamgleuf en terugsterwing siektesimptome toon. Eenhonderd en sewe bome is geïdentifiseer in die verskillende pomelo produksie gebiede. Enthout is van hierdie bome gesny en in die glashuis geïnkuleer op virusvrye Meksikaanse lemmetjie (sensitiewe indikator kultivar vir STV). Indeksing gedurende 2003 het slegs 19 isolate van die oorspronklike 107 geïdentifiseer wat potensiaal het om as kruisbeskermers gebruik te word. 'n Verdere biologiese indeksing is gedoen om die 19 isolate se virulensie te bepaal asook hoe hulle vergelyk met die huidige GFMS12 en GFMS35 STV isolate. Nartia sub-isolate van die ITSG en Beltsville is ook ingesluit in hierdie evaluering. Die boord isolate is ook geïndekseer op virusvrye Etrog sitroen om te bepaal of die isolate vry is van sitrus viroïde. Daar is gevind dat vyf van die isolate met viroïde besmet was, agt het groei gestrem en strawwe stamgleuf veroorsaak terwyl twee lae virus titer getoon het. Dus, van die 107 isolate is daar vier wat uiteindelik in die boord ge-evalueer gaan word

Introduction

Very little is known regarding the biological activity and transmissibility of *Citrus tristeza virus* (CTV) strains present in the citrus areas of southern Africa. Reports of severe outbreaks of decline caused by CTV have been received from Nkweleni, Zululand and various parts of the world. CTV severity has the potential to change without warning and can cause severe decline even in tolerant cultivars such as sweet orange. It is therefore imperative that the biological activity of our local strains be determined and prospective protective mild isolates be selected for cross-protection purposes. The Nartia mild isolate (GFMS 12), currently being used to pre-immunize white grapefruit in southern Africa has been found to be contaminated with severe stem pitting strains. It is important that this source be separated into its mild and severe forms and the mild isolates be characterized and tested for their cross-protection abilities.

Materials and methods

Candidate trees were selected in a survey in all the different grapefruit production areas on a continuous basis i.e. outstanding trees with good export fruit size, mild stem pitting and showing no decline. Trees were made from these parent trees, believed to be infected with non-virulent strains of CTV. Budwood from 107 trees were collected, trees were made and are kept in the glasshouse at CRI. A biological indexing study was done where buds were taken from these plants and inoculating them onto virus free Mexican lime plants which are sensitive to CTV. These host plants will develop symptoms characteristic of the biological activity of an isolate. After six months growth since inoculation was measured, stem pits were counted under a stereo microscope after removal of the bark. Pitting per square cm of stem was calculated. After the initial evaluation the procedure will be repeated by comparing the most promising isolates to each other and to the current GFMS 12 (standard for white grapefruit) and GFMS 35 (standard for red grapefruit) cross-protecting isolates as controls. Prospective mild isolates will be challenged with severe isolates to test their protective ability and ultimately evaluated in the field.

Results and discussion

Nineteen of the best field isolates were compared with each other in a biological evaluation test where they were inoculated onto virus-free Mexican lime indicator plants. GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit), GFMS 12/7, GFMS 12/9 (single aphid transfers from ITSC) and the six selected single aphid transfers from Beltsville (389-1, 389-3, 389-4, 390-3, 390-4 and 390-5) were included in the test. The host plants were evaluated for growth and stem pitting (Table 4.2.2.1). The virus titre was determined by means of ELISA (enzyme-linked immunosorbent assay) six months after inoculation.

Field isolates NV 16/2, NV 41/2, ORE 8 and Tshipise 19/5 with growth of 670 to 900 mm during six months, also displayed mild pitting (0-20 pits/m²), Beltsville sub-isolates (B389/1, B389/3, B389/4, B390/3, B390/4 and B390/5) had a growth of 830 to 1240 mm with mild pitting. Sub-isolates B389/3 and B390/4 had a very low ELISA reading and was confirmed in trees prepared for a field experiment (virus-free Marsh grapefruit and Star Ruby inoculated with the Beltsville isolates – exp. 679). Because of the poor multiplication and

movement of the virus, these two sub-isolates together with field isolates; Nyalazi 3/34 and Tshipise 8/17, will be eliminated from future evaluations. The ITSC sub-isolates (GFMS12/7 and GFMS12/9) had a growth of 670 – 830 mm also with mild pitting. The presence of a severe strain in the Nartia isolate (GFMS12) is once again confirmed in this study with growth that varied between 55 – 118 mm. The field isolates also appear to have a diversity of strains amongst them as in the case of the Nartia isolate (van Vuuren, *et al.*, 2000).

Further evaluation of the best isolates as cross-protectors for Marsh and Star Ruby grapefruit trees will be done in the orchard.

Citrus viroid indexing

The 19 selected field isolates were indexed onto virus-free Etrog citron plants to test for the presence of citrus viroids. Isolates of CVd-CEV (exocortis) and CVd-GRP III (gum pocket) were included as positive controls. These plants were kept in the glasshouse at 32°C. The following isolates tested positive for citrus viroids: Bedhlane AP5, Bedhlane AP17, Bedhlane AP18, Mouton and Douw. Therefore, they will be excluded from further evaluations.

Table 4.2.2.1 Comparison of growth, stem pitting (SP) development and virus titre (ELISA) in Mexican lime host plants that were bud-inoculated with the 19 selected field isolates and the different 'nartia' sub-isolates from Beltsville and the ITSC.

Selected field isolates	Growth (mm)	SP/cm ²	ELISA OD ₄₀₅
Tambankulu 1	456 abc	8.0 ab	1.761 b
Tambankulu 9	405 ab	70.2 d	1.744 b
New Venture 16/2	726 efgh	3.90 ab	1.724 b
New Venture 41/2	691 defg	2.18 a	1.747 b
ORE 4	618 cde	13.58 ab	1.758 b
ORE 5	586 cde	5.14 ab	1.733 b
ORE 7	663 defg	10.51 ab	1.777 b
ORE 8	685 defg	2.20 a	1.773 b
Crookes 8	663 defg	7.7 ab	1.767 b
Harmony E7	341 a	55.73 c	1.731 b
Esselen 6A6	535 bcd	17.24 b	1.737 b
Tshipise 8/17	1335 l	0.01 a	0.314 a
Tshipise 19/5	711 defg	1.71 a	1.725 b
Nyalazi 3/34	896 hi	0.01 a	0.296 a
GFMS12/7	668 defg	2.39 a	1.575 b
GFMS12/9	827 fghi	9.13 ab	1.064 ab
GFMS12	830 ghi	3.3 a	1.733 b
GFMS35	690 defg	0.53 a	1.738 b
B390/3	821 fghi	0.37 a	1.702 b
B390/4	1240 kl	0.01 a	0.327 a
B390/5	998 ij	0.06 a	1.725 b
B389/1	983 ij	0 a	1.714 b
B389/3	1148 jk	0.01 a	0.022 a
B389/4	1093 jk	0 a	1.720 b
Virus Free	1215 kl	0 a	0.016 a
Bedhlane AP5	***	***	***
Bedhlane AP17	***	***	***
Bedhlane AP18	***	***	***

Mouton	***	***	***
Douw	***	***	***

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers LSD).

** Stem pitting: 0-20 pits/cm² = mild; 21-50 pits/cm² = moderate; 51+ pits/cm² = severe.

*** Field Isolates contaminated with citrus viroids.

Conclusion

The fact that some of the field isolates appear mild upon the biological assay does not mean that they necessarily have good cross-protecting abilities. Field evaluation will ultimately establish the integrity of the protective abilities of these isolates.

Future research

The best field isolates, two ITSC sub-isolates and four Beltsville sub-isolates will be budded to virus-free Marsh and Star Ruby grapefruit trees, and planted in two grapefruit production areas as orchard experiments. Once they become established they will be evaluated for growth (canopy volume), stem pitting, yield and fruit size.

Literature cited

Van Vuuren, S.P., van der Vyver, J.B. & Luttig, M. 2000. Diversity among sub-isolates of cross-protecting citrus tristeza virus isolates in South Africa. Proc. 14th Conf. IOCV., 103-110.

4.2.3 Cross protection of Star Ruby grapefruit Experiment 423 by JHJ Breytenbach (CRI)

Opsomming

Gedurende 'n STV opname in die Nkwaleni vallei, is 10 moederbome gekies in 11-13 jaar oue Star Ruby boorde. Enthout is gesny van hierdie moederbome en geokuleer op Carrizo citrange onderstamme. Die boompies is uitgeplant in die Nkwaleni vallei en word jaarliks vir produksie, vruggrootte, boom grootte en stamgleuf ge-evalueer. Een en twintig bome van die oorspronklike 100 in die proef is gekies op grond van vroeë prestasie, vir biologiese indeksering in die glashuis. Biologiese indeksering van die 21 bome is voltooi. Evaluasie volgens groeitempo en stamgleuf, het slegs twee isolate (NV16/2 en NV41/2) ligte eienskappe getoon en word verder in 'n kruisbeskermingsprogram ge-evalueer. Die twee isolate word nou met 17 ander boord isolate wat gekies is, tesame met die Beltsville sub-isolate ge-evalueer en vergelyk met GFMS 12 (standard vir wit pomelos) en GFMS 35 (standard vir rooi pomelos).

Introduction

Field observations and glasshouse experiments have shown that Star Ruby (SR) grapefruit is more sensitive to CTV stem pitting isolates than other grapefruit selections. The Star Ruby industry is increasing and is very lucrative, more so than any other selection, but CTV remains the single limiting factor in the production of this selection. It can reduce the economic life span to between 5 and 7 years.

Materials and methods

During a survey of selected SR orchards in southern Africa in 1995 (Marais & Breytenbach 1996), 10 parent trees were selected in 11-13 year old SR orchards at Bolton Estates as potential sources of mild isolates. Budwood were cut from these trees and budded to Carrizo citrange rootstock. Each source was replicated 10 times and planted in a completely randomized design at New Venture during 1997. Yield, fruit size and degree of stem pitting will be monitored each year.

Results and discussion

As in the 2002 season, these trees were once again harvested by the owner before they were evaluated.

Conclusion

It was mentioned in the report of 2002 that there is not a control treatment in the trial and if no meaningful results are obtained in 2003, the trial will be terminated. The fruit was harvested for a second time in

succession and therefore no results were obtained. However, the evaluation of two isolates (NV16/2 en NV41/2) that showed potential in a biological indexing study in the glasshouse will continue (see section 4.2.2).

Future research

This experiment will be terminated and isolates with potential incorporated into experiment 49 (section 4.2.2).

4.2.4 Cross-protection of Marsh and Star Ruby grapefruit using Beltsville sub-isolates of *Nartia* mild strain

Experiment 679 by JHJ Breytenbach (CRI)

Opsomming

Daar is gevind dat die *Nartia* isolaat (GFMS 12) wat huidiglik gebruik word vir pre-immuisering van wit pomelos, gekontamineer is met strawwe stamgleuf *Sitrus tristeza virus* rasse. Twintig sub-isolate van die oorspronklike isolaat is in Beltsville MD, VSA, voorberei deur middel van enkel plantluis oordragings. Ses van die 20 sub-isolate wat 'n potensiaal as kruisbeskermings-agente 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 gepreïmuniseer met die ses Beltsville sub-isolate asook twee enkel plantluis oordragings sub-isolate van die ITSG (GFMS 12/7, GFMS 12/9), GFMS12, GFMS35 en boompies is virusvry gelaat as kontrole. Preïmunisasie is bevestig deur middel van ELISA. ELISA het uitgewys dat twee van die Beltsville sub-isolate nie voldoen aan sekere vereistes vir 'n goeie kruisbeskermings-isolaat nie deurdat hulle swak oordraagbaar is, stadig vermeerder en stadig beweeg in die plant. Die twee sub-isolate word nie verder ge-evalueer nie.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by 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 produced by CTV range from mild with no noticeable effect on the host to severe stem pitting and decline resulting in uneconomic production. The only practical means of controlling CTV disease at present is by mild strain cross-protection (syn. pre-immunization). A breakdown in the protection offered by the 'nartia' (GFMS 12) isolate owing to the presence of a possible severe strain within the complex (Marais, *et al.* 1996), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained and in this study they were evaluated for mildness and their potential as cross protecting isolates.

Materials and Methods

The 20 SAT sub-isolates of the 'nartia' isolate were prepared at the quarantine facility in Beltsville MD, USA and were imported back to South Africa. They were bud-inoculated separately to CTV-sensitive Mexican lime indicator plants. Growth and stem pitting were determined and the virus titre was measured by means of ELISA (enzyme-linked immunosorbent assay) for each sub-isolate six months after inoculation. The Mexican lime indicator plants that were used, was a differential host that develop symptoms characteristic of the biological activity of a sub-isolate. The six mildest sub-isolates (B389-1, B389-3, B389-4, B390-3, B390-4 and B390-5) were bud inoculated (pre-immunization) to virus-free Marsh and Star Ruby grapefruit on MxT rootstocks. They will be compared with GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit) (van Vuuren, *et al.*, 1993), GFMS 12/7 and GFMS12/9 (ITSC single aphid transfer sub-isolates) (van Vuuren, *et al.*, 2000). Pre-immunization was confirmed by ELISA six months after inoculation.

Results

Confirming pre-immunization by ELISA, it was revealed that trees pre-immunized with two of the sub-isolates (B389/3 and B390/4) contained a low virus titre (Table 4.2.4.1). Because of the poor virus multiplication and movement of these sub-isolates they will be excluded from further evaluation. The Star Ruby trees were planted in orchards in Swaziland at Tambuti Estates and the Marsh grapefruit trees at Riversbend in the Nkweleni area.

Table 4.2.4.1 ELISA readings of different CTV isolates and sub-isolates in Marsh and Star Ruby grapefruit three months after pre-immunization.

Sub-isolate	ELISA OD ₄₀₅	
	Marsh Grapefruit	Star Ruby
B389/1	0.231	0.353
B389/3*	0.010	0.003
B389/4	0.401	0.900
B390/3	0.340	0.415
B390/4*	0.021	0.005
B390/5	0.678	0.717
GFMS12/7	0.469	0.423
GFMS12/9	0.667	0.739
GFMS12	0.291	0.945
GFMS35	0.251	0.772
Virus free	0.013	0.011

* Sub-isolates that will be excluded from further evaluations because of their low ELISA readings.

Conclusion

These isolates appear mild upon biological assay but this does not mean that they necessarily have a good cross-protecting ability. Therefore the sub-isolates that showed potential in this study were planted as an orchard trial to test them against challenges from severe strains in nature. They will be evaluated for growth, yield and fruit size to see if they indeed produce more, and better quality fruit than GFMS12.

Future research

Evaluate the horticultural performance of trees over a 10-year cycle using the following parameters:

- Canopy volume,
- Stem pitting,
- Yield and fruit size.

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4.2.5 The response of Star Ruby to different CTV isolates

561015: Trial 1 by S.P. van Vuuren (ARC-ITSC)

Opsomming

In die proef waar verskillende *Sitrus tristeza virus* (STV) isolate as kruisbeskermings-agente vir Star Ruby geëvalueer word, het bome met isolate GFMS 35 en GFMS 78 die beste produksie oor 'n periode van vyf jaar gelewer. Die verskil in die kumulatiewe produksie van bome met die twee isolate was 0,5 kg. Bome met hierdie twee isolate het betekenisvol beter geproduseer as bome wat virusvry geplant is, die wat met GFMS 12 (Nartia) en GFMS 67 gepreïmmuniseer is en die met die twee strawwe isolate.

Berekening van die oeswaarde (vruggrootte en mark pryse) wys dat die oeswaarde van bome met GFMS 78 1% beter is as die met GFMS 35. Gedurende die vorige seisoen (2002) was bome met GFMS 35 5% beter as die met GFMS 78. Bome met GFMS 12a het die derde beste oeswaarde gehad en was 12% laer as die van GFMS 78. Isolaat GFMS 12a is van die oorspronklike goeie Star Ruby moederboom by die Sitrus Grondvesblok versamel. Die meerderheid van die Star Ruby bome in die bedryf wat tans in produksie is se oorsprong is vanaf die moederboom.

Volgens die status van die proef moet dit gesluit word. Die resultate toon dat isolate GFMS 35 (kruisbeskermings-agent tans vir Star Ruby gebruik word) en GFMS 78 uitstaande kruisbeskermers vir Star Ruby pomelo is in vergelyking met die ander isolate wat ge-evalueer is. Nietemin, dit sal voordelig wees dat die proef voortgesit word om te bepaal tot watter mate boom leeftyd en ekonomiese produksie verleng word.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources (de Lange, *et al.*, 1981). In South Africa, the benefit of optimum growth and production of virus-free trees cannot be utilized 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 isolate.

Of the commercial citrus cultivars grown in Southern Africa, grapefruit is the most sensitive to the disease which causes stem pitting, decline and production of small fruit. With the initiation of the Southern African Citrus Improvement Programme (CIP), all grapefruit selections were pre-immunized with the GFMS 12 CTV isolate (von Broembsen & Lee, 1988). This isolate originated from a 50-year-old *Nartia* grapefruit tree in the Western Cape. Bud-wood source trees at the Foundation Block 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 the 6-year-old Star Ruby bud-wood source 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 isolate and that segregation of the strains, where the severe strain became dominant, may 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-immunization of all red grapefruit in the interim.

The first step in searching for mild isolates for cross protection purposes is to look for old trees which are healthy and produce good quality fruit (Müller & Costa, 1987). The Star Ruby grapefruit industry in South Africa started in the late 1970s and therefore no trees older than 15 years existed at the time. To overcome this problem, the best producers in the oldest plantings at Malelane and Swaziland were selected. Isolates from these trees were evaluated in glasshouse tests and those with the best potential had to be evaluated in the field.

The objective of the study is to find superior CTV isolates for pre-immunization that will maximize the profitability (productive life and quality) of Star Ruby grapefruit.

Materials and methods

Virus-free Star Ruby trees on Swingle citrumelo rootstock was grown under aphid-free conditions. When the scions have developed to approximately 5 mm diameter, they were bud-inoculated with different isolates of CTV. These isolates were selected from healthy-looking trees and showed potential as cross-protecting isolates in glasshouse tests. The following treatments were applied in replicates of five:

1. GFMS 12 (derived from *Nartia* grapefruit A – standard at the time);
2. GFMS 12a (derived from good-looking Star Ruby mother tree at OFB – pre-immunized with GFMS 12);
3. GFMS 12b (derived from GFMS 12 pre-immunized mother tree at OFB, displaying severe stem pitting and small fruit);
4. GFMS 35 (derived from Rosé grapefruit at Komatipoort. Marsh grapefruit trees pre-immunized with this isolate performed better than trees with GFMS 12 over a 12-year period. Present pre-immunizing isolate for red grapefruit);
5. GFMS 65 (derived from a Star Ruby tree at Tambankulu Estates, Swaziland);
6. GFMS 67 (similar than 5);
7. GFMS 71 (derived from old bud wood source Star Ruby, Esselen Nursery, Malelane);
8. GFMS 73 (similar than 7);

9. GFMS 77 (similar than 7);
10. GFMS 78 (derived from 10-year-old planting, F. Esselen, Malelane);
11. GFSS 1 (derived from 5-year-old Marsh grapefruit tree with severe stem pitting, Nkwaleni Valley);
12. GFSS 5 (derived from 5-year-old Star Ruby grapefruit with severe stem pitting, F. Esselen, Malelane).

After infection was confirmed by ELISA, the trees were planted at Nelspruit according to a randomized block design.

The following data are taken:

Tree size is measured annually;

Fruit are harvested, graded in export sizes and weighed;

Tree health is monitored by evaluating stem pitting and decline.

To evaluate the approximate monetary value of each CTV isolate, a projection was made for a hectare planting (6 X 3 spacing). The average production over three years of such a planting was determined and the value calculated according to fruit size distribution of each treatment. The value of the crop in relation to fruit size was determined by calculating the average value per export box for ten years. The highest price equaled a value of ten while the other values were calculated accordingly. The value of the crop per hectare for each treatment was determined by multiplying the production of a specific fruit size (export box) by the value for that size.

Results and discussion

Growth, production and disease rating of the six-year-old Star Ruby trees on Swingle citrumelo rootstock are given in Table 4.2.5.1.

Tree size. Trees pre-immunized with GFMS 12a, GFMS 12b, GFMS 35, GFMS 73 and GFMS 76, as well as those that were planted virus-free, were significantly larger than the trees with GFMS 65 and the two severe isolates.

Yield at year six. Trees preimmunized with GFMS 35 produced the largest crop and was significantly larger than that of trees pre-immunized with GFMS 12, GFMS 67, GFMS 71, GFMS 73, the trees that were planted virus-free and those with the two severe isolates.

Yield efficiency. The highest yield efficiency (kg/canopy volume) was achieved by trees pre-immunized with GFMS 65 but was not significantly better than that of trees pre-immunized with isolates GFMS 35, GFMS 67, GFMS 77 and GFMS 78.

Stem pitting. Trunks of trees with isolates GFMS 35 and GFMS 78 showed no pitting. Except for the trees with the two severe isolates, severe pitting occurred in trees pre-immunized with GFMS 12 (Nartia), GFMS 12b, GFMS 65 and GFMS 67. No decline occurred in any treatment but one tree with GFSS 5 died.

Cumulative yield. The highest cumulative yield over four seasons was produced by trees pre-immunized by GFMS 35 but was not significantly better than that of trees pre-immunized with GFMS 12a, GFMS 12b, GFMS 65, GFMS 76, GFMS 77 and GFMS 78.

Crop value. High yields can reduce fruit size and therefore the value of the crop. However, trees with GFMS 35 also had the best crop value, 5% better than that of trees with GFMS 78 which was second best.

Table 4.2.5.1. The average tree size, production, yield efficiency and stem pitting rating of seven-year-old Star Ruby trees pre-immunized with different tristeza isolates. The cumulative yield for five years and the relative monetary value is presented. The control trees were planted virus-free.

Tristeza isolate	Tree canopy size (m ³)	2003 yield (kg)	Yield efficiency (kg/m ³)	Stem pitting rating	Cumulative yield 1999 - 2003	Relative crop value/ha/yr
Control	7.2 a	39 c	5.4 ef	0.6 abc	87 d	6658
GFMS 12	5.9 abc	46 bc	7.8 bcde	2.8 e	111 cd	8426
GFMS 12a	7.6 a	64 ab	8.4 bcd	0.2 ab	158 abc	11696
GFMS 12b	7.3 a	63 ab	8.6 bcd	2.5 e	153 abc	11534
GFMS 35	7.1 a	76 a	10.7 abc	0.0 a	174 a	13588
GFMS 65	4.0 bcd	57 abc	14.3 a	2.5 e	134 abcd	10147
GFMS 67	5.4 abc	55 bc	10.2 abcd	2.2 de	118 bcd	8862
GFMS 71	6.5 ab	53 bc	8.2 bcde	1.4 cd	123 bcd	9186
GFMS 73	7.6 a	51 bc	6.7 de	1.1 bc	123 bcd	9133
GFMS 76	7.5 a	59 abc	7.9 cde	1.2 bcd	136 abcd	10240
GFMS 77	5.5 abc	56 abc	10.2 ab	0.9 abc	148 abc	11197

GFMS 78	6.3	ab	65	ab	10.3	abcd	0.0	a	164	ab	12877
GFSS 1	3.4	cd	11	d	3.2	f	3.0	e	26	e	1533
GFSS 5	1.9	d	11	d	5.8	ef	2.6	e	22	e	1461

* 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 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

Trees pre-immunized with GFMS 35, the present cross protector, were superior after five production years. Isolates GFMS 12a and GFMS 78 showed promise.

Future research

Measure trees, rate disease occurrence, harvest and grade fruit.

Acknowledgements

Kobus Breytenbach (CRI) is acknowledged for his assistance.

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4.2.6 Field evaluation of promising mild isolates for Star Ruby in two climatic areas

561015: Trial 2 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Net soos in 2002 het die produksie van die 7-jaar oue Star Ruby pomelo bome wat met verskillende *Sitrus tristeza virus* isolate (GFMS 35, GFMS 67, GFMS 71, GFMS 73, LMS 6) geïnkuleer is nie van mekaar verskil nie. Kumulatief was die produksie en oeswaarde van bome met GFMS 35 betekenisvol beter as bome met GFMS 71 en die wat met 'n strawwe isolaat geïnkuleer is. Matige tot strawwe stamgleuf is waargeneem waar GFMS 12 (Nartia) en 'n strawwe isolaat gebruik is.

Introduction

(Refer to section 4.2.5).

The aim of the study is to find a better CTV isolate for Star Ruby grapefruit that will be stable in different climatic conditions.

Materials and methods

Virus-free Star Ruby grapefruit, budded onto Swingle citrumelo rootstock, were inoculated with five mild isolates of CTV (GFMS 35, GFMS 67, GFMS 71, GFMS 73, LMS 6). These trees will be compared to trees with GFMS 12 (standard at the time) and GFSS 5 (severe) isolates as well as trees planted virus-free. Subsequent to the initiation of this trial, GFMS 35 was approved to replace GFMS 12 as the pre-immunizing isolate for red grapefruit and LMS 6 showed promise as a pre-immunizing isolate for grapefruit in another trial (van Vuuren & van der Vyver, 2000).

The trees were planted at Nelspruit (1996) and Malelane (1998) in randomized blocks with five replications.

Results and discussion

Growth, stem pitting rating, production and cumulative production over four years are given in Table 4.2.6.1.

Nelspruit. Similarly to 2002, the yield of the 7-year-old trees of all the treatments with mild CTV isolates did not differ from each other. Cumulatively, the production of trees with GFMS 35 was significantly better than those with GFMS 71 and the known severe control. Trees with GFMS 35 had the highest crop value followed by trees that were planted virus-free and those pre-immunized with LMS 6. Moderate to severe stem pitting occurred where GFMS 12 and the severe isolate were used.

Malelane. No yield data was obtained at this site since the trees were harvested accidentally by the farm manager without taking records. The largest trees were those pre-immunized by GFMS 73 and LMS 6. They were significantly larger than trees with GFMS 35, those that were planted virus-free and those with the severe isolate. The occurrence of stem pitting was similar to the Nelspruit site.

Table 4.2.6.1. The average tree size, stem pitting rating and production of 7-year-old Star Ruby trees pre-immunized with different CTV isolates and planted at Nelspruit .

TRISTEZA	TREE SIZE (m ³)	PITTING RATING**	2003 YIELD (kg)	CUMELATIVE YIELD 1999 - 2003
Control	7.6 b	2.2 bcd	122.3 A	189.9 ab
GFMS 12	9.6 ab	3.0 d	101.8 A	173.2 ab
GFMS 35	7.2 b	2.0 abcd	118.8 A	219.2 a
GFMS 67	9.3 ab	1.6 abc	98.9 A	196.0 ab
GFMS 71	7.3 b	1.8 abc	87.9 A	128.0 bc
GFMS 73	10.7 a	1.0 a	111.1 A	148.4 ab
LMS 6	11.6 a	1.2 ab	123.0 A	162.0 ab
GFSS 5 (severe)	2.3 c	2.4 cd	30.7 B	46.7 c

* Figures in each column followed by the same letter do not differ significantly at the 5% level (LSD).

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

At this early stage the results at Nelspruit confirm that GFMS 12 is not suitable as a cross-protector for Star Ruby grapefruit, however, the results of the younger trees at Malelane do not complement the Nelspruit results at this stage. At Nelspruit the best production was achieved by trees pre-immunised by GFMS 35 (present pre-immunising isolate for red grapefruit) but at Malelane trees pre-immunised with this isolate are surprisingly poor.

Future research

Measure trees, rate disease occurrence, harvest and grade fruit.

References cited

(Refer to section 4.2.5).

4.2.7 **The response of different red grapefruit cultivars to *Citrus tristeza virus***
561015: Trial 3 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Vyf-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red, het baie eenvormig gereageer met vier STV isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Daar is aanduidings van interaksies tussen sommige STV isolate en pomelo seleksies, bv. Rio Red bome met GFMS 12 en Star Ruby, Rio Red en Ruben bome met GFMS 73 se kroon volumes was betekenisvol kleiner. Daar was geen verskil in die voorkoms van stamgleuf tussen die seleksies of isolate nie. Die kruisbeskermings-eienskappe van elk van die vier isolate vir die verskillende pomelo seleksies sal met tyd bekend word.

Introduction

(Refer to section 4.2.5).

The objective of this study is to evaluate new CTV isolates in different red grapefruit selections.

Materials and methods

Seven red grapefruit selections viz. Star Ruby, Flame, Rio Red, Nelruby, Henderson, Ruben and Oran Red were budded as scions on Swingle citrumelo rootstocks. Tristeza isolates GFMS 35, GFMS 67, GFMS 71 and GFMS 73 are evaluated in each scion and compared to the standard (GFMS 12) and a severe isolate (GFSS 5). Infection was confirmed by ELISA before they were planted in a randomized split plot with five replications at Malelane during December 1998.

Results and discussion

Tree size and stem pitting ratings of the red grapefruit selections that were pre-immunized with different CTV isolates are presented in Table 4.2.7.1 and Table 4.2.7.2 respectively.

Tree size: Overall, the canopy sizes of the trees pre-immunized with the different CTV isolates did not differ from each other. Trees with the severe isolate were significantly smaller. Of the selections, tree sizes of Nel Ruby and Ruben were significantly larger than those of Oran Red.

The results indicate interactions between Rio Red trees with GFMS 12, Star Ruby, Rio Red and Ruben trees with GFMS 73, where tree volumes were significantly reduced.

Production: No yield data was obtained since the trees were harvested accidentally by the farm manager without taking records.

Stem pitting: Overall the stem pitting did not differ among trees pre-immunized with the different CTV isolates or the selections. Stem pitting varied the least in trees of all the grapefruit selections where GFMS 35 was pre-immunized.

Table 4.2.7.1. The effect of different CTV isolates on tree size (canopy volume = m³) of 5-year-old red grapefruit selections

GRAPEFRUIT SELECTIONS	CTV ISOLATES						MEAN
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5		
STAR RUBY	10.5 ab	12.2 abc	11.9 ab	8.9 b	4.0 c	9.5 wx	
RIO RED	9.4 bc	15.0 a	12.9 ab	11.0 ab	5.2 bc	10.7 wx	
HENDERSON	9.8 ab	9.0 c	10.8 bc	9.0 b	6.8 b	9.1 wx	
NEL RUBY	12.1 ab	14.2 ab	13.6 a	12.5 a	6.0 bc	11.7 w	
FLAME	12.1 ab	11.5 bc	11.8 ab	10.9 ab	6.8 b	10.6 wx	
RUBEN	12.3 a	11.9 abc	13.9 a	9.8 ab	9.6 a	11.5 w	
ORAN RED	6.6 c	8.7 c	8.5 c	8.5 b	4.4 bc	7.3 x	
MEAN	10.4 y	11.8 y	11.5 y	10.3 y	6.1 z		

Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.7.2. The effect of different CTV isolates on stem pitting (rating^{**}) of 5-year-old red grapefruit selections

GRAPEFRUIT SELECTIONS	CTV ISOLATES											
	GFMS 12		GFMS 35		GFMS 67		GFMS 73		GFSS 5		MEAN	
STAR RUBY	1.0	a	1.4	a	1.0	a	1.0	a	3.0	b	1.5	x
RIO RED	1.6	abc	1.2	a	1.0	a	1.4	ab	1.2	a	1.3	x
HENDERSON	1.0	a	1.6	a	1.8	b	1.4	ab	1.2	a	1.4	x
NEL RUBY	2.0	c	1.8	a	1.0	a	1.6	b	1.4	a	1.6	x
FLAME	1.2	ab	1.8	a	1.4	ab	1.0	a	1.6	a	1.4	x
RUBEN	1.6	abc	1.0	a	1.2	ab	1.0	a	1.2	a	1.2	x
ORAN RED	1.8	bc	1.0	a	1.2	ab	1.0	a	1.6	a	1.3	x
MEAN	1.5	z	1.4	z	1.2	z	1.2	z	1.6	z		

Figures in each column followed by the same letter do not differ significantly at the 5% level (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 isolates were very similar at this stage and the cross-protection ability of each isolate for the selections can only be measured over time.

Future research

Measure trees, rate disease occurrence, harvest and grade fruit.

References cited

(Refer to section 4.2.5).

4.2.8 Evaluation of cross protecting traits of *Citrus tristeza virus* sub-isolates and the use of sub-isolates with specific characteristics to construct a superior isolate for the protection of grapefruit

561022: Trial 1 by S.P. van Vuuren & M. Luttig (ARC-ITSC)

Opsomming

Sewe sub-isolate van GFMS 12 (Nartia), vier enkel plantluis oordragings en drie gasheer skeidings, word ge-evalueer vir hul kruisbeskermings-eienskappe (virulensie, vermenigvuldiging, beweeglikheid) in vyf pomelo seleksies (Marsh, Star Ruby, Flame, Rio Red, Henderson) en vergelyk met die twee isolate wat tans vir pomelos in die bedryf gebruik word. Twee sub-isolate wat deur plantluis skeiding verkry is het goeie eienskappe vir kruisbeskerming getoon en was beter as beide die huidige kruisbeskermings-isolate. Sub-isolate 12/7 en 12/9 was hoogs oordraagbaar, vertoon eenvoudige enkel-string konformasie polimorfism (SSCP) profiele (n aanduiding van n enkel ligte ras) en het geen negatiewe effek op groei van al die pomelo seleksies gehad nie. Evaluasies van hierdie twee sub-isolate word voortgesit, afsonderlik en in kombinasie.

Introduction

The traits (biological activity (severity), multiplication, movement) for good cross protecting *Citrus tristeza virus* (CTV) isolates have been described (Lee, *et al.*, 1987). Many CTV strains exist in South Africa and, because of the abundance of the aphid vectors, occur naturally as mixtures in trees (McClellan, 1963; Racca, *et al.*, 1978). Vastly different biological activities have been reported for trees propagated from the same bud wood source but planted under different growing conditions (da Graça, *et al.*, 1984). Müller (1980) has proposed that the biological activity expressed by an isolate depend on which strain predominates. Some CTV strains are better adapted to warm conditions, whereas some prefer cooler temperatures (van Vuuren, 1982). Extremely hot growing conditions reduce CTV titre and can result in temporary thermotherapy (Garnsey, *et al.*, 1980). It has been shown that the host can favor the multiplication and movement of specific strains (Moreno, *et al.*, 1963). This will lead to different symptom expressions when isolates are constituted of several strains. When a severe strain is present and becomes the prevailing strain, it will result in serious consequences.

Because of the diversity of climatic conditions in which different grapefruit selections are cultivated in South Africa (Barry, 1996), it is important for a good cross-protector to adapt to all these variable factors. If that is not possible, it appears that it will be necessary to identify specific isolates for each grapefruit selection (Star Ruby, Flame, Rio Red, Marsh, etc.) as well as for the different production areas (*i.e.* for hot humid coastal areas, hot and humid inland areas, hot and dry inland areas, etc.).

Several sub-isolates were obtained from the GFMS 12 isolate by single aphid transfers as well as by host passage (van Vuuren, *et al.*, 2000; unpublished data). Apart from the difference of aphid transmissibility between the two groups, biological and molecular differences were also established among sub-isolates within each group (van Vuuren, *et al.*, 2000; Luttig, *et al.*, 2001; unpublished data). Each of these sub-isolates may have special cross-protecting properties and in combination may support or complement each other so that such an isolate is adaptable to different conditions. The traits of these sub-isolates regarding the two main factors viz. host and environment, should be established to enable the construction of an isolate that may be suitable for different hosts and environmental conditions.

It was found that sub-isolates from isolates GFMS 12 and GFMS 35 differ in transmissibility and movement. Where the aim was to identify single strains for cross-protection to minimize variability, it appeared that movement of the sub-isolates varied among hosts and in specific environmental conditions. The incomplete invasion of the whole plant by the protecting strain leaves the plant vulnerable to infection by severe strains introduced by aphid vectors. The sub-isolates of GFMS 12 appear to be more stable in variable conditions than those of GFMS 35 (unpublished data).

The objective of this study is to evaluate sub-isolates of GFMS 12, aphid and host separated, and construct a cross-protecting isolate from these sub-isolates, considering specific characteristics that will complement each other in all the grapefruit selections in different climatic conditions.

Materials and methods

Five virus-free grapefruit selections (Marsh, Star Ruby, Flame, Rio Red, Henderson) were established on Troyer citrange rootstock under aphid-free conditions according to normal nursery practices. When the scions had developed to a thickness of approximately 5 mm, they were bud-inoculated by two buds containing selected CTV sub-isolates (subs) of GFMS 12 (12/2, 12/5, 12/7, 12/9, S12, F12, H20). The subs were selected according to their biological activity in hosts as well as molecular differences (van Vuuren, *et al.* 2000; Luttig, *et al.*, 2001; unpublished data). These subs were compared to GFMS 12 (standard isolate for white grapefruit), GFMS 35 (standard isolate for red grapefruit) and un-inoculated plants as controls. Each scion/CTV combination was replicated five times and after inoculation the plants were cut back at two buds above the inoculation point to force new growth. They were kept at a temperature regime of 28-32°C in a glasshouse with additional lighting to exclude the effect of short daylight on growth (Roistacher and Nauer, 1985).

To establish multiplication and movement of the CTV in each host, enzyme-linked immunosorbent assay (ELISA) was performed on the subsequent flush at six weeks after inoculation to establish the presence and titre of the virus. The top two leaves of the flush were sampled for the test. The procedure was repeated at 12, 24 and 28 weeks after inoculation.

At six months the growth since inoculation was removed, measured and the bark stripped to evaluate stem pitting. Samples were taken to perform single-strand conformation polymorphism (SSCP) analysis to compare profiles of each isolate and sub in the different hosts.

The plants were allowed to re-grow, allowing two shoots on each scion. When the new shoots were approximately 3-5 cm long, one shoot was harvested for ELISA. The second shoot was left and growth and stem pitting were evaluated six months later.

A preliminary evaluation was done by constructing CTV isolates by bud-inoculating some subs, singly and in combination, into virus-free Mexican lime seedlings. After six months ELISA was done on the plants, the growth measured and stem pitting assessed.

Double strand RNA isolation and reverse transcription polymerase chain reaction (RT-PCR): Thirty mg bark from each sample were frozen with liquid nitrogen and pulverized, and total RNA was extracted using a small-scale isolation method (Zhou, 2001). Complimentary DNA was synthesized from total RNA by reverse transcription and PCR amplification using the Titan One Tube RT-PCR System (Roche Diagnostics, GmbH) and primers defined for amplification of gene fragment p27B and gene p25 (Luttig *et al.*, 2003).

Single-strand conformation polymorphism (SSCP) analysis: For SSCP analysis, a modified procedure described by Yap and McGee (1994) was followed. Gels were stained with silver nitrate (Beidler *et al.*, 1982).

Results and discussion

The plants were inoculated with the different CTV isolates and sub-isolates in January 2002.

Following on the 2002 report:

GROWTH: The length of the shoots of the grapefruit selections after the first six months and the second six months post inoculation with the different CTV isolates and subs, as well as the totals for the year were established.

Marsh: The plants inoculated with GFMS 12 (Nartia) grew the best and they were significantly larger than plants inoculated with subs 12/2, 12/5, 12/7 and F12 during the first six months. During the second six months, growth of plants with all the isolates and subs did not differ.

Star Ruby: Plants with sub 12/7 grew the best in the first and second six-month cycles. During the first six months they were significantly larger than trees with sub S12 and the second six months, better than sub S12 again as well as plants with GFMS 35.

Flame: All the isolates were stable in this grapefruit selection and no significant differences occurred in the growth of the plants.

Rio Red: Plants with subs 12/5 and 12/9 grew the best and were significantly larger than plants with the original GFMS 12 isolate during the second six months.

Henderson: During the first six months plants with sub F12 grew the best and was significantly better than that of plants with the original GFMS 12 isolate. During the second six months, plants with GFMS 35 and sub F12 grew significantly better than those with sub 12/5.

STEM PITTING: Overall the stem pitting was very mild in all the grapefruit selections with the different CTV isolates and subs. Only two subs (S12 and F12, both host separated) induced stem pitting in all the grapefruit selections.

TREE HEALTH: Two plants died, one Marsh and one Henderson, both inoculated with sub H12. It cannot be concluded with certainty that the virus of this sub was responsible for the tree death. However, it will take too long for confirmation and therefore this sub will be excluded in further evaluations.

SUMMARY OF SSCP ANALYSIS: A single-strand conformation polymorphism (SSCP) profile for a CTV isolate or sub may be simplistic (2 prominent DNA bands), intermediate (3-4 prominent DNA bands) or complex (several prominent DNA bands). A simplistic profile usually indicates mild isolates or subs in a specific host, while severe isolates contain complex band patterns. Therefore, a simplistic SSCP profile for a mild isolate or sub with potential cross-protection ability is desirable.

According to the SSCP profiles obtained for gene fragment p27B the 9 isolates and subs were grouped into complex (>4 DNA bands), intermediate (3-4 DNA bands) and simplistic (2 DNA bands) categories for the 5 grapefruit selections. Those with a simplistic SSCP profile when pre-immunised in a specific grapefruit selection will remain stable. Mild isolates usually yield a SSCP profile with only two DNA bands. Pre-immunised grapefruit selections with complex profiles may become vulnerable for severe strain challenges over time if a shift in strain dominance may occur. A strain with undesirable traits may become dominant, resulting in the breakdown of cross protection.

The isolates and subs were grouped based on their SSCP profile complexity:

Simplistic (stable) p27B SSCP profiles: Star Ruby, Rio Red, Henderson, Flame, Marsh with GFMS 12; Henderson and Flame with GFMS 35; Henderson with sub S12; Star Ruby, Rio Red, Marsh with sub 12/2; Rio Red, Henderson, Flame, Marsh with sub 12/5; Star Ruby, Rio Red, Henderson, Flame, Marsh with sub 12/7; Star Ruby, Rio Red, Henderson, Flame, Marsh with sub 12/9; Henderson, Flame, Marsh with sub F12; Flame with sub H12.

Intermediate p27B SSCP profiles: Star Ruby and Marsh with GFMS 35; Marsh with sub S12; Henderson with sub 12/2; Star Ruby with sub 12/5; Star Ruby, Rio Red, Marsh and Henderson with sub H12.

Complex p27B SSCP profiles: Rio Red with GFMS 35; Star Ruby, Rio Red, and Flame with sub S12; Flame with sub 12/2; Star Ruby and Rio Red with sub F12.

Conclusion

Subs MS 12/7 and MS 12/9 were highly transmissible, display simple SSCP bands and had no effect on growth of all the grapefruit selections. Therefore, evaluations of these two subs, singly and in combination should be continued.

Future research

Inoculate five grapefruit selections on Troyer citrange rootstock. ELISA tests at 6 and 12 weeks after inoculation. Evaluate growth and stem pitting at 6 months. Establish a field trial with the same treatments. Plant and maintain field trial.

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4.2.9 Evaluation of CTV isolates in Clementine

561004: Trial 1 by S.P. van Vuuren (ARC-ITSC)

Opsomming

'n Proef is op Addo gevestig om die invloed van *Sitrus tristeza virus* (STV) isolate op Clementine te bepaal en om geskikte ligte isolate te identifiseer wat in 'n kruisbekeringsprogram gebruik kan word. Die Marisol bome, op verskillende onderstamme, is nou sewe jaar oud en is met verskillende STV isolate gepre-immuniseer voordat hulle in die boord geplant is. Die effek van die verskillende STV isolate op vruggehalte (grootte) word uitgedruk in hul markwaarde. Die berekening van die produksiewaarde, gebaseer op produksie, vruggrootte en markpryse, toon aan dat bome wat met GFMS 12 (Nartia) ge-pre-immuniseer was, 'n 12% hoër inkomste gelewer het as bome wat met LMS 6 ge-pre-immuniseer was. Die verskil is 8% minder as in 2002 en dit is moontlik dat die huidige voordeel van bome met GFMS 12 oor die lang termyn verder sal

afneem. Nietemin, die verskil tussen bome met die twee isolate is nie betekenisvol nie en regverdig nie 'n verandering van die preïmmuniserings-isolaat nie. Die doel van die proef is bereik en word gesluit.

Introduction

The failure of sour orange as a rootstock for most 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 isolates is the most successful approach to reduce the effect of the disease (Müller & Costa, 1972; van Vuuren, *et al.*, 1993a, 1993b).

Since the establishment of the South African citrus industry on tolerant rootstocks, it was generally accepted that tristeza virus 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 effect 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 Programme 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 isolates with regard to cross protection is specific with regard to biological activity (Müller & Costa, 1987; Van Vuuren, *et al.*, 1993b) and therefore, mild CTV isolates specifically for tolerant cultivars should be identified.

This study was initiated to establish the effects of CTV on Clementine and to identify isolates that can be used beneficially as a pre-immunizing agent.

Materials and methods

Rootstocks. Rough lemon is the most commonly used rootstock in South Africa and the Wallace selection was recommended (H.J. Breed, personal communication). At the time the trial was initiated Volckameriana, Troyer citrange and Yuma citrange were equally used in the industry with the latter two gaining more interest. Yuma citrange is a semi-dwarf rootstock and it appears that transmissible pathogens (viruses and viroids) increase the dwarfing effect which make it suitable for high density plantings (Rabe, *et al.*, 1992). Gou Tou is a new rootstock selection and it was included being a sour orange hybrid showing CTV tolerance and supporting higher levels of *Phytophthora* and citrus nematodes (van Vuuren, *et al.*, 1991).

Scions. Virus-free Marisol Clementine was used as a scion for each rootstock.

CTV isolates. The following CTV isolates that showed promise in previous field and glasshouse trials were selected: GFMS 12 (pre-immunizing isolate for sweet orange at the time - standard), LMS 6, SM 34, SM 36 and SM 41. Control treatments included a known severe isolate (SOSS 2) and virus-free planted trees.

Tree preparation. The rootstocks were grown under insect-free conditions and budded according to normal nursery practices. When the scions had developed approximately 300 mm, the CTV isolates were bud-inoculated into the scions. The virus-free control trees were left un-inoculated. Three months were allowed for proper distribution of the CTV isolates in the plants. ELISA was performed to confirm infection whereafter the trees were planted in the field.

Sites and lay-out. The trees were planted at Addo in 1996 according to a split-plot design with five replications and at a spacing of 6 x 3 m.

Records. Canopy volumes of the trees were determined by measuring the tree canopy and calculating the volumes according to Burger *et al.* (1970).

Fruit was harvested, sized according to export requirements and weighed.

Yield efficiency has been calculated.

To evaluate the approximate monetary value of each CTV isolate, a projection was made for a hectare planting (6 X 3 spacing). The production of such a planting was determined and the value calculated according to fruit size distribution of each treatment. The value of the crop in relation to fruit size was determined by calculating the average value per export box for each size at all the major overseas markets. The highest price equaled a value of ten while the other values were calculated accordingly. The value of the

crop per hectare for each treatment was determined by multiplying the production of a specific fruit size (export box) by the value for that size.

Results and discussion

The effect of different CTV isolates on tree size of 7-year-old Clementines is presented in Table 4.2.9.1. Overall, the largest trees were those pre-immunized with GFMS 12 and LMS 6. The new mild isolates derived from sweet orange (SM 34, SM 36, SM 41) and the known severe isolate (SOSS 2) reduced tree size. The trees with SOSS 2 were significantly smaller than trees pre-immunized with GFMS 12 and LMS 6. This isolate affected trees on all the rootstocks.

The first crop was produced when the trees were three years old. The production for 2003 is presented in Table 4.2.9.2 and yield efficiency in Table 4.2.9.3. The cumulative production over a four-year period is shown in Table 4.2.9.4. The highest yield for 2003 was produced by trees pre-immunized by LMS 6, however, the highest cumulative yield was produced by trees with GFMS 12. In both cases the two treatments did not differ significantly from each other. Trees with these two treatments produced significantly more than trees with SOSS 2 (severe). Although the yield efficiency of the smaller trees (SM 41, SOSS 2) was higher during the first two production years, the larger trees produce currently as well. Trees on Yuma citrange rootstock had a significant higher yield efficiency.

The projection of the crop value for each treatment is shown in Table 5. The crop value (fruit size coupled with market prices) of trees with GFMS 12 was the highest, 42% higher than trees with SM 41, which was the lowest. The second and third highest crop values, which were also higher than those of trees planted virus-free, were obtained from trees with SM 34 and LMS 6 respectively.

Table 4.2.9.1. The effect of different CTV isolates on the growth (canopy volume = m³) of 7-year-old Marisol Clementine on different rootstocks.¹

CTV ISOLATES	ROOTSTOCKS ²						MEAN					
	RL		VOLCK		GOU TOU			YUMA		TROYER		
GFMS 12	34.8	ab	25.7	cd	23.6	cde	13.5	gh	23.0	cde	24.1	u
LMS 6	34.3	ab	21.0	defg	26.6	cd	12.3	gh	24.0	cde	23.6	u
SM 34	28.8	bc	25.5	cd	22.8	cdef	3.3	j	14.2	gh	18.9	uv
SM 36	25.9	cd	26.3	cd	24.8	cd	9.9	hi	16.3	efgh	21.6	uv
SM 41	25.6	cd	17.5	defg	10.8	h	4.2	ij	18.1	defg	15.2	uv
SOSS 2	20.9	defg	16.2	fgh	10.1	hi	2.5	j	10.5	hi	12.0	v
Control	35.6	a	23.7	cde	25.4	cd	11.8	gh	21.6	defg	23.6	u
MEAN	29.4	x	22.3	y	21.3	y	8.2	z	18.2	y		

¹ Figures in the body of the table that are followed by the same letter do not differ significantly at the 5% level. Rootstock and CTV isolate means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

² Rootstocks: RL = Rough lemon, Volck = Volckameriana, Gou Tou = Gou Tou sour orange, Yuma = Yuma citrange, Troyer = Troyer citrange.

Table 4.2.9.2. Production (kg) of 7-year-old Marisol Clementine on different rootstocks and pre-immunized with different CTV isolates.

CTV ISOLATE	ROOTSTOCKS ²		VOLCK	GOU TOU	YUMA	TROYER	MEAN					
	RL											
GFMS 12	87.3	Bcd	74.2	bcdef	63.9	cdefg	77.8	bcde	84.0	bcde	77.4	u
LMS 6	117.1	A	83.2	bcde	68.4	bcdef	48.3	fg	77.5	bcde	78.9	u
SM 34	94.6	Ab	88.3	abcd	73.0	bcdef	26.5	hij	57.4	efg	68.0	uv
SM 36	57.8	Efg	78.2	bcde	60.1	defg	38.9	ghij	62.7	cdefg	59.5	uv
SM 41	90.3	Abc	73.6	bcdef	39.1	ghij	14.7	j	69.6	bcdef	57.5	uv
SOSS 2	73.2	Bcdef	63.4	cdefg	45.5	fghi	17.3	ij	46.0	fghi	49.0	v
Control	92.8	Ab	66.2	bcdefg	53.5	cdefg	57.8	efg	72.9	bcdef	68.6	uv
MEAN	87.6	W	75.3	wx	57.6	y	40.2	z	67.2	xy		

¹ Figures in the body of the table that are followed by the same letter do not differ significantly at the 5% level. Rootstock and CTV isolate means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

² Rootstocks: RL = Rough lemon, Volck = Volckameriana, Gou Tou = Gou Tou sour orange, Yuma = Yuma citrange, Troyer = Troyer citrange.

Table 4.2.9.3. Yield efficiency (kg/m³) of 7-year-old Marisol Clementine trees on different rootstocks and pre-immunized with different CTV isolates.¹

CTV ISOLATES	ROOTSTOCKS ²		VOLCK	GOU TOU	YUMA	TROYER	MEAN					
	RL											
GFMS 12	2.5	fg	3.0	efg	2.7	efg	6.0	abc	3.7	defg	3.6	v
LMS 6	3.4	defg	4.2	cdefg	2.5	fg	4.2	cdefg	3.2	efg	3.5	v
SM 34	3.3	defg	3.5	defg	3.3	defg	7.9	a	4.2	cdefg	4.4	v
SM 36	2.2	g	3.2	efg	2.7	efg	4.0	cdefg	4.5	cde	3.3	v
SM 41	3.5	defg	4.4	cdef	3.8	defg	3.0	efg	4.0	cdefg	3.7	v
SOSS 2	3.4	defg	4.5	cdef	4.5	cdef	6.7	ab	4.3	cdefg	4.7	v
Control	2.6	efg	2.7	efg	2.6	efg	5.3	bcd	3.8	defg	3.4	v
MEAN	3.0	z	3.6	z	3.2	z	5.3	y	4.0	z		

¹ Figures in the body of the table that are followed by the same letter do not differ significantly at the 5% level. Rootstock and CTV isolate means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

² Rootstocks: RL = Rough lemon, Volck = Volckameriana, Gou Tou = Gou Tou sour orange, Yuma = Yuma citrange, Troyer = Troyer citrange.

Table 4.2.9.4. Cumulative production (kg) of 7-year-old Marisol Clementine on different rootstocks and pre-immunized with different CTV isolates.

CTV ISOLATE	ROOTSTOCKS ²						MEAN
	RL	VOLCK	GOU TOU	YUMA	TROYER		
GFMS 12	286 a	208 cdefgh	165 fghij	207 cdefgh	280 ab	225 v	
LMS 6	259 abc	214 bcdefgh	165 fghij	183 defghi	222 abcdefgh	209 vw	
SM 34	241 abcde	248 Abcd	166 fghij	75 kl	190 cdefghi	184 vw	
SM 36	226 abcdefg	252 Abcd	166 fghij	155 hij	184 defghi	197 vw	
SM 41	221 abcdefg h	214 bcdefgh	109 jkl	61 l	174 efghij	156 vw	
SOSS 2	215 bcdefgh	196 cdefghi	125 ijkl	57 l	127 ijkl	144 w	
Control	235 abcdef	159 Ghij	134 ijk	186 defghi	237 abcde	190 vw	
MEAN	240 y	213 Y	147 z	132 z	202 y		

¹ Figures in the body of the table that are followed by the same letter do not differ significantly at the 5% level. Rootstock and CTV isolate means that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

² Rootstocks: RL = Rough lemon, Volck = Volckameriana, Gou Tou = Gou Tou sour orange, Yuma = Yuma citrange, Troyer = Troyer citrange.

Table 4.2.9.5. The effect of different CTV isolates on the cumulative production (kg) of Marisol Clementine trees over a 4-year period and the average annual crop value per hectare.¹

CTV ISOLATES	CUMELATIVE PRODUCTION	CROP VALUE ²
GFMS 12	224.6 A	23836
LMS 6	208.5 Ab	21042
SM 34	184.0 Ab	19942
SM 36	196.5 Ab	19657
SM 41	155.7 Ab	13776
SOSS 2	143.9 B	14641
CONTROL	190.1 Ab	16178

¹ Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

² 578 trees/ha.

Conclusion

The effect of the different CTV isolates on fruit quality (size) is expressed in their market value. It is essential that trees pre-immunized with a mild isolate should produce a high yield of good quality. This will ensure the highest income to the producer. The projection of the crop value, based on yield, fruit size and market prices, shows that trees pre-immunized with GFMS 12 had a 12% better income than those pre-immunized with LMS 6. The difference is 8% less than 2002 and it is possible that the present benefit of GFMS 12 will further decrease in the long term. However, the difference between these two isolates is not significant and LMS 6 can remain as the pre-immunizing isolate for Clementine.

Future research

The objective of the trial has been achieved and it will be terminated.

Acknowledgements

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4.2.10 Evaluation of CTV isolates in Valencia

561004: Trial 2 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Die effek van verskillende *Sitrus tristeza virus* isolate word op drie Valencia bostamme (McCleean, McCleean Saadloos en Delta) ge-evalueer. Die drie-jaar oue bome het vrugte vir die eerste keer geproduseer. Van die bostamme, het McCleean saadloos bome betekenisvol beter geproduseer as McCleean en Delta bome. Bome wat gepreïmmuniseer is met LMS 6 was nie die grootste nie maar het die beste geproduseer en daarom ook die hoogste produksie doeltreffendheid gehad.

Introduction

(Refer to section 4.2.9).

The objective of this trial is to evaluate promising CTV isolates in three Valencia scions and identify suitable cross-protecting isolates.

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 had developed to approximate five mm in diameter, they were inoculated with isolates derived from sweet orange and showed promise in glasshouse tests. The isolates are LMS 6 (standard), SM 36, SM 41, SM 45 and GXI. Trees with these

isolates were compared to trees with a severe isolate (SOSS 2) as well as un-inoculated virus-free (VF) plants.

The trees were planted in 2000 according to a split plot design with five replications at Malelane. The effect of the CTV isolates on growth, production, fruit size and tree health will be determined.

Results and discussion

Tree size

Tree sizes were measured and the canopy volumes calculated according to Burger *et al.* (1970) (Table 4.2.10.1). Overall the canopy volumes of the three Valencia selections did not differ significantly. With the CTV isolates however, trees with the GXI isolate had the largest canopies and they were significantly larger than those of trees with isolates LMS 6, SM 34, SM 36 and the trees that were planted virus-free. The smallest trees were pre-immunized with SM 36. Although this isolate has a mild effect on Mexican lime indicator plants, it causes stem pitting in sweet orange.

Comparing the effects of the individual CTV isolates on the growth of the three scions show that mild isolates LMS 6, GXI, SM 36 were stable in all the scions. With the other isolates, isolate SM 34 was significantly better in McClean Seedless Valencia than in both the other scions, SM 41 and SM 45 were significantly better in McClean Seedless Valencia than in McClean Valencia.

Production

The three-year-old trees bear fruit for the first time this year and the production of trees of each treatment is shown in Table 4.2.10.2 and the yield efficiency in Table 4.2.10.3. Overall, the McClean Seedless Valencia trees yielded significantly better than McClean and Delta Valencia trees. Of the CTV isolates, trees with isolate LMS 6 produced significantly better than trees with mild isolates SM 36, SM 45, the severe isolate SOSS 3 and the trees that were planted virus-free. The production of trees with isolates GXI, SM 34 and SM 41 were lower than that of trees with LMS 6 but not significantly.

The McClean Seedless trees with isolates SM 36 and SM 45 produced significantly lower than trees with LMS 6. With McClean, trees with isolates SM 34, SM 36, SM 41, SM 45, SOSS 3 and the virus-free trees produced significantly lower than trees with LMS 6. The Delta Valencia trees with SM 36 and those that were planted virus-free yielded significantly lower than trees with LMS 6.

Delta Valencia had the lowest yield efficiency, significantly lower than that of McClean Seedless but not significantly lower than McClean. Trees with LMS 6 had the highest yield efficiency and were significantly better than trees with SM 41, SM 45, SOSS 3 and those that were planted virus-free.

Table 4.2.10.1. Tree size (canopy volume in m³) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free.

CTV ISOLATE	SCION**			MEAN
	McC	McC SL	DELTA	
LMS 6	7.5 def	9.1 abcdef	7.5 def	8.0 y
GXI	9.5 abcde	11.1 ab	11.4 a	10.7 x
SM 34	6.7 fgh	10.3 abc	7.3 ef	8.1 y
SM 36	4.3 hi	3.9 i	4.6 ghi	4.3 z
SM 41	8.0 cdef	11.1 ab	9.3 abcde	9.5 xy
SM 45	8.0 cdef	11.5 a	9.3 abcde	9.6 xy
SOSS 3	8.1 cdef	10.1 abcd	8.7 bcdef	9.0 xy
VF	7.6 def	7.2 efg	7.6 def	7.5 y
MEAN	7.5 w	9.3 w	8.2 w	

* Figures in the body of the table followed by the same letter do not differ significantly at the 5% level. Means followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Table 4.2.10.2. The production (kg/tree) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free*.

CTV ISOLATE	SCION**			MEAN
	McC	McC SL	DELTA	
LMS 6	29.4 a	32.9 a	21.9 bcdef	28.1 v
GXI	24.8 abcd	28.1 abc	18.5 bcdefg	23.8 vw
SM 34	17.7 cdefg	28.9 abc	11.4 efgh	19.3 vw
SM 36	14.9 cdefgh	20.7 bcdef	6.1 h	13.9 w
SM 41	17.4 cdefg	22.0 abcdef	11.5 defgh	17.0 vw
SM 45	13.9 cdefgh	19.1 bcdefg	10.0 efgh	14.3 w
SOSS 3	12.1 defgh	24.4 abcdef	10.2 efgh	15.6 w
VF	8.4 fgh	22.5 abcdef	7.3 gh	12.7 w
MEAN	17.3 z	24.8 y	12.1 y	

* Figures in the body of the table followed by the same letter do not differ significantly at the 5% level. Means followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Table 4.2.10.3. The production efficiency (kg/m³ canopy) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free*.

CTV ISOLATE	SCION**			MEAN
	McC	McC SL	DELTA	
LMS 6	4.7 ab	3.8 bc	3.3 bcd	3.9 u
GXI	2.6 cdefg	2.6 cdefg	1.8 defg	2.3 uvw
SM 34	2.7 cdefg	3.0 bcdef	1.7 defg	2.5 uvw
SM 36	3.9 bc	6.0 a	1.3 fg	3.7 uv
SM 41	2.2 cdefg	2.4 cdefg	1.2 fg	1.9 vw
SM 45	1.9 defg	1.6 defg	1.1 g	1.5 w
SOSS 3	1.5 efg	2.5 cdefg	1.4 fg	1.8 w
VF	1.2 g	3.3 bcde	1.0 g	1.8 w
MEAN	2.6 yz	3.1 y	1.6 z	

* Figures in the body of the table followed by the same letter do not differ significantly at the 5% level. Means followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Conclusion

The three-year-old trees bore fruit for the first time this year. Of the scions, McClean Seedless Valencia yielded significantly better than McClean and Delta Valencia trees. Trees with isolate LMS 6 were not the largest but yielded best.

Future research

Harvest, size and weigh fruit. Determine tree size.

References cited

(Refer to section 4.2.9).

4.2.11 Evaluation of CTV isolates in navel

561004: Trial 4 by S.P.van Vuuren (ARC-ITSC)

Opsomming

Verskillende *Sitrus tristeza virus* isolate word in Palmer nawel op verskillende kommersiële onderstamme (Growweskil suurlemoen, Troyer citrange, Swingle citrumelo, C35 citrange) in die Oos Kaap geëvalueer. Die vier-jaar oue bome met LMS 6 en SM 45 isolate was betekenisvol groter as bome wat met isolate SM 36 en SM 41 gepreïmmuniseer is asook die met die bekende strawwe isolaat. Die onderstamme het tans geen effek op boom grootte nie.

Introduction

(Refer to section 4.2.9).

The aim of this research was to obtain suitable isolates to cross protect navel in the Eastern Cape production area.

Materials and methods

CTV isolates are evaluated in Palmer navel on four commercial rootstocks for that area *viz.* Rough lemon, Troyer citrange, Swingle citrumelo and C35 citrange (J. Miller, personal communication). Three tristeza virus isolates, with the seedling yellows component, (SM 36, SM 41, SM 45) are being evaluated and compared to trees with LMS 6 (standard), a severe isolate (SOSS 2) and trees that were left un-inoculated. The trees were prepared according to standard nursery practices in an aphid-free environment.

The trees were planted at Addo in November 1999 according to a split plot design with five replications. The effect of the CTV isolates on growth, production, fruit size and tree health was determined.

Results and discussion

The crop was harvested accidentally by the contractor and therefore no yield records were taken.

Overall, trees with isolate LMS 6 (present pre-immunizing isolate) were the largest but not significantly than those with isolate SM 36 and the control trees (Table 4.2.11.1). Trees with the severe isolate were significantly smaller than trees with isolates LMS 6, SM 36, SM 45 and the control trees.

Overall the rootstocks did not affect tree size.

Table 4.2.11.1. The effect of different CTV isolates on the growth (canopy volume = m³) of 4-year-old Palmer navel on different rootstocks¹.

CTV ISOLATE	ROOTSTOCK ²				MEAN
	RL	SWINGLE	TROYER	C 35	
LMS 6	5.4 abc	5.1 abcde	5.1 abcde	6.3 a	5.5 u
SM 36	3.8 cdefg	3.2 fg	3.5 defg	3.2 fg	3.4 vw
SM 41	5.3 abcd	4.0 cdefg	3.2 fg	3.5 efg	4.0 w
SM 45	5.5 abc	4.3 bcdef	5.2 abcde	5.3 abcd	5.1 u
SOSS 2	3.5 efg	3.0 fg	2.3 g	3.1 fg	3.0 w
CONTROL	5.4 abc	4.4 bcdef	4.4 bcdef	6.1 ab	5.1 u
MEAN	4.8 z	4.0 z	4.0 z	4.6 z	

¹ Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

² Rootstocks: RL = Rough lemon, Swing = Swingle citrumelo, Troyer = Troyer citrange, C35 = C35 citrange.

Conclusion

Overall the rootstocks did not affect tree size, however, the CTV isolates affected size. Trees with isolates, LMS 6 and SM 45 were significantly larger and did not differ from trees that were planted virus-free.

Future research

Harvest fruit and measure trees.

References cited

(Refer to section 4.2.9).

4.2.12 Identification of suitable *Citrus tristeza virus* isolates for pre-immunizing Midseason sweet oranges and Turkey Valencia 561023: Trial 1 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Indekseringsresultate gedurende 2002 het getoon dat die *Sitrus tristeza virus* (STV) isolate vanaf Shamouti bome sonder entverbindings-abnormaliteit nie noodwendig lig is nie. Dit dui aan dat daar nie 'n korrelasie is tussen die STV strafheid en entverbindings-abnormaliteit by Shamouti bome nie.

Turkey Valencia het stamgleuf simptome ontwikkel na die inokulasie van sekere STV isolate en daarom wil dit voorkom asof dit meer gevoelig is vir STV as wat aangeneem word. 'n Ondersoek is geloots na die teenwoordigheid van stamgleuf in Turkey Valencia in boord bome in verskillende lokaliteite. Bome op verskeie onderstamme by die Sitrus Grondvesblok (ou oop blok bome), Addo Navorsingstasie en Crocodile Valley Landgoed is ondersoek vir die teenwoordigheid van stamgleuf. Stamgleuf is gevind in bome in al die lokaliteite ongeag die onderstam. Aangesien Turkey Valencia 'n vroeë Valencia is, vorm dit 'n belangrike deel van sitrus produksie in die bedryf en daarom bly dit 'n hoë prioriteit om 'n geskikte STV isolaat te vind om Turkey Valencia te pre-immuniseer.

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 Programme, all citrus, including sweet oranges, was pre-immunized with a CTV isolate originating from grapefruit for the interim until suitable isolates was found for the different types (von Broembsen & Lee, 1988). Subsequently a suitable isolate, LMS 6, has been identified for lime (van Vuuren, *et al.*, 1993). LMS 6 contains a mild form of seedling yellows which the grapefruit isolate does not have, and therefore it was also approved to replace GFMS 12 as the pre-immunizing isolate 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). Young Shamouti plantings in South Africa, where the mother material was pre-immunized with GFMS 12, show bud-union creasing symptoms (personal observation). The transmissible factor was probably transferred to the virus-free shoot tip grafted material when it was pre-immunized by budding, indicating that the plant material with the GFMS 12 isolate contains this transmissible factor. It will therefore be beneficial to establish if LMS 6 isolate contains this factor and to identify a suitable CTV isolate that will improve tree life and fruit quality of midseason cultivars.

Recently it was found that Turkey Valencia trees on Rough lemon and Volckameriana rootstocks developed similar bud-union creasing symptoms (personal observation)(Beeton, *et al.*, 2000). This effect of a transmissible factor can also be excluded by the application of a suitable isolate for pre-immunization.

Bud-union crease has a permanent girdling effect resulting in an excessive yield of poor quality. Tree growth as well as production are affected.

The objective of this study is to evaluate field as well as constructed CTV isolates to identify a suitable cross-protecting isolate without the bud-union crease factor for Midseason sweet oranges and Turkey Valencia.

Materials and methods

CTV severity of isolates: Healthy Mexican lime seedlings were bud-inoculated with material collected from healthy looking and diseased Shamouti sweet orange trees at Crocodile Valley Estates, as well as CTV isolates LMS 6, GFMS 12, SM 45, SM 46 and SM 47. Two buds from each source were budded on each indicator plant and replicated three times. After inoculation the plants were kept at a temperature regime of 24-28°C for six months. The plants were evaluated for growth and stem pitting.

Orchard investigation. Turkey Valencia and Shamouti sweet orange were grown on Troyer citrange and Rough lemon rootstocks under aphid-free conditions. When the scions have developed to approximately 5

mm in diameter, the following sources of CTV isolates will be bud inoculated to each scion/rootstock combination, replicated five times:

1. LMS 6 (standard);
2. Constructed isolate with aphid-transmitted LMS 6 sub-isolates (6/1, 6/4, 6/6)(van Vuuren, *et al.*, 2000; Luttig, *et al.*, 2001);
3. GFMS 12;
4. Constructed isolate with aphid transmitted GFMS 12 sub-isolates (12/2, 12/5, 12/7)(van Vuuren, *et al.*, 2000; Luttig, *et al.*, 2001);
5. Healthy Shamouti from Crocodile Valley Estates;
6. Diseased Shamouti from Crocodile Valley Estates;
7. Diseased Turkey Valencia from Crocodile Valley Estates;
8. SM 36, derived from Valencia sweet orange;
9. SM 45, derived from Valencia sweet orange;
10. SM 46, derived from Midseason sweet orange;
11. SM 47, derived from Valencia sweet orange;
12. Un-inoculated control.

Three months will be allowed for the virus to spread through the plants, and after confirming infection with ELISA, will be planted in an orchard according to a randomized block design.

Tree growth and bud union crease will be monitored. When the trees come into production, fruit will be sized and weighed.

Results and discussion

The comparison of the CTV severity of different CTV isolates with those present in the Shamouti trees were presented in the 2002 report.

Due to the presence of citrus viroids in the Turkey Valencia budwood source, the orchard evaluations had to be postponed (2002 report). The plants that were inoculated with the different treatments were kept for a year whereafter the bud-unions were inspected for abnormalities after removing the bark. No bud-union abnormalities were observed that could be attributed to a treatment. However, CTV stem pitting was observed on the Turkey Valencia where SM 36, SM 47 and diseased Shamouti treatments were applied. This led to investigations of Turkey Valencia trees on several rootstocks at the Citrus Foundation Block (old open block trees), Addo Research Station and Crocodile Valley Estates. CTV stem pitting was found in all the trees regardless the rootstock. The finding indicates that Turkey Valencia is more sensitive to CTV than other Valencia cultivars. STG was redone on Turkey Valencia and budwood from the virus-free source is currently being multiplied.

Conclusion

Various sources of Turkey Valencia had stem pitting symptoms and it appears that this cultivar is more sensitive to CTV than other Valencia cultivars. Since Turkey Valencia is an early cultivar, it forms an important part of citrus production, and therefore the identification of a suitable CTV isolate for cross-protection remains a high priority.

Future research

Multiply virus-free Turkey Valencia budwood and bud to established Troyer citrange rootstocks. Inoculate scions with the different CTV isolates for a field trial.

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4.2.13 Indexing for citrus leaf blotch virus which is associated with bud-union crease 561023: Trial 2 by S.P. van Vuuren (ARC-ITSC)

Opsomming

Die biologiese indeksering van sitrus bontblaar virus was onsuksesvol. Daar is geen bewyse van die teenwoordigheid van die virus in Suid Afrika nie. Volgens navorsers in Spanje is die virus nie maklik na indikator plante oordraagbaar nie moontlik as gevolg van 'n lae virus titer.

Introduction

Recently a graft transmissible pathogen causing citrus bud union crease (CBC) of Nagami kumquat on Troyer citrange was characterised for host range and symptomatology (Galipienso, *et al.*, 2000). Nules Clementine and Eureka lemon developed bud-union crease six months after propagation while Marsh grapefruit and Pineapple sweet orange plants still showed normal bud-unions after one year. The pathogen was characterised and citrus leaf blotch virus was identified (Galipienso, *et al.*, 2001). It was also shown that other citrus varieties carry the virus (Vives, *et al.*, 2001).

The aim of this study is to investigate the use of indicators for detection of bud-union crease in South Africa.

Materials and methods

Virus-free Nules Clementine and Nagami kumquat were budded on *Poncirus trifoliata*, Troyer citrange and Swingle citrumelo rootstocks in the glasshouse. When the scions were approximately 5 mm thick the plants were bud-inoculated with the following sources and kept in a glasshouse at 22-26°C:

1. Turkey Valencia/Troyer citrange, displaying CBC symptoms;
2. Turkey Valencia/Swingle citrumelo, displaying no CBC symptoms;
3. Turkey Valencia from OFB, bud wood source for 1 and 2 (pre-immunised with GFMS 12);
4. Turkey Valencia pre-immunised with LMS 6;
5. Turkey Valencia, virus-free;
6. Shamouti displaying CBC symptoms;
7. Shamouti without CBC symptoms;
8. GFMS 12;
9. CD 8 (citrus viroids: CEV/GRP III);
10. Control

The plants are monitored regularly for abnormal leaf symptoms. At six months the plants were cut back and a piece of bark of approximately 3 mm² across the bud union was removed and the union inspected for creasing.

Twelve months after inoculation the plants were inspected finally.

All the sources were indexed for the presence of citrus viroids (CVd) on Etrog citron indicator.

Results and discussion

The Nules Clementine and Nagami kumquat indicator plants have developed no abnormal symptoms during the first six months after inoculation. After they were cut back at six months, some plants developed tattered leaf edges as well as yellow blotches on some leaves. Small pits (without gumming and different to CTV stem pitting) were observed at the unions of some plants after a piece of bark was removed but did not correlate with any treatment. It appears that plants on Troyer citrange rootstock are more subjected to the development of disorders.

Conclusion

At this stage no conclusion can be drawn, however, it appears that plants on Troyer citrange rootstock are more subjected to the development of disorders. There is no evidence of citrus leaf blotch. According to researchers in Spain citrus leaf blotch is not easily transmitted to indicator plants possibly because of a low titre of the virus.

Future research

None at this stage.

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4.2.14 Screening of rootstocks for Citrus Blight tolerance

Experiment 32 by JHJ Breytenbach (CRI)

Opsomming

Die inokulasie van sitruskroei in Delta Valencia bome op verskillende onderstamme induseer 'n afname in boom grootte en oes opbrengs in vergelyking met ongeïnokuleerde bome. Serologiese analises van die 12-kd proteïene wat slegs in sitruskroei besmette bome voorkom, is gebruik om bome te identifiseer wat met sitruskroei besmet is. Uitslae bevestig die visuele simptome in die boord maar identifiseer ook die siekte in 'n vroeë stadium wanneer geen simptome nog waargeneem kan word nie. Onderstamme soos C35 citrange, Empress mandaryn en Carrizo citrange is die meeste ge-afekteer deur sitruskroei. Bome op M&T en Swingle onderstamme, wat in sitruskroei gebiede gebruik word om dooie bome te vervang, se boomgrootte is met 16 en 25 % repektiewelik, verminder in die proef. Onderstam X639 toon die meeste verdraagsaamheid

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, 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 three months after root-graft inoculations (Derrick, *et al.*, 1993).

Until the inception of the Citrus Improvement Programme in South Africa in 1973, practically all commercial citrus orchards were established on Rough Lemon rootstock. Rough Lemon remained the most popular rootstock up 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 can be used successfully in CB affected areas.

Materials and methods

A rootstock experiment to test the tolerance of various rootstocks to CB has been established in Letsitele at Bosveld Citrus. The trial comprises Delta Valencia on Empress mandarin, Troyer citrange, *P. trifoliata*, Volckameriana, Sampson tangelo, Swingle citrumelo planted in 1990, in 1992 trees on MxT, X639, Gou Tou, Orlando Tangelo. Zhu Luan and Marsh grapefruit were planted, 1995 Cleopatra mandarin, C35 and Sun Chu Sha were added, and during 1996 trees on Benton citrange and Sunki mandarin were included.

Virus-free Delta Valencia scion material was used for all the 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 non-grafted trees constituted as the controls. The donor trees were selected using standard diagnostic techniques such as water uptake and zinc accumulation in the xylem. The trees were treated with granular formulations of Temik and Ridomil and trunk paint applications of Aliette, every three months to exclude the effects of *Phytophthora* and citrus nematode infections.

The following data are 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

Trees on X639 (early planting) and Benton citrange (later planting) rootstocks show the least effect of CB (Table 4.2.14.1). The presence of CB in South Africa has influenced the rootstock choice in affected areas. Rootstocks such as X639, MxT 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. Results of this trial show that trees on MxT and Swingle citrumelo rootstocks are affected by CB. The C35 citrange rootstock appears to be the most sensitive to CB.

The mean production of the control trees are higher with less small fruit than that of the CB-inoculated trees (Table 4.2.14.2). The effect of CB on production and fruit size will be emphasized more when trees start to decline.

The water uptake test shows a decrease in the water uptake ability, due to the presence of occlusions by amorphous plugs, of CB inoculated trees on most rootstocks (Table 4.2.14.3). Exceptions are trees on Sampson tangelo, X639, Gou Tou and Zhu Luan rootstocks. Trees on Sunki mandarin and Benton citrange were too small to apply the test.

The presence of the 12-kd protein was much higher in the root-grafted trees than in the control non-grafted trees (Table 4.2.14.4). The latter can get infected by natural means. The 12-kd protein was higher in control trees on X639, C35 citrange and Sunki mandarin rootstocks. The high 12-kd protein in the trees on X639 rootstock does not correlate with the growth of trees on this rootstock, but correlates with the water uptake. More data in this regard is necessary to make a conclusion.

Table 4.2.14.1. Comparison of tree size (canopy volume) of CB inoculated and un-inoculated (control) Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Tree volume (m ³)		% Difference
		Control	Inoculated	
<i>P. trifoliata</i>	1990	29.5	21.0	- 29
Swingle citrumelo	1990	56.7	42.6	- 25
Empress mandarin	1990	26.1	18.7	- 28
Carrizo citrange	1990	27.7	19.8	- 28
Volckameriana	1990	52.4	40.3	- 23
Sampson tangelo	1990	35.5	31.6	- 11
MxT	1992	37.5	30.7	- 18
X639	1992	30.5	43.7	+ 43
Gou Tou	1992	45.1	47.0	+ 4
Orlando tangelo	1992	34.4	36.0	+ 5
Zhu Luan	1992	12.3	12.9	+ 5
Marsh grapefruit	1992	15.1	19.4	+ 28
Cleopatra mandarin	1995	13.0	10.6	- 18
Sun Chu Sha	1995	16.3	16.8	+ 3
C35 citrange	1995	16.1	8.6	- 46
Sunki mandarin	1996	6.4	7.3	+ 14
Benton citrange	1996	2.9	4.4	+ 52

Table 4.2.14.2. Comparison of yield (kg) and % small fruit (< count 105) of control and CB inoculated Delta Valencia trees on different rootstocks.

Rootstock	Year planted	Control		Inoculated	
		Production	% Small fruit	Production	% Small fruit
<i>P. trifoliata</i>	1990	118.7	1.4	178.8	0.1
Swingle citrumelo	1990	126.8	1.8	66.0	4.2
Empress mandarin	1990	200.5	0.3	88.3	0.5
Carrizo citrange	1990	44.5	3.8	23.5	4.7
Volckameriana	1990	117.7	1.4	77.8	3.2
Sampson tangelo	1990	37.9	1.6	60.1	0.8
MxT	1992	44.6	1.4	29.7	2.4
X639	1992	77.7	0.8	95.4	0.9
Gou Tou	1992	27.9	1.4	55.1	0.2
Orlando tangelo	1992	78.8	1.9	80.7	1.5
Zhu Luan	1992	32.5	2.2	20.1	6.0
Marsh grapefruit	1992	17.9	0.6	25.5	1.2
Cleopatra mandarin	1995	26.7	6.0	0	0
Sun Chu Sha	1995	24.5	0.4	30.8	3.9
C35 citrange	1995	55.8	0.2	22.3	5.8
Sunki mandarin	1996	10.6	3.8	12.7	2.4
Benton citrange	1996	15.6	3.2	13.2	5.3
Mean		62.3	1.9	55.0	2.7

Table 4.2.14.3 Comparison of water-uptake (seconds/10 ml) through the trunk xylem of CB inoculated and control Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Inoculated	Control
<i>P. trifoliata</i>	1990	41.4	41.2
Swingle citrumelo	1990	31.6	25.1
Empress mandarin	1990	31.4	25.8
Carrizo citrange	1990	35.8	29.3
Volckameriana	1990	35.2	23.3
Sampson tangelo	1990	31.6	35.4
MxT	1992	30.3	23.5
X639	1992	20.8	36.1
Gou Tou	1992	13.7	31.0
Orlando tangelo	1992	26.7	25.3
Zhu Luan	1992	26.7	29.0
Marsh grapefruit	1992	31.0	18.0
Cleopatra mandarin	1995	44.0	30.0
Sun Chu Sha	1995	36.4	25.5
C35 citrange	1995	55.4	35.2
Sunki mandarin	1996	Too small	Too small
Benton citrange	1996	Too small	Too small

Table 4.2.14.4. Comparison of protein (12-kd) serological tests of CB inoculated and control Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	% Infected with 12-kd protein	
		Inoculated	Control
<i>P. trifoliata</i>	1990	33.3	16.6
Swingle citrumelo	1990	33.3	16.6
Empress mandarin	1990	50.0	16.6
Carrizo citrange	1990	83.3	16.6
Volckameriana	1990	50.0	16.6
Sampson tangelo	1990	0	16.6
MxT	1992	66.6	0
X639	1992	16.6	33.3
Gou Tou	1992	16.6	16.6
Orlando tangelo	1992	0	16.6
Zhu Luan	1992	33.3	33.3
Marsh grapefruit	1992	16.6	0
Cleopatra mandarin	1995	50.0	50.0
Sun Chu Sha	1995	16.6	0
C35 citrange	1995	66.6	83.3
Sunki mandarin	1996	16.6	50.0
Benton citrange	1996	50.0	0

Conclusion

Rootstocks X639, MxT and Swingle citrumelo are being used to establish new plantings in CB-affected areas. Swingle citrumelo rootstock is mainly used to replace trees that have succumbed to CB in existing orchards. Results of this trial show that trees on MxT and Swingle citrumelo rootstocks are affected by CB. Trees on X639 appear to exhibit the most tolerance.

Future Research

Continue to monitor disease development, measure canopy volumes and take yield data.

Reference cited

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4.3 PROJECT: FRUIT AND FOLIAR DISEASES

Project Co-ordinator: G.C. Schutte (CRI)

4.3.1 Project summary

Fruit and leaf lesions of an unknown origin were investigated on Palmer nawels as well as Delta and Midnight Valencias north-east of Naboomspruit. Isolations from the plant material yielded nothing that would cause these symptoms. But Tim Grout found a publication by B.J. Dippenaar where he described similar symptoms in 1958 and a comparative study showed that it was grey mites that caused the lesions on fruit and leaves (4.3.2). Due to the extremely dry season during the 2002/2003, *Alternaria* brown spot was not an enormous problem, very few growers reported problems and only one set of isolates could be obtained from a site at Brits. The project is continuing and isolates will be collected until a suitable student can be found to continue the study (4.3.5). Mancozeb residue-analyses were done from selected spray programmes that formed part of programmes consisting of either 1, 2 or 4 treatments. Results showed that two and four treatments, irrespective of their treatment dates during the season, resulted in unacceptable levels of more than 0.10 ppm (4.3.3). Concomitantly, one mancozeb treatment sprayed as late as January resulted in no detectable levels of mancozeb. The die-back of Clementines in the Knysna region, can be ascribed to *Phytophthora* and not *Diplodia* as mentioned before (4.3.4). Various sources of inoculum were identified and spray programmes consisting of different fungicides with different modes of action were selected and sprayed onto the trees to contain further spread of the disease. Trials are continuing to determine if secondary pathogens such as *Fusarium* and anthracnose play any role in the complex through the fulfillment of Koch's postulates. The *Pseudocercospora* situation in Zimbabwe cannot be monitored due to the current political climate, but there are consultants that constantly monitor the situation on the occupied farms.

Projekopsomming

Vrug- en blaarletsels van 'n onbekende aard is op Palmer nawels asook Delta – en Midnight Valencias noord-oos van Naboomspruit opgespoor. Isolasië vanuit plantmateriaal het niks opgelewer nie totdat 'n publikasie van B.J. Dippenaar soortgelyke simptome in 1958 beskryf het en met verdere bestudering bevestig het dat dit toegeskryf kan word aan grysmyt. Weens die uitermatige droë seisoen wat ondervind is in 2002/2003 was die voorkoms van *Alternaria* bruinvlek ook baie beperk en kon slegs een stel isolate van die siekte in die Brits omgewing gemaak word. Die projek duur voort en isolate sal voortdurend versamel word totdat 'n geskikte student vir 'n uitgebreide studie gevind word. Mancozeb residu-analise is gedoen van geselekteerde spuitprogramme waar dit deel gevorm het van die spuitprogram hetsy as 1,2 of 4 bespuitings. Resultate toon dat twee en vier bespuitings, ongeag wanneer dit in die seisoen gespuit is, onaanvaarbare vlakke van meer as 0.10 dpm opgelewer het. Daarteen was dit slegs enkele bespuitings wat tot so laat as Januarie gespuit is, onopspoorbare residu opgelewer het nadat al die behandelings aan standard pakhuisbehandelings blootgestel was. Die terugsterwing wat op Clementines in die Knysna-omgewing waargeneem is, kan toegeskryf word aan *Phytophthora* en nie *Diplodia* soos voorheen gerapporteer is nie. Verskeie bronne van inokulum is geïdentifiseer en spuitprogramme wat uit verskillende swamdoders bestaan, is toegedien om die siekte hok te slaan. Proewe gaan voort om ander sekondêre patogene soos *Fusarium* en antraknoses se rolle te bepaal deur Koch se postulate te doen. Die *Cercospora* situasie in Zimbabwe kon nog nie gemonitor word nie weens die huidige ongunstige politieke klimaat wat daar heers, maar ons word op hoogte gehou deur agente wat voortdurend die geokkupeerde plase besoek.

4.3.2 Investigation and identification of unknown leaf and fruit symptoms on Palmer Navel, Midnight Valencias and Delta Valencias at Naboomspruit

by G.C. Schutte, T.G. Grout, M.C. Pretorius and L. Huisman & T. Goszczynska (PPRI)

Opsomming

Ongekende blaarval en gesonke letsels is op Navel lemoene en Delta Valencia op 'n plaas naby Naboomspruit waargeneem kort voor oes in Junie 2003. Boord besoeke is gedoen en vrug en blaarmonsters is getrek om die moontlike betrokkenheid van swamme, bakterieë en virusse te ondersoek. Eerste indrukke het getoon dat dit die gevreesde *Pseudocercospora* van Zimbabwe kon wees, maar weens die heersende droogte tydens 2003, is die vrese afgesweer. Isolasië deur 4 laboratoriums kon geen swamsiekte isoleer nie. So ook kon ander plantpatoloë geen virus en bakteriese siekte daarmee assosieer nie. Ander kundiges het die simptome geassosieer met spuitskade van een of ander aard, insekkskade en ook moontlike fitotoksiteit van onkruidodders. 'n Publikasie van 1958 deur B. J. Dippenaar het ons vermoede bevestig deurdat presiese letsels beskryf en gefotografeer is wat identies is van skade wat deur grysmyt veroorsaak is.

Introduction

Outbreak of new diseases is a great concern to us and to citrus growers as it may influence our access to certain markets. This may lead to quarantine measures and the eradication of orchards which no one can afford these days. Speedy analysis with subsequent effective spray programmes need to be implemented before such a disease can spread to adjacent farms.

Materials and methods

General analysis

Visits were made on the 23 June, 29 July, 4 and 11 September 2003 when fruit, twig and leaf samples were collected at the farm "Haakdoringkuil" near Naboomspruit where symptoms were seen. From these, sub-samples were sent to the Diagnostic Centre at CRI, in Nelspruit, the University of Pretoria, Plant Protection Research Institute in Pretoria as well as the Plant Protection Research Institute at Roodeplaat for general isolations.

Specialist analysis

To investigate the possibility of the occurrence of citrus leprosis, plant material from fruits, twigs and leaf lesions was examined by using electron microscopy to find rabdovirus particles. Tests were conducted by Mr. Kassie Kassdorf.

To investigate the possibility of the occurrence of citrus scab, isolations from lesions were done on several media, including Potato Dextrose Agar and Water Agar with streptomycin and (I) diiodine, (II) chloramphenicol. Isolations were done by Dr. Wilhelm Botha.

To investigate the possibility of the occurrence of citrus bacterial canker, tissue was sampled aseptically from 16 (sixteen) different points on fruits, twigs and leaves (plants delivered on 4 September 2003) and from 12 (twelve) points (4 per cultivar) from plants delivered on 11 September 2003). Plant macerate was plated on the following media: Tryptone Glucose Extract Agar (TGA), Nutrient Agar, King's B, KBC, Tween A, Milk-Tween and Milk-Tween minus cephalixin. Isolations were done by the Bacterial Diseases Unit staff under supervision of Ms Teresa Goszczynska.

Suspect colonies were purified on King's B medium. PCR on pure cultures of *Xanthomonas campestris* pv. ? was done according to the method described by Cubero & Graham (2002). Primers used were J-RXg and J-RXc2, specific for the type A (Asiatic) citrus canker. Expected product size – 179 bp.

Photographic records

A series of photographs were also taken to see how the lesions developed over time. Photographs of the lesion types on different parts of the tree are also presented.

Results and discussion

General analysis

Although common fungi such as *Colletotrichum gloeosporioides*, *Alternaria tenuissima*, *Epicoccum*, *Fusarium*, *Trichothecium* and *Cladosporium* were isolated, no serious diseases such as *Pseudocercospora* were isolated.

Specialist analysis

Rabdovirus was NOT found in tested plants. Citrus scab pathogen, *Elsinoë fawcettii* was NOT isolated.

Bacterial colonies with colony morphology typical for *Xanthomonas campestris* were isolated from only one sample (spp. unknown), 4C – lesion on fruit stalk (Fig. 7 and Fig. 2). Colonies were yellow, circular, 1-2 mm in diameter, surrounded by two zones: bigger clear zone of milk hydrolysis and smaller opaque zone of Tween 80 lipolysis (Goszczynska & Serfontein, 1998). Colonies appeared on Milk-Tween minus cephalixin after five days of incubation, on Milk-Tween after seven days.

PCR with primers J-RXg and J-RXc2, specific for the type A (Asiatic) citrus canker were done on 13 isolates. We could not find any culture of *Xanthomonas campestris* pv. *citri* in South Africa and researchers from other

countries **were not contacted** to obtain isolates. In consequence positive control was not used in PCR. Expected size (~179 bp) PCR product was amplified by primers J-RXg/J-RXc2 from DNA of three South-African isolates. One isolate gave slightly bigger than expected product (Fig. 8). All thirteen isolates were deposited with the Plant Pathogenic and Plant Protecting Bacteria (PPPPB) national culture collection for future references.

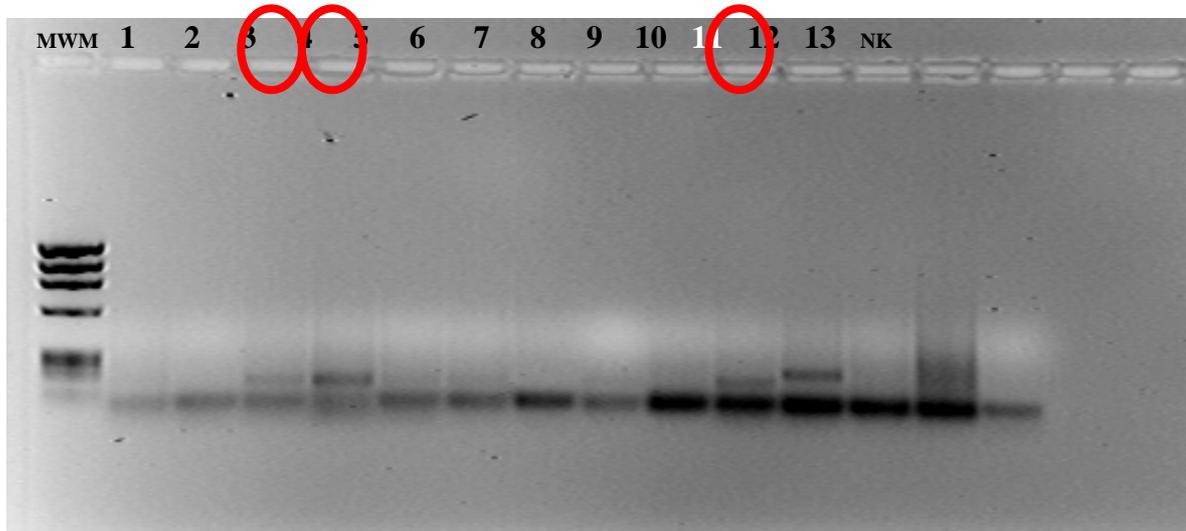


Fig. 4.3.2.1. Results of PCR amplification from 13 South African *Xanthomonas campestris* strains isolated from orange cultivar Delta Valencia. Primers used were J-RXg/J-RXc2, specific for the type A (Asiatic) bacterial canker. Three strains, 4c-3 (line 3), 4c-4 (line 4) and 4c-C (line 10) amplified PCR product of about 179 bp. These three lines are marked by red ovals. One strain, 4c-B produced slightly bigger product (line 11 marked in white).

Photographic records

A series of photographs taken of fruit over a period of a month, showed that only the small lesions visible at the time of harvest, developed into deep sunken lesions (Fig. 4.3.2.1, 4.3.2.2 & 4.3.2.3). Figure 4 is a close-up of the lesions as seen on Delta Valencias and Figures 5 & 6 shows comparisons of lesions as caused by grey mytes of citrus dated back to 1958 and described by Dippenaar (1958) and similar lesions on Delta Valencias.

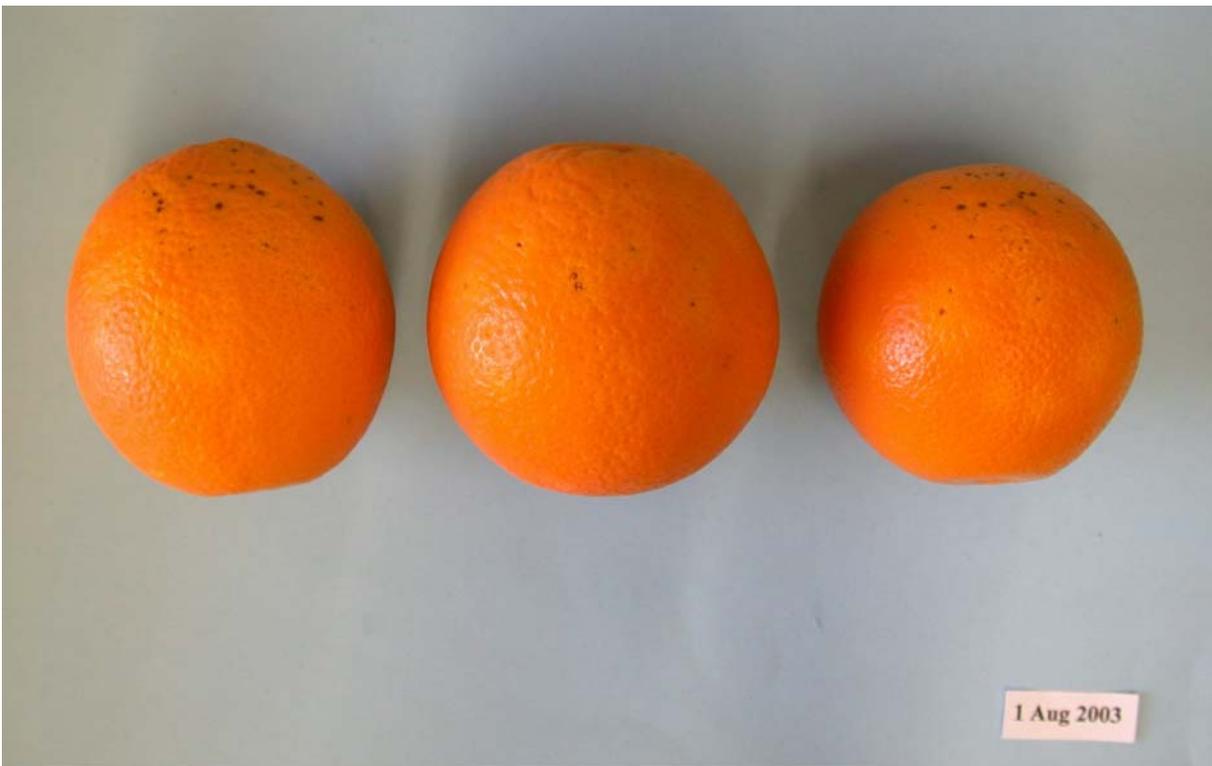


Fig. 4.3.2.2. Minute lesions on Delta Valencias as photographed on 1 August 2003.

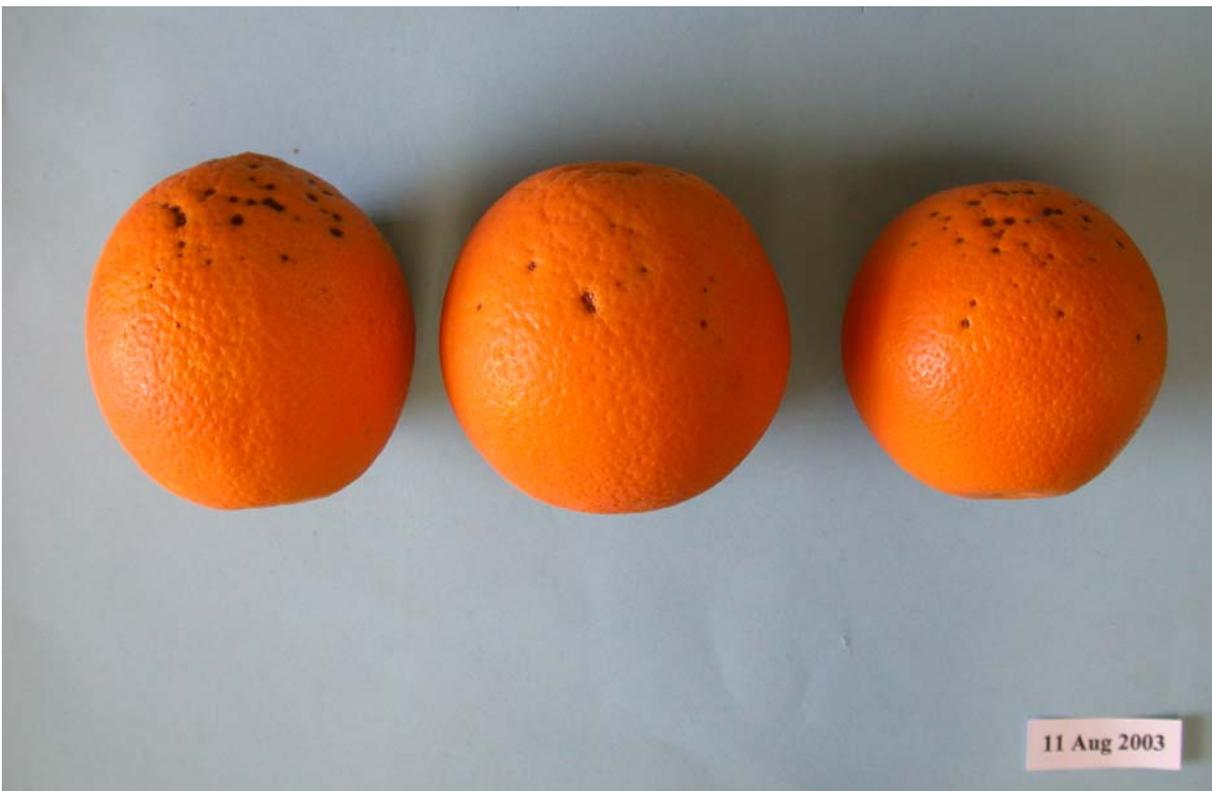


Fig. 4.3.2.3. Development of lesions on the same Delta Valencias as above here seen after 10 days showing sunken lesions with no additional lesion development.

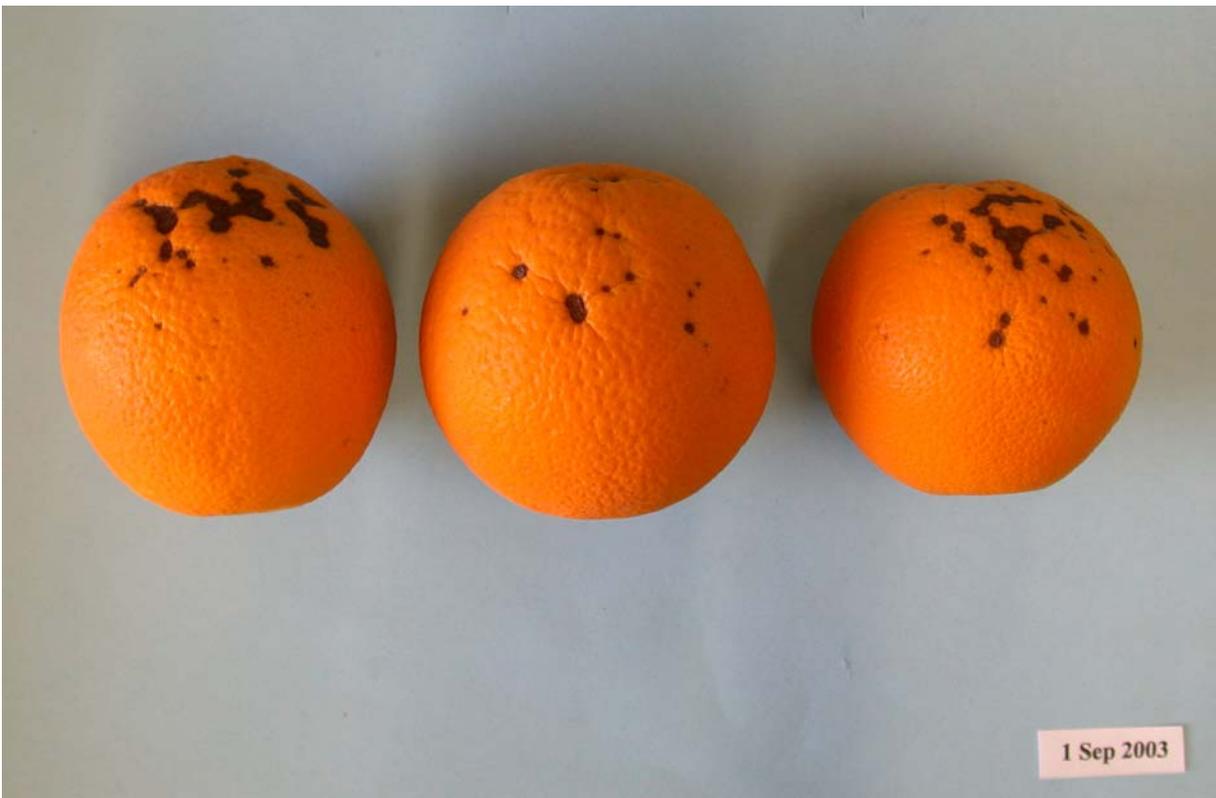


Fig. 4.3.2.4. Development of lesions on the same Delta Valencias as above here seen after 30 days, showing large sunken lesions with no additional lesion development.



Fig. 4.3.2.5. Delta Valencia fruit rind showing black, sunken lesions with no fungal growth visible.



Fig. 4.3.2.6. On the left is a photograph from Dippenaar (1958) showing shoots of Ascona lemon with oval-shaped ring blotch caused by grey mites and on the right is a similar lesion from Delta Valencia.

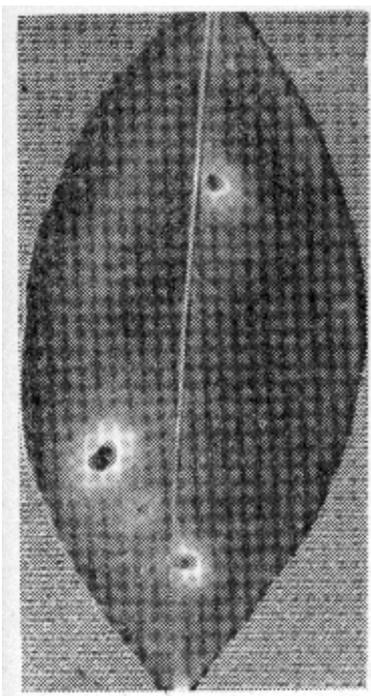


Fig. 4.3.2.7. On the left is a photograph also from Dippenaar (1958) showing a Navel leaf with necrotic blotches consisting of brown, dead tissue surrounded by a yellow halo. On the right are similar lesions on Delta Valentias.

Isolations were done from total 28 points on fruits, twigs, fruit stalks and leaf lesions. Occasionally some single bacterial colony was growing on one or another medium on which plant macerates of other 27 isolations were plated out. *Xanthomonas campestris* was isolated from only **ONE** sample only (number 4c). Otherwise, no bacterial or fungal growth was observed. This is unusual because saprophytic microorganisms are always present on plant material collected from orchards. However, the orchard was sprayed with copper oxychloride which may have influenced the population of microorganisms, both pathogenic and saprophytic. However, in some places like sample 4c, bacteria did survive and could be detected on diagnostic media. The bacteria, on Milk-Tween medium, have colony morphology typical of the species *Xanthomonas*

campestris. Colonies are yellow, circular, 1-2 mm in diameter, surrounded by two zones, bigger clear and smaller opaque. The bacteria in question are gram negative rods, aerobic, oxidase negative. They grow slowly on Milk-Tween, King's B and Nutrient Sucrose Agar media, but not on TGA or KBC. In PCR with primers specific for type A citrus bacterial canker three South African isolates produced PCR product of expected size (179 bp). It is not sure if they represents *Xanthomonas campestris* pv. *citri*.

For positive identification of *X. c.* pv. *citri*, strains of *X. c.* pv. *citri* (type A), *X. c.* pv. *aurantifolii* (type B), *X.c.* pv. *citrumelo* (citrus bacterial spot) have to be obtained from (for example) University of Florida. Such strains, or at least their DNA, can be used as positive control in PCR with citrus canker-specific primers. It is necessary to do a pathogenicity test with the South African isolates. The pathogenicity test will consist of inoculation of young, healthy plants with bacterial isolates of interest to see if disease symptoms will develop. The test will have to be done in a greenhouse under quarantine conditions. Plants used in a pathogenicity test must consist susceptible cultivars to bacterial canker. Symptoms of bacterial canker look different on different cultivars. For example on lemons and grapefruit fruit lesions are corky, raised and brownish, on some oranges – deep, crater like and black. Pathogenicity test should be done on at least two orange cultivars, lemon and grapefruit. With this in mind, it was decided not to continue with this approach as only one isolate was made out of 27.

Conclusion

Dippenaar (1958) made a thorough study of the concentric ring blotch disease of citrus in South Africa. The symptoms were redescribed and the cause was shown to be the grey mite, *Calacarus citrifolii* Keifer. In 1945 this problem manifested itself as a serious problem only on nursery trees. From time to time it would also attack young trees in the orchard after transplanting, but as the trees grew older, it always disappeared. In 1947 some growers in the Marico district had to cull a large percentage of their crop as unsuitable for export. Dippenaar (1958) stated that ring blotch affects only the young and actively growing parts of the tree. We observed the same phenomenon at Naboomspruit. Only young flush were attacked and defoliated. These twigs showed areas of dead tissue on vigorous shoots that eventually died, and black fungal fruiting bodies formed in the dead tissues of the blotched areas; usually anthracnose. This again matched our findings. Concomitantly, his description of the lesions on the leaves matched ours as to be blotches with brown dead material surrounded by a yellow halo. Although he mentioned that the blotching varied on the fruit, the necrotic form has often wrongfully been ascribed to the anthracnose fungus – a common secondary fungus. His description of sunken lesions on Washington Navels also matched with our findings. Therefore, we are convinced that the phenomenon as experienced at Naboomspruit, can be ascribed to grey mites causing necrotic blotch.

Future research

No further research on this phenomenon is planned and the situation will be monitored.

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4.3.3 Mancozeb residue analysis of spray programmes used for CBS control Experiment 720 by GC Schutte (CRI)

Opsomming

Mancozeb residu-analise is gedoen van geselekteerde spuitprogramme waar dit deel gevorm het van die spuitprogram hetsy as 1, 2 of 4 bespuitings. Resultate toon dat twee en vier bespuitings, ongeag wanneer dit in die seisoen gespuit is, onaanvaarbare vlakke van meer as 0.10 dpm opgelwer het. Daarteen was dit

slegs enkele bespuitings wat tot so laat as Januarie gespuit is, onopspoorbare residue opgelewer het nadat al die behandelings aan standard pakhuisbehandelings blootgestel was.,

Introduction

During 2003 we received a document regarding the restrictions of dithiocarbamates which placed our fruit production in jeopardy as dithiocarbamates have been used extensively for over 40 years for the control of citrus diseases. They also form an integral part of our anti-resistance strategy as they are they only fungicides to which no fungal resistance has ever been reported.

The current document "Restrictions on the Use of Plant Protection Products on Export Citrus" dated March 2003, recommends the following restriction on the use of dithiocarbamates (e.g. Mancozeb, Maneb, Dithane, Sancozeb) on citrus destined for Canada: "28 day pre-harvest interval and only where packhouses have wet lines, if fruit washed, preferably with high pressure sprayers".

The Canadian tolerance for dithiocarbamate residues on citrus is 0.1 ppm. Technology available at the time of establishing the current restriction did not cater for detection of this residue below 0.5 ppm. Newer techniques now available enable detection at lower levels and it is apparent that the current usage restriction is inadequate to ensure that residues in the range of 0.1 ppm to 0.5 ppm are avoided.

The recommended usage restriction for the use of dithiocarbamates on citrus destined for Canada is therefore amended to the following: "May not be applied after December and only where packhouses have wet lines and fruit is washed with clean (non-recycled) water".

Luckily my spray programmes for CBS control consisted of different spray programmes where the dithiocarbamate, mancozeb, was sprayed at different intervals during the susceptible period for CBS from October to January. Some of these were selected to determine the mancozeb residue levels to determine which programmes will be suited for the Canadian market.

In my field trials various spray programmes were evaluated against each other where different mancozeb applications were evaluated in combination with other fungicides. The aim of this study was to take mancozeb residues from selected programmes and residues determined to see if we can comply with the restrictions.

Materials and methods

The same application procedures were followed as described in experiment 720. The spray programmes and date the fruit was harvested, treated and submitted for analyses, is listed in Table 4.3.3.1.

Results and discussion

Residue analyses results of the spray programme that consisted of 4x mancozeb treatments showed that the residues were as high as 0.45 ppm if taken directly from the orchard. The same sample, if subjected to a high pressure descaler spray treatment, showed a 50% decrease in the mancozeb residues to 0.26 ppm. Tank mixtures of mancozeb with Flint and spray oil sprayed in November and December and preceded with a copper application in October followed with a final copper application in January (spray programme no. 3), showed that the accumulation of mancozeb residues nearly made the cut-off point of 0.10 ppm but still had a residue level of 0.17 ppm. Similar results were obtained where a mancozeb treatment as early as October followed by a tank mixture of Flint+copper+spray oil in November followed by the same sequence again in December and January (spray programme 5) resulted in 0.15 ppm mancozeb residues which is still above the cut-off point of 0.10 ppm. Where only one mancozeb in tank mixtures with Benlate and oil (spray programmes 6 & 7) was sprayed once off in either December or January, no residues were detected. It shows that one application of mancozeb sprayed as late as January can result in no mancozeb residues and that two or more applications can accumulate residues to be unacceptable for exports to certain markets like Canada and Japan.

Future research

There is a need to look for alternative control spray programmes where mancozeb can still be incorporated as it is one of the few fungicides where no resistance development has ever been recorded. It is also a cheap fungicide and is favoured by growers and chemical companies as part of their resistance strategy included in tank mixtures with other fungicides to prevent fungal resistance development due to frequent use.

Table 4.3.3.1. Mancozeb residue analysis of different citrus black spot spray programmes.

No	Spray dates				Mancozeb concentration* (ppm)
	14 October 2002	11 November 2002	9 December 2002	6 January 2003	
1	Mancozeb - ph	Mancozeb - ph	Mancozeb - ph	Mancozeb - ph	0.45
2	Mancozeb	Mancozeb	Mancozeb	Mancozeb	0.26
3	Copper	Flint + mancozeb + oil	Flint + mancozeb + oil	Copper	0.17
4	Mancozeb	Flint + copper + oil	Flint + copper + oil	Mancozeb	0.21
5	Mancozeb	Flint + copper + oil	Mancozeb	Flint + copper + oil	0.15
6		Flint + copper + oil	Benlate + mancozeb + oil		ND
7		Flint + copper + oil		Benlate + mancozeb + oil	ND

- ph = sample not subjected to pack house treatments

*Fruit samples taken were subjected to pack house procedures on 24 June 2003 at Karino Coop and taken to the SABS on 25 June 2003 for analysis

ND = Not detected

4.3.4. Investigation into die-back of Clementines in the Eastern Cape

Experiment 736 by G.C. Schutte (CRI)

Opsomming

Verskeie swamme is ge-isoleer vanaf 'nules' x Troyer onderstamme wat terugsterwing toon te Knysna. Hulle sluit in *Colletotrichum gloeosporioides*, *Glomerella cingulata*, *Phytophthora nicotianae* var. *parasitica*, *Phytophthora citrophthora*, *Fusarium oxysporum* en *F. solani*. Van hierdie is die eerste vier gebruik om Koch se postulate uit te voer en toon *Phytophthora nicotianae* var. *parasitica* die enigste ooreenkomstige simptome. *Glomerella cingulata* is die enigste ander swam wat vergommig tot gevolg gehad het, maar het nie verder ontwikkel in die tipiese simptome soos in die boord waargeneem is nie. *Phytophthora nicotianae* var. *parasitica* is suksesvol uit die geïnkuleerde glashuisboompies geïnkuleer en vervul die laaste vereiste van Koch se postulate en kan dus as een van die veroorsakende organismes beskou word.

Introduction

Tree die-back was observed in the Knysna region of the Eastern Cape on the Nules cultivar. No other Clementine cultivars such as Marisol, SRA and Orival seem to be affected by this unknown disease complex. All these cultivars were budded onto Troyer rootstocks and the rootstock is not affected. The die-back starts right on the scion (30 cm above ground level) as white fluffy mycelial growth and is accompanied by antracnose and *Diplodia* fungal growth (secondary wound pathogens). The aim of this experiment is to do isolations from the stems, identify the fungi and test them to fulfil Koch's postulates and then to identify the fungus/fungi and to evaluate certain fungicides for the control of the disease. Other possible causes will also be investigated such as soil and irrigation water.

Materials and methods

Isolations

During a visit on the 9 July 2003 to the orchards on the farm "Candlewood" of John Stanwix close to Knysna, soil, bark and water samples were drawn for analyses from an orchard with a high incidence of tree die-back. The orchard, consisting of 15 rows with 30 trees (\pm 2-3 m high) each that ran in a north-south direction. Isolations were made from the bark onto PDA and PARP media and taken to CRI in Nelspruit for identification.

Treatments

The selected orchard was sub-divided into five groups. These rows were marked and the following 4 treatments were applied at the following rates:

a) Benlate (100g/hl water)

- b) Flint (20g/hl water)
- c) Copstar (700 ml/hl water)
- d) Fighter (1l/hl water)
- e) Control

These treatments were applied with a 400 litre spray machine with one handgun to the trunks and branches of the tree with each tree receiving about 4 litres. They were sprayed to the point of run-off. Treatments were applied on the 10 July 2003, 18 September 2003, 20 November 2003 and 25 February 2004.

Tree rating

The trees were evaluated on the 10 July 2003 using two evaluation techniques, viz. the tree condition (according to a 10 point index where 1=healthy canopy and 10 = defoliated canopy) and lesion size on the stem (according to a 10 point index where 0% = no lesion and 100% = trunk completely covered with fungal growth). The orchard will be visited at certain intervals during the season to determine the status of tree health/decline and to see if the selected fungicidal sprays had any influence in controlling the disease.

Results and discussion

Isolations

Soil samples show a high incidence of *Pythium* and *Phytophthora* root rot, and isolates from the bark shows either *Fusarium* or the *Diplodia*/antracnose complex. To identify the fungus/fungi involved, isolates collected have to be subjected to fulfil Koch's postulates using the same rootstock and cultivar. To do this, Clementine x Troyer rootstocks were collected from the CFB at Uitenhage and taken to CRI in Nelspruit. Here *Colletotrichum gloeosporioides*, *Glomerella cingulata*, *Phytophthora nicotianae* var. *parasitica* and *Phytophthora citrophthora* isolates were tested for their pathogenicity on healthy trees. Three trees were inoculated on the trunk and on the lower part of the branches after those parts were surface sterilized with ethanol and the bark surface (20 mm²) scratched with a sterile scalpel. A 10mm² agar plug was aseptically removed from the petri dish and placed on the bark and trunk surface and sealed with Parafilm and left for 2 months to encourage fungal development in a glasshouse. After 6 weeks the first signs of gumming was observed with the *C. gloeosporioides* and *P. nicotianae* var. *parasitica* inoculations but not with any of the other fungi. After a while, the trees inoculated with *C. gloeosporioides* healed their wounds while the *P. nicotianae* var. *parasitica* fungus developed further (Fig. 4.3.4.1.). *P. nicotianae* var. *parasitica* was successfully isolated from the inoculated trees fulfilling Koch's postulates. *Fusarium* isolates were not tested to date.

Treatments and tree rating

The efficacy of the fungicides will be determined just before harvest in May/June by using the tree rating as discussed earlier. The same trees will be treated for a second season as well to determine if any of the fungicides were effective.

Future research

Frequent tree ratings will determine if there is a increase or decrease in tree health. For the time being fungicidal treatments will continue and orchard management will have to be improved by filtering the irrigation water, change over from drip to micro-irrigation, soil treatment with Ridomil followed by foliar applications with phosphonates and trunk treatments with one of the other fungicides.



Fig. 4.3.4.1. Trunk (T) and branch (B) symptom development on 'hules" after inoculation with *P. nicotianae* var. *parasitica*

4.3.5 Etiology, epidemiology and prediction modelling of *Alternaria* brown spot of citrus in South Africa

Experiment 716 by G.C. Schutte (CRI)

Opsomming

Met dié proef is daar slegs fondse beskikbaar gestel vir die opname van die siekte in Mandaryne tydens veldbesoeke. Weens die droë seisoen wat ondervind was tydens 2002/2003 is slegs een stel isolate uit Brits omgewing versamel wat ook baie moeilik te vinde was. Daar word nogtans voortgegaan met die uitbou van die versameling.

Summary

Some funds were made available for the collection of brown spot fungi for later studies. Some isolates were made from an orchard at Brits, but due to the extremely dry conditions experienced during the 2002/2003 season, no more isolates were collected. The collection will, however, continue.

4.4 PROJECT: SOILBORNE DISEASES

Project Co-ordinator: M.C. Pretorius (CRI)

4.4.1 Project summary

As part of an integrated pest management approach in controlling the citrus nematode, genetic resistance is a reliable replant strategy which is applied as common practice by citrus producers. Twenty years ago when the term 'replant problem' was a general term and a reality, 90% of the rootstocks used in South Africa were Rough Lemon, a highly susceptible rootstock to *viz.* citrus nematodes and *Phytophthora* spp. Today 80% of rootstocks used are Trifoliolate hybrids, of which Troyer, Carrizo citrange and Swingle citrumelo are the most common. It is clear that C35 and Swingle could successfully be utilized as replant rootstocks. It is interesting that the Australian trifoliolate which was always regarded as a good indicator of tolerance, performed poorly compared to the other trifoliolate rootstocks such as Pomeroy, Jacobsen and Rubidoux trifoliolate. The Mandarin rootstocks performed generally beyond expectations. On average, the Benton and Carrizo citrange rootstocks performed well (although the population counts were more than 2000 females per 10 g roots) compared to the poor performance of Yuma, Troyer citrange and X639. These rootstocks could be regarded as susceptible. As a result, Wallace Rough lemon could be regarded as a highly susceptible rootstock due to its bad performance. Wallace Rough lemon could definitely not be recommended to be used on replant soils infested with nematodes. No significant differences were detected regarding tree-height and stem diameter the past season which did not necessarily correlate with the nematodes' susceptibility of the rootstock (4.4.2).

None of the registered post plant nematicides have an effect on the eggs of the citrus nematode, *Tylenchulus semipenetrans*. These eggs can survive for up to 9 years in the soil and during favourable conditions the eggs hatch and the life cycle continues. It is therefore essential to follow an integrated nematode control strategy to assist producers in obtaining an economically viable control strategy for effective citrus nematode control. The combination of Rugby and a specific stimulating agent reduced the nematode population numbers from 15000 in March 2002 to 133 females/10g roots. It is believed that the Rugby treatment contributed towards the control of nematode populations after the stimulating process took place. More laboratory trials will be executed in the 2004 season in conjunction with Potchefstroom University to determine the potential of these products under normal field conditions (4.4.3).

Mammalian toxicity of nematicides and the fate of these materials in the environment has rekindled interest in biological control of nematodes. It is clear from previous results that these products must form part of an integrated control strategy because, as protocol, these products on their own were not effective in controlling the citrus nematode. Both the chemical treatments and 2 x Rugby 20 g/m² plus PL+ kept the female nematode counts below the threshold value of 1000 females/10 g roots. It is clear from the results that the PL+ plus Trichoderma treatments did not have any significant effect in reducing the nematode populations in both the soil and roots. The Nema-cur treatment performed poorly possibly because of AMD. The extreme drought situation may have had a negative influence on these agents to perform to their full potential in reducing the nematode populations in the soil and roots of citrus trees. Producers are still recommended to only treat a small irrigation block with this combination in order to determine the efficacy relative to their own orchard conditions. The drought situation also indicated that an effective irrigation system is required to optimize applications/treatments, bio control products and nematicides, in controlling the nematode numbers effectively. The use of PL+ through drip micro-irrigation systems where the environmental conditions are more suited for micro-organisms will also be investigated (4.4.4).

Nematodes other than *T. semipenetrans* currently known to be capable of damaging citrus tend to be very limited in distribution. *Hemicycliophora* appears to have a wide host range (ten of nineteen hosts tested) although the rutaceous host status is variable. The nematode feeds in large numbers at root tips whose roots typically develop round galls arising from hyperplasia. Three spp. that were detected from samples in the Gamtoos River Valley were identified by Dr. Ester v/d Berg, viz. *Hemicycliophora halophila*, *H. nortoni* and *H. typica*. The nematode populations are still relatively low because the trees are still young. It is, however, clear from the results that the citrus nematode female counts in the Swingle rootstock treatment are significantly lower than all the other rootstocks. No significantly different *Hemicycliophora* counts were present at this stage but it is believed that the nematode counts will increase as the trees grow older. The trial will be monitored regularly. *Hemicycliophora* was recorded in certain samples in the Western Cape and the Onderberg areas. This nematode is, however, always present on citrus roots in combination with the citrus nematode. It is therefore difficult to determine the damage caused by the presence of *Hemicycliophora* to the citrus tree. *Hemicycliophora* can be controlled with a post-plant chemical nematicide control strategy (4.4.5).

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is an aggressive root rot and fruit rot pathogen in citrus. The pathogen has a wide range of host species and can be isolated from soil in most of the older citrus orchards in southern Africa as well as in young nursery trees. The phosphonates are currently the most effective product that could be used to control *Phytophthora* spp. in existing infected orchards. However, in the nursery environment, limited availability of effective fungicides to control root pathogens such as *Phytophthora* and *Pythium* is an enormous concern to the nursery industry. Alternative products which include a combination of phosphonates and Product X and one biological control agent (PL+ - Trichoderma treatment) were used in this trial to search for more effective products and techniques to limit and control *Phytophthora* and *Pythium* root rot in nursery trees. It is clear from the results that none of the treatments reduced the percentage of *Phytophthora* infections to undetectable levels. Not even the Ridomil treatments reduced the infestation significantly to acceptable levels. The question asked is why this fungicide performed so poorly? Could it be as a result of resistance? It is clear that although the combination of the SAR product and Phytex did not control *Phytophthora* in the media, the effect it had on the seedlings' root health as demonstrated by its dry root mass gave good results. This matter should be investigated. In the dry root mass parameter, the phosphonate treatment did not result in a satisfactory result and the concentration could possibly be increased to achieve a better result. In the past it has been shown by several different institutions that the phosphonates control *Phytophthora* on citrus. The SAR product, in combination with the phosphonate, resulted in the treatments which performed best. This treatment could be investigated further. It has been shown that a loss of 20% of the feeder root system may not show in the canopy of trees irrigated and fertilized optimally. However, the most important aspect still remains, i.e. nurseries which may from time to time experience a problem with *Phytophthora*, need a product

that can effectively control this pathogen both in the plant and in the medium. Therefore, there will have to be an ongoing process to establish effective products to assist in this regard (4.4.6).

FMC Southern Africa approached CRI to conduct a demonstration trial to establish the efficacy of Rugby ME (liquid formulation) compared to Rugby G (granular formulation) on a contract basis. A new trial was laid out at Moosrivier Citrus (4.4.7).

Illovo Sugar approached CRI to evaluate Crop Guard, a non-organophosphate nematicide, to determine its effect in controlling the citrus nematode, *Tylenchulus semipenetrans*, on a contract basis (4.4.8).

It was established that although the phytoalexin scoparone is associated with resistance to stem cancer, it does not dictate resistance to root rot. It can therefore not be used as an indicator for resistance for root rot caused by *Phytophthora nicotianae*. As far as could be established, this finding has not been reported before in citrus. Levels of total soluble phenolics can therefore be used as a parameter in the screening of rootstocks for *P. nicotianae* resistance. A unique chemical compound has been discovered that is only associated with resistant rootstocks. This compound shows up as a yellow spot on TLC plates. If this compound is a viable marker for resistance, it will certainly be a breakthrough in rootstock resistance research. Such a unique compound that is only associated with resistant rootstocks could potentially be used in developing a high throughput screening technique for citrus rootstock resistance. Chemical characterisation of the compound is currently being conducted (4.4.9).

Projekopsomming

Genetiese weerstand is een van die mees betroubare faktore wat gevolg kan word as deel van 'n geïntegreerde beheer program vir die beheer van die sitrusaalwurm. Die resultate bevestig die feit dat C35 en Swingle wel as uitstekende herplant onderstamme beskou kan word. Australiese trifoliaat het swakker as verwagting gepresteer. Benton en Carrizo citrange het redelik presteer terwyl Yuma, Troyer citrange en X639 teleurgestel het. Schaub Rough lemon het beter as Carrizo en Benton citrange presteer en aansienlik beter as Wallace Rough lemon wat die swakste presteer het (4.4.2).

Soos reeds bekend het geeneen van die geregistreerde na-plant aalwurmdoders enige effek op die eiertjies van die sitrusaalwurm, *Tylenchulus semipenetrans*, nie. Die eiertjies kan tot nege jaar in die grond oorleef. 'n Geïntegreerde bestrydingsstrategie is dus noodsaaklik wat daartoe sal bydrae dat 'n ekonomies-vatbare bestuurstrategie geïmplimenteer kan word. In teenstelling met vorige resultate om die uitbroei van sitrusaalwurmeiertjies te stimuleer was hierdie jaar se resultate teleurstellend. Rugby teen 3x15 g/m² is steeds die mees effektiewe chemiese na-plant beheermaatreël teen sitrusaalwurm. Meer laboratorium werk sal in 2004 in samewerking met Potchefstroom Universiteit gedoen word. Die uiterste droë toestande wat tans in meeste van die somerreënval gebiede voorgekom het kon 'n negatiewe uitwerking op die resultaat van die middels se werking gehad het (4.4.3).

Die huidige tendens wêreldwyd om chemiese middels in die landbou te vervang plaas groot druk op navorsers om alternatiewe beheermaatreëls te ondersoek. Daarom is dit belangrik dat daar gestreef moet word na 'n geïntegreerdebeheerprogram om sodoende die sitrusaalwurm effektief te beheer. Huidige organofosfaat en karbamaat aalwurmdoders mag moontlik van die mark verdwyn a.g.v. hul toksisiteit. PL+ as 'n biologiesebeheeragent is volgens resultate nie altyd effektief om op sy eie aangewend te word nie. Die nuwe PL+ en Trichoderma produk het geen effek gehad op die beheer van aalwurmpopulasies in die wortels en grond nie. Die middel word dus tans nie aanbeveel nie. Die kombinasie van PL+ met 'n aalwurmdoder het bevredigende resultate gelewer. Jong visueel gesonde bome met 'n aalwurm probleem behoort op 'n kommersiële kleinskaal toegedien te word aangesien die gebruik van 'n biologiese agent as aalwurmdoder spesiale tegniese toegewytheid verg. Besproeiingsbestuur bv. skedulering, is uiters belangrik aangesien die biologiese agente lewende organismes is wat slegs onder uiters gunstige toestande sal oorleef (4.4.4).

Die sitrusaalwurm is steeds die enkele grootste aalwurm probleem tans in die Suid-Afrikaanse sitrusindustrie. Aalwurms wat die vermoë besit om skade te kan veroorsaak op sitrus is uiters beperk. In Suid-Afrika is *Hemicycliophora*, die skedeaalwurm, in verskeie boorde reeds so vroeg as 1963 al geïdentifiseer. Tans is dié aalwurm teenwoordig in die Gamtoosriviervallei, in sekere boorde in die Wes-Kaap asook in sekere boorde in Mpumalanga se Laeveld. Drie spesies is geïdentifiseer, nl. *Hemicycliophora halophila*, *H. nortoni* en *H. typica*. Die standaard na-plant chemiese aalwurmdoders geregistreer op sitrus sal die skedeaalwurm ook beheer. Vier van die gewildste onderstamme word tans in 'n *Hemicycliophora* geïnfecteerde boord gemonitor, naamlik Growweskiilsuurlemoen, Swingle citrumelo, Carrizo citrange en C35. Alhoewel die bome nog jonk is, en die situasie oor 'n langer termyn gemonitor sal word, het die Swingle onderstam die laagste aalwurm getalle in vergelyking met die ander onderstamme gehad (4.4.5).

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is 'n aggressiewe watergedraagde patoog wat wortelvrot en bruinvrot in boorde en kwekerye in suidelike Afrika veroorsaak. Meeste van die resultate verkry na aanwending van die middels, bly steeds wisselvallig. Alternatiewe maatreëls is daarom deur die CIP geïmplimenter om die oorsake van besmetting te beperk. Veiliger, meer effektiewe en ekonomies bekostigbare produkte moet gevind en ge-evalueer word om die voorkoms van *Phytophthora* te beperk. Ridomil as 'n chemiese standaard en 'n fosfonaat (Phytex), asook 'n kombinasie van 'n Produk X met Phytex en 'n Biologiese beheer agent, is in die proef ge-evalueer. Nie een van die produkte het die *Phytophthora* insidensie bevredigend beheer nie. Die swak prestasie van die Ridomil behandeling as 'n swamdoder het die vraag laat ontstaan of daar nie dalk weerstand teenwoordig is nie. Die kombinasie van Produk x en Phytex het wel 'n positiewe effek op die saailinge se droëwortelmasse en boomgewig gehad. Indien *Phytophthora* effektief beheer kan word en met Produk x se positiewe effek op die boomkwaliteit, kan dit 'n uitstekende kombinasie wees wat deur die kwekerybedryf gebruik kan word (4.4.6).

'n Demonstrasieproef is vir FMC Suid Afrika uitgevoer om die Rugby-korrelformulasie met die vloeibare Rugby-formulasie op 'n kontrakbasis te vergelyk (4.4.7).

CRI is deur Illovo Suiker genader om Crop Guard, 'n nuwe chemiese afvalprodukt van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te toets (4.4.8).

Daar is vasgestel dat die fitoaleksien scoparone wat geassosieer word met weerstand teen *Phytophthora citrophthora* stam kanker, nie verantwoordelik is vir weerstand teen *P. nicotianae* wortelvrot nie. Dit kan dus nie as 'n merker vir weerstand teen wortelvrot gebruik word nie. Sover vasgestel kon word, is dit nog nie voorheen in sitrus geraporteer nie. Die konsentrasie van totale fenoliese verbindings kan dus gebruik word as 'n parameter in die evalueering van sitrus onderstamme vir *P. nicotianae* weerstand. 'n Unieke verbinding is ontdek wat alleenlik teenwoordig is in weerstandbiedende onderstamme. Hierdie verbinding vertoon as 'n geel kol op dunlaag-chromatografie plate. As hierdie verbinding 'n werkbare merker vir weerstand is, sal dit beslis 'n deurbraak wees in sitrus weerstand navorsing. So 'n unieke verbinding, wat net geassosieer word met weerstandbiedende onderstamme, kan moontlik gebruik word om vinnige, effektiewe tegnieke te ontwikkel vir die sifting van sitrus onderstamme vir weerstand. Chemiese karakterisering van die verbinding word tans uitgevoer (4.4.9).

4.4.2 **Assessment of citrus rootstocks for citrus nematode resistance** Experiment 281 by M.C. Pretorius (CRI)

Opsomming

Genetiese weerstand is een van die mees betroubare faktore wat gevolg kan word as deel van 'n geïntegreerde beheer program vir die beheer van die sitrusaalwurm.

Twintig jaar gelede was die term "herplant probleem" 'n algemene term en realiteit in die sitrusbedryf. Negentig persent van die onderstamme was Growweskil suurlemoen gewees. Vandag is 80% van die onderstamme Trifoliaatbasters, waarvan Troyer, Carrizo, Citranges en Swingle citrumelo die mees algemene is.

Die proef bevestig dat aalwurmpopulasies nie maklik vestig op jong bome nie as gevolg van 'n ongunstige mikro-klimaat.

Die onderstamme tot en met C32 kan as hoogbestande onderstamme beskou word wat insluit C35, Swingle citrumelo, Pomeroy, Jacobsen en Rubidoux trifoliolate, sowel as F80-8 citrumelo en Sun Chu Sha. Die resultate bevestig die feit dat C35 en Swingle wel as uitstekende herplant onderstamme beskou kan word.

Australiese trifoliaat het swakker as verwagting gepresteer, terwyl die Mandaryn tipes bo verwagting goed presteer het. Benton en Carrizo citrange het redelik presteer terwyl Yuma, Troyer citrange en X639 teleurgestel het. Die onderstamme kan dus ook as gevoelige vir nematode infeksie geklassifiseer word.

Schaub Rough lemon het beter as Carrizo en Benton citrange presteer en aansienlik beter as Wallace Rough lemon wat die swakste presteer het en as uiters gevoelig vir aalwurmpopulasies geklassifiseer kan word. Wallace Rough lemon behoort onder geen omstandighede op herplant gronde met hoë aalwurm populasies aangeplant te word nie.

Introduction

As part of an integrated pest management approach in controlling the citrus nematode, genetic resistance is a reliable replant strategy which is applied as common practice by citrus producers. Twenty years ago when the term 'replant problem' was a general term, 90% of the rootstocks used in South Africa were Rough Lemon, a highly susceptible rootstock to pathogens related to the replant problem, viz. citrus nematodes and *Phytophthora* spp. Today 80% of rootstocks used are Trifoliolate hybrids, of which Troyer, Carrizo citrange and Swingle citrumelo are the most common.

The degree of resistance to citrus nematodes varies from rootstock to rootstock and from hybrid to hybrid. In susceptible rootstocks, the female citrus nematode penetrates the root cortex through the epidermal and hypodermal cells and into the parenchyma cells where they induce nurse cells and form permanent feeding sites.

The nematode populations on the citrange rootstocks do not increase as rapidly as on more susceptible rootstocks, e.g. Rough lemon. With time, however, the populations become as high as on the more susceptible rootstocks. Swingle citrumelo on the other hand used to be nematode resistant in South Africa until a couple of years ago when Miller *et al.* (1997) found the first Swingle orchards in Addo in which the citrus nematode overcame this resistance. This has also been reported in other places in the world, even in open nurseries in Florida. Fortunately the experience in South Africa is that this phenomenon very seldom occurs and in most cases where Swingle was used as a rootstock in replant soils, the trees are nematode-free, despite the soils being infested with nematode eggs after the removal of the previous citrus planting.

Nematode resistance is a dominant character controlled by two genes as resistance is frequently manifested in first generation hybrids. Since new rootstocks are frequently introduced into South Africa, it is necessary to screen these rootstocks for their levels of resistance against the nematode strains found in South Africa. This study was conducted at CRI as a pot experiment.

Materials and methods

The rootstocks screened in this trial are listed in Table 4.4.2.1. The trial was executed in 40 ℓ pots in the open at CRI in Nelspruit. The trees were watered by hand once or twice per week depending on the water requirements. The tree canopies are becoming so large that the root area is permanently shaded, creating more suitable conditions for the nematodes to establish. The trees received the same fertilizer, insect and *Phytophthora* rootrot control programme as the rest of CRI's glasshouse. As inoculation with eggs and juveniles failed to establish nematode populations during the previous years, the trees were re-potted in 2000 with nematode-infested soil collected from Crocodile Valley Citrus Co. The trees were left for eight months to establish and to allow nematode eggs in the soil to hatch and to infect the roots.

Root and soil samples were taken and the second stage juvenile population densities were determined by CRI's Diagnostic Centre according to the method of Whitehead and Hemming (1965), whereas the female population densities were determined according to the method of Van der Vegte (1973). This year trees were sampled by means of a small garden spade to collect as many roots as possible because the trees are much bigger than a year ago. However, this practice could not be repeated as often as we anticipated because it was too dramatic. The result was visible on the tree condition. The tree height and stem diameter were also measured.

Table 4.4.2.1. Rootstocks screened for resistance against the citrus nematode, *Tylenchulus semipenetrans*.

