



**CRI GROUP
ANNUAL RESEARCH
REPORT
2005**

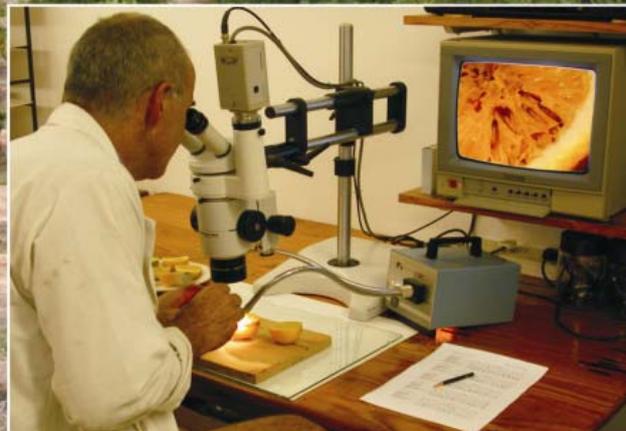


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1 INTRODUCTION

CEO (CRI): Vaughan Hattingh

This report records the research results and technical support services of the CRI group for the calendar year 2005. The 2005 year was noteworthy as being especially difficult for the industry, with generally very poor market performance, compounded by record export volumes. The Japanese market was over supplied, prices crashed and heavy losses were incurred. Access to the Chinese market remained severely constrained by complications with implementation of the export protocol. The USA market was temporarily closed due to phytosanitary concerns and although access was regained, it was in association with a more restrictive export protocol.

The adverse weather conditions in Spain and Morocco, preceding the 2005 Southern African export season, had severe knock-on effects in many of the southern African export markets. There was firstly freeze-damage, shortly after the soft citrus harvest in both Spain and Morocco and secondly hail damage to the Spanish lemon crop. The external hail damage to Spanish lemons provided Southern African lemon exporters with a market opportunity. However, this advantage was greatly out-weighed by the adverse effects of the freeze on the internal quality of Spanish and Moroccan oranges. This had a negative effect on consumer demand for oranges and, despite the reduced orange volumes supplied by Spain and Morocco, the first effect was to stall sales rates of Spanish, Moroccan and Egyptian oranges in the EU market. This had a knock-on effect of over-supplying the Russian market and increased supply to the Middle Eastern market, as alternative outlets for the Mediterranean oranges. The implications for Southern African navel export campaign, compounded by variable quality of the southern African product, were severe.

In addition to the difficult 2005 marketing season, the 2005 production season was also made difficult by drought conditions prevailing in much of the northern production areas. A disaster of potentially monumental proportions was narrowly averted by the timely alignment of CBS spray programmes with an unannounced and sudden revocation of EU residue tolerances (MRLs) for the fungicide carbendazim.

Fortunately, due to commendable insight shown by the industry, the support for industry research and technical services remained strong despite the financial difficulties experienced by the industry. Likewise, in support of its long-term strategic objectives, CRI achieved strong growth in its diversified (non-levy) income stream. This was principally due to the successful start-up of the citrus industry's technology commercialisation venture, River Bioscience. Another strong contributor, was the SA-EU Pesticide Initiative Programme (PIP), that the citrus industry had been instrumental in initiating.

CRI's in-house capacity was strengthened when the position of Manager Cultivar Development was filled by Dr. Graham Barry and the position of Area Extension Manager (Southern region) by Hannes Bester. CRI's organizational structure was accordingly amended by separating Cultivar Development from the Citrus Improvement Programme (CIP). The CRI Board endorsed a change in the structure and terms of reference of the CIP Advisory Committee and the formation of an advisory committee for Cultivar Development. Stephan Verreyne successfully completed a PhD in California and returned to SA, to take up the CRI position of Research Horticulturist with secondment to Stellenbosch University.

CRI continued to utilise the operational processes and procedures that had been successfully developed and implemented over the preceding years. A comprehensive revision of industry research priorities was undertaken. A strong increase in research focus on False Codling Moth (FCM) occurred in recognition of its phytosanitary status. The CRI Board appointed an advisory committee to provide guidance and recommendations on the fast-track commercialisation of the Sterile Insect Technique (SIT) for the area-wide control of FCM. Alarming, resistance to the post-harvest fungicide Imazalil was observed, leading to the development of a resistance management strategy for implementation in 2006. The need to enhance the industry's capacity in the fields of plant nutrition and plant pathology, were identified for action in 2006.

Despite the unfavourable economic climate within the industry, the Citrus Foundation Block (CFB) maintained a firm level of budwood sales, with only a marginal downturn in its objective of maintaining a break-even financial position. On the back of good financial performance by CFB in the preceding year, further infrastructural development projects were undertaken at CFB in 2005, as an investment in the future security of the industry.

The long-term nature of research makes it essential that momentum be maintained during times of economic difficulty in the industry. This is fundamental to the pursuit of CRI's long-term objective, as reflected in its mission "To maximise the long-term global competitiveness of the southern African citrus growers through the development, support, co-ordination and provision of Research and Technical services by combining strengths of all CRI Group partners". It is particularly encouraging to witness the continued level of strategic

foresight of the Citrus Growers Association of Southern Africa, as reflected in its unwavering support for industry Research and Technical Support Services. The burden of responsibility is upon each individual within the CRI group to ensure that this vote of confidence by the grower community is rewarded. The admirable progress reflected in the 2005 Annual Research Report reflects a high level of recognition of this by the CRI Group and bodes well for the future of the southern African citrus industry. The Board of Directors of CRI, the CGA, River Bioscience, the southern African citrus industry as a whole, the CRI Group Alliance Partner Organisations, and staff, are thanked for this commitment, confidence and achievement.

INLEIDING

Hierdie verslag sluit die resultate van navorsing sowel as tegniese ondersteuningsdienste in wat deur CRI-groep in die 2005 boekjaar uitgevoer en gelewer is. Die jaar sal onthou word as 'n besonders moeilike jaar vir die bedryf vanweë die swak prestasies van markte oor die algemeen tesame met rekord volume vrugte wat vir uitvoer beskikbaar was. Die oorvoorsiene Japanese mark het gelei tot swak pryse en ernstige verliese. Beperkte toegang tot die Chinese mark is nog steeds ondervind as gevolg van komplikasies met die implementering van die uitvoerprotokol. Die VSA-mark was ook tydelik gesluit weens fitosanitêre onsekerhede en alhoewel die mark weer later oopgestel is, was dit onderhewig aan 'n strenger uitvoerprotokol.

Ongunstige weersomstandighede in Spanje en Marokko het ook 'n nadelige effek op baie van die suider Afrikaanse uitvoermarkte gehad in die 2005-uitvoerseisoen. Eerstens was daar vries-skade in Spanje en Marokko kort na die sagte sitrus seisoen en tweedens was daar haelskade aan die suurlimoene in Spanje. Die haelskade het gelei tot markgeleenthede vir suider Afrikannse suurlimoen-uitvoerders. Die voordeel is egter grootliks oorskadu deur die ongunstige effek wat die koue op die interne kwaliteit van die Spaanse en Morokkaanse lemoene tot gevolg gehad het. Dit het gelei tot 'n negatiewe effek op die verbruikersaanvraag vir lemoene. Ten spyte van die verminderde volumes vanaf Spanje en Marokko, is die eerste effek gevoel met die stolling van die verkoopstempo van lemoene vanaf Spanje, Marokko en Egipte in die markte van die Europese Unie. Dit het tot gevolg gehad dat die Russiese mark oorvoorsien is en groter volumes ook na die Midde Ooste gestuur is. Hierdie twee markte moes dien as alternatiewe vir die lemoene wat vir die Middelerreense streke beplan was. Tesame met die wisselvalige kwaliteit van die suider Afrikaanse produk het dit ernstige implikasies vir die reklame van nawel uitvoer tot gevolg gehad.

Bydraend tot die moeilike 2005-bemarkingsseisoen, is die seisoen verder bemoeilik deur droogte toestande wat in meeste van die noordelike produksie areas geheers het. 'n Potensiele, enorme ramp is tennouernood afgeweer met die tydige aanpassing van die sitrus swartvlek (SSV) - bespuitingsprogramme met die onverwagte en skielike intrekking van die EU se residu toleransie (Maksimum Residu Vlakke) vir die fungisied, carbendazim.

Ondersteuning vir bedryfsnavorsing en tegniese dienste het 'n prioriteit gebly weens die bedryf se goeie insig ten spyte van die finansiële probleme wat ondervind is. Ter ondersteuning van sy lang termyn strategiese doelstellings het CRI ook sterk groei getoon in sy gediversifiseerde (nie-heffings) inkomste. Dit kan hoofsaaklik toegeskryf word aan die suksesvolle stigting van die sitrusbedryf se onderneming om tegnologie te kommersialiseer, naamlik River Bioscience. Nog 'n sterk bydraer was die SA-EU Plaagdoder Inisiatief Program (PIP) waar die sitrusbedryf ook fundamenteel was met die inisieringsproses.

CRI se kapasiteit is versterk met die aanstellings van Dr. Graham Barry as Bestuurder: Kultivarontwikkeling en Hannes Bester as Area Voorligtingsbestuurder van die suidelike streek. Die struktuur van CRI is ook diensooreenkomstig gewysig deurdat kultivarontwikkeling geskei is van die sitrusverbeteringsprogram. 'n Wysiging aan die struktuur en opdrag van die Sitrusverbeteringsprogram se raadgevende komitee en die stigting van 'n raadgevende komitee vir Kultivarontwikkeling is deur die CRI-Raad goedgekeur. Stephan Verreyne het teruggekeer na Suid-Afrika nadat hy sy PhD suksesvol in Kalifornië voltooi het. Hy beklee tans die CRI-posisie as Navorsingshortoloog en is gesekondeer aan die Universiteit van Stellenbosch.

CRI se werksaamhede het voortgegaan deur dieselfde operasionele prosedures en prosesse te volg wat suksesvol ontwikkel en geïmplementeer is oor die afgelope jare. 'n Omvattende hersieningsproses van die navorsingsprioriteite het weereens die belangrikheid van navorsing op VKM bevestig en versterk. Dit kan toegeskryf word aan die insek se fitosanitêre status. 'n Raadgevende komitee is deur die CRI-Raad aangestel om leiding te gee en aanbevelings te doen rakende die vinnige kommersialisering van die Steriele Insek Tegniek (SIT) vir area-wye beheer van VKM. Die ontdekking van weerstand teen die na-oes fungisied, Imazalil, is kommerwekkend. Hierdie ontdekking het gelei tot die ontwikkeling van 'n weerstandsbestuur strategie vir implementering in 2006. Die behoefte om die bedryf se vermoëns te versterk op die gebiede van Plantvoeding en Plantpatologie is geïdentifiseer. Die nodige aksies sal in 2006 geneem word.

Ten spyte van die ongunstige ekonomiese klimaat binne die bedryf het die verkope van voorplantingsmateriaal vanaf die Sitrusgrondvesblok bestending vertoon met slegs 'n marginale daling in die besigheid se winsgewendheid. Gerugsteun deur die goeie finansiële prestasies van die vorige jaar is verdere infrastrukturele ontwikkelingsprojekte by die Grondvesblok onderneem. Hierdie infestering word beskou as 'n belegging in die toekoms van die bedryf.

Dit is baie belangrik dat momentum behou moet word gedurende tye van moeilike finansiële omstandighede in die bedryf vanweë die langtermyn doelstellings van navorsing. Dit is veral van kardinale belang vir die voortsetting van CRI se langtermyn doelwitte soos gereflekteer in die missie – “Om die langtermyn globale mededingendheid van die suider Afrikaanse sitruskwekers te bevorder deur middel van ontwikkeling, ondersteuning, ko-ordinerende en voorsiening van navorsing en tegniese dienste deur die vermoëns van al CRI groep se vennote te kombineer”. Dit is veral bemoedigend om die volgehoue vlak van strategiese insig wat deur die Sitrus Kwekers Assosiasie van suider Afrika geopenbaar word, waar te neem in hul volgehoue ondersteuning vir bedryfsnavorsing en tegniese ondersteuningsdienste. Die las van verantwoordelikheid is daarom op elke individu binne die CRI groep om te verseker dat hierdie mosie van vertroue deur die kwekers beloon word. Die uitstekende vordering soos weergegee word in die Jaarlikse Navorsingsverslag van 2005 reflekteer die hoë vlak van erkenning deur die CRI Groep en voorspel net 'n goeie toekoms vir die suider Afrikaanse sitrusbedryf. Die Raad van Direkteure van die CRI, die CGA, River Bioscience, die suider Afrikaanse sitrus bedryf as 'n geheel, die CRI groep Alliansie Vennote Organisasies en personeel word bedank vir hierdie verbintenis, vertroue en prestasie.

2 MARKET ACCESS TECHNICAL CO-ORDINATION

Co-ordinator: Vaughan Hattingh (CEO)

2.1 PROGRAMME SUMMARY

The research required to include cold treatment as an FCM disinfestation treatment option in the **China** export protocol was successfully concluded. However, inclusion in the official protocol was delayed, pending signature by the relevant South African and Chinese government officials. The potential of non-host status for lemons, and the feasibility of irradiation as a post-harvest disinfestation treatment, were investigated as alternatives to cold treatment.

South Africa's submission to the **EU**, calling for a relaxation in the requisite CBS phytosanitary standards, remained outstanding, pending a response from the EU. Despite several requests from South Africa (SA) to expedite an EU response, no progress was made. Alternate mechanisms to facilitate resolution will need to be pursued in 2006. The phytosanitary status of FCM was heightened by 14 official interception reports received from Spain. Both pre-harvest pest management and post-harvest inspection standards will have to be intensified to reduce the risk of exporting FCM-infested fruit. Consequently FCM was identified by growers as the top industry research priority. The urgent further development and fast-track commercialisation of FCM SIT, and an increase in the extent of FCM research has ensued. Improved pre-harvest control of fruit flies and the potential non-host status of citrus for non-Mediterranean fruit flies, received attention as additional risk mitigation for exports to the EU. A task team was formed to develop an action plan in preparation for the potential future spread of the fruit fly *Bactrocera invadens* into Southern Africa.

A local confirmatory trial on cold treatment as a fruit fly disinfestation measure, was successfully concluded in the presence of a visiting **Japanese** MAFF scientist. This was done in support of SA's application to commence exporting Clementines to Japan. All supporting documentation was provided to Japan and a decision by Japanese government officials is awaited. SA proceeded with research aimed at validating a higher temperature, cold treatment condition, for the export of all citrus types to Japan. However, the dispute between SA and Japan regarding the nature of research required, remained unresolved.

The **USA** market was temporarily closed due to an alleged interception of a live FCM in SA citrus fruit in the USA. Intensive negotiations enabled re-opening of the market, but under more restrictive phytosanitary treatment conditions. SA requested reversion to a less restrictive cold treatment and a supporting data pack was supplied to USDA, together with an analysis of the data. This will be followed up in 2006. The CEO CRI met with influential groupings in the USA, aimed at reducing the risk of a recurrence of such events. A compulsory Good Agricultural Practice for the control of FCM control was agreed upon by USA and SA. The development of SIT for FCM control progressed rapidly and holds promise for improved future control of this pest.

Additional surveys were conducted in the Northern Cape in pursuit of establishing this as an officially recognised “CBS-free-area”. The development of a system, whereby farms in areas of low CBS prevalence

could potentially gain access to the **USA** market by being recognised as CBS-free-places-of-production, advanced through intensive surveying of 4 farms, as a test case to evaluate the system. SA has requested USDA to consider this system, but by year-end, no response had been obtained. This is to be pursued further in 2006. Grain Chinch Bug continued to be problematic in the USA export programme and evaluation of a novel pyrethrum product, for post-harvest control of the pest, was initiated.

A proposed FCM cold treatment protocol was received from **South Korea** for potential future export of lemons from SA to South Korea. A response, with an amended FCM risk mitigation procedure, as an alternative to cold treatment, was supplied to the Department of Agriculture (DoA) for submission to South Korea. The pursuit of access to **Thailand** experienced a setback in 2005 and a strategy to regain lost ground is needed for 2006. The pursuit of access to **Israel** for SA lemons progressed in 2005, through official interaction that will continue in 2006. Pursuit of gaining access to **Australia** was given momentum in early 2005, when the industry supplied a data package for submission to Australia. However, further bilateral interaction, through official Government channels, has not been able to elicit any further progress. Industry intervention will have to be considered as an alternative mechanism. As a follow on to the industry having initiated pursuit of regaining access to the **Iran** market in 2004, the market was opened in 2005 and this provides a promising future opportunity for the industry. Technical barriers to the opening of access to **Pakistan** were successfully overcome in 2005.

Responsibility for industry **food safety** issues was successfully transferred from CEO CRI to CGA Industry Affairs Manager in 2005. The CEO CRI remained responsible for directing and supervising the CGA Industry Affairs Manager's operational handling of pesticides issues. The industry achieved excellent results through this structure, by engaging with UK multiple retailers on their pesticide residue expectations. The CEO CRI and CGA Industry Affairs Manager were successful in averting a potential disaster, of proportions not previously experienced by the industry through timely implementation of changes to industry use of carbendazim. This was undertaken in anticipation of the 2006 revocation of its residue tolerance in the EU, despite failure of international trade organisations and SA Government bodies to recognise the threat.

PROGRAMOPSOMMING

Die navorsing benodig om koue behandeling as 'n VKM disinfestasië opsie in te sluit in die China uitvoerprotokol is suksesvol afgehandel. Die insluiting as deel van die amptelike protokol is egter verhoog hangende die ondertekening van die gewysigde protokol deur regeringsamptenare van Suid Afrika en China. As alternatiewe tot die koue behandeling is die nie-gasheer status van suurlemoene asook die geskiktheid van bestraling ondersoek.

Die voorlegging aan die EU waarin 'n verslapping van die voorgeskrewe swartvlek fitosanitêre standaard gevra word, is nog steeds hangende wagtend op terugvoering vanaf die EU. Geen vordering is gemaak ten spyte van verskeie navrae van SA om die terugvoering te bespoedig nie. Alternatiewe maniere om 'n oplossing te verkry sal in 2006 ondersoek moet word. Die 14 amptelike onderskeppingsverslae van VKM in Spanje het die fitosanitêre status van die plaag verder verhoog. Hierdie onderskeppings het tot gevolg gehad dat beide voor- en na-oes plaagbestuur asook na-oes inspeksie standaard verbeter sal moet word om die risiko verbonde aan die uitvoer van VKM-besmette vrugte te verlaag. VKM is ook deur die kwekers geïdentifiseer as die hoogste prioriteit vir bedryfsnavorsing. Die dringende verdere ontwikkeling en die vinnige kommersialisering van VKM-SIT asook 'n toename in die omvang van VKM navorsing het hieruit voortgevloei. As addisionele risikobestuur vir uitvoer na die EU is aandag ook aan die verbetering van voor-oes beheer van vrugtevlies asook die moontlike nie-gasheer status van sitrus vir nie-Mediterrane vrugtevlies geskenk. 'n Taakspan is saamgestel om 'n aksieplan te ontwikkel ter voorbereiding van die moontlike verdere verspreiding in suider Afrika van die vrugtevlies, *Bactrocera invadens*.

'n Plaaslike bevestigings eksperiment met koue behandeling as 'n disinfestasië metode vir vrugtevlies is suksesvol in die teenwoordigheid van 'n besoekende Japan MAFF wetenskaplike voltooi. Hierdie werk is gedoen as ondersteuning van SA se aansoek om Clementines na Japan uit te voer. Dokumentasie ter ondersteuning is ook aan Japan voorsien en daar word nou gewag op 'n besluit vanaf die Japanese owerhede. SA het ook voortgegaan met navorsing wat gerig is op die validasie van 'n hoër temperatuur as koue behandelingstoestand vir alle sitrus tipes wat na Japan uitgevoer word. 'n Oplossing vir die dispuut tussen SA en Japan rakende die aard van die navorsing benodig, kon nog nie gevind word nie.

Die VSA mark was tydelik gesluit weens die beweerde onderskepping van 'n lewende VKM in SA sitrus vrugte in die VSA. Intensiewe onderhandelinge het weer die opening van die mark moontlik gemaak maar onder strengere fitosanitêre maatreëls. 'n Data inligtingspakket, asook 'n analise van die inligting, is aan USDA voorsien as deel van SA se versoek dat 'n minder streng koue behandelingsmaatreël weer ingestel moet word. Hierdie aspek sal in 2006 opgevolg word. Die HUB CRI het met invloedryke groepe in die VSA

ontmoet om die risiko van 'n herhaling van so 'n gebeurtenis te verminder. Daar is tussen die VSA en SA ooreengekom dat verpligte goeie landboupraktyke vir die beheer van VKM ingestel en gevolg sal word. Die ontwikkeling van SIT vir die beheer van VKM het vinnig gevorder en dit hou baie belofte in vir toekomstige verbetering vir beheer van die plaag.

Addisionele siektevoorkoms opnames is in die Noord Kaap uitgevoer in die strewe daarna om die gebied as is 'n amptelike "swartvlek-vrye-area" te erken. 'n Sisteem, waar plase wat geleë is in areas van lae swartvlek voorkoms ook moontlik toegang tot die VSA mark kan verkry as erkende swartvlek vrye areas van produksie, is ontwikkel. Intensiewe opnames is op die 4 plase uitgevoer om die sisteem te evalueer. 'n Versoek is deur SA aan die USDA gerig om die sisteem te oorweeg maar teen jaareinde was geen terugvoering nog ontvang nie. Dit sal verder in 2006 opgevolg word. Graan stinkluise veroorsaak nog steeds probleme in die VSA uitvoerprogram. Die evaluasie van 'n nuwe pyrethrum produk, vir na-oes beheer van die plaag, is van stapel gestuur.

'n Voorgestelde VKM koue behandelingsprotokol is vanaf Suid Korea ontvang vir die moontlike toekomstige uitvoere van suurlemoene na Suid Korea. Terugvoering tesame met 'n gewysigde VKM risiko bestuursprosedure, as alternatief tot koue behandeling is aan die Department van Landbou (DvL) voorsien vir voorlegging aan Suid Korea. Pogings om toegang tot Thailand te verkry het 'n terugslag ondervind in 2005 en 'n strategie om verlore veld terug te wen word benodig vir 2006. Die strewe om toegang tot Israel te verkry vir SA suurlemoene het gevorder in 2005 deur amptelike interaksies. Hierdie prosesse sal voortgesit word in 2006. Vroeg in 2005 is daar momentum verkry vir toegang tot Australie deur die amptelike voorlegging van 'n inligtingspakket wat deur die bedryf voorsien is. Verdere bilaterale interaksies deur die amptelike regeringskanale kon egter geen vordering bewerkstelling nie. As 'n alternatief sal ander bedryfsaksies oorweeg moet word. Die Iranese mark, wat 'n belowende toekomstige geleentheid voorsien het vir die bedryf is in 2005 geopen na die pogings wat deur die bedryf in 2004 aangewend is. Tegnie se struikelblokke vir die opening van die Pakistaanse mark is ook suksesvol in 2005 oorkom.

Verantwoordelikheid van die bedryf se voedselveiligheidsaspekte is gedurende 2005 suksesvol vanaf die HUB CRI na die CGA se Bestuurder: Bedryfsake oorgedra. Die toesighoudende verantwoordelikheid vir die operasionele hantering van plaagdoder-residue aspekte word egter nog deur die HUB CRI behou. Hierdie reëling het die bedryf met uitstekende resultate beloon deurdat dit voorsiening gemaak het vir effektiewe skakeling met verskeie VK supermarkte oor hul plaagdoder residue verwagtinge. 'n Potensiele enorme ramp, soos nog nooit voorheen deur die bedryf ervaar nie, is afgeweer deur die tydige implementering van aanpassing in die gebruik van carbendazim in die bedryf. Dit is gedoen in afwagting op die EU se intrekking van die residu toleransie ten spyte van die onvermoë van internasionale handelsorganisasies en SA regeringsliggame om hierdie bedreiging te identifiseer.

2.2 CHINA

Whereas the 2005 export season was the first full year that SA citrus had access to the Chinese market, the quantities of fruit successfully exported were disappointingly small. As indicated in the Annual Research Report for the 2004 period, there was an urgent need to refine the China export protocol, before the potential value of the market could be realised. The principal constraint within the original protocol related to the requisite FCM risk mitigation measures. Accordingly, only fruit originating from "FCM-free production sites" could be considered for export to China. The process of gaining acceptance by China for inclusion of cold treatment, as an alternative FCM risk mitigation measure, was initiated in the previous report period. This resulted in an official large-scale efficacy validation trial, conducted in South Africa by Hendrik and Marsheille Hofmeyr (under supervision of the South African Department of Agriculture), in the presence of a delegation of Chinese scientists. This validation trial was conducted from 4 July 2005 to 8 August 2005 to the satisfaction of the visiting Chinese delegation. The cold treatment condition that was validated in this experiment consisted of 22d at $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ fruit pulp temperature, preceded by a 3d step-down cooling period. The trial results are reported under Section 3.2.12. The official inclusion of the cold treatment disinfestation option in the China export protocol, remained pending at the end of the current report period, due to a delay in the signing of the revised protocol by the relevant SA and Chinese Government officials.

Export of grapefruit, lemons and limes, under such severe cold treatment conditions is not generally feasible, making it important to investigate means of alleviating this constraint. One approach that received attention during the report period, was the possibility that lemons may be recognised as having a non-host status for FCM and thereby precluding the need for specific FCM risk mitigation measures for lemons. This research was conducted by Sean Moore and Bruce Tate and is reported under Section 3.2.11. Conclusive evidence of potential non-host status had not been obtained by the end of the current report period, but the investigation will be continued. The potential of irradiation as an alternative post-harvest disinfestation

treatment also received attention in terms of FCM disinfestation efficacy (Hofmeyr and Hofmeyr) as reported under section 3.2.7.

Another priority that arose in association with this FCM cold treatment requirement, was the need for handling procedures that would reduce the sensitivity of grapefruit and lemons to chilling injury. Considerable research has been conducted on this topic in the past and consequently the first step towards addressing this need, should be the collation of current knowledge on the topic into a guideline and this will receive attention in 2006.

2.3 EUROPE

South Africa's submission to the EU, proposing a relaxation of the quarantine measures associated with CBS on citrus fruit imported from South Africa, remained outstanding. The last batch of scientific data supporting South Africa's submission (as requested by the EU) was provided in July 2004. The EU standing committee for plant health acknowledged receipt of the data and gave an undertaking to subject it to appropriate evaluation and revert to South Africa. During the 2005 report period, on the citrus industry's request, the relevant EU body was periodically contacted by the South African Department of Agriculture, with requests to provide an update on the status of South Africa's submission. Despite receiving repeated assurances from the EU that the matter would receive their attention, no progress had been reported back to South Africa by the end of the report period. Alternate routes for encouraging progress with this matter will have to be investigated and these may include the utilisation of political and legal trade regulation mechanisms.

For the first time in 2005 South Africa received official notification from the EU that consignments of citrus fruit from South Africa had been rejected on arrival in the EU for the presence of FCM. In total 14 such interception notices were received in 2005, all from inspections conducted in Spain. These incidents highlighted the need to exercise considerably higher levels of care to avoid exporting fruit with a high risk of FCM, in particular to the EU, and especially when such fruit is destined for the citrus producing Mediterranean countries. Likewise, the need to implement more effective FCM control procedures in the orchards, has been highlighted by these events. This makes the rapid development and implementation of area-wide control of FCM with SIT, a top industry priority. In November 2005 CRI and CGA endorsed the formation of a FCM SIT Advisory Committee, to provide guidance and recommendations on a fast track approach to commercial implementation of SIT for FCM control. An extensive portfolio of FCM research was conducted in 2005 and is reported under Section 3.2.4. Cryptogram performed well under commercial field conditions and greater use of the product will be valuable in improving field control. Isomate, an FCM mating disruption treatment, also became available as a new FCM control option in 2005. Research on FCM received the highest rating during the 2005 research priority setting process. Consequently, there has been a large increase in the allocation of funds for FCM research in the 2006 budget.

The phytosanitary risk mitigation measures required for fruit flies, in association with citrus fruit exported to Europe, consists of effective pre-harvest control, post-harvest inspection and official certification that the fruit is free of non-Mediterranean fruit flies. The citrus industry has been very successful in effectively managing this through diligent implementation of intensive pre-harvest monitoring and control. To ensure that this is sustained, the standard industry fruit fly control recommendations were again reviewed in 2005 and published as Cutting Edge editions 26 & 27. Likewise, ongoing research, aimed at improving fruit fly monitoring and control practices, was continued in 2005 and is reported under Sections 3.3.3 & 3.3.6.

Since the fruit fly risk mitigation procedures required by the EU include certification that the fruit is free of non-Mediterranean fruit flies, additional research was conducted to verify that citrus is not a host for *Ceratitis cosyra* (Marula fruit fly). The results of this research will be reported once the investigation has been concluded.

An associated concern has been the announcement in 2005, that a new exotic and invasive fruit fly species has been introduced and has become established in East and West Africa. *Bactrocera invadens* originates from Indonesia and in 2005 it was reported to have become established in Kenya, Tanzania, Sudan, Benin, Uganda, Cameroon, Togo, Senegal, Ghana and Nigeria. A task team to coordinate the development of an action plan, in anticipation of this fruit fly species potentially migrating down into southern Africa, was formed as a sub-committee of the Market Access Working Group. The CEO of CRI represents the citrus industry on the task team. Contact is being maintained with entomologists in the effected African countries. An Africa-wide research project proposal was submitted to the United Nations Food & Agriculture Organisation for potential funding. Due to the quarantine nature of the insect, most of the relevant research must be conducted outside of southern Africa, in those African countries where the species has already become established.

2.4 JAPAN

The Japanese market is currently open for the export of fresh Grapefruit, oranges and lemons from South Africa and Swaziland but not for other types of citrus. There are currently two phytosanitary issues receiving attention, namely (1) an application to open the Japanese market for the export of South African Clementines and (2) the adoption of a revised cold treatment condition for the export of all citrus types from South Africa to Japan. Japan stipulated the remaining requirements for opening the market to Clementines and accordingly, Japan MAFF sent a scientist to South Africa from 8 June 2005 to 24 June 2005. The visiting scientist inspected the Clementine industry and observed the successful execution of a large-scale confirmatory trial, aimed at validating earlier research that had demonstrated efficacy of the cold treatment. The Clementine cold treatment conditions that were validated, consisted of a 14-day exposure to a mean temperature of -0.4°C , as reported under Section 3.3.7.

On departure from South Africa, the MAFF scientist advised South Africa that there would be a public hearing in Japan to obtain comments on MAFF's proposal to open the market for South African Clementines. On subsequent enquiries from South Africa regarding progress made with the process, it transpired that Japan MAFF also required a report from South Africa on the joint validation trial, in addition to the report from the MAFF scientist. CRI supplied such a report to the South African Department of Agriculture (DoA) on 6/12/05.

Japan and South Africa had previously communicated regarding the requirements for validating an amended cold treatment condition, for the export of all citrus types to Japan. No agreement was reached in 2004 on the nature of the data required. Nonetheless, the trial was initiated in 2004, in accordance with the South African proposal. The test consisted of medium scale (Phase 3) evaluation of cold treatment at 1°C for an extended (16 days) period, using mature larvae, this life stage having previously been shown to be the most cold tolerant life stage in Clementines. In response to Japan's expectation that SA would conduct a full set of phase 2, 3 and 4 trials on each citrus type, before amendment of the cold treatment conditions would be considered, SA conducted phase 2 trials on Grapefruit, oranges and lemons. This research is reported under Section 3.3.2 and showed that larvae were more cold tolerant than eggs, that young larvae were more cold tolerant than mature larvae in oranges. There were no statistically significant differences between the cold tolerance of larval life stages in grapefruit and lemons. These data verified that the 2004 phase 3 evaluations had been conducted with an appropriate life stage (young larvae).

The difference of opinion between SA and Japan, as to whether phase 4 should be conducted in only one of the fruit types, or if Japan is justified in insisting on phase 4 being repeated in all types of citrus, remained unresolved at the end of the report period. SA will either have to proceed in 2006 with Phase 4 on oranges only (in accordance with the SA position), or further research will have to be put on hold until this dispute is resolved.

2.5 USA

An agreement had been reached between SA and USA in 2004 to implement a suite of pre-harvest FCM control practices, described in a Good Agricultural Practice (GAP) protocol. The objective was to reduce FCM population pressure and thereby reduce the risk of post-harvest disinfestation treatment failure. A USDA APHIS delegation visited SA in 2005 prior to commencement of the 2005 exports, to review the proposed GAP. It was agreed that the CRI Production Guidelines, with some modifications, would be used as the detailed guideline for the GAP requirement. The Production Guidelines were consequently revised, an associated GAP document was compiled, communicated with affected industry parties and submitted to DoA for official implementation in the 2006 season.

The principle of reducing phytosanitary risk through pre-harvest pest management, progressed in 2005 with the successful commercialisation of the FCM Granulovirus, Cryptogran. Relevant research in this regard is reported under Section 3.2.2. Likewise, a new FCM mating disruption product for the control of FCM, Isomate, became commercially available in 2005.

The commercial implementation of SIT as an area-wide FCM population management tool, may prospectively be very valuable within such an integrated systems approach to FCM risk mitigation. Research aimed at making commercial implementation feasible, advanced rapidly in 2005 and is reported under Section 3.2.4. The CRI Board (endorsed by the CGA) formed an FCM SIT Advisory committee late in 2005. The committee was tasked with providing guidance and recommendations on the fast-track commercialisation of SIT for the control of FCM. As a first order of business the committee agreed upon a set of guiding principles and an action plan, with milestones for implementation of the FCM SIT technology.

The services of a specialist were secured by CRI to investigate the scope of funding/financing opportunities for commercialisation of FCM SIT.

In continued support for expanding the SA citrus supply base for exports to USA, the surveying of areas as potential CBS "Pest-free areas" was concluded for the Knysna and Mossel Bay regions (Section 4.3.17). The DoA reviewed the surveys conducted in the Northern Cape and adjacent Free State regions and called for an additional round of surveys to be conducted, with a greatly intensified sampling intensity. As a joint DoA-CRI effort, the collection of these samples was concluded by late 2005. The DoA Quarantine Laboratory in Stellenbosch is to conduct the analyses of the 450 samples in 2006.

An additional mechanism for potentially expanding SA's access to the USA market, through a mechanism of certifying CBS "Pest-free places of production", had originally been presented to USA in 2004 for consideration. The proposal was raised with a visiting APHIS delegation in early 2005, but despite assurances that the matter would receive attention, no progress had been made with USDA APHIS by year-end. The citrus industry accordingly requested high level attention be focused, within APHIS, on this unsatisfactory situation and initiated such measures, in collaboration with the USA Government Relations Facilitator working in the USA on a CGA contract.

Despite USDA APHIS not having responded to SA on its proposal to develop a "CBS-free-place-of-production" system, the SA DoA agreed to proceed with intensive sampling of 4 farms in the Tshipise and Weipe regions, as a practical evaluation of the scheme. As a joint DoA-CRI endeavour, the samples were drawn in December 2005 and submitted for analysis (4.3.14).

Grain chinch bug continued to be a source of numerous rejections in the 2005 USA export programme. Growers reported promising exploratory trial results with the use of packhouse dip treatments containing pyrethrum extracts. Fruit quality evaluation, compatibility with fungicide baths and insecticidal efficacy treatments were initiated and are reported under Sections 3.6.5, 3.6.6 & 4.5.8.

The 2005 USA export season was marked by exceptionally high FCM population pressure. High levels of interception during pre-export inspections were reported. In June 2005, USA advised SA that it was closing the SA citrus export programme, on the grounds of a report by a Californian border inspector, that SA citrus had been intercepted with live FCM. Intensive negotiations enabled a re-opening of the programme, but with an extended cold treatment (24d plus a regulated pre-treatment cooling) and a reduced tolerance for interception during pre-treatment/export inspection. The CEO CRI undertook a trip to USA and held discussions with USDA APHIS and Californian Department of Agriculture officials, aimed at implementing measures to reduce the risk of recurrence of such an event.

On successful completion of the remainder of the 2005 export season, an application to revert back to a 22d FCM cold treatment protocol was prepared for submission to USA. A supportive data package was compiled. On request of DoA, an accompanying analysis of the data package was provided. The information was officially submitted to USDA-APHIS by SA DoA.

During discussions with Californian Department of Agriculture officials in July 2005, aimed at re-opening the USA market, SA was requested to assist California with the following. Firstly, to supply USA entomologists with FCM samples to support reliable identification of larvae intercepted during fruit import inspections. Such samples were supplied by Alicia Timm. Secondly, SA was requested to provide USA with access to results of a trial conducted in collaboration with South Korea, to establish the disinfestation efficacy of cold treatment for California Red Scale (*Aonidiella aurantii*). The relevant data package was compiled, approval was obtained from CRI, CGA and DoA to provide these data, and the package was then officially forwarded to USDA-APHIS via SA DoA.

2.6 SOUTH KOREA

The development of a molecular identification technique for mealybugs had been under development, in collaboration with South Korea, since 2003. The South Korean authorities, prior to the commencement of the 2005 export season, accepted the adoption of the technique into the phytosanitary inspection process. The technique was successfully implemented in the 2005 export season.

South Africa has been pursuing the inclusion of lemons and Grapefruit into the South Korean export programme for some time. In 2005 the South Korean authorities notified South Africa that they would accept South African lemons, on condition that they are subjected to the 22d FCM cold treatment, as for oranges. However, lemons display extensive chilling injury when exposed to these conditions. An amended FCM risk

mitigation procedure was formulated and provided to DoA in December 2005, for presentation to South Korea, as a South African counter proposal. No response had been received from South Korea by year-end.

2.7 THAILAND

South Africa and Thailand were close to concluding an export protocol for citrus from South Africa in 2003. An SA response to outstanding technical PRA questions, was prepared at an industry-DoA workshop. However, there was a protracted delay in DoA submitting this to Thailand. Thailand reverted in 2005, indicating that they wished to revisit the Pest Risk Analysis (PRA) that had previously been conducted in 2003. Furthermore, the CEO CGA and SA Agricultural attaché in China, subsequently met with Thai Plant Health officials, and received an invitation for SA specialists to go to Thailand to discuss their requested reconsideration of the PRA. The industry agreed on a suitable response and requested DoA to act accordingly. However, by year-end, no such communication had been sent to Thailand by DoA.

2.8 ISRAEL

Israel had previously requested South Africa to provide additional technical data, to support SA's application to commence exporting lemons. The additional technical data was supplied to DoA and subsequently submitted to Israel. In December 2005, Israel provided SA with the outcome of its PRA on SA lemon exports. The provision of an SA response was scheduled for early 2006.

2.9 AUSTRALIA

Australia had requested additional data to support SA's application to potentially authorise commencement of SA citrus exports. The requisite data package was compiled and forwarded to DoA in February 2005, for submission to Australia. Whereas the DoA assured industry that the data package had been submitted to Australia, no response had been received from Australia by year-end. This was despite repeated requests to DoA from industry, to request Australian feedback on the status of the request and repeated assurances from DoA, that such requests had been sent to Australia. Alternate routes to facilitating progress on this matter will have to be utilised in 2006.

2.10 IRAN

CRI and CGA initiated the process of re-opening the Iranian market in 2004. Official bilateral interaction took place periodically, but SA export agents began positioning themselves to take advantage of the opportunity and involved themselves in this process. The Iranian market was opened in 2005, but the initial notification was provided to an exporter and not via the official Government or organised industry structures. Consequently, the start-up of the export programme was chaotic, but order was subsequently restored and this market represents a valuable future opportunity.

2.11 PAKISTAN

The Pakistani market was opened in 2005 after a delegation from the industry had visited the country. A formal request was put forward to the DoA to start liaison with the Pakistani authorities and a permit was issued to some exporters. None of the diseases identified on the permit as requiring specific mitigation measures occur in SA, but problems were experienced with the required post-harvest treatment (SOPP) since this chemical is not in use anymore. CRI provided a technical argument that the DoA discussed with the Pakistani Authorities and they agreed to the standard industry post-harvest treatment practices. Although all the technical problems were thereby successfully addressed, no citrus was apparently exported to this market.

2.12 OTHER MARKET ACCESS AND FOOD SAFETY ISSUES

The CEO CRI, as a CGA representative, has participated in the Annual General Assemblies of CLAM (the Mediterranean citrus industry's co-ordination forum) because of strong ties with phytosanitary market access issues within this forum. Consequently in 2005, the CGA, as the CLAM point of interaction with the SA citrus industry, was invited to become a formal Associate CLAM member. SA will be the first non-Med country to gain such association with CLAM, a body representing the single biggest citrus production and export grouping in the world. CLAM will accordingly send a delegation to SA in May 2006.

CRI and its predecessor OCC, historically handled industry food safety matters. During 2005 the operational handling of food safety, was effectively transferred to the CGA, in the person of Paul Hardman as Industry

Affairs Manager. CRI, through its CEO, retained supervisory responsibility for residue issues, although the CGA Industry Affairs Manager very successfully adopted the requisite operational function. This highly successful arrangement was demonstrated in 2005 by two significant food safety (pesticide residue) incidents. The CEO CRI and CGA Industry Affairs Manager engaged with major multiple retailers in UK and established a single SA desk for engagement with retailers on pesticide residue issues. The value of this is considerable in counteracting the potential imposition (by retailers) of pesticide usage restrictions that go beyond the scope of legal food safety regulations.

Furthermore, in continuation of a long-standing schedule of periodic consultation, the CEO CRI and CGA Industry Affairs Manager met with UK officials responsible for the regulation of pesticide residues on foodstuff. This meeting gave rise to information on an imminent residue crisis, associated with the use of the fungicide carbendazim. Direct engagement with appropriate EU officials ensued and additional data requirements were identified. A package of available data was compiled and submitted to the EU, but it was insufficient to avert the interim revocation of the MRL. Relevant field trials were initiated (with co-funding by PIP), to generate data in support of the future implementation of a revised EU MRL. Deficiencies in the EU consultation process were highlighted by these events and this will be taken up as a point of objection by the Southern Hemisphere Association of Fresh fruit Exporters (SHAFFE). As soon as the impending problem had been identified, a cautionary notice was issued, advising the industry to refrain from spraying carbendazim. If this had not taken place, a large proportion of the southern African citrus crop would have been unsuitable for export to the EU. This timely emergency intervention averted disastrous financial losses, of proportions that have not previously been experienced, and that would most certainly have crippled the industry.

3 PROGRAMME: INTEGRATED PEST MANAGEMENT

3.1 PROGRAMME SUMMARY

By Tim G. Grout (Research & Technical Manager)

As in the last few years, the research emphasis in the IPM programme during 2005 was on market access pests. False codling moth (FCM) once again received the most attention and the most research funding and it is gratifying to see one of the results of this research focus being successfully used by citrus growers, i.e., Cryptogran. The intensive research into the development of techniques required for the implementation of the Sterile Insect Technique against FCM has led to the first evaluation of this method over 35 ha and this control method will soon be implemented over a much larger area. Several other approaches to FCM control are also under investigation including biocontrol with larval parasitoids and entomopathogenic nematodes, and the possible use of female attractants in an attract-and-kill system. Research on the movement of FCM and the importance of alternative host plants was also conducted, in addition to the host status of lemons. The next most important phytosanitary pest is fruit fly and again various aspects were investigated with a view to improving control both now and in the future when organophosphates may no longer be permitted in baits. Much of this research is on-going and few conclusions can be drawn at this stage. Post-harvest cold disinfestation research involved the verification of the treatment of Clementines at -0.4°C in the presence of a Japanese observer and further trials with the objective of raising the treatment temperature to 1°C . Our indigenous mealybugs are also phytosanitary pests and further research was conducted on the parasitoids of oleander mealybug and a survey showed that this mealybug species has become the dominant species in several citrus production regions. Grain chinch bug has been responsible for rejections of fruit destined for the USA due to it sheltering in the navel opening of navel oranges. A survey was conducted to show the distribution of this hitchhiker and some preliminary tests were conducted with insecticides. The diminishing number of chemicals available for the control of certain pests such as mealybug mean that the contribution of natural enemies is important, particularly in the latter half of the season. Because ants disrupt biocontrol, ant control is also important and research was conducted on possible ant repellents. A few pests do not have any phytosanitary significance but can seriously reduce exports (such as citrus thrips) or reduce production (such as citrus psylla vectoring greening disease). Research was conducted on both these pests. Although no suitable IPM-compatible alternatives to abamectin were found that are not already registered for citrus thrips it was found that the amount of oil used with this product could be reduced or certain adjuvants used as a replacement for oil. Various plant protection products were evaluated against citrus psylla but none were as effective as endosulfan. Although a report is not included, a considerable amount of time was spent on GLP trials to develop a Maximum Residue Limit for methamidophos for use in the European Union. This research is continuing and a report will be provided next year.

PROGRAMOPSOMMING

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

Gedurende 2005, net soos in die afgelope jare, was die fokuspunt van navorsing in die IPB-program gerig op marktoegangsplae. Vals kodlingmot (VKM) het weereens die meeste aandag geniet en die meeste van die befondsing is ook daarvoor aangewend, daarom is dit baie bemoedigend om te weet dat een van die resultate van die navorsing suksesvol deur die sitrus kwekers aangewend word, naamlik Cryptogran. Intensiewe navorsing in die ontwikkeling van tegnieke benodig vir die implementering van die Steriele Insek Tegniek teen VKM het gelei tot die eerste evaluering van die metode op 35 ha. Hierdie metode van beheer sal binnekort oor 'n wyer area toegepas word. Verskeie ander aanslae op VKM-beheer word ook ondersoek, wat biologiese beheer met parasitoïede larwes en entomopatogeniese nematodes insluit, asook die moontlike gebruik van vroulike aanlokmiddels as deel van 'n lok-en-dood sisteem. Navorsing is ook op die beweging van VKM en die belangrikheid van alternatiewe gasheerplante uitgevoer aanvullend tot die gasheerstatus van suurlemoene. Die volgende belangrikste fitosanitêre plaag is vrugtevlieë en weereens is verskeie aspekte ondersoek met die oog op verbetering van beheer vir nou en in die toekoms veral vir wanneer organofosfate nie langer in lokaasmiddels gebruik kan word nie. Baie van die navorsing is aaneenlopend en min gevolgtrekkings kan op hierdie stadium gemaak word. Na-oes koue disinfestasië navorsing het behels die verifikasie van die behandeling op Clementines teen -0.4°C onder toesig van 'n Japanse inspekteur en is uitgevoer met die doelwit om die behandelingstemperatuur te verhoog na 1°C . Die inheemse witluis word ook gesien as fitosanitêre plaag en verdere navorsing is op die parasitoïede van die oleander witluis uitgevoer. 'n Opname het getoon dat hierdie witluis spesie nou die mees dominante spesie in verskeie sitrus produksie areas is. Graanstinkluis was ook verantwoordelik vir afkeurings van vrugte wat bestem was vir die VSA as gevolg van die skuiling van die plaag in die opening van nawel lemoene. 'n Opname om die verspreiding van hierdie ryloper te bepaal is ook gedoen. Die afname in die aantal chemikalieë wat beskikbaar is vir die beheer van sekere plaeg soos witluis beteken dat die samewerking van natuurlike vyande belangrik is, veral in die tweede helfte van die seisoen. Die beheer van miere is baie belangrik omrede dit verantwoordelik is vir die ontstigting van biologiese beheer. Navorsing is ook gedoen op moontlike mier afweermiddels. Navorsing is ook uitgevoer op 'n paar ander plaeg wat van geen fitosanitêre belang is nie, maar wat wel uitvoere ernstig kan benadeel soos sitrus blaaspootjies of selfs produksie kan verminder soos sitrus psylla wat vergroeningssiekte versprei. Alhoewel geen geskikte IPB-verenigbare alternatief vir abamectin gevind is nie, anders as dit wat reeds teen sitrus blaaspootjies geregistreer is nie, is dit gevind dat die hoeveelheid olie wat saam met die produk gebruik moet word verminder kan word en dat sekere hulpmiddels ook as plaasvervangers vir olie gebruik kan word. Verskeie plantbeskermingsprodukte is ook teen sitrus psylla geëvalueer, maar nie een was so effektief soos endosulfan nie. Alhoewel 'n verslag nie ingesluit is nie, is 'n aansienlike hoeveelheid tyd gespandeer op gaschromatografie proewe om 'n maksimum residu limiet te ontwikkel vir methamidofos vir uitvoere na die Europese Unie. Hierdie navorsing duur voort en 'n verslag sal volgende jaar gelewer word.

3.2 PROJEK: VALSKODLINGMOT

Projekkoördineerder: Hendrik Hofmeyr (CRI)

3.2.1 Projekopsomming

Valskodlingmotnavorsing het gedurende 2005 weer, soos in die verlede, intensiewe aandag aan 'n verskeidenheid van onderwerpe van verskeie navorsers ontvang. Die navorsing het gewissel van 'n genetiese studie, die evaluasie van chemiese en biologiese insekdoders, 'n breë ondersoek na lok- en afdryfmiddels, biologiese beheer met eier- en larweparasitoïede, asook nematodes, ekologiese ondersoeke en die steriele insek bestrydingstegniek, tot ondersoeke na 'n beter disinfestasiestegniek en die bevordering van sitrusuitvoere deur die ontginning van 'n nuwe afsetgebied.

Die aanvanklike navorsing wat tot die registrasie van die VKM-granulovirus as Cryptogran gelei het, is afgehandel (3.2.2). Etlike ondersoeke is uitgevoer om die produk se algemene gebruik te ondersoek en verdere werk sal voortaan waar nodig in 'n nuwe eksperimentreeks (791) uitgevoer word.

'n Groot aantal chemiese reukstowwe is in olfaktometerproewe gesif om middels te vind wat vir die aanlok van valskodlingmot (VKM) in lokvalle of in 'n bestrydingstegniek soos lok-en-vrek, gebruik sal kan word (3.2.3). Ten spyte van die groot aantal middels word vordering gemaak om geskikte verbindings te identifiseer wat meer navorsingsaandag moet ontvang.

Goeie vordering is met basiese navorsing sedert 2002 ten opsigte van die steriele insektegniek (SIT) gemaak. Dit het tot gevolg gehad dat daar gedurende 2005 begin is met die eerste praktiese toetsing van steriele insekloslatings (SIL) as 'n metode om VKM te bestry (3.2.4). Die waardevolle rol wat die

eierparasitoïed, *Trichogrammatoidea cryptophlebiae*, beide opsigself en in kombinasie met SIL kan speel, is in twee hokproewe bevestig (3.2.4).

Swam- en virusbesmettings wat groot probleme gedurende die massateling van VKM in insektariums kan veroorsaak, is ondersoek en metodes is gevind om die probleem grotendeels op te los (3.2.4 en 3.2.5). 'n Begin is gemaak met ondersoeke na larwe-parasitoïede, wat moontlik dikwels 'n bydrae tot VKM-onderdrukking maak, maar geen erkening geniet nie weens ontoereikende kennis omtrent die organismes (3.2.6). Aspekte soos identifikasie en teling is aangespreek en goeie vordering is gemaak.

Navorsing met gammabestraling om VKM in verpakte vrugte hok te slaan, is voortgesit (3.2.7). Die direkte (onmiddellike mortaliteit) en indirekte (vertraagde mortaliteit, F1-steriliteit) skadelike uitwerking van bestraling op volwasse larwes is ondersoek. Verskeie faktore word bespreek wat dié tegniek as 'n bruikbare disinfestasietegniek kan kelder.

Gasheerplante in en om sitrusboorde kan moontlik VKM-bevolkinggetalle bevorder. Opnames in die Oos-Kaap dui daarop dat verskeie wilde plante deur VKM besmet word en derhalwe moontlike ondersoeke in die toekoms sal regverdig (3.2.8).

'n Basiese probleem met virusse is dat hulle oor die algemeen 'n kort nablywend werking het en maklik deur ultravioletbestraling afgebreek word. Die granulovirus wat in Cryptogran gebruik word, is 'n organisme met 'n prakties bruikbare nablywende werking. Dié produk is voortdurend onderhewig aan ontwikkelingswerk om die produk te verbeter (3.2.9). Die biologiese beheer van VKM kan 'n hupstoot kry indien navorsing op entomopatogeniese nematodes suksesvol is. Spesies wat goed op VKM-larwes in die laboratorium aanteel is geïdentifiseer en heelwat meer werk sal in die toekoms hierop uitgevoer word (3.2.10).

VKM hou 'n bedreiging vir uitvoersitrus in wat nie onderskat moet word in terme van die skade wat die organisme vir uitvoermarkte kan berokken nie. Twee aspekte wat hiermee te doen het, het aandag gekry. Nuwe markte kan vir suurlemoene oopgaan indien die status daarvan as 'n gasheer vir VKM as onbeduidend bevestig kan word. Opnames is uitgevoer om die kwessie te ondersoek (3.2.11). 'n Suksesvolle disinfestasieproef is uitgevoer om die doeltreffendheid van kouedisinfestasië (vrugte 22 dae lank behandel teen $-0,6^{\circ}\text{C} \pm 0,6^{\circ}\text{C}$) vir verteenwoordigers van 'n potensieel goeie uitvoermark te bevestig (3.2.12).

'n Interessante DNS-studie dui daarop dat VKM-bevolkings relatief staties is nie (hul migreer min) en dat hulle samestelling geografies en van seisoen tot seisoen baie stabiel is. VKM op alternatiewe gasheerplante kan ook gereedlik met VKM in sitrusboorde kruisteel (die plant waarop die insek aanteel, beperk dus nie sy besmetting van ander plante nie) (3.2.13).

Twee produkte wat nie tans vir VKM-bestryding geregistreer is nie, is in 'n boordproef geëvalueer (3.2.14). Die resultate was belowend en die middels sal waarskynlik deur die verspreiders daarvan verder ontwikkel word.

Project Summary

During 2005 a variety of aspects relating to false codling moth (FCM) again, as in the past, received concerted attention from a number of researchers. The research varied from a genetic study, the evaluation of chemical and biological insecticides, a broad investigation into attractive and repellent odors, biological control with egg and larval parasitoids, as well as nematodes, ecological surveys and the sterile insect technique, to research into a better disinfestation technique for export fruit and the promotion of citrus exports by exploiting new markets.

The original research leading up to the registration of the FCM granulovirus as Cryptogran, was concluded (3.2.2). Various experiments to examine the product's general usage and further work will be conducted when necessary under a new experiment (791).

A great number of chemical odors were screened in olfactometer experiments in a search for products which could be used for attracting or repelling FCM, or in attract and kill techniques (3.2.3). In spite of the vast number of compounds, progress was made in identifying compounds that justify more research attention.

Basic research into the sterile insect technique (SIT) has made excellent progress since 2002. This made possible the first practical application of sterile insect releases (SIR) as a means to control FCM in 2005 (3.2.4). The potentially valuable contribution of the egg parasitoid, *Trichogrammatoidea cryptophlebiae*, both independently and in combination with SIR, was also confirmed (3.2.4).

Fungal and viral infections causing major problems when mass rearing FCM in insectaries, were investigated and methods were found to greatly alleviate these problems (3.2.4 and 3.2.5). A start was made with investigations into larval parasitoids, which probably contribute to FCM control, but are seldom appreciated due to inadequate knowledge of the organisms (3.2.6). Aspects such as identification and rearing were addressed and good progress was made.

Research on gamma irradiation as a method to rid export fruit of live FCM larvae was continued (3.2.7). The direct (immediate mortality) and indirect (delayed mortality development, F1 sterility) detrimental effects of irradiation on mature larvae were examined. Two factors are discussed which can nullify the development of this technique as a practical disinfestation protocol.

Host plants in and around citrus orchards may possibly contribute to the size of FCM populations. Surveys in the Eastern Cape showed that various wild plants are infested by FCM, and this may result in extending the research (3.2.8).

A basic problem with viruses is that they generally have a short residual action and are also easily degraded by ultraviolet radiation. Although the granulovirus used in Cryptogran has a useful residual action, it is still affected by ultraviolet radiation. Methods to improve this product are continuing (3.2.9). The biological control of FCM may be promoted if research on entomopathogenic nematodes is successful (3.2.10). Species that can be reared successfully on FCM larvae in the laboratory have been identified and more research is envisaged in future.

FCM hold a threat for citrus that should not be underestimated in terms of its ability to harm the export of oranges. Two aspects received attention that impinges on this fact. New markets can be created for lemons if its status as a host for FCM can be demonstrated as negligible. Surveys were conducted to examine this aspect (3.2.11). A successful cold disinfestation experiment was conducted to demonstrate the efficacy of this method (fruit treated for 22 days at $-0,6^{\circ}\text{C} \pm 0,6^{\circ}\text{C}$) to representatives of a potentially profitable export market (3.2.12).

An interesting DNA study shows that FCM populations are relatively static (they do not migrate easily) and that their composition is geographically and temporally very stable (3.2.13). FCM infesting alternate host plants can also easily outcross with FCM in citrus orchards (the plant that FCM develops on therefore does not limit its ability to infest other suitable plants).

Two products currently unregistered for FCM control were evaluated in an orchard experiment (3.2.14). Results were positive and development of the products will be continued by the suppliers.

3.2.2 Evaluation of the efficacy of a granulovirus (GV) for the control of false codling moth Experiment 169 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

’n Granulovirus (GV) van VKM-larwes is ’n paar jaar gelede geïdentifiseer. Met DNS-ontleding is die virus as ’n onbeskryfde isolaat van *Cryptophlebia leucotreta* GV (CrleGV) herken. Biotoetse teen pasuitgebroeide larwes het die potensiaal van die virus as ’n biologiese beheer produk bevestig. Vanaf die jaar 2000 is ’n groot verskeidenheid boordproewe met die virus aangepak. Gedurende 2004 is die virus by die Departement Landbou (Wet 36 van 1947; registrasienommer L7598) onder die naam Cryptogran geregistreer om teen VKM op sitrus gebruik te word. Gedurende 2005 is daar bevestig dat as Cryptogran teen ’n temperatuur van nie meer as 10°C geberg word, sal die raklewe minstens 12 maande wees. ’n Boordproef het die verenigbaarheid van Cryptogran as ’n tenkmengsel met ’n reeks chemiese plaagdoders vir ander plae, bevestig. Dit het die volgende produkte ingesluit: abamektien en olie; piriproxifen en olie; mankozeb, benomiel en olie en methidathion. Cryptogran is ook teen ’n reeks konsentrasies wat laer as die geregistreerde konsentrasie is, getoets. ’n Vermindering in konsentrasie van 10 ml tot 2 ml per 100 l water het tot ’n betekenisvolle of opsigtelike afname in werking gelei. Dit kom daarom voor asof ’n vermindering in die geregistreerde konsentrasie van Cryptogran nie moontlik is nie. In dieselfde proewe is die werking van Cryptogran met dié van Cryptex vergelyk. Cryptex is ’n nuwe VKM-virusprodukt wat in Switserland vervaardig word en binnekort dalk geregistreer sal wees. In een van die proewe het Cryptogran betekenisvol beter as Cryptex gevaar. In nog ’n boordproef is gepoeierde molasse as ’n alternatief vir vloeibare molasse getoets. Die VKM-besmetting was egter te laag vir betroubare resultate. Die proef is later in eksperiment 791 herhaal. Dié eksperiment (169) is nou afgesluit. Verdere werk op die nawerking van Cryptogran word tans onder ’n nuwe eksperiment (791) voortgesit.

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 (Moore, 2002). Through restriction enzyme analyses the virus was identified as a novel isolate of *Cryptophlebia leucotreta* GV (CrleGV) (Singh *et al.*, 2003). Bioassays against neonate larvae, confirming its potential as a biocontrol agent (Moore, 2002), have led to fairly extensive field trials since 2000 (Moore, 2002; Moore *et al.*, 2002, 2003 & 2004). These trials, in which FCM infestation was reduced by up to 80%, confirmed the concentration and coverage required to achieve the best results. During 2004 the virus was registered with the Department of Agriculture (Act 36 of 1947; registration number L7598) to be used against FCM on citrus, under the trade name, Cryptogran. Work conducted during 2005 on the shelf-life of Cryptogran and various field trials, are described in this report.

Materials and methods

Shelf-life

A total of 19 aliquots of Cryptogran were made. Each was 0.25 ml (250 µl), with a concentration of 1×10^{11} OBs/ml. One of these aliquots was immediately frozen at -40°C as the test standard. The other 18 were refrigerated at 10°C. At monthly intervals from one month after initiation of the trial, one vial was removed from the fridge and frozen at -40°C. This will continue for 18 months. However, at the time of writing this report, samples had only been incubated for 12 months. After incubation for the designated time, samples were bioassayed (dose-response) against neonate FCM larvae on artificial diet (two replicates). This was done alongside a bioassay with the equivalent sample that had been frozen at day zero. Bioassays were previously conducted with Cryptogran after seven, eight and 10 months (Moore *et al.*, 2004) at 10°C. For this report, bioassays were conducted after 12 months at this temperature. Probit analysis was used to analyse the results of the bioassays.

Compatibility field trial

A field trial was conducted to test the compatibility of Cryptogran with various pesticides which might be needed at the same time at which Cryptogran would normally be applied. Cryptogran was always applied at 10 ml per 100 l water with 500 ml molasses per 100 l water. The trial was applied in an orchard of Cara Cara Navel oranges on Penhill Farm in Sundays River Valley. The trial was laid out in a semi-commercial block format. Each treatment block (replicated twice) consisted of 81 trees. As trees were still young and small, an average of only 8 l of spray mix was applied per tree. Five Cryptogran treatments, each in a tank-mix with other products, were applied on 9 December 2004 (Table 3.2.2.1). An untreated control was retained.

After application, the trial was evaluated in the following manner. Seven data trees were selected in the middle of each block (i.e. a total of 14 data trees per treatment). Fruit drop (from data trees) was evaluated from three weeks after application, until there was a substantial decline in efficacy. Dropped fruit from each data tree was collected and analysed separately. Evaluations were done by inspecting and dissecting fruit in order to determine the cause of drop. FCM infestation was identified by the presence of the larva or its frass. Mean numbers of FCM infested fruit per tree per week for each treatment were compared using ANOVA and the Bonferroni multiple range test (or in one case the Students' t-test), using Statgraphics Plus for Windows Version 2.0 (Statistical Graphics Corporation, 1996).

Table 3.2.2.1. Treatments applied on 9 December 2004 for the control of FCM on Cara Cara Navel orange trees at Penhill Farm.

Treatment		Dosage in 100 l water
1	Untreated control	-
2	Cryptogran + molasses	10 ml + 500 ml
3	Cryptogran + molasses + abamectin + oil	10 ml + 500 ml 20 ml + 300 ml
4	Cryptogran + molasses + pyriproxyfen + oil	10 ml + 500 ml 30 ml + 300 ml
5	Cryptogran + molasses + benomyl + mancozeb + oil	10 ml + 500 ml 50 g + 200 g + 500 ml
6	Cryptogran + molasses + methidathion + Agral 90	10 ml + 500 ml

(wetter)	150 ml + 18 ml
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Cryptogran dosage and Cryptogran vs Cryptex field trials

Another FCM virus product, manufactured in Switzerland, is likely to soon be registered and available for use on citrus in South Africa. This product, which is named Cryptex, is manufactured with a distinctly different granulovirus to that which is in Cryptogran (Javier Ogembo, unpublished data).

Two field trials were conducted to test the efficacy of Cryptex against FCM, in comparison to the efficacy of Cryptogran. Simultaneously, Cryptogran was tested at a range of concentrations lower than the registered concentration.

The first trial was conducted in an orchard of Washington and Palmer Navel orange trees on Bernol Farm in Sundays River Valley. The orchard, which was planted at a density of 595 trees per hectare, was planted in 1998. The trial was laid out in a semi-commercial block format (twice replicated), as described above for the compatibility field trial. An average of 15.1 l of spray mix was applied per tree for each treatment. A total of seven treatments were applied on 1 December 2004 (Table 3.2.2.2). Five of these treatments were different concentrations of Cryptogran. The other two were Cryptex, at the concentration designated for registration i.e. 200 ml per hectare (Hennie Korf, personal communication; www.andermtt.co.za). One of these was applied with molasses and one was applied with sugar, as the suppliers were considering registering the product to be applied with sugar (Hennie Korf, personal communication). An untreated control was retained. The trial was also evaluated in exactly the same way as described above for the compatibility field trial.

Table 3.2.2.2. Treatments applied on 1 December 2004 for the control of FCM on Palmer and Washington Navel orange trees at Bernol Farm.

	Treatment	Dosage in 100 l water
1	Untreated control	-
2	Cryptogran + molasses	10 ml + 500 ml
3	Cryptogran + molasses	8 ml + 500 ml
4	Cryptogran + molasses	6 ml + 500 ml
5	Cryptogran + molasses	4 ml + 500 ml
6	Cryptogran + molasses	2 ml + 500 ml
7	Cryptex + molasses	2.25 ml + 500 ml
8	Cryptex + white sugar	2.25 ml + 500 g

The second trial was applied on Atmar Farm in Sundays River Valley in an orchard of mature Palmer Navel orange trees. This trial was intended to be a repeat of the dosage trial conducted earlier at Bernol Farm. The same seven treatments (Table 3.2.2.3) were applied to blocks of 63 trees (two blocks per treatment) on 2 February 2005. An average of 22.0 l of spray mix was applied per tree. Two Cryptex treatments were again included. However, this time both were applied with molasses. In one of the treatments, Cryptex was applied at the dosage proposed for registration (even though the concentration of the product was measured to be higher than stated). In the other treatment, Cryptex was applied at a lower dosage. This was done in order to rectify the concentration to that proposed for registration (equivalent to 200 ml per ha). FCM infestation of fruit was evaluated for a four-week period.

Table 3.2.2.3. Treatments applied on 2 February 2005 for the control of FCM on Palmer Navel orange trees at Atmar Farm.

	Treatment	Dosage in 100 l water
1	Untreated control	-
2	Cryptogran + molasses	10 ml + 500 ml
3	Cryptogran + molasses	8 ml + 500 ml
4	Cryptogran + molasses	6 ml + 500 ml
5	Cryptogran + molasses	4 ml + 500 ml
6	Cryptogran + molasses	2 ml + 500 ml
7	Cryptex + molasses	3.3 ml + 500 ml
8	Cryptex + molasses	0.41 ml + 500 ml

Molasses field trial

This trial was conducted to determine whether powdered molasses could be used as an alternative to liquid molasses. This trial was applied in an orchard of Palmer Navel orange trees on Orange Grove Farm in Sundays River Valley. The orchard, in which trees were spaced at 5.5 m x 2.5 m (rows x trees), was planted in 1995. Each treatment block (replicated twice) consisted of 60 - 65 trees. An average of 14 l of spray mix was applied per tree. Five Cryptogran treatments, each with a different formulation or concentration of molasses, were applied on 9 February 2005 (Table 3.2.2.4). An untreated control was retained.

Table 3.2.2.4. Treatments applied on 9 February 2005 for the control of FCM on Palmer Navel orange trees at Orange Grove Farm.

Treatment number	Product	Concentration per 100 l water	Additive	Dosage per 100 l water
1	Untreated	-	-	-
2	Cryptogran	10 ml	-	-
3	Cryptogran	10 ml	Molasses	500 ml
4	Cryptogran	10 ml	Molasses + Agral 90	250 ml + 18 ml
5	Cryptogran	10 ml	Kalorie 3000	450 g
6	Cryptogran	10 ml	Kalorie 3000 + Agral 90	450 g + 18 ml

Results and discussion

Shelf-life

Separate probit analyses were conducted with each of the two dose-response bioassay replicates using the 12-month refrigerated Cryptogran. In both replicates, the residual variances were found to be homogenous, indicating that the slopes of the lines were comparable. Again, in both of the replicates the control and treatment lines (dose-response relationships) were found to be parallel and their elevations were not significantly different. This is reflected in the LC₅₀ and LC₉₀ values calculated for both replicates (Table 3.2.2.5). The mean LC₅₀ (from the two replicates) calculated for "12-month old" Cryptogran was 5.00 x 10³ OBs/ml – marginally lower than that of "0-day old" Cryptogran: 6.07x 10³ OBs/ml. Calculated LC₉₀ values were even closer: 8.29 x 10⁴ OBs/ml for "12-month old" Cryptogran and 8.18 x 10⁴ OBs/ml for "0-day old" Cryptogran.

Table 3.2.2.5. Results of dilution series bioassays with neonate FCM larvae and Cryptogran refrigerated for 12 months at 10°C.

Treatment (Cryptogran concentration)	Corrected* larval mortality (%)			
	Replicate 1		Replicate 2	
	Control (0 days)	12 months	Control (0 days)	12 months
Distilled water control	-	-	-	-
1.219 x 10 ²	5.00	5.00	22.22	15.79
6.093 x 10 ²	10.00	15.00	27.78	26.32
3.046 x 10 ³	25.00	40.00	44.44	52.63
1.523 x 10 ⁴	55.00	45.00	66.67	73.68
7.616 x 10 ⁴	95.00	95.00	94.44	94.74
LC₅₀ (OBs/ml)	9.22x10³	7.49x10³	2.91x10³	2.51x10³
LC₉₀ (OBs/ml)	6.81x10⁴	1.10x10⁵	9.55x10⁴	5.58x10⁴

*Larval mortality corrected for control mortality.

Compatibility field trial

It is envisaged that under commercial conditions, the first treatment of Cryptogran 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. Methidathion (Ultracide) is also often used at this time for thrips and mealybug control. In some areas it might also be necessary to spray for the control of black spot. Mancozeb and benomyl with oil is likely to be the most popular treatment. Previously, laboratory trials revealed no reduction in efficacy of Cryptogran when any of these products were added at their registered concentrations (Moore *et al.*, 2004).

FCM infestation of fruit was relatively high at an average of 1.6 infested fruit per tree per week in the untreated control. All treatments significantly reduced fruit infestation by between 64% and 70% (Table 3.2.2.6). Although reduction in infestation was marginally greater where Cryptogran was not applied in combination with another product, none of the differences in efficacy recorded were significant (Table 3.2.2.6).

Table 3.2.2.6. FCM infestation of Cara-Cara Navel oranges on Penhill Farm subjected to various treatments (in combination with Cryptogran) applied on 9 December 2004.

Treatment		Fruit from data trees infested with FCM					Mean/tree /week ²	Reduction in infestation relative to control (%)
		3 WAT ¹	4 WAT	5 WAT	6 WAT			
1	Untreated control	24	15	25	26	1.61a	-	
2	Cryptogran	6	6	5	10	0.48b	70.0	
3	Cryptogran + abamectin	5	7	7	9	0.50b	68.9	
4	Cryptogran + pyriproxyfen	5	6	7	11	0.52b	67.8	
5	Cryptogran + benomyl + mancozeb	6	4	13	9	0.57b	64.4	
6	Cryptogran + methidathion	6	4	6	15	0.55b	65.6	

¹WAT = weeks after treatment.

²Values in the same column followed by the same letter are not significantly different (P>0.05; Bonferonni LSD multiple range test).

Weekly inspections of trees revealed no signs of phytotoxicity.

Cryptogran dosage and Cryptogran vs Cryptex field trials

FCM infestation was at a fairly high level in the trial orchard at Bernol Farm (an average of 1.83 infested fruit per tree per week). Despite this, all of the Cryptogran treatments significantly reduced infestation, by at least 56.3% (for the weakest concentration) (Table 3.2.2.7). There was no significant difference between any of the Cryptogran concentrations. However, the lowest concentration was the only one for which FCM infestation was not significantly lower than in the Cryptex treatments. Surprisingly, the 4 ml per 100 l concentration of Cryptogran resulted in the greatest reduction in FCM infestation. Cryptex did not work as well as did Cryptogran (Table 3.2.2.7). Sugar did not appear to be a viable alternative to molasses.

Table 3.2.2.7. FCM infestation of Palmer and Washington Navel oranges at Bernol Farm treated with a range of concentrations of Cryptogran and with Cryptex on 1 December 2004.

Treatment		Fruit from data trees infested with FCM					Mean/tree /week ²	Reduction in infestation relative to control (%)
		3 WAT ¹	4 WAT	5 WAT	6 WAT	7 WAT		
1	Untreated control	31	14	20	36	27	1.83a	-
2	Cryptogran 10 ml	5	4	7	11	13	0.57cd	68.8
3	Cryptogran 8 ml	5	4	9	10	13	0.59cd	68.0
4	Cryptogran 6 ml	8	2	7	11	8	0.51cd	71.9
5	Cryptogran 4 ml	9	2	8	7	4	0.43d	76.6
6	Cryptogran 2 ml	17	7	7	10	15	0.80bcd	56.3
7	Cryptex +	9	5	9	17	31	1.01abc	44.5

	molasses							
8	Cryptex + sugar	10	6	13	27	24	1.14ab	37.5

¹WAT = weeks after treatment.

²Values in the same column followed by the same letter are not significantly different (P>0.05; Bonferonni LSD multiple range test).

In the second trial, conducted on Atmar Farm, Cryptogran reduced infestation by between 29% (lowest concentration) and 45% (Table 3.2.2.8), indicating that the registered concentration should not be substantially lowered, if at all. Results were rather disappointing. This was ascribed to the poor coverage achieved with the non-oscillating tower mistblower used to apply the treatments. The two Cryptex treatments reduced infestation by 43% and not at all (Table 3.2.2.8).

Table 3.2.2.8. FCM infestation of Palmer Navel oranges at Atmar Farm treated with a range of concentrations of Cryptogran and with Cryptex on 2 February 2005.

Treatment	Fruit from data trees infested with FCM					Mean/tree /week ²	Reduction in infestation relative to control (%)
	3 WAT ¹	4 WAT	5 WAT	6 WAT			
1 Untreated control	16	14	11	8	0.88ab	-	
2 Cryptogran 10 ml	9	8	8	3	0.50c	42.9	
3 Cryptogran 8 ml	9	9	3	6	0.48c	44.9	
4 Cryptogran 6 ml	11	5	7	5	0.50c	42.9	
5 Cryptogran 4 ml	8	8	9	4	0.52c	40.9	
6 Cryptogran 2 ml	12	9	9	5	0.63bc	28.6	
7 Cryptex 3.3 ml	10	9	5	4	0.50c	42.9	
8 Cryptex + 0.41 ml	21	12	11	7	0.91a	-4.1	

¹WAT = weeks after treatment.

²Values in the same column followed by the same letter are not significantly different (P>0.05; Bonferonni LSD multiple range test).

Molasses field trial

Infestation in the trial orchard at Orange Grove Farm was very low, averaging only 0.21 infested fruit per tree per week in the untreated control (Table 3.2.2.9). Consequently, results must be considered inconclusive. Therefore, it will be necessary to repeat this trial. Despite this, all treatments reduced FCM infestation – some significantly.

Table 3.2.2.9. FCM infestation of Palmer Navel oranges at Orange Grove Farm treated with Cryptogran and molasses on 9 February 2005.

Treatment	Molasses added	Fruit from data trees infested with FCM				Mean/tree /week ²	Reduction in infestation relative to control (%)
		3 WAT ¹	4 WAT	5 WAT			
1 Untreated control	-	4	2	3	0.21a	-	
2 Cryptogran 10 ml	-	1	2	2	0.12ab	44.44	
3 Cryptogran 8 ml	Molasses	1	0	1	0.05b	77.78	
4 Cryptogran 6 ml	Molasses + Agral90	1	1	1	0.07b	66.67	
5 Cryptogran 4 ml	Kalorie 3000	2	1	2	0.12ab	44.44	
6 Cryptogran 2 ml	Kalorie 3000 + Agral 90	1	0	1	0.05b	77.78	

¹WAT = weeks after treatment.

²Values in the same column followed by the same letter are not significantly different (P>0.05; Bonferonni LSD multiple range test).

Conclusion

It was confirmed that the shelf-life of Cryptogran is at least 12 months, if kept refrigerated at a temperature of no more than 10°C. A field trial confirmed the compatibility of Cryptogran with a range of chemical pesticides, used for control of other pests. Cryptogran was tested at a range of concentrations, lower than the registered concentration. It appeared unlikely that the registered concentration could be lowered. Simultaneously, the efficacy of Cryptogran was compared with that of Cryptex, another FCM virus product. In one trial, Cryptogran performed significantly better than did Cryptex. In another field trial, powdered molasses was tested as an alternative to liquid molasses. Infestation was too low for results to be conclusive.

Future research

This experiment has now come to an end. However, further work on the field persistence of Cryptogran is currently being conducted under a new experiment (no. 791), led by Wayne Kirkman.

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

Experiment 648 by Christo Smit (Desense Pest Control, Citrusdal)

Opsomming

Gedurende die verslagtydperk is byna uitsluitlik gekonsentreer op beheerde olfaktometerproewe om 'n wye verskeidenheid van reukstowwe te sif ten opsigte van hulle aanloklikheid vir VKM. Gemengde geslagte van motte is gebruik om die siftingsproses te versnel.

Daar is ook begin met 'n proefprosedure wat ontwikkel is om die toepaslikheid van olfaktometerresultate onder beter beheerde toestande in opelugtoestande te bevestig. Dit is gedoen as tussenstap tot praktiese toepassing in vrugteboorde.

Die beskikbare voorrade verbindings in verskillende chemiese groeiperings waarin aantrekmiddels aangetref word, is grotendeels in siftingsproewe getoets.

(i) Terpene

- **Hemiterpene (C5):** 2-Metiel 3-buten-2-ol en 3-metiel 3-buten 1-ol en die versadigde 3-metiel butanol.
- **Monoterpene (C10):** Veral nerielasetaat, maar ook terpinielasetaat, geranielasetaat en terpineen.
- **Seskwiterpene (C15):** Veral farnesielaseton en bisaboleen; asook kubebeen, panasinseen, kopaeen, sedreen en elemol
- **Diterpene (C20):** Aurantiol
- **Plantolies** (waarin o.a. C15 en C20- terpene voorkom): Veral olies/ekstrakte vanaf kardamom en kamomiel, asook vanaf patchouli, kananga, ylang-ylang, Litsea cubeba, lemoenbloeisels (neroli), sandelhout, guaiak-hout en tot 'n swakker mate, lemoenessensolie.

(ii) Fenoliese (=benseen)verbindings

- **Metoksie benseene:** 2-metoksie bensiël alkohol (=Guaiakol) en 3-metoksie 2-hidroksie bensiëlalkohol (=3-metoksie katekol).
 - **Propeniël benseene:** Sinnamielisobutiraat en isobutiëlsinnamaat, anetol en 2-metoksie anetol.
 - **Propiël benseene:** Bensiëlpropionaat, fenieletielpropionaat en 3-feniëlpropionaaldehyd.
- **Aminobenseene:** Verskillende antranilate (=2-aminobensoate), o.a. veral metiel N-metielantranilaat en tot 'n mindere mate etiel-, butiel-, dekiel, geraniel- en sitronellielantranilate; ook die metielantranilaatanisaldehyd verbinding; ook van die nie-antranilate, S-alfa-metielbensiëlamien.

(iii) O-Heterosikliese verbindings (= suurstof-bevattende ringverbindings)

- Gamma Dodekalaktoon en 3-Formiel 6-metielkromoon

(iv) Onversadigde C6 tot C12 olefiene (medium lengte reguit kettings)

- **C6- verbindings** (o.a. blaarreukstowwe): Veral E2-Heksenal asook Z3-hekseniëlisobutiraat.
- **C8 tot C10 Verbindings** (vrugtereukstowwe): E2-Oktenal, 2,4-oktadienal, E2-nonenal en die E2,E4-, E2,E6- en E2,Z6- isomere van nonadienal.

(v) Onversadigde C18 tot C22+ verbindings: (oa. die sg poli-ene):

- Z3,Z6,Z9-Eicosatriene (C20) en skwaleen (C30)

(vi) Ander poli-onversadigde verbindings:

- Retiniëlasetaat, retiniëlpropionaat en retiniëlpalmiëtaat.

(vii) Ander

- Water, hidroksie-asetoon en dodekiëlasetaat.

(viii) Kombinasies

Indikasies van sinergisme is gevind in kombinasies van die volgende:

- Skwaleen in kombinasie met óf etielantranilaat óf lemoenessens/olie plus L-sisteïen.
- Retiniëlasetaat in kombinasie met E2,E6-nonadienal plus L-sisteïen of DL-iseleusien

Introduction

The primary aim of this year's experiments was to identify FCM attractive semiochemical odorants under controlled olfactometer conditions with the view to developing attract-and-kill techniques.

Material and methods

A Olfactometer screening

The olfactometer: Initial olfactometer developments were described in the 2004 CRI Annual Report. Instead of the plastic tubes used during 2004-2005, glass tubes were used exclusively during 2005. Dimensions were 75 mm long x 50 mm diameter. The tubes were divided into seven migration zones with marker rings 100 mm apart.

At the start of each experiment FCM were released at the starting point of each tube, i.e. in the space between the 0- and 1- rings. Blank control tubes were kept for comparison. Migration indexes were calculated by multiplying the percentages of moths present in every distance zone by the number (= distance travelled) of that zone. The sum of all these products were added and expressed as a percentage of the migration index of the blank control.

The capacity of the olfactometer apparatus (i.e. the number of tubes) was gradually increased from 10 to 16 tubes. The capacity of the suction and exhaust fans was also increased commensurately.

- **Fitting mesh stoppers at the ends of the olfactometer tubes:** Black PVC irrigation pipe 40 mm in diameter were cut in 50 mm lengths as stoppers for the glass tubes. A section of nylon insect mesh was placed over one end of a section of plastic pipe and pressed into the end of the 50 mm glass tube.
- **Preparing the olfactometer tubes:** The test FCM were obtained from Ceder Biocontrol Insectary, Citrusdal. The moths were immobilized before use by cooling in a household refrigerator. The glass tubes were held upright with the mesh stoppers at the bottom. Approximately 100 immobilized FCM of mixed sexes were measured off into each tube with a 5 ml measuring spoon. The moths therefore accumulated in the 0-1 zone. After loading, the upper end of each glass tube was closed with a second mesh stopper.
- **Fitting the prepared tubes into the olfactometer:** The temperature inside the olfactometer room is kept at 16°C to maintain moth immobilization. The exhaust ends of the tubes (0-1 zone) were attached to an exhaust manifold by means of 60 mm plastic pipe nipples. The intake ends of the glass tubes were fitted with experimental odorants in dispensers placed inside the intake mesh stopper. Each odorant dispenser consisted of a 20 mm x 20 mm piece of six-ply toilet paper to which 0,5 ml of a particular test odorant was added. The dispensers were then rolled into a 30 mm wide strip of heavy-duty aluminium foil. The emission rate could be regulated to a certain degree by opening or closing the end of each foil cylinder.

After preparing all tubes, the temperature of the test room was increased to 22°C and the intake and exhaust fans started. The immobilized moths became active at 20°C and a test was initiated at this stage.

- **Moth counts:** All tests were conducted in the dark, and a flashlight was used momentarily for observation and counting. A total of six observations were made at 10 to 15 minute intervals to inspect moth migration down the tubes toward the odorant source. The data were transferred to Microsoft Excel to calculate the distribution means in each of the seven zones.
- **Odorant sources:** Test odorants were obtained from Aldrich (USA), Fluka (Switzerland), Bedoukian (USA) and R C Treatt (Britain).

Results and discussion

The results are presented in terms of a Relative Dispersion Index (RDI), expressed as a percentage of the dispersion index of the control (olfactometer tubes with no odorants = 100%). When a compound was tested more than once, the mean RDI of the applicable number of replicates is mentioned in the tables. In general, a RDI of more than 120, was representative of an odorant that was very active in terms of FCM attraction. A RDI of 105-120 was regarded as intermediate and compounds with a RDI of less than 105 had little attractive capabilities.

The attractiveness of odorants of various chemical groupings for FCM were as follows:

1 Plant oils and extracts

Relatively high dispersion indices (RDI = >120) were obtained with Cardamom oil, chamomile oil, Cananga oil and Patchouli oil (Table 3.2.3.1). Most attention will be given to the further evaluation of these compounds. Litsea cubeba oil, ylang-ylang oil, Lippia oil, Sandalwood oil, Artemisia oil, neroli oil, ginger oil and guaiacwood oil were generally less active (RDI = 110-120), but will also be included on a smaller scale.

Table 3.2.3.1. Attractiveness for FCM of various plant oils and extracts.

Replicates	Odorant	RDI (Control =100%)
2	Cananga oil	119.2
2	Litsea cubeba oil	119.1
2	Chamomile oil	116.4
2	Ylang-ylang oil	114.6

2	Neroli oil (Orange blossom oil)	114.6
2	Opopanax oil	101.1
2	Chamomile oil	133.3
2	Cardamon oil	127.1
2	Patchouli oil	122.9
2	Cananga oil	121.2
2	Litsea cubeba oil	112.4
2	Ylang-ylang oil	110.5
2	Cardamon oil	135.6
2	Sandalwood oil	121.5
2	Patchouli oil	115.5
2	Ginger oil	114.3
2	Guaiacwood oil	113.1
2	Cedarwood oil	109.4
2	Lippia odorata oil	121.7
2	Agathosma glabrata (fresh leaves)	121.5
2	Artemisia sp. oil ("Lanyana")	117.9
2	Salvia africana (Mountain sage) oil	104.5
2	Erioccephalus sp (Cape Snowbush) oil	101.1
1	Lippia odorata oil	113.7
1	Artemisia sp. oil ("Lanyana")	110.8
1	Salvia africana oil (Mountain sage)	110.6
1	Erioccephalus sp oil (Cape Snowbush)	99.8
1	Tagetes minuta oil ("Tagette")	98.0
2	Orange oil 5%	105.3
2	Tagetes minuta oil ("Tagette")	101.5
2	Rose geranium oil	99.5
2	Apricot-peach nectar	94.5
2	Treacle/molasses	96.4
2	Orange essence 7	105.6

Potential advantages of plant oils include their lower price in general, they can be agriculturally produced and their lesser volatility which results in longer emission of vapours.

2 Terpenes

2.1 Hemiterpenes

The following hemiterpenes were relatively attractive and more attention will be given to them in further testing (Table 3.2.3.2):

(a) Unsaturated methyl butenyl hemiterpenes and related compounds: 2-Methyl 3-buten 2-ol, 3-Methyl 3-buten 1-ol, Isobutyl E2-butenate and Isobutyl tiglate.

(b) Related methyl butyl and isobutyl compounds: Cinnamyl isobutyrate, also isobutyl cinnamate and 3-Methyl butanol.

Table 3.2.3.2. The attractiveness for FCM of C5 hemiterpenes (methyl butenyl and related methyl butyl compounds).

Replicates	Odorant	RDI (Control =100%)
Isobutenyl alcohols/aldehydes		
1	2-Methyl 3-buten 2-ol	117.8
1	3-Methyl 3-buten 1-ol	108.2
1	E2-Methyl 2-butenal (=tiglic aldehyde)	104.3

2	Tiglic aldehyde	94.8
Isobutyl esters		
2	Isobutyl E2-butenoate	109.0
2	Isobutyl tiglate	106.6
2	Isobutyl angelate	97.8
1	Isobutyl cinnamate	115.2
1	Z3-Hexenyl isobutyrate	115.0
1	Cinnamyl isobutyrate* (induces sexual hiperactivity)	120.1
1	Isobutyl cinnamate	110.2
Isobutyl/methyl butyl aldehydes/alcohols		
2	3-Methyl butanol	113.8
2	3-Methyl butyraldehyde	86.6
2	2-Methyl butyraldehyde	89.3
2	2-Ethyl butyraldehyde	98.2
Isobutyl/methyl butyl aldehydes/alcohols/acids & related		
1	3-Methyl butanol	105.0
1	3-Methyl butyraldehyde	93.5
1	2-Methyl butyraldehyde	87.6
1	2-Ethyl butyraldehyde	95.5
1	2-Methyl butyric acid	86.7
1	1-Butanol	90.9

2.2 Monoterpenes

Activity of the following promising odorants will be confirmed (Table 3.2.3.3):

Neryl acetate, alpha-terpinyl acetate, geranyl acetate, alpha terpinene, with linalyl acetate and nerol as border cases.

Table 3.2.3.3. The attractiveness for FCM of C10 monoterpenes.

Replicates	Odorant	RDI (Control =100%)
Monoterpenes – acetate esters		
2	Neryl acetate	125.9
2	alpha-Terpinyl acetate	120.2
2	Geranyl acetate	115.3
2	Linalyl acetate	108.0
2	Menthanyl acetate	106.5
2	Myrcenyl acetate	105.2
2	Bergyl acetate	105.0
Monoterpenes (-OH, =O and CHO)		
2	Nerol	107.0
2	Carvone	104.0
2	Geraniol	101.5
2	Linalool	101.7
2	Citral	100.9
2	Verbenone	99.8
2	Verbenol	97.8
Mono terpenes [-H]		
2	alpha-Terpinene	117.2
2	Ocimene	101.8
2	Limonene	99.2

2	alpha-Pinene	96.5
2	beta-Pinene	96.5
2	Myrcene	95.3
2	Sabinene	91.5
2	Mono terpenes (-H and =O)	
1	Allo-ocimene (C10)	106.6
1	delta-Carene (C10)	105.0
1	Geranyl acetone (C10)	97.3
1	Menthone (C10)	92.1

2.3 Sesquiterpenes (C15)

Farnesyl acetone and bisabolene seem to be the better of the sesquiterpene attractants (Table 3.2.3.4). Alpha-cubebene and alpha-panasinsene are too expensive in pure form and will further be investigated in the natural plant oil form in which they occur, i.e. Litsea cubeba oil and Opopanax oil.

In the many other FCM attractant plant oils mentioned, all will contain among others, their specific sesquiterpenes and diterpenes, most of which are not available as pure laboratory chemicals. Examples are cadinene and cadinol in Canaga oil, cubebene in Litsea cubeba oil, ylangene in ylang-ylang oil and sinensal in citrus essence oil.

Table 3.2.3.4. The attractiveness for FCM of C15 Sesquiterpenes (with -H, -OH, =O and -CHO active groups).

Replicates	Odorant	RDI (Control =100%)
2	Alpha-Cubebene	111.5
2	Alpha-Panasinsene	110.0
2	Elemol	107.1
2	Cedrene	107.1
2	Alpha Copaene	106.3
2	Sinensal 40A	104.5
2	Farnesene	100.5
2	Nerolidol	92.9
2	alpha-Isomethyl ionone	92.7
2	alpha-Ionone	81.3
2	Farnesyl acetone (C15) 1x	128.4
1	Bisabolene (C15) 1 x	111.2
1	alpha Isomethyl ionone (C15)1x	107.7
1	Cedrene (C15) 1x	100.5
1	Bisabolene	125.6
2	Farnesyl acetone	119.3
2	Elemol (C15)	103.3
2	Sinensal 40A (C15)	99.5
2	Farnesol (C15)	96.1
2	Valencene (C15)	79.6
2	Cedrene 1x	93.2
2	Farnesol	102.0

The indications of better FCM attraction by the following odorants in paired tests, will have to be confirmed in more replications. Farnesyl acetone and bisabolene seem to be potentially the most useful of the sesquiterpene attractants. Alpha-cubebene and alpha-panasinsene are too expensive in pure form and will be further investigated in the natural plant oils in which they occur, i.e. Litsea cubeba oil and Opopanax oil.

2.4 Diterpenes (C20)

One of the two diterpenes that were commercially available, aurantiol, revealed a strong attraction for FCM and will be further evaluated (Table 3.2.3.5).

Table 3.2.3.5. The attractiveness for FCM of C20 diterpenes.

Replicates	Odorant	RDI (Control =100%)
2	Aurantiol (C20)	133.5
2	Dimyrcetol (C20)	99.8

3 Unsaturated olefins

3.1 C6 compounds

E2-hexenal and Z3-hexenyl isobutyrate will be further evaluated (Table 3.2.3.6).

Table 3.2.3.6. The attractiveness for FCM of leaf volatiles.

Replicates	Odorant	RDI (Control =100%)
C6 aldehydes, alcohols, acids and esters		
2	E2-Hexenal	126.8
2	E2,E4-Hexadienol	105.8
2	Hexanal	105.6
2	E2-Hexenyl acetate 1x	103.6
2	E2-Hexenol	102.4
2	E2,E4-Hexadienoic acid	92.1
C6 esters - unsaturated		
1	Z3-Hexenyl isobutyrate	129.5
1	Z3-Hexenyl butyrate	105.6
1	Z2-Hexenyl butyrate	84.3
2	E2-Hexenal	130.1

3.2 C8 - C12 compounds

The attractiveness of E2-Octenal and E2-nonenal, ?-2,4 –Octadienal, E2,E4-nonadienal, E2,E6- and E2,Z6-nonadienal (all C8 and C9 unsaturated aldehydes) for FCM has been fairly well established in repeated tests (Table 3.2.3.7).

Table 3.2.3.7. The attractiveness for FCM of fruit flavours.

Replicates	Odorant	RDI (Control =100%)
2	E2-Octenal	127.1
2	E2-Nonenal	126.4
2	E2,E4-Nonadienal	125.5
2	?-2,4-Octadienal	121.6
2	E2,Z6-Nonadienal	116.8
2	E2,E6-Nonadienal	111.7
2	?-2,4-Octadienal 1x	113.6
2	E2, E4-Nonadienal	133.7
2	E2,Z6-Nonadienal	113.2
2	Ethyl E2,Z4-Decadienoate	107.5
2	E2,E4-Decadienal	97.7
2	E2,Z6-Dodecadienal	88.3

2	E2,E6-Nonadienal	113.6
2	E2,E6-Nonadienal	125.4
2	E2-Nonenal	126.0
2	E2-Octenal	111.4
2	E2-Decenal	104.9
2	E2-Dodecenal	102.3
2	E2,Z6-Nonadienal	116.7
2	E2,E6-Nonadienal	106.4
2	E2-Nonenyl acetate	96.2
2	E2-Octenal	117.9
2	?-2,4-Octadienal	114.2
2	E2-Octenyl acetate	96.3
2	E2-Hexenal	130.1
2	E2-Octenal	116.5
2	E2-Decenal	101.3
2	E2-Undecenal	94.8
2	E2-Dodecenal	94.6

3.3 Long chain poly-unsaturates (polyenes C18 – C22+)

Z3,Z6,Z9-eicosatriene and squalene (2,6,10,15,19,23-hexamethyl 2,6,10,14,18,22-tetracosahexaene) is earmarked for further attention (Table 3.2.3.8). Polyenes are natural aggregation pheromones in many Lepidoptera species. This group will be investigated to find a more specific compound with stronger attraction for FCM. Squalene (a natural C30 oil) is fairly cheap and may be useful in combinations mentioned below, while the C20 polyene is very expensive. The reason why so much importance is attached to Z3, Z6, Z9-eicosatriene, is that it represents a fairly large group of natural Lepidoptera pheromones which attracts both sexes of certain Lepidoptera species. It thus acts as an aggregation pheromone in these species. It has not yet been reported to have been found in Tortricids but that does not mean that it may be without effect. However, these polyenes are not available in the pheromone market and have to be obtained by de novo custom synthesis order and are therefore very expensive.

Table 3.2.3.8. The attractiveness for FCM of polyenes.

Replicates	Odorant	RDI (Control =100%)
Polyenes		
4	Z3,Z6,Z9-Eicosatriene (C20)	109.9
2	Z3,Z6,Z9-Eicosatriene	106.0
2	Z3,Z6,Z9-Eicosatriene	112.7
2	Squalene (C30)	116.5
2	Squalene	106.5

3.4 Retinyl compounds (=C12 poly-unsaturate with double bonds at positions 2, 4, 6 and 8 + trimethyl cyclohexyl group)

The attractiveness of acetate, propionate and palmitate retinyl esters for FCM (including) was confirmed in all cases (Table 3.2.3.9). See also interactions with other odorants below.

Table 3.2.3.9. The attractiveness for FCM of C12 poly-unsaturates.

Replicates	Odorant	RDI (Control =100%)
2	Retinyl palmitate	126.6
2	Retinyl acetate	123.0
2	Retinyl propionate	114.3
2	Retinyl palmitate	106.3

2	Retinyl palmitate*	125.5
2	Retinyl acetate	117.3
2	Retinyl propionate	109.2
3	Retinyl acetate	131.7
2	Retinyl acetate	110.0

*Retinyl palmitate polymerised during the test series, concomitant deactivation occurred and attraction was lost.

4 Oxygen containing ring structures (“O-Heterocyclics”)

The better choices in this group which have to be further confirmed were gamma-dodecalactone, 3-formyl 6-methyl chromone and heliotropyl (Tables 3.2.3.10 and 3.2.3.11).

4.1 Non-benzyl heterocyclics

Table 3.2.3.10. The attractiveness for FCM of non-benzyl heterocyclics.

Replicates	Odorant	RDI (Control =100%)
2	gamma-Dodeca lactone	122.2
2	alpha-Angelica lactone	96.9
2	gamma-Decalactone	90.7
2	gamma-Undecalactone	87.9
2	omega-6-Hexadecalactone	86.6
2	delta-Dodecalactone	80.0
2	C16 “Aldehyde” A*	102.3
2	C19 “Aldehyde” B*	99.1
2	C29 “Aldehyde” C*	96.4
2	C18 “Aldehyde” D*	88.9

*Names protected for commercial reasons.

4.2 Oxygen containing rings + benzyl ring (benzyl heterocyclics)

Table 3.2.3.11. The attractiveness for FCM of benzyl heterocyclics.

Replicates	Odorant	RDI (Control =100%)
2	3-Formyl 6-methyl chromone	112.9
2	Heliotropyl acetate	112.1
2	6-Methyl coumarin	105.0
2	7-Methoxy coumarin	102.0
2	Dihydro coumarin	86.4
2	Coumarin	68.4

Gamma-dodecalactone, 3-formyl 6-methyl chromone and heliotropyl acetate will be further investigated.

5 Benzyl/phenyl compounds

5.1 Methoxy benzenes

Further attention will be given to 2-Methoxy benzyl alcohol (=Guaiacol) and 3-Methoxy 2-hydroxy benzyl alcohol (=3-Methoxy cathecol) (Table 3.2.3.12). In contrast with these methoxy benzyl alcohols, the methoxy benzaldehydes were ineffective.

Table 3.2.3.12. The attractiveness for FCM of methoxy benzenes.

Replicates	Odorant	RDI (Control =100%)
Methoxy benzyl alcohols(=methoxy-phenols)		
2	2-Methoxy benzyl alcohol (=Guaiacol)	109.3
2	2-Methoxy benzyl alcohol (=Guaiacol)	117.3
1	3-Methoxy 2-hydroxy benzyl alcohol (=3-Methoxy catechol)	117.1
1	2-Methoxy 4-methyl benzyl alcohol (=Methyl guaiacol)	98.6
Methoxy benzyl aldehydes		
1	4-Methoxy 3-hydroxy benzaldehyde	99.2
1	3-Methoxy 4-hydroxy benzaldehyde	98.4
1	3-Methoxy 2-hydroxy benzaldehyde	93.0
1	4-Methoxy 2-hydroxy benzaldehyde	90.5
1	5-Methoxy 2-hydroxy benzaldehyde	89.7
1	3-Methoxy 2-hydroxy benzaldehyde	97.2
2	3,4-Dimethoxy benzaldehyde	93.9
2	3,5-Dimethoxy 4-hydroxy benzaldehyde	92.5
Other methoxy benzenes		
2	2-Methoxy benzyl acetate	86.4
2	1-Methoxy 4-methyl benzene	80.1
1	1-Methoxy benzene (Anisole)	102.5

5.2 Benzyl propyl and benzyl propenyl compounds

In the propenyl group, the double bond can be positioned in the middle of the C3 group (which occurs in the cinnamyl group), or at the end (the allyl group, which occurs in the eugenol group). In general the eugenol group is less attractive towards FCM than the cinnamyl group.

Cinnamyl isobutyrate, Isobutyl cinnamate, 2-Methoxy anethole and Anethole (all benzyl propenyls), as well as 3-Phenyl propyl aldehyde (=Hydro-cinnamaldehyde), Benzyl propionate and Phenylethyl propionate (all benzyl propyls) were be further evaluated (Tables 3.2.3.13, 3.2.3.14 and 3.2.3.15). Methyleugenol and 2-Phenyl 2-butenyl aldehyde were borderline. In general the eugenol group was less attractive than the cinnamyl group.

The good attraction of cinnamyl isobutyrate was accompanied by a noteworthy induction of sexual hyperactivity of both sexes. This reminds one of another cinnamyl ester, i.e. ethyl cinnamate, which occurs in the aphrodisiac mixture of the related Oriental fruit moth and facilitates mating.

5.2.1 Propenyl benzenes

Table 3.2.3.13. The attractiveness for FCM of cinnamyl compounds (double bond at middle C of propenyl group).

Replicates	Odorant	RDI (Control =100%)
2	Isobutyl cinnamate	119.3
2	Cinnamic aldehyde	95.3
2	Ethyl cinnamate	87.0
2	Cinnamic alcohol	80.9
1	Cinnamyl isobutyrate* (Induce sexual hyperactivity)	120.1
1	Isobutyl cinnamate	110.2
1	Cinnamyl acetate	76.1
1	Ethyl cinnamate	75.9

1	trans-Cinnamic aldehyde (3-Phenyl 2-propenyl aldehyde)	70.4
Eugenol compounds (Double bond at end C of propenyl group)		
1	Methyl eugenol	108.8
1	Methyl iso-eugenol	77.6
1	Eugenol (= 2-Methoxy 4(1-propenyl) phenol)	76.2
1	Iso-eugenol	69.8
Cinnamyl related (2-propenyl)		
1	1,2-Dimethoxy 4(2-propenyl) benzene (=2-Methoxy anethole)	110.3
1	1-Methoxy 4(2-propenyl) benzene (= Anethole)	109.0
Eugenyl related (1-propenyl)		
1	2(1-Propenyl) phenol (=2-Allyl phenol)	96.2
1	1-Methoxy 4(1-propenyl) benzene (=Estragole)	92.1

5.2.2 Propyl benzenes

Table 3.2.3.14. The attractiveness for FCM of propyl benzenes.

Replicates	Odorant	RDI (Control =100%)
1	3-Phenyl propyl aldehyde (=Hydro-cinnamaldehyde)	118.2
1	Benzyl propionate	116.8
1	Phenylethyl propionate	115.2

5.2.3 Butenyl benzenes

Table 3.2.3.15. The attractiveness for FCM of butenyl benzenes.

Replicates	Odorant	RDI (Control =100%)
2	2-Phenyl 2-butenyl aldehyde	108.5

5.3 Amino benzenes

Methyl N-methyl anthranilate, Anisaldehyde-methyl anthranilate, Butyl anthranilate, Ethyl anthranilate, Decyl anthranilate, Citronellyl anthranilate (all amino benzoates), as well as S-alpha-methyl benzylamine and Octopamine (both benzyl amines) will be further evaluated (Table 3.2.3.16).

Table 3.2.3.16. The attractiveness for FCM of amino benzenes.

Replicates	Odorant	RDI (Control =100%)
2-Amino benzenes (= anthranilates)		
1	Methyl N-methyl anthranilate	125.5
1	Anisaldehyde-methyl anthranilate	124.2
1	Butyl anthranilate	114.6
1	Ethyl anthranilate	113.3
1	Decyl anthranilate	112.9
1	Citronellyl anthranilate	111.2
1	Phenylethyl anthranilate	108.5
1	Geranyl anthranilate	107.9
1	Cyclohexyl anthranilate	103.5
1	Menthyl anthranilate	101.5
1	Lilial-methyl anthranilate	99.9
1	Methyl anthranilate	96.9

1	Canthoxal-methyl anthranilate	87.5
1	Triplal-methyl anthranilate	82.2
2, 3, 4- Amino-benzoates		
1	Ethyl 2-aminobenzoate	105.0
1	Ethyl 3-amino benzoate	103.6
1	Ethyl 4-amino benzoate	100.5
1	Butyl 4-amino benzoate	99.6
1	4-Amino benzoic acid	92.8
Other aminobenzenes		
1	S-alpha-methyl benzylamine	112.6
1	Octopamine	108.5
1	4-Ethoxy 1-aminobenzene (= p-Phenetidine)	105.1
1	4-Isopropyl 1-aminobenzene	101.2
1	2-Amino phenylethanol	100.9
1	Ephedrine	99.8
1	R-alpha-Methyl benzylamine	99.0
1	m-Toluamide	92.1
1	Phenyl ethylamine	85.9
3	Ethyl 2-aminobenzoate (=Ethyl anthranilate)	109.9
2	Ethyl 2-aminobenzoate (=Ethyl anthranilate)	113.0

6 Other attractants

The activity of dodecyl acetate and hydroxy acetone will be confirmed in follow-up experiments (Table 3.2.3.17). Dodecyl acetate is an important part of the natural FCM sex pheromone. In the 2004 CRI Annual Report, a synergistic action of dodecyl acetate together with the commercially used FCM sex pheromone was indicated in olfactometer tests. The attractiveness of water vapour has also been recorded in previous tests.

Table 3.2.3.17. The attractiveness for FCM of saturated olefin esters ketones and water.

Replicates	Odorant	RDI (Control =100%)
C6 Olefin esters		
2	Ethyl hexanoate	80.8
2	Propyl hexanoate	81.9
2	Butyl hexanoate	94.3
2	Iso-amyl hexanoate	88.9
2	Hexyl hexanoate	79.7
C8 to C18 acetate series		
2	Octyl acetate	90.5
2	Decyl acetate	94.9
2	Dodecyl acetate	115.7
2	Tetradecyl acetate	89.5
2	Hexadecyl acetate	99.5
2	Octadecyl acetate	92.8
Ketones		
2	Hydroxy acetone	111.6
2	Pyruvic aldehyde	98.4
2	Ethyl pyruvate	88.4
Water		
2	Water	114.1

7 Interactions between groups

7.1 Ethyl anthranilate interaction

The synergistic properties of the ethyl anthranilate + squalene combination will be confirmed in follow-up experiments (Table 3.2.3.18).

Table 3.2.3.18. The attractiveness for FCM of ethyl anthranilate in combination with compounds in other chemical groups.

Replicates	Odorant	RDI (Control =100%)
Ethyl anthranilate : Retinyl acetate		
3	Ethyl 2-aminobenzoate (=Ethyl anthranilate)	109.9
3	Retinyl acetate	131.7
3	Retinyl acetate + Ethyl anthranilate	117.6
2	Ethyl 2-aminobenzoate (=Ethyl anthranilate)	113.0
2	Retinyl acetate + Ethyl anthranilate	120.0
Ethyl anthranilate: unsaturated olefins/Orange essence		
2	Ethyl 2-aminobenzoate (=Ethyl anthranilate)	113.0
2	Z3,Z6,Z9-Eicosatriene	112.7
2	Z3,Z6,Z9-Eicosatriene + Ethyl anthranilate	98.8
2	E2,E6-Nonadienal	125.4
2	E2,E6-Nonadienal + Ethyl anthranilate	110.9
2	Orange essence 7	105.6
2	Orange essence 7 + Ethyl anthranilate	110.9
2	Orange essence 7 + Butyl 4-aminobenzoate	110.0
2	Squalene	106.5
2	Squalene + Ethyl 2-aminobenzoate (Ethyl anthranilate)	123.8
2	Squalene +Butyl 4-aminobenzoate	105.2

7.2 Squalene interaction

Squalene + ethyl anthranilate, squalene + L-cysteine and squalene + cysteine together with orange essence or orange oil all demonstrated relatively high activity (Table 3.2.3.19).

Table 3.2.3.19. The attractiveness for FCM of Squalene in combination with compounds in other chemical groups.

Replicates	Odorant	RDI (Control =100%)
2	Squalene	106.5
2	Squalene + Ethyl 2-aminobenzoate (Ethyl anthranilate)	123.8
2	Squalene +Butyl 4-aminobenzoate	105.2
2	Squalene + L-Cysteine	118.0
2	Squalene + L-Cysteine + Orange essence 7	124.6
2	Squalene + L-Cysteine + Orange oil cold pressed	120.2
2	Squalene + L-Cysteine + Ethyl sorbate (fruity ester)	106.0
2	Squalene + DL-Isoleucine + Ethyl sorbate	99.4

7.3 Retinyl acetate/palmitate interaction

The synergism of the retinyl acetate + Z3, Z6, Z9-eicosatriene combination and between retinyl acetate and the combination of E2,E6-nonadienal with either L-cysteine or DL-Isoleucine will be further investigated in follow-up experiments (Table 3.2.3.20).

Table 3.2.3.20. The attractiveness for FCM of Retinyl acetate and Retinyl palmitate in combination with compounds in other chemical groups.

Replicates	Odorant	RDI (Control =100%)
2	Z3,Z6,Z9-eicosatriene	106.0
2	Z3,Z6,Z9-eicosatriene + retinyl acetate	115.7
2	Retinyl acetate	110.0
2	Retinyl acetate + E2,E6-nonadienal	112.0
2	E2,E6-nonadienal	113.6
2	Retinyl acetate + ethyl sorbate	95.5
1	L-cysteine	97.0
1	Retinyl acetate + E2,E6-nonadienal + L-cysteine	130.3
1	Retinyl acetate + E2,E6-nonadienal + DL-isoleucine	128.4
2	Retinyl acetate + L-cysteine + ethyl sorbate	110.3
2	Retinyl acetate +DL-isoleucine + ethyl sorbate	92.7
2	Retinyl palmitate	106.3
2	Retinyl palmitate + isobutyl tiglate	112.9
2	Retinyl palmitate + ethyl sorbate	99.2

7.4 Z3,6,9-Eicosatriene interactions

Synergism of the Z3, Z6, Z9-eicosatriene + Retinyl acetate will be further investigated in follow-up experiments (Table 3.2.3.21).

Table 3.2.3.21. The attractiveness for FCM of Z3,6,9-Eicosatriene in combination with compounds in other chemical groups.

Replicates	Odorant	RDI (Control =100%)
2	Z3,Z6,Z9-Eicosatriene	106.0
2	Z3,Z6,Z9-Eicosatriene + Retinyl acetate	115.7
2	Z3,Z6,Z9-Eicosatriene + Retinyl acetate + E2,E6-Nonadienal	111.3
2	Z3,Z6,Z9-Eicosatriene + Retinyl acetate + methyl anisole	108.9
2	Z3,Z6,Z9-Eicosatriene + Retinyl acetate + isobutyl tiglate	90.8

7.5 Water and combinations

No synergism was found with the following combinations (Table 3.2.3.22).

Table 3.2.3.22. The attractiveness for FCM of water in combination with other compounds.

Replicates	Odorant	RDI (Control =100%)
2	Water	114.1
2	Water + Orange essence 7	101.0
2	Water + 2-Methyl butyraldehyde	97.4
2	Water + Octanal	91.0

B Open air experiments with odorants

- **Traps for field experiments:** Yellow delta traps fitted with sticky pads from Chempack were used exclusively.
- **Layout of field experiments:** The traps were exposed on leaf litter in the shade under fairly dense Port Jackson trees. Two traps, each fitted with an odorant dispenser containing the same odorant, were placed parallel and about 300 mm apart next to each other on the dead leaf litter. Only one pair of traps was used per treatment. Approximately 200 FCM were deposited between the two traps on the leaf litter at sunset. Trap pairs were at least 10 m apart. For comparison, traps with no odorants were used as controls. Counts of trapped moths were conducted daily for three days after test initiation (Table 3.2.3.23).

In this unreplicated pilot experiment, the volatile plant oils from *Litsea cubeba* and *Cananga* attracted more FCM than the sex pheromone, the orange oil and the unsaturated olefins (Table 3.2.3.23).

Table 3.2.3.23. Field released false codling moth trapped with various odorants.

Odorant	Mean number of FCM per trap
Control	0.5
Litsea cubeba oil	11.0
Cananga oil	10.5
FCM sex pheromone	4.0
Orange oil	4.0
E2-Octenal	3.5
E2,E6-Nonadienal	3.0
E2,Z6-Nonadienal	2.5
Z3,Z6,Z9-Eicosatriene	2.0

Conclusion

The implementation of the adapted olfactometer and its commensurate increased capacity facilitates an increased rate of odorant screening. Approximately 50 odorants were identified which will receive more attention.

The use of the paired trap technique as an intermediate step to orchard experiments, will allow extrapolation of the best of the olfactometer indicated attractants to their practical use in orchards, e.g. for population monitoring purposes or for attract-and-kill techniques. During the coming season, attention will be given to a search for suitable toxicants to be used in combination with potential attractants.

Results from the olfactometer screening will clarify certain specific properties that the best attractants should have and thus enable a search to procure such odorants. One such case is the long chain polyene groups, a group of natural moth aggregating pheromones which attract both sexes, within which stronger FCM attractants will be sought. This search will also include other chemical groupings where the chemical structure of the strongest attractants will guide the selection and procurement of new test odorants.

Research will also include a search for the best synergists for specific odorants to maximise their attraction; this can be useful under natural orchard conditions where there will be strong competition between artificial lures and the natural odorants to which FCM normally reacts.

Acknowledgements

Ceder Biocontrol Insectary in Citrusdal is gratefully acknowledged for the ample supply of FCM material for test purposes.

3.2.4 Bestryding van valskodlingmot deur middel van Steriele Insekloslatings Proef 662 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

Research to suppress fungal and viral infections in FCM culture jars was conducted. An egg treatment consisting of 20 ml formalin plus 80 ml water, rinsed with distilled water, was accepted as the standard

treatment. This treatment can suppress fungal and viral infections to such a degree that that they are no longer a production threat.

A cutting bench was designed and manufactured to facilitate the cutting of large numbers of single face cardboard strips, used for the mass collection of FCM pupae. Perspex troughs used to mass collect FCM were refined and commissioned.

Two cage experiments were conducted to investigate the effect of supplementary releases of the egg parasitoid *Trichogrammatoidea cryptophlebiae* on sterile FCM releases. In both cases parasitoid releases were found to be beneficial for the suppression of FCM.

A pilot project was initiated at the end of the report period to suppress FCM commercially in 35 ha of citrus with sterile insect releases. The techniques are fully described, but few results of importance were collected up to the end of the report period.

Inleiding

Daar is breedvoerig in die CRI-jaarverslag vir 2004 oor die ontwikkeling van apparaat en tegnieke wat vir die teel en voorbereiding van VKM vir steriele insekloslatings (SIL) gebruik moet word, verslag gelewer. Dié navorsing is in 2005 op die volgende aspekte voortgesit:

- Navorsing is voortgesit om die VKM-teeltegniek só aan te pas dat swam- en virusbesmettings makliker gedurende die teelproses in die insektarium hokgeslaan kan word,
- Nuwe apparaat is ontwerp en veranderinge is aan bestaande toerusting aangebring.
- Hokproewe is uitgevoer om die potensiaal van eierparasitoïedloslatings om VKM-besmettings gedurende die beginfase van SI-loslatings te onderdruk, te bepaal.
- Die eerste loodsprojek om VKM op kommersiële skaal met behulp van SIL te bestry, is ingelei.

Die Deel van die verslag is in Engels geskryf aangesien die navorsing vir internasionale befondsing kwalifiseer.

Materiale en tegnieke

1 Further development of rearing techniques for the pilot project: The pilot project was initially planned for 2004-2005. Due to unforeseen difficulties with fungal and viral infections in the insectary of Ceder Biocontrol, the supplier of the required test insects, the pilot project had to be postponed. Towards the end of this study it became apparent that a successful surface sterilization routine had to include Formalin. It was the only product to suppress viral infection to any significant degree without suppressing moth production.

Fifteen batches of FCM were reared successively to support and consolidate previous results with Formalin (Table 3.2.4.1). Standardized 500 ml honey jars were used as rearing containers throughout to hold the enriched diet developed by CRI (Moore and Richards 2001). Egg paper squares, each containing 400-500 FCM eggs were inoculated into each diet jar after treatment with the test products. Each egg square was immersed momentarily in a solution of the test product in water, ensuring good coverage. In previous work it was established that Formalin was toxic to FCM eggs to a variable degree. The squares from some sub-batches were therefore rinsed by brief immersion in distilled water directly after treatment and then inoculated into the rearing jars. To evaluate production, 10 diet jars were selected at random from each batch when mature larvae started moving upwards into the standard cotton wool stoppers to pupate. The cotton wool stoppers were replaced with SFK cardboard stoppers to facilitate the removal of pupae for quantitative assessment. The jars were retained to enable recovery and counting of moths developing from larvae that had not moved upward into the stoppers but had pupated in the diet.

Table 3.2.4.1. Production of false codling moth using various treatments to surface-sterilize eggs before inoculation into diet jars (January to March 2005).

Batch	No. of jars	Treatment	Rinsed with water	% Jars with virus	Production / Comments
1	140	20 ml Formalin + 80 ml water	Rinsed	0	Mean production per 10 jars: 237 moths
	28	1 ml Jik + 99 ml water	Rinsed	53.6	Unacceptable viral infection
	28	1 ml Jik + 99 ml water	Unrinsed	35.7	Unacceptable viral infection

2	196	20 ml Formalin + 80 ml water	Rinsed	0.5	Mean production per 10 jars: 199 moths
3	50	10 ml Formalin + 90 ml water	Unrinsed	0.0	Reduced production – see batch 7
	140	20 ml Formalin + 80 ml water	Rinsed	1.4	Normal production
4	112	20 ml Formalin + 80 ml water	Rinsed	0.0	Normal production
5	168	20 ml Formalin + 80 ml water	Rinsed	0.0	Mean production per 10 jars: 183 moths
	28	0.2 g granular chlorine/100 ml water	Unrinsed	25.0	Unacceptable viral infection
6	112	20 ml Formalin + 80 ml water	Rinsed	3.6	Mean production per 10 jars: 237 moths. One jar with fungal infection
	10	20 ml Formalin + 80 ml water	Rinsed	10.0	Cotton wool stoppers replaced with metal screw lids and blotting paper septa. Mean production per 10 jars: 238 moths
7	112	20 ml Formalin + 80 ml water	Rinsed	0.0	Mean production per 10 jars: 233 moths
	14	10 ml Formalin + 90 ml water	Rinsed	7.1	Mean production per 10 jars: 203 moths
	14	10 ml Formalin + 90 ml water	Unrinsed	0.0	Mean production per 10 jars: 173 moths
8	112	20 ml Formalin + 80 ml water	Rinsed	2.7	Mean production per 10 jars: 222 moths
9	112	20 ml Formalin + 80 ml water	Rinsed	0.0	Normal compared to the above
10	122	20 ml Formalin + 80 ml water	Rinsed	0.0	Normal compared to the above
11	122	20 ml Formalin + 80 ml water	Rinsed	0.0	Normal compared to the above
12	112	20 ml Formalin + 80 ml water	Rinsed	3.6	Normal compared to the above
13	112	20 ml Formalin + 80 ml water	Rinsed	0.0	Normal compared to the above
14	112	20 ml Formalin + 80 ml water	Rinsed	1.8	Production responsibility transferred to Ceder Biocontrol Insectary: Diet ingredients pre-mixed with water
	392	20 ml Formalin + 80 ml water	Rinsed	0	Production responsibility transferred to Ceder Biocontrol Insectary: Dry diet ingredients measured into jars and water added
15	112	20 ml Formalin + 80 ml water	Rinsed	0	Production responsibility transferred to Ceder Biocontrol Insectary: Diet ingredients pre-mixed with water
	392	20 ml Formalin + 80 ml water	Rinsed	0.8	Production responsibility transferred to Ceder Biocontrol Insectary: Dry diet ingredients measured into jars and water added

Up to 2% product of the bleaching agent, Jik (3,5% a.i.), in water, was not previously able to suppress viral contamination in the rearing jars to an acceptable degree. This result was confirmed in batch 1 (Table 3.2.4.1). Rinsing the egg squares with clean water after treatment aggravated the infection. Similarly, larvae in 25% of the jars inoculated with eggs surface sterilized with granular chlorine became contaminated with virus (batch 5). Use of these two products was therefore discontinued. Eggs treated with Formalin at 10 ml product per 100 ml water and remaining unrinsed, gave good results (batches 3 and 7), but rinsing with water caused a resurgence of viral infection. The unrinsed treatment was, however, toxic and killed up to 38% of the treated eggs (CRI Annual Report for 2004, Table 3.2.5.11). In contrast, an egg treatment consisting of 20 ml of Formalin in 100 ml water, and rinsed with water afterwards, consistently and, in most cases completely, suppressed the virus. All the treatments tested were excellent fungal suppressors and

only one jar showed signs of fungal growth during the entire development process above. The egg treatment consisting of 20 ml Formalin plus 80 ml water, followed by rinsing with water, was therefore considered acceptable. Its use was recommended to Ceder Biocontrol who subsequently assumed responsibility for the rearing of the SIR insects.

2 Modifications to the moth collecting apparatus: The perspex troughs developed for the collection of the SIR moths, were ready for use in time for the 2004-2005 season but due to the postponement of releases the troughs stood unused until preparations were initiated for the start of the pilot project in July 2005. The rearing of the required weekly number of moths was initiated by Ceder Biocontrol and the troughs put to use. However, within two weeks of use the perspex trough lids from which the cardboard rolls holding the FCM pupae were suspended, had warped so badly that moths were escaping through the gaps in large numbers. The lids therefore had to be modified. Frames were constructed from aluminium angle metal and the perspex lids were riveted to these. This straightened out the warped lids and solved the problem (Fig. 3.2.4.1).



Fig. 3.2.4.1. Modified perspex lids in aluminium frames.

3 Preparation of SFK cardboard rolls as pupation site: Tightly rolled strips of single face corrugated SFK cardboard ("C" flute), 580 mm x 35 mm, is used as diet jar stoppers to provide pupation sites for mature larvae (see above). A basic jig was used in the past to cut the strips individually. This method was naturally laborious and had to be accelerated to enable preparation of the approximately 1 000 stoppers per week (for 60 weeks) required for the intended SIT pilot project. An apparatus based on paper cutting equipment used by USDA entomologists (Carpenter, pers. com.) was therefore developed. A steel jig holding nine carpet knife blades side by side and 35 mm apart in a subframe, was constructed (Fig. 3.2.4.2) and bolted to one end of a bench, 3 600 mm x 600 mm. The subframe could be swiveled upward to allow the insertion of a 580 mm wide strip of SFK cardboard. When swiveled downwards, the blades were pushed through the cardboard that was then pulled through the jig along the length of the benchtop (Fig. 3.2.4.3). A steel hinged "ladder" was then pivoted down to hold the 10 strips in position. Grooved "rungs" in the ladder enabled the strips to be cut to the required length. This arrangement worked well and has subsequently been in constant use.



Fig. 3.2.4.2. Steel jig holding nine carpet knife blades for the preparation of SFK cardboard diet jar stoppers.



Fig. 3.2.4.3. Bench with steel jig and positioning ladder to cut SFK cardboard into strips.

4 Supplementing SIR with egg parasitoid releases: There can be a certain amount of crop damage while sterile insects, and especially Lepidoptera, are released. This is due to a small percentage of fertile eggs that result from possible matings between the released, partially sterile males and wild fertile females in the orchard. It was demonstrated in previous laboratory research that sterile eggs are acceptable to the egg parasitoid, *Trichogrammatoidea cryptophlebiae*, in choice and no-choice situations. Two cage experiments were conducted to confirm these results under more natural conditions. The information is the subject of a forthcoming article and the data have not yet been statistically analyzed.

A Cage experiment 1: Effect of egg parasitoid releases in combination with FCM SIR

Sixteen navel orange trees were individually caged with insect proof netting. They were divided into four blocks of four trees each. One replicate of each of four treatments was allocated randomly to one tree in each block. Fifty fruit were labeled at random on each tree and the rest of the crop was removed. Ten pairs

of unirradiated (“normal”) FCM were released into each cage. In two treatments, 100 pairs of moths treated with 150 Gy gamma irradiation were added to each replicate (10:1 ratio). Parasitized FCM eggs were also introduced into certain cages. Egg parasitism decreases rapidly when FCM eggs are more than 48 hours old. A single release of parasitoids would not have survived for the duration of the experiment, and two releases were therefore made one and three days after the moth releases. The parasitoids started emerging from these eggs on the day of introduction. The parasitoid sex ratio was established (1 male to 1,4 females) and the number of parasitoid females released per cage (approximately 1 400) was calculated from subsamples of parasitized eggs that were retained.

All fruit were picked two days later and transported to the laboratory. The fruit were incubated at 26°C for seven days and then inspected. All eggs were inspected and classed as unparasitized or parasitized (Table 3.2.4.2). All larval strikes (constituting damage that could cause larval development and/or fruit decay) were noted and cut open for inspection.

Table 3.2.4.2. The supplementation of false codling moth SIR with egg parasitoid releases.

Treatment	% parasitized eggs	Mean number of larval strikes per fruit	Number of undamaged fruit out of 200
10 pairs normal FCM	0.0	0.83	35
10 pairs normal FCM + 1 400 egg parasitoid females	85.7	0.05	191
10 pairs normal FCM + 100 pairs irradiated FCM	0.0	0.50	101
10 pairs normal FCM + 100 pairs irradiated FCM + 1 400 egg parasitoid females	89.4	0.08	184

A large percentage of eggs was parasitized when parasitoids were introduced into the cages in the absence of irradiated moths. The number of undamaged fruit was also increased substantially compared to the control treatment. This result was obtained at a release ratio of 140 parasitoid females to one FCM female. Approximately the same result was obtained when the release ratio was reduced to approximately 9:1 (parasitoids to FCM) when irradiated FCM were released simultaneously.

The extent to which FCM eggs were parasitized differed very little between cages containing normal (fertile) moths only or a mixture of normal and irradiated (mostly fully sterile) moths. This trend confirms previous laboratory data (Carpenter *et al*, 2004) that sterile eggs are acceptable to egg parasitoids for parasitization. Fruit damage was substantially reduced by releasing irradiated moths into the cages containing normal moths. In these cages the reduction in damage was due to the influence of the irradiated P1 moths only and was not all that spectacular due to the fact that the experiment was not designed to allow the effect of F1 sterility to be demonstrated. However, there was an additional substantial reduction in damage when the egg parasitoids were added. These data suggest that there would be an increased benefit to release egg parasitoids in conjunction with SI-releases.

B Cage experiment 2: Ratio of egg parasitoids to FCM for effective parasitism

An experiment was conducted to establish whether an increase in egg parasitoids would affect the success rate of parasitism noticeably.

The same individually caged trees as before, were used. Fifteen trees were divided into five blocks of three trees each. One replicate of each of three treatments was allocated at random to a tree in each block. All fruit from these trees had been used and removed in the previous experiment. Navel orange fruit were therefore picked in another orchard and 30 fruit were suspended from each data tree using lengths of nylon fishing line, 400 mm long. A plastic button was looped through a length of line and both ends of the line were then threaded longitudinally through a fruit with the aid of a 150 mm long needle. The button was thus seated at the navel end of each fruit and prevented the line from tearing out. The two loose ends of fishing line from each fruit were tied together and the fruit was then suspended with cable ties to branches in and around each tree at random.

Twenty pairs of unirradiated FCM were released into each cage. This was followed by two parasitoid releases respectively one and three days later using the same method as in experiment 1. Again, the parasitoid sex ratio was established (1 male to 1.7 females) and the number of parasitoid females released

per cage (Table 3.2.4.3) was calculated from subsamples of parasitized eggs that were retained. All fruit were picked five days after moth release and transported to the laboratory. The fruit were incubated at 26°C for seven days and then inspected. All eggs were counted and classed as unparasitized or parasitized (Table 3.2.4.3). All larval strikes (constituting damage that could cause larval development and/or fruit decay) were noted.

Table 3.2.4.3. The effect of parasitoid release ratio on fruit damage.

Ratio of parasitoids to FCM (required/calculated number of parasitoid females released per cage)	% parasitized eggs	Mean number of larval strikes per fruit	Number of undamaged fruit out of 150
5:1 (100/99)	51.4	2.3	49
10:1 (200/180)	72.7	1.07	74
20:1 (400/372)	83.4	0.97	82

The percentage of parasitized eggs was noticeably improved by increasing the ratio of parasitoid females to FCM females. There was a commensurate decrease in the number of FCM damaged fruit. This result is much better than what could normally be expected in a commercial situation when parasitoids are released. This is despite the fact that the release ratio (max. 20:1) per tree in the cages was arguably much smaller than what could be achieved in the orchard where 25 000 parasitoids per ha are usually released simultaneously. Factors inside the mesh cages, such as the restricted number of fruit concentrated into each cage, to be explored by parasitoids for FCM eggs (which would increase the parasitoids' effectiveness) and the more temperate environment in the cages, probably promoted the parasitoids' effectiveness relative to the orchard situation. It may very well be economically unrealistic to increase the number of released parasitoids beyond that already recommended and used in practice. However, the above results do indicate that *T. cryptophlebiae* is independently capable of suppressing FCM to a commercially acceptable degree.

5 Kommersiële SIL-navorsingsprojek

'n Loodsprojek wat op die onderdrukking van VKM met behulp van SIL gemik is, is gedurende November 2005 van stapel gestuur.

Materiale en metodes

- **Die proefperseel:** Die proefperseel waarin VKM losgelaat word, is 35 ha groot en bestaan uit 14 boorde volwasse sitrusbome wat van 1,0 tot 3,93 ha wissel. Die blokke bome is in 'n 7 x 2 patroon gerangskik en bestaan uit nege aangrensende Washingtonnawelboorde, een Minneola Tangelo- en vier Valenciaboorde. 'n Enkele boord met nawelbome, nagenoeg 800 m vanaf die proefboord, word as kontrole gebruik. Die proefperseel is relatief geïsoleer – die naaste sitrusboorde is ongeveer 500 m weg en word van die proefperseel deur die Olifantsrivier geskei. VKM word in laasgenoemde aanplantings met behulp van paringsontwrigting en Cryptogran-bespuiting onderdruk.

- **Steriele Insekloslating:** Motproduksie is só beplan dat die motte slegs uitsonderlik meer as 48 uur oud is. Die motte word uit die mottrôle gehaal (raadpleeg Fig. 3.2.4.1) en in 'n koelkamer tot 4°C verkoel om hulle te inaktiveer. Hulle word in die koelkamer gehou tot die dag voor loslating. Op daardie dag word hulle met fluoriserende poeier gemerk en nagenoeg 467 motte word volumetries in elk van 75 Petribakkies afgemete, wat daarna in koelkissies verpak word. Dié werk word alles in die koelkamer uitgevoer. Die volgende dag word die koelkissies met motte in 'n groter koelkis, toegerus met vriesblokkies, verpak en vir bestraling na Infruitec, Stellenbosch, vervoer. Die motte word na bestraling na Citrusdal teruggeneem en in die proefperseel losgelaat.

Motloslating is gedurende die verslagtydperk met die hand vanaf 'n vierwielangedrewe motorfiets gedoen. Vyf en sewentig werksrye, eweredig in die perseel versprei, is vooraf gemerk en alle loslatings is in dié rye uitgevoer. Die motte van 'n Petribakkie is so eweredig as moontlik op elke derde boom in elke ry toegedien. Die motte is so hanteer dat hulle ten tye van die loslating alreeds grotendeels uit hulle verkoelde toestand ontdooi het. Dit het gehelp dat hulle aktief kon vlieg om skuiling te vind sodra hulle losgelaat is. Eenduisend bestraalde VKM (mannetjies en wyfies in 'n nagenoeg 1:1 verhouding) is sodoende twee keer per week per hektaar in die proefperseel losgelaat.

- **Lokvalondersoeke:** Dertien deltalokvalle, toegerus met Lorelei-feromoonvrystellers, is in die proefperseel versprei. Twee kontrolelokvalle word ook gebruik, wat in twee boorde, onderskeidelik in die

kontroleboord (verwysing hierbo) en in 'n Valenciaboord, 600 m ver, geplaas is. Alle lokvalle word twee keer per week ondersoek. Gedurende elke inspeksie word die lokvalbodems met nuwes vervang. Die gebruiktes word na die laboratorium vervoer waar die gevangde motte getel word.

- **Vrugvalopnames:** Vyf aangrensende databome in 'n ry by elke lokval wat in 'n nawelboord geplaas is, word vir vrugvalopnames gebruik. Elke lokval met vyf databome word as 'n datapunt beskou. Daar word sodoende 'n totaal van 12 datapunte gebruik – 11 in die proefperseel en een in die kontroleboord. Alle afvalvrugte word een keer per week opgetel, oopgesny en vir tekens van VKM-besmetting ondersoek.

- **Mededingendheid van bestraalde, losgelate motte:** Die kwaliteit van bestraalde motte moet voortdurend nagegaan word om te verseker dat hulle so goed as moontlik met wilde motte kan meeding. Dit word op twee maniere gedoen:

- (i) **Paringsplatforms:** Die vlieg- en opsporingsvermoë van bestraalde mannetjies en die roep(=aanlok)vermoë van bestraalde wyfies word met behulp van paringsplatforms getoets. 'n Aantal een- tot tweedae-oue, deels-ontvlerkte, bestraalde en onbestraalde wyfies word op paringsplatforms in die proefperseel geplaas waarin bestraalde motte as deel van die SIL-program losgelaat was. Die bestraalde wyfies word met spesiale poeier gemerk om van die onbestraaldes onderskei te word. Dié poeier fluoriseer onder ultravioletlig (UV-lig) en gemerkte motte kan duidelik van ongemerkte motte onderskei word. Elke wyfie wat *in copulo* gedurende die tydperk nagenoeg 08:00 tot 01:00 waargeneem word, word versigtig versamel om nie die paringsproses te versteur nie. Die gepaarde motte word die volgende oggend onder UV-lig ondersoek om vas te stel of hulle bestraal was en daarna gedissekteer om te bepaal of paring suksesvol was. Die herkoms van die mannetjies word ook ondersoek, aangesien dit een van drie tipes, t.w. 'n losgelate P1-mannetje (gemerk), 'n wilde mannetjie (ongemerk), of 'n F1-mannetje (ongemerk; raadpleeg [i] hieronder) kan wees.

- (ii) **Lokvalle:** Alle motte wat in die proefperseel losgelaat word, word voor bestraling met fluoriserende poeier bestuif. Dit stel die navorsers in staat om met behulp van lokvalle tussen bestraalde, losgelate mannetjies en ongemerkte mannetjies, wat nie in die boorde losgelaat was nie, te onderskei. Daar is geen lokmiddel vir wyfies beskikbaar nie en dus geen manier om losgelate wyfies te vang nie.

- **Opspoor van F1-steriele VKM:** Die sukses van die SI-tegniek is op die steriele F1-beginsel geskoei. Alle bestraalde wyfies is volkome steriel na behandeling met die 150 Gy-dosis wat gebruik word en kan geen nageslag hê nie al paar hulle met wilde, vrugbare mannetjies. Daar is egter 'n klein persentasie van die losgelate mannetjies wat slegs gedeeltelik deur die gamma-behandeling gesteriliseer word. Parings sulke mannetjies met wilde, vrugbare wyfies kan tot gevolg hê dat vrugte besmet word. Enige nageslag (F1) wat uit dié parings voortgebring word, is egter steriel. Sulke F1-motte kan geensins in die boord met die blote oog van wilde motte onderskei word nie. Dit beteken dus dat ongemerkte mannetjies wat in lokvalle gevang word, nie noodwendig slegs wilde mannetjies is nie, maar ook F1-indiwidue, wat uit vrugte ontwikkel het, kan wees. Daar word dus op twee maniere tussen dié mannetjies onderskei:

- (i) **Mikroskopiese tegniek:** Die interne geslagsorgane van ongemerkte mannetjies wat uit lokvalle of vanaf paringsplatforms verwyder word, word onder 'n mikroskoop verwyder en deur 'n spesiale proses met Giemsa-kleurstof gekleur (Carpenter, pers. kom.). Dié kleurstof reageer met chromatienmateriaal in spermbundels van vrugbare mannetjies en kleur duidelik homogenies. Die hoeveelheid chromatienmateriaal in die spermbundels van F1-steriele mannetjies is egter hoogs veranderlik en sal daarom nie homegenies kleur nie.

- (ii) **Herwinning van motte uit natuurlik-besmette vrugte:** Afvalvrugte word opgetel en indiwidueel in 500 ml plastiekbakkies met gaasdeksels in die laboratorium ingehok. Elke mot wat uit 'n vrug ontwikkel, word met 'n insektarium-geteelde kontrolemot van die teenoorgestelde geslag in 30 ml plastiekhokkies gepaar. Eiers wat gelê word, word onder 'n mikroskoop ondersoek om vas te stel of hulle vrugbaar is en in staat is om uit te broei. Herwonne wyfies wat in sulke parings eiers lê wat nie kan uitbroei nie, moet uiteraard die steriele F1-nageslag van 'n bestraalde mannetjie wees. Indien 'n insektarium-geteelde wyfie wat met 'n herwonne mannetjie gekruis word, nie vrugbare eiers lê nie, moet die mannetjie derhalwe ook die F1-nageslag van 'n bestraalde mannetjie wees.

Resultate en bespreking

Die proef was slegs ses weke oud met afsluiting van dié verslag. Op daardie tydstip was dit nog nie moontlik om enige van bogenoemde maatstawwe, die lokvalle en vrugvalopnames uitgesluit, toe te pas nie.

Min ongemerkte mannetjies is van die begindatum af in die proefperseel gevang (Fig. 3.2.4.5). Die klein vangste kan daarom nie tot aan die einde van Desember 2005 aan die invloed van die bestraalde motte toegeskryf word nie. In teenstelling met motaktiwiteit in die proefperseel is motgetalle wat die lokvaldrempelwaarde oorskry (selfs al word die vangste aangepas om vir die groter doeltreffendheid van die deltalokvalle wat gebruik word, te vergoed) amper weekliks in die kontroleboord gevang.

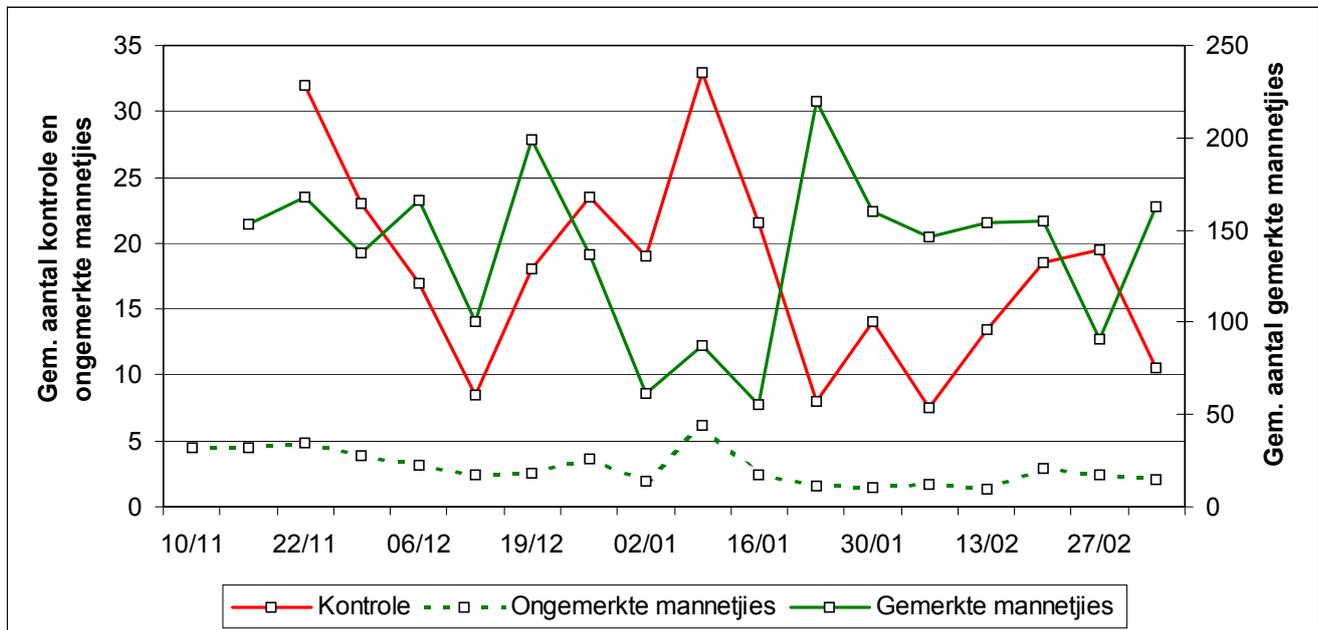


Fig. 3.2.4.4. Vangste van valskodlingmotmanneljies in kontrole- en SIT-proefboorde.

Slegs enkele vrugte het gedurende die proeftydperk in die kontrole- en proefboorde afgeval en geen afleidings kan derhalwe gemaak word nie.

Toekomstige navorsing

Die proef word voortgesit en sal omvattend in die volgende verslag bespreek word. Daar word intussen reëlins getref om Fase 2 van die SIT-projek gedurende die 2006-2007 seisoen in 'n heelwat groter gebied as die proef onder bespreking, van stapel te stuur.

Verwysings

- Carpenter, J. E., Bloem, S. & Hofmeyr, J. H. 2004. Acceptability and suitability of eggs of false codling moth (Lepidoptera: Tortricidae) from irradiated parents to parasitism by *Trichogrammatoidea cryptophlebiae* (Hymenoptera: Trichogrammatidae). *Biological Control*. 30: 351-359.
- Moore, S.D. & Richards, G.I., 2001. Improvement of the false codling moth mass rearing technique. 2005 Citrus Research International Jaarverslag, pp. 77-81.

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

Experiment 689 by Sean D. Moore & Wayne Kirkman (CRI)

Opsomming

'n Belangrike voorwaarde om sukses met die steriele insek tegniek (SIT) te behaal, is die ontwikkeling van 'n stelsel vir die massateling van uiters groot getalle insekte. Die getalle VKM wat met huidige stelsels vir parasitoïed- en virusproduksie geteel kan word, is ontoereikend. In hierdie eksperiment is 'n reeks proewe met verskillende behandelings vir die oppervlakontsmetting van eiers uitgevoer. Die hoofrede vir sulke proewe is om virus- en swambesmettings in larwes en dieët te verminder. Formalienbehandeling van VKM-eiers het die uitbraak van virus in larwes verhoed. Formalien het tot 'n mate uitbroeiing van eiers nadelig beïnvloed maar dit was nie 'n groot probleem nie veral in vergelyking met die ernstigheid van 'n virusbesmetting. Sporekill-behandeling van eiers het swambesmetting van dieët verminder. Verskeie oopbakteelproewe is met beperkte sukses uitgevoer. Die twee grootste probleme met hierdie metode van teling is die beheer van vogtigheid en asynvliegbesmetting. Een van die diëte wat redelike belofte gewys het, is 'n wortelbasisdieët. Die gebruik van formalien in kunsmatige dieët het larweontwikkeling nadelig

beïnvloed. Denim was 'n bruikbare plaasvervanger vir watte as bedekking vir larwe-teelflesse, maar net tot die stadium waar larwes begin pupeer het. Vermikuliet het gewerk as 'n substraat vir papievorming. 'n Nuwe VKM-kultuur is suksesvol van boordversamelde larwes vermeerder. Hierdie eksperiment sal in Maart 2006 tot 'n einde kom. Gedurende die oorblywende tydperk sal navorsing op die teling van larwes in oop bakke gefokus word.

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 control of insect pests in various parts of the world. It is important that this system also be tested against FCM on citrus in southern Africa, starting in the Western 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

Egg sterilisation

Trial 1:

Thirty jars of standard FCM larval diet were prepared. Thirty FCM egg sheets of average size and approximately 400 eggs each were sterilised in three different ways (Table 3.2.5.1). Ten egg sheets were dipped into each of the three treatments for approximately 2 seconds after which one egg sheet was placed into each jar of diet. Jars were then stoppered with cotton wool and placed in a rearing room at 27°C. Observations were recorded.

Table 3.2.5.1. Treatments for surface sterilisation of FCM egg sheets.

Treatment number	Number of rearing jars	Egg sheet treatment
1	10	10% formalin (of a 37% formalin solution)
2	10	25% formalin (of a 37% formalin solution)
3	10	40% formalin (of a 37% formalin solution)

Trial 2:

A trial was conducted to determine the effect of various egg surface sterilisation treatments on egg hatch. Twelve egg sheets were prepared with an average of 165 eggs on each sheet. Two sheets were subjected to each of six treatments (Table 3.2.5.2). The egg sheets were then placed into Petri dishes and kept at 27°C for two weeks. Thereafter, they were microscopically examined to determine egg hatch.

Table 3.2.5.2. Treatments for surface sterilisation of FCM egg sheets.

Treatment number	Egg sheet treatment	Duration of treatment
1	Distilled water	Dip (2 seconds)
2	Virkon (1%) + Sporekill (0.2%)	15 minutes
3	Sporekill (0.2%)	15 minutes
4	Formalin 10 %	15 minutes
5	Formalin 25%	15 minutes
6	Formalin 40%	15 minutes

Trial 3:

A trial was conducted to examine the effect of rinsing of egg sheets in sterile (autoclaved) distilled water after dipping in formalin and sodium hypochlorite. Twenty jars of diet were prepared in the standard way. Five egg sheets were subjected to each of four treatments (Table 3.2.5.3) and each jar of diet was inoculated with one egg sheet. The jars were kept at 27°C for two weeks and observations were recorded.

Table 3.2.5.3. Treatments for surface sterilisation of FCM egg sheets.

Treatment number	Number of jars	Egg sheet treatment
1	5	Dipped in 25% formalin
2	5	Dipped in 25% formalin and rinsed in sterile distilled water.
3	5	Dipped for 5 minutes in 0.17% sodium hypochlorite
4	5	Dipped for 5 minutes in 0.17% sodium hypochlorite and rinsed in sterile distilled water

Trial 4:

It became evident that the prevalence of fungal contamination of FCM diet in rearing jars was increasing in FCM cultures at CRI, PE and at River Bioscience. Contamination of the culture at CRI, peaked at 50% of jars. Consequently, a trial was conducted where egg sheets were soaked in a 0.15% Sporekill solution for 15 minutes before dipping in formalin. As viral contamination is potentially a greater problem than fungal contamination, it was imperative that the formalin treatment not be excluded. It was reasoned that the formalin treatment should be the last step in the process in order to avoid rinsing off of formalin residue and consequently, reduced viral control. The egg sheets used in 30 jars of diet were treated in this way, and kept at 27°C. Observations were recorded.

Larval diet

Trial 1:

A cosmopolitan carrot-based insect diet, developed by Tony Ware, was tested for FCM larvae. The diet was supplied in one fraction as a desiccated powder. The diet was bulked up with paper pulp. The paper pulp (2 g) was immersed in hot boiled water and agitated with a Kenwood Food Processor until its appearance was that of fine wet cotton wool. This was an emulsion of the protocol practiced in the Canadian codling moth SIR facility. The paper pulp was then strained from the water and added to 40 g of the dry powder in an open plastic tray (160 mm x 140 mm x 60 mm). Boiling hot distilled water was added and stirred until a thick paste was formed. Four trays of diet were prepared. After the diet had cooled, an egg sheet with approximately 1000 eggs, was suspended over each tray (after being surface sterilised with formalin). Trays were left uncovered and kept at 27°C. Observations were recorded.

Trial 2:

Certain rearing facilities, such as the SIR codling moth facility in Osoyoos, Canada, add formalin to the artificial diet to suppress viral contamination. A trial was conducted where formalin was added to the standard FCM artificial diet to examine the effect on the development of the larvae (Table 3.2.5.4). Diet was prepared in jars as usual. Each jar was inoculated an egg sheet with an estimated 300-400 eggs per sheet. The jars were kept at 27°C and observations were recorded.

Table 3.2.5.4. Treatments to examine the effect of formalin in the standard artificial diet on FCM larval development.

Treatment number	Number of jars	Diet	Egg sheet sterilisation
1	10	Standard	Dipped in 25% formalin
2	5	Standard + 150 µl formalin	Dipped in 25% formalin
3	5	Standard + 150 µl formalin	None

Trial 3:

The larval diet that the Canadian SIR facility uses for codling moth, works very successfully for open tray rearing of the designated species. However, the diet is very complex and expensive. Therefore, in order to test open tray rearing, cheaper and simpler variations of this diet were tested. The diets tested were a sort of hybrid between the existing standard diet (Moore, 2002; Moore & Richards, 2001) and the Canadian diet. Four variations of diet were prepared in open trays (Table 3.2.5.5) – one tray for each recipe. The ingredients were boiled for 5 minutes, and allowed to cool to 65°C before sorbic acid and nipagin were added.

Table 3.2.5.5. Ingredients of four artificial diets for open tray rearing of FCM larvae.

Diet no 1 ingredients	Mass (g)	Diet no 2 ingredients	Mass (g)	Diet no 3 ingredients	Mass (g)	Diet no 4 ingredients	Mass (g)
Maize meal	169	Maize meal	169	Soya flour	169	Soya flour	169
Wheat germ	17						
Brewers yeast	8.5						
Milk powder	3.1						
Sorbic acid	0.6						
Nipagin	1.3	Nipagin	1.3	Nipagin	1.3	Nipagin	1.3
Paper pulp	8						
Distilled water	400	Distilled water x 1.5	600	Distilled water	400	Sawdust	200
						Distilled water	450

The first two trays were prepared using approximately double and triple the amount of water that is normally used when diet is prepared in jars. This was done as the diet in the open trays was expected to dry out more rapidly than in the plugged jars. Paper pulp was added to bulk up the diet and make it more suitable for pupation to occur in the diet.

In the third tray, soya flour was used instead of maize meal as it was expected that the maize meal based diets might putrefy in open trays. This would be due to the diet only being boiled for 5 minutes, instead of being autoclaved.

In the fourth tray sawdust was added as an extra bulking agent.

After diet had cooled, an egg sheet with approximately 1000 eggs, was suspended over each tray (after being surface sterilised with formalin). The trays were kept open at 27°C and observations were recorded.

Trial 4:

A further trial was conducted to test the carrot-based diet developed by Tony Ware. Hot boiled distilled water (400 ml) was added to the dry powder (150 g). The diet was divided into 4 honey jars, which were inoculated with approximately 300 FCM eggs each. Three of the four eggs sheets were surface sterilised with formalin, one of them being rinsed (Table 3.2.5.6). The jars were kept at 27°C, and observations were recorded. Emerged moths were counted and sexed.

Table 3.2.5.6. Treatments for surface sterilisation of egg sheets used for inoculation of carrot-based diet in open trays.

Jar no	Egg sheet sterilisation
1	25 % formalin, unrinsed
2	25 % formalin, unrinsed
3	25 % formalin, rinsed in distilled water
4	No sterilisation

Trial 5:

A trial was conducted in which the ingredients of the standard diet (Moore, 2002; Moore & Richards, 2001) were prepared in open Tupperware dishes (160 mm x 140 mm x 60 mm). Paper pulp was added as a

bulking agent. Egg sheets with approximately 1000 eggs each were dipped in 25% formalin and suspended above each tray. Neonate larvae “abseiled” down onto the diet. The trays were kept at 27°C, and observations were recorded.

Trial 6:

Previously trials have been conducted where tap water was used instead of distilled water when preparing rearing jars for FCM (Moore & Kirkman, 2004). Results showed poor larval development and a high level of viral contamination. Since the use of formalin had almost eradicated virus in the culture, it was decided to repeat the trial. Fifteen rearing jars were prepared by adding distilled water to the dry diet ingredients, and 5 jars were prepared using tap water supplied by the Nelson Mandela Metropolitan Municipality (Port Elizabeth). Once cooled, jars were inoculated with FCM eggs. The jars were kept at 27°C, and observations were recorded.

Pupation medium

Trial 1:

Cotton wool as a pupation medium is one of the major expenses in the current method of FCM rearing. In this trial, corrugated cardboard was tested as an alternative pupation medium to cotton wool. The cardboard was a C-flute (Seyferts, Cape Town), which was cut into strips of 580 mm x 35 mm, rolled up tightly and inserted into round PVC lids. The lids were sealed at the top with a fine aperture mesh to prevent larvae from escaping. Once the cardboard was inserted into the PVC lid, it could fit snugly into the neck of the diet jar. A problem with this lid is that it could not regulate the rate of desiccation of the diet, as was the case with the cotton wool plugs. Therefore, the cardboard stoppers could only be inserted shortly before the start of pupation. Another alternative to cotton wool, for preventing rapid desiccation and preventing entrance of contaminants, was sought. Four jars of diet were prepared. Two were plugged with cotton wool and two were sealed with denim cloth, secured over the mouth of the jars with an elastic band. Surface sterilised egg sheets were added to the jars, which were then kept at 27°C until the larvae had developed to the 4th instar. The denim cloths and cotton wool plugs were then removed and replaced with the cardboard plugs. Observations of larval development were made and pupae were counted once pupation was complete.

Trial 2:

A small trial was conducted to test the suitability of vermiculite as a pupation medium. A rearing jar, in which the larvae had begun pupating, was removed and placed upright in a pie dish with a 1 cm layer of vermiculite. Once the larvae had all moved out of the diet and pupated, the vermiculite was removed and put in a 5.5% sodium hypochlorite solution in order to dissolve the silken cocoons. Some of the pupae were immersed for 2 minutes and others for 6 minutes, all of them being rinsed thereafter.