1	F80-8 citrumello	22	Changsha mandarin
2	Pomeroy trifoliolate	23	Natsudaidai
3	C-32 citrange	24	Rangpur x Troyer
4	Australian trifoliolate	25	Cairn RL
5	Sunki mandarin	26	Cleopatra mandarin
6	Konejime	27	1113 FD X Sunki
7	C. macrophylla	28	C. obovoideae
8	C35 citrange	29	Carrizo citrange
9	Calamandrin	30	Troyer citrange
10	Sun Chu Sha	31	Volckameriana
11	X639 citrange	32	Koethen citrange
12	Yuma citrange	33	Terra Bella citrumelo
13	Milan lemon	34	Roebidoux trifoliolate

14	Orlando tangelo	35	Japanese citron
15	C. amblycarpa	36	Benton citrange
16	Rusk citrange	37	F80-3 citrumelo
17	Jacobsen trifoliolate	38	1112 FD X Sunki
18	Schaub RL	40	Smooth Flat Seville
19	Swingle citrumelo	41	1116 FD X Sunki
20	Rangpur lime	42	Wallace RL
21	Shekwasa mandarin		

Results and discussion

This trial confirms the fact that nematode populations do not establish easily on young trees as a result of unsuitable soil micro-climate. It is only once the canopies are large enough to create a more acceptable micro-climate in the soil that the nematode populations increase. Two to three years ago it appeared as if this trial was a failure and, since then, the shading effect of the trees has allowed the nematode populations to increase to a level where last years nematode counts were 13000 females/10 g roots in the Wallace Rough lemon trees, compared to 250 in the C35 citrange rootstocks (Table 4.4.2.2).

For the purpose of this discussion the results displayed in Fig. 4.4.2.1 indicate the average female nematode populations on 41 different rootstocks over a period of 2 years. It is clear from these results that up to C32 citrange these rootstocks could be regarded as highly tolerant because the average female nematode populations were less than the threshold value of 1000 females per 10 g roots. It is clear that C35 and Swingle could successfully be utilized as replant rootstocks with great success.

It is interesting that the Australian trifoliolate which was always regarded as a good indicator of tolerance, performed poorly compared to the other trifoliolate rootstocks such as Pomeroy, Jacobsen and Rubidoux trifoliolate. The performance of the Mandarin rootstocks exceeded expectations. Although counts were recorded above the threshold value, they were less than 2000 females per 10 g roots which placed them in an acceptable category of tolerant rootstocks.

On average, the Benton and Carrizo citrange rootstocks performed well (although the population counts were more than 2000 females per 10 g roots) compared to the poor performance of Yuma, Troyer citrange and X639. These rootstocks could be regarded as susceptible. Schaub Rough lemon performed better than Carrizo and Benton citrange and out-performed Cairn and Wallace Rough lemon. As a result, Wallace Rough lemon could be regarded as a highly susceptible rootstock due to its bad performance. A few years ago Wallace Rough lemon rootstock was regarded as a rootstock with good *Phytophthora* tolerance qualities. From these results it is clear that Wallace Rough lemon could definitely not be recommended for use on replant soils infested with nematodes.

No significant differences were detected regarding tree-height and stem diameter (Table 4.4.2.3) the past season which did not necessarily correlate with the nematode-susceptibility of the rootstock. The extreme drought and high temperatures experienced during the season could have had an influence on tree growth as a whole. The initial above-ground tree condition could be misleading when compared with the situation in the root system of the trees infested with nematodes. A tree with a high nematode population infestation could still be visually healthy.

Table 4.4.2.2. Listing of 41 rootstocks in order of apparent susceptibility to female nematodes/10g roots and J2 juveniles/250mℓ soil.

Rootstock	September 2002		February 2003		December 2003	
	J2/250mℓ soil	♀ / 10g roots	J2/250mℓ soil	♀ / 10g roots	J2/250mℓ soil	♀ / 10g roots
Citrus amblycarpa	708 ab	3400 abcdefg	200 ab	1300 abcde	0 a	666 abcde
C32 citrange	733 ab	550 a	783 bcdef	1508 abcde	116 abc	800 abcdef
Pomeroy trifoliolate	283 ab	550 a	200 ab	583 abcd	416 abcdef	500 abc
Shekwasha mandarin	2050 abcde	3458 abcdefg	250 abc	871 abcde	0 a	3600 hijk
C35 citrange	250 ab	250 a	316 abcd	250 a	83 ab	333 ab
Swingle citrumelo	66 a	566 a	50 a	200 a	33 ab	133 a
Australian trifoliolate	900 abc	1850 abcde	333 abcd	1100 abcde	216 abcde	1433 abcdefg
Sunki mandarin	1366 abcd	1833 abcde	333 abcd	1141 abcde	500 abcdefg	2333 fghi
Jacobsen trifoliolate	1316 abc	933 abc	350 abcd	433 ab	0 a	733 abcdef
1113 FD x Sunki	1366 abcd	3100 abcdef	650 abcdef	388 ab	300 abcde	933 abcdef
F80-8 citrumelo	1050 abc	691 ab	550 abcde	483 ab	100 ab	733 abcdef
Changsha mandarin	716 ab	1233 abcde	283 abcd	500 abc	150 abc	1216 abcdefg
Troyer citrange	2616 bcde	3725 abcdefg	950 def	4316 gh	483 abcdefg	1900 bcdefg
Konjime	2333 abcde	6516 ghij	250 abc	1433 abcde	283 abcde	2800 ghi
Smooth Flat Seville	1716 abcd	2700 abcde	150 ab	2050 abcdef	283 abcde	1666 abcdefg
Carrizo citrange	1533 abcd	3125 abcdefg	800 bcdef	975 abcde	950 g	2266 efghi
Rangpur x Troyer	1900 abcd	4441 cdefgh	1066 ef	5600 h	383 abcde	1366 abcdefg
Calamandrin	2466 abcde	3625 abcdefg	16 a	283 a	166 abcd	1300 abcdefg
1112 FC x Sunki	1683 abcd	2166 abcd	66 a	683 abcd	183 abcd	866 abcdef
Citrus obovoidae	1500 abcd	4583 defghi	650 abcdef	2405 cdef	233 abcde	1483 abcdefg
F80-3 citrumelo	4466 e	4208 bcdefgh	583 abcdef	741 abcd	250 abcde	2000 cdefgh
Sun Chu Sha	333 ab	1050 abcd	33 a	608 abcd	0 a	366 abc
Japanese citron	1700 abcd	8716 j	366 abcd	1471 abcde	366 abcde	1683 abcdefg
1116 FC x Sunki	1550 abcd	1483 abcde	350 abcd	1066 abcde	666 defg	1400 abcdefg
Rubidoux trifoliolate (?)	466 ab	691 ab	416 abcde	416 ab	183 abcd	1100 abcdef
Cairn Rough lemon	2383 abcde	8116 ij	900 cdef	2716 efg	616 cdefg	4266 jk
Volckameriana	1550 abcd	2883 abcdef	233 abc	1185 abcde	83 ab	3783 ijk
X639 citrange	2500 abcde	6383 fghij	800 bcdef	2758 efg	533 bcdefg	1800 bcdefg
Koethen citrange	1350 abcd	3475 abcdefg	416 abcde	1400 abcde	183 abcd	1866 bcdefg
Wallace Rough lemon	7183 f	13533 k	466 abcde	3866 fgh	916 fg	4433 jk
Benton citrange	2000 abcde	2200 abcde	650 abcdef	1866 abcde	516 bcdefg	2200 defghi
Citrus macrophylla	1850 abcd	2900 abcdefg	316 abcd	1448 abcde	216 abcde	1733 abcdefg
Milan lemon	1050 abc	2658 abcde	266 abc	900 abcde	50 ab	1033 abcdef
Natsudaidai	3816 de	4841 efghi	1250 f	2241 bcdef	700 efg	4166 jk
Schaub Rough lemon	916 abc	3358 abcdefg	216 ab	720 abcd	0 a	1133 abcdef
Rangpur lime	2516 abcde	7550 hij	366 abcd	583 abcd	0 a	266 ab
Terra Bella citrumello	883 abc	1983 abcde	266 abc	545 abc	133 abc	1166 abcdefg
Yuma citrange	1750 abcd	2216 abcde	283 abcd	2491 defg	100 ab	4633 k
Orlando tangelo	3250 cde	4633 defghi	150 ab	530 abc	283 abcde	1500 abcdefg
Rusk citrange	1316 abc	4716 efghi	316 abcd	1866 abcde	333 abcde	600 abcd
Cleopatra mandarin	700 ab	1533 abcde	166	666 abcd	266 abcde	1116 abcdef

Means in the same column with common letters do not differ significantly at a 5% level according to the Fishers LSD comparison.

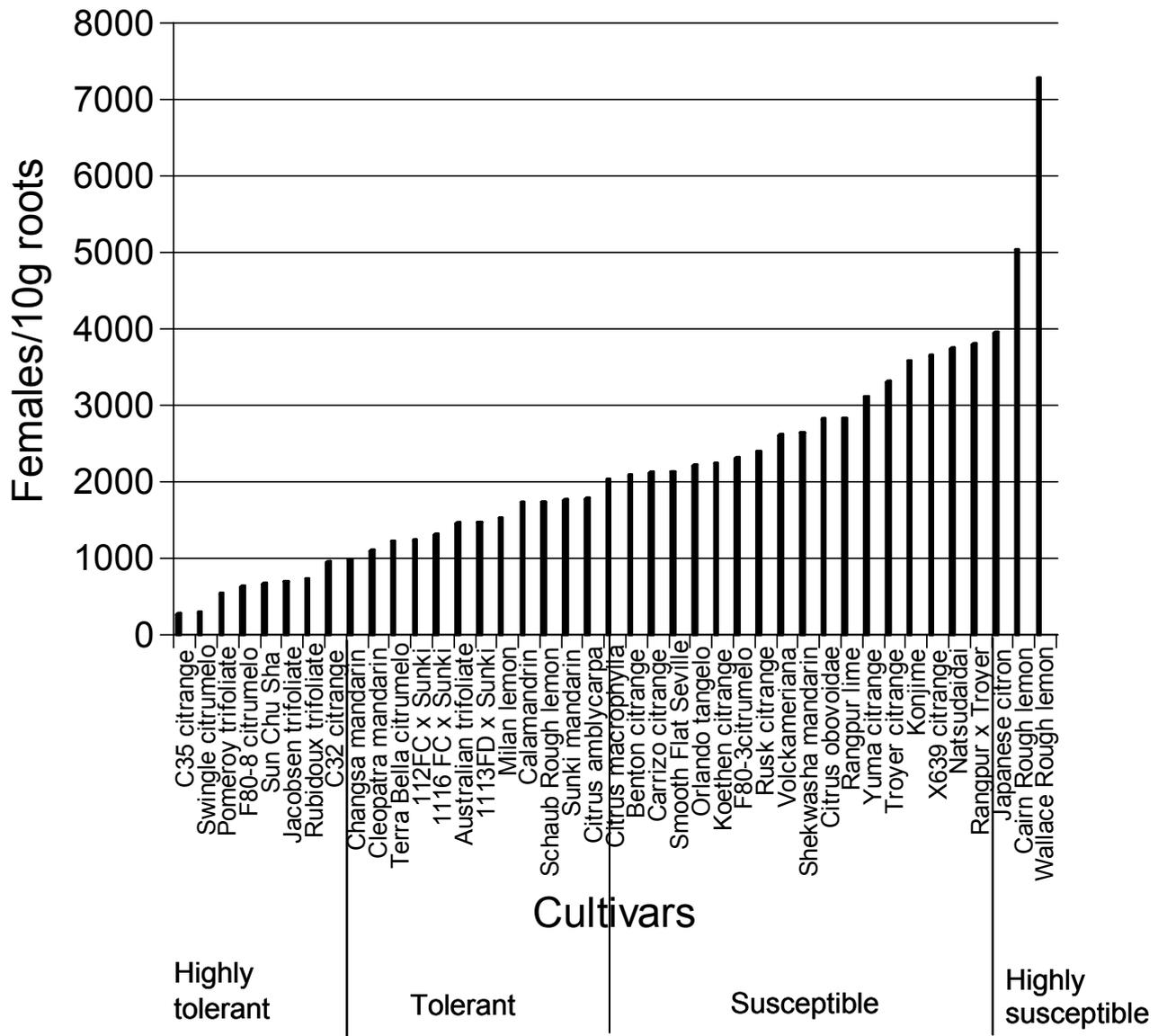


Fig. 4.4.2.1. Average female nematode populations on citrus rootstocks planted in 40 l containers over a period of two years.

Table 4.4.2.3. Differences in stem diameter and tree height due to treatments.

Rootstock	Treatment	December 2002		December 2003	
		Stem diameter	Tree height	Stem diameter	Tree height
F80-8 citrumelo	-N	26.97 a	1.5 a	30.33 a	1.6 a
	+N	24.82 a	1.3 a	27.33 a	1.46 a
Pomeroy trifoliolate	-N	24.67 a	1.4 a	28.66 a	1.63 a
	+N	20.94 a	1.1 a	24.33 a	1.23 a
C-32 citrange	-N	27.2 a	1.2 a	34.83 a	1.4 a
	+N	25.54 a	1 a	29.7 b	1.3 a
Australian trifoliolate	-N	24.28 a	1.4 a	30.5 a	1.33 a
	+N	23.47 a	1.2 a	29.06 a	1.26 a
Sunki mandarin	-N	27.07 a	1.3 a	28 a	1.36 a
	+N	26.72 a	1.3 a	27.46 a	1.33 a
Konejime	-N	29.35 a	1.5 a	35.33 a	1.66 b
	+N	28.53 a	1.6 a	33.9 a	1.53 a
Citrus Macrophylla	-N	23.21 a	1.3 a	24.63 a	1.43 a
	+N	18.73 a	1.2 a	20.0 a	1.33 a
C-35 citrange	-N	26.52 a	1.6 b	33.73 a	1.43 a
	+N	24.99 a	1.1 a	31 a	1.4 a
Calamandrin	-N	27.1 a	1.4 a	29.06 a	1.5 a
	+N	25.13 a	1.3 a	25.5 a	1.33 a
Sun Chu Sha	-N	27.5 a	1.4 a	27.96 a	1.46 a
	+N	24.74 a	1.1 a	24.93 a	1.15 a
X639 citrange	-N	28.23 a	1.3 a	31.03 a	1.43 a
	+N	27.68 a	1.2 a	29.6 a	1.23 a
Yuma citrange	-N	26.34 b	1.3 a	33.1 a	1.26 a
	+N	22.88 a	1.2 a	25 a	0.83 a
Milan lemon	-N	30.67 a	1.5 a	29.5 a	1.53 a
	+N	24.74 a	1.3 a	27.9 a	1.3 a
Orlando tangelo	-N	25.78 a	1.4 a	27.96 a	1.4 a
	+N	23.45 a	1.2 a	27.53 a	1.3a
Citrus amblycarpa	-N	25.05 b	1.4 b	27.83 b	1.43 a
	+N	21.44 a	1.1 a	23.5 a	1.1 a
Rusk citrange	-N	23.01 a	1.4 a	29.03 a	1.4 a
	+N	23.15 a	1.2 a	26.9 a	1.26 a
Jacobsen trifoliolate	-N	23.4 a	1.3 a	27.63 a	1.46 a
	+N	22.81 a	1.4 a	26.53 a	1.36 a
Schaub RL	-N	29.18 a	1.8 a	29.7 a	1.66 a
	+N	28.38 a	1.4 a	29.06 a	1.46 a
Swingle citrumelo	-N	24.89 a	1.5 a	34.83 b	1.46 a
	+N	24.69 a	1.3 a	32.16 a	1.36 a
Rangpur lime	-N	29.34 b	1.8 b	30.73 a	1.73 b
	+N	26.17 a	1.4 a	28.33 a	1.5 a
Shekwasa mandarin	-N	26.78 a	1.3 a	27.16 a	1.43 a
	+N	24.89 a	1.2 a	24.53 a	1.33 a
Changsha mandarin	-N	19.8 b	1.1 a	21.9 a	1.13 a
	+N	17.6 a	1.1 a	20.8 a	1.1 a
Natsudaikai	-N	13.75 a	1.1 a	28.83 a	1.43 a
	+N	12.87 a	1.1 a	26 a	1.36 a
Cairn RL	-N	11.67 a	1.1 a	22.46 a	1.2 a
	+N	10.88 a	0.9 a	19.5 a	1.03 a
Cleopatra mandarin	-N	13.91 a	1.1 a	31 a	1.3 a
	+N	12.53 a	1.2 a	27.33 a	1.2 a
1113FC x Sunki	-N	30.69 b	1.2 a	27.26 a	1.3 a
	+N	23.26 a	1.1 a	22.5 a	1.2 a
Citrus obovoideae	-N	26.44 a	1.3 a	28.73 a	1.26 a
	+N	25.4 a	1.2 a	26.33 a	1.23 a
Carrizo citrange	-N	28.14 a	1.6 b	34.5 a	1.5 a
	+N	24.59 a	1.3 a	34.1 a	1.33 a

Troyer citrange	-N	31.22 b	1.4 a	32.3 a	1.53 a
	+N	25.01 a	1.2 a	27.66 a	1.36 a
Volckameriana	-N	26.14 a	1.4 a	29.47 a	1.51 a
	+N	23.75 a	1.3 a	23.7 a	1.4 a
Koethen citrange	-N	32.65 b	1.5 a	32.63 a	1.63 a
	+N	27.43 a	1.5 a	29 a	1.6 a
Terra Bella citrumelo	-N	24.55 b	1.5 a	33.56 a	1.46 a
	+N	22.31 a	1.2 a	30.36 a	1.23 a
Roebidoux trifoliolate	-N	27.23 b	1.3 b	34.83 a	1.46 b
	+N	25.09 a	1.1 a	33.7 a	1.13 a
Japanese citron	-N	22.66 a	1.2 a	31.63 a	1.33 a
	+N	25.49 a	1.1 a	27.93 a	1.2 a
Benton citrange	-N	28.15 a	1.3 a	29.7 a	1.4 a
	+N	28.74 a	1 a	28.96 a	1.05 a
F80-3 citrumelo	-N	26.85 a	1.3 a	27.83 a	1.63 a
	+N	24.35 a	1.4 a	26.13 a	1.36 a
1112FD x Sunki	-N	27.8 a	1.3 a	34.16 a	1.53 a
	+N	25.04 a	1.4 a	32 a	1.5 a
Sampson tangelo	-N	26.38 a	1.3 a	27.5 a	1.4 a
	+N	22.73 a	1.1 a	25.03 a	1.26 a
Smooth Flat Seville	-N	29.41 a	1.3 a	34.33 a	1.56 a
	+N	29.82 a	1.4 a	30.7 a	1.26 a
116FD x Sunki	-N	28.57 a	1.3 b	30.16 a	1.6 a
	+N	26.63 a	1.2 a	29.23 a	1.5 a
Wallace RL	-N	31.87 a	1.4 a	31.96 a	1.7 a
	+N	28.62 a	1.6 a	30.3 a	1.5 a

* -N : Nematode free trees

** +N : Nematode infested trees

Means in the same column with common letters do not differ significantly at a 5% level according to the Fisher LSD comparison.

Conclusion

The information from this trial will be important for any further citrus rootstock breeding that may take place in South Africa. This trial will be monitored for another season.

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4.4.3 Stimulation of egg hatching of *Tylenchulus semipenetrans* eggs Experiment 547 by M.C. Pretorius (CRI)

Opsomming

Soos reeds bekend het geeneen van die geregistreerde na-plant aalwurmdoders enige effek op die eiertjies van die sitrusaalwurm, *Tylenchulus semipenetrans*, nie. Die eiertjies kan tot nege jaar in die grond oorleef en dan onder gunstige toestande uitbroei en daardeur die lewensiklus voortsit. 'n Geïntegreerde bestrydingsstrategie is dus noodsaaklik wat daartoe sal bydrae dat 'n ekonomies-vatbare bestuurstrategie geïmplimenteer kan word.

In teenstelling met vorige resultate om die uitbroei van sitrusaalwurmeiertjies te stimuleer was hierdie jaar se resultate teleurstellend. Beide Nema-cur en Moca-p se beheer van aalwurms was oneffektief, die Nema-cur behandeling is waarskynlik a.g.v. versnelde afbraak. Moca-p het effens beter resultate as Nema-cur opgelewer alhoewel nie aanvaarbaar nie. Rugby teen 3x15 g/m² is steeds die mees effektiewe chemiese na-plant beheermaatreël teen sitrusaalwurm. Meer laboratorium werk sal in 2004 in samewerking met

Potchefstroom Universiteit gedoen word. Die uiterste droë toestand wat tans in meeste van die somerreënval gebiede voorgekom het kon 'n negatiewe uitwerking op die resultaat van die middels se werking gehad het.

Introduction

Although they are aquatic animals, plant parasitic nematodes have evolved protective structures and metabolic adaptations which allow them to survive and flourish in what is often a harsh and competitive soil environment. 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.

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 high reproductive capacity of most plant parasitic nematodes is one of the features which makes 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. 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 three 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).

None of the registered post plant nematicides have an effect on the citrus nematode, *Tylenchulus semipenetrans*, eggs. These eggs can survive for up to 9 years in the soil and during favourable conditions the eggs hatch and the life cycle continues. It is therefore essential to follow an integrated nematode control strategy to assist producers in obtaining an economically viable control strategy for effective citrus nematode control. The purpose of this trial was to synchronize hatching of nematode eggs and to eradicate the nematode population in the soil by using one or two nematicide treatments. The initial trial results at Crocodile Valley were very promising and it was clear that the egg hatching stimulants had a positive effect in successfully stimulating the citrus nematode eggs to hatch.

Materials and methods

A trial site with a high nematode population in an eleven-year-old orchard at Bokfontein in the Brits area, was identified. Three single-tree replicates per treatment, were used. A total of 150 trees were monitored. Three liquid formulated nematicides, viz. Rugby EC; NemaCur EC and Mocap EC were used as the chemical control component. A combination of different treatments and dosages, which include the liquid nematicides in combination with the egg hatching stimulants, the egg hatching stimulants on their own, and a standard chemical nematicide application on its own, were monitored. These consisted of Rugby and NemaCur only and were compared to an untreated control. Three applications were conducted: November 2002, January 2003 and March 2003. Soil and root samples were taken before the trial was re-applied in September 2002, November 2002, January 2003 and March 2003. These were analysed by the Diagnostic Centre in Nelspruit. The treatments applied were as follows (Table 4.4.3.1).

Table 4.4.3.1. Treatments and time of application.

Treatment & Dosage/m ² (except the two Halls treatments = dosage/tree of 9 m ²)		Application			
		Sept 2002	Nov 2002	Jan 2003	Mar 2003
1.	Rugby 20mℓ/m ² + D-Limonene 30mℓ/m ²	R + S	S	R + S	S
2.	Rugby 20mℓ/m ² + Citronella 15mℓ/m ²	R + S	S	R + S	S
3.	Rugby 20mℓ/m ² + orange 50mℓ/m ²	R + O	S	R + S	S
4.	Rugby 20mℓ/m ² + Halls 1ℓ	R + S	S	R + S	S
5.	Rugby 20mℓ/m ² + Halls 2ℓ	R + S	S	R + S	S
6.	Rugby 10mℓ/m ² + D-Limonene 30mℓ/m ²	R + S	S	R + S	S
7.	Rugby 10mℓ/m ² + Citronella 15mℓ/m ²	R + S	S	R + S	S
8.	Rugby 10mℓ/m ² + Orange 50mℓ/m ²	R + S	S	R + S	S
9.	Rugby 10mℓ/m ² + Halls 1ℓ	R + S	S	R + S	S
10.	Rugby 10mℓ/m ² + Halls 2ℓ	R + S	S	R + S	S
11.	Rugby 10mℓ/m ² + 2 x D-Limonene 30mℓ/m ²	R + S	R + S	R + S	R + S
12.	Rugby 10mℓ/m ² + 2 x Citronella 15mℓ/m ²	R + S	R + S	R + S	R + S
13.	Rugby 10mℓ/m ² + 2 x Orange 50mℓ/m ²	R + S	R + S	R + S	R + S
14.	Rugby 10mℓ/m ² + 2 x Halls 1ℓ	R + S	R + S	R + S	R + S
15.	Rugby 10mℓ/m ² + 2 x Halls 2ℓ	R + S	R + S	R + S	R + S
16.	Nemacur 5mℓ/m ² + D-Limonene 20mℓ/m ²	N + S		N + S	
17.	Nemacur 5mℓ/m ² + Citronella 15mℓ/m ²	N + S		N + S	
18.	Nemacur 5mℓ/m ² + Orange 50mℓ/m ²	N + S		N + S	
19.	Nemacur 5mℓ + Halls 1ℓ	N + S		N + S	
20.	Nemacur 5mℓ + Halls 2ℓ	N + S		N + S	
21.	Nemacur 2.5mℓ/m ² + D-Limonene 30mℓ/m ²	N + S		N + S	
22.	Nemacur 2.5mℓ/m ² + Citronella 15mℓ/m ²	N + S		N + S	
23.	Nemacur 2.5mℓ/m ² + Orange 50mℓ/m ²	N + S		N + S	
24.	Nemacur 2.5mℓ/m ² + Halls 1ℓ	N + S		N + S	
25.	Nemacur 2.5mℓ + Halls 2ℓ	N + S		N + S	
26.	Nemacur 2.5mℓ/m ² + 2 x D-Limonene 30mℓ/m ²	N + S	N + S	N + S	N + S
27.	Nemacur 2.5mℓ/m ² + 2 x Citronella 15mℓ/m ²	N + S	N + S	N + S	N + S
28.	Nemacur 2.5mℓ/m ² + 2 x Orange 50mℓ/m ²	N + S	N + S	N + S	N + S
29.	Nemacur 2.5mℓ/m ² + 2 x Halls 1ℓ	N + S	N + S	N + S	N + S
30.	Nemacur 2.5mℓ/m ² + 2 x Halls 2ℓ	N + S	N + S	N + S	N + S
31.	Mocap 7.5mℓ/m ² + D-Limonene 30mℓ/m ²	M + S		M + S	
32.	Mocap 7.5mℓ/m ² + Citronella 15mℓ/m ²	M + S		M + S	
33.	Mocap 7.5mℓ/m ² + Orange 50mℓ/m ²	M + S		M + S	
34.	Mocap 7.5mℓ + Halls 1ℓ	M + S		M + S	
35.	Mocap 7.5mℓ + Halls 2ℓ	M + S		M + S	
36.	Mocap 3.75mℓ/m ² + D-Limonene 30mℓ/m ²	M + S		M + S	
37.	Mocap 3.75mℓ/m ² + Citronella 15mℓ/m ²	M + S		M + S	
38.	Mocap 3.75mℓ/m ² + Orange 50mℓ/m ²	M + S		M + S	
39.	Mocap 3.75mℓ + Halls 1ℓ	M + S		M + S	
40.	Mocap 3.75mℓ + Halls 2ℓ	M + S		M + S	
41.	Mocap 3.75mℓ/m ² + 2 x D-Limonene 30mℓ/m ²	M + S	M + S	M + S	M + S
42.	Mocap 3.75mℓ/m ² + 2 x Citronella 15mℓ/m ²	M + S	M + S	M + S	M + S
43.	Mocap 3.75mℓ/m ² + 2 x Orange 50mℓ/m ²	M + S	M + S	M + S	M + S
44.	Mocap 3.75mℓ/m ² + 2 x Halls 1ℓ	M + S	M + S	M + S	M + S
45.	Mocap 3.75mℓ/m ² + 2 x Halls 2ℓ	M + S	M + S	M + S	M + S
46.	2 x D-Limonene 60mℓ/m ²	S	S	S	S
47.	2 x Citronella 30mℓ/m ²	S	S	S	S
48.	2 x Orange 100mℓ/m ²	S	S	S	S

49.	2 x Halls 2ℓ	S	S	S	S
50.	2 x Halls 4ℓ	S	S	S	S
51.	Untreated control	-	-	-	-
52.	Rugby 3 x 15g/m ²	R	R	R	R

Results and discussion

It is known from previous trial results that the egg hatching stimulants had a positive effect on the hatching process of the citrus nematode. The results obtained during April 2003 after two applications, were, however, disappointing (Tables 4.4.3.2-4.4.3.7). The trial was not re-applied to save costs. However, it was re-sampled in January 2004 to determine the long term effect these treatments had, if any, on the hatching process of the citrus nematode.

The results were analysed in three groups to determine the effect of the egg hatching stimulants in combination with three liquid formulations of nematicides. Group A: Rugby EC at different dosages in combination with the egg hatching stimulants. Group B: The aim was to synchronise the egg hatching process and to control the nematode populations with a single or double nematode treatment. The treatments were as follows: Nema-cur EC and Rugby EC at different dosages in combination with the egg hatching stimulants. Group C: Mocap EC and Rugby EC at different dosages in combination with the egg hatching stimulants.

Table 4.4.3.2. *Tylenchulus semipenetrans* second stage juvenile counts J2/250 ml soil.

GROUP A: RUGBY EC – EGG STIMULATION					
J2 / 250 ml SOIL					
Treatment		Mar 2002	Sept 2002	Jan 2003	Apr 2003
1.	Rugby (20mℓ)m ² + D-Limonene (30mℓ) m ²	166 a	200 a	33 a	866 a
2.	Rugby (20mℓ)m ² + Citronella (15mℓ) m ²	1000 a	66 a	33 a	0 a
3.	Rugby (20mℓ)m ² + Orange (50mℓ) m ²	5966 ab	200 a	200 ab	433 a
4.	Rugby (20mℓ)m ² + Halls (1ℓ)	2500 ab	933 ab	66 a	733 a
5.	Rugby (20mℓ)m ² + Halls (2ℓ)	200 a	133 a	0 a	166 a
6.	Rugby (10mℓ)m ² + D-Limonene (30mℓ) m ²	266 a	500	400 ab	3166 ab
7.	Rugby (10mℓ)m ² + Citronella (15mℓ) m ²	266 a	566	466 ab	1800 a
8.	Rugby (10mℓ)m ² + Orange (50mℓ) m ²	3100 ab	1566	1133 abc	4666 abcd
9.	Rugby (10mℓ)m ² + Halls (1ℓ)	533 a	2100	2033 bcd	4233 abc
10.	Rugby (10mℓ)m ² + Halls (2ℓ)	500 a	1766	900 ab	4533 abcd
11.	Rugby (10mℓ)m ² + D-Limonene 2 x (30mℓ) m ²	300 a	400 a	133 a	1733 a
12.	Rugby (10mℓ)m ² + Citronella 2 x (15mℓ) m ²	633 a	700 a	166 ab	6466 abcd
13.	Rugby (10mℓ)m ² + Orange 2 x (50mℓ) m ²	2900 ab	766 a	66 a	600 a
14.	Rugby (10mℓ)m ² + Halls 2 x (1ℓ)	5733 ab	766 a	166 ab	3600 ab
15.	Rugby (10mℓ)m ² + Halls 2 x (2ℓ)	1500 ab	1600 ab	433 ab	633 a
46.	D-Limonene 2 x (30mℓ)m ²	4800 ab	7600 d	4433 e	3100 ab
47.	Citronella 2 x (15mℓ)m ²	2266 ab	6266 d	8066 f	15500 d
48.	Orange 2 x (50mℓ)m ²	2466 ab	6066 cd	3766 de	15133 cd
49.	Halls 2 x (1ℓ)	7166 b	6133 cd	8833 f	13866 bcd
50.	Halls 2 x (2ℓ)	14366c	5000 cd	2966 cde	9033 abcd
51.	Untreated control	19866 c	3566 bc	11733 g	9200 abcd
52.	Rugby 3 x (15g)m ²	100 a	366 a	66 a	333 a

Table 4.4.3.3. *Tylenchulus semipenetrans* female population counts ♀ / 10 g roots

GROUP A: RUGBY EC – EGG STIMULATION					
♀ / 10 g roots					
Treatment		Mar 2002	Sept 2002	Jan 2003	Apr 2003
1.	Rugby (20ml)m ² + D-Limonene (30ml) m ²	6200 bcde	3133 abcd	466 ab	3800 cdef
2.	Rugby (20ml)m ² + Citronella (15ml) m ²	15000 h	800 a	133 a	133 a
3.	Rugby (20ml)m ² + Orange (50ml) m ²	6733 bcde	2400 abc	1133 ab	1466 abc
4.	Rugby (20ml)m ² + Halls (1ℓ)	4733 abcd	5333 cdef	1066 ab	1266 abc
5.	Rugby(20ml)m ² + Halls (2ℓ)	3666 abc	1333 ab	533 ab	200 ab
6.	Rugby (10ml)m ² + D-Limonene (30ml) m ²	4666 abcd	666 a	966 ab	2866 bcde
7.	Rugby (10ml)m ² + Citronella (15ml) m ²	2400 ab	5400 cdef	933 ab	3733 cdef
8.	Rugby (10ml)m ² + Orange (50ml) m ²	4613 abcd	14733 h	2033 abc	5666 fg
9.	Rugby (10ml)m ² + Halls (1ℓ)	8000 cdef	2000 abc	5533 de	4466 defg
10.	Rugby (10ml)m ² + Halls (2ℓ)	4133 abc	2133 abc	4933 cde	2266abcd
11.	Rugby (10ml)m ² + D-Limonene 2 x (30ml) m ²	9666 efg	5533 cdef	1733 abc	1800 abcd
12.	Rugby (10ml)m ² + Citronella 2 x (15ml) m ²	15066 h	7400 efg	1866 abc	5266 efg
13.	Rugby (10ml)m ² + Orange 2 x (50ml) m ²	11666 fgh	4400 bcde	1400 ab	1200 abc
14.	Rugby (10ml)m ² + Halls 2 x (1ℓ)	20933 i	9533 g	1500 abc	1866 abcd
15.	Rugby (10ml)m ² + Halls 2 x (2ℓ)	6000 abcde	4466 bcde	400 ab	2333 abcd
46.	D-Limonene 2 x (30ml)m ²	14000 gh	8200 fg	15933 h	3266 cdef
47.	Citronella 2 x (15ml)m ²	9533 defg	6200 defg	13800 gh	4266 def
48.	Orange 2 x (50ml)m ²	6400 bcde	15466 h	3800 bcd	5533 efg
49.	Halls 2 x (1ℓ)	14800 h	15266 h	11200 fg	7066 gh
50.	Halls 2 x (2ℓ)	20933 i	5000 cdef	7800 ef	10333 l
51.	Untreated control	8266 cdef	7000 efg	13400 gh	9200 hi
52.	Rugby 3 x (15g)m ²	1133 a	200 a	0 a	66 a

Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 4.4.3.4. *Tylenchulus semipenetrans* second stage juvenile counts J2/250 ml soil.

GROUP B: NEMACUR EC – EGG STIMULATION					
J2 / 250 ml SOIL					
Treatment		Mar 2002	Sept 2002	Jan 2003	Apr 2003
16.	Nemacur (5ml)m ² + D-Limonene (30ml) m ²	333 a	6200 abcd	1900 ab	5300 a
17.	Nemacur (5ml)m ² + Citronella (15ml) m ²	1833 ab	3733 abcd	2633 ab	6500 ab
18.	Nemacur (5ml)m ² + Orange (50ml) m ²	800 a	1966 ab	766 a	10000 ab
19.	Nemacur (5ml)m ² + Halls (1ℓ)	1766 ab	1566 a	1833 ab	7433 ab
20.	Nemacur (5ml)m ² + Halls (2ℓ)	2066 ab	2933 abc	1700 ab	9866 ab
21.	Nemacur (2.5ml)m ² + D-Limonene (30ml) m ²	1533 ab	3233 abcd	2266 ab	10733 ab
22.	Nemacur (2.5ml)m ² + Citronella (15ml) m ²	2033 ab	3400 abcd	5066 bcd	18300 ab
23.	Nemacur (2.5ml)m ² + Orange (50ml) m ²	4666 ab	2500 ab	2666 ab	5033 a
24.	Nemacur (2.5ml)m ² + Halls (1ℓ)	5066 ab	6200 abcd	4066 abc	5766 ab
25.	Nemacur (2.5ml)m ² + Halls (2ℓ)	4566 ab	11700 e	6300 cde	11566 ab
26.	Nemacur (2.5ml)m ² + D-Limonene 2 x (30ml) m ²	2500 ab	5833 abcd	3133 abc	17233 ab
27.	Nemacur (2.5ml)m ² + Citronella 2 x (15ml) m ²	1400 ab	7966 de	1933 ab	6700 ab
28.	Nemacur (2.5ml)m ² + Orange 2 x (50ml) m ²	4700 ab	6100 abcd	1566 ab	14366 ab
29.	Nemacur (2.5ml)m ² + Halls 2 x (1ℓ)	733 a	1633 a	1933 ab	2633 a
30.	Nemacur (2.5ml)m ² + Halls 2 x (2ℓ)	3900 ab	2433 ab	2700 ab	21133 b

46.	D-Limonene 2 x (30mℓ)m ²	4800 ab	7600 cde	4433 bc	3100 a
47.	Citronella 2 x (15mℓ)m ²	2266 ab	6266 abcd	8066 de	15500 ab
48.	Orange 2 x (50mℓ)m ²	2466 ab	6066 abcd	3766 abc	15133 ab
49.	Halls 2 x (1ℓ)	7166 bc	6133 abcd	8833 ef	13866 ab
50.	Halls 2 x (2ℓ)	14366 de	5000 abcd	2966 abc	9033 ab
51.	Untreated control	19866 e	3566 abcd	11733 f	9200 ab
52.	Nemacur 3 x (15g)m ²	13000 cd	6566 bcd	20966 g	13333 ab

Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 4.4.3.5. *Tylenchulus semipenetrans* female population counts ♀ / 10 g roots

GROUP B: NEMACUR EC – EGG STIMULATION					
♀ / 10 g roots					
Treatment		Mar 2002	Sept 2002	Jan 2003	Apr 2003
16.	Nemacur (5mℓ)m ² + D-Limonene (30mℓ) m ²	5066 a	6266 abc	12133 efghij	3000 a
17.	Nemacur (5mℓ)m ² + Citronella (15mℓ) m ²	6200 a	5666 ab	5733 abc	9800 cd
18.	Nemacur (5mℓ)m ² + Orange (50mℓ) m ²	5600 a	11133 defg	2933 a	11200 de
19.	Nemacur (5mℓ)m ² + Halls (1ℓ)	4466 a	4933 a	9600 cdefgh	8833 bcd
20.	Nemacur (5mℓ)m ² + Halls (2ℓ)	4533 a	4066 a	9000 bcdefgh	10333 cde
21.	Nemacur (2.5ℓ)m ² + D-Limonene (30mℓ) m ²	8066 ab	10333 bcdef	7200 abcde	1673 3 fg
22.	Nemacur (2.5ℓ)m ² + Citronella (15mℓ) m ²	4533 a	6200 abc	12933 fghij	21400 g
23.	Nemacur (2.5ℓ)m ² + Orange (50mℓ) m ²	14866 e	4333 a	7533 abcde	4133 ab
24.	Nemacur (2.5ℓ)m ² + Halls (1ℓ)	15266 e	4533 a	9800 cdefgh	5200 abc
25.	Nemacur (2.5ℓ)m ² + Halls (2ℓ)	13733 cde	6066 abc	17000 jk	18200 fg
26.	Nemacur (2.5mℓ)m ² + D-Limonene 2 x (30mℓ) m ²	6466 a	10400 cdef	11000 cdefghi	15133 ef
27.	Nemacur (2.5mℓ)m ² + Citronella 2 x (15mℓ) m ²	4666 a	12000 efg	9866 cdefgh	6866 abcd
28.	Nemacur (2.5mℓ)m ² + Orange 2 x (50mℓ) m ²	12000 bcde	5933 abc	8133 abcdefg	5800 abc
29.	Nemacur (2.5mℓ)m ² + Halls 2 x (1ℓ)	4400 a	5133 a	5800 abc	2933 a
30.	Nemacur (2.5mℓ)m ² + Halls 2 x (2ℓ)	9266 abc	14866 fg	6733 abcd	6800 abcd
46.	D-Limonene 2 x (30mℓ)m ²	14000 cde	8200 abcde	15933 ijk	3266 a
47.	Citronella 2 x (15mℓ)m ²	9533 abcd	6200 abc	13800 hijk	4266 ab
48.	Orange 2 x (50mℓ)m ²	6400 a	15466 g	3800 ab	5533 abc
49.	Halls 2 x (1ℓ)	14800 de	15266 g	11200 defghi	7066 abcd
50.	Halls 2 x (2ℓ)	20933 f	5000 a	7800 abcdef	10333 cde
51.	Untreated control	8266 ab	7000 abcd	13400 ghijk	9200 bcd
52.	Nemacur 3 x (15g)m ²	13666 cde	22266 h	18333 k	15500 ef

Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 4.4.3.6. *Tylenchulus semipenetrans* second stage juvenile counts J2/250ml soil

GROUP C: MOCAP EC – EGG STIMULATION					
J2 / 250 mℓ SOIL					
	Treatment	Mar 2002	Sept 2002	Jan 2003	Apr 2003
31.	Mocap (7.5mℓ)m ² + D-Limonene (30mℓ) m ²	2266 a	2933 abcd	1500 abc	4966ab
32.	Mocap (7.5mℓ)m ² + Citronella (15mℓ) m ²	2000 a	1833 ab	666 a	7200 ab
33.	Mocap (7.5mℓ)m ² + Orange (50mℓ) m ²	6133 a	1766 ab	1966 abcd	333 a
34.	Mocap (7.5mℓ)m ² + Halls (1ℓ)	4166 a	1900 ab	866 ab	4833 ab
35.	Mocap (7.5mℓ)m ² + Halls (2ℓ)	4933 a	1166 a	1433 abc	20100 ab
36.	Mocap (3.7mℓ)m ² + D-Limonene (30mℓ) m ²	3333 a	3100 abcde	3200 abcde	11566 ab
37.	Mocap (3.7mℓ)m ² + Citronella (15mℓ) m ²	7600 ab	3433 abcde	6466 efgh	5800 ab
38.	Mocap (3.7mℓ)m ² + Orange (50mℓ) m ²	6633 a	666 a	7333 fgh	11133 ab
39.	Mocap (3.7mℓ)m ² + Halls (1ℓ)	1466 a	2066 ab	5133 defg	9433 ab
40.	Mocap (3.7mℓ)m ² + Halls (2ℓ)	2200 a	2600 abc	3566 abcde	15066 ab
41.	Mocap (3.7mmℓ)m ² + D-Limonene 2 x (30mℓ) m ²	3900 a	2700 abc	8833 hi	21533 b
42.	Mocap (3.7mℓ)m ² + Citronella 2 x (15mℓ) m ²	5100 a	1266 a	4366 bcdef	14733 ab
43.	Mocap (3.7mℓ)m ² + Orange 2 x (50mℓ) m ²	3766 a	5400 cdef	2466 abcd	4633 ab
44.	Mocap (3.7mℓ)m ² + Halls 2 x (1ℓ)	1533 a	3300 abcde	5166 defg	6766 ab
45.	Mocap (3.7mℓ)m ² + Halls 2 x (2ℓ)	2700 a	2100 ab	3866 abcdef	22066 b
46.	D-Limonene 2 x (30mℓ)m ²	4800 a	7600 f	4433 cdef	3100 a
47.	Citronella 2 x (15mℓ)m ²	2266 a	6266 ef	8066 gh	15500 ab
48.	Orange 2 x (50mℓ)m ²	2466 a	6066 def	3766 abcde	15133 ab
49.	Halls 2 x (1ℓ)	7166 a	6133 def	8833 hi	13866 ab
50.	Halls 2 x (2ℓ)	14366 bc	5000 bcdef	2966 abcde	9033 ab
51.	Untreated control	19866 c	3566 abcde	11733 i	9200 ab

Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 4.4.3.7. *Tylenchulus semipenetrans* female population counts ♀ / 10 g roots.

GROUP C: MOCAP EC – EGG STIMULATION					
♀ / 10 g roots					
	Treatment	Mar 2002	Sept 2002	Jan 2003	Apr 2003
31.	Mocap (20mℓ)m ² + D-Limonene (30mℓ) m ²	7533 abcd	8133 d	5133 abcd	6333bcdef
32.	Mocap (20mℓ)m ² + Citronella (15mℓ) m ²	10466 def	6000 bcd	2400 ab	5933 bcde
33.	Mocap (20mℓ)m ² + Orange (50mℓ) m ²	17733 gh	2800 ab	3400 abc	1466 a
34.	Mocap (20mℓ)m ² + Halls (1ℓ)	6266 abcd	4800 abcd	2066 a	2400 a
35.	Mocap (20mℓ)m ² + Halls (2ℓ)	4800 ab	2300 a	2066 a	5800 bcde
36.	Mocap (10mℓ)m ² + D-Limonene (30mℓ) m ²	4066 a	2333 a	3466 abc	12400 hi
37.	Mocap (10mℓ)m ² + Citronella (15mℓ) m ²	6133 abcd	4866 abcd	6466 bcde	10666 ghi
38.	Mocap (10mℓ)m ² + Orange (50mℓ) m ²	5066 abc	5266 abcd	5733 abcde	8666 efgh
39.	Mocap (10mℓ)m ² + Halls (1ℓ)	5133 abc	3666 abc	10833 fgh	3633 abcd
40.	Mocap (10mℓ)m ² + Halls (2ℓ)	8866 bcd	12200 e	6933 cdefg	3933 abcd
41.	Mocap (10mℓ)m ² + D-Limonene 2 x (30mℓ) m ²	7200 abcd	2066 a	9533 efgh	10733 ghi
42.	Mocap (10mℓ)m ² + Citronella 2 x (15mℓ) m ²	7133 abcd	4866 abcd	4400 abcd	17100 j
43.	Mocap (10mℓ)m ² + Orange 2 x (50mℓ) m ²	5666 abc	3200 ab	7100 cdefg	3333 abc
44.	Mocap (10mℓ)m ² + Halls 2 x (1ℓ)	6800 abcd	5533 abcd	6700 cdef	7666 defg
45.	Mocap (10mℓ)m ² + Halls 2 x (2ℓ)	8800 bcd	4866 abcd	5333 abcde	13133 ij
46.	D-Limonene 2 x (30mℓ)m ²	14000 efg	8200 d	15933 l	3266 abc
47.	Citronella 2 x (15mℓ)m ²	9533 cde	6200 bcd	13800 hi	4266 abcd
48.	Orange 2 x (50mℓ)m ²	6400 abcd	15466 e	3800 abcd	5533 abcde

49.	Halls 2 x (1ℓ)	14800 fg	15266 e	11200 gh	7066 cdefg
50.	Halls 2 x (2ℓ)	20933 h	5000 abcd	7800 defg	10333 fghi
51.	Untreated control	8266 abcd	7000 cd	13400 hi	9200 efghi

Means in the same column followed by the same letter are not significantly different ($P > 0.05$).

According to this year's data it is clear that none of the egg hatching stimulating treatments had a dramatic effect in increasing the nematode populations compared to the previous year's data. Initial trials conducted in the laboratory at CRI, and a field trial at Crocodile Valley Citrus Co., indicated that the possibility does exist to confirm the theory. It is clear from the results in Tables 4.4.3.1-4.4.3.7, that Rugby at 3 x 15 g/m² on its own is the most effective post-plant chemical control measure to control the citrus nematode, *Tylenchulus semipenetrans* (Tables 4.4.3.2, treatment 52). The combination of Rugby and a specific stimulating agent (Table 4.4.3.2, treatment 2) reduced the nematode population numbers from 15000 in March 2002 to 133 females/10g roots. It is believed that the Rugby treatment contributed towards the control of nematode populations after the stimulating process took place. The same results as above were recorded in treatment 5.

The nematicides (Rugby & Mocap) used in combination with these egg stimulating products did not reduce the nematode populations to acceptable levels to less than 1000♀/10g roots and may therefore be an indication that the stimulating products do have a small effect in stimulating the nematode eggs to hatch. The poor result of the Nemacur treatment (Table 4.4.3.5, treatment 52) is most likely due to AMD. The poor results could be due to the current drought situation and therefore the trial was sampled in January 2004 after good rains fell in the area during that month.

For comparison purposes, the trial data of January 2004 was not available for inclusion in this report due to unforeseen circumstances.

Conclusion

The fact that poor results were obtained in the field does not indicate that this research should be abandoned. More laboratory trials and consultation will be necessary to determine future strategies. It is essential to develop new, alternative and more effective measures to control the citrus nematode economically.

Future research

More laboratory trials will be executed in the 2004 season in conjunction with Potchefstroom University to determine the potential of these products under normal field conditions.

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4.4.4 Evaluation of biological control agents against *Tylenchulus semipenetrans* Experiment 318 by M.C. Pretorius (CRI)

Opsomming

Die huidige tendens wêreldwyd om chemiese middels in die landbou te vervang plaas groot druk op navorsers om alternatiewe beheermaatreëls te ondersoek. Alle grondorganismes leef in balans met mekaar in die natuur. Plant parasitiese nematodes spandeer 'n gedeelte van hul lewensiklus in die grond, wat 'n uiters komplekse omgewing is. Daar is heelwat faktore wat 'n invloed op hul lewensaktiwiteite uitoefen soos bv. grondfisiese faktore soos temperatuur, grondvog, gronddeurlugting, ander nematodes en ander grondorganismes. Daarom is dit belangrik dat daar gestreef moet word na 'n geïntegreerdebeheerprogram

om sodoende die sitrusaalwurm effektief te beheer. Huidige organofosfaat en karbamaat aalwurmdoders mag moontlik van die mark verdwyn a.g.v. hul toksisiteit.

PL+ as 'n biologiesebeheeragent is volgens resultate nie altyd effektief om op sy eie aangewend te word nie. Die nuwe PL+ en Trichoderma produk het geen effek gehad op die beheer van aalwurmpopulasies in die wortels en grond nie. Die middel word dus tans nie aanbeveel nie. Die kombinasie van PL+ met 'n aalwurmdoder het bevredigende resultate gelewer. Jong visueel gesonde bome met 'n aalwurm probleem behoort op 'n kommersiële kleinskaal toegedien te word aangesien die gebruik van 'n biologiese agent as aalwurmdoder spesiale tegniese toegewydheid verg. Indien sukses behaal word kan die beheerprogram op 'n groter skaal uitgebrei word. Besproeiingsbestuur bv. skedulering, is uiters belangrik aangesien die biologiese agente lewende organismes is wat slegs onder uiters gunstige toestande sal oorleef.

Die gebruik van PL+ deur mikro- en drupbespuitingsstelsel sal ondersoek word om die effektiwiteit van die produkte moontlik te verbeter.

Introduction

Mammalian toxicity of nematicides and the fate of these materials in the environment has rekindled interest in biological control of nematodes. After more than half a century of neglect, biological control is seen as a high priority area for research, and funding has increased world wide. However, biological control has now reached a critical point in its development. Only a few systems of utilizing antagonists of nematodes for nematode control have been widely adopted with obvious success, and this lack of progress towards the commercialisation of biological control has left many nematologists sceptical of its potential. The difficulties involved in developing a reliable crop protection system are often underestimated.

The relatively stable behaviour of animal populations in natural environments should serve as a constant reminder that in nature all organisms are subjected to a constant series of checks and balances. Populations of individual species do not increase indefinitely but tend to be constrained by the physical environment and by the community of organisms with which they co-exist. Plant parasitic nematodes spend at least some part of their lives in the soil, one of the most complex of environments. Their activities are not only influenced by variation in soil physical factors such as temperature, moisture and aeration, but also by a vast array of living organisms, including other nematodes, bacteria, fungi, algae, protozoans, insects, mites and other soil animals. This biological component of the soil ecosystem is particularly important in limiting, and more or less stabilizing, nematode populations. All organisms are competitors for resources such as space and oxygen, etc. and plant pathogens compete with plant parasitic nematodes for the same food source. It is clear from previous results that these biocontrol products must form part of an integrated control strategy because these products on their own were not effective in controlling the citrus nematode.

The work conducted in this trial was done in collaboration with Biological Control Products (BCP) to initially find an effective solution to replace the organophosphates with biological control agents. A new PL+ Trichoderma combination was evaluated on the previous PL+ treated treatments.

Materials and methods

A twelve-year-old replant orchard with Valencia orange on Rough lemon trees was selected at Bokfontein in the Brits area. The grower experienced a problem with low yields and small fruit size despite the trees being visually healthy. Citrus nematodes were present (>8000 females per 10 g roots) in numbers exceeding the threshold levels recommended for treatment of 1000 females per 10 g roots. Six treatments were replicated seven times in a randomised block design. The treatments were as follows:

Treatment	Dosages
1. Untreated control	-
2. Temik + Rugby	12.5 g/m ² + 20 g/m ²
3. Rugby	3 (20 g/m ²)
4. Rugby + PL Plus	20 g/m ² + 2 (PL 4 g + Agr 2 ml + BM 5%)
5. PL Plus (New)	3 (PL+ plus Trichoderma)
6. PL Plus (New)	3 (PL+ plus Trichoderma)
7. Nemacur	3 (20 g/m ²)

The original trial was retained with a new PL+ formulated *Paecilomyces* + *Trichoderma* combination treatment being applied during the 2003 season to the original PL+ treatments (Treatments 5 & 6).

According to the manufacturing company, BCP, this new PL+ tricho product would be more effective as a biological control agent in controlling the citrus nematode.

Soil samples were taken prior to each re-application. The samples were analysed by the CRI 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).

Three applications were conducted during the season. Due to the extreme drought situation that was experienced in most of the summer rainfall areas, the trial was only sampled twice during the season. A final sample was taken in January 2004 after good rains fell in the area. The final analysis will be added to the report when the data are analysed.

Results and discussion

Both the chemical treatments (2 + 3) and treatment 4 (2 x Rugby 20 g/m² + PL+) kept the female nematode counts below the threshold value of 1000 females/10 g roots. These results indicated significantly lower female population levels compared to the untreated control, 2x PL+ Trichoderma (treatments 5 & 6), and Nemacur (treatment 9) treatments (Table 4.4.4.1). It is clear from the results that the PL+ plus Trichoderma treatments (treatments 5 & 6) did not have any significant effect in reducing the nematode populations in both the soil and roots. The Nemacur treatment performed poorly, possibly because of AMD.

The trial was reapplied during the 2003/04 season for a final evaluation of the biological control agents. The extreme drought situation may have prevented these agents from performing at their full potential in reducing the nematode populations in the soil and roots of citrus trees. It is clear from the results in this trial that the chemical nematicide Rugby is still superior in reducing the nematode populations to very low numbers. The combination of Rugby and PL+ (chemical and biological combination) is the most effective treatment that can be utilized to effectively reduce nematode populations. Producers are still recommended to only apply a small irrigation block with this combination in order to determine the efficacy relative to their own orchard conditions.

The drought situation also indicated that an effective irrigation system is required to optimize applications/treatments of biocontrol products and nematicides, to control the nematode numbers effectively.

Table 4.4.4.1. Second stage larvae population counts J2/250 ml soil and female population / 10 g of roots at Brits.

Treatment	Oct 2001	Dec 2001	Mch 2002	Sept 2002	Feb 2003	May 2003
J2 / 250 cc SOIL						
1. Untreated control	18071 b	8471 b	5471 b	9042 b	2600 ab	4700 ab* (100%)
2. Temik (12.5g/m ²) + 2 x (Rugby 20g/m ²)	528 a	214 a	0 a	728 a	2442.86 ab	471.42 a (10)
3. Rugby 3 x (20g/m ²)	2157 a	1300 a	114 a	1057 a	2042.86 ab	1814.29 ab (38)
4. Rugby (20g/m ²) + 2 x (PL Plus)	300 a	42 a	114 a	2128 a	542.8 a	1942 ab (41)
5. 3 x (PL Plus)	13371 b	8457 b	3485 b	10085 b	5671.43 b	9985 bc (212)
6. 3 x (PL Plus)	27757 c	9785 b	4885 b	18871 c	10585.7 c	22042.9 d (469)
7. Nemacur 3 x (20g)	11042 b	6857 b	3685 b	17942 c	15814.3 d	15042.9 d (320)
♀ / 10g roots						
1. Untreated control	10371 b	6657 b	8228 b	12171 b	11871.4 c	6428.57 b (100)
2. Temik (12.5g/m ²) + Rugby 2 x (20g/m ²)	1114 a	85 a	1057 a	200 a	542.85 a	400 a (6)
3. Rugby 3 x (20g/m ²)	1342 a	137 a	1485 a	1800 a	457.14 a	400 a (6)
4. Rugby (20g/m ²) + 2 x (PL Plus)	1057 a	342 a	628 a	1942 a	542.85 a	714 a (11)
5. 3 x (PL Plus)	11300 bc	11600 c	6085 b	12857 b	9200 bc	7942 b (124)
6. 3 x (PL Plus)	15200 bc	17000 d	19828 c	22257 bc	8171.43 b	12200 c (190)
7. Nemacur 3 x (20g)	17114 c	18714 d	22485 c	18214 bc	6800 b	12657 c (197)

*May 2003 - results as a percentage of the control which was the new trial, new PL+ Trichoderma combination being applied during the 2003 season.

Future research

BCP developed a new combination of *Trichoderma* spp. and PL+. This product was included in the existing trial on the two PL+ treatments. According to BCP this combination would be more effective on its own in controlling nematodes. These treatments did not have any effect in reducing the nematode populations in the soil and roots of this trial site. This trial will therefore be terminated.

The use of PL+ through drip micro-irrigation systems where the environmental conditions are more suited for micro-organisms will also be investigated.

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4.4.5 Rootstock evaluation against *Hemicycliophora* in the Gamtoos River Valley Experiment 676 by M.C. Pretorius (CRI)

Opsomming

Die sitrusaalgwurm is steeds die enkele grootste aalgwurm probleem tans in die Suid-Afrikaanse sitrusindustrie. Aalgwurms wat die vermoë besit om skade te kan veroorsaak op sitrus is uiters beperk. In Suid-Afrika is *Hemicycliophora*, die skedeaalgwurm, in verskeie boorde reeds so vroeg as 1963 al geïdentifiseer. Tans is dié aalgwurm teenwoordig in die Gamtoosriviervallei, in sekere boorde in die Wes-Kaap asook in sekere boorde in Mpumalanga se Laeveld. Die skedeaalgwurm kom in kombinasie met die sitrusaalgwurm op wortels van sitrus voor. Die effek van die skedeaalgwurm op die sitrusboom word as gevolg van die kombinasie bemoeilik. Drie spesies is geïdentifiseer, nl. *Hemicycliophora halophila*, *H. nortoni* en *H. typica*. Die standaard na-plant chemiese aalgwurmdoders geregistreer op sitrus sal die skedeaalgwurm ook beheer, soos vasgestel in vorige proewe. Vier van die gewildste onderstamme word tans in 'n *Hemicycliophora* geïnfekteerde boord gemonitor, naamlik Growweskiisuurlemoen, Swingle citrumelo, Carrizo citrange en C35. Die parameters wat geëvalueer word is *Hemicycliophora* in die wortels, boomhoogte, stamdeursnee en 'n visuele gradering. Alhoewel die bome nog jonk is, en die situasie oor 'n langer termyn gemonitor sal word, het die Swingle onderstam die laagste aalgwurm getalle in vergelyking met die ander onderstamme gehad. Dit is bekend dat Swingle as 'n weerstandbiedende onderstam suksesvol in herplant situasies gebruik kan word.

Introduction

Nematodes other than *T. semipenetrans* currently known to be capable of damaging citrus tend to be very limited in distribution. A number of species of *Hemicycliophora* have been identified from the citrus rhizosphere. *Hemicycliophora arenaria* is a species native to plants in the desert valleys of southern California that causes damage in citrus nurseries (McElroy *et al.*, 1966). The nematode was closely studied (Van Gundy, 1959) and quarantined to prevent its spread to other areas of that state. It appears to have a wide host range (ten of nineteen hosts tested) although the rutaceous host status is variable. *Citrus limon*, *C. aurantifolia*, *C. reticulata* and *C. sinensis* are resistant (Van Gundy & Rackham, 1961). The nematode feeds in large numbers at root tips whose roots typically develop round galls arising from hyperplasia. Seedling growth in pot studies was reduced by 35%. *Hemicycliophora nudata* causes similar symptoms on citrus in Australia (Colbran, 1963). *H. arenaria* can be eradicated from root systems with hot water dips (10 min at 46°C), preplant soil fumigation with methyl bromide is very effective and a number of rootstocks resistant to the nematode are available (Van Gundy & McElroy, 1969). Three spp. that were detected from samples in the Gamtoos River Valley were identified by Dr. Ester v/d Berg, viz. *Hemicycliophora halophila*, *H. nortoni* and *H. typica*.

It is clear from previous trial results that post-plant chemical nematicides did control both the citrus nematode and *Hemicycliophora*. It is, however, essential to determine the effect of *Hemicycliophora* on rootstocks currently used by the citrus industry.

Materials and methods

Four popular rootstocks currently used by citrus producers were obtained from Paksaam nursery, viz. Rough lemon, Swingle citrumelo, Carrizo citrange and C35. An orchard infected with *Hemicycliophora* was identified and one of each rootstock was randomly planted next to a tree in a wagon wheel formation with ten replicates. A drip irrigation system was installed in the orchard. The producer assisted in ensuring that aboveground insects were controlled by means of stem applications every month, using Azodrin. These trees were fertilized by means of a liquid fertilizer mixture through the drip irrigation system.

The following parameters will be evaluated for the duration of this trial:

1. Soil analysis after one year, 18 months and thereafter twice per year to determine the nematode population status.
2. Visual rating (after 1 year and on a regular basis thereafter).
3. Stem diameter (after 1 year and on a regular basis thereafter).
4. Tree height (after 1 year and on a regular basis thereafter).

The trees were sampled and evaluated in February 2003 and ten months later in December 2003.

Results and discussion

The nematode populations are still relatively low because the trees are still young. However, it is clear from the results in Table 4.4.5.1 that the citrus nematode female counts in the Swingle rootstock treatment are significantly lower than all the other rootstocks. It is known that the Swingle rootstock is resistant to nematodes and therefore an excellent replant rootstock although not very popular. Nematode larvae will attack the root system of Swingle but will never complete its life cycle due to the characteristic nature of the rootstock. Because no host is therefore available, the citrus nematode populations in these situations will decline to very low or undetectable levels. The December 2003 results indicated that the Rough lemon and Carrizo Citrange rootstocks had the highest citrus nematode female counts. No significantly different *Hemicycliophora* counts were present at this stage but it is believed that the nematode counts will increase as the trees grow older. The trial will be monitored regularly.

Table 4.4.5.1. *Tylenchulus semipenetrans* and female population counts, and *Hemicycliophora* juvenile and adult population counts on 4 different rootstocks.

Treatments	<i>Tylenchulus semipenetrans</i>				<i>Hemicycliophora</i>			
	J2/250ml soil		♀ / 20 g roots		J2/250ml soil		♀ / 20 g roots	
	Feb 2003	Dec 2003	Feb 2003	Dec 2003	Feb 2003	Dec 2003	Feb 2003	Dec 2003
Swingle citrumelo	990 a	1270 a	320 a	240 a	196.66 a	174.5 b	360 ab	330 a
Carrizo Citrange	3130 a	4020 b	2020 ab	2580 b	60 a	44 a	90 a	250 a
Rough lemon	3330 a	2580 ab	1940 ab	2820 b	193 a	27.5 a	810 b	230 a
C35 citrange	1790 a	1610 a	2660 b	1460 b	126.5 a	98.5 ab	340 ab	230 a

Means in the same column followed by the same letter are not significantly different ($P>0/05$).

It is clear that all the trees increased in stem diameter and tree height (Table 4.4.5.2) although no huge differences were visible. The stem diameter of the Carrizo rootstock treatments was noticeably smaller as expected due to its characteristics, compared to the remainder of the rootstocks. No significant difference in the tree height and the visual rating was observed. The trial will be evaluated twice a year to determine the effect of both nematodes on the different rootstocks regarding nematode population, stem diameter, tree height and the visual appearance of the different trees in the trial.

Table 4.4.5.2. The effect of nematode populations on the stem diameter 10 cm above and under the bud union, tree height and visual tree rating on 4 different rootstocks.

Treatments	Feb 2003	Dec 2003	Feb 2003	Dec 2003	Feb 2003	Dec 2003	Feb 2003	Dec 2003
	Stem diameter (above budunion)	Stem diameter (above budunion)	Stem diameter (under budunion)	Stem diameter (under budunion)	Tree height	Tree height	Visual rating (3=good 0=dead)	Visual rating (3= good 0=dead)
Swingle citrumelo	14.91 b	15.17 ab	2265 b	22.32 b	1.29 c	1.27 b	2.7 bc	2.5 ab
Carrizo citrange	10.47 a	12.44 a	14.74 a	17.32 a	0.96 a	1.12 ab	2 a	1.9 a
Rough lemon	15.84 b	16.75 b	18.96 b	20.03 ab	1.17 bc	1.23 ab	2.9 c	2.6 b
C35 citrange	14.31 b	14.09 ab	20.98 b	20.78 ab	0.97 ab	1.03 a	2.1 ab	1.9 a

Means in the same column followed by the same letter are not significantly different ($P>0.05$).

Hemicycliophora was recorded in certain samples in the Western Cape and the Onderberg areas. This nematode is, however, always present on citrus roots in combination with the citrus nematode. It is therefore difficult to determine the damage caused by the presence of *Hemicycliophora* to the citrus tree. *Hemicycliophora* can be controlled with a post-plant chemical nematicide control strategy.

Conclusion

The trees are being monitored on a regular basis but it is too early to draw conclusions.

Future research

The trial is ongoing and will be evaluated for a number of years.

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4.4.6 Determining the effect of a biological control product and a phosphonate in combination with a chemical which induces systemic acquired resistance in *Phytophthora*-infested nursery seedlings

Experiment 722 by M.C. Pretorius (CRI)

Opsomming

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is 'n aggressiewe watergedraagde patogeen wat wortelvrot en bruinvrot in boorde en kwekerie in suidelike Afrika veroorsaak. Heelwat beheermaatreëls en middels is alreeds sedert die vroeër jare 1950s ondersoek om die probleem suksesvol te kan oplos. Kontakswamdoders sowel as sistemiesemiddels is ontwikkel. Meeste van die resultate verkry na aanwending van die middels, bly steeds wisselvallig. Alternatiewe maatreëls is daarom deur die CIP geïmplimenteer om die oorsake van besmetting te beperk.

Veiliger, meer effektiewe en ekonomies bekostigbare produkte moet gevind en ge-evalueer word om die voorkoms van *Phytophthora* te beperk. Ridomil as 'n chemiese standard en 'n fosfonaat (Phytex), asook 'n kombinasie van 'n Produk x met Phytex en 'n Biologiese beheer agent, is in die proef ge-evalueer. Nie een van die produkte het die *Phytophthora* insidensie bevredigend beheer nie. Daar was wel 'n onderdrukkende effek sigbaar maar nie aanvaarbaar genoeg nie. Die swak prestasie van die Ridomil behandeling as 'n swamdoder het die vraag laat ontstaan of daar nie dalk weerstand teenwoordig is nie. Die aspek sal beslis nagevors moet word. Die kombinasie van Produk X en Phytex het wel 'n positiewe effek op die saailinge se droëwortelmasse en boomgewig gehad. Indien *Phytophthora* effektief beheer kan word en met Produk x se positiewe effek op die boomkwaliteit, kan dit 'n uitstekende kombinasie wees wat deur die kwekerybedryf gebruik kan word. Die benadering behoort verder ondersoek te word.

Introduction

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is an aggressive root rot and fruit rot pathogen in citrus. The pathogen has a wide range of host species and can be isolated from soil in most of the older citrus orchards in southern Africa as well as in young nursery trees.

Most of the research on the chemical control of fungal root pathogens of citrus concerns *P. nicotianae* var. *parasitica* and *P. citrophthora*. Godfrey (1953) recommended that young citrus trees be painted with a fungicidal trunk paint to protect them against *Phytophthora*. Sleeth (1966) reported the effectiveness of

paints containing 1-5% copper. Timmer (1977) found that copper ammonium carbonate, cupric hydroxide and captafol were all active for at least 33 weeks when applied at a concentration of 60 mg/m². Captab and pyroxychlor were less effective and retained activity for only 17 weeks. The latter compound limited the expansion of root rot lesions but was not translocated from the treated area. Excision of effected tissue and painting with a copper fungicide slightly improved tree recovery (Timmer, 1977).

During the seventies a new class of systemic fungicides, the acylalanines, proved to be effective in the control of diseases caused by *Phytophthora* spp and certain other oomycetes under field and greenhouse conditions (Young, Seifried & Biehn, 1977; Darvas, Kotzé & Toerien, 1978). Metalaxyl controlled fruit, stem and/or root infections of citrus by *P. nicotianae* var. *parasitica* and *P. citrophthora* (Timmer, 1979; Farih, Menge, Tsao & Ohr, 1981) and drastically reduced or even eliminated *P. nicotianae* var. *parasitica* populations in treated soils (Farih et al, 1981). Low concentrations of the compound were highly inhibitory to mycelial growth as well as to the formation of sporangia, chlamydo spores and oospores (Farih et al, 1981).

Soon after the launching of the acylalanine fungicides, resistant strains were observed among the populations of various oomycete species (Clerjeau, Piganeau, Bompeix & Malfatti, 1984). After five years of application to avocados in South Africa, metalaxyl has shown a loss of effectiveness (Darvas & Becker, 1984). This probably was due to soil bacteria metabolising the compound (McKenzie & Margot, 1982), and not to the induction of resistant strains of *P. cinnamomi*. However, Pegg (1983) claims that strains tolerant to metalaxyl pre-exist in field populations of other *Phytophthora* spp. and that the selection of resistant strains results in the loss of disease control. Zentmeyer & Ohr (1978) reported the control of *Phytophthora* root rot by another systemic fungicide, fosetyl aluminium (aluminium tris-o-ethyl phosphonate). The effectiveness of this compound in the control of gummosis and root rot of citrus caused by *P. nicotianae* var. *parasitica* and *P. citrophthora* was proved by Farih et al (1981). The formation of sporangia, chlamydo spores and oospores was highly sensitive to fosetyl-Al, but zoospore and chlamydo spore germination, as well as germ tube growth were insensitive to low concentrations of the fungicide (Farih, Tsao & Menge, 1981). Fosetyl-Al can thus be regarded as an anti-sporulant.

Since fosetyl-Al has a low activity against mycelial growth *in vitro*, it was proposed that the compound might act indirectly by triggering a host resistance response (Zentmeyer & Ohr, 1978). In the plant, fosetyl-Al is degraded to H₃PO₃. The latter compound has a similar, though generally higher efficiency in reducing stem infection of *Persea indica* seedlings by *P. citricola* Sawada (Fenn & Coffey, 1984).

The phosphonates are currently the most effective product that could be used to control *Phytophthora* spp. in existing infected orchards. However, in the nursery environment, the limited availability of effective fungicides to control root pathogens such as *Phytophthora* and *Pythium* is an enormous concern to the nursery industry. Unfortunately, the phosphonates did not perform consistently well in the nursery environment. A few biological products were identified with fungicidal control, however, when commencing with their trials the companies concerned failed to provide CRI with the products. Alternative products which include a combination of phosphonates and Product X and one biological control agent (PL+ - *Trichoderma* treatment) were used in this trial to search for more effective products and techniques to limit and control *Phytophthora* and *Pythium* root rot in nursery trees.

Materials and methods

Rough lemon citrus seedlings from Esselen Nursery, Malelane, were used in the glasshouse at CRI. Ten trees per treatment were used. Trees were replanted in 5 litre plastic bags in a potting soil mixture. The trees were left for a month to settle in the bags and adapt to the glasshouse environment. Excluding the untreated control, the remaining trees were infected with *Phytophthora*-infested irrigation water. The treatments and time of application are shown in Table 4.4.6.1. A chemical fungicide (Ridomil), a phosphonate (Phytex), a chemical which induces systemic acquired resistance (SAR) (Product X), on its own and in combination with a phosphonate and a biological control product PL+ (*Paecilomyces* plus *Trichoderma* combination), were included in the trial.

Table 4.4.6.1. Treatments and time of application of trees manually infected with *Phytophthora*-infested irrigation water, excluding the untreated control.

	Treatment	Dosage / bag	Month of application					
			Aug	Sept	Oct	Nov	Dec	Jan
1	Untreated control (-P) Phytophthora	-	-	-	-	-	-	-
2	Treated control (+) Phytophthora	-	-	-	-	-	-	-
3	Ridomil	5 g / bag	X		XP*		X	
4	Phytex	2 ml / plant	X	X	XP	X	X	X
5	Phytex + Product 1X	2 ml + 17 g Product X	X	X	XP	X	X	X
6	Phytex + Product 2X	2 ml + 3 g Product X	X	X	XP	X	X	X
7	Phytex + Product 1X	1 ml + 17 g Product X	X	X	XP	X	X	X
8	Phytex + Product 2X	1 ml + 34 g Product X	X	X	XP	X	X	X
9	Product 1x	17 g	X	X	XP	X	X	X
10	Product 2x	34 g	X	X	XP	X	X	X
11	Bio Control (PL+)	1.87 g / 4 litre mixture	X	X	XP	X	X	X
12	Bio Control (PL+)	3.75 g / 4 litre mixture	X	X	XP	X	X	X

*All the trees were re-infected with *Phytophthora* in October to simulate a re-infestation of seedlings with *Phytophthora* and to determine the effect of the products applied in a situation such as this.

The trees were sampled in September, October, December and January. The following parameters were evaluated.

1. Stem diameter
2. Tree height
3. Visual tree rating
4. Wet root mass
5. Dry root mass
6. % *Phytophthora* infestation

Results and discussion

It is clear from the results in Table 4.4.6.1 that none of the treatments reduced the percentage of *Phytophthora* infections to undetectable levels. Not even the Ridomil treatments reduced the infestation significantly to acceptable levels. The Phytex treatment (4) had the lowest percentage of infected leaf pieces compared to the treated control (2). Most of the products did, however, suppress the incidence of *Phytophthora* compared to the treated control (2), but did not control the pathogen satisfactorily. The SARs product (9 & 10) on its own did not perform satisfactorily in controlling *Phytophthora*.

Table 4.4.6.2. Percentage *Phytophthora*-infected leaf pieces per treatment.

	Treatment	Sept 2003	Oct 2003	Dec 2003	Jan 2004	Feb 2004
1	Untreated control (-P)	0 a	0 a	0 a	0 a	0 a
2	Treated control (+ Phytophthora)	88.8 f	89.6 e	84.3 c	86.9 f	96.9 e
3	Ridomil	78.1 ef	20.3 bc	35.3 b	79.6 ef	67.2 d
4	Phytex	70.3 e	28.7 cd	48.7 b	58.6 bcde	39.9 b
5	Phytex + Product x	70.3 e	33 d	30.9 b	50.3 bcd	43.5 bc
6	Phytex + Product x	71 e	30.2 cd	28.7 b	35.9 b	52.2 bcd
7	Phytex + Product x	41 cd	23.6 bcd	43.6 b	85.5 f	63.7 cd
8	Phytex + Product x	48.4 d	15.5 b	39.3 b	52.4 bcd	61.7 cd
9	Product x	38.8 cd	23.3 bcd	27.5 b	65.8 cdef	59.5 bcd
10	Product x	21.6 b	20.7 bc	43.2 b	89 f	59.5 bcd
11	Bio Control	20 b	20.5 bc	47.4 b	46.5 bc	45.1 bc
12	Bio Control	30.9 bc	24.6 bcd	41.9 b	74.3 def	59.7 bcd

Means in the same column followed by the same letter are not significantly different (>0.05).

Table 4.4.6.3. Stem diameter, tree height and tree weight evaluated throughout the season, wet and dry root mass were determined when the trial was terminated.

Treatments	Dosage	Oct 2003	Feb 2003	Oct 2003	Feb 2003	Tree weight	Root (wet)	Root (dry)	
		Stem diameter	Stem diameter	Tree height	Tree height				
1	Untreated control	8.79 cde	8.89 e	0.82 cd	0.85 bc	145.79 de	59.54 fg	20.73 ef	
2	Treated control + <i>Phytophthora</i>	7.68 a	7.28 a	0.74 a	0.72 a	77.52 ab	23.31 a	9.05 ab	
3	Ridomil	2 g/pot	8.34 bc	8.32 cd	0.83 d	0.9 cd	120.61 c	49.12 def	17.14 de
4	Phytex	2mℓ / 100mℓ H ₂ O	8.14 ab	8.43 cde	0.78 abcd	0.82 b	94.68 b	35.18 bc	10.76 abc
5	Phytex + Product x	1ℓ Phytex + 17 g Product x	8.59 bcd	8.69 de	0.82 cd	0.93 d	147.84 e	59.41 fg	21.47 f
6	Phytex + Product x	1ℓ Phytex + 34 g Product x	8.32 bc	8.70 de	0.82 cd	0.89 cd	146.25 e	63.09 g	22.34 f
7	Phytex + Product x	500 mℓ Phytex + 17 g Product x	8.56 bcd	8.59 cde	0.83 d	0.89 cd	137.3 cde	52.1 ef	16.95 de
8	Phytex + Product x	1ℓ Phytex + 34 g Product x	8.95 e	8.23 cd	0.77 abc	0.84 bc	124.44 cd	44.68 cde	14.26 cd
9	Product x	17 g Product x + 1ℓ H ₂ O	8.93 e	8.62 cde	0.76 ab	0.83 b	97.08 b	39.33 bcd	11.9 bc
10	Product x	34 g Product x + 1ℓ H ₂ O	8.85 cde	8.34 cd	0.79 abcd	0.83 b	80.3 ab	29.37 ab	8.92 ab
11	Bio control	1.87 g/4ℓ H ₂ O	8.47 bcd	7.68 ab	0.78 abcd	0.80 b	68.79 a	23.42 a	7.66 a
12	Bio control	3.75 g/4ℓ H ₂ O	8.51 bcd	8.16 bc	0.86 bcd	0.89 cd	91.47 e	33.69 ab	11.7 abc

Means in the same column followed by the same letter are not significantly different (P>0.05).

The results in Table 4.4.6.3 indicate the significant difference in tree health and condition and the effect *Phytophthora* root rot (treatment 2) had on seedlings if compared to the untreated control treatment (1). *Phytophthora* is an aggressive pathogen that will destroy infected seedling roots in uncontrolled conditions. It is therefore of utmost importance to develop effective control measures to protect the nursery plant material against pathogens such as *Phytophthora* which can have a devastating effect on the citrus industry. The dry root mass parameter is an indication of the effect that *Phytophthora* has on the root system of a plant. The results indicated that treatment 11 fared even worse than the untreated control (1) although not significantly. However, treatments 5 & 6 indicated a “healthier” root system compared to the untreated control (1) even though no significant difference in percentage *Phytophthora* infection was evident. The same tendency occurred in the weight of the trees, i.e. treatments 5 & 6 weigh more than all the other treatments and significantly more than the untreated control. The SARs product (9 & 10) on its own did not perform satisfactorily at all.

Conclusion

It is clear that although the combination of the SAR product and Phytex did not control *Phytophthora* in the media, the effect it had on the seedlings’ root health as demonstrated by its dry root mass gave good results. Contrary to other systemic fungicides, phosphonates were shown to possess only weak, direct activity against some species of *Phytophthora* despite their ability to control these pathogens *in vivo*. The mode of action of the phosphonates is orientated towards the possibility of the fungus being the primary target of the compound, whereas the plant’s natural defence mechanisms play the main role in the inhibition of fungal growth. The fact that the percentage *Phytophthora* infection in the phosphonate treatments with the SAR product is still high, is understandable and there is no need for concern as these products are not fungicides but do have a positive affect on the plant health as indicated in the dry root mass and tree weight results (Table 4.4.6.3, treatments 5 & 6).

What is a matter of concern is the poor performance of the Ridomil treatments. The question asked is why this fungicide performed so poorly? Could it be as a result of resistance? This matter should be investigated.

In the dry root mass parameter, the phosphonate treatment did not result in a satisfactory result and the concentration could possibly be increased to achieve a better result. In the past it has been shown by

several different institutions that the phosphonates control *Phytophthora* on citrus. The SAR product, in combination with the phosphonate, resulted in the treatments which performed best. This treatment could be investigated further. The stem diameter and tree height results can be misleading with regard to the actual situation within the roots. It has been shown that a loss of 20% of the feeder root system may not show in the canopy of trees irrigated and fertilized optimally.

However, the most important aspect still remains, i.e. nurseries which may from time to time experience a problem with *Phytophthora*, need a product that can effectively control this pathogen both in the plant and in the medium. Therefore, this will have to be an ongoing process to establish effective products to assist in this regard.

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4.4.7 Evaluation of cadusafos on citrus trees in nematode infested soils Experiment 684 by M.C. Pretorius (CRI)

Opsomming

’n Demonstrasieproef is vir FMC Suid Afrika uitgevoer om die Rugby-korrelformulasie met die vloeibare Rugby-formulasie op ’n kontrakbasis te vergelyk.

Summary

FMC Southern Africa approached CRI to conduct a demonstration trial to establish the efficacy of Rugby ME (liquid formulation) compared to Rugby G (granular formulation) on a contract basis. A new trial was laid out at Moosrivier Citrus. The trial site was selected on 12 year old Valencia trees with $\pm 8000\text{Q}/10\text{g}$ roots. The trial was applied and sampled three times during the season. A final report will be sent to FMC for the work conducted during the 2002 and 2003 seasons.

4.4.8. Evaluation of Crop Guard against the citrus nematode, *Tylenchulus semipenetrans* Experiment 675 by M.C. Pretorius (CRI)

Opsomming

CRI is deur Illovo Suiker genader om Crop Guard, ’n nuwe chemiese afvalprodukt van suiker, se doeltreffendheid teen die sitrusaalwurm op ’n kontrakbasis te toets.

Summary

Illovo Sugar approached CRI to evaluate Crop Guard, a non-organophosphate nematicide, to determine its effect in controlling the citrus nematode, *Tylenchulus semipenetrans*, on a contract basis. Crop Guard is registered as a nematicide on peanuts. Two trials were laid out at Karino (Mpumalanga) and at Citrusdal, Western Cape. This trial is ongoing and Illovo Sugar requested that we keep the information confidential until the final results are available. A progress report was handed to Illovo Sugar for work done during the 2003 season.

4.4.9 Resistance of citrus rootstocks to root pathogens

Experiment by Prof. N Labuschagne (UP)

Opsomming

Daar is vasgestel dat die fitoaleksien scoparone wat geassosieer word met weerstand teen *Phytophthora citrophthora* stam kanker, nie verantwoordelik is vir weerstand teen *P. nicotianae* wortelvrot nie. Dit kan dus nie as 'n merker vir weerstand teen wortelvrot gebruik word nie. Verhoging in die konsentrasie van totale oplosbare fenoliese verbindings in sitrus wortels speel 'n rol in weerstand, of maak ten minste 'n deel uit van die meganisme van weerstand van sitrus onderstamme teen *P. nicotianae* wortelvrot. Sover vasgestel kon word, is dit nog nie voorheen in sitrus geraporteer nie. Die konsentrasie van totale fenoliese verbindings kan dus gebruik word as 'n parameter in die evalueering van sitrus onderstamme vir *P. nicotianae* weerstand. Behandeling met fosetyl-AI het totale fenoliese konsentrasies verder verhoog. Dit verskaf verdere bewyse dat die verhoging in totale fenoliese verbindings deel is van die meganisme van aksie van fosetyl-AI teen *Phytophthora* wortelvrot. Dus is 'n indirekte meganisme van aksie hier ter sprake.

'n Unieke verbinding is ontdek wat alleenlik teenwoordig is in weerstandbiedende onderstamme. Hierdie verbinding vertoon as 'n geel kol op dunlaag-chromatografie plate. As hierdie verbinding 'n werkbare merker vir weerstand is, sal dit beslis 'n deurbraak wees in sitrus weerstand navorsing. So 'n unieke verbinding, wat net geassosieer word met weerstandbiedende onderstamme, kan moontlik gebruik word om vinnige, effektiewe tegnieke te ontwikkel vir die sifting van sitrus onderstamme vir weerstand. Chemiese karakterisering van die verbinding word tans uitgevoer.

Introduction

Tolerance of citrus rootstocks to *Phytophthora* has been amply demonstrated and it offers an excellent means of reducing losses due to *Phytophthora* root and collar rot. However, the mechanisms of resistance were not known and no rapid screening method was available. The objectives of this project were to develop screening techniques for the identification of disease-tolerant citrus rootstocks, as well as to determine the biochemical mechanisms that govern this resistance.

In previous investigations, mostly by Afek and co-workers (1986; 1988), the phytoalexin scoparone has been implicated in the resistance mechanism of citrus against collar rot caused by *Phytophthora citrophthora*. *P. citrophthora* is seldom encountered in South Africa and citrus collar rot is not as prevalent in Southern Africa where the foremost problem is root rot caused by *P. nicotianae*. In the present study the focus was on the roots, which is the primary locus of infection for *P. nicotianae*.

Materials and methods

Two *Phytophthora*-susceptible citrus rootstocks (Rough lemon & Volckameriana lemon), two moderately susceptible rootstocks (Carrizo and C35) and four tolerant rootstocks (Swingle, Troyer, Sour orange and Macrophylla) were compared for differences in total phenolic production as well as for the productions of specific phenols, following inoculation with *P. nicotianae*. Extracted total phenolic concentration was determined with a colorimetric reaction using the Folin-Ciocalteu reagent (Bray & Thorpe, 1954; Swain & Hillis, 1959; Julkunen-Tiitto, 1985). The occurrence of phenolic compounds was visualized using fluorescence microscopy. Scoparone was extracted from citrus roots and analyzed using thin layer chromatography (TLC) and capillary electrophoresis (Aucamp et al., 2000). Unknown phenolic compounds were characterized using TLC and HPLC and they were subjected to bioassays with *Cladosporium cladosporioides* to determine their antifungal activity. Data were statistically analysed according to Duncan's multiple range test ($P = 0.05$), using the SAS-system (SAS User's guide, 1999).

Results and Discussion

Thin layer chromatography (TLC) and a novel technique, micellar electrokinetic capillary chromatography (MEKC) were compared as methods for screening citrus root extracts for scoparone and other resistance related compounds. Both procedures were firstly optimized to provide adequate separation of these specific compounds. TLC was found to be an easy and rapid technique to screen large numbers of samples. It is ideal for experiments such as time course studies. MEKC was found to be very sensitive and selective. It complements TLC analysis, very low levels of compounds can be detected, and it can be used to analyze complex root extract mixtures. TLC and MEKC analysis revealed very little or no scoparone in citrus roots when infected by *P. nicotianae*, confirming that scoparone which confers resistance to *Phytophthora* stem canker is not the main mechanism of resistance in the roots.

A novel technique was developed for the detection of fluorescent compounds that has been excreted from citrus roots upon infection by *P. nicotianae*. Zoospore suspensions of *P. nicotianae* were used to inoculate detached root pieces, after which phenolics were extracted from the suspensions and analysed using TLC. No scoparone was detected but TLC revealed up to seven different unknown fluorescent/phenolic compounds that might play a role in resistance. Using only small root pieces has the advantage of not destroying the whole plant for analysis. Sufficient amounts of phenolic compounds can easily be obtained with this procedure and it can therefore be used as a rapid technique in screening rootstocks for the production of resistance related compounds.

Phenolic research in citrus is mainly focused on the production of phenolic compounds in fruit, and on the influence on fruit and juice quality. Resistance responses are usually characterized by the early accumulation of secondary phenolic compounds that effectively isolate the pathogen at the point of infection. We studied total phenolic accumulation in response to *P. nicotianae* infection, to determine what role phenolic production plays in citrus rootstock resistance.

Extracted total phenolic concentration was determined with a colorimetric reaction using the Folin-Ciocalteu reagent. The use of ELISA plates in the Folin-Ciocalteu procedure was much quicker than the conventional test tube technique, more cost-effective due to smaller reaction volumes and yet sensitive for the determination of total phenolic concentration. It can therefore be used as a valuable tool especially when screening a large number of samples or during time-course studies of phenolic production.

The levels of total soluble phenolics were determined in citrus rootstocks tolerant and susceptible to *Phytophthora nicotianae* following inoculation with the pathogen. Phenolic concentrations were higher and increased more rapidly in the roots of *Phytophthora*-tolerant swingle, Macrophylla, Troyer and sour orange rootstocks 21 days after inoculation, whereas only minor increases occurred in susceptible rough lemon and volkamer lemon rootstocks. Total phenolic concentrations correlated well with tolerance or susceptibility to *P. nicotianae*. Treatment with the systemic fungicide fosetyl-Al further elevated phenolic levels in the roots. Total phenolic concentrations were two- to three times greater in inoculated rootstocks treated with fosetyl-Al than in uninoculated, untreated rootstocks, indicating that the elevation of total phenolics plays a role in the mode of action of this fungicide.

Compounds of phenolic nature can be visualised using fluorescence microscopy, due to their autofluorescent properties. Uninfected and infected roots of the three-rootstock categories were examined and fluorescence was mainly located in the first layers of cortex cells just beneath the epidermis as well as in the vascular system. A yellow-green fluorescence was observed under UV light with a blue excitation filter (365 nm), which depicts the presence of flavanoid type compounds. Under UV light with a green excitation filter (546 nm), fluorescence appears blue which illustrates the presence of hydroxy-cinnamic acid derivates.

Crude total phenolic extracts were subjected to TLC and high-pressure liquid chromatography (HPLC) analyses as well as bioassays to determine the antifungal activity of unknown compounds. HPLC analysis of crude phenolic extracts showed complex patterns of constitutively present as well as induced phenolic compounds. Although differences could be seen between susceptible and tolerant rootstocks, the phenolic mixture was still too complex for accurate evaluations.

Preliminary TLC analysis of the free acid extracts showed quite a number of unknown phenolic compounds. A distinct yellow fluorescent compound was detected in tolerant swingle that was absent in susceptible rough lemon rootstocks. Yellow fluorescence under UV light (360 nm) is characteristic of flavanoid type compounds. Tolerant swingle and sour orange produced substantial amounts of this unknown yellow fluorescing compound, followed by moderate concentrations in Troyer, Macrophylla, Carizzo and C35 rootstocks. It was totally absent in susceptible rough lemon and Volckameriana rootstocks. Bioassays were performed where TLC chromatograms were sprayed with a spore suspension of *Cladosporium*

cladosporioides. Several unknown phenolic compounds, including the previously mentioned yellow fluorescing compound, displayed antifungal activity.

Using preparative TLC and column chromatography, the yellow compound was separated and concentrated from other compounds for structure analysis and further bioassays with *P. nicotianae*. HPLC analysis was used to determine its purity. In order to identify this compound, we are currently using mass spectroscopy to determine the molecular weight and formula, infrared spectroscopy to establish what functional groups are present and nuclear magnetic resonance spectroscopy to determine the carbon-hydrogen framework.

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4.5 PROJECT: CITRUS BLACK SPOT

Project Co-ordinator: J.M. Kotzé

4.5.1 Project summary

Although the focus of CBS research is the EU market related problems and to improve disease control on the farm, there is a strong line of investigation to eliminate Black Spot in certain areas and to permanently reduce disease pressure in difficult-to-control areas. The research load is distributed among three groups: Plant pathology at University of Pretoria (UP) (Group 1), which provides the vital techniques and information to reach the research objectives; CRI (Group 2) which addresses the practical investigations relating directly to the grower; QMS (Group 3) doing field research for better control and forming a link between group 1 and the grower to test out new information.

Projekopsomming

Alhoewel die fokus van SSV navorsing gefokus is op die EU mark en verwante probleme asook om die siekte in die boorde te beheer, is daar ook ondersoek om swartvlek in sekere areas te elimineer asook die siektedruk te verminder in moeilike beheerbare gebiede. Die navorsingsgebied is oor drie groepe versprei: Plantpatologie by die Universiteit van Pretoria (UP) (Groep 1), wat essensiële tegnieke en informasie verskaf om die navorsingsdoelwitte te bereik; CRI (Groep 2) wat praktiese beheeropsies (chemies en fisies) ondersoek en ook direk skakel met die kwekers; QMS (Groep 3) wat navorsing doen vir beter beheer en ook 'n skakel vorm tussen groep 1 en die kweker om nuwe informasie te beproef.

4.5.2 Inoculation of citrus leaves with *Guignardia citricarpa*

Experiment PPL 6 by TJC Regnier, PM Labuschagne & L Korsten (UP)

Opsomming

Agt maande oue sitrus swart vlek-vrye Valencia blare van twee jaar oue bome is suksesvol geïnfekteer deur 'n piknidiodpoor suspensie van *Guignardia citricarpa*. 'n Maand na inokulasie kon die patogeen weer suksesvol geïsoleer word. Die patogeen kon egter nie van blare geïsoleer word wat 12 maande tevore geïnokuleer was nie. Die data dui daarop dat sitrus blare tussen agt en 12 maande weerstandbiedend raak teen piknidiospore infeksies. Die opeenhoping van oplosbare en selwand gebinde fenoliese verbindings is ondersoek in geïnokuleerde blare. Verhoogde vlakke van feruliese suur is geëstifiseer in die selwande by vier maande oue blare. Geen fitoaleksien verbindings soos scopoletin of scoparone kon gevind word in geïnokuleerde blare nie. Geen verskil in totaal oplosbare fenoliese verbindings en oplosbare polimere kon gevind word tussen geïnokuleerde en kontrole blare nie. Dit dui op die waarskeimlikheid dat die sel

waarskynlik van 'n fisiese selwand verdedigings meganisme gebruik maak in plaas van chemiese verbindings soos bv. fitoaleksien.

Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, is an economically important disease. In spite of years of research, many aspects still remain poorly understood. Little information is available concerning the quiescence of the pathogen and the reaction of trees to infection. It has been observed that fruit become resistant to pathogen infection between four and five months after flowering (Kotzé, 2000). To date, no data is available regarding the age of leaves at which they become resistant.

As part of the defense system, secondary plant metabolites are often studied as indicators of resistance mechanisms and have been the subject of intense biochemical studies (Harborne, 1991). The ability to rapidly deploy chemicals at the infection court may aid plants in their defense mechanisms, particularly if the metabolites prevent pathogens from accessing other tissues and/or maintain their latency. Several studies strongly suggest that esterification of phenols to cell wall materials is a common phenomenon in the expression of resistance (Fry, 1987) and is often associated with an accumulation of polymerised phenols (Grand *et al.*, 1987).

Secondary metabolites are thought to protect plants against pathogens, herbivores and abiotic agents such as light (UV radiation) (Dixon and Paiva, 1995, Karban and Baldwin, 1997). The leaf surface can allocate a large amount of carbon to the production of secondary metabolites that can be quantified and monitored. Most constitutive phenolic compounds in plants are present in conjugated form, i.e. linked to a sugar or as conjugated esters (Harborne, 1991). Some occur constitutively and could be toxic to pathogens (phytoanticipins), while others are produced in response to the invasion of the pathogen into the plant (phytoalexins). Associated responses include the accumulation of cell-wall appositions and esterification of phenols to cell wall materials (Fry, 1987).

This paper reports on the period of leaf susceptibility to *G. citricarpa* pycnidiospore infections and the associated chemical nature and accumulation of soluble free phenolic acids (non-conjugated), wall-bound phenolics and phenolic polymers (ester-bound, phenolic glycosides) in Valencia leaves.

Materials and methods

Plant materials

A preliminary trial was initiated in 2001, using six five-year-old Valencia trees from Mpumalanga. The trees were manually defoliated and incubated in a large walk-in growth chamber with a 12 h day-night cycle of 25 / 20 °C and 70 % relative humidity. Petioles of new flushes (*ca.* ten days old) on different branches were labelled with short plastic straws (Fig. 4.5.2.1). Five trees were used for spore inoculations and the sixth one was only treated with water (control). For each inoculation, a branch on each of the five trees were used as replicates.

A second trial was started in July 2002, using 30 two-year-old disease-free Valencia trees obtained from the Western Cape. The trees were manually defoliated and incubated in a greenhouse, with temperatures ranging between 12 to 30 °C. New flushes were monitored and ten new leaves (*ca.* ten days old) per tree were labelled for inoculations as described above. Three replicate trees were used per inoculation.



Figure 4.5.2.1. Petioles of newly developed leaves labelled with pieces of plastic drinking straws.

Fungal isolate

A *G. citricarpa* isolate (GC252), originally obtained from naturally infected fruit, were maintained in the Plant Pathology Laboratories fungal culture collection at the University of Pretoria. The isolate was sub-cultured onto half-strength potato dextrose agar (PDA) (Difco) and incubated at 20°C (\pm 2°C) under continuous fluorescent light for 18 to 21 days to stimulate sporulation. Pycnidiospores were harvested and a spore suspension of 10^3 spores ml⁻¹ was prepared in sterile water. Leaf inoculation was done within 4 h after preparation of the spore suspension.

Leaf inoculation

In the preliminary trial with only six trees, one branch of each tree were selected and covered with a plastic bag. Only the leaves in the plastic bag were inoculated by spraying the leaves abaxially and adaxially with the pycnidiospore suspension, until run-off. After 72h, the bags were removed and marked leaves were collected after a month. New leaves were inoculated each month. The leaf sample was divided into sub-samples for re-isolation, microscopy and phytochemical analysis.

The same inoculation procedure was followed for the trial consisting of 30 trees, however, the entire tree was covered with a bag and spray inoculated as mentioned previously. The control branches or trees were treated the same, but were inoculated with only sterile water.

Re-isolation

Collected leaves were surface disinfected for 1 min with 1 % sodium hypochlorite, rinsed with sterile water, blotted dry and sections (10 mm²) of the leaves aseptically plated to half-strength PDA. The plates were incubated for two weeks at 25 °C and developing colonies noted. The retrieved colonies were identified and for confirmation the polymerase chain reaction (PCR), according to the test method PPL009 of Meyer *et al.* (2001) was performed on selected re-isolated cultures in the ISO 17025 accredited Plant Pathology Laboratory, University of Pretoria. Three replicate leaves per tree were used.

Extraction of phenolics

Collected leaves (three per tree) were freeze-dried for 48 h. The dried material was ground with a mortar and pestle to a fine powder, sieved with a tea strainer and stored at room temperature for further use. Soluble phenolics were extracted from 0.05 g dried leaf material in duplicate with 1 ml mixture of methanol/acetone/water (7:7:1, v/v) (Regnier, 1994). The suspension was homogenised for 1 min before being placed on an orbital shaker at 200 rpm for 1 h at 4 °C. After centrifugation (12000 g, 5 min), the supernatant was collected and stored. The remaining precipitate was re-homogenised and centrifuged as above. The extraction was repeated three times in order to ensure a complete extraction of the soluble phenolic compounds. The three supernatants were combined and concentrated to 1 ml under vacuum. The two duplicate extracts per sample were combined and divided into four micro centrifuge tubes (0.5 ml per tube) in order to determine total soluble phenolic acids, non-conjugated (free) phenolic acids, methanol/acetone soluble ester-bound phenolic acids and methanol/acetone soluble glycoside-bound phenolic acids. The remaining alcohol insoluble residue (AIR) was dried overnight at 55 °C and used to extract the ester-bound cell wall phenolic acids (De Ascensao and Dubery, 2003).

The one tube of supernatant was directly used to determine the total soluble phenolic acid content with the Folin-Ciocalteu reagent (Swain and Hillis, 1959). The second tube of supernatant was acidified with 50 μ l of 1M Trifluoro-acetic acid and the solution was extracted three times with 1 ml anhydrous diethyl ether (Cvikrová *et al.*, 1993). The ether extract was reduced to dryness under vacuum and the resulting precipitate was re-suspended in 0.25 ml pure methanol. This solution was used to determine the amount of soluble non-conjugated phenolic acids with the Folin-Ciocalteu reagent.

The third tube of supernatant was hydrolysed in 50 μ l concentrated HCl for 1 h at 96 °C and extracted three times with 1 ml anhydrous diethyl ether. The ether extract was reduced to dryness and the resulting precipitate was re-suspended in 0.25 ml pure methanol. This solution was used to determine the soluble glycoside-bound phenolic content using the Folin-Ciocalteu reagent.

The fourth and last tube of supernatant was hydrolysed in 0.125 ml 2 M NaOH, sealed and allowed to stand for 4 h at room temperature in the dark. Thereafter, the tubes were cooled at -10 °C for 30 min and 60 μ l 1M HCl added. The phenolics were then extracted three times with 1 ml anhydrous diethyl ether. The ether extract was evaporated to dryness and the resulting precipitate was re-suspended in 0.25 ml pure methanol. This solution was used to determine the cell wall-bound phenolics using the Folin-Ciocalteu reagent.

Ester-bound phenols incorporated in the cell wall were extracted after extraction of the soluble ester-bound phenolic content. The remaining alcohol insoluble residue (AIR) from the above process was weighed into a glass tube (50 mg) and re-suspended in 1 ml 0.5 M NaOH before being sealed. The tubes were then placed in a water bath for 1 hour at 96 °C. Under these conditions, wall-esterified hydroxycinnamic acid derivatives were selectively released (Regnier, 1994). After saponification, the tubes were cooled at -10 °C for 30 min before addition of 40 µl concentrated HCl. The phenolics were then extracted three times with 1 ml anhydrous diethyl ether. The ether extract was reduced to dryness and the resulting precipitate was re-suspended in 0.25 ml pure methanol. This solution was used to determine the cell wall-bound esterified phenolic acid content with the Folin-Ciocalteu reagent. Extracts were diluted five times before being analysed by High-Performance Liquid Chromatography (HPLC).

Folin-Ciocalteu assay

The concentration of phenolic compounds in each fraction was determined using the Folin-Ciocalteu reagent (Swain and Hillis, 1959). The volumes were modified to facilitate the use of ELISA-plates. Four replicates of the extract (5 µl) was diluted to 175 µl with distilled water, added to 25 µl of Folin-Ciocalteu reagent (Sigma, SA branch) and mixed. After 3 min, 50 µl of aqueous sodium carbonate (20% w/v) was added, mixed thoroughly and incubated at 40 °C for 30 min. A blank of 5 µl methanol was used as control. The absorbance was read at 690 nm using an ELISA reader (Muliskan Ascent V1.24354 – 50973, Version 1.3.1). Gallic acid was used as a phenolic standard to construct a standard curve ranging from 0 to 400 µg, $r^2 = 0.9989$. The concentration of phenols in the various extracts was calculated from the standard curve and expressed as mg gallic acid equivalent g^{-1} dry weight.

Histochemistry

Fresh pieces of collected leaves were stored in glycerinaldehydes at 4 °C until required. Foliar material was sectioned at -15 °C with a cryostat microtome (Reichert HistoStat). The sections were first immersed in 15 % glycerine water, followed by immersion in Neu's reagent (1 % methanolic solution of 2-aminoethyldiphenyl borinate) for 1-2 min, and then mounted in 15 % glycerine water before being viewed with a fluorescent microscope. To locate catechins and condensed tannins, sections were stained with vanillin-HCl and viewed with a Nikon light microscope at 400 x magnification.

Results and Discussion

Effect of leaf age on resistance to pycnidiospore infections

In the preliminary trial, leaves of up to five months old were successfully infected with pycnidiospores and *G. citricarpa* could be re-isolated from the leaves after an infection period of one month (Table 4.5.2.1). The identity of the isolated colonies (Fig. 4.5.2.2) was confirmed with PCR as *G. citricarpa*.

In the follow-up trial, leaves of the two-year-old trees were successfully infected with a pycnidiospore suspension up to an age of eight months and the pathogen could be re-isolated from the leaves (Table 2). *G. citricarpa* was not re-isolated from leaves that were inoculated at 12 months. The data strongly suggests that citrus leaves become resistant to new infections with pycnidiospores between eight and 12 months. However, two important questions remain: for how long can the pathogen survive in infected leaves so that the leaves stay infected, and how does the leaf become resistant to the pathogen?

Since citrus leaves could remain on the tree for up to three years (Kotzé, 2000), more research is required to determine for how long can the pathogen be re-isolated. By studying the change in citrus leaf phenolic concentrations resulting from the inoculation, our study partially provided some answers to the second question.

Table 4.5.2.1. *Guignardia citricarpa* re-isolated from leaves of different ages artificially inoculated with pycnidiospores and re-isolated one month later

Leaf age at time of inoculation	Colonies obtained on half-strength potato dextrose agar ^a	
	Uninoculated	Inoculated
1	-	+
2	-	+
3	-	+
4	-	+
5	-	+

^a Mean of three leaves assessed from five replicate branches, with one branch per tree.

- = No *Guignardia* was isolated from the leaves.

+ = At least one *Guignardia* culture was isolated from the nine leaves.



Figure 4.5.2.2. *Guignardia citricarpa* growth on half-strength potato dextrose agar from artificially inoculated citrus leaves.

Table 4.5.2.2. Percentage *Guignardia citricarpa* re-isolated from leaves at different ages artificially inoculated with pycnidiospores and re-isolated a month later

Leaf age at time of inoculation	Colonies obtained on half-strength potato dextrose agar (%) ^a	
	Uninoculated	Inoculated
2	0	16.0
3	0	5.3
4	0	14.7
5	0	17.3
6	0	10.7
7	0	25.3
8	0	4.0
9	ND ^b	ND
10	ND	ND
11	ND	ND
12	0	0
13	ND	ND
14	0	0

^a Data represents the mean of three leaves assessed from three replicate trees.

^b ND = not determined.

Changes in the content of phenolic acids following inoculation with *G. citricarpa*

The amount of total soluble phenolic acids, non-conjugated acids, phenolic glycosides, ester-bound phenolic acids and phenolic acids esterified to the cell wall were quantified for both inoculated and control leaves, as depicted in Figs 4.5.2.3, 4.5.2.4, 4.5.2.5, 4.5.2.6 and 4.5.2.7 respectively.

The total soluble phenolics for the uninoculated leaves increased steadily between months two and seven, after which it decreased (Fig. 4.5.2.3). A similar pattern could be observed for inoculated leaves, although more variation in response occurred. This data is similar than that found by Castillo *et al.* (1992), which indicated that phenolics reach a maximum concentration in leaves during the logarithmic phase of growth, gradually decreasing until fully developed. This decrease is often associated with the dilution of metabolites due to cell growth.

Levels of non-conjugated phenolic acids represent ca. 15% of the total soluble phenolics (Fig. 4.5.2.4). A 200 % increase was observed after eight months for uninoculated leaves. Inoculation of leaves resulted in a slight decrease of the level of non-conjugated phenolic acids the first five months, followed by a slow increase thereafter. The data strongly suggests an orientation of secondary plant metabolism to allocation of these phenolics to further esterification. Uninoculated eight to 13 month old leaves showed a slight decrease in the level of non-conjugated phenolic acids. Free phenolic acids usually esterified to sugars are stored or integrated into the cell wall, such as the ferulic acid. This is a well-described phenomenon, forming part of the natural defense systems and leads to the formation of lignin-like polymer systems (Matern and Kneusel, 1988).

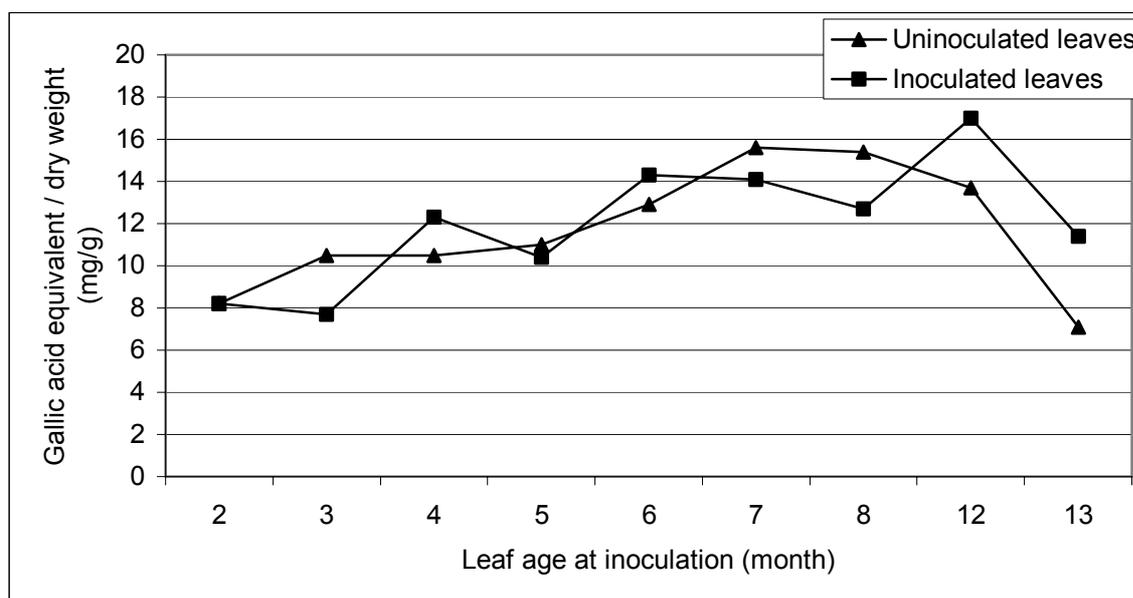


Figure 4.5.2.3. Effect of leaf age on the accumulation of total soluble phenolics between leaves of inoculated trees and uninoculated trees. Inoculation was performed at monthly intervals with a pycnidiospore suspension of *Guignardia citricarpa*.

The contents of glycoside-bound phenolic acids represent almost 50% of the pool of total soluble phenolics (Fig. 4.5.2.5). Between six and 13 months, the inoculated leaves presented a more variable content of glycoside-bound phenolic acids than the uninoculated leaves.

At two months, the pool of soluble ester-bound phenolic acids represented approximately 38% of total soluble phenolics (Fig. 4.5.2.6). The inoculated leaves presented a lower content of ester-bound phenolics up to five months, after which the inverse was observed.

The cell wall-bound phenolic acids represented 56% of the total phenolic compounds in three month-old leaves and 48% in 13 month-old leaves in the uninoculated leaves (Fig. 4.5.2.7). In the inoculated leaves, the cell wall-bound phenolic acids represented 60% of the total phenolic compounds in three month-old leaves and 62% in 13 month-old leaves. As the leaves get older, the amount of phenolic compounds bound to the cell wall decreased slowly in all the leaves. Inoculation therefore resulted in a strong increase in the amount of phenolic material bound to the cell wall.

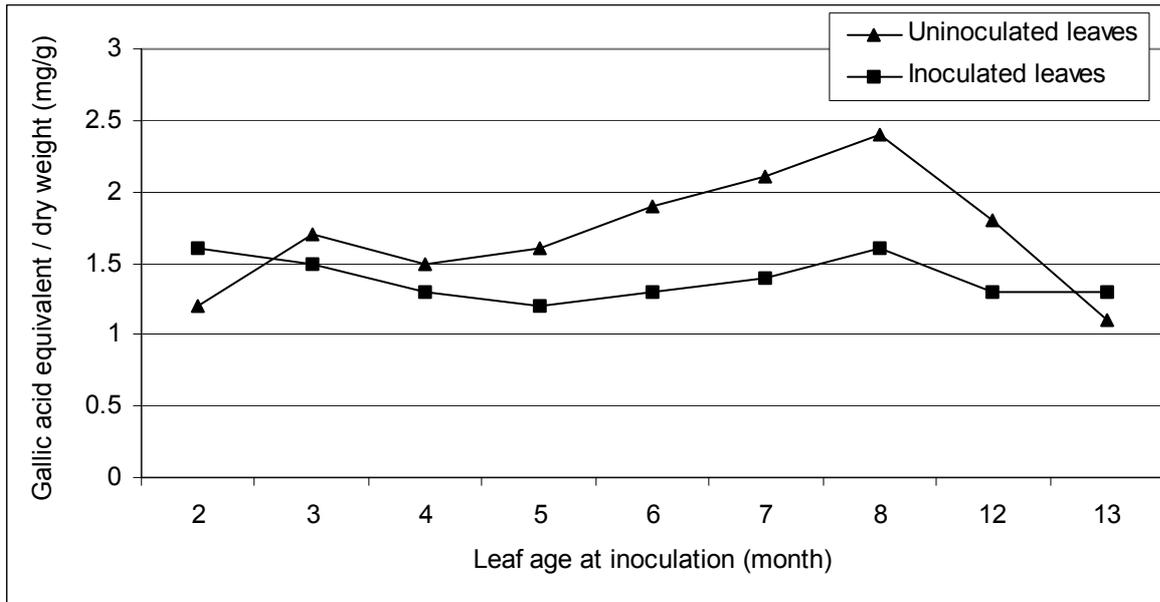


Figure 4.5.2.4. Effect of leaf age on the accumulation of non-conjugated soluble phenolic acids between leaves of inoculated trees and uninoculated trees. Inoculation was performed at monthly intervals with a pycnidiospore suspension of *Guignardia citricarpa*.

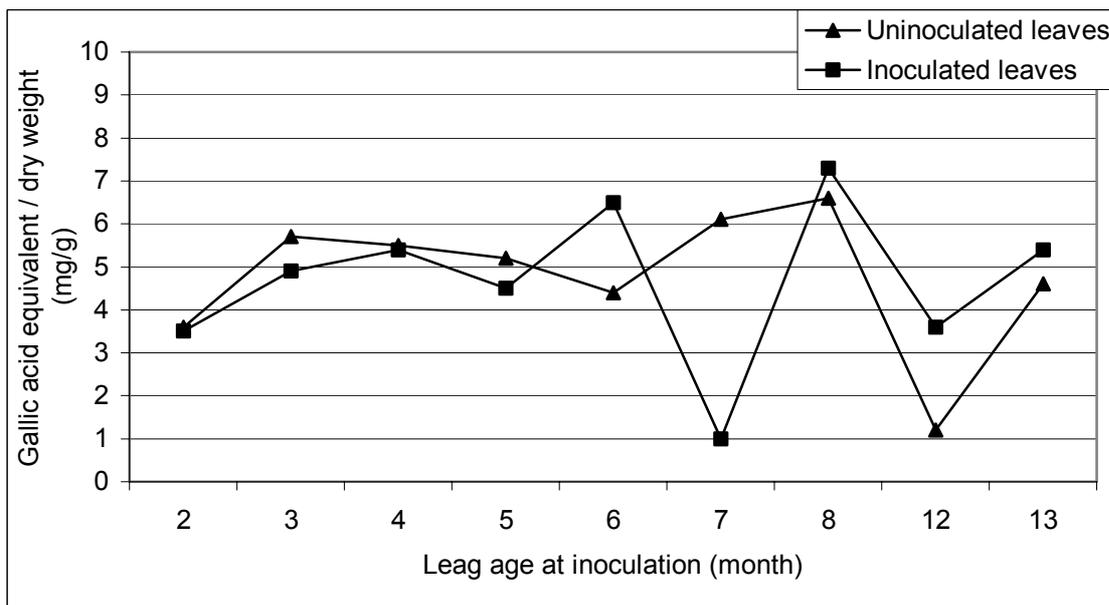


Figure 4.5.2.5. Effect of leaf age on the accumulation of soluble glycoside-bound phenolic acids between leaves of inoculated trees and uninoculated trees. Inoculation was performed at monthly intervals with a pycnidiospore suspension of *Guignardia citricarpa*.

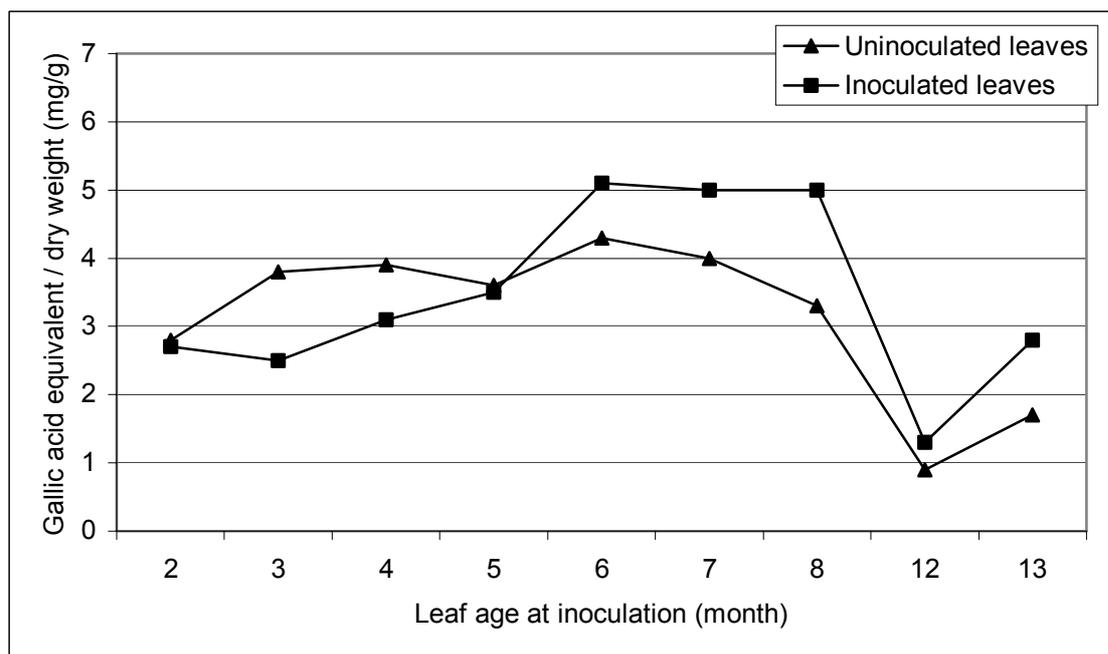


Figure 4.5.2.6. Effect of leaf age on the accumulation of soluble ester-bound phenolic acids between leaves of inoculated trees and uninoculated trees. Inoculation was performed at monthly intervals with a pycnidiospore suspension of *Guignardia citricarpa*.

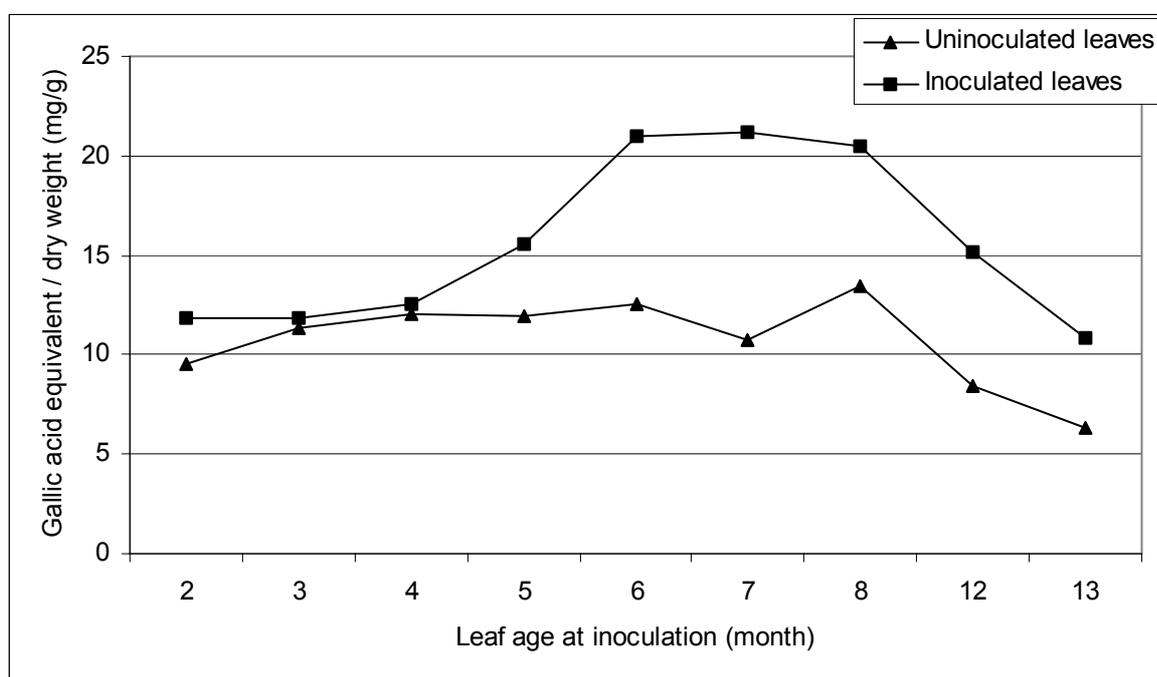


Figure 4.5.2.7. Effect of leaf age on the accumulation of cell wall-bound phenolic acids between leaves of inoculated trees and uninoculated trees. Inoculation was performed at monthly intervals with a pycnidiospore suspension of *Guignardia*.

The esterification of ferulic acids into the polysaccharide matrix of the cell wall appeared to take place in the four-month-old inoculated leaves. High levels of ferulic acid have also been indicated by histochemical studies.

Histochemical study of inoculated and non-inoculated leaves over time.

Localization of the phenolic metabolites in inoculated and uninoculated four month-old leaves visualized by fluorescence microscopy indicated the presence of hydroxycinnamic acids to be mainly in the cuticular layer of the leaf (Fig. 4.5.2.8).

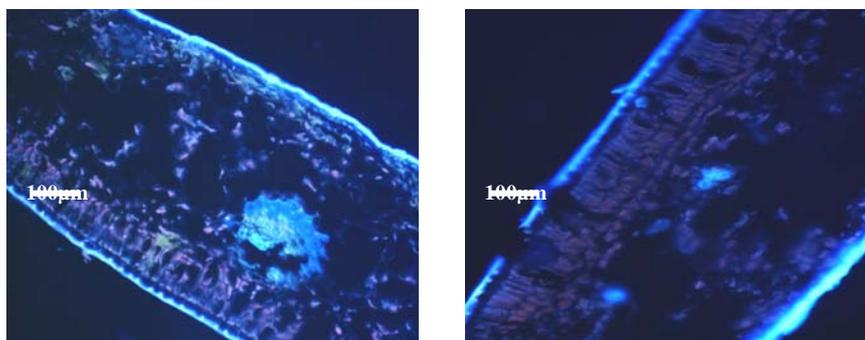


Figure 4.5.2.8. Section of four-month-old leaves treated with Neu's reagent under UV radiation.

Condensed tannins and catechin are often associated with a form of plant defense against insects and herbivores. They also contribute to the protection of the plant against pathogens as they have been found to be toxic above certain concentrations. No accumulation of these compounds was observed throughout the study.

Conclusion

Valencia leaves can be infected by pycnidiospores up to eight months, after which the leaves become resistant. A high variability in the number of leaf pieces that resulted in *G. citricarpa* colony growth was observed. Therefore, a growth or no growth rating was selected for the first trial and percentage colonies obtained for the second. The investigation proved that citrus leaves responded to the presence of the pathogen through accumulation of ferulic acid esterified into the cell wall in leaves from four months-old and onwards. This finding indicates a defense system of a physical nature, building up in the cell wall for protection.

Acknowledgments

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4.5.3 Colonisation of dead decaying leaves with *Guignardia citricarpa* Experiment PPL 7 by M Truter, PM Labuschagne & L Korsten (UP)

Opsomming

Die doel van hierdie studie was om te bepaal of pycnidiospore oorgedra kan word na dooie blare wat wissel van groen nuut geplukte blare, tot geel en bruin blare wat reeds afgeval het. Siekte vrye blare was vir twee maande saam met gesonde en swartvlek geïnfecteerde Valencia lemoene geïnkubeer onder verskeie omgewingstoestande. Die blare en vrugte is weekliks nat gemaak. Die blare is na twee maande versamel en mikroskopies ondersoek vir die teenwoordigheid van vrugliggame. Die teenwoordigheid van askospore is bepaal met die Kotzé-Quest inoculum monitor, en die teenwoordigheid van *Guignardia* sp., met polimerase ketting reaksie (PKR). Al die resultate was negatief vir die teenwoordigheid van *Guignardia citricarpa* en *G. mangiferae* op die blare wat in die Convirion en in die Wes Kaap geïnkubeer was. 'n Paar askospore is wel waargeneem op van die blare wat in Tzaneen en Burgersfort geplaas was, hoewel data daarop dui dat dit *G. mangiferae* mag wees. Die PKR kon nie *G. mangiferae* opspoor nie vanweë te lae inokulum vlakke.

Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, is an economically important disease of citrus in some of the summer rainfall regions of South Africa. Despite the free movement of CBS infected fruit in this country, the winter rainfall regions have remained disease free. Likewise, exports of citrus fruit from South Africa to Europe have continued unabated for the past 80 years and no reports of CBS have appeared from European countries.

Importing countries may legitimately restrict trade on the basis of phytosanitary risks provided that these measures are based on sound science. The European Union has imposed restrictions on citrus fruit imports from CBS affected areas.

A pest risk analysis on exports of fresh citrus from South Africa to Europe identified the need to clarify the potential for *G. citricarpa* pycnidiospores from fruit to infect leaf litter and whether this may lead to the production of ascospores. The aim of this investigation was therefore to determine whether pycnidiospores from CBS lesions on Valencia oranges could be transferred to detached disease free Eureka lemon leaves ranging from fresh detached green leaves to partially decomposed brown leaves under controlled and natural conditions.

Materials and methods

The investigation consisted of two parts.

1. Laboratory experiments under controlled temperature conditions in a Convirion at the University of Pretoria
2. Field trials under natural environmental conditions in the Western Cape, Gauteng, Mpumalanga and Limpopo Provinces in South Africa.

Convirion experiments

In the investigation under controlled temperature conditions, disease free Eureka lemon leaves (green, yellow and brown) from the Western Cape were collected and placed between two rectangular grids. Green, yellow and brown leaves were included in the study, since at any given time there may be fresh detached to advanced decaying leaves under a citrus tree. Valencia orange fruit with CBS lesions (red, hard and virulent spot) from Mpumalanga, was then placed directly on top of the leaves and secured with plastic tubing (Fig. 4.5.3.1). Prior to incubation, the lesions were microscopically examined for the presence of pycnidia fruiting

bodies and pycnidiospores. Leaves with no fruit and leaves with CBS disease free fruit were included as control.

Isolations were made from selected fruit with CBS symptoms, mainly the hard spot lesions. The fruit was surface disinfected with 3 % sodium hypochlorite, rinsed twice with sterile tap water and small pieces were aseptically removed from the lesions and plated onto potato dextrose agar (PDA) supplemented with 50 mg/l rifampicin. Pure cultures of *G. citricarpa* were prepared from developing cultures on PDA and the identity confirmed with PCR (Meyer *et al.*, 2001).

The leaves and fruit were incubated at 20, 25 and 30°C in different Convirons. The grids were sprayed with tap water until run-off on a weekly basis. After two months, citrus fruit was removed; leaf pieces cut from each treatment and incubated in moist chambers to induce development of fruiting structures. The leaf pieces were microscopically examined for the presence of pycnidia or pseudothecia resembling those of *G. citricarpa*. In order to confirm the presence of *G. citricarpa* or *G. mangiferae*, PCR was done on selected leaf material. DNA from a known *G. citricarpa* and *G. mangiferae* isolate were included as positive controls in the PCR tests. Three replicate grids were used per treatment and repeated twice. The trial was repeated for a third time, with incubation of the fruit and leaves at 25 °C only, due to limited facilities.



Figure 4.5.3.1. Citrus black spot-infected fruit tied to disease-free citrus leaves (green left, brown right) showing red, hard and virulent spot lesions.

After completion of the third trial, the grids that were stored at room temperature in paper bags were submersed in water for 5 min at 40 °C, drained and placed in the Kotzé-Quest inoculum monitor (K-QIM). Spore collection from the grids was done with a Vaseline coated standard microscope slide. After the two-hour operation, the microscope slides were stained with lactofuchin and ascospores counted in four transverse rows in the centre with each row separated by 2 mm.

Field trials

Round plastic grids (350 mm diameter) suitable for the K-QIM were used. Treatments consisted of green and brown disease free Eureka lemon leaves, disease free Valencia orange fruit and CBS infected Valencia orange fruit. Disease free oranges and citrus leaves were obtained from the Western Cape. Oranges with CBS red, hard and virulent spot lesions were obtained from Mpumalanga.

Each treatment consisted of three grids filled with either green or brown leaves with either three CBS disease free or three CBS infected fruit tied to each grid with plastic tubing. For the control, the fruit on top of the grids were omitted. The grids were placed on the ground underneath a tree (Fig. 4.5.3.2) in the field or in a garden at three sites in the Western Cape and at single sites in Gauteng, Mpumalanga and Limpopo. The grids were collected after two months. Grids, from which the fruit was removed, were submersed in water for 5 min at 40 °C, drained and placed in the K-QIM. Spore collection from the grids was done with a Vaseline coated standard microscope slide. After the two-hour operation, the grid were removed and stored in paper bags. The microscope slides were stained with lactofuchin and ascospores counted in four transverse rows in the centre with each row separated by 2 mm. The field experiment was repeated twice.



Figure 4.5.3.2. Valencia oranges with CBS hard spot lesions on disease free Eureka lemon leaves on top of round plastic grids, as used in the field in the Western Cape.

Results

Pycnidia containing pycnidiospores were found in 80% of the selected lesions microscopically examined on the CBS infected Valencia orange fruit. Of these *G. citricarpa* was isolated from 64 % of the selected lesion pieces. All isolates obtained tested positive for *G. citricarpa* with PCR.

Microscopic examination of selected leaves after the two-month incubation period with CBS infected fruit yielded fruiting bodies that appeared similar to that of *G. citricarpa*. However, no *G. citricarpa* cultures were obtained from the fruiting bodies. PCR tests conducted on these leaves containing the fungal fruiting bodies, were negative for *G. citricarpa* and *G. mangiferae* for all treatments (Table 4.5.3.1). On the basis of morphological characters the fruiting bodies and pycnidiospores were tentatively identified as being a *Phomopsis*-like fungus.

No ascospores were captured with the K-QIM from grids that were incubated in the conviron (Table 4.5.3.2) and from the Western Cape areas (Table 4.5.3.3). Although a few ascospores were collected from the leaves that were placed in Tzaneen and Burgersfort, it came from leaves that had either clean Valencia fruit from the Western Cape tied on top of it or no fruit.

Discussion

The results indicate that although there were pycnidiospores and viable *G. citricarpa* material present on the fruit at the start of the two-month incubation period, no colonisation of any of the leaves by *G. citricarpa* or *G. mangiferae* took place under favourable colonisation conditions.

At 20, 25 and 30 °C, visual fungal fruiting bodies were observed on the leaves after two months. However, these leaves were green and brown at the beginning of the experiment. Further investigation that included morphological examination of the fruiting bodies (pycnidia) and the spores, as well as PCR analysis, confirmed beyond doubt that the fruiting bodies in question were not *G. citricarpa* or *G. mangiferae*, but a *Phomopsis*-like fungus.

The fruit containing the inoculum became mummified with time and were completely covered by postharvest pathogens and saprophytes. Since *G. citricarpa* cannot compete with saprophytes, the inoculum present on the fruit dies. The same scenario occurs with leaf colonization. If *G. citricarpa* does not infect and colonise young leaves still attached to the tree, it is unlikely that *G. citricarpa* will be able to compete successfully with the existing microbial populations on the leaves. Leaves are colonised with various types of microbes throughout its life cycle, from mainly epiphytes and endophytes on young leaves changing to mainly saprophytes on dying and dead leaves.

In the disease cycle of CBS, two types of spores can play a role, namely pycnidiospores from pycnidia of the anamorph, *Phyllosticta citricarpa* (Mc Alpine) Aa found on fruit, leaves and with certain cultivars on twigs, and ascospores from pseudothecia found only on dead leaves (leaf litter) (Kiely, 1948; McOnie, 1964; Van der Aa, 1973; Kotzé, 2000).

It is well known that airborne ascospores from dead leaves are the main source of inoculum and are responsible for the spread of the disease (Kotzé, 2000). In general the water-borne pycnidiospores from fruit are regarded as unimportant in the dissemination of the pathogen, mainly due to limited spread achieved with water and the short viability period of the pycnidiospores (Kiely, 1948; McOnie, 1964; Van der Aa, 1973). Kiely (1948) used fresh spores from fruit lesions for longevity studies, and found that germination dropped by 60 % after four days and terminated after three months.

Strict packhouse procedures and existing quality regulations reduce the chance that fruit with CBS symptoms will be exported. Treatments in the packhouse also reduce and even exclude the survival of pycnidiospores on the fruit (Korf *et al.*, 2001). Nonetheless symptomatic fruit are occasionally encountered on exported fruit. In these lesions pycnidia sometimes develop. The pycnidia may be empty or may contain pycnidiospores, but their viability will depend on spore age, prevailing postharvest temperatures and packhouse treatments.

Conclusion

This study demonstrates that inoculum from CBS-infected Valencia orange fruit does not colonise detached disease-free Eureka lemon leaves (green, yellow or brown) under controlled conditions that are favourable for potential colonisation. These findings support the absence of any evidence in the literature to suggest that should there be viable pycnidiospores on fruit imported into Europe, these could infect leaf litter and lead to the formation of ascospores.

Acknowledgements

We would like to thank Citrus Research International and the Technology and Human Resources for Industrial Programme (THRIP) for financial support, Mr Peter Wahl of Capespan for providing disease-free fruit and leaves from the Western Cape and Dr Hennie Le Roux of CRI for supplying CBS-infected fruit from Mpumalanga.

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Table 4.5.3.1. Presence of *Guignardia citricarpa* and *G. mangiferae* in citrus leaf pieces after two months incubation using healthy leaves from different ages and fruit with citrus black spot symptoms or healthy fruit as determined by PCR

Treatment	Trial 1 ^a			Trial 2			Trial 3
	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C	25 °C
Green leaves only (control)	Negative ^b	Negative	Negative	Negative	Negative	Negative	Negative
Brown leaves only (control)	ND ^c	ND	ND	ND	ND	ND	Negative
Green leaves & healthy fruit	ND	ND	ND	ND	ND	ND	Negative
Green leaves & CBS infected fruit	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Brown leaves & healthy fruit	ND	ND	ND	ND	ND	ND	Negative
Brown leaves & CBS infected fruit	Negative	Negative	Negative	Negative	Negative	Negative	Negative

^aResults of each trial are the mean of three grid replicates.

^bNegative = No *G. citricarpa* or *G. mangiferae* were detected with PCR.

^cND = Not determined.

Table 4.5.3.2. *Guignardia* ascospores collected with the Quest inoculum monitor from citrus black spot free leaves that were incubated with Valencia fruit for two months in the conviron

Treatment	Total number of ascospores ^a						
	Trial 1			Trial 2			Trial 3
	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C	25 °C
Green leaves only (control)	0	0	0	0	0	0	0
Brown leaves only (control)	ND ^b	ND	ND	ND	ND	ND	0
Green leaves & healthy fruit	ND	ND	ND	ND	ND	ND	0
Green leaves & CBS infected fruit	0	0	0	0	0	0	0
Brown leaves & healthy fruit	ND	ND	ND	ND	ND	ND	0
Brown leaves & CBS infected fruit	0	0	0	0	0	0	0

^a Results of each trial are the mean of three grid replicates.

^b ND = Not determined.

Table 4.5.3.3. *Guignardia* ascospores collected with the Quest inoculum monitor from citrus black spot free leaves that were incubated with Valencia fruit for two months in various areas

Treatment	Total number of ascospores ^a					
	Incubation area of leaves and fruit					
	Tzaneen	Pretoria	Burgersfort	Constantia	Stellenbosh	Bellville
Green leaves only (control)	6	0	3	0	0	0
Brown leaves only (control)	2	0	1	0	0	0
Green leaves & healthy fruit	0	0	0	0	0	0
Green leaves & CBS infected fruit	0	0	0	0	0	0
Brown leaves & healthy fruit	3	0	2	0	0	0
Brown leaves & CBS infected fruit	0	0	0	0	0	0

^a Results are the mean of two trials each with three grid replicates.

4.5.4 *In vitro* germination and infection conditions of *Guignardia* spp.

Experiment PPL 8 by M Truter, TJC Regnier, PM Labuschagne & L Korsten (UP)

Opsomming

Sitrus swart vlek letsels op vrugte bevat piknidiospores van die ananmorf *Phyllosticta citricarpa*. *Guignardia citricarpa*, die teleomorf stadium van die swam, produseer ascospores op blaarafval en word beskou as die hoof inokulum bron in die siekte siklus. Piknidiospore was *in vitro* geëvalueer t.o.v. die ontkiemings tempo en appresorium vorming in verskillende oplossings en temperature. Die kiembuise en gevormde appresoriums was na 24, 48 en 96 h bepaal. Die optimum temperatuur vir piknidiospore ontkieming is tussen 20 en 24 °C (±2 °C). Die data kan gebruik word om die optimum kondisies vir ontkieming en infeksie in epidemiologiese studies te bepaal, en dit kan 'n beter begrip van die siekte ontwikkeling te weeg bring en gevolglik siekte beheer verbeter. Dit kan ook waardevolle inligting verskaf in 'n risiko bepalingstudie waar die waarskynlikheid van vrugte as bron van blaar inoculum bevraagteken word.

Introduction

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, is mainly spread by airborne ascospores. Despite their importance, little research has been done on the germination requirements of ascospores due to a lack of ascospores production on artificially growth media. Up to date, no production of ascospores of *G. citricarpa* on artificial growth medium has been established. Wager (1952) reported the presence of ascospores in culture, which Kiely (1948), Hudson (1962) and others were unable to do. McOnie (1964) found that the ascospores in culture were only limited to the non-pathogenic isolates, *G. mangiferae*, while the pathogenic isolates, *G. citricarpa*, produced only pycnidiospores *in vitro*.

Already in 1918, Darnell-Smith found special conditions for pycnidiospore germination and stated that spores older than three days took longer to germinate. Kiely (1948) used fresh spores from lesions for longevity studies, and found that germination dropped by 60% after four days and stopped after three months. It was, therefore, concluded that pycnidiospores were short-lived and of little importance in the dissemination of the disease.

Broderick and Rabie (1970) found in their studies that both black spot symptoms and sporulation of *G. citricarpa* on fruits and flavedo pieces increased significantly when exposed to light at 27°C. Similarly, fruit on the north side of a tree, usually have higher incidence of infected fruit than the other sides (Kotzé, 2000). Although Korf *et al.* (2001) followed a similar approach, they only investigated the viability of pycnidiospores under pack house conditions. In order to obtain a better understanding of disease development for improved management strategies, the influence of pH, temperature and different growth mediums on the *in vitro* germination of pycnidiospores was investigated.

Materials and methods

Fungal isolate and *in vitro* pycnidiospore production

A *G. citricarpa* culture, isolated by Dr Linda Meyer (Department of Microbiology and Plant Pathology, University of Pretoria, South Africa) and identified using PCR technology as described by Meyer *et al.* (2001), was maintained on half-strength potato dextrose agar (PDA) (Difco) at 22 °C (± 2 °C) under continuous fluorescent light for 18 to 21 days for sporulation. Pycnidiospores were harvested by gently rolling a wet sterile cotton swab over the mycelium to pick up spores. The spores were periodically transferred to sterile distilled water (SDW). The spore suspension was filtered through several layers of sterile gauze to eliminate mycelial fragments and adjusted to the desired concentration (10^3 spores ml⁻¹) using a haemocytometer.

Germination solutions

Freshly squeezed orange juice was filtered through several layers of gauze to remove solid particles. A 2 % orange juice (OJ) stock solution was prepared using 20 ml juice in 1000 ml distilled water. One hundred millilitres of the stock solution was filtered through a 0.45 µm filter, the pH adjusted to 4 and 6, and the solution autoclaved at 121°C for 15 minutes.

Fresh young Valencia orange leaves (30 g) were boiled in 1000 ml distilled water for 20 minutes and sieved to prepare a leaf extract (LE) stock solution. From the stock solution, a 2 % leaf extract solution was prepared as described for the orange juice solution.

ELISA plates

Disposable sterile multiple ELISA well plates (Corning ®) comprising of 96 wells, a flat bottom and well capacity of 370 µl were used for the *in vitro* experiments. Three wells per treatment were used and replicated twice. Each well was filled with 250 µl of the different germination solutions and SDW used as control. Twenty-five micro-litres of the pycnidiospore suspension (10^3 spores ml⁻¹) were added to the germination solutions in each well. The plates were incubated according to the following *in vitro* germination schedules.

In vitro pycnidiospore germination

(a) Influence of light, time and temperature on pycnidiospore germination

The ELISA plates containing SDW, LE or OJ and pycnidiospore suspensions were incubated at 10, 20 and 25°C (± 2°C) in continuous light or darkness. Germ tube and appressoria development was followed after 24, 48 and 96 hours. Pycnidiospore germination was assessed at 400 X magnification under an inverted microscope (Nikon TMS-F). The average percentage germ tube and appressoria formation in three randomly chosen microscope fields in each well was used to determine the germination potential of the pycnidiospores. A spore was considered germinated if the length of the germ tube was equal to or longer than the spore, or when an appressorium was present, either sessile or attached to a short or long germ tube (Fig. 4.5.4.1). Very low germination rates were encountered in the initial experiments with germination solutions at pH6. On the basis of these results this experiment was continued with germination solutions at pH4 only. However, for the cold storage experiment, germination solutions of pH4 and 6 were used.

(b) Influence of cold storage on pycnidiospore germination

ELISA plates with SDW, LE or OJ (pH 4 and 6) and spore suspensions were subjected to cold (± 4 °C) and warm (± 25 °C) incubation at different periods ranging from 1 to 30 days (Table 4.5.4.1). In brief, ELISA plates A, B and C were subjected to different cold periods initially and then incubated at 25 °C for three days in an attempt to determine survival potential and germination potential of the pycnidiospores. ELISA plates D, E and F were initially placed in cold storage for one day, then for different periods of time at 25 °C, and again in cold storage for three days (Table 4.5.4.1). Pycnidiospore germination and growth potential was assessed as described previously.

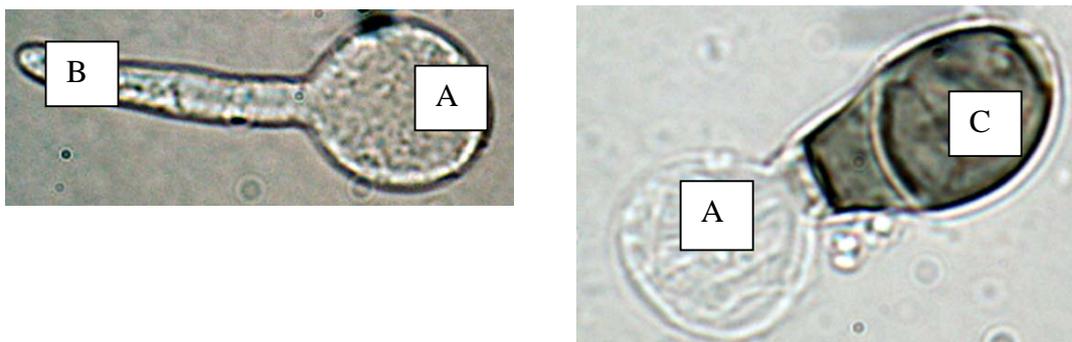


Figure 4.5.4.1. Pycnidiospores (A) of *Phyllosticta citricarpa* with germ tube (B) longer than the spore and appressorium (C).

Table 4.5.4.1. Description of cold (4°C) and warm (25°C) treatments over time (days) in ELISA plates

ELISA plate	Number of cold/warm/cold incubations (days)		
	4°C	25°C	4°C
A	3	3	0
B	20	3	0
C	30	3	0
D	1	3	3
E	1	20	3
F	1	30	3

Statistical analysis

Data were analysed using the statistical program GenStat (2000). The experiment (influence of cold storage) was designed as a split-plot design in two blocks. The six storage treatments were whole plots and six subplot treatments of the different solutions (per ELISA plate). Differences between treatments, solutions and the treatment-by-solution interaction were tested in an analysis of variance (ANOVA). The angular transformation of percentages was used to stabilise treatment variances. Means were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor & Cochran, 1980).

Results

Influence of light, time and temperature on pycnidiospore germination

Continues light treatment significantly enhanced pycnidiospore germination over time in the different germination media at pH 4 compared to the darkness treatment (Fig. 4.5.4.2). After 24 h incubation at 25 °C, more pycnidiospores germinated in the SDW (pH4) compared to that at 10 and 20°C. However, the germination percentage in OJ (pH4) was higher at 20°C compared to 25°C.

Influence of cold storage on pycnidiospore germination

When pycnidiospores were initially subjected to cold storage (3 to 30 days), less than 5% of the spores germinated in the ELISA plate assay A (SDW, OJ and LE at pH4), while only a few spores germinated in the OJ and LE solutions at pH6. In ELISA plate B at pH4, only a few spores germinated and none germinated in ELISA plate C. At pH6, no spores germinated in ELISA plates B and C. In ELISA plates D, E and F at pH4, spore germination was less than 40% in SDW and LE and more than 50% in OJ. At pH6, the germination in the three different solutions and treatments were less than 10%, and at the end of the warm treatment in D, E and F at pH4 no additional germination was observed.

Table 4.5.4.2. Influence of storage time and temperature on the germination rate (%) of *Phylosticta citricarpa* in different germination solutions at pH4 and 6

	SDW4	OJ4	LE4	SDW6	OJ6	LE6
ELISA plate	% Pycnidiospore germination ^y					
A	1	4	1	0	1	1
B	0	0	2	0	0	0
C	3	0	0	0	0	0
D	23	57	32	7	6	15
E	34	61	19	7	3	3
F	32	74	22	7	5	5

Germination solutions: SDW4 = sterile distilled water pH4; OJ4 = 2% orange juice pH4; LE4 = leaf extract pH4; SDW6 = sterile distilled water pH6; OJ6 = 2% orange juice pH6; LE6 = leaf extract pH6
^y = Data represents the mean of three replicates.

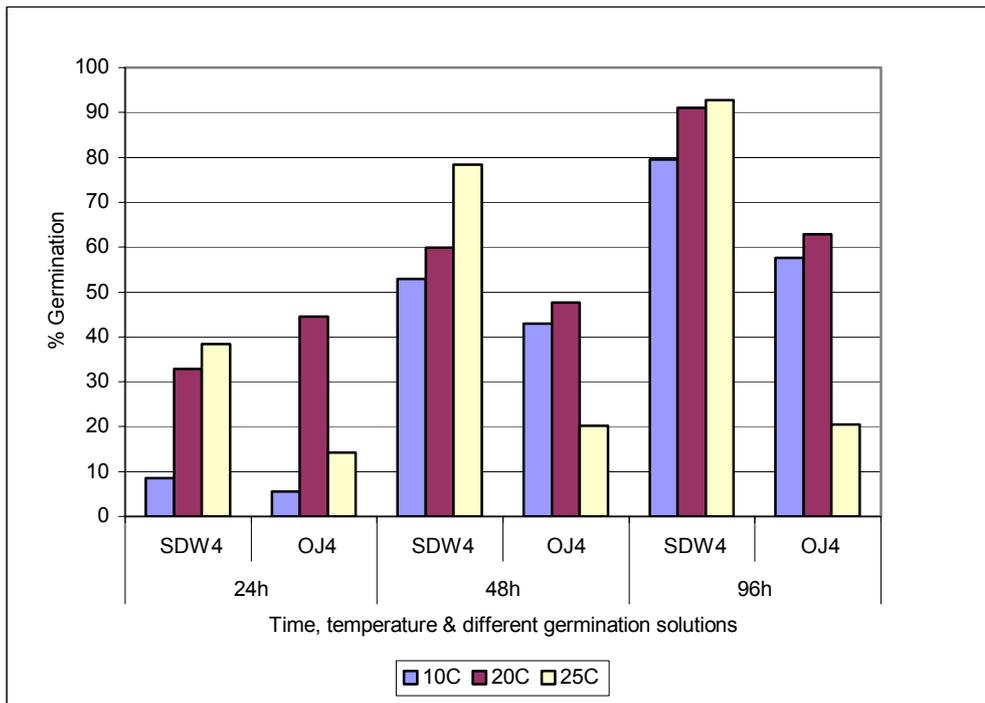


Figure 4.5.4.2. Effect of light, time, pH and temperature on *Phyllosticta citricarpa* pycnidiospore germination. (Germination solutions: SDW4 = sterile distilled water pH4; OJ4 = 2% orange juice pH4).

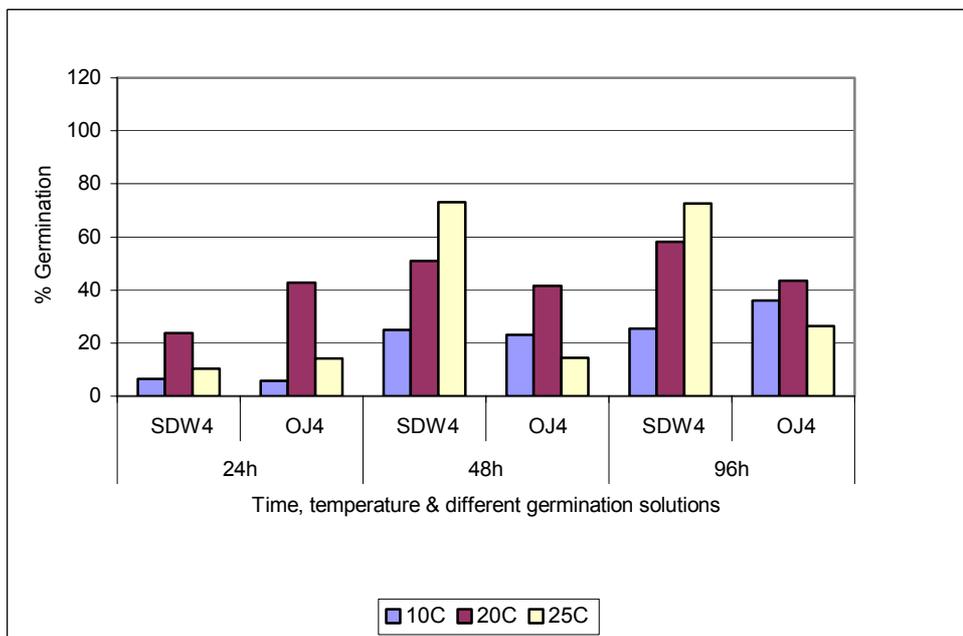


Figure 4.5.4.3. Effect of darkness, time & temperature on *Phyllosticta citricarpa* pycnidiospore germination. (Germination solutions: SDW4 = sterile distilled water pH4; OJ4 = 2% orange juice pH4).

Discussion

Influence of light, time and temperature on pycnidiospore germination

Differences in the germination rates of *P. citricarpa* pycnidiospores were observed. Although Broderick and Rabie (1970) found that pycnidiospore production on artificial medium could be enhanced by light and temperature, they did not investigate the influence of light on the germination potential of pycnidiospores. In our study we found that continuous light, temperature (20°C) and pH4 significantly increased the germ tube numbers and appressoria formation (Fig. 4.5.4.2) compared to the dark treatment (Fig. 4.5.4.3). No germination was observed at 4°C while very long germ tubes and only a few appressoria developed at 37°C.

At 10 and 20°C the appressoria tended to be sessile and at 25°C it was attached to a germ tube longer than the spore length (data not shown).

Influence of cold storage on pycnidiospore germination

Pycnidiospores seem to be affected by the acidity of the germination solution. Significantly more pycnidiospores germinated in the germination solutions at pH4 than at pH6 (Table 4.5.4.2). Furthermore, pycnidiospores subjected to prolonged cold storage periods did not germinate after the period at 25°C. No pycnidiospore germination latency has been investigated. Whether the pycnidiospores will still be viable after a prolonged revival time still needs to be determined. It seems as if the initial cold treatment of 3 – 30 days inhibited the pycnidiospore germination (Table 4.5.4.2). There were no significant differences in the germination rate after incubating treatments at alternating 4 °C and 25 °C temperatures compared to the initial recording.

Acknowledgements

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4.5.5 Development of semi-selective media for direct isolation of *Guignardia* spp. from plant material

Experiment PPL10 by GM Swart, T Beart & L Korsten (UP)

Opsomming

Verskeie swamdoders en minimale media met swamdoder toevoegings was ge-evalueer vir die ontwikkeling van 'n semi-selektiewe medium vir die isolasie van *Guignardia*. Isolate van beide *Guignardia* en *Colletotrichum* was by die studie ingesluit. Geen swamdoders behalwe tiabendasool kan verder gebruik word nie, aangesien hulle nie tussen die twee genera kan onderskei nie. Minimale media en plant ekstrak media was ook nie suksesvol nie. Hierdie is waarskynlik omdat die swamgenera baie nou verwant is. Nuwe benaderings om 'n selektiewe medium te ontwikkel behoort in die toekoms geëvalueer te word.

Introduction

Studying the epidemiology of citrus black spot caused by *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlp.) van der Aa) are crucially important to provide a better understanding of disease development and control. One of the problems encountered in epidemiology studies is the difficulty in isolating the pathogen, particularly from senescent material such as twigs and leaf litter. This is complicated by the fact that *Guignardia* is a slow grower, while ubiquitous contaminants such as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. grow quickly, and out compete and ultimately overgrow any

Guignardia which may have been isolated. It has also been shown that despite efforts to exclude these contaminants, up to 60% of isolates in a study by Glienke-Blanco *et al.* (2002) were identified as *C. gloeosporioides* and only as 27% *Guignardia* spp.

The use of selective or semi-selective media is an invaluable aid in the isolation and identification of fungi and bacteria. The aim of this study was therefore to develop a semi-selective medium for the isolation of *Guignardia* spp. from plant material.

Materials and methods

Fungal isolates

Two isolates of *Guignardia*, viz. GC68 (*G. citricarpa*) and GC6 (*G. mangiferae*) were used in this study. A citrus isolate of *C. gloeosporioides* obtained from a hard spot lesion (3HS2) was also included. Starter cultures were prepared by plating onto potato dextrose agar (PDA) (Biolab) and incubated for seven days. Five millimeter plugs were cut from these starter cultures and inoculated onto the various test media. Measurements were made daily with a digital vernier for seven days. Means were calculated and compared to determine which media could possibly be developed further.

Media

Several approaches were used in this study. The first was evaluation of different fungicides, and exclusion of contaminants by the use of specific growth compounds. Several fungicides were evaluated at 5 different concentrations, with five replicates per concentration. Fungicides evaluated included thiabendazole (TBZ) (Tecto), Imazalil (Fungazil), Chlorothalonil (Bravo), benomyl (Benlate), Prochloraz (Omega) and AR grade copper sulphate. All fungicides except copper sulphate were incorporated at different concentrations into PDA and copper sulphate into CYE medium (Sundin *et al.*, 1993). Rose bengal chloramphenicol agar (Biolab) was also included in this phase of the trial. The second phase involved the use of basal media supplemented with carbon and nitrogen sources reported to support abundant growth of *Guignardia* species (Frean, 1964; Brodrick, 1969). Several carbon and nitrogen sources were evaluated including starch, hesperidin and casein. Orange serum agar (Merck), a minimal medium for *Guignardia* (Sasaki *et al.*, 2002) and a leaf extract medium prepared using fresh citrus leaves and malt extract agar also formed part of this evaluation. Based on the results of these trials, the most promising fungicides were incorporated into the media which supported the best growth of *Guignardia*, while suppressing *C. gloeosporioides*.

Results and discussion

When growth of test isolates was compared at the maximum fungicide concentration evaluated at day seven, none of the products showed any potential for inclusion into a selective medium (Table 4.5.5.1). What is interesting to note is that benomyl and TBZ (both benzimidazole fungicides) completely inhibit both *C. gloeosporioides* and *G. mangiferae*, but not *G. citricarpa*. When the different concentrations of fungicides were evaluated, it was found that at certain concentrations TBZ appeared to have a discriminatory inhibition between the *Guignardia* and *Colletotrichum* isolates. For this reason, it was incorporated into all the minimal media and plant extract media evaluated. The Rose bengal medium supported the same level of growth for all isolates. Despite the selectivity of the minimal media and incorporation of TBZ, *C. gloeosporioides* could not be suppressed without concomitant suppression of both *Guignardia* isolates. The problems encountered in this study may be ascribed to the fact that both *Colletotrichum* and *Phyllosticta* (the anamorph of *Guignardia*) are very closely related, with very similar growth and nutrient requirements. Furthermore, problems were encountered with the *Guignardia* test cultures. Due to extensive subculturing required for the medium evaluation, the growth rate of starter reduced substantially eventually requiring more than double the time to reach the required colony size for medium evaluation.

Table 4.5.5.1. Comparison of different fungicides on growth of the different test isolates

Fungicide	Percentage growth relative to control *		
	<i>Colletotrichum gloeosporioides</i> (3HS2)	<i>Guignardia citricarpa</i> (GC 68)	<i>Guignardia mangiferae</i> (GC 6)
Prochloraz	0	0	0
Benomyl	0	50.9	0
Chlorothalonil	33.5	32.7	52
CuSO ₄	85.6	65.4	58
Thiabendazole	0	58	0
Imazalil	76.7	52.7	52

* Only the highest concentration of each fungicide at day 7 is shown.

Conclusion and future prospects

From these results it is clear that an alternative approach will have to be adopted for the development of selective medium. Due to the problems encountered with the *Guignardia* isolates, all future experiments will have to focus on methods to inhibit the growth of *C. gloeosporioides*. These methods will include the use of various growth factors inhibitors as well as combinations with fungicides, other selective media etc. Once some suitable options have been identified, these can be evaluated using the *Guignardia* test isolates. Following this, the medium can be tested using a range of fungi isolated from citrus to further test the efficacy of the medium

Acknowledgements

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4.5.6 Epiphytic and endophytic occurrence of *Guignardia citricarpa* on twigs Experiment PPL 11 by M Truter & L Korsten (UP)

Opsomming

Vorige verslae het aangedui dat dooie takkies 'n rol kan speel in die verspreiding van *Guignardia citricarpa*. Dooie takkies sonder blare was versamel op gereelde basis in 'n swart vlek besmette Eureka boord naby Brits. Die takkies was ondersoek vir swam vrugliggame en rypwording van die vrugliggame is geïnduseer. Geselekteerde takkies is bo jong Valencia vrugte vasgemaak in 'n boord naby Burgersfort in November 2002, waarna reënval en askospoor vrystelling gemonitor is. Meer swart vlek het voorgekom by vrugte waar die takkies teenwoordig was. Geen askospore was gevang met die Quest spoorvanger vanaf Oktober 2002 tot Januarie 2003 nie. Dooie takkies wat met *G. citricarpa* geïnfekteerd is, kan bydra tot die voorkoms van sitrus swart vlek in die afwesigheid van askospore.

Introduction

The epidemiology of citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, is determined by the presence of inoculum, climatic conditions, growth cycle of the tree, and age of the fruit in relation to susceptibility to infection and development of symptoms. The pathogen attack leaves, twigs and flowers of

citrus and remain as a latent infection (Sutton & Waterston, 1966). All infected material, to some extent, play a role in the epidemiology of CBS. Kotzé (1981) also reported that pycnidiospores may occur on dead twigs, however, the degree to which infected twigs contribute to the epidemiology of CBS is still vague. During 2002, the presence of infected dead twigs was observed in particularly old orchards. Although all the leaves from the orchard floor were removed before the critical infection period, the trees still had a high incidence of black spot. This study was undertaken to determine the ratio of infected dead to infected live twigs in an orchard and to determine to what extent, infected twigs contribute to inoculum levels in orchards and as a potential inoculum source.

Materials and methods

Dead twigs from ca. 20-year-old Eureka lemon trees, were collected from Brits in April, June, October and November 2002. Twigs with visible fruiting structures were examined with a stereomicroscope and those with fruiting bodies resembling *Guignardia* were selected. Twigs with no visible fruiting structures were surface disinfected by spraying with 98 % ethanol until run-off, air-dried and placed in moist chambers with varying moist and dry conditions to induce fruiting of fungi present. The twigs were kept in the moist chamber until fruiting of fungi occurred, after which the twigs were examined for fruiting bodies resembling those of *Guignardia*. The selected twigs (80 to 150 mm long) were tied with a cotton rope above (50 to 200 mm) young Valencia orange fruit on the northern side of each tree on 28-11-2002 in an orchard near Burgersfort. Five twigs were each tied above 10 randomly chosen fruit in three trees in an orchard that received no chemical sprays for CBS during 2001 to 2003. In addition all the leaf litter were removed from the orchard floor in October 2002. Ten fruit were marked in a fourth tree, and used as control. Any other dead twigs still present in the four trees were removed by pruning. A Quest spore trap was operated in the orchard from October 2002 to February 2003 to indicate the presence of ascospores. The twigs were removed from the trees in February and marked fruit assessed for CBS on 08-08-2003 according to a rating scale: 0 = clean; 1 = 1-5 spots per fruit; 2 = 6-50 spots per fruit; 3 = fruit extensively infected. A severity index was calculated for each tree by means of the following formula:

$$\text{CBS-index} = 100 \times \frac{(0n_0 + 0.25n_1 + 0.5n_2 + 0.75n_3)}{n_{\text{total}}}$$

Data were analysed using the statistical program GenStat (2002). Analysis of variance was used to test for differences between values and means were separated according to Fisher's protected *t*-test least significant difference.

Results and discussion

The CBS index of the treated fruit was higher than the control fruit in two of the three trees (Table 4.5.6.1). The orchard received 14, 33 and 99 mm rainfall during November and December 2002 and January 2003 respectively (Table 4.5.6.2). The rainfall was sufficient during these three months for 1, 2 and 5 days respectively, to cause good water run-off in the trees and subsequent spreading of pycnidiospores from the twigs to the fruit. No ascospores were captured with the spore trap during this period.

Ascospores from leaf litter on the orchard floor represent the main inoculum source for CBS (Kotzé, 1981). In the absence of available ascospores, pycnidiospores on dead twigs or infected fruit from the previous season becomes an important inoculum source of CBS. Fruit infection due to pycnidiospores, is more localised and usually only a few fruit is heavily infected. Fruit infected by ascospores, on the other hand, is more evenly distributed in the orchard, with fruit containing only a few black spots.

Table 4.5.6.1. Contribution of dead twigs containing *Guignardia* fruiting structures tied above young Valencia orange fruit, as inoculum source of Citrus Black Spot (CBS), as determined by a CBS index

Tree	Infected fruit (%)	CBS index*	Number of lost fruit
1	29.00	7.14 ab	3
2	0	0 b	2
3	25.00	9.38 ab	2
Control	0	0 b	2

* Values followed by the same letter do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

Conclusion

More CBS was detected where dead twigs were present. Dead twigs with *Guignardia* fructification can contribute to CBS in the absence of ascospores.

Acknowledgements

The authors would like to thank Citrus Research International and the Technology and Human Resources for Industrial Programme (THRIP) for financial support, and M Smith, Agricultural Research Council, Pretoria, for statistical analysis.

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Table 4.5.6.2. Rainfall (mm) received in the orchard

Month	Day																															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
November 2002 ^a																				2			12									
December 2002 ^b							15	2			2	2	9			3																
January 2003 ^c								12	40		12			18			1					16										

^aTotal rainfall during November 2002 was 14 mm.

^bTotal rainfall during December 2002 was 33 mm.

^cTotal rainfall during January 2003 was 99 mm.

4.5.7 Seasonal availability of ascospore inoculum of *Guignardia citricarpa* Experiment PPL 12 by M Truter & L Korsten (UP)

Opsomming

Die beskikbaarheid en rypwording van *Guignardia citricarpa* askospore is ondersoek in boorde in Burgersfort en Brits. Natuurlike blaarafval is maandeliks versamel en die beskikbare askospore op die materiaal gevang op 'n Vaseline bedekte mikroskoopplaatjie d.m.v. 'n Kotzé-Quest inokulum monitor. Natuurlike geïnfekteerde volwasse groen blare is maandeliks gepluk en in die boorde geplaas in roosters om die rypwording van askospore te ondersoek. Natuurlike blaarafval het nul tot 409 askospore opgelewer per gemiddeld van 20 blare, waarteen geen askospore gevang is van die geplukte groen blare na drie maande nie. Meer data word benodig voordat enige afleidings gemaak kan word. Die ondersoek sal voortduur tot einde 2005.

Introduction

Citrus Black Spot (CBS), caused by *Guignardia citricarpa* Kiely, affects the production of citrus in subtropical regions with a summer rainfall climate (Kotzé, 2000). Infection occurs through ascospores released from pseudothecia on dead infected leaves on the orchard floor. Thus far, information on ascospore release in South Africa was based on data obtained with a Burkhard volumetric sampler (Campbell & Madden, 1990) and from 1997 onwards with a Quest spore trap, which is based on the Burkhard volumetric sampler and locally manufactured by Quest Developments. The Quest spore trap is used in the field and can determine the inoculum present in the air for a couple of hours or up to eight days, but results are not always in time to implement control measures. To establish the potential inoculum at a specific time in a particular locality, a different type of sampler was required. A new apparatus was therefore designed and manufactured by Quest Developments in collaboration with Prof J.M. Kotzé to determine the inoculum present in samples of leaf material at any given time. Information obtained using the new Kotzé-Quest Inoculum Monitor (K-QIM) can be used to estimate the potential inoculum load available in orchards to cause new infections by *G. citricarpa* or other plant pathogens that are mainly disseminated through airborne spores.

Materials and methods

The availability and maturation of ascospores on citrus leaf litter was assessed from three areas in Burgersfort and one in Brits from October 2003, and it will continue until September 2005 with the aid of the K-QIM. The availability of ascospores was determined by collecting natural leaf litter at a monthly interval and processing it with the K-QIM. The leaf litter was submerged in water at 40 °C for 5 min, placed on paper towels for 5 min to remove excess water, and placed in plastic grids (10 mm mesh size) in the K-QIM. A standard microscope slide, coated with a thin layer of Vaseline, was used to collect spores. After a two-hour operation, the slide was stained with lactofuchin and ascospores resembling *Guignardia* were counted in four transverse rows in the centre with each row separated by 2 mm.

The maturation pattern of leaves and developing *Guignardia* fruiting structures was assessed by picking mature green leaves, placing it in grids (10 mm mesh size) and leaving it underneath trees in the orchard. Three grids were collected each month and processed with the K-QIM as described above.

Results and discussion

Natural leaf litter contained from zero to 409 ascospores upon collection as determined with the K-QIM (Table 4.5.7.1). Large variation in available ascospores occurred between different sampling dates and between farms sampled on the same date.

No ascospores could be captured from the green leaves that were detached and placed in grids on the orchard for one to three months. So far, only 15 grids were evaluated and more data is required before any conclusions can be drawn.

Table 4.5.7.1. Ascospores captured on a Vaseline coated microscope slides with the Kotzé-Quest inoculum monitor from citrus leaf litter collected from the orchard floor at three farms near Burgersfort

Collection date	Ascospores ^a		
	Zalo Citrus Estate	Motsepula Estate	Citrus Van Wyk
14 Nov 2003	0	0	89.29
12 Dec 2003	363	0	ND ^b
13 Jan 2004	0	409	0

^a Data represents the average ascospores counted from three replicate grids.

^b ND = not determined.

Future research

The process of inoculum maturity of *G. citricarpa* on leaf litter will be monitored over at least two growing seasons starting with the 2003/2004 season.

Acknowledgements

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4.5.8 Breaking the life cycle of *Guignardia citricarpa*: Removal or confinement of inoculum Experiment PPL 13A by M Truter & F C Wehner (UP)

Opsomming

Verwydering of vasvanging van die oorwinterde inokulum van *Guignardia citricarpa* was ondersoek in 'n 36-jarige Valencia boord naby Burgersfort. Al die blare op die boordvloer was met die hand verwyder en verbrand middel Oktober 2002. Agt aangrensende rye van 20 bome elk was gebruik vir die behandelings en het tydens die proeftydperk geen chemiese bespuiting vir swart vlek ontvang nie. Die boordvloer oppervlak van vier van die agt rye was bedek met 'n laag koringstrooi. By die ander vier rye was die blare verwyder van die boordvloer 'n maand later. Die omliggende boorde was met drie maal met chemiese middels gespuit vanaf Oktober 2002 tot Januarie 2003. Geen askospore van *G. citricarpa* was gevang met 'n Quest spoorvanger gedurende die drie maande nie. Evaluering van die boorde tydens die opeenvolgende seisoen het 'n sitrus swart vlek insidensie aangedui van 0.01 % in die strooi bedekte rye en 0.04 % in die onbedekte rye. Feitlik geen sitrus swart vlek was sigbaar op die chemiese behandelde vrugte nie.

Introduction

The most important inoculum source of citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, is airborne ascospores (Kotzé, 1981). Pseudothecia of the fungus develop on dead infected leaves on the orchard floor within 40 to 180 days after leaf drop, depending on the temperature and frequency of wetting. Once mature, ascospores are discharged during spells of rain (Kotzé, 1963) or irrigation (Smith, 1996). Exclusion of the pathogen is the ultimate aim in any disease control programme. The most critical period for infection occurs at fruit set and can persist for ca. four months. Therefore, infected leaf litter must be removed before and during this critical infection period, to reduce the available CBS inoculum. In South Africa the critical infection period is between October and January. Reduction of available inoculum in this period can be achieved by entire removal, inactivation or immobilisation of overwintering inoculum residing in infected leaf litter on the orchard floor. This report describes an experiment aimed at elucidating the efficacy of removal of leaf litter and mulching of the soil under infected trees for the control of CBS.

Materials and methods

The experimental site comprised an orchard near Burgersfort with 36-year-old Valencia orange on Rough Lemon rootstock. All the leaves on the orchard floor were manually removed and burned on 2002-10-22. A total of eight adjacent rows of 20 trees each were used and received no chemical spray for CBS. On 2002-11-14, the surface under the trees in four of the eight rows, was mulched with a layer of wheat straw, whereas leaves were removed again from the unmulched area in the other four rows. In the adjacent orchards, leaf litter were removed during September/October 2002. The producer was requested to keep four rows of trees without any litter removal and no chemical sprays, at the side of the orchard to be used as control, however, the request was denied. The orchards were sprayed with Copstar and oil on 2002-10-14, Flint and oil on 2002-11-26 and Benlate, Diathane and oil on 2003-01-08, except for the trees in the demarcated area (eight rows). A Quest spore trap was operated in the orchard during October 2002 to February 2003.

Trees were evaluated on 2003-08-08. Forty-eight evenly-distributed fruit on each tree were assessed for CBS according to a rating scale: 0 = clean; 1 = 1-5 spots per fruit; 2 = 6-50 spots per fruit; 3 = fruit extensively infected. A severity index was calculated for each tree by means of the following formula:

$$\text{CBS-index} = 100 \times \frac{(0n_0 + 0.25n_1 + 0.5n_2 + 0.75n_3)}{n_{\text{total}}}$$

Where n represents the total number of infected fruit falling into each of the categories.

The treatments were repeated during the 2003/2004 season in the same block used in the 2002/2003 season. All leaves on the orchard floor were manually removed and burned by 2003-11-06. The four rows in the demarcated area were mulched and leaves were again removed from the other four rows on 2003-12-03. Trees will be evaluated during July 2004 for black spot.

Data were analysed using the statistical program GenStat (2002). Analysis of variance was used to test for differences between values and means were separated according to Fisher's protected *t*-test least significant difference.

Results and discussion

No ascospores of *G. citricarpa* could be discerned on the discs of the spore trap. Due to the low disease incidence, no significant differences was detected between treatments with a CBS incidence of 0.01 % in the mulched rows and 0.04 % in the unmulched rows (Table 4.5.8.1). The mulched trees had a lower CBS incidence compared to the unmulched ones in terms of total infected fruit per treatment (results not shown). No significant differences were evident between fruit borne within the canopy and on the outside (Table 4.5.8.2). Differences were evident between fruit of different aspects of the tree, with CBS occurring only on the northern and eastern side of the tree (Table 4.5.8.3). No CBS was present on chemically sprayed fruit in the adjacent orchards at the time of assessment.

Table 4.5.8.1. Incidence and severity of citrus black spot in citrus orchard where leaf litter was either removed or removed together with mulching with wheat straw

Parameter	Mulched*	Unmulched*	Chemical sprayed*
Infected trees (%)	0.63	1.92	0.28
Infected fruit (%)	0.01	0.04	0.01
CBS-index	1.04	0.87	0.59

* Values do not differs significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

Table 4.5.8.2. Distribution of citrus black spot-infected fruit in trees in citrus orchards from which leaf litter was either removed or removed together with mulching with wheat straw

Position	Percentage infected fruit per infected tree*					
	Mulched trees			Unmulched trees		
	Total	Rating 1	Rating 2	Total	Rating 1	Rating 2
Top	0	0	0	2.08	2.08	2.08
Middle	2.08	0	2.08	2.08	2.08	2.08
Bottom	0	0	0	2.08	2.08	2.08
Internal	2.08	0	2.08	2.08	2.08	2.08
Peripheral	0	0	0	2.08	2.08	2.08

*Values do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

Conclusion

No significant differences were observed between treatments due to low rainfall during the critical infection period. The available ascospores were reduced in the orchard by leaf litter removal and could not be detected with a Quest spore trap. The treatments were repeated during the 2003/2004 season and results of the latter will be available after July 2004.

Table 4.5.8.3. Aspectual distribution of citrus black spot-infected fruit in trees in citrus orchards from which leaf litter was either removed or removed together with mulching with wheat straw

Aspect	Percentage infected fruit per infected tree*							
	Mulched orchard				Unmulched orchard			
	Mean	Top	Middle	Bottom	Mean	Top	Middle	Bottom
North	2.08	0	2.08	0	2.08	2.08	0	2.08
East	0	0	0	0	2.08	0	2.08	0
South	0	0	0	0	0	0	0	0
West	0	0	0	0	0	0	0	0

*Values do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \leq 0.05$).

Acknowledgements

The authors would like to thank Citrus Research International and the Technology and Human Resources for Industrial Programme (THRIP) for financial support, and M Smith, Agricultural Research Council, Pretoria, for statistical analysis.

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4.5.9 Field evaluation of chlorothalonil of Syngenta for the control of citrus black spot Experiment 706 by G.C. Schutte (CRI)

Opsomming

Drie verskillende konsentrasies (150 ml, 225 ml, and 300 ml/hl water) van Bravo 500 SC was ge-evalueer in 'n swartvlek besmette boord te Crocodile Valley Citrus Co. digby Nelspruit gedurende die 2002 en 2003 seisoen. Vier bespuitings is gedoen tot die punt van afdrup en is met handspuite toegedien. Weens 'n uitermatige droë jaar wat ondervind was, is geen resultate verkry nie. Inteendeel het al drie konsentrasies fitotoksisiteit tot gevolg gehad en het die bome blare en vrugte afgegooi kort nadat die behandelings in Oktober, November, Desember en Januarie toegedien is. Indien chlorothalonil oorweeg sou word vir verdere evaluasiedoeleindes teen swartvlek, sal laer konsentrasies oorweeg moet word.

Introduction

Chemical control is currently the most critical part of CBS control during the susceptible period from October to January in the summer rainfall regions. There is however, a constant need for new fungicides with different modes of action which growers can select to suit their specific needs. Sometimes growers fail to finish their spray rounds in time or get caught by untimely with rain spells and therefore need a fungicide with a curative action such as Benlate. The latter is also on the endangered list and may be withdrawn in the near future. So there is an urgent need to find a fungicide that is just as good or better than Benlate.

Materials and methods

The experiment was conducted in a commercial Valencia orange (*Citrus sinensis* (L.) Osbeck) grove (Brits 1) on rough lemon rootstock (*C. jambhiri* Lush.) with a history of CBS at Crocodile Valley Citrus Co. near Nelspruit during 2002 and 2003. The trees were 26 years old, 3 to 4 m high and planted in an 8 by 6 m spacing. The rows ran directly north to south. Each treatment was replicated 5 times in a randomized single-tree design. Spray dates and rates of applications are listed in Tables 4.5.9.2 and 4.5.9.3).

The fungicides were applied with a trailer-mounted high-volume, high-pressure (2,500 to 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, each receiving ~ 35 litres of spray mix per tree per application. At fruit maturity during June, 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. Data were analyzed by ANOVA, using Fisher's LSD test ($P = 0.05$).

The fungicides tested included chlorothalonil (Bravo, 50% SC) and the standard registered spray treatments consisting of copper oxychloride (Kopchlor, 85 % WP) and mancozeb (Sancozeb, 80% WP). The same application and evaluations were followed as described above. Applications were applied in mid-October, November, December and January.

Results and discussion

Due to the extremely dry year, no CBS lesions were detected in any of the treated or untreated trees. Concomitantly, chlorothalonil at the three rates tested of 150 ml, 225 ml, and 300 ml/hl water was phytotoxic and resulted in serious fruit and leaf drop (see Fig. 4.5.9.1).

Future research

The rates tested were too high and if any future field trials are considered, it should be at rates lower than those tested.



Fig. 4.5.9.1. Phytotoxicity experienced with a chlorothalonil (Bravo) application to Valencia oranges near Nelspruit during the 2002/2003 season.

4.5.10 Positioning of a single benomyl application in a strobilurin spray programme Experiment 707 by G.C. Schutte (CRI)

Opsomming

Twee proewe is uitgevoer op Clanors en Valencia lemone, albei te Crocodile Valley Citrus Co. Toedienings is gemaak in middel November en middel Januarie. Een behandeling is Benlate, Dithane en olie teen geregistreerde dosisse in middel November is opgevolg met 'n strobilurien (Flint), Dithane en olie behandeling in Januarie en andersom. Weens die uitermatige droë seisoen wat ondervind is, is geen resultate verkry nie en sal die proef oor gedoen moet word in die volgende seisoen.

Introduction

Fungal resistance development to strobilurins is an existing possibility. Resistance to fungicides such as benzimidazoles, dicarboximides and triazoles has been reported (De Wet, 1987; Schutte, 1995). Therefore, anti-resistance strategies using fungicides with different modes of action have to be investigated without losing the edge on effective control with less spray rounds. It was reported that a single Benlate application gave good CBS control at Lisbon Estates where resistance towards benzimidazoles were reported in the 1980's (Kellerman, 1976; Kellerman & Kotzé, 1977; Schutte *et al.*, 1996). Therefore we need to investigate how a single curative benomyl application will perform in a benzimidazole resistant environment and the placement thereof in a strobilurin spray programme.

Materials and methods

Two orchards (one mid-season and two Valencia oranges) 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 five times in single tree plots arranged in a randomized design. Guard trees were located between plots within rows. Two applications were done on the 10 December 2002 and 7 January 2003 at registered rates. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. The fungicides tested included mancozeb (Sancozeb, 80% WP), benomyl (Benlate, 50% WP), azoxystrobin (Ortiva, 25% SC), and the mineral oil, Sunspray 6E. At fruit maturity in July or August, CBS severity was rated on 100 fruits per tree according to a 3-point index that was described previously (Kotzé, 1963; Mc Onie, 1964; Mc Onie & Smith, 1964; Schutte, 1995): 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Data were analysed by ANOVA, using Fisher's LSD test ($P=0.05$).

Results

Due to the extremely dry year, no CBS lesions were detected in any of the treated or untreated trees. Residues of mancozeb were taken from this treatment and will be presented separately.

Future research

This trial will be repeated in 2003/2004 and carbendazim will be included.

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4.5.11 Evaluation of copper fungicides as replacements for mancozeb in a strobilurin spray programme for the control of CBS

Experiment 720 by G.C. Schutte (CRI) & C. van Ginkel (Vallei-Advies)

Opsomming

Verskeie nuwe en ou spuitprogramme is uitgevoer met en sonder verskillende koperformulasies met die oog om mancozeb residu te beperk en om die invloed van koper stippelvorming te bepaal. Weereens het die droë seisoen geen swartvlek resultate opgelewer nie alhoewel die proewe op verskillende plekke herhaal is. Die kontrole behandeling in Groblersdal het 95% skoon vrugte gehad wat uiters swak is om gevolgtrekkings te kan maak. Stippelvorming soos deur koperformulasies veroorsaak is, is bepaal. Resultate toon dat opeenvolgende koperbespuitings meer stippelvorming tot gevolg gehad het as spuitprogramme waar die koperbespuitings opgevolg is met mancozeb bespuitings. Vloeibare koperswamdoders wat gebruik is, het merkbaar minder stippelvorming as die benatbare poeiers gehad. Dit kan moontlik toegeskryf word aan die laer koper konsentrasies in dié middels. Opvolgproewe word vir die volgende seisoen beplan.

Introduction

Registered spray programmes for the control of CBS are expensive. For instance, strobilurin spray programmes cost growers about R14/tree/season consisting of 4 applications if sprayed according to the label. On the other hand, 4 mancozeb sprays cost the growers between R9-R10/tree/season. Moreover, mancozeb is not permitted on fruit destined for the USA. Countries like Canada, a 28-day pre-harvest interval is required and only where packhouses with wet lines, i.e., where fruit is washed or rinsed, is permitted. For all other countries a 21-day pre-harvest interval is permitted. However, mancozeb is more expensive than any of the currently registered copper fungicides, costing about R25-29/kg in comparison with R18-21/kg for copper fungicides. Furthermore, copper fungicides are registered at 35-day intervals in comparison with the 24-25 day intervals for mancozeb. Preliminary trials during the 2001/2002 season showed that copper oxychloride and cuprous oxide were effective against CBS if sprayed as 2 applications according to the old strobilurin label (November and January applications). No stippling was detected either. The question remains what will happen if the two strobilurin tank mixtures with copper are sprayed at monthly intervals during mid summer (November and January) which is also the critical period for copper stippling to occur (Brodrick, 1970).

Materials and methods

Two Valencia oranges orchards were selected at Crocodile Valley Citrus Co., Nelspruit and at Jacobus Badenhorst, Groblersdal to do the evaluations. A randomized 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 (Kotzé, 1963; Mc Onie, 1964; Mc Onie & Smith, 1964; Schutte, 1995), 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 randomized complete block design. Guard trees were located between plots within rows. Spray programs, dates and rates of application are listed in Table 4.5.x. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data. The fungicides tested included mancozeb (Sancozeb, 80% WP), copper hydroxide (Copstar, 12% SC), copper sulphate (Copflo Super, 18% SC), copper oxychloride (Demildex, 85% WP), cuprous oxide (Nordox, 75% WP), trifloxystrobin (Flint, 50% WG), and the mineral oil, Sunspray 6E. At fruit maturity in July or August, CBS severity was rated on 100 fruits per tree according to a 3-point index that was described previously (Kotzé, 1963; Mc Onie, 1964; Mc Onie & Smith, 1964; Schutte, 1995): 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Rind stippling were recored according to criteria described by Schutte *et al.* (1997). Data were analysed by ANOVA, using Fisher's LSD test ($P=0.05$).

Results and discussion

Efficacy of fungicide spray programmes on CBS

The trial site at Crocodile Valley Citrus Co. had no CBS infection and results were not recorded. Certain treatments were selected for mancozeb residue analyses and will be presented separately.

The trial site at Groblersdal also had a low CBS incidence and the control treatment had 94.8% clean fruit (in normal years this can drop to 20%) which was significantly different from all the fungicide treatments (Table 4.5.11.1). The control treatment's other categories (fruit with 1-3 CBS lesions = 3.8%; 4+ CBS lesions = 1.4%) were also significant different from all the other fungicide treatments, but again this can be attributed to an abnormal year for CBS infections. The only spray programme that was significantly different from the others, was the combination of Flint (10 g/hl water) and the cuprous hydroxide (100 g/hl water) and spray oil (0.25%) that resulted in 97.4% clean fruit and 2.0% fruit with four and more CBS lesions. The latter result was the worst of all the treatments in this trial.

Effect of copper containing spray programmes on copper stipple formation

From Table 4.5.11.1 it is interesting to note that spray programmes with alternating A:B:A:B (where A = Dithane/mancozeb; B = copper) sprays concept resulted in less copper stippling than those spray programmes where copper was sprayed in succession, viz. A:B:B:A, when applied during the susceptible period from October to January at monthly intervals. All four copper formulations, viz. copper hydroxide, copper sulphate, copper oxychloride and cuprous oxide in tank mixtures with Flint and spray oil, and altered with Dithane/mancozeb, were not significantly different from each other and did also not result in

stippling of the fruit rind. They resulted in 99.6%, 98.8%, 98.4% and 98.4% clean fruit respectively that was not significantly different ($P = 0.05$) from each other with regards to all three criteria used for evaluation.

Concomitantly, where a B:A:A:B spray sequence was followed or where liquid copper formulations using an A:B:B:A spray sequence as well as 4x liquid copper formulations using Copstar and CopFlo at the lower rates of 270 ml/hl water (registered rate = 350 ml/hl water) and 190 & 250 ml/hl water (future registered rate = 250 ml/hl water) respectively, were also not significantly different from the afore mentioned spray programmes or where the A:B:A:B sequence was followed.

Moreover, the four A:B:A:B spray sequence treatments as mentioned above were significantly different from the 4x Copstar applications (sprayed at the 2x rate of 700 ml/hl water), the 4x Copflo Super (sprayed at the 2x rate of 700 ml/hl water), the 4x Demildex (sprayed at the registered rate of 200 g/hl water) and the A:B:B:A spray sequence consisting of Flint with either Nordox or Demildex with spray oil. The 4x Demildex spray programme also resulted in the most fruit with mild as well as severe stippling, 23.8% and 4.6 % respectively.

Conclusion

Results showed that two copper applications should not be sprayed in succession.

Future research

No CBS recommendations can be made from these results and the trials will be repeated.

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Table 4.5.11.1. Effect of spray programmes for the control of CBS on Valencia oranges at Jacobus Badenhorst at Marble Hall during the 2002/2003 season.

Spray programme no.	Application dates				Percentage fruit in each class		
	14 October 2002	11 November 2002	9 December 2002	6 Jan 2003	No CBS	1-3 CBS	4 and more CBS
1	Dithane (200 g)	Benlate + Dithane + oil (50 g + 150 g + 0.5%)		Benlate + Dithane + oil (50 g + 150 g + 0.5%)	100 a	0 a	0 a
2	Dithane (200 g)	Flint + Dithane + oil (10 g + 150 g + 0.25%)		Flint + Dithane + oil (10 g + 150 g + 0.25%)	100 a	0 a	0 a
3	Dithane (200 g)	Dithane (200 g)	Dithane (200 g)	Dithane (200 g)	100 a	0 a	0 a
4	Dithane (200 g)		Benlate + Dithane + oil (50 g + 150 g + 0.5%)	Dithane (200 g)	100 a	0 a	0 a
5		Flint + Copflo Super + oil (10 g + 200 g + 0.25%)		Flint + Copflo Super + oil (10 g + 200 g + 0.25%)	100 a	0 a	0 a
6		Benlate + Dithane + oil (50 g + 150 g + 0.5%)		Benlate + Dithane + oil (50 g + 150 g + 0.5%)	100 a	0 a	0 a
7		Benlate + Dithane + oil (25 g + 150 g + 0.5%)		Benlate + Dithane + oil (25 g + 150 g + 0.5%)	100 a	0 a	0 a
8		Flint + copper oxychloride + oil (10 g + 100 g + 0.25%)		Flint + copper oxychloride + oil (10 g + 100 g + 0.25%)	100 a	0 a	0 a
9		Flint + Dithane + oil (10 g + 150 g + 0.25%)		Flint + Dithane + oil (10 g + 150 g + 0.25%)	100 a	0 a	0 a
10	Dithane (200 g)	Flint + Copstar + oil (10 g + 300 ml + 0.25%)	Flint + Copstar + oil (10 g + 300 ml + 0.25%)	Dithane (200 g)	100 a	0 a	0 a
11	Dithane (200 g)	Benlate + Dithane + oil (25 g + 150 g + 0.5%)	Benlate + Dithane + oil (25 g + 150 g + 0.5%)		100 a	0 a	0 a
12		Flint + Copstar + oil (10 g + 300 ml + 0.25%)		Benlate + Dithane + oil (50 g + 150 g + 0.5%)	99.6 ab	0.4 ab	0 a
13	Copstar (350 ml)	Copstar (350 ml)	Copstar (350 ml)	Copstar (350 ml)	99.2 ab	0.8 ab	0 a
14	Copflo Super (270 ml)	Copflo Super (270 ml)	Copflo Super (270 ml)	Copflo Super (270 ml)	98.4 ab	1.6 b	0 a
15		Flint + Nordox + oil (10 g + 100 g + 0.25%)		Flint + Nordox + oil (10 g + 100g + 0.25%)	97.4 b	0.6 ab	2.0 c
16	Control				94.8 c	3.8 c	1.4 bc

^yMeans 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.

Table 4.5.11.2. Effect of different copper containing spray programmes on stipple formation on Valencia oranges as sprayed at Crocodile Valley Citrus Co.

Spray programme no.	Application dates				Stippling (%)		
	14 October 2002	11 November 2002	9 December 2002	6 Jan 2003	No stippling	Mild stippling	Severe stippling
1	Dithane (200 g)	Flint + Copflo Super + oil (10 g + 200 g + 0.25%)	Dithane (200 g)	Flint + Copflo Super + oil (10 g + 200 g + 0.25%)	99.6 a	0.4 a	0.0 a
2	Dithane (200 g)	Flint + Copstar + oil (10 g + 300 ml + 0.25%)	Dithane (200 g)	Flint + Copstar + oil (10 g + 300 ml + 0.25%)	98.8 a	1.2 a	0.0 a
3	Dithane (200 g)	Flint + Demildex + oil (10 g + 150 g + 0.25%)	Dithane (200 g)	Flint + Demildex + oil (10 g + 150 g + 0.25%)	98.4 a	1.6 a	0.0 a
4	Dithane (200 g)	Flint + Nordox + oil (10 g + 100 g + 0.25%)	Dithane (200 g)	Flint + Nordox + oil (10 g + 100 g + 0.25%)	98.4 a	1.6 a	0.0 a
5	Copstar (350 ml)	Flint + Dithane + oil (10 g + 150 g + 0.25%)	Flint + Dithane + oil (10 g + 150 g + 0.25%)	Copstar (350 ml)	98.0 ab	2.0 ab	0.0 a
6	Dithane (200 g)	Flint + Copflo Super + oil (10 g + 200 g + 0.25%)	Flint + Copflo Super + oil (10 g + 200 g + 0.25%)	Dithane (200 g)	98.0 ab	2.0 ab	0.0 a
7	Copflo Super (250 ml)	Copflo Super (250 ml)	Copflo Super (250 ml)	Copflo Super (250 ml)	96.2 abc	3.8 abc	0.0 a
8	Copflo Super (190 ml)	Copflo Super (190 ml)	Copflo Super (190 ml)	Copflo Super (190 ml)	96.0 abc	3.6 abc	0.4 b
9	Dithane (200 g)	Flint + Nordox + oil (10 g + 100 g + 0.25%)	Flint + Nordox + oil (10 g + 100 g + 0.25%)	Dithane (200 g)	95.6 abcd	4.2 abc	0.2 b
10	Copstar (270 ml)	Copstar (270 ml)	Copstar (270 ml)	Copstar (270 ml)	95.2 abcd	4.8 abcd	0.0 a
11	Dithane (200 g)	Flint + Copstar + oil (10 g + 300 ml + 0.25%)	Flint + Copstar + oil (10 g + 300 ml + 0.25%)	Dithane (200 g)	93.2 bcd	6.8 bcd	0.0 a
12	Copstar (350 ml)	Copstar (350 ml)	Copstar (350 ml)	Copstar (350 ml)	93.0 bcd	7.0 cd	0.0 a
13	Dithane (200 g)	Flint + Demildex + oil (10 g + 150 g + 0.25%)	Flint + Demildex + oil (10 g + 150 g + 0.25%)	Dithane (200 g)	92.4 cd	7.6 cd	0.0 a
14	Copflo Super (500 ml)	Copflo Super (500 ml)	Copflo Super (500 ml)	Copflo Super (500 ml)	92.2 cd	7.8 cd	0.0 a
15	Copstar (700 ml)	Copstar (700 ml)	Copstar (700 ml)	Copstar (700 ml)	92.2 cd	7.8 cd	0.0 a
16	Dithane (200 g)	Flint + Nordox + oil (10 g + 100 g + 0.25%)	Flint + Nordox + oil (10 g + 100 g + 0.25%)	Dithane (200 g)	90.4 d	9.4 d	0.2 b
17	Demildex (200 g)	Demildex (200 g)	Demildex (200 g)	Demildex (200 g)	71.6 e	23.8 e	4.6 c

^yMeans in a column, based on 5 replicates, followed by the same letter are not significantly different (P = 0.05) according to Fisher's least significant difference test.

4.5.12 Evaluation of a gas-operated incinerator for the elimination of dead citrus leaves on the orchard floor

Experiment 710 by G.C. Schutte (CRI)

Opsomming

Die branderkonsep wat getoets is in 'n suurlemoenboord, het weens die hoë temperature wat ondervind is in Septembermaand, skroeiskade tot gevolg gehad. In 'n opvolgproef op 'n koel bewolkte dag, is daar egter geen skroeiskade opgedoen nie. Weens die raarheid van sulke dae in die periode net voor blom wanneer al die blare wat as inokulum kan dien, verbrand moet word, kan hierdie praktyk nie verder oorweeg word nie omrede dit 'n brandgevaar kan inhou. Alternatiewe metodes is ondersoek en word bespreek.

Introduction

An incinerator was obtained from the ILI in Silverton in Pretoria to evaluate the possibility of eliminating the primary source of CBS ascospores, viz. dead leaves from the orchard floor. This was a modified incinerator which varies from the standard one used to make firebreaks. The primary tasks were to evaluate critical points such as thickness of leaf mats that can be incinerated, damage to tree canopies by the fire, speed of vehicle, etc.

Materials and methods

A lemon orchard at Larten Estates with sufficient leaf drop was selected. Leaves were raked from under the trees and centrally placed within the rows. The thickness of the mat was about 10 to 20 cm thick. The incinerator was towed behind a bakkie at a speed of about 2 km/h. Six rows of gas flames (1 m wide) were used and temperatures of about 600°C were created.

Results

On the first day's trial run, the temperatures were very high (30+ °C) and the RH very low (<30%). This scenario created the ideal conditions for incineration, but there were many more side-effects such as:

- a) serious scorching was observed on the flush closest to the chimney of the incinerator (Fig .4.5.12.1).
- b) a fire hazard was created as some of the leaves in thick piles kept on smouldering long after the machine had passed.
- c) branches within the row created blockages and leaves could not be effectively incinerated as the procedure had to be stopped and cleaned continuously
- d) the bakkie could not cope with the slow speed of the process

A second trial run on a cool humid day did not result in any scorching, but these weather conditions are rare in September when it is also the most suitable time to execute such operations. In general this procedure will not be practical on a larger scale as the time of year when one should do the incineration is during the peak period for wild fires. Gas is also expensive and the operational speed of the whole process is too slow to allow sufficient incineration. Other ways to get rid of the primary source of inoculum have to be considered.

Conclusion

In the USA for instance there is currently a move to make more use of herbicides than tillage to control weeds. In the past tillage operations buried a large portion of the dead leaves that in their case, harboured the source of greasy spot (*Mycosphaerella citri* Whiteside). Not only was the primary source buried, but the weeds also interfered with ascospore dispersal (Mondal & Timmer, 2003). What about plough furrows? Or a vacuum machines to remove all the leaf litter? The first option is practical, but will be laborious to rake the leaves into the furrows that were made within the rows. The latter option seems to be the only practical alternative as leaves will be removed from the orchard and destroyed when environmental conditions are more suitable.

Future research

To search for alternative means for inoculum control.

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Fig. 4.5.12.1. Scorching of flush on a lemon tree due to uncontrolled incinerating practices in an attempt to eliminate dead leaves, the primary source of CBS infection.

4.5.13 Correlation between ascospore inoculum and infection potential in an orchard

Experiment QMS1.2 (PPL12) by W. van Broekhuizen & S.H. Swart (QMS)

Opsomming

Askospoorvystelling in Suid-Afrika word tans gemonitor met spoormoniteerders ontwikkel deur Quest Developments. Hierdie moniteerders vang luggedraagde askospore en gee sodoende 'n aanduiding van die inokulum in 'n boord teenwoordig. Hoewel askospore slegs vrygestel word nadat blaarafval onder die boom benat is, kom spoordraende strukture deur die jaar op hierdie materiaal voor. Daar is dus besluit om van Quest se nuwe inokulum-moniteerder gebruik te maak om vas te stel wat die potensiele inokulumkonsentrasie deur die jaar in 'n boord is. 'n Blaarmonster word in die moniteerder geplaas en lug daardeur gesirkuleer. Vrygestelde spore word op 'n vaseline bedekte mikroskoopplaatjie vasgevang en gee dan 'n aanduiding van die teenwoordigheid van askospoor inokulum in 'n boord. Elke maand word swartvlekgeïnfekteerde blare in die biologiese boord op Letaba Landgoed gepluk (simuleer blaarval) en in die boord aan natuurlike toestande blootgestel. Blare word elke vier weke getoets om die askospoor teenwoordigheid te bepaal. Elke maand se blare word vir ses maande gemonitor.

Introduction

It is generally accepted that ascospores are the primary source of inoculum for citrus black spot (CBS) infection. Perithecia are produced on dead leaves on the orchard floor and ascospores are discharged after leaf litter has become wet. Citrus leaves drop all year round and perithecia can develop within 50-180 days on leaf litter. These structures may occur anytime during the year but their presence and abundance depend on the frequency of moistening and drying as well as on prevailing temperatures. In South Africa ascospore discharges are at a peak during November to March (Kotzé, 1981). Information on ascospore release in South Africa is based on data obtained with Quest spore traps (Quest Developments). These spore traps are used in the field and can determine the inoculum present in the air (Swart, 2002). Since ascospore release is closely influenced by rain patterns, a new approach was needed to determine the inoculum

present in an orchard at any given time throughout the year. A new inoculum monitoring system was designed and manufactured by Quest Developments and used in this study to determine the ascospore inoculum in an orchard throughout the year. This spore trap circulates air through a collected leaf sample and trap discharged spores on a vaseline coated microscope slide. These slides can then be used to determine the inoculum potential of the collected sample.

With this study we would like to verify the most critical period of leaf fall contributing to enhanced ascospore inoculum potential as well as the time needed between leaf fall and ascospore maturation as influenced by seasonal differences.

Materials and methods

This project was initiated in August 2002 and has been ongoing since then. Each month, leaves were picked from CBS infected trees in a chemically untreated orchard at Letaba Estates near Tzaneen. Mature leaves were picked randomly in the orchard and placed between two plastic mesh grids (supplied by Quest Developments) to cover approximately 700 cm² and secure with cable ties. Each month, six sets of leaves were placed under three replicate trees in the orchard. Every four weeks, a set of these grids was collected from each tree and evaluated for ascospore presence. Leaves from each month will thus be evaluated over a six-month period.

Each grid was submerged in hot water (40°C ± 2°C) for 5 min, removed and allowed to drain for 5 min. It was then placed in the inoculum monitor (Quest Developments) and the power supply turned on for two hours.

Spores were trapped on a standard microscope slide coated with vaseline. The slide was removed after the running time, stained with lactophenol cotton blue and covered with a cover slip. Slides were evaluated under a light microscope at 400x magnification. The number of ascospores, resembling the morphology of *Guignardia citricarpa*, was determined by counting three lanes, covering the width of the microscope field. These lanes stretched in the length of the microscope slide, from the starting point to where the trapping process was stopped and represented a surface of approximately 5 mm².

Results and discussion

Preliminary results of the first cycle are summarised in Table 4.5.13.1. Preliminary results of the second cycle are summarised in Table 4.5.13.2.

Table 4.5.13.1. Summary of ascospore maturation – Cycle 1 (Leaves picked August 2002 – July 2003)

Month when leaves were picked		Aug '02	Sept '02	Oct '02	Nov '02	Dec '02	Jan '03	Feb '03	Mar '03	Apr '03	May '03	Jun '03	Jul '03
Average ascospores monitored per month	Sept '02	0											
	Oct '02	2	2										
	Nov '02	86	26	0									
	Dec '02	16	65	0	0								
	Jan '03	8	405	431	1	2							
	Feb '03	5	0	6	386	10	52						
	Mar '03		0	0	0	0	1	1					
	Apr '03			0	0	0	1	1	1				
	May '03				0	4	0	22	0	0			
	Jun '03					0	0	0	0	1	0		
	Jul '03						0	0	0	0	0	0	
	Aug '03							0	0	4	0	0	0
	Sept '03								0	1	0	0	0
	Oct '03									0	0	0	0
Nov '03										25	210	152	
Dec '03											14	6	
Jan '04												?	

? Ascospore counts not yet available.

Table 4.5.13.2. Summary of ascospore maturation – Cycle 2 (Leaves picked August 2003 – July 2004).

Month when leaves were picked		Aug '03	Sept '03	Oct '03	Nov '03	Dec '03	Jan '04	Feb '04	Mar '04	Apr '04	May '04	Jun '04	Jul '04
Average ascospores monitored per	Sept '03	0											
	Oct '03	0	0										
	Nov '03	1	71	0									
	Dec '03	83	20	41	15								
	Jan '04	?	?	?	?	?							

? Ascospore counts not yet available.

The last set of grids for the 2002/2003 season was placed in the orchard in July 2003. These leaves will be monitored till January 2004 to complete the data set for one year (Cycle 1).

Initial results indicate that there is a definite difference in seasonal availability of ascospores (Table 4.5.13.1). The majority of spores were released from November to February with very little spore release between March and August. Shorter periods were needed to produce ascospores during the warmer months of the year. Leaves picked in January needed one month before maximum spore release was monitored while leaves picked in September needed four months before maximum spore release was observed.

The first set of grids for the 2003/2004 season (Cycle 2) was placed in the orchard during August 2003. The last set will be placed in the orchard in July 2004 and will be monitored until mid January 2005 to complete the data sets for two consecutive years.

Future research

To conclude this study, a third cycle will be conducted during the 2004/2005 season. This cycle will be repeated in three different areas to include results from different climatic regions and to evaluate the effect of spray programs on ascospore availability on leaves in commercial orchards. One trial will be done in the previously selected biological orchard on Letaba Estates, one will be done in a commercial orchard on Mahela near Letsitele and one on Richmond Estate near Hoedspruit.

The first sets of grids will be placed under trees during March 2004 and the last ones in February 2005. Last results will be obtained in August 2005 to conclude this study on the correlation between ascospore inoculum and infection potential in an orchard.

Reference cited

- Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Disease* 65: 945-950.
- Swart, S.H. 2002. The value of ascospore release monitoring and correlation with climatic conditions conducive to infection in citrus orchards. 2nd Citrus Symposium, Stellenbosch 2002.

4.5.14 Epiphytic and endophytic occurrence of *Guignardia citricarpa* Experiment QMS 2.1b (PPL 11) by S H Swart & W van Broekhuizen (QMS)

Opsomming

Voorkoms van endofitiese besmetting in bome: Die UP eksperimente het gewys dat takkies uit besmette boorde vrugte besmet het, terwyl QMS gevind het dat die blare van dooie takkies vrugte kan besmet. Die resultaat is nie onverwags nie. In volle konteks sal dit help om beheer meer volledig aan te pas.

Introduction

During the 2003/2004 season lemon fruit in orchards at Group 91, close to Letsitele, Limpopo Province, had citrus black spot (CBS) lesions. These fruit were mainly found in clusters and was mostly associated with dead branches in the tree. This phenomenon was previously reported in a Valencia orchard on the farm Richmond, in the Hoedspruit area (PPL 11, 2002/2003 season). No CBS cultures were obtained from isolations made from branches, twigs or fruit stems obtained from the Valencia orchard at Richmond. Typical *Guignardia*-like cultures developed only when isolations were made from fruit with CBS lesions. In another investigation, plant material from an unsprayed Navel plot at Letaba Estates, close to Tzaneen, were

used. In this instance again no *Guignardia*-like cultures could be isolated from branches and twigs. *Guignardia*-like cultures were, however, obtained from fruit stems, fruit and leaves.

The aim of this investigation was:

1. To determine the source of inoculum in citrus trees
2. To determine the type (asco- or pycnidiospores) of inoculum
3. To determine the period between breaking of branches and inoculum production
4. Determine seasonal occurrence of ascospore inoculum in trees

Materials and methods

Dead branches with attached leaves were collected during March 2003 from lemon orchards at Group 91, close to Letsitele. Leaves were treated according to the protocol established for the ascospore inoculum monitor.

Six lemon trees in an orchard at Group 91 were selected and marked. Each month, starting April 2003 until December 2003, 12 branches (5 – 10 mm diameter) were snapped, but not totally broken of, in each tree. In July 2003 the first twig and leaf samples were put through the ascospore inoculum monitor. The last twigs and leaves were collected at the end of February 2004.

Results and discussion

Ascospores typical to that of *Guignardia spp* were disseminated from leaves obtained from dead branches in lemon trees during March 2003. We were unable to grow any *Guignardia*-like cultures on potato dextrose agar plus chloramphenicol. Leaves were not subjected to PCR analysis to determine *Guignardia spp* since this option was not included in the budget.

No ascospores were disseminated from leaves and twigs gathered in July, August, September, November and December. No pycnidia or perithecia could be found on these leaf samples. The absence of sexual and non-sexual sporulation structures could be due to abnormal dry weather conditions which occurred during the largest part of the growth season (September to January). Good rain occurred only during the last part of January and February. Ascospores were observed during March of the previous season and therefore ascospores might still be found on leaves gathered during February 2004 and possibly during March.

Conclusion

The technique of breaking branches needs refinement. Some branches lost most of their leaves during the ageing process. Other branches recovered and leaves did not die. The climatic conditions probably played the most important role in the ageing process of leaves in the trees, where dry conditions did not favor infection of leaves or formation of reproduction structures on dead leaves.

Future research

Current investigations will include the covering of broken twigs with vegetable bags in order to prevent leaves from falling to the ground. Leaves or *Guignardia*-like cultures obtained through isolation techniques should be identified with the PCR technique.

4.5.15 **Determine the curative action of different chemicals regarding citrus black spot infection** Experiment QMS 7.1 by W. van Broekhuizen & S.H. Swart (QMS)

Opsomming

Verskillende tegnieke is ondersoek om 'n metode daar te stel waartydens jong, swartvlek-vrye sitrus boompies aan swartvlek-infeksie blootgestel kon word. Na infeksie sou die werking van sommige strobuleriene en benlate vir die behandeling van swartvlek ondersoek word. Aangesien die oorspronklike infeksie nie suksesvol was nie, moet daar nog aan 'n metode gewerk word voordat die werking van sommige swamdoders ondersoek kan word.

Introduction

The curative actions of individual strobularin fungicides are unknown. The curative action of Benlate should also be verified, since it is speculated to be between two and six weeks. A technique based on spraying trees with different chemicals after infection was proposed to determine the curative action of these fungicides.

Materials and methods

1. Three techniques to manipulate young citrus trees from a nursery to produce fruits were attempted.
 - Trees were defoliated by breaking off leaves manually
 - Trees were ring barked to induce defoliation
 - Trees were subject to severe water stress in order to remove leaves

After new flush occurred, trees were sprayed with low buret urea to induce flowering.

2. If point 1 was achieved, young, citrus black spot (CBS) free trees will be placed under old trees with a known history of CBS during periods conducive to infection by the CBS pathogen. Trees will be treated with different chemicals at certain time intervals after infection took place. Fruit would be evaluated for CBS infection.

Results and Discussion

A very low number of trees produced flowers but none of these developed any fruit. Since no fruit set occurred, trees were unsuited to place in the CBS infected orchard.

During this normal infection period (October to February) no significant natural infection period occurred due to drought conditions.

Future research

After consultation with Prof Kotze, it was decided that fruit will be artificially inoculated with picnidiospores during March. CBS isolates will be grown on ½ Potato Dextrose Agar and picnidiospores harvested for inoculum. The picnidiospore suspension will be sprayed on fruit where after it will be covered with plastic bags and subject to conditions conducive for CBS infection. Curative action will be attempted in September 2004 if the above technique proves to be effective. The objective will be to:

- a) Evaluate the technique under field conditions.
- b) Determine if fruit are still sensitive to CBS infection after February, should climatic conditions are conducive to infection.
- c) Determine if there is a difference in resistance towards CBS infection due to tree age (young vs. old trees).

4.5.16 **Long term effect of different chemical spray programs on inoculum potential in a citrus black spot infected orchard** Experiment QMS2.3 by W. van Broekhuizen & S.H. Swart (QMS)

Opsomming

Daar is verskeie chemiese spuitprogramme beskikbaar vir die beheer van sitrus swartvlek (SSV). Sewe van hierdie programme is gekies en word met mekaar vergelyk om die effektiwiteit van sistemiese- en kontakswamdoders vir die beheer van SSV te bepaal. Sommige van hierdie middels besit semi-sistemiese en sistemiese eienskappe wat moontlik 'n bepaalde effek op bronne van inokulum mag hê. Spuitprogramme is

reeds in Oktober 2002 begin en in Februarie 2003 afgehandel. Vrugte sal in Julie 2003 geoes word waarna swartvlek simptome visueel daarop geëvalueer sal word. Blare sal ook ondersoek word met die Quest inokulum-moniteerder om te bepaal of daar verskille in potensiële inokulumvlakke is a.g.v. die verskillende chemiese behandelings.

Introduction

The control of CBS is mainly aimed at preventing fruit from being infected. According to Kotzé (1981) ascospores produced on dead leaves on the orchard floor are the primary source of CBS infection. Spores are discharged after leaf litter has become wet and are therefore closely influenced by the rainfall pattern. In South Africa the ascospore discharge is at a peak during November to March. Before November, the fruit is very susceptible but the amount of inoculum is relatively low and the rains are infrequent and of short duration. From January, when rains are more frequent and inoculum is abundant, fruit is believed to be no longer susceptible to CBS infection. Chemical spray programs are therefore aimed to protect fruit from infection between October to February. The objective of this study is to compare different spray programs consisting of contact and systemic fungicides for the control of CBS and the effect of these chemicals on leaf infection and potential inoculum production during the next season.

Materials and methods

A Valencia orchard with a previous record of high CBS incidence was selected on Letaba Estates near Tzaneen. Trees were sprayed according to various commercial spray programs (Table 1) using handguns at approximately 20 bar pressure (spray cart developed by Quest Developments). Single tree treatments were repeated six times in a completely randomised block design.

Table 4.5.16.1. Chemical spray programs for the control of citrus black spot

No	Trade Name	Active Ingredient	Formulation g/l or kg	Dosage g or ml/100l	Week sprayed (Week 1 = 8 October)							
					1	4	7	10	11	14	16	18
1	Untreated control			-	-	-	-	-	-	-	-	-
2	Flint (F)	trifloxystrobin	500 WP	10	D	D+F+Olie	-	D+F+Olie	-	-	D	-
3	Cabrio (C)			10	D	D+C+Olie	-	D+C+Olie	-	-	D	-
4	Ortiva (O)	azoxystrobin	250 SC	20	D	D+O+Olie	-	D+O+Olie	-	-	D	-
5	Benlate (B)	benomyl	500 WP	50	D	D+B+Olie	-	D+B+Olie	-	-	D	-
6	Dithane (D)	mancozeb	750/800 WP	200	D	D	D	-	D	D	-	D
7	Trimangol (T)	maneb/zinc oxide	435/4.7 SC	200	T	T	T	-	T	T	-	T
8	Coprox (Cu)	copper oxychloride	850 WP	200	Cu	Cu	Cu	-	Cu	Cu	-	Cu

D - 150g/100l

Olie - 300ml/100l

Results and discussion

Due to a very dry season with conditions unsuited for black spot infection, no symptoms could be found on fruit from the control or chemical treatments during a pre-harvest orchard inspection in July 2003. It was therefore decided to terminate the trial and not to evaluate harvested fruit. It was then decided to collect leaves from each treatment and evaluate it with the Quest spore monitor in order to try and determine different levels of inoculum between the various treatments. However, before sampling could take place, all the trees from the orchard were removed without giving us any prior warning. Thus, no results could be obtained from this trial.

For a final attempt to obtain some information for this project, leaves from commercial orchards on Letaba Estates, receiving similar spray programs as those applied in the trial, were collected and are being evaluated with the Quest spore monitor. Results will be used as an indication of the potential of each

program to suppress ascospore production and thus the long term effect of inoculum suppression within an orchard. Leaves were sampled in December 2003 but initial data were not satisfactory. Sampling will be repeated in March 2004 after which a final report on this project will be submitted.

Future research

This project has been re-approved for the 2004/2005 season and the spraying of orchards will commence in early September.

Reference cited

Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Disease* 65: 945-950.

4.5.17 Determine the incidence and importance of *Guignardia citricarpa* on nursery trees

Experiment: QMS5.3 by W. van Broekhuizen & S.H. Swart (QMS)

Opsomming

Die teenwoordigheid van swartvlek is deeglik in 'n kwekery in die Letsitele-omgewing naby Tzaneen ondersoek. Isolasië is uit verskillende skadunet-strukture, vanaf verskillende kultivars, gedurende verskillende tye van die jaar gemaak. Hoewel daar nog nie 'n korrelasie getref kon word tussen die voorkoms van die twee verskillende *Guignardia* spp. nie, het resultate wel daarop gedui dat die probleem regdeur die kwekery voorkom en nie tot sekere areas beperk is nie. Verder het resultate daarop gedui dat die siekte meer algemeen op ouer bome in die kwekery voorkom en meer gereeld op suurlemoen, Bahianina nawels en Turkey valensias voorkom as op Delta of Midnight valensias. Die tyd van die jaar wanneer isolasië gedoen word speel ook 'n belangrike rol aangesien die meeste positiewe isolasië gedurende die Mei-evaluasie voorgekom het.

Introduction

It has been noted that nurseries play an important role in the manifestation of citrus black spot disease. During the 2002/2003 season, several *Guignardia* isolates were obtained from citrus leaves in a local nursery close to Letsitele, Limpopo province. These isolates were all identified by the University of Pretoria, Plant Pathology Laboratories, as species belonging to *Guignardia mangiferae*.

The objective of this study was to develop a protocol in order to detect and quantify the incidence of *Guignardia* spp. on citrus trees in a nursery and to determine the composition of the population regarding the occurrence of *G. citricarpa* and *G. mangiferae*.

Materials and methods

Sampling period 1: Samples were collected during May 2003. Leaves were sampled from different rootstocks and scions combinations of the oldest citrus trees in several shade net structures of a nursery. Rootstocks included Rough lemon, Carrizo citrange, Swingle citromello, C-35, and X639. Scions included Eureka lemon, Bahianinha navel, Turkey valencia, Delta valencia and Midnight valencia. Twenty leaves with small black lesions were selected randomly per block of approximately 2500 nursery trees. Leaves were surface sterilised and five isolations per leaf were plated on Potato Dextrose Agar containing 200 ppm chloramphenicol. Culture media with isolations was incubated at 25°C and evaluated for development of typical *Guignardia*-like cultures. A random sample of *Guignardia*-like cultures were sent to the Plant Pathology Laboratories of the University of Pretoria where the *Guignardia* species was confirmed, using PCR techniques.

Sampling period 2: Samples were collected during July and August 2003. Where possible the same blocks of citrus trees were included that was used during the May sampling period, but since several blocks of trees had been sold, all blocks could not be included and other blocks were selected for this investigation. Due to the fact that rootstocks lose most of their leaves, it was decided to only use the oldest leaves on scions during this investigation.

Sampling period 3: Samples were taken during January 2004. This evaluation included the oldest leaves from Eureka lemon, Turkey Valencia, Delta Valencia, and Midnight Valencia scions. Blocks of trees containing the four cultivars were selected according to tree age. Three different blocks of each cultivar were

sampled. Cultures representing typical *Guignardia*-like growth will be sent to the Pathology Laboratories of the University of Pretoria where the *Guignardia* species will be determined, using PCR techniques.

Results and discussion

A total of 18 blocks of citrus trees in 8 shade net structures were sampled in a nursery during May 2003. Eighty three *Guignardia*-like isolates were found in 6 different blocks of citrus trees in 5 of the shade net structures included in this investigation. Results indicated that both leaves of rootstocks and scions could be infected with CBS. Indications were CBS isolates were more often obtained from Eureka lemon, Turkey Valencia and Bahianinha navel than from Delta Valencia or Midnight Valencia cultivars. According to PCR test results (TR 03/06/03) of Plant Pathology Laboratories, all isolates belonged to *G citricarpa*.

This data suggested that CBS occurred throughout the nursery and that it was not confined to specific shade net structures or blocks of trees.

During the 2nd sampling conducted during July and August 2003 very few CBS cultures were recovered. Only 2 isolations were obtained from Midnight valencia and 1 from Bahianinha navel trees. Much less positive isolations were made during the July and August sampling periods than during May, suggesting that the time of sampling could be crucial for CBS sampling in nurseries.

Isolation made during the 3rd period (January 2004) also resulted in a very low recovery of *Guignardia*-like cultures. From the four cultivars included in this evaluation CBS cultures were only obtained from Delta, Midnight and Turkey cultivars. Lemons tested negative on this occasion. It was however obvious that during this evaluation all positive blocks came from one shade net structure.

In general results from this project indicated that CBS is more likely to be found on the oldest trees in a nursery. Most positive isolations were made from the oldest leaves on either rootstock or scion. It seems that CBS is more frequently obtained from lemon, Bahianinha navel and Turkey Valencia than Delta or Midnight valencia. All isolates from 2002 were identified as *G. mangiferae*, using PCR technology while isolates obtained during May 2003 were identified as *G. citricarpa*.

Future research

Cultures from the last two cycles will be sent to the Plant Pathology Laboratories at the University of Pretoria for PCR identification.

Using previous results, a preliminary protocol can now be developed for the surveying of citrus nurseries for the presence of citrus black spot. It is clear that the disease can occur anywhere in a nursery and that sampling should occur in various structures. Since the most positive isolates were made during the May sampling period, this might be the best time to conduct a black spot survey. Most positive isolates were made from older leaves and should therefore be used for sampling. An important aspect that still needs clarification is the amount of leaves needed during sampling to act as a representative sample. This will be determined in the 2004/2005 season by picking different sample sizes and comparing the recovery rate of positive isolates to establish the ideal amount of leaves needed per sample when sampling a nursery for the presence of citrus black spot. To conclude this study, the final protocol will be tested in a second nursery.

4.6 PROJECT: POST-HARVEST PATHOLOGY

Project Co-ordinator: K.H. Lesar (CRI)

4.6.1 Project summary

Post-harvest citrus decay is controlled by the post-harvest fungicides, thiabendazole, imazalil, guazatine and SOPP. During the last 10-15 years there has been continuous pressure exerted from various quarters, political and regulatory, health groups, and pressure from certain markets to discontinue the use of post-harvest chemicals or decrease the MRLs of these compounds on citrus. At the beginning of the 2003 season the further use of one of the post-harvest chemicals, 2,4-D, came under serious threat of being discontinued when the EU announced that the MRL for this compound would be reduced from 2.0 mg/kg to 0.05 mg/kg. This caused considerable consternation and panic within the South African citrus industry. However, after numerous trials and negotiations with the EU MRL Commission, a reprieve was granted where the MRL was set at 1.0 mg/kg, however temporary the reprieve may be. This has highlighted the fact even further that it has become necessary to screen new chemicals against the citrus pathogens in order to find new, safe compounds that could assist in the overall decay control as well as preventing the onset of fungal pathogen resistance to the post-harvest fungicides.

As a result of the sudden notification (January 2003) that the MRL for 2,4-D was being decreased from 2.0 mg/kg to 0.05 mg/kg, before the start of the 2003 season, an appropriate series of 2,4-D efficacy trials at different concentrations were conducted on different citrus cultivars, together with the corresponding residue analyses. The reason for conducting these trials was because a full data package for 2,4-D had never been compiled by the South African citrus industry. Such a data package was necessary to determine the minimum concentration of 2,4-D that could be applied to export citrus, post-harvest, to retain a detectable residue of 2,4-D that would still be effective in keeping a live, green calyx (button) on the fruit, thus preventing abscission and subsequent infection by one of the post-harvest latent citrus pathogens. Valuable results were obtained from these trials. After intense negotiations between representatives from the South African citrus industry (Citrus Research International and Citrus Growers Association) and representatives from various other citrus producing countries, and the EU MRL Commission, and the submission of all the necessary data on 2,4-D, a workable solution to the problem was achieved (4.6.2).

A product, Citri A and Citri B, was evaluated as a possible alternative to the 2,4-D (Deccomone), which is currently used as post-harvest treatment in citrus packhouses. Valencia oranges with green, live calyxes (buttons) were treated with two concentrations of 2,4-D (Deccomone) in a water dip and wax as well as four different concentrations of a mixture of Citri A and B in a water dip. All the treatments were stored at ambient temperature and then evaluated, for live buttons and button abscission, on a weekly basis for a period of five weeks. The possible alternative product, Citri A & B did not perform as well as 2,4-D, showing poor fruit quality, abscised buttons and phytotoxicity on treated fruit over the five week storage period (4.6.3.1).

Three generic formulations of Imazalil 750 WSP and one generic formulation of guazatine SL were screened for efficacy against the post-harvest citrus pathogens, *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot). Two new formulations of the already registered UltraCure (guazatine) were also submitted for screening against these pathogens. All the formulations demonstrated good control of the two citrus pathogens compared to the standard recommended Fungazil 750 WSP and Deccotine SL. No phytotoxicity was observed on the treated fruit (4.6.3.2, 4.6.3.3, 4.6.3.4).

Two new formulations of the quaternary ammonium compound Quattro Kill and the quat F10 were screened *in vitro* and *in vivo* against *P. digitatum* and *G. candidum*. The three products indicated no fungicidal properties in inhibiting the two pathogen infections. However, the three quats did prove to be effective sanitizing agents, killing superficial fungal spores of the two pathogens from fruit surfaces in a solution. Pilot trials in a packhouse dumptank situation during production will have to be conducted to evaluate the product concentration test kits before final recommendations can be made. The quat, Terminator, was also evaluated *in vivo* only, against the two pathogens as above, for fungicidal properties. The results also indicated no fungicidal properties as with the above three quats. A pilot packhouse trial will also need to be done, as above, before the product can be recommended for use in a citrus packhouse dumptank as a sanitizing agent (4.6.3.5).

Three Sasol natural (Carnauba) citrus waxes, incorporating post-harvest fungicides, were submitted by Sasol SA (Pty) Ltd. for evaluation of the efficacy of the chemicals in the wax against post-harvest diseases. The three waxes were submitted with imazalil, thiabendazole and a combination of the two fungicides respectively. The results obtained were erratic. However, this evaluation will not be repeated as the scope in the market for waxes, incorporating fungicides is limited (4.6.3.6).

The incidence of the spread of *Phytophthora* brown rot infection within packed cartons of healthy citrus fruit was evaluated as the last part of the overall trial where the Israeli product, Canon PH (a phosphonate) was evaluated as a post-harvest control for *Phytophthora* brown rot. A few artificially infected *Phytophthora* navel oranges were strategically placed in packed cartons of treated and untreated (controls) navel oranges. The fruit was treated with the three phosphonates, Aliette, Phytex and Canon PH. The cartons were cold-stored under simulated shipping conditions and evaluated thereafter for incidence of spread of infection. All the treated cartons showed a degree of spread of the brown rot infection in the carton. This trial demonstrated that the incidence of *Phytophthora* brown rot infection did not warrant the post-harvest application of phosphonates for control of *Phytophthora* brown rot. This work has now been completed (4.6.3.7).

The post-harvest fungicide, Prochloraz (an imidazole), is currently registered in the southern African citrus industry in a brush-on total loss application system. Because this type of application system does not exist in citrus packhouses any longer, due to the introduction of the fungicide hot water bath dip application, the need arose to evaluate this compound for efficacy against the *Penicillium* organisms in a dip treatment for the purpose of registration of this compound. An *in vivo* evaluation of Prochloraz for efficacy against the post-harvest citrus pathogen *P. digitatum* (green mould) was conducted in a hot water bath dip treatment at 40°C. Prochloraz was evaluated at four different concentrations, and good control of the pathogen was

achieved, compared to the standard recommended fungicide imazalil sulphate (also an imidazole). This work is ongoing and more evaluations will be done before submitting for registration (4.6.3.8).

Forty-five *P. digitatum* (green mould) fungal spore samples from various production areas, were screened for resistance against the post-harvest fungicide Imazalil. All the spore samples were found to be sensitive to imazalil (4.6.3.9).

Projekopsomming

Naoes sitrus bederf word beheer deur die gebruik van die naoes swamdoders, thiabendazole, imazalil, guazatine and SOPP. Gedurende die afgelope 10-15 jaar is voortdurende druk uitgeoefen vanuit verskeie bronne, polities en voorskrewend, gesondheidsinstansies, en druk vanaf sekere markte om die gebruik van swamdoders te eindig, of die MRLs van swamdoders op sitrus te verminder. Tydens die begin van die 2003 produksie seisoen is die verdere gebruik van een van die naoesmiddels, 2,4-D onder gevaarlike druk geplaas om nie meer gebruik te word nie, deurdat die EU aangekondig het dat die MRL vir 2,4-D van 2.0 mg/kg tot 0.05 mg/kg verminder gaan word. Dit het gelei tot heelwat onsteltenis en benoudheid in die S. Afrikaanse sitrusbedryf. Nietemin, na heelwat proewe met 2,4-D en onderhandelinge met die EU MRL Kommissie, is 'n grasie van 1.0 mg/kg toegeken, hoe ook al tydelik die grasie mag wees. Dit het die feit nog verder beklemtoon dat dit nodig is om nuwe chemikalië te evalueer teen die sitrus patogene om nuwe, veiliger middels te kry wat kan bystaan in die algehele bederf beheer strategie en swamdoder weerstandbiedendheid voorkoming.

As gevolg van die skielike kennisgewing (Januarie 2003) dat die MRL vir 2,4-D van 2.0 mg/kg tot 0.05 mg/kg voor die begin van die 2003 produksie seisoen verminder sal word, is 'n nodige stel proewe met 2,4-D uitgevoer waar verskeie sitruskultivars met verskillende konsentrasies van 2,4-D behandel en die behandelde vrugte is ingedien vir ontleding vir die ooreenstemende residue. Die redes vir dié proewe is omdat 'n volle data pakket vir 2,4-D nooit deur die S. Afrikaanse sitrus bedryf saamgestel is nie. So 'n pakket was nodig om die minimum konsentrasie van 2,4-D te kan bepaal vir toediening op uitvoer sitrus, en 'n konsentrasie wat nogsteeds effektief is om 'n lewendige, groen blomkelk op die vrugte te kan hou en dus skeiding van die blomkelk te verhoed en enige daaropvolgende infeksie deur een van die naoes-latente sitrus patogene. Waardevolle resultate is deur hierdie proewe bekom. Na die indien van die nodige data oor 2,4-D en hewige onderhandelinge tussen verteenwoordigende partye vanuit die S. Afrikaanse sitrus bedryf (CRI en CGA), verskeie ander sitrusproduserende lande en die EU MRL Kommissie, is 'n uitvoerbare oplossing vir die 2,4-D probleem bereik (4.6.2).

'n Produk, Citri A en Citri B, is ge-evalueer as moontlike alternatiewe vir 2,4-D (Deccomone), wat huidiglik as naoes behandeling in sitruspakhuis gebruik word. Valencia lemoene, met groen, lewendige blomkelke, is met twee konsentrasies van 2,4-D (Deccomone) in 'n water doop en ook waks, sowel as vier verskillende konsentrasies van 'n mengsel van Citri A en B in 'n water doop behandel. Al die behandelings is opgeberg teen kamer temperatuur, en dan weekliks vir 'n tydperk van vyf weke vir lewendige blomkelk en blomkelk skeiding ge-evalueer. Die moontlike alternatiewe produk, Citri A en B het nie, in vergelyking met 2,4-D (die kontrole) so goed gevaar nie. Hier is swak gehalte vrugte, geskeide blomkelk en fitotoksiteit op die behandelde Citri A en B vrugte, oor die vyfweek tydperk van opberging, te sien (4.6.3.1).

Drie generiese formulasies van Imazalil 750 WSP en een generiese formulasie van guazatine SL is ge-evalueer vir effektiwiteit teen die naoes sitrus patogene, *Penicillium digitatum* (groenskimmel) en *Geotrichum candidum* (suurvrot). Twee nuwe formulasies van die alreeds geregistreerde UltraCure (guazatine) is ook ingedien vir evaluasie teen die twee patogene. Al die bogenoemde formulasies het goeie beheer van die twee patogene in verlyking met die standard Fungazil 750 WSP en Deccotine SL getoon. Geen fitotoksiteit is op behandelde vrugte gesien nie (4.6.3.2, 4.6.3.3, 4.6.3.4).

Twee nuwe formulasies van die kwatenêre ammonium verbinding Quattro Kill en die kwatenêre ammonium verbinding F10 is ge-evalueer *in vitro* en *in vivo* teen *P. digitatum* en *G. candidum*. Die drie produkte het geen swamdoderende eienskappe gewys nie. Nietemin, dié drie middels het gewys dat hulle wel doeltreffende ontsmettingsmiddels is deurdat hulle oppervlakkige swamspore van die twee patogene op die vrugskil in oplossing doodmaak. Loodsproewe sal moet in pakhuis dompeldaddens gedoen word om die produk konsentrasie toetsapparaat te evalueer voor die produkte aanbeveel word vir gebruik as saniteermiddels in sitrus pakhuis dompeldaddens (4.6.3.5).

Drie Sasol Carnuba (natuurlike) sitruswakse, wat naoes swamdoders inkorporeer, is ingedien deur Sasol SA (Edms) Bpk vir evaluasie van die effektiwiteit van die swamdoders in die waks, teen naoes infeksies. Die drie wakse het imazalil, thiabendazole, en 'n kombinasie van dié twee swamdoders ingekorporeer in die

waks. Die resultate van hierdie evaluasies was baie wisselvalig. Hierdie evaluasie sal nie herhaal word nie weens die beperkte mark vir die gebruik van wakse met swamdoders (4.6.3.6).

Die voorkoms van die verspreiding van *Phytophthora* bruinvrot infeksie in verpakte kartonne van gesonde sitrusvrugte is ge-evalueer. Hierdie evaluasie is die laaste deel van die proef waar die Israeli middel Canon PH ('n fosfonaat) ge-evalueer is as 'n naoes behandeling vir die beheer van *Phytophthora* bruinvrot. 'n Paar *Phytophthora* geïnkuleerde nawel lemoene is strategies geplaas in verpakte kartonne behandelde en onbehandelde (kontrole) nawel lemoene. Die vrugte is behandel met drie fosfonate, Aliette, Phytex en Canon PH. Die kartonne vrugte is dan koelopgeberg teen gesimuleerde verskepingstoestande en daarna ge-evalueer vir die voorkoms van die verspreiding van *Phytophthora* infeksie. Al die behandelde kartonne het mate van verspreiding van bruinvrot infeksie in die kartonne. Hierdie proef het gewys dat die voorkoms van *Phytophthora* bruinvrot verspreiding die naoes aanwending van fosfonate vir die beheer van *Phytophthora* bruinvrot nie regverdig nie (4.6.3.7).

Die naoes swamdoder Prochloraz ('n imidazole) is tans naoes geregistreer in die S. Afrikaanse sitrusbedryf in 'n aanborsel, totale verlies sisteem. Omdat hierdie soort van aanwendingssisteem nie meer in pakhuis gebruik word nie, weens die gebruik van die swamdoder warm water bad doop behandeling, was dit nodig om dié middel te evalueer vir effektiwiteit teen die *Penicillium* swamme in 'n doop behandeling vir die uiteindelijke registrasie van Prochloraz. 'n *In vivo* evaluasie van Prochloraz vir effektiwiteit teen die naoes patoogeen *P. digitatum* (groen skimmel) is uitgevoer in a warm waterbad doop behandeling teen 40°C. Prochloraz is ge-evalueer teen drie verskillende konsentrasies en goeie beheer van die patoogeen is getoon, in vergelyking met die standard swamdoder imazalil sulfaat (ook 'n Imidazole). Die werk is aangaande en verdere evaluasies sal uitgevoer word voor indiening vir registrasie (4.6.3.8).

Vyf-en-veertig *P. digitatum* (groenskimmel) swamspoormonsters vanuit verskeie produksie gebiede is ge-evalueer vir weerstandbiedendheid teen die naoes swamdoder, imazalil. Al die spoormonsters was sensitief teenoor imazalil (4.6.3.9).

4.6.2 **2,4-D Efficacy trials and Residue Analyses to establish compliance with EU MRL of 1.0 mg/kg** Experiment by K.H. Lesar (CRI)

Opsomming

Die suiderlike Afrika sitrus bedryf is in Januarie 2003 in kennis gestel dat die MRL vir 2,4-D in die EU verminder sal word vanaf 2.0 dpm tot 0.05 dpm geldig vanaf 1 Julie 2003. Dit het nodig geword om effektiwiteitsproewe en relevante residu ontledings met 2,4-D (Deccomone) uit te voer vir voorlegging van hierdie data aan die EU Residu Kommissie tydens die beplande vergadering tussen die Residu Kommissie en 'n Suid Afrikaanse delegasie teen die einde Mei 2003. Na hierdie vergadering plaasgevind het, het die SKV/CRI laat weet dat 'n ooreenstemmende EU MRL van 1.0 dpm deur die EU Kommissie oorweeg word. In tussentyd sal 'n EU MRL van 0.05 dpm deur alle EU lede toegepas word, indien deur 'n nasionale MRL vervang word.

Introduction

The southern African citrus industry was informed in January 2003 that the MRL for 2,4-D in the EU would be decreased from 2.0 ppm to 0.05 ppm as from 1 July 2003. Prior to the introduction of the registered formulation of 2,4-D (Deccomone), the unregistered formulations of 2,4-D, the amino and ester formulations, were being used. Consequently a full data package for 2,4-D was never compiled.

It thus became necessary to conduct efficacy trials and relevant residue analyses with 2,4-D (Deccomone) for submission of this data to the EU Residue Committee at the planned meeting between the Residue Committee and a South African delegation at the end of May 2003.

After this meeting had taken place, feedback from the CGA/CRI advised that a harmonized EU MRL of 1.0 ppm was being considered by the EU Committee. In the interim an EU MRL of 0.05 ppm would apply to all member states, unless replaced by a national MRL. The UK was then the only EU member state to set a national MRL of 1.0 ppm.

The export tolerance for 2,4-D residue on citrus to the EU was previously 2.0 ppm. It was thus relevant to advise on usage practices required to ensure compliance with the 0.05 ppm and 1.0 ppm levels. This information was attained from the following trials conducted with 2,4-D (Deccomone).

Materials and methods

Good, sound untreated lemons, Novas, grapefruit and navel oranges were acquired in bulk for these trials. All the fruit was sorted, washed in clean water and then surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was allowed to dry prior to being treated.

All the treatments were dipped in ambient solutions of 2,4-D (Deccomone) for 3 minutes. The fruit was then incubated in paper packets prior to evaluation or being submitted for residue analyses. The various trials conducted were either stored at ambient temperature for immediate submission for residue analyses or over a longer period for evaluation of efficacy of 2,4-D at various concentrations, as well as stored under longer simulated shipping conditions to evaluate the possible breakdown of the 2,4-D residues on the fruit, prior to the fruit arriving on the market.

Trial 1

The following treatments were used:

- A. 500 ppm 2,4-D in Deccowax dip (standard commercial recommendation).
- B. 20 ppm 2,4-D in water dip (based on the Spanish citrus industry application in a predegreening drench).
- C. 500 ppm 2,4-D in water dip (standard commercial recommendation).

Trial 2

Same samples as in Trial 1 (A, B & C) stored under simulated shipping conditions at 11°C for 3 weeks. The “first sample” evaluation for residue was conducted 5 days after treatment, as in Trial 1, then on “arrival” at the port 3 weeks after shipping at 11°C, and finally 1 week after storage at ambient prior to “dispatch” onto the market.

Trial 3 - Efficacy trial

Novas were treated with varying concentrations of 2,4-D, ranging from 20 ppm to 500 ppm in a 3 minute dip treatment. 10 Fruit (Novas) were used for each treatment. The fruit were then stored in paper packets at ambient temperature (23°C) for 3 weeks. The fruit was then evaluated weekly for live, non-abscised buttons (calyxes).

Trial 4 – Residue level / Surface area of fruit (fruit size)

Four citrus cultivars were treated with 200 ppm 2,4-D in a 3 minute dip treatment and submitted for residue analyses. The purpose of this trial was to determine the level of 2,4-D residue retained per surface area of fruit, or fruit size.

Trial 5

A second shipping trial was conducted using Novas. The Novas were treated with 20, 50, 100 and 200 ppm 2,4-D in a three minute dip treatment. The initial sample was sent for residue analyses and the remainder of samples was stored under shipping conditions at 11°C for 3 weeks. After 3 weeks storage an “arrival” sample was submitted for residue analyses. The final sample stored at ambient prior to “dispatch” onto the market was not submitted for residue analysis due to the final high cost of analyses.

Table 4.6.2.1. Evaluation of residue results of ‘first’ and ‘arrival’ samples of fruit

Treatments (ppm 2,4-D)	Residue Results (ppm)	
	“First” sample	“Arrival” sample
20	0.03	0.04
50	0.05	0.12
100	0.08	0.25
200	0.12	0.37

Trial 6 - 2,4-D Residue Analysis for new post-harvest concentration recommendations

Three samples of 10 lemons each were treated with 2,4-D at a concentration of 250 and 200 ppm as follows.

- Sample 1 250 ppm “Drench” (Pre-degreening).
 Sample 2 250 ppm “Drench” (Pre-degreening). Fruit was washed with sanitizing agent 2 days after drench and then retreated with 250 ppm 2,4-D in “fungicide bath” dip treatment.
 Sample 3 Repeat of 200 ppm dip treatment.

Results

Table 4.6.2.2. Lemon residue results for Trials 1 & 2.

Lemons - Residue results (ppm)			
Treatments	1st Sample	Arrival	Despatch
A	0.25	0.31	0.71
B	0.09	0.10	0.15
C	0.64	1.0	0.92

Table 4.6.2.3. Evaluation of number of live, non-abscised buttons on fruit.

Treatments (ppm 2,4-D)	Evaluation			
	Number of live buttons out of 10			
	Week 1	Week 2	Week 3	% Live buttons
Untreated control	4	0	0	13.0
20	2	2	2	20.0
50	2	2	2	20.0
75	4	2	2	27.0
100	4	4	4	40.0
125	6	6	6	60.0
150	6	6	6	60.0
200	6	6	6	60.0
300	8	6	6	67.0
400	8	6	6	67.0
500	8	6	6	67.0

The above results reveal favourable button retention from 125-500 ppm 2,4-D even though the Novas used were over-mature.

Table 4.6.2.4. Level of 2,4-D residue retained per surface area of fruit or fruit size.

Cultivar	Residue level (ppm)
Novas	0.09
Navels	0.38
Lemons	1.9*
Star Ruby grapefruit	0.16

The lemon result was abnormally high compared to the other cultivars. A repeat analyses produced a result of 2,0 ppm.

Table 4.6.2.5. 2,4-D residue analysis for new post-harvest concentration recommendations

Treatments	Residue results (ppm)
1	0.41
2	0.48
3	0.27 (previous 1, 9)

The results from these trials conducted with 2,4-D for efficacy and residue analyses indicate that it is not possible to effectively use 2,4-D as a post-harvest treatment and comply with a 0.05 ppm MRL.

Conclusion and recommendations

The current SA registered post-harvest usage of 2,4-D is at a concentration of 500 ppm. At this 2,4-D (Deccomone) dip treatment the residue level tested out at 0,64-1,0 ppm. Continued, careful usage as a single treatment at a concentration of 500 ppm would not present a problem in complying with the 1,0 ppm

MRL. However, in order to establish a safety margin for possible errors it was decided to decrease the recommended concentration of 2,4-D to 250 ppm to ensure compliance with the 1,0 ppm MRL. The above efficacy trials showed that 2,4-D (Deccomone) remains effective at 250 ppm.

The new recommendations for the post-harvest application of 2,4-D on export citrus in compliance with the 1,0 ppm MRL were made as follows.

1. **Degreening (Pre-degreening drench + packhouse treatment)**
Pre-degreening drench apply: 250 ppm Deccomone = 10 ℓ / 1000 ℓ water; PLUS
After degreening apply: 250 ppm in hot water fungicide bath = 10 ℓ / 1000 ℓ water; OR
250 ppm in the wax = 250 ml / 25 ℓ wax
 2. **No Degreening (Packhouse treatment only)**
250 ppm Deccomone in hot water fungicide bath = 10 ℓ / 1000 ℓ bath; OR
250 ppm Deccomone in the wax = 250 ml / 25 ℓ wax
 3. **Top up of fungicide bath**
Replace lost water + full strength Deccomone + 20ml Deccomone/Ton fruit (not 40ml as previously recommended)
- 4.6.3 **The screening of sanitizing agents, new chemicals and potential new fungicides *in vitro* and *in vivo* against the post-harvest citrus pathogens**
Experiment 123 by K.H. Lesar (CRI)

Opsomming

Patogeen bestandheid teen na-oes swamdoders op sitrus was altyd 'n bedreiging en vind plaas, inderdaad, tans met sekere van die patogene en swamdoders. Saam met die bestandheidsbedreiging is die aanhoudende druk vanuit verskeie bronne, soos voorheen genoem, om die toekomstige gebruik van die na-oes middels te eindig. Dit het die evaluering van nuw veilige chemikalië vereis teen sitruspatogene vir swamspoor vernietiging en swamododende eienskappe wat kan bydra tot ontsmetting en bederf beheer.

Introduction

Pathogen resistance to post-harvest fungicides in citrus has always been a threat and, in fact, is occurring with certain pathogens and fungicides at present. Together with the resistance threat is the continued pressure from various quarters, as already mentioned, to discontinue the future use of the post-harvest chemicals.

It has thus become necessary to screen new chemicals against citrus pathogens for fungal spore kill qualities and for fungicidal properties so as to find new safe compounds that could assist in sanitizing functions as well as decay control.

4.6.3.1 The screening of a product, Citri A and Citri B, as a possible alternative to 2,4-D (Deccomone)

A nitrogen based product from Holland consisting of two components, Citri A and Citri B, was submitted by ICA International for evaluation as an alternative product to 2,4-D (Deccomone) as a post-harvest application.

Materials and methods

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. Blemish free, sound fruit with live buttons (calyxes) was selected and randomized. The fruit was washed in clean water, surface sterilized by dipping in a 1% NaOCl solution for a few minutes, and then allowed to dry prior to being treated. The fruit was then divided into lots of 10 fruit per treatment. The prepared fruit was then treated with different concentrations of the standard, 2,4-D (Deccomone) in a dip treatment as well as with 2,4-D in citrus wax. The balance of the fruit was treated with different concentrations of a mixture of Citri A and B. Once the Citri A & B were mixed, a certain level of nitrogen was produced which penetrated the fruit cells by translocation, thereby boosting the fruit vigour and thus possibly being able to retain live buttons (calyxes) on citrus fruit.

All the fruit were dipped in the treatments at ambient temperature for three minutes. The treated fruit was then incubated in paper packets at 20°C and evaluated on a weekly basis for five weeks for button retention/abscission.

Results

Table 4.6.3.1.1. Calyx condition after various treatments.

	Treatments	Number of buttons abscised out of 10						
		Week 1	Week 2	Week 3	Week 4	Week 5	Alive/Dead	% Abscission
1.	Untreated control	Nil	1	4	6	10		100
2.	250 ppm 2,4-D dip	Nil	Nil	2	4	5		50
3.	500 ppm 2,4-D dip	Nil	Nil	2	3	4		40
4.	250 ppm 2,4-D in wax	Nil	Nil	Nil	Nil	Nil	6/4	Nil
5.	500 ppm 2,4-D in wax	Nil	Nil	Nil	Nil	1	9/0	10
6.	1,5ℓ Citri A + 1ℓ Citri B	1	All buttons dead				0/10	Nil
7.	1ℓ Citri A + 0.66ℓ Citri B	Nil	All buttons dead				0/10	Nil
8.	0.5ℓ Citri A + 0.33ℓ Citri B	Nil	All buttons dead				0/10	Nil
9.	0.25ℓ Citri A + 0.17ℓ Citri B	Nil	All buttons dead				0/10	Nil

Conclusions

The potential alternative product to 2,4-D, Citri A & B, indicated no live buttons on the fruit after storage and evaluation compared to the standard 2,4-D treatments. The Citri A & B treatments also indicated a high level of phytotoxicity. No further work will be done on this product. Note that 2,4-D demonstrated fairly good button retention even though the Valencias used were overmature.

4.6.3.2 The *in vivo* screening of three generic formulations of the post-harvest fungicide Imazalil

Three generic formulations of imazalil 750 WSP were submitted to CRI by AgChem Africa, Agritech and Hyper Agrochem for evaluation of efficacy against the post-harvest citrus pathogen *Penicillium digitatum* (green mould).

Materials and methods

The three products were compared separately with the standard Fungazil 750 WSP (Janssen Pharmaceutica) at the recommended commercial rate of 500 mg/kg. Each generic imazalil was evaluated at the rates of 250 mg/kg ($\frac{1}{2}x$), 500 mg/kg (x) and 1000 mg/kg ($2x$).

A spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 10^6 spores/ml spectrophotometrically.

Good, sound, untreated fruit were obtained in bulk. The fruit type used for the evaluations was navel and Valencia oranges from Crocodile Valley Citrus Co. for the Agritech and Hyper Agrochem products respectively, and lemons from Larten Estates for the AgChem Africa product. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 25 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 to each other. Each fruit was then infected with the pathogen by applying 35 μ ℓ of spore suspension to each injury site using a micropipette.

All the treatments (see Tables) with the relevant chemical being evaluated were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated 4 and 16 hours after inoculation (to simulate a short and long delay after harvesting prior to packhouse treatments) with the chemical compound being evaluated. Each treatment was immersed in the water bath for 3 minutes.

After treatment, the fruit was incubated in paper packets at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

Table 4.6.3.2.1. The results were identical for all three products.

Treatments	Percentage Decay
A Untreated control (<i>P. digitatum</i>)	100.0
B Treated control – Fungazil WSP (500 mg/kg) – 4 hrs after infection	0.0
C Treated control – Fungazil WSP (500 mg/kg) – 16 hrs after infection	0.0
D Test Imazalil – 250 mg/kg – 4 hrs after infection	0.0
E Test Imazalil – 250 mg/kg – 16 hrs after infection	0.0
F Test Imazalil – 500 mg/kg – 4 hrs after infection	0.0
G Test Imazalil – 500 mg/kg – 16 hrs after infection	0.0
H Test Imazalil – 1000 mg/kg – 4 hrs after infection	0.0
I Test Imazalil – 1000 mg/kg – 16 hrs after infection	0.0

No phytotoxicity was evident on the fruit treated at the highest concentration (1000 mg/kg) of any of the generic imazalils.

Conclusion

All generic formulations of imazalil demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard, recommended Fungazil 750 WSP. The results from these trials were submitted for the purpose of registration of these formulations.

4.6.3.3 The *in vivo* screening of a generic formulation of the post-harvest fungicide, guazatine

A sample of a generic formulation of the post-harvest fungicide, guazatine (G22) was submitted to CRI by P.W. Landbou dienste for evaluation of efficacy against the post-harvest citrus pathogens *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot) to determine the efficacy of the product in inhibiting infection caused by these pathogens.

Materials and methods

Spore suspensions of these pathogens were made up by suspending spores in sterile deionised water containing the surfactant or wetting agent, Tween 20. The spore suspensions were then adjusted to a concentration of 10⁶ spores/ml spectrophotometrically.

Good, sound, untreated lemons (Larten Estate) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 to each other. 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 treated 4 hours and 16 hours after inoculation (to simulate a short and long delay after harvesting prior to packhouse treatment), with the chemical compound being evaluated. Each treatment was immersed in the fungicide solution for 3 minutes. After treatment, the fruit was incubated in both paper packets and plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

G22 was effective against both pathogens (Tables 4.6.3.3.1 and 4.6.3.3.2).

Table 4.6.3.3.1. The effect of G22 on *P. digitatum*

LEMONS		
	Treatments	% Decay
A	Untreated control	100
B	Treated control (1000 mg/l) Deccotine	Nil
C	Treated control (500 mg/l) Imazalil Sulphate	Nil
D	500 mg/l G22 – 4 hrs	Nil
E	500 mg/l G22 – 16 hrs	10
F	1000 mg/l G22 – 4 hrs	Nil
G	1000 mg/l G22 – 16 hrs	10
H	2000 mg/l G22 – 4 hrs	Nil
I	2000 mg/l G22 – 16 hrs	Nil

Table 4.6.3.3.2. The effect of G22 on *G. candidum*

LEMONS		
	Treatments	% Decay
A	Untreated control	90
B	Treated control (1000 mg/l) Deccotine	Nil
C	500 mg/l G22 – 4 hrs	Nil
D	500 mg/l G22 – 16 hrs	Nil
E	1000 mg/l G22 – 4 hrs	Nil
F	1000 mg/l G22 – 16 hrs	Nil
G	2000 mg/l G22 – 4 hrs	Nil
H	2000 mg/l G22 – 16 hrs	Nil

No phytotoxicity was evident on the fruit treated at the highest concentration of G22 (2000 mg/l).

Conclusion

The generic formulation of guazatine (G22) submitted by P.W. Landboudienste, demonstrated good control of the citrus pathogens, *P. digitatum* and *G. candidum*, compared to the standard recommended Deccotine. These results were submitted for registration purposes.

4.6.3.4 The *in vivo* evaluation of two new generic formulations of the already registered post-harvest fungicide UltraCure (guazatine)

Two new samples “A” and “B” of the registered guazatine formulation UltraCure were submitted to CRI by Hyper Agrochem for evaluation of efficacy against infections caused by the post-harvest citrus pathogens *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot).

Materials and methods

Spore suspensions of both of these pathogens were made up by suspending spores, grown on Potato Dextrose agar plates, in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were then adjusted to a concentration of 10^6 spores/ml spectrophotometrically.

Good, sound, untreated Navel oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 25 fruit each, per treatment, and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 to each other. Each fruit was then infected with the pathogen by applying 35µℓ of spore suspension to each injury site using a micropipette.

Treatments

The fruit was treated with the following compounds at the given (recommended) concentrations.

Imazalil Sulphate WSP	=	500 ppm a.i.
Deccotine (guazatine)	=	1000 ppm a.i.
UltraCure A and B	=	500 ppm a.i. (½x) 1000 ppm a.i. (1x) 2000 ppm a.i. (2x)

All the treatments were dipped in water at ambient temperature. The inoculated fruit was treated 4 hours after inoculation with the chemical compounds. Each treatment was immersed in the water bath for 3 minutes.

After treatment, the fruit was incubated in paper packets as well as plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

Table 4.6.3.4.1. The effect of UltraCure “A” on *P. digitatum*

Treatments		Percentage Decay
1.	Untreated control	100
2.	Treated control – 500 ppm Imazalil Sulphate	Nil
3.	Treated control – 1000 ppm Deccotine (guazatine)	Nil
4.	UltraCure – 500 ppm	Nil
5.	UltraCure – 1000 ppm	Nil
6.	UltraCure – 2000 ppm	Nil

Table 4.6.3.4.2. The effect of UltraCure “B” on *P. digitatum*

Treatments		Percentage Decay
1.	Untreated control	100
2.	Treated control – 500 ppm Imazalil Sulphate	Nil
3.	Treated control – 1000 ppm Deccotine (guazatine)	Nil
4.	UltraCure – 500 ppm	100
5.	UltraCure – 1000 ppm	40
6.	UltraCure – 2000 ppm	20

Table 4.6.3.4.3. The effect of UltraCure “A” on *G. candidum*

Treatments		Percentage Decay
1.	Untreated control	90
2.	Treated control – 1000 ppm Guazatine	Nil
3.	UltraCure – 500 ppm	Nil
4.	UltraCure – 1000 ppm	Nil
5.	UltraCure – 2000 ppm	Nil

Table 4.6.3.4.4. The effect of UltraCure “B” on *G. candidum*

	Treatments	Percentage Decay
1.	Untreated control	100
2.	Treated control – 1000 ppm Guazatine	Nil
3.	UltraCure – 500 ppm	40
4.	UltraCure – 1000 ppm	30
5.	UltraCure – 2000 ppm	10

No phytotoxicity was observed on any of the above treatments at the highest concentration treated (i.e. 2000 ppm).

Conclusion

UltraCure sample “A” demonstrated good control of both of the citrus pathogens, *P. digitatum* and *G. candidum*, compared to the standard recommended post-harvest fungicides imazalil sulphate and Deccotine. The UltraCure sample “B” however did not demonstrate the same degree of activity against both of the citrus pathogens, and thus sample “B” cannot be considered for registration for use as a post-harvest treatment against post-harvest citrus pathogens.

4.6.3.5 The screening of two new formulations of the quaternary ammonium compound Quattro Kill as well as F10 and Terminator against post-harvest fungal spores causing disease of citrus fruit

QUATTRO KILL

New samples “A” and “B” of the QAC formulation Quattro Kill were submitted by Hyper Agrochem for evaluation against the post-harvest citrus pathogens, *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot), to determine whether the products have any fungicidal properties. The standard recommended post-harvest fungicides, imazalil sulphate and Deccotine (guazatine) were used as treated standards.

Materials and methods

Spore suspensions of both of these pathogens were made up by suspending spores, grown on Potato Dextrose agar plates, in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were then adjusted to a concentration of 10^6 spores/ml spectrophotometrically.

Good, sound, untreated Navel oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 25 fruit each, per treatment, and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 to each other. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette.

Treatments

The fruit was treated with the following compounds at the given (recommended) concentrations.

Imazalil Sulphate WSP	-	500 ppm a.i.
Deccotine (guazatine)	-	1000 ppm a.i.
Quattro Kill A and B	-	500 ppm product ($\frac{1}{2}$ x)
	-	1000 ppm product (1x)
	-	2000 ppm product (2x)

All the treatments were dipped in water at ambient temperature. The inoculated fruit was treated 4 hours after inoculation with the chemical compounds. Each treatment was immersed in the water bath for 3 minutes.

After treatment, the fruit was incubated in paper packets as well as plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Evaluation for Phytotoxicity

The following evaluation was done for any phytotoxic reaction to the rind of the fruit. Navel oranges were left standing in a 1000 ppm (i.e. 1ℓ/1000ℓ water) solution of the product for certain periods of time. This was a cold water treatment simulating a citrus packhouse dumptank washing system. A second treatment in a hot water dip at 40°C, simulating a fungicide bath was also conducted.

Results

Table 4.6.3.5.1. The effect of Quattro Kill “A” on *P. digitatum*

Treatments		Percentage Decay
1.	Untreated control	100
2.	Treated control – 500 ppm Imazalil Sulphate	Nil
3.	Quattro Kill – 500 ppm	90
4.	Quattro Kill – 1000 ppm	60
5.	Quattro Kill – 2000 ppm	60

Table 4.6.3.5.2. The effect of Quattro Kill “B” on *P. digitatum*

Treatments		Percentage Decay
1.	Untreated control	100
2.	Treated control – 500 ppm Imazalil Sulphate	Nil
3.	Quattro Kill – 500 ppm	80
4.	Quattro Kill – 1000 ppm	60
5.	Quattro Kill – 2000 ppm	20

Table 4.6.3.5.3. The effect of Quattro Kill “A” on *G. candidum*

Treatments		Percentage Decay
1.	Untreated control	90
2.	Treated control – 1000 ppm Guazatine	Nil
3.	Quattro Kill – 500 ppm	70
4.	Quattro Kill – 1000 ppm	70
5.	Quattro Kill – 2000 ppm	60

Table 4.6.3.5.4. The effect of Quattro Kill “B” on *G. candidum*

Treatments		Percentage Decay
1.	Untreated control	90
2.	Treated control – 1000 ppm Guazatine	Nil
3.	Quattro Kill – 500 ppm	70
4.	Quattro Kill – 1000 ppm	60
5.	Quattro Kill – 2000 ppm	50

No phytotoxicity was observed on any of the above treatments at the highest concentration treated (i.e. 2000 ppm).

Statement on phytotoxicity

Table 4.6.3.5.5. Phytotoxic reaction on Navel oranges by Quattro Kill "A".

Quattro Kill "A" concentration (ppm product)	Exposure time	Phytotoxicity
1000 Cold water	2 hrs	Nil
	4 hrs	Nil
	6 hrs	Slight
	8 hrs	Slight
	12 hrs	Severe
	16 hrs	Severe
	24 hrs	Severe
1000 Hot water	1 sec	Severe
	30 sec	Severe
	1 min	Severe
	2 min	Severe

Conclusion

Both Quattro Kill samples "A" and "B" demonstrated limited fungicidal activity against both of the citrus pathogens. Quattro Kill, however, has good sanitizing properties as demonstrated in a previous *in vitro* evaluation of the product. An appropriate test kit to measure the product concentration is now available. However, before final recommendation for the use of Quattro Kill in a citrus packhouse dumptank washing system, a pilot trial will have to be conducted in such a system where water samples will have to be evaluated for micro-organism populations, correlated with concentration measurements and topping up procedures.

Upon final recommendation of such a product, the fact that the product could "burn" sensitive fruit will have to be specified.

FORMULA 10 (F10)

The quaternary ammonium/biguanidine compound F10 from Health and Hygiene (Pty) Ltd. was submitted to CRI for screening for efficacy of the compound in the killing of the fungal spores of *P. digitatum* and *G. candidum* (*in vitro*) as well as evaluating the product against the abovenamed post-harvest pathogens to determine whether the product has any fungicidal properties (*in vivo*).

In vitro evaluation

Materials and methods

In order to study the effect of Chlorine and F10 on the germination of *P. digitatum* and *G. candidum*, spore suspensions of both these organisms were made by suspending spores, grown on Potato dextrose agar plates, in sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 10^6 spores/ml spectrophotometrically.

A stock solution of chlorine (HTH) was made up at a concentration of 200 ppm a.i. (i.e. available chlorine) as recommended for citrus packhouses. A stock solution of F10 was made up at a concentration of 1000 ppm (product i.e. 1 l/1000 l water) as recommended by the manufacturers.

For this evaluation, 1 ml of each fungal spore suspension was added to 9 ml of chlorine stock solution as well as to 9 ml of solution of the compound being evaluated. After the fungal spores had been exposed to the chlorine for time periods of 10 sec., 30 sec., 1 min., 5 min., and 10 mins, a 1 ml sample from each dilution was added separately to 0.1 ml of 0.1 molar sodium thiosulphate in order to then inactivate the chlorine.

Thereafter, 1 ml from each inactivated chlorine suspension was added to a PDA plate. Similarly a 1 ml sample from each time exposure of the test compound to the fungal spores was added to a PDA plate. A 1 ml sample of spore suspension was also added to PDA plates as controls.

All media plates were incubated at 25°C for 7 days or until such time as the control grew and the results were then recorded as spore germination or no spore germination.

Results

The results are indicated in Tables 4.6.3.5.6 and 4.6.3.5.7, relating to *P. digitatum* and *G. candidum*, respectively.

Table 4.6.3.5.6. Effect of chlorine and F10 on spore germination of *P. digitatum*

Product name	Concentration (ppm)	Exposure time (sec.)	Spore germination
F10	1000 (product)	10	+
		30	+
		60	-
		300	-
		600	-
Chlorine (HTH)	200 (a.i.)	10	+
		30	-
		60	-
		300	-
		600	-
<i>P. digitatum</i> control – positive + = spore germination - = no spore germination			

Table 4.6.3.5.7. Effect of chlorine and F10 on spore germination of *G. candidum*

Product name	Concentration (ppm)	Exposure time (sec.)	Spore germination
F10	1000 (product)	10	+
		30	-
		60	-
		300	-
		600	-
Chlorine (HTH)	200 (a.i.)	10	+
		30	-
		60	-
		300	-
		600	-
<i>G. candidum</i> control – positive + = spore germination - = no spore germination			

Conclusions

F10 demonstrated good fungal spore kill qualities of both post-harvest pathogens, which compared favourably with the standard, chlorine (HTH). Spore germination did however occur with both pathogens on exposure to both products at the lowest exposure rate. It is advisable to use a longer exposure period as demonstrated in the result tables above.

Prior to this product being recommended for use in citrus packhouse fruit washing systems, a pilot trial will have to be conducted to evaluate the monitoring of the product concentration (reliable test kit) and correlating these readings with micro-organism counts on water samples from the system.

In vivo evaluation

Materials and methods

Spore suspensions of both these pathogens were made up by suspending spores, grown on Potato Dextrose agar plates, in sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good, sound, untreated Navel oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 10 fruit each, per treatment, and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 (20 inoculations/treatment) on opposite sides to each other. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

Treatments

The inoculated fruit was treated with the following compounds at the given (recommended) concentrations.

Imazalil sulphate	-	500 g/kg
Guazatine (Deccotine)	-	1000 g/l
F10	-	500 ppm product (1/2x)
	-	1000 ppm product (x)
	-	2000 ppm product (2x)

All the treatments were dipped in water at ambient temperature. The inoculated fruit was treated 4 hours after inoculation with the chemical compounds. Each treatment was immersed in the water bath for 3 minutes.

After treatment, the fruit was incubated in paper packets as well as plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

Table 4.6.3.5.8. The effect of F10 on *P. digitatum*.

Treatments		% Decay
NAVELS		
A	Untreated control	100.0
B	Standard – 500 g/kg Imazalil Sulphate	0.0
C	F10 – 500 ppm product	100.0
D	F10 – 1000 ppm product	100.0
E	F10 – 2000 ppm product	100.0

Table 4.6.3.5.9. The effect of F10 on *G. candidum*.

Treatments		% Decay
NAVELS		
A	Untreated control	90.0
B	Standard – 1000 mg/l Deccotine	0.0
C	F10 – 500 ppm product	100.0
D	F10 – 1000 ppm product	100.0
E	F10 – 2000 ppm product	100.0

Conclusion

As can be seen from the above results, F10 did not demonstrate any fungicidal activity against either of the citrus pathogens. F10, however, has good sanitizing properties as can be seen from the *in vitro* results, which is what is required of such a product in a citrus packhouse washing system.

However, the product cannot be recommended for use in a dumptank system at this stage as the test kit, supplied with the product needs to be evaluated again due to the rapid evaporation (damping off) of the chloroform component of the "titration" test kit.

Once the test kit has been approved, under laboratory conditions, a pilot trial will have to be conducted in a citrus packhouse dumptank system during production to monitor and evaluate the test kit under practical conditions.

Once the test kit is approved, two further requirements are necessary before the product can finally be recommended for use in a citrus packhouse dumptank system, i.e. firstly, fruit samples must be submitted for residue analyses and, secondly, it must be established what the phytotoxicity status of the product is.

TERMINATOR

A new sample of the quaternary ammonium compound, Terminator, was submitted to CRI by Agricultural Protection Systems for re-evaluation. The product was evaluated for fungicidal properties 2-3 years ago. However, an appropriate test kit for measuring product concentration was never available. Therefore the product was never recommended for use in citrus packhouses. However, a test kit has subsequently been produced and the product was re-submitted for evaluation.

Materials and methods

Spore suspensions of both these pathogens were made up by suspending spores, grown on Potato Dextrose agar plates, in sterile deionised water containing the surfactant Tween 20. The spore suspensions were then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 10 fruit each, per treatment, and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 (20 inoculations/treatment) on opposite sides to each other. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

Treatments

The inoculated fruit was treated with the following compounds at the given (recommended) concentrations.

Imazalil Sulphate	-	500 g/kg
Guazatine (Deccotine)	-	1000 g/l
Terminator	-	100 g/l ($\frac{1}{2}x$)
	-	200 g/l (x) recommended conc. a.i.
	-	400 g/l (2x)
	-	1000 g/l (extra treatment)

All the treatments were dipped in water at ambient temperature. The inoculated fruit was treated 4 hours after inoculation with the chemical compounds. Each treatment was immersed in the water bath for 3 minutes.

After treatment, the fruit was incubated in paper packets as well as plastic bags at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Evaluation for Phytotoxicity

The following evaluation was done for any phytotoxic reaction to the rind of the fruit. Valencia oranges were left standing in a 200 ppm a.i. solution of the product for certain periods of time. This was a cold water treatment simulating a citrus packhouse dumptank washing system. A second treatment in a hot water dip at 40°C, simulating a fungicide bath was also conducted.

Results

Table 4.6.3.5.10. The effect of Terminator on *P. digitatum*.

Treatments		% Decay
A	Untreated control	100.0
B	Treated control – 500 g/kg Imazalil Sulphate	0.0
C	Terminator – 100 g/l	100.0
D	Terminator – 200 g/l	100.0
E	Terminator – 400 g/l	100.0
F	Terminator – 1000 g/l	100.0

Table 4.6.3.5.11. The effect of Terminator on *G. candidum*.

Treatments		% Decay
A	Untreated control	90.0
B	Treated control – 1000 g/l Deccotine	0.0
C	Terminator – 100 g/l	100.0
D	Terminator – 200 g/l	100.0
E	Terminator – 400 g/l	100.0
F	Terminator – 1000 g/l	80.0

Table 4.6.3.5.12. Phytotoxic reaction on Valencia oranges by Terminator.

Terminator concentration (ppm a.i.)	Exposure time	Phytotoxicity
200 Cold water	2 hrs	Nil
	4 hrs	Slight
	6 hrs	Slight
	8 hrs	Severe
	12 hrs	Severe
	16 hrs	Severe
	24 hrs	Severe
200 Hot water	1 sec	Severe
	30 sec	Severe
	1 min	Severe
	2 min	Severe

Conclusions

As can be seen from the above results, Terminator did not demonstrate any fungicidal activity against either of the citrus pathogens. Terminator, however, has good sanitizing properties but cannot be recommended for use in a dumptank system at this stage as an appropriate test kit to measure the product concentration is not yet available. As soon as an appropriate test kit is available and has been approved, under laboratory conditions, a pilot trial will have to be conducted in a citrus packhouse dumptank system during production to monitor and evaluate the test kit under practical conditions.

Once the test kit is approved, two further requirements are necessary before the product can finally be recommended for use in a citrus packhouse dumptank system, i.e. fruit samples must be submitted for residue analyses and, secondly, it must be specified on the product label that fruit must not be left standing in a solution of the recommended concentration of the product for an extended period of time as this could lead to the “burning” of sensitive fruit.

N.B. Terminator is not recommended for use in a **hot water** fungicide bath.

4.6.4 The evaluation of three Carnauba (natural) citrus waxes, incorporating post-harvest fungicides, for efficacy against post-harvest infections

Three Carnauba (natural) citrus waxes from Sasol SA (Pty) Ltd. were forwarded to CRI for evaluation. The three waxes contained 2000 ppm imazalil, 10 000 ppm Tecto (thiabendazole) and a combination of 2000 ppm imazalil and 10 000 ppm Tecto respectively. The waxes were evaluated for efficacy of the fungicides in inhibiting the post-harvest citrus pathogens, *P. digitatum* (green mould) and the latent pathogens, *Diplodia* stem end rot, Anthracnose and *Alternaria*.

Materials and methods

A spore suspension, *P. digitatum* (green mould) was made up by suspending spores in sterile deionised water containing the surfactant or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Co.) and lemons (Larten) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomized. The fruit was then divided up into lots of 10 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 to each other, giving a total of 20 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette. The inoculated fruit was treated 4 hours after inoculation with the chemical plus wax compound being evaluated. Each treatment was immersed in the wax for 3 minutes.

Waxes

- A. Carnauba + 2 000 ppm imazalil.
- B. Carnauba + 10 000 ppm Tecto (thiabendazole or TBZ).
- C. Carnauba + 2000 ppm imazalil + 10 000 ppm Tecto.

Treatments

- 1. *P. digitatum* inoculated lemons dip treated in A.
- 2. *P. digitatum* inoculated lemons dip treated in B.
- 3. *P. digitatum* inoculated lemons dip treated in C.
- 4. Uninoculated Valencias dipped in B.
- 5. Uninoculated Valencias dipped in C.
- 6. Untreated controls.
- 7. Treated controls – 500 ppm imazalil SO₄ in water dip.
- 8. Treated controls – 4 000 ppm Tecto (TBZ) in wax.

After treatment, the treatments 1, 2, 3, 6 & 7 were incubated in paper packets at 20°C for 7 days or until such time as the controls had grown. These treatments were then evaluated and the results recorded as percentage decay.

Treatments 4, 5, 6 & 8 were incubated in paper packets under simulated shipping conditions at 4.5°C for 4 weeks and 1 week at ambient (20°C) and then evaluated for latent pathogen infections.

Results

Table 4.6.4.1. The effect of the fungicides imazalil and Tecto in wax treated fruit on *P. digitatum*.

Treatments	% Decay
LEMONS	
Untreated control	100
Standard – 500 ppm imazalil SO ₄	Nil
Wax Emulsion A	20
Wax Emulsion B	100
Wax Emulsion C	30

Table 4.6.4.2. The incidence of latent pathogen infection after simulated shipping storage of Valencia treated oranges.

Treatments	% Decay
VALENCIAS	
Untreated control	Nil
Standard – 4 000 ppm Tecto in wax	Nil
Wax Emulsion B	Nil
Wax Emulsion C	Nil

Observations

No phytotoxicity was evident in any of the above treatments. In Table 4.6.4.1 wax emulsions A & C on the treated lemons showed 20-30% decay respectively, indicating that the concentration of Imazalil in the wax at 2 000 ppm is too low. The standard recommendation is 3 000 ppm. Wax emulsion B on the treated lemons showed 100% decay. This indicates a TBZ resistant strain of *P. digitatum* as TBZ (Tecto) is no longer effective against the *Penicillium* organisms.

In Table 4.6.4.2 no latent pathogen growth was evident in all the treatments, including the untreated control.

Conclusions

This work will not be continued as the scope in the market for citrus waxes, incorporating post-harvest fungicides, is limited.

4.6.5 The spread of *Phytophthora* brown rot infection in stored cartons of healthy citrus fruit

The incidence of the spread of *Phytophthora* brown rot in packed cartons of healthy citrus fruit is the last part of the trials conducted to evaluate the role of Canon PH, a phosphonate submitted by the Israeli company Luxembourg Industries as a post-harvest control for *Phytophthora* brown rot. The phosphonates Fosetyl-Al (Aliette) and Phytex were used as standards to compare with Canon PH to treat cartons of sound fruit in determining the incidence of spread of infection in the cartons.

Materials and methods

Good, sound, untreated navel oranges (Crocodile Valley Citrus Co.) were obtained in bulk. Blemish free fruit was selected and randomized for the purpose of inoculation and incidence of spread of infection in stored cartons. All the fruit was washed in clean water and then surface sterilized by dipping in a 1% NaOCl (sodium hypochlorite) solution. The fruit was then allowed to dry. Thereafter, all the fruit to be used was dipped for 1 minute in a 500 ppm Imazalil sulphate solution for the purpose of preventing the development of any *Penicillium* secondary infections that could possibly mask the *Phytophthora* infected fruit before and after treatment with the phosphonates.

The 'inoculum' was prepared and the 'inoculation' was conducted in the same way as in the first part of this trial.

Treatments

Twenty-four x 15 kg (50 fruit each) cartons of navel oranges were packed, i.e. twelve cartons for each of two storage regimes. The treatments were done as follows:

6 cartons	-	4 000 ppm a.i. Canon PH
6 cartons	-	4 000 ppm a.i. Phytex
6 cartons	-	4 000 ppm a.i. Aliette
4 cartons	-	control (untreated + inoculated fruit)
2 cartons	-	control (untreated)

All the treatments were immersed in a dip treatment at ambient temperature for 3 minutes. Three *Phytophthora* inoculated navel oranges were placed into the middle of each of the 18 cartons treated with the phosphonates as well as in the 4 untreated control cartons. The three fruit were strategically placed amongst the middle layers of fruit in each carton.

Storage

The cartons of treated fruit plus controls were stored under simulated shipping conditions at cold storage (11°C), and ambient (20°C) as follows:

11°C	3 x 3 cartons each of Canon PH, Phytex and Aliette + infected fruit 2 cartons control – untreated + infected fruit 1 carton control – untreated
20°C	3 x 3 cartons each of Canon PH, Phytex and Aliette + infected fruit 2 cartons control – untreated + infected fruit 1 carton control – untreated

The storage period for the fruit shipped at 11°C was 1+3+1, i.e.

1 week at ambient
3 weeks at shipping (11°C)
1 week at ambient

The cartons stored at ambient (20°C) were stored for three weeks before being evaluated.

Results

After storage of the cartons under shipping conditions (11°C), the average percentage of infected fruit was determined for each treatment, i.e.

	Average % infected fruit
Control	23
Canon	15.3
Aliette	14
Phytex	16.7

The cartons stored at 20°C for 3 weeks could not be evaluated due to the high incidence of secondary infection (*Penicillium*).

Conclusion

The results recorded in this trial work where the phosphonate Canon PH, from Luxembourg Industries (Israel) was evaluated as a post-harvest dip treatment for the control of the post-harvest decay *Phytophthora* brown rot (*Phytophthora nicotianae*), demonstrated some degree of activity against this pathogen. Canon PH was compared with three other phosphonates which are applied pre-harvest under South African conditions for the control of *Phytophthora* brown rot. These phosphonates also showed some degree of activity against this pathogen in a post-harvest dip treatment.

The treatment of fruit post-harvest with Canon PH (and the other phosphonates), prior to packing, also showed some degree of spread of the *Phytophthora* infection in packed cartons stored under simulated shipping conditions.

Phytophthora brown rot is a very aggressive pathogen which normally spreads rapidly from infected fruit to sound fruit within packed cartons of fruit under ideal conditions. Given the nature of this pathogen and the distance of South African citrus fruit from the markets, and thus the longer storage period of the fruit, the results in these trials do not warrant the registration and use of Canon PH (and other phosphonates) as a post-harvest application on citrus fruit for the control of *Phytophthora* brown rot.

The pre-harvest application of the phosphonates for post-harvest control of this infection still remains the best option.

4.6.6 **The *in vivo* evaluation of the post-harvest fungicide, Prochloraz, from AgChem Africa, against the *Penicillium* organisms for the purpose of registration in a dip treatment**

Prochloraz, an imidazole (same as imazalil), is currently registered in citrus packhouses in a brush-on total loss application system. However, as most post-harvest fungicides are applied in a citrus packhouse in a hot water fungicide dip treatment, it was decided to conduct trials with Prochloraz in a hot water fungicide dip treatment for efficacy against the *Penicillium* pathogens for the purpose of registering Prochloraz for control of the *Penicillium* organisms in a dip treatment.

Prochloraz was evaluated here against the post-harvest citrus pathogen *Penicillium digitatum* (green mould).

Materials and methods

Spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant or wetting agent, Tween 20. The spore suspension was then adjusted to a concentration of 10^6 spores/ml spectrophotometrically.

Good, sound, untreated Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into lots of 20 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit was then 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 to each other. Each fruit was then infected with the pathogen by applying 35 μ l of spore suspension to each injury site using a micropipette.

All the treatments were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse.

The inoculated fruit was treated 4 hours and 16 hours after inoculation (to simulate a short and long delay after harvesting prior to packhouse treatment), with the chemical compound being evaluated. Each treatment was immersed in the fungicide solution for 3 minutes.

After treatment, the fruit was incubated in paper packets at 20°C for 7 days or until such time as the controls had grown. The treatments were then evaluated and the results recorded as percentage decay.

Results

Table 4.6.6.1. Efficacy of Prochloraz.

	Treatments	% Decay
	Valencias	
1	Untreated control	90
2	Standard – 500 ppm Imazalil SO ₄ – 4 hrs after infection	Nil
3	Standard – 500 ppm Imazalil SO ₄ – 16 hrs after infection	Nil
4	250 ppm Prochloraz (AgChem Africa) – 4 hrs after infection	30
5	250 ppm Prochloraz (AgChem Africa) – 16 hrs after infection	90
6	500 ppm Prochloraz (AgChem Africa) – 4 hrs after infection	30
7	500 ppm Prochloraz (AgChem Africa) – 16 hrs after infection	70
8	1000 ppm Prochloraz (AgChem Africa) – 4 hrs after infection	Nil
9	1000 ppm Prochloraz (AgChem Africa) – 16 hrs after infection	50
10	1500 ppm Prochloraz (AgChem Africa) – 4 hrs after infection	Nil
11	1500 ppm Prochloraz (AgChem Africa) – 16 hrs after infection	50

Conclusion

Good control of *P. digitatum* was achieved with Prochloraz at 1000 and 1500 ppm within 4 hours of treatment after infection. However, a delay in treatment after 16 hours demonstrated poor control of the pathogen. Previous trials with Prochloraz indicated that fruit had to be treated within 10-12 hours maximum, after harvesting, to achieve good control of the *Penicillium* pathogens. This aspect will have to be verified as well as more efficacy trials need to be conducted on navel oranges and lemons before the data is submitted, together with residue analyses, for registration purposes.

4.6.7 Screening of *Penicillium* spore samples for resistance to the post-harvest fungicide imazalil

Forty-five *Penicillium* spore samples were screened for resistance to the post-harvest fungicide imazalil. Samples from the following cultivars, packhouse localities and production areas were screened.