In a similar trial, two jars with 5th instar larvae on the verge of pupation were placed upright in a 2 cm layer of vermiculite in a pie dish. Simultaneously, two other similar jars were suspended above a 2cm layer of vermiculite in a pie dish, so that larvae could spin down into the vermiculite to pupate. Once pupation was completed, the vermiculite was immersed in a 5.5% sodium hypochlorite solution to dissolve the silken cocoons, rinsed in water and the pupae were separated. Observations were recorded, and pupae were counted.

New laboratory culture

It is suspected that all FCM cultures in South Africa (of which there are currently four of reasonable size) might originate from the same source. Due to the possibility of genetic bottle-necking, it was decided that a new FCM culture should be established from field collected individuals. This culture could provide genetic diversity for the existing FCM cultures and act as a backup for them.

Between April and July 2005, eight mass collections of fruit were made from orchard 26 (Autumn Gold Navel orange trees) and 28 (Newhall Navel orange trees) on Woodridge Farm in the Sundays River Valley. Several hundred fruit that appeared to be infested were collected. These fruit were dissected carefully so as not to 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 vials were kept at 27°C. When moths emerged, they were placed on wax paper under a small sieve with a hole cut in the top. Cotton wool was plugged into the hole and kept wet, as a source of water for the moths. When sufficient egg numbers had

been laid on the wax paper, it was removed and replaced with a fresh sheet. Egg sheets were cut into squares of approximately 400 eggs each, surface sterilised and placed into jars with artificial diet. Jars were plugged with cotton wool and kept at 27°C until pupation. Initially, production was very low and only 10-20 moths were produced per jar. Moths were individually caught and placed under the sieves. The rearing protocol used was based on that developed by Ripley *et al.* (1939), improved by Theron (1948) and described by Moore (2002).

Results and discussion

Egg sterilisation

Trial 1

Visual observations were made on the development of larvae in the rearing jars. No viral contamination occurred in any of the jars. However, the use of 25% formalin resulted in a conspicuously lower level of larval development relative to eggs treated with 10% formalin. Little development of larvae was observed when eggs were treated with 40% formalin. This prompted more trials to be conducted to further test the effect of formalin on egg hatch.

Trial 2

As only two egg sheets were used per treatment, the data could not be statistically analysed. However, mortality of eggs was clearly increased through the use of 25% and 40% formalin (Table 3.2.5.7). Unless 25% formalin provides a significantly greater reduction in larval infection, its use over 10% formalin will not easily be justified. Sporekill is effective in reducing levels of fungal contamination of diet. However, it is relatively ineffective against virus. Formalin will therefore be preferred as an egg treatment unless a specific problem with fungal contamination occurs. Viral contamination is generally a bigger problem than fungal contamination.

Table 3.2.5.7. Egg hatch for different egg surface sterilisation treatments.

	Treatment	Duration	Eggs		% Egg hatch
			hatched	unhatched	
1	Distilled water	2 sec	133	25	84
2	Virkon (1%) + Sporekill (0.2%)	15 min	154	36	81
3	Sporekill (0.2%)	15 min	143	31	82
4	Formalin* (10%)	2 sec	131	40	77
5	Formalin* (25%)	2 sec	105	58	64
6	Formalin* (40%)	2 sec	70	61	53

*Stock solution = 37%.

Trial 3:

No contamination of any kind was observed in the jars with formalin treated eggs (Table 3.2.5.8). No difference in larval survival or development was observed between the rinsed and unrinsed formalin treatments. However, where eggs were treated with sodium hypochlorite (without rinsing), larvae in three jars experienced a virus outbreak and diet in one jar was infected with a fungus (Table 3.2.5.8). Where the eggs were rinsed after being treated with sodium hypochlorite, diet in four of the jars was infected with fungus (Table 3.2.5.8). Sodium hypochlorite was therefore considered as an unsuitable and ineffective egg treatment.

Table 3.2.5.8. Contamination and larval development for different egg surface sterilisation treatments.

Treatment number	Number of jars	Egg sheet treatment	Jars with virus contamination	Jars with fungal contamination	Larval development
1	5	Dipped in 25% formalin	0	0	Good development
2	5	Dipped in 25% formalin	0	0	Good

		and rinsed in autoclaved distilled water.			development
3	5	Dipped for 5 minutes in 0.17% sodium hypochlorite	3	1	Poor
4	5	Dipped for 5 minutes in 0.17% sodium hypochlorite and rinsed in autoclaved distilled water	0	4	Poor

Trial 4:

Although no direct comparison was made with jars where egg sheets had not been soaked in a Sporekill solution, fungal contamination declined dramatically, relative to separate batches in which eggs had not been treated with Sporekill. Only one of the 30 jars of diet, in which egg sheets had been subjected to the described treatment, was contaminated with fungus. Only one jar developed any viral contamination of larvae. When problems with fungal contamination arise, this practice is employed for the treatment of eggs used in the CRI and River Bioscience FCM cultures. However, further investigation is required to determine whether this double treatment has any detrimental effect on eggs.

Larval diet

Trial 1:

Hatched larvae dropped onto the diet and initial penetration by larvae appeared good. However, before larvae could complete their development, the diet shrunk and hardened – clearly to the detriment of the larvae. Therefore the level of development to adulthood was low. A major difficulty with rearing in open trays is the control of moisture in the diet. Sophisticated humidity control would be imperative in order to be able to successfully rear larvae in open trays.

Trial 2:

Initial penetration of all diets initially appeared good. However, survival of larvae in diet with formalin was ultimately poor. Larval development was conspicuously worse in diet with formalin than in the control diet without formalin. No viral contamination occurred in any of the jars. If it can be shown that the inclusion of formalin in the diet reduces viral contamination, it will be necessary to test lower concentrations of formalin, which do not negatively affect the larvae.

Trial 3:

Larvae in the first two trays developed viral infection symptoms. It is suspected that the diet was too moist at the time the neonate larvae penetrated (particularly in the second tray), creating anaerobic conditions within the diet. This would obviously stress the larvae, reducing their immunity and increasing the possibility of a viral epizootic. The first tray also dried out too rapidly. By the time pupation should have taken place, the diet was too dry and brittle. One positive finding was that the maize meal based diet did not putrefy, against expectations. When rearing larvae in open trays it is important to ensure that diet is not too moist at the time of larval penetration and not too dry at the time of pupation. This would be achieved, not only by insuring that the initial moisture in the diet is appropriate, but that humidity is well controlled in the rearing facility.

Similar results were obtained with the third tray of diet, where maize meal was replaced with soya meal. Larvae in the fourth tray did not become infected, but there was very little penetration and emergence.

Another problem that was encountered was vinegar fly (*Drosophila melanogaster*) infestation of the diets. Not only do these flies compete directly with the FCM, but they seem to affect the suitability of the diet for FCM, possibly by altering the pH. They have a very short life cycle and are very difficult to control. This appears to be a common problem with rearing in open trays.

Trial 4:

Larvae in jar no 2 developed viral infections early in development. Larvae in jar no 1 and 4 developed extremely fast and were large, but developed viral infection when the first larvae began to pupate.

Larvae in Jar no 3 developed well and were large at maturity. The first moths appeared 21 days after the egg sheets were introduced into the jar. No viral infection occurred in this jar. A total of 243 moths emerged from the jar: 175 males and 68 females i.e. a sex ratio of 2.6:1. An advantage with this diet is that no autoclaving is required. However, further investigation is necessary to test its suitability for rearing FCM larvae, particularly in light of the high levels of viral contamination.

Trial 5:

Initial penetration appeared good, but once again vinegar flies contaminated the diet. As was previously the case, the diet also dried out too quickly for larval development to occur.

Trial 6:

Larvae in the jars that were prepared with tap water developed as well those in which distilled water was used. No virus or fungal contamination occurred in the jars prepared with tap water, whilst two of the 15 jars prepared with distilled water developed fungal contamination. This trial should be repeated with water from Addo, where River Bioscience is situated. If such a commercial insectary could use tap water instead of filtered water, this could lead to major savings. However, the use of tap water instead of filtered water would be a high risk. It can be expected that the quality and make-up of tap water would not only differ from area to area but possibly even from day to day.

Pupation medium

Trial 1:

Numbers of larvae were not high in any of the jars. There did not appear to be any difference in larval development between any of the jars. However, the diet in the jars prepared with denim appeared slightly more desiccated than those prepared with cotton wool. Therefore, a thicker textile should be tested, in order to better retain moisture, or humidity in the rearing room should be kept at a higher level. There was little difference between the numbers of pupae from each treatment (Table 3.2.5.9). Further trials with these alternative media would therefore be warranted. One drawback might be the additional labour required in the preparation of the cardboard rolls and the replacing of the denim seals with the cardboard plugs.

Table 3.2.5.9. Comparison of FCM pupal counts from larval jars covered with denim and jars plugged with cotton wool.

Jar number	Jar covering	Number of pupae
1	Denim	49
2	Denim	45
3	Cotton wool plug	34
4	Cotton wool plug	55

Trial 2:

In the first trial it was found that 33 larvae pupated in the vermiculite, 4 pupated on the bottom surface of the pie dish and one larva pupated inside the jar. Although the sodium hypochlorite dissolved the cocoons, it was still necessary to separate most of the pupae from the vermiculite individually by hand, making it a very delicate and time-consuming process. Pupae which were in the sodium hypochlorite for 6 minutes survived as well as those which were in for only two minutes, with no apparent advantage.

There was little difference in the number of larvae pupating elsewhere than in the vermiculite, between the two treatments (Table 3.2.5.10), indicating that the position of the jar (upright or inverted) was not of paramount importance. Vermiculite appeared to be a suitable pupation medium for FCM larvae. However, its use would not be practical unless an easier and more effective means of separating the pupae from the vermiculite could be devised. This might be necessary for irradiation of pupae for SIT.

Table 3.2.5.10. Numbers of larvae from diet jars pupating in vermiculite.

Treatment number	Number of jars	Position of Jar	Number of pupae			
			On inner side of jar	On diet surface	Against jar lip (in vermiculite)	In vermiculite
1	2	Upright in vermiculite	7	9	2	287
2	2	Inverted and suspended above vermiculite	0	17	0	239

New laboratory culture

After three generations, there were sufficient moths to produce densely laid round egg sheets (60mm diameter). These sheets had enough eggs to place into two jars of diet at approximately 400 eggs per jar. When the fourth generation of moths emerged, they were paced on wax paper under a larger sieve (190mm diameter), which produced enough eggs for up to 12 jars of diet every second day. By this stage moth production was too high to warrant individual capturing of moths. Cotton wool plugs with pupae were placed into an emergence box, from which eclosing moths were caught in a 2 L glass jar and placed under the sieves.

The FCM culture is now well established after seven 7 generations. The size of the culture can be expanded whenever required. Individuals from this culture will be sent to the University of Stellenbosch to ascertain genetic variation between this recently field collected material and the long established cultures. This culture is also being used to supply eggs for trials investigating FCM larval parasitoids and the field persistence of CRYPTOGRAN. The establishment of this culture has also provided valuable experience in insect rearing.

Conclusion

Surface sterilisation of FCM eggs with formalin was very effective in preventing a virus outbreak in larvae. Formalin did have some detrimental effect on egg hatch, but this was not a major problem, particularly in comparison to the severity of a viral outbreak. Surface sterilisation of eggs with Sporekill reduced fungal contamination of diet. Moisture control and vinegar fly contamination appeared to be major problems with open tray rearing of FCM larvae. The addition of formalin to artificial diet, virus control, proved detrimental to larval development. Denim appeared to be a suitable substitute for covering bottles up to the point where larvae would begin to pupate. Vermiculite proved to be a suitable pupation medium for FCM larvae. A wild FCM culture has been successfully established.

Future research

This study will be concluded in March 2006. During the remaining time, a strong emphasis will be placed on testing larval diets in open trays.

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

Opsomming

Die doel van hierdie eksperiment is om die vermoë van parasitoïede van VKM-larwes te ondersoek en indien moontlik, om hulle vir die beheer van VKM te gebruik. Van April tot Junie 2005 is 'n groot aantal VKM-besmette vrugte versamel om parasitoïede in die hande te kry. Dit is gedoen om 'n laboratoriumkultuur van *Agathis bishopi* te vestig en die biologie daarvan te bestudeer. Altesaam 681 VKM-larwes is versamel. Geen parasitisme van 5^{de} instar larwes is waargeneem nie. Van die ander vier larwe-instars is altesaam 14.5% deur *A. bishopi* geparasiteer. In teenstelling met vorige bevindinge is geen tendens in parasitisme vir die verskillende instars gekry nie. Die mannetjie- tot wyfieverhouding is as 1:0.79 gemeet.

Met die teling van *A. bishopi* het net mannetjieparasitoïede in die F1-generasie vir die eerste vier dae uitgebroei. Wanneer twee wyfieparasitoïede in 'n flessie met VKM-larwes geplaas is, het 'n tweede generasie parasitoïede uit 81.6% van die flessies uitgekom. Dit was in teenstelling met enkelwyfies, waar 'n tweede generasie van parasitoïede uit net 46.8% van flessies uitgekom het. Die geslagsverhouding het ook verbeter van 23.5% tot 43.7% wyfies. Die stelsel wat tans vir die teling van parasitoïede gebruik word, is baie arbeidsintensief en die proses neem baie tyd in beslag. Toekomstige navorsing sal deur 'n nagraadse student uitgevoer word. Daar sal gefokus word op die biologie en verbetering in teeltnieke van *A. bishopi*. Translokasie en vrylatingsproewe met *A. bishopi* sal so gou as moontlik uitgevoer word.

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. However, the distribution, seasonal occurrence and effectiveness of these parasitoids are 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 the 2003/04 season weekly surveys of larval parasitoids were conducted in an Eastern Cape navel orange orchard with consistently high levels of FCM activity (Moore & Kirkman, 2003 & 2004). This survey confirmed the total dominance of *A. bishopi* in the Eastern Cape. In this report, the mass collection of *A. bishopi* parasitoids and laboratory rearing of them is recounted.

Materials and methods

Parasitoid collection

Two orchards of Navel oranges on Woodridge Farm in the Sundays River Valley (orchard 26: Autumn Gold Navel orange trees; orchard 28: Newhall Navel orange trees) with a reputation for FCM problems were selected for the trial. Nine mass collections of fruit were done during this period, in which several hundred fruit, which appeared to be infested, were collected. Later in the season, infested fruit were picked off trees in an attempt to recover younger instars of larvae. These fruit were dissected carefully so as not to 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.

Parasitoid rearing

Attempts were made to start an *Agathis bishopi* parasitoid culture, by exposing the parasitoids to FCM larvae in rearing jars. Parasitoids emerging from larvae collected on 16 February 2005 were caught and placed in a honey jar with an estimated 250 1st and 2nd instar FCM larvae. Honey was smeared to the inside of the jar as nutrition for the parasitoids.

When parasitoids emerged from subsequent collections, they were collected and placed in a 2 l glass jar, with a piece of cotton wool soaked in an equal mixture of honey and distilled water. Thereafter, one male and one female parasitoid were placed in a honey jar (with honey and water) in order that they could mate. Once the pair of parasitoids had spent 24 h together, they were placed in a jar with a few dozen (not precisely quantified) 1st or 2nd instar larvae in a thin layer of diet. Again, they were supplied with honey and water. After every 24 h they were transferred into a new jar of larvae. After parasitoids were removed from a jar, the jar was plugged with cotton wool, which could serve as medium for pupation. Once pupation was complete, the cotton wool plug was removed and placed in a 2 l glass jar and emergence was monitored.

In other trials, between two and five 1st instar larvae were exposed to a mated female parasitoid in small glass vials. Observations were recorded.

Subsequently, one male and one female parasitoid were introduced into each jar and were removed after various intervals, ranging from one to seven days and transferred to new jars. Once the larvae were ready to pupate, the metal lids were replaced by full cotton wool plugs as a pupation substrate. Once all larvae had pupated, the plugs were removed and placed individually into 2 l glass jars. Petri-dishes were placed over the jars and parasitoids were then captured as they settled on the surface of the Petri-dish. Emerging parasitoids were enumerated and compared.

Various sizes of wire mesh were used in an attempt to separate moths from parasitoids.

As the culture grew, glass bottles were replaced with 2 l plastic cordial bottles, as emergence chambers. A doorway was cut into each bottle through which the cotton wool plug was placed. A small hole was punched into the lid of the bottle, and a glass vial was secured onto the lid with Prestik. A small piece of cotton wool, soaked in water and honey, was placed in the vial as an attractant for the parasitoids. Bottles were covered with brown paper bags in an attempt to improve the movement of parasitoids into the vials. A lid was placed over the jar opening after removal of the cotton wool stopper, in order to facilitate capturing of parasitoids from pupae that had formed on the surface of the diet in the honey jar.

As the culture grew, it became impossible to keep each cotton wool plug separately, so a large number of plugs were placed into a single emergence box.

The glass jar into which parasitoids and moths emerged was replaced by a plastic bottle with holes in the top. Glass vials with small openings were put over these holes, and cotton wool soaked in honey and water was put into the vials to attract the parasitoids. This system was designed to enable separation of parasitoids from the moths.

Results and discussion

Parasitoid collection

A total of 681 larvae were collected from the eight mass collections of fruit conducted at Woodridge Farm from February to June 2005 (Table 3.2.6.1). As no parasitoids were recovered from 5th instar larvae from the first five collections (Tables 3.2.6.2 – 3.2.6.6), these mature larvae were ignored (not collected) in the subsequent surveys. The low rate of parasitoid recovery from 5th instar larvae was confirmed by findings from surveys conducted during previous seasons (Moore & Kirkman, 2003 & 2004; Sishuba *et al.*, 2002; Sishuba, 2003).

Table 3.2.6.1. FCM larvae collected from mass collected Navel oranges from Woodridge Farm (Sundays River Valley) from February to July 2005.

Date	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	
16/02/05	11	21	42	43	83	200
28/04/05	14	20	19	8	14	75
05/05/05	15	29	16	9	13	82
10/05/05	5	12	15	11	7	50
02/06/05	16	30	22	10	0	78
14/06/05	11	24	21	8	0	64
22/06/05	6	31	25	22	0	84
28/06/05	3	16	15	14	0	48
Total	81	183	175	125	117	681

As was the case during the preceding two seasons, *Agathis bishopi* was the only parasitoid species identified parasitizing FCM larvae in the Eastern Cape during the 2001/02 season (Sishuba, 2003; Sishuba *et al.*, 2002) and during the 2003/04 season (Moore & Kirkman, 2004). During the 2003/04 season, larvae were collected from the field on a weekly basis. It was thus possible to ascertain that larval parasitism peaked at 40.0% during one particular week in December and 37.5% during another week in April (Moore & Kirkman, 2004). However, mean parasitism of larvae for all the collections during a single month, did not differ much from the level of parasitism determined from the mass collections reported here (2004/05 season). Larval parasitism in these surveys ranged from 12-18% (Tables 3.2.6.2 – 3.2.6.9).

On 16 February 2005, 200 larvae were recovered from fruit, of which 7.0% were parasitised (Table 3.2.6.2). Parasitism of 1st and 2nd instar larvae was higher than for any of the other instars. A total of 33% of larvae died, therefore not producing moths or parasitoids. No differentiation was made between male and female parasitoids.

Table 3.2.6.2. Parasitoid emergence from larvae collected on 16 February 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>	% Larvae parasitised by <i>A. bishopi</i>
1	11	4	2	18.2
2	21	10	4	19.0
3	42	22	5	11.9
4	43	27	3	7.0
5	83	57	0	0
Total	200	120	14	7.0

Parasitoids emerging from larvae collected on 16 February 2005 were caught and placed in a honey jar with an estimated 250 1st and 2nd instar FCM larvae. Unfortunately, the FCM larvae became symptomatically infected with virus and a second generation of parasitoids was therefore not possible. This virus outbreak was an anomaly, as no virus was evident in any other FCM larvae in the laboratory culture. This was possibly indicative that *A. bishopi* can act as a vector of the granulovirus. Parasitoids have been shown to fill such a role in other cases, including some other braconid parasitoids (as is *A. bishopi*) (Levin *et al.*, 1983; Gould *et al.*, 1990).

On 28 April 2005, 75 larvae were recovered from fruit, of which 9.4% were parasitised (Table 3.2.6.3). Surprisingly, parasitism of 4th instar larvae was higher than for any of the other instars. This was not usual, but might not have been significant, due to the small number of 4th instar larvae collected. A total of 36% of larvae died. Differentiation was made between male and female parasitoids.

Table 3.2.6.3. Parasitoid emergence from larvae collected on 28 April 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	14	7	1	0	1	7.1	0	7.1
2	20	11	0	3	3	0	15.0	15.0
3	19	12	1	0	1	5.3	0	5.3
4	8	1	0	2	2	0	25.0	25.0
5	14	10	0	0	0	0	0	0
Total	75	41	2	5	7	2.7	6.7	9.4

On 5 May 2005, 82 larvae were recovered from fruit, of which 9.5% were parasitised (Table 3.2.6.4). Only 1st and 2nd instar larvae were parasitised. A total of 45.1% of larvae died.

Table 3.2.6.4. Parasitoid emergence from larvae collected on 5 May 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	15	6	0	1	1	0	6.7	6.7
2	29	14	6	0	6	20.7	0	20.7
3	16	6	0	0	0	0	0	0
4	9	4	0	0	0	0	0	0
5	13	8	0	0	0	0	0	0
Total	82	38	6	1	7	7.3	1.2	9.5

On 10 May 2005, 50 larvae were recovered from fruit, of which 10.0% were parasitised (Table 3.2.6.5). Surprisingly, none of the 1st instar larvae collected were parasitised, whereas parasitised larvae of the following three instars were found. As previously speculated, the lack of parasitism of 1st instar larvae may have been due to the small sample size. A total of 32% of larvae died.

Table 3.2.6.5. Parasitoid emergence from larvae collected on 10 May 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	5	2	0	0	0	0	0	0
2	12	8	1	0	1	8.3	0	8.3
3	15	8	2	0	2	13.3	0	13.3
4	11	9	0	2	2	0	18.2	18.2
5	7	2	0	0	0	0	0	0
Total	50	29	3	2	5	6.0	4.0	10.0

On 2 June 2005, 78 larvae were recovered from fruit, of which 11.5% were parasitised (Table 3.2.6.6). Parasitism was highest for 2nd and 3rd instar larvae. A total of 37.2% of larvae died.

Table 3.2.6.6. Parasitoid emergence from larvae collected on 2 June 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	16	5	1	0	1	6.3	0	6.3
2	30	16	0	4	4	0	13.3	13.3
3	22	11	3	0	3	13.6	0	13.6
4	10	8	0	1	1	0	10.0	10.0
5	0	0	0	0	0	0	0	0

Total	78	40	4	5	9	5.1	6.4	11.5
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On 14 June 2005, 64 larvae were recovered from fruit, of which 4.7% were parasitised (Table 3.2.6.7). This excluded 5th instar larvae, as none of the previous collections revealed any parasitism of this instar. Parasitism was highest for 1st instar larvae. A total of 57.8% of larvae died.

Table 3.2.6.7. Parasitoid emergence from larvae collected on 14 June 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	11	2	1	0	1	9.0	0	9.0
2	24	13	0	1	1	0	4.2	4.2
3	21	8	1	0	1	4.8	0	4.8
4	8	1	0	0	0	0	0	0
Total	64	24	2	1	3	3.1	1.6	4.7

On 22 June 2005, 84 larvae were recovered from fruit, of which 4.7% were parasitised (Table 3.2.6.8). This again excluded 5th instar larvae. Parasitism was again highest for 1st instar larvae and for the second consecutive collection, no parasitism of 4th instar larvae was found. A total of 25.0% of larvae died.

Table 3.2.6.8. Parasitoid emergence from larvae collected on 22 June 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	6	2	0	1	1	0	33.3	33.3
2	31	23	2	2	4	6.4	6.4	12.9
3	25	18	2	1	3	8.0	4.0	12.0
4	22	12	0	0	0	0	0	0
Total	84	55	4	4	8	4.8	4.8	9.6

The final fruit collection was conducted on 28 June 2005. Of the 48 larvae recovered from fruit, 8.3% were parasitised (Table 3.2.6.9). Surprisingly, no 1st instar larvae were found to be parasitised. However, this might have been due to the small sample size for these young larvae. A total of 35.4% of larvae died.

Table 3.2.6.9. Parasitoid emergence from larvae collected on 28 June 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	3	2	0	0	0	0	0	0
2	16	9	1	0	1	6.2	0	6.2
3	15	8	1	1	2	6.7	6.7	13.4
4	14	8	1	0	1	7.1	0	7.1
Total	48	27	3	1	4	6.2	2.1	8.3

In total, 14.5% of all larvae collected, were parasitised by *A. bishopi* (Table 3.2.6.10). This was not dissimilar to the survey conducted the previous year, which showed an average of 11.3% of larvae parasitised (Moore & Kirkman, 2003). There did not appear to be any trend in the relative rates of parasitism per instar (Table 3.2.6.10). However, it is likely that parasitism takes place fairly early on in the larval cycle. Even though *A. bishopi* has a relatively long ovipositor, it will not be able to reach a larva once it has penetrated further than a centimetre or two into the fruit. Unfortunately, the sex ratio was biased slightly in favour of males (Table 3.2.6.10). However, this might not be significant.

Table 3.2.6.10. Total parasitoid emergence from larvae collected from April to June 2005.

FCM Larval instar	Number of larvae	Number of moths emerging	Number of larvae parasitised by <i>A. bishopi</i>			% Larvae parasitised by <i>A. bishopi</i>		
			♂	♀	Total	♂	♀	Total
1	70	26	3	2	5	4.3	2.9	7.2
2	162	94	10	10	20	6.2	6.2	12.4
3	133	71	10	2	12	7.5	1.5	9.0
4	82	43	1	5	6	2.0	10.2	12.2
(5)	(34)	(20)	(0)	(0)	(0)	(0)	(0)	(0)
Total (excluding 5th instars)	447	234	24	19	43	8.1	6.4	14.5

Parasitoid rearing

One male and female parasitoid were placed in a honey jar to mate. After 24 h, they were placed in a jar with a few dozen 1st or 2nd instar larvae and transferred to a new such jar every 24 h. The F1 generation of parasitoids was monitored and recorded (Table 3.2.6.11).

Table 3.2.6.11. F1 (second generation) progeny from a pair of *A. bishopi* parasitoids.

Date	Occurrence	FCM larval instar in jar when exposed	F1 parasitoids	
			Males	Females
Day 1	<i>A. bishopi</i> female emerged			
Day 4	<i>A. bishopi</i> male emerged, placed with female for 24 hrs to mate			
Day 5	Jar 1 larvae exposed to parasitoids for 24 h	1 st	21	0
Day 6	Jar 2 larvae exposed to parasitoids for 24 h	1 st	14	0
Day 7	Jar 3 larvae exposed to parasitoids for 24 h	1 st	5	0
Day 8	Jar 4 larvae exposed to parasitoids for 24 h	2 nd	1	2

It is probable that *A. bishopi* can produce males parthenogenically i.e. from unfertilised eggs, whereas females would only hatch from fertilised eggs. The fact that female parasitoids only emerged from the larvae which were parasitized on the fourth day of parasitism (Day 8) (Table 3.2.6.11) indicated that mating might only have occurred at or just before Day 8. Another possible reason why only the fourth jar yielded females is that it was the only jar in which 2nd instar larvae were available to parasitoids. Some species of parasitoids tend to lay fertilised eggs (producing females) in larger hosts and unfertilised eggs (producing only males) in smaller hosts.

As it is known that in some species, females are ready to mate sooner than males, attempts were subsequently made to mate three-day-old males with females which had just emerged.

In other trials, between two and five 1st instar larvae were exposed to a mated female parasitoid in small glass vials. In all these trials, the larvae developed virus symptoms and died, possibly due to multiple oviposition wounds.

Subsequently, one male and one female parasitoid were introduced into each jar, and were removed after various intervals ranging from one to 7 days, and transferred to a new jar. It was found that the parasitoids became less fecund as they grew older. The first two jars into which the parasitoids were introduced, produced more parasitoids than the subsequent jars. Parasitised larvae turned pink at an earlier stage than did unparasitised larvae and pupated sooner (4th instar).

The percentage of jars producing parasitoids was too low (Table 3.2.6.12). It was therefore decided to add two pairs of parasitoids to each jar, which improved not only the parasitism rate but also the sex ratio (Table 3.2.6.12).

Table 3.2.6.12. Production of *A. bishopi* parasitoids in FCM larvae with different combinations of parasitoid parents.

	Combination of P1 parasitoids in jars*					Summary of P1**	
	2 males + 2 females	2 males + 1 female	1 male + 2 females	1 male + 1 female	Mated female	All jars with 1 female	All jars with 2 females
Number of jars	24	4	14	213	5	222	38
F1 parasitoids emerging							
Total parasitoids emerged	292	14	363	1276	19	1309	655
Male parasitoids emerged	164	14	205	968	19	1001	369
Female parasitoids emerged	128	0	158	308	0	308	286
% Males	56.2c	100a	56.5c	75.9b	100a	76.5	56.3
% Females	43.8a	0c	43.5a	24.1b	0c	23.5	43.7
Mean parasitoids per jar	12.2b	3.5ab	25.9c	6.0a	3.8ab	5.9A	17.2B
Total number of jars producing parasitoids	17	2	14	101	1	104	31
% jars producing parasitoids	70.8b	50.0c	100a	47.4c	20d	46.8A	81.6B

*Values in the same row (first five value columns) followed by the same letter are not significantly different ($P < 0.05$; Scheffe Multiple Range Test).

**Values in the same row (last two columns) followed by the same letter are not significantly different ($P < 0.05$; Students' T-test).

The main difficulty with the described method of rearing *A. bishopi*, was to separate the moths and parasitoids which emerged into the jars. The glass jars into which parasitoids and moths emerged were replaced by a plastic bottle with holes in the top. Glass vials with small openings in their plastic lids, were put over these holes and cotton wool soaked in honey and water was put into the vials to attract the parasitoids. Many parasitoids did enter the vials, but so did some moths, and many of the parasitoids remained in the plastic bottles. They therefore had to be removed manually. The individual capturing and transferring of parasitoids is extremely time consuming, and improvements are being sought for mass rearing purposes.

Conclusion

From April to June 2005, regular mass collections of parasitoids from FCM infested fruit were conducted. A total of 14.5% of 1st to 4th instar larvae were parasitised by *A. bishopi*. Sex-ratio of males to females was 1:0.79. In rearing *A. bishopi*, where two female parasitoids were placed into a jar with larvae, 81.6% of jars produced a second generation of parasitoids, as opposed to 46.8% of jars when only one female parasitoid was placed into the jar. Sex ratio of progeny was also improved. The system currently used for rearing parasitoids is extremely time consuming, and improvements are being sought for mass rearing purposes.

Future research

Future research will be conducted by a post-graduate student, who will focus on the biology and rearing of *A. bishopi*. Translocation and augmentation experiments will be conducted with *A. bishopi* as soon as possible.

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3.2.7 Gammabestraling van VKM-larwes vir die disinfestasië van verpakte sitrusvrugte Proef 719 deur Hendrik en Marsheille Hofmeyr (CRI)

Summary

Three experiments were conducted to study the influence of gamma irradiation on false codling moth larvae in diet jars. A dose of 100 Gy could not prevent mature larvae from developing to the moth stage. After treatment with 200 Gy larvae could develop into pupae, but these all died before eclosing. Two problems can defeat gamma irradiation as a method to safeguard oranges against live larvae, i.e. (i) an irradiation dose ratio of approximately 3:1 from the outside to the inside of a pallet full of fruit, which implies that the approved dose will have to be 3x higher than the effective dose and (ii) the improbability that inspectors will accept fruit containing live larvae, in spite of assurances that the treated larvae will eventually die without further development.

Inleiding

Die beweegredes vir bestralingsproewe wat op die uitwissing van VKM-larwes in lemoene gemik is, is in vorige verslae bespreek (2003 en 2004 CRI Jaarverslag). Uitstekende resultate is verkry met bestralingsdosisse wat van 200 Gy tot 400 Gy gewissel het. Verskillende ouderdomme VKM-larwes is in dieëfflesse bestraal en toegelaat om te ontwikkel. Geen larwe kon tot volwassenheid (motte) ontwikkel nie en die nagenoeg 5% van die larwes in verskillende ouderdomsgroepe wat wel daarin kon slaag om te pueer, het in die papiestadium gevrek. Gammastrale versprei oneweredig in palette verpakte sitrusvrugte en die buitenste vrugte ontvang 'n 3-3.5 keer groter dosis as die binneste vrugte. Dit kan vrugbeskadiging en/of verkorte rakteertyd tot gevolg hê, afhangende van watter bestralingsdosis gebruik word. Dit is daarom nodig om die laagste doeltreffende dosis te bepaal wat die doding van bestraalde larwes of uitgestelde mortaliteit van die papies of motte daarna, sal veroorsaak. Enige ander potensieel bruikbare nadelige invloed soos volledige P1- of F1-steriliteit, moet ook ondersoek word ingeval dit aanvaar sal word vir insluiting in 'n toekomstige protokol wat op die disinfestasië van vrugte deur die gammabestraling van VKM-larwes gemik is. Drie proewe is uitgevoer om die invloed van dosis van 200 Gy en kleiner op VKM-larwes en hul latere ontwikkelingstadia vas te stel.

Materiale en metodes

Drie proewe is opeenvolgens uitgevoer. Die proeftegniek het in die meeste opsigte ooreengestem en word sover moontlik saam bespreek. Drie tot vier teelflesse waarin die eerste larwes alreeds begin pupeer het, is deurgaans per behandeling gebruik. Kopkapsulemetings van 50-100 larwes wat ewekansig uit 'n bykomende teelfles in elke proef gehaal is, het getoon dat 99% van die larwes in die vierde en vyfde instar was en dus die ontwikkelingstadia verteenwoordig het wat die sterkste weerstand teen gammabestraling sou bied.

Die teelflesse is in Infruitec, Stellenbosch, se gammabestralende behandel. Die watterproppe is in die CRI-laboratorium, Citrusdal, verwyder en met riffelkartonproppe toegevoeg sodat enige papies wat ontwikkel het, versamel kon word. Die teelflesse is by 26°C gehou. Die papies is uit die kartonproppe verwyder, getel en toegelaat om verder te ontwikkel. Aantekening is gemaak van die aantal en geslag van die motte wat ontwikkel het. Paringstudies is vervolgens uitgevoer om die vrugbaarheidstatus van die motte vas te stel. Drie paringskombinasies is waar moontlik gebruik (genoeg motte kon nie altyd versamel word om alle kombinasies te toets nie), naamlik $T_{\text{♀}} \times N_{\text{♂}}$ (T = Mot van bestraalde larwe; N = Mot van onbestraalde larwe), $N_{\text{♀}} \times T_{\text{♂}}$ en $T_{\text{♀}} \times T_{\text{♂}}$. Tien paar motte is per kruising gebruik. Elke paar motte is in 'n 50 ml plastiekbakkie geplaas wat met 'n nat watterrolletjie voorsien is. Eiers is teen die wande, bodem en deksel van die bakkies gelê. Eierlegging is toegelaat totdat die wyfie in elke bakkie gevrek het. Die eiers in die bakkies is daarna mikroskopies ondersoek en getel om die persentasie mortaliteit te bepaal. Onbevugte wyfies lê dikwels eiers, maar dié ontwikkel uiteraard nie. Die wyfiemotte is derhalwe gedissekteer om vas te stel of hul gepaar het. Elke wyfie se bursa copulatrix is met behulp van twee tangetjies oopgeskeur en vir die aanwesigheid van spermatofore (spermabundels) ondersoek. Laasgenoemde is as bewys beskou dat paring plaasgevind het en dat onontwikkelde en/of onuitgeborede eiers wat daarna gelê is, die gevolg van steriliteit van een of beide van die ouerlike motte was.

In proewe 2 en 3 is drie herhalings van onderskeidelik 50 en 100 paar motte (afkomstig van larwes wat met 50 Gy behandel was) in sifhokkies geplaas sodat eiers gelê kon word wat vir teling tot die F1-generasie gebruik kon word. Daar is veronderstel dat slegs $T_{\text{♀}} \times T_{\text{♂}}$ parings (potensieel ontpop uit bestraalde uitvoervrugte) in 'n land waar VKM nie aanwesig is nie, sal kan plaasvind. Eiers wat op dié wyse in proef 3 versamel is, is vir verdere ontwikkeling in die teelflesse geplaas. Tien paar van die F1-motte is vervolgens in 50 ml plastiekbakkies met mekaar gekruis ($T_{\text{♀}} \times T_{\text{♂}}$) om vas te stel of F1-steriliteit deur bestraling met 50 Gy bewerkstellig kon word. Tien kontrolekruisings is ook op dieselfde wyse uitgevoer.

Resultate

Larwe-ontwikkeling vind in die teelflesse plaas en dit is dus onmoontlik om die aantal larwes te bepaal wat in die verskillende behandelings bestraal is. Die aantal papies wat uit die kontroleflesse herwin is, is derhalwe as die gemiddelde produksie per fles beskou.

In 'n vorige proef (CRI-jaarverslag vir 2004, Afd. 3.4.11) het 3.8% van alle uitgegroeide larwes wat met 200 Gy bestraal was, papies gevorm, maar geeneen kon in motte ontwikkel nie. In die drie proewe onder bespreking is 200 Gy en laer dosisses getoets. Geen ontwikkeling van larwes tot papies het by die 200 Gy-behandeling (proef 1) plaasgevind nie (Tabel 3.2.7.1). Daarbenewens is die ontwikkeling van 150 Gy-bestraalde larwes (proewe 1 en 2) in die papiestadium stopgesit. Slegs 11.7%, 8.8% en 26.4% van larwes wat onderskeidelik in die drie proewe met 100 Gy bestraal was, kon tot motte ontwikkel waarvan die oorgrote meerderheid mannetjies was.

Tabel 3.2.7.1. Ontwikkeling van gammabestraalde valskodlingmotlarwes tot papies en motte

Bestralingsdosis (Gy)	Aantal papies ontwikkel (% afname relatief tot kontrole)	Aantal motte ontwikkel (% afname relatief tot kontrole)	% afname in papies wat tot motte kon ontwikkel	Geslagsverhouding (♀♂)
Proef 1				
0	3198	3138	1,9	1:1,04
50	2797 (12.5)	2370 (24.5)	25.9	1:1.09
100	1735 (45.7)	367 (88.3)	88.5	1:19.4
150	116 (96.4)	0 (100.0)	100.0	-
200	0 (100.0)	0 (100.0)	-	-
Proef 2				
0	887	850	4.2	1:1.12

50	819 (7.7)	701 (17.5)	14.4	1:1.26
100	411 (53.7)	75 (91.2)	81.8	1:6.5
150	288 (67.5)	0 (100.0)	100.0	-
Proef 3				
0	1604	1514	5.6	1:1
50	1495 (6.8)	1401 (7.5)	6.3	1:1
75	950 (40.8)	768 (49.3)	19.2	1:1.5
100	575 (64.2)	400 (73.6)	30.4	1:7.9

In SIT-navorsing is bewys dat wyfies wat as motte bestraal word, vatbaarder vir bestraling as mannetjiemotte is. Motte wat uit bestraalde larwes ontwikkel, toon dieselfde neiging (Tabel 3.2.7.2). Dit is veral by die 50 Gy-behandeling in al drie proewe te sien waar 'n groot persentasie van die eiers onuitgebrou gebly het in parings waarby wyfies van bestraalde larwes afkomstig, betrokke was ($T_{\text{♀}} \times N_{\text{♂}}$ en $T_{\text{♀}} \times T_{\text{♂}}$). Eiermortaliteit was veel laer in die geval van parings waarby mannetjies betrokke was wat uit bestraalde larwes ontwikkel het ($N_{\text{♀}} \times T_{\text{♂}}$). Met 'n verhoging in dosis tot 100 Gy raak die bestralingsinvloed strawwer en geen eiers word deur $T_{\text{♀}}$ gelê nie, ongeag of hulle gepaar het of nie. Die 100 Gy-behandeling het ook 'n erg nadelige uitwerking op die mannetjies gehad, te oordeel aan die totale afwesigheid van spermatofore in wyfies wat met sulke mannetjies gekruis was ($N_{\text{♀}} \times T_{\text{♂}}$ en $T_{\text{♀}} \times T_{\text{♂}}$). Dit kan as gevolg van enige een van twee redes wees, naamlik (i) die mannetjies was nie fisies in staat om te paar nie of (ii) paring het wel plaasgevind, maar een of ander faktor in die mannetjies het ontbreek om die wyfies in staat te stel om spermatofore te vorm.

Tabel 3.2.7.2. Mortaliteit* van eiers wat deur die motte van gammabestraalde larwes gelê is.

Bestralingsdosis	Paringskombinasie			
	$N_{\text{♀}} \times N_{\text{♂}}$	$T_{\text{♀}} \times N_{\text{♂}}$	$N_{\text{♀}} \times T_{\text{♂}}$	$T_{\text{♀}} \times T_{\text{♂}}$
Proef 1				
0	(7.3)	-	-	-
50	-	81.7	30.1	79.8
100	-	Alle wyfies bevrug; geen eiers gelê nie	Alle wyfies onbevrug; eiers gelê, maar almal onontwikkeld	Alle wyfies onbevrug; geen eiers gelê nie
150	-	Papiës ernstig misvorm – geeneen ontwikkel tot motte nie		
200	-	Larwes vrek – geeneen ontwikkel tot papiës nie		
Proef 2				
0	(5.4)	-	-	-
50	-	63.7	32.7	70.4
100	-	Oorwegend mannetjies ontwikkel; te min wyfies vir paringstudie	Alle wyfies onbevrug; eiers gelê, maar almal onontwikkeld	Alle wyfies onbevrug; geen eiers gelê nie
150	-	Papiës ernstig misvorm – geeneen ontwikkel tot motte nie		
Proef 3				
0	(11.0)	-	-	-
50	-	33.3	45.4	85.1
75	-	Alle wyfies het gepaar, maar slegs een wyfie het eiers gelê (16), waarvan 87.5% nie uitgebrou het nie.	40% wyfies onbevrug; slegs een bevrugte wyfie se eiers broei uit waarvan 77.6% nie uitgebrou het nie.	50% wyfies het gepaar, maar geen eiers gelê nie
100	-	40% wyfies bevrug, maar geen eiers geê nie.	Alle wyfies onbevrug, eiers gelê, maar almal onontwikkeld.	Alle wyfies onbevrug, geen eiers gelê nie.

*Volgens Abbott.

Daar is in proef 2 beplan om die eiers van P1-motte wat in die 50 Gy-behandeling ontwikkel het, te gebruik om 'n F1-studie moontlik te maak. Eiermortaliteit in die bykomende drie herhalings van die $T_{\text{♀}} \times T_{\text{♂}}$ -kruisings was egter so hoog (96.8%) dat daar nie genoeg eiers bymekaar gemaak kon word om inenting in dieët moontlik te maak nie.

Genoeg eiers is in die 50 Gy T♀ x T♂-kruisings van proef 3 versamel om in dieëfflesse in te ent. Tien paar van die F1-generasie wat hieruit ontwikkel het is met mekaar gekruis in die hoop dat die motte steriel sou wees en dat die eiers nie sou uitbroei nie. Gemiddeld 69% van die eiers in die verskillende herhalings het egter uitgebroei, wat daarop dui dat F1-steriliteit nie bewerkstellig kon word deur die P1-larwes met 50 Gy te behandel nie.

Daar is voorheen gemeld dat die bestralingsdosis so laag as moontlik moet wees om enige nadelige invloed van gammabestraling op vrugkwaliteit te voorkom. Dié dosis sal afhang van welke maatstaf van doeltreffendheid in 'n gammadisinfestasiëprotokol vasgelê word. Die VSA keur tans 'n generiese 400 Gy-behandeling goed op alle gewasse. Dié behandeling is bedoel om plaë wat van fitosanitêre belang is, in vrugte uit te wis en dood alle bestraalde larwes sonder dat verdere ontwikkeling plaasvind. Die nagenoeg 3:1 afname in bestralingsdosis van die buitekant na die middel van 'n pallet gestapelde kartonne met vrugte, beteken egter dat lemoene met 'n dosis van nie laer nie as 1200 Gy behandel sal moet word om te verseker dat vrugte in die middelste kartonne die voorgeskrewe 400 Gy dosis ontvang. Dit is uit 'n vrugkwaliteitsoogpunt heeltemal onaanvaarbaar.

Daar is egter ook 'n verdere probleem. Selfs al kan daarin geslaag word om die generiese 400 Gy-dosis vir VKM-disinfestasië te verminder, sal 'n dosis van nie minder nie as 150 Gy toegepas moet word. Dit sal meebring dat inspekteurs wat lewendige larwes in bestraalde vrugte opspoor, sonder voorbehoud sal moet aanvaar dat die larwes nie verder as papies sal kan ontwikkel nie. Die moontlikheid dat so 'n toegewing gemaak sal word, is uiters skraal. Indien bestraalde larwes wat nie verder kan ontwikkel nie, as maatstaf geneem word, sal 'n dosis van 200-350 Gy (200 Gy – dié verslag; 350 Gy - Afd. 3.4.11, 2004 CRI-jaarverslag) gebruik moet word. Larwes wat egter selfs met dié dosisse behandel word, vrek nie onmiddelik, of selfs kort na, behandeling nie, maar kan nog etlike weke lank in 'n skyndoodfase bly lewe, voordat die dood uiteindelik intree. Dit bring mee dat die doeltreffende dosis nog hoër sal moet wees om onmiddelike doding te verseker, wat uiteraard ook heeltemal onaanvaarbaar sal wees.

As bogenoemde resultate in ag geneem word, lyk dit tans onseker of gammabestraling tot 'n disinfestasiëbehandling vir VKM in sitrusvrugte ontwikkel sal kan word. Voortgesette navorsing op dié aspek sal grotendeels afhang van die vermoë van verskillende kultivars sitrusvrugte om relatiewe hoë dosisse bestraling sonder enige nadele te weerstaan. Daarbenewens sal 'n bestralingstegniek ontwikkel moet word wat nie die huidige 3-3.5:1 dosisvermindering in palette vrugte lewer nie.

3.2.8 Investigation of alternative hosts for FCM

Experiment 743 by Sean Moore and Wayne Kirkman (CRI)

Opsomming

Dit is onseker watter invloed alternatiewe gasheerplante aangrensend aan sitrusboorde op VKM-bevolkings in daardie boorde het. Opnames is op 'n gereelde basis in drie streke in die Oos-Kaap uitgevoer. Vrugte, galle en vlesige gedeeltes van moontlike alternatiewe gashere vir VKM is versamel. Monsters van elk is vir tekens van VKM ondersoek. Ander monsters is aan dagoue VKM-larwes blootgestel en twee weke later vir tekens van besmetting, indringing en larwale ontwikkeling ondersoek. VKM-larwes is in *Ricinus communis*, *Schotia afro*, *Opuntia ficus-indica*, *Asparagus crassifolius*, *Solanum tomentosum*, *Passiflorai* sp. en *Albuca* sp. gevind. Hierdie bevindinge kan implikasies inhou vir die bestuur van plante in en om sitrusboorde. Die opname is voltooi.

Introduction

Much emphasis is placed on the importance of orchard sanitation to keep FCM under control. However, little is known of alternative hosts for FCM. In the presence of alternative hosts, even diligent orchard sanitation might only have a limited impact on reducing FCM levels.

A recent survey in the Western Cape revealed very few alternative hosts (Stephan Honiball, personal communication). This might not be the case in the Eastern Cape, as FCM infestation appears to peak a lot earlier in the season than is the case in the Western Cape, indicating a build-up on other hosts before citrus fruits are available in meaningful quantities. The discovery of alternative hosts could lead to better management of these hosts, where they occur within or adjacent to citrus producing areas. Stoffberg (1939) and Schwartz (1981) reported a number of cultivated hosts (other than citrus) and wild hosts for FCM (Table 3.2.8.1).

Table 3.2.8.1. Literature recorded cultivated hosts (other than citrus) and wild hosts of FCM Stofberg (1939) & Schwartz (1981).

Cultivated hosts	Wild hosts
<i>Quercus robur</i>	<i>Ziziphus mucronata</i>
<i>Prunophora armeniaca</i>	<i>Diospyros lycoides</i>
<i>Persia americana</i>	<i>Schotia brachypetala</i>
<i>Punica granatum</i>	<i>Podocarpus latifolius</i>
<i>Gossypium hirsutum</i>	<i>Diospyros mespiliformis</i>
<i>Psidium guajava</i>	<i>Dovyalis caffra</i>
<i>Litchi chinensis</i>	<i>Pseudolachnostylis maprouneifolia</i>
<i>Macadamia ternifolia</i>	<i>Mimusops zeyheri</i>
<i>Mangifera indica</i>	<i>Sclerocarya caffra</i>
<i>Juglans sieboldiana</i>	<i>Combretum apiculatum</i>
<i>Olea europeae</i>	<i>Ximenia caffra</i>
<i>Diospyros virginiana</i>	<i>Bequaertiodendron magalisonatum</i>
<i>Amygdalus persica</i>	<i>Syzygium cordatum</i>
<i>Prunophora domestica</i>	<i>Annona senegalensis</i>
<i>Sorghum halepense</i>	
<i>Annona cherimoya</i>	

In studies by Moore and Kirkman in 2004, FCM larvae were discovered in the fruit of *Ricinus communis*, the castor oil plant, and in *Crassula ovata*. A suspected FCM larva was found in the fruit of *Opuntia ficus-indica*, the prickly pear. These surveys were continued in 2005.

Materials and methods

At regular intervals during the year, four surveys were conducted within areas of wild vegetation (both on and adjacent to citrus farms), at three sites in the Eastern Cape (Table 3.2.8.2). Fruiting, gall-forming and succulent plants were noted. Plant parts bearing fruit and galls and fleshy parts were collected. A minimum of 10 such parts were collected for each plant species. At least five of the fruits and galls were dissected to check for FCM infestation, while at least five other seemingly healthy fruits and galls were exposed to 4–6 neonate FCM larvae each. These fruits and galls were kept at approximately 27°C and then dissected two weeks after exposure and inspected for the presence of FCM larvae or pupae. If these were found, an attempt was made to rear them to adulthood for identification. Moths were sent to either the Plant Protection Research Institute or the Transvaal Museum for identification. Plant species were identified, where possible, with the use of the following references: Gledhill (1981), Shearing (1994), Urton (1993), Van Wyk et al (1998), Von Breitenbach (1985), G. Kerley and M. Landman (personal communication).

Table 3.2.8.2. Details of the three sites used for investigating alternative hosts for FCM.

Area	Farm	GPS Coordinates
Kirkwood, Sundays River Valley	Welgelegen	S 33° 25' 15.2" E 25° 24' 33.3"
Sunland, Sundays River Valley	Woodridge	S 33° 28' 43.4" E 25° 41' 38.1"
Uitenhage	Citrus Foundation Block	S 33° 46' 26.9" E 25° 19' 36.7"

Results and discussion

Table 3.2.8.3. Results of a survey of alternative hosts for FCM done on 11 January 2005.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Woodridge	<i>Mesembryanthemum</i> sp.	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Schotia afra</i>	Pods and flowers	No infestation or signs of penetration.	No infestation or signs of penetration.

Woodridge	<i>Aloe sp.</i>	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Crassula ovata</i>	Stem and leaves	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Rhus longispinus</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Opuntia ficus-indica</i>	Fruit	No infestation or signs of penetration.	7 penetration marks found, but no larvae detected.
Woodridge	<i>Scutia myrtina</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Solanum tomentosum</i>	Fruit	No infestation or signs of penetration.	Signs of penetration, but no larvae found
Woodridge	<i>Lagoneiria sp.</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Rhoicoccus tridentata</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.

Table 3.2.8.4. Results of a survey of alternative hosts for FCM done on 25 January 2005.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Welgelegen	<i>Acacia caffra</i>	Galls	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Lyceum afra</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Datura ferox</i>	Pods	Signs of penetration in seeds, but no FCM larvae found.	No infestation or signs of penetration.
Welgelegen	<i>Schotia afra</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Ricinus communis</i>	Seeds	No infestation or signs of penetration.	No infestation or signs of penetration.

Table 3.2.8.5. Results of a survey of alternative hosts for FCM done on 6 April 2004.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Woodridge	<i>Ricinus communis</i>	Seeds	No infestation or signs of penetration.	2 larvae found, which were reared to adulthood and identified as FCM
Woodridge	<i>Asparagus crassifolius</i>	Fruit	No infestation or signs of penetration.	3 larvae found, which were reared to adulthood. Two were

				identified as FCM, one unidentified.
Citrus Foundation Block	<i>Passiflora</i> sp.	Fruit	No infestation or signs of penetration.	4 penetration marks, one 4 th instar larvae found, reared to adulthood and identified as FCM.
Woodridge	<i>Schotia afra</i>	Pods	No infestation or signs of penetration.	1 larva found, which was reared to adulthood and identified as FCM
Woodridge	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Putterlickia pyracantha</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Datura ferox</i>	Pods	Signs of penetration, no FCM found	No infestation or signs of penetration.
Woodridge	Fam: Mesembryanthemaceae	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	Unidentified	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Azima tetraacantha</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Acacia karroo</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Acacia karroo</i>	Galls	Many penetration marks in galls, no FCM found.	No infestation or signs of penetration.
Welgelegen	<i>Melia azedarach</i>	Fruit	No infestation or signs of penetration.	Many penetration marks, no FCM found.
Woodridge	<i>Viscum rotundifolium</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Clausena anisata</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Ficus natalensis</i>	Fruit	Some signs of penetration and frass, no larvae found	No infestation or signs of penetration.
Welgelegen	<i>Grewia robusta</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.



Fig. 3.2.8.1. Two FCM larvae were found in the pods of *Ricinus communis*.



Fig. 3.2.8.2. Two FCM larvae were found in the fruit of *Asparagus crassifolius*.



Fig. 3.2.8.3. One FCM larva was found in the fruit of *Passiflora* sp.



Fig. 3.2.8.4. One FCM larva was found in the pods of *Schotia afra*.

Table 3.2.8.6. Results of a survey of alternative hosts for FCM done on 26 May 2005.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Woodridge	<i>Azima tetracantha</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Opuntia ficus-indica</i>	Very ripe fruit	No infestation or signs of penetration.	5 x 3 rd instar and 2 x 5 th instar larvae found. 6 of the larvae were reared to adulthood and identified as FCM
Woodridge	<i>Putterlickia pyracantha</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Schotia afra</i>	Pods	No infestation or signs of penetration.	1 x pre pupa found, reared to adulthood and identified as FCM. Must have been infested before larvae were added in the lab.
Citrus Foundation Block	<i>Eucalyptus torelliana</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Azima tetracantha</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Asparagus crassicaulus</i>	Fruit	No infestation or signs of penetration.	One larva recovered which died before adulthood.
Citrus Foundation Block	<i>Jacaranda mimosifolia</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Clausena anisata</i>	Fruit	No infestation or signs of penetration.	One pod penetrated, no larva recovered.
Citrus Foundation Block	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	Unidentified	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Solanum tomentosum</i>	Fruit	No infestation or signs of penetration.	3 x 3 rd instar and 1 x 5 th instar larvae recovered. Three larvae reared to adulthood and identified as FCM
Welgelegen	<i>Solanum</i> sp.	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Pod	9 x <i>Dacus ciliatus</i> pupae found in pod	No infestation or signs of penetration.



Fig. 3.2.8.5. Six FCM larvae were found in the mature fruit of *Opuntia ficus-indica*.



Fig. 3.2.8.6. Three FCM moths reared from larvae found in the fruits of *Solanum tomentosum*.

Table 3.2.8.7. Results of a survey of alternative hosts for FCM done on 20 July 2005.

Farm	Species	Part of tree	Dissection at collection	Dissection two weeks after exposure to FCM larvae
Citrus Foundation Block	<i>Lycium cinerium</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Ficus natalensis</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Albuca</i> sp	Plant	No infestation or signs of penetration.	1 x 3 rd instar larvae found, reared to adulthood and identified as FCM
Citrus Foundation Block	<i>Aloe</i> sp	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	<i>Asparagus racemosus</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Citrus Foundation Block	Unidentified	Flower	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Ekebergia capensis</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Putterlickia pyracantha</i>	Pods	No infestation or signs of penetration.	No infestation or signs of penetration.
Welgelegen	<i>Lycium ferocissimum</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Azima tetracantha</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Lycium cinerium</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Rhoicissus digidata</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Aloe</i> sp.	Plant	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Crassula subaphylla</i>	Flowers	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Viscum rotundifolium</i>	Fruit	No infestation or signs of penetration.	No infestation or signs of penetration.
Woodridge	<i>Schotia afra</i>	Pods & flowers	Penetration marks in pods and flowers, no larvae found	No infestation or signs of penetration.



Fig. 3.2.8.7. One FCM larva was found in *Albuca* sp.

During these surveys, the invasive and fast growing weed, *Ricinus communis* (Table 3.2.8.5; Fig. 3.2.8.1), commonly known as the castor oil plant, was once again confirmed to host FCM. *Ricinus communis* has been reported as a host for FCM in Israel (Yael Argov, personal communication), and the Western Cape (Hendrik Hofmeyr, personal communication). This weed could contribute to FCM infestations in citrus orchards, as they are commonly found in areas where bush has been cleared and where there is an abundance of water. More attention should be paid by growers to eradicating this weed in and around their orchards.

In 2004 a larva was found in the fruit of *Opuntia ficus-indica*, the prickly pear (Fig. 3.2.8.5). Although it could not be reared to adulthood in order to confirm its identification, it closely resembled a fourth instar FCM larva. It has now been confirmed that *Opuntia ficus-indica* can host FCM, as seven larvae were recovered from the fruit, six of which were reared to adulthood and identified as FCM. These larvae were recovered from very mature fruit, which appear more susceptible to FCM infestation than green fruit. The prickly pear flowers during August and September, and fruit is set soon thereafter. Therefore it is possible that this plant could host FCM and help build up numbers leading to the typical peak in infestation in citrus during December. This plant occurs in abundance in the Uitenhage, Addo and Kirkwood areas. Growers could be advised to clear as many of the plants in the areas adjacent to citrus orchards as possible, and consider the abundance of prickly pear plants in an area before deciding to develop citrus orchards there.

In 2004, *Cryptophlebia peltastica* was identified attacking the pods of *Schotia afra* (Fig. 3.2.8.4). As FCM is very closely related to this moth and as FCM has previously been recorded attacking *Schotia brachypetala* (Schwartz, 1981) it was suspected that FCM could also be found attacking *S. afra*. In subsequent surveys, a FCM larva (Table 3.2.8.5) and a FCM pre-pupa (Table 3.2.8.6) were found in *S. afra* pods. The pre-pupa was more mature than any other FCM larval instars recovered during the survey, which would indicate that infestation had occurred in the field and not in the laboratory, subsequent to the pods being picked.

Various plant species showed signs of penetration, and four other plant species were confirmed to host FCM, namely *Asparagus crassicaudus* (Table 3.2.8.5 & 3.2.8.6; Fig. 3.2.8.2), *Solanum tomentosum* (Table 3.2.8.6), *Coccinea quinqueloba* (Table 3.2.8.5; Fig. 3.2.8.3) and *Albuca* sp. (Table 3.2.8.7).

Asparagus crassicaudus is a prickly shrub with cream-coloured sweetly scented flowers and red berries (Vanderplank, 1999). They have a high conservation rating, being rated as Albany Centre Endemic, and should therefore not be removed (M. Landman, personal communication).

Solanum tomentosum, commonly known as the 'slangappelbos' (Fig. 3.2.8.6), is a weed that often occurs in areas where bush has been cleared and there is an abundance of water. Growers would be advised to remove these weeds.

Coccinea quinqueloba, commonly known as the 'Ivy Gourd', is a perennial climber, with bright orange fruit, which is not uncommon and widely distributed (Vanderplank, 1999).

Albuca sp. (Fig. 3.2.8.7) are part of the family Hyacinthaceae. They have weak flowering stems, and pendulous white and green flowers, and are fairly common in the surveyed area.

Future research

No future research is planned.

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3.2.9 Investigating and improving field persistence of Cryptogran

Experiment 791 by Wayne Kirkman, Sean Moore (CRI) and Kierryn Gendall (Rhodes University)

Opsomming

Die doel van hierdie eksperiment was om probleme met die nawerking van Cryptogran te identifiseer, kwantifiseer en op te los en om nawerking van Cryptogran te verbeter deur moontlike verbeterde formulasie van die produk. 'n 25% vermindering van water en agar in die dieët wat vir dosis-mortaliteit bio-toetse met jong VKM-larwes gebruik is, het oorlewing van larwes waarskynlik verbeter. In sulke bio-toetse het Cryptogran begin afbreek na 1 tot 2 ure se blootstelling aan UV-bestraling. Dit is gemeet deur 'n vermindering in mortaliteit van jong VKM-larwes. Dit was nie moontlik om te bepaal of die nawelent van nawellemoene enige rol in die beskerming van virus teen UV-bestraling speel nie. In 'n boordproef op nawellemoene het dit voorgekom asof gepoeierde molasse nie 'n doeltreffende plaasvervanger vir vloeibare molasse vir toediening saam met Cryptogran is nie. Die bestryding van VKM was nietemin beter as wanneer Cryptogran sonder enige molasse toegedien is.

Navorsing sal voortgesit word om uit te vind of die nawelent van nawellemoene enige beskerming vir die virus teen UV-bestraling bied. Dosis-mortaliteit bio-toetse en boordproewe sal met Cryptogran en verskeie moontlike UV-beskermers uitgevoer word. Die effek van gesimuleerde reënval op Cryptogran sal gemeet en verduidelik word. Laastens sal proewe met Cryptogran uitgevoer word in 'n poging om die geregistreerde raklewe van die produk te verleng.

Introduction

Field trials have been conducted with Cryptogran for around six years now. Cryptogran is also in its second year of commercial use. Results from both field trials and commercial use have shown varying degrees of field persistence. A principal disadvantage of the use of baculoviruses in the field, is their short residual activity due to their inactivation by UV irradiation (Huber, 1990; Shapiro, 1995). This has also been demonstrated for CrleGV (Moore, 2002), the virus in Cryptogran. A prerequisite for the success of Cryptogran as a means of controlling false codling moth is to understand all of the factors affecting field persistence of the virus (not only UV irradiation) and to find ways to improve it. Environmental persistence can be improved by ensuring rain fastness and UV protection (Most & Quinlan, 1986). This experiment aims to identify, quantify and resolve persistence problems and to improve persistence through formulation.

Materials and methods

Dose-response bioassay protocol

Bioassays are an integral part of many studies with Cryptogran. Recently, dose-response bioassay results have been inconsistent, often with unacceptably high control mortality. Control mortality higher than 20% is deemed unacceptable (Moore, 2002) and the results of the bioassay are considered unreliable. In recent bioassays, larvae have also often not appeared to grow at the expected rate. In an attempt to identify where the problem might lie, bioassays were conducted with different combinations of diet (from two sources) and larvae (from the same two sources) (Table 3.2.9.1). The CRI culture was recently established from field collected FCM and was only a few generations old. One 25-well bioassay tray was prepared for each combination. Diet in the bioassay trays was untreated. Each well was inoculated with a neonate FCM larva. Seven days later, survival and growth of larvae were recorded.

Table 3.2.9.1. Combinations of diet and larvae from River Bioscience and CRI, used in control bioassays.

Treatment number	Diet	Larvae
1	CRI	River Bioscience
2	CRI	River Bioscience
3	CRI	CRI culture
4	River Bioscience	CRI culture
5	River Bioscience	River Bioscience
6	River Bioscience	River Bioscience

Bioassays were then conducted using diet with 25% less water and agar. Three bioassay trays were prepared and inoculated with one neonate larva per well i.e. a total of 75 larvae. Seven days later, survival and growth of larvae were recorded.

Another attempt at improving the diet was made by replacing the agar used in the diet, with gelatine or citrus pectin. Three bioassay trays were prepared with each of the gelling agents and inoculated with neonate larvae. Seven days later, survival and growth of larvae were recorded.

Effect of UV-irradiation on Cryptogran

Several bioassays were done to determine the effect of UV-irradiation on CrleGV, the virus used in Cryptogran. For each bioassay, five bioassay trays (25 well) of diet were prepared according to the recipe for dose-response bioassays with neonate FCM larvae, described by Moore (2002). Diet was then inoculated with Cryptogran. One tray of diet was left untreated, as a control. The trays were covered with gladwrap to prevent desiccation of the diet. They were then exposed to UV irradiation from a germicidal UV lamp or to natural sunlight, for varying periods of time (from 0-240 min). One neonate larva was then placed onto the diet in each well (i.e. 25 larvae per treatment). Trays were closed and sealed and incubated at 27°C for seven days. Thereafter, mortality of larvae was evaluated.

Variations of the protocol were used (e.g., lower and higher concentrations of virus and exposure of virus (in suspension) to UV-irradiation before inoculation of diet in bioassay trays) in order to establish the most appropriate trial protocol. Ultimately, it was found that exposure of the virus in suspension, before inoculation of diet, held the most promise. This was done by pipetting 15 ml of each virus suspension into a Petri dish and exposing it either to a germicidal UV lamp or to natural sunlight (between 11h00 and 15h00). After the virus suspensions were exposed for various lengths of time, they were inoculated onto the diet in the trays. After approximately 30 minutes under a laminar flow hood, the diet had dried sufficiently and was then inoculated with neonate FCM larvae.

Effect of the navel end on Cryptogran

Trials were conducted in order to measure the role of the navel end of navel oranges in protecting Cryptogran against breakdown, particularly that induced by UV irradiation. Trials were conducted to compare the persistence of virus in the navel end with virus on the sides of the fruit.

Sixty Autumn Gold navel oranges were dipped in distilled water and another 60 were dipped in Cryptogran and molasses at the registered concentration. The 60 Cryptogran-treated fruit were exposed to the sun for 9 hours a day for 6 days, in a similar position to that in which they would hang in the tree, i.e., navel end down. After the 6 days, both groups of 60 fruit were divided into a further two groups of 30 fruit each (Table 3.2.9.2). Three neonate FCM larvae were put onto the navel end of one untreated and one Cryptogran

treated group of fruit. An arena (polypropylene lid, attached to the fruit by a vaseline/wax mixture) was placed around the navel end of each fruit to confine them to the navel end. The other untreated and treated groups had three larvae placed onto the sides of the fruit, also confined by an arena. The fruit were evaluated for penetration and infestation after 14 days.

Table 3.2.9.2. Treatments to examine whether the navel end of navel oranges provides any protection of Cryptogran against UV degradation.

Treatment number	Treatment	Position of arena & larvae
1	Distilled water	Navel end
2	Distilled water	Side
3	Cryptogran, exposed to the sun for 6 days	Navel end
4	Cryptogran, exposed to the sun for 6 days	Side

The trial was repeated, incorporating certain changes. A higher concentration of Cryptogran was used; Prestik was used instead of the Vaseline and wax mixture to seal the arenas; five instead of three larvae were placed into the arenas on the sides of fruit; and treatments of Cryptogran unexposed to the sun were included (Table 3.2.9.3).

Table 3.2.9.3. Treatments in a second assay, to examine whether the navel end of navel oranges provides any protection of Cryptogran against UV degradation

Treatment number	Treatment	Position of arena & larvae
1	Distilled water	Navel end
2	Distilled water	Side
3	Cryptogran, no exposure to sun	Navel end
4	Cryptogran, no exposure to sun	Side
5	Cryptogran, exposed to the sun for 6 days	Navel end
6	Cryptogran, exposed to the sun for 6 days	Side

Field trials

A trial was conducted in a mature Palmer Navel orange orchard on Bernol Farm in Sundays River Valley. Five treatments of Cryptogran were applied, all at the registered rate (Table 3.4.9.4). One of the treatments was applied without any additives, another was applied with 0.5% molasses, as registered, one was applied with 0.25% molasses with Agral 90 (18 ml/100 l water) and the last two were applied with powdered molasses. The trial was laid out in a single-tree randomised block format, with 10 replicates per treatment. An average of 19.5 l of spray mix was applied per tree, using hand guns at a pressure of 20 bars. These sprays were applied on 22 March 2005.

Table 3.2.9.4. Treatments applied on 22 March 2004 for the control of FCM on Palmer navel orange trees at Bernol Farm.

Treatment number	Product	Concentration per 100 l water	Additive	Concentration per 100 l water
1	Untreated	-	-	-
2	Cryptogran	10 ml	-	-
3	Cryptogran	10 ml	Molasses	500 ml
4	Cryptogran	10 ml	Molasses + Agral 90	250 ml + 18 ml
5	Cryptogran	10 ml	Kalorie 3000	450 g
6	Cryptogran	10 ml	Kalorie 3000 + Agral 90	450 g + 18 ml

The trial was evaluated for a period of six weeks i.e., until harvesting began, in the following manner. Fruit drop (from data trees) was evaluated from three weeks after application. Dropped fruit from each data tree were collected and analysed separately. Evaluations were done by inspecting and dissecting fruit in order to determine the cause of drop. FCM infestation was identified by the presence of the larva or its frass. Mean numbers of FCM infested fruit per tree per week for each treatment were compared using ANOVA and the LSD multiple range test, using Statgraphics Plus for Windows Version 2.0 (Statistical Graphics Corporation, 1996).

Results and discussion

Dose-response bioassay protocol

Results were inconsistent and no trend could be identified (Table 3.2.9.5). Survival in all bioassays was unsatisfactory. Through years of conducting such bioassays with FCM, 80% has been set as the minimum acceptable level of survival. It was observed that survival of larvae appeared higher in cells where there was less diet and the diet was therefore not as moist. Consequently, bioassays were conducted using diet with 25% less water and agar (Table 3.2.9.6).

Table 3.2.9.5. Survival of neonate FCM larvae in bioassays using diet and larvae from River Bioscience and CRI.

Treatment number	Diet	Source of larvae	% Larval mortality
1	CRI	River Bioscience	24
2	CRI	River Bioscience	48
3	CRI	CRI	36
4	River Bioscience	CRI	28
5	River Bioscience	River Bioscience	40
6	River Bioscience	River Bioscience	28

Table 3.2.9.6. Survival of neonate FCM larvae in bioassays using untreated diet with 25% less water and agar than the standard diet.

Replicate	% Larval mortality
1	24
2	32
3	16

Improved and acceptable survival was achieved in one of the replicates (Table 3.2.9.6). However, further work is required to confirm if this change to diet is truly an improvement.

Diet prepared with gelatine set well, but putrefied after seven days, while the citrus pectin did not facilitate adequate gelling. Various other products are to be tested.

Effect of UV-irradiation on Cryptogran

Varying problems were experienced with many of the trials. These included excessive mortality of larvae, excessive survival of larvae, and evaporation and condensation within bioassay trays. Ultimately, it was found that exposure of the virus in suspension, before inoculation of diet, held the most promise. Useful results were initially obtained with from such a trial, where a concentration of 4.466×10^4 OBs/ml was exposed in suspension to a germicidal lamp for varying periods of time (Table 3.2.9.7) before being inoculated onto the diet. Results (particularly with the second replicate) indicated that exposure of at least 1 h was required to effect some detectable breakdown of virus, measured by a lowered level of mortality (Table 3.2.9.7).

Table 3.2.9.7. Impact of UV irradiation on Cryptogran (4.466×10^4 OBs/ml) measured by mortality of neonate FCM larvae in dose-response bioassays.

Number of treatment	Treatment	Exposure time to UV (min)	% Mortality of neonate larvae	
			Replicate 1	Replicate 2
1	Distilled water	0	16	24
2	Cryptogran	0	40	56
3	Cryptogran	30	40	64
4	Cryptogran	60	36	48
5	Cryptogran	120	40	48
6	Cryptogran	240	32	36

As the unexposed (to UV) treatment of 4.466×10^4 OBs/ml of Cryptogran induced no more than 56% mortality, it was considered that a higher concentration of virus might facilitate stronger results. Consequently, a concentration of 8.932×10^4 OBs/ml was used in the following bioassay (Table 3.2.9.8). In this bioassay, Cryptogran in Petri dishes was exposed to the UV irradiation of natural sunlight.

Table 3.2.9.8. Impact of UV irradiation on Cryptogran (8.932×10^4 OBs/ml) measured by mortality of neonate FCM larvae in dose-response bioassays.

Number of treatment	Treatment	Exposure time to UV (min)	% Mortality of neonate larvae	
			Replicate 1	Replicate 2
1	Distilled water	0	16	20
2	Cryptogran	0	64	68
3	Cryptogran	30	72	60
4	Cryptogran	60	68	68
5	Cryptogran	120	56	52
6	Cryptogran	240	60	52

The higher concentration of Cryptogran led to a higher level of mortality of larvae (68%), when the virus was not UV-irradiated (Table 3.2.9.8). With the change in protocol (i.e. irradiation of virus with natural sunlight instead of with a germicidal lamp), virus only appeared to be affected after 2 h of exposure to UV-irradiation. This is probably an indication that intensity of UV irradiation with the germicidal lamp was higher than that from sunlight.

An even higher concentration of Cryptogran induced up to 72% mortality of larvae (Table 3.2.9.9). However, the same trend was observed as in the previous bioassay i.e., detectable breakdown of virus occurred only after 2 h of exposure to sunlight (Table 3.2.9.9).

Table 3.2.9.9. Impact of UV irradiation on Cryptogran (1.340×10^5 OBs/ml) measured by mortality of neonate FCM larvae in dose-response bioassays.

Number of treatment	Treatment	Exposure time to UV (min)	% Mortality of neonate larvae	
			Replicate 1	Replicate 2
1	Distilled water	0	16	20
2	Cryptogran	0	72	64
3	Cryptogran	30	76	72
4	Cryptogran	60	72	76
5	Cryptogran	120	60	52
6	Cryptogran	240	52	40

Effect of the navel end on Cryptogran

Table 3.2.9.10. Navel orange fruit damage and FCM larval infestation in a fruit-dip bioassay, designed to examine the protective effect of the navel end for Cryptogran against UV irradiation.

Number	Treatment	Position of arena & larvae	Penetration marks	Fruit penetrated	No of larvae	Fruit infested
1	Distilled water	Navel end	24	20	16	15
2	Distilled water	Side	20	5	4	3
3	Cryptogran, exposed to the sun for 6 days	Navel end	21	16	18	13
4	Cryptogran, exposed to the sun for 6 days	Side	6	6	2	2

Unfortunately, many larvae escaped, as the Vaseline and wax mixture did not seal the arenas adequately. The results (Table 3.2.9.10) do not include penetration or infestation that occurred outside of arenas. It was also apparent from this trial that a higher concentration of Cryptogran should be used, and that more larvae should be placed onto fruit (at least into the side arenas). In addition, treatments of Cryptogran unexposed to the sun, should have been included for reliable comparison. This is because it was obvious that larvae penetrated more successfully through the navel end than through the side of the fruit. Therefore, in order to draw reliable conclusions, comparisons of penetration and infestation with and without exposure to sunlight, should have been made for the same positioning on the fruit (i.e. navel vs. navel and side vs. side). This was done in the subsequent trial. However, despite minimal escape of larvae, results were inconclusive.

Spray trials

By the sixth and final week of evaluation before harvesting began (i.e. eight weeks after treatment), the only treatment which still gave a significant reduction in infestation (50%) was the Cryptogran applied with 0.25% molasses and a wetter. Therefore, the results of this final week of evaluation have not been tabulated (Table 3.2.9.11). The aforesaid Cryptogran treatment caused a 68% reduction in infestation for this period, whereas the registered Cryptogran treatment resulted in a 71% reduction in infestation. However, if one includes the results of the following week, Cryptogran with 0.25% molasses and a wetter caused an average of 64% reduction in infestation and the registered Cryptogran caused a 56% reduction in infestation. The two treatments applied with Kalorie 3000 performed worse than the others. Therefore, powdered molasses does not seem to be a viable alternative to liquid molasses.

Table 3.2.9.11. Comparison of the efficacy of various treatments against FCM on navel orange trees at Bernal Farm (evaluated from 13 April – 10 May 2005).

Weeks after treatment Treatment	Infested fruit per tree per week					Mean infested fruit per tree per week*	Reduction in infestation relative to untreated control (%)
	3	4	5	6	7		
Untreated control	1.1	0.6	0.3	0.2	0.6	0.56a	-
Cryptogran	0.8	0.2	0.1	0.2	0.4	0.34ab	39.29
Cryptogran + 0.5% molasses	0.3	0.1	0	0.1	0.3	0.16b	71.43
Cryptogran + 0.25% molasses + Agral 90	0.2	0.1	0	0.1	0.5	0.18b	67.86
Cryptogran + Kalorie3000	0.5	0.2	0.2	0.0	0.4	0.26ab	53.57
Cryptogran + Kalorie3000 + Agral 90	0.5	0.2	0.2	0.1	0.3	0.26ab	53.57

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

Moore and Kirkman (2004) noted that the vast majority of FCM larvae infesting Navel oranges penetrate the fruit through the navel end. However, a few weeks before harvest, this behaviour changes and the majority of larvae eventually penetrate through the side of the fruit. This is exactly where the virus is highly exposed to UV irradiation from sunlight. It is speculated that this is the reason that control with Cryptogran applied shortly before harvest, does not persist for as long as it does earlier in the season.