Cultivars and packhouse environment	Production areas
Packhouse, cold rooms and crates	Wellington, Western Cape
Clementines	Fort Beaufort, Eastern Cape
Marsh grapefruit	Hectorspruit
Navels	Nelspruit areas
Lemons	Karino area
Blood oranges	Karino area
Valencias	Nelspruit areas
Packhouse	Burgersfort

Materials and methods

Spore suspensions of all the spore samples were made up by suspending the spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were then adjusted to a concentration of 10⁶ spores/ml by means of a Bausch & Lomb spectrophotometer.

Blemish free, sound, untreated Valencia oranges were selected and randomized for inoculation. The fruit was then divided up into lots of 10 fruit per treatment and then all the fruit was washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few 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 to each other. Each fruit was then infected with the spores, being screened, by applying 35 µl of spore suspension to each injury site using a calibrated micropipette. The inoculated fruit was then treated 4 hours after inoculation with imazalil.

After treatment the fruit was incubated in paper packets at 20°C for 7 days or until such time as the untreated controls had shown fungal growth. The treatments were then evaluated and the results recorded as percentage decay.

All of the inoculated treatments (excluding controls) were treated with 500 mg/l imazalil sulphate.

Results and conclusion

All the samples screened were found to be sensitive to imazalil. This screening process is ongoing and will be continuously and randomly conducted.

4.6.8 Evaluation of prochloraz for the control of CBS Experiment 729 by G.C. Schutte (CRI)

Opsomming

Prochloraz se MRV is vasgestel op 10 dpm vir die EU wat ons die kans gee om prochloraz weer te gebruik vir die beheer van nie net na-oes patogene nie, maar ook sitrusswartvlek. Daarom is besluit om prochloraz 450 EC teen die huidige geregistreerde konsentrasie van 330 ml/100l water (1x of 1500 dpm) te evalueer op swartvlek geïnfecteerde Valencias en ook konsentrasies van 165 ml/100l water (0.5x) en 660 ml/100l water (2x) te toets. Honderd vrugte is in 'n swartvlek geïnfecteerde boord getrek vir elke behandeling en vir 30 sekondes, 1 en 3 minute gedoop waarna isolasies uit die vrugte gemaak is. Resultate toon dat nie een van die konsentrasies swartvlek 100% kon beheer nie. Die proef is drie keer herhaal en het elke keer dieselfde resultate opgelewer. Konsentrasies so hoog as 1320 ml/hl water kon ook nie swartvlek na-oes beheer nie.

Introduction

For any prospective post harvest fungicide to be effective it is essential to determine how soon after harvest it must be applied; what the influence of fruit temperature is during the delay between infection and fungicide application, and what concentration of fungicide and exposure time is needed for effective control. Prochloraz was introduced in 1977 as a broad-spectrum fungicide. It has a high level of eradicant activity together with a significant protectant action against many of the pathogens responsible for the complex of post-harvest diseases. It was reported that prochloraz, applied as an aqueous non-recovery spray, could not control the development of stem-end rots of citrus fruit caused by *Diplodia* stem-end rot and *Phomopsis* stem-end rot. However, it was effective against anthracnose in avocados, citrus and mango fruits. It was however not stated if prochloraz was tested against conidia or perithecia of the latter fungi. How prochloraz will perform against latent infections imbedded in the fruit rind, is also not known.

Materials and methods

CBS infected fruit was obtained from an old neglected Valencia orchard at Bayer's experimental farm at Hectorspruit. Only fruit with red actively growing CBS lesions were picked. At CRI in Nelspruit they were selected into four batches, each containing 50 fruit and treated with prochloraz 450SC at rates of 165 ml, 330 ml and 660 ml /100 l water for 30, 60 and 180 sekonds. Thereafter the fruit was kept at room temperature for 1 day and isolations were made from the red actively growing CBS lesions. Five lesions per fruit were isolated and placed onto PDA and incubated for 14 days at 25°C. The amount of lesion development was recorded and calculated as a percentage.

In a separate trial the prochloraz rate was increased to 440 and 1320 ml/hl water and the same treatment and evaluation procedures were followed as described above.

Results

According to Table 4.6.8.1, prochloraz tested at the different rates did have a dosage response and even resulted in eliminating CBS after 180 seconds (Replicate 1). Exposure time also had an influence as less CBS was isolated from this fruit. However, when the trial was duplicated a different picture emerged and the exposure time and rates tested did have an effect on latent CBS. For instance the lowest rate of 165ml/hl water resulted in a higher rate from which CBS was isolated if compared with the previous trial. The same tendency was observed when the trial was repeated for a third time. Here it was clear that the highest rate of 660ml/hl water exposed for 60 and 180 seconds also had an effect on the isolation rate of CBS, but again none of the treatments resulted in 100% control of CBS.

Where the prochloraz rates were increased to 440 and 1320 ml/hl water, CBS development still occurred and resulted in 8 and 5% colony development. Therefore, prochloraz could not be considered as an effective treatment at the maximum exposure time of 180 seconds.

Table 4.6.8.1. Efficacy of prochloraz 450 EC for the control of latent CBS on Valencia oranges after exposure to different rates and time intervals.

Prochloraz concentration (ml / hl water)	Replicate 1 ^x			Replicate 2 ^y			Replicate 3 ^z		
	% CBS lesion development after different exposure time (seconds)			% CBS lesion development after different exposure time (seconds)			% CBS lesion development after different exposure time (seconds)		
	30	60	180	30	60	180	30	60	180
165	28	20	4	20	10	30	26	40	34
330	20	8	4	13	13	10	30	20	14
660	23	12	0	20	26	13	23	14	9
Control	24			22			18		

^x Treated with prochloraz on 25 June 2003, isolated on 27 June 2003 and evaluated on 17 July 2003

^y Treated with prochloraz on 8 July 2003, isolated on 9 July 2003 and evaluated on 2 August 2003

^z Treated with prochloraz on 6 August 2003, isolated on 8 August 2003 and evaluated on 29 August 2003

Discussion

It is clear that prochloraz is not effective as a post-harvest dip treatment for CBS even if the rate is increased from the 1500 ppm registered rate to 6000 ppm.

Future research

No further trials are planned for CBS control in this regard.

4.6.9 Evaluation of a fumigation chamber system for the application of alternative post-harvest disease control gas such as chlorine dioxide Experiment 730 by GC Schutte (CRI)

Opsomming

Laboratoriumproewe met chloordioksied teen 1000 dpm het goeie beheer van groen skimmel, suurvrot, witluis, rooidopluis en vrugtevlug larwes gegee na 'n blootstellingstydperk van 1 uur. Twee palette Valencia is van Croc Valley verkry en met die bogenoemde swamme geïnkuleer en gerandomiseer in die palette geplaas om die effek van chloordioksied op hulle oorlewingsvermoë na blootstelling aan die gas, te kyk in 'n houer waaraan waaiers geïnstalleer is vir die effektiewe sirkulasie van gas soos chloordioksied. Aan die binnekant van die houer is spesiale vinne aangebring om lugvloei deur spesiaal ontwerpte kartonne te kanaliseer. Resultate toon dat die gas (waarvan die konsentrasie gemeet kon word) swamspoorontwikkeling vir tot een week gehibiteer het, waarna die vrugte onomkeerbaar verrot het.

Introduction

Diplodia rot and *Penicillium* blue and green mould occurs in all citrus producing regions of the world and is the most common and serious post-harvest disease of citrus. Green and blue moulds are similar in many respects and all types of citrus fruit are susceptible. Millions of spores of the fungi are produced on the surface of mainly mechanically damaged fruit and these spores are present in the field, packing area, storage room, transit containers and the market place. Sour rot is the most objectionable and unpleasant of all the citrus decays. Ripe or over mature fruit are more susceptible to this decay than green or immature fruit and sour rot is more serious during and after prolonged wet seasons. Fungal resistance against post-harvest fungicides is a serious problem worldwide and there is a need to search for alternatives.

Brown & Wardowski (1984) reported that during a survey of various commercial chlorine applications showed that in many cases, proper control of pH and chlorine concentration was not maintained for maximum biocidal activity and minimum corrosion of equipment. Studies with chlorine dioxide showed that stability of this material decreased as storage temperatures were increased. Inoculum of green mould and sour rot in a soak tank was reduced by maintaining 5-10 µg/ml of available chlorine dioxide in the tank. Decay control of d'Anjou pears was also effective with chlorine dioxide at 10 µg/ml as dip treatment (Spotts & Peters, 1980). How effective gas treatments with chlorine dioxide will perform against post harvest fungi of prominent fungal diseases such as green mould and sour rot, has not been determined and has to be tested and be effective against both fungi before it can be considered as post harvest treatment.

Materials and methods

A mini container, measuring 3 x 2.44 x 2.45 m which is air tight and re-inforced to withstand a vacuum, was stacked with two pallets of untreated Navel oranges consisting of 70 cartons each were obtained from Crocodile Valley Estates. All cartons were sub-divided into different treatments that were subjected to green mould and sour rot inoculations. For superficial fungal infections with green mould and sour rot, all the fruit in each of the 10 cartons was wounded at two infection points which is equidistant from each other. A sterile rubber plug with nails will be dipped in either green mould or sour rot suspensions consisting of conidia (1×10^6 conidia/ml water). Three special gauges, to measure the chlorine dioxide levels in different parts of the container, were installed.

After the chlorine dioxide gas treatment at 500 and 1500 ppm for 1 hour, the container was opened. The pallets were removed and the boxes containing the fungal inoculated fruit were separated from the non-inoculated ones and stored at 25°C to promote fungal development.



Figure 4.6.9.1. Chlorine dioxide generator (left) with hoses through which the gas was pumped into the circulation system (middle right). The gauges used for measuring the chlorine dioxide levels can be seen here at the bottom right.

Results and discussion

Although the small scale lab trials killed all the fungi at 1000 ppm for 1 hour, the large scale trial gave initial control of the fungi as no sporulation occurred on the inoculated fruit for about 7 days after which the fruit was totally infected.

Conclusion

With all the right equipment at hand and probes to determine the actual gas concentrations before, within and behind the treated pallets, we still failed in controlling green mould and sour rot with chlorine dioxide at rates of 500 and 1500 ppm.

Future research

Other means of controlling post harvest diseases with gasses do exist. For instance, peroxyacetic acid, which is a strong oxidizer formed from hydrogen peroxide and acetic acid, showed activity against bacterial fruit blotch and other seedborne diseases of watermelon in the USA (Hopkins, Thompson, Hilgen & Lovic, 2003). The concentrated product (40% PAA) has a pungent odor and is very soluble in water with very little off-gassing and it leaves no toxic breakdown products or residue on the produce. Unlike chlorine and ozone, it is stable in water containing organic matter, which can greatly increase the longevity of the sanitizer, and it is not particularly corrosive to equipment. PAA is most active in acidic environments (pH 3.5 to 7). Activity declines rapidly at pH's above 7-8. High temperatures and metallic ion contamination also reduce its activity. We hope to obtain some of the chemicals for future research.

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5 PROGRAMME: CROP LOAD AND FRUIT QUALITY MANAGEMENT

5.1 PROGRAMME SUMMARY

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

With increasing competition on world-wide citrus markets, only top quality fruit fetches good prices. Assuming that the correct cultivar is being grown in the correct climate and that pests and diseases have been controlled, all other determinant factors of quality are horticultural. Research in 2003, was conducted on various aspects of improving internal and external pre-harvest quality as well as post-harvest rind condition research. Although open hydroponic irrigation systems are known to produce rapid tree growth and high yields, internal fruit quality is not always ideal and continues to receive research attention. As our early fruit arrives on the European market soon after well-coloured Mediterranean fruit, colour is an important quality factor. Various pre- and post-harvest methods of improving fruit colour have therefore been under investigation. Other pre-harvest quality investigations have included products to lower fruit acidity and a means of producing elongated lemons. Rind breakdown of Clementines has cost the citrus industry many millions of Rand over the last few years. Research by P. van Rensburg has resulted in recommendations to reduce this condition and P. Cronjé is now undertaking further research on the post-harvest causes of this problem. Other rind condition problems also received attention in 2003 and will continue to do so.

PROGRAMOPSOMMING

Deur Tim G. Grout (Navorsing & Tegnieuse Bestuurder: CRI)

Met toenemende mededinging op sitrusmarkte wêreldwyd, word goeie pryse slegs deur top-kwaliteit vrugte behaal. Met die veronderstelling dat die regte kultivar in die regte klimaat aangeplant word en dat peste en plaë onder beheer is, is alle ander faktore wat gehalte bepaal, tuinboukundig van aard. Navorsing is in 2003 onderneem op verskeie aspekte van toepassing op die verbetering van interne en eksterne voor-oes kwaliteit, asook na-oes skilgehalte. Alhoewel oop hidroponiese besproeiingsisteme bekend is om vinige boomgroei en hoë opbrengste te bevorder, is interne vruggehalte nie altyd na wense nie en navorsing in hiedie verband word voortgesit. Aangesien ons vroeë vrugte die Europese markte bereik kort ná goedgekleurde Mediterreense vrugte, is kleur 'n belangrike kwaliteitsfaktor. Verskeie voor- en na-oesmetodes om vrugkleur te verbeter is derhalwe ondersoek. Ander voor-oes kwaliteitsondersoeke het produkte om vrugsuurheid te verlaag en 'n metode om langer suurlemoene te produseer, ingesluit. Skilneestoring by Clementines het die sitrusbedryf miljoene Rand gekos die afgelope paar jaar. Navorsing deur P van Rensburg het gelei tot aanbevelings om hierdie toestand te verlig en P van Rensburg onderneem tans verdere navorsing oor die na-oes oorsake van die probleem. Ander probleme met skilgehalte het ook aandag geniet in 2003 en die werk sal voortgesit word in die toekoms.

5.2 PROJECT: FRUIT QUALITY ENHANCEMENT

Project Co-ordinator: Graham H. Barry (CRI at Stellenbosch University)

5.2.1 Project summary

The requirement of meeting minimum quality specifications and being able to successfully market citrus fruit products has now been superseded by the necessity to produce a product of superior quality, in terms of appearance and eating quality. When the supply of citrus products exceeds demand, product differentiation becomes increasingly important to ensure that sales rates are maintained. The goal of Citrus Research International's Fruit Quality Enhancement programme is to provide Southern African citrus producers with cultural practices that assist in producing superior product quality.

A single treatment of 1% MAP applied to Delta Valencia orange 6 weeks after full bloom resulted in lower fruit acidity than fruit from untreated, control trees. However, acidity was intermediate between that of fruit from the control and calcium arsenate-treated trees. MAP did not reduce acidity in Star Ruby grapefruit (5.2.2).

Premium prices are obtained in the Japanese market for elongated lemons with a length-to-diameter ratio (L:D) exceeding 1.25:1. Increasing the proportion of a lemon crop with elongated fruit will potentially improve grower returns. Goosen (2002) conducted a thorough study on the factors affecting fruit shape in lemon, from which the role of gibberellins was highlighted. In the apple industry, gibberellins are used to produce elongated fruit. Initial results using GAs on lemons resulted in variable results, and Goosen (2002) recommended that earlier application timings be attempted to increase L:D in lemon. Various GA sources (ProGibb®, Promalin®, Provide®, Perlant®, Falgro®) and a gibberellin biosynthesis inhibitor (Regalis®) were applied to Eureka lemon in the Gt. Drakenstein area of the Western Cape during flower differentiation (19

Jun 2003) and 6 weeks after full bloom (mid-Nov. 2003). Fruit will be harvested in April/May 2004 to determine whether any of these treatments produced more elongated fruit (5.2.3).

Little research has been conducted thus far on the effect of exogenously applied auxins on citrus rind colour. Experiments were done where Nules Clementine mandarin were treated with Maxim® and Corasil.E®, and Marisol Clementine and Miho Wase Satsuma mandarins were treated with Maxim and Citrimax®. Auxin treatment during stage I of fruit development did not consistently improve rind colour of Clementine and Satsuma mandarins. At maturity, 2,4-DP (Corasil.E or Citrimax) had a better effect on rind colour than 3,5,6-TPA (Maxim). However, the measured improvement in rind colour was not visible to the naked eye (5.2.4).

Rind colour development of citrus is a problem in South Africa, especially for early maturing cultivars, because autumn temperatures are too high for chlorophyll breakdown and carotenoid synthesis. Citrus fruit require night temperatures of <math><13^{\circ}\text{C}</math> for optimal colour development. It is unlikely that cool night temperature *per se* is the cause chlorophyll breakdown and carotenoid synthesis. Rather, the cool night temperature probably plays a direct role in halting vegetative growth. The latter is antagonistic to chloroplast conversion to chromoplasts, thereby delaying chlorophyll breakdown and carotenoid synthesis. Therefore, techniques to reduce vegetative vigour during late summer and early autumn may play a role in enhancing rind colour development. Endogenous gibberellins promote vegetative growth in plants. Gibberellin biosynthesis inhibitors effectively reduce vegetative growth and, in turn, may promote the conversion of chloroplasts to chromoplasts. Previous exploratory research (2002) on Navelina Navel orange trees treated with prohexadione calcium (Ph-Ca), a new gibberellin biosynthesis inhibitor with less persistence than triazoles, had fruit that were better coloured than the control by more than half a colour plate. However, subsequent treatment of Palmer Navel orange with Ph-Ca did not significantly improve rind colour, although there was a trend towards enhanced colour. The objective of this research was to determine the dose response of citrus trees to Ph-Ca. At the time of writing this research progress report data had not been collected. Fruit will be sampled from April to June 2004 and reported on in the 2004 report (5.2.5).

Various simulated shipping/holding regimes were applied to initially "orange" and "yellow" Palmer Navel orange fruit to study the effects of sub-zero temperature shipping and elevated holding temperature on final rind colour. Rind colour at shipping is the most critical factor affecting colour on arrival. Holding temperature plays an important role in managing rind colour after shipping (5.2.6).

The effects of tree nutrition and post-harvest treatments on concentration of the most important colour imparting carotenoids in physiologically mature citrus fruit forms the basis of the PhD research of Molipa Mosoeunyane at UNP. A "carotenoid library" has been prepared to identify certain pigment changes in citrus during fruit development. Molipa Mosoeunyane is also preparing a literature review on carotenoids in citrus and subtropical fruit, with particular interest in the type of carotenoids present in these crops, to possibly be able to identify easier means of, firstly, extraction and/or purification of the carotenoid pigments, and, secondly, to search for further indications of micronutrient involvement in triggering carotenoid biosynthesis. Furthermore, information is sought on addressing the question if a general increase in fruit carotenoid concentration is paralleled by an increase in the colour-imparting carotenoids (5.2.7).

Projekopsomming

Die behoefte om aan die minimum verlange kwaliteit vereistes te voldoen om suksesvol sitrus produkte te bemark word nou vervang deur die behoefte om produkte van superieure kwaliteit, met die klem op voorkoms en eetbaarheid te produseer. Sodra die aanbod van sitrus die aanvraag oortref word produk differensiasie al meer belangrik om gewaarborgde verkope te handhaaf. Die vrugkwaliteit verbeterings program van die CRI staan te doel om die Suider Afrikaanse sitrus produsent met verbouings praktyke te voorsien wat kan dien as hulpmiddel in die produksie van superieure kwaliteit produkte.

Een toediening van 1% MAP op Delta Valencia lemoenbome 6 weke na volblom het 'n laer vrug suur inhoud tot gevolg in vergelyking met die kontrole. Maar die vrug suur waardes van MAP was geleë tussen die kontrole en die kalsium arsenaat (hoogste waarde). MAP het nie enige effek op die suur inhoud van Star Ruby nie (5.2.2).

'n Premie word in die Japanse mark betaal vir suurlimoene met 'n lengte-deursnee verhouding (L:D) van 1.25:1 en meer. Om dus dië proporsie van 'n produsent se oes te verhoog kan dus meer winsgewend wees. 'n Deeglike studie van faktore wat die vrugvorm beïnvloed is deur Goosen (2002) gedoen waarin sy die rol van gibberellien beklemtoon. Gibberellien word in die appelbedryf gebruik om vrugverlenging te bewerkstellig. Die aanvanklike aanwending van gibberelline het wisselvallige resultate tot gevolg gehad en Goosen (2002) beveel aan dat toedienings op 'n vroeër stadium gedoen word om die L:D verhouding te verhoog. Die gibberellien behandeling is in die vorm van 'n verskeidenheid produkte (ProGibb®, Promalin®,

Provide®, Perlan®, Falgro®) asook 'n gibberellien biosintese inhibeerder (Regalis®) toegedien op Eureka suurlemoene Gt. Drakenstein area van die Wes Kaap gedurende blom differensiasie (19 Jun 2003) en 6 weke na volblom (mid Nov. 2003). Vrugte sal geoes word gedurende April/Mei 2004 waarna die effektiwiteit van die behandelings bepaal sal word (5.2.3).

Baie min navorsing is al gedoen om die effek van ouksien toedienings op sitrus skilkleur te bepaal. Eksperimente is gedoen waartydens Nules Clementine mandaryn behandel is met Maxim® en Corasil.E® en Marisol Clementine en Miho Wase Satsuma mandaryne behandel is met Maxim® en Citrimax®. Ouksien toedienings gedurende fase I van vrugontwikkeling het nie konsekwent skilkleur verbeter van die Clementine en Satsuma mandaryne nie. Tydens plukrypheid het 2,4-DP (Corasil.E® en Citrimax®) 'n beter effek as 3,5,6-TPA (Maxim®) op skilkleur gehad, maar die verbeterde kleur metings was nogtans nie sigbaar met die blote oog nie (5.2.4).

Skilkleur ontwikkeling van sitrus in Suider Afrika is veral 'n probleem in die vroeër kultivars a.g.v. die herfs temperature wat te hoog is vir chlorofil afbraak en karoteen sintese. Vir optimale kleur ontwikkeling van sitrus word 'n nagtemperatuur van <13°C verlang. Dit is heel moontlik nie die koue nag temperature *per se* wat vir die kleurontwikkeling verantwoordelik is nie. Dit blyk egter of die lae nag temperature vegetatiewe ontwikkeling stop. Laasgenoemde het 'n bekende antagonistiese uitwerking op chloroplast omskakeling na chromoplast en sodoende word chlorofil afbraak en karoteen sintese vertraag. Om die rede kan maatstawwe wat die oordadige vegetatiewe groei gedurende die laat somer en vroeg herfs beperk, 'n betekenisvolle rol speel in pogings om skilkleur te verbeter. Interne gibberellin bevorder vegetatiewe groei in plante en om daardie rede kan 'n gibberellin biosintese inhibeerder die omskakeling van chloroplast na chromoplast bevorder. Gedurende 2002 is daar voeler proewe met prohexadione kalsium (Ph-Ca) op Navelina Navel lemoen bome gedoen. Ph-Ca is 'n nuwe produk wat gibberellin biosintese inhibeer en het korter nawerkings effek as die triazole en die toediening daarvan het die vrugkleur verbeter met 'n halwe kleurkaart. Opvolg behandeling van Ph-Ca op Palmer Navel lemoenbome het egter in geen betekenisvolle verbetering van kleur tot gevolg gehad nie alhoewel daar aan die einde wel 'n neiging na kleur verbetering was. Die doel met die navorsing was om te bepaal wat die respons van die bome sal wees op verskillende dosisse Ph-Ca. Die data sal egter eers in 2004 beskikbaar wees a.g.v. vrugte wat vanaf April-Junie 2004 geoes word (5.2.5).

“Oranje” en “geel” Palmer Navel lemoene is aan 'n verskeidenheid gesimuleerde verskeppings/opbergings regimes onderwerp om die effek van sub-zero temperatuur verskeping en daaropvolgende verhoogde opbergings temperature, op finale skil kleur te ondersoek. Skil kleur by verskeping is as 'n uiters krities faktor geïdentifiseer. Opberg temperature speel 'n rol in die handhawing van skil kleur na verskeping (5.2.6).

Die effek van boom voeding en naoes behandeling op die konsentrasie van die mees belangrikste kleur bydraende karotene in fisiologiese volwasse sitrus vrugte, vorm die basis van Molipa Mosoeunyane se PhD navorsing by UNP. 'n “Karoteen biblioteek” is voorberei om sekere pigment veranderinge te identifiseer gedurende sitrus vrug ontwikkeling. Molipa Mosoeunyane berei 'n literatuur oorsig van karotene in sitrus en subtropiese vrugte voor en gee besondere aandag aan die tipe karotene teenwoordig in die gewasse om sodoende makliker maniere te vind om eerstens, ekstraksie en/of suiwering van die karoteen pigmente te doen en tweedens om inligting te vind wat 'n indikasie van mikro voedings elemente se betrokkenheid in die aanskakeling van karoteen biosintese kan gee. Meer informasie word ook gesoek op die vraag of 'n algemene verhoging in vrug karoteen konsentrasie hand aan hand geskied met verhoging in kleur bydrae (5.2.7).

5.2.2 Reduction of acidity of high acid citrus cultivars using alternatives to calcium arsenate Experiment ACID 01/02 by Graham H. Barry (CRI at Stellenbosch University)

Opsomming

Een toediening van 1% MAP op Delta Valencia lemoenbome 6 weke na volblom het 'n laer vrug suur inhoud tot gevolg in vergelyking met die kontrole. Maar die vrug suur waardes van MAP was geleë tussen die kontrole en die kalsium arsenaat (hoogste waarde). MAP het nie enige effek op die suur inhoud van Star Ruby nie.

Introduction

Following promising results in the 2002 season (see 2002 CRI Annual Research Report), monoammonium phosphate (MAP) and monopotassium phosphate (MKP) were further tested to determine guidelines for their use as alternatives to calcium arsenate to reduce acidity.

Materials and methods

Plant material and treatments. A Delta Valencia orange orchard at Paardekop, Citrusdal was used in this study. The trees used were selected for uniformity in tree size and health.

Treatments were applied 6 WAFB on 20 November 2002 when fruit diameter was approximately 16 mm. A randomized complete block design with eight single-tree replicates was used. Treatments included commercial treatment of calcium arsenate applied on 20 Nov. 2002, 1% MKP, 1% MAP and an untreated control. A medium-cover spray was used to apply spray material until just before run-off. On average, 7.5 L of spray material was applied per tree.

In a semi-commercial experiment, 1% MAP was applied in November 2002 to Star Ruby grapefruit in the Malelane grapefruit production region.

Data collection and statistical analysis. Fruit acidity of six replicates was determined every 6 weeks from 5 Mar. 2003 until maturity to map seasonal changes in acidity. Ten fruit of similar size were sampled from the east side of trees. At maturity on 26 Sept. 2003, samples of 12 fruit per replicate were taken for juice quality analysis. Juice content, Brix (by refractometer), titratable acidity (TA) and ratio were determined using standard procedures. Data were analysed using SAS.

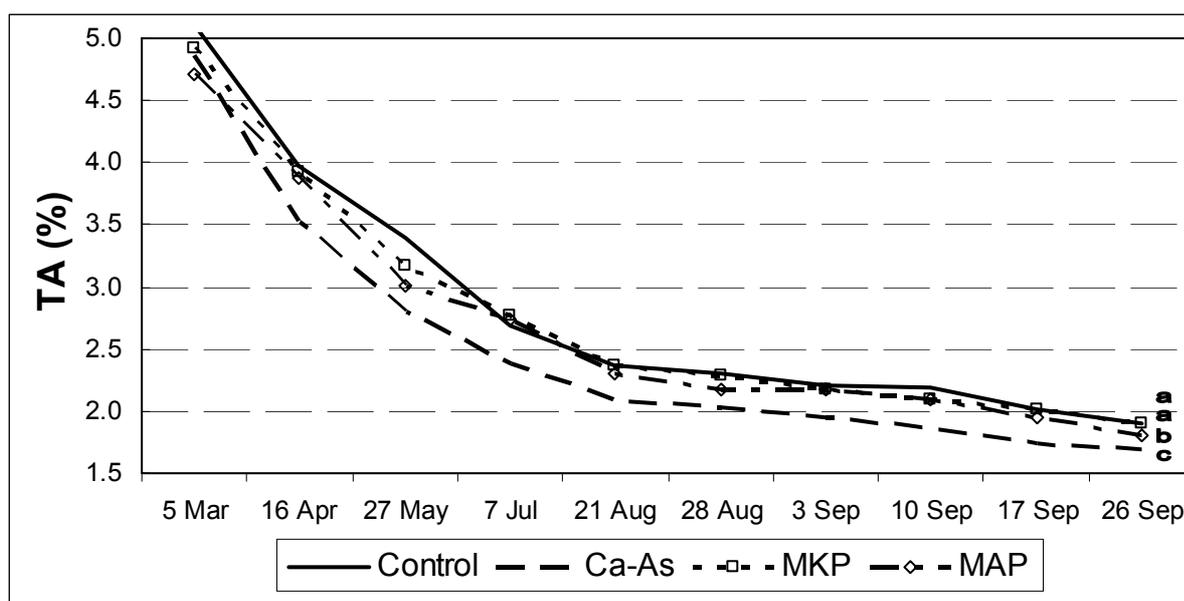
Fruit samples from the Star Ruby grapefruit semi-commercial experiment were taken at three-weekly intervals from 25 Mar. 2003 until maturity on 26 May 2003. Internal fruit quality of the fruit was tested.

Results and discussion

In this progress report, only the acidity data are presented (Figs. 5.2.2.1 and 5.2.2.2). A single treatment of 1% MAP applied to Delta Valencia orange 6 WAFB resulted in significantly lower fruit acidity than fruit from untreated, control trees. However, MAP is less effective than calcium arsenate in reducing acidity, and acidity of fruit from the MAP-treated trees was intermediate between that of fruit from the control and calcium arsenate-treated trees. MKP was less effective than MAP and did not consistently reduce acidity over time. The poor response of MKP is similar to what was observed during the previous season.

MAP did not reduce acidity in Star Ruby grapefruit (Fig. 5.2.2.2).

These data support last season's data showing that the application of 1% MAP 6 WAFB (± 20 Nov. in the Western Cape) consistently lowers acidity. However, 1% MAP is less effective than calcium arsenate in reducing acidity. Therefore, a higher application rate, i.e. up to 5% MAP, should be tested to ascertain whether a greater effect can be achieved.



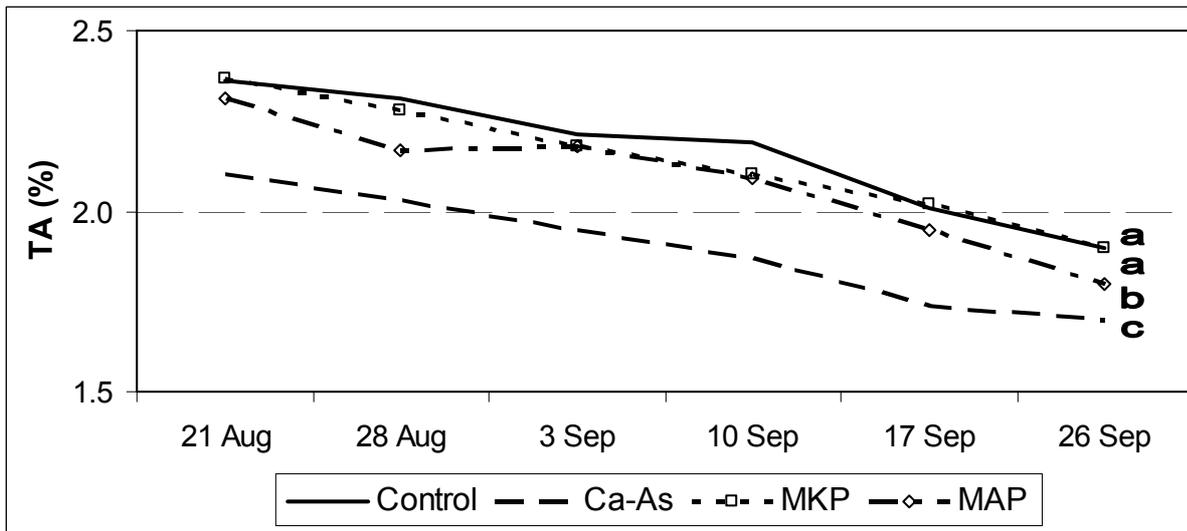


Figure 5.2.2.1. Effect of MKP and MAP on fruit acidity of Delta Valencia orange in comparison with calcium arsenate. Treatments were applied on 20 November 2002 and fruit were sampled six-weekly from early March to demonstrate changes in acidity over time and to overcome issues of sampling error. The lower graph shows only the last six sampling times to decrease the Y-axis scale and thereby allow the reader to more clearly see the MAP and MKP data points. (Means followed by different letters differed at $P=0.1$); $n=6$, except on 26 Sept. $n=8$).

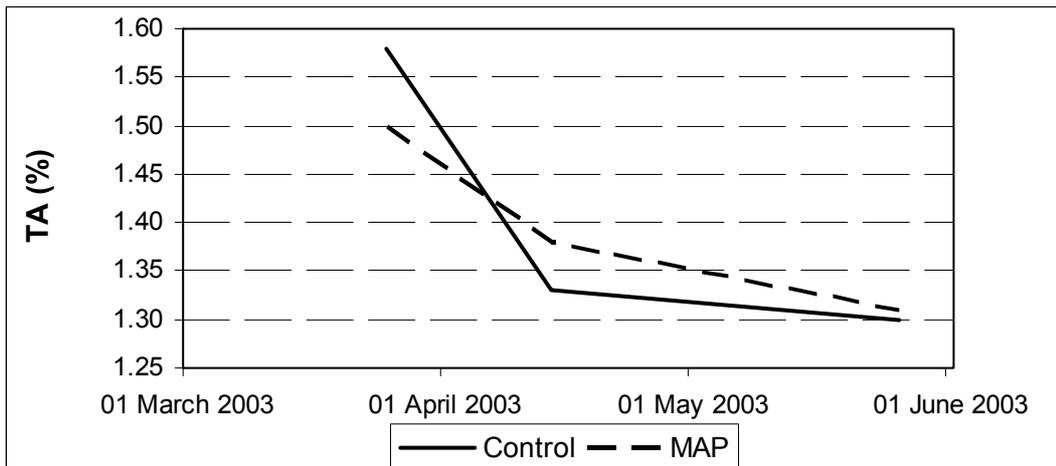


Figure 5.2.2.2. Effect of MAP on fruit acidity of Star Ruby grapefruit. Treatments were applied in November 2002 and fruit were sampled three-weekly from 25 March to 26 May 2003 to demonstrate changes in acidity over time and to overcome issues of sampling error ($n=5$).

5.2.3 Lemon fruit shape

Experiment SHAPE 01/03 by Graham H. Barry (CRI at Stellenbosch University)

Opsomming

'n Premie word in die Japanse mark betaal vir suurlemoene met 'n lengte-deursnee verhouding (L:D) van 1.25:1 en meer. Om dus dië proporsie van 'n produsent se oes te verhoog kan dus meer winsgewend wees. 'n Deeglike studie van faktore wat die vrugvorm beïnvloed is deur Goosen (2002) gedoen waarin sy die rol van gibberellie beklemtoon. Gibberellie word in die appelbedryf gebruik om vrugverlenging te bewerkstellig. Die aanvanklike aanwending van gibberellie het wisselvallige resultate tot gevolg gehad en Goosen (2002) beveel aan dat toedienings op 'n vroeër stadium gedoen word om die L:D verhouding te verhoog. Die gibberellie behandeling is in die vorm van 'n verskeidenheid produkte (ProGibb®, Promalin®, Provide®, Perlan®, Falgro®) asook 'n gibberellie biosintese inhibeerder (Regalis®) toegedien op Eureka suurlemoene Gt. Drakenstein area van die Wes Kaap gedurende blom differensiasie (19 Jun 2003) en 6

weke na volblom (mid Nov. 2003). Vrugte sal geoes word gedurende April/Mei 2004 waarna die effektiwiteit van die behandelings bepaal sal word.

5.2.4 Role of auxins in rind colour enhancement

Experiment COL 01/03 by Graham H. Barry (CRI at Stellenbosch University)

Opsomming

Baie min navorsing is al gedoen om die effek van ouksien toedienings op sitrus skilkleur te bepaal. Eksperimente is gedoen waartydens Nules Clementine mandaryn behandel is met Maxim® en Corasil.E® en Marisol Clementine en Miho Wase Satsuma mandaryne behandel is met Maxim® en Citrimax®. Ouksien toedienings gedurende fase I van vrugontwikkeling het nie konsekwent skilkleur verbeter van die Clementine en Satsuma mandaryne nie. Tydens plukrypheid het 2,4-DP (Corasil.E® en Citrimax®) 'n beter effek as 3,5,6-TPA (Maxim®) op skilkleur gehad, maar die verbeterde kleur metings was nogtans nie sigbaar met die blote oog nie.

Introduction

Ethylene plays a central role in chloroplast degradation and subsequent chromoplast biosynthesis, and, as such, is an important factor influencing rind colouration in citrus. Endogenous ethylene production is induced by exogenously applied auxins (Abeles and Rubinstein, 1964). Some physiological processes have similar responses to auxins and ethylene application, e.g. senescence. Thus, there appears to be an inter-play between auxins and ethylene where maturation and senescence are concerned.

Previously it was reported that auxins had no direct effect on citrus rind colour enhancement (Coggins and Hield, 1968; El-Zeftawi, 1978). However, Hirose et al. (1978) and Kamuro and Hirai (1981) found that an auxin, called ethychlozate (commercially known as Figaron® in Japan), originally tested for fruit thinning, had a positive effect on rind colour if applied later in the season. Ethychlozate accelerated degreening of Satsuma mandarin fruit, and increased the colour index of the rind (Tominaga and Daito, 1981). Ethychlozate shows different activities to NAA and 2,4-D in that it induces ethylene generation that occurs more slowly and endures longer. Iwahori et al. (1986) found that ethychlozate increased colouration of Ponkan mandarin fruit. This response allowed fruit to be harvested 7 to 10 days earlier than untreated fruit.

The mechanism by which ethychlozate affects fruit senescence is not yet fully understood. Unlike other auxins, ethychlozate is transported to the roots where it is metabolised (Kamuro and Hirai, 1981). The metabolites increase the uptake of water and minerals, and consequently enhance fruit quality (Kamuro and Hirai, 1981). However, Manago and Hirobe (1984) showed that water absorption and the absorption of N, P, Ca and Mg decreased for 1 to 3 weeks after ethychlozate application. They suggested that this causes a "dry effect", leading to higher sugar content in fruit. This response would cause ethylene production, which, in turn, would enhance senescence.

The objective of this experiment was to investigate the effect of exogenously applied auxins on citrus rind colouration.

Materials and methods

Plant material and treatments. In 2002, Nules Clementine mandarin from Boontjiesrivier, Citrusdal (32°42'S;19°03'E, elev. 150m) were treated with Corasil.E (100 ppm) and Maxim (10 ppm).

In 2003, Marisol Clementine and Miho Wase Satsuma mandarins from Môreilig, Wemmershoek (33°51'S;19°02'E, elev. 200m) were treated with Citrimax (100 ppm) and Maxim (10 ppm).

Data collection and statistical analysis. In 2002, the experiment was laid out as a randomized complete block design with 10 single-tree replicates. Thirty fruit were randomly sampled from the fruit harvested from all the trees per treatment. On 20 May 2002, after the fruit had been sampled, an area was marked on each fruit and rind colour was measured within the marked area with a colorimeter. Lightness, hue angle and chroma were measured. The fruit were then rated according to a colour chart. Measurements and ratings were repeated in the marked areas on the fruit on 20 Jun. and 9 Jul. 2002. During this period, fruit were stored at 22°C.

In 2003, the experiments were also laid out as randomized complete block designs with 10 single-tree replicates. Fifty Miho Wase Satsuma mandarin fruit were randomly sampled from the fruit harvested from all of the trees per treatment. Another 50 fruit were randomly sampled from fruit harvested from all of the trees

per treatment, after the fruit had been waxed. The fruit were then marked and their colour measured on 31 Mar. and 14 Apr. 2003. The fruit were stored at 22°C during this time. One hundred Marisol Clementine mandarin fruit were randomly sampled from the fruit harvested from all of the trees per treatment. Fruit were then marked and measured on 2 May and 16 May 2003, and were stored at 22°C during this time.

Results and discussion

Nules Clementine mandarin. There was no significant difference between fruit hue angles as measured on 20 May 2002 (Table 5.2.4.1). However, measurements taken on 20 Jun. 2002 showed a significant decrease in hue angle (better orangeness) in both Corasil- and Maxim-treated fruit. By 9 Jul. 2002, only Corasil-treated fruit had better colour than control fruit (Table 5.2.4.1).

Maxim-treated fruit had a higher colour rating (thus poorer colour) than control and Corasil-treated fruit on 20 May 2002, but by 20 Jun. 2002, both Maxim- and Corasil-treated fruit showed better colour than untreated fruit (Table 5.2.4.2). However, ratings on 9 Jul. showed no significant difference in colour rating between auxin-treated and untreated fruit.

Miho Wase Satsuma mandarin. There was a significant interaction between the auxin treatment and waxing for hue angle values (Table 5.2.4.3). Maxim had higher hue angles for waxed fruit than for unwaxed fruit where the opposite was true for Citrimax and the control treatments (Figure 5.2.4.1). Thus, waxed fruit treated with Maxim were less orange than unwaxed fruit. This was true before and after storage, but the interaction was stronger before storage (Table 5.2.4.3).

There was no difference in the brightness (chroma) between the treatments and control. Citrimax resulted in lower hue angles, and thus better orangeness, before and after storage, but as shown before, there was an interaction between the treatment and whether fruit were waxed for this variable. There was no significant difference in lightness between treated and non-treated fruit.

The 2-week storage period had no effect on the differences in brightness (or chroma) and little effect on the differences in lightness of the fruit. However, hue angle of the Citrimax-treated fruit was significantly lower than that of the control or Maxim-treated fruit, i.e. more orange.

Measurements taken soon after wax treatment showed a decrease in hue angle (more orange) and in lightness, but had no effect on chroma (brightness), compared to unwaxed fruit. After 2 weeks storage, however, there was a significant decrease in chroma (brightness) of the waxed fruit, where there was no difference in hue angle and lightness of the waxed compared to the unwaxed fruit.

Marisol Clementine mandarin. The auxins (Citrimax and Maxim) resulted in more orange rind colour than the untreated control as the hue angle of the treated fruit was significantly higher than that of control fruit (Table 5.2.4.4). Untreated fruit were also found to be paler or lighter than the treated fruit, as shown by the higher L values. However, untreated fruit had a brighter colour than the treated fruit as indicated by the higher chroma values (Table 5.2.4.4). Maxim tended to have a better effect on colour enhancement than Citrimax.

After 2 weeks storage, hue angle and lightness decreased slightly whereas chroma increased slightly. This indicates that rind colour improved in terms of orangeness, lightness (i.e. became darker) and brightness, respectively.

Table 5.2.4.1. Means and probability values of hue angle measurements of Nules Clementine mandarin on 20 May, 20 Jun. and 9 Jul. 2002.

Treatment	20 May	20 Jun	9 Jul
Control	72.9 NS	74.4 a	66.9 a
Corasil	71.8	70.8 b	65.1 b
Maxim	72.9	72.0 b	66.1 ab
P-value	0.2976	< 0.0001	0.03
LSD	1.61	1.31	1.34

Table 5.2.4.2. Means and probability values of colour rating of Nules Clementine mandarin on 20 May, 20 Jun. and 9 Jul. 2002.

Treatment	20 May	20 Jun	9 Jul
Control	2.0 b	1.5 a	1.05 NS
Corasil	2.0 b	1.2 b	1.02
Maxim	2.3 a	1.2 b	1.02
P-values	0.0009	< 0.0001	0.4358
LSD	0.2	0.15	0.06

Table 5.2.4.3. Means and probability values of chroma (C), hue angle (H) and lightness (L) of auxin-treated waxed and unwaxed Miho Wase Satsuma mandarin on 31 Mar. and 14 Apr. 2003.

Treatment x Wax	31 Mar			14 Apr		
	C	H	L	C	H	L
P-value	0.0829	< 0.0001	0.5822	0.4189	0.0842	0.6008
Treatment						
Control	68.3 NS	81.6 a	72.1 a	67.6 NS	72.1 b	67.7 ab
Citrimax	67.2	79.2 b	71.2 b	67.2	70.5 c	67.3 b
Maxim	67.7	81.5 a	71.7 ab	67.9	73.4 a	69.1 a
P-value	0.1963	< 0.0001	0.1076	0.3333	< 0.0001	0.0324
LSD (5%)	1.17	1.11	0.86	0.91	1.12	1.41
Waxed						
No	67.9 NS	81.5 a	72.9 a	68.3 a	72.1 NS	68.1 NS
Yes	67.6	80.1 b	70.4 b	66.9 b	72.0	68.0
P-value	0.6291	0.0026	< 0.0001	0.0004	0.9071	0.8756
LSD (5%)	0.95	0.91	0.71	0.74	0.91	1.15

Table 5.2.4.4. Means and probability values of chroma (C), hue angle (H) and lightness (L) of auxin-treated Marisol Clementine mandarin on 2 and 16 May 2003.

Treatment	2 May			16 May		
	C	H	L	C	H	L
Control	60.4 a	65.1 a	63.2 a	60.7 a	61.7 a	61.4 a
Citrimax	59.5 b	61.7 b	60.9 b	60.6 a	56.4 b	58.6 b
Maxim	57.8 c	60.6 b	58.5 c	59.4 b	56.1 b	58.1 b
P-value	< 0.0001	< 0.0001	< 0.0001	0.0031	< 0.0001	< 0.0001
LSD (5%)	0.80	1.34	0.80	0.81	1.63	0.83

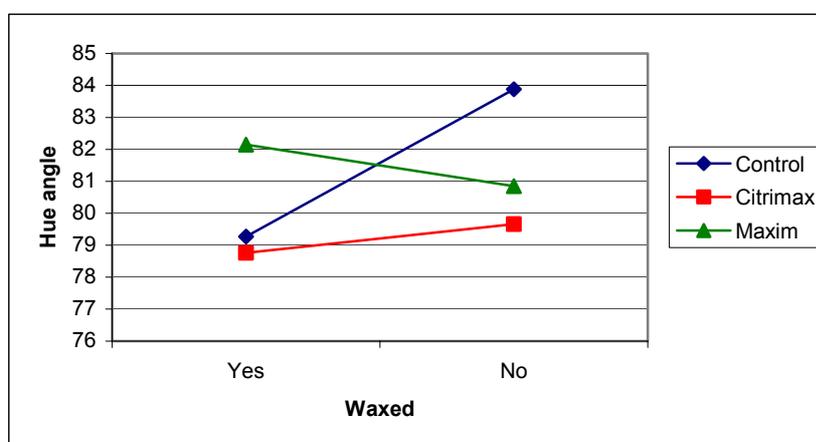


Figure 5.2.4.1. Wax and treatment interaction for hue angles of Miho Wase Satsuma mandarin 31 March 2003.

Conclusions

Little research has been conducted on the effect of auxins on citrus rind colour. Auxins may play a role through increased ethylene production in the presence of high auxin concentrations. Ethylene is known for its effect on rind colour development and is used commercially in post-harvest degreening.

In this experiment, auxin treatment during stage I of fruit development did not consistently improve rind colour of Clementine and Satsuma mandarins. At maturity, 2,4-DP (as Corasil.E or Citrimax) had a significantly better effect on rind colour than 3,5,6-TPA (Maxim).

However, although the probability and mean values showed a significant difference and improvement in hue angle, the difference in rind colour was not visible to the naked eye. Thus, preharvest auxin-treatment of citrus fruit did not appear to improve rind colour significantly, as observed by Hirose et al. (1978) and Kamuro and Hirai (1981) with ethylchlozate. Nevertheless, further research is proposed to determine carotenoid content of auxin-treated mandarin fruit.

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5.2.5 Physiological aspects of rind colour development

Experiment COL 01/02 by Graham H. Barry (CRI at Stellenbosch University)

Opsomming

Skilkleur ontwikkeling van sitrus in Suider Afrika is veral 'n probleem in die vroeër kultivars a.g.v. die herfs temperature wat te hoog is vir chlorofil afbraak en karoteen sintese. Vir optimale kleur ontwikkeling van sitrus word 'n nagtemperatuur van <13°C verlang. Dit is heel moontlik nie die koue nag temperature *per se* wat vir die kleurontwikkeling verantwoordelik is nie. Dit blyk egter of die lae nag temperature vegetatiewe ontwikkeling stop. Laasgenoemde het 'n bekende antagonistiese uitwerking op chloroplast omskakeling na chromoplast en sodoende word chlorofil afbraak en karoteen sintese vertraag. Om die rede kan maatstawwe wat die oordadige vegetatiewe groei gedurende die laat somer en vroeg herfs beperk, 'n betekenisvolle rol speel in pogings om skilkleur te verbeter. Interne gibberellin bevorder vegetatiewe groei in plante en om daardie rede kan 'n gibberellin biosintese inhibeerder die omskakeling van chloroplast na chromoplast bevorder. Gedurende 2002 is daar voeler proewe met prohexadione kalsium (Ph-Ca) op Navelina Navel lemoen bome gedoen. Ph-Ca is 'n nuwe produk wat gibberellin biosintese inhibeer en het korter nawerkings effek as die triazole en die toediening daarvan het die vrugkleur verbeter met 'n halwe kleurkaart. Opvolg behandeling van Ph-Ca op Palmer Navel lemoenbome het egter in geen betekenisvolle verbetering van kleur tot gevolg gehad nie alhoewel daar aan die einde wel 'n neiging na kleur verbetering was. Die doel met die navorsing was om te bepaal wat die respons van die bome sal wees op verskillende dosisse Ph-Ca. Die data sal egter eers in 2004 beskikbaar wees a.g.v. vrugte wat vanaf April-Junie 2004 geoes word.

Summary

Rind colour development of citrus is a problem in South Africa, especially for early maturing cultivars, because autumn temperatures are too high for chlorophyll breakdown and carotenoid synthesis. Citrus fruit

require night temperatures of $<13^{\circ}\text{C}$ for optimal colour development. It is unlikely that cool night temperature *per se* is the cause of chlorophyll breakdown and carotenoid synthesis. Rather, the cool night temperature probably plays a direct role in halting vegetative growth. The latter is antagonistic to chloroplast conversion to chromoplasts, thereby delaying chlorophyll breakdown and carotenoid synthesis. Therefore, techniques to reduce vegetative vigour during late summer and early autumn may play a role in enhancing rind colour development. Endogenous gibberellins promote vegetative growth in plants. Gibberellin biosynthesis inhibitors effectively reduce vegetative growth and, in turn, may promote the conversion of chloroplasts to chromoplasts. Previous exploratory research (2002) on Navelina Navel orange trees treated with prohexadione calcium (Ph-Ca), a new gibberellin biosynthesis inhibitor with less persistence than triazoles, had fruit that were better coloured than the control by more than half a colour plate. However, subsequent treatment of Palmer Navel orange with Ph-Ca did not significantly improve rind colour, although there was a trend towards enhanced colour. The objective of this research was to determine the dose response of citrus trees to Ph-Ca. At the time of writing this research progress report, data had not been collected. Fruit will be sampled from April to June 2004 and reported on in the 2004 report.

5.2.6 Post-harvest manipulation of rind colour

Experiment COL 02/02 by Angelique van Wyk (Stellenbosch University) & Graham H. Barry (CRI at Stellenbosch University)

Opsomming

“Oranje” en “geel” Palmer Navel lemoene is aan ’n verskeidenheid gesimuleerde verskeppings/opbergings regimes onderwerp om die effek van sub-zero temperatuur verskeping en daaropvolgende verhoogde opbergings temperature, op finale skil kleur te ondersoek. Skil kleur by verskeping is as ’n uiters krities faktor geïdentifiseer. Opberg temperature speel ’n rol in die handhawing van skil kleur na verskeping.

5.2.6.1 The effect of sub-zero shipping temperature on rind colour

Introduction

When shipping fruit to the USA it is necessary that the fruit undergo cold-sterilisation at -0.6°C for a minimum of 22 days to destroy any possible fruit fly or false codling moth infestation. Shipping temperature has a large effect on eventual fruit colour. Fruit shipped at -0.5°C showed no colour development (Le Roux, 1999). At sub-zero shipping temperatures fruit develop a yellow colour instead of the desired orange colour. In some cases, fruit shipped with an orange colour arrive at their destination with a pale yellow appearance. It is thought that carotenoid breakdown occurs at sub-zero shipping temperatures making fruit paler. Hue angle is the component of colour, which contributes most to perceivable colour as it indicates the actual colour of the object, i.e. yellow or orange. Lightness refers to how light or dark a specific colour is on a scale from black to white. Chroma is a complex component of colour and refers to the intensity of colour, or its departure from grey towards the purest possible specific colour.

The goal of this research is to identify the effect of sub-zero shipping temperature and its duration (2003 season) on rind colour development.

Material and methods

Sites and plant material. In the 2002 and 2003 seasons Palmer Navel, or Washington Navel or Bahianinha Navel sweet oranges (hereafter referred to as Palmer, Washington and Bahianinha, respectively) were selected from the ALG Packhouse in Citrusdal. The fruit were drenched with 2,4-D ($125\text{ mg}\cdot\text{L}^{-1}$), Guazatine® ($500\text{ mg}\cdot\text{L}^{-1}$) and Sporekill® ($120\text{ mg}\cdot\text{L}^{-1}$) and then transported to Stellenbosch where fruit were subjected to various cold storage regimes to simulate commercial shipping and holding conditions.

Treatments and experimental design. In 2002, 1200 fruit were sorted into two colour classes, “orange” and “yellow”, visually and all fruit were fully coloured with no green patches. Fruit were stored at either 4.5°C for 21 days or at -0.6°C for 28 days to simulate commercial shipping conditions for Navel oranges exported to Europe or the USA, respectively. Fruit were then stored at 4.5°C for 7 days to simulate post-shipping holding conditions, followed by an extended shelf-life at 15°C for 3 or 4 weeks, depending on shipping temperature and duration.

In 2003, 1200 fruit were sorted into the two colour classes (“orange” and “yellow”) using a colorimeter to ensure that the yellow (hue angle $\approx 60^{\circ}$) and orange (hue angle $\approx 75^{\circ}$) fruit were separated into two distinct colour classes. There were six replicates per treatment, each containing 40 fruit. Fruit were stored at 4.5°C for 21 days or at -0.6 for various periods of 26 days, 28 days, 30 days or 32 days. Fruit were then

exposed to a holding temperature of 4.5°C for 7 days followed by an extended shelf-life at 15°C for 3 or 4 weeks, depending on shipping temperature and duration.

Data collection. A circle was drawn at the equatorial position of 10 fruit using a permanent marker to ensure that consecutive colour measurements were made at the same position thereby minimising variation of rind colour from one position on the rind to another. Rind colour was measured on the same fruit at each sampling date. Ten fruit per replicate were used in 2002, and eight fruit per replicate were used in 2003. Rind colour was quantified objectively using a colorimeter by measuring hue angle, lightness and chroma. Visual observations relating to colour intensity and general appearance were also noted. In the 2002 season, rind colour was measured at intake (before cold-storage treatment), after 2 weeks shipping at 4.5°C or -0.6°C, after 3 weeks shipping at 4.5 or 4 weeks shipping at -0.6°C, after 7 days holding at 4.5°C, after 2 weeks holding at 4.5°C and after a 3 week shelf-life period at 15°C. In the 2003 season, rind colour was measured at intake, after shipping at 4.5°C or -0.6°C, after 7 days holding at 4.5°C and after 2 weeks shelf-life at 15°C.

Statistical analysis. Data were subjected to analysis of variance and means were separated using Fisher's LSD.

Results and discussion

2002 season. Fruit were visually sorted into the orange and yellow categories, and this did not ensure that there was no overlap in colour measurements. Hue angle increased during shipping at both 4.5°C and -0.6°C (Fig. 5.2.6.1). During the 1-week holding period at 4.5°C and 2-week shelf-life periods there was a decrease in hue angle; initially there was a rapid drop in hue angle followed by a slight increase on the last evaluation date. Changes in hue angle followed similar trends for both shipping temperatures, irrespective of initial fruit colour class. Fruit shipped at 4.5°C tended to have lower hue angles, i.e. were more orange than fruit shipped at -0.6°C. On the first evaluation date there was a small difference in hue angle between orange and yellow fruit (69° vs. 64.5°). During shipping and holding there was a slight overlap in hue angle of orange and yellow fruit rind, but "yellow" fruit tended to have a higher hue angle than "orange" fruit, i.e. less well-coloured or orange. At the final evaluation date, orange fruit shipped at 4.5°C had the best colour, whereas there was no significant difference among yellow fruit shipped at either 4.5°C or -0.6°C and orange fruit shipped at -0.6°C. Lightness followed a similar trend to that of hue angle (Fig. 5.2.6.2), i.e. lightness initially increased during shipping then decreased during the holding period. Fruit shipped at 4.5°C tended to have lower lightness values than fruit shipped at -0.6°C, and "orange" fruit tended to have lower lightness values than "yellow" fruit. Chroma also initially increased during shipping, then decreased during holding (Fig. 5.2.6.3.). Chroma was consistently lower for fruit shipped at 4.5°C than -0.6°C. However, initial colour class did not consistently affect chroma, although "orange" fruit tended to have slightly lower chroma than "yellow" fruit.

On the last evaluation date, initially orange fruit still had the most intense colour although there was no significant difference between yellow fruit shipped at either temperature and orange fruit shipped at -0.6°C. Orange fruit shipped at -0.6°C deteriorated to the same eventual colour level as yellow fruit shipped at 4.5°C.

2003 season. "Orange" fruit had significantly lower hue and lightness values than "yellow" fruit at all stages of sampling and at all shipping conditions (Figs. 5.2.6.4 and 5.2.6.5). Hue angle increased slightly during shipping and remained relatively constant then decreased slightly during shelf-life (3 weeks at 15°C) (Fig. 5.2.6.4). "Orange" and "yellow" fruit maintained their initial difference in hue angle throughout the shipping and holding periods; "orange" fruit remained more well-coloured than "yellow" fruit irrespective of shipping regime (temperature x duration). There were no consistent differences in hue angle among fruit of the same colour class shipped 4.5°C or -0.6°C for different durations. Lightness of "orange" fruit remained consistent during the post-harvest storage period whereas lightness of "yellow" fruit increased slightly during shipping, then decreased slightly during the holding and shelf-life periods (Fig. 5.2.6.5). There were no consistent differences in lightness among storage regimes, although lightness of fruit shipped at 4.5°C was significantly lower than fruit shipped at -0.6°C at the end of the storage period. Chroma of all fruit increased during shipping, then decreased during the holding and shelf-life periods to similar values to those measured at intake (Fig. 5.2.6.6). After the shipping period chroma of fruit shipped at 4.5°C was significantly lower than fruit shipped at -0.6°C.

Conclusions

Shipping at sub-zero (-0.6°C) or low (4.5°C) temperatures does not favour colour development. Thus fruit need to be well-coloured before shipping as no further colour development will occur. Shipping regime

cannot be used to improve the rind colour of poorly coloured fruit. Furthermore, initial rind colour is the most important factor determining rind colour on arrival at the market, irrespective of the shipping regime.

Low-temperature (<5°C) shipping of citrus fruit has previously been shown to restrict rind colour development (Le Roux, 1997). However, the current study highlights the role of low-temperature shipping on the loss of rind colour, possibly via carotenoid degradation. Gross (1987) showed that carotenoid degradation occurred below 6°C. Individual fruit with deep orange initial colour (~60° hue, <65 Lightness) prior to shipping at low temperatures are better able to withstand colour loss associated with low temperature shipping, whereas fruit with pale orange initial colour (~75° hue, >70 lightness) prior to shipping at low temperature will be pale after shipping. This response of individual fruit of varying colour to low temperature shipping results in variation in rind colour among fruit at the point of arrival in export markets.

The perceived loss of rind colour following shipping at sub-zero temperatures is not a result of increased “greenness” due to chlorophyll synthesis, but rather a result of decreased “orangeness” probably due to carotenoid degradation. Therefore, initial rind colour plays a critical role in product quality. Depending on market destination, and hence, shipping temperature, fully coloured yet pale fruit should not be packed for markets sensitive to rind colour.

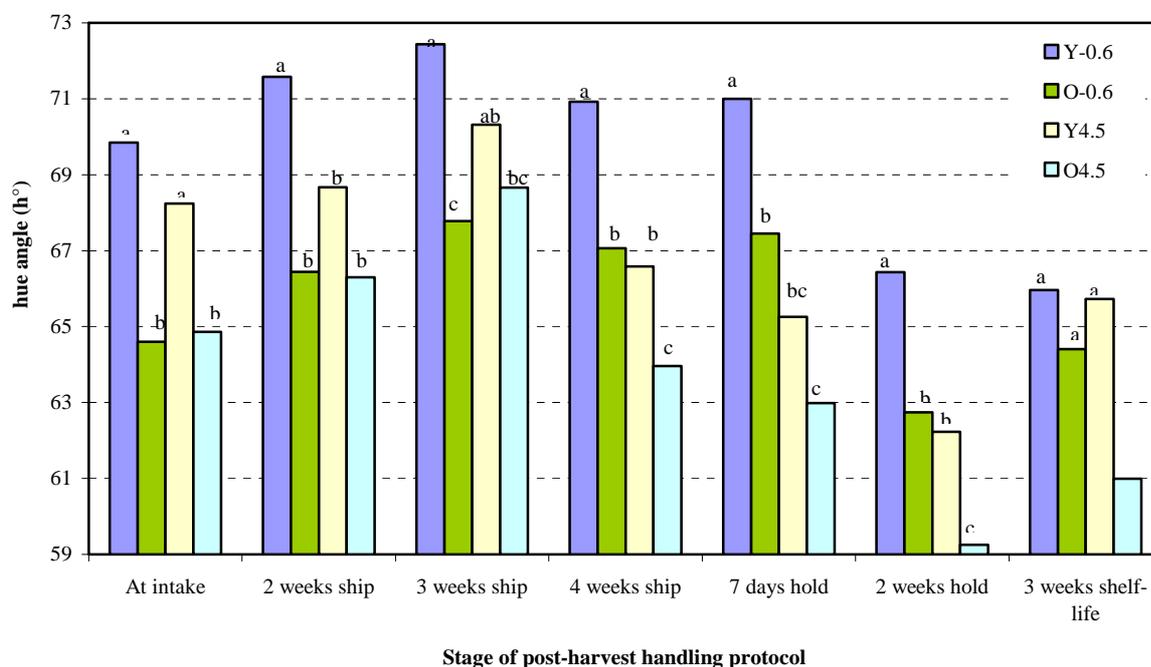


Figure 5.2.6.1. Change in hue angle over time in initially orange and yellow Palmer Navel orange fruit during shipping at -0.6 °C or 4.5 °C during the 2002 season.

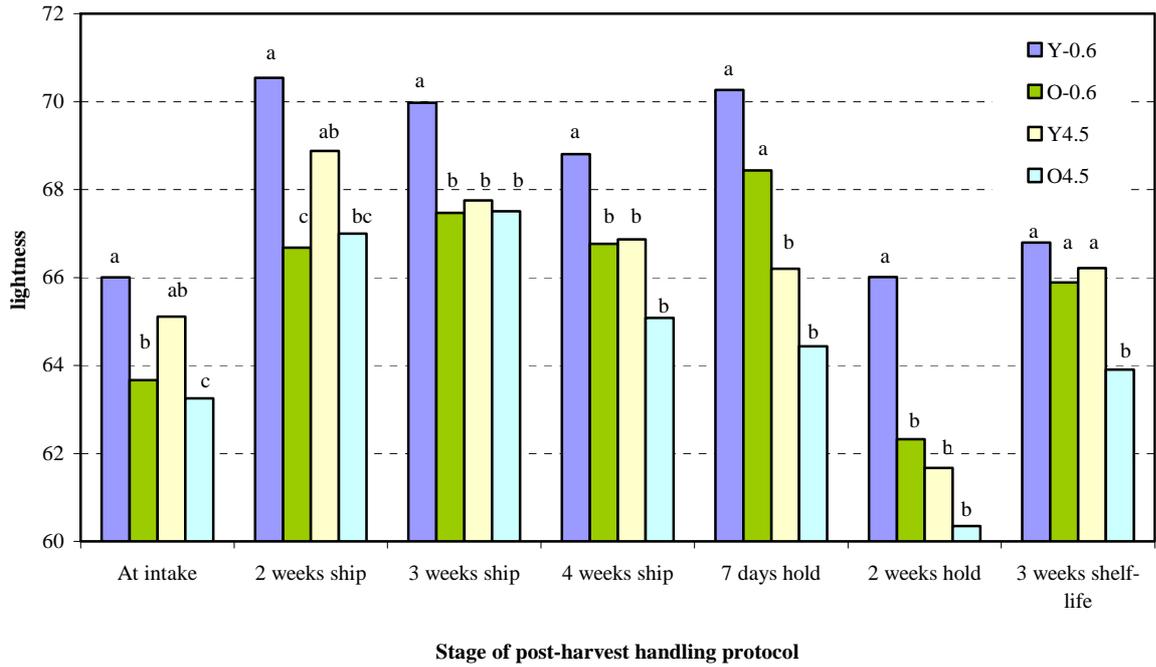


Figure 5.2.6.2. Change in lightness over time in orange and yellow Palmer Navel orange fruit during shipping at -0.6°C or 4.5°C during the 2002 season.

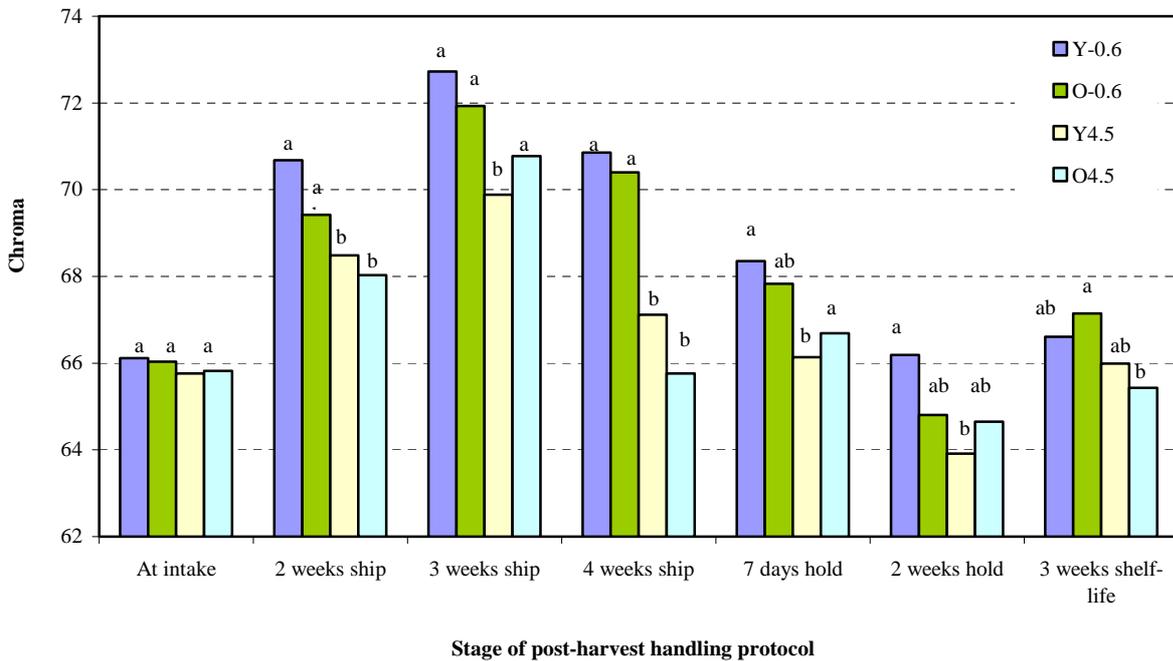


Figure 5.2.6.3. Change in chroma over time in orange and yellow Palmer Navel orange fruit during shipping at -0.6°C or 4.5°C during the 2002 season.

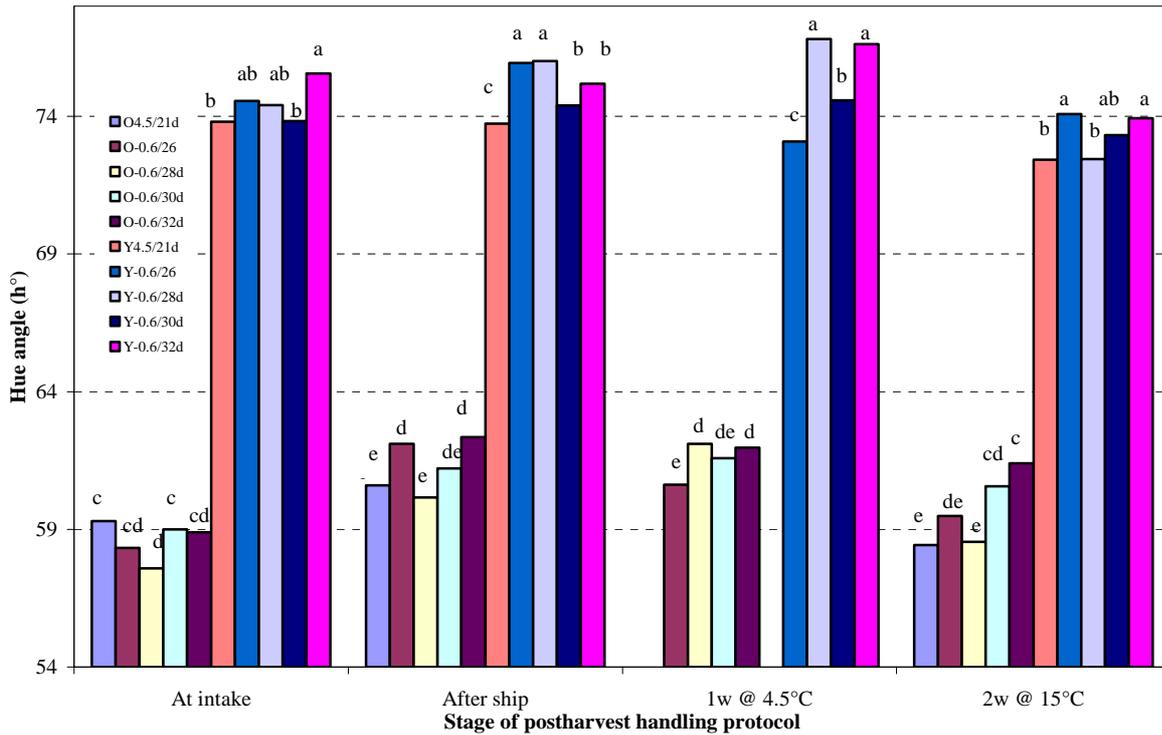


Figure 5.2.6.4. Change in hue angle in initially orange and yellow Palmer Navel orange fruit during shipping at -0.6°C or 4.5°C for varying durations followed by holding and shelf-life periods during the 2003 season.

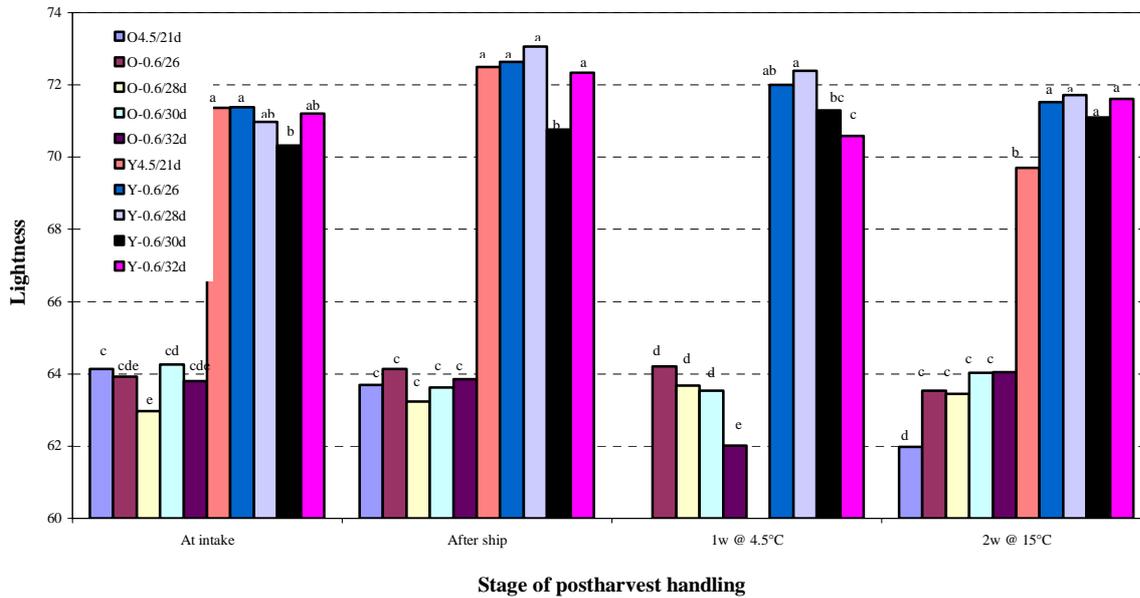


Figure 5.2.6.5. Change in lightness in initially orange and yellow Palmer Navel orange fruit during shipping at -0.6°C or 4.5°C for varying durations followed by holding and shelf-life periods during the 2003 season.

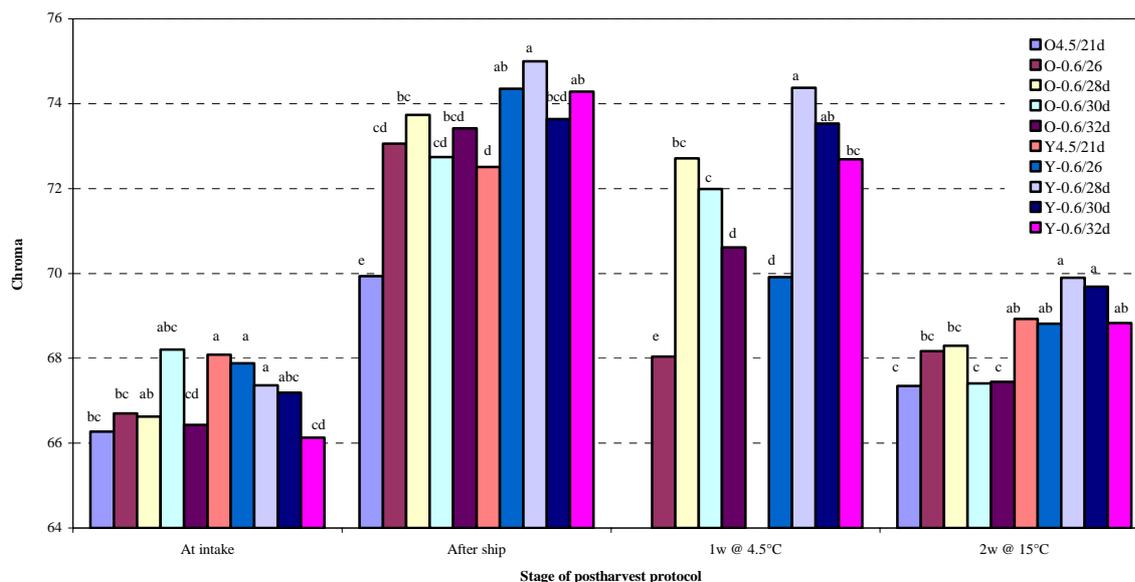


Figure 5.2.6.6. Change in chroma in initially orange and yellow Palmer Navel orange fruit during shipping at -0.6°C or 4.5°C for varying durations followed by holding and shelf-life periods during the 2003 season.

5.2.6.2 The effect of post-shipping holding temperature on rind colour

Introduction

The effects of shipping temperature on rind colour are relatively well-known, e.g. citrus fruit shipped at 11°C showed more colour development than fruit shipped at 4.5°C , whereas fruit shipped at -0.5°C showed no colour development. However, optimal holding temperature to promote colour development after shipping is less certain. Le Roux (1999) showed that fruit stored at 20°C (post-shipping) had increased colour development compared with fruit held at 4.5°C or 11°C .

Materials and methods

Sites and plant material. In the 2002 and 2003 seasons, Palmer Navel and Bahianinha Navel sweet oranges (hereafter referred to as Palmer and Bahianinha, respectively), were selected from the ALG Packhouse in Citrusdal.

Treatments and experimental design. In 2002 fruit were visually sorted into two colour classes, namely “yellow” and “orange”, and all fruit were fully coloured with no green patches. A circle, approximately 2 cm in diameter, was drawn at the equatorial position of each fruit using a permanent marker to ensure that consecutive colour measurements were made at the same position thereby minimizing variation of rind colour from one position on the rind to another. All fruit were drenched with 2.4-D ($125\text{mg}\cdot\text{L}^{-1}$), Guazatine ($500\text{mg}\cdot\text{L}^{-1}$) and Sporekill ($120\text{mg}\cdot\text{L}^{-1}$). Yellow and orange fruit were separated and packed into boxes, each box (replicate) contained 50 fruit, while 10 of these were marked and kept throughout the period for repetitive colour measurements. Both of the fruit colour groups were shipped at either -0.6°C or 4.5°C for 28 days (phytosanitary requirement for shipping fruit to the United States of America) or 21 days, respectively. There were six replicates of each treatment, i.e. Yellow/4.5, Orange/4.5, Yellow/ -0.6 and Orange/ -0.6 . Each treatment was subjected to a holding temperature of 4.5°C , 12.5°C or 20°C for 6 or 7 weeks depending on the shipping temperature. There were a total of 12 treatments when considering the various combinations of initial fruit colour, shipping temperature and holding temperature.

In the 2003 season the same treatments were maintained, except that the holding temperatures were changed to 4.5°C , 11°C and 15°C as data from the previous season showed a holding temperature of 20°C was ineffective in improving rind colour.

Rind colour development and general appearance. Rind colour was quantified objectively (lightness, chroma and hue angle) measured using a colorimeter. Visual observations relating to colour intensity and general appearance were also noted. In 2002, rind colour was measured when the fruit went into cold storage,

colorimeter readings were taken each week. Fruit were kept for a total period of 10 weeks to evaluate quality throughout this period. In 2003, rind colour measurements were taken at six evaluation dates being, before shipping, after shipping followed by three evaluations during holding and a final evaluation at the end of the holding period.

Statistical analysis. The results were analysed by analysis of variance.

Results

During the 2002 season, the hue angle of “orange” fruit increased consistently throughout shipping, at various temperatures, indicating consistent colour degradation (Figs. 5.2.6.7 and 5.2.6.8). Fruit kept at 4.5°C and 12.5°C after shipping showed an increase in hue angle until the third evaluation date, when hue values either decreased slightly (in “orange” fruit shipped at 4.5°C) or increased slightly (in “orange” fruit shipped at -0.6°C). In initially “orange” and “yellow” fruit at either shipping temperature, a holding temperature of 12.5°C showed best colour development.

Hue angle decreased constantly throughout shipping and after shipping of “orange” fruit exposed to post-shipment temperatures of 12.5°C. Fruit held at 12.5°C had the lowest eventual hue angle ($\approx 65^\circ$). Final hue values in “orange” fruit shipped and held at various temperatures varied over a range of 10° . In “orange” and “yellow” fruit, a holding temperature of 20°C resulted in poorest colour development. Colour remained relatively consistent at 4.5°C showing no degradation or improvement. The level of decay was lowest in fruit held at 4.5°C.

During 2003, the holding temperatures were chosen on the basis of the 2002 results. In an attempt to identify the ideal holding temperature, the ranges between the three holding temperatures were decreased and the 20°C holding temperature was replaced with a lower temperature as data indicated that 20°C was too high to favour good colour development. In the 2003 season, fruit held at 4.5°C showed the poorest colour development and fruit colour remained constant throughout the holding period (Fig. 5.2.6.9). There was the greatest improvement in colour of “yellow” fruit held at 11°C whereas “orange” fruit shipped at 4.5°C and held at either 11°C or 15°C showed the best colour development. There were no significant differences between these treatments.

Initially “orange” fruit generally remained more well-coloured throughout the holding period than “yellow” fruit, except at the last evaluation date where there was no significant difference between the O-0.6/4.5, Y-0.6/11 and Y4.5/11 treatments (Fig. 5.2.6.9). A trend can be seen where fruit colour is grouped, while all treatments are significantly different at the last evaluation date, except Y-0.6/11, Y4.5/11 and O-0.6/4.5 which are statistically similar and O4.5/15 and O4.5/11 which are statistically similar. Fruit held at 4.5°C stands out as having the poorest eventual colour in both “yellow” and “orange” colour classes. Fruit held at 11°C and 15°C, within colour classes were more similar throughout the entire holding period.

Discussion

Post-shipment holding temperature appears to play a greater role in colour development than shipping temperature. As holding temperature increased, the rate of decay increased and was highest at 20°C. The high rate of decay observed during holding in the 2002 season may have influenced results, especially at later evaluation dates. Holding temperature appears to have a substantial effect on eventual fruit colour, while the data suggests that shipping temperature has a minor effect on final fruit colour. The most colour development took place in the intermediate temperature range, i.e. 11 to 15°C. At 4.5°C there was a decrease in rind colour during the holding period.

Initial fruit colour plays a central role in final fruit colour after shipping. Initially “orange” fruit remained more well-coloured throughout the holding period than “yellow” fruit, in spite of shipping temperature, while a holding temperature of between 11 and 15°C appears to accelerate post-shipment colour development.

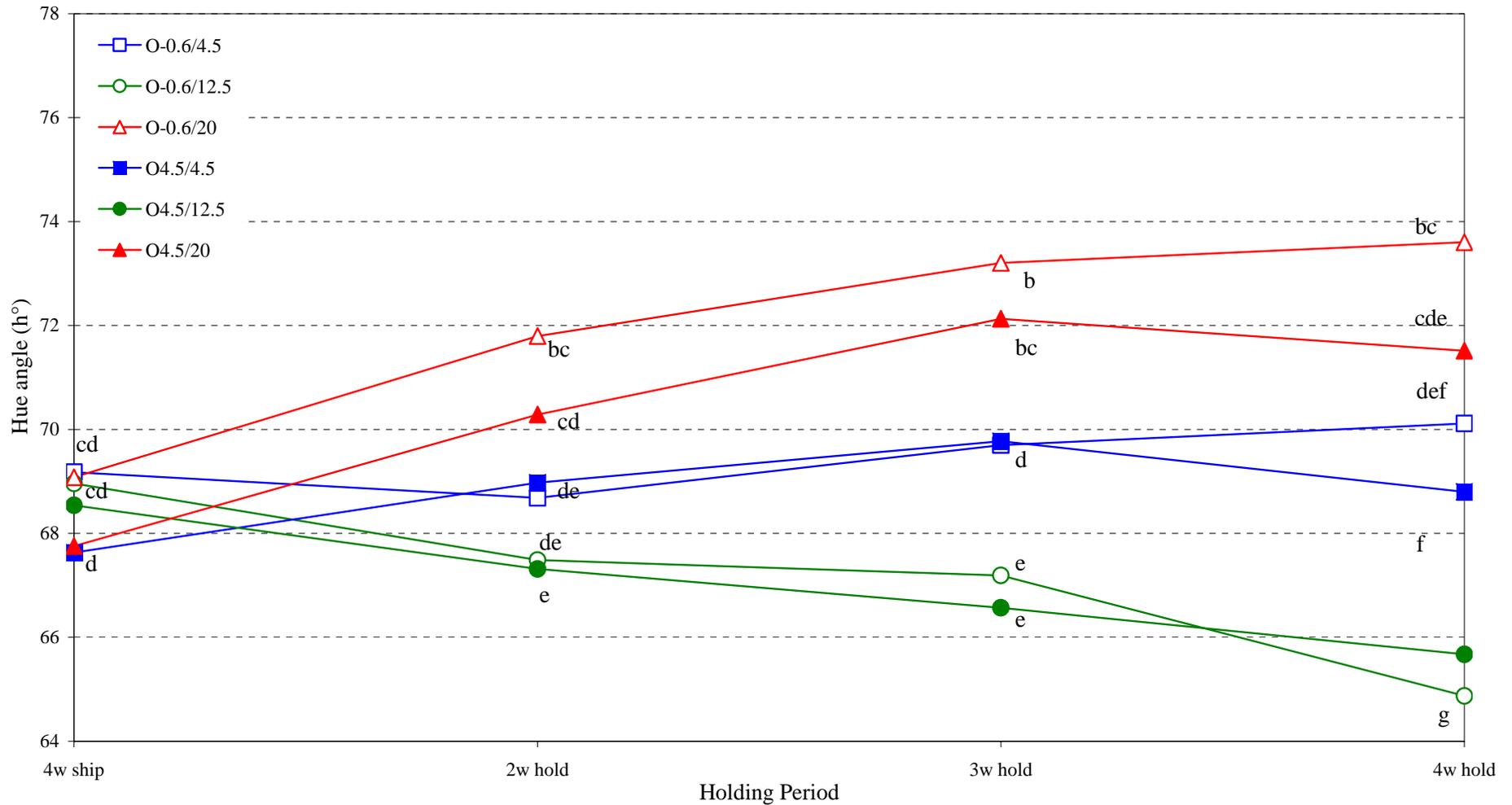


Figure 5.2.6.7. Change in hue angle of “orange” Palmer Navel orange fruit during holding, at 4.5, 15 and 20 °C after being shipped at either -0.6 °C or 4.5 °C in the 2002 season

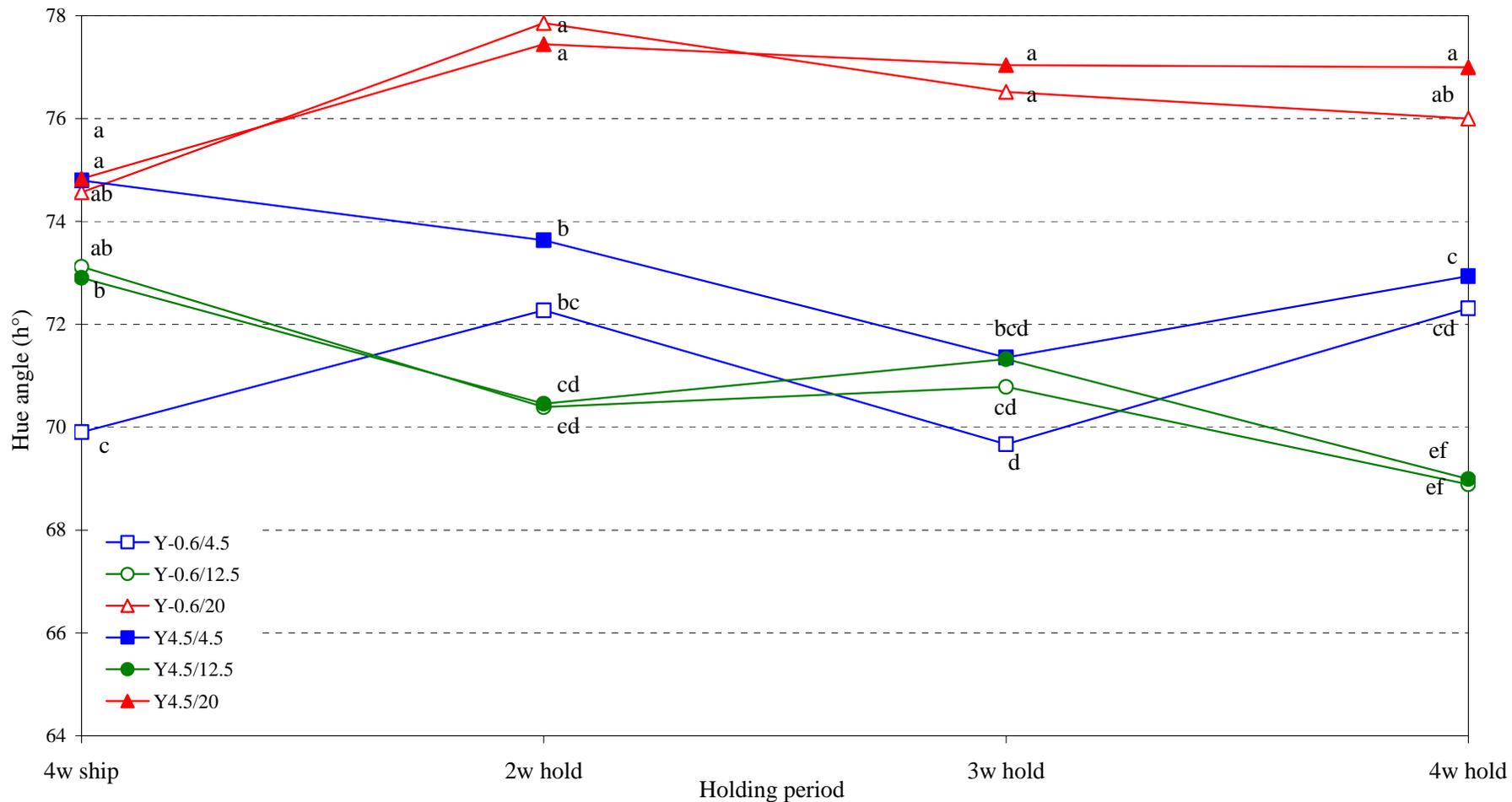


Figure 5.2.6.8. Change in hue angle of “yellow” Palmer Navel orange fruit during holding, at 4.5, 15 and 20 °C after being shipped at either -0.6 °C or 4.5 °C in the 2002 season

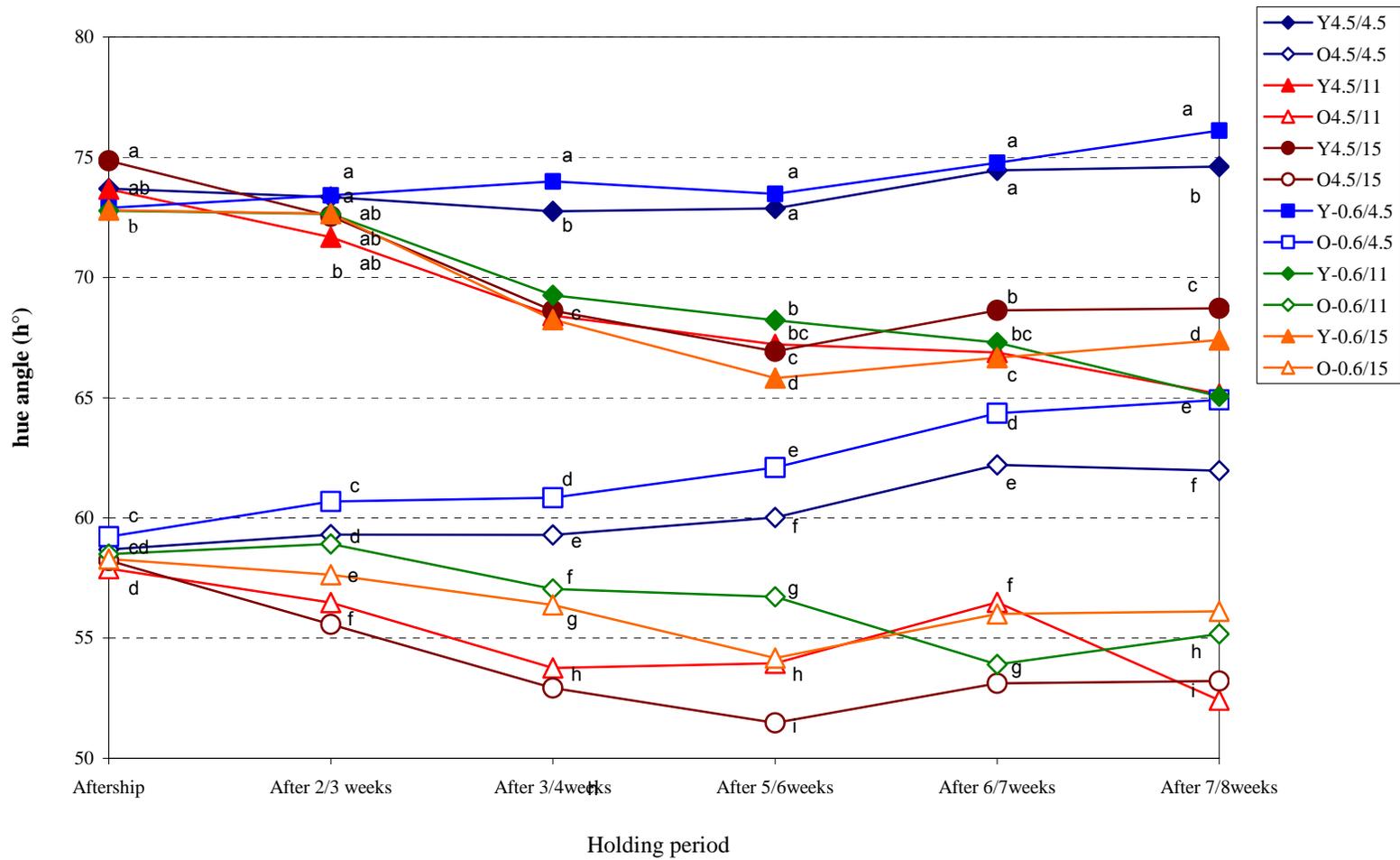


Figure 5.2.6.9. Change in hue angle of “orange” and “yellow” Palmer Navel orange fruit during holding, at 4.5, 15 and 20 °C after being shipped at either -0.6 °C or 4.5 °C in the 2003 season.

5.2.7 Effect of tree nutrition and post-harvest treatments on concentration of the most important colour imparting carotenoids in physiologically mature citrus fruit

Experiment UNP 1 by Isa Bertling, John Bower, Renate Oberholster, Molipa Mosoeunyane and Tony Bruton (University of Natal, Pietermaritzburg)

Opsomming

Die effek van boom voeding en naoes behandeling op die konsentrasie van die mees belangrikste kleur bydraende karotene in fisiologiese volwasse sitrus vrugte, vorm die basis van Molipa Mosoeunyane se PhD navorsing by UNP. 'n "Karoteen biblioteek" is voorberei om sekere pigment veranderings te identifiseer gedurende sitrus vrug ontwikkeling. Molipa Mosoeunyane berei 'n literatuur oorsig van karotene in sitrus en subtropiese vrugte voor en gee besonderse aandag aan die tipe karotene teenwoordig in die gewasse om sodoende makliker maniere te vind om eerstens, ekstraksie en/of suiwering van die karoteen pigmente te doen en tweedens om inligting te vind wat 'n indikasie van mikro voedings elemente se betrokkenheid in die aanskakeling van karoteen biosintese kan gee. Meer informasie word ook gesoek op die vraag of 'n algemene verhoging in vrug karoteen konsentrasie hand aan hand geskied met verhoging in kleur bydrae.

Summary

The student who took the project on as his PhD project, Molipa Mosoeunyane, is preparing a "carotenoid library". To be able to identify certain pigment changes in citrus during fruit development by postharvest means, carotenoid standards have to be categorized. As these standards are not commercially available, a set of carotene and xanthophylls standards was donated to us by Prof. Peter Molnar from Hungary. These standards were loaded onto our HPLC system and placed into our carotenoid library.

Simultaneously, Molipa Mosoeunyane is preparing a literature review on carotenoids in citrus and subtropical fruit, with particular interest in the type of carotenoids present in these crops, to possibly be able to identify easier means of, firstly, extraction and/ or purification of the carotenoid pigments, and, secondly, to search for further indications of micronutrient involvement in triggering carotenoid biosynthesis. Therefore, it is crucial to identify if certain types of carotenoids are the major colour imparting compounds for citrus in general. Furthermore, information is sought on addressing the question if a general increase in fruit carotenoid concentration is paralleled by an increase in the colour-imparting carotenoids.

5.3 PROJECT: CROP LOAD MANAGEMENT

Project manager: Graham H. Barry (CRI at Stellenbosch University)

5.3.1 Project summary

Daily fertigation could be considered as one of the most important recent advances to citricultural practices. Direct benefits of managing plant water relations and mineral nutrition through such a system includes, *inter alia*, i) increased precocity, i.e. earlier fruit bearing potential, ii) improved fruit yield, and iii) larger fruit size. However, there is also a biological "cost" to these benefits, viz. delayed rind colouration and sub-optimal sugar accumulation.

The advantages and disadvantages of daily fertigation are in the process of being quantified, and detailed physiological studies are being conducted to fine-tune current recommendations and industry practices. Part of this research aims to prove that reduced midday depression of leaf gas exchange by daily fertigation results in increased C fixation, thereby providing extra photosynthates, which are translocated to the fruit, and hence improved internal fruit quality in terms of juice content, Brix and acidity.

Differential irrigation treatments (1/2X, X and 2X) were applied to Nules Clementine mandarin trees from the end of stage I of fruit development until maturity to quantify the ecophysiological responses and benefits of daily fertigation, as well as the effects of this technology on crop load and fruit quality. At one site, Backsberg, the 1/2X treatment tended to produce fruit with higher Brix. However, this response was not repeated at the other site (Greendale). This research is not yet complete and another season's data is required.

Projek opsomming

Daaglikse toediening van bemesting deur middel van die besproeiingsstelsel ("fertigation"), is een van die belangrikste vorderings wat onlangs gemaak is in die verbouing van sitrus. Daar is direkte voordele aan verbonde deur die plant water verhouding en minerale voeding so te bestuur, naamlik, i) verkorting van die boom se jeugfase en dus die vermoë om vroeër vrugbaar te wees, ii) verhoging in opbrengs, en iii)

verbeterde vruggrootte. Daar is egter 'n negatiewe biologiese prys wat verhaal word, naamlik vertraagde kleurontwikkeling en sub-optimale suiker akkumulاسie.

Verskillende besproeiingsbehandelings (1/2X, X en 2X) is toegedien op 'nules Clementine' mandaryn bome vanaf fase I van vrugontwikkeling tot vrugvolwassenheid om die ekofisiologiese reaksies asook die voordele wat verkry word met die gebruik van die tegnologie op opbrengs en vrugkwaliteit te bepaal. By die Backsberg-perseel het die 1/2X-behandeling vrugte met 'n hoër Brix-waarde tot gevolg gehad. Die Greendale-proefperseel het egter nie dieselfde tendens getoon nie. Die navorsing is nog nie voltooi nie en een jaar se data sal nog ingewin word.

Die voor- en nadele verbonde aan daaglikse bemesting deur middel van die besproeiingstelsel is in die proses om gekwantifiseer te word. Gedetailleerde fisiologiese studies word tans onderneem om huidige aanbevelings en verbouingspraktyke te verfyn. 'n Deel van die navorsingsprojek poog om te bewys dat indien 'n verdere verlaging in die mid-dag daling van die gasuitruiling van die blaar bewerkstellig kan word met die toediening van bemesting deur die besproeiingstelsel (fertigation), 'n verhoogde koolstof vaslegging verkry kan word. Gevolglik sal vrugkwaliteit verbeter in terme van sapinhoud, Brix en sure as gevolg van die verhoogde translokاسie van fotosintetiese produkte na die vrug.

5.3.2 **Ecophysiological responses and changes in sugar accumulation due to altered plant water relations of *Citrus* trees**

Experiment TSS 01/02 by Jandré A. Prinsloo (Stellenbosch University) and Graham H. Barry (CRI at Stellenbosch University)

Opsomming

Verskillende besproeiingsbehandelings (1/2X, X en 2X) is toegedien op 'nules Clementine' mandaryn bome vanaf fase I van vrugontwikkeling tot vrugvolwassenheid om die ekofisiologiese reaksies asook die voordele wat verkry word met die gebruik van die tegnologie op opbrengs en vrugkwaliteit te bepaal. By die Backsberg-perseel het die 1/2X-behandeling vrugte met 'n hoër Brix-waarde tot gevolg gehad. Die Greendale-proefperseel het egter nie dieselfde tendens getoon nie. Die navorsing is nog nie voltooi nie en een jaar se data sal nog ingewin word.

Introduction

The daily fertigation method of irrigation and fertilisation promises to increase orchard productivity and ultimately profitability through improved and timeous application of water and mineral nutrients. However, these promises are not necessarily supported by facts. For example, increases in fruit yield and fruit size have been reported for citrus trees grown under daily fertigation. However, delayed rind colour development and inadequate sugar accumulation are being reported in the citrus industry. Therefore, it is essential to quantify the ecophysiological responses and benefits of daily fertigation, as well as the effects of this technology on crop load and fruit quality.

Materials and methods

Nules Clementine mandarin orchards at two commercial farms (Backsberg and Greendale) in Simondium, Western Cape Province, were used. Differential irrigation consisting of 2X, X and 1/2X was applied at end of stage I (mid-December) of fruit development. Six replicates each consisting of six trees were used at each orchard in a randomized complete blocks design.

Leaf water potential (WP) was determined by using a pressure chamber. Two leaves per replicate on the west side of trees were bagged before sunrise with small black bags and covered with tinfoil. Water potential measurements were taken from 11H00 at Greendale and from 12H00 at Backsberg. In December 2002, 10 fruit were tagged and fruit size measurements taken with an electronic caliper. From March 2003, 10 fruit were randomly harvested for internal fruit quality analysis. The fruit that were tagged for fruit growth measurements were harvested at maturity (7 May 2003) and also analyzed for internal fruit quality purposes. Fruit quality analysis consisted of fruit size and fresh weight, juice weight and percentage, Brix (using an electronic refractometer) and titratable acidity. All sampling was made from the middle four trees of each replicate to reduce possible influences from adjacent treatments. Soil samples were taken and weighed at each evaluation period to determine gravimetric soil water content (SWC).

Results

Water potential. At the beginning of the experiment (19 Dec 2002) at Backsberg, WP did not differ among treatments (Fig. 1), and was -1.15 MPa. From 22 Jan. 2003 (5 weeks after the start of the differential irrigation treatments), statistical differences among treatments were detected. Fluctuations among treatments were observed until 19 Feb. From this date until the last measurement taken on 30 Apr., the 2X treatment had the highest (least negative) WP of all treatments. This response would be expected as twice as much water was applied to these trees which would thus be less water stressed. In contrast, the 1/2X treatment had the lowest (most negative) WP and these trees were thus most water stressed. Water potential decreased to values between -1.30 and -1.55 MPa on 20 Mar., probably due to very warm conditions experienced during the previous week.

At Greendale, the 2X treatment had the highest WP throughout the experiment, except on 19 Feb. when it was the lowest (Fig. 5.3.2.2). Water potential fluctuated between -0.87 MPa (on 10 Jan. 2003) and -1.11 MPa (on 20 Mar.). The 1/2X treatment had the lowest WP except on 19 Feb. and on 3 Apr. From 19 Feb., WP of the 1/2X treatment was statistically lower than the other treatments. Treatment 1/2X reached its highest value of -1.0 MPa on 19 Feb. and its lowest value (-1.31 MPa) on 20 Mar. Water potential of the X treatment tended to be intermediate between the 1/2X and 2X treatments. Its highest WP (-0.97 MPa) was on 19 Feb. and its lowest WP was -1.2 MPa on 20 Mar.

Fruit size. At Backsberg, 1/2X treated trees had consistently smaller fruit throughout the experiment (Fig. 5.3.2.3). Fruit size increased from 25.0 mm to 57.1 mm over the duration of the experiment. The X treatment had the largest fruit from the start of the experiment, and fruit size increased from 27.1 mm to 58.7 mm. The 2X treatment started with an average fruit size of 25.9 mm and reached a maximum of 58.4 mm. There was no statistical difference in fruit size among treatments at the end of experiment.

At Greendale, the 1/2X treatment had the largest fruit from the onset of the experiment (Fig. 5.3.2.4). Average fruit size started at 26.6 mm and ended at 58.0 mm. The X treatment had the smallest fruit, starting at 16.5 mm and reaching 54.8 mm. No significant differences were observed in fruit size among treatments from 19 Feb. to 16 Apr.

Internal fruit quality. At Backsberg, there was no statistical difference in fruit weight among treatments, although there was >10 g difference in fruit weight between 1/2X and X treatments (Table 5.3.2.1). Although there was no significant difference in juice content, fruit from the 2X treatment had the highest juice percentage. This could be expected as more water is available to these trees. There was no significant difference in SSC, acidity or ratio among treatments. However, the 1/2X treatment tended to produce fruit with higher SSC than the other treatments.

Fruit sampled on a two-weekly basis at Backsberg (Table 5.3.2.3a) were all larger than the tagged fruit at end of season (Table 5.3.2.1). There was no statistical difference among treatments on 30 Apr. for all juice quality variables (Tables 5.3.2.3a to 5.3.2.3f).

Although not statistically different, at Greendale the 1/2X treatment tended to have the heaviest fruit (70.0 g; Table 5.3.2.2), and the 2X tended to have the lightest fruit with the highest juice percentage. SSC was significantly higher for the 1/2X treatment compared with the 2X treatment; 11.2 vs. 10.4°. The 1/2X treatment also tended to produce fruit with the highest acidity (0.91%), whereas acidity tended to be lowest for 2X (0.81%).

Fruit sampled on a two-weekly basis at Greendale (Table 5.3.2.4a) were all larger than the tagged fruit at end of season (Table 5.3.2.2). There was no statistical difference among treatments on 30 Apr. for all juice quality variables (Tables 5.3.2.4a to 5.3.2.4f).

Soil water content. At Backsberg, SWC was lowest for the 1/2X treatment and highest for the 2X treatment. SWC of the 1/2X treatment decreased from 11.3% before the treatments were applied to 9.9% on 5 Mar. (Fig. 5.3.2.5) and then increased to 10.8% on 16 Apr. before decreasing slightly to 10.4% on 30 Apr. There was a decrease in SWC for the 2X treatment from 19 Dec. 2002 to 5 Mar. 2003, but increased steadily to a value of 12.5% by 30 Apr. 2003.

At Greendale, the trend in SWC was less clear among treatments (Fig. 5.3.2.6), although the 2X treatment tended to have higher SWC and the 1/2X treatment tended to have lower SWC until 16 Apr. Due to rainfall, differences in SWC among treatments could not be sustained by the irrigation treatments.

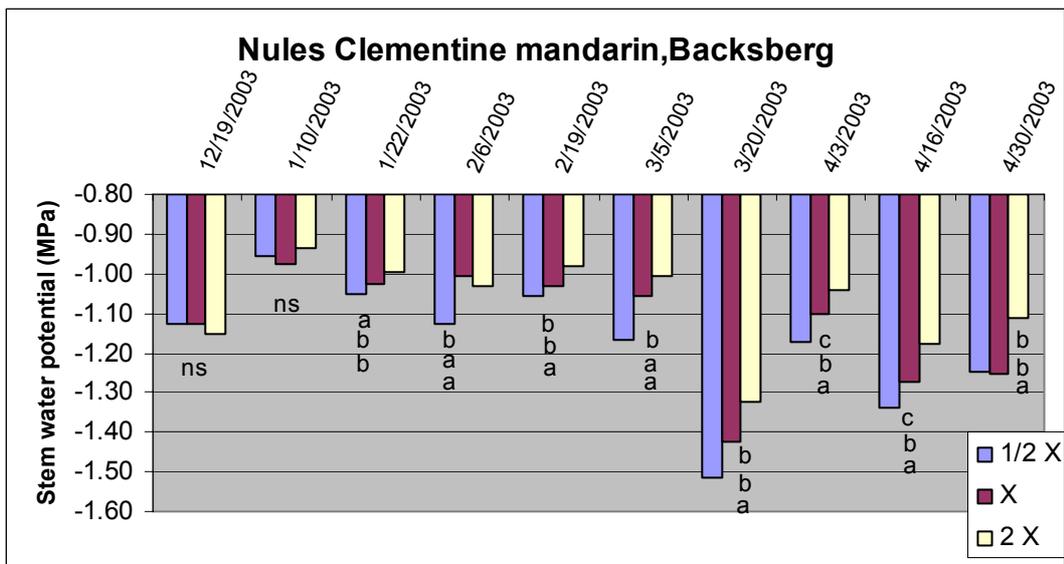


Figure 5.3.2.1. Stem water potential (MPa) measurements taken of Nules Clementine mandarin over a period of 5 months at Backsberg. Leaves were bagged on the west side of trees before sunrise and measurements were taken from 12h00. There were 6 replicates with 2 leaves in each replicate.

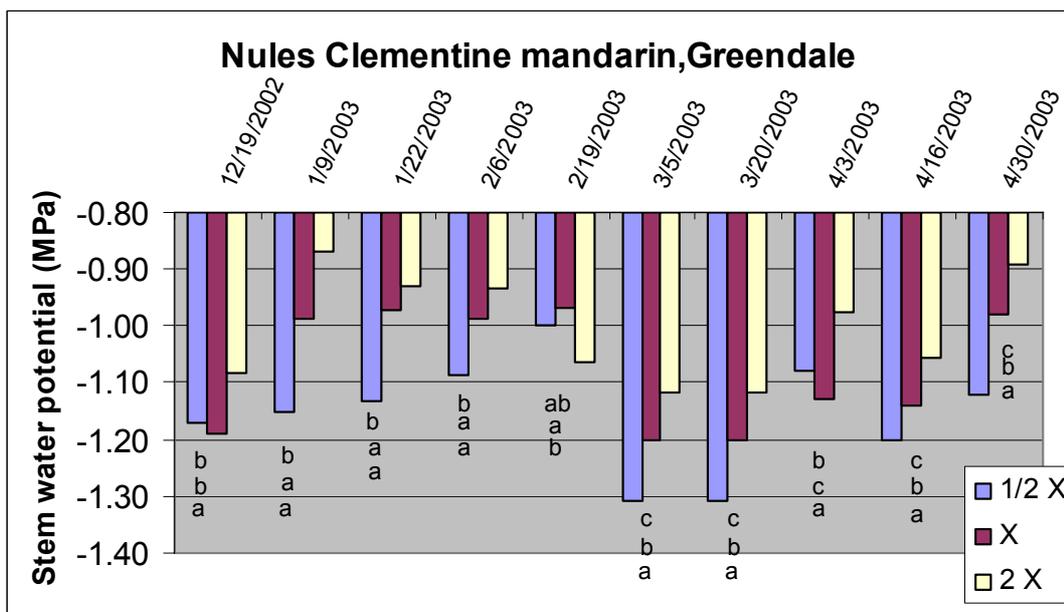


Figure 5.3.2.2. Stem water potential (MPa) measurements taken of Nules Clementine mandarin over a period of 5 months at Greendale. Leaves were bagged on the west side of trees before sunrise. Measurements were taken from 11h00. There were 6 replicates with 2 leaves in each replicate.

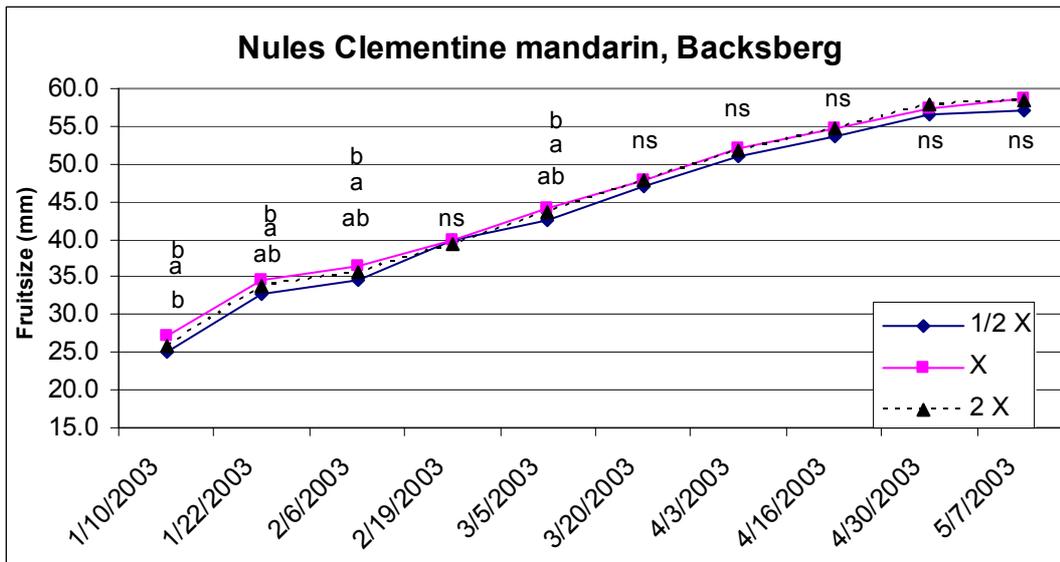


Figure 5.3.2.3. Tagged fruit size measurements of Nules Clementine mandarin over a period of 5 months at Backsberg.

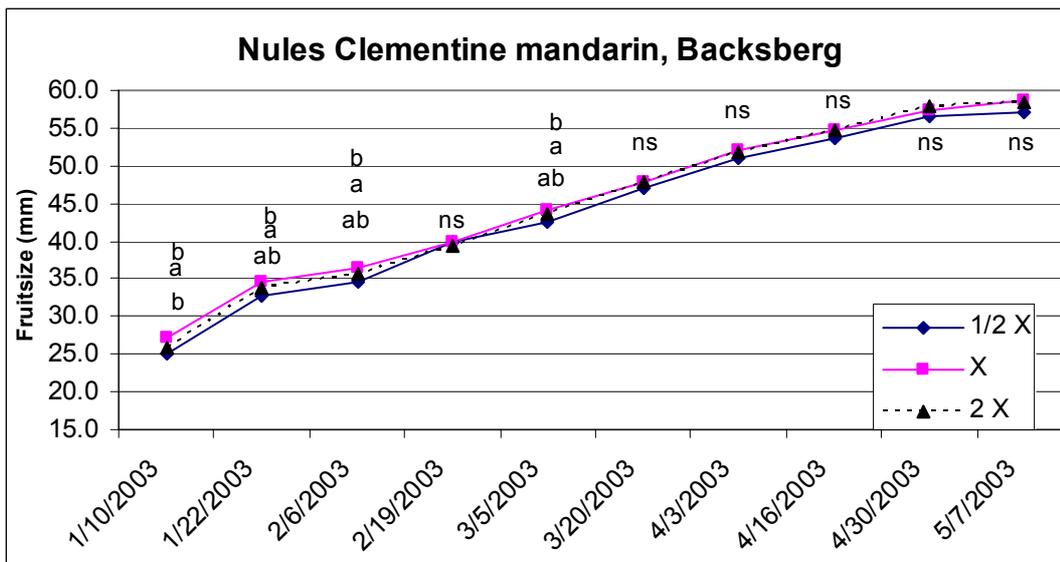


Figure 5.3.2.4. Tagged fruit size measurements of Nules Clementine mandarin over a period of 5 months at Greendale.

Table 5.3.2.1. Fruit quality of tagged Nules Clementine mandarin fruit harvested on 7 May 2003 at Backsberg.

Treatment	Fruit size		Fruit weight		Juice per		SSC		Acid		Ratio	
1/2 X	57.1	a	69.3	a	54.5	a	10.5	a	0.81	a	12.90	a
X	58.7	a	82.3	a	52.8	a	10.1	a	0.81	a	12.47	a
2 X	58.4	a	79.8	a	56.5	a	10.1	a	0.84	a	12.11	a
p-value	0.1364		0.1473		0.3195		0.4287		0.7633		0.4716	
lsd	5.3		14.1		5.1		0.74		0.08		1.33	

Table 5.3.2.2. Fruit quality of tagged Nules Clementine mandarin fruit harvested on 7 May 2003 at Greendale.

Treatment	Fruit size		Fruit weight		Juice %		SSC		Acid		Ratio	
1/2 X	58.0	a	70.0	a	52.4	a	11.2	a	0.91	a	12.60	a
X	54.8	b	69.2	a	53.2	a	10.6	ab	0.87	a	12.26	a
2 X	56.2	ab	66.9	a	55.0	a	10.4	b	0.81	a	12.96	a
p-value	0.1698		0.9784		0.2688		0.0331		0.4044		0.8262	
Lsd	2.6		37.2		3.8		0.61		0.18		2.34	

Table 5.3.2.3a. Fruit size of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	46.4	a	50.0	ab	53.0	a	57.2	a	61.3	a
X	44.7	b	49.1	b	53.1	a	56.5	a	61.2	a
2 X	46.2	a	50.8	a	53.2	a	56.6	a	60.3	a
p-value	0.0540		0.0517		0.9371		0.5849		0.3633	
lsd	1.50		1.37		1.38		1.51		1.44	

Table 5.3.2.3b. Fruit weight of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	52.7	a	63.3	a	72.3	a	86.1	a	101.5	a
X	47.7	a	60.0	a	73.0	a	83.3	a	101.9	a
2 X	52.0	a	64.8	a	72.2	a	83.9	a	96.9	a
p-value	0.3407		0.4052		0.9469		0.7101		0.5221	
lsd	7.6		7.5		5.8		7.6		10.0	

Table 5.3.2.3c. Juice percentage of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	41.0	a	47.4	a	50.2	a	52.1	a	52.2	a
X	41.0	a	48.0	a	50.5	a	51.9	a	52.3	a
2 X	42.0	a	48.5	a	50.2	a	51.6	a	52.3	a
p-value	0.8733		0.5269		0.8339		0.6719		0.9325	
lsd	4.6		2.1		1.4		1.1		0.945	

Table 5.3.2.3d. SSC of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	10.9	a	10.4	a	10.9	a	10.9	a	11.4	a
X	10.6	a	10.4	a	10.5	a	10.7	a	11.1	a
2 X	10.8	a	10.3	a	10.7	a	10.7	a	11.4	a
p-value	0.4694		0.9893		0.2460		0.6668		0.3798	
lsd	0.4		0.7		0.5		0.5		0.5	

Table 5.3.2.3e. Acidity of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	2.64	a	1.99	a	1.42	a	1.16	a	0.94	a
X	2.72	a	1.89	a	1.43	a	1.14	a	0.93	a
2 X	2.64	a	1.88	a	1.44	a	1.09	a	0.92	a
p-value	0.8412		0.6080		0.9337		0.3075		0.6201	
lsd	0.32		0.25		0.13		0.08		0.05	

Table 5.3.2.3f. Ratio of Nules Clementine mandarin fruit harvested on a two-weekly basis at Backsberg.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	4.12	a	5.28	a	7.71	a	9.48	a	12.11	a
X	3.96	a	5.49	a	7.37	a	9.45	a	12.03	a
2 X	4.14	a	5.55	a	7.43	a	9.86	a	12.42	a
p-value	0.7034		0.7346		0.5895		0.5204		0.5328	
lsd	0.51		0.78		0.75		0.83		0.77	

Table 5.3.2.4a. Fruit size of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	42.8	a	47.4	a	51.8	a	56.5	a	60.6	a
X	41.2	ab	46.1	a	50.7	a	55.5	a	60.0	a
2 X	40.8	b	46.6	a	51.3	a	56.2	a	60.7	a
p-value	0.0966		0.4384		0.5261		0.5975		0.7061	
lsd	2.0		2.0		2.0		1.90		1.76	

Table 5.3.2.4b. Fruit weight of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	43.9	a	55.1	a	71.2	a	88.6	a	104.1	a
X	40.1	a	52.5	a	66.6	a	84.8	a	102.7	a
2 X	38.3	a	53.9	a	70.2	a	88.6	a	103.6	a
p-value	0.6276		0.9301		0.8441		0.8899		0.9834	
lsd	12.9		14.0		17.3		17.0		16.4	

Table 5.3.2.4c. Juice percentage of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	44.3	a	49.3	a	50.1	a	52.3	a	52.7	a
X	43.5	a	46.1	a	49.1	a	50.8	a	52.7	a
2 X	41.8	a	48.0	a	49.0	a	52.1	a	54.9	a
p-value	0.6573		0.5099		0.8182		0.7340		0.0901	
lsd	6.2		5.8		3.9		4.1		2.3	

Table 5.3.2.4d. SSC of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	10.3	ab	10.2	a	10.6	a	10.7	a	11.7	a
X	10.7	a	10.3	a	10.7	a	10.5	a	11.3	a
2 X	9.7	b	10.4	a	10.6	a	10.7	a	11.4	a
p-value	0.0548		0.7121		0.8260		0.5130		0.1919	
lsd	0.8		0.7		0.4		0.4		0.4	

Table 5.3.2.4e. Acid of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	2.97	a	2.33	a	1.58	a	1.07	a	0.93	a
X	3.29	a	2.42	a	1.67	a	1.16	a	0.93	a
2 X	3.27	a	2.23	a	1.53	a	1.06	a	0.92	a
p-value	0.8246		0.8755		0.8253		0.4565		0.9712	
lsd	1.25		0.77		0.49		0.18		2.13	

Table 5.3.2.4f. Ratio of Nules Clementine mandarin fruit harvested on a two-weekly basis at Greendale.

Treatment	3/5/2003		3/20/2003		4/3/2003		4/16/2003		4/30/2003	
1/2 X	3.74	a	4.78	a	7.07	a	10.19	a	12.65	a
X	3.64	a	4.39	a	6.77	a	9.33	a	12.20	a
2 X	3.16	a	5.01	a	7.22	a	10.29	a	12.41	a
p-value	0.6689		0.7766		0.9030		0.5006		0.8278	
lsd	1.49		1.85		2.12		1.82		1.55	

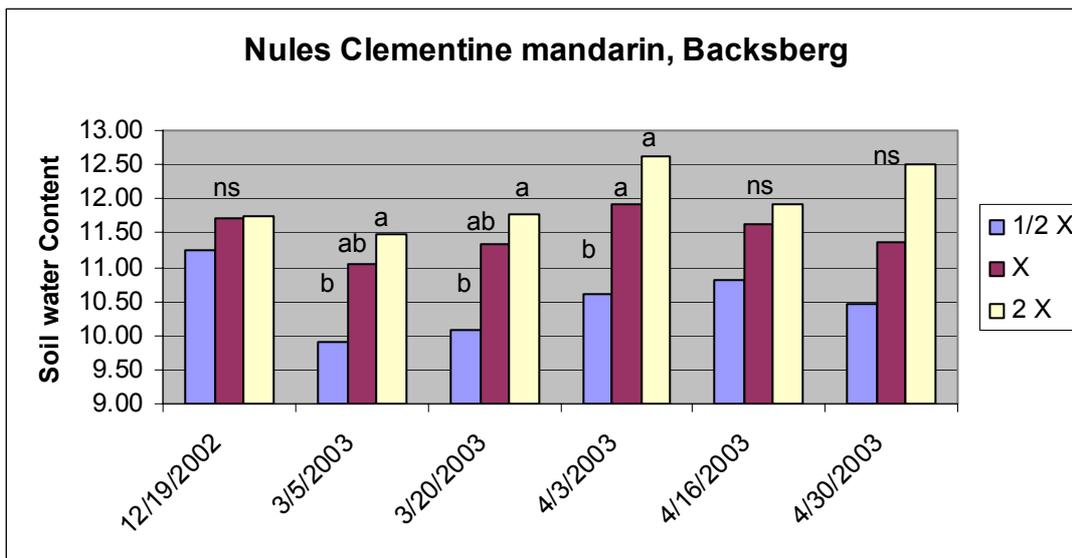


Figure 5.3.2.5. Gravimetric soil water content of Nules Clementine mandarin planted at Backsberg.

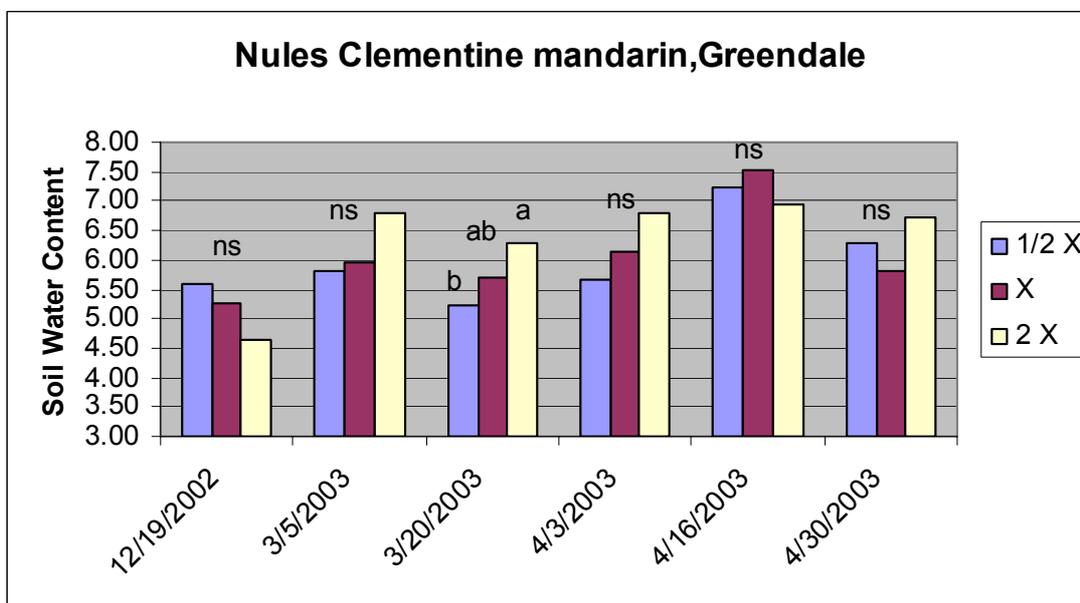


Figure 5.3.2.6. Gravimetric soil water content of Nules Clementine mandarin planted at Greendale.

5.3.3 Physiological responses of *Citrus* trees to altered plant water relations

Experiment FERT 01/02 by Ntombekhaya Ann Twalingca (Stellenbosch University) and Graham H. Barry (CRI at Stellenbosch University)

Opsomming

Die voor- en nadele verbonde aan daaglikse bemesting deur middel van die besproeiingstelsel is in die proses om gekwantifiseer te word. Gedetailleerde fisiologiese studies word tans onderneem om huidige aanbevelings en verbouingspraktyke te verfyn. 'n Deel van die navorsingsprojek poog om te bewys dat indien 'n verdere verlaging in die mid-dag daling van die gasuitruiling van die blaar bewerkstellig kan word met die toediening van bemesting deur die besproeiingstelsel (fertigation), 'n verhoogde koolstof vaslegging verkry kan word. Gevolglik sal vrugkwaliteit verbeter in terme van sapinhoud, Brix en sure as gevolg van die verhoogde translokasie van fotosintetiese produkte na die vrug.

Introduction

In any fruit industry, to meet and maintain the quality standards especially for export-market is a crucial and commercial factor, which necessitates farmers to be on par with the technologies towards improving fruit quality and most importantly the internal fruit quality. Reports on four different 'navel' orange cultivars on two

rootstocks, by ARC-ITSC Addo (unpublished data) for the seasons 1999-2000, 2000-2001 and 2001-2002 showed that daily fertigation (OHS) seems to be an effective tool in citrus to control, among other inputs, water and fertilizer management using a drip irrigation system.

The source-sink relationship is an important aspect to balance the distribution of photosynthates so as to support crop growth and not forsaking high distribution for flowering and fruit development since (Syvertsen, 1999) various plant organs (vegetative and reproductive) compete for available photosynthates. Nevertheless actively growing organs are strong sinks (Goldschmidt and Koch, 1996) while it is also true that competition for photosynthates is not only evident among different organs but is also apparent between fruit to fruit since fruitlet abscission is linked to carbohydrate status.

Water management, among other various cultural practices, may influence this balance in terms of sugar accumulation in fruit juice as an internal fruit quality trait. Translocation of photosynthates (Kadoya et al., 1981) from leaves to fruit involves a series of carbohydrate (CHO) metabolism thus contribute to accumulation of sugars in juice only when moisture supply is reduced at a certain fruit maturation stage.

The behaviour of stomatal conductance (gs) under water stressed conditions can be clearly defined if studies on factors influencing leaf gas exchange are carried out to illustrate the influence that leaf gas exchange has on midday depression in net carbon dioxide (CO₂) assimilation. Photosynthesis is directly and indirectly influenced by both environmental conditions like climate, soil physical and chemical conditions; cultural practices and plant physiological factors, which in turn with respiration determine the total amount of CHO supplied to other plant parts.

In well-managed citrus, severe water stress, vapour pressure deficit (VPD) and light (photosynthetically active radiation [PAR]) are some of the factors that can adversely affect photosynthesis and subsequently fruit quality.

This study therefore is to investigate, under reduced water stress conditions by daily fertigation, the physiological response of *Citrus* sp., specifically a decrease in midday depression in leaf gas exchange, which results in elevated carbon fixation. To elucidate the daily fertigation effect on internal fruit quality three experiments are being conducted on:

- (i) 'Midnight Valencia' orange (*C. sinensis* [L.]) on rough lemon rootstock (*C. jambhiri* [L.]);
- (ii) different 'navel' orange cultivars on rootstocks of differing vigour; and
- (iii) the use of the kaolin (Surround®) to enhance photosynthesis by reducing strong irradiance to the leaves.

Materials and methods

Site selection. This research is being conducted in three orchards at ARC-ITSC, Addo in the Eastern Cape Province of South Africa. The site is located at 33°34'S, 25°42'E and 85 m above sea level, under semi-arid, cool sub-tropical climatic conditions with the mean annual rainfall of approximately 396 mm with a fair distribution throughout the year. The mean maximum and mean minimum air temperatures in summer months are 27.7°C and 13.7°C, respectively, while in winter it is 23°C and 6.6°C.

Soils are high in sand (60%), silt (17%) and clay (23%) on an Oakleaf soil type with a pH of 7.8. Soils are also prone to potassium (K) and iron (Fe) deficiencies, and a poor water quality (pH 7 to 7.8 and high Cl and B) was determined (Addo – ARC-ITSC).

Progress Experiment 1: Effect of daily fertigation on photosynthesis and consequent effect on fruit quality of 'Midnight Valencia' orange.

Hypothesis. Daily fertigation increases net CO₂ assimilation by influencing stomatal opening, thus reducing midday depression.

Objective. To determine whether reduced water deficit stress by daily fertigation results in decreased midday depression in leaf gas exchange and hence elevated C fixation.

Plant material. Data are collected on 11-year-old 'Midnight Valencia' orange trees budded onto rough lemon rootstock in orchard D4 of ARC-ITSC at Addo Research Station, planted at a spacing of 6.6 x 4.0 m.

Treatments. Irrigation, at a discharge of 4 litres/hour with two drippers per tree is applied in 2, 4, and 8 weeks interval for each of the three major treatments; giving a total of 5 treatments of 4 replicates in a randomized block design.

- (i) Daily fertigation,
- (ii) Drip fertigation (double line irrigate at 2 week interval; single line at 2 week interval), and
- (iii) Conventional method (microjet with hand fertilization at 8 week interval; microjet and fertigation at 2 week interval).

Neutron probes are used for irrigation scheduling with measurements taken at 30, 60, 90 and 120 cm depths. Calculated concentrations of fertilizer (increased N and lowered K levels applied after harvest from June to November) according to leaf and water analysis as well as crop removal figures are applied and compared to conventional methods.

Measurements. For the past season (2002-2003) a sample of 20 fruit per tree were taken at maturity (late August) for yield and test data collection, i.e. using seven different counts to determine fruit size, total yield, creasing incidence, rind colour rating, TA (by titration), TSS (by refractometer), and TSS/TA ratio. During fruit growth and development, photosynthesis measurements (leaf gas exchange and stomatal conductance) were taken using a Portable Leaf Chamber Analyser (LCA-3).

Progress. Currently yield and test data collected are being statistically analysed. Unfortunately, all the photosynthesis data collected was detected to be incorrect due to the LCA-3 machine being faulty, which presently is being recalibrated at the Botany Department – University of Port Elizabeth.

Progress Experiment 2: Rootstock effect on midday depression.

Objective. To investigate the influence of rootstock on the photosynthetic characteristics of 'navel' oranges under reduced water stress conditions through daily fertigation.

Plant material. Different 10-year-old 'navel' orange cultivars (early maturing – Newhall and Navelina; mid maturing – Palmer and Bahianinha) budded on two rootstocks of differing vigour (rough lemon and Troyer citrange) and spaced at 6.0 x 3.0 m are used.

Measurements and progress. At maturity (late May 2003) a sample of 20 fruit from two-tree plots of three replicates in each cultivar/rootstock combination (orchard B1) were picked and tested (using seven different counts) for total yield, creasing incidence, rind colour rating, rind thickness, TA (by titration), TSS (refractometer), juice % and TSS/TA ratio.

Yield and test data were analysed statistically in which fruit obtained from all cultivars, and, compared to Troyer citrange, rough lemon rootstock (Figs. 5.3.3.1 and 5.3.3.2) showed that a higher percentage of fruit is distributed in the lowest counts (especially for Newhall), while a fair distribution of fruit is found throughout the counts more so on the medium-sized count. Table 5.3.3.1 shows that with the internal fruit quality of four different Navel cultivars budded on two rootstocks of differing vigour there is no significant difference on cultivars budded on Troyer in terms of yield, fruit mass, juice content, ratio and rind thickness; at the same time Troyer seem to have an influence in increasing TSS, TA, rind colour and on the negative creasing, to significant amounts in all cultivars when compared to rough lemon rootstock.

In terms of yield (Table 5.3.3.1) all cultivars behaved differently as a higher percentage of fruit between Palmer, Navelina and Bahianinha is distributed between counts of 72 and 56. Newhall on the other hand show that a larger percentage of fruit is obtained on the lowest count, irrespective of the rootstock, which could reflect a lot of unmarketable size, especially for export purposes. Meanwhile Bahianinha when budded on Troyer produce a lot of medium sized fruit unlike when under the rough lemon rootstock.

Nevertheless for meaningful results there is a need to collect data for at least two seasons to clearly indicate the effect of rootstock on Navel cultivars under daily fertigation, and the influence thereof on midday depression, in terms of yield and internal fruit quality.

Progress Experiment 3: The effect of kaolin on photosynthetic characteristics of Navel oranges.

Objective. To determine if sweet oranges under daily fertigation will lead to increased photosynthates produced if kaolin is applied onto the leaves to reduce the irradiance effect.

Plant material. 'Midnight Valencia' and 'Bahianinha Navel' oranges were used. Both cultivars are budded onto Swingle citrumelo rootstock, spaced at 6 x 3 m, and were planted in 1998.

Measurements. Two full cover sprays were applied in January and February 2004 and photosynthesis measurements will be conducted in early April 2004. Ten trees were treated with kaolin and ten trees served as an untreated control.

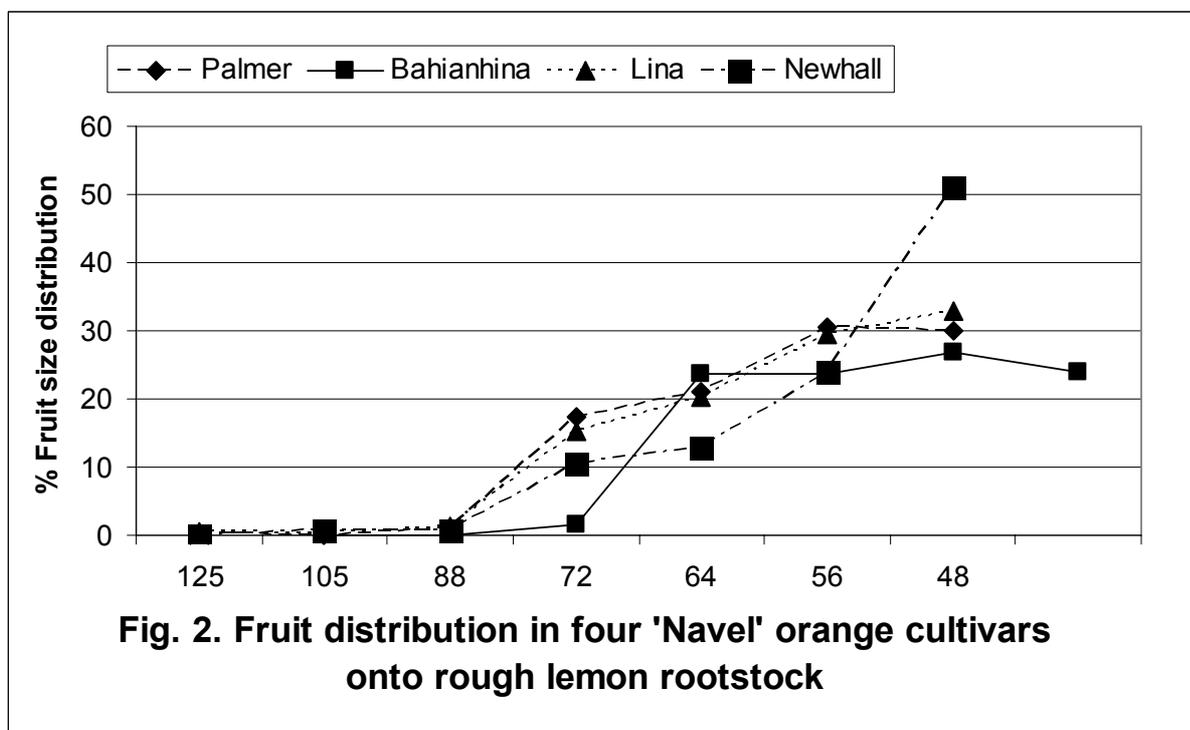
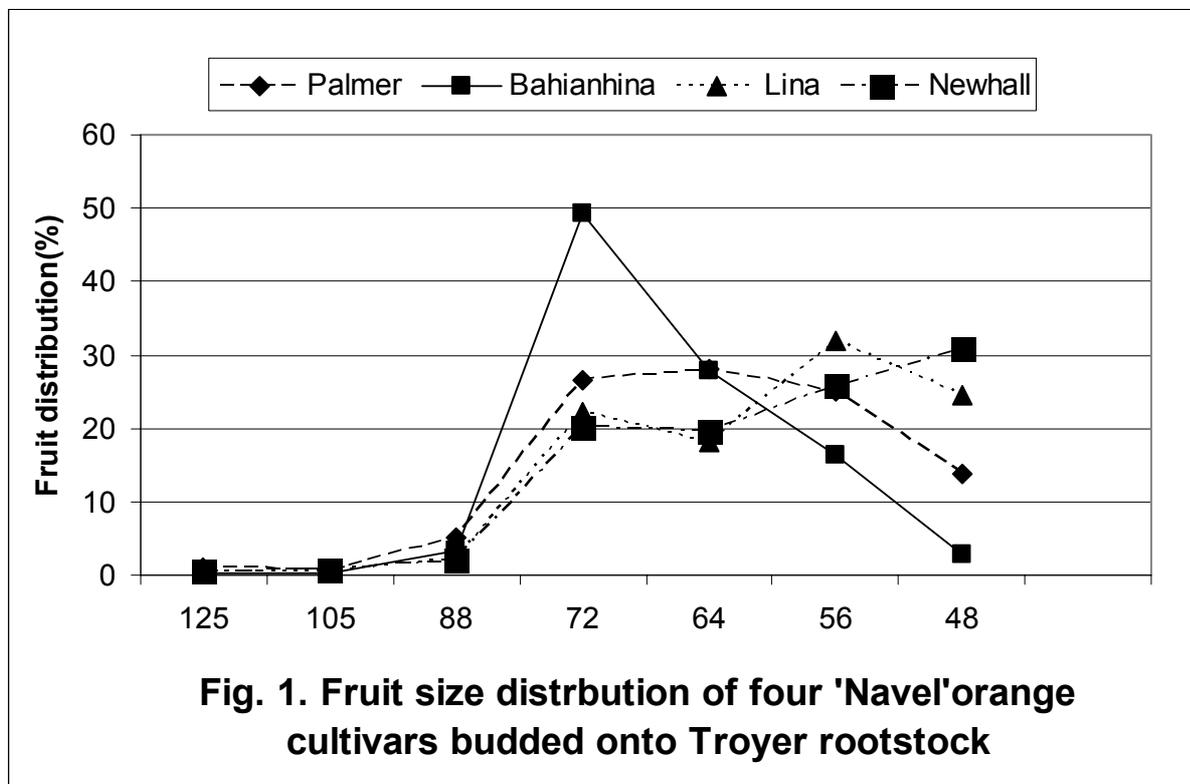


Table 5.3.3.1. Mean yield and fruit quality variables of four 'navel' orange cultivars on two rootstocks harvested in May 2003 at Addo Research Station, Eastern Cape (n=3, two-tree plots).