Conclusion

A 25% reduction in water and agar in the diet used for dose-response bioassays with neonate FCM larvae, appeared to improve the survival of larvae. In such bioassays it was determined that after 1-2 h of exposure of Cryptogran to UV irradiation, detectable degradation of virus occurred. This was measured by a reduction in the mortality of neonate FCM larvae. In a field trial on Navel oranges, powdered molasses did not appear to be an adequately effective alternative for liquid molasses as an adjuvant for Cryptogran.

Future research

Research will be continued to examine whether the navel end of Navel oranges provides any protection of the virus against UV irradiation. Dose-response bioassays will be conducted with Cryptogran and various potential UV protectants. Field trials will also be conducted with some of these adjuvants. The effect of simulated rainfall on Cryptogran will be measured and quantified. Finally, shelf-life trials will be conducted with Cryptogran in an attempt to extend the registered shelf-life of the product.

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3.2.10 Entomopathogenic nematodes for control of FCM

Experiment 793 by Antoinette P. Malan (SU) and Sean D. Moore (CRI)

Opsomming

Grondmonsters uit sitrusboorde in die Oos- en Wes-Kaap is vir die voorkoms van entomopatogeniese nematodes (EPNs) ontleed. Inseknematodes is in die laboratorium uit die grondmonsters versamel deur grond in toe plastiekhouders te plaas en *Galleria mellonella* larwes (wasmotlarwes) by te voeg. Infektiewe nematodelarwes (IL) is uit dooie *Galleria*-larwes na 'n periode of 2-3 weke versamel. Hierdie IL is gehersirkuleer deur *Galleria*-larwes en die IL gedurende die eerste week van vorming te versamel. Die IL is geberg in 150 ml water in plat 500 ml plastiek kultuurflesse by 14°C. Die IL is elke twee maande deur wasmotlarwes gehersirkuleer om hulle lewend te hou vir verdere studie. Die genus van EPNs is bepaal deur *Galleria* te inkuleer met IL, af te was na twee dae, vir 'n verdere twee dae te laat ontwikkel en dan te dissekteer in Ringer's oplossing. 'n Totaal van 46 grondmonsters is ontleed, waarvan drie vanuit die Oos-Kaap en twee vanuit die Wes-Kaap met nematodes besmet was. In totaal is nematodes uit 10,9% van die monsters versamel. In al vyf monsters is die nematodes geïdentifiseer as *Heterorhabditis* op grond van die biologie en morfologie van die eerste generasie in *Galleria*-larwes. Geen *Steinernema* is opgespoor nie.

Biotoetse in die laboratorium is uitgevoer om die mortaliteit van VKM-larwes en -papias te bepaal, sowel as die hoeveelheid infektiewe ILs wat in staat was om die insek binne 'n periode van 48 uur te penetreer. Die gemiddelde mortaliteit vir VKM-larwes was 94% vir SF41 (*Heterorhabditis zealandica*) en 81% vir SF134 (*H. bacteriophora*). Mortaliteit van VKM-papias ($\leq 44\%$) was oor die algemeen laag in vergelyking met die larwes ($\leq 94\%$). Die hoogste penetrasie van nematodes in VKM-larwes is verkry met SF41 en SF134. Penetrasie van VKM-papias was oor die algemeen laer in vergelyking met larwes. In beide SF41 en SF134 het daar meer ILs in die VKM-larwes as in die *Galleria*-larwes geopenetreer. Die SF134-isolaat van *H. bacteriophora*, asook SF41 en SF134, het oor die algemeen die beste resultate gelewer, terwyl die SF1-isolaat van *H. bacteriophora* die swakste gevaar het.

In die toekomst sal verdere monsters van sitrusboorde en natuurlike bosveld in die omgewing geneem word. EPNs wat in die monsters gekry word, sal gevries word om kontaminasie en verlies in genetiese eienskappe te verhoed. Die gasheer vir hersirkulering van EPNs sal ook verander word van *Galleria* na VKM-larwes. Dit sal die ontwikkeling van patogenisiteit vir *Galleria* verhoed. Proewe met EPN-isolate teen VKM-larwes en -papias sal voortgesit word. Dit sluit glashuis- en boordproewe in.

Introduction

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are obligate parasites of soil inhabiting insects. Together with their associated bacteria, they are lethal parasites, killing their insect host within 24-48 hours. The infective juvenile is the only free living stage in the soil and has been commercially produced in many countries for the biological control of a wide range of soil inhabiting and above ground insect stages.

From South Africa the first record of an EPN was by Harington (1953). He found large numbers of *Steinernema* in maize beetle life stages from Grahamstown, Eastern Cape. In 1988 three *Steinernema* and one *Heterorhabditis* isolates were reported from Natal and were evaluated for the control of the African sugarcane stalk borer, *Eldana saccharina*, in laboratory and field tests (Spaull, 1988; 1990). A further seven *Heterorhabditis* and 15 *Steinernema* were isolated for use against *E. saccharina* (Spaull, 1991). The only species identifications recorded for South Africa were *Heterorhabditis bacteriophora*, which was identified in France by using satellite DNA as diagnostic probes (Grenier *et al.*, 1996) and a new nematode, *S. khoisanus* (Khuong *et al.*, 1996) by means of morphological and molecular techniques.

Although there are many commercially available formulations of EPNs, it is not possible to use them for evaluation against FCM in South Africa. Except for *H. bacteriophora*, all other species are regarded as

exotic organisms and cannot be imported without a full environmental impact study. *Steinernema carpocapsae* and *S. feltiae* are being used very effectively against codling moth (Lacey *et al.*, 2005), as well as a wide range of other Lepidoptera, but have not yet been reported from South Africa.

The aim of the survey was to obtain endemic EPNs, locally adapted to citrus orchards, for use as biological control agents against FCM larvae and pupae.

EPN species differ in many aspects, such as morphology and behaviour. Initial bioassays with FCM larvae and pupae are therefore of the utmost importance. This is in order to evaluate which nematodes have potential to be used as biological control agents in the field. Different bioassays have been used to test the virulence of EPNs against different insects (Navon & Ascher 2000), but no standard assay is currently available. In a previous study the penetration rate of four *Steinernema* and two *Heterorhabditis* isolates were compared and it was demonstrated that penetration rate allowed for the comparison among nematode strains invading a host as an indicator of infectivity (Caroli *et al.* 1996).

In this study, four isolates of EPNs, one *Steinernema* (Nguyen *et al.*, 2006) and three *Heterorhabditis* were tested by means of a laboratory bioassay with regard to mortality and penetration rate against FCM larvae and pupae.

Materials and methods

Survey for nematodes in citrus orchards

Collection of soil: Soil samples of approximately 1 kg were randomly collected to a depth of up to 20 cm from moist, shaded areas and transported to the laboratory in plastic bags.

Trapping of nematodes: The soil samples were thoroughly mixed and five *Galleria mellonella* larvae (the greater wax moth) were added to 250 ml soil in a plastic container closed with a lid, and incubated in the dark at 25°C (Bedding & Akhurst, 1975). After a period of 6-7 days, dead larvae were removed and placed on a modified White's trap.

Storage and recycling: The IJs obtained from trapping were recycled through *Galleria* larvae and IJs collected within the first week of emergence. The suspension of IJs was stored in flat 500 ml culture flasks at a temperature of 15°C and recycled through *Galleria* every two months to keep them alive for further studies.

Identification: Preliminary identifications were made by means of biological and morphological features. *Galleria* were inoculated with 200 IJs each and kept at 25°C for a period of 2 days. Dead *Galleria* larvae were shaken in 50 ml of water to remove all nematodes from the surface and incubated for a further 2 days for the nematodes to develop. Cadavers were dissected in Ringer's solution and the presence of hermaphrodites or males and females in the first generation were used to determine the genus of EPNs.

Laboratory evaluation of nematodes for mortality and penetration rate of FCM larvae and pupae

Nematode source: The EPNs used in the study were collected during a survey conducted from 1993-1995. The survey was conducted from disturbed and undisturbed soils, for endemic EPNs for use as biological control agents. The infective juveniles were stored in water in flat culture flasks and recycled periodically through *Galleria* to keep them alive for future use. The nematode species and isolates used are shown in Table 3.4.10.1. Identifications were made in collaboration with the University of Florida by using molecular techniques.

Table 3.2.10.1. Entomopathogenic nematodes used against FCM larvae and pupae

Species ID	Isolate	Nearest Town	GPS reading	Habitat
<i>Heterorhabditis bacteriophora</i>	SF1	Elgin	34°08'.10S/019°01'.17E	Apple
<i>H. zealandica</i>	SF41	Patensie	33°41'.28S/024°35'.23E	Wit stinkhout
<i>Steinernema khoisanae</i>	SF87	Villiersdorp	33.57.06S/019°24.02E	Apple
<i>H. bacteriophora</i>	SF 134	Piketberg	33°20'.28'S/018°89'.03E	Apple

Insects: *Galleria* larvae were cultured under laboratory conditions and FCM larvae and pupae were obtained from a rearing facility in Port Elizabeth. Sentinel FCM larvae and pupae were used in this study.

Bioassay procedure: Twenty four well plates padded with filter paper were used for testing. To prevent escape of the larvae, a piece of glass was put in the lid and closed with a rubber band. The closed wells were put into a plastic container, lined with wet tissue paper, and closed with a lid to prevent drying for the

duration of the experiment. The plastic containers with the wells, were kept in a growth chamber at 25°C for the duration of the experiment.

Inoculum: The IJs used were collected within one week of emergence from *Galleria* larvae and used as inoculum within one month of storage at 14°C. The nematodes were kept at room temperature (18-22°C) for 24 hours before use. Each well was inoculated with 200 IJs in 50 µl of water. After inoculation with nematodes, FCM larvae or pupae were added to each of 12 wells and *Galleria* larvae were used as controls.

Mortality: After incubation of 48 hours the mortality of the larvae and pupae was determined. Pupae were touched and if no movement occurred, they were regarded as dead.

Penetration rate: After 48 hours, all the insects were washed and placed in 15 mm plastic petri dishes and kept in a closed plastic container, lined with wet tissue paper, for another 48 hours at 25°C. All dead insects were either dissected in Ringer's solution (sometimes after first being stored at -20°C) and the number of nematodes inside the insects counted.

Data analysis: Each experiment consisted of 12 replicates and was repeated three times (n=36). Different batches of nematodes and insects were used for each replication. Data were pooled from the three separate bioassays for each nematode and insect. The penetration rate was calculated by dividing the number of nematodes counted inside a cadaver by the initial inoculum. A one-way ANOVA was used to test for the main effect of the nematode isolates. If a significant F-value (P<0.05) was found, means were separated by Student t-range test.

Results and discussion

Survey for nematodes in citrus orchards

During the period 1 April - 31 December 2005 a total of 45 soil samples were analysed by means of laboratory trapping with *Galleria* for the presence of EPNs. A total of 34 soil samples from the Eastern Cape and 12 samples from the Western Cape were analysed. EPNs were recovered from three Eastern Cape and two Western Cape samples (Table 3.2.10.2). In total, EPNs were recovered from 10.9% of the samples. From the dissected cadavers, only hermaphrodites were recovered, indicating that all positive samples were infested with *Heterorhabditis* spp. These nematodes were successfully recycled every two months through *Galleria* and stored for future use.

Table 3.2.10.2. Entomopathogenic nematodes recovered from citrus soil samples.

Province	Area	Number of samples	Positive for EPNs	Genus identified
Eastern Cape	Addo	12	1	<i>Heterorhabditis</i> sp.
Eastern Cape	Ford Beaufort	2	0	-
Eastern Cape	Kirkwood	4	1	<i>Heterorhabditis</i> sp.
Eastern Cape	Summerville	3	0	-
Eastern Cape	Sundays River Valley	13	1	<i>Heterorhabditis</i> sp.
Western Cape	Citrusdal	4	1	<i>Heterorhabditis</i> sp.
Western Cape	Clanwilliam	1	0	-
Western Cape	Stellenbosch	2	0	-
Western Cape	Robertson	4	0	-
Western Cape	Wellington	1	1	<i>Heterorhabditis</i> sp.
Total number of soil samples analysed:		46	5 (10.9%)	

Laboratory evaluation of nematodes for mortality and penetration rate of FCM larvae and pupae

Effect on the mortality of hosts: Average mortality for *Galleria* larvae for three isolates was 100%, except for *S. khoisanae* which was 94%. The highest average mortality for FCM pupae was caused by *H. zealandica* (SF41) at 94%, followed by *H. bacteriophora* (SF81) at 81%, *S. khoisanae* (SF87) at 67% and *H. bacteriophora* (SF134) at 56%. With regard to mortality of FCM, SF 41 (*H. zealandica*) and the SF134 isolates of *H. bacteriophora* showed the most promising results (Fig. 3.2.10.1). Mortality of pupae was generally low, with the highest mortality for *H. zealandica* at 44%.

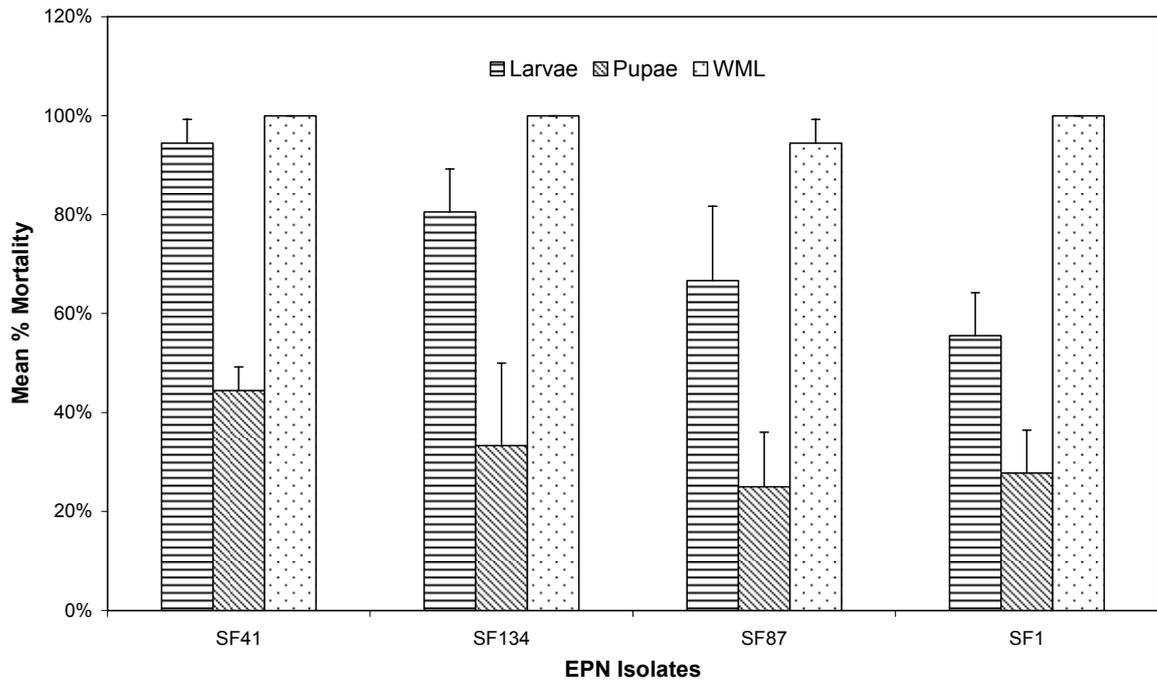


Fig. 3.2.10.1. Mortality of FCM larvae, pupae and *Galleria* (WML) for four isolates of EPNs

Penetration rate: With regard to invasion efficiency, the highest average penetration rate for FCM larvae and pupae was for SF41 (*H. zealandica*) and the SF134 isolate of *H. bacteriophora* (Fig. 3.2.10.2). These results coincide with those for mortality. In both *H. zealandica* and *H. bacteriophora* the penetration rate for FCM larvae was higher than that for *Galleria* larvae. The invasion efficiency for pupae was also the highest for *H. zealandica* and *H. bacteriophora* (SF134), but in general much lower.

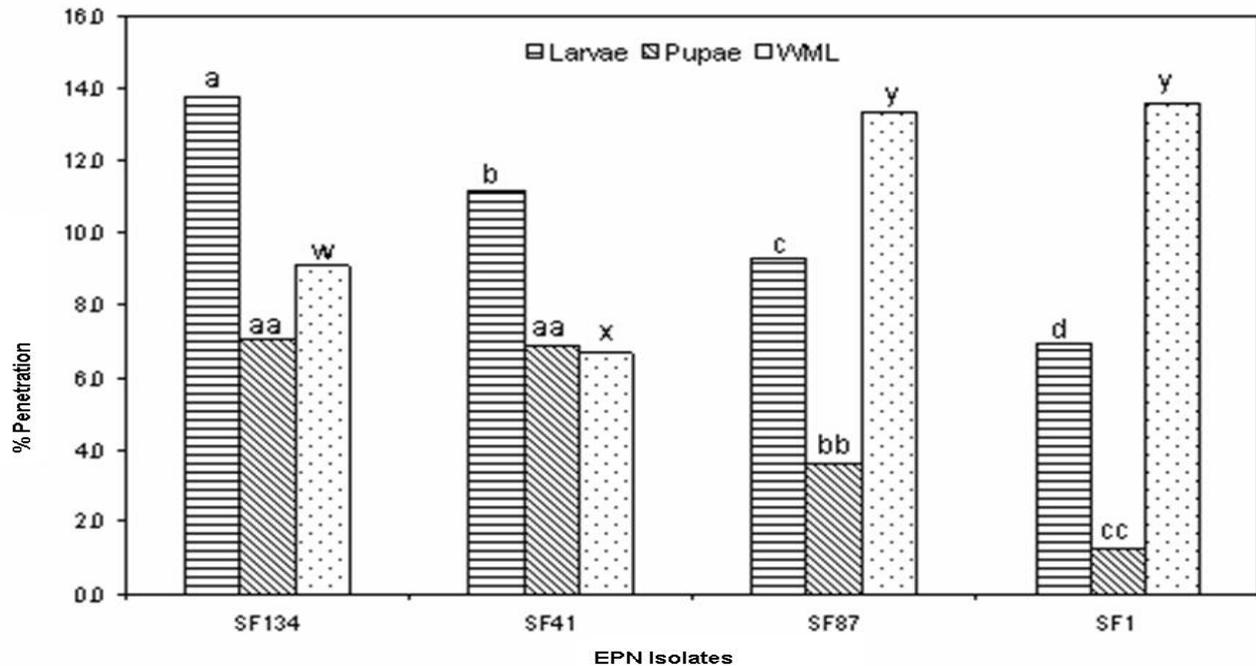


Fig. 3.2.10.2. Invasion efficiency of different isolates of EPNs for FCM larvae, pupae and *Galleria* (WML)

Conclusion

During the survey of citrus orchards EPNs were recovered in 10.9% of the samples analysed. All nematodes found in the soil samples were identified as *Heterorhabditis* spp. No *Steinernema* spp., which are generally more suitable for the biological control of Lepidoptera, were found.

From these preliminary results it can be concluded that *S. khoisanae* does not perform as well as expected, in comparison with the *Heterorhabditis* isolates, with regard to mortality and penetration rate of FCM larvae and pupae. To determine the penetration rate is very time consuming and there is a very high standard deviation between the replicates. Since the results of the invasion efficiency coincide with those of mortality, only mortality will be used in future as a first screening of isolates of EPNs for their efficiency to kill FCM larvae and pupae. EPN isolates which cause a high rate of mortality will be submitted to further laboratory bioassays.

Future research

To obtain EPNs, proven in other countries suitable against Lepidoptera, further samples from citrus orchards and surrounding natural vegetation should be taken. These nematodes, mostly from the genus *Steinernema*, such as *S. carpocapsae* and *S. feltiae*, have not yet been reported from South Africa. Species identification has to be done by means of molecular techniques. Isolates of EPNs found in the samples should be cryopreserved to prevent loss of genetic traits, accidental loss and contamination. The host for recycling should also be changed from *Galleria* to FCM larvae to prevent selection of pathogenicity for *Galleria*.

The screening of EPN isolates, in the US collection (SF1-35) and isolates collected during the citrus orchard survey, for pathogenicity to FCM larvae and pupae will be continued. Further laboratory bioassays will be conducted with EPN isolates against FCM larvae and pupae. Further to this, glasshouse and field trials will be conducted.

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3.2.11 The host status of lemons for FCM

Experiment 828 by Sean D. Moore, Bruce Tate & Wayne Kirkman (CRI)

Opsomming

Die Chinese mark is onlangs vir uitvoere van suider Afrikaanse sitrusvrugte oopgestel. Net vrugte van VKM-vrye boorde of vrugte wat op pad kouedisinfestatie ondergaan het, sal aanvaar word. Kouedisinfestatie van suurlemoene is nie moontlik nie. Die doel van hierdie eksperiment was om die teenwoordigheid van motte in suurlemoenboorde te meet en om die geskiktheid van suurlemoene op verskeie stadiums van ontwikkeling as gashere vir VKM vas te stel. Motte is gereed in feromoonlokvalle oor twee seisoene in suurlemoenboorde in Sondagsrivervallei gevang. Soortgelyke vangste is in suurlemoenboorde in Mpumalanga opgemerk. In laboratoriumsproewe is opsigtelike vlakke van besmetting van suurlemoene in alle kleurgrade gekry. Hierdie besmetting is geassosieer met 'n hoë vlak van vrugverrotting. Die hoogste besmettingsvlakke is in geel suurlemoene (T1) en in lemoene gekry. Lemoene is as 'n kontrole gebruik. Boordproewe wat uitgevoer is om hoë vlakke van vrugverrotting te voorkom, het net een besmette vrug opgelewer. Dit was 'n vierde instar larwe in 'n T4 (groen) vrug. Dit is moontlik, alhoewel onwaarskynlik, dat hierdie vrug in die laboratorium besmet geraak het gedurende die 11 dae ná boordversameling en vóór inspeksie. Verdere proewe om die plaagstatus van VKM op suurlemoene vas te stel, word benodig. Probleme wat in proewe ondervind is, soos verrotting en vrugval, moet eers opgelos word. Dit is dus nodig om 'n geskikter proefprotokol vas te stel.

Introduction

The Chinese market has recently opened for exports of South African citrus fruits. Originally, the relevant protocol stated that only fruit from FCM-free orchards would be admissible. The Chinese market has now accepted an alternative protocol of cold disinfestation of fruit in transit. The first protocol, in effect means that if any FCM adults are caught in pheromone traps in an orchard, even if the fruit is not attacked, then the fruit from that orchard cannot be packed for China. It is known that lemons are an unsuitable host for FCM (Newton, 1998), and reports of FCM infestation of lemons are few, are usually anecdotal and pertain mainly to over-ripe fruit. It is possible that pheromone traps could catch moths in lemon orchards even if the fruit is not being attacked. In light of this, the host status of lemons for FCM must be determined. It is possible that even if fruit are attacked, development to adulthood will not take place (except possibly in over-ripe fruit). This experiment proposes to determine the suitability of lemons, at various stages of development, as hosts for FCM. The cold disinfestation treatment for lemons is not a feasible option as lemons damage easily at such low temperatures.

Materials and methods

Trapping

Lorelei FCM pheromone traps were hung, according to registered recommendations (Hofmeyr, 2003), in lemon orchards in the Sundays River Valley. Sundays River Citrus Company (SRCC) Technical staff hung traps in 10 lemon orchards during the 2004/05 citrus season and again in nine of these 10 orchards during the ensuing season. FCM adults (males) caught in traps were monitored each week on the same day. Moths were removed from traps and recorded.

Detached fruit trials

In the first trial, lemon fruit were picked according to the Outspan colour plate standards for export fruit (Anonymous, 1995): 50 fruit each were selected for each colour standard from T1 (yellow ripe) to T6 (green). This represented stages of ripeness from the earliest stage at which lemons can be harvested for export, to being overripe. Fifty ripe oranges, being a highly suitable host for FCM, were used as a positive control. Fruit were placed in export grapefruit cartons (50 per carton) on a bed of washed filter sand as a pupation medium.

FCM eggs were obtained on sheets of wax paper, from the CRI-PE FCM culture (rectangular sheets) and from Ceder Biocontrol (round sheets). These eggs were divided equally among the fruits by cutting the rectangular wax sheets into longitudinal strips and the circular sheets into equally sized triangles. Approximately 240 eggs were placed onto each fruit. Fruit were placed in a temperature-controlled room at 23-25°C. Twenty-five fruit from each treatment were removed at 19-24 days and dissected to determine the larval stage achieved for each treatment. The remaining fruit were kept until pupation was completed, 31-34 days later, at which stage the sand was sieved to remove pupae for counting.

The experiment was repeated, this time using an estimated average of only 10 eggs per fruit.

The trial was conducted a third time, according to a similar protocol to that described for the first two replicates. However, in order to try and reduce the decay of fruit, the fruit were dipped in a Guazatine/Sporekill fungicide combination. This consisted of 4.8 ml Guazatine and 1 ml Sporekill (active ingredient: poly dimethyl ammonium chloride 120 g/l; Hygrotech International, South Africa) in a litre of water, dipped for 3 minutes. FCM egg sheets were also submerged in a Sporekill solution to surface-sterilize them. Eggs were divided equally among the fruit with approximately 19 eggs per fruit. Fruit were stored in a temperature controlled room at 25°C for 27 days, after which the sand was sieved to remove pupae.

Orchard trial

An orchard of Eureka lemons at various stages of development from T1 to T6 was used. Wax paper with FCM eggs on it, was cut into squares with an average of 64.3 eggs per square. One square was glued onto each of 40 lemons for each colour standard. Fruit were tagged. This was done on 10 November. Simultaneously, 40 Valencia orange fruit in an orchard at the Citrus Foundation Block (Uitenhage) were subjected to the same protocol. These served as a positive control. On 28 November, fruit were harvested and taken to the laboratory. Unfortunately, by this time many of the fruit (particularly lemons) had abscised and could not be recovered. As five of the Valencia orange fruit were already showing signs of decay, they were immediately inspected for signs of FCM penetration and dissected to look for infestation. Lemon fruit were retained in the laboratory (at approximately 27°C) for a further 11 days after collection (until 9 December) and then inspected for signs of penetration and dissected to check for infestation.

Results and discussion

Trapping

Moths were caught in all lemon orchards monitored during both the 2004/05 and 2005/06 season (Tables 3.2.11.1 and 3.2.11.2). On a number of occasions, trap catches even exceeded 10 moths per week, the registered threshold for action against the pest. Although no FCM damage or infestation was reported from any of these orchards and in all likelihood there was none, these orchards could not be declared as FCM-free. Fruit from these orchards would therefore be unacceptable for the Chinese market. All of these lemon orchards can be considered as being relatively small and were on farms which also had a number of Navel orange orchards, which are highly susceptible to FCM infestation.

It might be speculated that moths would less likely be caught in large homogenous lemon plantings. However, traps which were hung on The Outback Farm near Barkley Bridge in Sundays River Valley, which has a large homogenous area of lemon trees of approximately 80 ha, also caught moths, albeit in low numbers.

A similar report was received from Moosrivier, Schoeman Landgoed, in Mpumalanga (Kevin Language, personal communication). Lemon orchards on this farm, in which FCM were caught in pheromone traps, were also very large in size.

Table 3.2.11.1. FCM caught per trap per week in lemon orchards on 10 farms in the Sundays River Valley (Kirkwood and Addo areas) during the 2004/05 season.

FARM	Moths per trap per week																																			
	Nov 04				Dec 04			Jan 05				Feb 05				Mar 05			Apr 05				May 05				Jun 05		Jul 05					Aug 05		
	04	11	18	25	02	09	16	07*	14	20	27	03	10	17	24	03	10	17	07*	15	21	28	03	12	19	26	02	09	01*	07	14	21	28	04	11	
Kirkwood																																				
Waverley	0	2	2	5	2	5	4	6	3	3	8	3	10	9	25	8	12	16	19	2	6	4	2	-	3	-	-	-	-	-	-	-	-	-	-	-
Luthando	0	0	1	17	4	5	8	8	0	6	2	5	18	4	5	2	0	3	11	2	4	4	0	-	3	-	-	-	-	-	-	-	-	-	-	-
J Venter	0	0	2	29	8	23	0	23	0	5	8	1	5	-	5	15	4	11	3	-	3	3	0	-	1	-	-	-	-	-	-	-	-	-	-	-
L Muller												5	8	-	3	9	24	13	5	-	2	0	1	-	3	-	-	-	-	-	-	-	-	-	-	-
K Swart	0	0	1	0	8	9	0	3	1	4	3	2	0	-	17	11	9	14	5	8	3	4	4	-	7	-	-	-	-	-	-	-	-	-	-	-
Addo																																				
Penhill	0	2	1	2	3	3	3	5	3	6	4	7	5	7	10	0	21	9	1	5	6	3	3	1	5	3	5	2	5	4	1	-	2	1	5	
Halaron	0	0	0	7	1	2	1	13	7	4	5	3	3	2	3	13	0	3	12	9	5	4	5	2	0	3	4	0	0	0	3	4	0	2	2	
Rhodene	0	0	-	2	0	0	1	0	0	4	0	1	0	0	0	1	1	6	2	0	0	1	1	0	0	0	0	0	-	1	0	0	1	1	0	
Allnutt	0	0	0	0	2	1	2	3	0	3	3	1	9	4	2	5	13	3	11	4	4	0	0	3	2	2	2	1	1	2	0	4	2	0	2	
Walton	0	0	2	3	1	5	3	4	2	12	3	4	2	2	4	4	0	4	15	5	3	1	3	2	4	1	2	3	1	3	1	1	2	3	1	

* = trap reading was for more than one week; - = no reading was taken.

Table 3.2.11.2. FCM caught per trap per week in lemon orchards on 9 farms in the Sundays River Valley during early summer of the 2005/06 season.

FARM	Moths per trap per week					
	Nov 04			Dec 04		
	10	17	24	1	9	15
Kirkwood						
Waverley	-	0	1	1	3	3
Luthando	-	0	2	4	5	0
L Muller	-	0	1	6	3	1
K Swart	-	0	4	9	2	6
Addo						
Penhill	0	0	0	1	0	0
Halaron	0	0	0	5	11	6
Rhodene	0	1	0	2	2	4
Allnutt	0	0	0	0	2	0
Walton	0	0	1	11	5	1

- = no reading was taken.

Detached fruit trials

Larval infestation and numbers of pupae developing were higher for oranges and yellow ripe lemons (T1) for the first two experiments (Table 3.2.11.3 & 3.2.11.4), but results were highly variable with survival through to pupation across the colour range for lemons. This could be attributed to the high levels of decay of all fruit in these laboratory-based experiments, which favour the development of FCM larvae regardless of stage of fruit maturity. It is doubtful that similar conditions would apply under orchard conditions.

Table 3.2.11.3. FCM larvae recovered from lemon fruit of different colour standards at 19-24 days after placing eggs onto fruit (two replicates).

Treatment	No. fruit	Total larvae		Larval instars							
				2		3		4		5	
		Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Control oranges	25	116	125	5	14	27	49	56	51	28	11
T1 Yellow	25	47	27	-	4	14	12	21	8	12	3
T2	25	9	20	1	-	6	3	2	9	-	8
T3	25	2	14	-	2	-	4	1	6	-	2
T4	25	9	16	-	5	2	6	6	3	1	2
T5	25	7	17	-	1	5	6	2	1	-	9
T6 Green	25	2	8	-	1	2	2	-	4	-	1

Table 3.2.11.4. FCM pupae recovered from sand, emerging from lemon fruit of different colour standards (two replicates).

Treatment	No. fruit	Total pupae	
		Exp 1	Exp 2
Control oranges	25	310	26
T1 Yellow	25	36	9
T2	25	10	4
T3	25	6	0
T4	25	29	3
T5	25	4	3
T6 Green	25	16	4

Despite lower levels of decay during the early stages of the third laboratory trial, most fruit were severely affected by the end of the experiment. The few fruit that were still sound by the end of the experiment had not been penetrated by FCM larvae. Pupal numbers were highest for oranges and yellow ripe lemons (T1), a similar result to the previous two trials (Table 3.2.11.5). Despite the high levels of fruit decay, no larval infestation was found in T4 fruit.

Table 3.2.11.5. Total pupae recovered from each treatment (50 fruit per treatment).

Treatment	No. fruit	Total pupae
Control oranges	50	328
T1 – Yellow	50	62
T2	50	6
T3	50	3
T4	50	0
T5	50	4
T6 - Green	50	2

Orchard trial

A high percentage of the oranges were infested with FCM, confirming the usefulness of the protocol (Table 3.2.11.6). Only one lemon fruit was found with a live FCM larva inside. This was a fruit which was of T4 colour when the trial was initiated (Table 3.2.11.6). As the larva was already at the fourth instar stage and fruit were only kept in the laboratory for 11 days before inspection, it is unlikely that fruit would have been infested after collection i.e., in the laboratory. However, this possibility cannot be eliminated. Nevertheless, unlike in the laboratory trials conducted previously, the infested fruit was healthy.

Table 3.2.11.6. FCM infestation of lemon and Valencia fruit

Cultivar	Colour plate	Fruit recovered	% Fruit with penetration marks	Mean number of marks per fruit	Fruit infested	% infested	Comments
Valencia orange	T7	25	100	4.64	22	88.0	Average of 2.28 larvae per fruit
Eureka Lemon	T1	7	14.3	0.14	0	0	
	T2	5	0	0	0	0	
	T3	14	14.3	0.14	0	0	1 dead 1st instar larva found
	T4	15	13.3	0.13	1	6.67	4 th instar larva; entry under calyx
	T5	19	21.1	0.21	0	0	
	T6	21	14.3	0.14	0	0	

Conclusion

Entry of lemons into the Chinese market cannot easily be achieved through current protocols. Lemons cannot be cold disinfested in transit and due to consistent and sometimes high catches of moths in lemon orchards, it is unlikely that many lemon orchards could be declared FCM free. Laboratory trials revealed notable levels of infestation of lemon fruits of all degrees of colour. However, this was associated with a high level of decay of fruit. Highest levels of infestation were recorded for oranges, used as a positive control, and for yellow lemons (T1). Field trials, conducted in order to avoid high levels of fruit decay, revealed only one infested fruit. This was a fourth instar larva in a T4 (greenish) fruit. It is possible, although unlikely, that the fruit could have been infested in the laboratory during the 11 days after field collection and before inspection. Further trials are required to determine whether lemons can reasonably be declared non-hosts for FCM.

Future research

Future trials will be conducted to determine the host status of lemons for FCM. Problems such as fruit decay and abscission were experienced with the trial protocols employed thus far. Therefore, it will first be necessary to establish a more appropriate trial protocol.

Acknowledgements

Sundays River Citrus Company Technical personnel are thanked for trapping data.

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3.2.12 Evaluation of a cold treatment for the disinfestation of export citrus from false codling moth, *Cryptophlebia leucotreta* Experiment 835 by Hendrik and Marsheille Hofmeyr (CRI)

Opsomming

'n Kouedisinfestasiëproef is op versoek van die Republiek van China uitgevoer om te bewys dat valskodlingmotlarwes in lemoene wat vir 22 dae by $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ gehou word, almal deur die kouebehandeling gedood sal word. Dié navorsing is 'n herhaling van vorige eksperimente wat deurgaans bewys het dat geen larwes so 'n behandeling kan oorlewe nie. Twee herhalings van natuurlik-besmette nawelvrugte met onderskeidelik 1027 en 1006 larwes is gebruik. 'n Verdere behandeling wat uit 32 946

vierde en vyfde instar larwes in teelflesse bestaan het, asook 35 000 eiers op waspapier, is bygevoeg. Alle behandelde vrugte en teelflesse is na die kouebehandeling vir tot 26 dae by 26°C gehou sodat enige oorlewende larwes opgespoor kon word. Geen lewendige larwes of eiers is na die kouebehandeling gevind nie en daar kan derhalwe met sekerheid aanvaar word dat die behandeling doeltreffend is.

Introduction

This experiment was conducted at the request of the People's Republic of China (AQSIQ), as part of a South African initiative to facilitate the establishment of a FCM disinfestation protocol for the export of citrus from the RSA to China. It was the purpose of the investigation to demonstrate that a cold disinfestation treatment, consisting of treating citrus fruit for 22 days at $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$, would kill all FCM larvae infesting the fruit. Similar research had been conducted in the past (Myburgh 1963, Hofmeyr & Hofmeyr 1998) and the results from this research have already been implemented for many years – particularly to disinfest citrus fruit exported to the USA and Korea. However, the Chinese authorities requested that the efficacy be demonstrated in the presence of their own appointed officials.

Materials and methods

It is very seldom possible to collect large numbers of citrus fruit infested with FCM larvae within a day. This is because the crop damage caused by FCM usually occurs over a long time. Fruit infestation does not peak all at once, which would have made it possible to collect enough fruit to permit an experiment at probit-9 level. It was therefore proposed that the main experiment should consist of treating larvae in artificial diet. A secondary experiment, consisting of oranges that were naturally infested with larvae in the orchard, would be conducted on a smaller scale, to confirm the results.

1. REPLICATE 1

The main investigation was conducted on 10 000 larvae, reared in an insectary on artificial diet in rearing jars. For comparative purposes, 1000 navel orange fruit, naturally infested with FCM larvae, were collected from an infested orchard and included in the experiment.

1.1 Test insects

1.1.1 Larvae in rearing jars: One hundred and twenty-five rearing jars were prepared with a diet consisting mainly of maize meal in Ceder Biocontrol Insectary in Citrusdal. Each jar was inoculated with 400-500 FCM eggs on 31 May 2005 and closed with a cotton wool stopper. The jars were incubated at 26°C for larval development. They were removed 15 days later on 15 June when many larvae had developed to the final (fifth) instar.

- **Control 1:** One rearing jar was collected at random from the batch of 125 jars, the diet removed and a number of larvae collected at random. They were heat-treated in boiling water and placed into 70% ethanol for preservation. The larvae were subsequently sent to CRI in Nelspruit for head capsule measurements to determine the age distribution of the larvae.
- **Control 2:** Ten rearing jars were collected at random from the batch of 125 jars on 15 June and incubated at 26°C. The cotton wool stoppers were removed the same day and replaced with single-face cardboard ("SFK") stoppers. The SFK stoppers facilitated removal of the pupae for counting purposes and were replaced on five dates, i.e., 17 June, 20 June, 24 June, 28 June and 1 July. All pupae collected from the SFK tops were counted. Some larvae had pupated on the surface of the diet in the rearing jars. The moths developing from these larvae were counted upon eclosion. The total number of pupae and moths were counted (i) to determine the mean larval production per jar to enable calculation of the relative number of larvae used for the cold treatment and (ii) to establish the time to be allowed for any surviving cold treated larvae in the rearing jars to also pupate.
- **Cold treatment:** One hundred and fourteen rearing jars were packed into three standard 15 kg citrus cartons, each containing 38 jars (19 jars in each of two layers).

1.1.2 Larvae in naturally infested fruit

One thousand three hundred and sixty-six navel orange fruit with symptoms of FCM infestation were picked from trees in an orchard on the farm Kweekkraal in the Citrusdal district on 15 June 2005.

- **Control 1:** One hundred fruit showing symptoms of FCM infestation were cut open on 15 June and the larvae removed to determine (i) natural mortality and (ii) age distribution. The larvae were heat treated in boiling water and placed into 70% ethanol for preservation. As above, the larvae were sent to Nelspruit for head capsule measurements to determine the age distribution of the larvae.
- **Control 2:** On 15 June 100 fruit with symptoms of FCM infestation were incubated at 26°C in the laboratory to determine natural mortality. The fruit were individually placed onto vermiculite in 500 ml plastic containers to allow pupation of larvae escaping the fruit. To confine the larvae, each container was closed with a mesh lid. The containers were inspected every third day for cocoons and to remove decayed fruit. When a cocoon was found in a container, the fruit and vermiculite were removed and discarded and the cocoon placed back into the container for pupal eclosion.
- **Cold treatment:** One thousand one hundred and sixty-six navel orange fruit were packed into 22 standard 15 kg citrus cartons.

1.2 Coldroom

The three cartons with rearing jars and 22 cartons with naturally infested oranges were transported to the coldroom complex at the Post-harvest Research Department of the Agricultural Research Council (ARC) Research Institute at Infruitec in Stellenbosch on 15 June. The cartons were placed into coldroom No. 10 at -0.6°C for a pre-cooling period of 72 hours. The cartons with rearing jars were stacked pyramidwise (two at the bottom and one carton at the top). The cartons with oranges were loosely stacked in three rows of 6-7 cartons each.

1.3 Temperature management and data recording

Temperatures for Replicate 1 were monitored in three different ways:

- **Ambient temperature:** The air temperature in the coldroom was controlled and monitored from a main computer in a centrally located control room in the coldroom complex. Control instruments in the coldroom were calibrated on 9 December 2004. Temperature data were recorded every 30 minutes and the data were saved to a file automatically every 24 hours.
- **Citrus carton temperature:** Two Hobo dataloggers were used to monitor the air temperature inside each of two cartons containing rearing jars and fruit. Each Hobo was placed next to the jar or fruit containing the second (middle) Squirrel thermocouple in the middle of the particular citrus carton. They were removed from the cartons when Replicate 1 was concluded on 10 July and the temperature data were down-loaded at the CRI laboratory in Citrusdal.
- **Internal rearing jar and fruit temperature:** Three thermocouples from a Grant Squirrel SQ800 datalogger (A) were inserted into the diet of a jar in the centre of each carton. Similarly, three thermocouples from a second Grant Squirrel SQ800 datalogger (B) were inserted into a fruit in the middle of a carton at the top, middle and bottom of each row of cartons respectively.

The thermocouples of Squirrel A were removed from the rearing jars on 9 July (24 hours before termination of the Replicate 1 cold treatment) and transferred into the fruit from Replicate 2, which were placed into the coldroom on that day for the start of the 72-hour pre-cooling period.

Squirrel B was left *in situ* until the treatment of Replicate 1 was concluded 24 hours later on 10 July, and was then removed.

Squirrel datalogger A was removed at the end of the pre-cooling period of Replicate 2. Because of problems with the calibration of these instruments, they were returned to the Grant agent/supplier for down-loading of the data, recalibration and certification.

The coldroom was momentarily opened on 18 June at the end of the pre-cooling period of Replicate 1, to confirm the temperature in the rearing jars and fruit with a glass, pencil-type thermometer. The coldroom was then locked and remained so for 21 days until 9 July, when the coldroom was opened for a few minutes to introduce the fruit from Replicate 2. The 22-day cold treatment was concluded the next day on 10 June.

1.4 Incubation of larvae in rearing jars and fruit

The rearing jars and fruit were removed from the coldroom on 10 July and placed into a temperature controlled (TC) room at the ARC Infruitec Entomology Department for incubation at 26°C to allow any surviving larvae to revive and pupate. The cotton wool stoppers were replaced with SFK cardboard stoppers on the same day to simplify the search for pupae from any surviving larvae. The fruit was incubated for two days and the rearing jars for 26 days, respectively, before evaluation.

1.5 Evaluation of treatment efficacy

- **Larvae in rearing jars:** It was impractical to search for surviving larvae in the rearing jars, as the diet could not be removed from the jars without damaging any surviving larvae. Treatment failure was therefore considered to be the ability of a surviving larva to pupate.

The rearing jars were incubated until 25 July. The SFK cardboard stoppers were then removed, and closely inspected for cocoons. The stoppers were replaced and incubated until 5 August. The stoppers were then finally removed and the two cardboard layers (the one smooth, the other corrugated) were pulled apart and again inspected for pupae. The rearing jars were also inspected for any larvae that may have managed to survive the cold treatment and were able to pupate.

- **Larvae in naturally infested fruit:** The fruit were incubated until 12 July and were then individually and carefully cut open and inspected for larvae. All larvae were removed with a forceps, and placed into Petri dishes. These larvae were inspected for any sign of movement. Any larvae that could not immediately be confirmed as obviously dead, were placed into a rearing jar with fresh diet, and incubated at 26°C. The remaining larvae were discarded.

2. REPLICATE 2

This experiment was conducted in naturally infested fruit only, involving 1000 test insects.

2.1 Test insects

The effect of cold treatment on FCM larvae and eggs was investigated in Replicate 2.

2.1.1 **Larvae in naturally infested fruit**

A total of 1 298 oranges with symptoms of FCM infestation were picked from an orchard on the same farm as before (see Replicate 1) on 8 July.

- **Control 1:** Fifty-six fruit showing symptoms of FCM infestation were cut open and the larvae removed to determine (i) natural mortality and (ii) age distribution. The larvae were heat-treated in boiling water and placed into 70% ethanol for preservation. As before, the larvae were sent to Nelspruit for head capsule measurements.
- **Control 2:** Fifty fruit with symptoms of FCM infestation were incubated at 26°C in the TC room at ARC Infruitec to determine natural mortality. The fruit were again individually placed in plastic containers as before and incubated. The containers were inspected on several occasions to remove decayed fruit.
- **Cold treatment:** One thousand one hundred and ninety-two fruit were packed into 28 standard 15 kg citrus cartons.

2.1.2 **FCM eggs**

Two sheets with 24-hour old FCM eggs ("white" stage = undeveloped), and two sheets with 48-hour old eggs ("red" stage = partially developed) were obtained from Ceder Biocontrol Insectary. Each sheet, 200 mm in diameter, contained approximately 10 000 eggs. A quarter sheet with eggs was cut from a sheet of each egg age for control purposes. The eggs sheets were placed into a plastic container 220 mm x 220 mm x 50 mm and placed on a carton of fruit in the coldroom. The control sheets were placed into Petri dishes and incubated at 26°C for development in the CRI laboratory at Citrusdal.

2.2 Coldroom

The infested oranges were transported to the same coldroom (No. 10) used for Replicate 1 at ARC Infruitec, on 9 July. As mentioned above, treatment of the two replicates overlapped and the coldroom, still at -0.6°C , was opened for a few minutes to introduce the fruit from Replicate 2. Twenty-eight cartons were placed into the coldroom for the pre-cooling period of 72 hours and were stacked pyramidwise one carton deep.

2.3 Temperature management and data recording

- **Ambient temperature 1:** The same integrated temperature control equipment from ARC Infruitec was used as for Replicate 1. Data recording was also identical.
- **Ambient temperature 2:** The two Hobo dataloggers from Replicate 1 were introduced into the coldroom at the beginning of the cold disinfestation period of Replicate 2 on 12 July and placed on top of two cartons.
- **Internal fruit temperature:** The accuracy of the two Squirrel dataloggers used for Replicate 1 was questionable. The one remaining Squirrel datalogger (A) was therefore removed from the coldroom on 12 July (the start of the cold disinfestation period of 22 days). It was replaced with a Honeywell Q11 datalogger, supplied by Perishable Products Export Control Board, Cape Town. This instrument was calibrated twice and then installed in fruit in the coldroom. Six thermocouples were distributed in the stack of 28 cartons. Both Squirrel dataloggers were returned to the Grant agents, Monitoring and Control Laboratories (Pty) Ltd (MCL) in Johannesburg on 18 July to examine the accuracy of the instruments.

2.4 Incubation of larvae in fruit

The fruit was removed from the coldroom on 3 August and placed into the TC room at ARC Infruitec for incubation at 26°C for 48 hours to allow any surviving larvae to revive.

2.5 Evaluation of treatment efficacy

The fruit and eggs were removed from the TC room on 5 August. The fruit were individually cut open and inspected the same day. The exact cutting procedure as in Replicate 1 was conducted.

The treated and control eggs were also removed on the same day and examined for hatching under a stereomicroscope.

Results

1. REPLICATE 1

1.1 Temperature management and data recording

Data recorded by ARC Infruitec in coldroom no. 10 showed that the ambient air temperatures in the coldroom during treatment of Replicate 1 were, with one exception, well within the required range of $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$. On 24 June the main cooling compressor at the coldroom complex broke down and all operations were shut down. The compressor was fixed within the next 13 hours, but during that time the coldroom and internal fruit temperatures increased to more than 10°C . However, after cooling resumed, the internal fruit temperatures were reduced to below 0°C within the next approximately 12 hours.

An internal fruit temperature of -0.3°C was recorded with the pencil-type thermometer at the end of the pre-cooling period on 18 June. This confirms in general the readings recorded by the Squirrel dataloggers at that time.

Calibration of the Squirrel dataloggers by MCL showed that both Squirrel dataloggers were very accurate, with a maximum deviation (applicable to two from six thermocouples) of 0.2°C from 0°C . The recorded temperature data (fruit pulp and rearing medium) is therefore acceptable.

It was not possible to calibrate the Hobo dataloggers, as they do not possess external thermocouples. However, their readings corresponded closely with the reading of ambient temperature by ARC Infruitec, as well as the Squirrel dataloggers, and their readings should therefore be acceptable for both replicates.

All temperatures recorded from the rearing jars and oranges during treatment of Replicate 1 with the integrated ARC equipment, the Squirrel dataloggers and the Hobo dataloggers, were well within the range of $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$.

1.2 Larvae in rearing jars

- **Control 1:** Measurements of the head capsules from 70 larvae [see Section 1.1.1, Control 1] conducted in Nelspruit, showed that 98.6% of the larvae from the randomly selected control rearing jar had already developed to the fourth and fifth instars, when the 112 rearing jars were introduced into the coldroom (see Addendum 8). An appreciable number of the treated insects therefore consisted of instars more resistant to cold relative to the younger larvae (Myburgh, 1963).
- **Control 2:** A mean of 289 pupae and moths were recovered per rearing jar from the 10 jars selected at random from the 125 jars used for this experiment (see Addendum 9). The last moths emerged on 13 July, 28 days after incubation.
- **Cold treated larvae in rearing jars:** It is calculated from the number of larvae recovered per jar from Control 2, that a mean of 32 946 larvae had potentially been exposed to the cold treatment, 23 000 more than the protocol required. ***None of the larvae showed any sign of life during the inspections conducted on 25 July (15 days after incubation) and 5 August (26 days after incubation) and none had survived to pupate in either the rearing jars or the SFK cardboard tops.***

1.3 Larvae in naturally infested fruit

- **Control 1:** Head capsule measurements showed that 74.6% of the larvae in the fruit had developed to the final instar when cold treatment of the bulk of the fruit started. Of the 114 larvae cut from the fruit, only one was dead. Natural mortality was therefore 0.9%.
- **Control 2:** One hundred and four pupae were collected from 96 of the 100 data fruit incubated in the plastic containers. No pupae were produced from four fruit and no dead or live larvae were found when inspected. One hundred and two moths developed from the pupae. This data confirms that larvae from the cold treated fruit would have been quite fit to reproduce in the absence of cold treatment and that the experimental material was of good quality.
- **Cold treated larvae in fruit:** A total of 1 036 larvae were removed from the 1 166 cold treated fruit on 12 and 13 July. Due to the 0.9% natural mortality (see Control 1 above) a total of 1027 live larvae (1 000 larvae required as per protocol) would therefore have been potentially exposed in the 1 166 cold treated fruit. On the grounds of body colour alone, nine larvae were isolated from the total number of insects removed from the fruit on 12 July (eight larvae) and 13 July (one larva). They were put onto diet in two rearing jars, to allow more time for possible larval revival. None of these larvae showed any signs of movement after being removed from the fruit. The eight larvae from 12 July turned completely black within 24 hours. The remaining larva from 13 July (13 July), although obviously dead and showing no signs of life, had not yet turned completely black by 29 July, and although being largely dried out, was still reddish in colour. This demonstrates that colour alone is not a criterion that should be used to determine whether an FCM larva had survived the cold treatment. ***Not one live larva was found in the oranges during inspection in spite of the increased temperatures caused by the faulty cooling equipment*** (see Results, section 1.1).

No live larvae were found in both the rearing jars and oranges notwithstanding the brief increase in internal fruit temperatures due to the malfunctioning cooling equipment. This demonstrates that the cold treatment was still completely effective even though it was less stringently applied than required.

2. REPLICATE 2

2.1 Temperature management and data recording

Air temperatures in the coldroom recorded during treatment of Replicate 2 with the integrated ARC Infruitec equipment were, with one exception, within the range of $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$. On 23 July a solenoid valve in the cooling equipment of the coldroom became faulty and temperature management had to be conducted manually during the next 72 hours. This caused air temperatures in the coldroom to fluctuate abnormally in the range 1.2°C to -1.4°C . Deviations to below the lower required temperature of -1.2°C occurred, although not once for longer than one successive reading. However, internal fruit temperatures were not detrimentally influenced and were at all times within the required range of $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$.

Internal fruit temperatures recorded with the Honeywell datalogger were well within the range of $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$. Temperatures recorded with the two Hobo dataloggers confirmed the reading from the ARC Infruitec instruments.

2.2.1 Larvae in naturally infested fruit

- **Control 1:** Head capsule measurements showed that 86% of the larvae from the fruit had developed to the final instar when cold treatment of the bulk of the fruit started. Two dead larvae (from 51 larvae) were recovered from the sample of 51 fruit that were cut open. Natural mortality was therefore 3.9%.
- **Control 2:** Forty-nine pupae were collected from 43 of the 50 fruit in the containers. No pupae were produced from seven fruit and no dead or live larvae were found when inspected. Forty-nine moths emerged from the pupae. These data prove, as in Replicate 1, that larvae from the cold treated fruit would have been viable and able to develop to the next life stage in the absence of cold treatment and that the experimental material was of good quality.
- **Cold treated larvae in fruit:** A total of 1 047 larvae were cut from the 1 186 cold treated fruit. Due to 3.9% natural mortality (see Control 1 above) a total of 1 006 live larvae (1 000 larvae were required as per protocol) would therefore have been potentially exposed in the 1 186 cold treated fruit. As in Replicate 1, 12 larvae were collected that showed no sign of movement. They still maintained a fairly natural looking body colour (whitish for younger larvae and reddish for older larvae) and were placed into a jar with rearing medium for further investigation. They were inspected daily until 8 August and were confirmed dead. ***No live larvae were found in the oranges during inspection.***

2.2.2 FCM eggs

No egg development to the black stage (shortly before hatching) was observed. All eggs were unhatched, and were therefore killed by the cold treatment.

Conclusion

No live larvae were discovered following the cold treatment of navel orange fruit containing approximately 2 000 FCM larvae, as well as nearly 33 000 FCM larvae reared in diet. The experiment also included 35 000 eggs which did not develop and were killed by the cold treatment.

The cold treatment consisted of a 22-day cold storage period at $-0.6^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$. This procedure can therefore be considered a successful cold disinfestation treatment to kill FCM eggs, as well as larvae in naturally infested citrus fruit.

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3.2.13 Genetic variation in false codling moth populations

Experiment US-AT DNS by Alicia Timm (SU)

Opsomming

Patrone van genetiese variasie is ondersoek in geografiese, gasheer- en temporele populasies van die valskodlingmot *Thaumatotibia leucotreta* in Suid Afrika. Geamplifiseerde fragment-lengte polimorfisme (AFLP) ontledings met vyf selektiewe inleierpare en nukleotied-volgordebepaling van die sitochroom okidase II gene is gebruik om genetiese diversiteit te ondersoek. Geografiese populasies van *T. leucotreta* was geneties onderskeibaar met uitgebreide genetiese verskille merkbaar oor groot en klein geografiese afstande. In teenstelling was min genetiese variasie gevind tussen populasies versamel vanaf nege verskillende gasheer of tussen temporele populasies (maw populasies versamel vanaf dieselfde boord gedurende agtereenvolgende seisoene). Hierdie resultate stel voor dat (1) verspreiding tussen boorde swak is, (2) populasies mag voorkom wat plaaslik aangepas is in streke of provinsies, (3) genetiese populasiestruktuur is stabiel gedurende agtereenvolgende seisoene en (4) populasies op verskillende gasheer mag geredelik kruisteel. Hierdie resultate behoort in ag geneem te word by bestaande plaagbestuurprogramme en die ontwerp van nuwe beheerstrategieë.

Introduction

The false codling moth *Thaumatotibia leucotreta* is one of the most destructive and polyphagous agricultural pests in Africa. Despite the economic importance of this pest, little is known about its ecology and population genetics even though such information may provide valuable insight into factors such as insect dispersal, which may potentially have important implications for pest management. Therefore, as a means of contributing towards this knowledge, genetic variation in geographic and host populations of *T. leucotreta* was studied (Timm, 2005; Timm *et al.* submitted). As an extension of this study, temporal patterns of genetic variation among *T. leucotreta* populations were studied in order to provide an indication of the stability of population genetic variation over successive seasons. This report presents results obtained regarding genetic variation among temporal populations of *T. leucotreta* as well as final results of patterns of genetic variation among geographic and host populations.

Material and Methods

Insect material

For studies of variation among geographic populations of *T. leucotreta*, insect material was collected from Citrusdal, Stellenbosch, Retreat, Paarl, Elgin and Tulbagh in the Western Cape Province, Nelspruit, Hazyview and Malelane in Mpumalanga Province and the Sundays River Valley in the Eastern Cape Province. In order to examine genetic variation between populations from different hosts, insect populations were sampled from nine different hosts: citrus, acorns, avocados, apples, pears, plums, litchis, macadamias and star fruit. For analyzing temporal patterns of genetic variation in *T. leucotreta* populations, insect material was obtained from citrus orchards and acorns from specific localities in the Western Cape and Mpumalanga provinces during two or three successive seasons.

Molecular markers

Genomic DNA was extracted from the head and legs of moths or, in rare instances, the thorax of larvae using a modified CTAB-based protocol (Reineke *et al.* 1998). For studies of genetic variation in geographic, host and temporal populations of *T. leucotreta*, specimens were analyzed using amplified fragment length polymorphism (AFLP) analysis using the restriction enzymes *EcoRI* and *MseI* with five selective primer pairs. In addition, for the study of genetic variation in temporal populations, the cytochrome oxidase I and II genes were amplified using previously published primers (TY-J-1460 5'-TAC AAT TTA TCG CCT AAA CTT CAG CC-3'; TK-N-3785 5'-GTT TAA GAG ACC AGT ACT TG-3') (Simon *et al.*, 1994). From this region of the mitochondrial genome, the nucleotide sequence of approximately 600 bases of the cytochrome oxidase II gene was determined using a primer developed for this study (Tortco2 5'-CAA GTT TAA TTA AGT TAA TAA AC), which was designed to be specific for the amplification of the cytochrome oxidase II gene for the *Cryptophlebia-Thaumatotibia* complex.

Data analysis

For analysis of AFLP data, fragments were scored and recorded into a binary data matrix with "1" indicating fragment presence and "0" indicating fragment absence. Standard population genetic statistics were calculated using POPGENE version 1.31 population genetics software (Yeh & Yang 1997) and MVSP version

3.11c (Kovach 1999). Data generated using nucleotide sequence analysis were analysed using BioEdit sequence alignment editor (Hall 1999), DnaSP version 4.00 (Rozas *et al.* 2004) and MEGA version 3.00 (Kumar *et al.* 2004).

Results and discussion

Genetic variation among geographic populations

Analysis of 322 AFLP fragments indicated that genetic diversity within the South African *T. leucotreta* population was high ($H = 0.1490$). Genetic diversity appeared to be higher within the Western Cape population ($H = 0.1599$) than both the Eastern Cape population ($H = 0.1136$) and the Mpumalanga population ($H = 0.1390$). Estimates indicated that *T. leucotreta* populations sampled from the three different provinces showed significantly high levels of genetic variation ($G_{st} = 0.2929$). Populations sampled from the same province were generally more closely related to each other than to those collected from other provinces. These results may indicate that *T. leucotreta* populations have most likely become locally adapted and, as such, may therefore potentially vary in one or more biological factors such as insecticide resistance or virus susceptibility. Populations of *T. leucotreta* sampled from Mpumalanga and the Eastern Cape Provinces appeared to be more closely related to each other than to those from the Western Cape. This might be due to the original distribution patterns of *T. leucotreta* throughout South Africa and may indicate that these populations show greater similarities in biological aspects than to populations from the Western Cape. Therefore, it may be advisable to bear this in mind when designing and evaluating control practices targeted against *T. leucotreta*. Interestingly, estimates of population genetic differentiation were similar in Western Cape and Mpumalanga populations ($G_{st} = 0.2601$ and 0.2591 respectively) but significantly lower in Eastern Cape populations ($G_{st} = 0.2036$). These results indicate that populations from the Eastern Cape are more heterogeneous than those from the Western Cape and Mpumalanga Provinces. This may be due to a number of factors including differential moth dispersal ability, the proximity of alternative/uncultivated hosts or the effect of control practices on populations. However, in all three provinces from which *T. leucotreta* populations were sampled, highly distinct populations were found over local scales and in certain instances where populations were separated by less than one kilometre, individuals could be ascribed to one or other population on the basis of their AFLP profiles. These results indicate that *T. leucotreta* is most likely a poorly dispersing species with little movement between orchards and correlate well with results obtained locally for closely related species such as codling moth *Cydia pomonella* (Timm *et al.* 2006a), macadamia nut borer *T. batrachopa* and litchi moth *Cryptophlebia peltastica* (Timm *et al.* 2006b).

Interestingly, in this study the only region in which significantly high levels of gene flow were calculated between populations was Retreat, the only urban area from which *T. leucotreta* samples were collected. It was not possible to distinguish between Retreat populations situated up to 6 km apart. It is therefore suggested that, like the closely related *C. pomonella* and *G. molesta*, *T. leucotreta* individuals may vary genetically in their capacity to disperse over long distances, which may be related to the habitat in which they are found (Rothschild & Vickers 1991, Schumacher 1997, Keil 2001). In orchards, where only short distance flights are required for *T. leucotreta* to reach another host plant, and where host plants are long-lived, the most successful ecological strategy for the moth would be stay within the habitat. This would allow individuals to avoid the considerable risks associated with long-range dispersal. The same may not be relevant for *T. leucotreta* populations in urban environments, where the habitat is more variable than in orchards. A more thorough investigation of the genetic structure of *T. leucotreta* in urban or more natural populations may therefore be of considerable value for understanding the ecology of this species in these habitats.

Genetic variation among host populations

Estimates of genetic differentiation based on host populations were not significant and calculated as $G_{st} = 0.1193$. At regional and local scales, estimates of population differentiation were not significantly higher when populations from more hosts were included in analyses and it was not possible to distinguish among populations sampled from different hosts using cluster analysis. Therefore, no evidence was found to suggest that populations collected from citrus, apples, pears, plums, litchis, macadamias, star fruit and acorns were genetically differentiated despite early suggestions that *T. leucotreta* races having different host preferences may exist (Ford 1934, Omer-Cooper 1939).

Genetic variation among temporal populations

Results showed that similar patterns of genetic differentiation among populations were apparent during successive seasons ($G_{st} = 0.1988$ and 0.2234 in 2004 and 2005 respectively). For populations collected during both seasons it was possible to distinguish among populations and the estimated numbers of

migrants exchanged were calculated as being insufficient to counter the effects of genetic drift and prevent differentiation on a local scale. These results lend further support to the hypothesis that *T. leucotreta* usually occurs as genetically isolated populations and is generally a poorly dispersing species. It was, however, possible to distinguish between populations collected during successive seasons, although these differences were not as pronounced as those between geographic populations. In addition, populations collected from the same orchard during successive seasons were more closely related to each other than to those from populations collected from different orchards during the same season. These results indicate that the major proportion of *T. leucotreta* individuals and their subsequent offspring remain and mate within the same orchard during successive seasons.

The amount and distribution of genetic diversity present between populations collected during successive seasons from both cultivated and uncultivated hosts showed no statistical differences. These results may indicate that current methods of control may not be effective in removing significant amounts of the genetic variation present in orchards. Alternatively, individuals maintained on uncultivated hosts may enter orchards during the growing season, thereby increasing the total amount of genetic variation present. This may be supported by the fact that no evidence was found to suggest that genetically distinct host populations were present in either season. It was observed that populations from different, closely-situated hosts interbred between seasons. This confirms the hypothesis that populations from uncultivated hosts may have an important influence on those from cultivated hosts. Individuals from uncultivated hosts may play an important role in maintaining high levels of genetic diversity within orchards, thereby adding to the persistence of the pests in orchards. A further implication of these results is that uncultivated hosts may maintain populations at times when fruit is unavailable in the orchard, confirming suggestions that the proximity of other susceptible cultivated or wild fruits has a considerable influence on the degree of *T. leucotreta* infestation (Gunn 1921, Daiber 1981, Anderson 1986). In addition, populations maintained on uncultivated hosts may affect the efficiency of chemical control and the development of insecticide resistance by maintaining reservoirs of susceptible populations.

Conclusion

Populations of *T. leucotreta* occur as distinct populations over both small and large geographic scales and these patterns of genetic variation are stable between seasons. No evidence was found to suggest that *T. leucotreta* occurred as host populations and that individuals most likely migrate between hosts. These results and the limited dispersal that was found between orchards should be taken into consideration for the design and maintenance of effective control programs.

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3.2.14 Reduction of crop losses due to false codling moth, *Cryptophlebia leucotreta*, on navel orange trees with EXP105 120 SC

Contract experiment by Hendrik and Marsheille Hofmeyr (CRI)

Opsomming

’n Boordproef is uitgevoer om die doeltreffendheid van Tracer en ’n nuwe eksperimentele produk, EXP105, teen valskodlingmot in ’n nawellemoenboord vas te stel. Die produkte is begin-Maart teen 0,0048-0,0192% a.b. as hoë volume handbespuitings toegedien. Beide produkte het teen al die getoetste konsentrasies vier weke lank beter as ’n standaardbehandeling met Meothrin gevaar, alhoewel die verskille nie statisties beduidend was nie. Oor ’n sesweke-tydperk het die hoogste konsentrasie van EXP105 beduidend beter as Meothrin gevaar en vrugval weens VKM-besmetting is met 80% verminder (38% met Meothrin). Al die behandelings was ooglopend beter as Meothrin. Proefwerk met die produkte behoort voortgesit te word.

Introduction

False codling moth (FCM), *Cryptophlebia leucotreta*, remains an elusive pest on many citrus cultivars considered important for the export market. Damage from this pest is two-fold, as it can cause severe fruit losses in the orchard, as well as being a continuous major phytosanitary problem in export fruit due to sensitive markets such as the USA, Japan and Korea. Crop losses from FCM can be reduced during the season with control measures such as orchard sanitation, augmentative releases of the egg parasitoid, *Trichogrammatoidea cryptophlebiae*, and mating suppression. However, these methods are often not able to suppress FCM satisfactorily close to harvest and an additional measure such as treating citrus trees with an appropriate insecticide is often considered essential to ensure a better chance of fruit consignments being approved for export. Only a few insecticides are registered for FCM control, and they are mostly subject to FCM resistance and/or market restrictions. It is therefore imperative that an effective insecticide is registered that will be acceptable to clients of southern African citrus produce. The insecticide under report is still in a developmental stage and may only be referred to by an experimental number, EXP105.

Materials and methods

The experiment was conducted on the farm, Kweekkraal, Citrusdal, in an orchard with 15-yr old Washington navel orange trees. The planting distance was 6,0 m x 4,5 m and adjoining trees did not touch. No guard trees were used in between the data trees. The experimental lay-out was a block pattern and each treatment was replicated once in each of 10 blocks. Each replicate was a single tree.

All insecticide treatments were applied once only on 7 March 2005 as high volume, medium cover sprays at 20 bar pressure with adjustable hand guns. The mean temperature during application of the treatments was 29°C. It was partly cloudy but no rain fell within at least one week after application of the treatments.

No fruit drop data was collected during the first four weeks after application of the treatments to allow all fruit infested prior to treatment to drop off the data trees. Data collection commenced on 12 April 2005. All fruit dropping per tree from the fifth week onwards were collected once a week. The fruit were cut open and inspected for FCM infestation. A fruit containing an FCM larva, or symptoms of infestation such as typical larval damage (tunnels) or granular frass inside the fruit, was considered to be infested. The inspections

lasted for six weeks and were terminated when the infestation on the control trees dropped to below the economic threshold (one infested fruit per tree per week).

Data with regard to the number of infested fruit and the percentage fruit drop reduction per treatment were analysed statistically. The variables of importance were adapted with the aid of the SAS statistical package (version 8.2) (SAS, 1999). Analysis of variance with suitable effects and interactions were performed on the data and residual deviations were tested for non-normality (Shapiro and Wilk, 1965). There was not enough evidence ($P>0.05$) against normality; transformation was therefore not needed and interpretation of the data was continued. Student's t-LSD was calculated at a 5% level of significance to compare means of significant effects (Snedecor, 1967).

Results and discussion

The different treatments and results are mentioned in Table 3.2.14.1. Fruit drop in the experiment was relatively low during the first four weeks after application of the treatments and a mean of 1.5 infested fruit per control tree was lost per week in the period five to eight weeks after treatment. The established economic fruit loss threshold of one fruit per tree per week was nonetheless exceeded; indicating that commercial FCM control in the orchard would have been justified. The infestation subsequently increased and in the next (and final) two weeks a mean of 5.2 infested fruit per tree was lost per week, indicative of a severe infestation. The total mean fruit loss in the control during the whole of the experimental period was 2.7 fruit per tree per week.

Meothrin was used at the registered rate of 30 ml product per hl water. From experiments conducted with this product over the last few years, it had already become clear that FCM was not suppressed satisfactorily any more. During the past two years, complaints about poor results with the product were also increasingly received from citrus producers. Therefore, strong circumstantial evidence suggests that FCM, which had already developed resistance to the benzoyl urea insecticides, Alsystin and Nomolt, has also developed multiple resistance to the pyrethroids. In this experiment Meothrin consistently performed poorer than Tracer and EXP105 (all dosages) (Table 3.2.14.1).

Table 3.2.14.1. The reduction of crop losses due to false codling moth infestation with a single application of Tracer 480SC and EXP105 120SC.

Treatment	ml product /hl water (% a.i.)	Cumulative number of false codling moth infested fruit						% FDR ³
		12 April	19 April	26 April	3 May	10 May	17 May	
Control	-	7	24	37	60 a	115	164 a	-
Meothrin 200EC	30 (0.006)	3	10	21	31 b	70	101 b	38.4
Tracer 480SC	40 (0.0192)	3	6	14	21 b	42	67 bc	59.1
EXP105 120SC	40 (0.0048)	0	4	11	14 b	42	64 bc	61.0
EXP105 120SC	80 (0.0096)	2	7	12	24 b	39	58 bc	64.6
EXP105 120SC	160 (0.0192)	4	7	7	11 b	21	33 c	79.9
LSD (5%)		-	-	-	0.5989 ¹	-	0.7208 ²	-

¹ Data for weeks 1-4 (12 April to 3 May).

² Data for weeks 1-6 (12 April to 17 May).

³ %FDR: % fruit drop reduction in comparison to the control treatment.

The Tracer and EXP105 treatments performed similarly during the first four weeks post-treatment due to the relatively low FCM infestation. However, the efficacy of Tracer at 40 ml per hl water (0.0192% a.i.) and the two lower dosages of EXP105 at 40 ml and 80 ml product per hl water (0.0048% and 0.0096% a.i. respectively), decreased in the fifth and sixth week after treatment due to the severe infestation. The value of EXP105 at 160 ml product per hl water (0.0192% a.i.) only became apparent at that time and much better results were obtained than with the other treatments. The difference in treatment efficacy between Tracer and EXP105, both at 0.0192% a.i., during the last two weeks of the experiment, could be an indication that the EXP105 formulation possesses a longer residual action than Tracer.

The abovementioned conclusions are based on the unprocessed data only. Although the deductions are correct in broad terms, statistical analysis by Frikkie Calitz, (Department Biometry, ARC Infruitec/Nietvoorbij, Stellenbosch), shows that all differences between the various treatments are not invariably large or consistent enough to be statistically significant, probably due to underlying variation between various replicates in any particular treatment. This is not enough reason for great concern, as there is often variation in this type of experiment due to the almost inherent uneven infestation pattern of FCM from tree to tree. As more experience with the particular insecticide is gained in future research, the above differences may yet prove to be consistent.

Conclusion

The result with EXP105 120SC for FCM suppression is promising. Backed by information from a previous field-aged residue experiment, the product can possibly be developed into a treatment that will be useful for the suppression of FCM in the period 4-6 weeks before harvest. As such it will alleviate a major headache for citrus producers who are not currently successful in suppressing FCM to required levels with available programs.

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3.3 PROJECT: FRUIT FLIES

Project Co-ordinator: Tony Ware (CRI)

3.3.1 Project summary by Tim Grout

Fruit flies remain important phytosanitary pests and their status in some markets is increasing. Although control of fruit flies has generally been good where recommendations are followed, much of the research is aimed at ensuring good control in the future, particularly if more attention is given to Natal fruit fly. Further post-harvest research was conducted to determine which fruit fly larval life stage should be used in large-scale disinfestation treatments at the higher temperature of around 1°C (3.3.2) and a phase four verification trial of the cold treatment of Clementines at -0.4°C was conducted under Japanese supervision (3.3.7). The possibility that the organophosphates (OPs) may not always be permitted in fruit fly baits led to research on alternative toxicants. Field populations of fruit fly were too low for good results but indications are that imidacloprid may warrant further investigation (3.3.3). With the increasing phytosanitary significance of Natal fruit fly it would be valuable to have a means of determining in the field or packhouse whether fruit fly larvae found in a fruit are Medfly or Natal fruit fly. Research is being conducted with CSL in the UK to develop such a diagnostic kit. The first prototypes were not sensitive enough but further refinement is underway (3.3.4). In the future it may be valuable to prove that our fruit fly species cannot survive in certain conditions or do not occur in certain parts of the country. This is partly being addressed by research on the distribution of Natal fruit fly that has just started at Stellenbosch University but is also addressed by work on the development rates of the *Ceratitis* species (3.3.5). Some field research was conducted to test assumptions that Medfly and Natal fruit fly are equally attracted to Capilure or Questlure in Sensus traps. However, low numbers of flies and a possible flaw in the experimental design mean that this work needs to be repeated (3.3.6). Research was planned on the use of gamma irradiation as a post-harvest treatment for fruit flies after similar work on FCM (3.2.7). This was postponed until the type of irradiation results that would satisfy inspectors, for example live, sterile larvae vs. dead larvae could be confirmed. The need for outside cartons in a pallet to receive up to three times the dosage required for the inner cartons to achieve the correct dosage inside, is also receiving attention as it may have a detrimental effect on fruit condition. Cold disinfestation contract work was conducted on persimmons but is not reported on here.

Projekopsomming deur Tim Grout

Vrugtevlieë is belangrike fitosanitêre peste en hul status is besig om in sommige markte toe te neem. Alhoewel beheer oor die algemeen baie suksesvol is waar aanbevelings toegepas word, is navorsing meestal gerig om in die toekoms ook goeie beheer te verseker, veral as die Natalse vrugtevlieg internasionaal al hoe meer aandag gaan geniet. Verdere na-oes navorsing om te bepaal watter larwale stadia gebruik kan word in grootskaalse disinfestasië behandelings by hoër temperature van 1°C, is ook

uitgevoer (3.3.2) en 'n fase vier verifikasie proef vir koue behandeling van Clementines by -0.4°C is uitgevoer onder Japanse toesig (3.3.7). Ander gifstowwe moes ook ondersoek word omrede die aanduidings daar is dat organofosfate nie vir altyd toelaatbaar sal wees as vrugtevlug lokaas nie. Die vrugtevlug populasies in die veld was egter te laag om goeie resultate te verkry, maar resultate wat met imidacloprid verkry is regverdig verdere ondersoeke (3.3.3). 'n Diagnostiese toets wat dit sal moontlik maak om in die veld of pakhuis te kan onderskei tussen larwes van die Mediterreense- en Natalse vrugtevlug sal baie waardevol wees, veral gesien in die lig van die toenemende fitosanitêre belang van die Natalse vrugtevlug. 'n Navorsingsprojek, in samewerking met CSL in die VK, is tans aan die gang om so 'n toets te ontwikkel. Die eerste prototipes was egter nie sensitief genoeg nie en verfyning van die tegniek is in die proses (3.3.4). In die toekoms sal dit moontlik baie van waarde wees as daar bewys kan word dat van ons vrugtevlug spesies slegs oorleef in sekere omstandighede of selfs nie voorkom in sekere dele van die land nie. Hierdie aspek word gedeeltelik aangespreek deur navorsing wat tans by die Universiteit van Stellenbosch op die verspreiding van die Natalse vrugtevlug uitgevoer word, maar ook deur werk wat gedoen is op die ontwikkelingstempo's van *Ceratitis* spesies (3.3.5). Navorsing is ook in die veld gedoen om die aannames te toets dat Mediterreense- en Natalse vrugtevlug tot dieselfde mate gelok word deur Capilure of Questlure in Sensus lokvalle. Lae getalle van die vlieë en 'n moontlike fout in die eksperimentale uitleg het daartoe gelei dat die werk herhaal moet word (3.3.6). Navorsing is ook beplan rondom die gebruik van gamma bestraling as 'n na-oes behandeling vir vrugtevlug na gelang van soortgelyke werk met VKM (3.2.7). Die navorsing is egter uitgestel totdat die tipe bestralingsresultate verkry kon word wat vir die inspekteurs aanvaarbaar sou wees, byvoorbeeld lewende steriele larwes vs. dooie larwes. Die feit dat die buitenste laag kartonne in 'n palet tot drie maal die dosis moet ontvang as die binneste kartonne ten einde die korrekte dosis binne die palet te verseker, word ook ondersoek omrede dit 'n nadelige effek op die kwaliteit van die vrugte mag hê. Koue disinfestasië werk is ook met persimmons uitgevoer, maar dit word nie hier weergegee nie.

3.3.2 Sensitivity of Mediterranean fruit fly eggs and larvae in lemons, grapefruit and oranges to cold treatment of 1°C

Experiment 772 by Tony Ware, Bruce Tate, John-Henry Daneel, Peter Stephen and Rooikie Beck (CRI)

Report edited by Vaughan Hattingh and Tim Grout

Opsomming

Die navorsing was gerig op koue toleransie van Mediterreense vrugtevlug eiers, ses dae oue (jong)- en 8 dae oue (volwasse) larwes in suurlimoene, pomelos en lemoene wanneer die vrugte aan 'n temperatuur van 1°C blootgestel word. Resultate het getoon dat eiers oor die algemeen minder bestand was teen koue as die larwes, alhoewel die verskille nie altyd statisties beduidend was nie. Daar was egter genoegsame aanduidings dat eiers nie in fase 3 en 4 evaluasies gebruik moet word wanneer die lewensstadium wat die meeste teen koue bestand sal wees, bepaal moet word nie. Baie oorvleueling is gevind tussen jong en volwasse larwes se koue bestandheid, met geen statisties betekenisvolle verskille tussen die koue bestandheid van die lewensstadiums nie. In lemoene is daar geringe aanduidings gevind dat jong larwes meer koue bestand mag wees as volwasse larwes, wat daarop dui dat jong larwes in fase 3 en 4 evaluasies gebruik moet word. Die verskille wat waargeneem is in die koue bestandheid van jong, sowel as volwasse larwes in pomelos en suurlimoene was egter baie gering en nie statisties betekenisvol nie. Die aanduidings is dus dat beide stadiums in fase 3 en 4 evaluasies gebruik kan word.

Introduction

The inter-regional movement of fresh produce can be severely restricted if it has the potential to harbour pests and diseases that do not occur in the country to which they are destined for export. One such group of pests are the fruit flies (Diptera: Tephritidae) and host commodities may be required to undergo some form of post-harvest disinfestation treatment before acceptance for importation. Of all the fruit fly species, arguably the most important is the Mediterranean fruit fly *Ceratitis capitata* [Wiedemann] (White and Elson-Harris, 1992). From its probable East African origin it has spread to most corners of the world with the notable exceptions of Far East Asia and North America (although periodic invasions do occur in California and Florida). One of the reasons for the species' success in its worldwide establishment is its large host range (White and Elson-Harris, 1992).

There are a number of post-harvest treatments that can be used to ensure phytosanitary security. These include fumigation, irradiation, hot water and heated air treatments, controlled atmosphere and the use of pesticides. However, it is cold treatment that has become the treatment of choice, mainly because of its ease of application and the fact that many fruit types can withstand low temperature exposure without undue

deterioration of quality. Cold storage was originally used to inhibit decay and extend shelf life of fresh produce.

Disinfestation research is a pre-requisite if importation of fruit is to be allowed into some regions. The development of a cold treatment for disinfestation traditionally takes on the form of (phase 1) determining the developmental tempo of the various life stages of the pest in the target fruit; (phase 2) comparing the cold tolerance of the different life stages; (phase 3) a probit analysis to determine the treatment time required to effect disinfestation; and (phase 4) large scale verification of the disinfestation treatment's efficacy. This study investigated the phase 2 cold tolerance of Mediterranean fruit fly eggs, six-day-old (young) larvae and eight-day-old (mature) larvae in lemon, grapefruit and oranges at 1°C.

Materials and methods

Test insects: Plums infested with Mediterranean fruit fly were collected from the Western Cape Province on 6 December 1998. These were transported to Nelspruit where they were used to initiate the laboratory colony. Annual additions of "wild" flies were made to this colony in order to maintain genetic variability.

The adult fruit flies were maintained in a constant environment room where the temperature was maintained at 26°C (\pm 2°C) with a relative humidity of approximately 60% and a day/night photoperiod of 14/10 hours. Colonies of approximately 15 000 adults were housed in cylindrical cages made of gauze (length 140 cm; diameter 45 cm). The tops and bottoms of the cages consisted of plastic bowls. Yeast hydrolysate and white granulated sugar were supplied *ad lib*. The females oviposited through the gauze and eggs were collected in a water-filled bowl placed under the cage. In order to perpetuate the colony, eggs were collected at regular intervals and allowed to hatch on an artificial medium made from bran, carrot powder, sugar and yeast. The larvae were maintained on this diet and allowed to pupate in sand.

The following data were obtained on the laboratory colony. Eggs (600) were placed on wet filter paper and 96% hatched. Pupae (500) were placed in a cage and the number of flies emerging determined. The sex ratio was almost equal with 50.7% of the emerging flies being female. These flies laid 113 375 eggs or an average of 487 eggs per individual. The duration of each life stage under the rearing conditions was 3 days for eggs, 8 days for larvae, 6 days for pupae, while the adults lived for approximately 30 days.

Test fruit. Eureka lemons (*C. limon* Burm. F.) were supplied from Bakgat farm situated in the Schoemanskloof Valley near Nelspruit, Valencia oranges (*C. sinensis* [L.] Os.) came from Crocodile Valley Citrus Company near Nelspruit and most of the Marsh grapefruit (*C. paradisi* Macf.) were obtained from Neo-Nova Packhouse, Onderberg (all in Mpumalanga Province). All fruit was maintained in a cold room at approximately 4°C until required. Twenty-four hours before they were used they were removed from the cold room, allowed to attain ambient temperature and then treated with a fungicide (imazalil sulphate [0.5%] or guazatine [0.15%]).

Cold chamber: The cold chamber was a custom-built unit constructed from Isowall (polystyrene sandwiched between aluminium sheets). The unit was positioned on a concrete floor in a warehouse. The dimensions of the room were: length 4.0 m, breadth 2.95 m and height 2.42 m. The chiller unit in the room was situated near the ceiling opposite the sliding door. Heating coils were used to defrost the chiller unit. They were engaged every 8 hours for 15 minutes. During the defrost cycle the fans were switched off. The cold chamber temperature was controlled using a Carel CR72 Universal electronic controller. Fruit core temperature variation during the defrost cycle was less than 0.2°C. This was measured using a Grant 1200 series Squirrel meter/logger attached to two-wired resistance thermocouples. The thermoprobes were calibrated on melting ice and correction factors recorded for each individual probe before being inserted in fruit in the cold room. Thermoprobe readings were then downloaded on an hourly basis and after these were corrected a long-term mean for all probes and all hourly readings was determined for the complete cold storage period. Long-term means of the lowest and highest hourly temperatures were also determined. Minor temperature adjustments were made to the cold room throughout the treatment period to ensure that the long-term mean was maintained above 1°C. When warm fruit was loaded into the cold room at the required times, the recorded temperature would rise for a few hours before returning to the set temperature.

Inoculation procedure: The fruit was prepared for inoculation with fruit fly eggs by drilling through the calyx end towards the centre to a depth of approximately 2 cm, using a 6 mm wood drill bit. The drill bit was washed with fungicide after approximately every 10 fruit processed to ensure that fungal cross contamination was kept to a minimum. Yeast hydrolysate (200 μ l) was placed in the fruit as a food supplement. An inoculum of eggs was prepared by gathering the eggs from a basin of water placed beneath the cages within 12 hours of their oviposition. The eggs were then diluted in water until approximately 40 eggs were delivered into the fruit using an automatic pipette (Kartell Pluripet PL 200). The wound was then plugged with cotton

wool and covered with paraffin wax. Each fruit was then placed into a brown paper bag. The fruit were then randomly packed into plastic lug boxes that were placed at 26°C for 6 days or 8 days larval development.

Nine hundred fruit from each of the 3 citrus types were inoculated. The fruit (250) destined to determine the cold tolerance of the eggs were placed into the cold chamber that was set to maintain a temperature of 1°C. The rest of the fruit was placed at 26°C for 6 days (young larval development stage) or 8 days (mature larval development stage), after which they were transferred to the cold chamber. For each developmental stage, 50 fruit were removed from the cold chamber after 24 hours of treatment (and thereafter at 48 hour intervals) and placed at 26°C for a period of 8 days (eggs), 2 days (young larvae) and 24 hours (mature larvae). The fruit was then dissected and the number of survivors determined. The trial was repeated.

Statistical analysis: All data were subjected to Probit analysis (Finney, 1971) using POLO-PC Software (LeOra Software). The fiducial limit of the combined data set for each life stage was calculated to a 90% confidence level. Some of the data varied excessively from the mathematical model and confidence readings could not be obtained.

Results and discussion

The long-term means of hourly temperatures recorded within fruit in the two replicates are shown below.

Phase 2, replicate 1			Phase 2, replicate 2		
Mean overall	Mean lowest	Mean highest	Mean overall I	Mean lowest	Mean highest
1.614	1.045	2.377	1.888	1.270	2.464

The number of fruit flies surviving the cold treatment in grapefruit, oranges and lemons is shown in Tables 3.3.2.1, 3.3.2.2 and 3.3.2.3, respectively. No fruit fly eggs survived after 9 days exposure to cold treatment when inoculated into grapefruit and oranges and none survived 7 days exposure when inoculated into lemons. In contrast, some young fruit fly larvae survived in grapefruit and oranges after 11 days of cold treatment but there were no survivors in lemons after 9 days exposure. A few mature larvae in grapefruit survived 13 days of exposure, but no mature larvae in lemons and oranges survived 11 days of exposure.

Statistical analysis is reported in Table 3.3.2.4. Eggs were generally less cold tolerant than the larvae. The differences in cold tolerance of eggs and larvae were in some cases statistically significant (LT50 and LT90 in oranges and lemons). In some cases where the differences between cold tolerance of eggs and larvae were not statistically significant they were large enough for a LT value of a life stage to fall outside the range of fiducial limits of LT values for another life stage. This occurred in Grapefruit (egg LT50, LT90), oranges (egg LT99) and lemons (egg LT99), where eggs were less cold tolerant than larvae. Earlier studies by Ware (1999) on Clementine mandarins (at -0.5°C) also showed that eggs were less cold tolerant than larvae, but the differences were not statistically significant (overlapping fiducial limit ranges), although the egg LT90, LT95 and LT99 values did fall outside the range of fiducial limits of the larval LT values. The tendency for eggs to be less cold tolerant than larvae is therefore fairly consistent and can be considered to be applicable to Medfly in all citrus types.

The differences observed between the cold tolerance of young and mature larvae were far smaller than the differences between eggs and larvae. None of the differences in sensitivity of young and mature larvae were statistically significant, including earlier work by Ware (1999) on Clementine mandarins. In some cases where the differences between cold tolerance of young and mature larvae were not statistically significant, the differences were large enough for a LT value of one larval age class to fall outside the range of fiducial limits for the LT values of the other age class. This occurred in oranges (young and mature larval LT90 and LT99) and Clementine mandarins (mature larval LT90, LT95 and LT99 [Ware, 1999]). The differences between the cold tolerance of larval life stages in lemons and grapefruit were smaller, were not statistically significant and the LT values either fell within the range of fiducial limits for the LT values of the other life stage, or the variability of the data was too large to enable calculation of fiducial limits and statistical significance.

Conclusion

It is apparent that eggs should not be used in phase 3 and 4 evaluations, where the most cold tolerant life stage should be treated. In the case of Clementine mandarins (Ware, 1999) and oranges there was some evidence, although not statistically significant, to suggest that young larvae should be used in phase 3 and 4

evaluations. In the case of grapefruit and lemons there was no evidence to suggest that either young or mature larvae should preferentially be used in phase 3 and 4 evaluations.

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Table 3.3.2.1. The survival of Mediterranean fruit fly in grapefruit after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	50	1750	429	24.51
		1	50	1750	393	22.46
		3	50	1750	344	19.66
		5	50	1750	17	0.97
		7	50	1750	5	0.28
		9	50	1750	0	0
		11	50	1750	0	0
	2	0	50	2000	310	15.50
		1	50	2000	173	8.65
		3	50	2000	87	4.35
		5	50	2000	25	1.25
		7	50	2000	0	0
		9	50	2000	0	0
		11	50	2000	0	0
Young	1	0	50	1750	429	24.51
		1	50	1750	446	25.48
		3	50	1750	429	24.51
		5	50	1750	367	20.97
		7	50	1750	47	2.68
		9	50	1750	8	0.45
		11	50	1750	0	0
	2	0	50	2000	310	15.50
		1	50	2000	223	11.15
		3	50	2000	160	8.0
		5	50	2000	102	5.10
		7	50	2000	16	0.80
		9	50	2000	0	0
		11	50	2000	1	0.05
Mature	1	0	50	1750	429	24.51
		1	50	1750	475	27.14
		3	50	1750	434	24.80
		5	50	1750	392	22.40
		7	50	1750	95	5.42
		9	50	1750	5	0.29
		11	50	1750	0	0
	2	0	50	2000	310	15.50
		1	50	2000	222	11.10
		3	50	2000	215	10.75
		5	50	2000	543	27.15
		7	50	2000	123	6.15
		9	50	2000	0	0
		11	50	2000	3	0.15

Table 3.3.2.2. The survival of Mediterranean fruit fly in oranges after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	50	1750	319	18.23
		1	50	1750	371	21.20
		3	50	1750	405	23.14
		5	50	1750	5	0.28
		7	50	1750	2	0.11
		9	50	1750	0	0
		11	50	1750	0	0
	2	0	50	2000	338	16.90
		1	50	2000	554	27.70
		3	50	2000	76	3.80
		5	50	2000	59	2.95
		7	50	2000	23	1.15
		9	50	2000	0	0
		11	50	2000	0	0
Young	1	0	50	1750	319	18.22
		1	50	1750	498	28.45
		3	50	1750	440	25.14
		5	50	1750	445	25.42
		7	50	1750	156	8.91
		9	50	1750	22	1.25
		11	50	1750	1	0.06
	2	0	50	2000	338	16.90
		1	50	2000	576	28.80
		3	50	2000	584	29.20
		5	50	2000	297	14.85
		7	50	2000	208	10.40
		9	50	2000	137	6.85
		11	50	2000	4	0.20
Mature	1	0	50	1750	319	18.22
		1	50	1750	476	27.20
		3	50	1750	339	19.37
		5	50	1750	403	23.02
		7	50	1750	124	7.08
		9	50	1750	3	0.17
		11	50	1750	0	0
	2	0	50	2000	338	16.90
		1	50	2000	505	25.25
		3	50	2000	387	19.35
		5	50	2000	553	27.65
		7	50	2000	229	11.45
		9	50	2000	15	0.75
		11	50	2000	0	0

Table 3.3.2.3. The survival of Mediterranean fruit fly in lemons after exposure of eggs, young and mature larvae to 1°C for various periods.

Life stage	Replicate	Exposure (days)	Number of fruit	Number of eggs	Number of survivors	Survival (%)
Egg	1	0	50	1750	414	23.65
		1	50	1750	303	17.31
		3	50	1750	380	21.71
		5	50	1750	8	0.45
		7	50	1750	0	0
		9	50	1750	0	0
		11	50	1750	0	0
	2	0	50	2000	662	33.10
		1	50	2000	637	31.85
		3	50	2000	109	5.45
		5	50	2000	81	4.05
		7	50	2000	0	0
		9	50	2000	0	0
		11	50	2000	0	0
Young	1	0	50	1750	414	23.65
		1	50	1750	335	19.14
		3	50	1750	460	26.28
		5	50	1750	297	16.97
		7	50	1750	10	0.57
		9	50	1750	32	1.82
		11	50	1750	0	0
	2	0	50	2000	662	33.10
		1	50	2000	862	43.10
		3	50	2000	772	38.60
		5	50	2000	352	17.60
		7	50	2000	128	6.40
		9	50	2000	0	0
		11	50	2000	0	0
Mature	1	0	50	1750	414	23.65
		1	50	1750	397	22.68
		3	50	1750	249	14.22
		5	50	1750	134	7.65
		7	50	1750	38	2.17
		9	50	1750	3	0.17
		11	50	1750	0	0
	2	0	50	2000	662	33.10
		1	50	2000	646	32.30
		3	50	2000	435	21.75
		5	50	2000	535	26.75
		7	50	2000	162	8.10
		9	50	2000	5	0.25
		11	50	2000	0	0

Table 3.3.2.4. LT₅₀, 90 and 99 (90% fiducial limits) for fruit fly eggs, young and mature larvae in grapefruit, oranges and lemons, when exposed to cold treatment of 1°C.

Cultivar	Life stage	LT ₅₀	LT ₉₀	LT ₉₉
Grapefruit	Egg	3.3 (2.2-4.2)	4.8 (4.0-6.2)	6.4 (5.2-12.2)
	Young	5.5 (-)	7.2 (-)	8.8 (-)
	Mature	6.6 (-)	7.8 (-)	8.9 (-)
Orange	Egg	3.3 (2.1-3.9)	4.8 (4.0-6.5)	6.5 (5.2-12.3)
	Young	6.6 (5.0-7.5)	9.3 (8.4-11.3)	12.3 (10.5-19.0)
	Mature	6.9 (6.3-7.2)	8.1 (7.7-8.8)	9.3 (8.6-10.9)
Lemon	Egg	3.0 (1.9-3.6)	4.7 (3.9-6.3)	6.7 (5.3-13.1)
	Young	5.2 (4.1-5.9)	7.3 (6.4-8.6)	9.5 (8.2-13.4)
	Mature	5.9 (3.6-6.7)	7.7 (6.7-9.9)	9.6 (8.2-18.6)

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3.3.3 Fruit fly bait sprays – alternatives to organophosphates Experiment 773 by Bruce Tate, Tim Grout and Tony Ware (CRI)

Opsomming

Vrugtevlieë is van die belangrike peste van sitrus en die beheer daarvan is hoofsaaklik gebaseer op die gebruik van organofosfaat gifstowwe in proteïen hidrolisaat lokaasmiddels. Die moontlikheid bestaan dat hierdie groep chemikalieë in die toekoms verbied gaan word of selfs dat streng beperkings op residu-vlakke geplaas gaan word en daarom moet alternatiewe middels ondersoek moet word. Van die middels wat reeds in hierdie navorsing, onder beheerde toestande, teen *Ceratitits capitata* getoets is, blyk imidacloprid die belowendste te wees. Veldtoetse waartydens 'n lokaasmengsel wat imidacloprid bevat ge-evalueer is, deur aanwending op takke van sitrusbome, was egter nie baie suksesvol nie. Die rede hiervoor word toegeskryf aan lae vrugtevlieg getalle. Verdere toetse sal dus op ander gewasse uitgevoer moet word, waar vrugtevlieg getalle hoër sal wees.

Introduction

Fruit flies are not only of economic importance but are also of phytosanitary significance. Management of fruit fly in citrus is largely based on the use of baits containing one of two organophosphates (mercaptotion and trichlorfon). Residues from these products are becoming increasingly less acceptable to European markets so there is an urgent need to find alternative toxicants. There are three registered non-organophosphate treatments (GF 120 NF, the M3 bait station and Last Call FF) but the industry considers these not to be cost-effective alternatives. CRI has, therefore, undertaken to investigate alternatives that satisfy this criterion. Some research on this topic was co-funded by PIP in 2004 (A27)

Materials and methods

Cage tests

Citrus seedlings of approximately 1,2 m in height were each treated with exactly 1 ml of bait mixture at any one of the concentrations tested. The bait was divided into 100 x 10 µl drops placed on the leaves using a micropipette. The bait was allowed to dry for 3 hours after which each tree was suspended within a gauze cage (1,1 x 0,6 x 1,85 m (LxBxH)). In general, the highest rate tested was 2-3 times that registered for citrus. All products were tested in combination with a 2% hydrolysed protein bait (Hym lure) that was also used as a control spray (Table 3.3.3.1). The floor of each cage was covered with plastic to facilitate the counting of dead flies. For six days after their emergence, adult laboratory-reared Mediterranean fruit flies (*Ceratitits capitata* [Wiedemann]) were fed granulated sugar and water *ad lib.* (i.e. were protein starved). Approximately 100 flies (± equal numbers of each sex) were released into each cage shortly after the trees

were positioned. Exactly 24 hours later all the dead flies were removed and counted. The total numbers of flies in the cages were assessed and the percentage mortality determined.

Field test to compare imidacloprid with mercaptothion in Hymlure bait

A comparison of imidacloprid (Confidor) and the standard toxicant mercaptothion in protein hydrolysate bait mixtures was made in a citrus orchard near Nelspruit. Confidor (350 g/l SC) at 4 ml was mixed with Hymlure protein hydrolysate at 400 ml/hl water and compared with mercaptothion (500 g/l EC) at 175 ml and 400 ml Hymlure/hl water. The bait mixtures were applied at 10 ml per branch using a hand operated spray bottle of 500 ml capacity, that delivered 1 ml spray mixture with each squirt. Twelve oval plastic basins (650 x 500 x 250 mm (LxBxH)) of approximately 40 l capacity with the bottoms removed and replaced with plastic gauze to prevent any liquid accumulation, were mounted on steel spikes at each end to hold them in close proximity to the treated branches which were manipulated to fit into the basins. Six branches were treated per bait in an alternating pattern where no treatments were closer than 10 m apart. Protection against ant predation of dead flies was done using Ant-Bar (polybutene) applied around the base of the treated branches and on the metal spikes holding the basins. Preparation for the trial was done the day before the bait was applied so that the bait application could be made early the following day. This comparison was conducted four times.

Results and discussion

Cage tests

Imidacloprid showed the most promise as it resulted in more than 50% kill after 24 hours at 4 ml product per 100 litres bait mixture (Table 3.3.3.1). The related product thiacloprid had very little effect with the highest mortality obtained being 11%. Acetamiprid, another chloronicotinyl, caused mortality levels between the two former products with no effect below 25 ml product/hl. Chlorfenapyr and fipronil both showed a slow increase in mortality with dosage when evaluated after 24 hours (Table 2.10.1). Although mortality improved with the 48-hour evaluation (Table 3.3.3.2), the control mortality for both these series of bioassays was unacceptably high so these results are not reliable. Proteus was similar to thiacloprid in having a negligible effect in both evaluations. The experimental product 0316423 caused 59% mortality after 24 hours at the highest dosage used, but this would probably not be cost effective. Fluvalinate caused some mortality at the lowest dosage tested, then some at the higher dosages with no effect in between (Table 3.3.3.1). This may be due to a repellent effect at the intermediate dosages and contact mortality at the high dosages. Based on the 24-hour bioassay results, field tests of bait mixtures containing imidacloprid were conducted.

Table 3.3.3.1. Percentage mortality of Mediterranean fruit fly after 24 hours for products (and their dilution rates) in protein hydrolysate baits screened in cages. 2% Hymlure (protein hydrolysate) was used as the control treatment.

Product	Formulation	Dilution (ml/100 l water and 2% Hymlure) and mortality (%) below						Control mortality (%)
		0.3	1.0	3.0	10	30	60	
Chlorfenapyr	360 g/l SC	0.3	1.0	3.0	10	30	60	-
	Mortality:	2.3	1.1	17.9	29.0	32.0	37.0	10.3
Fipronil	200 g/l SC	0.1	0.3	1.0	3	10	30	-
	Mortality:	9.4	8.5	40.0	34.0	59.0	50.0	8.6
Imidacloprid	350 g/l SC	0.5	1.5	4.0	12	35	105	-
	Mortality:	1.0	42.0	58.0	60.0	71.0	85.0	1.0
Thiacloprid	480 g/l SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	6.0	2.6	0.0	11.4	10.0	5.7	4.4
Acetamiprid	222 g/l SL	3.125	6.25	12.5	25	50	150	-
	Mortality:	2.0	0	0	21.0	35.6	58.5	0
Fluvalinate	240 g/l EW	1.875	3.75	7.5	15	30	90	-
	Mortality:	26.0	2.0	6.0	2.0	23.0	23.0	12.0
Proteus	170 g/l OD	6.25	12.5	25	50	100	300	-
	Mortality:	12.0	5.4	14.0	8.5	8.1	14.3	2.0
0316423*	SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	1.3	0.0	1.0	10.3	16.3	59.6	0

*Experimental Bayer CropScience product – Ketanol group

Table 3.3.3.2. Percentage mortality of Mediterranean fruit fly after 48 hours for products (and their dilution rates) in protein hydrolysate baits screened in cages. 2% Hym lure (protein hydrolysate) was used as the control treatment.

Product	Formulation	Dilution (ml/100 ℓ water and 2% Hym lure) and mortality (%) below						Control mortality (%)
		0.3	1.0	3.0	10	30	60	
Chlorfenapyr	360 g/l SC	0.3	1.0	3.0	10	30	60	-
	Mortality:	51.1	26.6	74.5	83.14	92.8	87.2	80.5
Fipronil	200 g/l SC	0.1	0.3	1.0	3	10	30	-
	Mortality:	69.4	79.8	84.1	84.5	89.1	96.6	44.4
Imidacloprid	350 g/l SC	0.5	1.5	4.0	12	35	105	-
	Mortality:	11.62	72.44	91	92.2	92.5	97.8	2
Thiacloprid	480 g/l SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	12.1	5.2	0	13.9	16.2	17.2	14.3
Acetamiprid	222 g/l SL	3.125	6.25	12.5	25	50	150	-
	Mortality:	5	1	7.4	46	64.3	84.1	2.2
Fluvalinate	240 g/l EW	1.875	3.75	7.5	15	30	90	-
	Mortality:	39.4	26.7	29	0	49.5	75.6	26.4
Proteus	170 g/l OD	6.25	12.5	25	50	100	300	-
	Mortality:	42.6	17.4	29	41.5	12.6	48	3.2
0316423*	SC	0.185	0.55	1.66	5	15	45	-
	Mortality:	2.5	17	38.3	44.3	51	84.4	1.3

*Experimental Bayer CropScience product – Ketanol group

Field tests

The first two tests yielded no flies in the basins despite moving the basins to a different site. The third test yielded only 3 *Ceratitis* spp. flies caught in 6 basins for the imidacloprid baits, and 4 flies for the mercaptothion baits. The fourth test was delayed and moved to an orchard known to have large numbers of flies, and the application volume was increased to 20 ml per branch. The yield here was an improvement over previous runs but still poor with 6 flies killed in total for imidacloprid and 22 for mercaptothion (Table 3.3.3.3).

Table 3.3.3.3. Dead fruit flies collected in basins beneath branches sprayed with bait containing either imidacloprid (0.0014% a.i.) or mercaptothion (0.0875% a.i.) as a toxicant.

Test no.	Product	Volume/branch	Med. flies		Marula flies		Natal flies		Totals
			♂	♀	♂	♀	♂	♀	
3	Imidacloprid	10 ml	0	1	2	0	0	0	3
3	Mercaptothion	10 ml	2	2	0	0	0	0	4
4	Imidacloprid	20 ml	2	3	0	0	1	0	6
4	Mercaptothion	10 ml	11	10	0	0	1	0	22

Conclusion

Fly numbers were too low for these tests to be conclusive but mercaptothion still killed more flies. The basin technique may be unsuitable for chemicals that do not cause rapid knock-down. An alternative technique will need to be developed to test slow-acting toxicants such as fipronil in the field.

Future research

This trial needs to be repeated in untreated fruit orchards such as guavas with extremely high fly numbers, using a different fly-collection technique and including fipronil as a toxicant.

3.3.4 **Development of a rapid diagnostic test to distinguish Medfly larvae from other larvae** Experiment 774 by Tim Grout and John-Henry Daneel (CRI)

Summary

The phytosanitary significance of Natal fruit fly is increasing in Europe and the value of being able to readily distinguish between Natal fruit fly and Mediterranean fruit fly in the larval life stage is therefore also increasing. It was decided that a rapid identification technique that could be used by a layman in an orchard or packhouse was required in order to take decisions before packing on where to market the fruit. Central Science Laboratories (CSL) in England was selected to do this work as they had a lot of experience in developing lateral flow diagnostic kits for viruses and other pathogens. Several batches of fruit fly larvae and other insect larvae that may be confused with fruit flies were sent to CSL on several occasions for the development of the technique. Two prototype lateral flow kits based on antigens A01 and A02 were provided to CRI for evaluation using two buffer options. However, these did not show sufficiently consistent results when tested with *Ceratitis* larvae or pupae. CSL is now trying to improve the ELISA reaction and make the disappearance of the "T" line in positive tests more obvious. A full report will be provided when a kit is received that can be tested on different fruit fly populations all over the country.

Opsomming

Die fitosanitêre belang van die Natalse vrugtevlieg is besig om in Europa toe te neem en daarmee saam ook die dringendheid om met sekerheid te kan onderskei tussen larwes van die Natalse- en Mediterreense vrugtevlieg. Die ontwikkeling van 'n vinnige en maklike identifikasie tegniek, wat selfs in boorde en pakhuis uitgevoer kan word, is gesien as die oplossing om voor pak die teikenmark te bepaal. Die Central Science Laboratories (CSL) in Engeland is baie kundig op die gebied van ontwikkeling van laterale vloei diagnostiese toetse vir virusse sowel as ander patogene. Verskeie monsters van vrugtevlieg larwes asook ander inseklarwes, wat moontlik met vrugtevlieë verwar kan word, is op verskillende tye aan CSL gestuur vir die ontwikkeling van so 'n tegniek. Twee prototipes van die toets wat gebaseer is op antigene A01 en A02 in kombinasie met twee buffer opsies is aan CRI voorsien vir evaluering. Die herhaalbaarheid van die toets met *Ceratitis* larwes of papies het egter nie bevredigende resultate gelewer nie. CSL is nou in die proses om die ELISA reaksie verder te verfyn sodat die verdwyning van die "T" lyn meer ooglopend waargeneem kan word in positiewe reaksies. 'n Volledige verslag sal beskikbaar gestel word sodra 'n tegniek ontvang is waarmee al die verskillende vrugtevlieg populasies landwyd getoets kan word.

3.3.5 **Establishing developmental thresholds for different life stages of Natal fruit fly and marula fruit fly for climate modelling purposes** Experiment 797 by Tim Grout and Kim Stoltz (CRI)

Summary

It is possible that some of our markets may in future only accept fruit from areas that are free of Natal fruit fly. In order to show where citrus can be grown in the absence of Natal fruit fly, or where Natal fruit fly would not establish in Europe, models will require information on the developmental thresholds of different life stages. This research is therefore being conducted in environmental chambers at CRI in Nelspruit. Up to 31 December 2005, results had been obtained for *Ceratitis capitata*, *C. rosa* and *C. cosyra* at 18, 22 and 26°C. Egg to egg development times for these three species at 18°C were, 41, 46, and 47 days, at 22°C they were 29, 32 and 33 days, and at 26°C they were 20, 26 and 23 days, respectively. The time interval between emergence of adults from pupae and the first eggs being laid for *C. capitata*, *C. rosa* and *C. cosyra* was 7, 12 and 14 days at 18°C, 4, 8 and 4 days for 22°C, and 3, 7 and 4 days at 26°C, respectively. The longevity of other life stages and intervals were very similar when species were compared at the same temperature. Developmental rates must still be determined at 14 and 30°C before developmental thresholds can be calculated. A full report will then be provided in the next annual research report.

Opsomming

Die moontlikheid bestaan dat sommige markte in die toekoms slegs vrugte sal aanvaar van areas wat vry is van die Natalse vrugtevlieg. Vir die ontwikkeling van modelle om te bepaal waar sitrus verbou sal kan word in die afwesigheid van die insek, of in watter dele van Europa die insek nie sal kan vestig nie, word inligting benodig rakende die ontwikkelingsdrempelwaardes van die verskillende lewensstadia. Hierdie navorsing is uitgevoer in omgewingskamers van CRI in Nelspruit. Resultate van *Ceratitis capitata*, *C. rosa* en *C. cosyra* is gerapporteer by 18, 22 en 26°C tot en met 31 Desember 2005. Eier tot eier ontwikkelingstye vir hierdie drie spesies was onderskeidelik 41, 46 en 47 dae by 18°C; 29, 32 en 33 dae by 22°C en 20, 26 en 23 dae by 26°C. Die tyd wat verloop het tussen die verskyning van die volwassenes en die lê van die eerste eiers vir

C. capitata, *C. rosa* en *C. cosyra* was onderskeidelik 7, 12 en 14 dae by 18°C, 4, 8 en 4 dae by 22°C en 3, 7 en 4 dae by 26°C. Die lewensduur en die intervalle van die ander stadia het baie ooreengestem as die spesies by dieselfde temperatuur vergelyk is. Ontwikkelingstempo's moet egter nog by 14°C en 30°C bepaal word alvorens die ontwikkelingsdrempelwaardes bereken kan word. 'n Volledige verslag sal in die volgende jaarlikse navorsingsverslag beskikbaar gestel word.

3.3.6 Natal Fruit Fly – Field Control other than OP substitutes

Experiment 801 by John-Henry Daneel, Tony Ware, Tim Grout and Bruce Tate (CRI)

Opsomming

Dieselfde metode is vir die beheer en monitering van die twee ekonomieë, belangrikste vrugtevlieë in die sitrusbedryf, oor die jare toegepas. Meer onlangse waarnemings het egter getoon dat dit nie noodwendig so moet wees nie. Verdere navorsing om hierdie verskille te ondersoek word gerugsteun deur die aanduidings dat die kwarantynstatus van die Natalse vrugtevlieë, *Ceratitis rosa*, verhoog gaan word in die toekoms. Die hoë getalle van vrugtevlieë spesies wat gedood is in boorde deur die gebruik van berokking het baie ooreengestem met die resultate van Capilure en Questlure in Sensus droë lokvalle. Die getalle van beide die spesies was egter te laag om hierdie verwantskap verder te ondersoek. Vergelykings van trap-lure kombinasies, in gevalle waar bekende getalle van laboratorium geteelde vrugtevlieë vrygelaat is, het getoon dat Sensus-Capilure dikwels meer manlike Mediterreense vrugtevlieë as manlike Natalse vrugtevlieë gelok het. Die alternatiewe proteïen hidrolisate, Camouflage en PI-ST, het ook baie beter gevestig as die Hym lure lokaas wat geregistreer is vir die beheer van Natalse vrugtevlieë. Hierdie resultate ondersteun dus die verdere ondersoek na kombinasies van hierdie proteïen hidrolisate met meer aanvaarbare gifstowwe as organofosfate.

Introduction

Although the Mediterranean fruit fly *Ceratitis capitata* is common in parts of the Mediterranean, the Natal fruit fly *C. rosa* is not and there are concerns that the phytosanitary status of this pest may increase in the future. The same control measures have successfully been used for both these *Ceratitis* species in the past and both have been monitored by use of Capilure (trimedlure plus extender) or Questlure in dry Sensus traps. However, recent unpublished research by Ware and Daneel indicated that Natal fruit fly may not be as attracted to the trap attractants or the baits used for control as Medfly is. The trap thresholds used in Sensus traps have never been correlated with actual numbers of flies present in citrus orchards so it is not known whether low numbers of Natal fruit fly in a trap are due to low populations in an orchard or due to poor attractiveness to the trap. Before embarking on research on control methods specifically for Natal fruit fly it was decided to try to quantify the attractiveness of the traps that would be used to determine the efficacy of control methods. For this reason the tree fumigation research below was conducted and the research to compare different trap types and lures. As there is only one registered protein hydrolysate bait on the market in South Africa, research was also conducted on other candidate products to evaluate whether they were more effective against Natal fruit fly. One of the objectives of this research was to investigate less expensive attract and kill methods than the M3, but the M3 has subsequently been modified and is being produced at a lower price so there is no longer a great need for this research.

Materials and methods

Tree fumigation

For this trial two sites were used, the packhouse orchard of Bahianinha navel oranges at Crocodile Valley Citrus Co. (Site 1) and a Delta Valencia orchard at the Lowveld College of Agriculture (Site 2). At site 1, thirteen traps (7 Questlure and 6 Capilure) were put out to monitor the orchard on a weekly basis and at site 2 eleven traps (7 Quest and 4 Capilure) were used. At both sites all traps were 30 metres apart and recharged with fresh lure every 6 weeks.

In February 2005, a citrus tree at CRI – Nelspruit was enclosed with a gazebo frame covered with plastic and plastic was placed on the ground under the tree. One hundred Medfly (50 male and 50 female) were released into the cage. The tree was then fogged for a period of 1min 38 seconds. From the released flies, 71 dead flies were retrieved (31 male and 40 female). It was then decided to lengthen the fogging period to 2 min. for the research below.

Two weeks before the fogging started seven trees were selected at both sites and were pruned to fit into a gazebo 3 m x 3 m x 2.6 m. All vegetation was removed from under each tree and on the day of fogging (2 March at site 2 and 9 March at site 1) it was ensured that no irrigation took place. Two plastic sheets were

placed under each tree to collect the flies. The tree was covered with a gazebo with mosquito netting and a plastic sheet 6 x 6m was placed on the top of the gazebo over the mosquito netting. Each tree was fogged for 2 minutes using a Swingfog Hot Fogging Machine with pyrethrin/piperonyl butoxide (5 g/20 g per l) and Dede vap (dichlorvos EC 1000 g/l) in a 1000 ml plus 8 ml mixture, respectively (Figure 3.3.6.1). The gazebo was removed 5 min later and the dead fruit flies were collected, identified and counted. The fogging was repeated before harvest; site 1 on 19 April and site 2 on 14 July.



Figure 3.3.6.1. Fogging a citrus tree under a gazebo frame covered with mosquito netting and plastic.

Comparing Sensus traps with Capilure, Ceratitislure, or Questlure with McPhail traps and Biolure

Laboratory-reared Mediterranean fruit flies were marked with Dayglo fluorescent dye (Figure 3.3.6.2) and packaged in lots of 500 (equal numbers of males and females). Protein was withheld, although the adults were allowed water and granulated white sugar *ad lib*. The flies were released 2-3 days after eclosion. Natal fruit fly and Marula fruit fly were treated in the same way.

The flies were transported to the release site, a harvested mango orchard at Neos Estates in the Onderberg area of Mpumalanga Province. A single lot of Mediterranean fruit flies was placed under a tree in a selected row (designated the release row). The lid of the holding container was removed and the flies were allowed to disperse naturally. Marula fruit flies were released under the adjacent tree and Natal fruit fly under the following tree. The sequence of releases was repeated 18 times and a total of 9 000 individuals of each species were released. Releases were made between 10h00 and 11h00 during fine weather. The trial was repeated three times on 27 May, 9 June and 15 July 2005.

Traps were emptied before each release of the flies. Sensus traps were placed in trees that were two rows east of the release row. A Sensus trap containing Questlure was placed in the first tree. Three trees further down the row a Sensus trap with Ceratitislure was hung and a further three trees down the row a Sensus-Capilure trap was used. The sequence was repeated until six traps of each Sensus-lure combination were positioned. The arrangement of Sensus traps with lures was repeated two rows west of the release row. A modified plastic McPhail trap containing Biolure was placed 12 trees in from the first tree in the fourth row from the release row on the east side. A second Biolure trap was placed in a similar position to the first but in the twelfth tree from the last tree in the fourth row from the release row on the west side. All lures were replaced every six weeks.

The traps were monitored and emptied daily for the first six days after a release of flies and weekly thereafter. All flies caught were taken to the laboratory where they were checked for the presence of dye and identified to species and sexed. The recovery rate for the flies and trap types could then be determined.



Figure 3.3.6.2. Adult male Medfly and pupa marked with fluorescent powder

A comparison of alternative protein hydrolysate products

Buminal is no longer manufactured and only Hym lure remains as a registered protein hydrolysate for inclusion in fruit fly baits. Although there is no scientific evidence that Hym lure is ineffective, there is a feeling in the field that the product is not doing a satisfactory job. Green Trading CC has produced some candidate alternative products and these were tested.

Two millilitres of neat product was placed into a sponge in Sensus traps. A dichlorvos tablet was used as the toxicant in each trap. Four traps of each product (Questlure, Buminal, Hym lure, Camouflage, PI, PI&ST) were hung out in a variety orchard (grapefruit, Empress mandarins, Ellendale, Clementine, and Satsuma) at the Lowveld College of Agriculture near Nelspruit. The traps were emptied each week and the number, species and sex of flies caught were determined. The positions of the traps were moved each week and the trial was terminated after 5 weeks.

Results and discussion

Tree fumigation

The fumigation of seven trees per site resulted in negligible numbers of Medfly and Natal fruit fly being recovered relative to the numbers of Marula fruit fly (Table 3.3.6.1). This trend is supported by the Questlure trap counts in Figures 3.3.6.4 and 3.3.6.6 for the March fumigations, weakly supported by the July fumigations but not apparently supported by the April fumigations. The high numbers of Marula fly in March at site 2 are especially well represented in the trap counts in Figure 3.3.6.6 so it is unlikely that the differences in fumigation results are due to differences between the species in diurnal behaviour. Unfortunately, Natal fruit fly was the least abundant species in these trials and the single fly recovered under one tree in one of the fumigations could not be used to determine any relationship between numbers of flies caught in traps and numbers collected from fumigated trees.

Table 3.3.6.1. Percentage trees infested and total numbers of fruit flies collected after fogging

Site & date	<i>Ceratitis capitata</i>				<i>Ceratitis rosa</i>				<i>Ceratitis cosyra</i>			
	Males		Females		Males		Females		Males		Females	
	% Trees infested	Total flies	% Trees infested	Total flies	% Trees infested	Total flies	% Trees infested	Total flies	% Trees infested	Total flies	% Trees infested	Total flies
1:9/3	0	0	0	0	0	0	0	0	29	3	57	5
1:19/4	0	0	0	0	0	0	0	0	43	7	29	3
2:3/3	0	0	0	0	14	1	0	0	71	32	86	17
2:14/7	14	2	14	1	0	0	0	0	29	3	0	0

The data in Table 3.3.6.2 suggest that the numbers of Medfly caught in traps with Capilure relative to the numbers of flies recovered under fumigated trees tend to be higher than the Capilure trap count for Natal fruit fly when a single fly was recovered after fumigation. However, more data are required to substantiate this. The fact that Capilure does not attract Marula fruit fly (White and Elson-Harris, 1992) is supported by the data in Table 3.3.6.2 and Figures 3.3.6.3 and 3.3.6.5.

Table 3.3.6.2. Comparison of mean number of flies trapped per day during the week that fumigation took place with the mean number of flies recovered per tree after fumigation

Site	Date	Fly source	<i>Ceratitis capitata</i>		<i>Ceratitis rosa</i>		<i>Ceratitis cosyra</i>	
			Males	Females	Males	Females	Males	Females
1	9 Mar	Capilure	0.93	0.00	0.26	0.00	0.00	0.02
		Questlure	0.08	0.10	0.08	0.10	0.18	0.31
		Tree fog	0.00	0.00	0.00	0.00	0.43	0.71
	19 Apr	Capilure	1.12	0.00	0.17	0.00	0.00	0.00
		Questlure	0.16	0.20	0.06	0.08	0.02	0.04
		Tree fog	0.00	0.00	0.00	0.00	1.00	0.43
2	3 Mar	Capilure	0.67	0.00	0.42	0.00	0.00	0.08
		Questlure	0.07	0.12	0.12	0.19	2.17	1.86
		Tree fog	0.00	0.00	0.14	0.00	4.57	2.43
	14 Jul	Capilure	1.61	0.00	0.07	0.00	0.00	0.00
		Questlure	0.06	0.43	0.02	0.08	0.04	0.18
		Tree fog	0.29	0.14	0.00	0.00	0.43	0.00

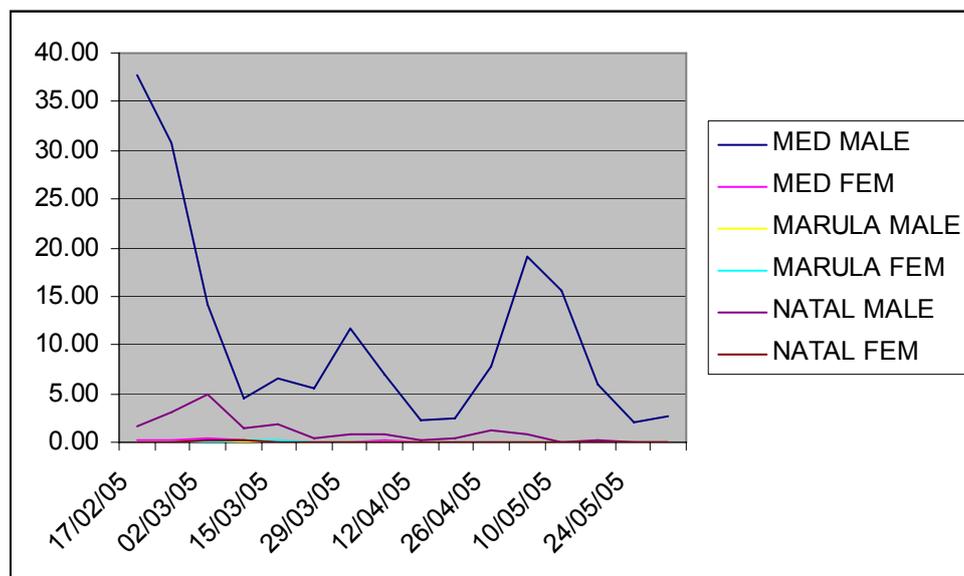


Fig. 3.3.6.3. Mean number of fruit flies caught per trap per week with Capilure at site 1.

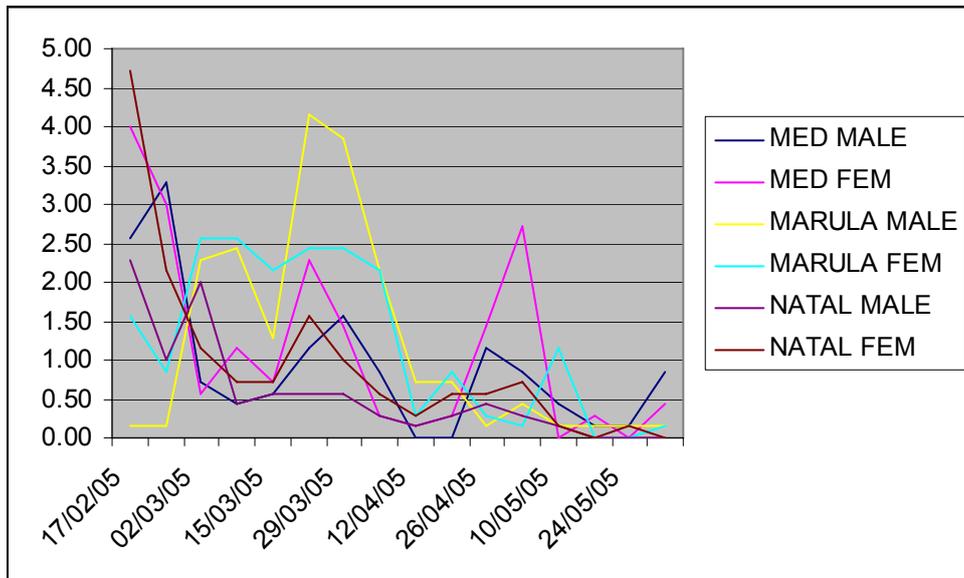


Fig. 3.3.6.4. Mean number of fruit flies caught per trap per week with Questlure at site 1.

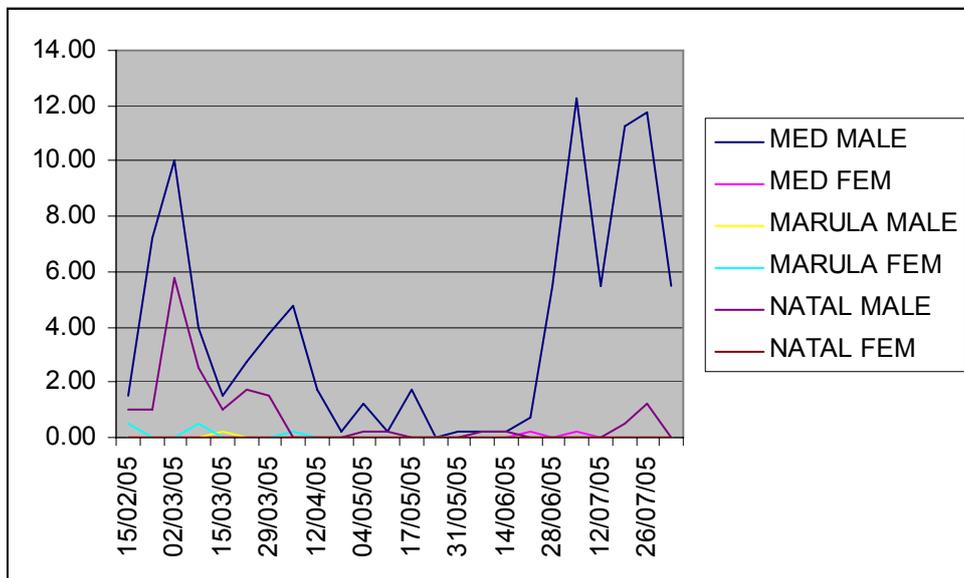


Fig. 3.3.6.5. Mean number of fruit flies caught per trap per week with Capilure at site 2.

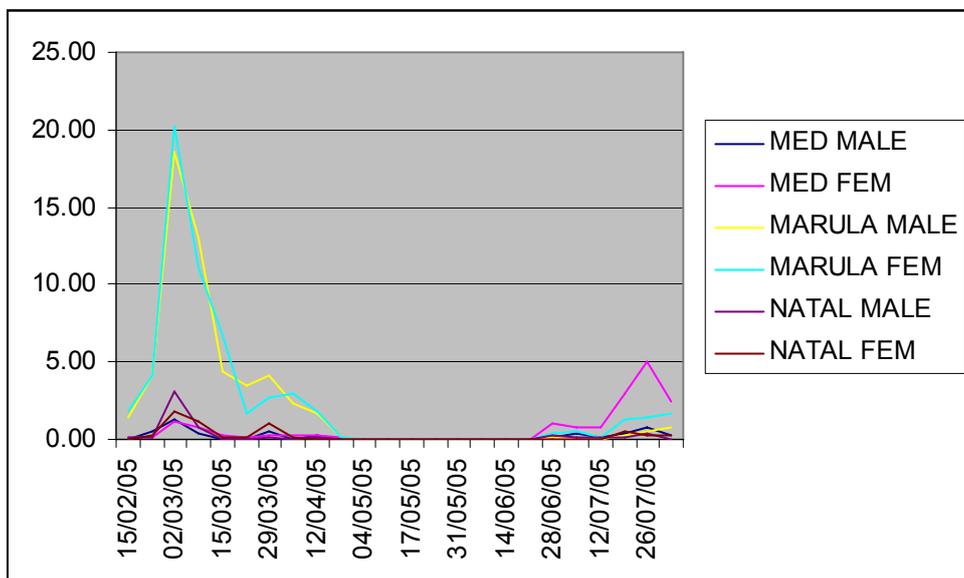


Fig. 3.3.6.6. Mean number of fruit flies caught per trap per week with Questlure at site 2.

Comparing Sensus traps with Capilure, Ceratitislure, or Questlure with McPhail traps and Biolure

Of the 81 000 fruit flies released, 7 356 flies were recaptured. Mediterranean fruit flies made up 69.4% of this number, Natal fruit flies 16.6% and Marula fruit flies 14%. The Sensus-Ceratitislure combination caught 18.8%, the Sensus-Capilure 16.7%, the Sensus-Questlure 5.7% and the McPhail-Biolure 58.8% of the flies that were recovered (Table 3.3.6.3). Marula fruit fly was neither attracted to the McPhail-Biolure traps nor the Sensus-Questlure traps.

Table 3.3.6.3. Mean numbers of marked-released-recaptured fruit flies/trap-lure combination expressed as a percentage of the total flies recovered.

Species	Sensus-Capilure	Sensus-Ceratitislure	Sensus-Questlure	McPhail-Biolure
<i>C. capitata</i>	16.4	11.3	2.1	33.5
<i>C. rosa</i>	0.3	0.7	3.5	24.1
<i>C. cosyra</i>	0	6.8	0.1	1.2
Total	16.7	18.8	5.7	58.8

Sensus-Capilure was the most effective trap-lure combination for Mediterranean fruit fly males and McPhail-Biolure for Mediterranean fruit fly females (Table 3.3.6.4). The McPhail-Biolure was the most successful trap-lure combination for both sexes of Natal fruit fly while the Sensus-Ceratitislure combination was the best combination for Marula fruit fly males and the McPhail-Biolure for Marula fruit fly females (although numbers were relatively low).

Table 3.3.6.4. Sex and percentage recovery of mark-release-recapture fruit flies in the various trap-lure combinations

Species	Mean no. flies trapped/release	Sex	Sensus-Capilure	Sensus-Ceratitislure	Sensus-Questlure	McPhail-Biolure
<i>C. capitata</i>	337.3	♂	50.6	31.8	0.7	16.9
	424.1	♀	6.3	6.7	5.4	81.6
<i>C. rosa</i>	160.3	♂	2	3.4	12.6	82
	182.7	♀	0.2	1.5	11.8	86.5
<i>C. cosyra</i>	86.9	♂	0	93.8	0.5	5.8
	10.9	♀	0	4.6	8.4	87

Both Questlure and Biolure are food attractants designed to attract female fruit flies. Previous research (Ware *et al.*, 2003 – CRI Group Annual research Report) showed that Questlure generally attracts one Mediterranean fruit fly male for every four Mediterranean fruit fly females while the ratio for Natal fruit fly was 1 male: 2 females. These results were obtained from naturally occurring populations of which the demographics were unknown. In this mark-release-recapture exercise Questlure was shown to have attracted 1 Mediterranean fruit fly male to 8.5 Mediterranean fruit fly females and 1 Natal fruit fly male to 1.03 females (although the released Natal fruit fly females were not yet old enough to oviposit so may have been less interested in protein attractants than wild flies of variable age). The relative numbers of flies trapped in the McPhail-Biolure trap combination were similar to those obtained from the Sensus-Questlure combination (Tables 3.3.6.3 and 3.3.6.5). Few Marula fruit flies were trapped in these trap-lure combinations indicating that they are unsuitable for the monitoring of this species.

Table 3.3.6.5. Mark-release-recapture of three fruit fly species in Sensus-Questlure and McPhail-Biolure traps (%)

Species	Sex	Sensus-Questlure	McPhail-Biolure
<i>C. capitata</i>	♂	4	8
	♀	34	49
<i>C. rosa</i>	♂	30	19
	♀	31	22
<i>C. cosyra</i>	♂	1	1
	♀	1	1

Comparisons between the numbers of flies trapped in the various trap-lure combinations indicate that the McPhail-Biolure combination was the most effective (Table 3.3.6.3). These traps caught 10 times the

number of flies that the Sensus-Questlure traps did, 3.5 times that of the Sensus-Capilure and 3.1 times that of the Sensus-Ceratitislure trap-lures. The result is considered conservative as the McPhail-Biolure traps were placed at twice the distance from the release row as the Sensus traps and the flies would have had to pass through the barrier of Sensus traps before finding the McPhail traps. A negative aspect of the McPhail trap was that approximately 50% of the insects caught were NOT tephritids.

As can be seen from Table 3.3.6.4, most of the flies caught in the Sensus-Capilure traps were Mediterranean fruit fly and very few Natal fruit fly. However, as these traps were in the proximity of the McPhail-Biolure traps that were more attractive to male Natal fruit fly than male Medfly (Table 3.3.6.5), the relative attractiveness of Sensus-Capilure to Medfly and Natal fruit fly males cannot be determined from these data. Few Marula fruit flies were caught using Sensus-Capilure, a result known from literature (White & Elson-Harris, 1992).

Some aspects of this work should be repeated with more physiologically mature flies and in the absence of competitive traps before a possible adjustment to the treatment threshold for Natal fruit fly is considered.

A comparison of alternative protein hydrolysate products

A total of 2247 flies were trapped over the five-week sampling period. The species distribution was 8.5% Mediterranean fruit fly, 41.2% Natal fruit fly and 50.3% Marula fruit fly. The sex ratio of all the flies was 1 male to 1.71 females. Among the species this ratio was 1: 3.8 for Mediterranean fruit fly, 1: 1.32 for Natal fruit fly and 1: 1.9 for Marula fruit fly. There was a difference between species in the ratio of males and females trapped (Table 3.3.6.6).

Table 3.3.6.6. Sex ratio (male: female) fruit fly trap catches using several different protein hydrolysate lures.

Products	Mediterranean fruit fly	Natal fruit fly	Marula fruit fly
Quest	1: 4.79	1: 1.95	1: 1.87
Buminal	1: 3.00	1: 1.17	1: 1.95
Camouflage	1: 3.38	1: 1.45	1: 1.53
Hym lure	1: 1.67	1: 1.81	1: 3.08
PI	1: 4.33	1: 1.38	1: 1.64
PI & ST	1: 4.75	1: 0.97	1: 1.94

Figure 3.3.6.7 shows that Buminal outperformed the other products in attracting both males and females of Marula fruit fly. Hym lure and PI were similar in their attraction to this species. The addition of ST to PI improved the attractiveness of PI. Camouflage was superior to both Hym lure and PI but inferior to Buminal.

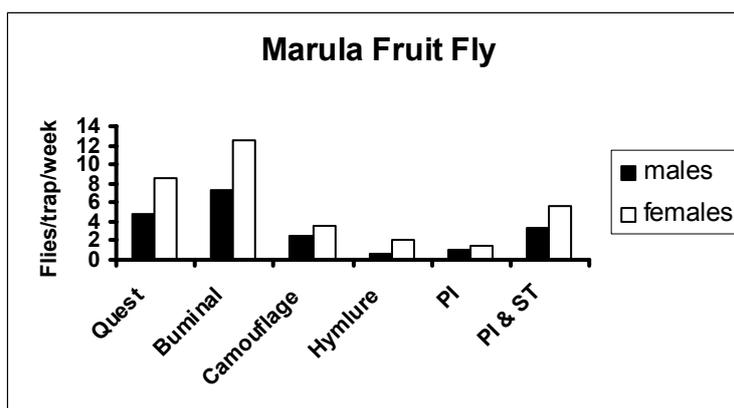


Fig. 3.3.6.7. Marula fruit fly catches in Sensus traps containing various protein hydrolysate formulations.

Natal fruit fly trap catches are shown in Figure 3.3.6.8. Questlure appeared to be more attractive to females (but less attractive to males) than Buminal. In comparison with the Buminal Natal fruit fly trap catches, Hym lure attracted about half the number of flies. There was a large increase in the number of flies caught when ST was added to PI. This product outperformed Questlure and should be considered as a replacement for Buminal. Camouflage was similar to Hym lure but better than PI in attracting Natal fruit fly.

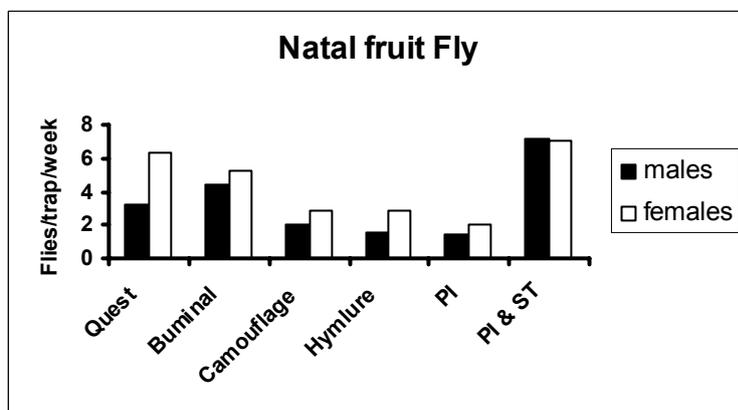


Fig. 3.3.6.8. Natal fruit fly catches in Sensus traps containing various protein hydrolysate formulations.

Mediterranean fruit fly trap catches are illustrated in Figure 3.3.6.9. Questlure outperformed the other products. Camouflage caught the next most flies. There was little difference between the Buminal and Hymlure trap catches. The addition of ST to PI did little in increasing the attractiveness of the PI base product.

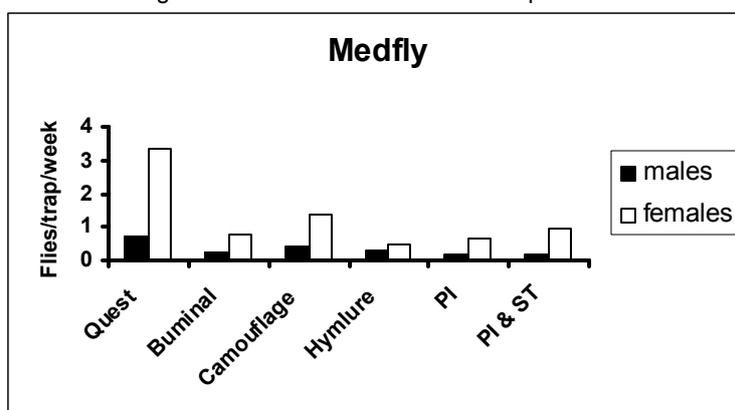


Fig. 3.3.6.9. Mediterranean fruit fly catches in Sensus traps containing various protein hydrolysate formulations.

Conclusions

The relative abundance of fruit fly species knocked down from orchard trees by use of fumigation showed similar trends to the use of Capilure and Questlure in Sensus dry traps. However, numbers of Medfly and Natal fruit fly were too low to explore this relationship further. Comparisons of trap-lure combinations when known numbers of laboratory-reared flies were released indicated that Sensus-Capilure caught many times more male Medfly than male Natal fruit fly in the presence of other trap-lure combinations, but this work needs to be repeated with more mature Natal fruit flies.

The alternative protein hydrolysates Camouflage and PI-ST were superior to the registered Hymlure bait for Natal fruit fly and should be investigated further.

Future research

Further research on the correlation between actual numbers of Natal fruit fly in the orchard and trap catches should be conducted in an orchard with high numbers of Natal fruit fly. Further research on alternative baits should be conducted.

3.3.7 Verification of Cold Treatment Disinfestation of Medfly-infested Clementines destined for Japan

By A. Ware, B. Tate, P. Stephen, J-H. Daneel and R. Beck (CRI) and T. Misumi (MAFF)

Opsomming

'n Japanse waarnemer is na Suid Afrika gestuur om die resultate van vorige navorsing oor koue behandeling te verifieer. Die navorsing is gerig op die ontwikkeling van 'n behandeling vir die ontsmetting van Clementines van Medvlieg wat van Suid Afrika na Japan uitgevoer sou word. Die twee proewe wat

uitgevoer is, het bevestig dat die ontsmettingsperiode van 14 dae genoegsame fitosekureit vir Japan bied om die toevallige invoer van Medvlieg deur Clementine uitvoere van Suid Afrika te verhoed. Nie een van die 45 900 larwes wat behandel is met 'n gemiddelde temperatuur van -0.37°C oor 13 dae en 22 uur het oorleef nie.

Introduction

The Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) requested that they be allowed to observe a large scale, cold treatment disinfestation trial of Medfly-infested Clementines in order that they may have enough data to defend, in an open forum, the South African proposal to export Clementines into Japan. A detailed itinerary was received from MAFF and the research was initiated in accordance thereof on 24 May 2005. The Japanese observer, Mr Takashi Misumi, arrived in South Africa on 8 June 2005. From the 12-15 June he inspected the soft citrus production area in the Western Cape. On the 22 June he inspected a grapefruit production area in the Onderberg near Malelane (Neos Estates) and a packhouse (Neo Nova) at Hectorspruit. The rest of the time was spent at the Citrus Research International (Pty) Ltd laboratories in Nelspruit where he observed, and participated in, the research conducted to verify previous research findings.

Materials and methods

The rearing technique used for Mediterranean fruit fly has been reported previously in a report submitted to MAFF (Ware et al. 1999). Nules Clementine mandarins were sourced from Naranja Packhouse in the Burgersfort area on 20 May 2005. They were kept at 4°C until required. The inoculation procedure was similar to that reported previously (Ware et al. 1999). Differences to the previously published method were: (1) Instead of a cork borer, an electric drill was used to make holes in the fruit. This method is not only more time efficient but the skin is removed, thereby diminishing the chance of fungal spores entering the fruit. (2) A small amount ($200\mu\text{l}$) of yeast hydrolysate enzymatic (catalysed brewers yeast - ICN Biomedicals INC) mixed in water was placed in each fruit. The addition of this small amount of protein was found to improve larval survival. (3) In the place of Clementine peel pieces, cotton wool was used to plug the wound. This was found to be firstly more efficient and secondly a more sterile method that resulted in better quality fruit.

After six days at 26°C , the treatment fruit was transferred to the cold chamber. The untreated control fruit remained at 26°C for a further two days after which it was dissected in the laboratory. The placement of the thermoprobes, which was the same for both replicates, is shown in Figure 3.3.7.1. A precooling period of 44 hours was required for replicate 1 and 64 hours for replicate 2. The fruit was removed from the cold chamber two hours before the 14-day treatment period had been completed and placed into a holding room set at a nominal 26°C . The early removal of the fruit ensured that the treatment period was not exceeded thereby avoiding potential invalidation of the experiment. The data logger was then stopped and all the thermoprobe data processed.

The trial commenced according to the schedule supplied by MAFF even though confirmation on the starting point of treatment had not been received. In the absence of this instruction the starting point that currently applies to commercial shipping of oranges, grapefruit and lemons from South Africa to Japan, was used as a starting point in the trial. Accordingly, the cold disinfestation treatment was deemed to begin once 50% or more of the thermoprobes had reached -0.6°C (corrected for individual thermoprobe variance using the method previously published (Ware et al. 1999)). The cold room temperature was then adjusted so that a long-term average temperature of greater than -0.4°C was obtained. At the request of Mr Misumi the starting point of the second replicate was modified and the trial was deemed to have begun once 50% or more of the thermoprobes had registered a corrected temperature of -0.4°C or less. Both experiments were terminated two hours before the completion of the 14 days treatment.

Each person dissecting the fruit removed a single lug box of fruit (approximately 100 fruit) from the holding room at a time. Messrs. Tate, Daneel, Stephen and Ware dissected the untreated control fruit from the first replicate and the cold treated fruit from the second replicate experiment and examined them for the presence of live larvae using stereomicroscopes. Mr Misumi helped process the cold treated fruit from the first replicate and the untreated control fruit from the second replicate.

A video detailing the rearing of fruit fly, inoculation procedure, calibration process and dissection of fruit, was given to the Japanese observer (Mr Misumi) and will be shown at the CRI citrus research symposium in 2006.

Results and discussion

The calibration factors of the individual thermoprobes are shown in Table 3.3.7.1. In the second of the replicated experiments, thermoprobe number 5 was replaced during the calibration process and the calibration factor for this probe was calculated from 2 sets of readings. The Japanese observer was present for the calibration of the second replicate.

Table 3.3.7.1. Calibration factors used to correct individual thermoprobe variance from 0°C

Thermoprobe Number	Replicate	Run 1	Run 2	Run 3	Run 4	Run 5	Average	Correction
1	1	0.17	0.13	0.15			0.15	-0.1
2	1	0.18	0.13	0.12			0.14	-0.1
3	1	0.08	0.08	0.08			0.08	-0.1
4	1	-0.01	0.00	-0.01			-0.01	0.0
5	1	1.58	1.61	1.65			1.61	-1.6
6	1	1.61	1.53	1.57			1.57	-1.6
7	1	1.38	1.37	1.40			1.38	-1.4
8	1	1.21	1.20	1.26			1.22	-1.2
9	1	0.90	0.89	0.91			0.90	-0.9
10	1	0.82	0.82	0.81			0.81	-0.8
11	1	0.49	0.51	0.50			0.50	-0.5
12	1	0.20	0.20	0.23			0.21	-0.2
13	1	1.38	1.39	1.46			1.41	-1.4
14	1	1.17	1.09	1.17			1.14	-1.1
15	1	0.70	0.62	0.69			0.67	-0.7
16	1	0.46	0.41	0.41			0.43	-0.4
1	2	0.15	0.11	0.19	0.21	0.26	0.19	-0.2
2	2	0.31	0.11	0.17	0.26	0.30	0.23	-0.2
3	2	0.12	0.06	0.09	0.03	0.02	0.06	-0.1
4	2	-0.07	-0.07	-0.09	-0.10	-0.14	-0.09	0.1
5	2				2.01	2.11	2.06	-2.1
6	2	1.75	1.81	1.80	1.89	1.94	1.84	-1.8
7	2	1.62	1.65	1.65	1.76	1.79	1.70	-1.7
8	2	1.30	1.47	1.33	1.40	1.46	1.39	-1.4
9	2	1.13	0.95	0.95	1.00	1.04	1.01	-1.0
10	2	0.73	0.68	0.95	0.80	0.80	0.73	-0.7
11	2	0.50	0.49	0.41	0.47	0.46	0.47	-0.5
12	2	0.02	0.03	0.00	0.04	0.00	0.02	0.0
13	2	1.70	1.71	1.69	1.80	1.88	1.76	-1.8
14	2	1.35	1.30	1.29	1.34	1.40	1.34	-1.3
15	2	0.86	0.85	0.89	0.90	0.92	0.89	-0.9
16	2	0.47	0.45	0.45	0.47	0.49	0.47	-0.5

The control fruit was dissected after 6 days larval growth at 26°C. Messrs. Tate and Ware processed the 510 inoculated fruit from the first replicate over two days and determined that there were an average of 9.31 live larvae/fruit. Messrs. Tate, Daneel, Stephen, Misumi and Ware processed the fruit from the second replicate and determined that there was an average of slightly less than 13.0 live larvae per fruit (n=519).

Mr. Misumi also determined the number of larvae in each life stage (first instar 44 (36%); second instar 37 (30%) and third instar 42 (34%); n=6 fruit). This translates to 64% of the insects being in the targeted life stage (young larvae) and more than 17 000 individuals of this life stage were subjected to the cold treatment.

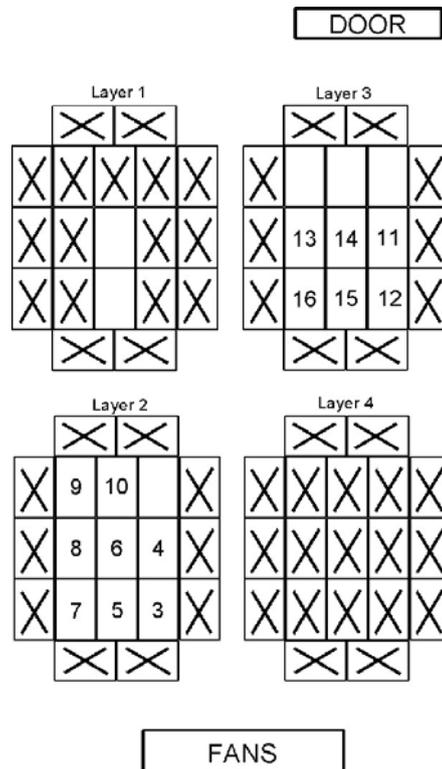


Figure 3.3.7.1. Placement of thermoprobes. Layer 1 was at the bottom and layer 4 at the top.

All temperature measurements reported are corrected values. The individual probe average and standard deviation is presented in Figure 3.3.7.2 (replicate 1) and Figure 3.3.7.3 (replicate 2). The mean daily average, the daily high and daily low thermoprobe readings for the first replicate are illustrated in Figure 3.3.7.4 and for the second in Figure 3.3.7.5. The long-term mean of replicate 1 was -0.373°C with a standard deviation of 0.49°C and the mean of replicate 2 was -0.374°C with a standard deviation of 0.34°C . The corrected data sets are presented in electronic format and accompany this report.

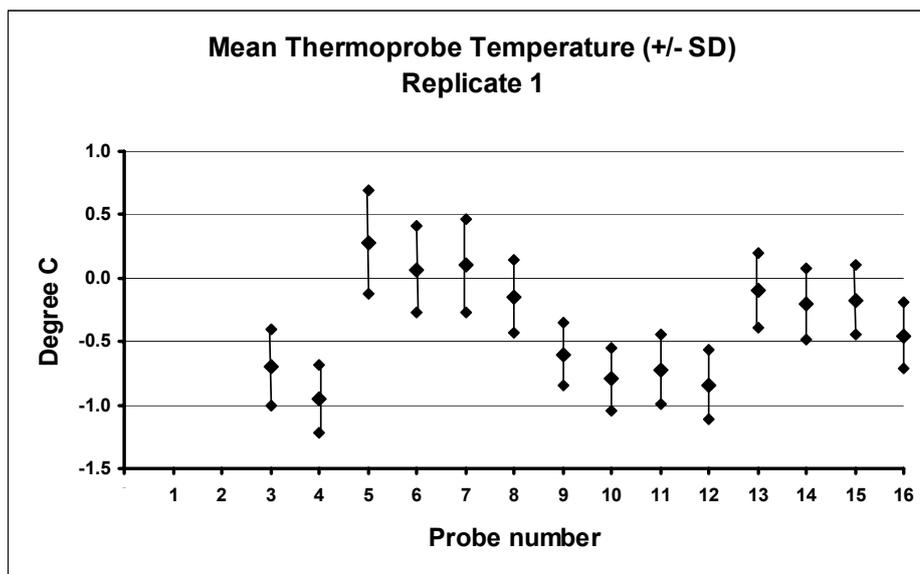


Figure 3.3.7.2. Thermoprobe mean temperatures (corrected) measured in the Clementine fruit core (replicate 1)

It is evident from these readings that two sides of the room (with probes 3,4,9,10,11,12) were colder than the other side (probes 5,6,7,8,13,14,15,16) (Figures 1 and 2). No such pattern was discernable in replicate 2 (Figures 1 and 3).

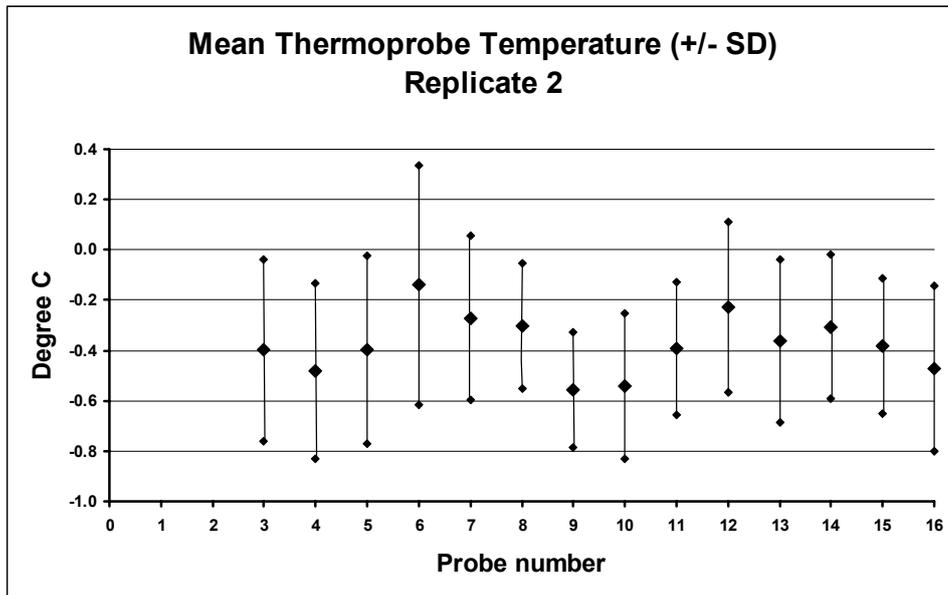


Figure 3.3.7.3. Thermoprobe mean temperatures (corrected) measured in the Clementine fruit core (replicate 2)

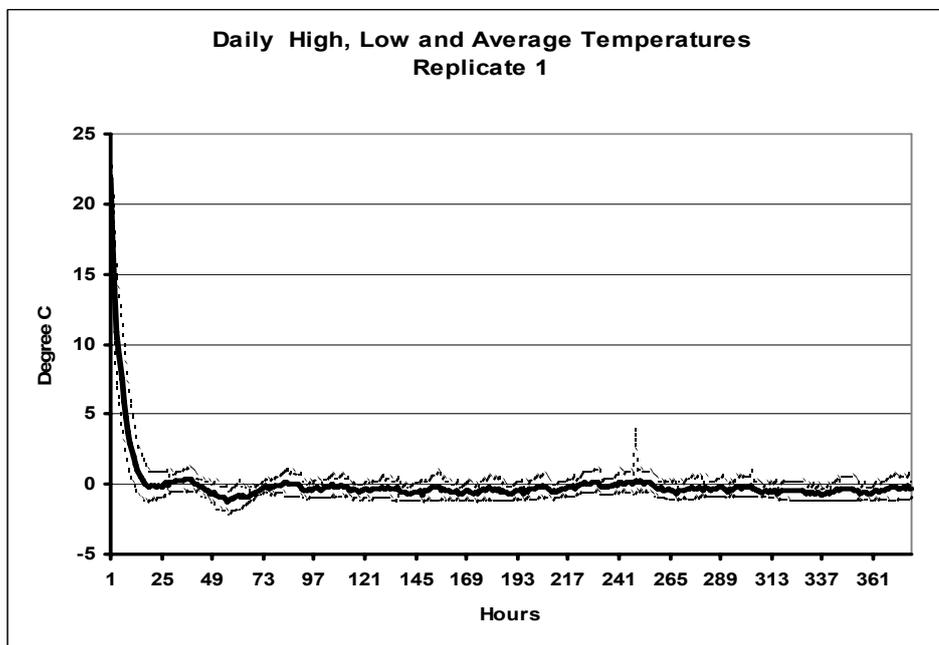


Figure 3.3.7.4. Daily high, low and mean temperatures (corrected) measured in the core of the fruit over the trial period (replicate 1).