Source of variation	Yield (kg/tree)	Fruit mass (g)	Juice content ^x	TSS ^w	TA ^v	Ratio ^t	Rind thickness	Creasing	Rind colour
Cultivar (C)									
Newhall	60.10 c	270.18 a	49.14 a	11.04 a	1.06 a	10.88 ab	4.78 a	1.00 a	2.17 b
Navelina	75.31 bc	271.23 a	49.54 a	11.18 a	1.05 a	10.76 ab	5.02 a	3.50 a	2.42 b
Palmer	108.52 ab	264.72 a	47.05 a	11.15 a	1.02 a	11.10 a	4.70 a	3.00 a	4.08 a
Bahianinha	118.95 a	239.17 a	47.97 a	10.73 a	1.13 a	9.51 b	5.00 a	2.17 a	3.42 a
LSD ^z	33.79	50.05	2.01	0.81	0.14	1.56	0.57	3.58	0.94
Rootstock (R)									
Troyer citrange	85.75 ns	254.33 ns	48.84 ns	11.94 a	1.14 a	10.61 ns	4.77 ns	3.92 a	2.71 a
Rough lemon	95.69	268.32	48.01	10.29 b	0.99 b	10.51	4.98	0.92 b	3.33 a
LSD	23.90	35.39	1.42	0.57	0.09	1.10	0.41	2.53	0.67
P-value^y									
C × R	0.9114	0.9582	0.4415	0.8844	0.4295	0.4656	0.7703	0.5716	0.9780
C	0.0061	0.5055	0.0700	0.3907	0.3344	0.1740	0.5759	0.4921	0.0017
R	0.3912	0.4146	0.2351	< .0001	0.0510	0.8453	0.2736	0.2300	0.0643

^z Least significant difference ($P \leq 0.05$).

^y Probability values for interaction and main effects.

^x Juice content, w/w.

^w Total soluble solids.

^v Titratable acidity.

^t Ratio of TSS-to-TA.

5.4 PROJECT: RIND CONDITION

Project Co-ordinator: Frans Kruger (ARC-ITSC)

5.4.1 Project summary

An amount of R 408 881 (R 388 881 for projects + R20 000 for co-ordination) was allocated towards this project during 2003. At that stage, the project consisted of 7 experiments to be conducted by 4 researchers and their co-workers. The largest stake of the research funding (R 266 800) was earmarked for Piet van Rensburg of Citricom followed by Keith Lesar of the CRI (R 51 931), Taniith Freeman of the ARC-ITSC (R 50 150) and John Bower of UNP (R 20 000).

A number of major changes have taken place over the last year. Piet van Rensburg resigned as co-ordinator and was replaced by Frans Kruger. Paul Cronje joined the CRI and submitted a number of project proposals to be started during the 2004 season. He, nevertheless, helped complete some of Piet van Rensburg's research and conducted an *ad hoc* experiment during 2003. Taniith Freeman left the ARC-ITSC and her trials were taken over by Frans Kruger. It is envisaged that Regina Cronje, who is joining the ARC-ITSC during April 2004, will manage segments of these trials in future. John Bower informed the committee that he was not to use the R20 000 allocated towards updating his creasing prediction model during 2003.

Reports have been included on Piet van Rensburg's trials concerning chilling injury and rind breakdown conducted in 2002. Two reports were also submitted by Paul Cronje in collaboration with Piet van Rensburg. The first concerns the effect of harvest delays and degreening on rind breakdown in 'nules Clementine' (5.4.2). The second has to do with the relationship between controlled atmosphere and puffiness in 'Orovol Clementine' (5.4.3). Paul also conducted research on the evaluation of possible alternatives to replace 2,4-D presently used for calyx retention purposes.

A summary for year 2 of the ARC-ITSC's project dealing with the relationship between fruit mineral composition and physiological disorders such as rind pitting, stem end rind breakdown and chilling injury is included (5.4.5). Although the project started off as a survey, it now also includes a number of small trials aimed at clarifying certain aspects observed during the survey. The survey is further to be extended during year 3 so as to obtain additional data from other areas that may prove to be helpful in 'seeing the bigger epidemiological picture'.

A combined report is included on projects 5250/11b (ARC-ITSC) and 419b (CRI) dealing with chilling injury in 'Marsh Grapefruit' (5.4.6). The first part of the report deals with the effect that different wax types have on the incidence of chilling injury. The second part concerns the application of PGR's to 'Marsh Grapefruit' by the ARC-ITSC and then measuring the incidence of chilling injury by the CRI and rind pitting by the ARC-ITSC. However, due to the absence of rind pitting in a Marsh Grapefruit orchard where different PGRs were applied (see report 5250/11a for the possible reasons for this), it was decided to rather induce chilling injury in the grapefruit harvested by the ARC-ITSC. This proved to be the right decision because the phytosanitary mitigation temperatures used by Keith Lesar failed to induce chilling injury.

Projekopsomming

'n Bedrag van R 408 881 (R388 881 vir projekte en R 20 000 vir koördinerings) is vir hierdie projek gedurende 2003 toegeken. Op daardie stadium het die projek uit 7 proewe bestaan wat deur 4 navorsers en hul medewerkers uitgevoer moes word. Die grootste deel van die navorsingsbefondsing (R 266 800) was toegewys aan Piet van Rensburg van Citricom gevolg deur Keith Lesar van CRI (R 51 931), Taniith Freeman van die LNR-ITSG (R 50 150) en John Bower van UNP (R 20 000).

'n Aantal veranderinge het gedurende die afgelope jaar plaasgevind. Piet van Rensburg het bedank as koördineerder en is vervang deur Frans Kruger. Paul Cronje het by CRI aangesluit en 'n aantal projekvoorleggings gemaak wat gedurende die 2004-seisoen sal begin. Hy het egter 3 *ad hoc* proewe gedurende 2003 uitgevoer. Taniith Freeman het die LNR-ITSG verlaat en haar proewe is oorgeneem deur Frans Kruger. Daar word voorsien dat Regina Cronje, wat gedurende April 2004 by die LNR-ITSG sal aansluit, gedeeltes van hierdie proewe in die toekoms sal bestuur. John Bower het die komitee in kennis gestel dat hy nie die R 20 000 wat toegewys is vir die opdatering van sy kraakskil voorspellingsmodel gedurende 2003 sal gebruik nie.

Verslae is ontvang van Piet van Rensburg se proewe rakende koue skade (Citricom 1), peteca en skil afbraak (Citricom 4). Twee verslae is ook ingehandig deur Paul Cronje in samewerking met Piet van Rensburg. Die eerste handel oor die effek van oes verdragings en kunsmatige opkleuring op skil afbraak by 'nules Clementine' (5.4.2). Die tweede het te doen met die verhouding tussen beheerde atmosfeer en

pofferigheid by 'Oroval Clementine' (5.4.3). Paul het ook 'n derde (ongenommerde) verslag ingehandig wat handel oor die evaluasie van maontlike alternatiewe vir 2,4-D wat tans gebruik word vir blomkelk retensie doeleindes.

'n Opsomming vir jaar 2 van die LNR-ITSG se projek wat handel oor die verhouding tussen vrug mineraal samestelling en fisiologiese afwykings soos gepokteskil, stingelend skil afbraak en koue skade is ingesluit (5.4.5). Alhoewel die projek begin het as 'n opname, sluit dit nou 'n aantal klein proewe in wat daarop gemik is om aspekte wat opgemerk is gedurende die opname te verklaar. Die opname is ook verder uitgebrei gedurende jaar 3 om addisionele data van ander gebiede te verkry wat sal help om die "groter epidemiologiese prentjie" te vorm.

'n Gekombineerde verslag is ingedien vir projekte 5250/11b (LNR-ITSG) en 419b (CRI) oor koue skade by 'Marsh pomelo' (5.4.6). Die eerste deel van die verslag handel oor die effek wat verskillende waks tipes het op die voorkoms van koue skade. Die tweede deel gaan oor die aanwending van PGR're op 'Marsh pomelo's deur die LNR-ITSG en dan die bepaling van die voorkoms van koue skade by die CRI en gepokteskil by die LNR-ITSG. As gevolg van die afwesigheid van gepokteskil in 'n 'Marsh pomelo' boord waar verskillende PGR're aangewend is (sien verslag 5250/11a vir maontlike verklarings hiervoor) is besluit om eerder koue skade te induseer by die pomelo's wat by die LNR-ITSG geoes is. Dit was die regte besluit aangesien die fitosanitêre oorgangstemperature wat deur Keith Lesar gebruik is nie koue skade geïnduseer het nie.

5.4.2 Rind breakdown of 'nules Clementine' mandarins

Experiment 488a by P.J.R. Cronjè (CRI) and P.J.J. van Rensburg, P.J.J (Citricom)

Opsomming

Skilafbraak het 'n negatiewe finansiële impak op die uitvoer van Clementine manadryne sedert 1993. Studies wat gemik was om maatstawwe te ontwikkel om dië fisiologiese afwyking te voorkom het gefokus op boord praktyke. Daar is gevind dat 'nules' meer sensitief is as 'Oroval' en dat vrugte aan die buitekant van die blaardak minder skilafbraak ontwikkel as skadu vrugte. Gebruik van die ouksien 3,5,6-TPA het ook getoon dat dit voorkoms van skilafbraak verlaag. Naoes toediening van etileen het 'n negatiewe invloed en daarteen het osoon 'n positiewe invloed op skilafbraak. Verliese word egter steeds ondervind en daar is begin met proewe wat fokus op die naoes praktyke.

Gedurende 2003 is 'n proef op 'nules Clementine' gedoen om ondersoek in te stel na die invloed van tydperk tussen pluk en verkoeling asook na ontgroening op skilafbraak. Vrugte is vir 7, 11 en 14 dae na pak terug gehou voor dit verkoel is na -0.6°C vir 22 dae. Daar was twee stelle van die vrugte en een stel is ontgroen en die ander nie.

Die resultate wys 'n verhoging van skilafbraak by elke behandeling (7, 11 en 14 dae voor verkoeling) as daar ontgroen is. Die voorkoms van skilafbraak het ook toegeneem soos dae voor verkoeling toeneem. Die resultate dui daarop dat enige faktor wat daarvoor bekend is dat dit veroudering van 'n vrug versnel bv. hoë temperature en etileen, die ontwikkeling van die fisiologiese afwyking verhoog.

Introduction

Rind Breakdown (RB) is a postharvest disorder that affects mainly Clementine easy peelers. This particular rind disorder is thought to be a result of the collapse of the oil gland structure leading to the oil leaking out into the adjacent areas of the rind (Van Rensburg et al., 1995). RB occurs over the whole surface of the fruit and not in one particular area. The initial symptom is the development of small circular depressions in the peel, which corresponds to collapsed oil glands. Regions between the oil glands sometimes collapse, but to a lesser extent than the oil glands themselves. The tissue around the collapsed oil gland discolours within days after collapse and becomes bronze/brown. The number of these brown spots could range from one or two on a fruit to the most severe incidences where spots cover the whole fruit.

Previous studies (Van Rensburg, 2002) reported that certain factors are probable causes in the development of RB:

- Cultivar differences: 'nules Clementine' was more sensitive than 'Oroval Clementine'.
- Growing conditions: Fruit exposed to full sunlight were less susceptible than shaded fruit. Late harvested fruit are more susceptible than early harvested fruit. Less susceptible fruit had higher K and Ca levels and auxin (3,5,6-TPA) as a pre-harvest application during fruit development decreases the occurrence of RB.

- Postharvest conditions: A Postharvest ethylene treatment increases the incidence of RB. Ozone application in a postharvest situation decreased RB as well as decay and puffiness.

Van Rensburg et al. (2004) grouped these factors into the secondary causes. The primary cause would be the climatic conditions such as a warm winter and autumn that could increase the incidence of RB. The secondary factors then act as “triggers” with symptoms becoming visible after 3 to 4 weeks of storage.

The occurrence of RB in Clementines is not an isolated instance in citriculture of a physiological disorder that affects the rind of the citrus fruit. Similar symptoms of oil gland collapse have been reported: rind breakdown of 'navelate' sweet orange (Agustí et al., 2001), postharvest pitting of grapefruit (Petracek et al., 1995) and rind-oil spot or “Kohansho” of 'Encore' (Chikaizumi 2000; 2003). These disorders probably all have different causes but develop in roughly the same way. The rind gland structure collapses, which leads to oil leaking into and damaging the albedo and flavedo. Some of these disorders develop while the fruit are still on the tree and not postharvest e.g.; rind-oil spot of 'Encore' and rind breakdown of 'navelate' sweet orange. A few other rind disorders also add to the confusion in identifying these disorders correctly e.g. chilling injury, oleocellosis (peel collapse occurs primarily in regions between oil glands), peteca spot of lemon and creasing (peel collapse occurs primary in albedo), stem end breakdown (damage occurs almost exclusively near stem-end and typically encircles the stem-end) and physical and chemical burns.

RB has a significant economic impact on citrus production in South Africa. Consequently there is a need for better understanding of the impact of production practices and postharvest handling procedures on the disorder. Research into the preharvest factors has been reported by Van Rensburg (2002). Thus the experiment for 2003 focused on postharvest conditions between picking of the fruit and shipping. The aim of this study was to test the protocol time allocated for handling the fruit before initiation of cooling as well as the influence of ethylene application.

Materials and Methods

'nules Clementines' mandarin fruit were harvested on 31 May 2003 in the Stellenbosch area. Six bins of fruit were brought to the SunCape packhouse on 2 June, 3 of the bins were sent to Stellenpak in Simonduim for degreening (+degreening). The other 3 was kept at ambient packhouse temperature (-degreening). After 3 days of degreening the fruit were divided into another 3 treatments; 14 days, 11 days and 7 days before initiation of cold storage. The fruit was packed on a commercial packline and stored at ambient temperature ($\pm 20^{\circ}\text{C}$) in the packhouse for the designated period before cold storage. The days before cooling were counted from the day of harvest until initiation of cooling. The cold storage took place in integral containers at SunCape packhouse in Stellenbosch. The temperature was set at -1.6°C for 4 days, followed by -0.6°C for 22 days and 4.5°C whereupon evaluation started. The first evaluation consisted of counting all the fruit per carton and visual inspection for any rind disorders. The evaluations were repeated every week for 7 weeks. Fruit that had a recognisable rind disorder (RB or decay) were counted and removed from the carton. For each of the 6 treatments there were ten replications comprising a 15 kg carton. For each treatment cartons of the same fruit size distribution were chosen. Data were collected every week and analysed with PROC GLM using SAS, release 6.12 (SAS Institute, Carry, NC, U.S.A).

Results

In Figure 5.4.2.1 the RB means are presented over a seven-week period and a polynomial line was fitted for each treatment. An increase in the delay period prior to cooling lead to a significant increase in the incidence of RB after 4 weeks of storage at 4.5°C . Degreening tends to increase the incidence of RB consistently, although not significantly at a 1% level.

Figure 5.4.2.2 displays the data collected for fruit decay during the evaluation period for the 6 treatments. For each of the treatments a sigmoidal curve was fitted through the means. During the first three weeks of evaluation there was very little decay but a sharp increase was observed between weeks 3 and 5. The 7-day delay treatment resulted in the highest incidence of decay followed by 14-day delay and then 11-day delay. The degreening treatment enhanced the rate of decay development for all the treatments but these differences were not significant. No explanation is offered for these results.

Figure 5.4.2.3 shows the means of all six treatments during the 7-week evaluation time. A polynomial and a sigmoidal line were fitted through the RB and decay data, respectively. These figures illustrate the rate of the disorder development over time in storage. RB incidence had a sharp increase during the first week's whereafter a steady but less rapid rise occurred during the rest of the storage period. Decay showed a typical lag phase during week 1 to 3, a sudden increase towards week 4 and then a steady rate of decay development for the remainder of the experiment.

From the graphical representation of the data it becomes clear that if fruit with uniform maturity and quality attributes underwent different postharvest treatments would impact on the occurrence of postharvest disorders and decay.

Conclusion

Postharvest management of fruit is critical in maintaining fruit quality at a level for adequate shelf-life period. What should be remembered in the handling of big volumes of fruit is the variability of the fruits sensitivity towards the development of a disorder. This always needs to be taken into consideration and the postharvest practices adopted to accommodate the most sensitive fruit. Therefore it is necessary to understand the direct influence of the different postharvest practices on fruit and the possible disorders that could occur if the handling protocols are not upheld. Two factors, *viz.* delays in cold storage and degreening, were shown to impact negatively on occurrence of RB and decay.

Higher temperatures and exposure to ethylene both enhance fruit respiration, thereby hastening senescence and limiting subsequent shelf life. Stress associated with senescence could also enhance the development of physiological disorders like RB. It is therefore critical to expose the fruit to a minimum amount of time in the degreening rooms and to introduce the fruit as quickly as possible into the cold chain.

The need to preserve fruit quality by lowering the fruit temperature as quickly as possible is underlined by the results. These results should be taken into consideration when planning postharvest protocols.

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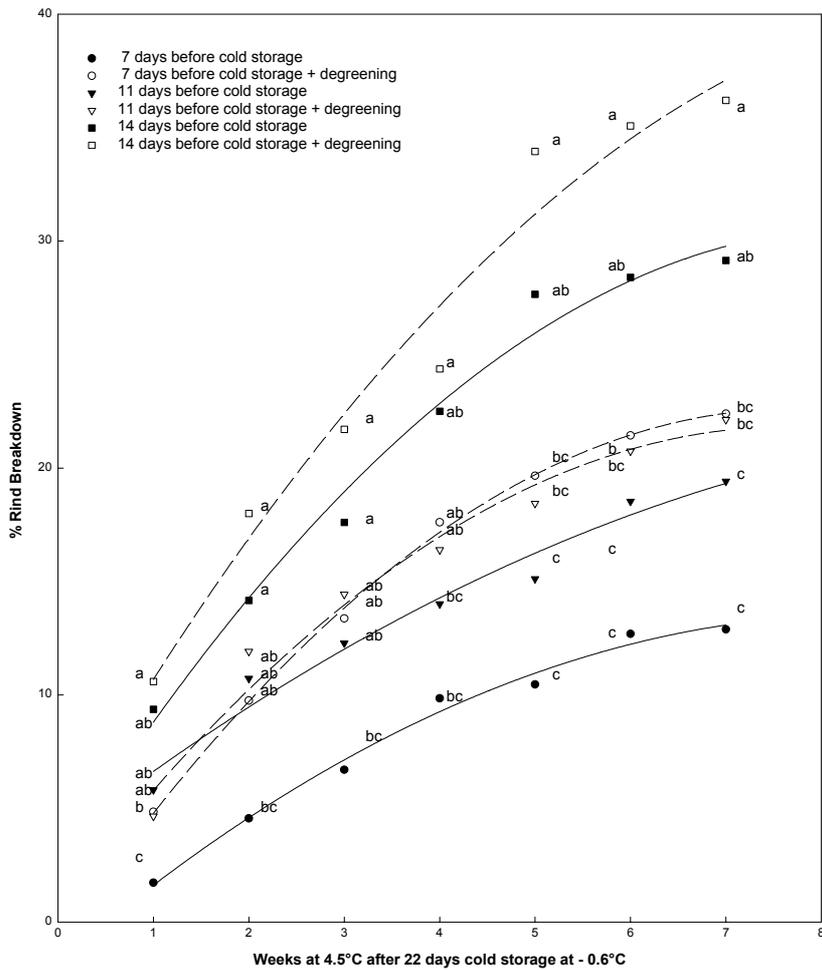


Fig. 5.4.2.1. The effect of delayed cold storage and degreening on occurrence of Rind Breakdown in 'hules Clementine' during 2003. Values represent the means of ten replications, those with different letters within the same evaluation week differ significantly at $P < 0.01$. Solid lines represent no-degreening and broken lines degreening treatments.

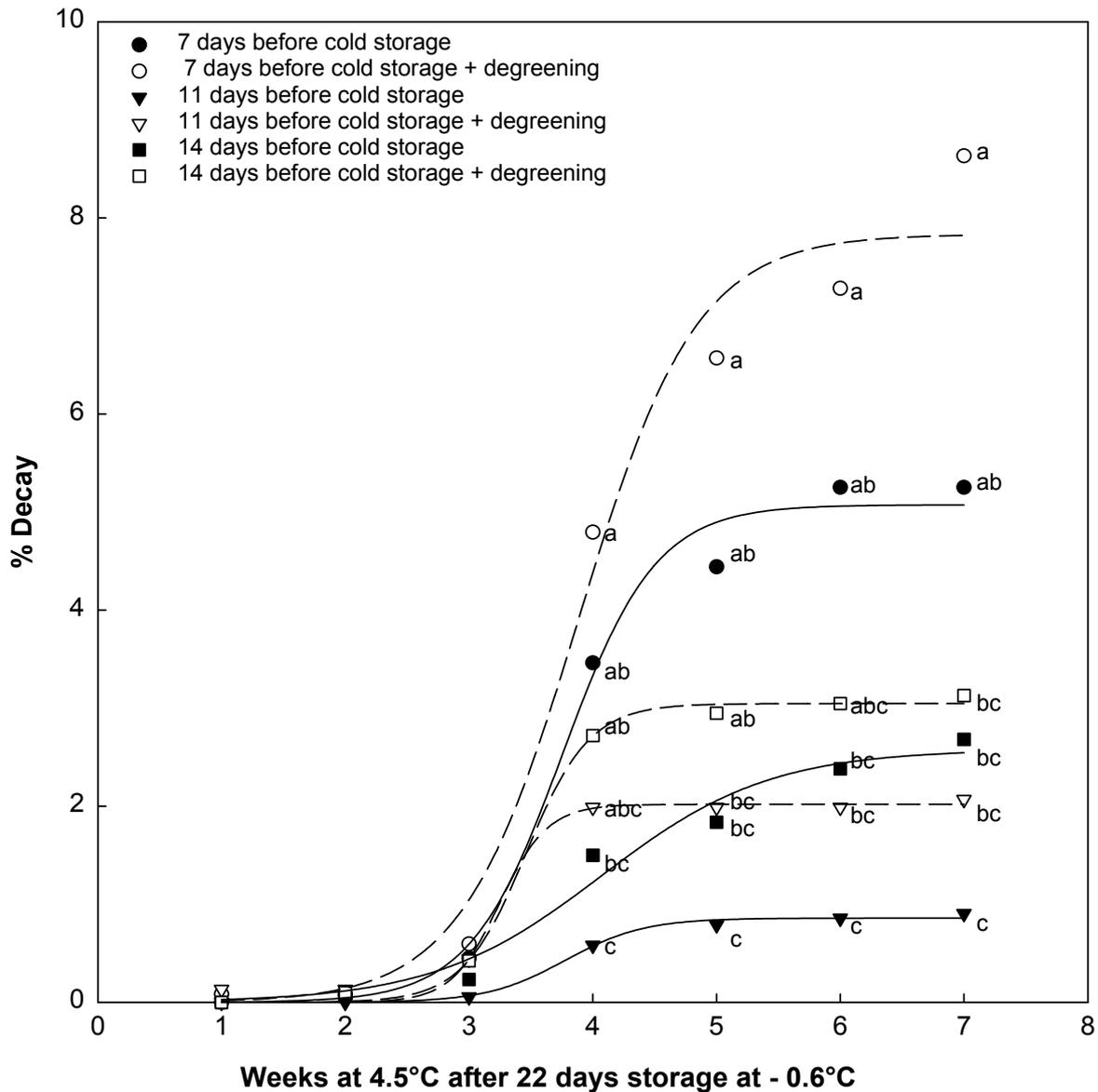


Fig. 5.4.2.2. Effect of delayed cold storage and degreening on occurrence of decay of 'hules Clementine' during 2003. Values represent the means of 10 replications, those with different letters within the same evaluation week differ significantly at $P < 0.01$. Solid lines represent no-degreening and broken lines degreening treatments.

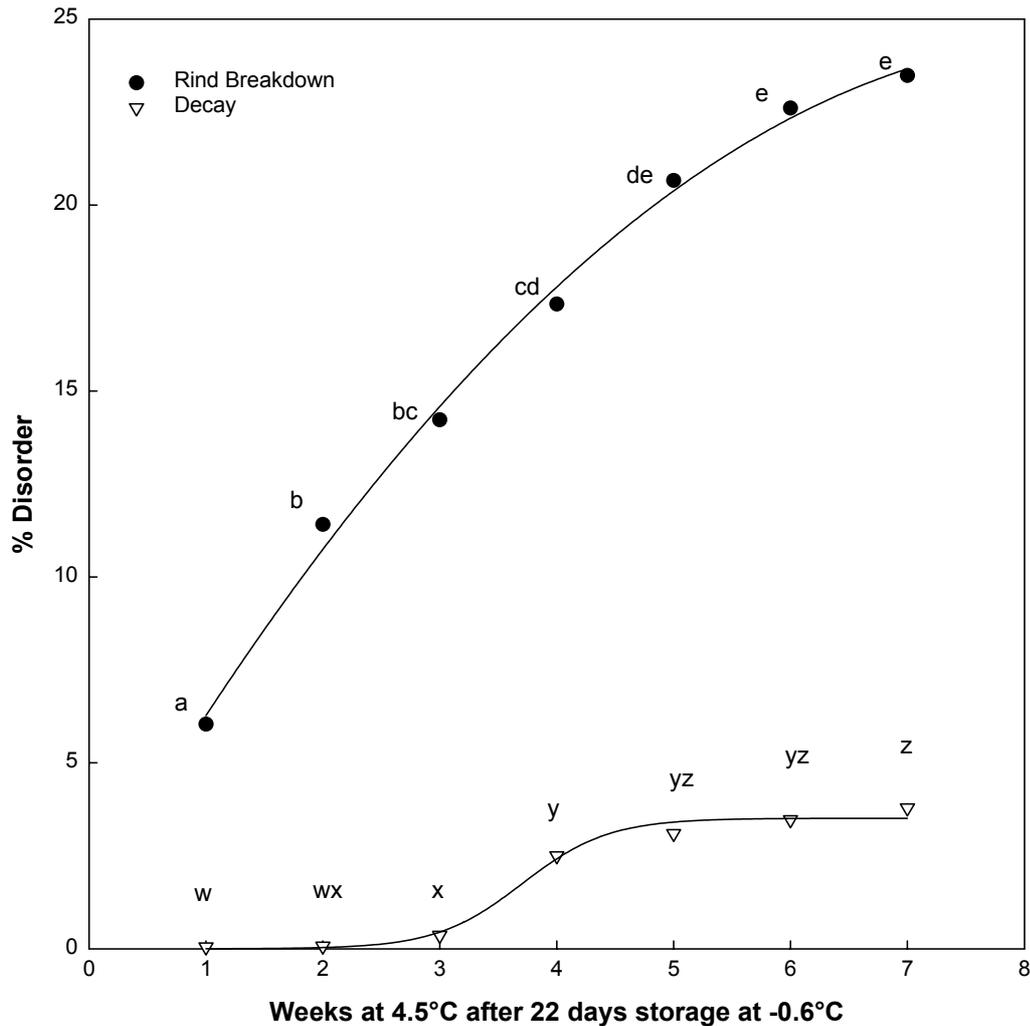


Fig. 5.4.2.3. The increase of the mean occurrence of Rind Breakdown and decay for all the treatments over a 7 week cold storage period. Values represent the means of all treatments, and those with different letters differ within a disorder significantly at $P < 0.01$.

5.4.3 Controlled atmosphere storage trial

Experiment 488b by Paul J.R. Cronje (CRI) and P.J.J van Rensburg (Citricom)

Opsomming

Die omringende toestande gedurende verskeping na die VSA (-0.6°C) het 'n negatiewe uitwerking op skil kwaliteit van Clementine mandaryne. Vrugte wat aan al die kwaliteit vereistes voldoen voor verskeping arriveer met verskeie naes fisiologiese afwykings soos pofferigheid, "regreening" en skil afbraak. In die proef is die effek van hoë CO₂ vlakke op skil kwaliteit ondersoek. 'Oroval Clementine' is by 5%, 10% en 20% CO₂ en 60% RH (by omringende CO₂ vlakke) opgeberg in koelkamers by 'n temperatuur van 9°C. Na 6 weke is die vrugte beoordeel vir pofferigheid en kleurverlies (geen skilafbraak is gesien nie, heel moontlik agv 'Oroval' wat minder sensitief is as 'nules'). Merkbare verhoging in pofferigheid is by 20% CO₂ gevind asook 'n kleur verlies. In 2004 gaan data oor die toestand aan boord van uitvoer skepe ingewin word. Dit sal aan die navorsers meer realistiese grense verskaf om die effek van CO₂, relatiewe humiditeit en temperatuur op vrugkwaliteit te bepaal.

Introduction

The strict phytosanitary requirements imposed by the USA concerning any fruit imports have a detrimental effect on fruit quality. Citrus from Southern Africa must be shipped at -0.6°C for 22 days to comply with quarantine protocols. Of the various citrus species exported, Clementine mandarin develops some of the most serious postharvest disorders such as rind breakdown, puffiness, loss of colour and chilling injury. Puffiness as a disorder has not received much research attention and only a few known studies were done on this disorder in Japan during the 1960's and in Spain in the 1980s. This research focused on puffiness that develops pre-harvest and not during shipping, but as the symptoms are related the relevance towards understanding the problem is obvious. Kuraoka (1962, 1966) found that the separation between the pulp and peel is related to the disintegration of the deepest cell layers of the albedo that gives rise to aerial spaces that result in cracked and low resistant albedo of mature fruit. The symptoms increase as peel grows just after the pulp has completed its development (Garcia-Luis et al., 1985). This belated peel growth takes place only in a few mandarin cultivars, such as Satsuma mandarins (Kuraoka, 1962) or 'Oroval Clementine' mandarin. These authors related the cause regulation of water exchange through the peel (Kawase et al., 1981). Accordingly, high relative humidity together with high temperatures at fruit colour-break increases the appearance and intensity of puffing particularly after a period of drought (Agusti et al., 2002). By the application of GA_3 (Garcia-Luis et al., 1985) and auxin (Agusti et al., 1994) the authors were successful in preventing the commercial incidence at harvest by more than 80%. Murata (1997) describes puffiness as a separation of the peel from the pulp during storage and states that as the result of advanced maturity, vigour of the tree and high humidity in the storage room, the rind becomes thickened and separates from the pulp.

Postharvest puffiness develops during the shipping period. Fruit that are within all quality requirements e.g. colour, sugar content and acid are packed, pre-cooled and shipped to the USA at normal protocol (-0.6°C for 22 days). The symptoms that are seen after shipping includes loss of colour or "regreening", fruit that are misshapen and bulging out into open areas between packed fruit. The rind starts pulling away from the pulp where segments meet and develops from a few millimetre separation to as much as 2 cm. These symptoms have also been seen to develop under the same postharvest conditions as rind breakdown, reported to be secondarily triggered by long ethylene exposure and long storage times (Van Rensburg et al., 2004). As a result of years of experience with export of South African citrus, people involved with the handling, packing and shipping of the fruit began to suspect that high CO_2 levels during shipping could be the source of the disorder. However due to a lack of any evidence a pilot experiment was done to study the effect of high CO_2 levels on puffiness of Clementine mandarin.

Materials and Methods

'Oroval Clementine' fruit were harvested from a commercial orchard in the Paarl area during the last week of June 2003. The fruit underwent all normal post harvest treatments and were packed on 25 June at SunCape packhouse in Stellenbosch. Fruit was transported to the cold storage facilities of the Department of Horticulture, University of Stellenbosch. The fruit were divided into 5 treatments: Control, Relative Humidity 60% (ambient CO_2), CO_2 concentrations of 20%, 10% and 5%. In all of the treatments fruit of equal size distribution were selected. The fruit were stored at 9°C in these specified conditions. These treatments were done to induce rind breakdown but after 6 weeks of storage no rind breakdown symptoms were observed. During the trial a difference in the number of puffy fruit was observed between the treatments. Fruit was evaluated according to; colour, puffiness, distance rind pulled away from pulp and the distance that the segments were separated from each other at the centre of the fruit. Data were analysed using SAS, release 6.12 (SAS Institute, Carry, NC, U.S.A).

Results and Discussion

On individual fruit that had severe symptoms of puffiness a loss of colour was seen. However, only the 20% CO_2 treatment gave a significant increase in the colour value (Fig 5.4.3.1). Similarly the 20% CO_2 treatment was the only treatment that showed a significant increase in puffiness (Fig 5.4.3.2). The peel of the 20% CO_2 treated fruit was also the furthest separated from the pulp (Fig 5.4.3.3) and the segments were the most separated from each other (Fig 5.4.3.4).

These results show a definite influence of high CO_2 levels on the occurrence of puffiness. The high concentrations of CO_2 used (20%, 10% and 5%) were chosen, as the researchers did not know what the actual concentrations are during commercial shipping. Experiments are planned for 2004 that will give us a more realistic range of CO_2 concentration during shipping.

The increase in severity of puffiness (Fig. 5.4.3.3 and 5.4.3.4) and the loss of colour (Fig. 5.4.3.1) in 20% CO₂ could be either a result of combined mechanisms or to different mechanisms reacting on the same stimulus of high CO₂ levels.

Future Research

During 2004 monitoring of CO₂, H₂O and temperature will be recorded on ship data loggers that would supply us with a better understanding of the actual situation during shipping. These values of prevailing conditions would then be used to study their effect on the rind condition of export citrus.

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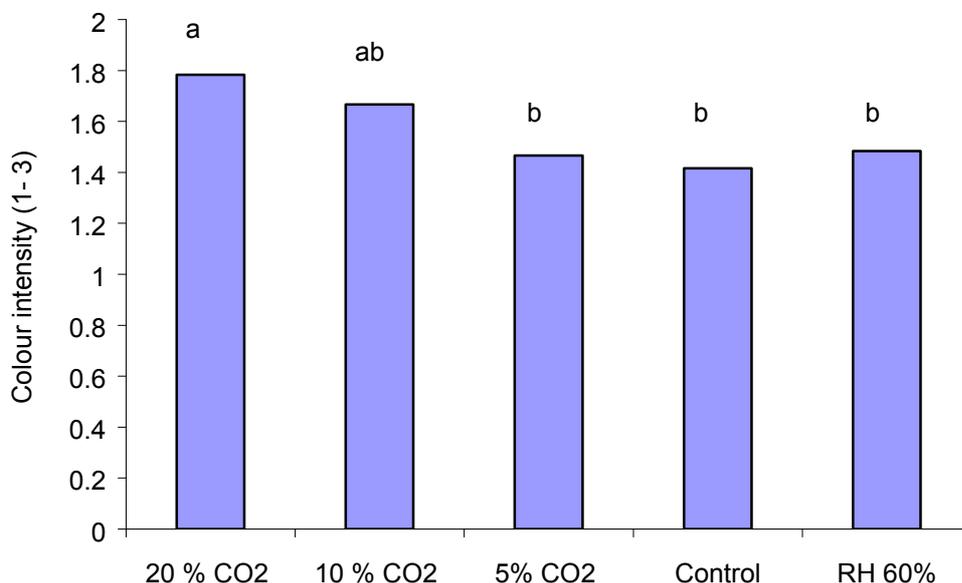


Fig. 5.4.3.1. The effect of different CO₂ concentrations and 60% Relative Humidity on external fruit colour of 'Oroval Clementine'. Fruit were evaluated in three classes, 1 equals most orange and 3 least orange. Columns sharing the same letter are not significantly different ($p > 0.05$) by Student's t-test.

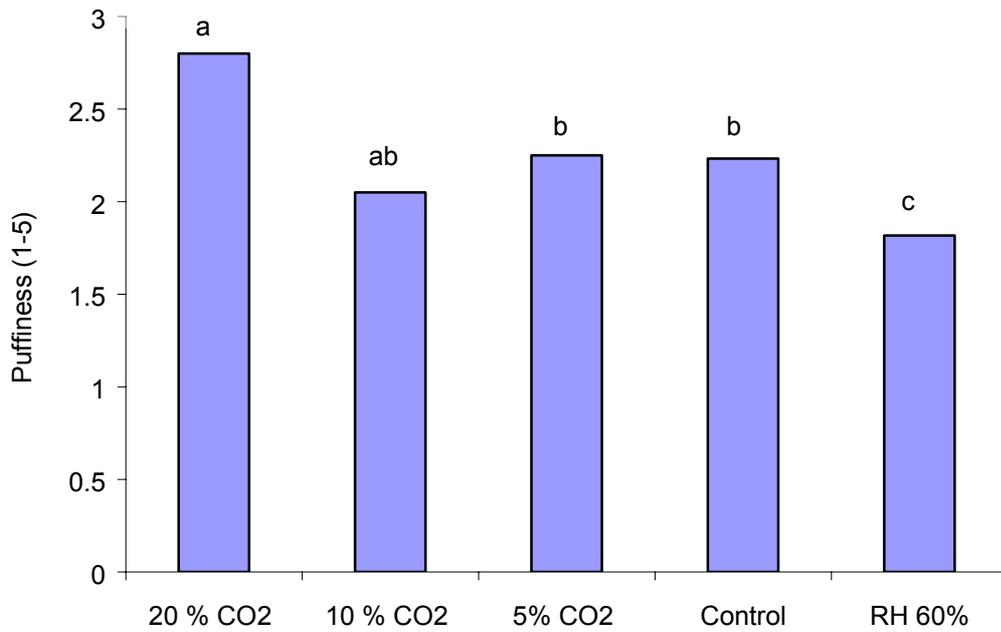


Fig. 5.4.3.2. The effect of different CO₂ concentrations and 60% Relative Humidity on **puffiness of 'Oroval Clementines'**. Fruit were evaluated in five classes of severity; 1 no puffiness and 5 most puffiness. Columns sharing the same letter are not significantly different ($p>0.05$) by Student's t-test.

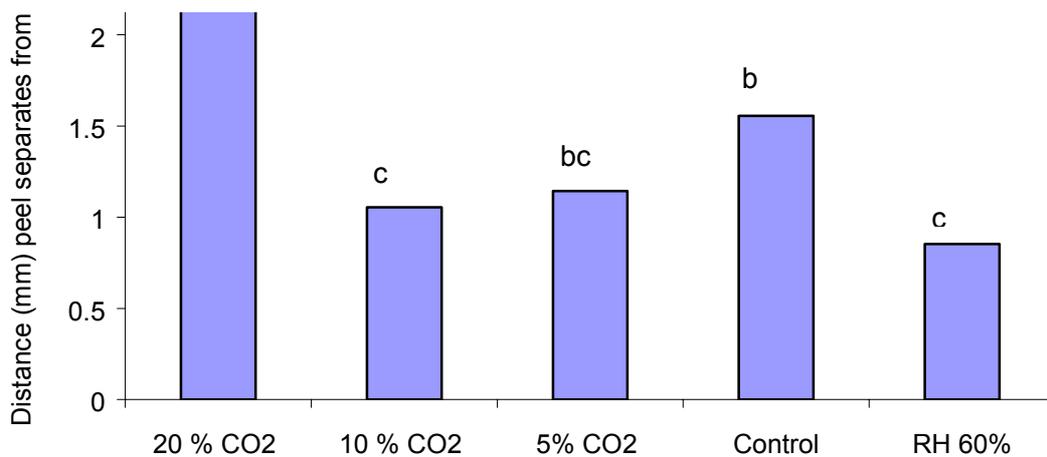


Fig. 5.4.3.3. The effect of different CO₂ concentrations and 60% Relative Humidity on the distance the **peel separates from the pulp** of the 'Oroval Clementine'. Columns sharing the same letter are not significantly different ($p>0.05$) by Student's t-test.

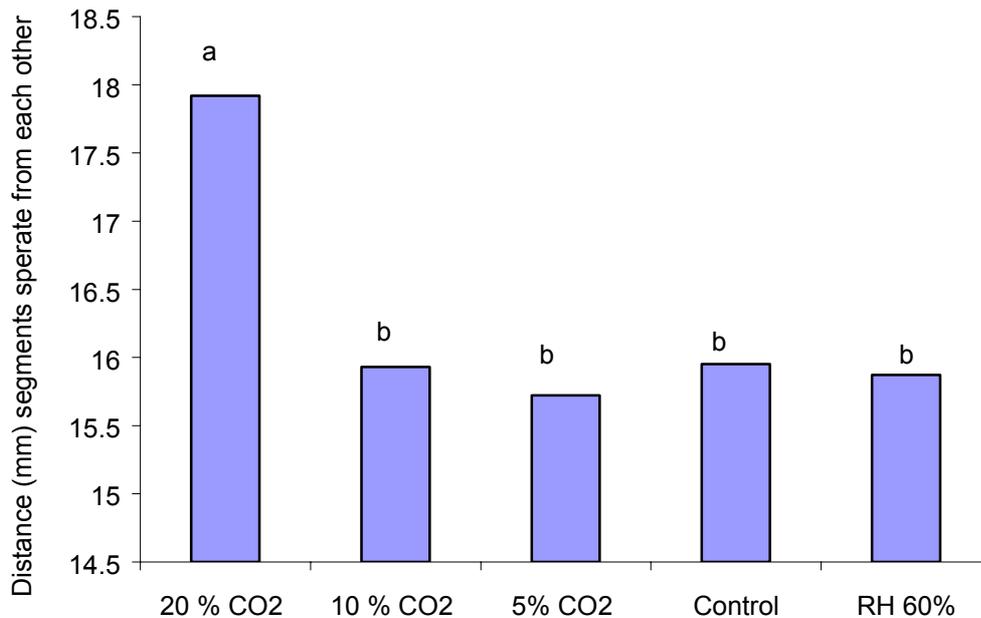


Fig. 5.4.3.4. The effect of different CO₂ concentrations and 60% Relative Humidity on the distance that **segments separate from each other** at the centre of the 'Oroval Clementine'. Columns sharing the same letter are not significantly different ($p > 0.05$) by Student's t-test.

5.4.4 Testing agrochemicals for their effectiveness in calyx retention of citrus fruit when applied as a post-harvest treatment

Experiment by Paul Cronjè (CRI)

Opsomming

Gedurende 2003 het die Europese Unie die MRL (maksimum vlak van opsporing) vir 2,4-D verander van 2 dpm na 0.05 dpm. Dit het die gebruik van 2,4-D as inhibeerder van blomkelk (calyx) absisise onmoontlik gemaak vir uitvoer na enige Europese land. 'n Proef is gedoen om bekende landbou chemikalie te toets wat moontlik vir dieselfde doel aangewend kan word. Drie anti-etileen middels is gebruik (a.g.v. die bekende positiewe uitwerking van etileen op absisise); AVG, 1-MCP en NAA. 1-MCP het die beste resultate gelever maar daar was seker negatiewe simptome wat met die gebruik gepaardgaan het, veral by te hoë konsentrasies. Ontgroening blyk om problematies te wees as dit saam met die middels gebruik word. Die simptome wat gesien was is 'n uitdroging van die blomkelk al vind absisise nie plaas nie en as na ontgroening toegedien word verskyn uitermatig uitdroging van die vrug om die stingel ent. AGV het die fermheid van die vrugte ietwat verbeter. Geen aanbevelings kan nou al gedoen word nie maar opvolg proewe sal gedurende 2004 geskied.

Introduction

The phenomenon of natural abscission is almost unique for the plant kingdom. The advantages of abscission for a plant could be to perpetuate its fruit containing its seeds, removal of useless structures such as flowers after pollination and to remove old leaves that would shade new leaves (Salisbury and Ross, 1992). Abscission is an active developmental process, which occurs at a specific zone, the abscission zone. The target cells in the abscission zone, which are involved in the process of separation, are located in a specific layer, the separation layer, which is organized in a few cellular layers. These cells are not active until the time has come for the mature or senescent organ to abscise. Three major physiological stages can be identified in the process of abscission. The **first** is the stimulus by either natural senescence or an external factor (heat stress, hormonal treatments, etc.). The **second** stage is a signal characterised by several internal factors, such as decrease in the endogenous auxin at the abscission zone, protein degradation, chlorophyll breakdown and increased ethylene production. The **third** and last stage in the response is characterised by specific nucleic acid and protein synthesis. This synthesis is responsible for the *de novo* synthesis of the hydrolytic enzymes, cellulase and polygalacturonase, which are responsible for the degradation of the cell walls. In general it is accepted that the increase in ethylene production during this stage is followed by increased sensitivity of cells to ethylene (Goren, 1998).

The major plant hormones, which are involved in the control of the abscission process, are auxin, ethylene and more seldom abscisic acid (ABA). The abscission process is divided into two phases with respect to sensitivity to auxin and ethylene (Addicott, 1982). In the **first phase**, auxin is responsible for the elongation of the cells, which precedes the following cell wall degradation. At this phase the cells are insensitive to ethylene and auxin can delay the separation process. Besides cell division, the major anatomical change is cell elongation, which is induced by auxin. At the **second phase**, when the level of auxin decreases below a certain threshold and auxin loses its delaying effect, the cells react to ethylene, and auxin can even stimulate the process by inducing more ethylene synthesis (Goren, 1996).

The auxin, 2,4-D (2,4-dichlorophenoxy acetic acid), is a plant growth regulator that has been widely used in citriculture around the world since the 1950's (Steward *et al.*, 1952). When used as a pre-harvest spray, 2,4-D reduces fruit drop. As a post-harvest packhouse treatment it is used as a dip, drench or in the wax to retard calyx abscission. Retention of the calyx reduces the fungal decay that was a serious storage problem prior to the introduction of the technology involving the isopropyl ester of 2,4-D (Eiset and Lyon, 1981; Dewolfe *et al.*, 1959). This control is ascribed to repressing the entry of mycelium of *Alternaria* stem-end rot into fruits. Commercially the sodium salt 2,4-D (Deccomone®) is applied to the fruit in a dip treatment at 500 ppm.

During 2003 European authorities implemented new legislation regarding 2,4-D. This new legislation would be mandatory for all EU members from 1 July 2003 and changed the level of detection from 2 ppm to 0.05 ppm. This unexpected development had a negative impact on citrus export from South Africa to our traditional markets in the EU. As a suitable replacement strategy doesn't exist the aim of this experiment was to test several agrochemicals that could possibly replace the post-harvest application of the auxin 2,4-D in order to prevent abscission of the calyx.

Materials and methods

First experiment (June/July 2003)

Navel oranges (*Citrus sinensis*) from a commercial farm were used for the experiment. The fruit were harvested during week 25 in the Piketberg area of the Western Cape on the farm of A.J van Santen. The fruit received no chemical treatment after harvest and were transported in two harvesting bins to the Novo Packhouse in Paarl. Thirty fruit were randomly packed by hand into a plastic tray (84 trays were packed). Each tray of 30 fruit represented a replication. For each treatment there were 6 replications. Thirteen treatments were applied using 4 different chemicals 2,4-D, 1-MCP, AVG and NAA.

Aminoethoxylvinyl glycine, AVG (ReTain®). AVG is a natural amino acid that forms during fermentation. It competitively inhibits the action of ACC synthase thereby blocking preventing inversion of SAM to ACC and thus ethylene biosynthesis in the plant tissue.

1-Naphthylacetic acid, NAA (Planofix®). NAA is an auxin used in certain apple and pear cultivars to prevent premature fruit drop and for thinning of certain apple cultivars.

1-Methylcyclopropene, 1-MCP (SmartFresh™). 1-MCP is used to significantly extend the storage and shelf-life of fruit and flowers by blocking the action of ethylene. 1-MCP attaches to the receptor proteins so that they no longer recognise ethylene, resulting in an inhibition of synthesis of internal ethylene and a lack of perception of external ethylene.

Different concentrations of AVG and 1-MCP were tested. Degreening (the commercial application of ethylene to induce peel colouration of cultivars lagging behind internal maturity) was used in combination with the chemicals to test this interaction. After sorting the fruit into the trays they were first drenched with a combination of fungicides (Ortho-Phenylphenol, Thiabendazole, Imazalil®), thereafter the 2,4-D, AVG and NAA were mixed into different containers. The fruit were dipped in the drench for 5 minutes. Those fruit selected for the 1-MCP gas treatment were transported to the Capespan Technology Development facility in Stellenbosch for the treatments. These treatments took place in airtight containers with a small electric fan for air circulation and took 24 hours. Those treatments that received Degreening were put into the Degreening room at Novo packhouse for 3 days. After the various treatments all the trays were stored at ambient temperature on the packhouse premises to induce environmental stress, which should lead to a higher rate of ethylene production, senescence and thus formation of the abscission layer at the calyx.

Data collection and Statistical analysis

The first evaluation was done three weeks after the treatments were completed and repeated 1 week thereafter. Evaluation consisted of testing each fruit for a loose calyx by rubbing over the calyx with the hand. After the second evaluation quality analyses (% juice, % acid, °Brix and TSS) were conducted on

representative samples in the quality laboratory of Novo packhouse. Fruit firmness at the equatorial position was quantified by using a densimeter with a 5 mm diameter tip (0-100 scale, high value = firmer fruit); three measurements per fruit were made. Data were analysed using the SAS, release 6.12 (SAS Institute, Carry, NC, U.S.A).

Second experiment (September 2003)

The second experiment was conducted to test the effect of 1-MCP at different concentrations compared to 2,4-D on calyx retention, as the first experiment showed 1-MCP to be the promising treatment.

Midnight Valencia oranges were picked in the Piketberg area on the farm of A.J. van Santen and received at Novo packhouse on 1 September. The fruit did not receive any chemical treatment. The fruit were selected by hand and packed into cartons, 6 treatments of 6 replications each and a replication consisting of 25 fruit. All the cartons were taken to Capespan Technology Development in Stellenbosch and those that were randomly selected treated with 1-MCP at 100 ppb, 250 ppb and 500 ppb. The other treatments were No Chemical treatment (only wax), 2,4-D in drench and untreated control (no wax or 2,4-D or 1-MCP). All of these 5 treatments were waxed later on, but one treatment received neither any chemical nor wax. The 1-MCP treatment lasted for 24 hours, hereafter all the fruit were transported to Novo packhouse. All the fruit were drenched with normal fungicides (Ortho-Phenylphenol, Thiabendazole, Imazalil®), waxed and packed back into the cartons. The cartons were put into an integral container set at 4°C; the same as normal shipping conditions to Europe. Fruit were stored at this temperature for 3 weeks, followed by another 3 weeks at 15°C.

Data collection and Statistical analysis

The evaluations for abscission were done the same way as in the first experiment during this period. Colour measurements were done with a chromameter (Minolta NR 4000, Osaka, Japan). Three measurements were done per fruit on the equatorial region. After this evaluation internal analyses of a representative sample per treatment were done (% juice, % acid, °Brix and TSS). Data were analysed using SAS, release 6.12 (SAS Institute, Carry, NC, U.S.A).

Results

First experiment (June/July)

The results confirmed the necessity of searching for alternatives for 2,4-D as it was the best chemical available for the prevention of calyx abscission (Fig 5.4.4.1).

Although 2,4-D application had the lowest % calyx abscission, 1-MCP before degreening and AVG without degreening resulted in a statistically similar amount of abscission. AVG with degreening and NAA did not have any positive influence on calyx retention but the AVG application did have a positive reaction on fruit firmness (Fig 5.4.4.2). The 1-MCP application after degreening resulted after in some unforeseen side effects and showed symptoms that resembled stem-end browning. These symptoms, which were also seen when the 1-MCP was applied before degreening although the calyxes from 1-MCP were not abscised they were dry. Application of NAA resulted in very high abscission. This confirms the complex relationship between auxin concentration, ethylene and abscission.

Second experiment (Sept/Oct 2003)

The results from the first experiment showed that the high concentrations of 1-MCP used could be a problem. The experiment was repeated for that reason and only included 2,4-D and 1-MCP at 3 lower concentrations. Valencia type oranges were used as the Navel season had ended. The results confirmed that high concentrations (500 ppb vs. 100 ppb) enhanced the abscission process (Fig 5.4.4.3). Statistically, 100 ppb, 2,4-D, No Treatment and Only Wax were grouped together but 2,4-D had less than 1% abscission compared to the others at 2 to 3%. During the first experiment, a difference in colour was noted but no measurements were taken. During the second experiment colour measurement was taken with the chromameter. The results (Fig. 5.4.4.4) are presented as to Lightness, Chroma and Hue. Lightness: Colour is separated according to how dark or light it is; a high light value means a darker colour. Hue: This value shows what colour you have; green is a high and yellow a low value. Chroma: High chroma value means a more vivid colour and a low chroma a duller colour.

In Figure 5.4.4.4 the un-waxed fruit (No Treatment) were significantly darker more vivid and yellow than the rest. Contrary to expectation, no reduction in colour due to 1-MCP was seen. The differences between the

waxed and un-waxed fruit are probably due to the properties of the wax. Although not evaluated statistically, there did not appear to be any treatment effects on internal quality (Tables 5.4.4.1 & 5.4.4.2). No off-flavours were detected in the juice of the treatments.

After these evaluations and considering these findings of increased abscission as a symptom of senescence, the ethylene production of the 500 ppb and the control (Only Waxed) fruit were determined with a gas chromatogram (Varian 3300, Walnut Creek, CA, USA), using 25 fruit divided into 5 replications each per treatment. In addition the total ACC concentration was determined according to the method of Lizada and Yang (1979). The average production of ethylene for the 1-MCP treated fruit was $1.5179 \mu\text{l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ and the control fruit had no detectable levels. The average total ACC concentration of the 1-MCP and control fruit was 0.0975 and $0.1925 \text{ nmol}\cdot\text{g}^{-1}$ fresh weight respectively.

Discussion

Even though none of the chemicals tested could be used at this stage in replacing 2,4-D to prevent calyx abscission in citrus fruit, some interesting results were observed.

AVG (ReTain®) had a definite fruit firming effect (although not significant in all cases) and should be tested as a possible preharvest spray treatment (as normally used in the deciduous fruit industry) in order to manipulate the firmness of the fruit. Fruit firmness is a problem during high rainfall seasons and leads to an increased postharvest loss of produce. Our results did not concur with previous findings (Einset, Lyon and Johnson, 1981) that AVG and AOA (a related compound) inhibit the abscission of the calyx. The reason could be either a wrong formulation or concentration that was used. Our use of AVG enhanced calyx retention but only without degreening (not a normal commercial practise for early season citrus cultivars). The difference in % abscission between AVG applied with or without degreening could indicate that in the presence of exogenous ethylene, ACC synthase is re-activated and thus leads to a higher internal ethylene level and results in abscission.

The high percentage of abscission seen after application of NAA illustrates the complex nature of the auxin/ethylene balance in abscission. Normally a low concentration application of auxin reduces ethylene formation and promotes growth by cell elongation and division. In contrast, high concentrations induce phenomena such as epinasty, premature leaf abscission and inhibition of root and shoot elongation with intensified green leaf pigmentation, followed by plant senescence. Concomitantly, ethylene formation is stimulated. These effects are described as auxin overdose and provide the basis for the use of synthetic analogs of auxin herbicides (Grossmann and Hansen, 2001; Sterling and Hall, 1997). The interplay of ethylene and auxin in the regulation of abscission is complex, including effects of ethylene on auxin transport and conjugation. The hormonal control of abscission probably involves gene activation but the details await elucidation (Spiegel-Roy and Goldschmidt, 1996)

The results of the first 1-MCP experiment correspond with previous studies e.g. *Citrus* sp. (Porat et al., 1999; 2001) and flowers such as *Rosa* sp. (Cameron and Reid, 2000), and *Pelargonium* sp. (Serek et al., 1994; 1995), but the second experiment's results do not concur. In both the studies, ethylene was administered along with 1-MCP and resulted in the inhibition of abscission. The results from our second experiment could indicate that if no exogenous ethylene is present the 1-MCP bound to the receptor sites thereby preventing the binding of ethylene. Consequently, no signal is received to control ethylene metabolism and the tissues continue to produce ethylene as they fail to perceive the quantities already being synthesized (Mullins et al., 2000). This argument could explain the high ethylene production ($1.5179 \mu\text{l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) from the 1-MCP treated fruit compared to control fruit (none detected) and would explain why the 500 ppb 1-MCP resulted in such high abscission percentage.

The observation (no data shown) of the first experiment on the loss of colour development are in agreement with Porat et al., 1999; Sisler et al. (1999) Mullins et al. (2000) and Porat et al. (2001) who all showed the inhibition of degreening. But it seems from the second experiment results that once colour break has taken place 1-MCP do not have a marked influence.

An explanation for the difference in colour loss could be that if exogenous ethylene is applied (degreening process) the 1-MCP could inhibit breakdown of chlorophyll and carotenoid synthesis. If 1-MCP is applied after adequate colour development (as in experiment two) the influence on the colour is not a factor.

To conclude, according to what is known about the working of ethylene and abscission 1-MCP and AVG should have prevented abscission of the calyx. Unfortunately the results show that this complex plant mechanism is not so readily manipulated and 2,4-D is the best product to inhibit calyx abscission of citrus fruit.

Future research

Follow-up experiments for next season are proposed for better documentation of the physiological effects, e.g. respiration, ethylene production and internal quality, of 1-MCP on the non-climacteric citrus fruit.

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Table 5.4.4.1. Effect of postharvest treatments of various anti-ethylene chemicals on quality analyses of 'navelate' (*Citrus sinensis*) oranges.

Treatments	Juice (%)	° Brix	Acidity (%)	Ratio
+ 2,4-D Degreen	48	10	0.70	14.14
+ 2,4-D No Degreen	52	10	0.75	13.13
No 2,4-D No Degreen	45	10	0.66	14.78
No 2,4-D Degreen	50	10	0.75	13.21
AVG (83g) Degreen	46	10	0.63	15.63
AVG (166g) Degreen	48	11	0.73	14.86
AVG (83g) No Degreen	48	11	0.70	15.55
1-MCP (500ppb) Degreen	48	10	0.65	15.94
1-MCP (1000ppb) Degreen	53	10	0.55	17.9
1-MCP (1000ppb) No Degreen	50	10	0.72	13.68
1-MCP (1000ppb) After Degreen	46	9	0.61	14.36
NAA Degreen	49	9	0.36	14.66
NAA No Degreen	44	10	0.68	14.41

Table 5.4.4.2. Effect of postharvest treatments of 1-MCP on internal quality of 'Midnight' oranges (*Citrus sinensis*).

Treatment	Juice %	°Brix	Acidity %	Ratio
100 ppb 1-MCP	52	12.7	1.87	6.79
250 ppb 1-MCP	48	12.3	1.86	6.63
500 ppb 1-MCP	50	12.3	1.89	6.51
No Treatment	52	12.9	1.92	6.72
2,4-D	53	12.2	1.89	6.44
Only Wax	42	11.7	1.79	6.55

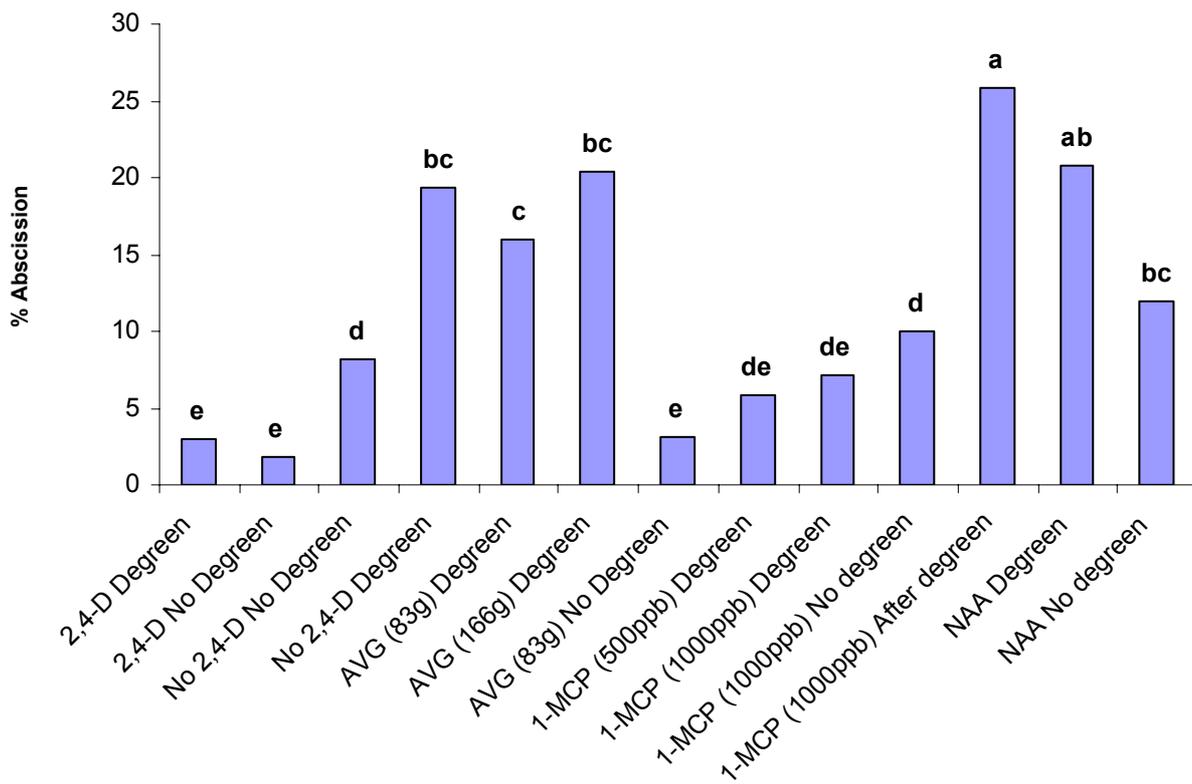


Figure 5.4.4.1. Percentage calyx abscission of 'Navelate' orange after treatment with three chemicals that influence ethylene production. Columns sharing the same letter are not significantly different ($p > 0.05$) by Student's t-test.

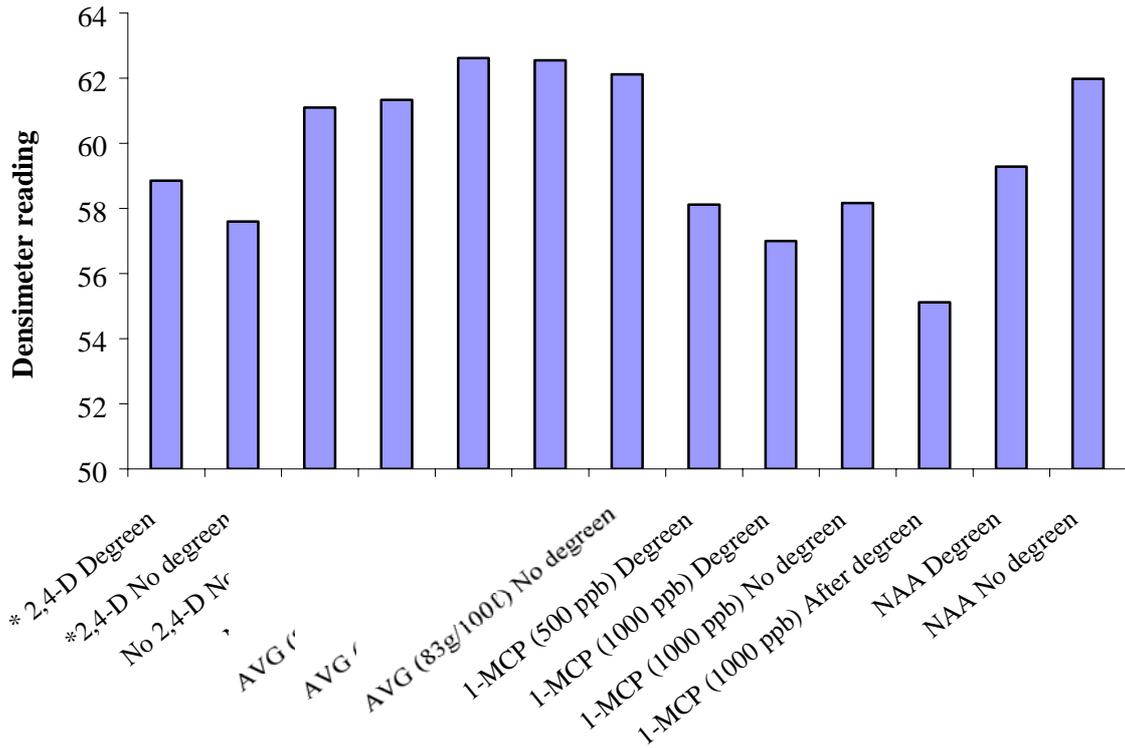


Figure 5.4.4.2. Fruit firmness measurements of 'navelate' orange after application of various chemicals influencing ethylene production. Data presented are the mean of measurements taken on 10 fruit per replication. Columns sharing the same letter are not significantly different ($p>0.05$) by Student's t-test.

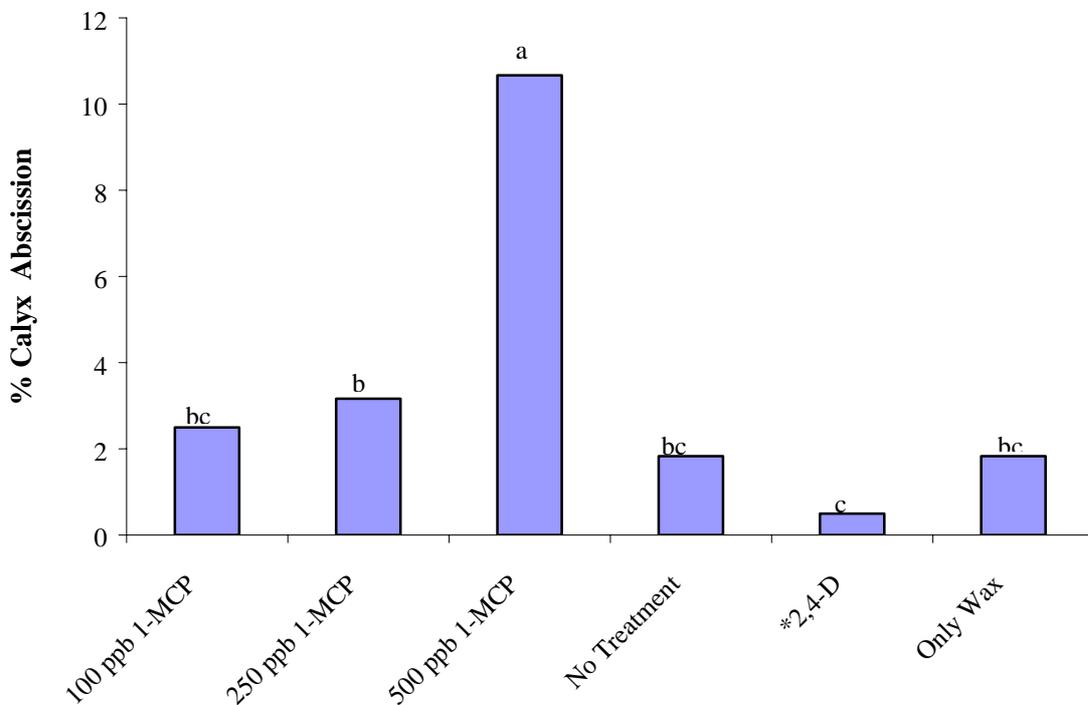


Figure 5.4.4.3. Abscission % of Midnight Valencia orange calyxes after treatment. Columns sharing the same letter are not significantly different ($p>0.05$) by Student's t-test.

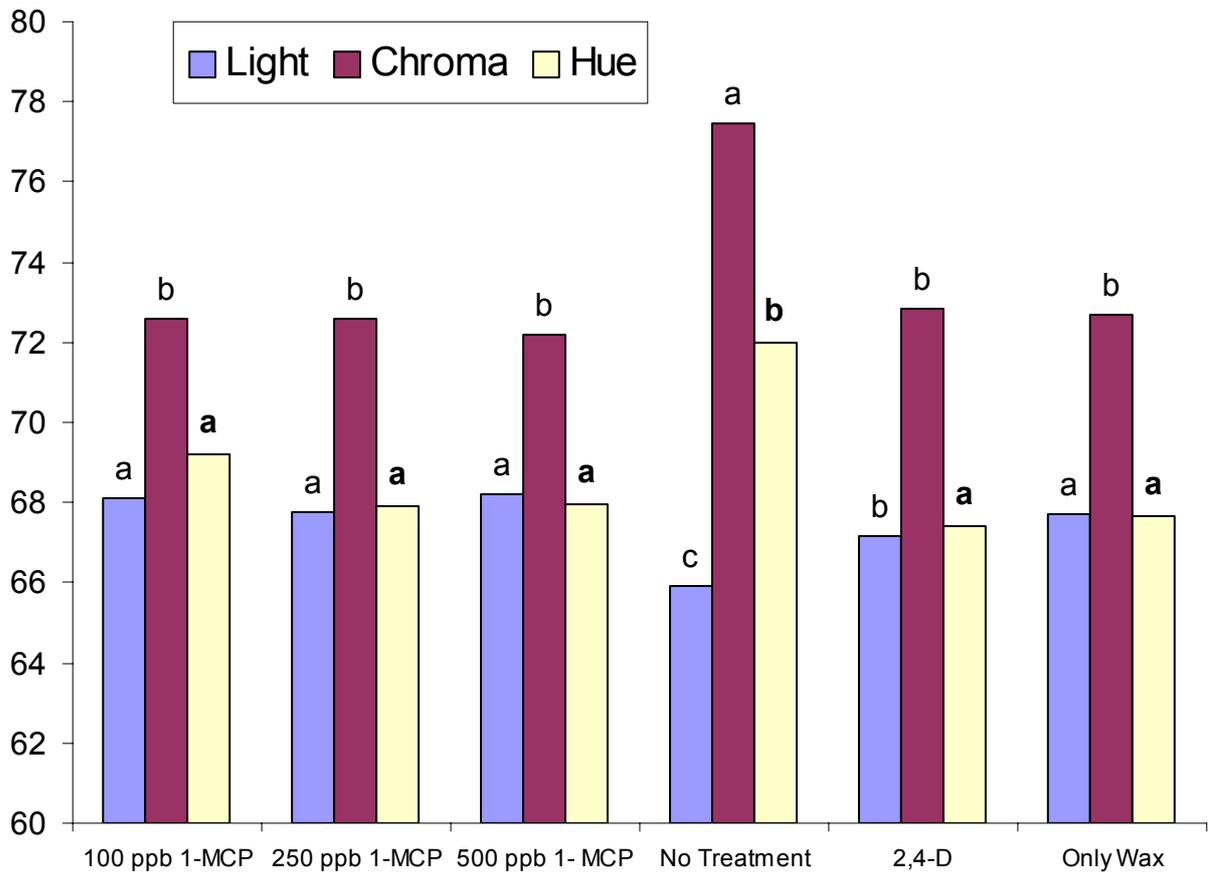


Figure 5.4.4.4. Effect of different concentrations of 1-MCP on the colour of Midnight Valencia's. Columns sharing the same letter are not significantly different ($p > 0.05$) by Student's t-test.

5.4.5 Role of rind mineral content in post-harvest rind pitting and stem end rind breakdown of citrus fruit

Experiment 5250/11a by F.J. Kruger & P. Chibi (ARC-ITSC)

Opsomming

Gepokte skil en stingelend afbraak is fisiologiese probleme wat die skil van uitvoer sitrusvrugte affekteer. Die huidige projek is in 2002 geloods as gevolg van die hoë voorkoms van gepokte skil wat by Transvaal Suiker Beperk (TSB) voorgekom het teen die draai van die eeu. Dit is later uitgebrei na Crocodile Valley Citrus Bpk. op grond van die hoë voorkoms van veral stingelend afbraak wat hier voorkom.

Ons navorsingsbenadering is om vas te stel of daar 'n verwantskap bestaan tussen die minerale samestelling van die skil en die voorkoms van fisiologiese afwykings. Om dit te bereik is die elementsamestelling in die vrugskil van 3 pomelos (Star Ruby, Rosé en Marsh) en een lemoen (Valencia) tydens groei en volwassewording bepaal.

Die resultate was uiters interessant. Die beweeglike en onbeweeglike elemente het verskillende verspreidingspatrone getoon. Byvoorbeeld, die skil van buitevrugte het ongeveer een derde meer (onbeweeglike) kalsium en boor bevat as binnevrugte. Verder het die skadukant van buitevrugte betekenisvol laer konsentrasies boor bevat as die gedeelte wat aan die son blootgestel was.

In teenstelling met die onbeweeglike elemente, was beweeglike elemente meer gekonsentreerd in skaduryke areas. Byvoorbeeld, binnevrugte van die Rosé kultivar het meer fosfor bevat as buitevrugte. By buitevrugte het die skadukant van die vrug oor die algemeen hoër konsentrasies van dié element bevat as die sonkant. Dit was ook die geval met kalium in die Star Ruby kultivar. Die beweeglike element samestelling van oostelike an westelike vrugte het ook verskil.

Die doel van die huidige studie is om vas te stel of daar 'n verwantskap bestaan tussen fisiologiese skil afwykings soos gepokte skil, stingelend skilafbraak en koueskade aan die een kant en die minerale samestelling van die skil aan die ander kant. In 'n tweede proef (5250/11b) was die doel om vas te stel of die aanwending van plant groei reguleerders moontlik kan bydra tot 'n verlaging in die voorkoms van skilafwykings. In laasgenoemde eksperiment is 1050 Marsh vrugte teen 2°C opgeberg vir 6 weke. Helfte van die vrugte is vanaf die noordekant van die bome geoes terwyl die ander helfte van die suidekant afkomstig was. In altwee gevalle is die gedeelte van die vrug wat aan die son blootgestel was gemerk. Tydens evaluering is gemerk dat die sonkant van die vrugte meer koueskade het as die skadukant (Dit is teenoorgesteld teenoor dit wat in avokados gevind is, maar stem saam met sitrus literatuur wat meld dat binnevrugte minder koueskade kry as buitevrugte.) Dit was egter interessant om waar te neem dat die vorm van die letsels verskil het. Alhoewel letsels meer algemeen aan die sonkant voorgekom het en hulle geneig was om saam te smelt, was die letsels redelik vlak. Daarenteen was die meer geïsoleerde letsels wat op die skadukant voorgekom het, groter en dieper as aan die sonkant. Soos die geval is met avokados waar koueskade deur 'n reeks faktore, insluitend die sogenaamde hiteskokproteïene beïnvloed word, is koueskade in sitrus ook 'n komplekse verskynsel. In terme van die huidige resultate is one hipotese dat die manifestasie van die simptome moontlik verband hou met diestrukturele eienskappe van die skil wat op sy beurt deur element samestelling beïnvloed word.

Ongelukkig (vanuit 'n navorsingsoogpunt) het geen gepokte skil tydens die 2002/3 seisoen by TSB voorgekom nie en gevolglik was dit nie moontlik om die voorkoms van fisiologiese afwykings met die mineraalinhoud van die skil te vergelyk nie. 'n Terugwerkende opname is egter gedoen waartydens die blaar elementontledings van die laaste 5 seisoene vergelyk is. Uit die opname het dit geblyk dat die blaarstikstofinhoud van die mees problematiese boorde so hoog as 3.5% was tydens 2001 (die jaar waartydens gepokte skil uitsonderlik hoog was by TSB). Bemestingspraktyke het sedertdien egter verbeter en die blaarstikstofinhoud van laasgenoemde boorde het verminder na 1.55 % gedurende die 2002 en 2003 seisoene. Geen gepokte skil het gedurende laasgenoemde seisoene voorgekom nie.

Met die oog daarop om bogenoemde hipotese te toets en toepaslike skil stikstof norme te ontwikkel, is besluit om 'n oordosis bemesting aan elk van die genoemde kultivars toe te dien. 'n Addisionele 2 kg KAN, verdeel in twee 1 kg dosisse, is tydens April en Julie aan 8 bome in die 4 boorde toegedien. 'n Verdere 2 bome in elke boord het slegs 1 kg gedurende Julie ontvang terwyl 4 kontrole bome in elke boord slegs die normale TSB toediening ontvang het.

Veranderinge in die stikstofinhoud van die blare is vanaf September 2003 tot Januarie 2004 aangeteken. Die stikstofinhoud van die vrugskil is gedurende Januarie 2004 bepaal en vergelyk met die waardes wat in die ooreenstemmende tydperk van die vorige seisoen bepaal is.

By Marsh het die blare van bome wat die dubbeldosis ontvang het die hoogste stikstofinhoud getoon, gevolg deur die bome wat die enkeldosis ontvang het en die kontroles. Dit was ook die geval by Star Ruby, alhoewel die onderskeid nie so duidelik was soos by Marsh nie. By Rosé was die blaarstikstof waardes van bome wat die dubbeldosis ontvang het betekenisvol hoër. Die enkeldosis bome se blaarstikstofvlakke was egter soortgelyk aan dié van die kontroles. In geval van Valencia was daar geen opmerkbare tendense nie.

By Marsh was die rangorde van die skilstikstofvlakke dieselfde as dié van die blare. Die skil stikstofinhoud van vrugte uit bome wat die twee addisionele stikstofvoedings ontvang het, was hoër as dié wat net een toediening ontvang het en dié was weer hoër as die kontroles. By Star Ruby het die 4 bome wat addisionele bemesting ontvang het, meer stikstof in die skil gehad as die kontroles, alhoewel die skil stikstofvlak nie presies ranggekorreleer met die blaar stikstof inhoud was nie. By Rosé en Valencia was daar nie 'n korrelasie tussen stikstof toedieningsdosis en skil stikstofvlakke nie.

Gedurende die 2004 seisoen sal die 32 bome in die 4 blokke geoes en die vrugte opgeberg word. Dit sal hopenlik die formulering van blaar en skil stikstofnorme, gemik op die voorkoming van gepokte skil, moontlik maak.

Wat stingelend skilafbraak betref, is 'n opname by Crocodile Valley Citrus Bpk. gedoen met die doel om die simptome te tipeer en die oorsake van stingelend skilafbraak te bepaal. Daarbenewens is 'n monster Clemlate vrugte met erge skilafbraak simptome vanaf 'n Wes-Kaapse produksiegebied ontvang. Hierdie vrugte is ook aan elementontledings onderwerp. Die mees opmerkbare verskil tussen gesonde en geïmpakteerde vrugte was dat die ysterinhoud van die skil van gesonde vrugte aansienlik hoër was dié van geïmpakteerde vrugte was.

Die oorsake en implikasies van laer ystervlakke by geïmpakteerde vrugte is op hierdie stadium moeilik bepaalbaar. Ons huidige hipotese is dat 'n aantal opeenvolgende droë seisoene moontlik mag bydra tot die

probleem, veral as besproeiingspraktyke ontoereikend is. Ironies genoeg mag onvoldoende stikstof tydens sekere kritiese tye moontlik ook die opname van yster negatief beïnvloed.

Na aanleiding van die resultate is 'n blaar yster toediening gedurende November 2003 in 'n Benny Valencia boord by Crocodile Valley Citrus Bpk. uitgevoer. Die produk Micrel (130 g Fe per kg) is teen 'n konsentrasie van 80 g / 100 liter opgemaak en teen 'n volume van 20 liter per boom toegedien. Aan die einde van Januarie was die blaar ysterinhoud van bome wat die behandeling ontvang het ongeveer 'n derde hoër as dié van ander boorde in die omgewing.

'n Volledige verslag sal opgestel word sodra die werk voltooi is.

Summary

Post-harvest rind pitting (PRP) and stem end rind breakdown (SERB) are physiological disorders affecting the rind of export citrus fruit. The present project was launched during 2002 as a result of the high incidence of PRP that occurred at Transvaal Suiker Beperk (TSB) during the turn of the century. It was later extended to Crocodile Valley Citrus Co. due to the high incidence of, especially, stem end rind breakdown at the latter farm.

The research approach taken was to establish whether a relationship exists between the mineral content of the rind and the incidence and intensity of rind pitting. An intensive survey of changes in the concentration of minerals in the rind of the fruit was conducted during the growth and maturation phases of three grapefruit cultivars (Star Ruby, Rosé and Marsh) as well as Valencia oranges.

The results were quite interesting. The mobile and immobile mineral element composition of the fruit of most cultivars was found to vary in relation to the position of the fruit within the tree canopy. For instance, outside fruit were found to have approximately a third more calcium and boron (immobile elements) in the rind than inside fruit. Also, the shaded side of outside fruit was found to contain significantly lower concentrations of boron than the side exposed to the sun.

In contrast, mobile elements such as potassium and phosphorus were shown to have precisely the opposite pattern to immobile elements. For instance, inside fruit of the Rosé cultivar contained more phosphorus than outside fruit. In outside fruit, the shaded side contained higher concentrations of this element than the sunny side of the fruit. There were also differences in the mobile element composition of eastern and western fruit.

The aim of the present trial is to determine whether a relationship exists between rind disorders such as postharvest rind pitting, stem end rind breakdown and chilling injury on the one hand and the mineral composition of the fruit on the other. In a second experiment (5250/11b) the aim was to establish whether the application of certain plant growth regulators contributes towards reducing the incidence of, amongst others, chilling injury in grapefruit. To do this, 1050 Marsh fruit were stored at 2°C for 6 weeks. Half of the fruit for the latter trial were obtained from the northern side of the tree and the other half from the southern side. The sides of the fruit that were exposed to the sun were clearly marked. When scoring the chilling injury, it was interesting to notice that chilling injury was more prevalent on the sunny side of the fruit than on the shady side. (This is exactly the opposite of what we found in avocados but it is consistent with citrus literature where it is reported that inside fruit are less susceptible to chilling injury than outside fruit). It was, however, interesting to note that the structure of the lesions differed. Although sunny side lesions were more prevalent and coalesced, they were shallow. On the other hand, the more isolated shady side lesions appeared to be larger and deeper than those on the sunny side. As is the case with avocados, where chilling injury is influenced by a range of physiological factors including the so called heat shock proteins, chilling injury in citrus is a complex phenomenon. In terms of the present results, our hypothesis is that the manifestation of the symptoms depend on structural skin characteristics that in turn is influenced by its elemental composition.

Unfortunately (from a research point of view) no rind pitting occurred at TSB during the 2002 – 2003 season and it was therefore not possible to compare the incidence of rind pitting with the mineral content of the rind. A retrospective survey was therefore conducted during which the leaf mineral analyses of the last number of seasons were compared. These yielded some interesting results, the most important of which was that the leaf nitrogen content of the orchards that displayed the highest incidence of rind pitting was as high as 3.5% in 2001, the year during which rind pitting was exceptionally severe. Fertilising practices have since been improved and the leaf nitrogen content of the latter orchards has been reduced to around 1.5% during the 2002 – 2003 season. No rind pitting occurred at TSB during the latter seasons.

In an attempt to test the above hypothesis, whilst also developing appropriate rind nitrogen content norms, it was decided to over-fertilise a number of trees in a single orchard of each of the above cultivars. Eight trees in the 4 orchards were therefore given an additional 2 kg of LAN, split into two 1 kg dosages, during April and July 2003. A further two trees in each of the four orchards received only 1 kg applied as a single dose on the second application date. A further four trees in each orchard served as controls. Changes in the nitrogen content of the leaves were recorded from September 2003 until January 2004. The nitrogen content of the fruit rind was hereafter determined and compared with the values recorded in the corresponding period during the previous season.

With Marsh, the trees that received the double dose were found to have the highest leaf nitrogen content followed by the trees that received the single dose and the controls. This was also the case with Star Ruby, although the distinction was less clear than in Marsh. In Rosé, the double dosage values were highest but the single application produced similar leaf nitrogen levels to the controls. In the case of Valencia, no trend was discernible. In the case of Marsh, the rind nitrogen content closely followed the trend recorded for leaf nitrogen content. The rind of fruit from trees that received 2 additional nitrogen applications had a higher nitrogen content than those that received only one application and these were in turn higher than that of the controls. With Star Ruby, the four trees receiving nitrogen had higher rind nitrogen levels than the controls, although the rind nitrogen level was not exactly rank correlated with the leaf nitrogen content. In Rosé and Valencia there did not seem to be a correlation between nitrogen application dose and rind nitrogen levels. During the 2004 season, the 32 trees in the four blocks will be harvested and the fruit subjected to conditions that are suitable for the development of rind pitting. This will hopefully enable the formulation of pitting specific leaf and rind nitrogen content norms.

In terms of stem end rind breakdown, a survey was conducted at Crocodile Valley Citrus Co. aimed at characterising the symptoms and determining the causes of SERB. In addition, a sample of Clemlates showing severe symptoms was obtained from a Western Cape production area. The fruit rind was subjected to mineral analysis. The most obvious difference between healthy and affected Clemlates concerned the iron content of the rind. The Fe content of healthy fruit was found to be considerably higher than that of affected fruit.

The reasons for insufficient Fe uptake into the fruit may be quite difficult to determine. Our current hypothesis is that a number of successive dry seasons may contribute to the problem, especially if inadequate irrigation practices are followed. Ironically, nitrogen deficiency during certain critical periods may also negatively influence the uptake of iron.

An experimental iron application has subsequently been made to a Benny Valencia orchard at Crocodile Valley Citrus Co. The product used was Micrel (130 g Fe per kg) at a rate of 80 g/100 litres and a volume of 20 litres per tree. At the end of January 2004, the leaf iron content of the trees in the treated orchard was approximately a third higher than those of the surrounding orchards. This and other orchards will be closely monitored during the 2004 season. A full report will be given when the work is complete.

5.4.6 Evaluation of methods aimed at reducing chilling injury in grapefruit

Experiment 419b by K.H. Lesar (CRI)

Experiment 5250/11b by F.J. Kruger (ARC-ITSC)

Opsomming

Hierdie studie het uit twee proewe bestaan. Tydens die eerste is die effek van verskillende wakse op die ontwikkeling van koueskade nagevors. In die tweede is die invloed van verskillende vooroes plant groeireguleerders op koueskade bestudeer.

Die eerste proef is met Marsh pomelos vanaf TSB (Hectorspruit) uitgevoer. Die pomelos is geoes, behandel met verskillende natuurlike (Carnauba) waks tipes en verpak vir koueopberging teen lae temperature. Die eerste evaluasie na kouesterilisasie, waartydens die behandeling vooraf verkoel is teen -1.8°C vir 72 ure en dan verskeep is teen -1.0°C vir 22 dae, het gewys dat die vrugte wat met Citrosol waks (Spaanse waks) behandel is, 1,1% koueskade ontwikkel het. Geen koueskade was sigbaar by enigeen van die ander behandelings nie. Die oorblywende behandeling is opgeberg teen 11°C vir 4 en 8 weke. Evaluasie na 4 weke het gewys dat koueskade op die Citrosol vrugte na 1,7% gestyg het. Na 8 weke het dit tot 6,3% verhoog. Nog steeds was daar geen koueskade in enige van die ander behandelings nie.

Tydens die tweede proef is Marsh pomelo bome te TSB (Hectorspruit) gespuit met Maxim (3,5,6-TPA), Corasil E (2,4-DP) en Deccomone (2,4-D). Vrugte van hierdie proefperseel is tydens Maart 2003 geoes en

onder fitosantêre sterilisasie/uitvoer toestande (-1.8°C vir 72 uur gevolg deur 1°C vir 14 uur gevolg deur 11°C vir 8 weke) by die CRI opgeberg. Monsters is ook by die LNR-ITSG teen 2°C vir 8 weke opgeberg. Die CRI monsters het geen koueskade ontwikkel nie. Die LNR-ITSG vrugte het egter koueskade getoon. Uit die resultate wil dit voorkom dat koueskade effens verminder is deur die hoër konsentrasies van Maxim en Corasil asook deur 'n kombinasie van 2,4-D, GA en CaNO₃. Deccomone op sy eie het egter die heel beste resultate gelever.

Introduction

During the last four or five years of South African citrus production and export, it has become evident that grapefruit, specifically Marsh grapefruit, has become exceptionally prone to rind breakdown, especially in the form of chilling injury (CI) during the cold storage period of the fruit in transit to the market place. This incidence of excessive levels of rind breakdown has resulted in high losses of this fruit in the market place. Trials conducted by CRI during 2001 where Marsh grapefruit was stored at different temperature regimes (4, 8 and 11°C) for long storage periods, revealed that decay levels were very low even after 16 weeks storage at all the above temperatures. However, excessive levels of rind breakdown in the form of Van Dongen spot and CI resulted in this fruit having a storage life of only 4 to 6 weeks at 4°C and 8°C. Conversely, the storage life of the fruit at 11°C was good up to 16 weeks storage with low total loss (2.9%). This situation of low storage life at the lower temperatures was possibly due to wet climatic conditions during 2000/2001 as well as the previous (1999/2000) growing seasons, causing the fruit to be more sensitive and susceptible to rind breakdown (CI). This high incidence of rind breakdown has made it a priority to determine the reasons for the occurrence of CI and methods to prevent CI or enhance fruit resistance against CI.

Materials and methods

The effect of citrus wax types on rind breakdown (chilling injury) on Marsh grapefruit stored under conditions simulating export to Japan

Marsh grapefruit was harvested and packed at TSB Hectorspruit on 29 May 2003. The fruit received the standard packhouse fungicide treatments as well as the different wax treatments for this trial. The fruit was transported to CRI on 2 June 2003 for simulated cold sterilization and shipping storage. The treatments were pre-cooled at -1.8°C for 72 hours and shipped at -1.0°C for 22 days (i.e. cold sterilization). The balance of the treatments were then stored at 11°C for 4 weeks and 8 weeks. After each storage period, the fruit was placed on the shelf at 20°C for 1 week before the incidence of decay and CI was determined.

The wax treatments were as follows:

- (i) Citrashine Polyorange (Control)
- (ii) Citrashine Stafresh (Carnauba)
- (iii) 2 + 3000 ppm Imazalil 800 EC
- (iv) 2 + 4000 ppm Tecto 500 SC
- (v) 2 + 100 ppm GA (Fallgro)
- (vi) Citrashine (New Carnauba)
- (vii) Sasol – Carnauba Natural
- (viii) Sasol – Lowveld Gleam
- (ix) Sasol – Carnauba Citrus
- (x) Citrosol – Natural Carnauba

The effect of 3,5,6-TPA, 2,4-DP and 2,4-D on CI in Marsh grapefruit

Marsh grapefruit at TSB Hectorspruit was sprayed with Maxim (3, 5, 6 TPA), Corasil (2,4-DP) and Deccomone (2,4-D) (5 replicates, 6 trees per replicate) during October 2002. The treatments were as follows:

- Treatment 1. Control
- “ 2. 10mg/L Maxim
- “ 3. 20mg/L Maxim
- “ 4. 50ml/100L Corasil
- “ 5. 100ml/100L Corasil
- “ 6. 15ppm Deccomone
- “ 7. 2% Ca Nitrate + 5ppm Ga + 15ppm Deccomone

On 28 May 2003, a sample of 1050 fruit were harvested and transported to the ARC-ITSC at Nelspruit where the fruit were stored at 2°C for 6 weeks. This was followed by external and internal quality analysis.

On 29 and 30 May the trees in treatments 1,4,5,6 and 7 were stripped, packhouse treated and transported to the CRI. (Due to logistical and labour constraints, treatments 2 & 3 could not be harvested). At the CRI, the fruit were stored under sterilisation and shipping simulation conditions (-1.8°C for 72 hours followed by 1°C for 14 days and finally 11°C for 8 weeks).

Results and discussion

The effect of citrus wax types on rind breakdown (chilling injury) on Marsh grapefruit stored under conditions simulating export to Japan

The first evaluation, after cold sterilization where the treatments were pre-cooled at -1.8°C for 72 hours and then shipped at -1.0°C for 22 days, indicated that the fruit treated with the Citrosol wax (Spanish wax) developed 1,1% CI. No CI was evident in any of the other treatments (Table 5.4.6.1).

Table 5.4.6.1. Effect of citrus waxes on CI on Marsh grapefruit after pre-cooling at -1.8°C for 72 hrs and shipping at -1.0°C for 22 days (cold sterilization)

Treatments	Percentage waste	Percentage Rind Breakdown (Chilling Injury)
1. Citrashine Polyorange (Control)	0.0	0,0
2. Citrashine Stafresh (Carnauba)	0.0	0,0
3. 2 + 3000 ppm Imazalil 800 EC	0.0	0,0
4. 2 + 4000 ppm Tecto 500 SC	0.0	0,0
5. 2 + 100 ppm GA (Fallgro)	0.0	0,0
6. Citrashine (Carnauba)	0.0	0,0
7. Sasol – Carnauba Natural	0.0	0,0
8. Sasol – Lowveld Gleam	0.0	0,0
9. Sasol – Carnauba Citrus	0.0	0,0
10. Citrosol – Natural Carnauba	0.0	1,1

The final evaluations are recorded in Table 5.4.6.2. The results indicate that after 4 weeks, CI increased to 1,7 % in the Citrosol treated fruit. After 8 weeks this increased to 6.3%. The others treatments remained CI free.

Table 5.4.6.2. Effect of citrus waxes on CI in Marsh grapefruit after precooling and storage for 4 and 8 weeks.

Treatments	Controls (8 weeks @ 11°C)		4 Weeks @ 11°C		8 Weeks @ 11°C	
	% Waste	%CI	% Waste	%CI	% Waste	%CI
1. Citrashine Polyorange (Control)	0,4	0,0	0,0	0,0	0,0	0,0
2. Citrashine Stafresh (Carnauba)	0,0	0,0	0,0	0,0	0,0	0,0
3. 2 + 3000 ppm Imazalil 800 EC	0,6	0,0	0,4	0,0	0,0	0,0
4. 2 + 4000 ppm Tecto 500 SC	0,0	0,0	0,0	0,0	0,0	0,0
5. 2 + 100 ppm GA (Fallgro)	0,5	0,0	0,0	0,0	0,3	0,0
6. Citrashine (Carnauba)	0,0	0,0	0,0	0,0	0,0	0,0
7. Sasol – Carnauba Natural	0,3	0,0	0,0	0,0	0,0	0,0
8. Sasol – Lowveld Gleam	0,0	0,0	0,0	0,0	0,0	0,0
9. Sasol – Carnauba Citrus	0,5	0,0	0,0	0,0	0,0	0,0
10. Citrosol – Natural Carnauba	0,5	0,0	0,0	1,7	0,5	6,3*

* 6,3% is made up of 1,8% CI and 4,5% Van Dongen Spot (also chilling injury)

Indications are that the Citrosol (Spanish) wax has promoted rind breakdown (chilling injury) on the Marsh grapefruit after long storage of the fruit.

Conclusion

This work will need to be repeated on Marsh grapefruit from another production area, to be able to confirm these results prior to any recommendations being made.

The effect of 3,5,6-TPA, 2,4-DP and 2,4-D on CI in Marsh grapefruit

No CI symptoms were detected in the fruit stored under sterilisation plus shipping simulation conditions (-1.8°C for 72 hours followed by 1°C for 14 days and finally 11°C for 8 weeks) at the CRI.

Chilling injury did, however, develop in fruit stored at 2°C for 6 weeks at the ARC-ITSC. The data is presented in Table 5.4.6.3.

Table 5.4.6.3. Chilling injury recorded in Marsh grapefruit treated with different PGRs

Treatment	Mass (g)	Diameter (mm)	Length (mm)	Chilling (0-3)	TSS °Brix	Acid (%)
Control	295.8 a	80.6 a	77.7 a	0.873 a	11.1 a	1.05 a
Maxim (10 mg/l)	305.6 a	81.2 a	78.1 a	0.840 a	12.1 a	1.00 a
Maxim (20 mg/l)	299.4 a	77.9 a	77.0 a	0.513 bc	11.7 a	1.06 a
Corasil (50 ml/100l)	330.3 ab	84.2 a	82.0 a	0.767 ab	11.0 a	1.05 a
Corasil (100 ml/100l)	314.9 a	82.8 a	74.8 a	0.452 c	11.1 a	1.03 a
Deccomone (15 ppm)	374.8 b	84.3 a	84.0 a	0.387 c	11.9 a	1.08 a
Deccomone (15 ppm) GA (5 ppm) CaNO ₃ (2%)	332.3 ab	83.1 a	81.2 a	0.472 c	12.3 a	1.12 a

From the results it would appear that chilling injury was reduced by the higher concentrations of Corasil (2,4 DP), Maxim (3,5,6-TPA) and the 2,4-D + GA + CaNO₃ combination. However, Corasil (2,4-D) on its own gave the best results and it more than halved the chilling injury symptoms. As the latter fruit were also larger than those from the other treatments, the possible confounding effect of fruit size was taken into account when interpreting the results. However, fruit size did not seem to have confounded the chilling injury results.

Conclusion

It would appear that the application of 2,4-D to grapefruit during the early season might contribute towards reducing postharvest chilling injury in mature fruit. However, we would first like to discuss the results with industry members so as to establish the commercial viability before conducting further trials.

Acknowledgements

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5.4.7 Factors influencing Rind Breakdown in citrus fruit

Experiment by P.J.J. van Rensburg (Citricom), P.J.R. Cronjé (CRI), Giuliana Gambetta (Univ. de la Republica, Montevideo, Uruguay) & Mariette Bruwer (Green Marketing)

Opsomming

Skilafbraak kom hoofsaaklik by Clementines, maar ook somtyds ook by Satsumas voor, indien die vrugte oor lang afstande na die oorsese market uitgeoer word. Die meganisme waarvolgens skade veroorsaak word, is dat die olieselle in die oliekliere van die vrug se skil bars en die olie dan in die albedo vrystel word, waar dit die selle oksideer. Die ge-oksideerde selle word dan bruin en dit is sigbaar op die oppervlak van die skil as ronde bruin kolletjies.

Hierdie studie is uitgeoer om die oorsake en oplossings vir die skilafbraak, op hoofsaaklik Clementines, te vind. Boordtoedienings van minerale elemente en plantgroeireguleerders, pakhuisbehandelings en na-oes opbergingsproewe is uitgeoer om verskillende hipoteses te toets.

Data dui aan dat belangrikste oorsaak van skilafbraak makro klimaatsfaktore is. 'n Warm winter voor die seisoen en 'n warm herfs in die seisoen, was die hooforsaak van die skilafbraak. Daar was egter gevind dat daar tweedens ook snellers nodig was om die reeds sensitiwe vrugte te laat skilafbraak kry. Die snellers is deur wetenskaplike navorsing met die vrugte gevind.

Wetenskaplike navorsing op Skilafbraak by Clementines is volgens 'n oorhoofse navorsings- en voorkomingstrategie uitgevoer. Die modelle wat vir die studies gebruik is, het sensitiewe met weerstandbiedende vrugte en situasies vergelyk. Die minerale en biochemiese samestelling van die skille in die scenarios is ook vergelyk en vrugte is onder verkoeling opgeberg en periodiek vir die voorkoms van bederf en ander skilprobleme ge-evalueer. Monsters is by die geleenthede geneem en biochemiese en minerale analyses is daarop uitgevoer om vas te stel waarom sommige skille sensitief was, al dan nie.

Die studies het bewys dat:

1. Die Nules Clementine seleksie was meer sensitief om skilafbraak te kry as die Oroval seleksie.
2. Vrugte wat blootgestel was aan hoë lig toestande het meer karotene bevat as vrugte wat aan lae lig blootgestel was en was meer bestand teen skilafbraak en het 'n langer rakleef tyd gehad. Dit is dus belangrik dat vrugte wat in die lae lig areas in die boom voorkom, vinnig gepluk, verskep en bemark moet word. Bleek geel vrugte kan ook in die pakhuis verwyder word om die skilafbraak risiko te verminder. Karoteenvlakke in die vrugte kan verhoog word deur die bome gereeld te snoei en lig in die lae lig areas van die boom in te laat.
3. Etileen behandeling van die vrugte het die risiko van skilafbraak, bederf en beklemtoning van "waterspot" verhoog. Twee dae etileenbehandeling het nie die vrugte betekenisvol benadeel nie, maar langer behandelings word nie aanbeveel nie. Etileen behandelings het teen verwagtinge in, nie die karoteenvlakke in die vrugte verhoog nie.
4. Die vrugte van die latere oeste het 'n neiging om meer sensitief vir skilafbraak te wees as die vrugte wat vroeër gepluk word en die rakleef tyd daarvan is ook betekenisvol korter.
5. Kleinere vrugte is meer sensitief as groter vrugte.
6. Een van die hoofvindrings wat by skilafbraak-weerstandbiedende vrugte gevind is, was dat die skille van vrugte wat weerstandbiedend vrugte in al die modelle hoër vlakke van baie van die minerale elemente bevat het, as die vrugte vanaf sensitiewe scenarios. As 'n mens die voedingstatus van die bome verbeter en optimal hou, sal die kans van skilafbraak verminder.
7. Proewe is uitgevoer om vas te stel of daar metodes is om die vrugte meer weerstandbiedend teen skilafbraak te maak. Die resultate dui aan dat Maxim (3,5,6 trichloro-2-pyridiloksiel asynsuur of 3,5,6 TPA) 'n hoogs betekenisvolle effek op die vrugte gehad en die rakleef tyd daarvan met twee tot vier weke verleng het. Dit het die bederf, skilafbraak, en powwerige vrugte drasties verminder. Corasil E (2,4 DP) het nie die effek gehad nie. Die produk bied 'n baie goeie manier om die skilafbraak probleem op te los, die vrugte se skille se weerstand teen skilprobleme te verbeter en die rakleef tyd van vrugte te verleng.
8. Die effek van osoon op die voorkoms van skilafbraak is getoets in 'n groot statistiese proef waar vier houers met osoon opwekkers tegevoer is, in vergelyking met vier houers sonder opwekkers. Van die houers is verskep teen 4,5°C en ander teen 11°C. Die osoon behandelde vrugte het minder skilafbraak en betekenisvol minder powwerige vrugte ontwikkel. Laasgenoemde is 'n nuwe verskynsel en veroorsaak verliese van tot 40% in sommige gevalle. Daar is in die proewe ook bewys dat die skil onder sekere toestande kan aanhou groei, na verskeping. Die gebruik van osoon kan dus die voorkoms van skilafbraak, bederf en powwerige vrugte betekenisvol verminder.
9. Die vinnige verkoeling van vrugte met geforseerde lug het nie die skilafbraak vergerger nie.
10. Die effek van verskepingstemperatuur op skilafbraak het duidelik aangedui dat skilafbraak deur hoër temperature veroorsaak word. Die skade het byna reglynig vermeerder vanaf -0.5°C tot 11°C. Karoteenvlakke het konstant met tyd in opberging vermeerder.
11. Aan die fisiologiese kant, was 'n betekenisvolle bevinding dat vrugte wat teen hoër temperature opgeberg word, 'n tipiese verhoging in respirasie sewe tot agt weke na oes toon. Dit beaam die resultate van ander navorsers en kan verduidelik hoekom vrugte eers sewe tot agt weke na oes ineens stort en skilafbraak ontwikkel.

Parallel aan die navorsing, is 'n skade-beheer-strategie ontwikkel en ge-implimenteer om die voorkoms van skilafbraak te voorkom, en as dit wel voorkom, om dit te hanteer. Sedert die implimentering van die strategie is skilafbraak verliese in die Suid Afrikaanse bedryf vanaf \$ 2 M na minder as \$ 50 000 verminder.

Introduction

Rind Breakdown (RB) is a rind defect which affects mainly the Clementine easy peeler, but occurs sporadically on Satsuma mandarins that are shipped over long distances to overseas markets. It is caused by collapsing oil cells in the oil glands of the fruit rind, and is visible as brown spots or pitting on the rind

surface (Figure 5.4.7.1). Rind Breakdown is a term loosely used for a range of fruit rind disorders, which can be seen as collapsed cells in the fruit rind that discolours with age. Some of these appear pre-harvest on fruit on the tree, like Rind Breakdown on navel oranges (Klotz et al., 1967; Agusti et al., 2001) and mandarins (Almela et al., 1992). Others appear post harvest, like post harvest pitting on grapefruit (Petracek et al. 1995) and Fallglo mandarins (Petracek, et al. 1998). The Rind Breakdown on Clementines, however, is different in that the pitting appears as randomly distributed, but regularly shaped, round spots on the rind of mandarin fruit (Figure 5.4.7.1).

Various studies on Rind Breakdown have been conducted during the last eight years in South Africa to find the cause and solutions. Tendencies indicate that, on a macro scale, RB occurs in seasons when we experience a warm winter, followed by a warm autumn. However, “triggers” are then needed for the sensitised fruit to experience RB (Fig 5.4.7.3) This study was conducted through a well structured research effort, to find factors which are involved in the occurrence of RB in Clementines and possibly other citrus fruit (Figure 5.4.7.4). Rind Breakdown had a major impact on the South African citrus industry during the 1992, 1993, 1999 and 2000 seasons, although it occurred at lower levels in the other seasons. Apart from fruit losses, large losses in the export and marketing chains have to be added to this. A new phenomenon, puffy fruit, was also recorded in the trials (Fig. 5.4.7.3). The puffy fruit occurs post harvest on Clementine and Satsuma mandarins during cold storage. The rind of the fruit was found to start growing during storage, causing the fruit to appear larger than normal and puffy, i.e. the rind separates from the segment walls. No explanation for the phenomenon can be given at this stage, but it is known that it causes substantial financial losses in the markets, particularly on Clementine and Satsuma mandarins, and possibly other citrus types as well.



Figure 5.4.7.1. Typical Rind Breakdown on Clementine fruit, that is visible as brown spots on the fruit rind. The yellow fruit developed RB vs. the darker orange fruit that did not.

Various hypotheses were studied to ascertain factors controlling the occurrence of Rind Breakdown. The main emphasis was on studying the post harvest reaction of fruit originating from scenarios that produced sensitive vs. resistant fruit. The analysis of storage trial results and mineral and pigment content was used to ascertain which factors were controlling Rind Breakdown. The models hypotheses were:

- a. Fruit treated with two synthetic auxins, i.e. 3,5,6 TPA (3,5,6 trichloro-2-pyridiloxycetic acid) and Corasil E (2,4 DP or 2,4 dichloropropionic acid): Fruit treated with Maxim seemed to have a deeper red colour at maturity, than untreated fruit and high carotene levels in the fruit rind was thought to give the fruit resistance against RB.
- b. Fruit growing in high light, compared to fruit growing in low light areas on the tree. The first being resistant and the latter sensitive to RB. Carotene levels in the fruit rind were thought to be involved in resistance to RB.

- c. Fruit from the Nules vs. the Oroval Clementine selection: Fruit of the Oroval was thought to be less sensitive than the Nules to RB, due to fruit rind colour and carotene content differences.
- d. Fruit harvested during the early vs. the latter part of the picking season: Fruit from the first picking period was thought to last longer in storage before it developed RB.
- e. Fruit treated for different durations with ethylene: The longer ethylene treatment was thought to have sensitized the fruit to RB.
- f. The use of ozone in storage to prevent the occurrence of RB. It is thought that ethylene might play a role in the occurrence of RB and ozone was to be used to break down the ethylene in storage.
- g. Fruit shipped at different shipping temperatures: It was initially thought that RB was a type of chilling injury.

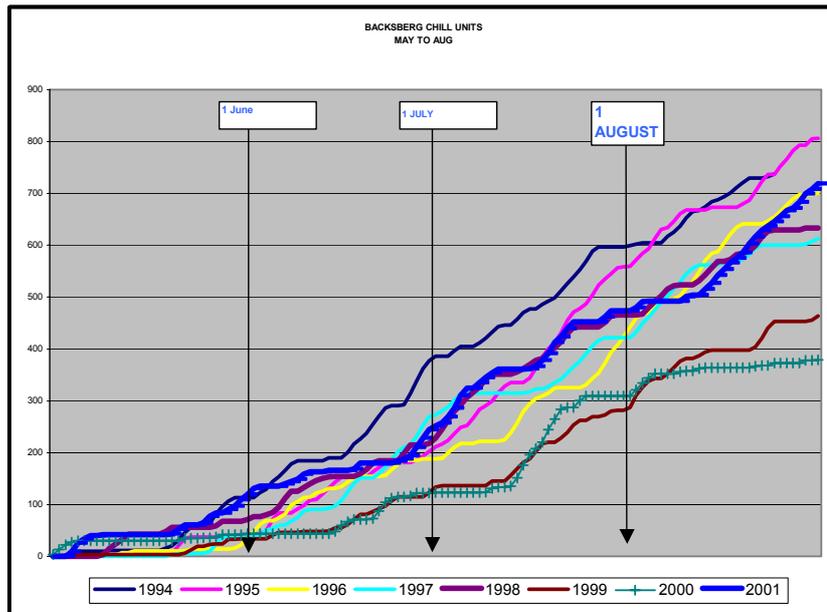


Figure 5.4.7.2. The winter chill units in the Paarl Clementine production areas in winter. Note: The winters of 1999 and 2000 had low chill units and high levels of RB was experienced in 2000 and 2001.



Figure 5.4.7.3. Typical fruit and rind deviations recorded in trials: Top right hand corner – Rind breakdown, puffy fruit and decay.

Materials and methods

General trial design

Throughout the research, trials were consistently designed and executed in the same way so as to be able to compare the results. Mature Clementine trees of eight to ten years old were used in these trials. Where trees were treated with spray applications, they were sprayed to the point of run off (about 8 litres solution per tree). PGR applications were applied at the end of the physiological fruit drop period and this was always compared with an untreated control. Where fruit was selected from a position on a tree, or from a tree that produced sensitive fruit vs. resistant fruit, these were harvested separately, but at random, from replicate trees and thereafter handled separately until it was packed in an individual box. Fruit that was packed for post harvest treatments was picked in the orchard, randomized afterwards, treated and then packed in boxes.

At harvest, fruit from each of the two-tree plots (eight replicates) of a treatment was harvested. Fruit per treatment was kept together and clearly identified. The fruit was then transported to a packhouse where the fruit was left at ambient temperature for a day to allow the field heat in the fruit to dissipate before it was treated. It was then drenched with a commercial fungicide solution and degreened for three days. (In the specific degreening trial, fruit was kept in the degreening room for different periods). The degreening conditions in the degreening room were 95% relative humidity, ethylene at 3 ppm and the CO₂ percentage in the room below 0.3%. After degreening, the fruit was allowed to stand at ambient temperature for 12 hours before it was packed in a commercial packhouse. A normal fungicide treatment with imazalil was applied in the packing line and a polyethylene wax was used to wax the fruit. The fruit was packed, each treatment separately, in 15 kg cartons and cartons of one commercial size was selected for the experiment. Ten to fifteen single-carton replicates were used in all the trials. Some sample boxes of remaining fruit sizes were stored under similar conditions, for photographic evaluation. After packing, fruit was palletised and transported at ambient temperature to the storage facilities. On arrival, the fruit was packed in commercial integral containers, which were used as storage facilities to simulate shipping conditions.

The trial fruit was kept at a constant temperature of 4.5 or 11°C and monitored periodically. (In the specific shipping temperature trial, different shipping temperatures were used.) Evaluation of fruit was executed at set intervals mostly every two weeks and shelf life and rind condition deviations recorded. The first evaluation was done at the start of the trial, while the last evaluation was executed predominantly 12 weeks after the trials started. The deviations recorded were Rind Breakdown, decay and puffiness (Figure 5.4.7.3). The same 15 replicate cartons per treatment, were used for each of the evaluations. Fruit which had any deviation was discarded at each evaluation. Fruit sampling for mineral and carotene analysis: one or two fruit were taken from each one of a separate set of replicates and placed in a new paper bag, until a ten fruit sample had been collected. The fruit was then sent to be analyzed to determine pigment content (Chlorophyll a and b; and total carotene content – three replicates) and mineral content (Micro and macro elements – five replicates) at a commercial analytical laboratory. Data were analyzed, using the Statgraphics statistical package. Only statistically significant data were reported, unless otherwise indicated. Most of the trials were replicated over two seasons.

Results and discussion

(i) The effect of Plant Growth Regulators (PGRs) on the post harvest occurrence of Rind deviations and the shelf life of Clementine fruit

The use of plant growth regulators (PGRs) to enhance resistance was prompted by observations that certain PGRs had an influence on the fruit colour at maturity. Fruit treated with Maxim always has a deeper red-orange colour, compared to untreated fruit (Van Rensburg, P.J.J - personal observation). The further observation found in operational research, was that well coloured red/orange fruit did not get RB during storage (van Rensburg, et al. 2000). These observations prompted the investigation of the effect of auxin applications on the occurrence of RB, as part of testing models of fruit with RB sensitivity vs. fruit with RB resistance.

The first storage trial (trial A) indicated that the **Rind Breakdown (RB) and decay occurrence on Clementine fruit, was highly significantly reduced by the Maxim** (3,5,6 TPA, or 3,5,6 trichloro-2-pyridiloxycetic acid) treatment (applied in early January, about one month after the end of the natural fruit drop period) and that only the highest concentration Maxim (20mg/l) proved effective (Figure 5.4.7.5). After four and eight weeks of storage at 11°C, the control fruit had 10.4 and 27.5% RB, respectively, while the figures for the Maxim treated fruit was 1.1 and 4.9%, respectively (Figure 5.4.7.5). This indicates a highly significant reduction in RB that can cause highly significant reduction in losses in the export markets. Untreated fruit was in good condition four weeks after packing, but collapsed and showed relatively high

levels of Rind Breakdown (10.4%), which made it commercially unmarketable at eight weeks, and un-usable (21% RB) ten weeks after packing. The fruit treated with Maxim was in a good marketable condition after eight weeks (1.4% RB), while the fruit could still be repacked with minor fruit loss (4.9% RB) ten weeks after packing (Figure 5.4.7.5). This implies that the **Maxim increased the shelf life by at least two weeks and perhaps even four weeks**. The effect of Maxim on the condition of the fruit could possibly be brought about by an increase in the carotene content in the rind of treated fruit. Apart from the RB effect, the **Maxim also reduced the puffiness and decay of fruit in storage highly significantly (data not shown)**.

The next trial (trial B): **Application of Maxim to trees at the normal fruit size enhancement stage (at the end of the natural fruit drop period) significantly reduced RB, decay and puffiness of the fruit**. Both the 10 and 20 mg/l applications effectively reduced these fruit rind deviations, as the treatment was applied earlier than in trial A (Table 5.4.7.1). The results of the three rind deviations could be combined, as these were recorded from the same boxes of fruit. If that is done, the results become more dramatic. Control fruit then showed combined deviations (RB, decay and puffy fruit) of 38.2%, while Maxim treated fruit (10 mg/l) had only 7.3% damage, eight weeks after packing (Table 5.4.7.1). **The effect of Maxim on the lengthening of the shelf life of the fruit by at least two weeks was again apparent** (Table 5.4.7.1).

The next trial (trial C) included a comparison between Maxim, Corasil E (2,4 DP) and the untreated Control. The results in this trial mimic the results of trials A and B, as **the application of 10 mg/l Maxim to trees, at the normal fruit size enhancement stage at the end of the natural fruit drop period, significantly reduced RB in the fruit** (Figures 5.4.7.7 and 5.4.7.8). The effect of **Maxim on the lengthening of the shelf life of the fruit, by at least two weeks, was again apparent. Results indicate that the commonly used Corasil E (2,4 DP) did not have the same effect as Maxim and could not decrease the decay or Rind Breakdown (Figure 5.4.7.8 and 5.4.7.9)**. Results from an analysis of the rinds of fruit treated with Maxim at harvest may give us an indication of the reason why this fruit is more resistant to RB than non treated fruit. **The rinds of Maxim-treated fruit had significantly higher levels of Nitrogen (12%), Potassium (7%), and Boron (24%), but, interestingly, lower levels of Calcium (18%) and Magnesium (11%) than untreated fruit (Table 5.4.7.2)**.

The results indicated above, thus provides us with a scenario where we can stimulate resistance of fruit to RB, by applying a PGR in the orchard. A future biochemical analysis of treated vs. untreated fruit could possibly yield more answers to finding the factors that cause RB. As far as practical RB prevention is concerned, the Maxim application to trees provides a very good method to build the resistance of the rind of the Clementine fruit and to prevent or reduce the occurrence of RB, puffiness and decay successfully.

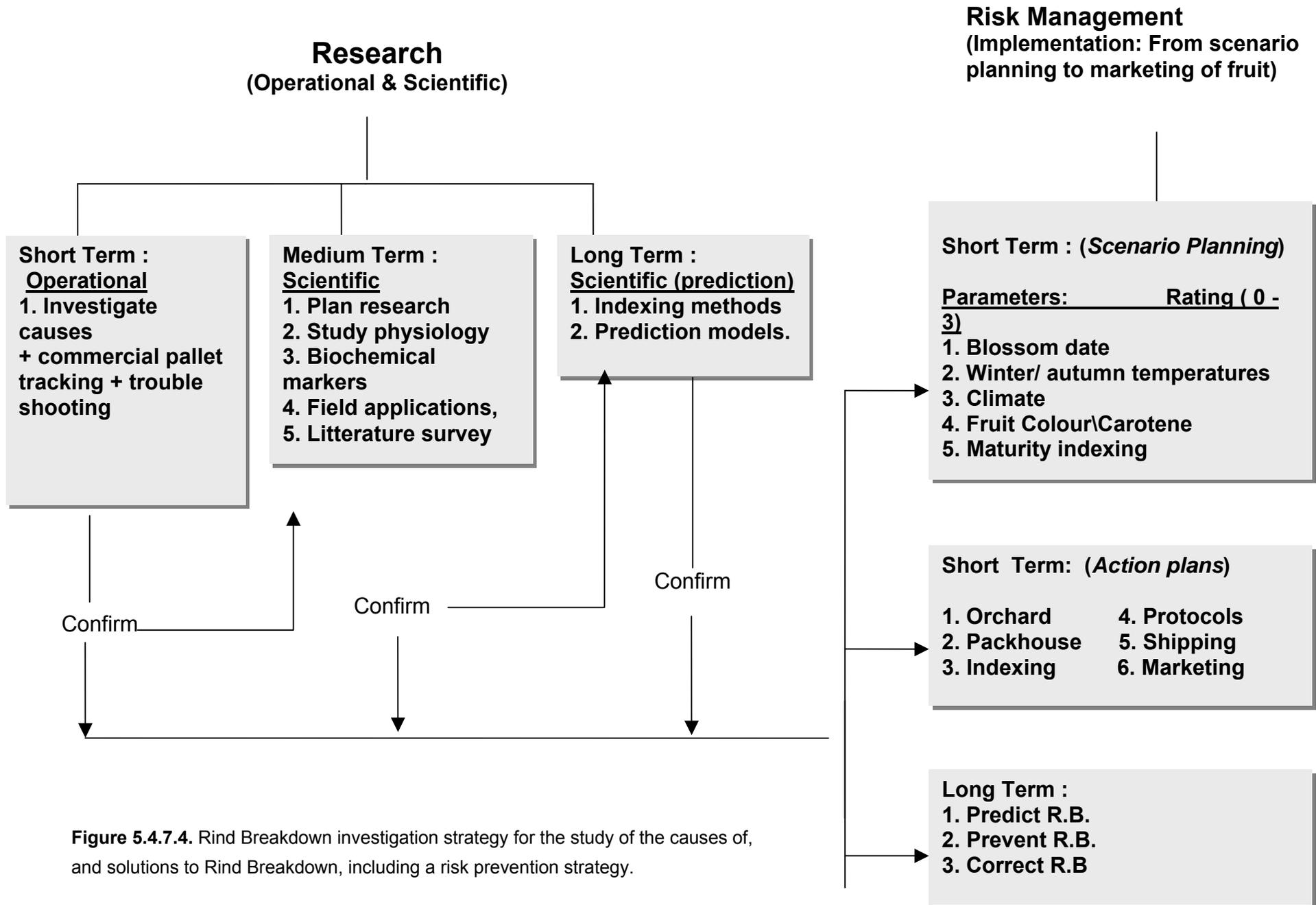


Figure 5.4.7.4. Rind Breakdown investigation strategy for the study of the causes of, and solutions to Rind Breakdown, including a risk prevention strategy.

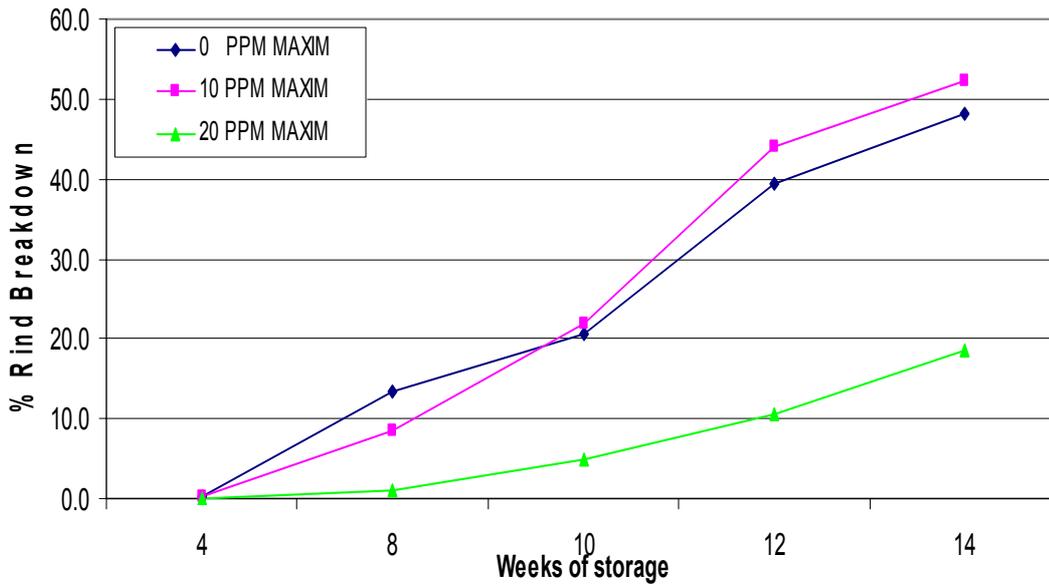


Figure 5.4.7.5. The effect of Maxim (3,5,6 TPA) tree sprays on the post harvest occurrence of Rind Breakdown on fruit, during storage at 11 °C (Trial A).

Table 5.4.7.1. The effect of Maxim (3,5,6 TPA) tree sprays on the post harvest occurrence of Rind Breakdown, decay and puffy fruit, during storage at 11°C (Trial B).

TREATMENT	MAXIM CONCENTRATION (PPM)	STORAGE WEEK	RB % PER WEEK	DECAY % PER WEEK	PUFFY % PER WEEK
1	0	4	0.2	2.0	0.0
2	10	4	0.0	1.0	0.0
3	20	4	0.0	0.4	0.0
4	0	8	13.2	10.9	11.9
5	10	8	2.7	1.8	2.8
6	20	8	5.2	1.1	3.1
7	0	10	20.2	3.0	1.0
8	10	10	12.5	1.9	0.3
9	20	10	13.2	0.9	0.1
	SE ±		1.3	0.8	0.6
	Significance		***	***	***

***Significantly different according to Fishers LSD test (P<0.001).



Figure 5.4.7.6. Boxes of fruit that were kept for observation, eight weeks after packing. Fruit in the top box was untreated and that in the bottom box treated with a 10 mg/l Maxim spray application to the trees at the end of the physiological fruit drop period. Results from trial B.

Table 5.4.7.2. The effect of PGR treatment on the mineral composition of the rind of Clementine fruit (Trial B).

Main Factor	N	P	K	Ca	Mg	Na	Cu	Zn	Mn	Fe	Al	B
A. Storage Time:												
0	1.8 b	0.2 c	2.58 ab	0.82 a	0.15 a	150.08 a	6.09 bc	16.48 a	10.67 ab	64.06 a	105.33 a	25.73 a
4	2.02 c	0.23 d	2.8 c	0.99 bc	0.19 b	185.25 a	6.62 c	42.11 b	12.99 cd	98.78 b	129.5 b	31.68 bc
6	2.13 d	0.21 c	3.56 d	1.09 c	0.24 c	190.33 a	6.35 bc	54.13 b	13.95 d	126.08 c	147.75 c	33.21 c
8	2.13 d	0.18 b	2.89 c	1.03 bc	0.22 c	206.42 ab	5.83 b	36.92 b	12 bc	220.25 e	192.75 d	34.03 c
10	1.62 a	0.14 a	2.47 a	0.95 b	0.19 b	178.82 a	4.71 a	105.17 d	10.53 ab	152.08 d	189.67 d	29.62 b
12	1.71 b	0.15 a	2.71 bc	0.95 b	0.2 b	246 b	4.72 a	80.11 c	10.02 a	131 cd	186.92 d	30.33 b
SE	±0.03	±0.01	±0.07	±0.04	±0.01	±18.94	±0.2	±6.36	±0.56	±8.36	±5.37	±0.92
Significance	***	***	***	***	***	*	***	***	***	***	***	***
B. Treatment:												
Control	1.72 a	0.18	2.73 a	1.07 b	0.21 b	207.16	5.31	61.72	11.59	157.1	171	26.86 a
Maxim	1.93 b	0.19	2.92 b	0.91 a	0.19 a	167.5	5.22	62.36	12.35	141.48	167.56	33.31 b
SE	±0.03	±0.004	±0.06	±0.03	±0.01	±15.47	±0.16	±5.19	±0.45	±6.83	±4.39	±0.75
Significance	***	NS	***	**	***	NS	NS	NS	NS	Ns	NS	***

*Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).

**Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).

***Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

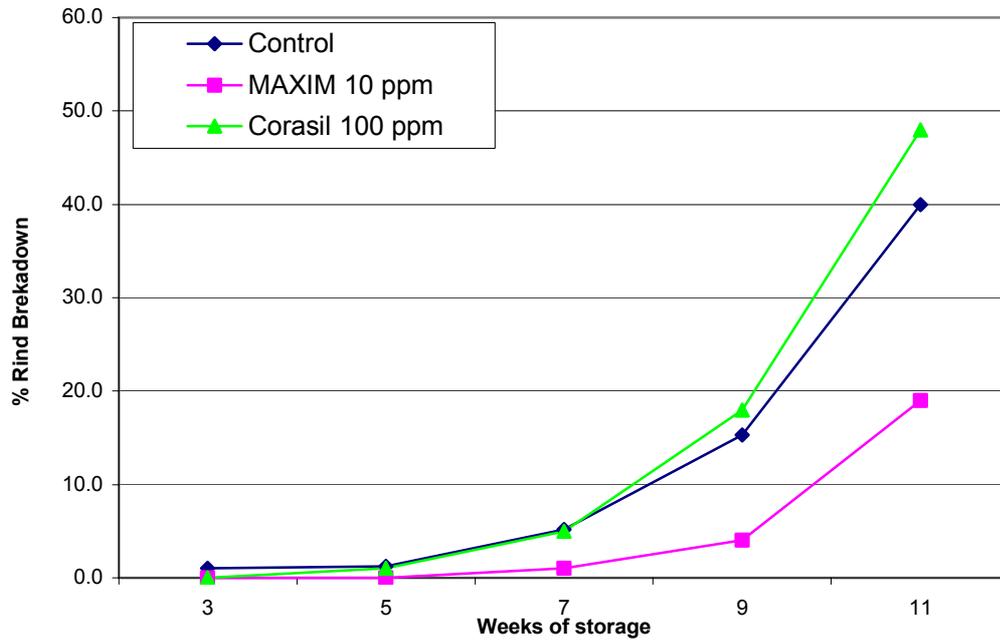


Figure 5.4.7.7. The effect of Maxim (3,5,6 TPA) on the development of Rind Breakdown on Clementine fruit during storage at 11°C (Trial C).

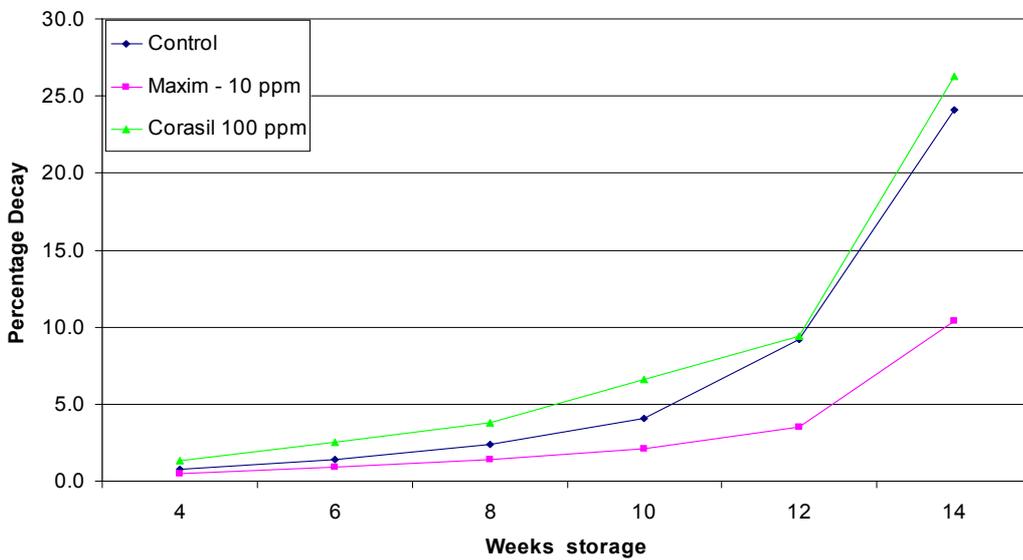


Figure 5.4.7.8. The effect of Maxim (3,5,6 TPA) on the development of decay on Clementine fruit during storage at 11°C (Trial C).

(ii) The effect of the growing position of the fruit on the tree, on the post harvest occurrence of rind deviations and the shelf life of Clementine fruit.

Three trials were executed to study the differences between fruit which is RB resistant, vs. fruit which is RB sensitive. These trials were based on the observation that fruit growing inside the tree, under low light (LL) conditions, was pale yellow in colour, compared to the darker orange coloured fruit growing on the outside, under high light conditions (HL). Furthermore, operational research showed that darker coloured fruit was less prone to RB, than the pale yellow fruit (Figure 5.4.7.1) (van Rensburg & Bruwer et al., 2000). The first factorial trial (A) tested the effect of Boron applications to enhance the fruit rind's resistance to, as well as the effect of fruit position on the tree, on RB. The results of this trial showed that there was a highly significant difference in the RB incidence between fruit growing under LL vs. HL conditions. The fruit from the LL position produced 48.7% RB vs. 9.5% RB on fruit from the HL position, after eight weeks of storage at 11°C (Table 5.4.7.3). In the second and third trials (B and C), the results were similar with the LL fruit showing significantly higher levels of RB than the HL fruit, from eight weeks after the start of storage (Figures 5.4.7.9 and 5.4.7.10). **The fruit from the LL position on the tree also developed RB at a faster rate and reached higher RB levels earlier, than the HL fruit in all trials** (Figures 5.4.7.9 and 5.4.7.10), indicating that the fruit from the HL position had a longer shelf life. The analysis of the rind of fruit harvested from the LL vs. the HL position, indicated that the HL fruit's rinds contained higher levels of potassium, manganese, iron, copper and zinc (Table 5.4.7.4). This could be an indication that the **HL fruit rinds' better nutritional status could possibly have made it less prone to contracting RB**. The physiological implications of these results are not known. The results of the analysis of the Third trial (C), indicate that the **HL fruit rinds contained higher levels of Nitrogen, Calcium, Magnesium, Manganese and Zinc, but lower levels of Potassium, Copper, Iron and Aluminium (Table 5.4.7.6). This could again point to the generally better nutritional status of the rinds of outside fruit**, but it was only the Zinc and Manganese that were consistently higher in outside fruit in both trials (Tables 5.4.7.4 and 5.4.7.6).

The analysis of the rind pigments, and especially the carotene content in trial B, did not yield any significant results, contrary to the expectation, as the rind of fruit growing in an LL position was thought to have less carotenes than that of the HL position (Table 5.4.7.5). This could be due to the fact that the orange/red pigment that we see in well coloured fruit, was not being picked up in the analytical method we used, as big colour differences were visible on the fruit (Figure 5.4.7.1). In trial C, the results of an analysis of the rinds of LL fruit indicated that LL fruit had less Chlorophyll than HL fruit. No differences in Carotene content were found (Table 5.4.7.7). This points to the carotenes not being involved in the resistance of the HL to RB. The Chlorophyll content of the fruit decreased with time in storage, as was expected (Table 5.4.7.7).

One explanation for the higher RB occurring on LL fruit could be their post harvest reaction. **Fruit from the LL areas respired at a higher rate and thus used energy at a higher rate**. From experience we know that the fruit lasts for a certain period and then collapses and RB occurs. In the past the occurrence of RB has been linked to a rise in respiration. (Van Rensburg & Bruwer 2000). We can thus say that the higher respiration of the LL fruit, could cause it to reach the "climacterium" quicker and that this is the reason why RB occurs more rapidly and at higher levels in LL fruit.

These results have important practical implications. They indicate that the need exists to expose fruit growing inside the tree to sunlight, which will increase the resistance of the fruit to RB. The trees thus have to be pruned to increase the light penetration into the shaded areas of the tree. Furthermore, it could be recommended that fruit from the LL position be harvested first to prevent them from contracting RB, as they have a potentially shorter shelf life. Also, in years where fruit is highly prone to RB, the risk of exporting fruit that is RB sensitive could be prevented by not exporting fruit growing in LL conditions, or by discarding pale yellow LL fruit in the packhouse.

Table 5.4.7.3. Effect of fruit position and Boron on rind breakdown occurrence (Trial A).

	MAIN FACTOR – BORON	RB (%)			
		Inside fruit (II)	Outside Fruit (HL)	MAIN FACTOR	INSIDE VS OUTSIDE
1.	Control ^z	42.5	3.6	Inside Fruit	48.7
2.	Borax *12.5 g/tree 1x	45.0	10.0		
3.	Borax *12.5 g/tree 2x	59.4	7.2	Outside fruit	9.4
4.	Borax *12.5 g/tree 3x	57.2	8.8		
5.	Solubor	39.4	17.2		
Significance		NS	NS		***
SE ±		± 7.7	± 4.8		±3.3

* Solubor and Borax are commercial formulations used for boron application.

*** Significantly different according to Fishers LSD test (P<0.001).

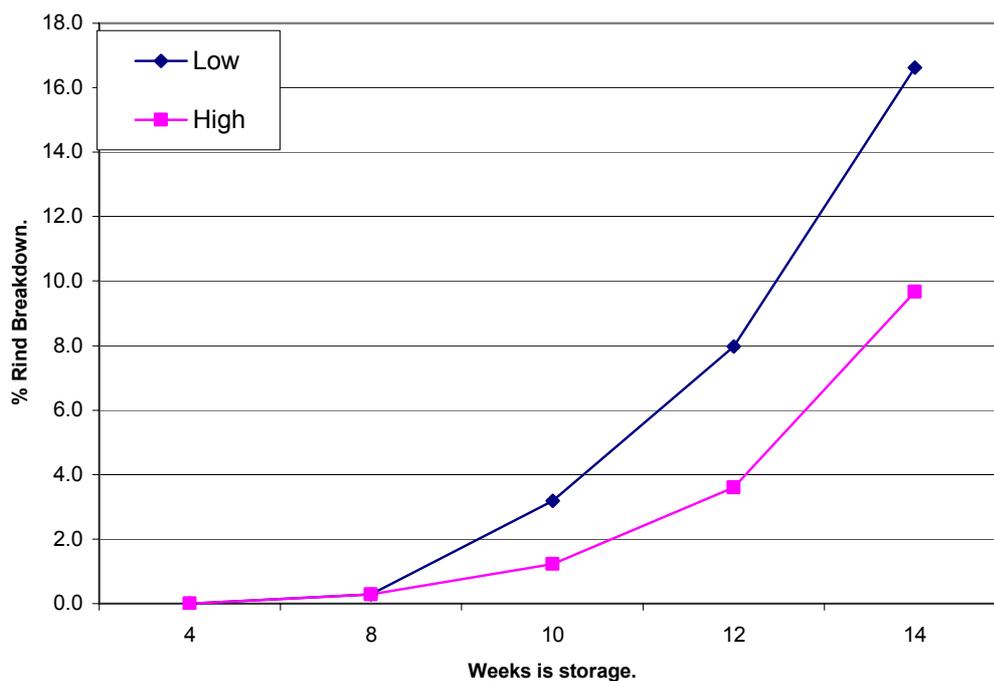


Figure 5.4.7.9. The effect of light level (and fruit position in the tree) on development of Rind Breakdown in Clementines. Low light (LL) fruit normally grows on the inside of the tree and high light (HL) on the outside of the tree (Trial B).

Table 5.4.7.4. The effect of light levels on mineral content in Clementine fruit (Trial B).