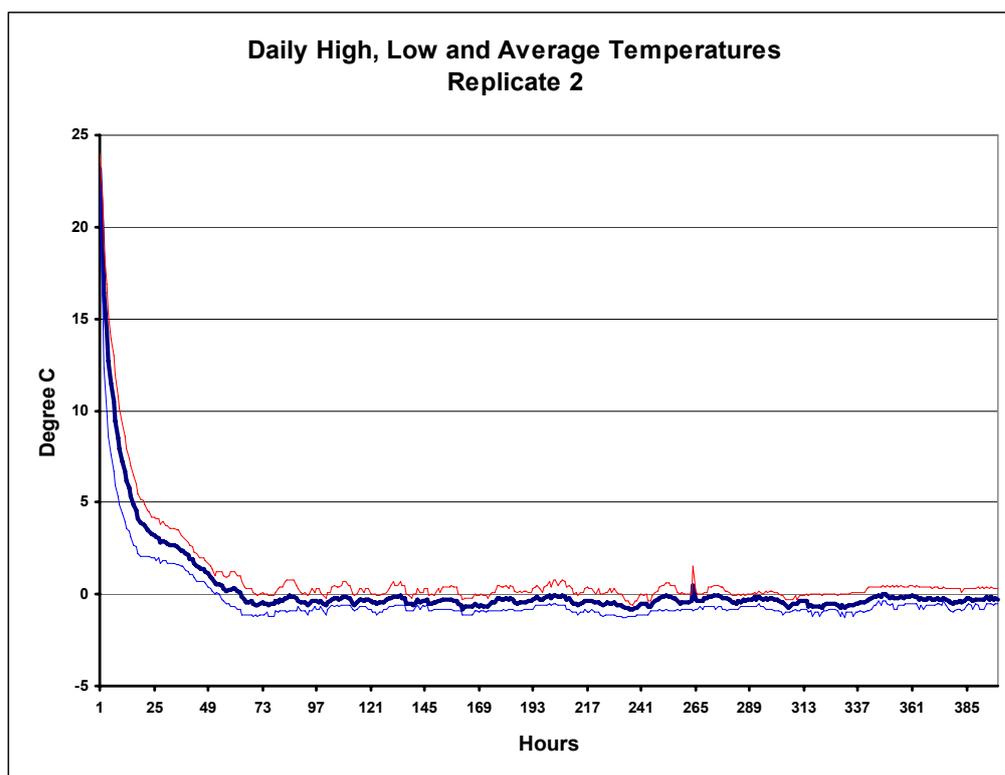


Figure 3.3.7.5. Daily high, low and mean temperatures (corrected) measured in the core of the fruit over the trial period (replicate 2).

No live tephritid larvae were found during the three days it took to process each of the replicates. However, a single fruit contained a few larvae of *Drosophila melanogaster* Meigen. Some adult flies had found their way to the fruit through a small hole in the wall of the 26°C holding room. They are easily distinguished from *C. capitata* by their relatively small size and the two prominent spiracle tubes (Figure 3.3.7.6).

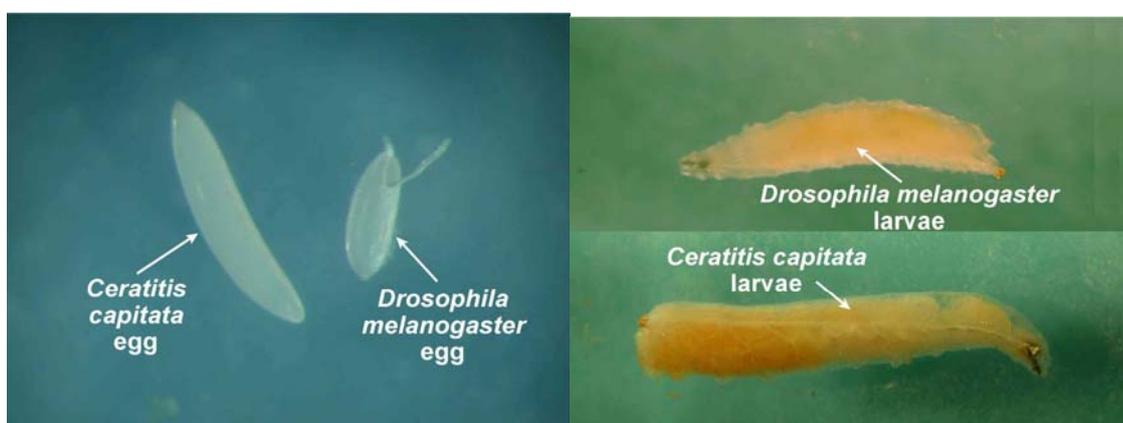


Figure 3.3.7.6. *Drosophila melanogaster* and *Ceratitidis capitata* eggs and larvae

No larvae from the approximately 45 900 treated survived the cold treatment, thereby verifying the previous research results (Ware & Joubert 2000) and satisfying the phytosanitary requirements of Japan that there be no survivors in 30 000 individuals treated (Table 3.3.7.2).

Table 3.3.7.2. The survival of young Mediterranean fruit fly larvae after 14 days cold treatment.

Replicate	Control		Treated fruit			
	Number of fruit	Number of live insects	Number of fruit	Estimated number of larvae treated	Number of survivors	Mortality (%)
1	510	4750	2024	18823	0	100
2	519	6724	2090	27077	0	100
Total	1029	11474	4114	45900	0	100

Conclusion

There were no survivors. The 14-day disinfestation period at an average temperature of -0.37°C thereby satisfies the phytosanitary security requirements of the Japanese (no survivors in 30 000 individuals treated).

References cited

Ware, A., Joubert, J. and Stephen, P. 1999. Cold sterilization of Mediterranean fruit fly-infested mandarins. pp. 72-85. Outspan Citrus Centre Annual Research Report, Nelspruit.

Ware, A. and Joubert, J. 2000. Cold sterilization of Mediterranean fruit fly-infested mandarins: repeat of phase 4 large-scale trial (addendum to main report of November 1999). pp. 51-52. CRI Annual Research Report, Nelspruit.

3.4 PROJECT: COSMETIC PESTS

Project coordinator: Tim G Grout (CRI)

3.4.1 Project summary

Cosmetic pests are sometimes overlooked in the emphasis on phytosanitary pests and market access issues but if control of these pests fails, a large proportion of the fruit will be unmarketable. There is a need for alternatives to organophosphates for the control of these pests and this aspect has been under investigation for citrus thrips (3.4.2) and bollworm (3.4.4) for some time. The thrips research has now been concluded and although there can now be a reduction in the amount of oil used with abamectin, no new IPM-compatible thripicides were found. This means that resistance management is critical for products such as abamectin. An Australian nuclearpolyhedrovirus for bollworm is looking very promising as an alternative treatment for this pest and there may soon be enough trial results for registration on citrus (3.4.4). Progress is still slow in developing a rearing technique for the parasitoid of citrus thrips but at least it seems that a reliable technique has been developed for rearing the citrus thrips themselves that was the main stumbling block (3.4.3). Research was requested to investigate the cause of necrotic lesions on Empress mandarins and some other cultivars near Burgersfort. It was thought that they may be caused by stinkbugs or fruit piercing moths but various investigations ruled out involvement by these insects and it is now thought that it may be a pathological problem.

Projekopsomming

Die belangrikheid van kosmetiese plae word soms in die skadu gestel deur fitosanitêre peste en marktoegangsaspekte. Onvoldoende beheer van die plae sal egter daartoe lei dat 'n groot gedeelte van vrugte nie bemark sal kan word nie. 'n Behoeftes ontstaan om alternatiewe vir organofosfate te vind vir die beheer van die plae en die aspek word al vir 'n geruime tyd ondersoek vir sitrus blaaspootjie (3.4.2) en bolwurm (3.4.4). Alhoewel die navorsing met blaaspootjies voltooi is, is geen nuwe IPB-verenigbare middels gevind nie. Die bestuur om ontwikkeling van weerstand teen bestaande middels soos Abamectin te verhoed, is dus van kardinale belang. Die navorsing het egter bewys dat dit nou moontlik is om minder olie saam met Abamectin te gebruik. In Australië is 'n "nuclearpolyhedrovirus" as alternatiewe behandeling teen bolwurm ontwikkel, wat belowende resultate toon. Genoegsame toetsresultate mag binnekort beskikbaar wees wat registrasie op sitrus moontlik sal maak (3.4.4). Die vordering vir die ontwikkeling van 'n tegniek om parasitoïede van sitrus blaaspootjie te teel vorder nog steeds nie na wense nie. Dit blyk egter dat die grootste struikelblok oorkom is deurdat 'n betroubare tegniek gevind is vir die teling van blaaspootjies self (3.4.3). Navorsing om die oorsaak te bepaal van nekrotiese letsels wat op Empress mandaryne asook op ander kultivars gevind is naby Burgersfort, is ook aangevra. Die oorsaak is aanvanklik toegeskryf aan stinkbesies of vrugtesteekmotte maar verskeie ondersoeke het hierdie moontlikheid uitgeskakel. Die vermoede bestaan nou dat die simptome moontlik patogenies van aard mag wees.

3.4.2 OP alternatives for citrus thrips, compatible with bio-intensive IPM or organic production Experiment 713 by Tim G Grout, Peter Stephen and Bruce Tate (CRI)

Opsomming

Verdere navorsing in die soektog na alternatiewe vir organofosfate is uitgevoer vir die beheer van die sitrus blaaspootjie, *Scirtothrips aurantii*, in sitrusboorde as deel van geïntegreerde plaagbestuur. Een van die twee proefpersele moes geëlimineer word weens 'n gebrek aan blaaspootjies en by die ander perseel was die getalle baie laag. Vorige resultate wat getoon het dat die konsentrasie olie wat saam met Abamectin gebruik moet word, verminder kan word van 0.3% na 0.15% sonder die verlies aan effektiwiteit teen sitrus blaaspootjie, is bevestig. Die natter WetCit teen 100 ml/hl, kan ook as alternatief vir olie saam met Abamectin gebruik word. Expellar, met 10 g/l rotenone teen 500 ml/hl water was nie effektief teen blaaspootjie nie. Hunter, gemeng met suiker was effektief as 'n lokaasmiddel vir blaaspootjies maar het voorheen sekere nagevolge tot gevolg gehad. Geen verskille in effektiwiteit is gevind tussen bruin en wit suiker wanneer dit gemeng was met braakwynsteen nie, maar verdere bevestiging is egter nodig in die teenwoordigheid van hoër sitrus blaaspootjie populasie digthede. Die navorsing is nou gestaak maar evaluasies sal hervat word sodra nuwe belowende produkte beskikbaar gestel word.

Introduction

Although the pest status of citrus thrips has declined in recent years relative to false codling moth and fruit fly, the control of this cosmetic pest remains essential for export quality fruit. In most citrus production regions, populations of citrus thrips have not been reaching the levels that they were 10 years ago and this may be partly due to increased numbers of various natural enemies in summer, thanks to less disruptive spray programmes. The citrus industry is now relying heavily on abamectin plus oil for citrus thrips control and the selection pressure for resistance is high. Alternative, IPM-compatible thripicides are required. In addition, the cost of oil used with abamectin is escalating and alternative adjuvants should be investigated. Last year, a lower concentration of oil was effective when used with abamectin (Grout et al. 2005) and the adjuvant NuFilm 17 was also found to be a suitable alternative to oil. Some of these aspects required further investigation this year. The question of whether unrefined sugar can be used with tartar emetic instead of white sugar also required further investigation.

Materials and methods

Two sites were used in the vicinity of Burgersfort where populations of citrus thrips have been high in the last three years. A Valencia orchard at Danie Schutte's farm and another Valencia orchard at Frans Winterbach's farm were chosen. At Winterbach, the orchard was divided into two zones and each zone subdivided into nine treatment blocks. Each block was three rows wide and at least seven trees long. Treatments were allocated randomly to the blocks in each zone. Treatments were applied by hand at high pressure (30 bar) on 5, 7 and 12 October 2005. Spraying was protracted due to problems with wind and a delay in obtaining the Expellar. At Schutte a similar randomised block layout was used but the blocks were four rows wide and 11 trees long. Sprays at that site were applied on 24 and 25 October 2005 (see Table 3.4.2.1 for treatment details). Both sites were then checked for citrus thrips infestation in the control blocks every week until numbers of thrips were considered adequate for an evaluation. Surprisingly, numbers of citrus thrips did not increase at Winterbach and were too low to warrant reapplication of treatments so that site had to be abandoned. The increase in citrus thrips at Schutte was also slower than expected but a single evaluation was conducted on 15 November 2005 using eight data trees from the central two rows in each block and inspecting 20 outside fruit on each tree. The number of fruit infested with larvae and the number infested with adults were recorded separately in addition to the number of fruit showing any sign of scarring by citrus thrips. The results were expressed as percentages and transformed to the arc sine of the square root before conducting a two-way ANOVA. Means were compared further using Student Newman Keuls' test at $\alpha = 0.05$.

Results and discussion

At both sites, the Expellar 500 ml/hl treatment foamed excessively in the spray tank. Due to low numbers of citrus thrips, only a single evaluation was possible at Schutte and reapplications could not be justified at either site. The lowering of the oil concentration with abamectin to 150 ml/hl water instead of 300 ml/hl did not influence efficacy against citrus thrips significantly ($P > 0.05$ Table 3.4.2.1). This confirmed the results obtained at two sites last year (Grout et al. 2005) so it appears that the oil concentration can be lowered to 150 ml/hl if suppression of red scale crawlers is not required. The adjuvant EXP1500 at 300 ml/hl and WetCit at 100 ml/hl both appeared to be suitable alternatives to spray oil at 300 ml/hl against citrus thrips.

No significant difference was found between unrefined brown sugar and white sugar when used with tartar emetic (Table 3.4.2.1). Hunter plus sugar showed similar efficacy to tartar emetic plus sugar but had previously (Grout et al. 2005) resulted in pest repercussions, so is not ideal for IPM. The only significant difference among the treatments was Expellar (rotenone), which resulted in significantly more larvae than any of the other sprays and more adults than two of the abamectin sprays and one of the tartar emetic bait sprays. Expellar had once before been evaluated against citrus thrips (Grout et al. 2004) in a similar situation to this with relatively low populations of citrus thrips. Although there were no significant differences amongst the treatments based on larval infestation, the Expellar treatment had the highest numbers of citrus thrips. It therefore appears that Expellar need not be evaluated further for citrus thrips control.

This brings to an end the series of trials aimed at finding IPM-compatible thripicides as alternatives to abamectin plus oil and tartar emetic plus sugar. No new unregistered products are suitable for citrus thrips control in an IPM situation. This means that resistant management for abamectin is critical and growers must use it as little as possible and only for citrus thrips control. If new, promising products are developed, they will be evaluated.

Table 3.4.2.1. Efficacy of treatments against citrus thrips on Valencias at Schutte's farm near Burgersfort, evaluated on 15 November 2005.

Treatments (applied 24-25 Oct 2005)	Fruit with larvae (%)	Fruit with adults (%)	Fruit with thrips scars (%)
Untreated control	17.2 a	5.3 a	25.9 a
Biomectin (abamectin 18 g/l EC) 20 ml plus Orchex 300 ml/hl	0.0 c	0.0 c	3.8 b
Biomectin (abamectin 18 g/l EC) 20 ml plus Orchex 150 ml/hl	0.0 c	0.6 bc	4.4 b
Biomectin (abamectin 18 g/l EC) 20 ml plus EXP1500 300 ml/hl	0.9 c	0.6 bc	9.4 b
Biomectin (abamectin 18 g/l EC) 20 ml plus WetCit 100 ml/hl	1.3 c	0.0 c	7.8 b
Tartar emetic (995 g/kg SP) 200 g plus white sugar 200 g/hl	2.5 c	1.3 bc	8.8 b
Tartar emetic (995 g/kg SP) 200 g plus brown sugar 200 g/hl	1.6 c	0.3 c	3.8 b
Hunter (chlorfenapyr 360 g/l SC) 20 ml plus white sugar 200 g/hl	1.9 c	1.9 bc	8.8 b
Expellar (rotenone 10 g/l EC) 500 ml/hl	9.7 b	3.4 ab	10.6 b

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

Despite the low infestations of citrus thrips in this trial, other parts of the valley were highly infested with citrus thrips and populations could not be controlled with pyrethroids. A small trial was therefore conducted at one of these farms to compare the efficacy of old and new batches of Klartan with Dicarzol plus sugar applied by the grower. Mean pre-spray fruit infestation by larvae was 47%; 6 days after treatment, fruit infestation by larvae after Dicarzol (0.0125% a.i.) plus sugar was 35% and after Klartan (0.0072% a.i.) was 28%. Cross-resistance between these two products has been shown to occur in the *Scirtothrips citri* species in California. This means that in this area, spray options that can be used a few weeks after petal fall for high population densities of citrus thrips have become very limited. Reversion of resistance to pyrethroids normally occurs where pyrethroids are only sprayed once a year, allowing for continued usage. But in this region reversion does not seem to occur, possibly due to pyrethroids being used on nearby crops at different times of the year. Although macadamias are not grown in this area, they are increasingly becoming infested with citrus thrips and the use of pyrethroids for stink-bug control is selecting for resistance in the citrus thrips. Citrus thrips is therefore becoming difficult to control in citrus growing adjacent to macadamias.

Conclusion

The requirement for 0.3% oil with abamectin can be reduced to 0.15% or replaced with WetCit 0.1% if a suppressive benefit of oil on red scale crawlers is not required. There was no difference in efficacy between tartar emetic with white sugar and tartar emetic with brown sugar with these relatively low thrips populations. Expellar is not considered suitably efficacious for citrus thrips control.

Future research

No further work will be conducted until new, promising materials become available.

References cited

- Grout, T.G., Stephen, P.R. & Tate, B.A. 2004. OP alternatives for citrus thrips, compatible with bio-intensive IPM or organic production. pp. 62-66. In: CRI Group Annual Research Report 2003. Nelspruit.
- Grout, T.G., Stephen, P.R. & Tate, B.A. 2005. OP alternatives for citrus thrips, compatible with bio-intensive IPM or organic production. pp. 27-32. In: CRI Group Annual Research Report 2004. Nelspruit.

3.4.3 Develop a rearing technique for the citrus thrips parasitoid *Goetheana incerta* Experiment 809 by Tim G Grout and Kim Stoltz (CRI)

Summary

Since our citrus thrips *Scirtothrips aurantii* was discovered in Australia, researchers there have been rearing it on *Bryophyllum* phyllodes in small plastic containers without water or soil. Our attempts to collect *S. aurantii* from *Bryophyllum* spp. at PPRI in Pretoria were unsuccessful. Four species of *Bryophyllum* were obtained from PPRI and established as pot plants in Nelspruit but attempts to transfer *S. aurantii* from other plants to *Bryophyllum* failed. After growing in pots in the open for approximately a year, *S. aurantii* discovered the plants and started damaging them. Decreasing severity of thrips damage was in the order of *B. pinnatum*, *B. daigremontianum*, *B. delagoense*, *B. proliferum*. Citrus thrips were collected from damaged plants and are being reared successfully on *B. pinnatum* phyllodes in environmental chambers but numbers are still too low to introduce parasitoids. A full report will be provided when the technique for rearing *Goetheana* has been developed.

Opsomming

Sedert ons sitrus blaaspootjie, *Scirtothrips aurantii*, in Australië gevind is, het navorsers begin om dit te teel op blaarstele van *Bryophyllum* in klein plastiek houers, sonder water of olie. Pogings wat plaaslik aangewend is deur die Plantbeskermingsnavorsingsinstituut (PBNI) in Pretoria om *S. aurantii* van *Bryophyllum* spp. te versamel was onsuksesvol. Vier spesies van *Bryophyllum* is van PBNI bekom en in potte by Nelspruit gevestig, maar pogings om *S. aurantii* van ander plante na *Bryophyllum* oor te dra was ook onsuksesvol. Potte is vir ongeveer 'n jaar lank buite geplaas waarna *S. aurantii* die plante ontdek en begin beskadig het. Die ernstigheid van die blaaspootjie skade het afgeneem in die orde van *B. pinnatum*, *B. daigremontianum*, *B. delagoense*, *B. proliferum*. Sitrus blaaspootjies is vanaf die beskadigde plante versamel en word nou suksesvol geteel op blaarstele van *Bryophyllum pinnatum* in omgewingskamers. Die getalle is egter nog steeds te laag vir die introduksie van parasitoïede. 'n Volledige verslag sal beskikbaar gestel word na ontwikkeling van 'n telingstegniek vir *Goetheana*.

3.4.4 Evaluation of the *Helicoverpa armigera* nuclearpolyhedrovirus (HearNPV) for control of bollworm on citrus Experiment 782 by Sean D. Moore, Wayne Kirkman and Peter Stephen (CRI)

Opsomming

Gedurende 1996-1998 is 'n Suid-Afrikaanse isolaat van *Helicoverpa armigera* nukliêre polihedrovirus (HearNPV) teen bolwurm (*Helicoverpa armigera*) met uitstekende resultate op sitrus getoets. Desondanks is navorsing daarop nie voortgesit nie omdat die virus nie kommersieel beskikbaar was nie. 'n Australiese maatskappy het onlangs 'n HearNPV-produk begin vervaardig. Die produk is oorspronklik as Vivus bekend maar word nou Vivus Gold genoem. Gedurende die 2004/05 seisoen is Vivus in boordproewe met HearNPV-SA, Mevinphos en Dipel vergelyk. In al drie proewe het die virus behandelings bolwurm beskadigde vrugte betekenisvol verminder. In die proef wat op Vergenoeg Plaas uitgevoer is, is vrugskade minder vir die virus behandelings as vir die standaard behandelings. In een van die proewe het die Dipel en Vivus behandelings ook oesverlies betekenisvol verminder. Gedurende die 2005/06 seisoen, is twee proewe in nawel lemoen boorde in die Oos Kaap uitgevoer. Vivus Gold is met 'n nuwe *Bacillus thuringiensis* (Bt) produk (X-Bt), Dipel en Dursban vergelyk. In albei proewe het Vivus Gold en X-Bt goeie nawerking getoon maar die virus se uitklop aksie was vinniger. Dipel het geen aktiwiteit na sy oorspronklike uitklop aksie gehad nie. Voor finale gevolgtrekkings gemaak kan word moet vrugskade- en oesevaluasies uitgevoer word. Hierdie data kan dalk bydra tot 'n aansoek vir registrasie van die bolwurm virus op sitrus. Verdere werk sal nodig wees met X-Bt.

Introduction

From 1996-1998 a South African isolate of *Helicoverpa armigera* nuclearpolyhedrovirus (HearNPV) was tested against bollworm (*Helicoverpa armigera*) on citrus, with excellent results (Moore *et al.*, 2004b). Despite this, work on this experiment was terminated, as the virus was not commercially available. Recently, an Australian company, Ag-Biotech Australia, began commercially producing HearNPV. The product was originally known as Vivus and is now known as Vivus-Gold (an improved formulation). It is available in a suspension at 2×10^9 viral occlusion bodies (OBs) per ml. During the 2004/05 season, three trials were conducted with Vivus. Bollworm control in these trials was described in the last annual report (Moore *et al.*, 2004a). Results of these trials, regarding reduction in fruit damage and crop loss, are reported here. Further trials, with Vivus-Gold, were conducted during the 2005/06 season. If similarly good results are achieved with this commercial product in a second season, its registration for the control of bollworm on citrus in South Africa will be considered. If HearNPV could replace the organophosphate, carbamate or pyrethroid usually used for bollworm control in spring, growers will be greatly assisted towards a reduction in the use of chemicals and the implementation of a bio-intensive IPM programme.

Materials and methods

2004/05 season

One Valencia orange orchard in Mpumalanga and two navel orange orchards in the Eastern Cape were selected for conducting field trials during the 2004/05 season. The Mpumalanga trial was conducted in an orchard of 18-year-old Valentine Valencia oranges on Crocodile Valley Citrus Co. near Nelspruit. These trees were planted at a spacing of 8 x 7.5 m (rows x trees). However, every third tree had been removed. There were therefore 227 trees per hectare. The first Eastern Cape trial was conducted in an orchard of 19-year-old Robyn navel orange trees on rough lemon rootstock on Vergenoeg Farm in the Gamtoos River Valley. These trees were planted at a spacing of 6 x 3 m (rows x trees) giving 555 trees per hectare. The final trial was applied in an orchard of 6-year-old Palmer navel orange trees on Lionel Jurgens's farm near Addo in Sundays River Valley. Ten trees, randomly selected in each orchard, were inspected for bollworm infestation, weekly from early in September 2004. Treatments were applied as soon as the first bollworm larvae were observed. The two Eastern Cape trials were laid out in a single-tree randomised design, with each treatment replicated 10 times. The Mpumalanga trial was laid out in a double-tree randomised design, as adjacent trees were so close that they were inseparable. As every third tree had been removed, trees were grouped in twos. Seven double replicates were used per treatment, although each tree was evaluated as a separate replicate, making 14 replicates per treatment.

At Crocodile Valley, three treatments were applied: Vivus (15 ml/100 l), Vivus (30 ml/100 l) and Mevinphos (100 ml/100 l). An average of 12.0 l of spray mix was applied per tree. The rates of virus per hectare (of Vivus) were therefore approximately 8.2×10^{11} OBs and 1.6×10^{12} OBs. An untreated control was retained. Applications were made on 8 September 2004. At this stage the average length of larvae was already about 12.5 mm. Larvae were therefore probably too large to expect optimum efficacy of Vivus.

Four treatments were applied in each of the other two trials (Vergenoeg and Jurgens): Mevinphos (100 ml/100 l); Dipel (12.5 g/100 l) and Kynobuff (100 ml/100 l); HearNPV-SA; Vivus (15 ml/100 l); Vivus (30 ml/100 l).

In the Vergenoeg trial, an average of 16 l of spray mix was applied per tree. Therefore the rate of virus per hectare of HearNPV-SA was approximately 3×10^{12} OBs; the rates of virus per hectare of Vivus were approximately 3×10^{12} OBs and 6×10^{12} OBs. This trial was applied on 29 September 2004.

In the Jurgens trial, an average of 9 l of spray mix was applied per tree. Therefore the rate of virus per hectare of HearNPV-SA was approximately 1.6×10^{12} OBs; the rates of virus per hectare of Vivus were approximately 1.6×10^{12} OBs and 3.2×10^{12} OBs. This trial was applied on 2 October 2004.

Techniques and results of evaluations of bollworm infestation, after applications, are given in the last annual report (Moore *et al.*, 2004a). From January to March 2005, fruit damage caused by bollworm was evaluated. Twenty randomly-selected fruit from each tree were inspected and categorised as clean, blemished, or culled (non-exportable) according to stipulated standards (Anonymous, 1995). The crop load was then estimated by placing a 0.125 m^3 frame at uniform height (1 m above the ground) into both the northern and southern sides of each tree, and counting the number of fruit within the frame. Fruit counts from both aspects of the tree were added together and the mean counts calculated for each treatment. This was not conducted in the Mpumalanga trial.

In all trials, data were analysed using ANOVA and means compared using the Bonferroni LSD multiple range test at the 95% significance level. Proportions (percentages/100) were subjected to an arc sine transformation, where necessary, in order to normalise the data. A zero percentage was counted as 1/(4n) and 100% as (n - 0.25)/n before transformation (Bartlett, 1947; Snedecor & Cochran, 1980).

2005/06 season

Two navel orange orchards in the Eastern Cape were selected for conducting field trials during the 2005/06 season. A trial site was also sought in Mpumalanga. However, bollworm infestation in this region was too low. The first trial was conducted in an orchard of eight-year-old Palmer navel orange trees on rough lemon rootstock on Scheepersvlakte Farm in the Sundays River Valley. These trees were planted at a spacing of 5.8 x 2.5 m (rows x trees) giving 690 trees per hectare. The second trial was applied in an orchard of 53 year-old Washington Navel orange trees interplanted with 24 year-old trees of the same variety, on Tierhok Farm in the Gamtoos River Valley. These trees were planted at a spacing of 7 x 3.5 m (rows x trees) giving 408 trees per hectare. Ten trees, randomly selected in each orchard, were inspected for bollworm infestation, weekly from early in September 2005. Treatments were applied as soon as the first bollworm larvae were observed. The trials were laid out in single-tree random design, with each treatment replicated 12 times.

The same six treatments were applied at both sites: Dursban (100 ml/100 ℓ; Dipel (12.5 g/100 ℓ) and Kynobuff (100 ml/100 ℓ); Vivus Gold (15 ml/100 ℓ); Vivus Gold (30 ml/100 ℓ); X-Bt (20 ml/100 ℓ); X-Bt (40 ml/100 ℓ). Untreated control trees were also used.

In the Scheepersvlakte trial, an average of 12.5 ℓ of spray mix was applied per tree. Therefore the rates of virus per hectare were approximately 2.6×10^{12} OBs and 5.2×10^{12} OBs. This trial was applied on 29-30 September 2004.

In the Tierhok trial, an average of 16.3 ℓ of spray mix was applied per tree. Therefore the rates of virus per hectare were approximately 2.0×10^{12} OBs and 4.0×10^{12} OBs. This trial was applied on 6 October 2004.

Trials were statistically analysed as described for the 2004/05 season trials.

Results and discussion

2004/05 season

The only treatment in the Crocodile Valley trial to significantly reduce fruit damage was Mevinphos (Table 3.4.4.1). This was because the treatments were applied later than they should have been, i.e. larvae were already approximately 12 mm in length (Moore *et al.*, 2004a). Not only could a significant amount of damage already have occurred at the time of spraying, but it is imperative that the virus is applied against small larvae, not yet 10 mm long, as is the case with Dipel (and other *Bacillus thuringiensis* products). Larger larvae require larger dosages of pathogen for death to occur. Despite this, the 2X concentration of Vivus significantly reduced fruit cull, to a similar extent as did Mevinphos (Table 3.3.8.1). Influence on yield was not measured.

Table 3.4.4.1 Bollworm induced fruit damage and crop size of navel oranges at Crocodile Valley Estate, Vergenoeg Farm and Jurgen's Farm.

Site	Treatment	Mean ± SE*		
		Fruit with bollworm damage (%)		Estimated yield/0.125 m ³
		Scarred	Culled	
Crocodile Valley				-
	Control	14.3a ± 0.7	15.0a ± 2.4	-
	Mevinphos	4.3b ± 1.3	1.4b ± 0.9	-
	Vivus 1X	10.7a ± 1.7	8.6ab ± 2.9	-
	Vivus 2X	10.7a ± 2.3	2.1b ± 1.5	-
Vergenoeg				
	Control	8.0a ± 1.5	1.0a ± 0.7	98ab ± 9.9
	Mevinphos	2.5b ± 1.1	0.5a ± 0.5	118ab ± 10.0
	Dipel	5.0ab ± 1.7	1.0a ± 0.7	85a ± 11.8
	HearNPV-SA	0b ± 0	0.5a ± 0.5	100ab ± 12.0

	Vivus 1X	2.5b ± 2.5	0a ± 0	91ab ± 11.9
	Vivus 2X	1.5b ± 1.5	0a ± 0	99ab ± 13.1
Jurgens	Control	32.0a ± 2.5	20.0a ± 2.2	78a ± 12.0
	Mevinphos	10.0b ± 2.2	3.5b ± 1.5	102ab ± 9.6
	Dipel	12.5b ± 1.3	4.5b ± 1.7	119b ± 17.3
	HearNPV-SA	13.5b ± 2.6	4.5b ± 1.6	104ab ± 16.6
	Vivus 1X	16.5b ± 2.2	5.5b ± 1.9	121b ± 11.6
	Vivus 2X	14.5b ± 2.6	3b ± 1.3	130b ± 14.0

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

In the Vergenoeg trial, all treatments with the exception of Dipel, significantly reduced fruit damage (Table 3.4.4.1). Total fruit damage was lowest for the HearNPV treatment, although this was not significant and also not relevant in light of the fact that no fruit were culled for either of the Vivus treatments (Table 3.4.4.1). Although both Mevinphos and Dipel had a more rapid knockdown effect than did the virus treatments ((Moore *et al.*, 2004a), these two standard treatments did not result in greater reduction in fruit damage. The virus treatments therefore obviously have an additional impact before mortality occurs, probably by reducing the feeding of the larvae. The Vivus 2X treatment did not appear to be any more effective than the Vivus 1X.

Bollworm infestation in the Jurgens trial orchard was higher than at the other two trial sites. Despite this high level of pest pressure, all of the treatments significantly reduced both fruit damage and fruit cull (Table 3.4.4.1). There was no significant difference between any of the treatments, although the lowest fruit cull recorded and the highest yield were for the Vivus 2X treatment. Estimated yield was higher for all treatments than for the untreated control, although only significantly so for Dipel and the two Vivus treatments (Table 3.4.4.1).

2005/06 season

At Scheepersvlakte, knockdown of bollworm by one week post-application was similar for Dipel and Vivus Gold (Table 3.4.4.2). However, Dipel had no activity beyond this initial knockdown, whereas the virus treatments continued to reduce bollworm infestation to a non-detectable level (for the higher concentration) at three weeks post-treatment (Table 3.4.4.2). A second application of Dipel at one or two weeks after the first treatment, as per registration, would have been appropriate. The other Bt treatments (X-Bt) resulted in a slower knockdown than did Dipel, but persisted longer than the latter (Table 3.4.4.2).

Table 3.4.4.2 Efficacy of various treatments in controlling bollworm on navel orange trees.

Site	Treatment	Blossom/fruitlet clusters infested with bollworm (%) (mean ± SE)*		
		1 week later	2 weeks later	3 weeks later
Scheepersvlakte		7 Oct	14 Oct	21 Oct
	Untreated control	42.5c ± 4.6	31.67c ± 3.7	6.67ab ± 1.9
	Dursban	4.17a ± 1.9	1.67a ± 1.1	0a ± 0
	Dipel	23.33b ± 4.0	23.33bc ± 3.3	10.83b ± 2.6
	Vivus Gold	16.67ab ± 3.3	8.33a ± 2.4	1.67a ± 1.7
	Vivus Gold	23.33b ± 2.1	7.50a ± 2.5	0a ± 0
	X-Bt	30.00bc ± 2.5	13.33ab ± 4.0	2.5a ± 1.8
	X-Bt	29.17bc ± 4.2	15.00ab ± 4.0	1.67a ± 1.1
Tierhok		13 Oct	20 Oct	27 Oct
	Untreated control	52.50c ± 3.0	38.33d ± 5.6	6.67b ± 3.5
	Dursban	1.67a ± 1.1	4.17a ± 1.9	0a ± 0
	Dipel	21.67ab ± 4.0	23.33bc ± 3.3	14.17b ± 3.8
	Vivus Gold	40.83bc ± 5.3	8.33ab ± 2.7	0a ± 0
	Vivus Gold	30.00b ± 5.1	8.33ab ± 2.7	1.67a ± 1.1
	X-Bt	40.83bc ± 5.8	28.33cd ± 5.1	1.67a ± 1.1
	X-Bt	35.83bc ± 5.6	27.50cd ± 3.0	1.67a ± 1.1

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

At Tierhok, knockdown with Dipel was better than the other biological treatments and better than at the Scheepersvlakte site (Table 3.4.4.2). However, as at Scheepersvlakte, Dipel had no persistence. Both Vivus Gold and X-Bt showed good persistence, but knockdown with the virus was more rapid. It is therefore likely that fruit damage will be less severe for the virus treatments than for the Bt treatments. This will be determined when fruit damage and yield are evaluated early in 2006.

Conclusion

Reduction in fruit damage from the use of Vivus (in the 2004/05 season trials) was significant and sufficient to indicate commercial potential for the product. Before final conclusions can be drawn regarding the 2005/06 trials, evaluations of fruit damage (and possibly yield too) must be conducted. However, based on evaluations of infestation, Vivus appears to be satisfactorily effective. The only question which remains is whether control of bollworm was sufficiently rapid to acceptably reduce damage. Further work is required with X-Bt.

Future research

During 2006, bollworm damage to fruit will be evaluated. This information might be sufficient to facilitate an application for registration of Vivus for use on citrus. Further work of a similar nature, will be required with X-Bt.

References cited

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3.4.5 Identify the pest piercing fruit and causing fruit drop near Burgersfort Experiment 831 by Peter Stephen and Tim Grout (CRI)

Opsomming

Gedurende 2004 is Empress mandaryne en nog een of twee ander kultivars in die Burgersfort omgewing gevind met vrugskade en -verliese kort voor oes. Die beskadige vrugte het donker bruin nekrotiese letsels van 5-10mm getoon met 'n verdikking in die middel. Die vrugte is tydens die winter deur plantpatoloë van CRI geïnspekteer en die bevindinge was dat die oorsaak nie patologies van aard was nie. In 2005 het die IPB komitee versoek dat navorsing gedoen moet word oor die onderwerp. Bome en vrugte is gedurende die nag geïnspekteer maar geen motte is gevind nie. Bome is ook intensief gedurende die dag ondersoek, bespuit met piretroïdes om verdagte insekte te versamel deur lakens onder die bome te gooi. Geen plaë is egter gevind nie. Bome is ook met skadu-nette bedek om insekte uit te sluit maar dit het nie die skade verminder nie. Afleidings van die afgelope seisoen se spuitprogramme in die dele wat die ergste geïmpakteer is, het getoon dat stinkbesies baie goed beheer was. Die gevolgtrekking is gemaak dat 'n insek soos 'n stinkbesie of vrugtesteekmot nie verantwoordelik vir die skade was nie. Verdere bespuitings met 'n strobilurin of 'n swamdodermengsel is op sommige van die bome gedoen en na vier weke het dit geblyk dat die bome minder skade vertoon het as die onbehandelde bome. Dit is later gevind dat antraknose verantwoordelik is vir die skade. Effektiewe beheer is verkry deur bespuitings met koper en strobilurin.

Introduction

In 2004, several cultivars in the Burgersfort environs suffered fruit damage and loss shortly before harvest. This was said to include navel oranges and different mandarins. CRI plant pathologists inspected Empress mandarins damaged in this way in winter 2004 but decided the cause was not pathological. Damaged fruit had dark brown necrotic patches 5-10 mm across with a protuberance in the centre where it appeared the fruit had been pierced. Dr Carel Buitendag searched for a possible insect pest responsible for this damage but could only find low numbers of shield bugs (Pentatomidae). In this region, losses from this pest are

significant and growers are spraying chemicals to reduce damage but are not sure what is responsible. Growers requested that research be conducted to determine what pest was causing the damage. For this reason the following investigation was conducted with the two most likely pests being shield bugs or fruit piercing moths.

Materials and methods

During April 2005, two Empress mandarin sites (A and B) that had shown a high incidence of severe damage in the past two seasons were selected for investigation. On 13 April, two trees at Site A were sprayed with a knockdown spray of dichlorvos and pyrethrum to ascertain whether shield bugs were present. At both sites, groups of trees were enclosed in shadecloth to exclude pests such as shield bugs and fruit piercing moths, and dropped fruit under these enclosed trees were monitored. The sites were also visually surveyed for the presence of sucking insects, and sweep net surveys were done using a 45 cm diameter sweep net. On 13 April at site A, two people using torches conducted a night survey from 20h00 to 21h00. This survey was mainly aimed at finding fruit piercing/sucking moths. Trees at both sites were sprayed with fungicides by the farm management, and counts were done to determine if this had an effect on the development of symptoms.

Results and discussion

The results of the various surveys are shown in the table below. Numbers of insects were generally low due to the spray programmes used by the growers to try to control the pest. The sweep net survey catch was for both sites conducted on two different dates.

Order	Description	Knockdown spray Site A	Sweep net (A & B)	Night survey Site A
Diptera	Various flies	8		
Coleoptera	Coccinellid spp	1		
Hemiptera	Stainer bugs, Shield bugs	3	2	
Lepidoptera	Moths and butterflies	1 (larvae)		1 (small moth)
Neuroptera	Green lacewing adults	1		1
Arachnida	Spider (<i>Herselia</i> sp.)	1		

Of the 5 Hemiptera found, none was a recognised pest species. Even if these insects were causing damage, their numbers were too low to relate to the high incidence of damage seen. The night survey by two people using torches yielded 1 green lacewing and 1 small moth. These surveys as well as the general observations made in the orchards, showed a very low incidence of pests and other arthropods.

Fruit on the trees enclosed by shade net continued to develop symptoms at a similar rate to exposed trees and fruit drop was similar to the exposed trees. At site A, where 5 trees were enclosed, a total of 421 fruit dropped during a 4-week period. Of these dropped fruit, 62% had the typical symptoms.

The results of fruit counts done on trees 3 to 4 weeks after receiving fungicidal sprays are shown in the figure below.

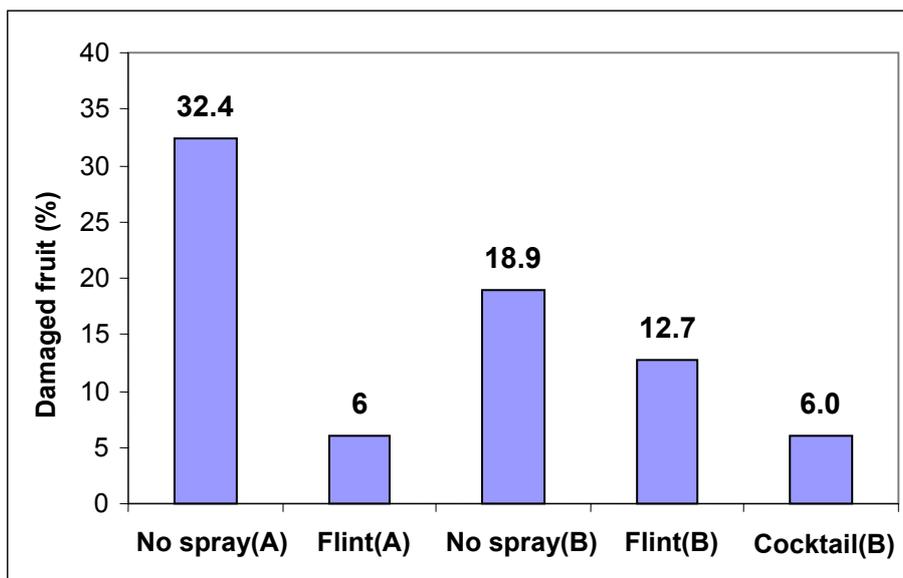


Figure 3.4.5.1. Reduction in damage caused by fungicide sprays. The “cocktail” comprised Benomyl 50 g/hl, Orius 100 ml/hl and Solitair 20 ml/hl.

These results, coupled with an examination of spray records for the orchards concerned, showed that it was highly unlikely that the damage could be attributed to insect activity. The general reduction of damage by the fungicidal sprays indicates that it is more likely that the cause is a pathogen and further investigation by Dr Tian Schutte has now shown that the damage is due to anthracnose.



Figure 3.4.5.2. Coloured Empress mandarin fruit with typical lesion.

Conclusion

After investigation at two sites near Burgersfort it was concluded that the necrotic lesions that resulted in fruit drop were not likely to be caused by an insect but were more likely to be due to an anthracnose infection.

Future research

No further investigation of this problem is to be conducted. Growers should apply suitable fungicides such as coppers and strobilurins for the control of anthracnose.

3.5 PROJECT: BIOCONTROL DISRUPTION

Project co-ordinator: Tim Grout (CRI)

3.5.1 Project summary

Although in some citrus production regions it appears that biological control and IPM are no longer important due to frequent sprays for mealybug and other phytosanitary pests, this will not always be the case. When organophosphates can no longer be used for mealybug control, biological control will once again increase in importance. For this reason, research on the biocontrol of mealybug is being conducted (3.5.2) and ant control will be essential to ensure that these natural enemies can be effective. As ants are useful predators on the orchard floor, research is being conducted on repellents that could keep them out of the trees without reducing their numbers on the ground (3.5.2). This work is in its early stages but some prospective repellents have been found and will be evaluated further. Although chemicals that are being registered for use in citrus for the first time are routinely tested against five key natural enemies, lacewings are not included in these tests. As these general predators are known to be less susceptible to some pesticides than some of the other natural enemies, bioassays need to be conducted on the commonly-used pesticides in citrus in order to know how best to conserve them. A culture of one of the most common green lacewings was established (3.5.3) but bioassays will only be conducted in 2006 when the numbers in the culture are adequate.

Projekopsomming

Dit wil voorkom asof biologiese beheer en IPB nie as belangrik beskou word in sekere sitrus produserende areas nie weens die gereelde bespuitings teen witluise en ander fitosanitêre plaë. Dit gaan egter nie altyd die geval wees nie en die belangrikheid sal weer toeneem sodra organofosofate nie langer vir die beheer van witluise beskikbaar sal wees nie. Dit is ook die rede waarom navorsing tans op die biologiese beheer van witluise uitgevoer word (3.5.2). Die beheer van miere is ook belangrik om sodoende die effektiwiteit van hierdie natuurlike vyande te verseker. Aangesien miere nuttige predatore op die vloer van die boord is, is navorsing op afweermiddels uitgevoer wat hul uit die bome sal hou sonder om hul getalle op die grond te verminder (3.5.2). Hierdie werk is nog in 'n baie vroeë stadium, maar moontlike afweermiddels is reeds gevind wat verder geëvalueer sal word. Chemikalieë wat vir die eerste maal op sitrus geregistreer is, is getoets teen vyf belangrike natuurlike vyande. Gaasvlerkies was egter nie in die toetse ingesluit nie. Dit is bekend dat hierdie algemene predatore minder sensitief is as van die ander vir sommige plaagdoders, maar biologiese ondersoek is egter nog steeds nodig met van die mees algemene plaagdoders om sodoende te kan bepaal wat is die beste bewaringsopsies vir hierdie natuurlike vyande. 'n Kultuur van een van die mees algemene groen gaasvlerkies is gevestig (3.5.3) maar biologiese ondersoek sal eers in 2006 in aanvang neem sodra voldoende getalle beskikbaar is.

3.5.2 To develop an ant repellent that will keep ants out of citrus trees without destroying their nest Experiment 798 by Tim G Grout, Sean D. Moore, Wayne Kirkman and Kim Stoltz (CRI)

Opsomming

Die ideale IPB oplossing vir mierbesmetting in sitrus bome sal wees om hul uit die bome te hou, maar hul nog steeds toe te laat om op sitrus plaë op die grond te aas. Dit is ook die doel in die soektog na 'n afweermiddel vir miere. Die aanvanklike siftingsproses van 'n wye reeks produkte is met *Anoplolepis custodiens* voltooi. Twee proewe is ook met *Pheidole megacephala* gedoen. Alhoewel die resultate deur klimaat en ander faktore beïnvloed is, kon verskeie produkte reeds geëlimineer word. Verdere proewe sal met die belowende produkte uitgevoer word.

Introduction

Ants are attracted to honeydew-producing homopteran insects in citrus trees and often protect these pests from their natural enemies. In addition, ant activity in trees can disrupt the natural enemies of non-honeydew-producing pests such as red scale *Aonidiella aurantii* and result in an increase in population density of this pest. However, ants on the orchard floor can play a beneficial role in preying on late larval instars or pupae of various citrus pests including fruit flies, false codling moth and other lepidoptera, and citrus thrips. Rather than destroying the nests on the orchard floor it would therefore be beneficial to stop the ants from gaining access to the tree. This has been done in the past with sticky ant bands (Samways & Tate 1985) and chemical-mechanical barriers such as The Protector but these are labour-intensive to maintain and the re-treating of The Protector sometimes results in pyrethroid insecticide being sprayed up into the tree. If natural products could be found that could be applied to tree trunks to repel ants without killing them they would be more cost effective than physical barriers. This research was initiated in Mpumalanga by

Grout and Stoltz using the pugnacious ant *Anoplolepis custodiens* then the same approach was used against *Pheidole megacephala* by Moore and Kirkman in the Eastern Cape.

Materials and methods

Various ingredients were identified and tested as potential ant repellents but details of successful treatments are being kept proprietary. The same technique to screen repellents was used for both ant species. A food source that combined both sugar and protein was used to attract ants. This was a combination of fish paste with either honey or golden syrup. The attractant mixture was placed in the middle of a plastic Petri dish and a prospective repellent was spread around the perimeter of the Petri dish. The Petri dish was then placed near an active ant nest opening and observations taken after a period of time.

Anoplolepis custodiens

Experiments started on 16 February 2005 using 5 replicates (each in the vicinity of one nest opening) per repellent and 65 mm diameter Petri dishes. On this occasion, 10 products were compared with a baited control. All treatments and the control were arranged equidistantly around each nest opening and observations made after approximately 1 hour when repellents were either rated as being effective or not. Six further comparisons were conducted with between 5 to 11 products and 5 to 8 replicates until mid-April when pugnacious ant populations declined. The experiments were continued from 24 November 2005 until 18 January 2006 with a further 5 comparisons being conducted. In the last 2 comparisons the delay before observations were made was extended to 3 hours to see whether ants would adapt to the presence of the repellent.

Pheidole megacephala

Five Petri dishes for each treatment were used. Petri dishes were then placed around active ant (*Pheidole megacephala*) nests until significant activity was observed in control dishes (i.e. dishes with honey and fish paste but without any treatment). Dishes were then categorised as clear (C), light infestation (L), medium infestation (M), heavy infestation (H) or disturbed (D). These were purely subjective and relative ratings. Some Petri dishes were disturbed and overturned, making it impossible to include them in the analysis. This was caused by wind.

Two trials were conducted as described. Both were conducted on the property of the CSIR in Port Elizabeth. The first was conducted with five different ingredients. Treatments were placed in the sun in the vicinity of *P. megacephala* nests for 1 h 45 min between 15h20 and 17h05 on 1 November 2005. Six different ingredients were used in the second trial. Treatments were exposed for 24 h from 09h00 on 3 November to 09h00 on 4 November 2005. This extensive duration of exposure was necessary as the weather on 3 November was initially cool and overcast. Therefore ant activity was minimal.

Results and discussion

Anoplolepis custodiens

Ant activity varied between the 12 trials due to differences in soil temperature, recent rainfall and competition from other ants. Several products seemed to repel ants when the Petri dish was first placed near the nest but with time the ants adapted to the repellent and crossed it, e.g., Cayenne pepper. Some products were so pungent that they seemed to affect the behaviour of ants on other treatments so the space between treatments had to be increased. Not all products were available as liquid so did not always form a continuous barrier around the edge of the Petri dish. Some of these products may have therefore been easier for the ants to avoid and they could perhaps have been more effective if mixed with some solvent. Most products were evaluated on more than one occasion unless they did not show any repellent properties at all. Products that did not repel in 40% or more of the replicates were rejected. The following products have been rejected as repellents for pugnacious ant in this initial screening stage: 5-methyl-3-heptanone, Cayenne pepper, Cinnamon, Coffee, Dodecalactone, Dodecyl aldehyde, Farnesyl acetone, Garlic, Geranyl acetone, Heliotropylacetate, Indalone, Ionone, Iso-boryl-methacrylate, Lemon grass, Lemon juice, Penny royal, Salcyl aldehyde, Soapy water, Tagetes oil, Thyme, Tobacco, Undecene, Verbenane.

The technique will now be changed so that promising repellents are applied to more absorbent material and evaluated over longer periods of time.

Pheidole megacephala

Two of the treatments (Treatments 1 and 5) used in the first trial eliminated *P. megacephala* from the Petri dishes altogether (Table 3.5.2.1).

Table 3.5.2.1. Levels of infestation of *P. megacephala* in baited and treated Petri dishes (trial 1).

Treatment number	Infestation level of ants for each Petri dish replicate*					Ant infestation (x/5)	Treatment identified with potential for further testing
	1	2	3	4	5		
1	C	C	C	C	C	0	Yes
2	L	L	C	C	M	3	
3	C	L	C	M	L	3	
4	L	L	L	M	L	5	
5	C	C	C	C	C	0	Yes

*C = clear (no infestation), L = light infestation, M = medium infestation, H = heavy infestation, D = disturbed replicate.

Only one of the treatments (Treatments 11) used in the second trial eliminated *P. megacephala* from the Petri dishes altogether (Table 3.5.2.2). However, a second treatment (Treatment 9) appeared promising, as only one of the Petri dishes was lightly infested. Unfortunately, two of the replicates had been disturbed.

Table 3.5.2.2. Levels of infestation of *P. megacephala* in baited and treated Petri dishes (trial 2)

Treatment number	Infestation level of ants for each Petri dish replicate					Ant infestation (x/5)	Treatment identified with potential for further testing
	1	2	3	4	5		
6	H	L	L	H	M	5	
7	M	H	M	M	M	5	
8	D	H	H	D	H	(3/3)	
9	L	C	D	D	C	(1/3)	Yes
10	L	M	H	M	H	5	
11	C	C	C	C	C	0	Yes

*C = clear (no infestation), L = light infestation, M = medium infestation, H = heavy infestation, D = disturbed replicate.

Conclusion

At this stage of the investigation a few products are worth investigating further to determine whether their repellent effect lasts long enough to be practical.

Future research

Research will continue with promising products and if they are effective for a month or longer they will be tested against the other ant species.

Reference cited

Samways, M. J. and B. A. Tate. 1985. A highly efficacious and inexpensive trunk barrier to prevent ants from entering citrus trees. *Citrus Subtrop. Fruit J.* 618: 12-14.

3.5.3 Determine the non-target effects of key pesticides used in citrus to lacewing predators of citrus thrips

Experiment 810 by Peter R Stephen and Tim G Grout (CRI)

Summary

Several species of green lacewings are found in citrus orchards and all are general predators of aphids, mealybugs, soft scales, citrus thrips and mites. In order to conserve these predators and derive maximum benefit from them, we must know their susceptibility to commonly used pesticides. This information will also be important if insectaries want to rear and sell lacewings for augmentative releases in orchards. A colony of *Chrysoperla pudica* was started from field-collected adults and maintained on a diet of fruit fly eggs and *Ephestia* sp. moth eggs for larvae and a mixture of honey and yeast for adults. Separate boxes were used for each day's egg production and these were largely filled with shredded paper to provide refugia for larvae. The complete life cycle from egg to egg took 30 days at 23°C. Numbers of lacewings were inadequate for bioassays to begin in 2005 but these were conducted in early 2006 and the results will appear in the 2006 report. No further research on this natural enemy is planned.

Opsomming

Verskeie spesies van groen gaasvlerkies, wat algemene predatore van plantluise, witluise, sagte dopluise, sitrus blaaspootjies en myte is, word in sitrus boorde gevind. Vir hul bewaring en ook om maksimum voordeel uit hul te trek, moet hul sensitiwiteit teenoor van die algemene plaagdoders bekend wees. Hierdie inligting sal ook van belang wees vir insektariums wat gaasvlerkies wil teel en verkoop as vermeerderingsvrylatings in die boorde. 'n Kolonie van *Chrysoperla pudica* is begin vanaf volwassenes wat in die veld versamel is. Larwes word aangehou op 'n dieet van vrugtevlug eiers en *Ephestia* sp. moteiers, terwyl volwassenes aangehou word op 'n mengsel van heuning en giste. Afsonderlike dose, vir daaglikse eierproduksie, wat hoofsaaklik gevul was met snipper papier om in skuilpleke vir die larwes te voorsien, is gebruik. Die volledige lewenssiklus (eier-tot-eier) was 30 dae by 23°C. Daar is eers met die ondersoek vroeg in 2006 begin omrede die getalle te laag was om reeds daarmee in 2005 te begin. Die resultate sal in die 2006-verslag verskyn en geen verdere navorsing word beplan nie.

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 2005. In the first, live trapping was conducted for parasitoids of oleander mealybug (3.6.2). Several parasitoids of *Leptomastix* sp., *Coccidoxenoides perminutus* and some unidentified parasitoid species were found. In the second experiment, a chemical control trial was conducted in spring to compare the efficacy of various products as preventative control measures for mealybug (3.6.3). Ultracide, Parathion, Selecron, Tokuthion and Applaud were the most effective treatments, with little difference between these products. In the third experiment, mealybug surveys conducted in different regions revealed that oleander mealybug was present in all but one of the orchards surveyed (3.6.4). Oleander mealybug was the dominant mealybug species in the orchards surveyed in the Citrusdal area. In the fourth experiment, results of surveys of grain chinch bug indicated that fruit orchards in the following areas were at risk of infestations: Ceres, Kouebokkeveld, Piketberg, Porterville, Riebeek-Kasteel, Langkloof and Malmesbury (3.6.5). Grain Chinch bugs do not appear to favour citrus fruits as shelters, but occur there incidentally. Preliminary bioassays of possible post-harvest treatments for the control of grain chinch bugs were conducted (3.6.6).

Projekopsomming

Vyf eksperimente is onder hierdie projek gedurende 2005 uitgevoer. In die eerste een is parasiete van oleanderwitluis gelok en lewendig gevang (3.6.2). Verskeie *Leptomastix* sp. parasiete en *Coccidoxenoides perminutus* en sekere ongeïdentifiseerde parasiete is gekry. In die tweede eksperiment is 'n chemiese beheer proef in die lente uitgevoer om die werking van verskeie produkte as voorkomende beheer middels vir witluis te vergelyk (3.6.3). Ultracide, Parathion, Selecron, Tokuthion en Applaud is die mees effektiewe behandelings, met min verskil tussen hierdie produkte. In die derde eksperiment het wiltuis opnames, wat in verskillende streke gedoen is, gewys dat oleanderwitluis in alle boorde behalwe een teenwoordig was (3.6.4). Oleanderwitluis is die dominante spesie in die boorde wat in die Citrusdal area geïnspekteer is. In die vierde eksperiment het resultate van opnames van graanstinkluis aangedui dat vrugte boorde in die areas onder risiko van besmetting is: Ceres, Kouebokkeveld, Piketberg, Porterville, Riebeek-Kasteel, Langkloof en Malmesbury (3.6.5). Dit wil voorkom dat graanstinkluise nie sitrusvrugte verkies as skuilplek

nie maar net toevallig daar voorkom. Voorlopige toetse van moontlike na-oes behandelinge vir die beheer van graanstinkluise was uitgevoer (3.6.6).

3.6.2 Investigating biocontrol agents of mealybug species other than citrus mealybug Experiment 692 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Die plaagstatus en derhalwe die belangrikheid van *Paracoccus burnerae* op sitrus, het in Suid-Afrika verhoog, veral in die Oos-Kaap. Belangrike en doeltreffende parasitoïede van 'n paar witluisspesies wat op sitrus voorkom, is geïdentifiseer. Soortgelyke inligting word egter nog vir *P. burnerae* benodig. 'n Proef is uitgevoer om sulke inligting te versamel. Geen parasitisme van *P. burnerae* is in enige van die opnames gekry wat gedurende die 2004/05 seisoen uitgevoer is nie. Dit is nie duidelik waar die probleem met die proef protokol gelê het nie. Nietemin is groter saailinge gedurende die 2005/06 seisoen gebruik om *P. burnerae* in boorde te sit. Saailinge is ook die keer nie in hokke gesit nie en is vir twee weke pleks van een week in boorde gehou. As gevolg hiervan is 'n redelike getal *Leptomastix* sp. en *Coccidoxenoides perminutus* parasiete gekry, sowel as verskeie parasiete wat nie geïdentifiseer is nie.

Hierdie eksperiment word voortgesit. Nadat die belangrikste parasitoïedspesies van *P. burnerae* geïdentifiseer is, sal die moontlike gebruik van hierdie spesies vir aanvullende loslatings nagevors word. 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*). However, it has been shown that the oleander mealybug, *Paracoccus burnerae*, is approximately 100 times less suitable a host for *C. perminutus* than is *P. citri* (Hattingh & Tate, 1997). What makes this a serious situation justifying further investigation is that *P. burnerae* is regarded by certain important markets, e.g. USA and Korea (and potentially many others), as being a phytosanitary pest; and that *P. burnerae* has increased in dominance in citrus orchards, at least in the Eastern Cape. 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 & Gilliomee (2002) qualified and quantified the species of parasitoids in the natural enemy complexes attacking citrophilous mealybug, *Pseudococcus calceolariae*, longtailed mealybug, *Pseudococcus longispinus*, and *P. citri*. However, this has not been done for *P. burnerae*.

Materials and methods

2004/05 season

A starter culture of *P. burnerae* was obtained from Welma Pieterse (Department of Agriculture, Stellenbosch). The mealybugs were transferred onto Clementine mandarin seedlings, which were kept under Growlux (UV-B) lights in the laboratory. Due to flaws in the trial protocol employed during the 2003/04 season (Moore & Kirkman, 2004), certain changes were made in an attempt to remedy these. Instead of the small seedlings used previously for exposing mealybug in orchards, larger Carrizo seedlings (approximately 40 cm from soil to tip) were used. Seedlings were obtained from the Citrus Foundation Block (CFB). Mealybug infested leaves from the Clementines in the laboratory culture were removed and placed onto 12 Carrizo seedlings to facilitate movement of the mealybug onto them.

Two orange orchards, in which there were conspicuous levels of mealybug infestation, were selected. Both of these were in the Sundays River Valley (SRV). One of them was in an organic orchard of Washington navel orange trees on Rosedale Farm. The other was an orchard of mature Palmer navel orange trees on Sun Orange Farm. 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. Cages were slightly larger than those used during the previous season (Moore & Kirkman, 2004) in order to facilitate larger seedlings than used previously. Six 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. Results from the two surveys conducted during September to November 2004 at the two sites are recorded in a previous report (Moore & Kirkman, 2004).

2005/06 season

During the previous two seasons, difficulties were experienced in maintaining the oleander mealybug culture in the laboratory under artificial lights. During this season the mealybug culture was established on potted Valencia trees and kept in a greenhouse at the Nelson Mandela Metropolitan University (NMMU).

A similar protocol for trapping parasitoids as was used during previous seasons, was again employed, with the following changes. Firstly, larger potted mealybug infested trees were used. This was to prevent desiccation of trees and improve their survival and suitability for mealybug. Secondly, trees were not covered with mesh. This was done in case the mesh in any way hindered access to the trees for certain parasitoids. Lastly, the trees were kept in the orchards for two weeks instead of one week. This was to allow more time for parasitism to occur.

One infested tree was placed in an orchard of Delta Valencia orange trees (orchard 4) on Tierhok Farm in the Gamtoos River Valley (GRV) on 27 October 2005. Another was placed into an orchard of Washington navel orange trees on Rosedale Farm in SRV on 2 November 2005.

After collection, the life stages of the mealybug on the tree from Tierhok Farm were counted, and the tree was then placed in a large emergence box. As the tree from Rosedale Farm was heavily infested, a leaf with a representative sample of life stages was placed in an emergence box after recording the life stages. The rest of the tree was placed in a large emergence box, to recover and identify any other parasitoids. Once the emerged parasitoids had died, they were recovered.

Results and discussion

2004/05 season

In the surveys conducted during the 2003/04 season, the seedlings placed into the orchards did not always survive very well. Due to the small size of the seedlings and the small size of the bags into which they were potted, trees desiccated too rapidly. It was therefore not uncommon to retrieve seedlings from orchards after the one week exposure period, which were dead or dying. This was thought to possibly be affecting the survival of the mealybug on the seedlings and the attractiveness of the mealybug and their host plants to parasitoids. Consequently, the trial protocol was changed for the 2004/05 season, i.e. larger seedlings and pots were used, resulting in far better survival of seedlings. Despite this, no parasitism of *P. burnerae* was recorded. It is not clear where the fault in the protocol lay. The natural enemy complex of mealybug is known to be very effective and is known to peak during January and February in the Eastern Cape (Hattingh & Moore, 2003) (although this does pertain chiefly to *P. citri*). It is therefore highly improbable that there was a dearth of parasitoids in the trial orchards, both of which were subjected to natural enemy friendly management (i.e. IPM or organic). From the results of the very first survey conducted (Moore & Kirkman, 2003) it was seen that a meaningful level of parasitism could be obtained with this general protocol. However, it is possible that if the infested seedlings are left in an orchard for longer than one week, the probability of capturing parasitoids could be significantly improved.

2005/06 season

Two surveys were completed during early summer of the 2005/06 season – one at each site. The revised protocol was far more effective than those used previously, with both surveys producing parasitoids (Table 3.6.2.1). Both *Leptomastix* sp. and *Coccidoxenoides perminutus* were recovered from *P. burnerae*. These identifications will be confirmed by the Biosystematics Division of the PPRI. A number of unidentified parasitoids will also be forwarded to them for identification. The only other survey from which parasitoids were recovered was conducted in November 2003 (Moore & Kirkman, 2003). In this survey, the same species of parasitoids were identified. However, only one *C. perminutus* individual was recovered compared to several *Leptomastix* sp. It is therefore interesting to note that 16 *C. perminutus* individuals were found in the two surveys of this study (Table 3.6.2.1). It is particularly interesting in light of the finding that *P. burnerae* is a comparatively unsuitable host for *C. perminutus* (compared to *P. citri*) (Hattingh & Tate, 1997).

Table 3.6.2.1. Mealybug placed into emergence boxes and parasitoids collected from emergence boxes in 2005 season survey.

	Exposure period of mealybug in orchard	Tierhok Farm (GRV)	Rosedale Farm (SRV)
		27/10/05 – 09/11/05	02/11/05 – 16/11/05
Mealybug life-stages counted before placement in emergence box	Egg sacs	12	7
	Crawlers (1 st & 2 nd instars)	31	56
	Sub-adults (3 rd instar)	6	70
	Adults	10	13
Parasitoids collected from emergence box	<i>Leptomastix</i> sp.	2	2
	<i>Coccidoxenoides perminutus</i>	10	6
	Unidentified	6	3
<i>P. burnerae</i> males collected from emergence box		15	5

Conclusion

No parasitism of *P. burnerae* was recorded during the surveys conducted during the 2004/05 season. It was not clear where the fault in the protocol lay. However, during the 2005/06 season larger seedlings were used for placing *P. burnerae* into orchards, seedlings were not caged and were left in orchards for two weeks instead of one. Consequently, several *Leptomastix* sp. and *C. perminutus* parasitoids, as well as some unidentified species of parasitoid were recovered.

Future research

This experiment is ongoing. After the most important parasitoid species of *P. burnerae* have been identified, it will be possible to investigate the rearing and augmentative release of such parasitoids. If other species of mealybug, such as *P. longispinus*, are found to be sufficiently important, biocontrol of these species will also be investigated.

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3.6.3 Preventative and corrective chemical treatments for control of mealybug on citrus

Experiment 755 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

'n Chemiese beheer proef om die werking van verskeie produkte as voorkomende beheer middels vir witluis te vergelyk is in die lente uitgevoer. Ultracide, Parathion, Selecron, Tokuthion en Applaud is die mees effektiewe behandelings, met min verskil tussen enige van die produkte. Ten spuite van 'n hoë vlak van besmetting in die onbehandelde kontrole (77% van vrugte besmet) het al die behandelings besmetting betekenisvol verminder. Die werking van Folimat, Chlorpyrifos en Mevinphos is teleurstellend, met witluis besmetting in die laaste twee behandelings betekenisvol hoër as vir Ultracide, Parathion, Selecron, Tokuthion en Applaud. Geen verdere werk sal op hierdie eksperiment uitgevoer word.

Introduction

The pest status of mealybugs on citrus has increased during the last few years. This is partly due to the increased levels of oleander mealybug, *Paracoccus burnerae* (Moore & Kirkman, 2003). Consequently, chemical treatment of mealybug is becoming more necessary than has been the case for several years. There is a lack of clarity as to how the efficacy of the various registered and available pesticides compare with one another. This coupled with rumours and misinformation about the various products makes decision making difficult for growers. The last trial to compare the various products registered for mealybug control was conducted approximately ten years ago (Hattingh *et al.*, 1995). Consequently, it is considered important and urgent to determine the relative efficacy of all products, both as preventative and corrective treatments. During the 2003/04 season a preventative treatment trial was conducted (Moore & Kirkman, 2004). Ultracide, Parathion and Tokuthion all gave 100% control. However, due to the very low level of infestation, even in the untreated control, the results of this trial were not considered as an accurate comparative measure of the efficacy of the various treatments. The trial has therefore been repeated, during the 2004/05 season, against a high level of infestation.

Materials and methods

A preventative treatment trial was applied during October 2004. An orchard of six year old Lane Late navel oranges on Atmar Farm in Sundays River Valley, which had a history of high mealybug infestation, was selected for the trial. The trial was laid out in a random block design with 12 single-tree replicates per treatment. The same nine treatments that were used in the preventative treatment trial at Vergenoeg Farm during the previous season (Moore & Kirkman, 2004) were again applied (Table 3.6.3.1). An untreated control was retained for comparison. An average of 17.5 l of spray mix was applied per tree using hand-held spray guns. Treatments were applied on one of two dates (15 or 25 October), depending on the recommended timing for application of the specific treatment, i.e. before or after full petal drop. Treatments were evaluated on 11 January 2005. This was done by randomly inspecting 10 fruit per tree. Fruit were separated from the calyx in order to inspect thoroughly in this region.

Table 3.6.3.1. Treatments applied on 15 October 2004 for the preventative control of mealybug in a Lane Late navel orange orchard on Atmar Farm.

Number	Treatment (concentrations per 100 L water)	Timing of application	Date of application
1	Untreated control	-	
2	Ultracide (150 ml) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
3	Parathion (150 ml) + Agral 90 (18 ml)	50-90% petal drop	15 October 2004
4	Selecron (100 ml) + Agral 90 (18 ml)	50-90% petal drop	15 October 2004
5	Tokuthion (50 ml) + Agral 90 (18 ml)	50-90% petal drop	15 October 2004
6	Folimat (50 ml) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
7	Chlorpyrifos WG (64 g) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
8	Applaud (30 g) + Agral 90 (18 ml)	100% petal drop +	25 October 2004
9	Mevinphos EC (165 ml) + Agral 90 (18 ml)	100% petal drop +	25 October 2004

Results and discussion

Mealybug infestation in the untreated control was very high (77% fruit infested). Despite this, all of the treatments significantly reduced the level of infestation (Table 3.6.3.2). Ultracide, Parathion, Selecron, Tokuthion and Applaud were the most effective treatments, with little difference between these products. The efficacy of Folimat, Chlorpyrifos and Mevinphos was disappointing, with mealybug infestation in the latter two treatments being significantly higher than for Ultracide, Parathion, Selecron, Tokuthion and Applaud.

Table 3.6.3.2. Mealybug infestation of Lane Late navel oranges on 11 January 2005, subjected to different treatments during October 2004.

Treatments (Dosages per 100 ℓ water)	Fruit infested (%)
Untreated control	77 d
Ultracide (150 ml) + Agral 90 (18 ml)	0 a
Parathion (150 ml) + Agral 90 (18 ml)	4 a
Selecron (100 ml) + Agral 90 (18 ml)	1 a
Tokuthion (50 ml) + Agral 90 (18 ml)	4 a
Folimat (50 ml) + Agral 90 (18 ml)	11 ab
Chlorpyrifos WG (64 g) + Agral 90 (18 ml)	18 bc
Applaud (30 g) + Agral 90 (18 ml)	4 a
Mevinphos (165 ml) + Agral 90 (18 ml)	28 c

Conclusion

All treatments applied to preventatively control mealybug, significantly reduced infestation. Ultracide, Parathion, Selecron, Tokuthion and Applaud were the most effective treatments, with little difference between these products.

Future research

No further work will be conducted on this experiment.

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3.6.4 A survey of the mealybug species complex on citrus throughout South Africa Experiment 792 by Sean D. Moore and Wayne Kirkman (CRI)

Opsomming

Gedurende die 1990s, is die sitruswitluis, *Planococcus citri*, ongetwyfeld die dominante witluis spesie op sitrus in Suid Afrika. Dit is onlangs gewys dat oleanderwitluis, *Paracoccus burnerae*, nou die dominante witluis spesie op sitrus in die Oos-Kaap geword het. Hierdie studie is aan die gang gesit om te bepaal of hierdie tendens ook elders in die land nou voorkom. Gedurende Februarie 2005 is witluis opnames in 10 boorde van verskillende kultivaars in verskillende streke van Suid Afrika uitgevoer. Vyftig vrugte van elke boord is mikroskopies ondersoek. In al die boorde wat ondersoek is, behalwe een, is oleanderwitluis gekry. In die boorde wat in die Citrusdal area ondersoek is is oleanderwitluis bevestig as die dominante spesie. Omdat Citrusdal die hoof area vir uitvoere van sitrusvrugte VSA toe is, is hierdie bevinding 'n bekomernis. Gedurende Desember 2005 is 'n verdere ses boorde in Mpumalanga vir witluis ondersoek. Probleme is met die identifikasie van die spesies wat gekry is ondervind. Dit is vermoed dat een van die spesies Karoodoringwitluis is. Daarom is monsters vir taksonomiese bevestiging na die NIPB toe gestuur. Hierdie opname sal tot einde Maart 2007 voortgesit word.

Introduction

During the 1990s, when research was being conducted on augmentation of *Coccidoxenoides perminutus* parasitoids, the citrus mealybug, *Planococcus citri*, was undeniably the dominant mealybug species on citrus throughout South Africa (Hattingh *et al.*, 1998). It has recently been demonstrated that oleander mealybug, *Paracoccus burnerae*, has become the dominant mealybug species on citrus in the Eastern Cape (Moore & Kirkman, 2003). There are two factors which make this a serious situation:

1. *P. burnerae* is regarded by certain important markets, e.g. USA and Korea (and potentially many others), as being a phytosanitary pest (Hattingh & Moore, 2003).

2. *P. burnerae*, is approximately 100 times less suitable a host for *C. perminutus* than is *P. citri* (Hattingh & Tate, 1997), making augmentation of *C. perminutus* unsuitable for *P. burnerae* control.

Consequently, a study has been initiated to investigate the parasitoid complex of *P. burnerae* (experiment 692; Moore & Kirkman, 2003 & 2004). What is also important is to determine whether the trend recorded in the Eastern Cape, of *P. burnerae* becoming the dominant mealybug species, has occurred elsewhere in the country too. This experiment proposes to determine this.

Materials and methods

Samples of mealybug-infested fruit (50 fruit per orchard) were collected from 10 orchards in four different production regions of South Africa, during February 2005. Samples were microscopically inspected to identify as many specimens within each sample, up to species level. Where any uncertainty existed, individuals from samples were sent to the Biosystematics Unit of the Plant Protection Research Institute (PPRI) (of the Agricultural Research Council [ARC]) in Pretoria for identification.

During December a total of six samples of mealybug-infested fruit were collected from different sites in Mpumalanga. Identifications of mealybug species were again conducted through microscopic inspection. However, as there was a large deal of uncertainty regarding some of the identifications, samples of mealybug from each inspection were preserved (in glycerol and glacial acetic acid) and sent to the Biosystematics Unit of the PPRI-ARC for identification.

Results and discussion

Although the results of the survey conducted during February 2005 (Table 3.6.4.1), represent only a small portion of the citrus orchards in each of the areas surveyed, at only one stage during the season, it is interesting to note: firstly, the presence of oleander mealybug in all but one of the orchards surveyed and secondly, the dominance of oleander mealybug in the Citrusdal area. These observations indicate that the increase in dominance of oleander mealybug might not be restricted to the Eastern Cape. The high level of oleander mealybug found in Citrusdal is a concern, as Citrusdal is the main area exporting citrus fruit to the USA. There is zero tolerance for oleander mealybug on fruit destined for the USA.

Table 3.6.4.1. Mealybug species occurring on citrus fruit collected during February 2005 from orchards in different parts of South Africa.

Area	Farmer (or Farm)	Cultivar	Individuals of each species out of total (%)*	
			Oleander mealybug	Citrus mealybug
Citrusdal	Van Wyk	Navel orange	56.25	43.75
	Laubser	Delta Valencia orange	46.43	53.57
	S. van der Merwe	Clementine mandarin	58.42	41.58
	H. van der Merwe	Navel orange	100	0
	Burger	Delta Valencia orange	81.25	8.75
	Visser (Hexrivier)	Navel orange	0	100
Marble Hall	Kruger	Newhall navel orange	43.48	56.52
	Engelbrecht	Eureka lemon	3.92	96.08
Nelspruit	Crocodile Valley	Navel orange	10.58	89.42
Letsitele	?	Marsh grapefruit	5.03	94.97

*Percentage for dominant species in each orchard is in bold.

Some difficulty was experienced in identifying the species of mealybug found on fruit during the December 2005 surveys (Table 3.6.4.2). Confirmation of identifications has not yet been received from the PPRI. These final results will be reported in the next annual research report.

Table 3.6.4.2. Mealybug species occurring on citrus fruit collected during December 2005 from orchards in Mpumalanga.

Area	Farmer (or Farm)	Cultivar	Individuals of each species out of total (%)*		
			Oleander mealybug	Citrus mealybug	Karoo thorn mealybug
Loskop Valley	Schoeman	Navel oranges	100	0	0
	?	Navel oranges	25.9	74.1	0
Komatipoort	Vergenoeg (TSB)	Star Ruby grapefruit	0	0	100?
	Vergenoeg (TSB)	Marsh grapefruit	100?	0	0
	Vergenoeg (TSB)	Rosé grapefruit	100?	0	0
	Vergenoeg (TSB)	Marsh grapefruit	100?	0	0

*Percentage for dominant species in each orchard is in bold.

Conclusion

During February 2005, mealybug surveys were conducted in 10 orchards from different regions in South Africa. Oleander mealybug was present in all but one of the orchards surveyed. Oleander mealybug was the dominant mealybug species in the orchards surveyed in the Citrusdal area. During December 2005 a further six orchards were surveyed in Mpumalanga. Difficulty was experienced with identification of species. Samples have therefore been sent to the PPRI for taxonomic confirmation.

Future research

This survey will be continued until the end of March 2007.

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3.6.5 A survey of grain chinch bug in fruit orchards in the Western Cape

Experiment IT 2300/55 by Pia Addison (SU)

Opsomming

Hierdie eksperiment vorm deel van 'n plaag-risiko opname. Die doel is om te bepaal in watter areas en by watter vrugsoorte, hoofsaaklik in die Weskaap, graanstinkluis 'n betekenisvolle risiko vir produsente se uitvoervrugte inhou. Agt sitrus boorde uit 'n totaal van 56 boorde (hoofsaakliks sagtevrugte) is in Swellendam, Olifantsrivier area en Piketberg gemonitor. Monitoring het vir twee weke in elke boord plaasgevind. Dit is gedoen deur middel van kartonbande, vruginspeksies en inspeksies van onkruid, dekgewasse en aangrensende natuurlike plantegroei. Uit al 56 boorde, het die resultate van die 2004/05 seisoen se opname getoon dat die grootste gevaar van graanstinkluis besmettings in die volgende areas bestaan: Ceres, Kouebokkeveld, Piketberg, Porterville, Riebeek-Kasteel, Langkloof en Malmesbury. 'n Risiko het ook in die Citrusdal en Clanwilliam areas bestaan. Dit lyk nie asof graanstinkluise sitrusboorde verkies as skuilplek nie, maar kom toevallig daar voor. Dit word aangedui deur die relatief lae getalle van graanstinkluise wat in sitrusboorde opgelet is. Dit is daarom ook moeilik om betekenisvolle afleidings te maak oor faktore wat teenwoordigheid van graanstinkluise beïnvloed. Hierdie eksperiment word dus nie voorgesit nie.

Introduction

The grain chinch bug (GCB) *Macchiademus diplopterus* Dist. (Hemiptera: Lygaeidae) is endemic to the Western Cape and as such is classified as a quarantine pest. It is a pest of wheat and moves into adjacent orchards when the wheat is harvested, looking for overwintering sites. These include the stalk and calyx area of fruit. Although not a pest of fruit, the presence of live GCB in packed fruit cartons has led to the rejection of fruit cartons destined for the USA market. A detailed survey of GCB in orchards has not previously been done. The last survey of GCB and other lygaeids was conducted before 1973 and took place mostly in natural vegetation (Slater & Wilcox, 1973). As this species is a known phytosanitary pest, particularly of pears from the Ceres area and citrus from the Olifants River region, but also from fruit coming from other fruit-growing areas of the Western Cape, it is important to establish its exact distribution and abundance in these areas. In conducting a survey of fruit orchards throughout the Western Cape, several questions could therefore be addressed:

- Identification of the pest/pest complex,
- Is the pest specific to pear orchards or does it infest other fruit types at similar levels?
- Are certain areas more prone to infestations than others?

This project is part of a pest-risk assessment, which aims to establish which areas and fruit kinds in the Western Cape pose a significant phytosanitary risk for exporting producers. A total of 56 orchards were sampled during the 2004/05 season, of which eight orchards were citrus orchards in the following areas: Piketberg, Swellendam, Citrusdal and Clanwilliam.

Materials and methods

Monitoring took place using the following methods:

- single-faced corrugated cardboard bands were placed around the stems of 25 evenly-spaced trees and left on the trees for two weeks, after which they were collected and inspected;
- five fruit/tree (125 fruit/orchard) were inspected once during the two-week sampling period (underneath the calyx and in the navel end);
- weeds were sampled in the orchard rows (10 swipes, 2 per row) using a flat, plastic container;
- wheat or weeds adjacent to orchards were sampled (10 swipes) using a flat, plastic container.

Sampling took place on the following dates (the dates bands were collected are indicated): Piketberg (10 May 2005), Swellendam (17 May 2005), Citrusdal and Clanwilliam (7 June 2005).

The following, additional data were collected from the orchards:

- GPS coordinates of orchards;
- Situation of each orchard in relation to adjacent vegetation;
- Any other stinkbug species found in the bands.

Orchards in Piketberg were Valencia orange; orchards in Swellendam were soft citrus; orchard A in Citrusdal was Valencia orange; orchard B in Citrusdal was navel orange; orchard A in Clanwilliam was a mixed cultivar orchard; orchard B in Citrusdal was navel orange. Mapping was done using the software package, ArcView.

Results and discussion

From all 56 orchards, the results from the 2004/05 season surveys indicate that the following areas are most at risk from grain chinch bug infestations: Ceres, Kouebokkeveld, Piketberg, Porterville, Riebeek-Kasteel, Langkloof and Malmesbury (Fig. 3.6.5.1). Citrusdal and Clanwilliam were also at risk (Fig. 3.6.5.2). No grain chinch bugs were sampled from Swellendam during this survey. Fruits harvested much later in the growing season (such as citrus) were not heavily infested (a total of 11 chinch bugs were the most caught in bands from an orchard in Piketberg), relative to deciduous fruits. Fruit infestations were noted from one orchard in Piketberg (2.4%) and one orchard in Citrusdal (0.8%). No correlation appears to exist between bugs found in cardboard bands and those found in fruit (Fig. 3.6.5.2). No grain chinch bugs were found on weeds within any of the orchards. Bluegum and pine trees adjacent to the two Clanwilliam orchards did shelter grain chinch bugs, although no fruit infestations occurred in the orchards. No other lygaeid species were found in bands during this survey.

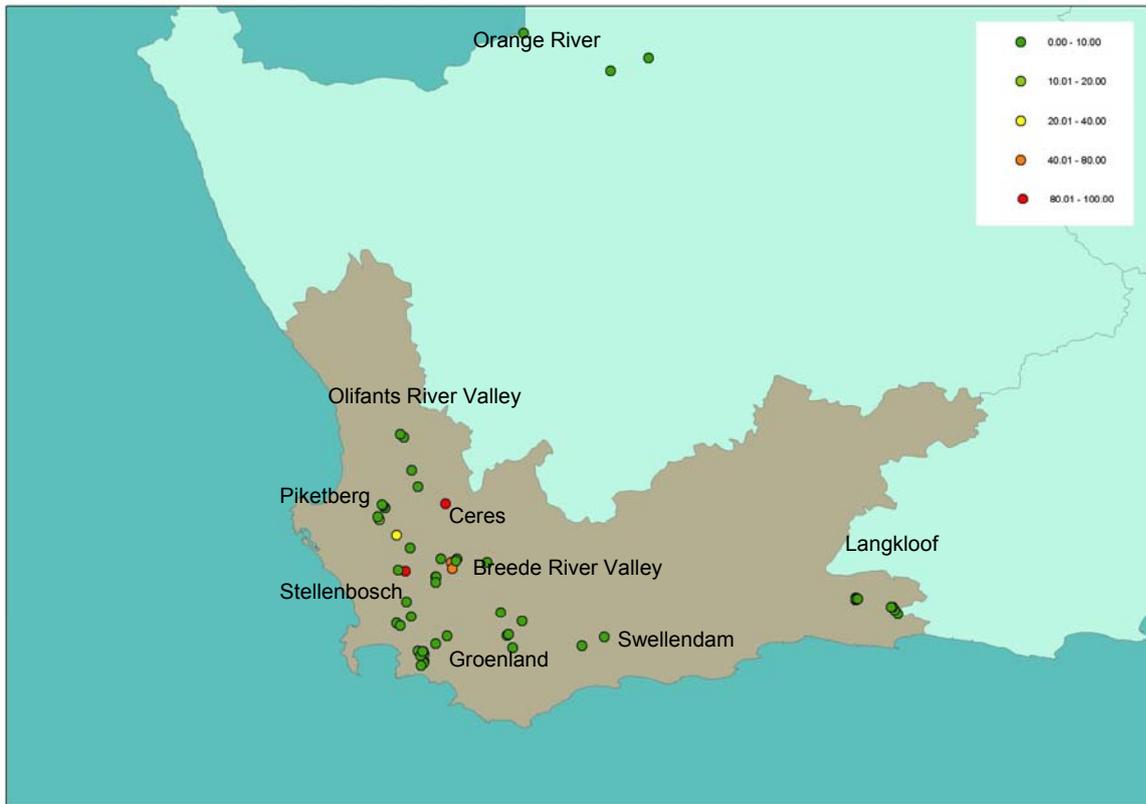


Fig. 3.6.5.1. Map of survey sites indicating percentage deciduous and citrus fruit infested with grain chinch bug (green = uninfested; red = highly infested).

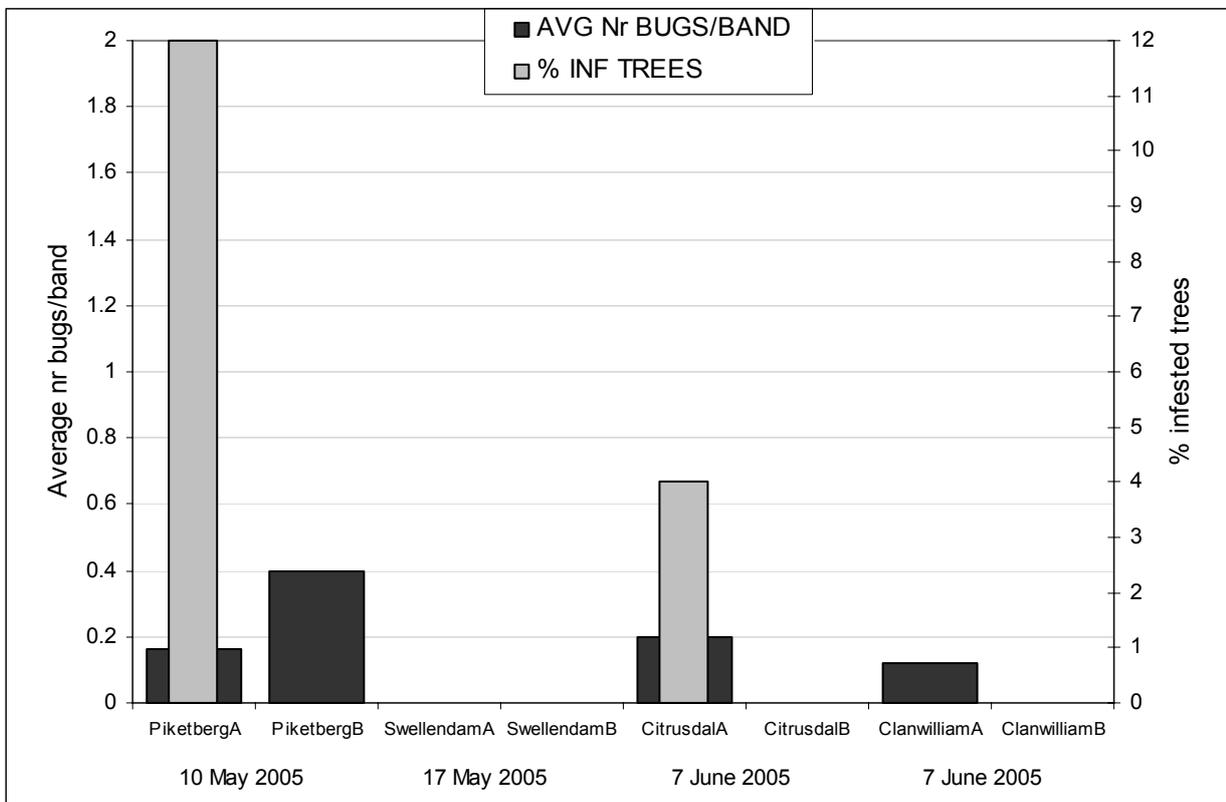


Fig. 3.6.5.2. Infestation of grain chinch bug in four citrus growing areas of the Western Cape. Each column represents one orchard monitored for two weeks close to harvest. The dates given are when the bands were collected after two weeks.

Conclusion

This survey indicates that although population movement in orchards may be relatively low, a significant phytosanitary risk still exists with this pest. Weeds, a potential food source, do not appear to play a major role in attracting bugs into orchards. Therefore, the incidental nature of this pest makes it difficult to draw significant correlations regarding its occurrence in commercial orchards.

Future research

No further research will be done on this experiment as it was thought more worthwhile to find suitable post-harvest disinfestation treatments for this pest.

Reference cited

Slater, J.A. & Wilcox, D.B. 1973. The chinch bugs or Blissinae of South Africa (Hemiptera: Lygaeidae). *Memoirs of the Entomological Society of southern Africa*. 12: 15 – 105.

3.6.6 Post-harvest chemical control of grain chinch bug

By Pia Addison (SU) written by Tim Grout (CRI)

Opsomming

Indien die koringstinkbesie, *Macchiademus diplopterus* (Distant), in uitvoer kartonne VSA toe ontdek word, kan die sitrus afgekeur word, al is die koringstinkbesie nie in Suid Afrika 'n plaag op sitrus nie. Proewe, vir moontlike na-oes behandelinge teen die koringstinkbesie, is dus op die Universiteit van Stellenbosch uitgevoer. Erador, met aktiewe bestandele, piretrum en azadirachtin, was teen 200 ml/hl gedeeltelik effektief, en Xterminator, met aktiewe bestandele piretrum, was teen 500 ml/hl effektief. Verdere navorsing sal uitgevoer word.

Introduction

Due to grain chinch bug, *Macchiademus diplopterus* (Distant) (GCB), occasionally being found as a hitchhiker on citrus destined for the USA and the fruit being rejected as a result, a means of post-harvest treatment for this insect is required. The first toxicant to be investigated was natural pyrethrum as it acts quickly and does not present a residue problem. Two formulations were investigated in the research below.

Materials and methods

Trials were conducted using the formulated products Xterminator (containing 20 g/l pyrethrum) and Erador (with 5.44 g/l pyrethrum and 0.003 g/l azadirachtin) in solutions in laboratory bioassays. Field collected GCBs were used for the trials. The bugs were placed (using a fine paint brush) on their backs onto some tape that was secured onto a glass microscope slide. Each slide, either with 10 or five bugs on it, was considered one replicate. The slides with the bugs were then dipped into the different solutions for 1 minute, and then dipped in tap water after 1 minute.

The first trial on 21 July 2005 compared Xterminator at 500 ml/hl tap water with Xterminator 500 ml/hl chlorinated water, chlorinated water alone and tap water alone. Ten bugs per replicate were used, with five replicates. Observations on mortality were made after 1.25, 4.75 and 21 hours.

The second trial on 17 August 2005 compared Xterminator at 500 ml/hl chlorinated water with Erador 100 ml/hl chlorinated water, Erador 200 ml/hl chlorinated water and chlorinated water alone. Five bugs per replicate, with five replicates, were dipped into the different solutions for one minute, then dipped in tap water after one minute. Observations on mortality were made after 1, 19, 24, 41 and 46 hours.

Results were analysed by two-way ANOVA. All data were normally distributed so there was no need for transformation. Means were further compared using Student-Newman-Keuls test at $\alpha = 0.05$.

Results and discussion

The results observed after approximately one hour showed a rapid knockdown with both products (Tables 3.6.6.1 and 3.6.6.2) followed by a recovery after a few hours as the insect detoxified the pyrethrum. This is a fairly common reaction to natural pyrethrum and is why it is usually used with piperonyl butoxide. However,

the counts after 21 hours in Table 3.6.6.1 and after 46 hours in Table 3.6.6.2 give a better reflection of final mortality rates.

Xterminator 500 ml/hl with the higher concentration of pyrethrum was significantly more effective than Erador 200 ml/hl ($P < 0.05$). Further experiments therefore excluded Erador.

Table 3.6.6.1. Mortality of GCB in the first trial conducted at Stellenbosch University on 21 July 2005.

Treatments	Mortality (%) after:		
	1.25 hr	4.75 hr	21 hr
Tap water	0	0	22 a
Chlorinated water	0	0	32 a
Xterminator 500 ml/hl tap water	100	22	98 b
Xterminator 500 ml/hl chlorinated water	100	6	100 b

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

Table 3.6.6.2. Mortality of GCB in the second trial conducted at Stellenbosch University on 17 August 2005.

Treatments	Mortality (%) after:				
	1 hr	19 hr	24 hr	41 hr	46 hr
Chlorinated water	4	4	4	28	44 a
Xterminator 500 ml/hl chlorinated water	100	36	40	92	100 c
Erador 100 ml/hl chlorinated water	100	28	40	76	80 bc
Erador 200 ml/hl chlorinated water	92	28	40	64	68 b

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

Conclusion

Results with Xterminator 500 ml/hl are promising and should be taken further.

3.7 PROJECT: PRODUCTION PESTS

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

3.7.1 Project summary

Ten years ago, research on production pests centred on the control of red scale, but the status of this pest has declined with the availability of imidacloprid, pyriproxyfen, good quality horticultural oils and *Aphytis* for augmentation. Latterly, greening disease or Huanglongbing, has become more important as it has been discovered in more citrus production areas and inexpensive control of the vector with monocrotophos stem-treatments is no longer possible. Apart from some contract research on red scale that cannot be reported on here, all research in this project focussed on the control of citrus psylla, the vector of greening disease. Often in the past, research has not been possible on citrus psylla because the numbers of insects have been too low. For this research the opposite was true and none of the prospective IPM-compatible alternatives were as effective as the registered endosulfan spray, which in itself could not provide adequate control (3.7.2). It was hoped that an attract-and-kill approach may be possible for this vector and two attractants that had previously shown some promise were evaluated further, but without success (3.7.3). However, horizontal, sticky yellow traps were found to catch three times as many citrus psylla on their upper surface as any vertical surface and horizontal traps also trapped significantly less natural enemies than vertical traps in one evaluation (3.7.3). These results will lead to further investigation of mass trapping as an alternative control method for citrus psylla, perhaps in combination with IPM-compatible sprays.

Projekopsomming

Tien jaar gelede was die navorsing van produksie plae gefokus op die beheer van rooi dopluis maar die belangrikheid van hierdie plaag het egter afgeneem as gevolg van die beskikbaarheid van imidacloprid, pyriproxyfen, goeie kwaliteit olies en *Aphytis* vir vermeerdering. Die belangrikheid van vergroeningsiekte, ook bekend as Huanglongbing, het onlangs toegeneem met die ontdekking van die siekte in meer sitrus produserende areas en omdat monocrotophos as 'n stambehandeling vir goedkoop beheer van die vector,

nie meer moontlik is nie. Afgesien van kontraknavorsing wat nog op rooi dopluis gedoen word en nie hier bespreek gaan word nie, word al die navorsing gefokus op die beheer van sitrus bladvlooi, die vektor van vergroeningsiekte. In die verlede was navorsing dikwels nie moontlik op sitrus bladvlooi nie aangesien die getalle te laag was maar tydens die navorsing was die teenoorgestelde egter waar en nie een van die moontlike IPB-verenigbare alternatiewe was so effektief soos die geregistreerde endosulfan bespuiting nie. Die endosulfan bespuitings kon egter ook nie genoegsame beheer verskaf nie (3.7.2). Daar is gehoop dat 'n lok-en-dood benadering moontlik sou wees vir die vektor en twee lokmiddels wat voorheen belowend vertoon het, is verder geëvalueer, maar sonder enige sukses (3.7.3). Daar is gevind dat die boonste oppervlakte van horisontale, taai, geel valle tot drie maal meer sitrus bladvlooi gevang het as enige vertikale oppervlak. Die horisontale valle het ook merkbaar minder natuurlike vyande as die vertikale valle gevang in een van die evaluasies (3.7.3). Hierdie resultate sal nou in verdere ondersoeke van massa-uitvang, as 'n alternatiewe beheer metode vir sitrus bladvlooi gebruik word, moontlik in kombinasie met IPB-verenigbare spuitstowwe.

3.7.2 IPM-compatible treatment options for citrus psylla *Trioza erytreae* Experiment 586 by Tim Grout, Bruce Tate and Peter Stephen (CRI)

Opsomming

Daar bestaan 'n dringende behoefte vir alternatiewe blaar spuitmiddels anders as organofosfate vir die beheer van sitrus bladvlooi. Alhoewel die insek skaars was in die lente en vroeë somer, is hoë besmettings gedurende die middel somer op 'n plaas, suid van Nelspruit gevind. Twee proewe om moontlike spuitmiddels te evalueer is uitgevoer. As gevolg van die hoë besmettingsvlakke het die geregistreerde endosulfan behandeling nie genoegsame beheer verskaf nie en geeneen van die alternatiewe behandelings was naastenby so effektief soos endosulfan nie. Die effektiwiteit van 1% olie en kaolien was swakker in vergelyking met verlede jaar. Neem het dieselfde mate van onderdrukking as abamectin en olie tot gevolg gehad. Verdere navorsing sal op moontlike kombinasies van die behandelings en massa-uitvang gerig wees.

Introduction

Periodically, citrus psylla populations increase dramatically and urgent control methods are required because the greening disease (*Liberobacter africanum*) that is transmitted by this vector can spread rapidly. In the past, organophosphates were often used to control these vectors but monocrotophos is no longer available in South Africa and the use of dimethoate has been reduced to one preblossom treatment on bearing trees due to the MRL in EU being lowered markedly. The neonicotinoid stem and soil treatments are effective but foliar spray options are few. The currently registered products are given in Nel et al. (2002) but not many of these can be used late in the season due to residue problems. The only recent work that has been conducted on the chemical control of citrus psylla has been that by the authors last year (Grout and Stephen 2005). An evaluation of various treatments was therefore planned for the 2005-6 season but as was the case last year, citrus psylla populations were very low in spring and summer and a site could only be found in January 2006. However, the results are written up here for convenience.

Materials and methods

No suitably infested citrus orchards were found until January 2006 when extremely high populations of citrus psylla were found in an orchard of Empress mandarins on Brackenhill farm south of Nelspruit. A randomised block design was used with the orchard being split in half and each half being subdivided into seven treatment blocks. Each block comprised three rows and was at least seven trees long. Sprays were applied by hand using a high-pressure (30 bar) spray machine and applying 6-10 l spray mixture per tree on 5 January 2006. Weather conditions were dry, partly cloudy with a maximum temperature of 28°C. The treatments used are shown in Table 3.7.2.1. They were evaluated 8 days after treatment on 13 January 2006 with a further partial evaluation on 23 January. Eight data trees were primarily selected from the central row in each block and if there were insufficient trees in the centre row, the sides of trees closest to the central row were used as well. Counts were based on 10 branch terminals per data tree having new leaves suitable for citrus psylla. Each terminal was rated for the presence or absence of fresh eggs, live nymphs or adults. Data were transformed to the square root of the arc sine, then analysed by two-way ANOVA and means further compared using Student-Newman-Keul's test at $P=0.05$.

A second trial was conducted at the same site using exactly the same techniques after the whole orchard was sprayed with Phosdrin to slightly reduce the citrus psylla numbers. Treatments were applied on 16 February 2006 and evaluated on 24 February. Treatment details are provided in Table 3.7.2.3. Statistical analysis of results was as described above.

Results and discussion

Numbers of citrus psylla were so high in this orchard that they were no longer ovipositing along the leaf margins but anywhere on the leaves. One-sided, yellow sticky traps (165 X 165 mm) were regularly catching over 300 psyllids per week. None of the treatments had any significant effect ($P>0.05$) on egg infestation (Table 3.7.2.1) and only the standard treatment of endosulfan had a significant effect on nymph infestation. There were more differences between treatments in adult infestation and Nemesis plus oil was significantly less infested than other treatments. For this reason, a second evaluation was conducted on 23 January to compare Nemesis with the control and the endosulfan standard. However, although the infestations of eggs and adults were the lowest in the Nemesis treatments they were not significantly different from the untreated control (Table 3.7.2.2). Neither the endosulfan treatment nor the Nemesis treatment caused a significant reduction in nymphal infestation. After the site was sprayed out and the treatments reapplied there were more differences in efficacy between the treatments (Table 3.7.2.3), although none could be considered to provide commercial control. Endosulfan was once again significantly better than all other treatments against nymphal life stages. Fruitcote and 1% medium grade horticultural oil appeared to be less effective than in the trial of the previous year (Grout & Stephen 2005) and were not significantly different from the control. The abamectin plus oil and the Bio-Neem treatments showed significantly better efficacy than the control against nymphs but did not reduce egg infestation significantly.

The differences between the results in this season and the previous season can probably be attributed to the difference in infestation levels. This emphasises the need to maintain citrus psylla populations at low levels because once they get out of control they are difficult to stop without using very disruptive treatments. Last season, abamectin plus 0.3% oil was significantly different from the control when all types of infestation were considered (Grout & Stephen 2005) but this season the combination was significantly more effective than the control only when nymphal infestation was compared. Although none of the treatments were as effective as the old standard endosulfan, perhaps they could be used in combination with mass-trapping or other cultural techniques to maintain citrus psylla populations at extremely low levels.

Table 3.7.2.1. The effect of various treatments against different life stages of citrus psylla at Brackenhill farm near Nelspruit.

Foliar treatments 5 Jan 2006	Infestation of shoot terminals 8 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	62.5 a	80.0 a	75.6 ab
Endosulfan (475 WP) 250 g/hl	77.5 a	21.3 b	68.8 b
Fruitcote (kaolin) 3 kg/hl	75.0 a	55.0 a	88.1 a
Biomectin (abamectin 18 EC) 20 ml plus BP Medium horticultural oil 300 ml/hl	69.4 a	51.3 a	75.6 ab
BP Medium horticultural oil 1%	73.8 a	46.9 a	80.6 ab
Nemesis (pyriproxyfen 100 EC) 30 ml plus BP Medium oil 300 ml/hl	53.1 a	45.6 a	51.9 c
Bio-Neem (azadirachtin 1.5 EC) 500 ml/hl	67.5 a	46.3 a	87.5 a

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

Table 3.7.2.2. The longer-term effect of endosulfan and Nemesis plus oil against different life stages of citrus psylla at Brackenhill farm near Nelspruit.

Foliar treatments 5 Jan 2006	Infestation of shoot terminals 18 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	81.9 ab	86.3 a	53.1 ab
Endosulfan (475 WP) 250 g/hl	92.5 a	76.9 a	66.9 a
Nemesis (pyriproxyfen 100 EC) 30 ml plus BP Medium oil 300 ml/hl	73.8 b	89.4 a	40.6 b

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

Table 3.7.2.3. The effect of various treatments against different life stages of citrus psylla in a second trial at Brackenhill farm near Nelspruit.

Foliar treatments 16 Feb 2006	Infestation of shoot terminals 8 days after treatment		
	Eggs (%)	Nymphs (%)	Adults (%)
Untreated control	63.8 a	41.9 a	51.3 a
Endosulfan (475 WP) 250 g/hl	35.6 b	4.4 c	27.5 c
Fruitcote (kaolin) 3 kg/hl	48.1 ab	40.0 a	46.9 ab
Biomectin (abamectin 18 EC) 20 ml plus BP Medium horticultural oil 300 ml/hl	44.4 ab	17.5 b	42.5 abc
BP Medium horticultural oil 1%	56.9 ab	37.5 a	52.5 a
Nemesis (pyriproxyfen 100 EC) 30 ml plus BP Medium oil 300 ml/hl	55.0 ab	26.9 ab	38.8 abc
Bio-Neem (azadirachtin 1.5 EC) 500 ml/hl	43.1 ab	18.1 b	29.4 bc

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

Conclusion

Numbers of citrus psylla were extremely high in these trials and the registered endosulfan spray could not provide adequate control. However, none of the alternatives tested were even as effective as the endosulfan.

Future research

Further research will be conducted with some of the alternatives in combination with mass-trapping.

References cited

- Grout, T.G. and Stephen, P.R. 2005. IPM-compatible treatment options for citrus psylla *Trioza erytraeae*. pp. 196-197. In: CRI Group Annual Research Report 2004. Nelspruit.
- Nel, A., Krause, M. and Khelawanlall, N. 2002. A guide for the control of plant pests. 39th edition. National Dept. Agric., Pretoria.

3.7.3 Development of an attract-and-kill system for citrus psylla Experiment 794 by Tim Grout and Bruce Tate (CRI)

Opsomming

Daar is sistemiese plantbeskermingsprodukte vir die beheer van sitrus bladvlou besikikbaar, maar aanwending van die produkte op draende bome mag moontlik nie prakties wees laat in die seisoen nie. Die aantal effektiewe blaar bespuitings is beperk en daarom sou 'n lok-en-dood sisteem vir sitrus bladvlou ideaal wees. In vorige navorsing wat deur CRI uitgevoer is, het dit geblyk dat linalool en suurlemoenolie wel sitrus bladvlou kan aantrek. Die middels is verder ondersoek maar nie een was egter beduidend effektief nie. Daar is gevind dat die boonste oppervlaktes van horisontale, taai, geel valle drie maal meer insekte gevang het as enige ander vertikale oppervlak. Horisontale valle het ook aansienlik minder natuurlike vyande gevang as vertikale valle in een van die twee evaluasies. Hierdie resultate sal nou in verdere ondersoeke van massa-uitvang, as 'n alternatiewe benadering tot die lok-en-dood van sitrus bladvlou, gebruik word.

Introduction

Since 2001, when citrus psylla populations went out of control, the need for new IPM-compatible control options was highlighted because the greening disease (*Liberobacter africanum*) that is transmitted by this vector began to spread rapidly. Subsequently, the availability of chemical control options has declined. Monocrotophos is no longer available and the use of dimethoate has been reduced to one preblossom treatment on bearing trees due to the MRL in EU being lowered markedly. Systemic control options can be used as soil or stem treatments with little disruption of IPM but these are often not practical late in the season due to residues in fruit. During summer growth flushes, sprays for citrus psylla can cause serious disruption to natural enemies of various pests and a more biorational technique such as an attract-and-kill system or mass trapping would be more appropriate, minimising or avoiding insecticide residues. Psyllids

are highly attracted to yellow traps (Samways et al. 1986) but so are many other insects including natural enemies. Mass trapping with yellow sticky traps could therefore be detrimental to important natural enemies in the citrus ecosystem. It was therefore decided to evaluate sticky yellow traps in different vertical and horizontal planes to see whether catches of psyllids could be maximised while the non-target effects on natural enemies were minimised. Earlier research (Grout & Stephen 2002) had also indicated that linalool and lemon oil may have been attracting citrus psylla so these products needed to be further evaluated as possible attractants for attract and kill purposes.

Materials and methods

All comparisons involving yellow traps were conducted in an Empress mandarin orchard at Brackenhill Farm south of Nelspruit. The first two trials involved comparing four trap orientations: the upper surface of a horizontal trap, the lower surface of a horizontal trap, the surface of a vertical trap facing southeast and the surface of a vertical trap facing northwest. For these trials, traps were made up from pieces of zinc-galvanized iron (280 x 280 x 0.5 mm) with a self-adhesive yellow material (MacTac vinyls) (MACal 9800) attached to one side as described by Samways et al. (1986). A demarcated count area of 160 X 160 mm was drawn in the centre of the yellow surface. The traps were covered with cling wrap and painted with Fly Tac adhesive. They were then clamped back-to-back in a horizontal position or a vertical position and held approximately 2 m above ground with the use of a wooden stand (Figure 3.7.3.1). The stand with the four trap orientations was placed between two trees in the row. Six such trap stands were used and these were approximately 9 m apart. The first trapping period was 7-14 December 2005 and the second trapping period was 21-28 December. The numbers of citrus psylla, parasitic hymenoptera and coccinellid beetles within the demarcated count areas on the traps were counted with the use of a stereomicroscope.

Due to promising results in the horizontal position, a simpler method of holding a trap horizontally at the top of a wooden stave (Figure 3.7.3.2) was developed for further evaluations with a view to possibly using this for mass trapping. An inexpensive yellow, rigid, plastic corrugated material called Correx™ was therefore used to make traps slightly larger than the demarcated count area of 160 X 160 mm. These traps were used to compare the yellow colour alone as an attractant with traps having a 10 mm length of dental roll that was cylindrically-wrapped with plastic packaging tape and impregnated with 0.66 g of either Linalool (0.6 ml) or lemon oil extract (0.5 ml - obtained from a fruit processing factory) placed in the middle of the upper surface (Figure 3.7.3.3). Four traps were used per treatment and placed between the trees in the row. Traps were once again approximately 9 m apart in the row and more than two rows apart across the rows. The positions of the traps within the orchard were completely randomised. Traps were coated in the same way as before but were only evaluated for citrus psylla. Three evaluation periods were used: 11-14 January, 1-8 February and 15-22 February. The orchard was sprayed with a short-residual organophosphate (mevinphos) at the end of January to reduce citrus psylla populations.

Statistical analysis was by means of two-way analysis of variance after normalising data with a square root ($x+0.5$) or natural log ($x+1$) transformation, when necessary. Where significant differences ($P<0.05$) were found in the ANOVA, means were further compared by using Student-Newman-Keul's test.



Figure 3.7.3.1. Stand for trap orientation trials.



Figure 3.7.3.2. Simple stand used for horizontal trap comparisons of attractants.

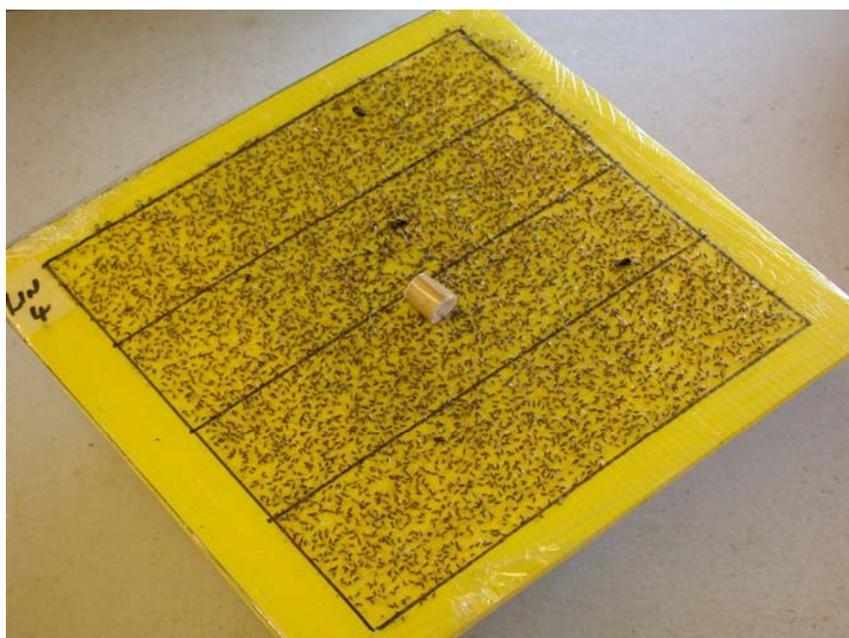


Figure 3.7.3.3. Correx™ yellow sticky trap showing thousands of citrus psylla and a wrapped portion of dental roll in the centre.

Results and discussion

It was not surprising that numbers of trapped psyllids on the lower side of the horizontal traps was low as the trap was probably silhouetted against the sky and the colour was not visible (Tables 3.7.3.1 and 2). However, it was surprising that the numbers of trapped psyllids on the upper horizontal surface were three times higher than the mean number on either vertical surface. Perhaps adult psyllids fly above the tree canopy and come down to land on new foliage, or in this case, the upper surface of the trap. If large horizontal traps are used and numbers of psyllids are low, the impact of mass trapping on the population may be significant. The use of horizontal traps rather than vertical traps for monitoring purposes would be three times more efficient.

The effect of trap orientation on natural enemies was not as obvious as with citrus psylla. In the first comparison, the only significant difference was lower numbers of hymenoptera on the lower side of the horizontal trap (Table 3.7.3.1). In the second comparison, the vertical surfaces caught significantly more parasitoids and coccinellids than the horizontal surfaces (Table 3.7.3.2). The overall numbers of all insects in this period were higher than in the former period. This may be the result of differences in rainfall as the second trapping period had much less rain than the former period (Figure 3.7.3.2 and Table 3.7.3.3). The slight differences in temperature were unlikely to have influenced insect flight. These results do therefore indicate that the use of horizontal traps would be less detrimental to alate natural enemies.

Table 3.7.3.1. Effect of trap orientation on numbers of citrus psylla and natural enemies caught between 7 and 14 December 2005.

Trap position	Mean no. psyllids/trap	Mean no. parasitic hymenoptera/trap	Mean no. coccinellids per trap
Horizontal: upper	547.5 a	50.7 a	0.7 a
Horizontal: lower	5.0 d	8.5 b	1.0 a
Vertical: NW facing	196.2 b	42.3 a	0.8 a
Vertical: SE facing	136.5 c	32.2 a	1.2 a

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

Table 3.7.3.2. Effect of trap orientation on numbers of citrus psylla and natural enemies caught between 21 and 28 December 2005.

Trap position	Mean no. psyllids/trap	Mean no. parasitic hymenoptera/trap	Mean no. coccinellids per trap
Horizontal: upper	1573.3 a	39.3 b	0.7 b
Horizontal: lower	15.5 c	23.3 b	1.8 b
Vertical: NW facing	606.3 b	73.3 a	5.6 a
Vertical: SE facing	498.0 b	70.0 a	6.7 a

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

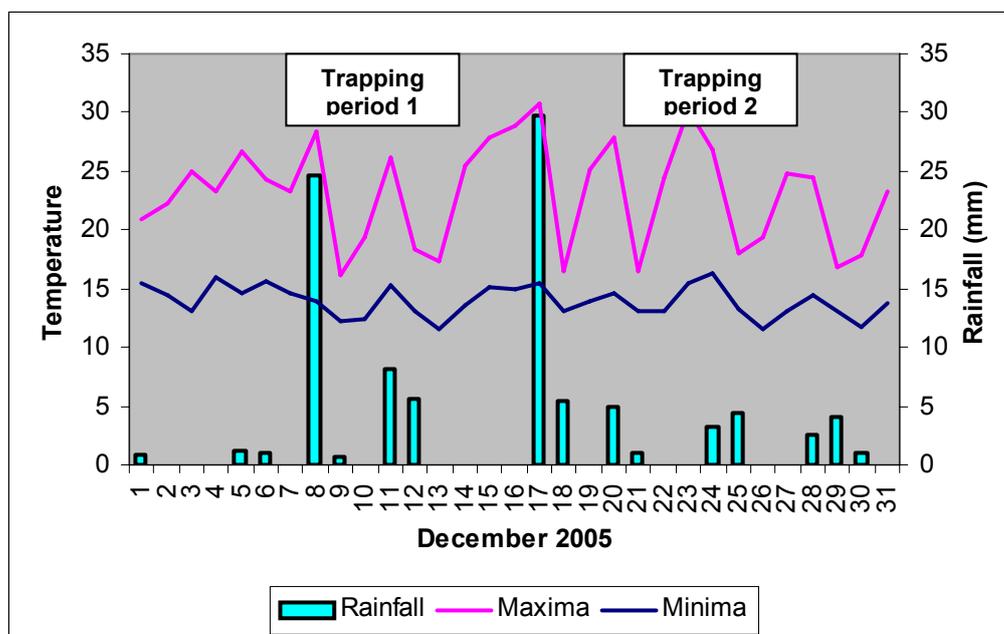


Figure 3.7.3.2. Daily Maxima and Minima temperatures in °C and rainfall during December 2005 in Nelspruit.

Table 3.7.3.3. Summarised weather data for the two periods when trap orientation was investigated.

Period	Mean Max (°C)	Mean Min (°C)	Total rain (mm)
7-14 Dec	24.9	15.2	39
21-28 Dec	26.4	15.7	14.2

None of the three evaluations of Linalool and lemon oil showed any indication of these products being attractive to citrus psylla (Table 3.7.3.4). Numbers of citrus psylla trapped on yellow Correx in the first evaluation must be a record. The mean number of psyllids per trap per day in this evaluation was 1359 or 5.3 per cm². Numbers declined after the orchard was sprayed but still remained out of control. Without an effective attractant other than colour, further research will involve a mass trapping approach rather than attract and kill. The lightweight yellow Correx material will be ideal for mass trapping and is produced in 600 x 400 mm sheets. The combination of large traps and IPM-compatible foliar sprays will be evaluated.

Table 3.7.3.4. Results of three evaluations of two prospective attractants on horizontal, Correx yellow, sticky traps.

Attractants	11-14 January 2006 Mean psylla/trap	1-8 February 2006 Mean psylla/trap	15-22 February 2006 Mean psylla/trap
Correx yellow only	4175 a	219.8 a	327.8 a
Correx yellow + lemon oil	4225 a	459.0 a	184.0 a
Correx yellow + linalool	3832 a	252.8 a	220.3 a

No significant differences ($P>0.05$) were found between treatments in any evaluation.

Conclusion

Horizontal, sticky yellow traps caught three times as many citrus psylla on their upper surface as any vertical surface. Horizontal traps also trapped significantly less natural enemies than vertical traps in one of two evaluations. Neither linalool nor lemon oil increased the attractiveness of yellow traps to citrus psylla and no further research will be conducted on these products.

Future research

Mass trapping with horizontal, lightweight Correx traps will be evaluated further in combination with IPM-compatible treatments.

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4 PROGRAMME: DISEASE MANAGEMENT

4.1 PROGRAMME SUMMARY

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

In 2005, most research funds were spent on graft transmissible diseases (GTDs) with citrus black spot (CBS) being the second-highest funded project. This is appropriate as GTDs have a long-term effect on citrus production and tree lifespan, whereas CBS infections can be controlled in the short term. The presence of the important GTDs is tested for biologically in most cases with the use of indicator plants but research is underway to develop faster, serological indexing methods. PCR techniques have now been developed: for the whole family of Clostoviridae (of which citrus tristeza virus (CTV) is a member), to distinguish between mild and severe strains of CTV, to detect Asian and African greening disease, to detect the responsible organisms for citrus variegated chlorosis and citrus canker, and to detect citrus leprosis virus and phytoplasmas. Research to improve the cross protection of grapefruit to CTV continued and further field results were obtained. The considerable progress achieved over the last few years in improving management of CBS continued and the use of the Kotzé Inoculum Monitor and a Wet-Dry incubation process now allow for detection of CBS at low levels such as in citrus nurseries. Various trials were conducted on the chemical control of CBS with some including the use of Sporekill. Further progress was also made in the forecasting of CBS infections. Research continued on the control of both the citrus nematode and the sheath nematode, with indications of which rootstocks are most tolerant to infestation. Control of *Phytophthora nicotianae* in nurseries and as the cause of post-harvest brown rot received research attention. Green and blue moulds are responsible for 90% of the losses due to post-harvest pathogens and although resistance of these pathogens to TBZ has been known for many years, resistance to imazalil is now increasing. This has led to widespread sampling and research on new fungicides and fungicide combinations. The combination of Sporekill with copper hydroxide or copper oxychloride for the control of *Alternaria* brown spot has allowed the copper concentration to be halved and eliminated copper-induced stippling. The cause of the unknown disease causing dieback of Clementines in the Knysna region was identified as *Phytophthora citrophthora* and the efficacies of fungicide treatments are being evaluated. Although a report is not included, a considerable amount of time was spent on GLP trials to develop Maximum Residue Limits for carbendazim and guazatine for use in the European Union. This research is continuing and a report will be provided next year.

PROGRAMOPSOMMING

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

Die meeste van die navorsingsfondse is in 2005 op ent oordraagbare siektes gespandeer, met sitrus swartvlek (SSV) as die tweede hoogste befondste projek. Die rede hiervoor is omdat ent oordraagbare siektes 'n langtermyn effek op sitrus produksie het sowel as op die leeftyd van die bome terwyl SSV-besmettings op die korttermyn beheer kan word. Alhoewel indikator plante gewoonlik gebruik word om biologies te toets vir die teenwoordigheid van die belangrikste ent oordraagbare siektes is navorsing in die proses om vinniger serologiese indekseringsmetodes te ontwikkel. PKR tegnieke is ook nou ontwikkel vir die hele familie Clostoviridae (Sitrus tristeza virus (CTV) is 'n lid van die familie) asook om tussen matige en ernstige lyne van CTV te onderskei, om Asiatiese sowel as Afrika vergroenings siekte op te spoor, om die organismes verantwoordelik vir sitrus veelkleurige chlorose en sitrus kanker op te spoor en ook om sitrus leprosie virus en fitoplasmas op te spoor. Navorsing om die kruisbeskerming van pomelos teen CTV te verbeter het voortgegaan en verdere resultate is verkry. Die aansienlike vordering wat oor die laaste paar jare verkry is in die verbetering van die bestuur van SSV het voortgegaan. Die opsporing van SSV, selfs as dit teenwoordig is in lae konsentrasies soos in sitruskwekerye, is nou moontlik vanweë die gebruik van die Kotzé Inokulum monitor en 'n Nat-Droog inkubasie proses. Verskeie proewe is op die chemiese beheer van SSV uitgevoer wat ook die middel Sporekill ingesluit het. Verdere vordering is ook gemaak met die voorspelling van SSV-infeksies. Navorsing op die beheer van beide die sitrus - en die skede-aalwurm het voortgegaan met indikasies van watter onderstokke meer bestand teen besmetting is. Die beheer van *Phytophthora nicotianae* in kwekerye en ook as die oorsaak van na-oes bruinvrot het aandag geniet. Groen en blou skimmels is verantwoordelik vir 90% van die verliese as gevolg van na-oes patogene en alhoewel weerstand van hierdie patogene teen TBZ al vir baie jare bekend is, is hul weerstand teen Imazalil ook besig om toe te neem. Dit het aanleiding gegee tot wydverspreide opnames en navorsing op nuwe fungisiedes en kombinasies van fungisiedes. Die kombinasie van Sporekill met koper hidroksied of koper oksichloried as beheermiddel van *Alternaria* bruinvrot het daartoe gelei dat koper konsentrasies halveer kon word en koper geïnduseerde stippeling geëlimineer is. Die oorsaak van die onbekende siekte wat verantwoordelik was vir terugsterwing van Clementines in die Knysna omgewing is geïdentifiseer as *Phytophthora citrophthora* en die effektiwiteit van swamdoder behandelings word nou geëvalueer. Alhoewel 'n verslag nie hierby ingesluit is nie, is 'n aansienlike hoeveelheid tyd gespandeer op gaschromatografie werk om die maksimum residu

vlakke te bepaal vir karbendazim en guazitien vir verskeping na die Europese Unie. Hierdie navorsing gaan voort en 'n verslag sal volgende jaar ingesluit word.

4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project Co-ordinator: S.P. van Vuuren (CRI)

4.2.1 Projekopsomming

Vinnige betroubare serologies indekseringsmetodes soos ELISA, Kol Klad en Polimerase-ketting reaksie (PKR) bestaan vir die meeste siektes, maar die tegnieke moet ge-optimeer word vir plaaslike gebruik. Vordering in die verband was as volg: 1) 'n Breë spectrum PKR sal hoogs uiteenlopende rasse van *Citrus tristeza virus* (CTV) kan opspoor en kan effektief gebruik word na virus-reiniging in die Sitrus Verbeteringskema. 2) 'n Multipleks PKR is gevestig wat beide 'n hoogs gekonserveerde sekvens, sowel as 'n sekvens wat ligte en strawwe rasse van CTV kan onderskei, teiken. Daar kan dus in een toets alle bekende CTV rasse opgespoor en as ligte of strawwe rasse onderskei word. 3) 'n Groep van 23 PKR'e wat saam gebruik kan word om CTV isolate te kan klassifiseer op genotipe vlak, is gevestig. 4) 'n "Micro-array" skyfie is ontwerp en gesintetiseer om tussen CTV-T30 (lig) en CTV-T36 (straf) rasse te kan onderskei in 'n loodsproef om die haalbaarheid van die toets te evalueer. 5) Die PKR tegnieke wat in 2004 teen "*Candidatus Liberibacter africanus*" (Afrika vergroening) gevestig is, is in 2005 gebruik vir roetine opsporing van die bakteriële in verskeie sitrusmonsters. Die metode se vermoë om "*Candidatus L. asiaticus* (Asiatiese vergroening) te kan opspoor is ook gedemonstreer. 6) Die vestiging van 'n PKR vir die opsporing van *Xylella fastidiosa* ("Citrus Variegated chlorosis") is bereik en die tegniek is beskikbaar vir kwarantyn behoeftes. 7) Twee PKR sisteme is gevestig vir die opsporing van "Citrus leaf blotch virus", 'n eksotiese saadoordraagbare virus, ge-assosieer met entverbinding onverenigbaarheid-simptome. 8) 'n PKR teen die DNA van "Apple stem grooving virus" (voorheen "Citrus tatter leaf virus") is gevestig. 9) Twee PKR'e is gevestig teen *Xanthomonas campestris* pv. *citri* (Sitruskanker) en is beskikbaar vir kwarantyn behoeftes. 10) Twee PKR tegnieke om cDNA van Sitrus leprose virus op te spoor is gevestig. 11) PKR vir die algemene opsporing van fitoplasmas is gevestig (afdeling 4.2.2). Biologiese indeksering is gebruik om die CTV strafheid in die moederbome van die Sitrus Grondvesblok te bepaal (afdeling 4.2.3). Geen nuwe cultivars is gedurende die jaar by die 213 cultivars van die genebron gevoeg nie (afdeling 4.2.4). Die Marsh en Star Ruby bome in Swaziland en die Nkweleni Vallei dui aan dat van die sub-isolate groei strem, en met ander is daar interaksies tussen die bostamme (afdeling 4.2.5). Star Ruby boompies wat met dieselfde isolate en sub-isolate as in die proewe in afdeling 4.2.5 geïnkuleer is, is in die Kakamas omgewing geplant (afdeling 4.2.6). Nuwe belowende ligte isolate wat in verskillende pomelo produksiegebiede versamel is word in Marsh en Star Ruby boompies geïnkuleer (afdeling 4.2.7). CTV isolate wat vanaf Star Ruby en Rosé pomelobome versamel is, is as kruisbeskermers vir Star Ruby ge-evalueer oor 'n 8-jaar periode. Bome met isolate GFMS 35 en GFMS 78 het die beste gevaar en het 'n 12% beter kumulatiewe produksie as bome met die ander isolate gehad (afdeling 4.2.8). Sewe-jaar oue bome van sewe rooi pomelo seleksies het baie eenvormig gereageer met vier CTV isolate as kruisbeskermings-agente (afdeling 4.2.9). Pogings om 'n isolaat met kruisbeskermings-eienskappe vir alle pomelo seleksies in al die verskillende klimaatstoestande te konstrueer was nie suksesvol nie. Die oordraagbaarheid het afgeneem sodra sub-isolate gemeng is, wat 'n swak eienskap vir kruisbeskerming is (afdeling 4.2.10).

'n Proef is gevestig waar gepreïmmuniseerde en virusvrye bome van sewe Clementine seleksies vergelyk word. Dit wil voorkom of al die Clementine seleksies nie dieselfde reageer op CTV besmetting nie (afdeling 4.2.11). In 'n nawel proef presteer bome wat met LMS 6 isolaat gepreïmmuniseer is die beste (afdeling 4.2.12). Daar is gevind dat Turkey Valencia blykbaar meer gevoelig is vir CTV en daarom word daar gepoog om 'n geskikte CTV isolaat vir die soetlemoen seleksie te kry (afdeling 4.2.13). Die effek van verskillende ligte CTV isolate word in drie Valencia bostamme ge-evalueer. Boom grootte van McClean Valencia was betekenisvol kleiner as die van Delta – en McClean Saadloos Valencias (afdeling 4.2.14). Ligte CTV isolate vanaf soetlemoenbome word in vyf Valencia bostamme ge-evalueer (afdeling 4.2.15).

Sewentien onderstamme word ge-evalueer vir sitruskroei ("Blight") toleransie. Tans kan afgelei word dat ondertamme C35, Empress mandaryn en Carizzo citrange die meeste ge-afekteer word terwyl Swingle citrumelo, X639, Sun Chu Sha en Cleopatra mandaryn die meeste toleransie toon (afdeling 4.2.16).

'n Literatuur oorsig om die moontlikheid van transgeniese weerstand teen vergroening te ondersoek is gedoen en dek relevante aspekte van vergroening soos die veroorsakende organisme, vektore en huidige beheermaatreëls. Natuurlike weerstand word bespreek, gevolg deur vier baie breë kategorieë van transgeniese benaderings wat relevant mag wees tot vergroening, naamlik: die gebruik van gasheer verdedigingsmeganismes, die gebruik van patogene gene, weerstand teen die vektor en weerstand met nie-patogene of vektor middels (afdeling 4.2.17). Daar word gepoog om vergroenings-weerstandbiedendheid te verkry deur plante uit gesonde chimeras van vegroende vrugte te kweek. Twee klone is geïdentifiseer as

simptoomloos na blootstelling aan silla (afdeling 4.2.18). Die her-evaluering van metodes om vergroening in besmette bome in bestaande boorde te beheer word voortgesit (afdeling 4.2.19).

Project summary

Quick, reliable serological indexing methods such as ELISA, Dot Blot and Polymerase chain reaction (PCR) exist for most diseases and in many cases the techniques should be optimized for local use. Progress in this regard during the past year includes the following: 1) A broad spectrum PCR was established that will detect highly divergent *Citrus tristeza virus* (CTV) variants and is ideal for testing plant samples following virus elimination in the Citrus Improvement Scheme. 2) A multiplex PCR directed against both a conserved region as well as a sequence region differing amongst severe and mild strains of CTV was established. This PCR is capable of detecting all known sequence variants of CTV, and can differentiate them into mild or severe strains in one test. 3) A group of 23 PCRs, capable of classifying CTV to genotype level when used together, were established. 4) A micro-array chip capable of differentiating CTV-T30 (mild) and CTV-T36 (severe) genotype sources was designed as a proof-of concept technique for identifying unique CTV sequence variants. 5) The PCR system established in 2004 to detect "*Candidatus*" *Liberibacter africanus* (African greening) was used in routine diagnosis and detected the bacteria in various samples. The ability of the technique to detect "*Candidatus*" *L. asiaticus* (Asian greening) was also demonstrated. 6) PCR for the detection of *Xylella fastidiosa* (Citrus Variegated Chlorosis), an important exotic disease of citrus, has been established and is ready for quarantine use. 7) Two PCR systems to detect Citrus leaf blotch virus, an exotic, seed transmissible virus of citrus was established. 8) A PCR to Apple stem grooving virus (formerly Citrus tatter leaf virus) DNA was established. 9) Two PCRs for the detection of *Xanthomonas campestris* *pv. citri* (Citrus canker) was established and is available for quarantine use. 10) Two PCR systems to detect Citrus leprosis virus DNA were established. 11) PCR for the universal detection of phytoplasmas was established (section 4.2.2). Biological indexing was used to determine the severity of CTV in mother trees at the Citrus Foundation Block (section 4.2.3). No new cultivars were added to the 213 cultivars in the gene source this year (section 4.2.4). The Marsh and Star Ruby trees that were planted in Swaziland and the Nkwaleni Valley show indications that some sub-isolates reduce growth and with others, there are interactions with the scions (section 4.2.5). Star Ruby trees that were pre-immunized with the same isolates and sub-isolates as those trials in section 4.2.5 were planted this year in the Kakamas region (section 4.2.6). New promising mild isolates that were collected in different grapefruit production areas, are being used to pre-immunize Marsh and Star Ruby trees (section 4.2.7). CTV isolates that were collected from Star Ruby and Rosé grapefruit trees were evaluated in Star Ruby over an 8-year period. Trees with isolate GFMS 35 and GFMS 78 were the best and their cumulative production was 12% better than that of the other isolates (section 4.2.8). Seven-year-old trees of seven red grapefruit selections reacted very similarly to four CTV isolates as cross-protecting agents (section 4.2.9). Attempts to construct a CTV isolate from sub-isolates that will have cross-protecting characteristics for all the grapefruit selections in all the grapefruit production areas, failed. When the sub-isolates were mixed, transmissibility decreased, which makes them unsuitable as good protectors. Evaluations of the two sub-isolates are continued in several field trials (section 4.2.10).

A trial to assess the effect of CTV on fruit size in Clementine was established where pre-immunized trees of seven Clementine selections are compared to trees planted virus-free. It appears that the different Clementine selections do not react the same to CTV infection (section 4.2.11). In a navel trial, trees pre-immunized with LMS 6 (the present pre-immunizing agent for the industry) performed the best (section 4.2.12). It was found that Turkey Valencia is apparently more sensitive to CTV than other Valencia cultivars. CTV isolates that were derived from sweet orange trees will be evaluated as cross-protectors (section 4.2.13). The effect of different CTV isolates, derived from sweet orange, is being evaluated in three Valencia scions. Tree size of McClean was significantly smaller than those of Delta and McClean Seedless (section 4.2.14). Mild CTV isolates derived from sweet orange trees will be evaluated in five Valencia scions (section 4.2.15).

Seventeen rootstocks are being evaluated for Citrus Blight tolerance. Presently it can be deduced that rootstocks C35, Empress mandarin and Carizzo citrange are the most sensitive, while Swingle citrumelo, X639, Sun Chu Sha, and Cleopatra mandarin are the most tolerant (section 4.2.16).

A literature review to assess the possibility of utilizing a transgenic approach to control citrus greening was done and covers relevant aspects of the disease, causal organisms, vectors and current control strategies. Natural resistance is discussed followed by four very general transgenic approaches, which may have application to greening, namely, utilization of host defence mechanisms, use of pathogen derived genes, resistance to the vector and resistance through non-host or non-vector genes (section 4.2.17). Attempts are made to obtain greening resistance from healthy chimeras of greening infected fruit by ovule rescue. Two clones have been obtained that remained symptomless after exposure to psylla (section 4.2.18). The re-evaluation of methods to control greening infection in existing orchards is continued (section 4.2.19).

4.2.2 Establish diagnostic capabilities to graft transmissible pathogens of Citrus at CRI-UP, with emphasis on *Citrus tristeza virus* variants

Experiment 783 by Prof. G. Pieterse, Katherine Stewart and Baby Phaladira (CRI at UP)

Opsomming

’n Nuwe plantvirologie program is deur Citrus Research International by die Universiteit van Pretoria van stapel gestuur as gevolg van die feit dat Dr. van Vuuren, Sitrusbedryf Viroloog binne ’n paar jaar gaan aftree. Die aanvanklike doelwit by die sentrum is die vestiging van ’n kapasiteit om ent-oordragbare patogene (hoofsaaklik viruse) te kan opspoor en identifiseer, aangesien dit ’n noodsaaklike komponent is in die beheer of bestuur van plantsiektes, sowel as dat dit enige navorsingsaktiwiteite op hierdie patogene vooruitgaan. Vordering met hierdie doel in 2005 is as volg: 1) ’n Polimerase-ketting reaksie (PKR) opsporings-toets is gevestig teen lede van die *Closteroviridae* Familie (waarvan *Citrus tristeza virus* (CTV) ’n lid is). Hierdie breë spektrum PKR sal hoogs uiteenlopende rasse van CTV kan opspoor en kan effektief gebruik word na die virus-uitskakeling stappe in die Sitrus Verbeteringsskema. 2) ’n multipleks PKR is gevestig wat beide ’n hoogs gekonserveerde sekwens, sowel as ’n sekwens wat tussen ligte en strawwe rasse van CTV kan onderskei, teiken. Daar kan dus in een toets alle bekende CTV rasse opspoor en as ligte of strawwe rasse onderskei. Hierdie toets is dus geskik vir gebruik in die roetiene evaluasie van CTV kruisbeskerende rasse. 3) A groep van 23 PKR’e wat saam gebruik kan word om CTV isolate te kan klassifiseer op genotipe vlak is gevestig. Terwyl al die bogenoemde PKR’e CTV cDNA suksesvol kan opspoor, moet ’n effektiewe RNA ekstraksie en tru-transkriptase (RT) stap vir geïnfecteerde sitrusplante nog ontwikkel word. 4) ’n “Micro-array” skyfie is ontwerp en gesintetiseer om tussen CTV-T30 (lig) en CTV-T36 (straf) rasse te kan onderskei in ’n loodsproef om die haalbaarheid van die toets te evalueer. Verskeie kleurings-reaksie koppelings word tans ge-evalueer voordat die tegniek getoets en uitgebrei kan word om sekwens verskille tussen rasse te kan opspoor. 5) Die produksie van CTV antiserum en die ontwikkeling van ’n ELISA tegniek vir die grootskaalse toets vir STV is vertraag deur die vereiste om eers etiese komitee goedkeuring by UP sowel as Onderstepoort te verkry om met lewendige diere te werk. Virus suiwering en immunisasie sal dus tydens 2006 plaasvind. 6) Die PKR tegnieke gevestig in 2004 teen “Candidatus” *Liberibacter africanus*, die oorsaak van Sitrus vergroening, is in 2005 gebruik vir die roetiene opsporing van die bakterie in verskeie sitrus monsters. Die metode se vermoë om “Candidatus” *L. asiaticus* (Asiatiese vergroening) te kan opspoor is ook gedemonstreer. Verskeie metodes om ekstraksies voor te berei vir die PKR is ge-evalueer sodat groter getalle monsters gelyktydig getoets kan word. ’n Metode wat omtrent ’n verdubbeling in monstergetalle toelaat is gekies. Verdere studies om die getalle verder te vergroot sowel as om ’n monsternemings-protokol te ontwikkel word beplan. 7) Die vestiging van ’n PKR vir die opsporing van *Xylella fastidiosa* (die oorsaak van “Citrus variegated chlorosis”) is bereik en die tegniek is nou beskikbaar vir kwarantyn behoeftes. 8) Twee PKR sisteme is gevestig vir die opsporing van “citrus leaf blotch virus” (CLBV), ’n eksotiese, saad-oordragbare virus, geassosieer met entlas onverenigbaarheids-simptome. Ekstraksie en RT stappe kon nie ge-evalueer word nie aangesien die virus nie in Suid-Afrika voorkom nie. Dit behoort egter soortgelyk te wees aan dié wat vir CTV sal werk. 9) ’n PKR teen die DNA van “Apple stem grooving virus” (voorheen “citrus tatter leaf virus”) is gevestig en soos met CTV kort dit nog die ontwikkeling van ’n ekstraksie en RT stap. 10) Twee PKR’e is gevestig teen *Xanthomonas campestris* pv. *citri*, die patogeen verantwoordelik vir Sitruskanker wat nie in Suid-Afrika voorkom nie sedert ’n baie suksesvolle uitwissingsveldtog in die vroeë 1900’s. Die PKR’e is dus beskikbaar vir kwarantyn opsporingsbehoefte. 11) Twee PKR tegnieke om cDNA van sitrus leprose virus (CiLV) op te spoor is gevestig, maar kort ’n RNA ekstraksie en RT stap voor die toets van ge-infecteerde plante uitgevoer kan word. 12) PKR vir die algehele opsporing van fitoplasmas is gevestig en is ook roetiene-gewys gebruik vir die opsporing van hierdie patogene in wingerdmateriaal. Die tegniek kan gebruik word vir die opsporing van Australiese sitrus terugsterwing en ander fitoplasmas van sitrus. Tydens 2006, ten einde die doel van ’n omvattende diagnostiese kapasiteit vir Sitrus ent-oordragbare patogene, sal opsporingstegnieke teen verdere viruses en ander ent-oordragbare patogene gevestig word en reeds-gevestigde tegniek verfyn word, veral met betrekking tot ’n ekstraksie en RT stap.

Introduction

Detection and diagnosis of pathogens in a rapid, reliable fashion, is fundamental to the development of strategies for their management and control, whether by phytosanitary legislation, certification schemes, vector control, resistance selection and breeding, or cultivation practices. Development of such techniques is also an essential first-step in most research actions involving such pathogens. A large number of rapid tests are PCR-based and are described in the literature with primer sequences deposited in Genbank or available in published articles. The first aim of this two-year project (of which the current report is on the first year’s work) is therefore to establish a diagnostic capability (ELISA- or PCR-based) locally to detect the major graft transmissible diseases of citrus. Furthermore to address the control of *Citrus tristeza virus* (CTV), the most important virus of citrus in South Africa by cross protection, and to exploit the probable underlying

mechanism of RNA-silencing, it is imperative that information on the variability of this virus be obtained locally. The new technique of micro-array analysis is ideally suited to this. The second aim of this project is therefore to generate information on the variability of the local mild strain cross-protecting CTV populations used in South Africa using micro-array analysis.

Materials and methods

Diverse PCR protocols were established. By the establishment of these techniques, “materials and methods” actually constitute the results, and are therefore included within “results and discussion” of each individual PCR technique.

Results and discussion

Detection of *Citrus tristeza virus* (CTV)

This is the most important virus of citrus and its detection on different taxonomic levels (virus Family, genus, species and strains) is required to develop increasingly effective methods of control. For example, a method capable of detecting all members of the *Closteroviridae*, the family to which CTV belongs, allows theoretical detection of all variants of CTV, irrespective of whether their sequences are known in advance or not. This technique would be suited for use in the certification scheme in testing plants following virus-elimination. In contrast, detection of specific strains of CTV will allow studies on strain cross-protection dynamics to be performed, and allow ever increasingly effective cross-protection strains to be selected or improved strategies to be employed. Use of CTV ELISA expands the repertoire of tests, allowing the testing of large numbers of samples simultaneously, and is ideal for the routine screening of citrus mother-block trees.

1. Aim: Establish and assess PCR for the group-specific detection of closteroviruses (for the wider range detection of CTV strains)

In order to increase the probability of detecting a wide range of CTV strains, including as yet undiscovered divergent sequence variants, a PCR designed to target a highly conserved region amongst closteroviruses was established. Degenerate, multiplex primers designed by Dovas & Katis (2003), were synthesized locally. The dHSP nest3G primer, which contains an unusual compound (6H,8H-3,4-dihydropyrimido[4,5-c]oxazin-7-one) could not be produced locally. As this primer overlaps significantly with one of the other primers, and only supplements the detection ability of the multiplex PCR system, the PCR was assessed without the dHSP nest3G primer. The amplification component of the PCR was evaluated using the published cycling conditions (Dovas & Katis, 2003) against CTV T30 (mild) and T36 (severe) full-length DNA clones (obtained from Dr. Gowda, University of Florida). Both CTV sources yielded amplification products in both the first and second round PCRs. CTV RNA was extracted from a CTV-T30 infected citrus tree as well as RNA from a healthy citrus tree. These were used to evaluate the single tube one-step reverse transcriptase RT-PCR reaction. No amplification products could be detected after the first round (possibly due to reverse transcriptase inhibition of Taq DNA polymerase or PCR inhibitors or an inhibitor of PCR from the citrus host), but correctly sized amplicons were obtained against CTV-T30 DNA in the nested reaction, along with some non-specific bands (Fig. 4.2.2.1). The PCR remains to be optimized and experiments to reduce the possible inhibitory effect of reverse transcriptase on Taq DNA polymerase are scheduled.

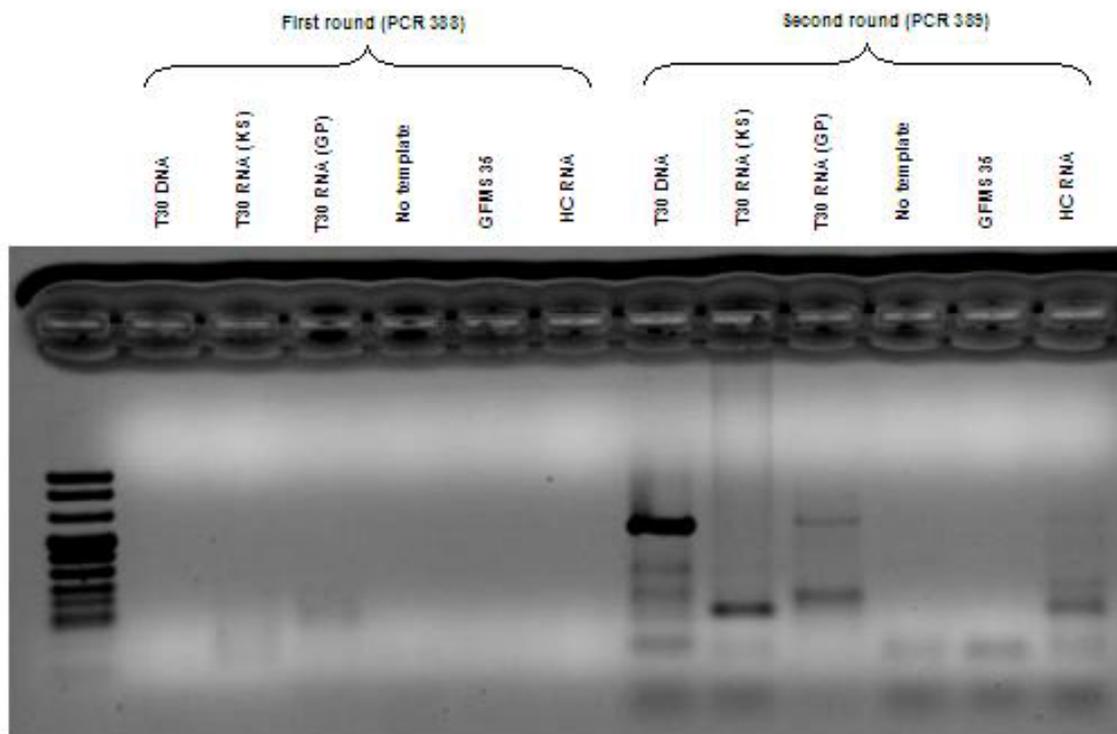


Figure 4.2.2.1. Electrophoresis gel of amplification products of first and second round products of a nested PCR to detect closteroviruses.

2. Aim: Establish a functional, routine use CTV-specific PCR

Two systems to detect CTV routinely AND to differentiate between mild and severe forms of the virus have been established.

Primers designed by Sambade, *et al.* (2003), were synthesized locally and amplification conditions and reagents adapted for local use (CRI Annual Research Report 2004). These primers are capable of detecting and differentiating Spanish severe and mild sources of CTV in a reverse-transcribed, nested-PCR (RT-N-PCR) based on differences in the p23 gene. The PCR was used to demonstrate the presence of both severe and mild strains of CTV present in mild isolate protecting sources GFMS 12 and GFMS 35.

The second PCR is based on primers designed by Huang, *et al.* (2004). These primers are capable of detecting all CTV isolates with one primer set, while a second primer set allows for the differentiation of severe and mild sources of CTV in a reverse-transcribed, multiplex nested-PCR (RT-N-mPCR). Primers were synthesized locally and amplification conditions and reagents adapted for local use. Some optimisation of conditions was required, but the system has been successfully demonstrated against mild and severe CTV DNA (Fig. 4.2.2.2). Conditions used were: PCR was performed in a 25 μ l reaction mixture containing 200 ng cDNA, 2 mM $MgCl_2$, 400 μ M of each dNTP, 500 μ M of each primer of a set, NH_4 buffer (Bioline) (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8, 0.1% Tween 20) and 2.5 U of *Taq* DNA polymerase (Bioline). Amplification was achieved under the following conditions: 2 min at 94°C, 35 cycles of 20 s at 94°C, 20 s at 56°C and 30 s at 72°C with a final extension of 10 min at 72°C. The amplified product was detected by horizontal agarose electrophoresis followed by ethidium bromide staining using standard molecular techniques (Sambrook, *et al.* 1989).

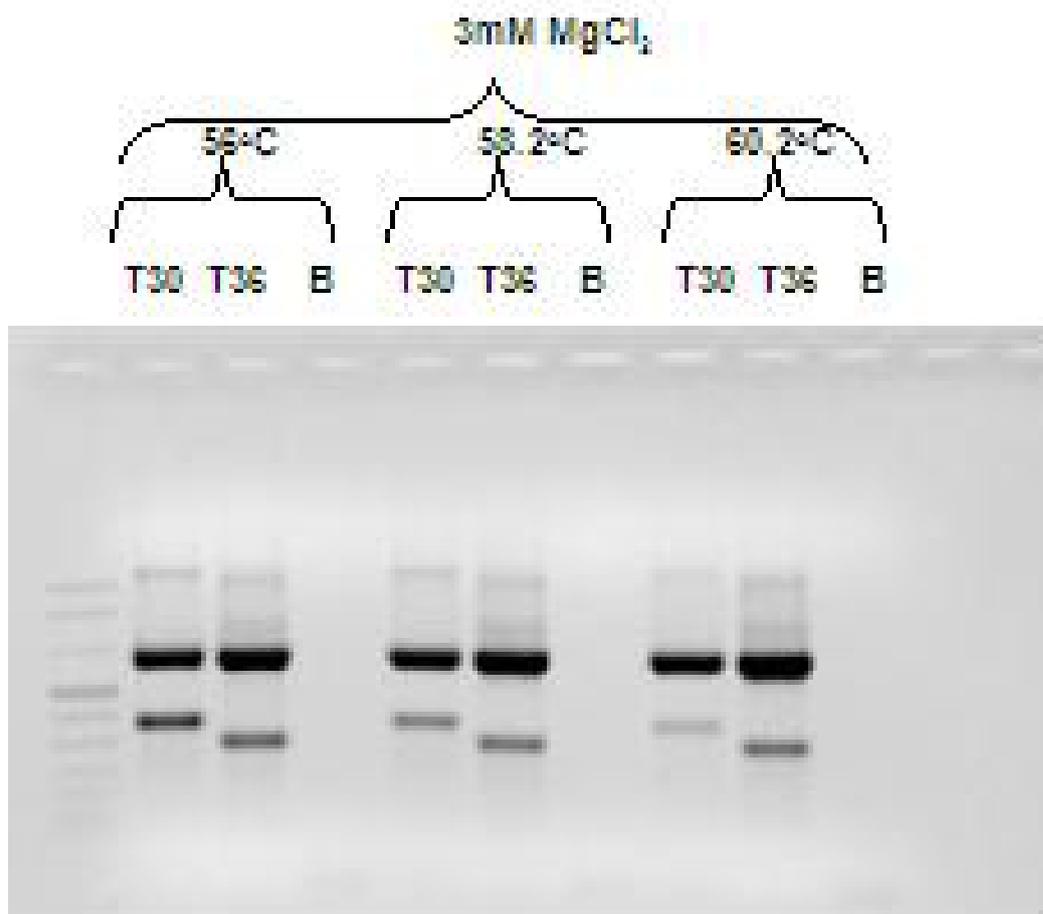


Figure 4.2.2.2. PCR to detect all CTV strains and simultaneously differentiate amongst mild and severe forms. In any group of three above the first lane is of CTV-T30, the second of CTV-T36 and the third no template control.