Treat- ment			N %	P %	K %	Ca %	Mg %	Na %	Mn %	Fe %	Cu %	Zn %	B %
MAIN EFFECTS													
A	Light level	High light	1.4	0.10	1.5	0.6	0.2	224.1	6.0	27.9	4.4	7.5	24.7
		Low light	1.3	0.13	1.4	0.6	0.2	205.3	5.1	26.0	3.9	6.7	24.6
SE ±			0.03	0.002	0.03	0.02	0.006	7.9	0.2	0.6	0.1	0.2	0.4
Significance			NS	*	*	NS	NS	NS	**	*	**	*	NS
B	Storage Weeks	4	1.2	0.11	1.6	0.6	0.2	169.7	5.1	31.3	4.2	6.7	21.5
		8	1.3	0.13	1.5	0.6	0.2	220.5	5.5	32.5	3.3	4.6	22.2
		10	1.4	0.12	1.3	0.6	0.1	222.1	5.9	27.0	4.8	8.0	27.0
		12	1.4	0.11	1.3	0.6	0.1	246.4	5.6	17.0	4.3	9.0	27.9
		SE ±			0.05	0.003	0.05	0.03	0.008	11.2	0.3	0.9	0.2
Significance			*	**	***	NS	NS	***	NS	***	***	***	***
INTERACTIONS													
A X B			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Significantly different according to Fishers LSD test (P<0.05).

**Significantly different according to Fishers LSD test (P<0.01).

***Significantly different according to Fishers LSD test (P<0.001).

Table 5.4.7.10. The effect of light levels on the pigment content of the rind of Clementine fruit (Trial B).

TREATMENT	LIGHT LEVEL	WEEK	CHLOROPHYLL	CAROTENE	CAROTENE: CHLOROPHYLL
1	Low	4	1.1	104.1	110.0
2	High	4	0.6	116.3	232.1
3	Low	8	1.2	136.9	116.8
4	High	8	1.0	115.9	114.7
5	Low	10	0.3	153.5	534.3
6	High	10	0.2	180.1	673.8
7	Low	12	0.5	174.6	460.4
8	High	12	0.7	187.9	445.2
SE ±			0.2	7.4	68.7
Significance			***	***	***
Main Effects:					
A	Light level	Low	0.7	142.3	305.4
		High	0.6	150.0	366.5
SE ±			0.1	3.7	34.4
Significance			NS	NS	NS
B	Weeks	4	0.9	110.2	171.1
		8	1.1	126.4	115.7
		10	0.2	166.8	604.1
		12	0.6	181.2	452.8
SE ±			0.1	5.2	48.6
Significance			***	***	***
Interactions					
A X B			NS	*	NS

* Significantly different according to Fishers LSD test (P<0.05).

*** Significantly different according to Fishers LSD test (P<0.001).

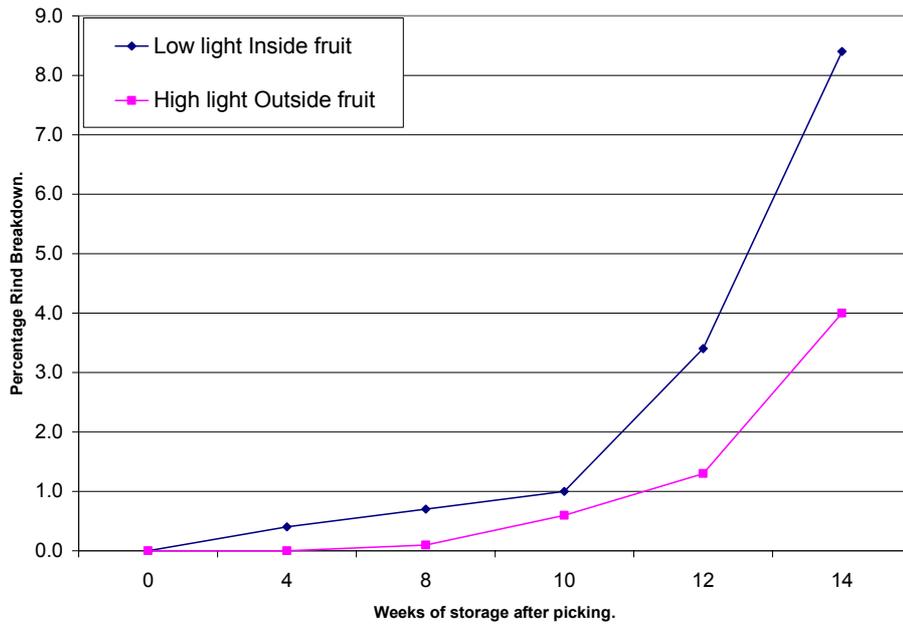


Figure 5.4.7.10. The effect of light level on development of Rind Breakdown in Clementines (Trial C).

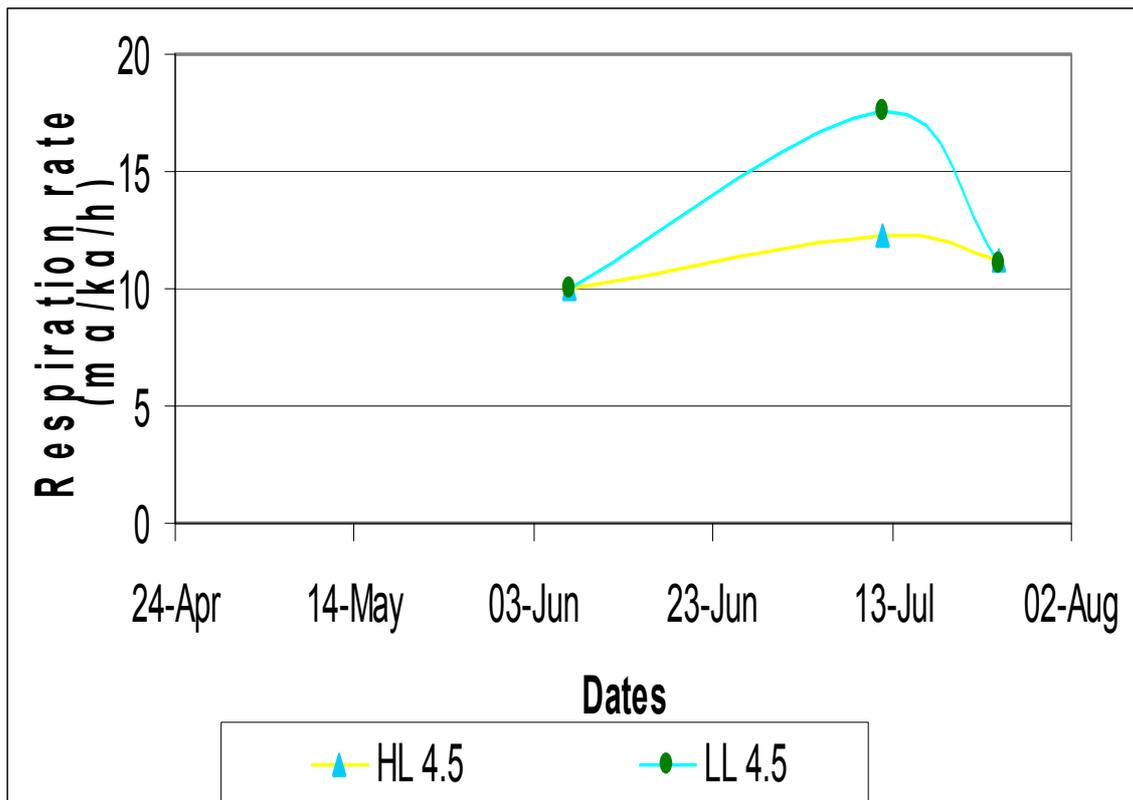


Figure 5.4.7.11. The effect of light levels on the post harvest fruit respiration rates in storage at two temperatures. The "LL 20" indicates fruit originating from low light areas of the tree and kept at 20°C. The "HL 4.5" indicates fruit from high light areas stored at 4.5° C.

Table 5.4.7.6. Effect of the light level on fruit on the mineral composition of the rind of Clementine fruit (Trial C).

Main Factor	N	P	K	Ca	Mg	Na	Cu	Zn	Mn	Fe	Al	B
A. Storage Time												
0	1.91 de	0.2 b	2.71 bc	0.72 a	0.13 a	126.33 a	4.97 abc	11.58 a	10.48 c	62.75	108.17 a	28.05 a
4	1.87 cd	0.21 b	2.47 ab	0.93 bc	0.19 bc	199.17 bc	5.81 d	12.28 a	9.71 bc	76.37 a	119.67 a	27.78 a
6	2.05 e	0.2 b	3.27 d	1.05 d	0.22 d	168.5 ab	5.17 bcd	32.28 b	10.25 c	121.33 b	141 b	30.27 ab
8	1.83 bcd	0.2 b	2.92 cd	1.01 cd	0.21 cd	162 ab	5.53 cd	38.17 b	10.33 c	199.67 c	202.67 c	29.6 ab
10	1.75 bc	0.14 a	2.25 a	1.02 cd	0.2 cd	173.17 b	4.07 a	24.75 ab	9.07 ab	118.67 b	157.5 b	31.83 b
12	1.71 b	0.16 a	2.62 bc	0.84 b	0.19 bc	245.5 d	4.62 abc	33.88 b	8.18 a	204.17	230.83 d	30.03 ab
14	1.42 a	0.14 a	2.19 a	0.94 bc	0.17 b	235.17 cd	4.53 ab	7.15 a	8.48 a	cd 240 d	223.67 d	36.05 c
SE	±0.05	±0.01	±0.13	±0.03	±0.01	±15.12	±0.34	±6.15	±0.35	±13.61	±6.74	±1.27
Significance	***	***	***	***	***	***	*	**	***	***	***	**
B. Treatment												
High Light	1.85 b	0.18	2.46 a	1.00 b	0.21 b	193.29 a	4.67 a	30.46 b	9.99 b	134.86 a	163.81 a	30.63 a
Low Light	1.74 a	0.18	2.8 b	0.85 a	0.16 c	180.95 a	5.25 b	15.28 a	9.01 a	157.41 b	174.33 b	30.41 a
SE	±0.03	±0.01	±0.07	±0.02	±0.004	±8.08	±0.18	± 3.29	±0.19	± 7.27	±3.6	±0.68
Significance	**	NS	**	***	***	NS	*	**	***	*	*	NS

*Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).

**Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).

***Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

Table 5.4.7.7. Effect of light level on pigment content of fruit (Trial C).

MAIN EFFECT	CHLOROPHYLL	CAROTENE	CAROTENE: CHLOROPHYLL
A. Storage Week			
0	1.30 b	30.0	27.3
4	1.14 ab	39.1	80.2
8	0.87 a	28.1	69.8
SE ±	1.2	3.9	11.8
Significance	*	NS	NS
B. Light Level			
Low Light	0.67 a	28.3	42.9
High Light	1.54 b	36.5	25.2
SE ±	0.10	3.2	9.6
Significance	***	NS	***

* Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).

*** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

(iii) The post harvest sensitivity of fruit of two Clementine selections to Rind Breakdown

The comparison between the Nules and Oroval is one of the models used to study differences between Clementine fruit that is sensitive to RB vs. fruit that is resistant to RB. Based on previous indications in operational research, it was thought that the deeper red colour of the Oroval's fruit rind was caused by higher carotene levels in the fruit rind and that this was the factor that made fruit of the Oroval selection more resistant to RB than the Nules selection (van Rensburg & Bruwer, 2000). Furthermore, on a practical level, it was necessary to find out whether the different Clementine selections should be handled differently (due to selection differences in sensitivity to RB), in the export chain.

Results indicate that the **Oroval Clementine fruit did develop relatively low levels of RB (1.6%) compared to the Nules Clementine selection (12.5%)** in Trial A (Figure 5.4.7.12). Furthermore, the RB started to develop later on the Oroval than on the Nules Clementine selection. In trial B the difference between the Nules and Oroval development of RB was 31% vs. 11.7%, respectively, clearly confirming the **sensitivity of the Nules cultivar** (Fig 5.4.7.14).

The pigment analyses indicate that the **Nules contained higher (total) carotene and lower (total) chlorophyll levels than the Oroval Clementine** (Table 5.4.7.8). The result of the analysis of the fruit rinds in the second trial indicate that the Oroval fruit had higher levels of Chlorophyll and lower levels of Carotenes (Table 5.4.7.10). **These results thus refute the hypothesis that the fruit with the higher carotene levels (better coloured fruit) has more resistance against RB than the lesser coloured fruit.**

An analysis of the rind mineral content indicated that the **RB-resistant Oroval selection contained higher levels of Potassium, phosphor and magnesium, copper, zinc and boron, while the levels of manganese were lower, than the Nules Clementine** (Table 5.4.7.9). In the second trial, the analyses show that the Oroval fruit's rinds had higher levels of **Nitrogen, phosphor, potassium, calcium, zinc, iron, aluminium and Boron, while the levels of magnesium and copper were lower** (Table 5.4.7.11). The higher levels of phosphor, potassium and boron were constant in both trials (Tables 5.4.7.9 and 5.4.7.11). **The generally better nutritional status of the Oroval fruit's rind thus might have made it more resistant to RB than the Nules.**

The **respiration rate of the Nules Clementine did not differ from the Oroval Clementine** when measured in storage at 4.5°C (Figure 5.4.7.13).

On a practical level, these results thus indicate that there is a significant difference in RB sensitivity between these two cultivars and that they should be packed and handled differently in the export chain. Fruit of the

Oroval should be picked later and stored longer than the Nules Clementine, as the Oroval selection has a potentially longer shelf life than the Nules Clementine selection.

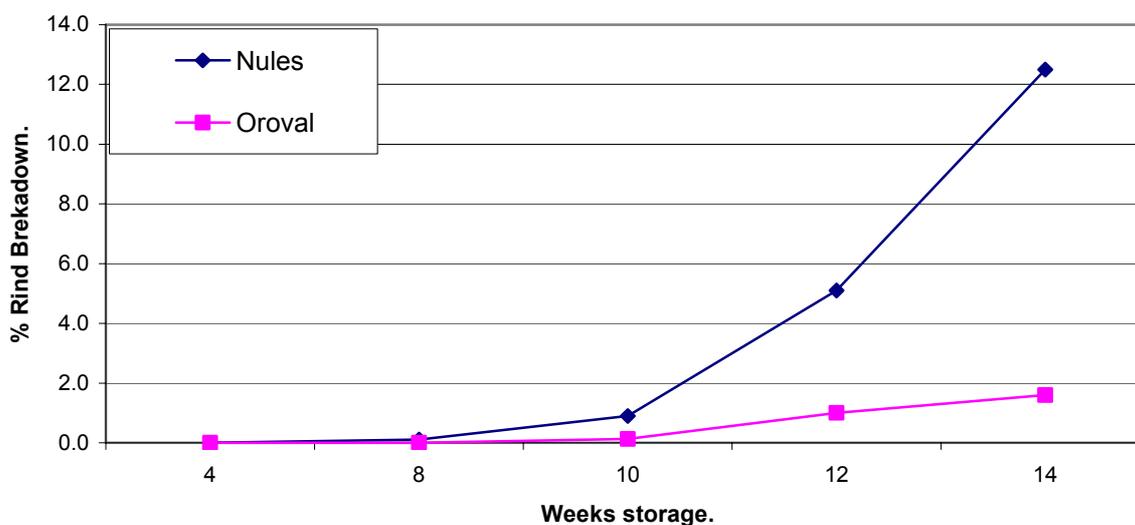


Figure 5.4.7.12. The influence of the Clementine mandarin selection on the post harvest occurrence of Rind Breakdown on the fruit.

Table 5.4.7.8. The influence of the cultivar selection on the pigment content of Clementine fruit.

Treatment		Chlorophyll Concentration	Carotene Concentration	Chlorophyll : Carotene	
MAIN EFFECTS:					
A	Cultivar:	Nules	1.22 b	132.1b	134.7
		Oroval	0.75 a	122.5a	166.6
SE ±		0.15	4.1	17.7	
Significance		*	*	NS	
B	Storage Weeks	4	1.22	98.75 a	132.6 a
		8	1.24	116.53 b	99.9 a
		10	0.72	137.72 c	128.8 a
		12	0.77	156.21 d	241.2 b
SE ±		0.21	5.8	25.0	
Significance		NS	***	**	
INTERACTIONS					
A X B		NS	NS	NS	

* Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).

** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).

*** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

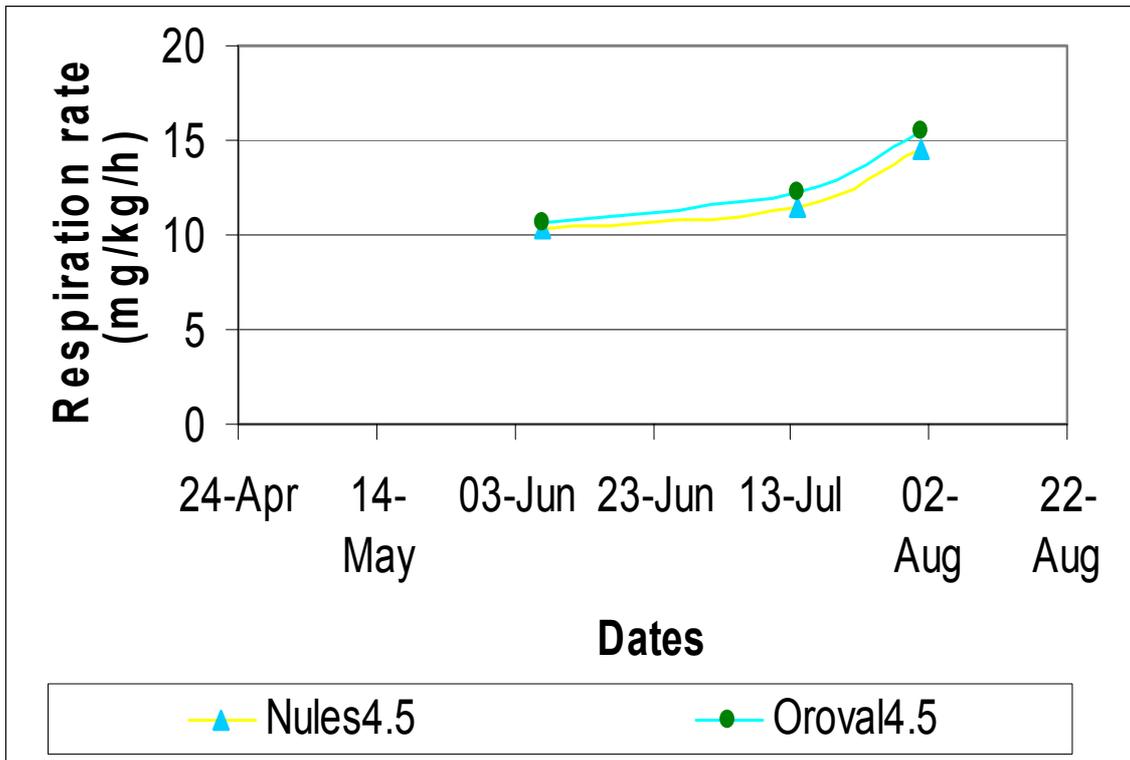


Figure 5.4.7.13. The characteristic respiration rate of the Nules vs. the Oroval cultivar in storage. “Oroval 4.5” indicates fruit from high light areas stored at 4.5°C.

Table 5.4.7.9. The effect of the type of Clementine selection on the mineral content of the fruit rind.

Treatment	Cultivar	Storage Week	N %	P %	K %	Ca %	Mg %	Na %	Mn %	Fe %	Cu %	Zn %	B %
1	Nules	4	1.31	0.076a	1.62	0.70	0.20	164.8	4.4	33.2c	4.0c	6.6b	20.6
2	Oroval	4	1.20	0.092b	2.08	0.63	0.20	155.6	3.2	35.8c	4.0c	7.0bc	22.2
3	Nules	8	1.42	0.092b	1.63	0.62	0.14	231.4	5.0	41.4d	3.2ab	5.0a	21.0
4	Oroval	8	1.43	0.122d	2.07	0.57	0.21	219.0	3.8	46.2e	2.8a	5.4a	24.2
5	Nules	10	1.27	0.068a	1.17	0.71	0.13	196.0	4.8	31.8bc	3.0a	6.6b	25.2
6	Oroval	10	1.34	0.108c	1.92	0.65	0.16	220.6	4.2	28.2b	4.2c	8.8d	29.0
7	Nules	12	1.43	0.076a	1.18	0.66	0.12	178.0	3.8	16.2a	3.2ab	6.8bc	25.8
8	Oroval	12	1.46	0.114cd	1.75	0.63	0.17	151.6	3.6	13.8a	3.8bc	7.8cd	29.2
SE ±			0.06	0.004	0.10	0.05	0.01	18.6	0.3	1.4	0.2	0.4	0.9
Significance			NS	***	***	NS	***	*	**	***	***	***	***
MAIN EFFECTS:													
A	Cultivar	Nules	1.359	0.078	1.40	0.67	0.149	192.6	4.5	30.7	3.4	6.3	23.2a
		Oroval	1.357	0.109	1.96	0.62	0.188	186.7	3.7	31.0	3.7	7.3	26.2b
SE ±			0.030	0.002	0.05	0.02	0.007	9.3	0.2	0.7	0.1	0.2	0.4
Significance			NS	***	***	NS	***	NS	***	NS	*	***	***
B	Storage Weeks	4	1.25a	0.084	1.85	0.67	0.200	160.2	3.8	34.5	4.0	6.8	21.4a
		8	1.43b	0.107	1.85	0.60	0.179	225.2	4.4	43.8	3.0	5.2	22.6a
		10	1.31a	0.088	1.54	0.68	0.148	208.3	4.5	30.0	3.6	7.7	27.1b
		12	1.45b	0.095	1.46	0.65	0.147	164.8	3.7	15.0	3.5	7.3b	27.5b
SE ±			0.04	0.003	0.07	0.03	0.010	13.2	0.2	1.0	0.2	0.3	0.6
Significance			**	***	***	NS	**	**	*	***	**	***	***
INTERACTIONS													
A X B			NS	*	NS	NS	NS	NS	NS	**	**	*	NS

*Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).

**Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).

***Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

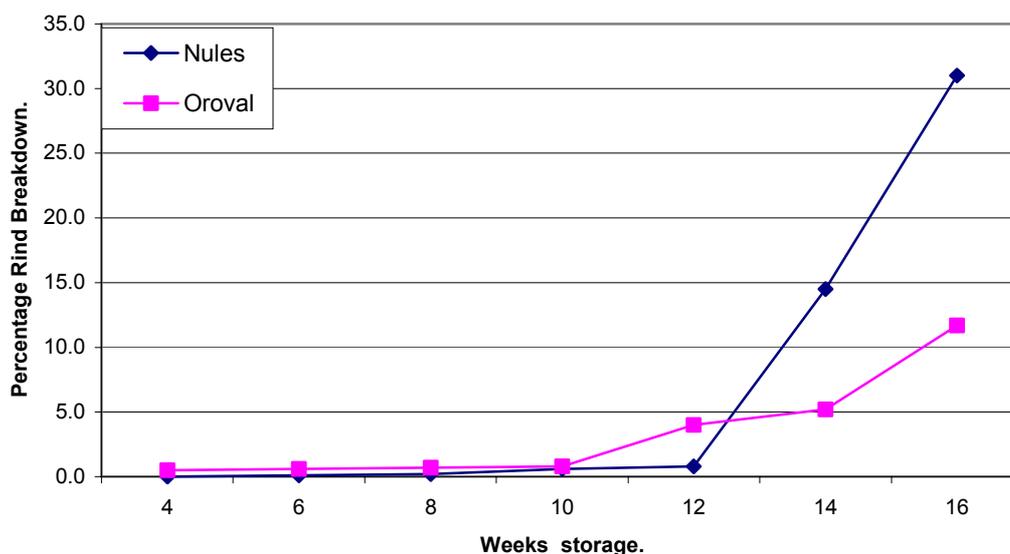


Figure 5.4.7.14. The effect of Clementine selection on development of Rind Breakdown in Clementines (Trial B).

Table 5.4.7.10. Effect of cultivar selection on the rind pigment content of fruit (Trial B).

MAIN EFFECT	CHLOROPHYLL	CAROTENE	CAROTENE: CHLOROPHYLL
A. Storage Week			
0	3.00 b	15.1 a	8.2
2	0.95 a	14.5 a	20.8
4	0.91 a	34.5 ab	53.0
8	0.73 a	20.1 c	23.0
12	1.12 a	23.6 b	36.0
SE ±	0.36	2.00	5.0
Significance	***	***	**
B. Cultivar			
Nules	1.0 a	24.2 b	35.8
Oroval	2.0 1.68 b	18.9 a	21.1
SE ±	0.23	1.3	3.
Significance	*	**	**

- * Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).
- ** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).
- *** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

Table 5.4.7.11. Effect of Clementine selection on the mineral composition of the rind of Clementine fruit (Trial B).

Main Factor	N	P	K	Ca	Mg	Na	Cu	Zn	Mn	Fe	Al	B
A. Storage Time												
0	1.97 cd	0.24 d	3.26 d	0.87 a	0.2 b	143.69 a	6.37 bcd	18.73 a	14.95 c	56.64 a	112.88	18.53 a
4	2.04 d	0.23 d	3.09 cd	1.03 b	0.21 bc	129.75 a	6.83 d	49.33 b	15.71 c	82.91 b	133.25 b	21.83 b
6	2.06 d	0.2 c	3.61 e	1.14 c	0.27 d	129.5 a	5.84 b	44.33 b	14.8 c	119.13 c	146.13 b	22.36 b
8	1.89 c	0.2 c	2.92 bc	1.02 b	0.23 c	189.5 ab	6.03 bc	69.38 c	14.63 c	169.88 e	198.88 d	23.4 b
10	1.59 b	0.18 b	2.43 a	0.98 b	0.22 c	243.75 b	4.65 a	58.61 bc	11.45 b	122.25 c	173.63 c	21.94 b
12	1.64 b	0.18 b	2.61 ab	0.87 a	0.21 bc	332.49 c	4.35 a	132.37	11.49 b	141.84 d	241.99 e	21.84 b
14	1.33 a	0.15 a	2.28 a	0.8 a	0.15 a	280.32 bc	6.79 cd	10.63 a	9.82 a	214.1 f	249.24 e	30.36 c
SE	±0.03	±0.01	±0.11	±0.02	±0.01	±29.36	±0.30	±5.21	±0.40	±5.89	±6.49	±0.71
Significance	***	***	***	***	***	***	***	***	***	***	***	***
B. Clementine selection:												
Nules	1.78	0.18 a	2.5 a	0.89 a	0.22 b	144.46 a	6.58 b	33.43 a	13.3 a	120.53 a	157.74 a	22.04 a
Oroval	0.21	0.21 b	3.27 b	1.02 b	0.21 a	269.54 b	5.09 a	76.1 b	13.23 a	138.54 b	201.12 b	23.75 b
SE	±0.02	±0.003	±0.06	±0.01	±0.004	±16.1	±0.15	±2.86	±0.21	±3.22	±3.56	±0.4
Significance	NS	***	***	***	**	***	***	***	NS	***	***	**

*Means in a column followed by the same letter are not significantly different according to Fishers LSD test ($P>0.05$).

**Means in a column followed by the same letter are not significantly different according to Fishers LSD test ($P>0.01$).

***Means in a column followed by the same letter are not significantly different according to Fishers LSD test ($P>0.001$).

(iv) The influence of the time of harvest on the post harvest shelf life of Clementine mandarin fruit

Operational research, concerning the occurrence of RB on commercially exported Clementine fruit, indicated that fruit that was picked and shipped early in the harvesting season, arrived overseas (after a sea journey of two weeks), without any RB. In contrast, fruit that was picked and shipped at the end of the season (more than four weeks after the first harvesting period) arrived with high levels of RB, after the two week shipping journey. It thus appeared as though the shelf life expectancy of the fruit decreased when the harvest was extended. The current study was conducted to test this hypothesis, but also to study the differences between fruit from resistant vs. sensitive situations, to enable us to find markers that can explain the differences in sensitivity of Clementine fruit to RB.

The fruit used in these trials was generally resistant to RB and the fruit had to be stored for a long period before it developed RB. **Fruit from the first harvest of the season had a longer shelf life than that of the last harvest and only** started developing significant levels of RB after 11 weeks in storage, while the last harvest's fruit developed RB after eight weeks in storage (Figure 5.4.7.15).

An interesting result in the first trial was that the rind of **Clementine fruit from the first harvest, had higher (total) carotene and chlorophyll levels and a lower carotene to chlorophyll ratio, than fruit of the last harvest** (Table 5.4.7.12). Normally, Clementine fruit appears to be greener and less orange/red in colour intensity at the beginning of the season. The "greenness" at the beginning of the season is supported by the higher (total) chlorophyll content that was measured in fruit from the early part of the season (Table 5.4.7.12). However, the higher carotene content, measured at the beginning of the season, does not confirm visual observations, which indicate that the fruit is generally more red/ orange at the end of the season. This again implies that the currently used analytical method, might not measure the pigment that causes the red colour in fruit. The fact, however, that there were higher levels of carotenes in the RB resistant fruit, indicates that the carotene levels in the fruit rind might play a role in fruit resistance to RB in this model. **In the second trial, there were no differences in the carotene and chlorophyll content found in the rinds of fruit from the first, second and third harvest** (Table 5.4.7.14). The chlorophyll content of the rind of the fruit decreased with time, as was expected (Table 5.4.7.14).

Fruit from the first harvest period had higher levels of Nitrogen, phosphor, potassium, calcium, manganese, iron, copper, zinc and boron, compared to fruit harvested at the end of the harvest season (Table 5.4.7.13). This could be due to re-mobilization of nutrients out of the fruit, at the start of fruit senescence. As in previous trials, it appears that the **resistance of the early harvested fruit, to RB could partly be explained by the better nutritional status of the fruit's rind**. In the second trial the rind deviation tendencies were clearer. The fruit from the first harvest started developing RB after 16 weeks, that of the second harvest after 14 weeks and that of the last harvest, after 12 weeks. The harvest dates were two weeks apart (Figure 5.4.7.16). The same, and even clearer, tendencies were found with the appearance of decay (Figure 5.4.7.17). **The results thus indicate that the shelf life expectancy of the fruit decreased by a week with every week that the harvest was extended (Figure 5.4.7.17).**

Practical implications: These results confirm the hypothesis that with an extension of the harvest by one week, one week of shelf life is lost. This implies that it does not matter if the fruit is stored on the tree, or in cold storage after picking – all fruit from a tree has the same shelf life from the point when the fruit becomes harvestable. It also implies strongly that one should handle and market fruit picked at different stages in the season differently, i.e. ship and sell fruit picked in the latter part of the season faster, than fruit picked in the beginning of the season.

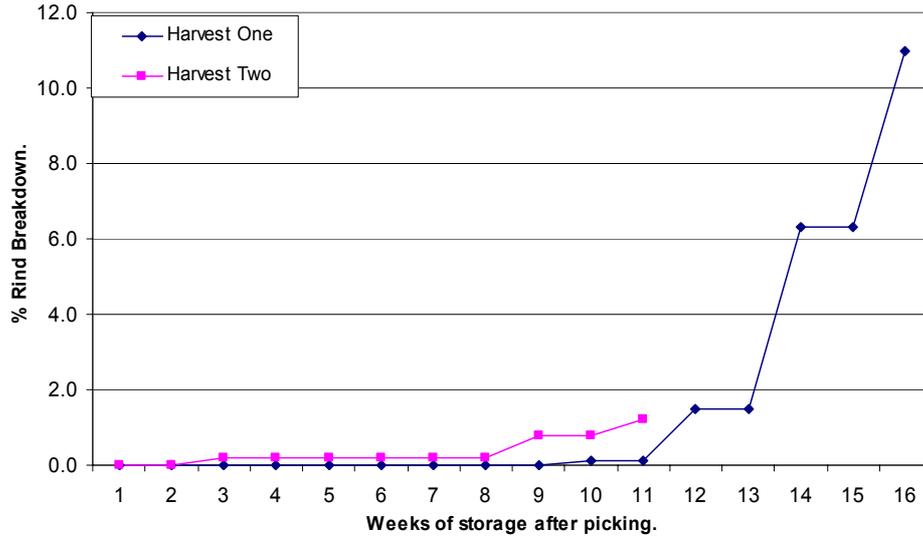


Figure 5.4.7.15. The influence of the harvest date on the post harvest shelf life of Clementine mandarin fruit. Harvest one was at the start of the season, harvest two, four weeks after the start of the season.

Table 5.4.7.12. The effect of harvest date on the pigment content of the rind of Nules Clementine fruit (Trial A).

TREATMENT			CHLOROPHYLL CONCENTRATION	CAROTENE CONCENTRATION	CAROTENE: CHLOROPHYLL
MAIN EFFECTS:					
A	Harvest¹ stage	First harvest	0.6	149.4	382.4
		Last harvest	0.2	124.3	705.4
SE ±			0.1	5.1	44.8
Significance²			**	**	***
B	Storage Weeks	8	0.4	131.9	577.1
		10	0.5	135.4	556.6
		12	0.3	143.2	498.4
SE ±			0.1	6.2	54.9
Significance			NS	NS	NS
INTERACTIONS					
	A X B		NS	***	**

¹ The first harvest was at the beginning of the harvest season, while the second was at the last date of harvest for the season, five weeks later.

² ** Significantly different according to Fishers LSD test (P<0.01).

*** Significantly different according to Fishers LSD test (P<0.001).

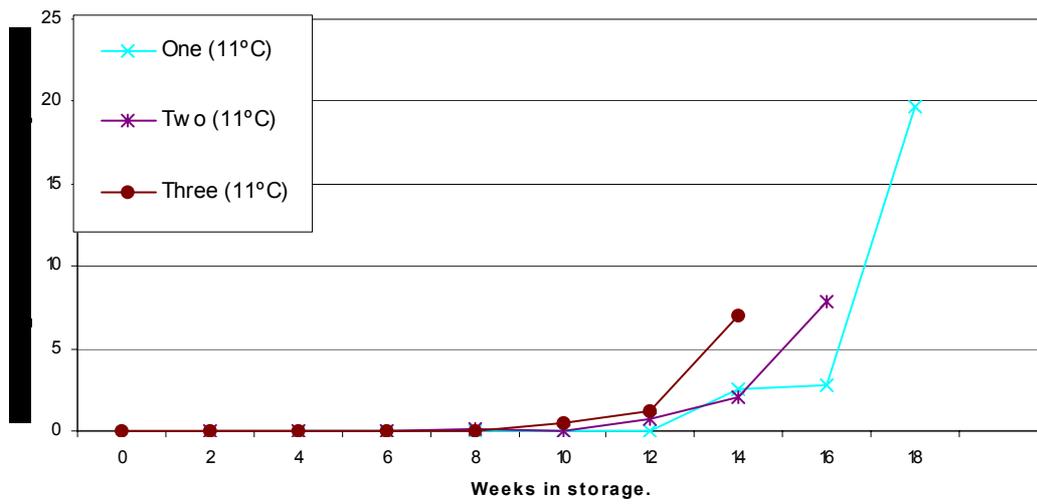


Figure 5.4.7.16. The effect of harvest date on the development of Rind Breakdown in Clementines (Trial B - 2002). “One” is at the beginning of the harvest period and “two” two weeks later and “three” a further two weeks later.

Table 5.4.7.13. The effect of harvest date on the mineral content in the rind of Nules Clementines fruit (Trial A).

Treat-ment			N %	P %	K %	Ca %	Mg %	Na %	Mn %	Fe %	Cu %	Zn %	B %
MAIN EFFECTS:													
A	Harvest¹	First harvest	1.2	0.12	1.5	0.5	0.2	290.3	4.3	24.7	3.9	6.7	27.9
		Last harvest	1.0	0.11	1.3	0.4	0.1	261.3	3.3	17.0	3.3	5.1	21.9
SE ±			0.02	0.002	0.04	0.02	0.01	11.2	0.1	0.8	0.1	0.2	0.5
Significance			***	NS	***	***	*	NS	***	***	***	***	***
B	Storage Weeks	8	1.1	0.11	1.5	0.4	0.2	229.9	3.8	26.0	3.4	4.3	21.0
		10	1.1	0.12	1.3	0.5	0.2	287.7	4.0	24.6	4.2	6.4	25.0
		12	1.2	0.11	1.4	0.5	0.2	309.9	3.6	12.0	3.3	7.0	27.9
SE ±			0.03	0.002	0.05	0.02	0.01	13.7	0.2	0.9	0.1	0.3	0.6
Significance²			*	**	NS	**	NS	***	NS	***	***	***	***
INTERACTIONS													
	A X B		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

¹The first harvest was at the beginning of the harvest season, while the second was at the last date of harvest for the season, five weeks later.

²
 *Significantly different according to Fishers LSD test (P<0.05).
 **Significantly different according to Fishers LSD test (P<0.01).
 ***Significantly different according to Fishers LSD test (P<0.001).

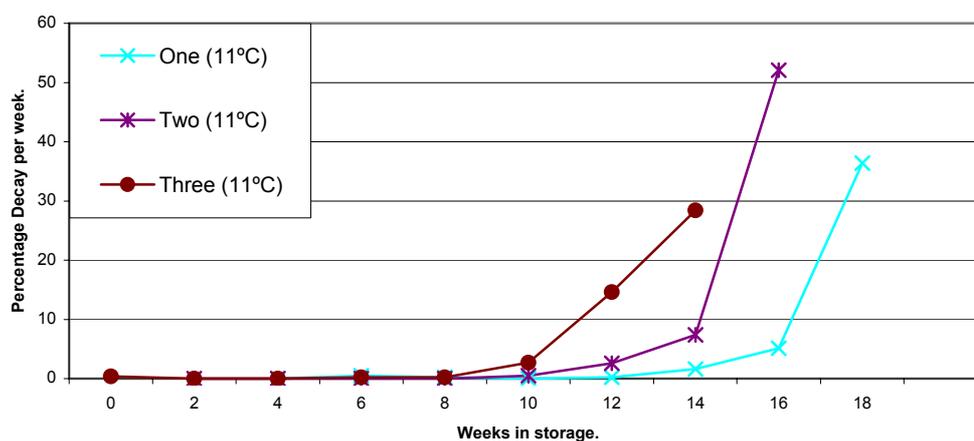


Figure 5.4.7.17. The Effect of Harvest date on Decay in Clementines (Trial B - 2002). “One” is at the beginning of the harvest period and “two” two weeks later and “three” a further two weeks later.

Table 5.4.7.14. The effect of harvest date on the mineral content in the rind of Nules Clementines Fruit (Trial A).

MAIN EFFECT	CHLOROPHYLL	CAROTENE	CAROTENE: CHLOROPHYLL
A. Storage Week			
8	3.40 c	22.3	49.1 c
10	1.02 b	28.7	31.6 b
12	0.62 a	28.6	12.4 a
SE ±	0.52	2.4	0.37
Significance	**	NS	***
B. Harvest			
One	1.78	26.4	24.5
Two	1.50	30.0	24.8
Three	1.75	23.2	24.7
SE ±	0.55	2.4	0.37
Significance	NS	NS	NS

** Significantly different according to Fishers LSD test (P<0.01).

*** Significantly different according to Fishers LSD test (P<0.001).

Table 5.4.7.15. Effect of ethylene treatment duration on pigment content of fruit (Trial B).

MAIN EFFECT	CHLOROPHYLL	CAROTENE	CAROTENE: CHLOROPHYLL
A. Storage Week			
0	0.84	17.3 a	7.9 a
2	0.95	38.9 c	68.3 c
4	1.06	45.5 d	82.1 a
8	1.71	23.8 ab	37.9 b
12	2.52	28.0 b	21.4 ab
SE ±	0.20	3.0	9.0
Significance	**	***	***
B. Ethylene Treat days			
0	1.59	26.9	24.1
2	0.93	31.5	58.6
4	1.32	32.4	43.7
6	1.81	31.7	47.7
SE ±	0.22	2.7	8.1
Significance	NS	NS	NS

** Significantly different according to Fishers LSD test (P<0.01).

*** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

(v) The effect of ethylene treatments on the development of Rind Breakdown in Clementines

An ethylene treatment is used post harvest on Clementine fruit to enhance the fruit colour and make it attractive to the consumer, who prefers red/orange looking fruit. In degreening, the fruit's colour can then change from green, with a slight orange undertone, to completely orange in a period of between 3 and 5 days, depending on a range of factors. The hypothesis that was tested in these trials, was that the degreening process enhances the colour and carotene content of the fruit and that this will lend resistance to the fruit against RB (due to the higher carotene content). Fruit was kept in the degreening room for 0, 2, 4 or 6 days and then packed and stored at normal shipping temperatures for the evaluation period.

The data from the trials indicate that the sensitivity of fruit to RB increased with an increase in the duration of the ethylene treatment. In the first trial, fruit that was treated for 0, 2, 4, or 6 days with ethylene, developed roughly 8, 12, 16 and 18% RB respectively (Figure 5.4.7.18). The incidence of decay on the fruit also increased with an increase in duration of the ethylene treatment, similarly to the RB results (data not shown). **This indicates that the ethylene treatment shortens the shelf life of the Clementine fruit increasingly, with an increase in the time period that the fruit spends in the ethylene chamber.** The results also indicate that there seems to be a maximum time for the ethylene treatment, after which the RB incidence increases dramatically. After two days of treatment the final RB incidence was 10% vs. the >31% RB for treatments of four days and longer. This indicates that the **maximum time of the ethylene treatment should be limited to two days.** The **ethylene treatment increased the appearance of waterspot on the fruit** (Figure 5.4.7.19). This was rather the result of the ethylene treatment that darkened the appearance of the waterspot mark and that made it more visible and recordable. The results of the carotene analysis of both trials indicate that, although the fruit colour was increased dramatically from green to red/ orange, **no differences in carotene levels between the different treatments were measured** (Table 5.4.7.13 and 5.4.7.14 and Figure 5.4.7.20, starting point of graph). These results could possibly be explained by the fact that the green chlorophyll molecules mask the carotene pigments before the ethylene treatment. The carotene levels then stay at a set level during the degreening process, but become increasingly visible with an increase in the duration of the ethylene treatment, as the chlorophyll pigments are broken down. The other explanation might be that the analytical method used, did not measure the red

pigment that is seen visually, as found in other trials. A very interesting result was that the **carotene levels increased drastically**, as time in storage at 4.5°C increased. (Figure 5.4.7.20). The respiration rate of the fruit stored at 20° C “spiked” after eight weeks of storage of the fruit. Those kept at 4.5 ° C, started to increase their respiration after 10 weeks (Figure 5.4.7.21). That coincided with the appearance of RB. It is clear that the fruit has a set shelf life. Thereafter the fruit “dies” – and this is characterised by a peak in the respiration and the appearance of RB.

The results of the second trial mimic those of the first trial. There was again a clear maximum duration for the ethylene treatment. The two day treatment with ethylene gave a final RB% of 10%, while the longer treatments gave >30% RB (Figure 5.4.7.22). The same applies to the decay – 5% RB at two days and >12.3% for longer than 4 days (Figure 5.4.7.23). This indicates that the **maximum duration of the ethylene treatment should be limited to two days.** The same accentuation of the waterspot was also recorded as in the previous trial. There was a clear increase in accentuation of the waterspot with an increase in ethylene treatment duration (Figure 5.4.7.24).

Ethylene is a plant growth regulator that enhances senescence and this might explain the results of the diminishing shelf life and increase of the RB and decay, caused by the ethylene treatment. These results thus disprove the above hypothesis and indicate that the **ethylene treatment does not influence the carotene content of the fruit’s rind, and actually increases the sensitivity of fruit to contracting RB, decay and waterspot.** The practical implication of these results is that the ethylene treatment used in the degreening process for Clementines, should be as short as possible and preferably not longer than two days.

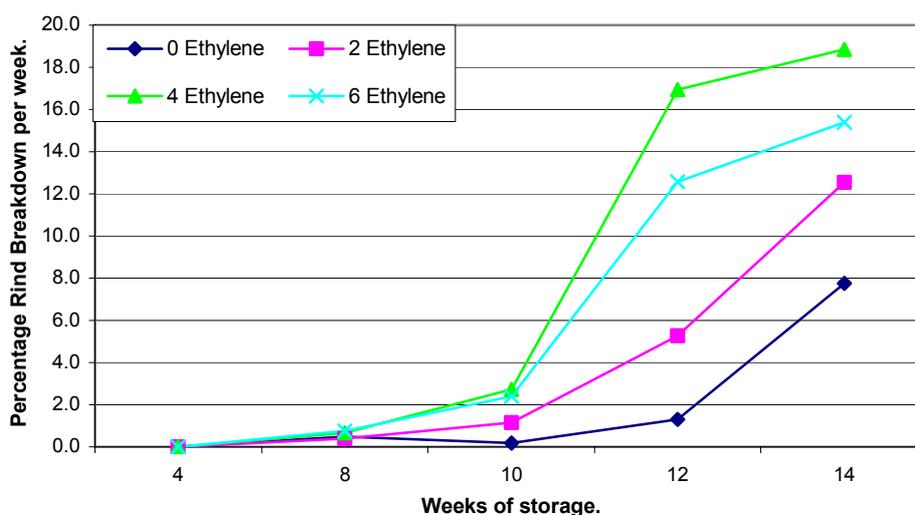


Figure 5.4.7.18. The effect of ethylene treatments on the development of Rind Breakdown in Clementines (2001). The 0, 2, 4 and 6 ethylene indicates 0, 2, 4 and 6 days of post harvest ethylene treatment.

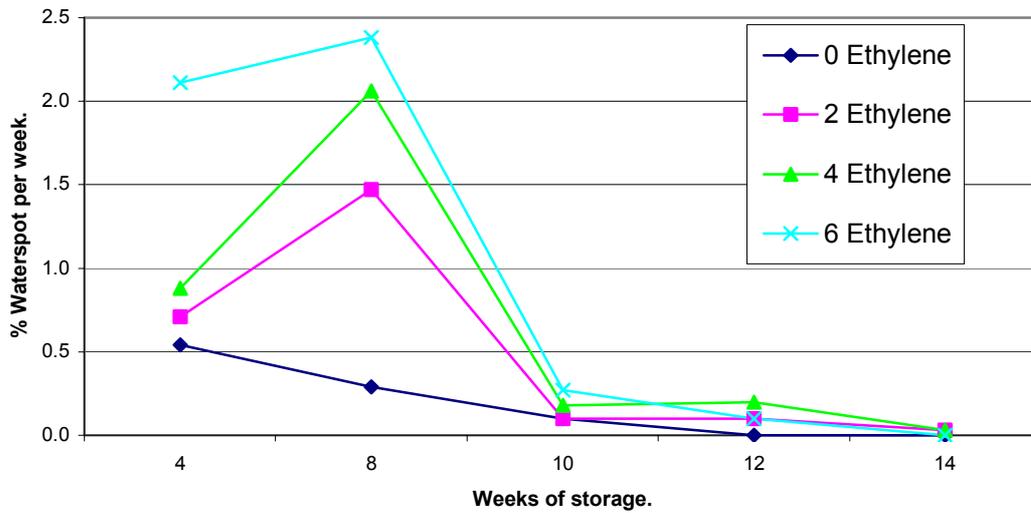


Figure 5.4.7.19. The effect of Ethylene treatments on the development of Waterspot in Clementines (2001). The 0, 2, 4 and 6 ethylene indicates 0, 2, 4 and 6 days of post harvest ethylene treatment.

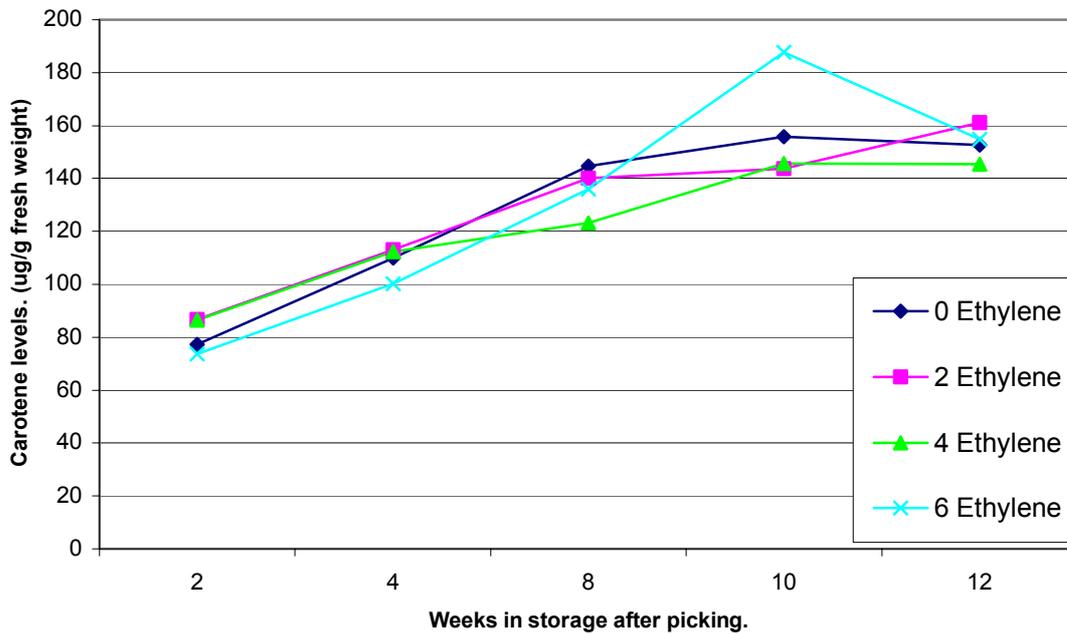


Figure 5.4.7.20. The effect of Ethylene on the Carotene levels in Clementines. (2001). The 0, 2, 4 and 6 ethylene indicates 0, 2, 4, and 6 days of post harvest ethylene treatment.

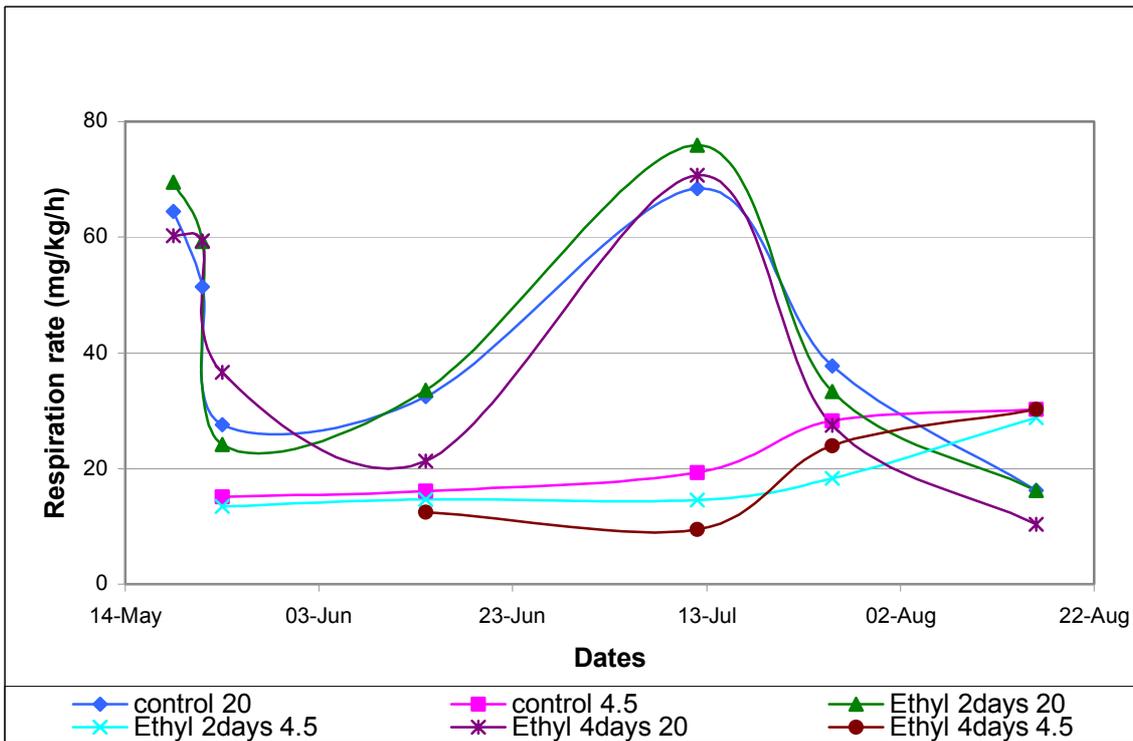


Figure 5.4.7.21. The effect of ethylene use and shipping temperatures on the carotene pigment development of Clementine fruit post harvest. Legend: Ethyl 4 days 20 – indicate ethylene-treated fruit (four days treatment), that was stored at 20°C. Packing of fruit was on 15 May.

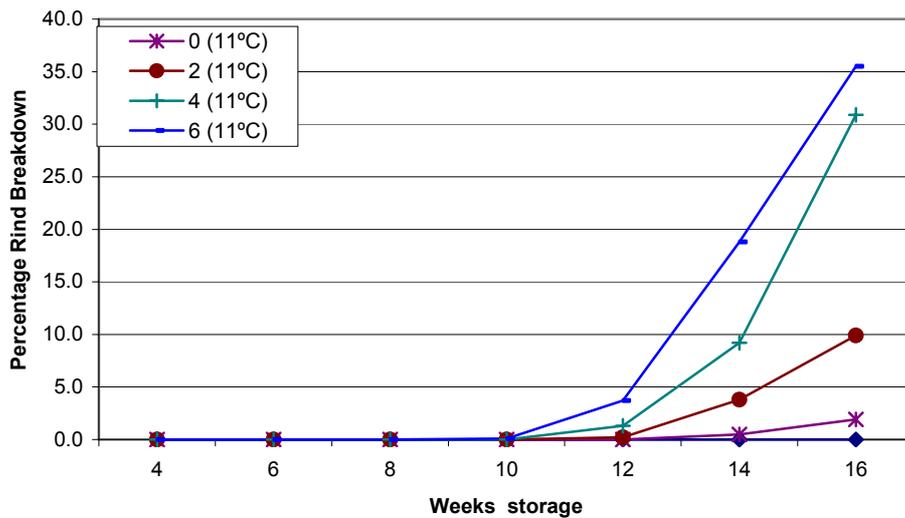


Figure 5.4.7.22. The effect of ethylene treatments on the development of Rind Breakdown in Clementines. The 0, 2, 4 and 6 indicates 0, 2, 4 and 6 days of post harvest ethylene treatment of fruit Trial B.

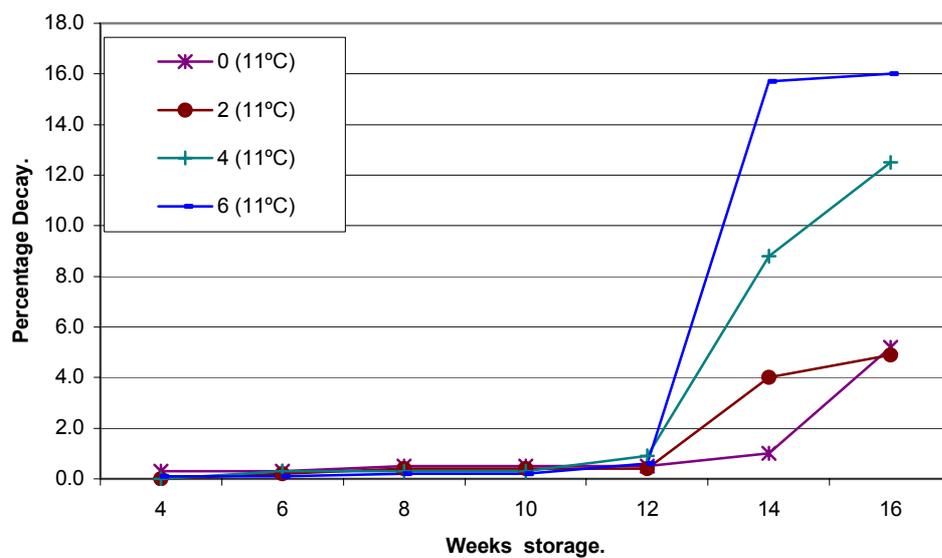


Figure 5.4.7.23. The Effect of Ethylene treatments on Decay in Clementines. The 0, 2, 4 and 6 indicates 0, 2, 4, and 6 days of post harvest ethylene treatment (Trial B).

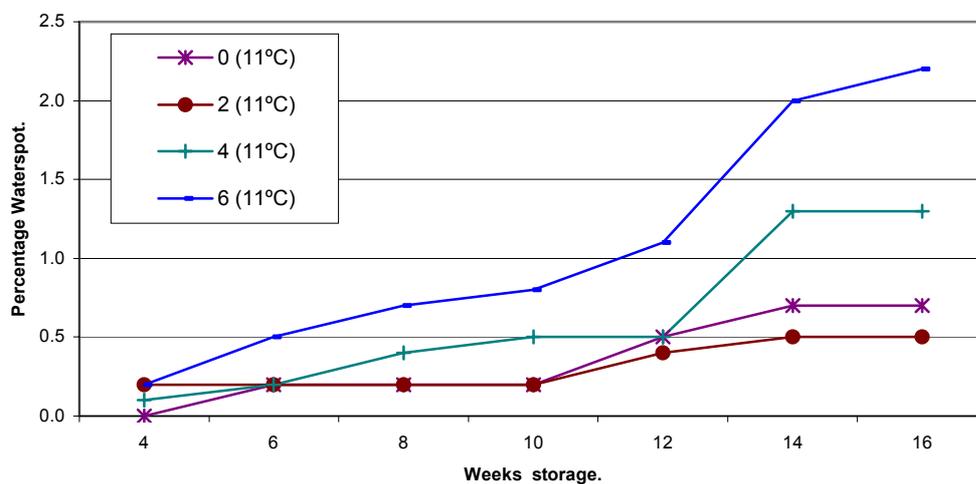


Figure 5.4.7.24. The Effect of Ethylene treatments on the development of Waterspot in Clementines (2002). The 0, 2, 4 and 6 indicates 0, 2, 4, and 6 days of post harvest ethylene treatment.

(vi) The effect of using ozone in cold storage, to influence the occurrence of Rind Breakdown on Clementine fruit

Ozone treatment is used under storage conditions to prevent the build up of ethylene gas in the storage rooms. The ozone breaks the ethylene down, to form carbon dioxide and water vapor. In the degreening trials the negative effects of ethylene on RB became known (Section v, above.). This trial was subsequently designed to investigate the possibility of controlling RB, by limiting the ethylene during shipping and storage, with ozone. Eight commercial integral shipping containers full of commercially-packed Clementine fruit, were shipped to the United Kingdom. Four of these were fitted with ozone generators that released a controlled amount of ozone into the air in the container. The other four were shipped without the ozone-releasing generators. After the voyage of 14 days, sample boxes were taken from each container and placed in two containers, with the fruit from the ozone containers again going into an ozone container, and the fruit from the non-ozone containers going into a non-ozone container. The fruit was then stored for a further five weeks at 4.5°C in these containers and evaluated afterwards.

The results indicate a significant effect of the ozone treatment. At the first evaluation, it was found that there were low levels of RB and decay on all fruit and therefore no clear results on these parameters. This was the same for fruit shipped at either 4.5 or 11°C. However, the ozone had a substantial effect on the puffiness of the fruit. It decreased the puffiness from 5.3% to 1.9% (-180% Table 5.4.7.16). This shows a clear benefit of the ozone treatment.

The results became more noticeable after further storage. **Fruit that was shipped and stored under ozone conditions, at 4.5°C had highly significantly less RB (-175%), decay (-56%) and puffy fruit (-70%)** (Table 5.4.7.17). In explanation, the ozone has the ability to break down the ethylene and kill all the fungal spores in the environment in the storage area. The whole area is thus saturated with an “anti-fungal” ethylene neutralizing agent. This has the direct effect that the fruit will last longer and will show less decay, Rind Breakdown and puffiness in storage.

Fruit that was shipped and stored under ozone conditions, at 11°C also had less puffy fruit (-63%) than that of the control container’s fruit (Table 5.4.7.18). The difference between the results of the fruit that was stored at 11 vs. 4.5°C is surprising as it was expected that the fruit stored at the higher temperature would have had more decay. No explanation for this is presented.

These findings are significant because they might provide a method to significantly enhance the shelf life of the fruit by preventing the occurrence of these post harvest disorders. Ozone could also be seen as a further important step in the chain of controlling fungal decay from the orchard to the market, and even in storage in the receiving countries!

Table 5.4.7.16. The effect of shipping fruit in presence of ozone on the shelf life of Clementines (main effect [average] of 4.5 and 11° C. U.K. - 2001)

	Main effects:		RB	Decay	Puffy
A	Ozone	no ozone	0.8	0.5	5.3 b
		plus ozone	0.7	0.6	1.9 a
	SE		± 0.1	± 0.1	± 0.5
	Significance		NS	NS	***
B	Grower number	61	0.4 a	0.8 b	1.8 a
		62	1.1 b	0.3 a	5.4 b
	SE		± 0.1	± 0.1	± 0.5
	Significance		***	***	***
	Interactions				
	A X B		NS	NS	NS

*** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

Table 5.4.7.17. The effect of storage of Clementines in the presence of Ozone for seven weeks at 4.5°C on the shelf life of Clementines (UK). The results of the two 4.5°C containers with grower nos. 62 and 73s fruit on board (UK 2001).

TREATMENT	OZONE	GROWER	RB %	DECAY %	PUFFY %
1	No ozone	62	0.5a	2.1	7.9a
2	No ozone	73	3.8b	2.9	25.6c
3	Plus ozone	62	0.4a	1.5	8.7a
4	Plus ozone	73	1.2b	1.8	12.3b
SE			±0.3	±0.4	±1.6
Significance			*	***	***
MAIN EFFECTS:					
A	Ozone	No ozone	2.2	2.51b	17.8
		Plus ozone	0.8	1.61a	10.5
SE			±0.24	±0.25	±1.3
Significance			***	**	***
B	Grower no.	62	0.43	1.80a	8.3
		73	2.54	2.32b	19.0
SE			±0.24	±0.25	±1.3
Significance			***	NS	***
INTERACTIONS:					
A X B			***	NS	***

- * Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.05).
- ** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).
- *** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

Table 5.4.7.18. The effect of long term ozone storage at 11°C on the shelf life of Clementines. The results of the two 11°C containers with grower numbers 61 and 62s fruit on board (U.K. - 2001).

Treat-ment	Ozone	Grower	RB	Decay	Puffy
1	No ozone	61	3.2	0.47 a	2.3 a
2	No ozone	62	2.9	1.56 b	8.1 c
3	Plus ozone	61	2.9	0.41 a	1.0 a
4	Plus ozone	62	1.7	2.17 b	5.5 b
	SE		± 0.57	± 0.24	± 0.6
	Significance		NS	***	***
	Main effects:				
A	Ozone	No ozone	3.0	1.03	5.2 b
		Plus ozone	2.3	1.31	3.2 a
	SE		± 0.4	± 0.18	± 0.5
	Significance		NS	NS	***
B	Grower	61	3.0	0.46	1.6 a
		62	2.3	1.88	6.8 b
	SE		± 0.4	± 0.18	± 0.5
	Significance		NS	***	***
	Interactions				
	A X B		NS	NS	NS

*** Means in a column followed by the same letter are not significantly different according to Fishers LSD test ($P > 0.001$).

(vii) The effect of the shipping temperatures on the incidence of Rind Breakdown on Clementine fruit

The hypothesis early in the project investigation, was that the RB that was experienced, was due to chilling injury (CI). It appeared as though the areas between the oil cells were damaged, as was experienced with CI on other citrus fruit types. This trial was conducted to test the effect of different shipping and post shipping storage temperatures on the sensitivity of fruit to RB. Fruit was kept under simulated shipping conditions and at shipping temperatures of -0.5; 4.5; 6.0; 7.5; 9 and 11°C. Thereafter it was stored at either 4.5° or 11°C. A second trial was conducted on Minneolas that are prone to developing RB. Minneola fruit were stored at either 11 or -0.5°C or at -0.5°C under controlled atmosphere conditions. Because CI was thought to be involved, a second trail was conducted to simulate CI stimulating conditions. Fruit was very rapidly pre-cooled for 72 hours at 4.5°C, under forced air cooling conditions, and this was compared with fruit cooled under slow static cooling conditions at 11°C.

The results clearly point to RB not being a CI type of damage. To the contrary, the incidence of **RB increased very significantly with an increase in shipping temperature**, from 3.9% RB at -0.5°C to 18% RB at 11°C (Table 5.4.7.19). These results also indicate that **there was no difference in the RB sensitivity of fruit, whether it was stored at 4.5°C or 11°C after the shipping phase** (Table 5.4.7.19). This implies that the Clementine fruit is only RB sensitive during the initial shipping phase, but not thereafter. The results of the **Minneola trial clearly point to higher shipping temperatures causing the appearance of RB and that fruit could be safely shipped at low temperatures, with minimum chance of developing RB** (Table 5.4.7.20). **The results of both trials point to RB being a high storage temperature phenomenon, as on Clementines, as well as Minneolas.** The results from the pre-cooling trial indicated that fruit kept under both the rapid cooling, low temperature regime, as well as the slow cooling, higher temperature regime, experienced RB to the same extent (Table 5.4.7.21). This indicates that **RB is not induced by CI stimulating conditions and that CI is an unlikely cause of RB.**

The practical implication of these results is that Clementine fruit should be shipped at lower temperatures to prevent the fruit from contracting RB. Thereafter, the temperature at which the fruit is stored, is not an important factor that will influence the fruit developing RB symptoms.

Table 5.4.7.19. Effect of shipping and storage temperatures on the post harvest reaction of Clementine fruit.

MAIN EFFECTS:		
A. SHIPPING TEMPERATURE (°C)		
Temperature	Rind Breakdown	Decay
-0,5	3.9 a	3.4 ab
4.5	7.1 ab	2.6a
6.0	9,2 ab	3.3 ab
7.5	11.2 abc	4.0 ab
9.0	12.6 bc	6.3 b
11.0	18.0 c	5.0 ab
SE	± 2.6	±1.1
Significance	**	NS
B. STORAGE TEMPERATURE °C		
Temperature	Rind Breakdown	Decay
4.5	11.0a	2.4a
11.0	9.7a	5.8b
SE	±1.6	±0.7
Significance	NS	***
INTERACTION		
A X B	NS	NS

** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.01).

*** Means in a column followed by the same letter are not significantly different according to Fishers LSD test (P>0.001).

Table 5.4.7.20. The effect of cold steri temperatures on Rind Breakdown in Minneolas (± SE).

Treatment	RB %	
	Light	Severe
1. Control – stored at 11°C	10.9 – 2.1	16.4 ± 2.7
2. Steri – stored at –0.5°C	8.5 ± 1.6	1.58 ± 1.1
3. Steri + CA – stored at –0.5°C and under CA conditions	12.1 ± 1.8	2.5 ± 1.1

Table 5.4.7.21. The effect of forced air cooling vs static cooling on the occurrence of Rind Breakdown, oleocellosis and decay on Clementines.

Main Factor	Oleocellosis %		RB %	Post harvest decay %
	Light	Severe		
A.				
1. Static air at 11°C	5.6	1.0	6.6	0
2. Forced air at 4.5°C + static at 11°C	11.1	2.2	6.6	0.3
3. Forced air at 11°C + static at 11°C	7.2	3.1	4.2	0.4
Significance	NS	NS	NS	NS
SE	± 3.3	± 0.6	± 1.8	± 0.3
B.				
1. Inside fruit	10.2	3.4	9.4	0.1
2. Outside fruit	5.7	0.8	2.1	0.4
Significance	NS	**	**	NS
SE	± 2.7	± 0.5	± 1.5	± 0.2
Interaction between A and B	NS	*	NS	NS

*, ** Significantly different from Control (P<0.5 and 0.01 respectively)

Conclusions

Rind Breakdown (RB) occurs mainly on Clementines, but also on Satsuma mandarins that are shipped over long distances to overseas markets. The mechanism of damage is through the collapse of oil cells in the oil glands of the fruit rind. The oil then oxidizes the albedo tissue and the oxidized tissue becomes visible as regularly shaped brown spots on the rind surface. The results of eight year's trials on RB, have indicated the following.

Factors influencing RB: The general tendency indicates that Rind Breakdown develops during years when a relatively mild winter precedes the season and the fruit matures during a relatively warm autumn. The results indicate that fruit is more prone to developing RB under the following conditions :

- fruit of the Nules Clementine
- fruit growing under low light conditions on the inside of the tree
- fruit that is harvested late in the season
- fruit that is treated for longer than two days with ethylene
- fruit that is shipped at relatively high temperatures

On the positive side, fruit could be made more resistant to RB by :

- using Maxim orchard applications to trees
- making sure that the nutritional status of the trees is optimal
- by pruning trees to let sunlight enhance the colour of fruit
- by picking and shipping fruit from low light areas on the tree early in the season
- by discarding pale coloured fruit in the packhouse
- by utilizing ozone in fruit storage
- by utilizing low shipping temperatures.

A finding in all trials was that the Clementine fruit tended to collapse and develop RB very rapidly in storage once a certain critical storage period (generally between four to eight weeks after picking and storage), had been reached. This indicates a limited potential shelf life inherent to Nules Clementine fruit.

Biochemical/physiological level: The results of the different hypotheses studied indicate that the Clementine fruit's resistance or sensitivity could be explained by at least two factors. Clementine fruit that was harvested early in the season had higher levels of carotenes in the fruit and was more resistant to RB than the fruit at the end of the season. This result might indicate that the carotene levels in the fruit rind might possibly play a role in the resistance of the Clementine fruit to RB. In the case of the most RB resistant models/ scenarios, the general nutritional status of the fruit rind was found to be better than the RB sensitive comparisons. It points to the nutritional status of the rind being an important factor that controls RB resistance of the fruit's rind.

Operational research: Export data were used to find correlations between damaged fruit and specific farm, packhouse and export chain operations.

Prediction: Rind Breakdown was predicted, based on macro climatic conditions – a warm winter and a warm autumn.

Technology transfer and implementation: As part of the overall strategy, a co-ordinated effort was made to interpret the research results and to transfer the technology to the end users.

Advice was given and consultation conducted on production, picking and packhouse procedures, shipping and export conditions and indexing for fruit sensitivity. Identification of rind problems was done for exporters, based on fruit samples and photographic evidence from the markets. Publications in the form of newsletters to growers have been published in collaboration with exporters.

RB prevention strategies have been developed for the industry, which have been distributed to producers, packhouses and export agents. Various specific trouble shooting exercises, both local and overseas, have been conducted for producers, packhouses, exporters and importers. Visits to receivers of fruit in overseas markets have been made to ascertain damage types and incidence and advice was supplied on the handling of fruit with rind deviations. Risk management exercises have been conducted.

Future research

The research should in future focus on the post harvest and shipping phase of the export operation. The conditions (temperatures and heat build up, CO₂ and ethylene concentrations and relative humidity) and fluctuations in these conditions in the export chain, should be investigated. The hypotheses tested in the above experiments, have been an attempt to find a biochemical basis for Clementine fruit resistance or sensitivity to RB. The above knowledge that was created, may possibly be applicable to the post harvest problems on other cultivars.

Acknowledgements

The co-authors are thanked for their contributions to the successful research effort. The Citrus Growers Association of Southern Africa and LGS Exports (SA) are thanked for the funding of the research and time allocated to the project, the Cape Citrus, Capespan and Colors exporting companies for contributing fruit, William Bourbon-Leftley for packing the experiments, and a range of citrus producers for making their farms available for the experiments. Thank you also to the co-authors who spent a huge amount of time, energy and focus on this project.

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6 PROGRAMME: CULTIVAR AND ROOTSTOCK DEVELOPMENT

6.1 PROGRAMME SUMMARY

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

The Cultivar and Rootstock Development programme comprises two projects: Breeding and Evaluations. Although the planned research in the Breeding project was undertaken by the ARC-ITSC during 2003, an agreement could not be concluded on the ownership of intellectual property of new cultivars. For this reason, the report on Breeding research was not submitted for publication in this research report nor were a few ARC-ITSC contributions to the Evaluations project. Although some new cultivars such as Salustiana and several Valencia selections look promising, the search continues for early Satsumas and Clementines. No proof of incompatibility between the Fukumoto navel and Citrange rootstocks has yet been found in southern Africa. The search for new rootstocks to correct various problems and improve quality is starting to produce some good results, but many of the trees are still young.

Programopsomming

Die Kultivar en Onderstam Ontwikkelingsprogram bestaan uit twee projekte: Teling en Evaluering. Alhoewel die beplande navorsing in die Telingsprojek in 2003 deur die LNR-ITSG onderneem is, kon uitsluitel nie verkry word oor die eienaarskap van intellektuele eiendom van nuwe kultivars nie. Om hierdie rede is die verslag oor Telingsnavorsing, asook 'n aantal bydraes van die LNR-ITSG tot die Evalueringsprojek, nie voorgelê vir publikasie in hierdie navorsingsverslag nie. Hoewel sommige nuwe kultivars soos Salustiana en verskeie Valencia-seleksies belowend lyk, duur die soektog na vroeë Satsumas en Clementines voort. Geen bewys van onverenigbaarheid tussen Fukumoto-nawel en Citrange onderstamme is tot dusver in Suider-Afrika gevind nie. Die soektog na nuwe onderstamme om verskeie probleme reg te stel en gehalte te verbeter begin om goeie resultate te lewer, maar baie van die bome is nog jonk.

6.2 PROJECT: EVALUATIONS

Project Co-ordinator: Thys du Toit (CRI)

6.2.1 Project summary

The purpose of the Cultivar and Rootstock evaluation project is to evaluate new scion and rootstock cultivars, compare these cultivars with existing commercial cultivars and make recommendations to the South Africa citrus industry.

On the recommendation of the growers an internal appointment was made in the CRI by the transfer to Nelspruit of Johan Joubert, who has been made responsible for inland evaluations. We are still privileged to have the services of Chris Alexander who carries out evaluations in the West and East Cape on a contract basis. The ARC have not submitted reports for the projects under their jurisdiction.

Market demand is a major factor to consider when determining which cultivars should be developed, and, to this end, input from export agents in respect of market trends is of utmost importance. In general the gap in the market is for early maturing soft citrus and navels, as well as a lengthened and well spread out maturity period in specific cultivar groups to facilitate keeping fruit in the market for the longest possible period. Late, seedless mandarins, high quality late navels and seedless high quality valencias have been identified as important cultivars.

The following summaries per cultivar group examine whether we meet market requirements:

Satsumas: None of the Satsuma selections evaluated are earlier than existing selections, although the selections can be used as replacements for current commercial selections. The search for earlier maturing selections and evaluation on the later selections continues.

Clementines: The Esbal shows promise as an early Clementine, but fruit size is smaller than Nules. External colour and fruit size continues to be a problem in the inland areas. A more spread out harvesting period is very important to ease the sharp peak of the Nules Clementine. Evaluations continue.

Mandarins: Numerous selections have been evaluated and a few selections look promising. However the trees are still young causing unreliable evaluation results, from which recommendations cannot yet be made.

Navels: The Fukumoto is a promising early navel, but in the USA incompatibility with citrange rootstocks has been experienced. No proof has yet been found to show that this problem will also be present in South Africa. The cultivar will be classed as experimental until its rootstock compatibility can be proven. Planting of this rootstock is therefore currently at the grower's own risk. The Cara Cara has good internal quality in the intermediate and cool inland areas, but will have to be marketed more effectively. Late navel selections did not deliver good results and will continue to be evaluated.

Midseasons: A commercial Salustiana planting in the Sundays River Valley is showing excellent result and can be harvested in June. Other selections also look promising, but problems are experienced with thorniness and fruit splitting. Red-pigmented selections require cold areas for internal colour development.

Valencias: A few selections, such as the Alpha, Delpont and Glen Ora, look promising when compared to commercial selections such as the Midnight. However, these cultivars must still be evaluated under semi-commercial conditions for better comparison.

Lemons: No significant results were obtained, except for the seedlessness found in the Villafranca. This cultivar will have to be compared to the Eureka Seedless under semi-commercial conditions.

Rootstocks: The search for better rootstock combinations for higher internal quality, reduction of external problems such as creasing, disease resistance and compatibility is important to the citrus industry. Trees in all the evaluation trials are still very young, but so far good results are being obtained from certain scion combinations with the following, fairly new, rootstocks: Benton citrange, C35 citrange, Minneola X Trifoliata, F900/3, F80, C61 and C32 citrange. Better results are anticipated as these trees mature.

Projekopsomming

Die doel van die Kultivar en Onderstam- evaluasieprojek is om nuwe bostam- en onderstam kultivars te vestig en te evalueer en te vergelyk met bestaande kommersiële kultivars en aanbevelings te maak aan die Suid-Afrikaanse Sitrusbedryf.

Op aanbeveling van die produsente is 'n interne aanstelling in die CRI gemaak deur die verskuiwing van Johan Joubert na Nelspruit vir die hantering van al die Noordelike en binnelandse evaluasie projekte. Ons is nog steeds bevoorreg om vir Chris Alexander op kontrak te gebruik vir die Wes- en Oos - Kaap evaluasies. Die LNR se evalueeringsverslae van projekte wat deur hulle hanteer word, is nie aan ons verskaf nie.

Met die ontwikkeling en evalueering van nuwe kultivars is dit baie belangrik om te bepaal wat die behoefte is van die mark en daaraan te voldoen en die insette vanaf die bemarkers is hiervoor baie belangrik. Oor die algemeen is die mark op soek na vroeë sagtesitrus en nawels en 'n goeie verspreiding van 'n spesifieke kultivargroep wat solank as moontlik op die mark beskikbaar is. Laat saadlose manderyne, goeie kwaliteit laatnawels en saadlose hoë kwaliteit valencias is baie belangrik.

Die volgende opsomming per kultivar groep bepaal of ons aan die behoefte voldoen:

Satsumas : Geen van die geëvalueerde Satsuma seleksies is vroeër nie, maar van die seleksies kan wel as plaasvervangers vir die huidige kommersiële seleksies gebruik word. Die soektog na vroeër seleksies gaan voort asook die evaluasies op die later seleksies.

Clementines : Die Esbal lyk belowend as 'n vroeër Clementine maar die vrugte is kleiner as Nules. Vrugkleur en vruggrootte bly 'n probleem in die binneland. 'n Beter oesverspreiding op Clementines is baie belangrik om die Nules piek af te plat. Evaluasies gaan voort.

Mandaryne: 'n Hele klomp seleksies is geëvalueer en 'n paar is belowend, maar die bome is nog jonk met onbetroubare resultate waaruit aanbevelings nie nou gemaak kan word nie.

Nawels: Die Fukumoto is 'n belowende vroeë navel, maar probleme met onvereenigbaarheid op citrange onderstamme kom in die VSA voor, maar geen bewys is tot dusver gevind dat dit ook in die RSA 'n probleem sal wees nie. Totdat sekerheid verkry is, word die kultivar as eksperimenteel beskou en op die produsent se eie risiko aangeplant. Die Cara Cara lewer goeie interne gehalte in die intermediêre en koel binnelandse areas, maar sal beter bemark moet word. Die laat seleksies het nie goeie resultate gelewer nie en sal verder geëvalueer moet word.

Midseisoeners : n Kommersiële Salustiana aanplanting in die Sondagsrivier lewer uitstekende resultate en

kan in Junie geoes word. Ander seleksies lyk ook belowend maar probleme met doringagtigheid en split kom voor. Rooi gepigmenteerde seleksies het koue nodig vir die interne kleur ontwikkeling.

Valencias : 'n Paar seleksies soos die Alpha, Delport en Glen Ora lyk belowend en vaar goed teenoor kommersiële seleksies soos die Midnight, maar moet onder semi-komersiële toestande geëvalueer word vir 'n beter vergelyking.

Suurlemoene : Geen noemenswaardige resultate behalwe dat die Villafranca saadloos toets en sal verder vergelyk moet word met die Eureka SL onder semi-komersiële toestande.

Onderstamme : Die soeke na beter onderstam kombinasies vir hoër interne gehalte, ekstenne probleme soos kraakskil, siektebestandheid en verenigbaarheid is baie belangrik vir die bedryf. Al die evaluering proewe is nog baie jonk, maar op die stadium vaar die volgende redelike nuwe onderstamme goed met sekere bostam kombinasies nl: Benton Citrange, C35 Citrange, Minneola x Trifoliata, F80/3, F80, C61 en C32 Citrange. Beter resultate word verwag met die toename in ouderdom van hierdie proefbome.

6.2.2 **Sub-Project: Cultivar and rootstock evaluation in the Cape region** Project Co-ordinator: C.J. Alexander (Private)

6.2.2.1 **Subprojekopsomming**

Die verslag behels werk wat deur Chris Alexander, gehelp deur Zongezile Zondi, in die suiderlike sitrusproduksiegebiede van die Oos en Wes-Kaap gedoen is. Van die proefpersele was nie in die begroting ingesluit nie, maar is wel geëvalueer om te voorkom dat data verlore gaan. Dankie aan al die kwekers wat proefbome op hulle plase aanhou wat geëvalueer kon word.

Sub-project summary

This report comprises work conducted by Chris Alexander and assisted by Zongezile Zondi in the southern citrus production areas of the Eastern and Western Cape. Some of the sites, although not included in the budget were evaluated to ensure that data was not lost. Thank you to all growers who co-operated by having trees available for evaluation on their farms.

6.2.2.2. **Evaluation of Satsuma mandarins in the Cape areas** Experiment 57 by C J Alexander (CRI)

Opsomming

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 te voorkom. Bepaal of die Miyagawa en Okitsu Wase seleksies 'n verbetering op die huidige reeks is wat tans kommersieel geplant word

Geen van die satsuma seleksies het aan die doelstelling van 'n vervroegde seisoen beantwoord nie. Daar is egter alternatiewe vir die huidige kommersiële reeks seleksies wat die seisoen van die Miho Wase tot na die Owari-seleksie kan verleng. Die Miyagawa en Okitsu Wases het swakker in die Wes-Kaap in semi en kommersiële aanplantings gevaar as die Oos-Kaap, rypwording is na die Miho Wase. Kommersiële aanplantings van hierdie seleksies kan oorweeg word om na die Miho Wase geoes te word. Okitsu se gehalte is beter as Miyagawa Wase. Ohtsu, Aoshima en Ueno was ook geëvalueer, gehalte onaanvaarbaar en rypwording April of Mei, kommersiële Dobashi Beni nog nie in produksie nie. Evaluasies van Dobashi Beni en die later seleksies moet nog voortgaan.

Introduction

The objective of the Satsuma project is to provide high quality, well coloured fruit early in the southern hemisphere marketing season, to capitalise on market opportunities between the late northern hemisphere season and early Southern African citrus season and to overcome anticipated production peaks by extending the harvest season earlier. The Satsuma X Nova, Ohtsu, Aoshima and Ueno were evaluated although not included in the budget.

Materials and methods

The trial trees are either planted or topworked within commercial orchards, or established on a semi-commercial scale, with Miho Wase control where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Due to budget cuts, the sites evaluated are limited, providing fewer results.

Fruit quality was compared with the following standards previously considered most acceptable by the market place, based on visual and organoleptic tests: 48% juice; 9.0% TSS; 0.7 – 1.5% acid; 7.5:1 ratio; colour T3 of set 36 of Outspan Blemish standards. Zero seeds/fruit. A list of the selections and sites evaluated is given in Table 6.2.2.2.1.

Table 6.2.2.2.1. Satsuma trial sites evaluated during 2002.

Selection	Area	Site	Plant Date	Root stock	No of trees
Satsuma X Nova	Addo	ITSC	2000	CC	3
Miyagawa Wase	Patensie	Patensie Acht	1995	TC	semi com
Miyagawa Wase	Wolseley	Whitebridge	95/96	CC	commercial
Okitsu Wase	Patensie	Patensie Acht	1995	TC	semi com
Okitsu Wase	Wolseley	Whitebridge	95/96	CC	commercial
Ohtsu	Uitenhage	CFB	95/97	TC/SC	1/6
Aoshima	Uitenhage	CFB	1994	TC	2
Ueno	Uitenhage	CFB	1997	TC/SC	3/3
Miho Wase (control)	Patensie	Patensie Acht	1995	TC/SC	semi com
Miho Wase (control)	Wolseley	Whitebridge	1989		commercial

Results and Discussion

A discussion of each selection follows with internal quality results presented in Table 6.2.2.2.4. which need to be referred to when reading the text.

ITSC Satsuma X Nova. (Refer Mandarin section in previous reports – Satsuma X Ellendale). The trees had a fair crop of medium large fruit size, varying between T2-3 and 4-5 on different trees on 15 April. The fruit is flat with a smooth rind. There were about 2 seeds per fruit. Quality was good with fairly high sugars and acid with a good balance and mature. No tests were done.

Miyagawa and Okitsu Wase. Trees were evaluated at two sites. The Patensie trees were planted adjacent to each other, Miho Wase on both Troyer citrange and Swingle citrummelo rootstocks (75 and 25% respectively). All had good yields, Miho Wase superior to Miyagawa and Okitsu in terms of yield and percentage grade I fruit. Miyagawa had the largest fruit size. Forty seven internal quality tests were done at for all the selections, the averages on specific dates given in Table 6.2.2.2.4. During February, none of the tests met export standards (high acid), all acceptable by 3 March (and thereafter), except colour due to a considerable drop in acid. Looking at the test averages, TSS was similar for all three selections, Miyagawa 0.1% higher acid, all tests good. Colour was similar. Eating quality was fair, mature and ready to be harvested (except colour) at the end of March, Okitsu a slightly better taste and Miyagawa the poorest but little difference. Okitsu had some slightly nose fruit. Refer Table 6.2.2.2.2 for figures.

Table 6.2.2.2.2. Average yield per tree, percentage fruit in grade 1, percentage fruit per count in grade 1 for various Satsuma selections harvested at Patensie during 2003.

Selection	Ave yield /tree (kg)	Export fruit % (grade 1)	Percentage fruit per count (grade 1)								% in count 1 - 3
			1 xxx	1 xx	1 x	1	2	3	4	5	
Miho Wase	58.3	72.0		8	18	19	34	14	5	2	67
Miyagawa Wase	45.3	68.4		18	30	19	22	8	2	1	49
Okitsu Wase	40.4	61.2		9	20	17	34	14	4	2	65

Wolseley. Picking of the Miho Wase controls commenced on 3 April and the other selections a week later, all partly picked by 15 April. Miho Wase had a higher yield, good-excellent crop, Okitsu and Miyagawa fair-good. Okitsu had medium large to large fruit size, followed by Miyagawa and Miho Wase the smallest, medium to medium large. Fruit colour was similar at T4-6 on 3 April. Eating quality in early April was disappointing poor (lacking sugars) for Miyagawa and only slightly better for Okitsu, Miho Wase variable but better. Both Miyagawa and Okitsu were fair quality on 15 April, Miho Wase good. Okitsu had acceptable average tests on both dates, Miyagawa unacceptable (low TSS), while Miho Wase was excellent, although smaller fruit size. Acid levels of all selections were similar. The fruit was seedless except one Miho Wase. All had some sunburn and Okitsu a larger tree. No oleocellosis was recorded in early April. Refer Table 6.2.2.2.3 for figures.

Please note that the data presented for the Wolseley site during 2000, 2001 and 2002 is incorrect and should be swapped around (Miyagawa and Okitsu Wase).

Table 6.2.2.2.3. Average yield per tree and percentage fruit per count for various Satsuma selections harvested at Wolseley during 2003.

Selection	Ave yield*	Percentage fruit per count								% in count 1 - 3
		1 xxx	1 xx	1 x	1	2	3	4	5	
Miho Wase	3.5			1	3	11	27	38	20	41
Miyagawa Wase	2.9	1	3	12	16	24	25	15	4	65
Okitsu Wase	2.9	3	6	16	19	24	20	10	2	63

* Average yield – number of 10kg carton equivalents.

In most cases at both sites Miyagawa and Okitsu Wases were fairly round with a fairly smooth rind.

Dobashi Beni. Wait until semi commercial orchard comes into production.

Ohtsu. The trees fair to good crop on Troyer of medium large fruit size, excellent and similar size on Swingle. Both were colour T6-7 on 23 April and on Troyer T5-6 on 14 May. Eating quality on Swingle in April was not so good, overmature and slightly puffy. Eating quality on Troyer was fair in May not quite mature, trees next to a windbreak. Both selections had unacceptable tests in May. Fruit is flat and smooth with odd seed (mixed blocks).

The trees on Swingle is in many respects similar to Ueno, overall slightly better than Ueno although the latter had slightly better tests.

Aoshima. The CFB trees had an excellent crop of medium large fruit size, T3-4 on 14 May and open cores. Eating quality was poor with low sugars and acid while the test was just unacceptable due to slightly low acid (large fruit). The fruit was mature but puffy and overmature on 16 June (not sampled). Peelable and oily in May and seedless. The colour was earlier than Ohtsu, but the Ohtsu next to a windbreak. Fruit shape is flat with a smooth rind.

Ueno. The CFB trees had excellent crops of medium large fruit size, colour T6-7 on 23 April, T1-2 and T2-4 for Troyer and Swingle respectively on 14 May. Fruit were about peak maturity in April, open cores, poor quality, puffy and overmature in May. The test on Troyer (no Swingle test) in May had low juice. Fruit is flat and smooth with odd seed (mixed blocks).

Conclusion

Satsuma X Nova. Production was acceptable with good fruit size. Maturity is early with good quality. This selection needs to be established in other areas as a potential early Satsuma. The seed status in a non pollinated situation needs to be established.

Miyagawa and Okitsu Wase. Production was generally good. Fruit size varied between sites, either similar or larger than Miho Wase. Okitsu and Miyagawa matured after Miho Wase by one to two weeks, around end March to mid April. Internal quality varied between sites and Miyagawa generally the poorest. Bearing in mind some of the variable results, both these selections can be planted commercially with caution, the only real advantage to date being slightly larger fruit size than Miho Wase and later maturing. Evaluations complete.

Please note that the data presented for the Wolseley site during 2000, 2001 and 2002 is incorrect and should all be swapped around (Miyagawa and Okitsu Wase).

Dobashi Beni. Wait until semi commercial orchard comes into production.

Ohtsu. The yields were good, with generally medium large fruit size. Internal quality was poor. Matures from about mid April to the third week of May. There is too little information available to make recommendations.

Aoshima. The yield was excellent with good fruit size, quality poor with a just unacceptable test. Maturity early May. There is too little information available to make recommendations.

Ueno. The yields were excellent, with medium large fruit size. Fruit quality was poor. There is too little information available to make recommendations.

Table 6.2.2.4. Internal fruit quality data for Satsuma mandarins for the Eastern and Western Cape areas tested during the 2003 season.

Selection	Root-stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Miyagawa Wase ⁴	TC	Patensie Acht	10-24/02	7		54.3	10.1	1.93	5.2	
Miyagawa Wase	TC	Patensie Acht	03/03	6		55.4	11.7	1.38	8.5	
Miyagawa Wase ⁶	TC	Patensie Acht	28/03-09/04	5-6		54.2	11.3	0.96	11.8	0
Miyagawa Wase ⁶	TC	Patensie Acht	16-17/04	6		53.5	10.6	1.00	10.6	
Miyagawa Wase ²	CC	Whitebridge	03/04	5-6	2/1X	59.9	8.9	0.98	9.1	0
Miyagawa Wase ²	CC	Whitebridge	15/04	6	2/1X	61.8	8.7	0.94	9.3	0
Okitsu Wase ⁴	TC	Patensie Acht	10/02-24/02	7		53.5	10.1	1.72	5.9	
Okitsu Wase	TC	Patensie Acht	03/03	6		54.0	10.6	1.20	8.8	
Okitsu Wase ⁶	TC	Patensie Acht	28/03-09/04	4-6		50.7	11.4	0.91	12.5	0
Okitsu Wase ²	TC	Patensie Acht	17/04	6		52.4	10.7	0.90	11.9	
Okitsu Wase ²	CC	Whitebridge	03/04	5	2/1X	61.5	9.5	1.04	9.1	0
Okitsu Wase ²	CC	Whitebridge	15/04	5-6	2/1X	61.5	9.0	0.93	9.7	0
Ohtsu	TC	CFB	14/05	5-6	1X	46.4	10.6	0.66	16.1	1.5
Ohtsu	SC	CFB	14/05	3-4	1XXX	43.2	8.9	0.70	12.7	1.1
Aoshima	TC	CFB	14/05	4-5	1XX	52.5	9.3	0.69	13.5	0
Ueno	TC	CFB	14/05	1-3	1X	45.1	9.6	0.77	12.5	0.6
Miho Wase ⁴	TC	Patensie Acht	10/02-24/02	7		54.9	10.6	1.70	6.2	
Miho Wase	TC	Patensie Acht	03/03	6		55.8	11.1	1.32	8.4	
Miho Wase ⁶	TC	Patensie Acht	28/03-09/04	4-6		53.4	11.3	0.95	11.9	0
Miho Wase ⁶	TC	Patensie Acht	14-15/04	6		53.6	10.7	0.91	11.8	
Miho Wase ²		Whitebridge	03/04	5-6	1/3	59.7	11.5	1.05	11.0	0
Miho Wase ^{2*}		Whitebridge	15/04	4-5	3/1	63.4	11.3	0.93	12.2	0.2

² average of 2 tests

⁴ average of 4 tests

⁶ average of 6 tests

* one fruit had 3 seeds

Future research

Evaluate the ITSC Satsuma X Nova as an early maturing selection and establish in other areas. Evaluate Dobashi Beni when semi commercial block comes into production. As there growing interest in the planting of late maturing Satsumas, establish new sites including all these selections. In the interim re-evaluate the old discarded sites that included Ohtsu, Aoshima and Ueno.

6.2.2.3 Evaluation of Clementine mandarins in the Cape areas

Experiment 63 by C J Alexander (Private)

Opsomming

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. Early Marisol lyk op die stadium nie baie belowend as 'n goeie vroeër Clementine nie – evalueer nog een jaar. Esbal produksie en gehalte was baie goed met kleiner vruggroote as Nules wat dalk probleme veral in koeler gebiede kan skep. Cadoux het goeie produksie maar klein vruggroote. Te min inligting oor die Tardif de Janvier I en II en Hernandina. Die Sidi Aissa en Ain Toujdate het goeie produksie, maar vruggrootte aan die kleiner kant. CELL lyk baie soos die Clemlate met geen voordele op die stadium, evalueer nog een jaar. Clementarde het te veel variasie tussen persele gehad. Jong Nour bome is dig met swak produksie en dalk vertraagde vrugkleur. Verder evaluasies is nodig, veral die laat seleksies.

Introduction

The objective of the project is to find suitable superior Clementine selections to help flatten the existing midseason production peaks by extending the harvest season both earlier and particularly later in accordance with market needs.

Materials and methods

The trial trees are either planted or topworked within commercial orchards, or established on a semi commercial scale, using Marisol, Nules or Clemlate as controls where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Due to budget cuts, fewer sites were to be evaluated than in the past, which would have provided fewer results. However to gain as much information as possible and also because some of the trial trees had been removed, as many sites as practically possible were evaluated. Fruit quality was compared with the standards previously considered most acceptable in the market place: 48% juice; 9.5% TSS; 0.7 – 1.5% acid; 8.0:1 ratio; colour T3 of set 36. Seed maximum average 3.0 seeds per fruit. Oleocellosis evaluations were done on some samples – the percentages given are rated for oleo present but exportable, class 1 or not exportable, class 2 fruit. A list of selections and sites evaluated is given in table 6.2.2.3.1.

Table 6.2.2.3.1. Clementine trial sites evaluated during 2003.

Selection	Area	Site	Plant Date	Root stock	No of trees
Early Marisol	Stellenbosch	L'Avenir	1999	CC	3 topwork
Esbal	Uitenhage	CFB Block 7	1995	TC	1
Esbal	Clanwilliam	Twaktuin	1998	CC	com topw
Esbal	Citrusdal	Brakfontein	1997	CC	3 topwork
Esbal	Citrusdal	Brakfontein	1998	CC	5 topwork
Cadoux	Addo	ITSC Addo	1998	CC	
Tardif de Janvier I	Uitenhage	CFB Psylla house	1999	TC	1
Tardif de Janvier I	Buffeljagsrivier	Sovereign	1999	SC	4 topwork
Tardif de Janvier II	Uitenhage	CFB Block 4	2001	CC	4
Sidi Aissa	Uitenhage	CFB Block 8	1997	TC	6
Sidi Aissa	Buffeljagsrivier	Sovereign	1999	SC	3 topwork
Sidi Aissa	Citrusdal	Brakfontein	1998	CC	5 topwork
AinToujdate	Uitenhage	CFB Block 8	1997	CC	6
AinToujdate	Buffeljagsrivier	Sovereign	1999	SC	7 topwork
AinToujdate	Citrusdal	Brakfontein	1998	CC	5 topwork
CELL	Uitenhage	CFB Block 7	1995	TC	2
CELL	Buffeljagsrivier	Sovereign	1999	SC	5 topwork
CELL	Citrusdal	Brakfontein	1997	SC	3
CELL	Citrusdal	Brakfontein	1998	CC	5 topwork
Clementarde	Uitenhage	CFB Block 8	1997	CC	6
Clementarde	Buffeljagsrivier	Sovereign	1999	SC	8 topwork
Clementarde	Citrusdal	Brakfontein	1998	CC	5 topwork

Hernandina	Buffeljagsrivier	Sovereign	1999	SC	8 topwork
Nour	Uitenhage	CFB Block 8	1999	SC	2
Nour	Addo	Summersby	1999	CC	commercial
Nour	Buffeljagsrivier	Rotterdam	1999		1 topwork
Nour	Buffeljagsrivier	Sovereign	1999	SC	7 topwork
Nour	Stellenbosch	L'Avenir	1999	CC	4 topwork
Nour	Citrusdal	Brakfontein	1988	CC	5 topwork
Marisol	Stellenbosch	L'Avenir	1992	CC	commercial
Arrufatina	Stellenbosch	L'Avenir	1992	CC	commercial
Nules (control)	Uitenhage	CFB Block 4	2001	SC	3
Nules (control)	Addo	Summersby	1998	CC	commercial
Nules (control)	Buffeljagsrivier	Sovereign	1990	TC	commercial
Nules (control)	Clanwilliam	Twaktuin	1992	TC	commercial
Nules (control)	Citrusdal	Brakfontein	1995	SC	commercial
Clemlate (control)	Buffeljagsrivier	Rotterdam	1999		commercial
Clemlate (control)	Buffeljagsrivier	Sovereign	1999	SC	3 topwork
Clemlate (control)	Citrusdal	Brakfontein	1997	SC	3

Results and discussion

A discussion of each selection follows with rootstock, production, fruit size and fruit colour presented in Table 6.2.2.3.2, oleocellosis evaluations in Table 6.2.2.3.3 and internal quality results in Table 6.2.2.3.4 which need to be referred to when reading the text.

Early Marisol. The trees had a fair to good crop of medium small to medium fruit size (50-60 mm), colour T4-5-6 on 10 April, Arrufatina T3-6 and Marisol T2-5. The fruit had fairly high acid, probably masking the sugars, but watery, two to three weeks short of maturity. The fruit picked easily. Fruit shape was roundish with a slightly pebbly to smooth rind and less sunken navel end than, but like Arrufatina. The fruit was fairly easily peeled with a thin rind, breaking up, juicy, pale flesh colour and seedless. Tree size is small. The Arrufatina had a good crop of larger fruit size, a flatter fruit, similar rind texture and peelability. The flesh was typically coarse and dry, yellow orange and seedless. The acid was also fairly high, but lower than the Early, probably good sugars and reasonable quality one to two weeks short of maturity. Overall earlier than the Early. The Marisol had a good crop of small to medium fruit size and the earliest colour of all. The Early internal quality test was good, lower sugars and higher acid than Arrufatina.

Esbal. Trees were evaluated at three sites. Only the older trees at Citrusdal are reported on. The tree at the CFB had fair eating quality in mid May, mature, with a good test although acid getting low and some seed in a mixed block.

The Clanwilliam trees had a better crop and smaller fruit size and slightly better colour than Nules (Esbal sprayed with Corasil or Maxim). Eating quality was good on 30 April, at or close to peak maturity. Nules also had good quality but lower acid and granulation starting, also ready to be picked. Of the test samples, 12% Esbal showed signs of granulation whereas 58% Nules were dry or had signs of granulation. Tests of both were good, Nules a higher TSS and slightly lower acid, also with odd seed. It was reported from overseas that the Esbal acid held well whereas the Nules acid dropped fast. Oleo on 30 April, 5% class1, Nules zero.

The Citrusdal trees had a better crop of smaller fruit size than nearby Nules and earlier colour on 30 April. Eating quality was good, slightly tart, one week short of peak maturity. Nules were good and sweet, with sufficient acid, about peak maturity, picking started. By 28 May the Esbal was slightly puffy and looked slightly overmature, quality still quite good (Nules already picked). The tests of both were very good, Nules slightly higher TSS and lower acid with signs of granulation. There was very little drop in Esbal acid over four weeks.

The fruit shape is fairly round to round, mostly slightly pebbly in the Western Cape, seedless when not cross pollinated, fairly easily peeled and oily. The tree tends to be upright growing and vigorous.

Table 6.2.2.3.2. Comparison of the production, fruit size and rind colour of various clementine selections at the different trial sites on different dates in the Cape areas during 2003.

Area	Selection	Root stock	Production	Fruit Size	Colour transparency - date								
					23 April	30 April	14 May	22 May	28 May	10 June	16 June	23 June	12 July
Uiten-hage	Esbal	TC	Good	Medium	6, 6-7		1-3						
	Tardif de Janvier I	TC	Fair	Medium large							1-2		
	Tardif de Janvier II		Odd - poor	Medium	8		7-8				4		
	Sidi Aissa	TC	Fair	Medium small	7-8, 8		5-6				1		1
	Ain Toujdate	CC	Good	Medium small	7		5-6				1		1
	CELL	TC	Poor	Medium large	8						5		1-2
	Clementarde	CC	Poor	Medium large	8		6-7				5-6		3-4
	Nour	CC	Poor	Medium large	8						5		1-2
	Nules	SC	Poor	Medium large	8		7-8				3		
Buffeljas-rivier	Tardif de Janvier I	SC	zero										
	Sidi Aissa	SC	Zero										
	Ain Toujdate	SC	Zero										
	CELL	SC	Zero – poor	Medium large				7					
	Clementarde	SC	Poor – excellent	Small - medium				6 ?					
	Hernandina	SC	Odd	Variable				6-7					
	Nour	SC	Zero – fair	Small – medium, variable				4-5					
	Clemlate	SC	Zero										
	Nules	TC	Few, picked					3-4					
	Nour (Rotterdam)		Odd					6-7, 7					
Clemlate (Rotterdam)		Good	Medium, medium small				7						

Table 6.2.2.3.2. continued

Area	Selection	Root-stock	Production	Fruit Size	Colour transparency – date								
					23 April	30 April	14 May	21 May	28 May	10 June	16 June	23 June	12 July
Clan-william	Esbal	TC	good-excellent, partly picked	Variable, mainly medium - medium large (56-58-62 mm)		3-5							
	Nules	TC	Fair	Mainly medium large - large (67-70-73 mm)		4,5,6, odd 3							
Citrusdal (older trees)	Esbal	CC	Good - excellent	Medium		4-5			1, some 2-3				
	CELL	SC	Poor	Medium large - large		7			T6 some T5	T4, some T3, T5-6		T2-3, some T1, fairly even	
	Clemlate	SC	Poor	Medium large - large		7, odd 6			T6 some T5, odd T4	T4-5, some T3		T3-4. Variable T1-5	
	Nules	SC	Fair	Medium large - large		T5-6, T6-7			picked				
Citrusdal (younger trees)	Marisol	CC							picked				
	Sidi Aissa	CC	Zero										
	Ain Toujdate	CC	Zero										
	CELL	CC	Good	Medium large		7-8			5-6	3-5		1-2	
	Clementarde	CC	Odd	Medium large		6-7							
	Nour	CC	Odd - poor	Medium - medium large		7-8			5-6, 6	5		1-2 and , 4-5	

Brackets – most fruit in this size range in mm.

Table 6.2.2.3.3. Oleocellosis evaluations of the various Clementine selections at Citrusdal on different dates.

Site	Selec-tion	30 April	28 May			10 June			23 June	
		% no oleo	% No oleo	% Class 1	% Class 2	% No oleo	% Class 1	% Class 2	% No oleo	% Class 1
Citrusdal Younger trees	CELL		33	67		76	24		100	
	Nour		40	56	4	80	20		100	
Citrusdal Older trees	Esbal	100	63	37						
	CELL	100	40	60		72	24	4		
	Nules	100								
	Clemlate	100	4	80	16	64	36		92	8

Cadoux. Trees were evaluated at the ITSC Addo on 19 May. The yield was good with mainly small fruit and some medium and odd large fruit. Fruit colour was T1-3 and Nules T2-3. The rind was smooth and most fruit had seed (Nules seedless). The test was excellent, much higher sugars and acid than Nules.

Tardif de Janvier I and II. The T de J I at Sovereign was not yet in production. The T de J I at the CFB had good eating quality (sugars and acid) and mature on 16 June. No tests were done. The T de J II had poor eating quality on 14 May with low sugars and acid, fair in June and mature. The test in June had borderline TSS and unacceptably low acid and seedy (mixed block). Older Nules had earlier colour and better quality.

The T de J I had flatter and coarser fruit than Tde J II, both peelable but oily and seedless.

Sidi Aissa and Ain Toujdate. The trees at Buffeljagsrivier and Citrusdal had no fruit or were not in bearing. The Ain Toujdate at the CFB had a slightly better crop than the Sidi Aissa with similar fruit size and colour. The tests were good although the juice percentages were not high and acid relatively low, slightly lower than Nules. The Ain Toujdate tasted later than the Sidi Aissa. The Sidi Aissa internal maturity was earlier than the colour, overmature by 16 June. Maturity estimated late May.

Both selections had round fruit shape with a coarseish rind, fairly easily peeled and oily and some seed (mixed block).

CELL. Trees were evaluated at four sites. The crops were generally poor except the younger Citrusdal trees. The CFB fruit had a poor taste on 16 June, ready towards the end of June. The test had good sugars, but unacceptably low acid on 16 June. Fruit colour was behind Nules and also lower acid. There was some seed (mixed block).

The Buffeljagsrivier fruit had good eating quality like Nules, slightly raggy and difficult to peel, around peak maturity on 22 May, counts 2-3. The acid test was similar to Nules (majority picked) but much poorer colour. Slightly coarse flesh.

The younger Citrusdal trees had fair eating quality on 30 April but still immature, fair quality on 10 June and past peak by 1-2 weeks. The acid levels remained constant over a 4 week period (0.97%) and high TSS. The flesh was coarse/dry from 28 May, Nules also. There were a lot of green styler ends in early June, less later on.

The older Citrusdal trees had a similar crop and fruit size as Clemlate. Eating quality was acceptable on 30 April, low acid but colour too late. Clemlate had a slightly better taste, Nules around peak maturity and good. On 28 May the fruit was raggy and good but lowish acid, Clemlate better better quality, Nules all picked. The fruit was past peak on 10 June and felt overmature, Clemlate at peak. The tests were good, fairly similar to Clemlate and Nules lower acid. Both had similar colour, later than Nules, Clemlate more green styler ends late June. Both selections unattractive.

Fruit shape is generally round with a slight nippleand pebbly rind and sometimes a ring on the styler end. Fairly easily peeled but can be oily. The trees are dense with a black stem. The fruit is seedless with no cross pollination. Fruit size at the sites varied mainly between counts 1X, 1 and 2 (also 3 at Buffeljagsrivier). There were some green styler ends, slightly worse than Clemlate, but disappearing with fruit maturity.

Clementarde. The CFB fruit had fair eating quality on 16 June, with highish acid and fair again on 12 July. The test in June was good, similar to Nules with a good acid level of 0,8% There were some seed (mixed block).

The trees at Buffeljagsrivier (poor orchard) had a variable crop and fruit size between counts 2-4. Eating quality was very good on 22 May with an excellent test. There were some green styler ends and the fruit unattractive.

There were only odd fruit at Citrusdal. On 30 April quality was fairly good but still slightly tart. No further evaluations or tests were done as there were too few fruit.

The Clementarde were round with pebbly rind, fairly easily peeled and oily, with a prominent styler end ring, orange flesh and odd to some seed in mixed blocks. The trees are dense with a dark stem. Estimated maturity varied widely between sites from end May at Buffeljagsrivier to early July at Uitenhage.

Hernandina. Trees were only evaluated at Buffeljagsrivier, although it is a poor orchard and there were only a few fruit for evaluation and no test was done. The quality was good on 22 May with sufficient acid.

Fruit shape is round with a nipple, a pebbly rind with large oil cells, fairly easily peeled to brittle and slightly oily. The flesh was coarse and no seeds (mixed block). Some fruit had a ring on the styler end with some green styler ends. Stems are dark.

Nour. Trees were evaluated at five sites and all trees still young. The few fruit at the CFB had fair quality on 16 June, colour later than Nules. The test was good in June, fractionally higher acid than Nules. Peak maturity about end June to mid July.

The young, semi commercial trees at Addo had a very poor crop – trees vigorous. The test on 19 May was good but colour very poor. Nules had higher TSS and slightly higher acid.

The tree at Rotterdam had only a few fruit with similar colour to Clemlate on 22 May. There were too few fruit to evaluate properly or test.

Buffeljagsrivier trees had variable crops and fruit size (counts 2-5), colour ahead of CELL and Hernandina. There were plenty of green styler ends on 22 May and fruit unattractive. Eating quality was good, but slightly tart with coarse flesh. The test was very good, fairly similar to CELL. Peak maturity around end May/early June.

Citrusdal trees had fairly good quality and sufficient acid on 30 April. By 28 May the fruit picked easily, lacking flavour and not quite mature with plenty of green styler ends. On 10 June the fruit tended to be tasteless, poorer than CELL. Maturity around early June. By 23 June, the fruit was overmature, but not as bad as the selections. Odd fruit had sunken navel ends. The tests were good and acid only dropping (but good) 0,18% between 28 May and 23 June. The flesh was coarse. There were a lot of green styler ends in early June, less later on.

The fruit is generally round with a nipple, pebbly rind, orange flesh, seedless when not pollinated, fairly easily peeled and oily. The green styler ends varied between the sites, but may disappear with maturity. The trees are vigorous and dense with a dark stem.

Conclusion

Early Marisol. The trees bore a reasonable crop but relatively small fruit size. The acid level was higher than Arrufatina and later colour than Marisol. Maturity towards the end of April. This selection does not look so promising as an early clementine but needs one final evaluation.

Esbal. Production is good with fruit size smaller than Nules. The smaller fruit size is of concern and will have to be manipulated, especially in the cooler Cape production areas. Fruit colour is ahead Nules, maturity is around 1st week of May. Internal quality is good with slightly higher acid than Nules. Finalise evaluations next season.

Cadoux. Production was good, but fruit size too small. Internal quality was excellent. The selection does not look promising due to the small fruit size and a final evaluation needs to be done.

Tardif de Janvier I and II. Due to the limited information available, no recommendations can be made. Further evaluations are necessary.

Sidi Aissa and Ain Toujdate. Production of both⁴⁸¹ selections at the CFB was fair or good, with

medium small fruit size. Maturity is estimated around end May, but further evaluations are necessary and at the other two

sites.

CELL. Production was good at only one site, generally good fruit size. Eating quality is not always as good as shown by the tests. Colour tends to be retarded and flesh slightly coarse. Acid levels were good in Citrusdal. Maturity around the end of May. Clemlate had slightly better quality. The CELL appears similar to the Clemlate with no distinct differences. Finalise evaluations next season.

Clementarde. Production was poor to variable and variable fruit size between sites. Maturity varied between sites and some styler ends were green. Internal quality was very good. Due to the variability of the results no conclusions can be drawn and further evaluations are necessary.

Hernandina. There were only odd fruit with good quality. Further evaluations are necessary.

Nour. Production was poor, although the trees are still young, fruit size variable between sites. The fruit quality was generally good, with good tests. Maturity varied between sites from early to late June, although the colour may be retarded. There were a lot of green styler ends initially. The trees are vigorous and dense. Further evaluations are necessary.

A lot more evaluations need to be done on the later maturing Clementine selections (CELL, Clementarde, Hernandina and Nour) to see if any are superior to the Clemlate selection. There appear to be a lot of similarities between these selections and Clemlate.

Table 6.2.2.3.4. Internal fruit quality data of the various Clementine selections for the Eastern and Western Cape during the 2003 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Marisol Early	CC	L'Avenir	10/04	5-6	3	67.6	10.2	1.21	8.4	0
Esbal	TC	CFB	14/05	3-4	2	52.4	10.7	0.80	13.4	2.2
Esbal	CC	Twaktuin	30/04	5-6	4	62.8	11.1	0.99	11.2	0
Esbal	CC	Twaktuin	30/04	5-6	2	58.9	11.2	0.92	12.2	0
Esbal	^o CC	Brakfontein	30/04	4-5	2	61.4	11.6	1.15	10.1	0
Esbal	^o CC	Brakfontein	28/05	1-2	2	62.6	12.3	1.11	11.1	0
Cadoux	CC	ITSC Addo	19/05	1-3	2	59.6	13.2	1.03	12.8	2.6
Sidi Aissa	TC	CFB	14/05	5-6	2	50.1	11.6	0.85	13.6	4.5
Sidi Aissa	TC	CFB	16/06	1	2	48.9	11.9	0.74	16.1	3.6
Ain Toujdate	CC	CFB	14/05	5-6	2	49.0	11.4	0.86	13.3	3.7
Ain Toujdate	CC	CFB	16/06	1	2	50.4	11.7	0.77	15.2	1.1
CELL	TC	CFB	16/06	5	2	54.2	11.1	0.65	16.9	3.7
CELL	SC	Sovereign	22/05	7	2	56.3	12.3	0.92	13.4	2.0
CELL	^y TC	Brakfontein	28/05	5-6	1	58.3	12.0	0.97	12.4	0
CELL	^y TC	Brakfontein	10/06	4	1X	55.0	12.4	0.96	12.9	0
CELL	^y TC	Brakfontein	23/06	1-2	1	55.1	12.2	0.97	12.6	0
CELL	^o SC	Brakfontein	30/04	7	1	56.1	11.1	1.13	9.8	0.3
CELL	^o SC	Brakfontein	28/05	6	1X	60.6	11.6	0.92	12.6	0
CELL	^o SC	Brakfontein	10/06	4	1X	59.9	12.2	0.88	13.9	0.2
CELL	^o SC	Brakfontein	23/06	2-3	2X	56.0	11.6	0.87	13.3	0.1
Clementarde	CC	CFB	16/06	5	3	56.9	11.2	0.80	14.0	0.3
Clementarde	SC	Sovereign	22/05	6-7	2/3	56.7	13.6	0.96	14.2	0.6
Tardif de Jan II	CC	CFB	16/06	1	1	54.2	9.7	0.61	15.9	5.1
Nour	SC	CFB	16/06	5	3	57.6	11.2	0.88	12.7	0.9
Nour	CC	Summersby	19/05	7-8	2	61.4	10.5	0.80	13.1	0
Nour	SC	Sovereign	22/05	4	3	57.9	12.9	0.91	14.2	0.8
Nour	^y TC	Brakfontein	28/05	6	1	58.0	11.8	1.09	10.8	0
Nour	^y TC	Brakfontein	10/06	4-5	1X	54.6	11.5	1.03	11.2	0
Nour	^y TC	Brakfontein	23/06	4	1	55.2	11.1	0.91	12.2	
Marisol	TC	L'Avenir	10/04	5	3	67.1	10.6	1.35	7.9	0
Arrufatina	CC	L'Avenir	10/04	4-5	2	64.6	11.2	0.90	12.4	0
Nules	SC	CFB	16/06	3	1XX	55.8	11.3	0.80	14.1	1.3
Nules		Summersby	19/05	3-4	1	56.3	12.8	0.89	14.4	0
Nules	CC	ITSC Addo	19/05	2-3	1X	58.3	10.8	0.73	14.8	0
Nules	¹ TC	Sovereign	22/05	2-3	2/3	65.8	13.3	0.92	14.5	0
Nules	TC	Twaktuin	30/04	5-6	2	62.5	12.0	0.90	13.3	0.1
Nules	TC	Twaktuin	30/04	4-5	1X	58.9	12.3	0.89	13.8	0.2
Nules	^o SC	Brakfontein	30/04	4	1/2	59.1	12.4	0.97	12.8	0
Clemlate	^o SC	Brakfontein	30/04	7	1	57.8	11.6	1.15	10.1	1.8
Clemlate	^o SC	Brakfontein	28/05	5-6	1X	58.7	12.0	0.94	12.8	1.9
Clemlate	^o SC	Brakfontein	10/06	5	1XX	56.3	12.3	0.91	13.5	2.2
Clemlate	^o SC	Brakfontein	23/06	3-4	2X	55.4	12.3	0.91	13.5	1.3

^y younger trees ^o older trees planted near or next to 3 Fortuna trees ¹ commercial orchard, last pick

Future research

Continue evaluations on mainly the later maturing selections.

6.2.2.4 Evaluation of Mandarin hybrids in the Cape areas

Experiment 73 by C J Alexander (Private)

Opsomming

Die doel van die mandaryn projek is om hoër gehalte, goed gekleurde, saadlose mandaryn seleksies te ondersoek. Hulle moet ook goeie produksie en vrug grootte hê en die sagte sitrus seisoen kan versprei, beide vroeër en veral later. Die meeste van die ITSG en ander seleksies is nog jonk en moet verder evalueer word. Nova SL is soortgelyk aan Nova, maar nie heeltemal saadloos in gemengde blokke nie; B17 lyk belowend maar het hoër suur en split; B24 is saadloos, het groot vrug grootte en minder suur as B17; M37 het 'n lemoen tipe geur en was goed oorsee ontvang. H25 het te min gedra; H36 smaak soos 'n Nouvelle met middelmatige gehalte; K33 is feitlik saadloos, maar lyk nie belowend nie; Roma het swak produksie, maar goeie vrug grootte en gehalte; Michal is vroeg gekleur, maar bots met clementines; Ora het goeie produksie, maar grensgeval in vrug grootte, dalk vertraagde vrugkleur en vol saad. Murcott x Clem het goeie tot te groot vrug grootte, 'n mooi voorkoms en matige gehalte. Die Bay Gold het swak produksie met goeie vrug grootte en aantreklik, maar hoër suur en lyk nie in koeler gebiede belowend nie; Hadas het goeie produksie, maar swak gehalte.

Introduction

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.

Materials and methods

The trees were either planted or topworked within commercial orchards where possible (to prevent cross pollination), or established on a semi commercial scale. Comparisons were made with a range of existing commercial selections or clementines where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following standards for clementines and mandarins previously considered most acceptable by the market place: 48% juice; 9.5% TSS; 0.7 – 1.5% acid; 8.0:1 ratio; colour T3 of set 36. Seed maximum average 3.0 seeds per fruit. A list of selections and sites evaluated is given in Table 6.2.2.4.1.

Table 6.2.2.4.1. Mandarin hybrid trial sites evaluated during 2003.

Selection	Area	Site	Plant Date	Root stock	No of trees
Nova Seedless	Uitenhage	CFB	1999	CC	2
Nova Seedless	Patensie	Paksaam	1999	SC	semi com
Nova Seedless	Buffeljagsrivier	Rotterdam	1999	CC/SC	14
Nova Seedless	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
ITSC B17	Addo	Dunbrody	1998	CC	semi com t/w
ITSC B17	Fort Beaufort	Baddaford	1998	TC	3
ITSC B17	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
ITSC B17	Citrusdal	ALG	1999	BN/RL	5
ITSC B24	Addo	Dunbrody	1998	CC	semi com t/w
ITSC B24	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
ITSC B24	Citrusdal	ALG	1999	BN/RL	5
ITSC M37	Fort Beaufort	Baddaford	1998	TC	4
ITSC M37	Citrusdal	ALG	1999	BN/RL	5
ITSC H25	Citrusdal	ALG	1999	BN/RL	5
ITSC H36	Citrusdal	ALG	1999	BN/RL	5
ITSC K33	Citrusdal	ALG	1999	BN/RL	5
ITSC K33	Citrusdal	Hexrivier	1997	RPL	1
Roma	Citrusdal	ALG	1998	BN/RL	5
Michal	Citrusdal	ALG	1998	BN/RL	5
Ora	Citrusdal	ALG	1998	BN/RL	5
Murcott x Clem	Uitenhage	CFB	1999	TC	2
Murcott x Clem	Citrusdal	Brakfontein	2000	RL	3 topwork
Bay Gold	Uitenhage	CFB	1999	TC	2

Bay Gold	Grabouw	Whitehall	1998	SC	5
Bay Gold	Clanwilliam	Jansekraal	2001	CC	semi com
Hadas	Uitenhage	CFB	1999	TC	2
Nova	Patensie	Paksaam	1999	SC	commercial
Nova	Buffeljagsrivier	Rotterdam	1994	TC	commercial

Results and discussion

A discussion of each selection follows. Various tables also need to be referred to when reading the text. Table 6.2.2.4.3 is a comparison of the various selections at ALG, Citrusdal; Table 6.2.2.4.4. shows oleocellosis results and Table 6.2.2.4.5 internal quality data.

Nova Seedless. Trees were evaluated at four sites. The CFB trees carried a good crop of medium small fruit size, colour T5 on 14 May, T1 on 16 June. There was a lot of sunburn in late April and odd split fruit. Eating quality was fair, acid a bit low. Estimated maturity is early June but acid may be too low. The early test was good, June test just acceptable (borderline acid). There were some seed (mixed block). The seed counts for 50 fruit picked in June are as follows: zero = 36%; 1 seed/fruit = 32%; 2 = 20%; 3 = 10%; 5 = 2%; average 1.1 seeds per fruit.

At Patensie the young trees (Nova Seedless and control) only had a few fruit with medium to medium large fruit size, colour T7-8 on 22 April. There was odd fruit split and no differences were observed between the selections. The tests on 12 May were similar and unacceptable due to low TSS. The control had 0.2 seeds/fruit and the Seedless zero seeds.

Interplanted trees at Buffeljagsrivier had from zero to good crops, medium to medium large fruit size (both selections around count 2), colour T3-4 on 22 May, Nova T1-3 (partly picked). Quality was fair to good with variable acid, not as good as control. The tests were good although the Nova had much lower acid. Both at or just past peak maturity. The Seedless had 32% malformed navel ends (exportable), the Nova zero, both seedless.

The fruit at Citrusdal had already been harvested by the end of April. Trees were pale with some leaf drop, probably stress related. There are signs of the scion overgrowing the rootstock which needs to be evaluated next season.

Fruit shape varied between the sites from round to flattish, rounder than Nova at Buffeljagsrivier, smooth rind orange internal colour, peelable to difficult and oily. In solid blocks the fruit was seedless but not with cross pollination.

ITSC B17. Trees were evaluated at four sites. The topworked semi commercial block at Addo bore a good crop of medium large fruit size, colour T 4-5 on 30 May, and T1 on 25 June. The colour and quality were good in June with good tests. The acid level dropped only fractionally between the tests, remaining over 1.0%. Maturity around third week of June. There were occasional seed (0.8 – 1.3 seeds/fruit) and splitting. Refer to Table 6.2.2.4.2. below for fruit size data.

Table 6.2.2.4.2. Average fruit size and count distribution (200 fruit) of ITSC B17 and B24 measured at Dunbrody Estates, Addo on 30 May 2003.

Selection	Fruit size (mm)	Percentage fruit per count						
		Over size	1XXX	1XX	1X	1	2	3
ITSC B17	70.9		1	38.5	40	18.5	2	
ITSC B24	79.3	14.5	45.5	23.5	9	6.5	1	

Trees at Fort Beaufort bore a fair crop of medium fruit size, colour T1 on 26 June. Quality was good, acid slightly high and mature. The test was very good, acid 1.16%. There was some seed (mixed block).

The semi commercial topworked trees at Citrusdal were pale with some leaf drop (probably stress) and had a variable crop of zero to fair with mainly medium large and some medium fruit size, averaging count 1X and 1XX. Fruit colour was mainly T3-4 on 13 May, T1 with a good deep orange colour on the greener trees on 10 June, T1 on 24 June and deep red on 15 July. The fruit had too high acid in June and good flavour although sometimes tart in July. Based on the eating quality peak maturity was mid July but this could be misleading due to tree condition. The tests were only acceptable by about 3rd week of June as the acid level was too high. Cores were open from 13 May. There were odd seed and there was a fair amount of stylar end splitting. Good looking fruit.

Fruit of the ALG trees were probably at peak maturity around mid June but the fruit was always tart with a good flavour, looking overmature on 23 June. The fruit was up to test on 10 June with borderline juice levels, thereafter the juice too low.

Fruit shape was flattish sometimes with a slight neck in Citrusdal, smooth to slightly pebbly in Citrusdal, fairly easily peeled and oily with a thin rind, deep orange internal colour and open cores, mainly seedless to odd seed. There was some fruit split, worse at Addo.

ITSC B24. Trees were evaluated at three sites.

The topworked semi commercial block at Addo had a good crop of medium large to extra large fruit size, colour T1-5 on 30 May, picked by 25 June. Quality was fair, not as good as B17. The tests were very good, slightly lower sugars and 0.28% lower acid than B17 but a higher ratio. Deep orange flesh colour. Maturity probably 2nd to 3rd week of June. The fruit was seedless. Refer to Table 6.2.2.4.2. above for fruit size data.

The semi commercial topworked trees at Citrusdal were pale with some leaf drop (probably stress) and had only odd large fruit, too few to evaluate.

Fruit at ALG had fair quality on 10 June, B17 better, about peak maturity. On 23 June the fruit was slightly soft and looked slightly overmature, better quality than earlier but not much flavour. The tests were good except for exceptionally low juice (oversize fruit), 1.7% lower TSS and 0.43% lower acid than B17 but a higher ratio. The navel ends were large but closed and excellent orange flesh colour. Seedless.

Fruit shape was flat sometimes fluting on the stem end. The rind was smooth at Addo and pebbly at Citrusdal. Internal colour was good with slightly open to open cores, fairly easily peeled and oily, completely seedless at all sites. The fruit was not as tart as B17.

ITSC I22. Trees were removed by grower.

ITSC M37. Trees were evaluated at two sites. Fort Beaufort trees had a fair crop of medium fruit size, colour T1-3 on 26 June. Eating quality was good with good tests but seedy (average 4.6 – 8.0 seeds per fruit in a mixed block). Estimated maturity end June.

Fruit at Citrusdal had a good almost orange like flavour on 10 June, flavour a little weaker on 23 June, peak maturity mid to late June. Some buttons coming off late June. The tests were very good except borderline juice late June. A sample sent overseas (picked 15 July) for evaluation was generally well received. There were possible signs of *Alternaria* which need to be confirmed.

Fruit shape was round/flattish with a smooth rind, slightly pebbly at Citrusdal. Internal colour was deep orange with closed to slightly open cores, a soft fruit and flesh, difficult to fairly easily peeled and oily, sometimes messy. Some splitting at Fort Beaufort. Seed counts exceeded the export limits (mixed blocks).

ITSC H25. Trees evaluated at Citrusdal only had odd fruit, too few to evaluate properly. The quality was mediocre with an unacceptable test.

ITSC H36. The quality was fair to good on 10 June with a slight Nouvelle-like taste, soft, around peak maturity but rind colour not too well developed although developing a good colour later. By 23 June the fruit looked overmature, picking fairly easily, a good balance, not much although a different flavour. Overmature on 15 July. The tests were good, not outstanding, acid and juice too low in July. The juice levels are not high. 50% of the fruit were too seedy, varying between zero to 20 seeds per fruit. Fruit shape is round with a pebbly rind, fairly easily peeled and oily. The flesh colour is deep orange with a slightly pink albedo, closed to slightly open cores.

ITSC K33. The Hexrivier tree had a poor to fair crop of medium large fruit size, colour T3-4 with green stem ends on 23 June, T2-3 on 15 July. Quality was fair to good in June with no real flavour and slightly tart also in July. Maturity probably end June? The test in July was quite acceptable, acid 1.28% This site/selection was previously reported on as ITSC 1445.

The fruit at ALG on 10 June had good quality but quite high acid and slightly soft, still good but high acid on 23 June and 15 July. Peak maturity probably end June. The test on 23 June was unacceptable due to low juice and the acid was almost too high. The July test had high juice and TSS but acid just too low which does not correspond with the taste.

Fruit shape is round with a green nipple (almost Minneola like) and pebbly rind, not very attractive. Peelability is fairly easy to difficult, messy and oily. Flesh colour is a good orange and closed to slightly open cores and seedless to odd seed in a mixed block. The tree has thorns and possibly cold sensitive.

Roma. The fruit was slightly dry with good quality and slightly high acid on 10 June, good on 23 June past peak maturity on 15 July. Except for the low juice the tests had high sugars with little change in acid over 5 weeks. Peak maturity around end June, good rind colour. There were some seed. Fruit shape is flattish, occasional slight nipple, pebbly rind and occasional navel. Peelability was fairly easy, oily and peel sometimes breaking up into pieces. Flesh colour was a good orange and open cores. The trees are dense with small thorns and leaves.

Michal. The fruit had fair quality although lacking flavour, slightly dry and overmature by 10 June. The juice levels were too low, acid acceptable on 10 June, too low on 25 June. There was some seed. Fruit shape is roundish with a smooth rind. Peelability was fairly easy and oily with a thin rind. Flesh colour orange with open cores. Trees are vigorous.

Ora. The quality was not so good on 10 June but had a good balance on 23 June although lacking flavour around peak maturity. Rind colour was slightly retarded. The fruit was past peak by 16 July and picked easily. The tests were good in late June, juice too low in July. Fruit shape is flattish with a fairly smooth rind, fairly easily peeled and oily. Flesh colour is orange with closed cores and seedy. Trees are vigorous and tall with some thorns.

Murcott x Clementine. Trees were evaluated at two sites. The CFB trees had a poor crop of medium to large fruit size, colour T1-4 on 16 June, T1 on 12 July. Eating quality was fair with good tests and mature end June/early July.

The Citrusdal trees had from a poor to fair crop of medium large to large fruit size (count 1XX), colour T5-6 on 28 May and T1-2 on 10 June with a good orange colour becoming deep orange red although paler on the sunny side. Quality was fairly good in May and June, juicy although not much flavour, peak maturity about 1st week of June but becoming slightly soft. This quality is retained to mid July. The tests done in May and early June were both good.

Fruit shape is round to slightly flat, a smooth to slightly pebbly rind, fairly easily peeled and oily. Flesh colour is a deep orange with slightly open core, seed varying from zero to seedy (mixed blocks). Eating quality does not match the good appearance. The trees are vigorous.

Bay Gold. Trees were evaluated at three sites. The CFB trees had a good crop of large fruit size, colour T1 on 16 June. Quality was poor with low sugars and high acid and overmature. It was the same on 12 July. The test in June was quite acceptable but borderline juice (very large fruit). The fruit were all granulated and seedy (mixed orchard).

The Grabouw trees had a fair crop of variable medium small to large fruit size, good T1 colour on 12 June although some with green tinges on the stem end. The fruit was soft and juicy but slightly tough segment walls, overmature, slightly puffy and easily picked. The fruit had high acid and no flavour (the trees are planted in a satsuma orchard and subject to stressing). The test had unacceptably low TSS and very high acid. It is difficult to establish the maturity date as it was overmature in June but extremely high acid. Some fruit had odd spots on them and there was some seed.

At Clanwilliam there were odd medium small fruit, past peak in mid July, colour T1 and slightly tart, too few to evaluate. The trees are pale and responding slowly to urea treatments.

The fruit is attractive with an obovoid shape (almost Minneola-like) with a smooth rind, fairly easily peeled with some albedo adhering to the fruit, sometimes oily, pale orange flesh, slightly open to open cores, some creasing and sunburn, seedless to seedy. The trees have a fairly pale leaf colour.

Hadas. Trees at the CFB had a good crop of medium large fruit size, colour T4-6 on 16 June, T1 on 12 July. Quality in June was poor with high acid and low sugars (probably masked by the acid), improving slightly in July. The tests fell short of Ellendale standards due to low ratio. The TSS was good, acid exceeding 1.60% on both dates. The fruit was mature by 12 July. The fruit shape is flattish with a smooth rind, peelable and oily and open cores in July, seedy in a mixed block.

Table 6.2.2.4.3. Comparison of production, fruit size and colour of various Mandarin hybrids topworked on Bahianinha/Rough lemon at ALG, Citrusdal and evaluated during 2003.

Selection	Production	Fruit Size	Colour transparency			Maturity	Comment	Ave Seed
			10 June	23 June	15 July			
B17	Fair-good	Medium+	1-3 deep	1 deep red	1	mid June?	Good flavour but tart, juice low	0.8 mainly 0
B24	Poor	Large - extra large	4	1 excellent orange/red	1 excellent red	mid June	Acceptable tests, lack flavour	0
M37	Poor	Medium - medium large	5	3-4	1	mid-end June	Good, orange like flavour. Fruit sent overseas	12.4
H25	Odd	Medium	1			Early June?	Soft, poor quality difficult to peel, very oily. Leaf drop in July	0
H36	Fair – good	Medium large - large	5-6	3,4,5 getting deep orange	1 deep orange/red	Early June	Fair to good tests and flavour like Nouvelle	8.5
K33	Fair – not all branches bearing	Medium	5-6	4 green nipples	2-3 slightly green nipples, pale orange	End June	High sugars, acid high. Green stem end	0.6 mainly 0
Roma	Zero - poor	Medium large	4 good orange	1 good orange	1 good orange	End June	High sugars, a little dry	15.3
Michal	Excellent	Medium small-medium	1 some 2-3 looks over-mature, deep red	1 excellent looks over-mature	1	Over-mature by 10 June		seedy
Rishon	Odd	Small - large	1 red			Way over-mature by 10 June		0
Ora	Good-excellent	Medium - medium large	5-6	3,4,5	1, some 2-3, pale orange	3 rd week June	Good sugars and acid not high	13.6

The trees are topworked next to each other in a navel block.

Table 6.2.2.4.4. Oleocellosis evaluations of the various mandarin hybrid selections on different dates.

Site	Selection	28 May	10 June			23 June			22 July	
		% no oleo	% no oleo	% Class 1	% Class 2	% No oleo	% Class 1	% Class 2	% No oleo	% Class 1
Citrusdal	MurcXCI	100	100							
Citrusdal Ebenhae	B17		96	4		100			100	
Citrusdal ALG	B17					100			100	
	M37					91	9		80	20
	H36					91	9		85	15
	K33					100			100	
	Roma					91	9		90	10
	Michal						80	20		
Ora						100			100	
Grabouw	Bay Gold		97	3						

Conclusion

Nova Seedless. Production and fruit size varied between sites. Maturity varies per area from about 3rd week of May to early June. Colour was later at Buffeljagsrivier, similar to Troyer at Citrusdal. Eating quality was fair to good, Nova slightly better (but older trees). Internal quality was acceptable to good, but acid can be low. Seedless in solid blocks, some seed in mixed blocks. There is some fruit split. The Nova Seedless in

general appears quite acceptable (low acid can be a problem) and not too different to a Nova, but further evaluations are necessary to confirm this.

ITSC B17. Production at the various sites was generally fair to good with medium to medium large fruit size. Fruit colour was very good. Fruit sugars are high while the acid also tends to be high. Maturity around 2nd to 3rd week of June but the acid still high. The selection looks promising but has the drawback of fruit split and high acid. Further evaluations are necessary, especially on the semi commercial blocks. The selection should also be evaluated in hotter areas with lower acid levels.

ITSC B24. Production at the various sites varied from poor to good, the fruit size tending to be very large. Fruit quality was fair with good tests (low juice at Citrusdal), good rind and flesh colour and seedless. Maturity varied from before mid to later June with lower acid and sugars but higher ratio than B17. Further evaluations are necessary, especially on the semi commercial blocks.

ITSC M37. Production varied from poor to fair, fruit size medium to medium large. Fruit quality was good sometimes a weak flavour, an orange like flavour and good tests. Maturity around mid to late June, seedy in mixed blocks. Fruit sent overseas was well received. Further evaluations are necessary and it needs to be established whether the fruit is seedy in the absence of cross pollination and whether *Alternaria* is present.

ITSC H25. There were only odd fruit of medium fruit size, poor quality. Too few to evaluate properly. Further evaluations are necessary.

ITSC H36. Production was fair to good with fairly large fruit size and seedy. Maturity is around early June although retarded rind colour and fair quality with Nouvelle like flavour. Further evaluations are necessary and it needs to be established whether the fruit is seedy in the absence of cross pollination.

ITSC K33. Production was poor to fair with medium or medium large fruit size. Fruit quality was fair with good sugars but fairly high acid, peaking around end June. The fruit are not attractive and have green stem ends. The selection does not look promising except for its virtual seedlessness but further evaluations are necessary as the trees are still young.

Roma. Production was poor (young trees) with good fruit size. Quality was good, maturing at the end of June with good rind colour. This selection looks promising except for seed (mixed block), growth habit and thorns. Further evaluations are necessary.

Michal. Production was excellent and fruit size around medium. Colour was excellent, fair quality but slightly dry and overmature in June. Fruit size may be a bit small and the fruit will clash with Clementines. Discontinue evaluations.

Ora. Production was excellent with just acceptable fruit size. Maturity around 3rd week of June but lacked flavour. Rind colour may be a little retarded. The fruit is seedy. Further evaluations are necessary as this was the first good crop.

Murcott x Clementine. Production varied from poor to fair with good to large fruit size. Fruit quality was fair with good tests, maturity late June to early June for the CFB and Citrusdal respectively. Seed counts varied from zero to seedy (mixed blocks). The quality does not match the good appearance but a heavier crop may improve the fruit size (smaller) and quality. Further evaluations are necessary and it is necessary to establish whether the fruit is seedless in the absence of cross pollination.

Bay Gold. Production was fair to good with good fruit size, developing good colour and attractive. Quality is poor and acid levels too high. Seed counts varied from seedless to seedy. There was some creasing and sunburn. The selection does not look promising and further evaluations are necessary but it should be tried out in hotter areas to reduce acid levels.

Hadas. Production and fruit size were good but not good eating quality. Tests were unacceptable due to a low ratio. The fruit was seedy, maturity around mid July. Evaluations to continue.

Table 6.2.2.4.5. Internal fruit quality data of mandarin hybrid selections for the Eastern and Western Cape during the 2003 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Nova Seedless	CC	CFB	14/05	3-5	1	57.2	11.9	0.83	14.3	1.4
Nova Seedless	CC	CFB	16/06	1	1/1X	58.7	10.5	0.71	14.8	0.8
Nova Seedless	SC	Paksaam	12/05	4		55.9	8.7	0.97	9.0	0
Nova Seedless	TC	Rotterdam	22/05	3	2	61.7	11.7	0.97	12.1	0
Nova	SC	Paksaam	12/05	4		52.2	8.7	0.90	9.7	0.2
Nova	TC	Rotterdam	22/05	2	1/2	60.3	11.5	0.79	14.6	0
Rishon	BN/RL	ALG	25/06			34.2	9.5	0.49	19.4	
ITSC B17	CC	Dunbrody	30/05	5	1X	57.3	12.4	1.33	9.3	0.9
ITSC B17	CC	Dunbrody	30/05	4-5	1XX	57.1	11.4	1.12	10.2	0.8
ITSC B17	CC	Dunbrody	25/06	1	1	60.3	13.2	1.26	10.5	0.8
ITSC B17	CC	Dunbrody	25/06	1	1XX	55.1	11.7	1.08	10.8	1.3
ITSC B17	TC	Baddaford	26/06	1	1X	58.1	13.2	1.16	11.4	2.1
ITSC B17	RPL	Ebenhaeser	13/05	3-4	1	62.5	12.2	1.95	6.3	1.0
ITSC B17	RPL	Ebenhaeser	10/06	1	1X	59.9	12.7	1.83	6.9	1.2
ITSC B17	RPL	Ebenhaeser	24/06	1	2X	58.6	12.3	1.27	9.7	0.7
ITSC B17	RPL	Ebenhaeser	15/07	1	1XX	55.7	12.2	1.07	11.4	0.83
ITSC B17	BN/RL	ALG	10/06		1	48.4	12.3	1.43	8.6	
ITSC B17	BN/RL	ALG	25/06			45.9	12.2	1.21	10.1	
ITSC B17	BN/RL	ALG	16/07			38.7	14.0	1.46	9.6	
ITSC B24	CC	Dunbrody	30/05	3-4	1XX	58.3	11.2	0.97	11.5	0
ITSC B24	CC	Dunbrody	30/05	3	1XXX	57.1	11.0	0.93	11.8	0
ITSC B24	BN/RL	ALG	10/06		1XXX	41.4	11.1	1.07	10.4	
ITSC B24	BN/RL	ALG	25/06			36.5	11.3	0.91	12.4	
ITSC B24	BN/RL	ALG	16/07			33.3	10.9	0.83	13.1	
ITSC M37	TC	Baddaford	26/06	1-3	2	67.0	13.7	0.91	15.1	4.6
ITSC M37	TC	Baddaford	26/06	1-3	1XX	60.7	13.4	0.83	16.1	8.0
ITSC M37	BN/RL	ALG	10/06		1/1X	51.7	12.0	0.90	13.3	
ITSC M37	BN/RL	ALG	25/06			48.6	13.0	0.95	13.7	
ITSC M37	BN/RL	ALG	16/07			54.1	12.8	0.82	15.6	
ITSC H25	BN/RL	ALG	10/06		1	42.9	9.4	0.71	13.2	
ITSC H36	BN/RL	ALG	10/06		1/1X	48.6	10.8	0.81	13.3	
ITSC H36	BN/RL	ALG	25/06			50.0	10.7	0.75	14.3	
ITSC H36	BN/RL	ALG	16/07			44.4	11.3	0.69	16.4	
ITSC K33	BN/RL	ALG	25/06			40.5	12.8	1.49	8.6	
ITSC K33	BN/RL	ALG	16/07			56.1	13.5	0.68	19.9	
ITSC K33	RPL	Hexrivier	15/07	2-3	1XXX	54.9	10.7	1.28	8.4	1.7
Roma	BN/RL	ALG	10/06		1X	45.9	12.8	1.04	12.3	
Roma	BN/RL	ALG	25/06			42.1	12.7	0.91	14.0	
Roma	BN/RL	ALG	16/07			43.2	13.0	0.90	14.4	
Michal	BN/RL	ALG	10/06		2	47.6	10.0	0.75	13.3	
Michal	BN/RL	ALG	25/06			41.3	10.4	0.62	16.8	
Ora	BN/RL	ALG	25/06			51.5	11.1	0.88	12.6	
Ora	BN/RL	ALG	16/07			46.9	11.5	0.81	14.2	
Murcott x Clem	TC	CFB	16/06	3	1XX	52.8	10.6	0.86	12.3	7.8
Murcott x Clem	TC	CFB	12/07	1	1XX	54.3	12.9	0.90	14.3	1.6
Murcott x Clem	RL	Brakfontein	28/05	5	1XX	60.0	11.6	1.06	10.9	12.3
Murcott x Clem	RL	Brakfontein	10/06	1-2	1XX	59.6	12.3	1.11	11.1	10.6
Bay Gold	TC	CFB	16/06	1	1XXX	48.2	10.4	1.13	9.2	6.5

Bay Gold	SC	Whitehall	12/06	1-2	1X	54.7	9.1	1.83	5.0	6.4
Hadas	TC	CFB	16/06	4-5	1	56.6	11.0	1.66	6.6	8.2
Hadas	TC	CFB	12/07	1	2	57.9	12.7	1.61	7.9	9.3

Future research

Continue evaluating all the above selections except Michal. Gather as much semi commercial data of Nova Seedless, ITSC B17 and B24 as quickly as possible as there is a lot of interest in these selections. Establish ITSC B17 and Bay Gold in hotter areas in an attempt to reduce acid levels. Confirm seed status in the absence of cross pollination of ITSC M37, H36 and Murcott x Clementine and check out whether there is *alternaria* on ITSC M37 and Bay Gold.

6.2.2.5 Evaluation of navels in the Cape areas

Experiment 74 by C J Alexander (CRI)

Opsomming

Die doel van die proef is om kultivars te bekom om die seisoen vroeër en later te versprei en produksie pieke te verminder. Ook 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. Die Atwood is nie noodwendig vroeër nie en die Dream het enkele pitte gehad. Fukumoto lyk vroeg, maar het blykbaar verenigbaarheids probleme in die VSA. Letaba Early en Painter Early II lyk vroeg. Die Washington (SGB material) het goed gevaar, maar met groot vrugte en kan swak gehalte gee op jong bome. Cambria (SGB material) word in Julie ryp, met swak gehalte. Coetzee Late het swak gehalte en is onaantreklik, Renken Late het swak gehalte maar is redelik aantreklik en Mouton Late het swak gehalte maar het fyn en sagte vleis. Verdere evaluasies van al die seleksies is nodig.

Introduction

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.

Materials and methods

The trees were either planted or topworked within commercial orchards, or established on a semi commercial scale. Palmer, Tuligold, Lina and Newhall navels were used as controls. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is based on subjective tasting. Fruit quality was compared with the following standards previously considered most acceptable by the market place (higher standard for Navelates in brackets): 48% juice; 9.0% (10.0%) TSS; 0.6 – 1.8% (0.85 – 1.50%) acid; 7.5:1 (8.0:1) ratio; colour T3 + 20% T4 of set 34 (T3). Zero seeds/fruit.

A list of selections and sites evaluated during 2003 is given in Table 6.2.2.5.1.

Table 6.2.2.5.1. Navel trial sites evaluated during 2003.

Selection	Area	Site	Plant Date	Root Stock	No of trees
Atwood	Uitenhage	CFB	1999	TC	2
Atwood	Sunland	Paterson	2000	CC	5 topwork
Dream	Uitenhage	CFB	1999	CC	2
Dream	Sunland	Paterson	2000	CC	5 topwork
Fukumoto	Uitenhage	CFB	2001	CC	2
Fukumoto	Sunland	Paterson	2000	CC	5 topwork
Letaba Early	Sunland	Paterson	2000	CC	5 topwork
Painter Early II	Fort Beaufort	Riverside	1996	TC	4
Washington	Uitenhage	CFB	1994	TC	2
Washington	Addo	Willowtree	1998	CC	commercial
Washington	Patensie	Melville	1996	RL	commercial
Washington	Patensie	Ripplehill	1999	RL	commercial
Washington	Fort Beaufort	Riverside	2000	TC	commercial
Cambria	Uitenhage	CFB	1999	CC	2

Cambria	Patensie	Patensie Acht	2000		commercial
Coetzee Late Navel	Citrusdal	Hexrivier	1997	RPL	6 topwork
Renken Late Navel	Citrusdal	Hexrivier	1997	RPL	7 topwork
Mouton Late Navel 1	Citrusdal	Hexrivier	1997	RPL	3 topwork
Mouton Late Navel 2	Citrusdal	Hexrivier	1997	RPL	2 topwork
Lina (control)	Fort Beaufort	Riverside			
Newhall (control)	Sunland	Paterson	1999	CC	commercial
Palmer (control)	Patensie	Melville	1996	RL	commercial
Palmer (control)	Fort Beaufort	Riverside			
Californ Lane L (control)	Citrusdal	Hexrivier	1997	RPL	semi comm
Tuligold	Fort Beaufort	Riverside	1995	TC	5

Results and discussion

A discussion of each selection follows with internal quality results presented in Table 6.2.2.5.5 which need to be referred to when reading the text.

Atwood. The young trees at the CFB had a poor crop of medium large fruit size, colour T5-6 on 14 May, T1 on 16 June. The sugars were low and acid fair, mature in late May better in June. The tests were acceptable except for a seed with quite a drop in acid. Older Washington (different rootstock) were T6 in May, good quality and about 2 weeks short of maturity. The trees at Addo had a good crop of medium large to large fruit size, T7 on 24 April. The fruit was slightly raggy, fair quality about 2-3 weeks short of full maturity. The test was just acceptable (borderline TSS). Newhall controls were T6-7 with good quality and a better test, 1-2 weeks short of maturity.

Fruit shape is generally round, a smooth to slightly pebbly rind, and small navel ends. Peelability is difficult and oily, orange flesh and closed core. The Atwood navel originates in California and is reported to be virtually indistinguishable from its Washington parent except for slightly earlier colouring, smooth rind and good hanging ability.

Dream. The young trees at the CFB had a fair crop of medium large fruit size, colour T5-6 on 14 May, T1 on 16 June. Quality was fair, maturing early June, overmature by 12 July. The tests met Navelate standards in May and June, except colour initially. The acid held well into early July but there was some creasing even in May. The fruit had on average zero to 0.3 seeds/fruit. Older Washingtons had higher acid. The trees at Addo had a good crop of medium to medium large fruit size, mainly T7 on 24 April. The fruit had variable quality, fair to good maturity about early May. The test was acceptable. Newhall controls were T6-7 with good quality, 1-2 weeks short of maturity.

The fruit shape is round with a smooth to slightly pebbly rind, navel ends, oily and difficult to peel, orange flesh and slightly open core. The Dream navel originates in Florida and is reported to have lower acid than other navels, maturing early and hangs well.

Fukumoto. The open block trees at the CFB are small with no fruit on. Trees at Addo had a good crop of medium large fruit size, colour T6-7 on 24 April, the same as Newhall. The quality was nearly as good as Newhall. The test met Navelate standards (except colour) and slightly better than Newhall with a slightly lower acid. Maturity the end of April. The fruit shape is round with a slightly pebbly rind, some navel ends, oily and fair to difficult to peel. The flesh has a good orange colour and slightly open core.

It has been reported from California (Dr L. Marais) that Fukumoto is giving compatibility problems on trifoliolate and its hybrids as a rootstock. C35 is the worst followed by Carrizo then trifoliolate. This needs to be confirmed under South African conditions.

Letaba Early. There was a fair to good crop at Addo with medium large to large fruit size, colour T7 on 24 April (Newhall) T6-7. The quality was good, better than Newhall, the test acceptable with 0.16% lower acid than Newhall, maturing the end of April. Fruit shape was mainly round with some elongated fruit, a slightly pebbly rind and variable navel ends. Peelability was difficult and oily, the flesh a good orange colour and slightly open cores.

The various early selections established at Kruisrivier (Heidelberg) are not yet in production.

Painter Early II (Cliff Early). The young trees were compared with slightly older Tuligold, Lina and Palmer navels. Fruit colour and the tests were virtually identical to Tuligold on 8 April. On 8 May colour was T3 (Tuligold T2, Lina T4, Palmer T6) TSS similar to Tuligold but higher (acceptable) juice and lower acid. The Lina had higher TSS but juice too low. Of the few fruit left on 26 June, the quality was still good. Maturity is

estimated around third week of April. Fruit shape is round with a smooth rind, externally similar to Washington.

Sundays River Early, Fenix Early and Krajewski Early. The daughter trees had zero to odd fruit and were not evaluated.

Washington. Trees were evaluated at six sites. The CFB trees had a poor crop of medium fruit size (next to a windbreak), colour T6 on 14 May, T1 on 16 May. Quality was good with very good tests, maturity estimated late May/early June. Commercial trees at Addo had a good crop of large fruit size, colour 30% T2-3 and 60% T4-5 on 30 May. The quality was fair, good looking fruit, tests differing considerably between dates which does not make sense, from acceptable to good, peak maturity around mid June. Picking commenced on 3rd June and finished on 15 July. There were 11.5% fruit with protruding navels (class1) and 7.5% with malformed navels (class 1), 2% with navels. The packhouse figures of class 1 fruit had a count distribution of of 11% count 36; 24/40; 24/48; 20.5/56; 12/64; 7/72; 1/88; 0.5/105. 12% of the fruit were not send for packing due to rough skin and wind, thrips, silver mite and mealybug damage. Results of a 200 random fruit sample are given in Table 6.2.2.5.2.

Table 6.2.2.5.2. Yield, fruit size, count distribution and percentage class 1 fruit (100 fruit random sample; 200 in 2004) of Washington navels on Carrizo citrange planted in 1998 at Willowtree Farm, Addo.

Year in production	Yield per tree (kg)	Ave fruit size (mm)	Percentage fruit per count							Percentage in class 1
			36	40	48	56	64	72	88	
1	1.1									50
2	9.6	83.6		4	26	52		18		66
3	24.1*	85.2	2	14	33	35.5	12	3	0.5	30

* Class 1 & 2

A test was done at Patensie (Melville) between adjacent Palmers and Washingtons on 21 May. Both tests were similarly good, Washington larger fruit. Packhouse figures were 35.6% grade 1 with a count distribution of 6% count 40; 12/48; 22/56; 19/64; 25/72; 12/88; 3/105; 1/125. Yield 62.5 kg/tree.

The trees at Ripplehill bore a good crop of medium large fruit size, colour T7 on 21 May, T1-3 on 7 July. Quality was poor (young trees) and tests unacceptable (low TSS and acid). Maturity probably mid June. Two hundred fruit were evaluated in July. 68% fell between counts 56-72 and 32% counts 30-48, average 84.0 mm. There were 15.5% class 1; 2% class 2 and worse Protruding navel ends and 7.5% class1 and 0.5% class 2 Malformed navels. A single test done at Persaverance in May had good quality, except for low juice. Young commercial trees at Fort Beaufort had a good crop of medium large fruit size, colour T1-2 on 26 June. Quality was good and fruit mature, the tests good, count 56 of Navelate quality.

The Washington fruit are round with a smooth rind, some protruding and malformed navel ends. Eating quality and tests varied from poor to good, depending on the orchard. Some creasing was seen at some sites. Peelability was peelable to difficult and oily, orange flesh and slightly open core.

Cambria. The trees at the CFB had a poor crop (a lot of budwood removed) of small fruit size, colour T6 on 16 June. Quality was poor with an unacceptable test (low TSS). Maturity estimated at early July. The young trees at Patensie had a fair crop of medium small fruit size, T3-5 on 7 July. Eating quality was poor with low sugars and acid, maturity estimated towards the end of July. The tests in July were acceptable, but colour not yet good enough. The count distribution of 200 fruit measured was: 4.5% count 105 and 88; 25.5/72; 37/64; 28.5/56; 4.5/48-40, average 78.7 mm. This is a lot larger than the visual evaluation on the trees. There were 5.5% class 1 Protruding navel ends and 3.5% malformed navels.

The Cambria is round and elongated fruit with a smooth rind, difficult to peel and oily with orange flesh and a closed core.

Evaluation of late maturing Coetzee Late, Renken Late, Mouton Late 1 and Mouton Late 2 navels at Hexrivier, Citrusdal. These trees have not performed well and some of the fruit has been stolen, leaving little to evaluate. The orchard, on sandy soil also suffered severe frost damage. All selections had shoot growth on the roostocks. The results must be treated with caution due to the small sample size. Trees and fruit were evaluated and tested on 23 June and 15 July. Adjacent Californian Lane Lates were used for comparative purposes. Refer Table 6.2.2.5.3. below for yield, fruit size and colour.

Table 6.2.2.5.3. Visual yield, fruit size and colour of various late navel selections in 2003 topworked onto Rangpur Lime at Hexrivier, Citrusdal.

Selection	Yield	Fruit size	Colour	
			23 June	15 July
Coetzee Late	Zero – poor	Medium large – large	4-6	3
Renken Late	Odd – poor	Medium – medium large	2-4	1-3
Mouton Late 1	Zero – poor		4-5	
Mouton Late 2	Zero			
Californian Lane Late	Zero – poor	Medium large – large	4	1-3

Coetzee Late. Quality on 23 June was poor to fair, lacking flavour. The fruit looked overmature although maturity could be later. The fruit had a thick rind, difficult to peel and coarse flesh. The test in July was poor. Fruit shape is slightly elongated, with a pebbly rind. Small to protruding navel ends, odd malformed, cores closed to slightly open. The fruit had some shoulders and green heads with slight ribbing and unattractive, although in July the fruit started developing a good orange colour.

Renken Late. Quality on 23 June was poor to fair, lacking sugars and raggy. Maturity around early July. Both tests were poor. The fruit was difficult to peel with coarse flesh and closed to slightly open cores with a good rind thickness. Fruit shape is round, with a smoothish to slightly pebbly rind, Palmer-like navel ends with odd slight protruding and malformed navel ends. The fruit is fairly attractive developing a good orange rind colour.

Mouton Late 1. The few fruit evaluated lacked flavour on 23 June but had incredibly tender flesh probably at peak maturity. There were too few fruit to test. Fruit shape is elongated with a coarse rind. Navel ends were mainly closed. Rinds were thickish with a fine flesh.

Mouton Late 2. There was no fruit.

Californian Lane Late (control). Quality on 23 June was poor and fruit slightly raggy, at or close to peak maturity. Fruit shape was roundish to slightly elongated with a slightly pebbly rind, not as deep a colour as the other selections, fairly attractive with some slight green heads. Navel ends were variable, some Palmer-like others protruding and malformed. The flesh was coarse with better colour than the other selections, a closed core and sometimes slightly thick rind.

Table 6.2.2.5.4. Oleocellosis evaluations of the test samples of various navel selections.

Site	Selection	Date	% No oleo	% Class 1	% Class 2
Paterson	Atwood	24 April	67	33	
	Dream		84	8	8
	Fukumoto		92	8	
	Letaba Early		92		8
	Newhall		67	33	
Melville	Washington	21 May	85	15	
Ripplehill			60	40	
Persaverance			90	10	
Hexrivier	Coetzee late	15 July	59	8	33
	Reken Late		95	5	
	Calif Lane Late		95	5	

The above results are only on a small sample.

Conclusion

Atwood. The trees had a poor to good crop between sites of good to large fruit size. Fruit quality was poorer than the controls and not necessarily earlier. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Dream. Production and fruit size were fair to good. Contrary to last years results, the Dream has lower acid than Washington and similar colour at the CFB, similar to Newhall at Addo. The quality tests are fair to good but the fruit does have some seed. Further evaluations are necessary before any recommendations can be made.

Fukumoto. The trees at Addo had good production and fruit size with good quality, almost as good as Newhall. The test was good, maturity estimated the end of April, although the colour could be late. Further evaluations are necessary before any recommendations can be made. Fukumoto should not be propagated on trifoliolate or its hybrid rootstock until confirmed otherwise in South Africa.

Letaba Early. Production was fairly good with generally large fruit size. Fruit quality was good with lower acid than Newhall, maturity the end of April. Based on the limited data available the selection looks promising (except colour) but further evaluations are necessary before recommendations can be made.

Painter Early II. The selection is early maturing, peaking around third week of April. Further trial plantings need to be established and further evaluations needed.

Washington. Production was generally good, with medium large to large fruit size. Colour was acceptable to borderline at maturity which is around mid June. Fruit quality varied between sites from poor to good. Although the trees are still young, the Washington can be considered for commercial planting although the fruit tends to be on the large side and quality not always good on young trees. More commercial production data needed.

Cambria. Production varied and fruit size from small to larger, maturity in July. Eating quality was poor, poor to acceptable tests. Further evaluations of trees from Foundation Block material are necessary before recommendations can be made.

Coetzee Late navel. Production was poor with good to large fruit size. Quality was poor. There was some ribbing and fruit unattractive. As there were only a few fruit and frost damage, further evaluations are necessary before recommendations can be made.

Renken Late Navel. Production was poor with good fruit size. Quality was poor, maturity early July. The fruit is fairly attractive. As there were only a few fruit and frost damage, further evaluations are necessary before recommendations can be made.

Mouton Late Navel 1. Production was poor and fruit lacked flavour. The fruit had fine and very tender flesh, maturity around late June. As there were only a few fruit and frost damage, further evaluations are necessary before recommendations can be made.

Mouton Late 2. No fruit.

Table 6.2.2.5.5. Internal fruit quality data for navel orange selections for the Eastern and Western Cape areas during the 2003 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Atwood	RL	CFB	14/05	5-6	48	53.2	9.6	1.15	8.3	0.1
Atwood	RL	CFB	16/06	1	48	53.4	9.5	0.76	12.5	0
Atwood	CC	Paterson	24/04	7	56	50.7	9.1	0.92	9.9	0
Dream	CC	CFB	14/05	5-6	72	50.8	10.4	1.04	10.0	0.3
Dream	CC	CFB	16/06	1	56	52.7	11.0	0.89	12.4	0.2
Dream	CC	CFB	12/07	1	56	50.2	10.5	0.84	12.5	0
Dream	CC	Paterson	24/04	7	72	53.6	9.8	1.04	9.4	0
Fukumoto	CC	Paterson	24/04	6-7	56	52.1	10.4	0.94	11.1	0
Letaba Early	CC	Paterson	24/04	7	56	50.3	9.8	0.85	11.5	0
Painter Early II	TC	Riverside	08/04	4-5			9.6	1.01	9.5	
Painter Early II	TC	Riverside	08/05	3		49.8	10.0	0.81	12.3	
Washington	TC	CFB	14/05	5-6	72	54.3	11.5	1.20	9.6	0
Washington	TC	CFB	16/06	1	56	54.5	11.2	1.01	11.1	0
Washington	CC	Willowtree	28/05		small		10.8	0.98	11.0	
Washington	CC	Willowtree	28/05		large		10.2	0.91	11.2	
Washington	CC	Willowtree	30/05	3	56	49.6	9.3	0.94	9.9	
Washington	CC	Willowtree	30/05	3	48	48.7	9.1	0.86	10.6	
Washington		Persaverance	21/05	6-7	64	47.0	10.3	1.03	10.0	0
Washington		D Rautenbach	21/05	7	48	49.2	7.3	0.70	10.4	0
Washington		D Rautenbach	04/07	1	72	50.5	7.9	0.59	13.4	0
Washington		D Rautenbach	04/07	1	56	47.8	7.5	0.58	12.9	0
Washington		Melville	21/05	5	48	56.4	10.3	0.81	12.7	0
Washington	TC	Riverside	26/06	1	56	51.8	11.2	0.94	11.9	0
Washington	TC	Riverside	26/06	1	48	50.7	10.2	0.82	12.4	0
Cambria	CC	CFB	16/06	6	88	50.5	8.9	1.02	8.7	0

Cambria		Patensie Acht	04/07	4-5	72	56.7	9.8	0.92	10.7	0
Cambria		Patensie Acht	4/07	4-5	56	56.0	9.7	0.92	10.5	0
Coetzee Late	RPL	Hexrivier	15/07	4	40	41.5	8.3	0.88	9.4	0
Renken Late	RPL	Hexrivier	23/06	4	64	42.1	8.7	0.79	11.0	
Renken Late	RPL	Hexrivier	15/07	2-3	56	44.7	8.9	0.71	12.5	0
Tuligold	TC	Riverside	08/04	4-5			9.8	1.01	9.7	
Tuligold	TC	Riverside	08/05	2		43.7	10.1	0.93	10.9	
Lina	TC	Riverside	08/05	4		44.5	10.9	0.85	12.8	
Newhall	CC	Paterson	24/04	6-7	72	51.7	10.2	1.01	10.1	0
Palmer		Melville	21/05	5	72	50.9	10.3	0.84	12.3	0
Californ Lane Lat	RPL	Hexrivier	23/06	4-5	48	44.1	8.1	0.85	9.5	0
Californ Lane Lat	RPL	Hexrivier	15/07	3-4	48	45.3	8.0	0.72	11.1	0

Future research

Evaluate all sites and selections and accumulate data from commercial Washington and Cambrias (CFB material).

6.2.2.6 Evaluation of Midseason oranges in the Cape areas

Experiment 77 by C J Alexander (Private)

Opsomming

Die doel van die proef is om midseisoen seleksies wat beter in die koeler streke sal aard in terme van vrug grootte, gepigmenteerde vleis en saadloosheid, te vind. Die aanvaarbaarheid van die Salustiana se vrug grootte onder kommersiële toestande word ook bevestig. Kommerciële Salustianas in die Sondagsriviervallei het 'n uitstekende oes van goeie vrug grootte, aanvaarbare tot goeie gehalte gehad en word vanaf vroeg Junie ryp. Die Sanguinello in Fort Beaufort het goeie produksie en vrug grootte gehad, redelike gehalte, maar vleiskleur swakker as Tarocco. Tarocco produksie en gehalte het gewissel tussen persele met goeie vrug grootte. 'n Kommerciële boord in die Oos-Kaap het blykbaar goed presteer. Die Tarocco Gallo het swakker gehalte as beide 57/1E/1 en Tarocco gehad. Daar was min verskil tussen die ander seleksies: Maltaise Half – goeie produksie en vrug grootte, redelike gehalte; Maltaise Half II – kleiner vrug grootte, swak gehalte; Maltaise Line G – soortgelyk aan Half II, maar effens vroeër; beide Barlerin en Bokhobza het goeie produksie, maar kleinerige vrug grootte, redelik suur, maar Bokhobza het rooi vleiskleur; Raratonga goeie vrug grootte en redelike eetgehalte. Verdere evaluasies is nodig.

Introduction

The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless. Confirm fruit size acceptably of the non-pigmented Salustiana in commercial orchards that are in production.

Materials and methods

The trees were either planted or topworked within commercial orchards, or established on a semi commercial scale where possible. Field evaluations and laboratory analyses were conducted in the Eastern and Western Cape areas. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following Tomango standards previously considered most acceptable by the market: 52% juice; 9.0% TSS; 0.7 – 1.8% acid; 7.0:1 ratio; colour T3 + 20% T4 of set 34. Seed maximum average 6.0 seeds per fruit.

A list of selections and sites evaluated is given in Table 6.2.2.6.1.

Table 6.2.2.6.1. Midseason orange trial sites evaluated during 2003.

Selection	Area	Site	Plant Date	Root Stock	No of trees
Salustiana	Addo	Dunbrody	1997	CC	commercial
Salustiana	Fort Beaufort	Baddaford	1996	TC	10
Sanguinello	Fort Beaufort	Baddaford	1996	TC	10
Tarocco	Uitenhage	CFB	1994	TC	2
Tarocco	Fort Beaufort	Baddaford	1996	TC	10
Tarocco	Adelaide	Saxfold Park	1996	TC	commercial
Tarocco	Ashton	Excelsior	2002	TC	com topw
Tarocco Gallo	Uitenhage	CFB	1999	CC	2
Tarocco 57/1E/1	Uitenhage	CFB	1999	CC	2
Maltaise Half	Uitenhage	CFB	1997	TC	2
Maltaise Half II	Uitenhage	CFB	1997	TC	2
Maltaise Line G	Uitenhage	CFB	1997	TC	3
Maltaise Line G	Fort Beaufort	Baddaford	1998	TC	5
Maltiaise Barlerin	Uitenhage	CFB	1997	TC	2
Malktaise Bokhobza	Uitenhage	CFB	1997	TC	2
Raratonga	Uitenhage	CFB	1999	TC	2

Results and Discussion

A discussion of each selection follows with yield, fruit size, colour and estimated maturity and internal quality results for the various selections presented in Tables 6.2.2.6.3. and 6.2.2.6.4. respectively which need to be referred to when reading the text.

Salustiana. Trees were evaluated at two sites. The commercial trees at Addo had an excellent crop of medium large fruit size, colour T2-5 on 30 May and T1 (partly picked) on 25 June. Picking commenced week ending 7 June. Quality was fair in May with slightly high acid, good in later June, maturing early to mid June. The tests were good in May, slightly lower TSS and acid in June, all meeting the standards. There was some creasing and odd oleocellosis. Fruit size measurements were slightly larger than the visual evaluation.

Average fruit size and count distribution results of fruit measured are given in Table 6.2.2.6.2. below.

Table 6.2.2.6.2. Average fruit size and count distribution for Salustianas measured at Dunbrody Estates, Addo on 22 May 2002 and 30 May 2003.

Year	Production tons/ha (6 x 3m)	Export % Class 1 & 2	Ave fruit size (mm)	Percentage fruit per count distribution					
				≥40	48	56	64	72	88
2001	51	78							
2002	82	82	79.1	2	9	23	32	28	6
2003	Est 55-60		80.8	8.5	11.5	25	32	19	4

Sample size 100 in 2002; 200 in 2003.

Fruit quality at Fort Beaufort was fair to good with a good test, colour and seedless.

The fruit shape is round with a smooth rind. The fruit was peelable but oily, orange flesh and slightly open to open cores and seedless.

Sanguinello. Fruit quality at Fort Beaufort was fair with a good test on 26 June although acid 1.13% The eating quality not quite as good as Salustiana probably due to the higher acid. Pigmentation was not as good as Tarocco. Maturity end June. Fruit shape is round with a smooth rind and peelable but oily. The flesh was orange red with a slightly open core and no seed.

Tarocco. Trees were evaluated at two sites. The CFB quality was fair to good, better than Tarocco Gallo and 57/1E/1, although the trees are older. The test on 16 June was good, maturing around mid June. Fruit quality at Fort Beaufort was poor, the count 72 test acceptable and count 48 unacceptable (fairly low TSS and too low acid), maturity around early to mid June. There was some pigmentation. Commercial Taroccos at Adelaide (not evaluated) were reported to do well, peaking around counts 64 - 72. The fruit is cold stored for 5-6 days to enhance flesh pigmentation before packing. The Western Cape commercial block is not yet in production.

Tarocco fruit shape is round to necked with a smooth rind. The fruit is peelable but oily, open cores and few seeds. There was some creasing at the CFB.

Tarocco Gallo. Fruit quality at the CFB was poor on 16 June, fair on 12 July. Tests in June and July were unacceptable with too low TSS, maturity around mid June. The fruit shape is round with a smooth rind, attractive, peelable and oily, orange flesh and closed cores with no seed. There was some splitting.

Tarocco 57/1E/1. Fruit quality at the CFB was fair with high acid on 16 June, better taste than Gallo, fair and overmature on 12 July. The tests were good although the acid levels were quite high, still 1.29% in July, the TSS and acid higher than Tarocco and Gallo. Maturity around end June. There was some creasing and odd seed. The fruit shape is round with a smooth rind, peelable and oily, orange flesh and slightly open to open cores.

Maltaise Half. Quality at the CFB was fair with a high acid on 16 June, mature around end June/early July. The test was acceptable in June, juice just too low on 12 July and a drop in TSS and acid. The fruit shape is round with a smooth rind, peelable and oily, slightly open to open cores and orange flesh with odd seed.

Maltaise Half II. Quality at the CFB was poor with slightly high acid on 16 June, the same on 12 July and overmature. The tests however were good. Maturity around end June/early July. The fruit shape is round with a smooth rind, peelable and oily, open cores and orange flesh. There was some creasing and odd seed.

Maltaise Line G. Trees were evaluated at two sites. Fruit quality at the CFB was poor with high acid on 16 June and 12 July. The test in June was good, but the juice level too low in July because of a thick rind. Mature in mid June. The fruit at Fort Beaufort had fair quality with soft rag on 26 June. The test was good, maturing mid to late June.

The fruit shape is round and smooth, peelable but oily, orange flesh and closed to slightly open cores, zero to odd seed. There was some creasing at the CFB.

Maltaise Barlerin. The quality was not so good on 16 June due to high acid, fair on 12 July and mature. The tests were good although the acid was still 1.22% in July. Maturity around mid July. Fruit shape is round with a smooth rind, peelable but oily, slightly open to open cores and orange flesh. There were odd seed.

Maltaise Bokhobza. Quality was poor with very high acid on 16 June, still poor on 12 July but mature. The tests were good, acid still 1.16% in July and reddish juice. Maturity estimated at mid July. Fruit shape is round with a smooth rind, peelable but oily and open cores. Flesh colour is orange with some red pigmentation. There was creasing and some seed.

Raratonga. The young trees were evaluated in the psylla house at the CFB which may not give a true reflection of the cultivar. The quality was fair on 16 June, maturity estimated around early July. No tests were done. Fruit shape is round with a coarse rind and peelable but oily. Flesh colour is orange with open cores and no seed. There were some split fruit.

Table 6.2.2.6.3. Yield, fruit size, fruit colour, estimated maturity and comments of various midseason oranges evaluated during 2003.

Selection (year planted)	Yield	Fruit size	Colour			Estimated maturity	Comment
			16 June	26 June	12 July		
CFB							
Tarocco ⁹⁴	Poor	Medium large	1			mid June	Fair – good quality
Tarocco Gallo ⁹⁹	Excellent	Medium large	1-3		1	mid June	Poor in June, fair in July, good looking fruit
Tarocco 57/1E/1 ⁹⁹	Excellent	Medium	1		1	end June, overmature in July	Fair, taste better than Gallo
Maltaise Half ⁹⁷	Good	Medium large	2-3		1	End June/early July	Fair quality
Maltaise Half II ⁹⁷	Good	Medium small	1-3		1	End June/early July overmature by 12 July	Poor quality
Maltaise Line G ⁹⁷	Excellent	Medium small	1-2		1	mid June	Poor quality
Maltaise Barlerin ⁹⁷	Good	Medium small	1-4		1	mid July	Poor, high acid, fair in July
Maltaise Bokhobza ⁹⁷	Good	Medium small	1-3		1	mid July	Poor, high acid, bad creasing
Raratonga ^{ps 99}	Fair	Medium large	4			Early July June	Fair quality
Dunbrody							
Salustiana ⁹⁷	Excellent	Medium large	2-5 (30 May)			Early-mid June	Fair quality
Baddaford							
Salustiana ⁹⁶	Good	Medium large		1		End June	Fair -good
Sanguinello ⁹⁶	Good	Medium large		1		End June	Fair. Not as red as Tarocco
Tarocco ⁹⁶	Good	Medium large		1		Early-mid June	Poor
Maltaise Line G ⁹⁸	Fair	Medium		1		mid-late June	Fair, soft flesh

Conclusion

Salustiana. Production and fruit size were good, good colour, fair to good quality and maturity from early to late June. Based on these results the Salustiana can be planted in the Addo and Fort Beaufort areas, although midseasons do tend to develop smaller fruit size especially when trees get older.

Sanguinello. Production and fruit size was good. Quality was fair with a good test, maturing end of June. There was some flesh pigmentation. Due to the lack of pigmentation, commercial plantings are not recommended at this stage. Further evaluations are necessary.

Tarocco. Production was poor to good with good fruit size at the evaluated sites. Quality and tests varied between the sites from poor to fair to good, mature around mid June. Further evaluations are necessary as they contradict last years evaluations, while commercial orchards in the Eastern Cape were reported to perform well. Further evaluations are necessary.

Tarocco Gallo. Production and fruit size was good but quality poor, maturing around mid June. Further evaluations are necessary.

Tarocco 57/1E/1. Production was excellent with medium fruit size and fair quality, higher sugars and acid than Tarocco, maturing around end June. Further evaluations are necessary.

Maltaise Half. Production and fruit size was good with fair quality and maturing end June/early July. Further evaluations are necessary.

Maltaise Half II. Production was good but fruit size a little small. Quality was poor, maturing end June/early July. Further evaluations are necessary.

Maltaise Line G. Production was good to excellent, fruit size a bit on the small side. Quality was poor to fair, generally good tests. Maturity around mid to late June. Further evaluations are necessary.

Maltaise Barlerin. Production was good but fruit size medium small. Tests were good, but acid fairly high, maturing about mid July. Further evaluations are necessary.

Maltaise Bokhobza. Production was good and fruit size medium small. The test was good although poor taste and some flesh flesh pigmentation. The fruit had creasing, maturity mid July. Further evaluations are necessary.

Raratonga. Production was fair with good fruit size and fair quality. Maturity is estimated at early July. Further evaluations are necessary.

Table 6.2.2.6.4. Internal fruit quality data of the various midseason orange selections for the Eastern and Western Cape during the 2003 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Salustiana		Dunbrody	25/06	1	48	54.2	9.7	0.88	11.0	0
Salustiana	TC	Baddaford	26/06	1	72	52.4	10.4	0.86	12.1	0
Sanguinello	TC	Baddaford	26/06	1	56	54.4	11.3	1.13	10.0	0
Tarocco	TC	CFB	16/06	1	56	55.7	10.4	1.05	9.9	0.3
Tarocco	TC	Baddaford	26/06	1	72	53.2	10.4	0.77	13.5	
Tarocco	TC	Baddaford	26/06	1	48	53.9	9.4	0.63	14.9	0.3
Tarocco Gallo	CC	CFB	16/06	1	56	49.4	8.9	0.94	9.5	0
Tarocco Gallo	CC	CFB	12/07	1	56	52.2	8.6	0.94	9.1	0
Tarocco 57/1E/1	CC	CFB	16/06	1	72	53.7	11.0	1.36	8.1	0.4
Tarocco 57/1E/1	CC	CFB	12/07	1	72	52.2	10.7	1.29	8.3	0.7
Maltaise Half	TC	CFB	16/06	1-2	72	54.0	10.1	1.15	8.8	0.2
Maltaise Half	TC	CFB	12/07	1	72	51.8	9.6	1.01	9.5	0.2
Maltaise Half II	TC	CFB	16/06	1-2	88	56.4	10.8	1.13	9.6	0.3
Maltaise Half II	TC	CFB	12/07	1	72	54.0	10.9	0.96	11.4	0.2
Maltaise Line G	TC	CFB	16/06	1	105	55.1	10.2	1.00	10.2	0.3
Maltaise Line G	TC	CFB	12/07	1	72	50.6	10.7	0.95	11.3	0
Maltaise Line G	TC	Baddaford	26/06	1	72	53.4	10.6	0.91	11.6	0
Maltaise Barlerin	TC	CFB	16/06	1	88	57.1	10.8	1.36	7.9	0.6
Maltaise Barlerin	TC	CFB	12/07	1	88	57.5	11.2	1.22	9.2	0.2
Maltaise Bokhobza	TC	CFB	16/06	1-2	88	54.1	10.8	1.18	9.2	1.4
Maltaise Bokhobza	TC	CFB	12/07	1	72	53.1	11.6	1.16	10.0	1.3

Future research

Continue with evaluations. Incorporate semi-commercial plantings of Tarocco, Tarocco Gallo and Tarocco 57/1E/1 selections if possible. Finalise Salustiana and other selections (except Taroccos) if sufficient data.

6.2.2.7 Evaluation of Valencia oranges in the Cape areas

Experiment 75 by C J Alexander (Private)

Opsomming

Die doel van die Valencia proef is om vroeër en groter, saadlose seleksies met verbeterde vrugset te soek, en die verenigbaarheid van Turkey op Rangpur Lime onderstam te ondersoek. Die Mouton Early en Turkey het gedeeltelik aan hierdie vereistes voldoen. Die Mouton Early lyk belowend as 'n vroeë seleksie, maar die

vrugte is grof. Die Turkey kan as 'n vroeër seleksie geplant word, maar die interne gehalte is 'n kwessie. Die verenigbaarheid van Turkey op Rangpur Lime is twyfelagtig. Evaluasies moet voortgaan.

Introduction

The aim is to evaluate earlier maturing Valencia cultivars in terms of their earliness, rootstock compatibility, colour, fruit size and seediness.

Materials and methods

The trees were either planted or topworked within commercial orchards, or established on a semi commercial scale where possible. Field evaluations and laboratory analyses were conducted in the Western Cape. In some instances there may be differences recorded between field and laboratory analyses. These are noted. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following valencia standards previously considered most acceptable by the market (Delta and Midnight in brackets): 48% (52%) juice; 9.0% (10.5%) TSS; 0.6 – 1.8% (0.85 – 1.5%) acid; 7.0:1 (7.5:1) ratio; colour T3 + 20% T4 of set 34. Seed maximum average 9.0 seeds per fruit (Delta 0, Midnight 1).

A list of selections and sites evaluated is given in Table 6.2.2.7.1.

Table 6.2.2.7.1. Valencia orange trial sites evaluated during 2003.

Selection	Area	Site	Plant Date	Root Stock	No of trees
Mouton Early upper orchard	Citrusdal	Sewe Oliene	1997	RL	5 topwork
Mouton Early lower orchard	Citrusdal	Sewe Oliene	1999	RL	commercial
Turkey	Citrusdal	Hexrivier	1995	RPL	commercial
Turkey lower (control)	Citrusdal	Sewe Oliene	1999	RL	semi com
Midnight upper (control)	Citrusdal	Sewe Oliene	1986	RL	1
Valencia upper (control)	Citrusdal	Sewe Oliene	1986	RL	commercial
Valencia lower (control)	Citrusdal	Sewe Oliene	1954	RL	commercial
Delta valencia (control)	Citrusdal	Hexrivier	1994	RL	commercial

Results and Discussion

A discussion of each selection follows with internal quality results presented in Table 6.2.2.7.4. which needs to be referred to when reading the text.

Mouton Early. Trees were evaluated in two orchards on the same farm – upper and lower orchards, with their own controls. Results are presented in Tables 6.2.2.7.2. and 6.2.2.7.3. below.

Table 6.2.2.7.2. Yield, fruit size, colour transparency, taste, test comments and estimated maturity of three Valencia selections at Sewe Oliene (upper orchard), Citrusdal, evaluated on 24 June and 15 July 2003.

Selection	Yield	Fruit size	Colour		Taste	Test	Estimated maturity
			24/6	15/7			
Mouton Early	Good	Medium–medium large. 83% count 72/88	T2-3	T1 deep orange	June – at or close to peak, slightly raggy, slightly sweet, lowish acid. Fair. July – past peak by 2-3 weeks, tender, lack flavour, low acids, slightly soft. Fair.	June – TSS too low, acid good. July – acceptable.	End June. Look overmature in July.
Midnight	Good	Medium–medium large. 73% count 72/88	T3 yellow	T1-2 pale	June – at least 3 weeks to go, slightly raggy, lacks flavour, more acid. July – 2-3 weeks to maturity, raggy, tart, not quite mature. Fair.	June – TSS too low, acid too high. July – unacceptable as valencia as ratio too low.	End July.
Valencia Late	Fair–good,	Medium small to	T4-5	T1-3	June – far from mature, high acid.	June – acid too high. July –	Mid August.

	variable	medium. 40% count 72/88			July – at least 4 weeks to go, high acid.	ratio too low.	
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Table 6.2.2.7.3. Yield, fruit size, colour transparency, taste, test comments and estimated maturity of three valencia selections at Sewe Oliene (lower orchard), Citrusdal, evaluated on 24 June, 2003.

Selection	Yield	Fruit size	Colour	Taste	Test	Estimated maturity
Mouton Early	Odd	Medium large	T3-4	Poor, lacks TSS, fairly acid. Frosted.	Low TSS	End June? Looks slightly overmature
Turkey	Poor – fair	Mainly medium large	T2-3 pale	Lacks flavour, low TSS, fairly acid. Frosted.	Low TSS	End June?
Old Valencia Late trees	Fair	Medium–medium large	T3-4	Far from mature, high acid	Not tested	After end July?

Fruit shape is round with a slight shoulder, thickish and pebbly rind (daughter trees better), not very attractive. Midnights and valencias have a similar rind thickness, but smoother rind. Flesh colour was a good orange, slightly open cores. Mostly seedless, no oleo.

Turkey. The Turkey was evaluated at one site to determine its suitability to the Citrusdal area and compatibility on Rangpur Lime rootstock. Unfortunately the orchard suffered an unusually severe frost occurrence in June and the fruit was not fit for packing. The trees carried a fair to good crop of good, medium large fruit size (mostly count 56 to larger), colour T1-3 on 23 June, improving to T1 on 15 July, adjacent Delta valencias a similar crop of medium to (count 105-72) fruit size, colour T4 in June, T3-5 in July. The quality was poor with sufficient acid but lacking sugars. The Deltas had higher acid and far richer, later maturing. The Turkey did not meet export standards in June or July due to low juice (thickish rinds and frost) and low TSS. The Delta in July also had unacceptably low TSS. Neither selection had oleo.

Fruit shape is mainly round to slightly elongated with a fairly smooth to slightly pebbly rind. Seed varies from zero to 1.1 seeds/fruit on average (67% of the fruit with no seed). Some trees have suckers growing out the rootstocks at ground level and other trees odd nodules on the rootstocks. There were no signs of rootstock incompatibility evident under the bark. The bud unions are fairly smooth.

Conclusion

Mouton Early. Older topworked and young trees were evaluated, the latter damaged by severe frost. Production was good on the old trees with good fruit size, slightly larger than Midnight. Maturity is early, around end June, colour good. Fruit quality was poor to acceptable, virtually seedless. The fruit has a coarse rind and is not very attractive. The Mouton Early is early maturing and should rather be planted on a heavier soil or citrange rootstock to induce a smoother rind. The selection is protected and virus free material is available. Commercial plantings not yet recommended. Evaluations to continue.

Turkey. Production was fairly good and fruit size was good. The fruit is much earlier maturing than Delta. Fruit quality was poor. The fruit was not exportable due to severe frost. The selection can be planted commercially but quality aspects must be taken into account and a better quality inducing rootstock be considered. The compatibility of Turkey on Rangpur Lime seems to be questionable as there is sucker and nodule growth on the rootstocks. Other rootstocks should rather be used.

Table 6.2.2.7.4. Internal fruit quality data for Valencia orange selections for the Western Cape during the 2003 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Mouton Early up	RL	Sewe Oliene	23/06	1-2	72	51.4	8.8	1.08	8.1	0.1
Mouton Early up	RL	Sewe Oliene	15/07	1	88	51.7	9.5	1.01	9.4	0
Mouton Early lo ^f	RL	Sewe Oliene	23/06	3-4	72	46.0	8.5	0.89	9.6	0
Midnight	RL	Sewe Oliene	23/06	2-3	88	56.4	8.6	1.73	5.0	0.1
Midnight	RL	Sewe Oliene	15/07	1-2	88	56.0	9.3	1.49	6.2	0.2

Valencia Late	RL	Sewe Oliene	23/06	4	105	53.2	9.1	1.74	5.2	2.0
Valencia Late	RL	Sewe Oliene	15/07	3	105	55.3	9.0	1.58	5.7	1.9
Turkey ^f	RL	Sewe Oliene	23/06	2-3	72	50.8	8.3	1.26	6.6	1.4
Turkey ^f	RPL	Hexrivier	23/06	1-2	56	47.8	7.7	1.36	5.7	0.5
Turkey ^f	RPL	Hexrivier	15/07	1	56	47.1	8.2	1.33	6.2	1.1
Delta Valencia	RL	Hexrivier	15/07	2-3	88	53.9	7.4	1.55	4.8	0

up = upper orchard

lo = lower orchard

^f = Severe frost damage

Future research

Evaluate both selections and accumulate commercial Turkey data. Evaluate Turkey on six different rootstocks in Citrusdal.

6.2.2.8. Evaluation of Genoa Lemon on various rootstocks in Citrusdal

Experiment 588 by C J Alexander (Private)

Opsomming

Die doel van die proef is om die prestasie van die Genoa suurlemoen in die Citrusdal area te beproef, asook die prestasie op tien verskillende onderstamme. Die bome het hul tweede drag gedra, maar die bome is ongelyk in terme van boomgrootte wat moontlik aan swak bome by uitplanting toegeskryf kan word. Growweskiisuurlemoen en Volckameriana het die grootste bome gehad, Benton en Trifoliate X Sweet orange die kleinste. Volckameriana, Japanse Citroen en Rangpur het suiers en of knoppies op die onderstam gehad, Swingle "benched". Produksie was oor die algemeen swak, Carrizo geen vrugte. Die trifoliaat en citrange tipes het die kleinste vruggrootte gehad. Vrugkleur het nie met die vorige seisoen s'n ooreengestem nie. Net Japanse Citroen het nie die sap uitvoer persentasie behaal nie. Saadtellings was heelwat laer die seisoen. Daar was tot 10% nie uitvoerbare vrugte weens hoër skouers, Oleoselosis die ergste by Rangpur en Japanse Citroen. Growweskiisuurlemoen en Rangpur het effens growwe skille gehad, M X T die gladste en Tifoliate X Sweet orange die mees aantreklikste. Verdere evaluasies is nodig.

Introduction

The Genoa is a newly acquired slightly earlier maturing lemon selection. The trial was established in Citrusdal to determine the performance of this selection in the area on ten different rootstocks and in so doing possibly provide an alternative to the currently planted lemon selections and on different rootstocks.

Materials and methods

The Genoa lemon was budded to ten different rootstocks and planted at Hexrivier, Citrusdal in January 2000 in adjacent rows. The trees evaluated according to certain criteria, including tree size, production, fruit size, rind colour, juice percentage, rind thickness, rootstock and scion diameter and rootstock/scion compatibility. One hundred fruit per rootstock were used for evaluations, except Trifoliate X and Benton citrange where only 50 fruit were used as there were too few fruit. A list of the rootstocks used is given in Table 6.2.2.8.1. below.

Table 6.2.2.8.1. List of roostocks, rootstock selection and number of trees planted and evaluated with Genoa Lemon as scion at Hexrivier, Citrusdal during 2003.

Rootstock	Selection	No of trees planted	No of trees evaluated
Cairn Rough lemon	163	21	16
Volckameriana	575	18	13
Trifoliate X	1242	17	9
Benton citrange	980	16	5
Trifoliate X Sweet orange	1287	16	14
Japanse Citroen	184	16	13
Minneola x trifoliate	1238	23	13
Swingle citrumelo	715	18	11
Rangpur Lime	225	6	4
Carrizo Citrange	608	6	4

Results and discussion

The trees were evaluated on the 13 May and tree heights measured on 15 July 2003. The trees had not grown out as anticipated this past season and some trees are a lot smaller than others. The possible reason for this is that some of the trees were poorer quality at planting. Only the better/larger trees have been used for evaluation purposes – refer Table 6.2.2.8.1. One hundred randomly picked fruit were used for evaluation purposes, except Trifoliata X and Benton citrange as there were too few fruit (50 fruit sample). Carrizo citrange had no fruit. The results are presented in the following tables.

Table 6.2.2.8.2. Visual evaluation of tree production, tree vigour and bud union appearance. Rootstocks arranged in order of planting.

Rootstock	Production	Tree vigour	Bud union
Cairn Rough lemon	Zero – good	Vigorous	Smooth
Volckameriana	Poor, variable	Vigorous	Fairly smooth, some fluting and odd nodules on rootstock
Trifoliata X	Zero – poor	Medium, slightly pale leaves The results are presented in the following tables	Smooth
Benton citrange	Zero – good	Variable	Smooth
Trifoliata X Sweet orange	Zero – fair	Medium	Smooth
Japanese Citroen	Fair – good	Fairly vigorous	Smooth but some nodules showing and odd sucker growth
Minneola X trifoliata	Zero – poor	Fairly vigorous	Smooth
Swingle citrumelo	Zero – fair	Variable	Slightly benched
Rangpur Lime	Poor – fair	Fairly vigorous	Smooth but some nodules and suckers
Carrizo citrange	Zero	Medium	Smooth

Stem and scion diameters were not measured as the trees were allowed to branch just above the bud union in the nursery.

Table 6.2.2.8.3. Average tree height for 2002 and 2003 and fruit colour transparency of Genoa lemon on various rootstocks. Rootstocks arranged according to ascending fruit colour.

Rootstock	Ave tree height 2002 (m)	Ave tree height 2003 (m)	Percentage fruit per colour transparency					
			T3	T4	T5	T6	T7	T8
Tri x Sweet orange	2.27	2.76		6	52	42		
Minneola x trifoliat	2.54	2.94		2	56	42		
Volckameriana	2.87	3.10		3	54	42	1	
Cairn Rough lemon	2.90	3.09		5	44	51		
Swingle citrumelo	2.38	2.78		3	45	51	1	
Japanese Citroen	2.58	2.98		1	50	49		
Rangpur Lime	2.58	2.90		4	40	56		
Trifoliata X	2.58	2.89			58	42		
Benton citrange	2.37	2.76			54	44	2	
Carrizo citrange	2.40	2.98						

Table 6.2.2.8.4. Average fruit size and percentage fruit per count of Genoa lemon on various rootstocks. Rootstocks arranged according to descending fruit size.

Rootstock	Ave fruit size (mm)		Percentage fruit per count								
	2002	2003	≤216	189	162	138	113	100	88	75	≥64
Japanese Citroen	70.8	63.7			7	11	26	33	17	4	2
Rangpur Lime	71.6	63.1	3		2	15	29	33	10	6	2
Volckameriana	71.8	63.1		5	5	14	28	19	18	7	4
Cairn Rough lemon	72.4	62.9		5	7	12	31	22	11	10	2
Benton citrange	69.8	61.9		4	8	28	22	24	8	4	2
Trifoliata X	64.6	61.0			10	16	46	24	4		
Tri x Sweet orange	67.6	58.8	5	12	15	21	30	14	2	1	
Swingle citrumelo	64.6	57.5	6	16	21	28	18	10	1		
Minneola x trifoliata	63.2	57.1	11	15	20	23	25	4	2		
Carrizo citrange	64.0										

Table 6.2.2.8.5. Fruit sample count, juice percentage, seed counts, sample rind colour and rind thickness of the Genoa lemon on various rootstocks for 24 June 2002 and 13 May 2003. Rootstocks arranged according to descending juice percentage.

Rootstock	Fruit Count		Sample Juice %		Average seed per fruit		Sample rind colour		Rind thickness (mm)	
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003
Rangpur Lime	88	100	46.7	47.0	4.8	1.4	3	5	6.0	5.0
Tri x Sweet oran	100	113/138	46.3	46.6	5.5	4.3	3	5-6	6.0	4.4
Benton Citrange	88	113	47.4	45.4	5.5	3.8	4	5	6.5	4.3
Cairn Rough lem	88	100	46.0	45.2	5.1	1.9	3	5	6.8	5.0
Trifoliata X	113	113	42.9	44.3	6.9	3.0	3	5	6.5	4.8
Swingle citrumel	113	113/138	45.0	43.8	7.9	3.7	3	5-6	5.8	4.4
Minneola x trifol	113	113/138	45.0	43.0	7.9	5.0	4	5	6.3	4.1
Volckameriana	88/75	113	45.2	43.0	5.5	3.3	3-4	5	6.8	4.7
Japanese Citroen	88	100	46.6	36.7	4.8	1.8	3-4	5-6	6.3	4.6
Carrizo citrange	100		44.4		3.5		2-3		6.2	

Table 6.2.2.8.6. Analyses of high shoulders, Oleocellosis and wind damage. Rootstocks arranged from least to most high shoulders.

Rootstock	High Shoulders					Oleocellosis					Wind damage								
	0	1	2	3	4	0	1	2	3	4	5	0	1	2	3	4	5	6	7
Volckamer	59	21	8	8	4	71	26	3				58	26	9	3	2		2	
Rangpur Lim	49	29	13	8	1	53	31	10	2	3	1	40	28	16	10	2	2	1	1
Swingle citru	41	43	9	7		86	12	1	1			45	26	10	5		2	1	1
Tri X Sw or	40	32	18	9	1	92	7	1				51	17	14	8	4	4	1	1
Trifoliata X	36	36	20	6	2	84	14	2				50	26	10	8	4		2	
Japanese Sit	46	23	15	10	6	55	35	7	2	1		50	24	17	6		3		
Minn X trifol	43	22	18	10	7	79	18	3				46	23	17	4	3	5	1	1
Cairn RL	34	30	16	12	8	81	17	2				72	11	8	1	1	5	2	
Benton citr	40	26	12	12	10	86	10	4				56	24	12	4	2	2		

Evaluations based on Outspan Colour Prints for Blemish and Appearance standards.

High Shoulders (Set 39): Prints 0 - 3 are exportable.

Oleo (Set 28): Prints 0 - 3 are exportable. Evaluated after eight days.

Wind Scars (Set 8): Prints 0 - 3/4 are exportable.

Botrytis (Set 43): Only Rough lemon and Volckameriana had minor blemish, 4% and 3% respectively, all exportable.

All had smooth fruit, although Rough lemon and Rangpur Lime were slightly coarser - reasonably smooth, Minneola X trifoliata very smooth and Trifoliata X Sweet orange very nice looking fruit.

Conclusion

This was the second year of production, with a few fruit last season. Unfortunately the trees are variable in size, only the best trees used for evaluation purposes. Rough lemon and Volkameriana are the largest and most vigorous trees, Benton and Swingle variable and Trifoliate X, Trifoliate X Sweet orange and Carrizo medium vigour. Benton and Tri X Sweet orange had the smallest trees. Most had smooth bud unions, except Volkameriana, Japanese Citroen and Rangpur which had some nodules/sucker growth. Swingle slightly benched. Production was generally poor, Japanese Citroen the best and Carrizo no fruit. Fruit size was smaller than last season, but the fruit was sampled earlier this year. Trifoliate types and citranges had the smallest fruit size. Tri X Sweet orange, M X T, Volkameriana and Rough lemon had the best fruit colour, Rangpur, Trifoliate X and Benton the poorest. This does not necessarily correspond with last season. Only Japanese Citroen did not make the minimum juice percentage of 40.0% whereas all met the standards last season. On average the seed counts this year were considerably lower than last season, the rind thickness not necessarily the same. Up to 10% of the fruit had high shoulders, only Swingle all exportable. All rootstocks had some Oleocellosis, some fruit not exportable, Rangpur and Japanese Citroen the worst. All had some wind damage, some fruit not exportable, M X T, Trifoliate X Sweet orange and Rangpur the worst. Rough lemon and Rangpur had slightly coarser fruit, M X T the smoothest and Trifoliate X Sweet orange the most attractive.

Future research

Continue evaluations.

6.2.2.9. Establishment of new and evaluation of existing cultivars at Lancewood, Knysna area Experiment CJA-1 by C J Alexander (Private)

Opsomming

Die doel van die proef is om die huidige proef te Lancewood te laat herleef, evalueer en nuwe, potensieële seleksies in die area te vestig. Die bome op Lancewood was in 'n swak toestand en is gesnoei en geëvalueer. Die drag was baie skaars en die resultate is nie van veel waarde op die oomblik nie. Volgende seisoen behoort daar betekenisvolle resultate te wees. 'n Lys potensieële seleksies is aan die plaaslike kwekers voorgelê vir vestiging in die gebied maar dit is nog nie aanvaar nie en dus nie gevestig nie.

Introduction

The Knysna area produces mandarins for export, but due to climatic constraints the cultivar range is restricted. Due to previous budget restraints, cultivar establishment and evaluations were terminated. It was requested that the trial site at Lancewood be revived and trees evaluated and new cultivars be established in an effort to find promising cultivars for the area.

Materials and methods

The trial trees are topworked within a commercial block of Miho Wase satsumas, (used as control) at Lancewood. Field evaluations were conducted on the trees and fruit maturity based on subjective tasting. A number of existing and new cultivars were screened through literature or experience for the establishment of potential cultivars in the area.

The selections evaluated at Lancewood are presented in Table 6.2.2.9.1. and proposed selections for establishment in Table 6.2.2.9.2.

Results and discussion

Evaluations

The trees were evaluated on the 21 April. The trees had not grown out well as there was no supervision from Capespan or CRI as there was no commitment from them for a few years. A large number of the trees had either died or reverted back to the rootstock. The trees were partly pruned on this date and fruit evaluated but there were too few to test or evaluate later. The trees were also subjected to water stress. The trees have since been pruned or rootstock growth removed. The results of the evaluation are given after the table below.

Table 6.2.2.9.1. Cultivars evaluated at Lancewood, Knysna area during 2003.

Selection	Topwork Date	Rootstock	Initial no of trees established
Ueno satsuma	1998	TC	10
B64 – Afourer	1998	TC	7
Or 2	1998	TC	6
Mor 22	1998	TC	6
Bay Gold	1998	TC	6
Nectar	1998	TC	7

Ueno. The trees only had a few fruit, medium large to large fruit size, colour transparency T7 on 21 April. The quality was not good with borderline sugars and high acid and very slightly raggy. The fruit was fairly easily peeled and slightly oily with orange flesh. The colour would mature in about two weeks, internal quality about 3 weeks. Nice looking fruit, flat and smooth rind.

Miho Wase (control). The trees had an excellent crop of medium to medium large to large fruit size, colour T2, 3, 4 on 21 April. The quality was reasonable, with sufficient sugars, acid not too high and cores open. Fruit shape was flattish. The fruit was slightly puffy, past peak maturity and should have been harvested at least two weeks ago.

Afourer (B64). There were only odd, flat fruit of medium fruit size and colour T7-8 on 21 April.

Or 2. There were only odd medium small to small fruit of colour T8 on 21 April.

Mor 22. Only a few fruit of medium small fruit size and ribbed, colour T8 on 21 April.

Bay Gold. A few fruit of medium and medium large fruit size, colour T7-8 and 8 on 21 April.

Nectar. There were only odd, very small, possibly out of season fruit, colour T8 on 21 April.

As there were only odd fruit no tests nor further evaluations were done. The trees are topworked on the side row of a Miho Wase satsuma block.

Establishment

The list of available STG cultivars was screened as well as discussions held with ARC - Addo to determine which new or existing selections would have possible potential in the area. A list of such cultivars/selections was drawn up and submitted to the Knysna Co-op growers for discussion so that establishment can go ahead. Establishment has not materialised due to the non-release of some of the material and no commitment from the growers yet. The list is given in the table at the end of the text.

Conclusion

The trees at Lancewood bore few fruit and no conclusions can be drawn and the results should be read with caution. Growers in the Knysna area to make place available for some or all of the new selections to be established.

Future research

Continue with establishment and evaluate cultivars at the existing sites (where applicable) in the forthcoming season.

Table 6.2.2.9.2. Proposed list of cultivars/selections to be established in the Knysna area

No	Selection	Estimated timing	Comments	Reason for planting
Satsumas				
1	WH/B/2-36	March	Early. Satsuma x Nova, firm, small thorns, thin leaves, mandarin flavour. Flat, smooth. Colour break mid March, degreened by end March. Odd seed.	Early maturing
2	Dobashi Beni	End April	Similar timing and characteristics to Owari except redder rind colour.	Better rind colour
3	Ueno	From mid-late April onwards	Later than Miho Wase and Owari. Originally evaluated as an early selection. Two trees in production at Lancewood.	Alternative to Owari?
4	Ohtsu	Early May	Matures after Owari. Odd seed in mixed block	Later than Owari
5	Aoshima	Early/late May	Flat and smooth. Later than Owari. Odd seed in mixed block.	Later than Owari
Clementines				
	Nour		Late Clementine, looks similar to slightly better than Clemlate.	Not too impressive at this stage
Mandarins				
1	ITSC I22	End June	Honey Gold. Medium fruit size, self incompatible	Good fruit, may clash with clementines.
2	Roma	June	Perhaps too early? good size and quality. Mid to late June in Citrusdal. Dense tree.	Good quality.
3	Murcott x Clem	End June	Fair quality, good size and colour. Alternaria sensitive at Addo.	Good timing and size
4	ITSC M37	3 rd week June	Good tests, orange like taste, good size in Citrusdal. Small/medium and creasing at Addo.	Good quality, promising overseas comment
	ITSC B17	June/July	Valley Gold. High sugars, high acid, excellent colour.	Acid too high
5	ITSC B24	mid-late June	African Sunset. Seedless. Good size. Lower acid than B17	Good size
6	ITSC B18	June	A good time after clementines, easily peeled, seedy in mixed block, good taste, soft rag, fruit size bit small, appearance a bit rough, very good to eat.	Lateish, good quality, but maybe too small
7	WH/S/1-2	mid May	Clem. Most promising hybrid ex Addo. End of Nules season Good taste, better than clementine. Size questionable.	Possibility but don't know about size at this stage
	WH/B/2-27 (was /S/2-27)		Kuno X. Excellent internal & rind colour, seedless. 11/5/01 T 1-3 fair flavour with some acid. 1.31 acid on 16/5 5/6/01 T1 firm, fairly acidic. 2/7/01 Tough walls, still acidic 18/6/02 T1 acid, thin rind, not puffy, some seed	Acid may be too high
8	ITSC K3/J5	End May to mid June	Robinson x Nouvelle. Medium to large. Seed status unknown. 22/5/01 smooth, good flavour, thornless. 18/7/01 overripe 4/6/02 seedy, smooth, firm, easily peeled, medium large, round. 2003 no fruit	May be a bit early?
9	ITSC K3/C30	Early-mid June	I22 x Nova 22/5/01 T1 Smooth, good colour. Alternaria? 17/7/01 overripe	Timing?
10	ITSC O7		CE x Djeroek alternate bearing. Hangs well. 9/5/02 T 1-3 13.0 TSS; 1.04 acid firm, early deep orange colour	May be too early?

11	ITSC J15		1999 & 2000 looks good and good quality 22/5/01 T6-7 18/7/01 T1 ok 4/6/02 good colour, large firm seedy? 24/4/03 T8 thin, smooth rind, looks good, late. Less raggy than J5	Seed?
12	ITSC K33	June/July	Few thorns, leaves curl, large/long, acid could be too high, green nipples. Acid could be too high?	
	Nova SL	May/June	Same as Nova, except lower seed count with cross pollination	
	Rishon	Early-mid May	Seedless	Too early
	Orah	3 rd week June	Seedy. Or derived from Orah.	
	B64	Mid July	(Afourer) Citrusdal – good quality but tart, size a bit small, good colour, few seed. Fruit is supposed to be larger than a Nules.	
	ITSC H36	3 rd week June	Citrusdal. Good size, colour a bit poor, look and taste like a Nouvelle.	
	Nectar	Early-mid July	Citrospan cultivar. Trees at Lancewood.	Worth looking at
	Murcott Seedless		No information.	
Lemons				
	Yen Ben		Suitable in New Zealand. Good production, small fruit, seedy.	Small fruit
	Lemonade		Very low acid. Quite round. Is there a market for this kind of fruit?	
Rootstocks				
	Citrange C35		Nules - slightly earlier colour and good yield for Nules.	
	F80-3 citrumelo		Nules – high yield.	May be some rootstock trees at the CFB
	F80-9 citrumelo		Nules – good colour and high juice %	
	MXT		Nules – high yield. Good colour and quality. Earliest colour and good quality for Miho Wase.	
	Swingle		Can have slightly higher acid, good sugars and delayed rind colour. Good for replant.	

Please note that the selections with no numbers are questionable or not recommended but included for information only.

6.2.2.10 Evaluation of Turkey Valencias on different rootstocks

Experiment CJA - 4 by C J Alexander (Private)

Opsomming

Die doel van die proef is om die uit te vind wat die mees geskikte onderstam vir die Turkey Valencia in die Citrusdal gebied is. Ongelukkig het die area ongewone swaar ryp gehad wat die bome en vrugte beskadig het. Evaluasies moet voortgaan.

Introduction

The aim is to evaluate the early maturing Turkey Valencia on different rootstocks to establish which is the most suitable rootstock in the Citrusdal area as incompatibility problems have been encountered on rough lemon.

Materials and methods

The trees were planted in a block on 7 different rootstocks in September 1999. Each block is in an adjacent block of four rows. Rootstocks (selections) used and number of trees of each selection are given in table 6.2.2.10.1. below. Fruit maturity is also based on subjective tasting. Fruit quality was compared with the following valencia standards previously considered most acceptable by the market: 48% juice; 9.0% TSS; 0.6 – 1.8% acid; 7.0:1 ratio; colour T3 + 20% T4 of set 34. Seed maximum average 9.0 seeds per fruit.

Table 6.2.2.10.1. Rootstocks (selections) and number of trees per selection of Turkey valencias on 7 different rootstocks planted at Sewe Oliene, Citrusdal in September, 1999.

Rootstock	Selection	No of trees
Cairn Rough Lemon	163	27
Carrizo citrange	608	26
Citrango C32		10
Citrumelo F 80.0		6
Rangpur Lime	184	26
Swingle citrumelo	715	13
1209		26

Results and Discussion

The internal quality results are presented in Table 6.2.2.10.3. which need to be referred to when reading the text. Unfortunately the orchard suffered unusually severe frost causing damage to the trees and fruit during June and July and consequently only Rough lemon rootstock was tested. Tree size, bud union bench and visual tree frost damage is presented in Table 6.2.2.10.2. and internal quality in Table 6.2.2.10.3. below.

Table 6.2.2.10.2. Tree size, bud union bench and visual frost damage to trees for Turkey Valencias on seven different rootstocks evaluated on 16 July 2003 at Sewe Oliene, Citrusdal.

Rootstock	Tree size	Bud union bench	Frost damage
Cairn Rough Lemon	Medium large	Smooth	Zero to slight
Carrizo citrange	Medium large	Slight	Slight to moderate
Citrango C32	Medium	Slight	Severe
Citrumelo F 80.8	Medium	Very slight	Slight
Rangpur Lime	Medium	Fairly smooth	Variable slight to moderate
Swingle citrumelo	Medium	Slight to benched	Slight
1209	Medium small	Slight	Moderate to severe

Fruit size on Rough lemon approximately count 64. Rough lemon had of the largest tree size and suffered the least frost damage, Citrango C32 the most damage.

Table 6.2.2.10.3. Internal fruit quality data for Turkey Valencia oranges on different rootstocks at Sewe Oliene, Citrusdal during the 2003 season.

Selection ^f	Rootstock	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Turkey	Rough lemon	23/06	2-3	72	50.8	8.3	1.26	6.6	1.4

^f= All trees suffered severe frost damage. Only Rough lemon tested, not meeting export standards due to low TSS and ratio.

Conclusion

Due to the severe frost damage, trees could not be evaluated properly. Rough lemon suffered the least frost damage to the trees.

Future research

Evaluate next and the following seasons.

6.2.2.11 Establishment of new cultivar trials

Experiment CJA-3 by C J Alexander

Opsomming

Die doel van die proef is om nuwe, potensiële kultivars in die veld te vestig. Sodra die bome in drag is, evalueer en versamel inligting. Besig om met 'n kweker op Citrusdal te onderhandel om een vroeër satsuma, sestien mandaryn kruisings, elf nawels en nege Valencia seleksies te vestig.

Introduction

The objective of the project is to establish new, potential cultivars in the field. Once in production, gather data and disseminate information to ensure that the local citrus industry keeps abreast with local performance of any new, superior or alternative cultivars compared to the existing cultivar range.

Materials and methods

Go through the lists of cultivars available in South Africa and determine what new cultivars/selections are suitable to be established in the Cape areas. Establish at suitable sites by means of topworking existing trees or having nursery trees made, for later planting out. Five trees of each selection per cultivar to be budded or topworked next to each with a suitable control.

Results and discussion

Cultivar lists from the ARC and CFB were scrutinised for potential cultivars to be established. The table below is a list of cultivars to be established in the Citrusdal area. The list is still under discussion with the grower concerned. Once agreed upon trees will be budded in 2004 on a suitable rootstock, probably Carrizo citrange, for later planting out. Permission has not yet been granted by the ARC for the release of their material for trial purposes.

No	Selection	Control	Timing	Reason for inclusion
Satsumas				
1	ITSC WH/B/2-36	Miho Wase	March	Satsuma x Nova. Possibly earlier maturing than Miho Wase
Mandarin hybrids				
1	ITSC WH/S/1-2	Nules	End of clementine season	Most promising clementine hybrid at Addo
2	ITSC K3/J5	Nules	End May-mid June	Robinson x Nouvelle. Good size and flavour
3	ITSC K3/C30	Nules	Early-mid June.	I22 x Nova. Smooth, good colour
4	ITSC WH/B/2-27	Nules		Kuno X. Excellent internal and rind colour, seedless.

5	ITSC O7	Nules	Early	Hangs well, deep orange colour
6	ITSC J15	Nules	Late	Looks good with good colour
7	ITSC WH/B/2-3			
8	ITSC C20 (1443)	Nules	Mid-end July	Medium large, firm, smooth.
9	ITSC C27	Nules		
10	ITSC J8/P42	Nules	Early-mid June	Medium size, round, orange red, smooth, good flavour, very soft rag
11	ITSC X 83			
12	ITSC WH/B/4-13	Nules	Mid-late June	Sweet Spring X. Large, firm
13	ITSC B18	Nules	June	A good time after clementines easily peeled, good quality, soft rag.
14	Murcott Seedless			
15	Honey Gold	Nules	End June	ITSC I22. Medium size, rich flavour, self incompatible. Good overseas comment. May clash with clementines. Protected.
16	Minneola x Temple	Nules or Minneola		

Navels Midseason				
1	Santa Catarina 1	Palmer or Washington	Mid maturing	Maturity similar to Washington
2	Santa Catarina 2 or 3	Palmer or Washington	Mid maturing	Sometimes without navel
3	Kirkwood Red	Cara Cara	Mid maturing	Red pigmentation
4	ITSC K3/E60	Palmer or Washington	Mid maturing	
5	ITSC X 78 –34-9 (1322)	Palmer or Washington	Mid maturing	Best production of ITSC selections, fairly good quality, no creasing.

Navels Late				
1	Coetzee Late Navel	Robyn or Lane Late	Late maturing about the same as Robyn	Alternative to Robyn
2	Glen Ora Late	Robyn or Lane Late	Late maturing, matures 4-6 weeks later than Palmer	Good quality. Small navel end, good colour and size and less creasing prone
3	Witkrans	Robyn or Lane Late	Late maturing	Good production, medium large fruit size. Round to oval shape, small navel ends. Good quality
4	Cambria	Robyn or Lane Late	Late maturing	Slightly elongated, smooth rind
5	Royal Late	Robyn or Lane Late	Late maturing	Slightly elongated, smooth rind
6	Australian Late	Robyn or Lane Late	Late maturing	Autumn Gold, Californian Lane Late or Powell as a control

Valencias Early				
1	Limpopo Seedless	Turkey	Early maturing, two weeks earlier than Turkey	A most promising seedless selection
2	Mouton Early	Turkey	Early maturing, before Turkey	An early maturing, virtually seedless selection
3	Benny 1 or Benny 2	Turkey	Early maturing	Good production and fruit size

Valencias Midseason				
1	Ruby Valencia	Valencia Late or Delta	Mid maturing	Red pigmented, otherwise same as Old Clone Valencia
2	Rietspruit	Valencia Late	Mid maturing	A most promising, seedless,

	(Alpha)	or Delta		midseason selection
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Valencias Late				
1	Glenora Soetbas	Valencia Late	Very late maturing	Ultra Late, seedless
2	Henrietta	Valencia Late	Late maturing	Seedless
3	Letaba Oranje	Valencia Late	Late maturing	Large, seedless
4	Midknight (1)	Midknight	Late maturing	New Midknight selection, seedless, soft rag

Conclusion

The trial site is still in the process of negotiation and should be established in 2004. Completeness of the trial depends on the availability of the material from the ARC.

Future research

Continue with establishment of new selections that become available.

6.2.2.12 Navel Rootstock trial

Experiment CJA-2 by C J Alexander

Opsomming

Die doel is om 'n nawelonderstam proef te vestig om te bepaal watter onderstam die mees geskikste is vir optimale produksie en gehalte in 'n nawel area. Daar is 'n toename in nawel aanplantings in die Citrusdal gebied waar die proef beplan is. Die proef is nog onder bespreking met 'n kweker in die gebied en daar word beplan om 13 onderstamme te vestig met Washington Nawel as bostam.

Introduction

The objective of the project is to establish a navel rootstock trial in order to determine which rootstock is the most suitable for optimum navel production and quality in a navel producing area. There is a resurgence in navel plantings in the Citrusdal area.

Materials and methods

Go through the lists of rootstocks available and suitable for navel production. Procure seed and have a nursery bud Washington navel to the rootstocks. Plant the trees in a random block design of 3 x 4 tree plots, including a commercially planted rootstock as a control.

Results and discussion

Research reports were scrutinised for potential rootstocks to be established. A list has been drawn up and a grower in the Citrusdal area has been approached and the trial is still under discussion with the grower concerned. Once agreed upon seedlings will be made and budded in 2004/5 for later planting out. The table below is the list of rootstocks to be established, depending on seed availability.

No	Rootstock	Description
1	Rough lemon	Control. Used extensively for navels in the area. Good yields and large fruit size, but low internal quality.
2	Carrizo citrange	Relatively new rootstock, induces good internal quality, smaller fruit with a smoother rind, possibly creasing prone. Plantings in the area for other cultivars on the increase.
3	Swingle citrumelo	Good replant rootstock, induces good internal quality, possibly creasing prone and cold tolerant. Plantings in the area for other cultivars on the increase.
4	F80/3 citrumelo	Slightly smaller tree size than Swingle, good yields and fruit size. Cold tolerant. Good production and colour for Cara Cara navels at Addo.
5	Citrango 35 (C-35)	Promising rootstock with slightly smaller tree size than Carrizo. Average yield, good quality and high juice for Cara Cara navels at Addo.
6	Minneola x trifoliata	Smaller tree size than Rough lemon/Troyer citrange. <i>Phytophthora</i> and nematode tolerant. Good yields with Cara Cara navels at Addo.
7	X 639	Medium tree size, cold hardy, good yields, fruit size and quality, smooth rinds, may be prone to creasing (like Swingle). Tolerant to nematodes.
8	Terra Bella	Average yield and good quality (slightly high acid) and high juice with Midknights at Addo. Slightly smaller tree than C35 for lemons.
9	61 AA3	Cleopatra mandarin x <i>P. trifoliata</i> . Excellent production for Midknights
10	Sunki x Flying Dragon (1117)	The best performer with M ^c Clean navels at Addo.
11	Rangpur x Shekwasha (1106)	The second best performer with M ^c Clean navels at Addo.
12	Forner-Alcaide 5	New Spanish rootsock (if available). Cleopatra mandarin x Rubidoux trifoliata. Semidwarfing. Navelina has higher yields than Carrizo with similar quality. CTV resistant, tolerant to lime induced chlorosis, flooding tolerant, resistant to citrus nematode and <i>phytophthora</i> .
13	Forner-Alcaide 13	New Spanish rootsock (if available). Cleopatra mandarin x Rubidoux trifoliata. Semidwarfing. Navelina has higher yields than Carrizo with similar quality. CTV resistant, flooding tolerant, susceptible to citrus nematode.

Conclusion

The trial site is still in the process of negotiation and seed should be planted out in 2004.

Future research

Continue with establishment and evaluation of the trial once in production.

6.2.3 Sub-Project: Cultivar evaluation in the Northern and inland region

By J. Joubert (CRI)

6.2.3.1 Sub-Project summary

The 2003 season was a good season bearing in mind that most of the new trials established are in production and bearing fruit now. We can start comparing results and produce recommendations that indicate the long-term nature of cultivar development. Good results on fruit size and future seedless selections were obtained. Recommendations are made with reservation taking into account the generally young tree age, the climatic and farm-management changes that take place from year to year. Thank you to all growers who co-operated by having trees available for evaluation on their farms.

Sub-Projekopsomming

Die 2003 seisoen het goed verloop veral as daar inaggeneem word dat 'n groot hoeveelheid van die nuwe proewe wat aangeplant is nou vrugte begin dra. Ons kan dus binnekort inligting begin vergelyk en aanbevelings maak wat na die langtermyn aard van kultivar ontwikkeling verwys. Daar is goeie resultate verkry t.o.v. vruggrootheid en moontlike saadlose kultivars. Aanbevelings word met voorbehoud gemaak, weens die langtermyn aard van kultivars, met inagneming van oor die algemeen jong bome asook klimaat- en plaasbestuurveranderinge wat van jaar tot jaar voorkom. Baie dankie aan al die produsente wat gewillig is om proefpersele op hul plase aan te hou en te versorg vir kultivar evaluering.

6.2.3.2 Evaluation of Clementine mandarins in Mpumalanga Experiment 72 by J.Joubert (CRI)

Opsomming

'n Proef is saamgestel om te bepaal of sekere Clementine-manderyne kommersieel vir uitvoer in die intermedieëre en koel binnelandse sitrusproduserende streke van die land in reaksie op markbehoefte, geproduseer kan word. Daar word gesoek na uitstaande seleksies met betrekking tot interne vruggehalte, eksterne vrugkleur en vruggrootte verspreiding.

Clementarde en Nour het 'n vruggrootte probleem (klein vruggies) en met albei kultivars word die vrugkleur vertraag as die interne kwaliteit aanvaarbaar toets vir uitvoere. Ain Toujdate, LL en Sidi Aissa toon ook almal eksterne kleurvertraging as die interne kwaliteit optimaal toets.

Introduction

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. Also to find superior selections in terms of internal fruit quality, colour and fruit size.

Materials and methods

Field evaluations and laboratory analysis were conducted on Ain toujdate, Clementarde, L.L., Nour and Sidi Aissa Clementine selections.

The minimum export requirements for the internal fruit quality of Clementines (Capespan) was compared during the 2003 season: 48% Juice; 9.5% TSS; 0.7% Acid (Min); 1.5% Acid (Max) Ratio 8.0:1; Colour T2 and 20% T3 of set 36.

Table 6.2.3.2.1. List of Clementine trial sites evaluated in Burgersfort area during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Ain Toujdate	Mpumalanga	Zalo Citrus	CC, SC	1999	5 each
Clementarde	Mpumalanga	Zalo Citrus	CC, SC	1999	5 each
LL	Mpumalanga	Zalo Citrus	CC, SC	1999	5 each
Nour	Mpumalanga	Zalo Citrus	SC	1999	5 each
Sidi Aissa	Mpumalanga	Zalo Citrus	CC, SC	1999	5 each

Results and discussion

Ain Toujdate

Trees were evaluated at L Lötter, Burgersfort during the 2003 season.

The fruit size on Carrizo citrange varied from count 1XX to 3 (better fruit size) and on Swingle citrumelo from count 1X to 4, with an average yield on the trees. Ain Toujdate, on Swingle citrumelo, had higher internal fruit quality compared to Carrizo citrange. When we started the evaluations Swingle citrumelo had more green fruit than Carrizo citrange, but at the end of the evaluations the external fruit colour was on average the same (T1-T2). Maturity was at the end of May. On both rootstocks there were a few seeds in the fruit because of cross-pollination from some other cultivars.

Clementarde

Trees were evaluated at L Lötter, Burgersfort during the 2003 season.

There was a good yield on the trees with small to medium fruit size (count 1-5). On Swingle citrumelo the internal fruit quality was better compared to Carrizo citrange. On both rootstocks there was a colour problem (T5) when the internal quality was ready for harvesting at the end of May.

L.L.

Trees were evaluated at L Lötter, Burgersfort during the 2003 season.

L.L. had an average yield and on Carrizo citrange better than Swingle citrumelo. Medium to large fruit size (count 1XXX-3) with Swingle citrumelo slightly larger fruit compared to Carrizo citrange. Swingle citrumelo had the highest internal fruit quality. Maturity was estimated between the third and last week of May.

Nour

Trees were evaluated at L Lötter, Burgersfort during the 2003 season.

The yield on the trees was average with a medium fruit size (count 1-4). All the trees evaluated were planted on Swingle citrumelo and the internal fruit quality was good. Maturity was estimated at the end of May but the external colour was slightly delayed (T3-T6).

Sidi Aissa

Trees were evaluated at L Lötter, Burgersfort during the 2003 season.

There was an average yield on the trees with medium to large fruit size (count 1XX-3). The trees planted on Swingle citrumelo had on average 20 seeds per fruit (more susceptible to cross pollination). The internal fruit quality of Carrizo citrange was better than Swingle citrumelo (especially juice %). Estimated maturity is very much similar to Ain Toujdate.

Conclusion and recommendations

Ain Toujdate

When the internal quality is optimal there is a slight delay in the external colour of the fruit (T4-T5). The harvest time moves from mid May to the end of May (2 weeks later). Evaluations continue.

Clementarde

Fruit size appears to be a problem but the production was better (good yield). External fruit colour is also delayed at maturity and seems to be a major problem (T5). Evaluations continue.

L.L.

There is a slight delay in external colour but at the end of May the external fruit colour was acceptable (T2-T3). The fruit size increased (medium to large). The internal quality is very good. Evaluations continue.

Nour

Production (medium yield) and fruit size (count 1-4) was a problem. External fruit colour was also delayed at optimum maturity (T3-T6). Evaluations continue.

Sidi Aissa

Delay in external colour (T4-5) when internal quality is optimal. Very similar to Ain Toujdate. Evaluations continue.

Table 6.2.3.2.2. Internal fruit quality data for Clementine mandarin selections for the inland areas during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Ain Toujdate	CC	14/4/03	Zalo Citrus	1X-3	43.9	14.01	1.2	11.87	15.6	T5-6
Ain Toujdate	CC	15/5/03	Zalo Citrus	1XX-3	52.3	14.49	1.2	11.78	3.4	T4-5
Ain Toujdate	CC	28/5/03	Zalo Citrus	1-2	52.7	15.06	1.3	11.77	14.9	T1-2
Ain Toujdate	SC	14/4/03	Zalo Citrus	2-4	46.2	14.61	1.5	9.68	13.3	T7-8
Ain Toujdate	SC	15/5/03	Zalo Citrus	1X-3	52.7	15.46	1.0	15.46	11.9	T4-5
Ain Toujdate	SC	28/5/03	Zalo Citrus	1-4	56.3	15.66	1.6	9.91	15.8	T1
Clementarde	CC	14/4/03	Zalo Citrus	3-5	51.2	13.16	1.5	8.66	5.3	T8
Clementarde	CC	15/5/03	Zalo Citrus	2-4	58.0	14.29	1.3	10.99	4.1	T6-7
Clementarde	CC	28/5/03	Zalo Citrus	1-4	56.9	14.49	1.2	12.08	2.7	T4-5
Clementarde	SC	14/4/03	Zalo Citrus	2-5	52.8	13.46	1.7	8.06	2.8	T8
Clementarde	SC	15/5/03	Zalo Citrus	1-4	56.3	14.29	1.3	11.25	1.5	T6-7
Clementarde	SC	28/5/03	Zalo Citrus	2-3	57.7	14.76	1.2	12.10	2.8	T4-7
LL	CC	14/4/03	Zalo Citrus	1X-3	50.4	13.91	1.2	11.99	13.0	T7

LL	CC	15/5/03	Zalo Citrus	1XX-2	55.9	14.19	1.0	14.33	2.3	T4-5
LL	CC	28/5/03	Zalo Citrus	1XX-2	53.8	14.76	1.1	13.92	13.6	T2-3
LL	SC	14/4/03	Zalo Citrus	1X-2	45.9	14.11	1.2	11.38	18.9	T7
LL	SC	15/5/03	Zalo Citrus	1XXX-2	52.1	14.86	1.2	12.81	3.3	T3-5
LL	SC	28/5/03	Zalo Citrus	1X-2	53.0	15.26	1.1	13.50	10.0	T1-3
Nour	SC	14/4/03	Zalo Citrus	2-4	51.0	12.54	1.1	11.00	3.1	T8
Nour	SC	28/5/03	Zalo Citrus	1-3	57.3	13.99	1.2	12.17	1.8	T3
Sidi Aissa	CC	14/4/03	Zalo Citrus	1X-2	51.9	12.04	1.2	10.12	10.2	T6-7
Sidi Aissa	CC	15/5/03	Zalo Citrus	1XX-2	54.8	12.94	1.1	11.87	3.8	T3-5
Sidi Aissa	CC	28/5/03	Zalo Citrus	1XX-1	53.0	13.04	1.2	10.78	10.6	T1-2
Sidi Aissa	SC	14/4/03	Zalo Citrus	1-3	42.2	14.71	1.9	7.95	21.2	T7-8
Sidi Aissa	SC	15/5/03	Zalo Citrus	1X-2	48.8	16.03	1.6	9.77	20.1	T4-5
Sidi Aissa	SC	28/5/03	Zalo Citrus	1XX-3	48.8	15.56	1.6	9.91	20.8	T1-2

6.2.3.3 Evaluation of late maturing Mandarins in the inland areas

Experiment 73 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.

Bay Gold, Hadas en Primasol se produksie en interne kwaliteite sal toeneem namate die bome ouer word. Hierdie proef is nog 'n besondere jong aanplanting.

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. No internal fruit quality analysis was conducted for the Burgersfort area due to young tree age, but internal fruit analysis was conducted for the Marble Hall area during the 2003 season.

Table 6.2.3.3.1. List of Clementine mandarin trial sites evaluated during the 2003 season.

Selection	Area	Site	Rootstock	Tree age	No. of trees
A25	Mpumalanga	Moosrivier Estate	CC	2001	10
Bay Gold	Mpumalanga	Moosrivier Estate	CC	2001	9
Cami	Mpumalanga	Moosrivier Estate	CC	2001	5
C27	Mpumalanga	Moosrivier Estate	CC	2001	5
Hadass	Mpumalanga	Moosrivier Estate	CC	2001	9
M26	Mpumalanga	Moosrivier Estate	CC	2001	10
Primasole	Mpumalanga	Moosrivier Estate	CC	2001	9
Roma	Mpumalanga	Moosrivier Estate	CC	2001	10
A25	Mpumalanga	Zalo Citrus	CC	2001	5
Bay Gold	Mpumalanga	Zalo Citrus	CC	2001	5
Cami	Mpumalanga	Zalo Citrus	CC	2001	4
C27	Mpumalanga	Zalo Citrus	CC	2001	4
Hadass	Mpumalanga	Zalo Citrus	CC	2001	5
M26	Mpumalanga	Zalo Citrus	CC	2001	5
Primasole	Mpumalanga	Zalo Citrus	CC	2001	5

Results and discussion

Trees are growing well at Burgersfort and evaluations will commence in 2004. At Moosrivier some trees had enough fruit to evaluate. For the rest of the Cultivars evaluations will commence in 2004.

Bay Gold

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season.

There was a good yield on the trees with fruit size varying from count 1X-1XXX. The external colour was delayed (T5) when internal quality was optimal. Average seeds per fruit was 22.7%. Some fruit developed sheep nose with high shoulders. The internal colour of the fruit was very good (dark orange). Maturity seems to be first week to mid June.

Hadas

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season.

Average yield on the trees with medium to large fruit size (count 1XX-3). Poor internal quality with very high acids (2.03% -2.51%), but juice percentage and TSS values were average. External colour delay (T6). Maturity might be mid June.

Primasol

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season.

Some of the problems were poor internal quality with low juice% (25.1%-32.2%) and acid% (0.28%-0.34%). The external colour of the fruit varied between T1 and T3. Yield was poor bearing in mind that it was the first season the trees were bearing fruit. Maturity seems to be mid to end of May.

Conclusion and recommendations

Bay Gold

Evaluate later in the season to establish if the internal quality and external colour will improve. Trees are still too young for scientific conclusions to be made. Evaluations continue.

Hadas

Take in consideration that the internal and external qualities will be affected due to the reason that the trees are bearing fruit for the first season. With the second evaluations (2004) the internal quality (acid%) might improve. Evaluations continue.

Primasol

Very low juice percentage and acid percentage with good external colour. Evaluations continue to determine if these qualities will improve.

Table 6.2.3.3.2. Internal fruit quality data for Clementine mandarin selections for the cool inland areas during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Bay Gold	CC	17/3/03	Moosrivier	1X-1XXX	49.3	10.25	1.41	7.3	18.3	T6
Bay Gold	CC	13/5/03	Moosrivier	1X-1XXX	47.5	10.21	1.06	9.6	18.4	T7
Bay Gold	CC	27/5/03	Moosrivier	1XX-1XXX	48.6	11.23	1.15	9.8	22.7	T5
Hadass	CC	17/3/03	Moosrivier	2-1XX	54.0	9.12	2.51	3.6	9.3	T6
Hadass	CC	13/5/03	Moosrivier	3-1XX	54.9	9.08	2.13	4.3	10.1	T7
Hadass	CC	27/5/03	Moosrivier	2-1XX	54.7	9.91	2.06	4.8	5.9	T6
Primasol	CC	17/3/03	Moosrivier	1XXX	32.2	7.90	0.28	28.2	5.3	T5
Primasol	CC	13/5/03	Moosrivier	1XXX	27.0	8.18	0.32	25.6	9.3	T2-3
Primasol	CC	27/5/03	Moosrivier	1XXX	25.1	8.18	0.34	24.1	5.8	T1-2

6.2.3.4 Evaluation of navels in the intermediate and cool inland areas

Experiment 74 by J. Joubert (CRI).

Opsomming

Wingsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleowerstand, kleiner nawelente om witluis teë te werk, *Alternaria*-besmetting, windbestandheid, blomdatum, en binnedrag) en vruggehalte (skilkleur vroeg in die seisoen, sappehalte, granulasie, lae suur) te verbeter, asook om die oes- en bemarkingseisoen (vroee-, middel- an laatrypwordende seleksies) te verleng.

Cara Cara lewer goeie resultate wat interne kwaliteit, vruggrootte en kleur aanbetref, maar daar moet seker gemaak word vir die bemarkbaarheid van die vrugte.

Fukumoto toon moontlike onvereenigbaarheids eienskappe met CC, C35 en gering op SC in California. Daar is nog geen gevalle in RSA aangemeld nie. Hierdie onsekerheid moet eers opgeklar word voordat kultivar kommersiëel aangeplant word.

Introduction

To optimise profitability by improving productivity (fruit set and size); packout percentage (creasing and oleo resistance, smaller navel ends to counter mealybug, *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).

Materials and methods

Field evaluations and laboratory analysis were conducted on Atwood, Autumn Gold, Cara Cara, Chislett, Dream, Fukumoto, Powel Summer and Tule Gold selections at two sites in the inland areas.

Internal quality data was compared with the minimum average export requirements (Capespan) for navels during the 2003 season: 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 6.2.3.4.1. List of navel trial sites evaluated during the 2002 season.

Selection	Area	Site	Rootstock	Tree age	No. of trees
Atwood	Mpumalanga	Moosrivier Estate	CC	2001	5
Autumn Gold	Mpumalanga	Moosrivier Estate	CC	1997	6
Cara Cara	Mpumalanga	Moosrivier Estate	CC	1997	Semi-Com.
Chislett	Mpumalanga	Moosrivier Estate	CC	1997	6
Dream	Mpumalanga	Moosrivier Estate	CC	2001	9
Fukumoto	Mpumalanga	Moosrivier Estate	CC	2001	4
Tule Gold	Mpumalanga	Moosrivier Estate	CC	2001	9
Atwood	Mpumalanga	Zalo Citrus		2001	5
Autumn Gold	Mpumalanga	Zalo Citrus	CC, SC	1999	10
Bahianinha	Mpumalanga	Zalo Citrus	SC	2001	6
Cara Cara	Mpumalanga	Zalo Citrus		2001	9
Dream	Mpumalanga	Zalo Citrus		2001	9
Fukumoto	Mpumalanga	Zalo Citrus		2001	5
Powel Summer	Mpumalanga	Zalo Citrus	CC, SC	1999	10
Tule Gold	Mpumalanga	Zalo Citrus		2001	9

Results and discussion

Atwood

Trees were evaluated at Moosrivier, Mpumalanga during the 2003 season.

Average yield on the trees with very large fruit size (count 36-56). External colour looks good (T2-T4) with acceptable internal quality (acids on the low side). As the trees get older the number of fruit per tree will increase and the fruit size will improve. Maturity at end of May to first week in June.

Autumn Gold

Trees were evaluated at Moosrivier, Mpumalanga and L.Lötter (Zalo Citrus), Burgersfort during the 2003 season.

Autumn Gold is one of the late maturing navel. Medium to large fruit size with acceptable internal quality. Acid levels tend to be higher at Zalo Citrus on Swingle citrumelo (1.34 – 1.92), but are still acceptable. On Swingle citrumelo there was also some splitting problems on the fruit. Maturity seems to be end of June (Moosrivier) to the middle of July (Burgersfort).

CaraCara

A semi-commercial orchard was evaluated at Moosrivier Estates, Mpumalanga and L.Lötter (Zalo Citrus), Burgersfort during the 2003 season.

Trees at Zalo Citrus are still young with a poor yield (first evaluation) and there was enough fruit for two evaluations. Fruit size was on the large side (count 40-64) with good internal quality (low acid %). Maturity mid May.

At Moosrivier the production was average and fruit size medium to large (count 40-72). Fruit set could be increased with GA₃ or girdling in future. Internal pigmentation was acceptable. Internal fruit quality was good but acid% tends to be low (0.7%). Maturity in mid to end May.

Chislett

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season.

External fruit colour might be a problem (T5-6). The juice% of the fruit is also on the low side (38.6% - 46.8%). The fruit size varied from small to medium (count 40 – 105) with no universal trend. There was some fruit with splitting problems. Chislett is also a late maturing navel with maturity probably end of June to mid July.

Dream

Trees were evaluated at Moosrivier, Mpumalanga and L.Lötter (Zalo Citrus), Burgersfort during the 2003 season.

Poor yield and medium to very large fruit size (count 36-72) due to young tree age. Internal quality is acceptable with high juice% (50.4% – 53.3%). The external colour of the fruit seems to delay the harvest time of the fruit. Maturity end of May.

Fukumoto

Trees were evaluated at Moosrivier, Mpumalanga and L.Lötter (Zalo Citrus), Burgersfort during the 2003 season.

Trees are still too young and there are not enough fruit for internal quality analysis. Trees look semi-dwarfed. In California incompatibility on citrange rootstock was detected, but so far no problems were reported here, although trees are still young.

Powel Summer

Trees were evaluated at L.Lötter (Zalo Citrus), Burgersfort during the 2003 season.

Powel Summer also a late maturing navel with good internal quality early in the season (mid May) but there was a slight delay in the external colour (T6) of the fruit. The fibre content of the fruit was on the high side with a normal juice% (46% – 54.1%). Fruit size was medium to large (count 40 – 88). Maturity later in the season because of external colour delay approximately end of June to mid July.

Tule Gold

Trees were evaluated at Moosrivier, Mpumalanga and L.Lötter (Zalo Citrus), Burgersfort during the 2003 season.

There was a poor yield at Zalo Citrus compared to a fairly good yield at Moosrivier. At Zalo Citrus there were some splitting and sheep nose fruit with lower acid% and a slight delay in external colour. Moosrivier had higher acids with better external colour. Both trials were planted on Carizo citrange. Fruit size was on the larger side between count 40 and 64. Maturity will be at the end of May.

Conclusion and recommendations

Atwood

Fruit size (count 36-56) appears to be a problem but the trees are still young and might improve. Acid levels are on the low side. Evaluations continue.

Autumn Gold

Fruit size seems acceptable with higher acid levels at Zalo Citrus. Split fruit on Swingle citrumelo. Evaluations continue.

Cara Cara

Cara Cara looks very promising in the Marble Hall area. Fruit set manipulation and de-greening can be used to optimise production and appearance.

Status: semi-commercial.

Chislett

External fruit colour and low juice% seems to be a problem but the production was good. Split fruit on some trees. Evaluations continue.

Dream

Fruit size and production might be a problem but trees are still young. Internal quality is acceptable with high juice% contents. Evaluations continue.

Fukumoto

An early Navel selection with high potential. Recommended on a semi-commercial scale. Must be included into rootstock trials to determine the best combination due to incompatibility problems. Evaluations continue.

Powel Summer

There is a delay in external colour of the fruit when the internal quality is optimal. Degreening can be used to optimise appearance of the fruit. High fibre content but good internal quality. Evaluations continue.

Tule Gold

Trees are still young and the production was a problem with some splitting and sheep nose fruit at Zalo Citrus. At Moosrivier the yield was better with larger fruit size and higher acids. Evaluations continue.

Table 6.2.3.4.2. Internal fruit quality data for navel selections in the intermediate and cool inland areas during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Atwood		13/05/03	Moosrivier	36-48	50.5	9.51	0.88	10.81	0	T5-6
Atwood		27/05/03	Moosrivier	36-56	54.0	9.61	0.89	10.80	0	T3
Autum Gold	CC	16/04/03	Moosrivier	56-72	44.5	10.98	0.96	11.44	0	T7
Autum Gold	CC	13/05/03	Moosrivier	40-72	45.5	11.33	0.70	16.19	0	T7
Autum Gold	CC	27/05/03	Moosrivier	40-72	49.2	11.73	0.82	14.30	0	T4
Autum Gold	CC	14/04/03	Zalo Citrus	56-105	48.3	8.97	1.16	7.73	0	T8
Autum Gold	CC	15/05/03	Zalo Citrus	40-72	51.3	10.43	0.88	11.85	0	T6
Autum Gold	CC	28/05/03	Zalo Citrus	40-72	52.7	10.93	0.99	11.04	0	T4-5
Autum Gold	CC	19/06/03	Zalo Citrus	48-72	54.2	10.81	0.88	12.28	0	T3-4
Autum Gold	SC	14/04/03	Zalo Citrus	72-125	45.4	12.54	1.92	6.53	0	T8
Autum Gold	SC	15/05/03	Zalo Citrus	56-72	50.2	12.79	1.48	8.64	0	T6
Autum Gold	SC	28/05/03	Zalo Citrus	56-72	50.4	12.74	1.47	8.67	0	T6
Autum Gold	SC	19/06/03	Zalo Citrus	56-88	52.1	13.22	1.34	9.87	0	T3-4
CaraCara	CC	16/04/03	Moosrivier	48-72	47.2	10.68	0.61	17.51	0	T2
CaraCara	CC	13/05/03	Moosrivier	48-64	50.0	11.03	0.67	16.46	0	T1-2
CaraCara	CC	27/05/03	Moosrivier	40-64	53.5	11.13	0.70	15.90	0	T1-2
CaraCara	CC	14/04/03	Zalo Citrus	40-64	49.7	8.97	0.75	11.96	0	T7-8
CaraCara	CC	15/05/03	Zalo Citrus	40-56	53.1	9.61	0.72	13.35	0	T1-2
Chislett	CC	16/04/03	Moosrivier	40-105	38.6	10.15	0.82	12.38	0	T7-8
Chislett	CC	13/05/03	Moosrivier	48-72	44.7	10.93	0.82	13.33	0	T6
Chislett	CC	27/05/03	Moosrivier	40-64	46.8	11.23	0.85	13.21	0	T5-6
Dream	CC	13/05/03	Moosrivier	36-56	52.0	9.61	0.87	11.05	0	T5-6
Dream	CC	27/05/03	Moosrivier	36-56	55.7	10.11	0.90	11.23	0	T3
Dream	CC	14/04/03	Zalo Citrus	36-64	50.4	9.17	0.84	10.92	0	T7-8
Dream	CC	15/05/03	Zalo Citrus	40-72	53.3	9.71	0.84	11.56	0	T5-6
Dream	CC	28/5/03	Zalo Citrus	36-64	53.3	9.71	0.84	11.56	0	T4-5
Powel Summer	CC	14/04/03	Zalo Citrus	56-72	46.6	9.81	1.03	9.52	0	T8
Powel Summer	CC	15/05/03	Zalo Citrus	40-72	51.4	10.43	0.90	11.59	0	T6
Powel Summer	CC	28/05/03	Zalo Citrus	40-64	44.5	10.21	0.86	11.87	0	T6
Powel Summer	CC	19/6/03	Zalo Citrus	40-64	52.7	10.71	0.83	12.90	0	T3-4
Powel Summer	SC	14/04/03	Zalo Citrus	56-72	47.3	9.71	1.14	8.52	0	T8
Powel Summer	SC	15/05/03	Zalo Citrus	40-72	51.3	9.91	0.93	10.66	0	T6
Powel Summer	SC	28/05/03	Zalo Citrus	40-88	50.9	10.53	0.95	11.08	0	T6
Powel Summer	SC	19/06/03	Zalo Citrus	48-78	54.1	10.71	0.92	11.64	0	T3-4
Tule Gold	CC	13/05/03	Moosrivier	48-64	54.2	10.43	0.80	13.04	0	T3-4
Tule Gold	CC	27/05/03	Moosrivier	40-56	53.8	10.11	0.85	11.89	0	T1-2
Tule Gold	CC	14/04/03	Zalo Citrus	40-56	51.1	8.87	0.67	13.24	0	T6-7
Tule Gold	CC	15/05/03	Zalo Citrus	40-64	55.2	9.71	0.72	13.49	0	T3-4
Tule Gold	CC	28/05/03	Zalo Citrus	40-64	55.6	9.61	0.77	12.48	0	T3-4

6.2.3.5 Evaluation of Valencia selections in the inland areas
Experiment 75 by J. Joubert (CRI).

Opsomming

Winsgewendheid 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 skilbaarheid; verleng plukseisoen deur vroeg- (Junie/middel Julie) en laatrypwordende seleksies (vir warm streke).

Alpha (Rietspruit) lyk steeds baie belowend en is feitlik saadloos met goeie interne kwaliteit en aanvaarbare vruggrootte. Delport staan ook uit t.o.v. goeie produksie, interne kwaliteit, vruggrootte en eksterne kleur. Die

vrugte van G5 was almal saadloos, maar die fisiese smaak was waterig. Glen Ora toon baie potensiaal met goeie vruggrootte, feitlik saadlose vrugte en goeie produksie.

Introduction

The aim is 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).

Materials and methods

Field evaluations and laboratory analysis were conducted on Alpha (Rietspruit), Benny (control), Broedershoek, Delicia, EEL-T, G5, Glen Ora, Kleinhans, Maroco Late, McClean SL, Midnight, Mouton, Pope Late, Portsgate, Ruby Valencia, Turkey (control) at various inland sites/areas.

Table 6.2.3.5.1. Internal fruit quality data was compared with the minimum export requirements (Capespan) for Valencia types during 2003 season.

Variety	% Juice	% TSS	Min % Acid	Max Acids	%	Ratio	Colour
Valencia	48	9.75	0.68	1.6%		8.5:1	Colour plate 3 of set no. 34
Midnight	52	10.5	0.85	1.5%		7.5:1	Colour plate 3 of set no. 34
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 6.2.3.5.2. List of Valencia selections trial sites evaluated during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Alpha (Rietspruit)	Mpumalanga	Esselen Nursery	CM	1995	1
Delicia (Delpport)	Mpumalanga	Esselen Nursery	CC		1
EEL-T	Mpumalanga	Esselen Nursery	Troyer		1
Glen Ora	Mpumalanga	Esselen Nursery	CC	1997	3
McClean SL	Mpumalanga	Esselen Nursery	CC	1997	1
Midnight	Mpumalanga	Esselen Nursery	C35		1
Portsgate	Mpumalanga	Esselen Nursery	TB	1995	1
Ruby Val	Mpumalanga	Esselen Nursery	RL	2000	2
Turkey	Mpumalanga	Esselen Nursery	CC		1
Alpha	Limpopo	Group 91	CC	1996	24
Benny (control)	Limpopo	Group 91	CC	1996	23
Broedershoek	Limpopo	Group 91	CC	1996	9
Delicia (Delpport)	Limpopo	Group 91	CC	1996	21
G5	Limpopo	Group 91	CC	1996	21
Kleinhans	Limpopo	Group 91	CC	1996	7
Glen Ora	Limpopo	Group 91	CC	1996	2
Maroco Late	Limpopo	Group 91	CC	1996	5
Mouton	Limpopo	Group 91	CC	1996	12

Pope Late	Limpopo	Group 91	CC	1996	7
Portsgate	Limpopo	Group 91		1996	23
Turkey (control)	Limpopo	Group 91		1996	18
Valencia Sport	Limpopo	Group 91		1996	12

Results and discussion

Alpha

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2003 season.

At Group 91 Alpha Valencia was compared to Turkey and Benny (both early maturing Valencia's). External fruit colour of Alpha was similar to Turkey and one colour plate better than Benny on the 12th of June 2003. Internal fruit quality also compared well with higher juice% and slightly higher acid content (1.24%). Alpha was almost seedless (0.3 seeds/fruit) compared to Benny with 7.7 seeds/fruit and Turkey with 11.8 seeds/fruit. Maturity is expected to be mid to end of June.

At Esselen Nursery Alpha was compared to Turkey. The external colour of the fruit was similar and the internal quality was very similar with Alpha having virtually no seeds (0.5 seeds/fruit) compared to Turkey (8.4 seeds/fruit). Estimated maturity mid July.

Benny

Trees were evaluated at Group 91, Limpopo Province during the 2003 season.

Very good yield with small to medium fruit size (64-88). Good external colour (T2) with optimal internal quality. On average 7,7 seeds per fruit evaluated with maturity at mid June.

Broedershoek

Trees were evaluated at Group 91, Limpopo Province during the 2003 season.

Fruit size seems to be on the small side (count 64-88) and there is no universal yield distribution between the trees. With some of the fruit the acid% was on the high side (1,44%) but with good external colour (T1-T2). Maturity seems to be end of June to mid July.

Delicia

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2003 season.

Very good yield at Esselen nursery on the trees with medium to large fruit size (count 40-72). Bearing of fruit is similar to grapefruit and well protected from sunburn. Good internal quality. Maturity in mid July.

At Group 91 there was a medium to large fruit size (count 56-72) with exceptional internal colour and good production on the trees. Good internal quality with optimal external colour (T1-T2). Acids slightly on the high side with harvest (1.21%) but acceptable if compared with the minimal requirements for export. Maturity mid July.

EEL-T

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2003 season.

Universal fruit size (count 56-88) with good yield on the trees. Acids on the low side (0.88-1.03%) with watery taste and on average 5.2-5.8 seeds per fruit. Good external colour (T1) and maturity might be at the beginning of July.

G5

Trees were evaluated at Group 91, Limpopo Province during the 2003 season.

The yield on the trees was average with no universal bearing pattern between the trees. There were no seeds in the fruit that was evaluated and tested for internal quality. The fruit had a slightly watery taste but the internal quality was good. Maturity seems to be at the beginning to mid of July.

Glen Ora

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2003 season.

Glen Ora is a late maturing Valencia mutation discovered in Burgersfort, Mpumalanga. Internal fruit quality is good and can be even better on quality inducing rootstocks. Fruit that were tested were virtually seedless. The next generation of bud wood tested should be totally seedless.

At Esselen nursery fruit size varied from medium to large (count 56-88) compared to Group 91's small to medium fruit size (count 64-105). There was some fruit with a thin rind and severe splitting at Esselen nursery. Group 91(T1-T2) had a slight colour advantage to Esselen nursery (T1-T4) when evaluated. Maturity mid of July.

Kleinhans

Trees were evaluated at Group 91, Limpopo Province during the 2003 season.

Small, medium and large fruit on the trees with average internal quality and thick rind. Good external colour (T1-T2) with maturity at end of June to mid July.

Mc Clean SL

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2003 season.

Mc Clean SL is an improved selection on the current Mc Clean Valencia used in the industry. The tree at Esselen Nursery had good production with small to medium fruit size. Internal quality was very good and external colour (T1) was ready in mid July. Average 0.6 – 0.8 seeds per fruit.

Mouton

Trees were evaluated at Group 91, Limpopo Province during the 2003 season.

Fruit size seems to be a problem with small to medium fruit (count 72-105) on the trees. Internal quality is adequate and external colour also acceptable. Maturity at end of July.

Pope Late

Trees were evaluated at Group 91, Limpopo Province during the 2003 season.

Reasonable external colour with high internal quality. Fruit size is on the small side (72-105) but with high TSS% in the fruit (Sweet taste). Maturity around mid of July.

Portsgate

Trees were evaluated at Group 91, Limpopo Province and Esselen Nursery, Mpumalanga during the 2003 season.

Portsgate is a seedless Valencia mutation, discovered in the Hoedspruit area, Limpopo Province. Yields were good at both sites this season with medium fruit size (fruit size on smaller side at Group 91). Internal fruit quality was very good at both sites and made the minimum export standards for Deltas. All fruit evaluated and internal quality tested was totally seedless. Maturity was mid July.

Ruby

Trees were evaluated at Esselen Nursery, Mpumalanga during the 2003 season.

There seems to be a fruit size problem (count 125) on the trees but with acceptable internal quality (TSS% levels on the low side between 7.85 and 8.90%) and acceptable external colour (T2). The internal colour of the fruit was similar to Cara Cara (reddish). Maturity at mid July.

Valencia Sport

Small fruit size (count 105) with good internal quality and on average 2.3 – 5.3 seeds per fruit. External colour between T1 and T2 with maturity. Maturity mid July.

Conclusions and recommendations

Alpha

Remains semi-commercial, until clean material has been proven true to type in the 2004 season. The Alpha Valencia is an option for early Valencia's, especially for the hot windy areas. Fruit set, size, internal fruit quality and external fruit colour appear to be good in these areas and the fruit are virtually seedless. Evaluations continue.

Broedershoek

Problems with small fruit size, yield and high acid% internally. Evaluations stopped.

Delicia

Advantages are fruit size (count 40-72), good yield, internal colour, internal quality, external colour and protection of fruit against sunburn. Acid % on the high side. Evaluations continue.

EEL-T

Acid% is low (watery taste), but with good yield, fruit size and external colour. Evaluations continue.

G5

The fruit was seedless but there was a problem with a watery taste although the internal quality applies with the minimum export standards. Evaluations continue.

Glen Ora

Most promising late Valencia with medium to large fruit size, excellent production and virtually seedless. Remains experimental until true to type bud wood is available. Evaluations continue.

Kleinhans

Problems with fruit size and thick rind on the fruit. Evaluations stopped.

Mc Clean SL

Recommended to extend the seedless range (Not completely seedless). Also an alternative in areas where Delta fruit set and size is a problem. Recommended on a semi-commercial scale. Evaluations continue.

Mouton

Small fruit size. Evaluations stopped.

Pope Late

Very good taste but fruit size on the small side. Evaluations stopped.

Portsgate

Fruit seems to be virtually seedless with good internal qualities. Remains semi-commercial until clean material has proven to be true to type. Evaluations continue.

Ruby

Fruit size on the small side and low TSS% levels, but trees are still young and might improve. The internal colour (reddish) and taste seems to be an advantage. Evaluations continue.

Valencia Sport

Fruit size on the small side. Might improve by girdling the trees or applying Gipps. Stop evaluations.

Table 6.2.3.5.3. Internal fruit quality data for Valencia orange selections for the inland areas during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Benny	CC	12/06/03	Groep 91	64-88	58.4	10.73	1.18	9.1	7.7	T2
Broedershoek	CC	12/06/03	Groep 91	72-88	57.9	10.93	1.44	7.6	3.3	T2-3
Broedershoek	CC	16/07/03	Groep 91	64-88	59.3	11.25	1.19	9.5	2.3	T1
Delicia	CC	12/06/03	Esselen	40-64	63.9	10.51	1.11	9.5	0.4	T3,4,5
Delicia	CC	17/07/03	Esselen	40-72	64.6	11.51	0.96	12.0	0.5	T1-2
Delicia	CC	12/06/03	Groep 91	56-72	61.6	8.88	1.30	6.8	0.5	T5
Delicia	CC	16/07/03	Groep 91	56-72	60.3	9.82	1.21	8.1	1.6	T1-2
EEL-T	Troyer	10/06/03	Esselen	64-72	58.0	10.51	1.03	10.2	5.2	T4
EEL-T	Troyer	17/07/03	Esselen	56-88	59.0	10.95	0.88	12.4	5.8	T1
G5	CC	12/06/03	Groep 91	56-72	57.2	10.73	1.27	8.4	0.0	T2
G5	CC	16/07/03	Groep 91	56-105	60.8	11.64	1.16	10.0	0.0	T1
Glenora Late	CC	10/06/03	Esselen	56-72	60.4	9.98	1.28	7.8	1.1	T6
Glenora Late	CC	17/07/03	Esselen	56-88	63.3	10.85	1.24	8.8	1.2	T4-5
Glenora Late	CC	12/06/03	Groep 91	64-105	60.7	9.81	1.20	8.2	0.8	T1-2
Glenora Late	CC	16/07/03	Groep 91	48-72	60.6	10.35	1.00	10.4	0.0	T1-2
Kleinhans	CC	12/06/03	Groep 91	64-88	57.5	10.01	1.23	8.1	1.8	T2-3
Kleinhans	CC	16/07/03	Groep 91	64-88	57.2	10.75	0.98	11.0	4.3	T1
McClellan SL	CC	10/06/03	Esselen	72-88	59.5	11.60	1.10	10.5	0.8	T3
McClellan SL	CC	17/07/03	Esselen	72-105	61.2	12.46	0.99	12.6	0.6	T1
Midnight	C35	10/06/03	Esselen	56-72	60.1	9.58	1.05	9.1	0.6	T4-5
Midnight	C35	17/07/03	Esselen	48-72	61.0	9.74	0.94	10.4	0.2	T1
Mouton	CC	12/06/03	Groep 91	72-105	58.3	10.63	1.39	7.6	6.2	T2
Mouton	CC	16/07/03	Groep 91	72-88	59.6	11.74	1.16	10.1	5.3	T1
Pope Late	CC	12/06/03	Groep 91	72-105	59.8	10.73	1.40	7.7	4.3	T2
Pope Late	CC	16/07/03	Groep 91	72-88	61.4	11.54	1.12	10.3	3.8	T1
Portsgate	CC	10/06/03	Esselen	64-88	60.4	10.91	1.06	10.3	0.0	T4-5
Portsgate	CC	17/07/03	Esselen	64-88	62.1	11.64	0.89	13.1	0.0	T1
Portsgate	CC	12/06/03	Groep 91	64-88	57.5	10.83	1.24	8.7	0.0	T1-2
Portsgate	CC	16/07/03	Groep 91	64-88	60.7	11.54	1.07	10.8	0.0	T1
Rietspruit	CC	10/06/03	Esselen	64-88	61.9	11.50	1.18	9.7	1.4	T1-2
Rietspruit	CC	17/07/03	Esselen	56-88	63.5	12.24	1.12	10.9	0.5	T1
Rietspruit	CC	12/06/03	Groep 91	48-72	59.7	10.21	1.46	7.0	0.0	T1-2
Rietspruit	CC	16/07/03	Groep 91	48-72	66.6	11.25	1.24	9.1	0.3	T1
Ruby	CC	10/06/03	Esselen	64-125	57.6	7.85	1.01	7.8	4.8	T2
Ruby	CC	17/07/03	Esselen	72-105	61.8	8.90	0.90	9.9	4.9	T2
Turkey	CC	10/06/03	Esselen	64-88	57.3	13.02	0.97	13.4	8.4	T1-2
Turkey	CC	17/07/03	Esselen	64-88	59.9	13.60	0.92	14.8	8.4	T1-2
Turkey	CC	12/06/03	Groep 91	72-88	58.9	12.44	1.20	10.4	11.8	T1-2
Valencia Sport	CC	12/06/03	Groep 91	72-105	56.0	9.71	0.96	10.1	2.3	T2
Valencia Sport	CC	16/07/03	Groep 91	72-88	60.3	10.95	1.26	8.7	5.3	T1

6.2.3.6 Evaluation of midseason oranges in the inland areas

Experiment 77 by J. Joubert (CRI)

Opsomming

Geskikte middelseisoen lemoene moet gevind word om die belangrike middelseisoenleemte (Junie/Julie) in die warmer binnelandse streke te vul. Vind 'n bron van gepigmenteerde lemoene en bepaal hul aanpasbaarheid vir 'n wye reeks klimaatstoestande. Markaanvaarbaarheid van die kultivars sal ook vasgestel word.

Raratonga het potensiaal getoon met saadlose vrugte, maar het 'n probleem met dorings. Tarocco Gallo was ook feitlik saadloos met goeie interne kwaliteit, maar heelwat split vrugte en dorings. Tarocco 57 het meer split vrugte en dorings gehad as Gallo.

Introduction

To find suitable midseason orange cultivars to fill the important "midseason" gap (June/July) in the warmer inland areas. To source pigmented oranges and test their adaptability to a broad range of climatic conditions. Market acceptance of these cultivars will also be assessed.

Materials and methods

Field and laboratory analyses were conducted on Barlerin and various Tunisian Maltaise Orange selections, Crookes Shamouti, Grosse Sanquine, Raratonga, Sanquinella, Tarocco Gallo and Tarocco 57 this season.

Internal quality data were compared with the minimum export requirements (Capespan) for midseason oranges during the 2003 season.

Variety	% Juice	% TSS	% Acid Min	% Acid Max	Ratio	Colour
Tomango	52.0	9.0	0.7	1.8	7.0:1	Set 34 no. 3
Shamouti	44.0	9.0	0.6	1.8	7.0:1	Set 34 no. 3
Sanguinello	48.0	9.0	0.6	1.8	7.0:1	Set 34 no. 3
Salustiana	52.0	9.0	0.7	1.8	7.0:1	Set 34 no. 3

Table 6.2.3.6.1. List of midseason orange trial sites evaluated during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Barlerin Maltese (MMBA)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 4, 4,4,4.
Crookes Shamouti	Kwazulu Natal	Riversbend	CC, C35, X639	1999	22, 23, 7.
Grosse Sanquinne (MGS)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	3, 3, 3, 3, 3.
Raratonga (MRAR)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 4, 4, 4, 4.
Sanquinella (MSAN)	Kwazulu Natal	Riversbend	CC, C35, SC.		12, 14, 16.
Tarocco Gallo (MTG)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 3, 4, 4, 4.
Tarocco 57 (MT57)	Kwazulu Natal	Riversbend	CC, C35, SC, X639, Yuma	1999	4, 4, 4, 3, 4.
Tunisian Maltaise (BKM)	Mpumalanga	Moosriver Estates	MxT, CC	1997	5 each
Tunisian Maltaise (HMM ²)	Mpumalanga	Moosriver Estates	CC, SC	1997	5 each
Tunisian Maltaise (MLM)	Mpumalanga	Moosriver Estates	CC, SC, MxT	1997	5 each

Results and discussion

Barlerin Maltaise (TMM)

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on three rootstocks.

Tree size on CC, SC, X639 and Yuma was medium compared to C35 with small tree size. Yield was good on CC and SC, average on C35 and Yuma and poor on X639. CC had large fruit size compared to SC and Yuma with medium fruit size but small fruit size on C35 and X639. External colour was delayed on Swingle citrumelo compared to C35, X639 and Yuma. Internal fruit quality was acceptable on all five rootstocks. Estimated maturity is mid June.

Crookes Shamouti

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on three rootstocks.

Tree size on CC and X639 was small to medium but on C35 all trees were on the small side. Production on all three rootstocks was good with large fruit size (count 56-88) on CC compared to medium to small fruit size on C35 and X639 (count 64-125). X639 had a slight external colour advantage over C35 and CC. Internal quality was acceptable with acids on the low side but still above the minimal requirements for export (0.6% Capespan). On all three rootstocks the rind was on the thick side. Maturity seems to be mid to end of June.

Grosse Sanquinne

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on five rootstocks.

Grosse Sanquinne has a typical midseason upright growth, with medium tree size for CC, SC, X639 and Yuma except for C35 with a small tree size. The yield on CC, C35 and SC was average but poor on X639 and Yuma. Fruit size was medium to large. Internal quality was good with slightly higher acid levels. External fruit colour was very similar on all the rootstocks. Estimated maturity is first week to mid June.

Raratonga

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on five rootstocks.

Raratonga is a midseason orange from the USA and was imported because it is a large fruited variety. Tree characteristics are vigorous and very thorny. The tree size varies from medium to large on all rootstocks. CC, SC and Yuma had a poor production, X639 very poor production and C35 an average production. The fruit size varies between medium and large for all rootstocks. Fruit was totally seedless. Maturity seems to be mid to end of June.

Sanquinello

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on three rootstocks.

Production on all three rootstocks (CC,C35,SC.) were good with medium to large fruit size (count 64-88). The internal quality was good with a good taste (sweet) on CC. External colour (T2-T3) was good and maturity early in the season compared to the other Midseason oranges. Maturity end of May to first week in June.

Tarocco Gallo

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on five different rootstocks.

From the visual evaluation it appears to have less thorns, especially on C35 and Yuma citrange. Yields on Yuma were poor to average, on C35 and SC average, on CC and X639 good. Overall fruit size was medium to large with some small fruit. Internal fruit quality was good with acid levels on the low side (0.63%-0.77%). Splitting was a problem on especially C35 and Yuma. C35 had an exceptionally good taste (Very sweet). Virtually no seeds present in the fruit (0.1% per fruit). Estimated maturity is first week to mid June.

Tarocco 57

Trees were evaluated at Riversbend, Kwazulu Natal during the 2003 season on five different rootstocks.

Production on all rootstocks were average to good with medium to large fruit size (CC,C35,SC,X639,Yuma). On all the rootstocks there was moderate to severe splitting . C35 and X639 were very thorny. External colour was good with good internal quality. More seeds present per fruit than with Tarocco Gallo (0.0%-1.9%). Maturity seems to be first week to mid June.

Tunisian Maltaise (BKM)

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season on four different rootstocks.

The yield on CC and MxT was exceptional compared to SC and X639 with a good production. Fruit size was small to medium with severe splitting on CC, SC and MxT and medium to large fruit size with sheeponose on X639. Juice% on all four rootstocks trends to be on the low side. External colour was good with early maturity first week to mid June.

Half Maltaise (HMM²):

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season on CC and SC rootstocks.

Yields were poor on both rootstocks and fruit size varied from medium to very large fruit (count 36-72). There were some Sheeponose fruit on CC and fruit with very thick rind on SC. Internal fruit quality was acceptable with low juice% (46.4%-48.6%). Maturity is also expected to be first to second week June.

Tunisian Maltaise (MLM):

Trees were evaluated at Moosrivier Estate, Mpumalanga during the 2003 season on CC, MxT and SC rootstocks.

Production on the trees were very good but with medium to very large fruit size on all three rootstocks. The fruit on CC and SC had a course rind with Sheep nose and on MxT the fruit had a thick peel. Juice% on all three rootstocks was on the low side (49.0%-50.0%) with good internal quality and acceptable external colour. Maturity mid June.

Conclusion and recommendations

Barlerin Maitaise (TMM):

Market acceptability must be determined for blood/semi-blood oranges. Fruit size and external colour could be a problem. Evaluation continue.

Crookes Shamouti:

Acid% on C35 on average low with a significant difference in fruit size distribution between the rootstocks. Evaluation continue.

Grosse Sanquinne

Limited information is available to make any recommendations. Evaluation continue.

Raratonga:

Thorns seems to be a problem especially with harvesting. Fruit was seedless with some advantages. Evaluation continue.

Sanquinella:

Good production and fruit size with exceptional good taste on CC. Evaluation continue.

Tarocco Gallo:

Tarocco is one of the best midseason varieties available at this stage. Splitting was a problem on C35 and Yuma. Almost no seeds present in most of the fruit. Thorns seems to be a problem but compared to Tarocco 57 fewer thorns was present. Evaluation continue.

Tarocco 57:

Compared to Gallo all rootstocks had splitting problems and on C35 and X639 trees were very thorny. More seeds present per fruit than Gallo. Evaluation continue.

Tunisian Maltaise (BKM):

Splitting problems on CC, SC and MxT with Sheep nose fruit on X639. Juice% are low. Stop evaluations.

Half Maltaise (HMM²):

Poor production on CC and SC with a very large variety of fruit sizes (count 36-72). Stop evaluations.

Tunisian Maltaise (MLM):

Some problems were fruit with a course rind, Sheep nose and low juice%. Stop evaluations.

Table 6.2.3.6.2. Internal fruit quality data for midseason orange selections for the inland areas during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Barlerin Maltese	CC	08/05/03	Riversbend	56-105	57.6	8.97	0.91	9.86	2.4	T6-7
Barlerin Maltese	CC	04/06/03	Riversbend	56-88	57.3	9.51	0.85	11.19	1.5	T5-6
Barlerin Maltese	C35	08/05/03	Riversbend	72-125	58.9	9.85	1.01	9.75	2.3	T6
Barlerin Maltese	C35	04/06/03	Riversbend	64-88	58.9	10.25	0.84	12.20	1.9	T3-4
Barlerin Maltese	SC	08/05/03	Riversbend	64-105	56.3	9.12	0.91	10.02	1.8	T7-8
Barlerin Maltese	SC	04/06/03	Riversbend	64-88	57.3	9.55	0.84	11.37	2.9	T5-6
Barlerin Maltese	X 639	08/05/03	Riversbend	72-125	58.3	9.65	0.98	9.85	2.3	T7-8
Barlerin Maltese	X 639	04/06/03	Riversbend	72-105	58.8	10.21	0.87	11.74	3.3	T4-5
Barlerin Maltese	Yuma	08/05/03	Riversbend	64-88	55.9	9.12	0.97	9.40	1.0	T6-7
Barlerin Maltese	Yuma	04/06/03	Riversbend	64-88	56.3	9.45	0.87	10.86	3.4	T5-6
Crookes Sham	CC	08/05/03	Riversbend	56-88	49.2	9.07	0.78	11.63	1.2	T7-8
Crookes Sham	CC	04/06/03	Riversbend	56-88	50.0	10.11	0.67	15.09	1.3	T5-6
Crookes Sham	C35	08/05/03	Riversbend	72-125	50.3	10.53	0.69	15.26	1.7	T7-8
Crookes Sham	C35	04/06/03	Riversbend	64-105	49.3	10.98	0.66	16.64	2.1	T5-6
Crookes Sham	X 639	08/05/03	Riversbend	72-125	50.7	9.70	0.83	11.69	1.4	T6-7
Crookes Sham	X 639	04/06/03	Riversbend	64-105	52.2	10.83	0.77	14.06	1.4	T4-5
Gross Sanguine	CC	08/05/03	Riversbend	64-105	57.3	10.58	0.88	12.02	0.1	T5
Gross Sanguine	CC	04/06/03	Riversbend	56-105	54.8	11.38	0.76	14.97	0.3	T3-4
Gross Sanguine	C35	08/05/03	Riversbend	64-88	57.8	10.73	0.86	12.48	0.3	T5
Gross Sanguine	C35	04/06/03	Riversbend	48-72	56.5	11.33	0.78	14.53	0.4	T4-5
Gross Sanguine	SC	08/05/03	Riversbend	64-88	53.1	10.68	0.73	14.63	0.2	T5
Gross Sanguine	SC	04/06/03	Riversbend	48-88	57.4	10.98	0.73	15.04	0.6	T3-4
Gross Sanguine	X 639	08/05/03	Riversbend	72-88	57.4	11.43	0.83	13.77	1.5	T4-5
Gross Sanguine	X 639	04/06/03	Riversbend	56-125	54.4	11.73	0.74	15.85	0.4	T3-4
Gross Sanguine	Yuma	08/05/03	Riversbend	56-88	54.3	10.35	0.86	12.03	0.3	T5
Gross Sanguine	Yuma	04/06/03	Riversbend	48-72	54.7	10.01	0.71	14.10	0.0	T3-4
Raratonga	CC	08/05/03	Riversbend	56-72	58.1	8.62	0.93	9.27	0.0	T7
Raratonga	CC	04/06/03	Riversbend	56-72	54.2	9.12	0.79	11.54	0.0	T5-6
Raratonga	C35	08/05/03	Riversbend	56-88	57.0	10.00	0.88	11.36	0.0	T6
Raratonga	C35	04/06/03	Riversbend	72-88	57.2	10.11	0.82	12.33	0.0	T3-4
Raratonga	SC	08/05/03	Riversbend	56-88	58.2	9.75	0.95	10.26	0.0	T6
Raratonga	SC	04/06/03	Riversbend	64-72	59.0	9.81	0.87	11.28	0.0	T4
Raratonga	X 639	08/05/03	Riversbend	48-88	58.9	8.77	0.92	9.53	0.0	T7
Raratonga	Yuma	08/05/03	Riversbend	56-88	58.1	9.65	0.90	10.72	0.0	T6-7
Raratonga	Yuma	04/06/03	Riversbend	56-88	58.4	9.71	0.82	11.84	0.0	T3-4
Sanguinella	CC	08/05/03	Riversbend	72-105	59.4	10.63	1.09	9.75	0.7	T6
Sanguinella	CC	04/06/03	Riversbend	64-105	58.7	11.08	0.92	12.04	1.6	T3
Sanguinella	C35	08/05/03	Riversbend	64-105	59.7	10.68	1.11	9.62	1.4	T5
Sanguinella	C35	04/06/03	Riversbend	72-88	59.9	10.93	1.01	10.82	1.5	T3
Sanguinella	SC	08/05/03	Riversbend	72-125	60.2	10.53	1.06	9.93	0.6	T5

Sanguinella	SC	04/06/03	Riversbend	88	60.3	11.53	1.07	10.78	0.5	T2
Tarocco Gallo	CC	08/05/03	Riversbend	64-88	60.5	9.45	0.73	12.95	0.0	T5
Tarocco Gallo	CC	04/06/03	Riversbend	56-88	60.3	9.61	0.65	14.78	0.0	T4-5
Tarocco Gallo	C35	08/05/03	Riversbend	64-88	59.6	10.78	0.77	14.00	0.1	T6
Tarocco Gallo	C35	04/06/03	Riversbend	48-88	59.5	10.25	0.68	15.07	0.0	T4
Tarocco Gallo	SC	08/05/03	Riversbend	72-125	61.4	9.85	0.73	13.49	0.0	T6-7
Tarocco Gallo	SC	04/06/03	Riversbend	64-88	60.5	10.43	0.63	16.56	0.0	T3-4
Tarocco Gallo	X 639	08/05/03	Riversbend	64-88	61.1	8.97	0.75	11.96	0.0	T6-7
Tarocco Gallo	X 639	04/06/03	Riversbend	56-88	60.4	9.55	0.65	14.69	0.0	T4
Tarocco Gallo	Yuma	08/05/03	Riversbend	72-125	60.5	9.70	0.73	13.29	0.0	T7
Tarocco Gallo	Yuma	04/06/03	Riversbend	64-105	57.7	9.95	0.72	13.82	0.1	T6-7
Tarocco 57	CC	08/05/03	Riversbend	72-105	61.6	9.65	0.93	10.38	0.9	T4-5
Tarocco 57	CC	04/06/03	Riversbend	48-72	61.6	9.55	0.75	12.73	1.4	T3
Tarocco 57	C35	08/05/03	Riversbend	72-88	61.2	10.00	0.93	10.75	0.4	T4-5
Tarocco 57	C35	04/06/03	Riversbend	88-125	61.4	9.71	0.89	10.91	0.0	T4
Tarocco 57	SC	08/05/03	Riversbend	56-105	61.5	9.90	0.94	10.53	0.6	T6
Tarocco 57	SC	04/06/03	Riversbend	64-88	60.6	10.25	0.79	12.97	1.9	T3
Tarocco 57	X 639	08/05/03	Riversbend	72-125	62.7	9.40	0.85	11.06	0.8	T5
Tarocco 57	X 639	04/06/03	Riversbend	64-88	62.2	9.55	0.76	12.57	1.2	T3
Tarocco 57	Yuma	08/05/03	Riversbend	72-125	61.3	9.60	0.86	11.16	0.4	T7
Tarocco 57	Yuma	04/06/03	Riversbend	64-105	61.3	9.75	0.69	14.13	1.3	T3-4
Tunisian Maltese (BKM)	CC	19/05/03	Moosrivier	72-105	56.5	11.73	1.07	10.96	2.8	T5-6
Tunisian Maltese (BKM)	CC	17/06/03	Moosrivier	72-105	56.3	12.17	0.96	12.68	3.8	T1-2
Tunisian Maltese (BKM)	MxT	19/05/03	Moosrivier	72-125	55.0	12.30	1.20	10.25	4.3	T5-6
Tunisian Maltese (BKM)	MxT	17/06/03	Moosrivier	72-105	52.6	12.89	1.08	11.94	3.5	T1-2
Tunisian Maltese (BKM)	SC	19/05/03	Moosrivier	88-125	55.6	12.03	1.16	10.37	3.6	T5-6
Tunisian Maltese (BKM)	SC	17/06/03	Moosrivier	72-125	55.0	12.27	1.06	11.58	3.5	T1-2
Tunisian Maltese (BKM)	X 639	19/05/03	Moosrivier	40-72	50.5	9.61	0.72	13.35	0.9	T5-6
Tunisian Maltese (BKM)	X 639	17/06/03	Moosrivier	48-72	50.0	9.95	0.74	13.45	1.3	T1-2
Half Maltese (HMM2)	CC	19/05/03	Moosrivier	56-36	46.6	10.93	0.91	12.01	1.3	T5-6
Half Maltese (HMM2)	CC	17/06/03	Moosrivier	40-72	48.4	11.57	0.93	12.44	1.9	T1-2
Half Maltese (HMM2)	SC	19/05/03	Moosrivier	36-72	46.4	10.43	0.86	12.13	0.3	T5-6
Half Maltese (HMM2)	SC	17/06/03	Moosrivier	36-64	48.6	10.88	0.98	11.10	0.8	T1-2
Tunisian Maltese (MLM)	CC	19/05/03	Moosrivier	40-72	50.0	9.91	0.80	12.39	1.7	T5-6
Tunisian Maltese (MLM)	CC	17/06/03	Moosrivier	56-72	49.1	10.58	0.83	12.75	1.3	T1-2
Tunisian Maltese (MLM)	MxT	19/05/03	Moosrivier	40-105	47.8	10.43	0.92	11.34	1.2	T5-6
Tunisian Maltese (MLM)	MxT	17/06/03	Moosrivier	64-88	49.4	10.98	0.95	11.56	1.7	T1-2
Tunisian Maltese (MLM)	SC	19/05/03	Moosrivier	56-72	49.0	10.63	0.83	12.81	0.4	T5-6
Tunisian Maltese (MLM)	SC	17/06/03	Moosrivier	56-72	49.3	10.98	0.83	13.23	0.8	T1-2

6.2.3.7 Evaluation of Lemons 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 verskaffing van Maart tot September te verseker, dit is vroeë en laatrypwordende seleksies. Probleme wat met lang blomtyd geassosieer is, moet verminder word. Goeie vrugkwaliteit (kleur, skildikte, 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 beantwoord.

Verna met feitlik geen sade en Villafranca met geen sade per vrug toon potensiaal. Albei variëteite het goeie vruggrootte en produksie gehad, maar verdere evaluasies is nodig.

Introduction

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).

To compare the tree characteristics and performance of new cultivars with the commercially grown Eureka to establish if they fulfil the above objectives.

Materials and methods

Field evaluations were conducted on Eureka SL (control), Eureka (Israel), Fino 49 & 95, Genoa, Limoneira 8A, Lisbon (control), Verna and Villafranca SL on various rootstocks.

Table 6.2.3.7.1. List of lemon trial sites evaluated during the 2002 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Eureka SL (ARC)	Mpumalanga	Esselen	RL	2000	5
Eureka (Israel)	Mpumalanga	Tekwane	RL	1998	4
Fino 49	Mpumalanga	Tekwane	RL	1998	4
Fino 95	Mpumalanga	Tekwane	RL	1998	4
Genoa	Mpumalanga	Tekwane	RL	1998	4
Limoneira 8A	Mpumalanga	Tekwane	RL	1998	3
Lisbon	Mpumalanga	Tekwane	RL,SO	1998	2,2
Verna	Mpumalanga	Tekwane	RL	1998	4
Villafranca	Mpumalanga	Tekwane	RL	1998	3

Results and discussion

Eureka SL (ARC):

Trees at Esselen Nursery are also looking good. Only bud wood from the Citrus Foundation Block must be used to contain certain problems arising from different sources.

Tekwane Estates:

Trees were evaluated at Tekwane Estates, Mpumalanga during the 2003 season.

Villafranca had no seeds per fruit compared to Verna with virtually no seeds (0.9 seeds per fruit). All the other cultivars had similar qualities with thick rind, oval shape fruit, good yield, large fruit size and a number of seeds per fruit.

Conclusions and recommendations

Eureka SL (ARC):

High potential for discerning markets. Fruit set and size must be manipulated. Only use bud wood from the Citrus Foundation Block. Evaluation continues.

Tekwane Estate Cultivar Trial:

Trial recovered well after severe pruning but there is still limited information available and the status remains experimental. Evaluation continues.

Table 6.2.3.7.2. Internal fruit quality data for Lemons during the 2003 season at Tekwane Estates.

Selection	Root-stock	Date harvested	Site	Juice %	Ave. Seed
Eureka seedless (ARC)	Fruit accidentally harvested				
Eureka seedless (Israel)	RL	14/05/03	Tekwane	34.0	9.2
Fino 1	RL	14/05/03	Tekwane	39.1	14.7
Fino 2	RL	14/05/03	Tekwane	38.1	13.9
Genoa	RL	14/05/03	Tekwane	37.1	19.3
Lisbon	RL	14/05/03	Tekwane	40.7	9.5
Lisbon	SO	14/05/03	Tekwane	38.2	14.9
Limineira	RL	14/05/03	Tekwane	40.1	9.9
Verna	RL	14/05/03	Tekwane	37.7	0.9
Villafranca	RL	14/05/03	Tekwane	43.1	0.0

6.2.4 Sub-Project: Rootstock evaluation in the Northern and inland region

By J.Joubert (CRI)

6.2.4.1 Sub-Projekopsomming

Die oogmerk van die onderstamprojek is om 'n bron van sitrusonderstamme te vind, dit te evalueer en te kommersialiseer. Dit moet hoë kwaliteit bevorder, asook siektebestand en verenigbaar met verskillende grond- en klimaatstoestande regoor Suidelike Afrika wees. Oesopbrengs per hektaar moet geoptimaliseer word deur van okuleerhout/onderstamverenigbaarheid, tuinboukundige prestasie te verbeter en beter interne kwaliteit, vrug grootte en produksie te induseer. Dit is daarom noodsaaklik dat produsente die beste moontlike keuse maak wanneer 'n onderstam gekies word, aangesien dit direkte invloed op beleggingsopbrengs sal hê. Baie dankie aan al die produsente wat gewillig is om proefpersele op hul plase aan te hou en te versorg vir kultivar evalueering.

Sub-Project summary

The objective of the rootstock project is to source, evaluate and commercialise citrus rootstocks, which are high quality inducing, disease resistant and compatible with different soil and climatic conditions throughout Southern Africa. Furthermore, that yield efficiency per hectare be optimised through the following: testing scion/rootstock compatibility, improving horticultural performance, inducing higher internal fruit quality, fruit size and production. It is therefore imperative that producers make the best possible choice when choosing a rootstock, as this will have a direct effect on the return on investment. Thank you to all growers who co-operated by having trees available for evaluation on their farms.

Abbreviations used in text:

SYMBOL		ROOTSTOCK
1.	AT	Australian trifoliolate
2.	BC	Benton citrange
3.	C	Calamandarin
4.	CA	C.amblycarpa
5.	CC	Carrizo citrange
6.	CM	C.macrophylla
7.	ChM	Changsa mandarin
8.	CLM	Cleopatra mandarin
9.	CO	C.obovoideae
10.	C32	citrange (trifoliolate orange x Ruby sweet orange)
11.	C35	citrange (trifoliolate orange x Ruby sweet orange)
12.	C61	Sunki x macrophylla
13.	FD	Flying Dragon

14.	FF6	Sunki x MTO trifoliolate orange
15.	F80/3	citrumelo
16.	F80/9	citrumelo
17.	GT	Gou Tou
18.	HRS 802	Siamese pummelo x trifoliolate orange
19.	HRS 809	Changsa x english large flowered trifoliolate orange
20.	HRS 812	Sunki mandarin x Beneke trifoliolate orange
21.	IRL	Indian rough lemon
22.	JC	Japanese citron
23.	JT	Jacobsen trifoliolate
24.	K	Konejime
25.	KC	Koethen citrange
26.	ML	Milan Lemon
27.	MXT	Minneola x trifoliolate
28.	N	Natsudaidai
29.	O	Orlando tangelo
30.	PT	Pomeroy trifoliolate
31.	RC	Rusk citrange
32.	RL-C	Rough lemon Cairn
33.	RL-S	Rough lemon Schaub
34.	RL-W	Rough lemon Wallace
35.	RP	Rangpur lime
36.	RT	Roebidoux trifoliolate
37.	RXT	Rangpur x troyer
38.	SC	Swingle citrumelo
39.	SCS	Sun chu sha
40.	SFS	Smooth flat Seville
41.	SM	Shekwasha mandarin
42.	SO	Sour orange
43.	ST	Sampson tangelo
44.	Sunki 1112	Flying dragon x Sunki (1112)
45.	Sunki 1113	Flying dragon x Sunki (1113)
46.	Sunki 1116	Flying dragon x Sunki (1116)
47.	TB	Terrabella
48.	TC	Troyer citrange
49.	Volk	Volkameriana
50.	X639	Cleopatra mandarin x trifoliolate
51.	YC	Yuma citrange
52.	61 AA3	Cleopatra mandarin x <i>P. trifoliata</i>
53.	75 AB 12/13	Mc Carthy grapefruit x <i>P. trifoliata</i>
54.	79 AC 6/2	Cleo x Swingle citrumelo

6.2.4.2 **Evaluation of Delta Valencia rootstocks at Moosrivier Estates, Groblersdal, Mpumalanga**
Experiment 94 by J. Joubert (CRI)

Opsomming

Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplantgronde, moet oor 'n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word.

Op hierdie stadium van die proef, met slegs die tweede evaluasie voltooi, wil dit blyk of BC en C35, gevolg deur ML en RP van die onderstamme is wat optimum produksie en vruggrootte gaan lewer met aanvaarbare interne kwaliteit in kombinasie met Delta Valencia's.

Introduction

To evaluate the performance of Delta Valencia on 42 different rootstocks in replant soils, over an eight-year production cycle in the Marble Hall area.

Materials and Methods

A randomised block comprising of 22 data rootstocks of two replicates of five trees each, the remainder (20) were planted in a non-randomised design comprising of 10 trees per stock. Delta Valencia on the following rootstocks are being evaluated: F80/8, F80/3, C32, C35, AT, X639, RL-C, RL-S, RL-W, PT, HRS812, K, ChM, N, RxT, CLM, Sunki 1113, CM, C, SCS, GT, CO, CC, TC, Volk, KC, TB, ML, OT, CA, RC, JT, RT, JC, BC, Sunki 1112, ST, SC, RP, SM, SFS, Sunki 1116.

Table 6.2.4.2.1. List of Delta Valencia trial sites evaluated in the Marble Hall area during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Delta Valencia	Mpumalanga	Moosrivier Estate	F80/8	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	PT	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	C32	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	AT	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	HRS812	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	K	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	CM	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	C35	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	C	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	SCS	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	X639	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	GT	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	ML	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	OT	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	CA	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	RC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	JT	1998	2
Delta Valencia	Mpumalanga	Moosrivier Estate	RL-S	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	SC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	RP	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	SM	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	ChM	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	N	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	RxT	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	RL-C	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	CLM	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	Sunki 1113	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	CO	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	CC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	TC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	Volk	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	KC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	TB	1998	5

Delta Valencia	Mpumalanga	Moosrivier Estate	RT	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	JC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	BC	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	F80/3	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	Sunki 1112	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	ST	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	SFS	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	Sunki 1116	1998	5
Delta Valencia	Mpumalanga	Moosrivier Estate	RL-W	1998	5

Results and discussion

Internal fruit quality in this trial was very promising with all the rootstocks meeting the minimal export standards, but on F80/8, PT, OT, CA, SC, RP, SM, RxT, CLM and Sunki the acid levels were on the high side. There were no differences noted between rootstocks with regards to external fruit colour, except for N and SFS with a T1-T2 colour plate rating. The optimum fruit size distribution for Delta Valencia's is between count 64 and count 88. According to the fruit size distribution of the trees most of the rootstocks were bearing fruit that were on the smaller side except for CM, C35, Volk and BC.

Benton citrange produced the heaviest crop with an average of over 61.7 kg per tree, followed by ML (60.5 kg/tree), RP (59.4 kg/tree), C35 (58.4 kg/tree) and RL-C (55.7 kg/tree). The smallest crop load of all the rootstocks in this trial were produced by ST (18.5 kg/tree) followed by C (18.7 kg/tree), CA (21.1 kg/tree) and JC (23.4 kg/tree). Maturity on these rootstocks seems to be first week to mid July.

Conclusion and recommendations

Evaluations of Delta Valencia on various rootstocks are still premature. As the production of the trees increase we hope to see an improvement in fruit size. Some of the rootstocks to recommend at this stage seems to be BC, ML, RP and C35. BC and C35 had the best fruit size/production combination compared to the rest of the rootstocks in the trial.

Table 6.2.4.2.2. Internal fruit quality of Delta Valencia on different rootstocks at Moosrivier Estate, (Marble Hall) during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Delta Valencia	F80/8	21/07/03	Moosrivier	64-88	57.9	12.69	1.53	8.29	0.0	T1
Delta Valencia	PT	21/07/03	Moosrivier	64-125	55.9	12.99	1.60	8.12	0.0	T1
Delta Valencia	C32	21/07/03	Moosrivier	64-105	58.8	11.87	1.35	8.79	0.0	T1
Delta Valencia	AT	21/07/03	Moosrivier	64-88	55.2	12.17	1.18	10.31	0.0	T1
Delta Valencia	HRS812	21/07/03	Moosrivier	64-88	60.1	12.27	1.40	8.76	0.0	T1
Delta Valencia	K	21/07/03	Moosrivier	72-88	58.4	12.59	1.47	8.56	0.0	T1
Delta Valencia	CM	21/07/03	Moosrivier	64-88	57.1	10.88	1.18	9.22	0.0	T1
Delta Valencia	C35	21/07/03	Moosrivier	64-88	59.5	11.97	1.28	9.35	0.0	T1
Delta Valencia	C	21/07/03	Moosrivier	72-105	56.8	12.99	1.58	8.22	0.0	T1
Delta Valencia	SCS	21/07/03	Moosrivier	64-125	54.3	12.94	1.47	8.80	0.0	T1
Delta Valencia	X639	21/07/03	Moosrivier	64-105	57.5	12.54	1.47	8.53	0.0	T1
Delta Valencia	GT	21/07/03	Moosrivier	72-125	56.6	13.64	1.71	7.98	0.0	T1
Delta Valencia	ML	21/07/03	Moosrivier	64-88	57.9	10.43	1.42	7.35	0.0	T1
Delta Valencia	OT	21/07/03	Moosrivier	72-105	55.6	13.34	1.68	7.94	0.0	T1
Delta Valencia	CA	21/07/03	Moosrivier	64-105	55.6	11.08	1.77	6.26	0.0	T1
Delta Valencia	RC	21/07/03	Moosrivier	64-105	59.1	12.74	1.48	8.61	0.0	T1
Delta Valencia	JT	21/07/03	Moosrivier	56-125	57.0	12.79	1.23	10.40	0.0	T1
Delta Valencia	RL-S	21/07/03	Moosrivier	72-88	58.1	11.87	1.38	8.60	0.0	T1
Delta Valencia	SC	21/07/03	Moosrivier	72-88	58.3	12.74	1.52	8.38	0.0	T1
Delta Valencia	RP	21/07/03	Moosrivier	56-88	61.8	11.77	1.59	7.40	0.0	T1
Delta Valencia	SM	21/07/03	Moosrivier	72-88	56.7	12.84	1.68	7.64	0.0	T1
Delta Valencia	ChM	21/07/03	Moosrivier	88-105	56.8	11.87	1.36	8.73	0.0	T1
Delta Valencia	N	21/07/03	Moosrivier	72-105	56.7	12.13	1.44	8.42	0.0	T1-2

Delta Valencia	RxT	21/07/03	Moosrivier	72-105	57.6	12.27	1.51	8.13	0.0	T1
Delta Valencia	RL-C	21/07/03	Moosrivier	64-105	57.1	10.25	1.29	7.95	0.0	T1
Delta Valencia	CLM	21/07/03	Moosrivier	56-125	54.9	12.13	1.53	7.93	0.0	T1
Delta Valencia	Sunki 1113	21/07/03	Moosrivier	64-105	58.7	12.37	1.47	8.41	0.0	T1
Delta Valencia	CO	21/07/03	Moosrivier	72-88	62.2	11.38	1.47	7.74	0.0	T1
Delta Valencia	CC	21/07/03	Moosrivier	64-88	57.5	12.47	1.39	8.97	0.0	T1
Delta Valencia	TC	21/07/03	Moosrivier	72-105	57.9	12.27	1.45	8.46	0.0	T1
Delta Valencia	Volk	21/07/03	Moosrivier	72-105	58.0	11.23	1.31	8.57	0.0	T1
Delta Valencia	KC	21/07/03	Moosrivier	72-105	58.2	11.67	1.37	8.52	0.0	T1
Delta Valencia	TB	21/07/03	Moosrivier	64-125	57.8	12.27	1.43	8.58	0.0	T1
Delta Valencia	RT	21/07/03	Moosrivier	64-105	55.9	12.64	1.41	8.96	0.0	T1
Delta Valencia	JC	21/07/03	Moosrivier	64-88	57.4	11.28	1.36	8.29	0.0	T1
Delta Valencia	BC	21/07/03	Moosrivier	56-88	58.6	11.53	1.25	9.22	0.0	T1
Delta Valencia	F80/3	21/07/03	Moosrivier	72-105	58.5	11.33	1.34	8.46	0.0	T1
Delta Valencia	Sunki 1112	21/07/03	Moosrivier	64-105	56.0	12.94	1.56	8.29	0.0	T1
Delta Valencia	ST	21/07/03	Moosrivier	64-105	59.2	11.97	1.39	8.61	0.0	T1
Delta Valencia	SFS	21/07/03	Moosrivier	72-88	57.9	10.53	1.16	9.08	0.0	T1-2
Delta Valencia	Sunki 1116	21/07/03	Moosrivier	56-88	59.7	11.57	1.45	7.98	0.0	T1
Delta Valencia	RL-W	21/07/03	Moosrivier	56-88	54.8	12.69	1.33	9.54	0.0	T1

Table 6.2.4.2.3. Fruit Size distribution per rootstock of Delta Valencia trees on different rootstocks at Moosriver Estate (Marble Hall) during the 2003 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	F80/8	48	0.08	Delta	ChM	48	0.00
Delta	F80/8	56	0.76	Delta	ChM	56	0.31
Delta	F80/8	72	7.39	Delta	ChM	72	3.20
Delta	F80/8	88	21.14	Delta	ChM	88	7.33
Delta	F80/8	105/125	56.62	Delta	ChM	105/125	51.55
Delta	F80/8	144	14.01	Delta	ChM	144	37.60
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	PT	48	0.00	Delta	N	48	0.00
Delta	PT	56	0.00	Delta	N	56	0.00
Delta	PT	72	1.05	Delta	N	72	2.51
Delta	PT	88	3.08	Delta	N	88	8.83
Delta	PT	105/125	39.59	Delta	N	105/125	45.32
Delta	PT	144	56.27	Delta	N	144	43.34
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C32	48	0.07	Delta	RxT	48	0.21
Delta	C32	56	2.24	Delta	RxT	56	0.53
Delta	C32	72	10.82	Delta	RxT	72	4.98
Delta	C32	88	17.68	Delta	RxT	88	11.98
Delta	C32	105/125	45.38	Delta	RxT	105/125	50.58
Delta	C32	144	23.81	Delta	RxT	144	31.71
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	AT	48	0.15	Delta	RL-C	48	0.00
Delta	AT	56	1.04	Delta	RL-C	56	2.67
Delta	AT	72	4.16	Delta	RL-C	72	16.09
Delta	AT	88	13.08	Delta	RL-C	88	26.16
Delta	AT	105/125	46.95	Delta	RL-C	105/125	47.29
Delta	AT	144	34.62	Delta	RL-C	144	7.78

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	HRS812	48	0.00	Delta	CLM	48	0.00
Delta	HRS812	56	2.77	Delta	CLM	56	0.44
Delta	HRS812	72	15.36	Delta	CLM	72	3.18
Delta	HRS812	88	26.29	Delta	CLM	88	7.14
Delta	HRS812	105/125	45.92	Delta	CLM	105/125	41.71
Delta	HRS812	144	9.66	Delta	CLM	144	47.53
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	K	48	0.00	Delta	Sunki 1113	48	0.00
Delta	K	56	0.44	Delta	Sunki 1113	56	2.56
Delta	K	72	2.32	Delta	Sunki 1113	72	7.12
Delta	K	88	7.69	Delta	Sunki 1113	88	15.92
Delta	K	105/125	49.06	Delta	Sunki 1113	105/125	44.00
Delta	K	144	40.49	Delta	Sunki 1113	144	30.40
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	CM	48	0.40	Delta	CO	48	0.11
Delta	CM	56	6.86	Delta	CO	56	0.00
Delta	CM	72	24.72	Delta	CO	72	3.06
Delta	CM	88	29.74	Delta	CO	88	6.80
Delta	CM	105/125	34.45	Delta	CO	105/125	53.79
Delta	CM	144	3.83	Delta	CO	144	36.24
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	0.08	Delta	CC	48	0.00
Delta	C35	56	5.14	Delta	CC	56	0.72
Delta	C35	72	18.99	Delta	CC	72	6.69
Delta	C35	88	27.29	Delta	CC	88	12.24
Delta	C35	105/125	39.32	Delta	CC	105/125	56.28
Delta	C35	144	9.18	Delta	CC	144	24.07
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	C	48	0.00	Delta	TC	48	0.00
Delta	C	56	0.00	Delta	TC	56	0.45
Delta	C	72	1.25	Delta	TC	72	5.43
Delta	C	88	4.10	Delta	TC	88	13.13
Delta	C	105/125	38.32	Delta	TC	105/125	52.23
Delta	C	144	56.33	Delta	TC	144	28.75
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SCS	48	0.00	Delta	Volk	48	0.53
Delta	SCS	56	0.29	Delta	Volk	56	8.30
Delta	SCS	72	2.42	Delta	Volk	72	16.59
Delta	SCS	88	5.33	Delta	Volk	88	16.82
Delta	SCS	105/125	37.83	Delta	Volk	105/125	42.01
Delta	SCS	144	54.12	Delta	Volk	144	15.75
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	0.00	Delta	KC	48	0.00
Delta	X639	56	0.00	Delta	KC	56	2.95
Delta	X639	72	0.21	Delta	KC	72	14.96
Delta	X639	88	2.74	Delta	KC	88	21.29
Delta	X639	105/125	42.05	Delta	KC	105/125	48.14

Delta	X639	144	55.00	Delta	KC	144	12.66
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	GT	48	0.00	Delta	TB	48	0.77
Delta	GT	56	0.13	Delta	TB	56	1.70
Delta	GT	72	0.38	Delta	TB	72	2.39
Delta	GT	88	4.77	Delta	TB	88	9.54
Delta	GT	105/125	40.64	Delta	TB	105/125	60.73
Delta	GT	144	54.08	Delta	TB	144	24.87
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	ML	48	0.62	Delta	RT	48	0.00
Delta	ML	56	7.19	Delta	RT	56	0.19
Delta	ML	72	19.42	Delta	RT	72	1.04
Delta	ML	88	23.84	Delta	RT	88	5.31
Delta	ML	105/125	41.19	Delta	RT	105/125	51.61
Delta	ML	144	7.74	Delta	RT	144	41.84
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	OT	48	0.00	Delta	JC	48	0.00
Delta	OT	56	1.24	Delta	JC	56	1.31
Delta	OT	72	2.48	Delta	JC	72	9.90
Delta	OT	88	5.37	Delta	JC	88	19.05
Delta	OT	105/125	41.32	Delta	JC	105/125	52.57
Delta	OT	144	49.59	Delta	JC	144	17.18
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	CA	48	0.00	Delta	BC	48	0.35
Delta	CA	56	1.11	Delta	BC	56	5.94
Delta	CA	72	6.28	Delta	BC	72	19.86
Delta	CA	88	17.01	Delta	BC	88	25.24
Delta	CA	105/125	51.57	Delta	BC	105/125	39.02
Delta	CA	144	24.03	Delta	BC	144	9.58
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	RC	48	0.23	Delta	F80/3	48	0.00
Delta	RC	56	3.52	Delta	F80/3	56	0.57
Delta	RC	72	10.02	Delta	F80/3	72	4.25
Delta	RC	88	18.56	Delta	F80/3	88	8.86
Delta	RC	105/125	43.30	Delta	F80/3	105/125	43.73
Delta	RC	144	24.35	Delta	F80/3	144	42.59
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	JT	48	0.00	Delta	Sunki 1112	48	0.00
Delta	JT	56	0.70	Delta	Sunki 1112	56	0.07
Delta	JT	72	7.37	Delta	Sunki 1112	72	1.18
Delta	JT	88	15.44	Delta	Sunki 1112	88	3.46
Delta	JT	105/125	49.47	Delta	Sunki 1112	105/125	34.19
Delta	JT	144	27.02	Delta	Sunki 1112	144	61.10
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	RL-S	48	0.00	Delta	ST	48	0.00
Delta	RL-S	56	0.60	Delta	ST	56	0.63
Delta	RL-S	72	5.19	Delta	ST	72	5.04

Delta	RL-S	88	12.57	Delta	ST	88	24.58
Delta	RL-S	105/125	54.63	Delta	ST	105/125	48.74
Delta	RL-S	144	27.01	Delta	ST	144	21.01
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SC	48	0.00	Delta	SFS	48	0.00
Delta	SC	56	0.65	Delta	SFS	56	1.59
Delta	SC	72	3.79	Delta	SFS	72	8.06
Delta	SC	88	10.34	Delta	SFS	88	17.06
Delta	SC	105/125	50.05	Delta	SFS	105/125	49.77
Delta	SC	144	35.18	Delta	SFS	144	23.52
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	RP	48	0.07	Delta	Sunki 1116	48	0.00
Delta	RP	56	2.19	Delta	Sunki 1116	56	0.63
Delta	RP	72	13.01	Delta	Sunki 1116	72	9.26
Delta	RP	88	24.78	Delta	Sunki 1116	88	23.90
Delta	RP	105/125	49.05	Delta	Sunki 1116	105/125	48.06
Delta	RP	144	10.89	Delta	Sunki 1116	144	18.15
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	SM	48	0.00	Delta	RL-W	48	0.00
Delta	SM	56	0.38	Delta	RL-W	56	0.92
Delta	SM	72	2.00	Delta	RL-W	72	9.75
Delta	SM	88	7.76	Delta	RL-W	88	22.94
Delta	SM	105/125	50.44	Delta	RL-W	105/125	45.41
Delta	SM	144	39.42	Delta	RL-W	144	20.99

Table 6.2.4.2.4. Production per tree of Delta Valencia's on different rootstocks at Moosriver Estate (Marble Hall) during the 2003 season.

Cultivar	Rootstock	Kg/tree
Delta Valencia	F80/8	48.4
Delta Valencia	PT	43.1
Delta Valencia	C32	54.9
Delta Valencia	AT	25.3
Delta Valencia	HRS 812	50.7
Delta Valencia	K	25.1
Delta Valencia	CM	57.4
Delta Valencia	C35	58.4
Delta Valencia	C	18.7
Delta Valencia	SCS	34.9
Delta Valencia	X639	47.2
Delta Valencia	GT	26.3
Delta Valencia	ML	60.5
Delta Valencia	OT	23.4
Delta Valencia	CA	21.1
Delta Valencia	RC	50.1
Delta Valencia	JT	26.3
Delta Valencia	RL-S	54.3
Delta Valencia	SC	40.0
Delta Valencia	RP	59.4
Delta Valencia	SM	28.8
Delta Valencia	ChM	34.9

Delta Valencia	N	46.2
Delta Valencia	RxT	36.2
Delta Valencia	RL-C	55.7
Delta Valencia	CLM	31.7
Delta Valencia	Sunki 1113	48.4
Delta Valencia	CO	31.1
Delta Valencia	CC	37.0
Delta Valencia	TC	50.6
Delta Valencia	Volk	56.7
Delta Valencia	KC	39.0
Delta Valencia	TB	49.5
Delta Valencia	RT	36.8
Delta Valencia	JC	23.4
Delta Valencia	BC	61.7
Delta Valencia	F80/3	49.9
Delta Valencia	Sunki 1112	43.9
Delta Valencia	ST	18.5
Delta Valencia	SFS	51.4
Delta Valencia	Sunli 1116	32.5
Delta Valencia	RL-W	34.8

6.2.4.3 Evaluation of Star Ruby grapefruit and Delta Valencia in Limpopo Province Experiment 137 by J. Joubert (CRI)

Opsomming

Die prestasie van verskillende onderstamme op herplante grond is in 'n intermediêre sitrusproduksiegebied geëvalueer

Op hierdie stadium van die evaluasies toon CC, MxT en RL-C onderstamme goeie resultate wat vruggroottes verspreiding en produksie aanbetref in kombinasie met Star Ruby. Ander onderstamme wat ook in berekening gebring kan word is BC, SC, KC en F80/3.

Onderstamme wat potensiaal toon met Delta Valencia's in hierdie area is SC, MxT, F80/3 en C35. Verdere ontleding kan tot voordeel van die Sitrusbedryf wees vir die toekoms.

Die Midnight Valencia gedeelte van die proef kon nie geëvalueer word nie a.g.v. onvoorsiene omstandighede.

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, 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 seed was collected locally and abroad and propagated by an accredited nursery using normal practices. Buds of Star Ruby grapefruit, Mid-knight Valencia and Delta Valencia were grafted onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates in Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per rootstock (total of 20 trees per rootstock).

Table 6.2.4.3.1. List of trial sites evaluated in the Letsitele area during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Delta Valencia	Limpopo	Letaba Estate	F80/9	1997	10
Delta Valencia	Limpopo	Letaba Estate	SC	1997	10
Delta Valencia	Limpopo	Letaba Estate	CC	1997	10
Delta Valencia	Limpopo	Letaba Estate	F80/3	1997	10
Delta Valencia	Limpopo	Letaba Estate	C35	1997	10
Delta Valencia	Limpopo	Letaba Estate	KC	1997	10
Delta Valencia	Limpopo	Letaba Estate	MxT	1997	10
Delta Valencia	Limpopo	Letaba Estate	BC	1997	10
Delta Valencia	Limpopo	Letaba Estate	X639	1997	10
Delta Valencia	Limpopo	Letaba Estate	RL-C	1997	8
Star Ruby	Limpopo	Letaba Estate	BC	1997	10
Star Ruby	Limpopo	Letaba Estate	CC	1997	10
Star Ruby	Limpopo	Letaba Estate	C35	1997	10
Star Ruby	Limpopo	Letaba Estate	F80/3	1997	10
Star Ruby	Limpopo	Letaba Estate	F80/9	1997	7
Star Ruby	Limpopo	Letaba Estate	KC	1997	10
Star Ruby	Limpopo	Letaba Estate	MxT	1997	10
Star Ruby	Limpopo	Letaba Estate	RL-C	1997	9
Star Ruby	Limpopo	Letaba Estate	SC	1997	10
Star Ruby	Limpopo	Letaba Estate	X639	1997	9

Results and discussion

Star Ruby:

All of the rootstocks made the minimum export requirements for Star Ruby Grapefruit with Juice% between 55.1% and 59.2%, TSS% between 9.67% and 12.33%, Acid% between 1.39% and 1.51% and average seeds per fruit between 0.1 and 0.5. The external colour was between T1-T2 for CC, C35, F80/9, KC, MxT, RL-C, SC, X639 and T2 for F80/3 and T2-T3 for BC.

The fruit size distribution varied from RL-C, CC and MxT with larger fruit size to SC, F80/3, KC and BC with medium fruit size to C35, X639 and F80/9 with smaller fruit size.

The production of Star Ruby Grapefruit varied from SC (126.9 kg/tree) with the highest production followed by F80/3 (119.0 kg/tree), MxT (110.2 kg/tree), RL-C (99.5 kg/tree) and F80/9 (91.3 kg/tree). Maturity seems to be mid to end of April.

Delta Valencia:

All the Delta Valencia rootstocks except for RL-C, made the minimum export standards for Delta Valencia. RL-C (control) failed to make Delta standards due to low TSS (9.46%). SC had a slight external colour (T1) advantage to the other rootstocks, which varied from colour plate T1 to T3.

The fruit size varied from C35 with larger fruit to F80/9 and F80/3 with medium fruit to SC, CC, KC, MxT, BC, X639 and RL-C with smaller fruit.

Production on the Delta Valencia's varied from SC (82.3 kg/tree) to RL-C (66.9 kg/tree), MxT (65.9 kg/tree), F80/3 (58.9 kg/tree) and CC (58.1 kg/tree). Maturity seems to be mid to end of July.

Conclusion and recommendations

Star Ruby:

There are indications that CC, MxT and RL-C compared by fruit size distribution and production per tree might be superior combinations with Star Ruby Grapefruit, but evaluations will continue to determine the value of the other rootstocks in this area. There are some of the other rootstocks (BC, SC, KC, F80/3) with excellent interior quality and acceptable fruit size to take in consideration.

Delta Valencia:

Some of the rootstocks evaluated with good qualities were SC, MxT, F80/3 and C35. These rootstocks compared excellent over against fruit size, external colour and production per tree. Evaluations will continue.

Table 6.2.4.3.2.a. Internal fruit quality of Star Ruby grapefruit trees on different rootstocks at Letaba Estates (Letsitele) during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
TSR	BC	29/4/03	Letaba	36-64	57.8	11.23	1.51	7.44	0.4	T2-3
TSR	CC	29/4/03	Letaba	32-56	55.3	10.70	1.42	7.54	0.5	T1-2
TSR	C-35	29/4/03	Letaba	32-48	55.1	10.70	1.47	7.28	0.1	T1-2
TSR	F80/3	29/4/03	Letaba	36-56	57.8	10.60	1.42	7.46	0.3	T2
TSR	F80/9	29/4/03	Letaba	32-56	58.7	12.33	1.50	8.22	0.4	T1-2
TSR	KC	29/4/03	Letaba	36-48	59.2	10.40	1.27	8.19	0.1	T1-2
TSR	MxT	29/4/03	Letaba	36-40	54.7	10.80	1.43	7.55	0.3	T1-2
TSR	RL-C	29/4/03	Letaba	36-48	58.1	9.67	1.39	6.96	0.2	T1-2
TSR	SC	29/4/03	Letaba	32-48	56.6	10.40	1.44	7.22	0.1	T1-2
TSR	X 639	29/4/03	Letaba	32-56	58.5	11.53	1.44	8.01	0.4	T1-2

Table 6.2.4.3.2.b. Fruit Size distribution per rootstock of Star Ruby grapefruit trees on different rootstocks at Letaba Estates (Letsitele) during the 2002 season.

Cultivar	Rootstock	Size	% Fruit
Star Ruby	RL-C	27	7.67
Star Ruby	RL-C	32	5.36
Star Ruby	RL-C	36	12.22
Star Ruby	RL-C	40	23.60
Star Ruby	RL-C	48	37.21
Star Ruby	RL-C	64	14.00
Cultivar	Rootstock	Size	% Fruit
Star Ruby	SC	27	1.81
Star Ruby	SC	32	2.86
Star Ruby	SC	36	10.78
Star Ruby	SC	40	21.64
Star Ruby	SC	48	47.06
Star Ruby	SC	64	15.85
Cultivar	Rootstock	Size	% Fruit
Star Ruby	F80/3	27	2.96
Star Ruby	F80/3	32	2.69
Star Ruby	F80/3	36	8.81
Star Ruby	F80/3	40	22.42
Star Ruby	F80/3	48	45.14
Star Ruby	F80/3	64	17.98
Cultivar	Rootstock	Size	% Fruit
Star Ruby	CC	27	4.59
Star Ruby	CC	32	5.52
Star Ruby	CC	36	15.43
Star Ruby	CC	40	26.78
Star Ruby	CC	48	36.49
Star Ruby	CC	64	11.19
Cultivar	Rootstock	Size	% Fruit
Star Ruby	KC	27	1.27
Star Ruby	KC	32	2.05
Star Ruby	KC	36	9.82

Star Ruby	KC	40	28.69
Star Ruby	KC	48	43.79
Star Ruby	KC	64	14.37
Cultivar	Rootstock	Size	% Fruit
Star Ruby	BC	27	1.67
Star Ruby	BC	32	3.35
Star Ruby	BC	36	11.51
Star Ruby	BC	40	22.59
Star Ruby	BC	48	44.18
Star Ruby	BC	64	16.70
Cultivar	Rootstock	Size	% Fruit
Star Ruby	MxT	27	3.10
Star Ruby	MxT	32	4.17
Star Ruby	MxT	36	16.78
Star Ruby	MxT	40	27.30
Star Ruby	MxT	48	36.87
Star Ruby	MxT	64	11.79
Cultivar	Rootstock	Size	% Fruit
Star Ruby	C35	27	0.48
Star Ruby	C35	32	1.48
Star Ruby	C35	36	11.09
Star Ruby	C35	40	15.34
Star Ruby	C35	48	50.50
Star Ruby	C35	64	21.11
Cultivar	Rootstock	Size	% Fruit
Star Ruby	X639	27	0.45
Star Ruby	X639	32	1.28
Star Ruby	X639	36	9.47
Star Ruby	X639	40	18.16
Star Ruby	X639	48	52.70
Star Ruby	X639	64	17.95
Cultivar	Rootstock	Size	% Fruit
Star Ruby	F80/9	27	0.48
Star Ruby	F80/9	32	0.36
Star Ruby	F80/9	36	6.81
Star Ruby	F80/9	40	8.42
Star Ruby	F80/9	48	57.49
Star Ruby	F80/9	64	26.45

Table 6.2.4.3.2.c. Production per tree of Star Ruby Grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2003 season.

Cultivar	Rootstock	Kg/tree
Star Ruby	RL-C	99.5
Star Ruby	SC	126.9
Star Ruby	F80/3	119.0
Star Ruby	CC	78.1
Star Ruby	KC	66.3
Star Ruby	BC	90.3
Star Ruby	MxT	110.2
Star Ruby	C35	83.1
Star Ruby	X639	89.9
Star Ruby	F80/9	91.3

Table 6.2.4.3.3.a. Internal fruit quality data for Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
DV	F80/9	27/07/03	Letaba	48-88	58.0	10.99	1.10	9.99	0.0	T1/2/3
DV	SC	27/07/04	Letaba	64-88	61.8	10.79	1.15	9.38	0.0	T1
DV	CC	27/07/05	Letaba	72-88	62.4	10.69	1.00	10.69	0.0	T1-2
DV	F80/3	27/07/06	Letaba	48-72	59.4	10.79	0.98	11.01	0.0	T1-2
DV	C-35	27/07/07	Letaba	64-72	62.2	12.18	1.23	9.90	0.0	T1-2
DV	KC	27/07/08	Letaba	64-105	60.8	10.79	1.02	10.58	0.0	T1-2
DV	MxT	27/07/09	Letaba	64-72	63.0	11.09	1.18	9.40	0.0	T1/2/3
DV	BC	27/07/10	Letaba	72-88	62.9	10.99	1.12	9.81	0.0	T1-2
DV	X639	27/07/11	Letaba	64-88	62.1	10.49	1.05	9.99	0.0	T1-2
DV	RL-C	27/07/12	Letaba	72-88	59.0	9.46	1.48	6.39	5.7	T1/2/3

Table 6.2.4.3.3.b. Fruit Size distribution per rootstock of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2003 season.

Cultivar	Rootstock	Size	% Fruit
Delta	F80/9	48	0.55
Delta	F80/9	56	6.24
Delta	F80/9	72	18.32
Delta	F80/9	88	22.32
Delta	F80/9	105/125	42.19
Delta	F80/9	144	10.38
Cultivar	Rootstock	Size	% Fruit
Delta	SC	48	0.03
Delta	SC	56	1.82
Delta	SC	72	10.66
Delta	SC	88	20.82
Delta	SC	105/125	49.57
Delta	SC	144	17.11
Cultivar	Rootstock	Size	% Fruit
Delta	CC	48	0.07
Delta	CC	56	2.86
Delta	CC	72	10.54
Delta	CC	88	21.51
Delta	CC	105/125	51.77
Delta	CC	144	13.25
Cultivar	Rootstock	Size	% Fruit
Delta	F80/3	48	0.18
Delta	F80/3	56	3.61
Delta	F80/3	72	13.91
Delta	F80/3	88	24.24
Delta	F80/3	105/125	46.73
Delta	F80/3	144	11.33
Cultivar	Rootstock	Size	% Fruit
Delta	C35	48	0.67
Delta	C35	56	5.86
Delta	C35	72	23.34
Delta	C35	88	27.82
Delta	C35	105/125	36.87
Delta	C35	144	5.43

Cultivar	Rootstock	Size	% Fruit
Delta	KC	48	0.11
Delta	KC	56	0.60
Delta	KC	72	5.09
Delta	KC	88	14.26
Delta	KC	105/125	54.33
Delta	KC	144	25.61
Cultivar	Rootstock	Size	% Fruit
Delta	MxT	48	0.09
Delta	MxT	56	1.69
Delta	MxT	72	9.60
Delta	MxT	88	17.66
Delta	MxT	105/125	53.89
Delta	MxT	144	17.06
Cultivar	Rootstock	Size	% Fruit
Delta	BC	48	0.33
Delta	BC	56	1.53
Delta	BC	72	12.53
Delta	BC	88	21.68
Delta	BC	105/125	49.38
Delta	BC	144	14.56
Cultivar	Rootstock	Size	% Fruit
Delta	X639	48	0.06
Delta	X639	56	0.30
Delta	X639	72	6.34
Delta	X639	88	15.60
Delta	X639	105/125	55.53
Delta	X639	144	22.18
Cultivar	Rootstock	Size	% Fruit
Delta	RL-C	48	0.00
Delta	RL-C	56	0.10
Delta	RL-C	72	2.81
Delta	RL-C	88	8.36
Delta	RL-C	105/125	53.96
Delta	RL-C	144	34.77

Table 6.2.4.3.3.c. Production per tree of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2003 season.

Cultivar	Rootstock	Kg/tree
Delta	F80/9	43.9
Delta	SC	82.3
Delta	CC	58.1
Delta	F80/3	58.9
Delta	C35	49.0
Delta	KC	55.3
Delta	MxT	65.9
Delta	BC	54.7
Delta	X639	35.9
Delta	RL-C	66.9

6.2.4.4 Evaluation of Valencia and navel varieties on different rootstocks in the Vaalharts area
Experiment 146 by J.Joubert (CRI).

Opsomming

Die prestasie van Valencia en Navel variëteite op verskillende onderstamme te ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Die beste produksie, vruggrootte, interne kwaliteit en eksterne kleur moet verkry word vir uitvoere na bv. die VSA markte.

Bahianinha op F80 het die beste produksie per boom gelewer, maar a.g.v. die jong boom ouderdomme kan daar nog geen aanbevelings gemaak word nie.

Die Delta Valencia's op al die onderstamme het nie aan die minimum uitvoer standaardte voldoen nie. Verdere evaluasies sal gedoen word om moontlik die oorsaak van die probleem op te spoor (Oes datum).

Met die Midnights was die bome ook nog jonk en die evaluasies moet voortgaan. C35 het die beste vruggrootte gehad, maar het nie aan die uitvoer standaardte voldoen nie. BC het die beste produksie getoon, maar het ook nie aan die uitvoer standaardte wat TOV% vlakke aanbetref voldoen nie.

Introduction

The progress of Valencia and Navel varieties on different rootstocks must be investigated and to determine the best rootstock for specific the Vaalharts area. To optimise the best production, fruit size, internal quality and external fruit colour for export to markets such as the USA.

Materials and methods

Field evaluations and laboratory analysis were conducted on Bahianinha navels on the following rootstocks: C32, C35, F80, IRL, KC, MxT, RL-W, RxT, SFS, TB, X639 and Delta Valencia's on the following rootstocks: BT, C35, CC, F80, SFS, X639 and Midnight Valencia's on the following rootstocks: C35, CC, X639 and Royal Late on the following rootstocks: C32, C35, CC, GT, RC.

Table 6.2.4.4.1. List of Valencia and Navel trial sites evaluated in the Vaalharts area during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No. of trees
Bahianinha	Northen Cape	Vaalharts	C32	1998	6
Bahianinha	Northen Cape	Vaalharts	C35	1998	6
Bahianinha	Northen Cape	Vaalharts	F80	1998	6
Bahianinha	Northen Cape	Vaalharts	IRL	1998	5
Bahianinha	Northen Cape	Vaalharts	KC	1998	6
Bahianinha	Northen Cape	Vaalharts	MxT	1998	6
Bahianinha	Northen Cape	Vaalharts	RL-W	1998	3
Bahianinha	Northen Cape	Vaalharts	RxT	1998	6
Bahianinha	Northen Cape	Vaalharts	SFS	1998	5
Bahianinha	Northen Cape	Vaalharts	TB	1998	6
Bahianinha	Northen Cape	Vaalharts	X639	1998	6
Delta Valencia	Northen Cape	Vaalharts	BC	1998	6
Delta Valencia	Northen Cape	Vaalharts	C35	1998	6
Delta Valencia	Northen Cape	Vaalharts	CC	1998	6
Delta Valencia	Northen Cape	Vaalharts	F80	1998	6
Delta Valencia	Northen Cape	Vaalharts	SFS	1998	6
Delta Valencia	Northen Cape	Vaalharts	X639	1998	6
Midnight	Northen Cape	Vaalharts	C35	1998	6
Midnight	Northen Cape	Vaalharts	CC	1998	6
Midnight	Northen Cape	Vaalharts	X639	1998	6
Royal Late	Northen Cape	Vaalharts	BC	1998	6
Royal Late	Northen Cape	Vaalharts	C32	1998	6
Royal Late	Northen Cape	Vaalharts	C35	1998	6
Royal Late	Northen Cape	Vaalharts	CC	1998	6
Royal Late	Northen Cape	Vaalharts	GT	1998	6
Royal Late	Northen Cape	Vaalharts	RC	1998	6

Results and discussion

Bahianinha:

IRL and RL-W did not meet the minimal export standards for internal quality (Capespan) with low Juice%, TSS% and Acid% levels. KC had a acceptable Juice% and Acid% level, but to low TSS% level. The fruit size average for all the rootstocks peeked at count 56, accept for RL-W at count 48, followed by ether count 48 or count 72. The production on the different rootstocks varied from F80 (12.3 kg/tree) with the highest followed by C32 (10.9 kg/tree), MxT (10.3 kg/tree), IRL (8.6 kg/tree) and TB (8.0 kg/tree). SFS had the lowest production per tree (4.1 kg/tree). Maturity seems to be end of May to first week in June.

Delta Valencia:

The Juice% level for all the rootstocks was below the minmal export standars for internal quality (Capespan) with BC and SFS also below the TSS% level. The Delta's all peeked at count 105/125 with their fruit size followed ether by count 88 or count 144. BC produced 11.4 kg/tree followed by X639 with 5.5 kg/tree. Maturity in this area might be end of June.

Midknight:

C35 was the only rootstock not meeting the minimal export standards for the internal quality (Capespan) with TSS levels on the low side. The fruit size on CC and X639 were on the smaller side (count 105/125) and with C35 the peak was with slightly larger fruit size (88). X639 had the highest production per tree (4.5 kg/tree). Maturity at the end of June.

Royal Late:

The Royal navels as with the Delta's seems to have a problem with their Juice% and Acid% levels that is below the export standards. BC peaked on count 56, GT and RC peaked on 72, C35 and C32 peaked on count 105/125 fruit size. C32 produced 7.3 kg/tree and C35 6.0 kg/tree with GT producing the lowest kg/tree (4.0 kg/tree). Maturity seems to be end of June.

Conclusion and recommendations

Bahianinha and Royal Late Navels:

Trees are still too young and there is not enough information to make any recommendations.

Delta and Midnight Valencia:

Not enough info for any conclusions.

Table 6.2.4.4.2. Internal fruit quality data for Valencia and Navel trees on different rootstocks at Vaalharts during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Bahianinha	C32	22/05/03	Vaalharts	36-56	50.7	10.43	0.86	12.13	0.0	T3-4
Bahianinha	C35	22/05/03	Vaalharts	40-56	50.5	10.01	0.74	13.53	0.0	T3-4
Bahianinha	F80	22/05/03	Vaalharts	36-72	52.6	10.83	0.91	11.90	0.0	T5
Bahianinha	IRL	22/05/03	Vaalharts	36-48	47.9	7.47	0.67	11.15	0.0	T4
Bahianinha	KC	22/05/03	Vaalharts	36-48	49.6	9.71	0.78	12.45	0.0	T4-5
Bahianinha	MxT	22/05/03	Vaalharts	40-64	52.2	10.53	0.86	12.24	0.0	T3-4
Bahianinha	RL-W	22/05/03	Vaalharts	36-48	47.8	8.18	0.70	11.69	0.0	T4
Bahianinha	RxT	22/05/03	Vaalharts	40-72	51.1	9.91	0.73	13.58	0.0	T4-5
Bahianinha	SFS	22/05/03	Vaalharts	40-64	52.2	9.91	0.89	11.13	0.0	T5
Bahianinha	TB	22/05/03	Vaalharts	36-64	51.2	9.91	0.84	11.80	0.0	T4
Bahianinha	X639	22/05/03	Vaalharts	40-72	50.6	10.01	0.75	13.35	0.0	T3-4
Delta Valencia	BC	26/06/03	Vaalharts	48-88	44.4	9.71	1.02	9.52	0.0	T1
Delta Valencia	C35	26/06/03	Vaalharts	56-125	46.0	11.23	1.33	8.44	0.0	T1
Delta Valencia	CC	26/06/03	Vaalharts	56-105	50.8	11.53	1.32	8.73	0.0	T1-2

Delta Valencia	F80	26/06/03	Vaalharts	56-72	44.5	10.53	1.05	10.03	0.0	T1-2
Delta Valencia	SFS	26/06/03	Vaalharts	56-88	42.4	9.81	1.08	9.08	0.0	T1
Delta Valencia	X639	26/06/03	Vaalharts	64-125	46.6	11.73	1.29	9.09	0.0	T1
Midnight	C35	26/06/03	Vaalharts	56-72	46.5	10.01	1.20	8.34	0.0	T1-2
Midnight	CC	26/06/03	Vaalharts	56-88	49.4	11.53	1.47	7.84	0.0	T1
Midnight	X639	26/06/03	Vaalharts	65-88	51.5	11.13	1.42	7.84	0.0	T1
Royal Late	BC	26/06/03	Vaalharts	40-72	38.8	10.53	0.63	16.71	0.0	T1
Royal Late	C32	26/06/03	Vaalharts	56-88	43.6	13.14	0.83	15.83	0.0	T1
Royal Late	C35	26/06/03	Vaalharts	56-72	42.0	11.83	0.62	19.08	0.0	T1-2
Royal Late	CC	26/06/03	Vaalharts	40-88	30.9	10.93	0.57	19.18	0.0	T1
Royal Late	GT	26/06/03	Vaalharts	48-72	40.8	11.53	0.74	15.58	0.0	T1
Royal Late	RC	26/06/03	Vaalharts	40-72	34.3	10.11	0.58	17.43	0.0	T1-2

Table 6.2.4.4.3. Fruit size distribution per rootstock for Valencia and Navel trees on different rootstocks at Vaalharts during the 2003 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	SFS	48	15.38	Midnight	CC	48	0.00
Bahianinha	SFS	56	47.44	Midnight	CC	56	3.17
Bahianinha	SFS	72	19.23	Midnight	CC	72	15.87
Bahianinha	SFS	88	10.26	Midnight	CC	88	20.63
Bahianinha	SFS	105/125	6.41	Midnight	CC	105/125	49.21
Bahianinha	SFS	144	1.28	Midnight	CC	144	11.11
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	RxT	48	23.40	Delta	C35	48	0.00
Bahianinha	RxT	56	60.64	Delta	C35	56	4.88
Bahianinha	RxT	72	10.64	Delta	C35	72	12.20
Bahianinha	RxT	88	3.19	Delta	C35	88	22.56
Bahianinha	RxT	105/125	2.13	Delta	C35	105/125	45.12
Bahianinha	RxT	144	0.00	Delta	C35	144	15.24
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	KC	48	24.16	Delta	X639	48	0.00
Bahianinha	KC	56	55.03	Delta	X639	56	0.00
Bahianinha	KC	72	13.42	Delta	X639	72	4.83
Bahianinha	KC	88	4.03	Delta	X639	88	6.76
Bahianinha	KC	105/125	3.36	Delta	X639	105/125	47.83
Bahianinha	KC	144	0.00	Delta	X639	144	40.58
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	MxT	48	8.58	Delta	CC	48	0.00
Bahianinha	MxT	56	43.78	Delta	CC	56	1.10
Bahianinha	MxT	72	33.91	Delta	CC	72	6.63
Bahianinha	MxT	88	9.01	Delta	CC	88	14.92
Bahianinha	MxT	105/125	4.29	Delta	CC	105/125	49.17
Bahianinha	MxT	144	0.43	Delta	CC	144	28.18
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	TB	48	2.87	Delta	F80	48	0.72
Bahianinha	TB	56	48.85	Delta	F80	56	2.88
Bahianinha	TB	72	29.31	Delta	F80	72	20.86
Bahianinha	TB	88	13.79	Delta	F80	88	20.86
Bahianinha	TB	105/125	5.17	Delta	F80	105/125	38.13
Bahianinha	TB	144	0.00	Delta	F80	144	16.55
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	IRL	48	48.89	Delta	SFS	48	0.00
Bahianinha	IRL	56	40.74	Delta	SFS	56	2.52

Bahianinha	IRL	72	8.15	Delta	SFS	72	13.45
Bahianinha	IRL	88	1.48	Delta	SFS	88	19.33
Bahianinha	IRL	105/125	0.74	Delta	SFS	105/125	36.97
Bahianinha	IRL	144	0.00	Delta	SFS	144	27.73
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	RL-W	48	50.00	Delta	BC	48	0.00
Bahianinha	RL-W	56	35.00	Delta	BC	56	7.49
Bahianinha	RL-W	72	13.33	Delta	BC	72	21.04
Bahianinha	RL-W	88	0.00	Delta	BC	88	26.22
Bahianinha	RL-W	105/125	1.67	Delta	BC	105/125	35.45
Bahianinha	RL-W	144	0.00	Delta	BC	144	9.80
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	F80	48	7.60	Royal Late	C35	48	1.10
Bahianinha	F80	56	38.40	Royal Late	C35	56	6.59
Bahianinha	F80	72	23.95	Royal Late	C35	72	19.23
Bahianinha	F80	88	19.01	Royal Late	C35	88	27.47
Bahianinha	F80	105/125	9.89	Royal Late	C35	105/125	34.07
Bahianinha	F80	144	1.14	Royal Late	C35	144	11.54
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	C32	48	29.41	Royal Late	C32	48	2.87
Bahianinha	C32	56	40.27	Royal Late	C32	56	4.31
Bahianinha	C32	72	19.00	Royal Late	C32	72	15.79
Bahianinha	C32	88	8.60	Royal Late	C32	88	30.14
Bahianinha	C32	105/125	2.71	Royal Late	C32	105/125	38.76
Bahianinha	C32	144	0.00	Royal Late	C32	144	8.13
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	C35	48	32.85	Royal Late	BC	48	9.09
Bahianinha	C35	56	48.18	Royal Late	BC	56	38.64
Bahianinha	C35	72	9.49	Royal Late	BC	72	25.76
Bahianinha	C35	88	4.38	Royal Late	BC	88	12.12
Bahianinha	C35	105/125	3.65	Royal Late	BC	105/125	9.85
Bahianinha	C35	144	1.46	Royal Late	Benton	144	4.55
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Bahianinha	X639	48	21.49	Royal Late	GT	48	0.00
Bahianinha	X639	56	55.37	Royal Late	GT	56	13.76
Bahianinha	X639	72	18.18	Royal Late	GT	72	28.44
Bahianinha	X639	88	3.31	Royal Late	GT	88	22.02
Bahianinha	X639	105/125	0.83	Royal Late	GT	105/125	22.94
Bahianinha	X639	144	0.83	Royal Late	GT	144	12.84
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	C35	48	0.00	Royal Late	RC	48	4.24
Midknight	C35	56	15.52	Royal Late	RC	56	29.66
Midknight	C35	72	20.69	Royal Late	RC	72	37.29
Midknight	C35	88	32.76	Royal Late	RC	88	20.34
Midknight	C35	105/125	25.86	Royal Late	RC	105/125	5.93
Midknight	C35	144	5.17	Royal Late	RC	144	2.54
Cultivar	Rootstock	Size	% Fruit				
Midknight	X639	48	0.00				
Midknight	X639	56	0.61				
Midknight	X639	72	4.88				
Midknight	X639	88	9.76				
Midknight	X639	105/125	55.49				
Midknight	X639	144	29.27				

Table 6.2.4.4.4. Production per tree for Valencia and Navel trees on different rootstocks at Vaalharts during the 2003 season.

Cultivar	Rootstock	Kg/tree
Bahianinha	SFS	4.1
Bahianinha	RxT	4.6
Bahianinha	KC	7.8
Bahianinha	MxT	10.3
Bahianinha	TB	8.0
Bahianinha	IRL	8.6
Bahianinha	RL-W	5.1
Bahianinha	F80	12.3
Bahianinha	C32	10.9
Bahianinha	C35	7.0
Bahianinha	X639	5.8
Midknight	C35	2.1
Midknight	X639	4.5
Midknight	CC	2.0
Delta	C35	4.8
Delta	X639	5.5
Delta	CC	5.3
Delta	F80	4.5
Delta	SFS	3.7
Delta	BC	11.4
Royal Late	C35	6.0
Royal Late	C32	7.3
Royal Late	BC	6.4
Royal Late	GT	4.0
Royal Late	RC	5.0

6.2.4.5 Evaluation of Valencia and Grapefruit varieties on new imported rootstocks in the Malelane and Swaziland area

Experiment 416 by J.Joubert (CRI)

Opsomming

Die prestasie van Valencia- en Pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplante grondtipes moet ondersoek word. Die produksie, vruggrootte, interne gehalte en skilkleur moet verbeter word, terwyl vruggrootte verminder moet word.

Marsh op C61 produseer die meeste vrugte en die interne kwaliteit voldoen aan die minimum standaarde vir uitvoere. Sunki 809 het nie uitvoer standaarde behaal met 'n te lae TOV% vlak.

Met Star Ruby het C32 die meeste vrugte geproduseer, maar Sunki 802, 809 en 812 het die beste verspreiding gehad.

Introduction

The progress of Valencia and Grapefruit varieties on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved and at the same time fruit size must be decreased.

Materials and methods

Identical methods were used for both sites. 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 grafted onto 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 802, Changsa x English large flowered trifoliolate orange, 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 PVA and, making use of a back actor, 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.

Delta Valencia was grafted 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). Mid-knight Valencia was grafted onto Sunki x Beneke HRS 812. The trees were planted at Esselen Nursery in March 1999.

Table 6.2.4.5.1. List of grapefruit and Valencia trial sites evaluated in the Swaziland and Malelane area during the 2003 season.

Selection	Area	Site	Rootstock	Tree Age	No.of trees
Marsh	Swaziland	Tambuti	812	1999	1
Marsh	Swaziland	Tambuti	809	1999	2
Marsh	Swaziland	Tambuti	C61	1999	4
Oroblanco	Swaziland	Tambuti	C61	1999	5
TSR	Swaziland	Tambuti	C32	1999	4
TSR	Swaziland	Tambuti	802	1999	2
TSR	Swaziland	Tambuti	809	1999	1
TSR	Swaziland	Tambuti	812	1999	2
TSR	Swaziland	Tambuti	C61	1999	5
TSR	Swaziland	Tambuti	C35	1999	4
Midnight	Malelane	Esselen	Sunki 812	1999	5
Delta	Malelane	Esselen	Sunki 812	1999	5
Delta	Malelane	Esselen	Sunki 802	1999	5
Delta	Malelane	Esselen	FF-6	1999	5

Results and discussions

Marsh:

Internal quality looks good, except Sunki 809 where the TSS% levels seems to be on the low side. Fruit size distribution peaks between count 36 and 48. Marsh on C61 produced 53.6 kg/tree compared to Sunki 812 with 39.5 kg/tree and Sunki 809 with 37.3 kg/tree. Maturity first week to mid May.

Star Ruby:

The internal quality on all the rootstocks comply with the minimal export standards (Capespan) with more or less the same external colour (T1-T2). The fruit size peak for most of the rootstocks were count 48 (C61, Sunki 809, C32, C35), but for Sunki 812 it was count 40 and Sunki 802 it was count 36. The production was the highest for C32 with 86.1 kg/tree followed by C35 with 77.1 kg/tree. The other rootstocks was on average between 5.0 and 34.7 kg/tree. Maturity seems to be first week of May.

Delta and Midnight Valencia:

Sunki 802 was the only rootstock with an acid% level on the low side for internal quality minimal export standards with the Delta's. On the Midnight side Sunki 812 had a problem with low juice% levels for export. Delta Valencia peaked at count 105/125 on all three rootstocks with Sunki 812 and FF-6 producing 58.5 and 58.4 kg fruit per tree. Midnight peaked between count 56 and 72 with Sunki 812 producing 46.3 kg fruit per tree. Maturity mid of July.

Conclusion and recommendation

Marsh:

Marsh grapefruit on HRS 812 is the most vigorous combination. There are not enough info to make recommendations. Trees are still young and evaluations continue.

Star Ruby:

Star Ruby on HRS 802 as the most vigorous scion/rootstock combination, but with C32 the best producing combination. C35 produced the second most fruit with the Star Ruby combination. Evaluations continue.

Delta and Midnight Valencia:

Sunki Beneke is proving to be a good alternative rootstock for both Midnight and Delta Valencia with regard to fruit quality. Trees are still young and internal quality might improve as trees grow older. Fruit size still on the small side, but evaluations continue.

Future Research

Trees have recovered well after transplanting at Tambuti Estates and are in healthy condition. The first fruit will be harvested in 2003. Evaluations are to continue in the 2004 season using the following parameters: tree size, rootstock and scion diameters and general horticultural performance of scion rootstock combinations, fruit size and fruit quality will be assessed.

Table 6.2.4.5.2.a. Internal fruit quality data for grapefruit on different rootstocks at Tambuti Estates during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Marsh	812	05/05/03	Tambuti	32-56	56.6	9.65	1.29	7.48	4.4	T5-6
Marsh	809	05/05/03	Tambuti	23-48	54.3	8.32	1.14	7.30	3.8	T5-6
Marsh	C61	05/05/03	Tambuti	23-56	52.9	10.78	1.45	7.43	4.1	T5-6
Oroblanco	C61	05/05/03	Tambuti	23-36	39.7	10.98	0.73	15.04	0.3	T7
TSR	C32	05/05/03	Tambuti	32-48	55.7	11.18	1.33	8.41	1.0	T1-2
TSR	802	05/05/03	Tambuti	23-40	55.1	9.95	1.32	7.54	0.1	T1-2
TSR	809	05/05/03	Tambuti	36-48	57.1	11.38	1.33	8.56	0.6	T1-2
TSR	812	05/05/03	Tambuti	27-36	55.9	9.55	1.23	7.76	0.8	T1-2
TSR	C61	05/05/03	Tambuti	23-56	57.4	10.88	1.26	8.63	0.3	T1-2
TSR	C35	05/05/03	Tambuti	32-56	53.8	11.18	1.40	7.99	0.2	T1-2

Table 6.2.4.5.2.b. Fruit size distribution per rootstock at Tambuti Estate during the 2003 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 61	27	0.32	Marsh	C 61	27	0.00
Star Ruby	C 61	32	1.45	Marsh	C 61	32	0.55
Star Ruby	C 61	36	10.29	Marsh	C 61	36	1.79
Star Ruby	C 61	40	22.83	Marsh	C 61	40	12.77
Star Ruby	C 61	48	51.29	Marsh	C 61	48	64.56
Star Ruby	C 61	64	13.83	Marsh	C 61	64	20.33
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 812	27	8.73	Marsh	Sunki 812	27	0.00
Star Ruby	Sunki 812	32	7.14	Marsh	Sunki 812	32	0.00
Star Ruby	Sunki 812	36	26.98	Marsh	Sunki 812	36	4.69
Star Ruby	Sunki 812	40	30.95	Marsh	Sunki 812	40	25.00
Star Ruby	Sunki 812	48	23.02	Marsh	Sunki 812	48	62.50
Star Ruby	Sunki 812	64	3.17	Marsh	Sunki 812	64	7.81
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 809	27	0.00	Marsh	Sunki 809	27	0.45
Star Ruby	Sunki 809	32	0.00	Marsh	Sunki 809	32	1.35
Star Ruby	Sunki 809	36	7.84	Marsh	Sunki 809	36	25.23
Star Ruby	Sunki 809	40	22.55	Marsh	Sunki 809	40	48.20
Star Ruby	Sunki 809	48	61.76	Marsh	Sunki 809	48	24.32
Star Ruby	Sunki 809	64	7.84	Marsh	Sunki 809	64	0.45

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 802	27	6.45	Oroblanco	C 61	27	12.10
Star Ruby	Sunki 802	32	3.23	Oroblanco	C 61	32	6.67
Star Ruby	Sunki 802	36	41.94	Oroblanco	C 61	36	24.69
Star Ruby	Sunki 802	40	25.81	Oroblanco	C 61	40	16.54
Star Ruby	Sunki 802	48	22.58	Oroblanco	C 61	48	31.85
Star Ruby	Sunki 802	64	0.00	Oroblanco	C 61	64	8.15
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	C 32	27	1.11				
Star Ruby	C 32	32	1.66				
Star Ruby	C 32	36	14.22				
Star Ruby	C 32	40	23.92				
Star Ruby	C 32	48	47.74				
Star Ruby	C 32	64	11.36				
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	C 35	27	0.18				
Star Ruby	C 35	32	0.18				
Star Ruby	C 35	36	13.43				
Star Ruby	C 35	40	16.83				
Star Ruby	C 35	48	46.28				
Star Ruby	C 35	64	23.10				

Table 6.2.4.5.2.c. Production per tree of Grapefruit on different rootstocks at Tambuti Estates during the 2003 season.

Cultivar	Rootstock	Kg/tree
Star Ruby	C 61	34.7
Star Ruby	Sunki 812	25.0
Star Ruby	Sunki 809	30.5
Star Ruby	Sunki 802	5.0
Star Ruby	C 32	86.1
Star Ruby	C 35	77.1
Marsh	C 61	53.6
Marsh	Sunki 812	39.5
Marsh	Sunki 809	37.3
Oroblanco	C 61	27.6

Table 6.2.4.5.3.a. Internal fruit quality of Midnight and Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2003 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Midnight	Sunki 812	25/07/03	Esselen	48-72	50.2	11.67	1.09	10.71	0.8	T1
Delta	Sunki 812	25/07/03	Esselen	56-72	60.8	12.37	1.11	11.14	0.0	T1
Delta	Sunki 802	25/07/03	Esselen	105-56	60.1	11.77	0.97	12.13	0.0	T1
Delta	FF-6	25/07/03	Esselen	64-88	62.0	12.74	1.06	12.02	0.0	T1

Table 6.2.4.5.3.b. Fruit size distribution per rootstock at Esselen nursery during the 2003 season.

Cultivar	Rootstock	Size	% Fruit
Midknight	Sunki 812	48	6.09
Midknight	Sunki 812	56	24.93
Midknight	Sunki 812	72	29.86
Midknight	Sunki 812	88	18.99
Midknight	Sunki 812	105/125	18.99
Midknight	Sunki 812	144	1.16
Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 812	48	0.66
Delta	Sunki 812	56	5.89
Delta	Sunki 812	72	19.47
Delta	Sunki 812	88	26.31
Delta	Sunki 812	105/125	40.17
Delta	Sunki 812	144	7.50
Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 802	48	0.00
Delta	Sunki 802	56	2.02
Delta	Sunki 802	72	9.84
Delta	Sunki 802	88	13.34
Delta	Sunki 802	105/125	52.02
Delta	Sunki 802	144	22.78
Cultivar	Rootstock	Size	% Fruit
Delta	FF-6	48	0.08
Delta	FF-6	56	0.55
Delta	FF-6	72	4.00
Delta	FF-6	88	9.01
Delta	FF-6	105/125	52.12
Delta	FF-6	144	34.25

Table 6.2.4.5.3.c. Production per tree of Midknight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2003 season.

Cultivar	Rootstock	Kg/tree
Midknight	Sunki 812	46.3
Delta	Sunki 812	58.5
Delta	Sunki 802	36.9
Delta	FF-6	58.4

7 Citrus Improvement Programme (CIP) By Thys du Toit and Louise Jackson (CRI)

7.1 Summary

Citrus Foundation Block: A total of 2 379 375 buds were supplied by the Citrus Foundation Block during 2003, which is 95 393 more than in 2002. Star Ruby grapefruit and Bahianinha navel were the most popular cultivars. Demand for seed reduced by 221 liters when compared to the previous year and for the 2nd consecutive year there was a large demand for seed from overseas countries, mainly China. Carrizo Citrange remains the most popular rootstock cultivar. New cultivars received from the ITSC for establishing, evaluating and increasing, included 28 new scion cultivars and 5 new rootstock cultivars. A 2nd insect controlled greenhouse was erected to house a further 30 000 increase trees.

Nursery Accreditation: The accreditation scheme guidelines were revised and implemented in the nurseries. During 2003, 23 nurseries were audited, of which 17 were fully accredited, 3 provisionally accredited, one new nursery approved and two were not accredited.

Tree certification: As a result of EurepGAP accreditation requirements, an increase in demand for tree certificates was experienced and a total of 595 certificates were processed and issued during 2003.

Statutory Improvement Programme: An application was submitted to the Department Genetic Resources to register the SACIP and to obtain assistance to write a legal document which will be the basis for a statutory Citrus Improvement Programme.

SA Breeding Programme and International Cultivar Liaison : Negotiations between the GCA, CRI and ARC for joint ownership of ITSC cultivars emanating from their breeding programme ended in a deadlock. Consequently no reports were received from the ITSC for inclusion in this annual report. No international liaison took place pending the outcome of the abovementioned negotiations. The CIP Advice Committee will examine cultivar development in the Southern African citrus industry and make recommendations with regard to the future strategies.

Cultivar evaluation: An internal appointment was made in the CRI and Johan Joubert assumed responsibility for all northern and inland evaluation projects. Chris Alexander is used on contract to manage projects in the West and East Cape. The evaluation guidelines were revised in consultation with the evaluators. The ARC's report has not been submitted due to the reasons stated above.

An overview of operations in the CIP:

Citrus Foundation Block

Budwood supply during 2003 compared to 2 preceding years, 10 most popular cultivar selections

2003			2002			2001		
Selection	Buds	%	Selection	Buds	%	Selection	Buds	%
TOTAL	2379375		TOTAL	2283982		TOTAL	1923589	
Star Ruby	212450	8.9%	Turkey	383970	16.8%	Turkey	350692	14.7%
Bahianinha	210220	8.8%	Bahianinha	262490	11.5%	Midnight	157860	6.6%
Turkey	203200	8.5%	Palmer	175410	7.7%	Palmer	142352	6.0%
Palmer	173730	7.3%	Star Ruby	164810	7.2%	Bahianinha	140540	5.9%
Nadorcott 1	169500	7.1%	Midnight	145450	6.4%	Cara Cara	120980	5.1%
Midnight	127450	5.4%	Nadorcott 1	91300	4.0%	Star Ruby	97882	4.1%
Eureka	117450	4.9%	Delta	84230	3.7%	Nadorcott 1	84500	3.6%
Newhall	102460	4.3%	Miho Wase	69400	3.0%	Delta	84480	3.6%
Miho Wase	102100	4.3%	Lane Late Cal.	69015	3.0%	Eureka	83555	3.5%
Lina	82050	3.4%	Eureka SL	66400	2.9%	Du Roi	55350	2.3%

Budwood supply per area during 2003 compared to 2 preceding years:

Area	2003	%	2002	%	2001	%
Eastern Cape	546660	23.0%	441902	19.3%	473369	24.6%
Western Cape	365665	15.4%	272850	11.9%	181534	9.4%
Northern Cape	116990	4.9%	156905	6.9%	152215	7.9%
Kwazulu Natal	36030	1.5%	20600	0.9%	38250	2.0%
Limpopo	930650	39.1%	804120	35.2%	712026	37.0%
Mpumalanga	244590	10.3%	376205	16.5%	192190	10.0%
North West	101100	4.2%	121350	5.3%	109665	5.7%
Mozambique	5400	0.2%	0	0.0%	8500	0.4%
Swaziland	10600	0.4%	49400	2.2%	43550	2.3%
Other African States	0	0.0%	1400	0.1%	0	0.0%
Zimbabwe	21700	0.9%	39250	1.7%	12290	0.6%
Total	2379385		2283982		1923589	

Seed supplied per rootstock selection, local and export, during 2003

Area Name	C35	CC	CM	FD	MXT	RLC	RLS	SC	TC	VA	X639	YC	Total
Eastern Cape	20	41				41		18	28	8	8		164
KwaZulu Natal					2			6	4				12
Limpopo	46	162			54	95	10	262	61		46	18	754
Mpumalanga	1	10			8	40		13	1		3		76
North West Province	2	16			4	2		3			4		31
Northern Cape								5					5
Swaziland									4				4
Western Cape	14	122				16			24.5		18		194.5
Zimbabwe						2		3					5
Southern African Total	83	351	0	0	68	196	10	310	122.5	8	79	18	1245.5
Carribbean										12			12
China		715							515				1230
Other African States									2				2.0
Reunion		8		0.5									8.5
South America								4					4
Thailand			6							8			14
USA		70											70
Vietnam										20			20
Export Total	0	793	6	0.5	0	0	0	4	517	40	0	0	1360.5
Grand Total	83	1144	6	0.5	68	196	10	314	639.5	48	79	18	2606

New cultivars received from the ITSC after shoot tip grafting:

Cultivar	Selection	Owner / Agent
Grapefruit	Henderson Mutant A13	ARC
Grapefruit	Henderson Mutant A17	ARC
Grapefruit	Pomelit 2	ARC
Lemon	Elongated Eureka	ARC
Lemon	Triploid	Sunworld
Mandarin	A3	ARC
Mandarin	B18	ARC
Mandarin	C27	ARC
Mandarin	Clara	Citrospan
Mandarin	Gold Nugget	Sunworld
Mandarin	Honey Gold	CGA
Mandarin	Murcott SL	CGA
Mandarin	Nova Mutant	CGA
Mandarin	Tacle	Citrospan
Midseason	Shamouti	CGA
Midseason	Tarocco Scire	Citrospan
Midseason	Tarocco Scire Nuc.	Citrospan
Midseason	Tarocco Tapi	Citrospan
Navel	Coetzee Late	CGA
Navel	Krajewski	CGA
Navel	Leng	CGA
Valencia	Benny 1 + 2	KGB / CTS
Valencia	Henrietta	B van Rooyen
Valencia	Jassie	CGA

Cultivar	Selection	Owner / Agent
Valencia	Kirkwood Red	CGA
Valencia	Letaba Orane	B van Rooyen
Valencia	Midknight 1	CGA
Valencia	Mouton Early	Open
Valencia	Skilderkrans	Open
Rootstock	Sweet rough lemon 72	ARC
Rootstock	Carrizo 669	ARC
Rootstock	South Orange hybrid	ARC
Rootstock	Troyer 609	ARC
Rootstock	Sunki X Beneke	ARC

New Development

A second 3904 m² greenhouse, which can accommodate 30000 increase trees in 10 litre containers, has been erected. This will enable the CFB to produce category 1 and 2 cultivar increase material from within insect and rain proof structures.

Nursery Accreditation

On instruction from the CIP Advisory Committee the guidelines for the South African Citrus Nursery Accreditation Scheme were revised by Dr Hennie le Roux, Dr Faan van Vuuren, Peter Kingston and Thys du Toit, and implemented in the nurseries.

Thys du Toit visited 23 nurseries twice each during 2003 for audit purposes, and the following nurseries were accredited:

Nursery	Address	Telephone	Accreditation
Apapanzi	Posbus 147, Kirkwood, 6120	042 2300790	Full
B F Joubert	Posbus 193, Kirkwood, 6120	042 230 0309	Full
Capricorn	P O Box 1925, Tzaneen, 0850	0834563148	Full
Casmar	Posbus 3, Mooinooi, 0325	0145 743152	Full
Du Roi	Posbus 66, Letsitele, 0885	015 345 1650	Full
Esselen	Posbus 100, Malelane, 1320	013 7900 160	Full
H J Joubert	Posbus 207, Montagu, 6720	0236 142237	Full
Letsitele	Posbus 1, Letsitele, 0885	015 3451600	Full
Miskraal	P O Box 106, Kirkwood, 6120	042 230 1461	Full
Ngwenya	Posbus 36, Malelane, 1320	013 790 3004	Full
Nucellar	P O Box 69, Simondium, 7670	021 860 1333	Full
Paksaam	Posbus 16, Patensie, 6335	042 28 30201	Full
Stargrow	Posbus 12536, Die Boord, 7613	021 921 2232	Full
Tweeling	Posbus 190, Kirkwood, 6120	042 230 1408	Full
Vaalharts	Posbus 317, Hartswater, 8570	053 474 0565	Full
Waterfall	P O Box 339, Adelaide, 5760	046 6840738	Full
Witkrans	Posbus 17, Boshhoek, 0301	014 57 33036	Full
Baddaford	P O Box 51, Fort Beaufort, 5720	046 645 2814	Provisional
Green Gables	P O, Summerville, 6107	042 234 0421	Provisional
Sondagsrivier	Posbus 304, Kirkwood, 6120	042 230 0349	Provisional
Westfalia	P O Box 14, Duiwelskloof, 0835	015 309 0026	Provisional (New)

Tree Certificate

The certification of orchards is one of the prerequisites for Eurepgap registration, and as a result the demand for tree certificates has increased considerably. During 2003 a total of 595 tree certificates were processed and issued to nurseries for handover to their clients.

Statutory Improvement Programme

The Procedural Guide of the Southern African Citrus Improvement Programme was revised in accordance with comments received from the CIP Advisory Committee. This document was submitted to the Director, Genetic Resources, together with a request for assistance in rewriting the document into a legal format, which would be acceptable for the registration of the CIP as a statutory programme.

The South African breeding programme and international cultivar liaison

Negotiations between the GCA, CRI and ARC for joint ownership of ITSC cultivars emanating from their breeding programme ended in a deadlock. Consequently no reports were received from the ITSC for inclusion in this annual report. No international liaison took place pending the outcome of the abovementioned negotiations. The CIP Advice Committee will examine cultivar development in the Southern African citrus industry and make recommendations with regard to the future strategies.

Cultivar evaluation

An internal appointment was made in the CRI and Johan Joubert assumed responsibility for all northern and inland evaluation projects. Chris Alexander is used on contract to manage projects in the West and East Cape. The evaluation guidelines were revised in consultation with the evaluators. The ARC's report has not been submitted due to reasons stated above.

SITRUS VERBETERINGSPROGRAM

Deur Thys du Toit en Louise Jackson (CRI)

Opsomming

Sitrus Grondvesblok: Die totaal van 2 379 375 okkuleerhout voorsien vanaf die Sitrus Grondvesblok in 2003 was 95 393 meer as in 2002 met Star Ruby Pomelo en Bahianinha Nawel die mees populêre kultivars. 'n Vermindering in die aanvraag van saad met 221 liter in vergelyking met die vorige jaar en 'n tweede agtereenvolgende jaar 'n groot aanvraag vir saad uit die buiteland, hoofsaaklik China. Carrizo Citrange bly die mees populêre onderstam kultivar. Vanaf die ITSG is 28 nuwe bostam kultivars en 5 nuwe onderstam kultivars ontvang vir vestiging, evaluering en vermeerdering. 'n Tweede insekbeheerde kweekhuis is opgerig waarin 30 000 vermeerderingsbome gevestig gaan word.

Kwekery Akkreditasie: Die akkreditasieskema se riglyn is hersien en in die kwekerye geïmplementeer. 23 Kwekerye is geoudit waarvan 17 ten volle geakkrediteer is, 3 voorwaardelik, een nuwe kwekery goedgekeur en twee afgekeur is.

Boomsertifikate: As gevolg van EurepGAP akkreditasie vereiste, is 'n toename vir die aanvraag van boomsertifikate ondervind en is in die tydperk 595 geprosesseer en uitgereik.

Statutêre Verbeteringsprogram: Aansoek is gedoen by die Departement Genetiese Hulpbronne om die SASVP te registreer en hulp te verleen met die wetlike samestelling van 'n dokument ter voorlegging vir 'n Statutêre Verbeteringsprogram.

SA Telingsprogram en Internasionale kultivar bemiddeling: Die CGA en CRI se onderhandeling met die LNR oor mede eienaarskap van die ITSG kultivars uit hul telingsprogram het op 'n dooie punt uitgeloop. Dit is die rede waarom geen verslae vanaf die ITSG ontvang is vir publiserings nie. Geen internasionale bemiddeling is onderhandel nie omdat ons gewag het vir sekerheid oor bogenoemde ooreenkoms. Die SVP Advieskomitee sal die benadering tot kultivar ontwikkeling binne die sitrusbedryf oorweeg en strategiese aanbevelings oor die toekomstige rigting maak.

Kultivar evaluering: 'n Interne aanstelling is gemaak in die CRI van Johan Joubert wat al die Noordelike en binnelandse evaluasie projekte hanteer. Chris Alexander se dienste word op kontrak gebruik om die Wes- en Oos-Kaap se projekte te hanteer. Die evaluasie riglyn is in samewerking met die evalueerders hersien. Die LNR se evalueringsverslae van projekte wat deur hulle hanteer word, is nie aan ons verskaf nie as gevolg van die rede wat reeds genoem is.

in Oorsig van werksaamhede binne die SVP:

Sitrus Grondvesblok

Okuleerhout voorsiening in 2003 in vergelyking met die vorige twee jaar.
Die 10 mees gewildste kultivars seleksies.

2003			2002			2001		
Seleksie	Oku.	%	Seleksie	Oku.	%	Seleksie	Oku.	%
TOTAAL	2379375		TOTAAL	2283982		TOTAAL	1923589	
Star Ruby	212450	8.9%	Turkey	383970	16.8%	Turkey	350692	14.7%
Bahianinha	210220	8.8%	Bahianinha	262490	11.5%	Midnight	157860	6.6%
Turkey	203200	8.5%	Palmer	175410	7.7%	Palmer	142352	6.0%
Palmer	173730	7.3%	Star Ruby	164810	7.2%	Bahianinha	140540	5.9%
Nadorcott 1	169500	7.1%	Midnight	145450	6.4%	Cara Cara	120980	5.1%
Midnight	127450	5.4%	Nadorcott 1	91300	4.0%	Star Ruby	97882	4.1%
Eureka	117450	4.9%	Delta	84230	3.7%	Nadorcott 1	84500	3.6%
Newhall	102460	4.3%	Miho Wase	69400	3.0%	Delta	84480	3.6%
Miho Wase	102100	4.3%	Lane Late Cal.	69015	3.0%	Eureka	83555	3.5%
Lina	82050	3.4%	Eureka SL	66400	2.9%	Du Roi	55350	2.3%

Okuleerhout verskaf in 2003 per area in vergelyking met die vorige twee jaar:

Area	2003	%	2002	%	2001	%
Oos-Kaap	546660	23.0%	441902	19.3%	473369	24.6%
Wes-Kaap	365665	15.4%	272850	11.9%	181534	9.4%
Noord-Kaap	116990	4.9%	156905	6.9%	152215	7.9%
Kwazulu Natal	36030	1.5%	20600	0.9%	38250	2.0%
Limpopo	930650	39.1%	804120	35.2%	712026	37.0%
Mpumulanga	244590	10.3%	376205	16.5%	192190	10.0%
Noord-Wes	101100	4.2%	121350	5.3%	109665	5.7%
Mozambique	5400	0.2%	0	0.0%	8500	0.4%
Swaziland	10600	0.4%	49400	2.2%	43550	2.3%
Ander Afrika State	0	0.0%	1400	0.1%	0	0.0%
Zimbabwe	21700	0.9%	39250	1.7%	12290	0.6%
Totaal	2379385		2283982		1923589	

Saad verkope per onderstam seleksie en streek in 2003

Area	C35	CC	CM	FD	MXT	RL	RLS	SW	TC	VA	X639	YC	Total
Oos Kaap	20	41				41		18	28	8	8		164
KwaZulu Natal					2			6	4				12
Limpopo	46	162			54	95	10	262	61		46	18	754
Mpumalanga	1	10			8	40		13	1		3		76
Noord-wes Provinsie	2	16			4	2		3			4		31
Noord Kaap								5					5
Swaziland									4				4
Wes Kaap	14	122				16			24.5		18		194.5
Zimbabwe						2		3					5

Totaal Afrika	Suider-	83	351	0	0	68	196	10	310	122.5	8	79	18	1245.5
Carribbean											12			12
Sjina			715							515				1230
Ander Afrika State										2				2.0
Reunion			8		0.5									8.5
Suid Amerika									4					4
Thailand				6							8			14
VSA			70											70
Vietnam											20			20
Totaal Uitvoer		0	793	6	0.5	0	0	0	4	517	40	0	0	1360.5
Groottotaal		83	1144	6	0.5	68	196	10	314	639.5	48	79	18	2606

Nuwe kultivars ontvang vanaf die ITSG na groeipuntenting vir vestiging, evaluering en vermeerdering:

Kultivar	Seleksie	Eienaar / Agent
Mandaryn	A3	LNR
Mandaryn	B18	LNR
Mandaryn	C27	LNR
Mandaryn	Clara	Citrospan
Mandaryn	Gold Nugget	Sunworld
Mandaryn	Honey Gold	CGA
Mandaryn	Murcott SL	CGA
Mandaryn	Nova Mutant	CGA
Mandaryn	Tacle	Citrospan
Midseison	Shamouti	CGA
Midseison	Tarocco Scire	Citrospan
Midseison	Tarocco Scire Nuc.	Citrospan
Midseison	Tarocco Tapi	Citrospan
Nawel	Coetzee Late	CGA
Nawel	Krajewski	CGA
Nawel	Leng	CGA
Pomelo	Henderson Mutant A13	LNR
Pomelo	Henderson Mutant A17	LNR
Pomelo	Pomelit Sel 2	LNR
Suurlemoen	Elongated Eureka	LNR
Suurlemoen	Triploid	Sunworld
Valencia	Benny 1 + 2	KGB / CTS
Valencia	Henrietta	B van Rooyen
Valencia	Jassie	CGA
Valencia	Kirkwood Red	CGA
Valencia	Letaba Oranje	B van Rooyen
Valencia	Midknight - 1	CGA
Valencia	Mouton Early	Oop
Valencia	Skilderkrans	Oop
Onderstam	Sweet Rough Lemon 72	LNR
Onderstam	Carizzo 669	LNR
Onderstam	Sour Orange Hybrid	LNR
Onderstam	Troyer 609	LNR
Onderstam	Sunki X Beneke	LNR

Nuwe uitbreiding

Om voorsiening te maak vir al die kategorie 1 en 2 kultivars onder insekbeheerde en geen reën toestande is 'n tweede kwekhuys van 3904 m² opgerig waarin 30 000 vermeerderings bome in 10 L plantsakke gevestig sal word.

Kwekery Akkreditasie

Die Suid-Afrikaanse Sitruskwekery Akkreditasieskema riglyn is in opdrag van die Sitrus Verbeteringsprogram Komitee hersien deur Dr Hennie le Roux, Dr Faan van Vuuren, Peter Kingston en Thys du Toit, en in die kwekerye geïmplementeer.

23 Kwekerye is twee maal in 2003 besoek deur Thys du Toit en die volgende is geakkrediteer:

Kwekery	Adres	Telefoon	Akkreditasie
Appapanzi	Posbus 147, Kirkwood, 6120	042-2300790	Volle
BF Joubert	Posbus 193, Kirkwood, 6120	042-2300309	Volle
Capricorn	Posbus 1925, Tzaneen, 0850	0834563148	Volle
Casmar	Posbus 3, Mooiooi, 0325	014-5743152	Volle
Du Roi	Posbus 66, Letsitele, 0885	015-3451650	Volle
Esselen	Posbus 100, Malelane, 1320	013-7900160	Volle
HJ Joubert	Posbus 207, Montagu, 6720	023-6142237	Volle
Letsitele	Posbus 114, Letsitele, 0885	015-3451600	Volle
Mistkraal	Posbus 106, Kirkwood, 6120	042-2301461	Volle
Ngwenya	Posbus 36, Malelane, 1320.	013-7903004	Volle
Nucellar	Posbus 69, Simondium, 7670	021-8601333	Volle
Paksaam	Posbus 16, Patensie, 6335	042-2830201	Volle
Stargrow	Posbus 189, Citrusdal, 7340	022-9212232	Volle
Tweeling	Posbus 190, Kirkwood, 6120	042-2301408	Volle
Vaalharts	Posbus 317, Hartswater, 8570	053-4740565	Volle
Waterfall	Posbus 339, Adelaide, 5760	046-6840738	Volle
Witkrans	Posbus 17, Boshoeck, 0301	014-5733036	Volle
Baddaford	Posbus 51, Fort Beaufort, 5720	046-6452814	Voorwaardelik
Green Gables	PK Summerville, 6107	042-2340421	Voorwaardelik
Sondagsrivier	Posbus 304, Kirkwood, 6120	042-2300349	Voorwaardelik
Westfalia	Posbus 14, Duiwelskloof, 0835	015-3090011	Nuwe kwekery

Boomsertifikate

EurepGAP registrasie vereis die sertifisering van 'n boord en daarom is 'n toename in die aanvraag na boomsertifikate ondervind en is in die tydperk 595 aan die kwekerye uitgereik vir oorhandiging aan die betrokke produsente.

Statutêre Verbeteringsprogram

'n Dokument ter beskrywing van die Suider-Afrikaanse Sitrusverbeteringsprogram is hersien nadat insette van die SVP Komiteedele ontvang is. Hierdie dokument is aan die Direkteur, Departement Genetiese Hulpbronne gestuur en aansoek is gedoen om die program te registreer en hulp te verleen met die wetlike samestelling van 'n dokument ter voorlegging vir 'n Statutêre Verbeteringsprogram.

Die Suid-Afrikaanse telingsprogram en Internasionale kultivar bemiddeling

Die CGA en CRI se onderhandeling met die LNR oor mede eienaarskap van die ITSG se kultivars uit hul telingsprogram het op 'n dooie punt uitgeloop. Dit is die rede waarom geen verslae vanaf die ITSG ontvang is vir publiserings nie. Geen internasionale bemiddeling is onderhandel nie omdat ons gewag het vir sekerheid oor bogenoemde ooreenkomste. Die SVP Advieskomitee sal die benadering tot kultivar ontwikkeling binne die sitrusbedryf oorweeg en strategiese aanbevelings oor die toekomstige rigting maak.

Kultivar evaluering

'n Interne aanstelling is gemaak in die CRI en Johan Joubert het verantwoordelikheid aanvaar vir al die Noordelike en binnelandse evaluasie projekte. Chris Alexander se dienste word op kontrak gebruik om die Wes- en Oos-Kaap se projekte te hanteer. Die evaluasie riglyn is in samewerking met die evalueerders hersien. Die LNR se evalueringsverslae van projekte wat deur hulle hanteer word, is nie aan ons verskaf nie as gevolg van die rede wat reeds genoem is.

8 INTERNATIONAL VISITS

8.1 V. HATTINGH

8.1.1 Visit to Brussels - 25 - 28 May 2003

Late in 2002 it became apparent to the SA citrus industry that the continued acceptability of 2,4D residues on citrus exported to the EU was under threat. Numerous measures were taken in an attempt to avert the replacement of 2,4D MRLs in EU Member States by an EU MRL of 0.05 ppm, as this would make it impossible to continue using 2,4D on citrus exported to Europe. By April 2003 it had become clear that extraordinary intervention was going to be required to avert a crisis.

VH mobilised an international trade delegation, including representation from SHAFTE (Southern Hemisphere Association of Fresh Fruit Exporters), CLAM (representing the Mediterranean citrus industries), Freshfel (representing fresh fruit trade in the EU) and COLEACP (representing African, Caribbean and Pacific country exporters) to meet with various EU officials in Brussels. All internationally available and relevant data on 2,4D was acquired and collated in a data package for presentation to the EU by the trade delegation. The trade delegation met with the chairperson of the EC MRL Committee responsible for the formulation of EU legislation relating to MRLs. Further meetings were held with representatives from EU Directorate Generals of Trade and Development. The SA Embassy's Agricultural representative and the SA Ambassador to the EU were briefed. Representatives from the Swaziland and Zimbabwe embassies were also briefed.

The EC MRL Committee took extraordinary steps to reach a decision in support of the adoption of an EU MRL for 2,4D residues on citrus and to implement these measures with unprecedented haste. These meetings, together with a multitude of other actions, have resulted in the adoption of temporary import MRLs (officially and unofficially) for 2,4D on citrus exports to the EU until revised EU legislation can be entered into force towards the end of 2003.

8.1.2. Visit to UK and Spain - 5 – 11 October 2003

Objective

The Committee for Coordination of Mediterranean Citrus (CLAM) holds an annual general assembly to provide a platform for exchange of industry information between the Mediterranean citrus industries. Producers, importers and exporters from Spain, France, Italy, Greece, Cyprus, Turkey, Israel, Egypt and Morocco regularly participate. From elsewhere Florida and Argentina routinely participate. South Africa was a standing member prior to 1997. In 2001, the Secretary General of CLAM approached the author and Justin Chadwick with a request to reinstate South African participation. The author subsequently attended the CLAM General Assembly in Montpellier in October 2002. This led to the strengthening of relations with international citrus industry contacts and the establishment of several new contacts. This network proved to be valuable in resolving the 2,4D residue crisis in 2003. The CGA consequently requested the author to maintain this network by again participating in the General Assembly in Madrid, 09 & 10 October 2003.

The UK Pesticide Safety Directorate coincidentally announced the development of an imminent MRL crisis in the UK shortly before the CLAM meeting. Consequently a stop-over in the UK was included to discuss this issue with various parties (6 to 8 October 2003).

MRL issues

An announcement describing the imminent MRL crisis in the UK was recently distributed by the Fresh Produce Consortium (FPC) and is appended to this report. Both the UK Pesticide Safety Directorate and Fresh Produce Consortium sent out notifications to industries encouraging them to revert to FPC and PSD with an indication of essential usages and temporary UK MRLs required. This led to considerable confusion among citrus producers, exporters and importers. In consultation with the FPC and PSD it was agreed that it would be advantageous if a consolidated industry position on this issue could be lodged with PSD. The author consequently conducted an analysis of the situation and produced a list of essential UK temporary MRLs required, together with motivations for the basis of their establishment. Likewise, the deciduous fruit industry was encouraged to do the same and Lindi Benic provided a similar list for deciduous fruit to be discussed with the PSD.

Discussions were first held with Andrew Richardson from Capespan who is very knowledgeable on this issue. Further discussions were held with Bayer CropScience in Cambridge to obtain the inputs of an Agricultural Chemical company directly involved in both the UK National and EU MRL regulatory issues.

A meeting was held with PSD in York to present a consolidated list of temporary UK MRLs required for citrus and deciduous fruit exports from these southern African industries. It was firstly confirmed that the southern African industries' interpretation of the situation and requirements was correct. The relevance of the process followed in analysing the industries' needs in this regard was verified with PSD. It was evident from these discussions that significant direct cost to the southern African fruit industries in addressing the specific issue of temporary UK MRLs are unlikely to occur.

The details of further actions required were agreed upon as being:

- (1) the industries need to access additional supportive data for a number of chemicals;
- (2) the southern African subtropical fruit industry needs to be encouraged to conduct the same exercise.

Although the issue of temporary UK MRLs has now been adequately addressed in terms of the southern African citrus and deciduous fruit industries, there was agreement that unless some of the currently proposed amendments to EU MRL legislation are amended, the consequent implementation thereof would seriously disrupt southern African fruit exports to the EU. It was agreed that the support of various parties (eg. CLAM, SHAFPE and Freshfel) would be sought in calling for amendments to current proposals for amendments to EU MRL legislation. The required amendments to draft EC Regulation 2003/0052(COD), were identified as being:

- (1) Extend the period for completion of required active ingredient – crop combinations from 1 year after first Annex listing of the active ingredient as currently proposed, to 4 years, before adoption of LOD for unsupported combinations.
- (2) On adoption of the new legislative framework, transfer existing 76/895, 90/642, 95/2, National Member State MRLs and Codex MRLs into temporary EU MRLs.

PSD was informed in the meeting that SA had similarly lodged an official objection to the proposed EU MRL legislative amendments through the WTO notification procedure. It was agreed to maintain an open channel of communication and to continue collaborating on these issues.

A meeting about these issues was also held with Mr Doug Henderson, Chief Executive of Fresh Produce Consortium and it was agreed that a cooperative approach to addressing the issue would be beneficial. The situation was also brought to the attention of the CLAM General Assembly. It was agreed that the author would provide the CLAM Secretary General with details of the required adjustments to the currently proposed (amended) EU MRL legislative framework and that CLAM would take appropriate action.

CLAM

The primary objective of the CLAM General Assembly is to exchange information between citrus industries to be able to better coordinate citrus exports, particularly to the European markets. Detailed and comprehensive statistics on production, processing, exports, market segmentation and timing were provided by all participating industries. A copy of the reports has been supplied to the CGA (Justin Chadwick) and a copy lodged in the CRI library in Nelspruit.

Some points of interest arising from the meeting were as follows:

1. Whereas, citrus production from southern Africa was listed as 1.7 million tons, some interesting comparisons were China 11.6, Mexico 6.1, India 4.7, Pakistan 2, Iran 3 and Mediterranean citrus industries 17.1 (Spain 6, Italy 2.8, Egypt 2.5, Turkey 2 and Morocco 1.3). The Med industries account for 65% of world exports, but only have 25% of world production.
2. Overall CLAM exports continued to grow, primarily due to Turkey and Egypt, which are showing strong growth in both production and exports. Whereas Israel's decline appears to have levelled off, the Italian volumes continue to decline.
3. The primary growth in destination markets was Russia, Poland and other E European countries (Easy peelers, oranges, grapefruit and lemons). Spain now exports approximately equal volumes of easy peelers to W and E Europe.
4. Attention is being given to the development of late season orange production in the Mediterranean countries and this could impact in future on the southern hemisphere's entry into the market at the beginning of the season.
5. Although blood oranges and Shamoutis continue to show a strong decline, blood oranges remain an important niche opportunity for the Italian industry.
6. Mediterranean countries' production is expected to be 5% lower than last year.
7. Spain's citrus processing volumes (Satsumas) were adversely affected last year by China's activity in this arena.

8. Spanish exports to USA were 60 000 tons last year.
9. Spain is in the process of replacing Marisols with an improved cv.
10. Spain is in the process of removing approx 50% of Fortuna plantings due to *Alternaria* sensitivity.
11. Spain may export oranges to Japan, but does not currently export due to unfavourable exchange rate and the complicated export programme. Spain has been trying to open the Japanese market to Clementine exports for 8 years.
12. Spain and Israel are trying to open the Chinese market for exports. They are concerned that the Chinese do not appear to be willing to accept cold as a fruit fly disinfestation treatment.
13. The protracted drought in Morocco was broken during this season.
14. Italy has high levels of organic citrus production.
15. The Italian citrus industry is planting two new cultivars: Tagla and Clara.
16. The Italian citrus industry is being severely affected by Tristeza virus.
17. Italian production has been adversely affected by bad weather and volcanic dust fallout in Sicily.
18. Israel reports that despite earlier optimism about the potential of Winola, they are encountering serious production problems with the cultivar. Israel also has production problems with the Mor and Or cultivars.
19. A large new dam has been built in SE Turkey that will support further citrus plantings in the region.
20. The Japanese market opened up for Argentina in 2003.
21. Argentinian exports of grapefruit to the EU increased by 25% in 2003 despite a 30% reduction in grapefruit production.
22. Ten E European countries are in the process of being included in the EU. This will mean that requirements for exporting to W European EU Member States will also apply to E European exports.
23. The European Commission is expected to become more involved in overcoming phytosanitary barriers restricting exports from the EU.
24. It was decided that CLAM's technical committee will actively take up issues relating to MRLs and other EU regulations potentially impacting on citrus trade, including traceability. This committee will also take the EurepGAP system under consideration. The southern African citrus industry was formally invited to actively participate in the CLAM technical committee, but it is expected that the industry should first become a member of CLAM.
25. The CLAM technical committee has coordinated evaluation of area wide fruit fly control strategies. These include Sterile male fly releases and field sterilization of male flies with the use of Insect Growth Regulator (lufenuron) impregnated feeding stations. The EU is providing Euro 12m pa to fund this project.
26. A private company offering SIT fruit fly control (Insecta) has received a Euro 2.5m pa EU grant to improve SIT technologies for fruit fly control in the Mediterranean.
27. It is envisaged by some parties that the EU Common Agricultural policy will move funds away from direct subsidies towards research and support services.
28. There was agreement to continue close cooperation between CLAM and the FAO inter-governmental working group on citrus. Currently South Africa does not have representation on this FAO working group. The coordinator invited the southern African citrus industry to participate and indicated that it is not necessary for representation to be directly from government.

Conclusion and recommendations

1. The UK temporary MRL situation seems now to have been adequately addressed.
2. Direct interaction with PSD, FPC and Freshfel on MRL issues should be maintained.
3. Urgent lobbying to muster support for opposing the impending changes to the EU MRL regulatory framework is required to avoid serious disruption to supplying the EU market over the next 4 to 5 years. Appropriate actions have been detailed in the report.
4. The CGA should consider becoming a member of CLAM to enable the industry to benefit from joining forces with the Mediterranean citrus industries in addressing various issues affecting the citrus trade. Accessing a means of influencing EU regulatory matters through internal EU channels is clearly more effective than trying to do so as a non-EU industry or trading country.
5. International networking of this nature should be maintained and extended in the future to enable southern Africa to muster the support of consequent contacts, particularly with regard to trade regulatory issues that continue to increase in importance in the international fresh product trade environment.

8.2 S.D. MOORE

Visit to the USA

Introduction

From 19–31 July I was in Burlington, Vermont, USA, for the Society for Invertebrate Pathology Annual Meeting, which ran from 26–30 July. This trip followed a visit to the Sterile Insect Release (SIR) programme in British Columbia, Canada, which was fully sponsored by the International Atomic Energy Agency (IAEA). A joint report with Hendrik and Marsheille Hofmeyr has been submitted on the visit to Canada.

The Commonwealth Scientific Council (CSC) promised sponsorship to cover flights to and from the USA and congress fees. However, to date no payment has been received despite claims being submitted and regular reminders being forwarded. Accommodation, S & T and additional transport were paid by CRI.

Itinerary

Date	Place	Institution	Activity	Mode of transport
12 July 2003	Cape Town	Cape Town International Airport	Travel to Vancouver via London	Air
13 July 2003	Vancouver	Vancouver International Airport	Entering Canada	Air
13 July 2003	Kelowna	Airport	Visit SIR	Air
19 July 2003	Kelowna	Airport	-	Air
19 July 2003	Vancouver	Vancouver International Airport	Exiting Canada to USA (to attend scientific congress – not sponsored by IAEA)	Air
19-31 July 2003	Burlington	Vermont State University	Society for Invertebrate Pathology Meeting	Air
31 July 2003	Vancouver	Vancouver International Airport	Transit from USA to return to South Africa (via London)	Air

Purpose of trip

The FCM granulovirus (GV) has been developed by CRI to a point where its commercial use is imminent. The annual congress of the Society for Invertebrate Pathology, held in Burlington, is a meeting of world leaders in the field of insect pathology and microbial (including viral) control. The objective of this visit was to gain knowledge from experts in relevant and specific areas, and to invite a peer review of the work conducted thus far on the FCM GV.

Programme

The congress, which focused on research conducted on various aspects of all pathogens infecting a range of invertebrate hosts, was attended by researchers from all over the world. In total there were approximately 250 participants. The society consists of six specialist divisions: viruses, bacteria, fungi, microsporidia, nematodes and microbial control. The meetings were also structured around these areas of interest. The congress consisted of plenary addresses, symposia, workshops, contributed oral presentations and contributed poster presentations. There were five contributed paper sessions for viruses, more than for any other topic.

Details of symposia attended

Conservation microbial control

Conservation of *Neozygites fresenii* in cotton. D. Steinkraus (USA).

Managed field margins as refugia for *Pandora neoaphidis*. P.A. Shah & J.K. Pell (UK).

Hedgerows, flies, aphids and winter survival of Entomophthorales. J. Eilenberg & C. Nielsen (Denmark).

Conservation of natural enemies of weeds and plant pathogens. H.C. Evans (UK).

Workshop: Microbial control products: What's in the pipeline?

Biologic. Albert Pye (USA).

LUBIOLOSA. Jurgen Langewald (Benin).
Certis. Michael Dimock (USA).
Valent Bioscience. Andrew Raath (Australia).
Calliope. Antoine Bonhomme (France).

Is bigger always better? A comparison of industrial-scale vs. cottage industry-scale production of microbial pesticides

Do we have it in the bag? – Production of *Metarhizium anisopliae*. J. Langewald (Benin), N.E. Jenkins (UK), B. Ali (Trinidad & Tobago), M. Bruntrup (Germany) & D. Moore (UK).
Entomopathogenic nematode production. D.I. Shapiro-Ilan (USA).
Production of biopesticides in developing countries: the roles of cottage industry, NGOs, state sector enterprises and private commercial producers in Asia. D. Grzywacz (UK), U. Ketunuti (Thailand) & H. Warburton (UK).
Commercialising mycoinsecticides. S.T. Jaronski (USA).
“Evolutionary ecology” of the microbial pesticide industry: Does size really matter? M.B. Dimock (USA).

Insect resistance mechanisms to viruses: Beyond the midgut

Clues from viral genomes to insect anti-viral immune responses. B.A. Webb (USA).
Luteovirus transmission barriers in aphids. S. Gray, F. Gildow, D. Cox-Foster & M. Caillaud (USA).
Apoptosis as a defense response against virus infection in insects. T.E. Clarke, L. Heaton & R.J. Clem (USA).
Virucidal activity against HzSNPV in plasma of *Heliothis virescens*. H.J.R. Popham, K.S. Shelby & S.L. Brandt (USA).
Intra-stadial developmental resistance of gypsy moth to its own baculovirus. D. Cox-Foster, M. Grove, S. Su, J. McNeil & K. Hoover (USA).

Host altered behaviour: Host mediated or pathogen induced

Changes in host behaviour: host altered or pathogen induced? H. Roy (UK).
Manipulation of host behaviour by entomopathogenic fungi. A.E. Hajek, J.E. Losey, C. Gilbert (USA).
Host manipulation by insect baculoviruses. J.S. Corey (UK).
Alteration of host physiology and mating behaviour resulting from virus replication. J.P. Burand (USA).
Manipulation of sexual reproduction by the intracellular bacteria *Wolbachia*. S. Bordenstein.
Disease resistance in crowds, density dependent prophylaxis in the Egyptian armyworm. S.C. Cotter, R.S. Hails, J.S. Corey & K. Wilson (UK).
Behaviour of nematode-infected insects and of scavengers to nematode-killed insects. H.K. Kaya & L. Luong (USA).

You are what you eat: Multitrophism in invertebrate pathology systems

Plant mediation of bacterial disease and lethality in insects. G.W. Felton, I. Ali & S. Young (USA).
Influence of transgenic BT plants on the performance of *Macrocentrus singulum*, a parasitoid of *Ostrinia nubilalis*. S.L. Sked, D.D. Calvin, C. De Moraes & N. Ostiguy (USA).
The influence of host plant on the ecology of insect-baculovirus interactions. J.S. Corey (UK).
The influence of host plant on the ecology of insect-baculoviruses. K. Hoover, G. Felton & R. Plymale (USA).
Tri- and tetratrophic level effects on entomopathogenic nematodes. A.M. Koppenhofer (USA).
Interactions between nematodes, insects and other microorganisms in forest ecosystems: An assortment of symbiotic associations in detrital food webs. S.P. Stock.

Epizootiological modelling

Entomophaga maimaiga and the Gypsy Moth: Insights from a model. R.M. Weseloh (USA).
The dynamics of inoculum persistence in the infection of the Colorado potato beetle with *Beauveria bassiana*. F.A. Drummond & E. Groden (USA).
Combining mechanistic and statistical modelling to predict epidemics in insect populations. G. Dwyer, B. Elder & M. Coram (USA).
Modelling *Nosema* disease in honey bee colonies. D.W. Onstad, D.W. Crowder & Z. Huang (USA).

Formal contribution by Sean moore to the programme:

I presented a paper in the Viruses I Contributed Papers session. The abstract for that paper, which appears in the formal congress proceedings is as follows:

Control of false codling moth on citrus with a South African isolate of *Cryptophlebia leucotreta* granulovirus (CrleGV-SA)

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False codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Olethreutidae), is a fruit pest of citrus, macadamias, stone fruits, avocados and litchis, in southern Africa. Chemical control of *C. leucotreta* is problematic for a number of reasons. Recently, a novel isolate of the *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) was described by restriction endonuclease analysis. In surface dose bioassays on artificial diet, LC₅₀ and LC₉₀ values with neonate larvae were estimated to be 4.095×10^3 OBs/ml and 1.185×10^5 OBs/ml respectively. LT₅₀ and LT₉₀ values with neonate larvae were estimated to be 4 days 22 h and 7 days 8 h, respectively. Detached fruit (navel orange) bioassays with neonate larvae indicated that virus concentrations that are likely to be effective in the field range from 1.08×10^7 to 3.819×10^{10} OBs/ml. In surface dose bioassays with fifth instar larvae LC₅₀ and LC₉₀ values were estimated to be 2.678×10^7 OBs/ml and 9.118×10^9 OBs/ml respectively. LT₅₀ and LT₉₀ values were estimated to be 7 days 17 h and 9 days 8 h, respectively. These values are relevant for *in vivo* mass production of CrleGV-SA. In four field trials, unformulated crude CrleGV-SA consistently reduced *C. leucotreta* larval infestation by around 60% for between five and nine weeks. CrleGV-SA formulated with molasses and a wetter reduced *C. leucotreta* infestation by around 80% over a nine-week period in two trials. These results were consistently better than those achieved with the insect growth regulator, triflumuron. Reasons for these impressive results and prospects for future use of CrleGV-SA in integrated pest management are discussed.

Value of visit

The information presented and discussed at this congress has great potential value for the southern African citrus industry. The particular area of interest to my current research is the use of baculoviruses for the control of lepidopteran pests of agricultural crops – in particular, control of FCM with a granulovirus. In the programme, there were more contributed paper sessions on viruses (5) than on any other single topic. This highlighted the high proportion of research work being conducted on insect viruses. Much of this work is applied or applied-orientated, as shown by some of the microbial control symposia topics e.g. conservation microbial control and production of microbial pesticides. The latter workshop highlighted the advantages and difficulties of both large-scale high-tech production and small-scale low-tech production of microbial pesticides. An encouraging message which came out of this symposium was the attainability of commercial production systems within a third world environment. Developed countries such as Switzerland, France and Australia have large high-tech production companies operating successfully. Whereas developing countries such as Thailand and India have similarly successful production plants, which operate on a much smaller and less sophisticated scale.

Several very valuable informal discussions were held with researchers and commercial producers of baculoviruses. Consequently, information was obtained which will assist in the commercial production and improved formulation of the FCM GV. Potential problems, which should be addressed or avoided, were also highlighted.

This meeting was therefore very valuable – not only in the formal dissemination of good information, but in informal networking between delegates, and the establishment of valuable contacts throughout the world.

8.3 **REPORT ON A SCIENTIFIC VISIT TO THE STERILE INSECT RELEASE PROGRAM IN CANADA AT THE INVITATION OF THE INTERNATIONAL ATOMIC ENERGY AGENCY**

By Hendrik (C6/SAF/03012PV) and Marsheille Hofmeyr (C6/SAF/03028PV), and Sean Moore (C6/SAF/03009PV)

A group of six people were invited to visit the Sterile Insect Release (SIR) programme in British Columbia, Canada, i.e. Hendrik and Marsheille Hofmeyr, Sean Moore (all of CRI), Dr Brian Barnes (Infruitec, ARC) as well as two fellowship visitors from South Africa, Stephan Honiball (Ceder Biocontrol, Citrusdal) and Daleen Henrico (Hortec, Grabouw). The authors are pleased to submit their report on their visit to the Codling Moth (CM) Okanagan-Kootenay SIR (OK-SIR) program at Osoyoos from Monday 14 July to Friday 19 July 2003.

(a) Background to visit

As scientists of CRI, we are mainly involved in research to determine the feasibility of SIR for the control of a major citrus pest in southern Africa, the false codling moth (FCM), *Cryptophlebia leucotreta*. It is an indigenous pest that has developed a variable degree of resistance to all registered pesticides. Only a cultural control method such as orchard sanitation, and augmentative releases of the egg parasitoid, *Trichogrammatoidea cryptophlebiae*, offer any hope for FCM suppression in the future. It is therefore of the utmost importance that alternative methods of control, such as SIR, are developed and evaluated. FCM is currently reared by three organisations in South Africa, viz. a small rearing unit in Port Elizabeth, maintained by Sean Moore for research purposes, and two commercial rearing units. These are Ceder Biocontrol in Citrusdal, Western Cape, managed by Stephan Honiball, which have been supplying egg parasitoids to the fruit industry for several years, and a recently erected facility, Du Roi Insectaries, in the Limpopo Province, who have yet to become commercially active.

Research on FCM SIT was initiated in 2002 by Drs Stephanie Bloem and Jim Carpenter, and supported by CRI. Until now, the work has progressed extremely well and according to plan. F1 sterility has been investigated in laboratory and field cage studies, while the prospect of SIR augmentation with egg parasitoids has also received detailed attention. We are therefore probably approximately a season away from the first sterile FCM pilot releases, which will be attempted in Citrusdal. This means that two factors will become of fundamental importance during the next few years, viz. (i) the mass rearing of FCM on a scale sufficient to supply the material necessary for the large scale application of SIT, and (ii) the physical release of sterile moths in the orchards.

The IAEA invitation to visit the Codling Moth SIR facility at Osoyoos was therefore extremely relevant and timely.

(b) Itinerary

Saturday 12 July: Depart Cape Town for Kelowna via Heathrow and Vancouver.

Sunday 13 July: Arrive Vancouver, board local air transport, depart for and overnight at Kelowna, British Columbia.

Monday 14 July: We were met by OK-SIR staff members Bob Fugger (General Manager) and Lynne Lashuk (SIR Agrologist). Lynn Lashuk explained the history and progress of the sterile insect technique (SIT) programme for codling moth control in British Columbia. The Okanagan Valley area has been broken into three zones. Zone 1 is approximately 5900 acres, Zone 2 approximately 4400 acres and Zone 3 approximately 2300 acres. Releases of sterile moths have been conducted in Zone 1 for nine years now. During the last season, 97% of orchards in Zone 1 were damage free. Consequently, only 15% of the sterile moths produced in the SIR facility are now released in Zone 1. The remaining 85% of moths – 800 sterile moths per acre, twice a week – are released in Zones 2 and 3, which have not been part of the programme for as long as Zone 1. In these two zones it is still necessary to supplement sterile male releases with chemical control and mating disruption.

We also visited an apple farm near Kelowna with Lynn, where an SIR field officer demonstrated the release of sterile CM with ground-based moth application apparatus.

SIR General Manager, Bob Fugger, met us at the main office in Kelowna, and we toured the administrative and technical facilities.

Met with Drs. Stephanie Bloem (IAEA Consultant) and Jim Carpenter (USDA-ARS) and departed for Osoyoos (SIR Zone 1). That evening (20:00 - 03:00) we assisted Bloem and Carpenter with trap counts in a mating trial with field released sterile male moths. The competitiveness of moths subjected to different doses of irradiation (0, 150 and 250 Gy) was compared.

Tuesday 15 July: We visited the SIR insectary at Osoyoos. The SIR Plant Manager, Lorne Tomlin, gave us a detailed guided tour of the production facility. This included examination of moth oviposition and egg collection, diet preparation and inoculation (with eggs/neonate larvae), surface sterilisation of eggs, eclosion and collection of moths, irradiation of moths and packaging of irradiated moths.

A total of 30 staff members are employed in the production facility. The facility operates seven days a week, 24 hours a day, in three shifts. Larvae are reared on an elaborate synthetic diet, which is prepared in 60 gallon quantities, several times a day. A total of around 2 100 trays of diet are prepared each day (5 days a

week). On each tray approximately 1 500 moths are reared. Consequently a total of approximately 2 million moths are produced per week, and about 300 million moths per year.

Probably the two most important contaminants with which problems are experienced during the rearing process are the *Cydia pomonella* granulovirus (CpGV) and vinegar flies (*Drosophila melanogaster*). Periodic air quality tests are conducted with agar plates in order to test for fungal spores.

Occasionally new codling moth genetic material is introduced into the culture from field collected material. This is done by collecting larvae from apples, allowing them to pupate, sexing the pupae, and only introducing male moths into the culture. Only male moths are used in order to eliminate the possibility of transovarially introduced pathogens and in order to avoid the problem of ovipositional adaptation of female moths to laboratory conditions.

Wednesday 16 July: A second visit was paid to the SIR insectary, this time hosted by Scott Arthur (Maintenance Supervisor) and Val Pleasance (Quality Control).

The Quality Control Manager, Val Pleasance, instructed us on, and demonstrated quality control procedures in the facility. This included sexing of larvae and adults (ratio should be 1:1), inspection of diet for dead larvae, testing of diet pH (should be 4.6), measurement of egg hatch (should be 80%) and determination of percentage mating taking place in the emergence room (a mean of 19% has been measured).

The Maintenance Supervisor, Scott Arthur, gave a detailed overview and tour of the SIR building equipment and operations. The computerisation of the monitoring and control systems for the environmental management of all rooms was demonstrated.

Most rearing rooms were fitted with a triple air filtration system, the last filter being a hepa-filter with a 0.3 μ aptitude.

Later on the Okanagan Valley (Osoyoos – Kaleden) was toured for a topographical familiarization with the SIR area – hosted by Bloem and Carpenter. During the late afternoon Carpenter, Bloem and Shannon Taggart (contract field worker) demonstrated the operation of an all-terrain vehicle (ATV, “quad bike”) fitted with a sterile moth release device. The opportunity was afforded to test drive the ATV and operate the release device.

Thursday 17 July: Drs. Carpenter and Bloem hosted a visit to the Similkameen Valley, the site of the first SIT field release trials conducted by Dr. Jinx Proverbs during the 1970s. This valley was selected for the programme because of its geographic isolation and its reasonably small size. There are approximately 640 ha of apples and pears in the valley with 95% of the trees in a 2 600 ha area. About 90% of all neglected trees in the valley were destroyed during 1972. Trial releases in the valley were conducted from 1976 to 1978 in 320 - 526 ha. Moths were sterilised with 350 Gy and released 2 – 3 times a week from May until early September. The trial was highly successful and by 1978 damage did not exceed 0.5% (the economic threshold level) in any one of the 157 orchards included in the trial. Possibly the most influential factor inhibiting the development of SIT thereafter was the cost. The implementation of SIT was calculated at that time to be \$225/ha compared to \$95/ha for chemical control.

Friday 18 July: The SIR insectary was revisited for follow-up discussions on insect rearing procedures. Shift Supervisor, Joanne Parker, took us through parts of the production facility that we had either not yet seen in operation or on which we needed more clarity of understanding. This included a visit to the laboratory in which codling moth eggs were surface sterilised and a visit to the oviposition room.

A meeting with the Production Manager, Lorne Tomlin, allowed us a final opportunity to clarify certain issues. Staff issues, such as motivation and remuneration were discussed, as were funding issues and the future of the programme in the Okanagan Valley.

Saturday 19 July: Departed for Heathrow (JHH and MH) and USA (SM) via Kelowna and Vancouver.

Sunday 20 July: Departed Heathrow for Cape Town.

(c) Overview of visit

The entire visit from our departure from South Africa, to our arrival back, was well planned and our compliments to all concerned, including Brian Barnes, Drs Bloem and Carpenter, the staff at Osoyoos and the I.A.E.A.

Codling Moth rearing: The OK-SIR facility is of first world standard, and will be difficult to duplicate in South Africa. The economic reality in South Africa is that little assistance for the establishment of such a facility will be forthcoming from the national government, while the contribution, if any, from provincial level will be completely inadequate. Running and maintenance costs will have to be carried by the grower community as, for example, a levy on property tax from house owners (as in British Columbia) will probably not be feasible. It will therefore be a major challenge to try to emulate the extremely high standard set by OK-SIR with inadequate funds and, unfortunately, a labour force which, on average, very often has questionable loyalties and inadequate motivation. Many techniques will have to be adapted to suit local conditions. This will very likely involve possible changes to the diet used, the level of sanitation applied, and the ability to react to crises without interrupting the flow of sterile insects and the rearing process.

False Codling Moth rearing: SM is involved in the modification of the artificial diet used for rearing FCM. This will change the current sterile diet (enclosed in rearing jars), to an asterile (open tray) diet, and will eliminate a major problem with the current technique – both in terms of the reduced risk of fungal and bacterial contamination and also in labour requirements.

Some techniques used in the OK-SIR facility may eventually prove to be applicable to the FCM rearing unit, especially with regard to the use of lighting and the management of humidity levels in the rearing rooms that facilitates drying out of the diet and eclosion of the pupae. The air flow collection system as used in the OK-SIR process is very interesting, but may also prove to be too expensive to install locally. As a matter of interest, and keeping in mind that our views are those of outsiders not intimately involved in the conceptual studies and decisions that led to the design of the current process, this aspect is probably the least impressive of the OK-SIR rearing process. According to data supplied, approximately 20% of moths are already mated by the time of collection, while a further 18% of moths are lost being unable to reach the ceiling mounted extraction vents. The last mentioned is regarded as a positive feature, as it eliminates uncompetitive moths. However, releasing uncompetitive moths can also be regarded as a plus factor, as it may reduce predation of fit individuals upon release in orchards, by acting as alternative prey for predators. It would indeed be interesting to investigate the possibility that either one, or both, loss factors can be reduced by increasing the (perceived) weak output of the black luminescent light used in the extraction vents or by replacement with alternative more(?) attractive light sources such as UV lamps.

We regard this as a probable topic for research in the FCM insectary, where the current collection technique of “newly” emerged moths results in similar numbers of mated females.

Sterile insect release technique: The method used to distribute moths in the orchards using “quad bikes” was very interesting. The cold immobilized insects are propelled out of an air pump applicator by an air stream, and fall to the ground where they lie for many minutes (personal observation) before taking wing – usually only after being physically disturbed. This technique will also have to be investigated and probably modified for local use. This is mainly because of our local high summer temperatures (resulting in very hot soil which will kill moths falling to the ground in the sun) and suspected behavioural differences between CM and FCM.

Unfortunately we were unable to see aerial moth releases in action, which may prove to be a viable alternative to ground based machinery.

Similarities between the Okanagan-Kootenay (OKV) and Similkameen Valleys (SV) (both British Columbia), and the Olifantsrivier Valley (OV) (Citrusdal): The Olifantsrivier Valley in which Citrusdal is situated, is very similar to the SV, where the original research on F1 sterility was conducted by Proverbs. Both valleys are closed at one end without public access routes at the blind end. They are also relatively narrow – the SV more so than the OV. The surrounding mountains in all three areas (OKV/SV and OV) are barren and largely devoid of host plants for CM and FCM respectively. Orchards in all three valleys are planted near the rivers or lakes that run down all of them, and also on the foothills of the mountains. The plantings range from larger and closely arranged orchards to relatively isolated orchards located up to a few kilometres away from their nearest neighbours. The surface area of plantings in the OV is larger than in the OKV/SV, containing more than 6 000 ha of citrus, compared to the ca. 2 500 ha in Zone 1 of the OKV/SV (Osoyoos – Summerland). Plantings in the OV is concentrated in a slightly smaller geographical area, being approximately 80 km long, in contrast to Zone 1’s 100 km. It therefore seems that there is little, at least from a geographical point of view, that will prevent SIR being applied successfully in Citrusdal.

(d) Value of visit

SM’s area of responsibility in the investigation and development of the sterile insect technique in South Africa is the improvement of methods for mass rearing of false codling moth. In light of this, the visit to the SIR facility in Canada was invaluable. First hand detailed information was obtained from many of the people

involved in the production of sterile codling moths and in the implementation of the programme in apple orchards. A detailed manual of all production, operational and quality control protocols, a detailed recipe of the diet used for rearing codling moth and samples of the diet which can be tested for FCM were obtained. We have consequently obtained a very accurate impression of what is required to implement a similar programme for false codling moth control in South Africa. Two factors which stood out as being crucial to the success of the Canadian programme were the unwavering adherence to best practice protocols and a daily and detailed quality control agenda. The facility in Canada is extremely "high-tech", and consequently it will not be possible to replicate locally much of what has been established there. It is nevertheless possible to determine what can be replicated and what can be adapted to suit our unique needs and financial restraints. We are convinced that much of what had been learned during our week's visit to Canada will be applied and tested in our research in the foreseeable future.

(e) Conclusion

The visit to the OK-SIR facility in Osoyoos, as planned and managed by Lorne Tomlin and his staff, can only be regarded as exceptional. The tours of the facility and all information supplied were conducted in a professional manner. Everything was done in a way that created an atmosphere of openness and all questions raised were answered fully, where necessary, supported by literature.

It needs to be emphasized that we have yet to see a business staffed with workers as obviously dedicated to their work as those in the OK-SIR rearing facility. The success of the unit is not due to the overt technical design and procedures, but to the level of enthusiasm showed by all and sundry, many of whom are involved in repetitive work, which must inevitably become tedious. It is our experience that this almost invariably leads to a lowering of standards with predictable results, especially where insect rearing is concerned. That Lorne Tomlin and his staff, ably managed by Bob Fugger, manage to circumvent this problem so successfully, is commendable and an example to us all!

In conclusion we would like to thank the I.A.E.A. for the invitation to visit Canada. It is much appreciated.

9 KNOWLEDGE TRANSFER

9.1 Summary

Knowledge Transfer Groups (KTGs)

Most of the KTGs, previously known as the citrus study groups, are functioning well. An intensive SWOT analysis was conducted in 2002 to determine the opportunities and threats of each of the areas. This assisted in accurately determining the research priorities for each area with the result that in most areas minimal changes in priorities took place between 2002 to 2003. Fruit fly received a higher priority rating in most of the areas because of threats from the EU. Rind disorders also received a high priority rating.

Each KTG has a technical committee where the research needs are determined. The KTGs are requested to inform the Extension Manager should there be any need for technical support. In the more established citrus growing areas, technical support is often not requested. In areas such as the Lower Orange River there is a higher demand. One such example in this area was the problem of fruit set. Soil scientists from CAL, an irrigation specialist from OHSSA, several horticulturists from the Cape and a root rot pathologist from CRI visited the orchards before holding a fruit set workshop with the producers. The problems were identified and recommendations were made. The results produced an increase in yields from 15 tons/ha to more than 50 tons/ha.

Keith Lesar was requested to visit many of the areas prior to the picking season to inform them on the latest developments with regard to post harvest pathology and post harvest treatments. Numerous talks were also given on IPM related aspects and Dr. Tony Ware visited 21 of the 22 KTGs to make them aware of the importance of fruit fly control during the 2004 season. Talks on false codling moth by Hendrik Hofmeyr and Dr. Sean Moore rated highly, and Thys du Toit gave a talk at the citrus symposium in Letsitele explaining the Citrus Improvement Programme. In the northern areas Dr. Tian Schutte discussed citrus black spot with several groups, and MC Pretorius gave talks on *Phytophthora* and nematodes. Dr. Graham Barry was involved with solving the fruit set problem in the Lower Orange River. Dr. Tim Grout gave a talk on red scale in Vaalharts, and the Spring Pest Complex meeting held in Nelspruit was also addressed by him. The EM emphasized the dangers of exotic diseases by giving talks on this topic to citrus growers, not only at the Letsitele citrus symposium, but also to several KTGs.

Scientists from other groupings were also involved in knowledge transfer. During a workshop in Nelspruit Gerhard Mostert gave a much appreciated talk on drought strategies, and both Dr. Hannes Coetzee (CAL)

and Japie Kruger (OHSSA) were involved in workshops with different KTGs. Dr. Fanus Swart was also involved in CBS control strategy meetings. All requests made by the different KTGs were adhered to and feedback on the research proposals which were approved were given to all areas.

Grower talks and presentations

Date	Place	Topic	Type of meeting
G.H. BARRY			
June 2003	Kakamas	Fruit set strategy	Benede Oranjerivier Study group
November 2003	Stellenbosch	Fruit quality enhancement	West Cape CTA
T.G. GROUT			
19 May 2003	Stellenbosch	SIR workshop for FCM and Natal fruit fly	Workshop
6-9 July 2003	Pretoria	Entomological Society congress	Congress
25 July – 7 August 2003	Mozambique & Zimbabwe	Talked to various individuals in Mozambique and Zimbabwe	Extension
28 August 2003	Nelspruit	Chaired Fruit fly discussion	Workshop
4 Sept 2003	Nelspruit	Talked on thrips, mealybug & Lepidoptera at Pest & Disease workshop	Grower meeting
J H HOFMEYR			
23 September 2003	Malelane Sitrus	Betryding van valskodlingmot	Produsente
K.H. LESAR			
6 February 2003	Protea Country Lake JHB	Packhouse Forum Meeting	Waste issues, treatments, waxing, new products, new research, etc.
19 February	Hygrotech Stellenbosch	Meeting with Hygrotech and ICA International	Trials conducted for registration of Sporekill
20 February	Study Group Stellenbosch	Packhouse Meeting. E. Cape & W.Cape	Waste issues, treatments, waxing, new products, new research, etc.
6 March	Nkwaleni Natal	Packhouse Meeting and Packhouse advisory visits	As Above
13 March	Marble Hall	As Above	As Above
20 March	NDA Pretoria	Chemical (Imazalil) residue working group	Strategy re report back of high Imazalil residues on Japan grapefruit
25-28 March	E.Cape	Packhouse meetings and Packhouse advisory visits	Waste issues, treatments, waxing, new products, new research, etc.
3 April	Malelane Co-op	Packhouse Meeting	Fact finding meeting to address the issue of high Imazalil residue on Japan grapefruit and future plan of action
12 May	J.J Joubert Schoemans kloof	Packhouse visit and orchard evaluation	Report on severe oleo problem on navels during degreening
14 May	Katco. E.Cape	Packhouse Visit	Packhouse issues. Treatments etc.
14 May	Riverside. E.Cape	As Above	As Above
15 May	SRCC E.Cape	Soft Citrus working group meeting	All issues related to soft citrus
26-29 August	Central Natal (Richmond etc.)	Packhouse Meeting and advisory packhouse visits	Waste issues, treatments, waxing, new products, new research, etc.

9 October	Malelane Co-op	Waste Workshop	Fact finding re high waste during season
6 November	Crocodile Valley Nelspruit	Packhouse Advisory and pre-harvest spray trials	Packhouse advise and organising of spray trials for post-harvest waste control
S.D. MOORE			
11 February 2003	Fort Beaufort	IPM: Practical decision making	Advanced course for growers
9 April 2003	Kirkwood	FCM and budmite management	Grower study group
25 June 2003	Addo	Management of the spring pest complex: recent research findings	Eastern Cape Citrus Technical Association
7 July 2003	Pretoria	Augmentation of natural enemies (Chairman)	Entomological Society of Southern Africa (workshop)
8 July 2003	Pretoria	FCM control with a granulovirus	Entomological Society of Southern Africa (oral session)
8 July 2003	Pretoria	Biological control of FCM (Poster)	Entomological Society of Southern Africa (poster session)
27 July 2003	USA	FCM control with a granulovirus	Society for Invertebrate Pathology
1 September 2003	Knysna	Biology and management of red scale, mealybug, thrips and FCM	Wenkem agents training
17 September 2003	Kirkwood	Management of red scale, mealybug and thrips	Grower technical meeting
18 September 2003	Addo	Management of red scale, mealybug and thrips	Grower technical meeting
M.C. PRETORIUS			
1-4Julie 2003	Strand	16 th symposium of the Nematological society of southern Africa	Symposium
July 2003	Nelspruit	Wenkem Growersday	Nematodes and Phytophthora
Sept 2003	Spain	FMC workshop	Nematodes
G.C. SCHUTTE			
29 July 2003	Loskop	Black spot & Alternaria	Grower
31 July 2003	Nelspruit	Black spot	Grower
8 October 2003	Tzaneen	Black spot	Grower
A.B. WARE			
11 Nov 2003	Citrusdal	Fruit Fly meeting	Study Group
12 Nov 2003	Swellendam	Fruit Fly meeting	Study Group
13 Nov 2003	Patensie	Fruit Fly meeting	Study Group
13 Nov 2003	Knysna	Fruit Fly meeting	Study Group
14 Nov 2003	Katrivier	Fruit Fly meeting	Study Group
14 Nov 2003	SRCC	Fruit Fly meeting	Study Group
18 Nov 2003	Vaalharts	Fruit Fly meeting	Study Group
20 Nov 2003	Marble Hall	Fruit Fly meeting	Study Group
20 Nov 2003	Rustenburg	Fruit Fly meeting	Study Group
25 Nov 2003	Zimbabwe	Fruit Fly meeting	Study Group
28 Nov 2003	Burgersfort	Fruit Fly meeting	Study Group
14 Jan 2004	Komatipoort	Fruit Fly meeting	Study Group
14 Jan 2004	Malelane	Fruit Fly meeting	Study Group
20 Jan 2004	Hoedspruit	Fruit Fly meeting	Study Group
20 Jan 2004	Letsitele	Fruit Fly meeting	Study Group
21 Jan 2004	Limpopo	Fruit Fly meeting	Study Group
3 Feb 2004	Nkwaleni	Fruit Fly meeting	Study Group
3 Feb 2004	Richmond	Fruit Fly meeting	Study Group
4 Feb 2004	Pongola	Fruit Fly meeting	Study Group
11 Feb 2004	Nelspruit	Fruit Fly meeting	Study Group

In many of the KTGs new chairpersons were elected during 2003. The listing below will assist growers who would like to become involved with the KTG in his/her area:

KTG (AREA)	NAME	TEL	E-MAIL
Lower Orange River	Francois Reyneke	082 771 6758	sabeth@mweb.co.za
Burgersfort	Elbert de Kock	013 231 7757	moronesitrus@intekom.co.za
Citrusdal	Dirk Visser	082 550 0158	dirkvisser@kingsley.co.za
Groblersdal	Chris van Ginkel	082 662 8426	valleiadvies@lantic.net
Hoedspruit	Org Boshoff	082 560 6309	grotro@worldonline.co.za
Katrivier	Lawrie Pringle	083 232 7943	technical@katco.co.za
Komatipoort	Dirk Horn	083 259 3359	sommerreg@soft.co.za
Knysna	John Stanwix	082 789 5051	knycit@mweb.co.za
Letsitele/Constantia	Pieter Vermaak	082 491 7743	nlc@mweb.co.za
Limpopo	Bennie Nicholson	083 306 0552	alicedal@lantic.net
Malelane	Leon Esselen	013 790 0160	esselenk@mweb.co.za
Marble Hall Midnight SG	John Howard	082 562 1203	
Nelspruit	Graham Piner	083 610 6095	crocval@mweb.co.za
Nkwaleni	Bester Snyman	082 896 2856	bsnyman@netactive.co.za
Paarl/Stellenbosch	Corrie Muller	083 631 7727	corriem@worldonline.co.za
Patensie	Ian Grieb	082 823 3960	iangrieb@gamnet.co.za
Pongola	Antoine Roulliard	082 651 1378	mvurshini@cybertrade.co.za
Richmond	Trever Dukes	082 945 7317	katnatal@futurenet.co.za
Rustenburg	Johan-Chris Grobler	082 922 1579	witkrans1@mweb.co.za
Sundays River Valley	Dave Gerber	072 292 2151	summersby@telkomsa.co.za
Swaziland	Gerd Hoppner	09268 3232311	hoppner@iysis.co.sz
Swellendam	Sarel Neetling	082 551 2357	thornlands@worldonline.co.za
Vaalharts	Tom Fouche	082 783 4842	marithaminie@mweb.co.za
Zimbabwe	Graham Crutchley		fruitveg@mweb.co.za

SA Fruit Journal

The SA Fruit Journal is becoming one of the most important extension tools for transferring knowledge to the growers. This publication is received with great enthusiasm by the producers. Unlike the Annual Report which is read by only a few growers, a large percentage of citrus producers enjoy reading this journal. Most of the problems with the distribution of this publication have been solved and the CGA is not aware of any exporters who do not receive it. Extension has a column in each issue called the Extension Briefs. This is used to update growers on technical aspects relevant to the time of the year in which the specific issue appears.

CRInet by Tim G. Grout

During 2003, the number of messages circulated on CRInet increased to 85 (Table 9.1.1) and membership grew to 155. It is proving to be a useful tool for the sharing of opinions on unusual fruit symptoms or phenomena. All members benefit from these discussions and any member may submit a message to the group, although they are moderated to prevent the circulation of junk mail.

Table 9.1.1. Numbers of messages circulated per month on CRInet since its inception in August 2001.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2003	1	4	6	14	22	4	3	6	5	6	11	3
2002	3	6	1	6	2	2	1	9	5	14	17	2
2001								2	4	2	4	

Cutting Edge

The role of the Cutting Edge has declined and has to a large extent been taken over by the Extension Briefs in the SA Fruit Journal and the CRInet. As more growers become familiar with the electronic media and link up with the CRInet the Cutting Edge will gradually phase out. At this stage urgent messages for the citrus industry are sent via the Cutting Edge.

CRI Website

Use of CRI's website (www.citrusres.com or www.cri.co.za) is increasing slowly. The value of the website lies in the downloads available to members. The public part of the website has been updated but further changes are planned to include announcements of grower meetings and more graphics. September 2003 was the busiest month of the website when the number of page requests exceeded 6 500. This may have been due to the latest MRL restrictions being posted on the site at this time. Monthly page requests are usually in the region of 3 000 to 4 000 (Figure 9.1.1). The search engines providing the most hits on our site are google.com followed by yahoo.com.

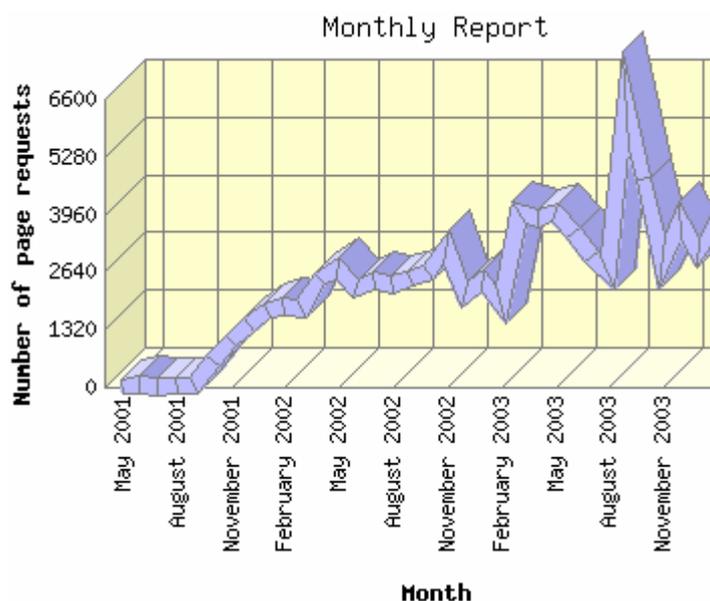


Figure 9.1.1. Growth in number of page requests on the CRI website until December 2003

Symposium

No Citrus Research Symposium was held during 2003. Talks were given at the annual Letsitele Citrus Symposium on the Citrus Improvement Programme (Thys du Toit) and on Exotic citrus diseases (Hennie le Roux). A venue was selected for the 2004 Citrus Research Symposium. It will be held at Modimolle (Nylstroom) from 24-27 May 2004. The timing may not suit all the different groupings and therefore future symposiums will be held at the end of August.

Egypt

Egypt was visited to conduct a *Phytophthora* and nematode survey of the El Magraby farms near Alexandria. It is expected that a boom in new citrus plantings in Egypt will take place as there is large interest from foreign parties to invest in new cultivars in this country. A full report is available from Hennie le Roux at CRI .

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