The Huang, *et al.* (2004) PCR has been tested using CTV RNA as template in a one-step reverse-transcriptase PCR (RT-PCR). In this format no amplification products were obtained (results not shown) with either CTV-DNA or CTV-RNA as template. An experiment to test the possibility that a component of the RT step was inhibiting the amplification step was conducted (Fig.4.2.2.3) and this demonstrated that the M-MLV RT enzyme does inhibit the amplification step. A study of the literature showed that this phenomenon has been reported in the past (Sellner, *et al.*, 1992) and that the ratio of reverse transcriptase to DNA polymerase is critical in a one-step RT-PCR reaction. Experiments to overcome this problem are underway.

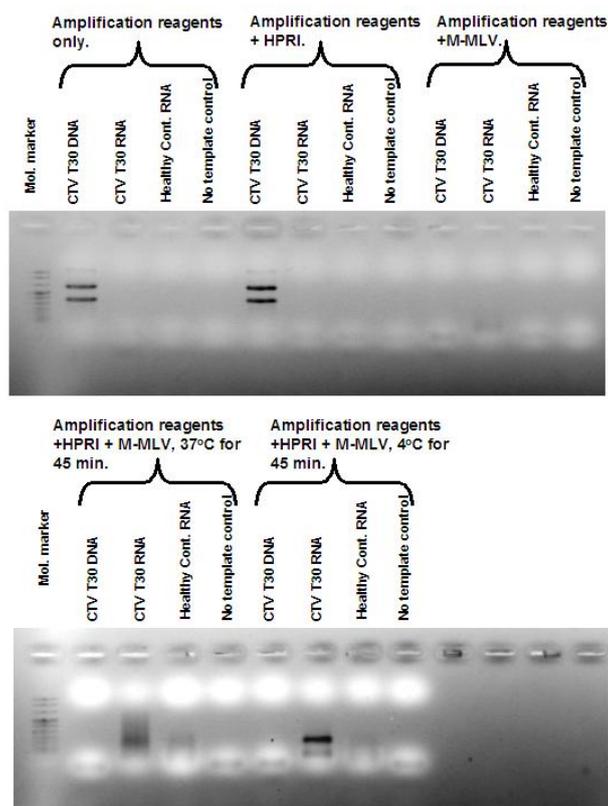


Figure 4.2.2.3. PCR to determine the possibility that a reverse transcriptase component inhibits the amplification step in a one-step RT-PCR.

3. Aim: Development of CTV strain differentiation and detection techniques

Two approaches to strain differentiation are being followed: 1) use of a set of differentiating primers in PCR to classify CTV isolates to genotype level, and 2) development of a micro-array chip to detect variability of sequences amongst isolates.

a) Classification of genotypes.

Known differences amongst CTV strain sequences are exploited using primers designed by Hilf & Garnsey (2000). The battery of 23 primer pairs allows the characterisation of strains to genotype level. Positive controls of the various CTV genotypes, in the form of cloned DNA or PCR amplicons were obtained from international collaborators for CTV-T36 (Fig. 4.2.2.4A), CTV-T30 (Fig. 4.2.2.4B), CTV-VT and CTV-T3. The set of 23 primer pairs were synthesized locally and their respective PCRs were established and optimised using the known positive controls (all conditions are available on request). All the PCR systems, with the exception of two directed against CTV-VT genotype, were successfully established and optimised (Fig. 4.2.2.4).

To utilize the technique routinely however, it is required that a step that converts RNA to DNA be utilised, the so-called reverse transcriptase step.

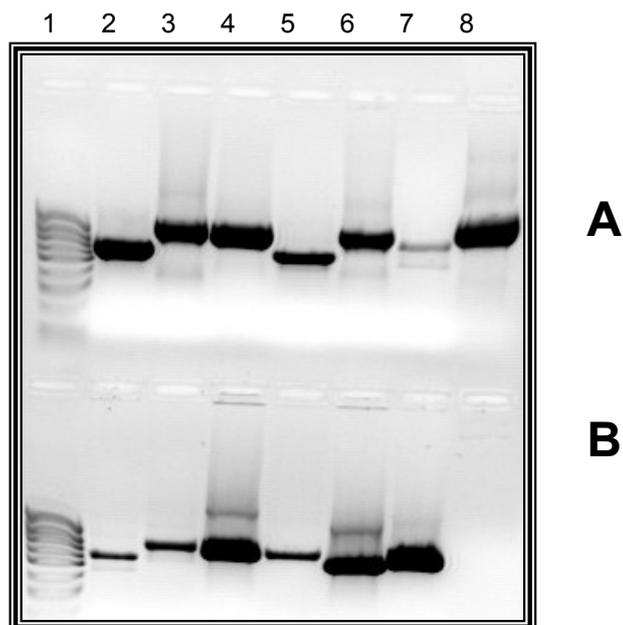


Figure 4.2.2.4. Genotyping PCRs (primers by Hilf, et al., 2002) of T30 (mild) and T36 (severe) strains for the 5' half of the CTV genome. A: CTV-T30 specific primers: lane 1 = Molecular markers, lanes 2 – 8 = primer pairs T30 1+/- to T30 7+/- . B: CTV-T36 specific primers: Lane 1 = Molecular markers, lanes 2 – 8 = primer pairs T36 1+/- to T36 7+/- .

A number of different protocols for one-step (where the reverse-transcriptase step is performed in the same reagent mixture as the PCR) and two-step RT-PCRs (where the reverse-transcriptase step is separate from the amplification step) were tested. While the PCR controls (DNA clone) consistently yield high-quality amplicons, field samples (RNA samples) do not amplify or when they do, the amplicons are at an extremely low concentration. A number of alternate cDNA synthesis protocols were tested including use of a superior reverse transcriptase enzyme (Superscript II RNase H Invitrogen) with no significant improvement in final amplicons yields. Amongst extraction methods, RNA was extracted using the Promega SV Total RNA extraction kit, standard dsRNA isolations as well as simply using an extraction buffer. None of these methods significantly improved the efficiency of amplification. Either the extraction method or the cDNA synthesis or both are inefficient and experiments are currently being conducted to solve this problem.

From the literature on RT-PCR it was evident that excess concentrations of MMLV reverse transcriptase are inhibitory to the functioning of Taq DNA polymerase (Sellner, *et al.*, 1992). An experiment was done where DNA from a cloned plasmid was used in a RT-PCR. The DNA does not require the RT step as it was already in DNA form but serves as a means of measuring any potential inhibition of the DNA polymerase activity due to M-MLV (See Fig. 4.2.2.2 above). Results showed that using 10 U or more of MMLV (USB) inhibits the functioning of DNA polymerase at 0.5 U. This result has helped in preventing inhibition of the enzymes in RT-PCRs and allowed faint bands to be more visible where none had been seen before.

Bovine Serum Albumin at 20 ug/ml was used as an additive to increase the efficiency of the RT-PCR system. A range of different amounts of RNA template was added to check the range of amplification. The concentration of amplicon remained the same with template amounts from 0.5 µl to 5 µl. Different concentrations of magnesium chloride were tried to determine the most optimal condition, which proved to be 1.5 mM.

An alternative to RNA extraction, use of Immunocapture to concentrate and purify virus particles, followed by RT-PCR was assessed. The immunocapture step was verified by performing an ELISA (Fig. 4.2.2.5) using a thin walled PCR tube simultaneously to the tubes destined for RT-PCR. It was found through the ELISA signal that sufficient amounts of CTV were binding to the tube. Therefore the type of tube used as well as the viability of the antiserum was suitable for this step. Thereafter cDNA was synthesized and PCR done. The products were of the expected size, however, remained poorly amplified.

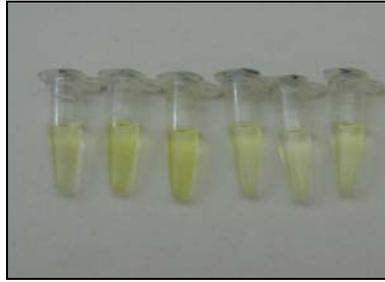


Figure 4.2.2.5. ELISA using the CTV specific antisera of Dr. Lee (Univ. Florida). Sixty minute substrate reaction time: Tube 1= T30 strain infected plant ($OD_{405nm} = 1.290$); Tube 2 = GFMS 12 Infected plant ($OD_{405nm} = 1.786$); Tube 3; GFMS 35 infected plant ($OD_{405nm} = 1.905$); Tube 4 = Virus free plant ($OD_{405nm} = 0.145$); Tube 5 = Buffer control ($OD_{405nm} = 0.108$); and Tube 6 Uncoated control ($OD_{405nm} = 0.112$).

b) Differentiation of strains using DNA micro-arrays.

In a proof-of-concept study, oligonucleotides were designed that could distinguish between CTV-T30 and CTV-T36 strains based on the 5' half of the CTV genome. The two strains were aligned and areas of variability were identified. ArrayDesigner™ was used to design the best probe of 18-25 bp within any given 70mers region from the CTV-T36 strain complete genome (CTU16304). The programme rated oligonucleotides based on the parameters: GC content, T_m , free energy and structural properties such as repeats, secondary structures and hairpins to find the best probes.

The designed oligonucleotides were assessed comparing them to known sequences on Genbank and using the Blast function. All oligonucleotides were 100% homologous to the targeted strain (T36). Oligonucleotides were selected which differed either by 1-20%, 21-40% and above 41%, compared to the T30 strain. Included were 2 oligonucleotides to regions conserved in both strains. External controls included were Rabies virus and West Nile virus primers.

Templates of the CTV-T36 and CTV-T30 strains were amplified using a number of different primer sets to target most of the 5' half of the genomes. The templates were purified using the Qiagen PCR Purification kit and the concentrations of the products quantified. Slides were spotted at the Micro-array facility by an Array Spotter Generation III (Molecular Dynamics). Primers designed for specific genotypes (Hilf & Garnsey, 2002) were also included on the Micro-array slide. Amplicons derived from the template of the CTV-T36 and CTV-T30 clones were pooled at equivalent concentrations and the Cy3 labelling step performed.

The labelling has been performed using Klenow reactions, using varying concentrations of DNA (100 ng to 500 ng) of pooled PCR fragments as well as single products. The labelling procedure was modified at various steps to increase efficiency. Hybridisation was performed using labelled products of T30 and T36. The slide was scanned and showed a haze of red spots, suggesting that the DNA concentration and or the dye incorporation were not optimal at concentrations high enough to produce a good hybridisation between the labelled DNA and the oligonucleotides. Further experiments to label the T30/T36 products are currently being performed. These include using 5'-end labelled Cy3 primers in a PCR reaction, and the incorporation of F3-dUTP label using Taq polymerase.

4. Aim: Production of antisera and the development of a TAS ELISA for the large-scale routine detection of CTV

Grapefruit cv. Flame cane tissue (5 kg) inoculated with CTV mild strain GFMS 35 was obtained from Mr. Thys du Toit (Citrus Foundation Block (CFB), Uitenhage), from plants kept in insect-free screen-houses. Bark shavings were prepared and 35 x 100g packages were stored at $-30^{\circ}C$, pending virus purification of CTV. The purification method of Lee, *et al.* (1987), will be utilized. Reagents for purification have been ordered and the permission to use required rotors and centrifuge tubes in external departments secured. The services of veterinarians at ARC-Onderstepoort Veterinary Institute (ARC-OVI) have been secured and experimental animals ordered. The envisaged immunisation has been reviewed by ARC-OVI ethics committee and permission granted to use the animals for the immunisation. Permission to perform these experiments on live animals has also been obtained from the UP Ethics committee. Virus purifications and the immunization programme are scheduled for the following few months.

Detection of “Candidatus” *Liberibacter africanus*, the causal organism of African Greening

Aim: Establish a functional, routine PCR system for greening organisms

Three primers sets were synthesized locally and PCRs established to detect “Candidatus” *Liberibacter* sp. (CRI Annual report 2004). Two of the PCR systems, developed by Harakava, *et al.* (2000) and Hocquellet, *et al.* (1999) respectively, are able to amplify sequences from both “Candidatus” *Liberibacter asiaticus* and “Candidatus” *L. africanus* albeit from different regions of the genome. The two bacteria are differentiated either by restriction enzyme digestion of the PCR amplicon in the Harakava, *et al.* (2000), system, or by differences in size of resulting amplicons in the Hocquellet, *et al.* (1999) system. Detection of “Candidatus” *L. africanus* in both systems was demonstrated (CRI Annual report 2004). During the current report year the detection of “Candidatus” *L. asiaticus* with the Hocquellet, *et al.* (1999) system was demonstrated using DNA obtained from France (Saillard, personal communication) Using the Hocquellet, *et al.* (1999) PCR, *L. africanus* infections could be demonstrated in samples collected from Nelspruit, Rustenburg, Eastern Cape and the Western Cape during routine tests in 2005. Unfortunately only small numbers of samples can be tested on any given occasion. Experiments to find a more efficient extraction procedure for the above PCR systems, which would allow larger sample numbers to be tested were done. Total DNA extraction using CTAB (hexadecyltrimethylammonium bromide) (Doyle & Doyle, 1990), with modification by J. Brown, University of Arizona) has been the standard extraction method used thus far to prepare samples for greening PCR. While this method is reliable, it is time consuming, and due to rotor tube limitations with centrifugation steps, only 6 samples can be prepared in parallel. In order to overcome this, the CTAB method was modified (Angelini, *et al.*, 2001) to allow more samples to be extracted, and the procedure shortened. Unfortunately most centrifugation steps remained. The use of the GenElute™ Plant Genomic DNA miniprep kit (Sigma-Aldrich), according to the manufacturer’s instructions, was also assessed. As an alternative, direct PCR on plant material was also assessed using 1) direct blotting of sample onto NitroPure (Micron Separation inc.) nitrocellulose transfer membranes, as successfully used for DNA viruses (Pietersen & Smith, 2002), and 2) PCR on samples macerated in four different extraction buffers.

“Candidatus” *L. africanus* could only be detected using the two CTAB DNA extraction methods (Fig. 4.2.2.6). The modified CTAB method allows homogenization of more samples in the initial step, and allows testing of up to 18 samples in parallel as centrifugation steps can be staggered. Sample numbers, however, remain relatively low due to the continued requirement of large volume centrifugation steps. Furthermore it appears as though PCR performed on templates using this extraction, yield less amplicons (observation not quantified). Further extraction procedures must be assessed to allow true large-scale testing for “Candidatus” *L. africanus* and a sampling and collection protocol has to be developed.

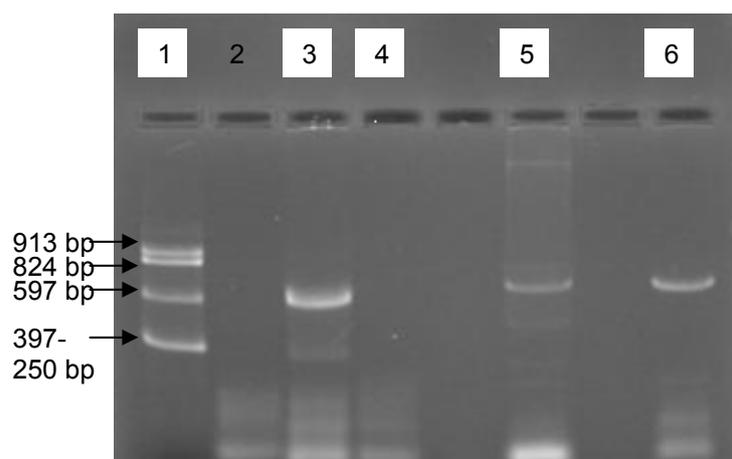


Figure 4.2.2.6. Detection of “Candidatus” *Liberibacter africanus* using A2 and J5 primers in a PCR (Hocquellet *et al.*, 1999). Expected amplification product size, 500 bp. Lanes 1: DNA size marker; 2: no template negative control; 3: 282 positive control sample (CTAB extract); 4: Eureka lemon healthy control; 5 & 6: Buffelsfontein samples (Modified CTAB extract).

Detection of further graft transmissible pathogens of Citrus

1. *Xylella fastidiosa* (Citrus variegated chlorosis).

Primers, designed by (Pooler & Hartung, 1995), for the PCR detection of *Xylella fastidiosa*, the causal organism of Citrus variegated chlorosis (CVC), were synthesized locally. The PCR was established and assessed using these primers and a DNA amplicon template obtained from Fundicitrus, Brazil, as positive control (Fig. 4.2.2.7). As the organism does not occur in South Africa, extraction procedures, on infected samples could not be evaluated. PCR conditions used were: PCR was performed in a 50 µl reaction mixture containing 2.5 mM MgCl₂, 200 µM of each dNTP, 500 µM of each primer of a set, NH₄ buffer (Bioline) (16 mM (NH₄)₂SO₄, 67 mM Tris-HCl, pH 8.8, 0.1% Tween 20) and 2.5 U of *Taq* DNA polymerase (Bioline). Amplification was achieved under the following conditions: 4 min at 94°C, 35 cycles of 60 s at 94°C, 60 s at 64°C and 90 s at 72°C, with a final extension of 10 min at 72°C. The amplified product was detected by horizontal agarose electrophoresis followed by ethidium bromide staining using standard molecular techniques (Sambrook, et al., 1989).

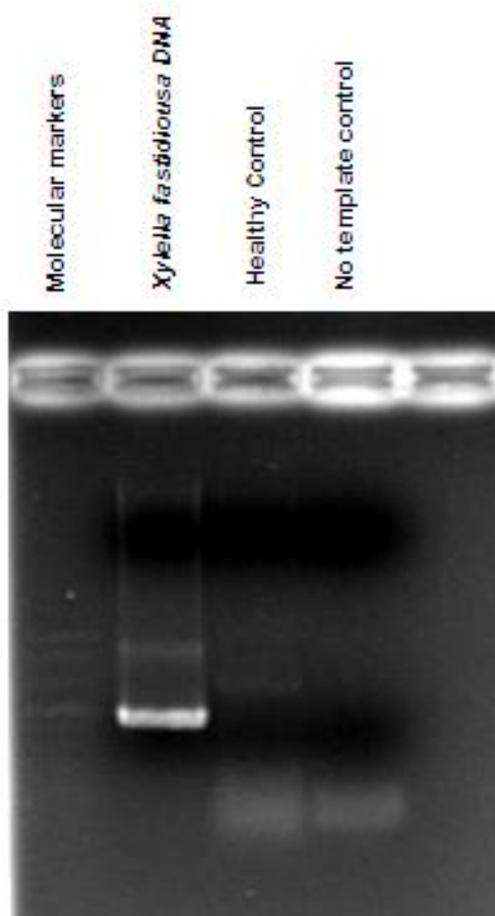


Figure 4.2.2.7. Agarose gel of electrophoresis of PCR amplification products obtained with *Xylella fastidiosa* specific primers.

2. Citrus leaf blotch virus (CLBV)

Two primers sets, designed by Galipienso, *et al.* (2004), for the detection of CLBV were synthesized locally. The two primers target the coat protein gene (CP) and RNA-dependant-RNA-polymerase gene (RdRp) of CLBV respectively. Plasmids, containing the CP and RdRp genes were obtained from Vives, IVIA, Spain, to serve as positive controls. Using these, the two PCR systems could be established and evaluated. PCR with both primer sets were performed in 50 µl reaction mixtures containing 3 mM MgCl₂, 400 µM of each dNTP, 200 µM of each primer of a set, NH₄ buffer (Bioline) (16 mM (NH₄)₂SO₄, 67 mM Tris-HCl, pH 8.8, 0.1% Tween 20) and 2.5 U of *Taq* DNA polymerase (Bioline). Amplification was achieved under the following conditions: 40 cycles of 20 s at 94°C, 20 s at 50°C and 30 s at 72°C, with a final extension of 5 min at 72°C. As part of a pilot study to assess the feasibility of a CLBV-based project for Me. J. Meyer (a potential PhD student), various citrus samples suspected of containing CLBV were tested in a RT-PCR version. While expected-size amplicons were obtained from a number of these, direct sequences of these amplicons

yielded inconclusive results suggestive of mixed amplicon sequences. Ms. Meyer therefore did a second round of PCR with the primers directed against the coat protein gene of CLBV using Turkey Valencia and Tomango samples as well as a PCR directed against Flexiviruses in general. The amplicons were cloned after purification of each of two amplicon bands obtained. The insert of a number of clones were sequenced. Clones 1 and 3 were directed against components of the citrus plant itself, while the remaining clones appeared to have homology with various plasmid/bacteria regions. No virus-like sequences were observed. Both the coat protein and Flexivirus primers therefore amplify non-specifically and cannot be utilized for the detection of CLBV without considerable modification and optimization.

3. Apple stem grooving virus (formerly Citrus tatter leaf virus)

A primer set (CTLV-AM and CTLV-AP)(Yoshikawa, *et al.*, 1992), designed to detect Citrus tatter leaf virus (now named apple stem grooving virus (ASGV)) was synthesized locally. ASGV amplicons from PCR's using the same primer set were obtained from Takao Ito, National Institute of Fruit tree Science, Japan. These were used to establish and assess a PCR based on the conditions of Ito, *et al.* (2002). Amplicons of the expected size were obtained (Fig. 4.2.2.8). Known "tatter leaf" infected citrus sources were obtained from K. Breytenbach and will be used to test the RNA extractions and reverse transcriptase steps of the protocol.



Figure 4.2.2.8. Agarose gel of electrophoresis of PCR amplification products obtained with apple stem grooving virus (formerly citrus tatterleaf virus) specific primers vs. CTLV DNA obtained from Japan.

4. Xanthomonas campestris pv. citri (Citrus canker)

Two primer sets, designed by Cubero & Graham (2002) and Hartung, *et al.* (1993) respectively, to detect *Xanthomonas campestris pv. citri* by PCR were synthesized locally. Appropriate amplicons were obtained from Dr. J.E. Thomas, QDPI, Australia to serve as positive controls in establishing and evaluating the PCR's. Amplicons of the expected size were obtained in both PCR systems (Fig. 4.2.2.9). Reagents and cycling conditions for both system were the same. The PCRs were performed in 50 μ l reaction mixtures containing 3 mM MgCl₂, 200 μ M of each dNTP, 1 μ M of each primer of a set, NH₄ buffer (Bioline) (16 mM (NH₄)₂SO₄, 67 mM Tris-HCl, pH 8.8, 0.1% Tween 20) and 2.5 U of *Taq* DNA polymerase (Bioline). Amplification was achieved under the following conditions: 35 cycles of 20 s at 92°C, 20 s at 58°C and 45 s at 72°C. As the organism does not occur in South Africa, extraction procedures could not be evaluated.

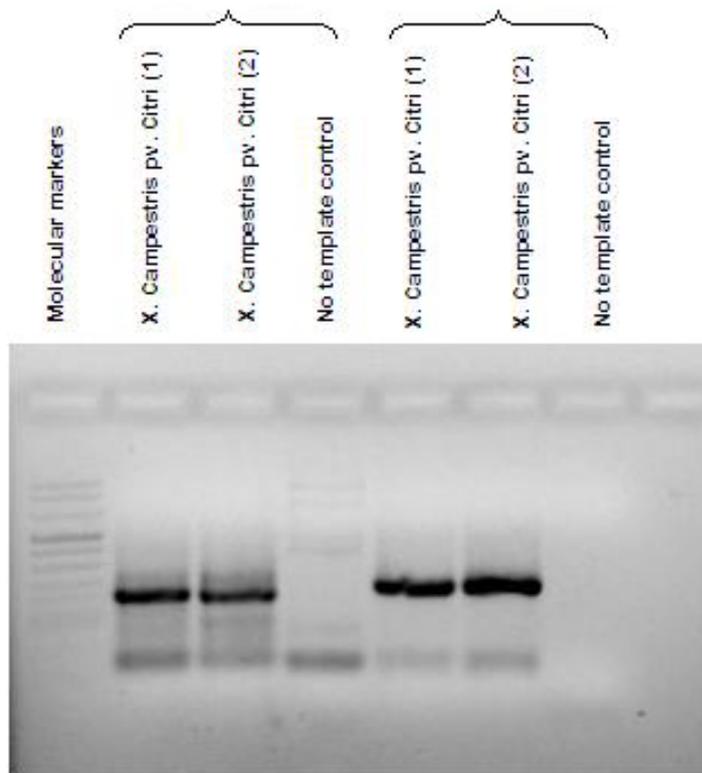


Figure 4.2.2.9. Agarose gel of electrophoresis of PCR amplification products obtained with two sets of citrus canker (*Xanthomonas campestris pv. citri*) specific primers vs. *X. campestris pv. citri* DNA obtained from Australia.

5. Citrus leprosis virus

Two PCR systems to detect Citrus leprosis virus (CiLV) were established in the last quarter of 2005. Two primer sets (MPF/MPR and RepF/RepR) designed by Locali, *et al.* (2003), were synthesized locally. Positive controls, in the form of non-infectious cDNA to CiLV and cognate primer-specific amplicons were obtained from Juliana Freitas-Astua (Centro APTA Citros "Sylvio Moreira"/IAC, Brazil). Both PCR systems were successfully established (Fig. 4.2.2.10). As CiLV does not occur in South Africa, extraction procedures, and the reverse transcriptase step of an RT-PCR could not be evaluated. Tests on a sample from Burgersfort, Mpumalanga, (UPCRI 05-0257), showing grey mite induced symptoms, which are rather similar to those of CiLV (Fig. 4.2.2.11) were negative.

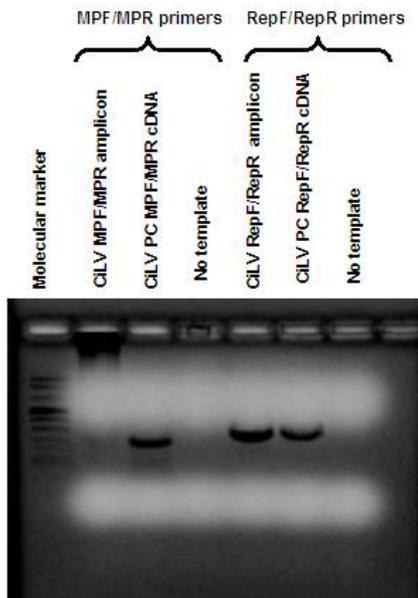


Figure 4.2.2.10. Electrophoresis gel of amplification products of two PCR primer systems to detect Citrus leprosis virus (CiLV) against positive control CiLV DNA from Brazil.

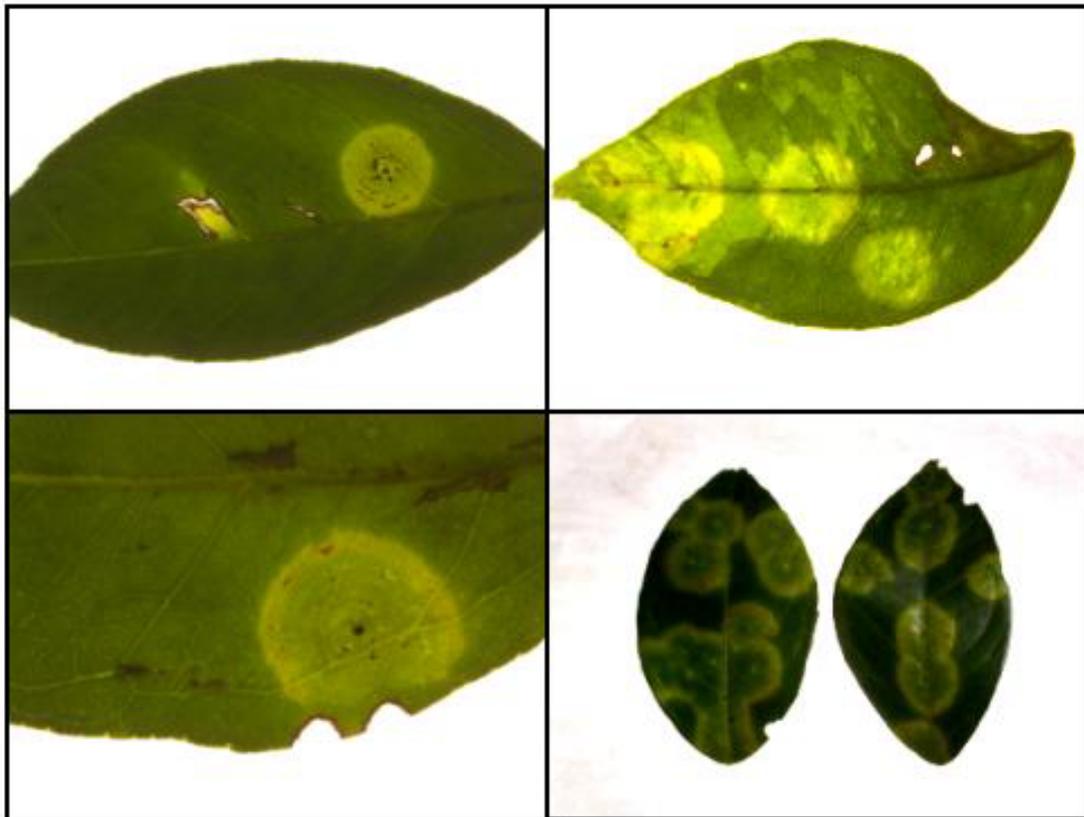


Figure 4.2.2.11. Leaf symptoms of sample UPCRI 05-0257 from Burgersfort, Mpumalanga.

6. Phytoplasmas of citrus (e.g. Australian die-back)

A primer pair P1 and P7 (Deng & Hiruki, 1991; Smart, *et al.*, 1996) directed to a highly conserved region in the 16S rRNA to 23s rRNA of phytoplasmas, followed by a nested set of primers, 16r723f and m23Sr (Padovan, *et al.*, 1995) were synthesized locally and established and optimized against the DNA of a phytoplasma of grapevine, Stolbur, imported from France. The PCR worked well and has been utilized in studies on grapevine over the past season. This PCR should theoretically detect phytoplasmas such as Australian citrus die-back phytoplasma as well.

Conclusions

Techniques for the detection and identification of the major graft transmissible pathogens of citrus in South Africa are being established at the newly established CRI plant virology programme at the University of Pretoria.

During the past year significant progress has been made in the detection of CTV on different taxonomic levels. As each technique has unique inherent advantages and disadvantages, specific techniques need to be utilised at different stages in the certification programme. For example after virus elimination a technique capable of detecting ALL CTV isolates is required, while for screening for cross protecting strains requires a technique to differentiate severe and mild forms of the virus. Similarly, in cross-protection breakdown studies, it will be important to detect specific sequence variants in order to assess the dynamics of these within plants, hence the requirement of the micro-array system.

Techniques to exotic viruses and other graft-transmissible pathogens are required to ensure that these pathogens are not introduced to South Africa, or if this happens they are identified rapidly and eradicated. Some examples of these for which detection systems were established include citrus leprosis virus, Citrus variegated chlorosis (*Xylella fastidiosa*), Citrus leaf blotch virus and Citrus canker (*Xanthomonas campestris* pv. *citri*).

Unexpected difficulties have been experienced in the detection of RNA genome pathogens from citrus, but attention to improved extraction procedures and reverse transcriptase steps will be given in 2006.

Future research

Good progress has been made in establishing PCR detection methods to a number of graft- transmissible pathogens of citrus. However, in cases where the pathogen has an RNA genome (e.g. the viruses to which PCR were established), the RNA extraction and/or reverse transcriptase step is proving problematic. Similar techniques utilized in the same laboratory on grapevines work well, and therefore it is concluded that some aspect of the citrus host affects the steps preceding amplification. This aspect will be addressed in the upcoming research period.

Limitations in the number of samples that can be tested for the bacteria associated with greening, along with uncertainty as to sampling protocols, necessitate that these aspects be addressed before the technique can be applied routinely on a large scale. Aspects of the epidemiology of greening (e.g. Alternate host range, seasonal *in planta* distribution and levels) need to be understood better to improve current control strategies (e.g. Planting healthy material, vector control and inoculum removal).

While many of these aspects have been done in the past, use of the PCR detection technique would allow expanded experiments to be conducted. PCR to a number of other citrus pathogens need to be established (e.g. Psorosis, the p12 protein associated with Citrus blight, etc.), and will also be addressed in the new research period.

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4.2.3 Diagnostic services for graft transmissible diseases

Experiment 796 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Die raamwerk van siektevrye plantmateriaal is 'n fitosanitêre program wat gebaseer is op 'n diagnostiese vaststelling van die teenwoordigheid van skadelike patogene, die eliminering daarvan en die onderhou en verspreiding van gesonde voortplantingsmateriaal. Indeksering, of die vasstelling of oordraagbare siektes teenwoordig is in plantmateriaal, word tans hoofsaaklik deur biologiese indikatorplante gedoen. Serologiese tegnieke soos ELISA en Kol Klad word gedoen om pre-immunisering met *Citrus tristeza virus* (CTV) te bevestig terwyl laasgenoemde tegniek ook gebruik word om die teenwoordigheid van Sitruskroei te bevestig. Laboratorium tegnieke vir indksering (bv. PKR) van verskeie sitrussektes word tans ge-optimeer

en sal baie tyd bespaar (sien 4.2.2). Behalwe vir indeksering wat op Sitrus Verbeteringskema materiaal gedoen word, word daar ook indeksering op materiaal wat vanaf kwekers ontvang of versamel word gedoen. Dit is nodig om die oorsaak van siektetoestande te bevestig om sodoende sinvolle aanbevelings te maak vir beheer. Spesifieke virusvrye indikatore is in die glashuis gekweek vir elk van die entoordraagbare siektes waarvoor ge-indekseer word. Die moederbome by die Sitrus Grondvesblok is ge-indekseer om te bepaal of enige strawwe CTV rasse in die moedermateriaal voorkom. Enthout wat vanaf kwekers ontvang of versamel is, is op verskeie indikatorplante ge-inokuleer en by optimale temperature in die glashuis gehou sodat siektesimptome kan ontwikkel indien dit teenwoordig is.

Introduction

As with any commercial crop, citrus species are subjected to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria and in some cases unknown organisms. The GTD affect the vigour, longevity of the trees, as well as the yield and quality of fruit. The framework of disease-free planting material is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and has been used in South Africa since 1977 (de Lange, *et al.*, 1981). Mainly biological indexing is used for the detection of GTD in STG material while ELISA is used to confirm pre-immunisation (Roistacher, 1991). Since *Citrus tristeza virus* (CTV) and its vector, *Toxoptera citricida*, is endemic in South Africa, virus-free material should be protected by pre-immunisation with a suitable virus isolate (Müller & Costa, 1987). Currently three CTV isolates are used in the southern African Citrus Improvement Scheme (CIS) according to the scion material to be protected (van Vuuren, *et al.*, 1993a; van Vuuren, *et al.*, 1993b; van Vuuren, *et al.*, 2000; von Broembsen & Lee, 1988). The STG and pre-immunisation procedures have been improved to suite South African conditions (Fourie & van Vuuren, 1993). Re-indexing to establish that mother material at the Citrus Foundation Block remains free of graft transmissible diseases and that the pre-immunising agent remained mild is done on an annual basis. Indexing for graft transmissible diseases where growers have problems of disease infections is necessary to recommend proper control measures. Indexing includes biological as well as serological techniques (ELISA, Dot Blot, PCR) (Roistacher, 1991).

Materials and methods

Virus-free indicator plants for the different graft transmissible diseases (GTD) are propagated from seed in an insect-free glasshouse and kept in stock until needed (except for Citrus leaf blotch virus, which does not occur in South Africa, GTD are not seed transmissible). When budwood from the source to be indexed is received, two buds are budded on each of three indicator seedlings for each disease. Hereafter the plants are cut back to force new growth and kept in the glasshouse at a temperature required for symptom expression of the specific disease. Positive and negative control samples are included. A minimum time of six months is required for the indexing.

Since field material is usually not suitable for the laboratory techniques (ELISA, PCR, etc.), material can be collected from the glasshouse plants three months after inoculation. Results can be obtained quicker and are a confirmation of the biological result.

When indexing is completed the client is informed of the result.

Results and discussion

Mother trees at the Citrus Foundation Block (CFB) were indexed to establish the CTV severity (Table 4.2.3.1). No severe CTV was detected but the Mexican lime indicators showed the presence of moderate stem pitting in some mother trees. These trees were suspended as budwood sources.

Table 4.2.3.1. The number of trees of selections from different cultivars at the CFB indexed on Mexican lime and the number of trees suspended due to increased severity of CTV.

Cultivar	Number of selections	Number of trees	Number of trees suspended
Midseasons	3	12	0
Navels	19	80	10
Valencias	10	52	4
Satsumas	1	6	0
Clementines	3	15	0
Limes	3	12	1

Lemons	3	18	0
Grapefruit	1	6	5
Total	43	201	20

Citrus material that was sent in by growers or collected during visits was indexed for specific diseases as indicated in Table 4.2.3.2. The results are also indicated.

Table 4.2.3.2. Indexing for graft transmissible diseases for various clients.

Client	Disease	Indexing	Result
Citrusdal grower	Psorosis	Biological	Awaiting results
Hoedspruit grower	CTV severity	Biological	Severe CTV
CRI (C. Alexander)	Apple stem grooving	Biological	Positive
Hoedspruit grower	Decline	Blight Protein test	Positive
CRI (Growers request) (2 cultivars)	CTV	Biological	Awaiting results
	Psorosis	Biological	Awaiting results
	Viroids	Biological	Awaiting results
	Apple stem grooving	Biological	Awaiting results
	Impietratura	Biological	Awaiting results

Citrus material that underwent shoot tip grafting is indexed firstly for CTV and CVd. When indexed negative for these two graft transmissible diseases, the material is released to the Citrus Foundation Block to establish mother trees. Indexing for psorosis virus, apple stem grooving virus (formerly citrus tatterleaf virus) and impietratura disease continues.

Indexing of CTV and CVd is presented in Table 4.2.3.3. The results are not yet available.

Table 4.2.3.3. The number of shoot tip grafted plants indexed for CTV and CVd.

Cultivar	Number of plants
Navel	3
Midseason	1
Valencia	1
Reticulata	1

The number of trees indexed for psorosis, apple stem grooving virus and impietratura is given in Table 4.2.3.4. All the plants indexed negative for psorosis virus and impietratura while one plant tested positive for apple stem grooving virus.

Table 4.2.3.4. The number of shoot tip grafted trees that were indexed for psorosis virus, apple stem grooving virus and impietratura disease.

Cultivars	Number of selections indexed
Navels	5
Midseasons	3
Valencias	10
Clementines	4
Lemons	6
Satsumas	1
Pumelos	2
Reticulatas	13
Grapefruit	2
Rootstocks	1

Conclusion

The diagnosis of graft transmissible diseases is a continuous service and results are reported to the parties involved.

Future Research

- Annual indexing of mother trees at the Citrus Foundation Block (every year for CTV severity and every third year for CVd).
- Indexing of STG plants for tristeza virus, citrus viroids, psorosis virus, apple stem grooving virus (tatter leaf) and impietratura disease.
- Index suspected budwood from growers and institutions using ELISA, PCR, Dot Blot and biological indicators.

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4.2.4 Citrus virus-free gene source

Experiment 790 by J.H.J. Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Groeipunt enting word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene. Virusvrye boompies van verskillende cultivars en seleksies word in 'n insekvrue tunnel by CRI bewaar. Die virusvrye bron by die LNR-ITSG is gedupliseer by CRI en 'n totaal van 213 cultivars en seleksies is gevestig. Virusvrye materiaal word gepre-immuniseer met 'n geskikte *Citrus tristeza virus* isolaat voordat dit aan die Sitrus Grondvesblok te Uitenhage verskaf word.

Introduction

The overall objective of the southern African Citrus Improvement Programme is to enhance the productivity of the industry by making the highest quality propagation material available. Graft transmissible agents have detrimental effects on the growth and production of citrus trees since they are responsible for stunting, decline and small fruit. Shoot tip grafting (STG) is the standard method for the elimination of pathogens (Navarro, *et al.*, 1975). Some pathogens are more difficult to eliminate and heat therapy should be incorporated with the STG process (Roistacher, 1977). The STG technique was developed by Murashige, *et al.* (1972) and improved by Navarro, *et al.* (1975) and de Lange (1978).

Materials and methods

In vitro cultured rootstocks

The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the testa of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile deionized water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige and Skoog, 1962). Germination takes place at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they can be stored at 4°C in darkness.

Scion preparation

Method 1. Buds of the plant that should go through STG are budded on a standard rootstock in the glasshouse. After the buds have grown and matured (approximately 3 – 4 months), the contaminated plants are defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested and surface sterilised on a flowbench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile deionized water.

Method 2. Budsticks from the mother plant are cut in 50 mm lengths and surface sterilised in 70% ethanol for 5 seconds followed by immersion for 10 minutes in 1% NaOCl containing a wetting agent. After 3 rinses in sterile deionized water the budsticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32°C and exposed to 16 h light. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG

The seedling rootstock is aseptically decapitated about 10 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth point with primordia is placed on the horizontal cut of the incision. The grafted plant is transferred to sterile MS liquid medium and cultured at a constant 28°C exposed to 16 h light/day.

STG plant care

The shoot tip will start growing three to four weeks after STG. The seedling rootstock with the shoot tip is now grafted onto a vigorous-growing virus-free rootstock in the glasshouse. After grafting it is closed by a plastic bag for 8 days.

Virus indexing

Elimination of graft transmissible pathogens is confirmed by indexing the STG material on sensitive biological indicators as described by van Vuuren and Collins (1990). Virus-free plants are multiplied and kept in an aphid-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunized (van Vuuren and Collins, 1990) with suitable *Citrus tristeza virus* (CTV) isolates prior to release to the Citrus Foundation Block at Uitenhage.

Results and discussion

For STG

Contaminated cultivars and selections were transferred from the ARC-ITSC to CRI.

In addition 28 new cultivars and selections were received from clients. STG has been initiated on the latter additions.

For enzyme-linked immunosorbent assay (ELISA) (Bar-Joseph *et al.* 1979)

Cultivars and selections that went through STG previously but of which the CTV status is unknown were transferred from a tunnel at the ARC-ITSC to a glasshouse at CRI. ELISA has to be performed on these 103 cultivars and selections to establish if CTV is present (Table 4.2.4.1). Those where the virus is present, STG has to be redone and those that are negative can be incorporated in the virus-free source.

Establishing and maintaining a virus-free gene source

The virus-free gene source at the ARC-ITSC was duplicated in an aphid-free tunnel at CRI. The cultivars and the number of selections of each cultivar are listed in Table 4.2.4.1.

Table 4.2.4.1. The following virus-free and contaminated cultivars have been successfully established at CRI.

Cultivar	Number of selections			
	Virus-free	Possibly contaminated	New for STG	Total
Clementine	15	15	4	34
Diverse (Citron, Sour orange, etc.)	1	2	0	3
Ellendale	4	0	0	4
Grapefruit	16	3	0	19
Kumquat	1	1	0	2
Lemon	20	3	0	23

Cultivar	Number of selections			
	Virus-free	Possibly contaminated	New for STG	Total
Lime	1	3	0	4
Midseason	22	2	3	27
Navel	33	19	15	67
Pummelo	7	1	0	8
Reticulata	31	33	3	67
Rootstock	20	14	0	34
Satsuma	8	2	0	11
Valencia	34	5	3	42
Total	213	103	28	344

Conclusion

A total of 213 virus-free selections of 14 cultivars have been successfully established in an insect-free tunnel. Additions to the source are a continuous process. Control of insects in the tunnel is crucial.

One hundred and thirty one selections will be added to the virus-free source after ELISA.

Twenty eight selections were received as new additions and are in the process of STG and indexing.

Future research

- Apply STG and ELISA on the current contaminated sources of CRI and clients.
- Receive and maintain new additions.
- Maintain the virus-free source in the insect-free tunnel.
- Pre-immunise virus-free sources that are required by the Citrus Foundation Block.
- Keep records of each source.

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4.2.5 Cross-protection of Marsh and Star Ruby grapefruit using Beltsville sub-isolates of *Nartia* mild strain

Experiment 679 by JHJ Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Daar is gevind dat die *Nartia* isolaat (GFMS 12) wat huidiglik gebruik word vir pre-immuisering van wit pomelos en pompelmoese, gekontamineer is met 'n strawwe *Citrus tristeza virus* ras. Twintig sub-isolate van twee *Nartia* isolate (A=GFMS 12, C=GFMS 14) en die Mouton 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 gepre-immuniseer met die ses Belstville sub-isolate asook twee enkel plantluis oordraging sub-isolate van die ITSG (GFMS 12/7, GFMS 12/9), GFMS 12 en GFMS 35. Boompies is

virusvry gelaat as kontrole. Preïmmunisasie is bevestig deur middel van ELISA. ELISA het uitgewys dat twee van die Beltsville sub-isolate nie voldoen aan sekere vereistes vir 'n goeie kruisbeskermings-isolaat nie deurdat hulle 'n lae persentasie oordraagbaarheid het, en stadig vermeerder en beweeg in die plant. Hierdie twee sub-isolate word nie verder ge-evalueer nie. Die Marsh boompies is uitgeplant by Riversbend in die Nkwaleni vallei en die Star Ruby is uitgeplant by Tambuti landgoed in Swaziland gedurende 2003. Die boompies se boomvolumes is geneem 18 maande na plant. Dit is egter nog te vroeg om afleidings te maak vanaf die vroeë resultate. Nietemin, daar is aanduidings dat van die sub-isolate groei strem en dat sub-isolaat 389/4 baie goed vaar in Star Ruby maar swak in die Marsh boompies, wat die gevolg kan wees van klimaat verskille of die invloed van die gasheer. Met tyd sal dit egter duidelik word of van die sub-isolate beter beskermers is vir pomelo as die huidige twee isolate, GFMS 12 en GFMS 35.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by 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 (Marais, *et al.*, 1996). The only practical means of controlling CTV disease at present is by mild strain cross-protection (van Vuuren, *et al.*, 1993). A breakdown in the protection offered by the 'hartia' A (GFMS 12) isolate owing to the presence of a severe strain within the complex (van Vuuren, *et al.*, 2000), motivated the separation of strains by single aphid transmission (SAT). Twenty SAT sub-isolates were obtained from two 'hartia' isolates (A=GFMS 12 and C=GFMS 14, van Vuuren, *et al.*, 1993) and the Mouton isolate. In this study the sub-isolates are being evaluated for mildness and their potential as cross-protecting isolates in the field.

Materials and methods

The 20 SAT sub-isolates of the 'hartia' A and C isolates (A=GFMS 12 and C=GFMS 14) and the Mouton 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 which are differential hosts that develop symptoms characteristic of the biological activity of a sub-isolate. Growth and stem pitting were determined and the virus titer was measured by means of enzyme-linked immunosorbent assay (ELISA) for each sub-isolate six months after inoculation.

The six mildest sub-isolates (GFMS 14 subs: B389-1, B389-3, B389-4; Mouton subs: B390-3, B390-4, 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), GFMS 12/7 and GFMS12/9 (ITSC single aphid transfer sub-isolates from GFMS 12) and trees that are planted virus-free. Pre-immunization has been confirmed by ELISA six months after inoculation. The Star Ruby grapefruit trees were planted in Swaziland at Tambuti Estates and the Marsh grapefruit trees at Riversbend in the Nkwaleni valley during 2003.

Results and discussion

The heights and diameters of the trees were measured and the canopy volumes calculated. The results are presented in Table 4.2.5.1. Although there are significant differences between the isolates and sub-isolates in the Marsh grapefruit trees, it is still too early to draw any conclusion from the data as the trees were only planted in September 2003. However, there are indications that some of the sub-isolates (B389/4, B390/5) retard growth at an early age in the Marsh trees. Sub-isolate B389/4 also gave contradictory results where it performs the best in the Star Ruby trees but poorly in the Marsh trees. This, however, can be the influence of the host or due to climatic differences.

Table 4.2.5.1. Canopy volumes of Marsh and Star Ruby grapefruit trees pre-immunized with different CTV isolates and sub-isolates, 18 months after planting at two sites.

Treatment	Tree volumes (m ³)*	
	Marsh (Riversbend)	Star Ruby (Tambuti)
B389/1	3.90 ab	4.02 ab
B389/4	2.36 cd	4.68 a
B390/3	2.96 abcd	3.90 ab
B390/5	1.82 d	3.34 ab
GFMS12/7	3.22 abc	3.50 ab
GFMS12/9	3.36 abc	4.10 ab

GFMS12 (Marsh control)	2.67 bcd	3.80 ab
GFMS35 (Star Ruby control)	3.56 abc	3.04 b
Virus-free (Control)	4.08 a	3.50 ab

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

The trees are still young and no conclusions can be made at this stage. However, there are indications that growth is retarded in Marsh by some sub-isolates. There are also indications that a sub-isolate performs good in a host at one site but poorly in another host at a second site. This can be a host or climatic effect.

Future research

Evaluate the horticultural performance of trees over a 10-year period using the following parameters:

- Canopy volume.
- Stem pitting.
- Yield and fruit size.

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4.2.6 Cross-protection of Marsh and Star Ruby using Beltsville sub-isolates of Nartia mild strain for the Orange River Valley

Experiment 738 by JHJ Breytenbach & SP van Vuuren (CRI)

Opsomming

As gevolg van die teenwoordigheid van 'n strawwe ras in die Nartia isolaat (GFMS 12) was dit nodig om isolate te verdeel in sub-isolate deur middel van enkel plantluis oordragings. Hierdie sub-isolate is voorberei vanaf twee Nartia isolate (A=GFMS 12, C=GFMS 14) en 'n Mouton isolaat by die kwarantyn fasaliteit in Beltsville, VSA, en ingevoer terug na Suid Afrika. Nadat die sub-isolate deur biologiese indeksering ge-evalueer is om tussen die ligte en strawwe rasse te onderskei, is gevind dat slegs vier (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) potensiaal het vir verdere evaluering. Twee belowende Nartia sub-isolate afkomstig van die LNR-ITSG (GFMS 12/7, GFMS 12/9) is ingesluit in die proef asook GFMS 12 (standaard vir wit pomelos) en GFMS 35 (standaard vir rooi pomelos). Virusvrye Star Ruby boompies is in 'n glashuis voorberei en gepre-immuniseer met die isolate en sub-isolate. 'n Virusvrye behandeling is ingesluit as kontrole. Omdat *Citrus tristeza virus* deur die gasheer en klimaat beïnvloed word, is dit nodig om isolate in die verskillende sitrus produserende streke te evalueer. Nadat pre-immunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing uitgeplant gedurende September 2004, en sal jaarliks ge-evalueer word vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte. Die boompies se groottes is gemeet 12 maande na uitplant. Dit is egter nog te vroeg om afleidings te maak vanaf die vroeë resultate.

Introduction

The severe effect of *Citrus tristeza virus* on grapefruit production makes pre-immunisation with mild strains essential (Marais, *et al.*, 1996). A breakdown in the CTV protection offered by the GFMS 12 (Nartia A) isolate, owing to the presence of severe strains within the complex, motivated the separation of the strains in isolates by single aphid transmission (SAT). SAT from two Nartia isolates (A=GFMS 12, C=GFMS 14, van Vuuren, *et al.*, 1993) and a Mouton isolate were prepared at the quarantine facility in Beltsville MD, USA. After re-importation these sub-isolates underwent biological indexing to differentiate between the severe and mild forms. Some sub-isolates had no potential since virus concentration and movement of the virus were poor as well as the development of unacceptable severe stem pitting (Breytenbach, *et al.*, 2002). Four of the sub-isolates showed potential and will be evaluated as cross-protectors. Promising SAT sub-isolates of Nartia obtaining from the ARC-ITSC will also be included in this experiment (van Vuuren, *et al.*, 2000). As CTV exhibits host and geographical specificity, it is imperative that mild protecting isolates be tested in the different production areas (da Graça, *et al.*, 1984).

Materials and methods

Virus-free Star Ruby budwood was budded to virus-free MxT rootstocks. When the scions had developed to approximately 5 mm thickness they were bud inoculated with the sub-isolates of GFMS 14 and Mouton (B389-1, B389-4, B390-3, B390-5), ITSC sub-isolates GFMS 12/7, GFMS 12/9) and compared to the two standards (GFMS 12 for white grapefruit; GFMS 35 for red grapefruit) and trees that were left virus-free. After pre-immunisation was confirmed by ELISA, they were planted in the Kakamas area according to a randomized block with five replications.

Results and discussion

Although there are significant differences between the growth of trees with the isolates and sub-isolates (Table 4.2.6.1), it is still too early to draw any conclusion from these data as the trees were only planted in September 2004.

Table 4.2.6.1. Canopy volumes of Star Ruby trees pre-immunised with different CTV isolates, 12 months after planting.

Treatments	Tree volumes (m ³)*	
B389/1	0.17	ab
B389/4	0.13	abc
B390/3	0.11	bc
B390/5	0.20	a
GFMS12/7	0.16	abc
GFMS12/9	0.17	ab
GFMS12 (Marsh control)	0.17	ab
GFMS35 (Star Ruby control)	0.11	bc
Virus-free (Control)	0.07	c

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

The trees are still young and no conclusions can be made at this stage.

Future research

Evaluate horticultural performance i.e. growth (tree size) health (stem pitting) and yield data (fruit size and kg/tree).

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4.2.7 Cross-protection of Marsh and Star Ruby by using the best field isolates collected in the different grapefruit production areas of southern Africa Experiment 742 by JHJ Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Enthout is gesny in die verskillende pomelo gebiede vanaf 108 uitstaande pomelo bome wat moontlik ligte isolate van *Citrus tristeza virus* (CTV) huisves. Die isolate is gevestig op onderstamme in die glashuis by CRI. Hierna is die verskillende isolate op Meksikaanse lemmetjie geïnkuleer (biologiese indeksering), om te

bepaal of hulle wel lig is. Na die eerste biologiese indeksering het slegs 19 isolate potensiaal getoon en is gekies vir verdere evaluering. Hierdie 19 isolate is 'n tweede keer geïnkuleer op Meksikaanse lemmetjie en vergelyk met GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9 en die vier beste Beltsville sub-isolate (GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5). Die geïnkuleerde plante is ge-evalueer vir groei en stamgleuf. Virus titer is bepaal deur ELISA. Die mees belowendste van hierdie 19 veld isolate, wat vry is van viroïede (Tabankulu 1 – versamel vanaf Star Ruby in Swaziland; New Venture 41/2 – versamel vanaf Star Ruby in die Nkwaleni Valle; ORE 8 – versamel vanaf Marsh in die Hoedspruit gebied; Tshipise 19/5 – versamel vanaf Marsh in Tshipise), word gebruik om virusvrye Marsh en Star Ruby boompies mee te preïmuniseer. Die isolate word vergelyk met GFMS 12 (standard vir wit pomelos), GFMS 35 (standard vir rooi pomelos), asook die vier beste Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR sub-isolate (GFMS 12/7, GFMS 12/9). Hierdie bome sal in twee pomelo produksie streke uitgeplant word sodra pre-immunisering bevestig is deur middel van ELISA.

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. 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).

This experiment is a follow-up of the glasshouse trial (exp. 49) where 108 CTV isolates were collected in different grapefruit production areas from productive old grapefruit trees. After an initial screening in the glasshouse, 19 isolates showed potential as cross protectors. These 19 isolates were then compared to the present pre-immunizing isolates. The most promising of these 19 field isolates, that are free of citrus viroïds, will be bud inoculated to virus-free Star Ruby and Marsh grapefruit trees, the Beltsville and ITSC sub-isolates of Nartia and compared to the current GFMS 12 (standard for white grapefruit) and GFMS 35 (standard for red grapefruit) isolates as controls. The trees will be planted in two grapefruit production areas.

Materials and methods

Virus-free Troyer citrange rootstocks were propagated and budded with virus-free Star Ruby and Marsh grapefruit budwood. When the scions had developed to approximately pencil thickness, they were inoculated with the selected CTV isolates in the scions. The following CTV isolates were used: the four most promising isolates selected from the original 108 field isolates (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwaleni Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), the four best Beltsville sub-isolates GFMS 14 subs: B389-1, B389-4; Mouton subs: B390-3, B390-5) and two ITSC sub-isolates (GFMS 12/7, GFMS 12/9). They will be compared to GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit) isolates and trees that will be left virus-free. After three months, positive pre-immunization will be confirmed by ELISA whereafter the trees will be planted in two grapefruit production areas according to a randomized block design with five replicates. Growth, production and tree health will be monitored.

Results and discussion

Virus-free rootstocks were budded with virus-free Marsh and Star Ruby grapefruit. Trees were pre-immunized with the different mild strains. Pre-immunisation will be confirmed by ELISA.

Conclusion

This experiment is still in the phase of applying all the different treatments.

Future research

Pre-immunize virus-free Marsh and Star Ruby trees with the different isolates and sub-isolates. Plant trees in two grapefruit production areas. Evaluate horticultural performance *i.e.* growth (tree size), production (fruit size and kg/tree) and health (stem pitting).

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4.2.8 The response of Star Ruby to different CTV isolates

Experiment 784 by S.P. van Vuuren, J.H.J. Breytenbach (CRI), B.Q. Manicom (ITSC)

Opsomming

Die onvermoë van GFMS 12 (Nartia) *Citrus tristeza virus* (CTV) isolaat as 'n kruisbeskermings-agent vir Star Ruby pomelo, het geroorsaak dat GFMS 35 isolaat as 'n kruisbeskermer gebruik word in die *interim*. Ses nuwe CTV isolate (GFMS 65, GFMS 67, GFMS 71, GFMS 73, GFMS 77, GFMS 78) wat verkry is van Star Ruby en Rosé pomelobome, is ge-evalueer en vergelyk met GFMS 12, GFMS 35, twee isolate wat vanaf Star Ruby bome by die Sitrus Grondvesblok (SG) versamel is (GFMS 12a, GFMS 12b), twee strawwe isolate (GFSS 1, GFSS 5) en bome wat virusvry geplant is. Bome wat die beste produksie gehad het oor 'n ses-jaar tydperk was gepre-immuniseer met isolate GFMS 35 en GFMS 78. Hierdie bome het gemiddeld 12% beter geproduseer as bome wat met die ander isolate gepre-immuniseer was. Berekening van die oeswaarde (vrug grootte gekoppel met mark pryse), was bome met GFMS 35 en GFMS 78, 15% beter as bome met GFMS 12a wat derde beste was. Laasgenoemde isolaat is versamel van 'n goeie moederboom by die SG wat met GFMS 12 gepre-immuniseer was. Daar is min verskil in die prestasie van die bome met die verskillende ligte isolate oor 'n agt-jaar periode, maar met 'n 22% beter oeswaarde per hektaar per jaar, toon CTV isolaat GFMS 35, wat die huidige isolaat is vir pre-immunisering van rooi pomelos, tesame met GFMS 78, dat hulle beter is as die ander isolate. Die doel van die eksperiment is bereik en word gestaak.

Introduction

Shoot tip grafting is a technique to eliminate all graft-transmissible agents from citrus bud-wood sources in South Africa (Fourie and van Vuuren, 1993). However, the benefit of optimum growth and production of virus-free trees cannot be utilised because of the abundance of the aphid vector, *Toxoptera citricida* (Kirkaldy) of *Citrus tristeza virus* (CTV) (Schwarz, 1965). Virus-free plantings will get infected with various strains of CTV within a few years after planting. Many strains of CTV exist and they usually occur as mixtures in a host (McClellan, 1963). Factors such as the type of strain, host plant and environment will determine dominance of a specific strain and it may change if any of the factors change *viz.* co-infection of a new strain or extreme temperatures (da Graça, *et al.*, 1984; Oberholzer, 1959). The only method to overcome this problem is by cross-protection, a deliberate infection of virus-free material with a known CTV isolate (Müller and Costa, 1987).

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. Since the initiation of the southern African Citrus Improvement Programme (CIP), all grapefruit selections are pre-immunised with the GFMS 12 CTV isolate (von Broembsen and Lee, 1988). This isolate originated from a 50-year-old Nartia (Marsh type) grapefruit tree in the Western Cape Province. Bud-wood source trees at the Citrus Foundation Block (CFB) at Uitenhage, which is the only source for propagating certified trees, are evaluated visually each year for decline and stem pitting symptoms. In addition, each tree of every selection is biologically indexed on an annual basis to establish the CTV severity. When there are indications of severe CTV, such a tree is terminated as a bud-wood source.

In 1993 it was found that 6-year-old Star Ruby bud-wood 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 and van der Vyver, 2000). Consequently, GFMS 35 (derived from a Rosé grapefruit tree) has been approved for the pre-immunisation of all red grapefruit in the interim (Luttig, *et al.*, 2002).

The first step in searching for mild isolates for cross protection purposes is to look for old trees that are healthy and produce good quality fruit (Müller and Costa, 1987). The Star Ruby grapefruit industry in South Africa started in the late 1970's and therefore no trees older than 15 years existed at the time. To overcome this problem, the best producers in the oldest plantings at Malelane, Mpumalanga Province, and Swaziland were selected. Isolates from these trees were evaluated in glasshouse tests and those with the best potential were evaluated in the field.

This report is on the field evaluation of the best isolates identified, and the objective of the study was to find superior CTV isolates for pre-immunisation that will maximise the profitability (productive live and quality) of Star Ruby grapefruit.

Materials and methods

Virus-free Star Ruby trees on Swingle citrumelo rootstock were grown under aphid-free conditions using standard nursery practices. When the scions have developed to approximately 5 mm diameter, they were bud-inoculated with different CTV isolates. 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 Star Ruby mother tree at CFB, showing mild stem pitting – pre-immunised with GFMS 12);
3. GFMS 12b (derived from GFMS 12 pre-immunised mother tree at CFB, displaying severe stem pitting and small fruit);
4. GFMS 35 (derived from Rosé grapefruit at Komatipoort. Marsh grapefruit trees pre-immunised with this isolate performed better than trees with GFMS 12 over a 12-year period (van Vuuren and da Graça, 2000). Present pre-immunising isolate for red grapefruit);
5. GFMS 65 (derived from a Star Ruby tree at Tambankulu Estates, Swaziland);
6. GFMS 67 (similar to 5);
7. GFMS 71 (derived from old budwood source Star Ruby, Esselen Nursery, Malelane);
8. GFMS 73 (similar to 7);
9. GFMS 77 (similar to 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, Nkweleni Valley);
12. GFSS 5 (derived from 5-year-old Star Ruby grapefruit with severe stem pitting, F. Esselen, Malelane).
13. Control. Trees planted virus-free.

After ELISA confirmed infection, a field trial was planted at Nelspruit in a randomised block.

The following data were taken:

- Tree size was measured and calculated as a cylinder and half sphere according to Burger *et al.* (1970).
- Fruit were harvested, graded in export sizes (Anon, 1990) and weighed.
- Tree health was monitored by evaluating stem pitting and decline.

The approximate monetary value of the fruit of each CTV isolate was calculated and a projection of income 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 equalled 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 8-year-old Star Ruby trees on Swingle citrumelo rootstock are given in Table 4.2.8.1.

Tree size. In the previous report it was stated that the canopy volumes of the trees of all the treatments were even, except for trees pre-immunized with GFMS 65 and the two severe isolates. This year trees with GFMS 12 and GFMS 67 were added to the latter group. Canopy sizes of these trees were significantly smaller when compared to trees pre-immunized with GFMS 12a, GFMS 35, GFMS 73, as well as those that were planted virus-free.

Yield at year eight. Trees planted virus-free produced the largest crop and were significantly larger than trees pre-immunized with GFMS 65, GFMS 67 and those with the two severe isolates.

Yield efficiency. The highest yield efficiency (kg/canopy volume) was achieved by trees pre-immunized with GFMS 67 but was not significantly better than that of the other treatments.

Stem pitting. Trunks of trees with isolates GFMS 35 and GFMS 78 showed very mild external 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. Decline. Less decline occurred in trees pre-immunized with GFMS 35, GFMS 65, GFMS 71, GFMS 73 and GFMS 77. However, it was not significantly less than that of trees with the two severe isolates and GFMS 67.

Cumulative yield. The highest cumulative yield over six seasons was produced by trees pre-immunized by GFMS 35 and GFMS 78, but was not significantly better than that of trees pre-immunized with the other mild isolates, except GFMS 65.

Crop value. High yields can reduce fruit size and therefore the value of the crop. However, trees with GFMS 35 and GFMS 78 also had the best crop value, 15% better than that of trees with GFMS12a8, which was third best.

There is little difference in the performance of the trees with the different mild isolates over an eight-year period, but with a 22% better crop value per hectare per year, isolates GFMS 35 and GFMS 78 were superior.

Table 4.2.8.1. The average tree size, production, yield efficiency and tree health (stem pitting and decline) rating of 8-year-old Star Ruby trees pre-immunized with different CTV isolates. The cumulative yield for 6 years and the relative monetary value is presented. The control trees were planted virus-free*.

Treatment	Tree canopy size (m ³)	2005 yield (kg)	Yield efficiency (kg/m ³)	Stem pitting rating*	Decline Rating***	Cumulative yield (kg) 1999 - 2005	Relative crop value R/ha/yr
Control	13.8 a	91.6 a	6.4 a	1.4 a	2.6 a	252.6 ab	16427
GFMS 12	10.1 cd	71.6 ab	7.1 a	3.4 d	2.6 a	252.8 ab	15294
GFMS 12a	14.6 a	73.1 ab	5.1 a	1.4 a	2.6 a	310.6 ab	18990
GFMS 12b	12.7 abc	69.8 ab	5.3 a	3.0 cd	2.8 a	298.6 ab	18224
GFMS 35	14.9 a	84.1 ab	5.7 a	1.4 a	2.2 a	341.2 a	21870
GFMS 65	8.7 d	55.8 bc	6.2 a	2.8 cd	2.2 a	253.9 ab	15372
GFMS 67	10.7 bcd	55.5 bc	7.7 a	2.4 bc	3.2 a	242.7 b	16510
GFMS 71	12.6 abc	83.8 ab	6.7 a	2.0 ab	2.2 a	287.7 ab	17622
GFMS 73	13.9 a	73.6 ab	5.1 a	2.0 ab	2.2 a	275.4 ab	16704
GFMS 76	13.3 ab	69.6 ab	5.1 a	1.8 ab	2.8 a	285.8 ab	17350
GFMS 77	13.2 ab	75.6 ab	5.5 a	2.0 ab	2.0 a	290.8 ab	18045
GFMS 78	13.6 ab	79.3 ab	5.9 a	1.4 a	2.6 a	336.9 a	21662
GFSS 1	8.0 de	31.0 c	3.7 a	4.4 e	3.0 a	82.3 c	4550
GFSS 5	5.3 e	21.2 c	9.3 a	3.0 cd	3.0 a	64.1 c	3924

* 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.

*** Decline rating: 1 = no decline; 2 = one branch or less; 3 = approximately one quarter of the tree or less; 4 = more than a quarter but less than half of the tree; 5 = more than half of the tree.

Conclusion

- Trees pre-immunised with GFMS 35, the present cross-protecting isolate for Star Ruby in the CIS, gave the best results. This isolate was followed by isolates GFMS 78 and GFMS 12a. The latter was derived from the original mother tree for Star Ruby budwood at the CFB.
- Calculating the total crop value (fruit size and market prices), the crop value of trees with GFMS 35 and GFMS 78 averaged 22% better than the rest of the mild isolates.
- GFMS 35 is recommended for use as a pre-immunising isolate for Star Ruby grapefruit in the southern Africa Citrus Industry. This isolate contains no strains that cause severe stem pitting (Luttig, *et al.*, 2002) and the chances of detrimental strain shifts within Star Ruby trees, which are planted in different climatic areas, are minimised.

Future research

The objective of this trial has been achieved and it will therefore be terminated. Data will be published in the Proc. 16th Conf. of the International Organization of Citrus Virologists.

Acknowledgements

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4.2.9 The response of different red grapefruit cultivars to *Citrus tristeza virus*

Experiment 785 by S.P. van Vuuren, J.H.J. Breytenbach (CRI), B.Q. Manicom (ITSC)

Opsomming

Sewe-jaar oue bome van sewe rooi pomelo seleksies, nl. Star Ruby, Rio Red, Henderson, Nel Ruby, Flame, Ruben en Oran Red op Swingle citrumelo onderstam, het baie eenvormig gereageer met vier ligte *Citrus tristeza virus* (CTV) isolate (GFMS 12, GFMS 35, GFMS 67, GFMS 73) as kruisbeskermings-agente. Tussen die CTV isolate wasbome met GFMS 35 die grootste en tussen die pomelo seleksies was Oran Red die kleinste. Dit is moontlik dat Oran Red 'n genetiese dwerg eienskap het. Daar is aanduidings van interaksies

tussen sommige CTV isolate en pomelo seleksies (Rio Red met GFMS 12; Flame met GFMS 35). Die strawwe isolaat (GFSS 5) het nie die kroon volume van die Ruben bome ge-afekteer nie wat 'n aanduiding is dat die seleksie tolerant is teen CTV.

Introduction

(Refer to section 4.2.7).

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, Nel Ruby, Henderson, Ruben and Oran Red were budded as scions on Swingle citrumelo rootstocks. Tristeza isolates GFMS 35, GFMS 67, GFMS 71 and GFMS 73 evaluated in each scion and compared to the standard (GFMS 12) and a severe isolate (GFSS 5). ELISA confirmed infection before they were planted in a randomised split plot with five replications at Malelane during December 1998.

Results and discussion

Tree size, yield, yield efficiency and stem pitting ratings of the red grapefruit selections that were pre-immunized with different CTV isolates are presented in Table 4.2.9.1, Table 4.2.9.2, Table 4.2.9.3 and Table 4.2.9.4 respectively.

Tree size: Overall, trees pre-immunised with GFMS 35 were the largest. They were significantly larger than trees pre-immunized with GFMS 12 and the severe isolate. Of the selections, trees of Nel Ruby, Flame and Ruben were the largest and were significantly larger than the Henderson and Oran Red trees. The Oran Red trees were the smallest, however, it is possible that Oran Red has a genetic dwarfing characteristic. It also appears that Ruben has some tolerance to CTV since the severe isolate did not affect tree size (Table 4.2.9.1). The body of the table shows some interactions between selections and some of the mild isolates (Rio Red with GFMS 12; Flame with GFMS 35). The severe isolate (GFSS 5) did not reduce the canopy volume of Ruben, indicating tolerance of the selection to CTV.

Production: No significant difference occurred between the mild CTV isolates. With the grapefruit selections, the yield of the Star Ruby trees were significantly lower than those of Rio Red, Nel Ruby, Flame and Ruben trees (Table 4.2.9.2). It was equal to that of the Oran Red trees, which had a significantly smaller canopy size (Table 4.2.9.1). This was due to a significantly lower yield efficiency of the Star Ruby trees, mainly induced by GFMS 35 (Table 4.2.9.3). This could be that the trees were in a more vigorous state or decline because of disease. However, the trees did not display decline and disease pressure is also not reflected in the stem pitting status (Table 4.2.9.4).

Stem pitting: Overall the stem pitting did not differ among trees pre-immunized with the different mild CTV isolates (Table 4.2.9.4). The Ruben trees had the least stem pitting and display some tolerance to CTV. Generally the trunks of the trees are smooth with occasional pits, however, stem pitting increased since last year. With the severe isolate Star Ruby, Rio Red and Henderson trees had an average rating of 3 and higher. Mild isolates with similar ratings were: GFMS 12 in Oran Red, GFMS 67 in Henderson, GFMS 73 in Henderson and Nel Ruby.

Table 4.2.9.1. The effect of different CTV isolates on tree size (canopy volume = m³) of 7-year-old red grapefruit selections*.

Grapefruit selections	CTV Isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	21.5 a	21.6 a	22.0 a	18.4 a	13.5 b	19.4 xy
Rio Red	18.8 b	22.7 a	20.0 ab	21.4 ab	14.3 c	19.4 xy
Henderson	18.8 a	15.1 a	18.7 a	17.2 a	16.7 a	17.3 yz
Nel Ruby	21.5 a	22.4 a	23.4 a	24.4 a	13.7 b	21.1 x
Flame	22.4 a	17.8 bc	22.1 ab	22.2 ab	16.5 c	20.2 x
Ruben	22.5 a	21.3 a	20.1 a	18.7 a	20.6 a	20.6 x
Oran Red	14.0 bc	17.0 a	16.2 ab	16.2 ab	13.0 c	15.3 z
Mean	19.9 x	19.7 x	20.4 x	19.8 x	15.5 b	

* Figures in each row in the body of the table that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.2. The effect of different CTV isolates on the production (kg/tree) of 7-year-old red grapefruit selections*.

Grapefruit selections	CTV isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	108.3 a	101.3 a	116.4 a	116.2 a	46.0 b	97.6 z
Rio Red	119.0 b	160.7 a	146.3 ab	139.5 ab	75.4 c	128.2 wxy
Henderson	113.9 ab	88.4 b	125.6 a	131.4 a	98.0 b	111.5 Yz
Nel Ruby	124.8 ab	155.9 a	130.1 ab	163.6 a	88.7 b	132.6 wx
Flame	150.8 a	109.4 b	125.2 ab	152.6 a	97.2 b	127.0 wxy
Ruben	150.9 a	140.6 a	152.9 a	126.4 a	126.7 a	139.5 w
Oran Red	118.7 ab	123.6 a	123.7 a	128.4 a	93.1 b	117.6 xy
Mean	126.6 x	125.7 x	131.5 x	136.9 x	89.3 y	

* Figures in each row in the body of the table that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.3. The effect of different CTV isolates on the yield efficiency (kg/m³ canopy) of 7-year-old red grapefruit selections*.

Grapefruit selections	CTV isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	5.1 ab	4.7 bc	5.4 ab	6.4 a	3.4 c	5.0 z
Rio Red	6.5 ab	7.1 a	7.3 a	6.5 ab	5.3 b	6.5 xy
Henderson	6.0 ab	5.9 b	6.8 ab	7.9 a	6.0 ab	6.5 xy
Nel Ruby	5.6 a	7.0 a	5.6 a	6.5 a	6.6 a	6.3 y
Flame	6.7 a	6.3 a	5.7 a	6.8 a	6.0 a	6.3 xy
Ruben	6.8 a	6.6 a	7.9 a	7.9 a	6.2 a	7.1 wx
Oran Red	8.5 a	7.3 a	7.7 a	8.1 a	7.3 a	7.8 w
Mean	6.5 xyz	6.4 yz	6.6 xy	7.1 x	5.8 z	

* Figures in each row in the body of the table that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.9.4. The effect of different CTV isolates on stem pitting (rating**) of 7-year-old red grapefruit selections*.

Grapefruit selections	CTV Isolates					Mean
	GFMS 12	GFMS 35	GFMS 67	GFMS 73	GFSS 5	
Star Ruby	2.6 a	2.6 a	2.3 a	2.0 a	3.0 a	2.5 yz
Rio Red	2.5 ab	1.9 a	2.3 ab	2.7 b	3.0 b	2.5 yz
Henderson	1.8 a	2.6 ab	3.4 b	2.9 b	2.5 ab	2.6 z
Nel Ruby	2.5 ab	2.5 ab	1.7 a	3.0 b	2.3 ab	2.4 yz
Flame	2.1 a	2.7 a	2.5 a	2.6 a	2.4 a	2.5 yz
Ruben	2.0 ab	1.7 a	2.7 b	2.2 ab	1.8 ab	2.1 y
Oran Red	2.8 b	2.3 ab	2.0 ab	1.8 a	2.2 ab	2.2 yz
Mean	2.3 x	2.3 x	2.4 x	2.5 x	2.5 x	

* Figures in each row in the body of the table that are followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

** Stem pitting rating: 1 = Smooth trunk; 2 = Occasional visible pits; 3 = Mild pitting; 4 = Moderate pitting; 5 = Severe pitting.

Conclusion

The reaction of the different grapefruit selections to the different CTV isolates was very similar at this stage. It appears that stem pitting development is increasing in some selection / isolate combinations. 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 4.2.7).

4.2.10 Constructing a superior *Citrus tristeza virus* isolate for cross-protection of grapefruit selections

Experiment 786 by S.P. van Vuuren (CRI)

Opsomming

Gedurende 2003 is sewe sub-isolate van GFMS 12 (Nartia), vier enkel plantluis oordragings en drie gasheer skeidings, 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 is voortgesit, afsonderlik en in kombinasie, in Marsh, Star Ruby, Flame, Rio Red en Henderson pomelo seleksies. Die studie het getoon dat alhoewel die twee sub-isolate lig was maar oordraging het verminder wanneer hulle gesamentlik geïnokuleer was. Dit maak bome kwesbaar vir strawwe rasse wat deur plantluis gedra word. GFMS 35 was ligter as GFMS 12 en net so lig soos die twee sub-isolate, maar met 'n hoër oordragingstempo in al die pomelo seleksies. Die twee sub-isolate word afsonderlik verder ge-evalueer in verskeie veld proewe (sien 4.2.5, 4.2.6 en 4.2.7). Die doel van die proef is bereik en word gesluit.

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 favour 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, 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 minimise 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).

Two aphid-transmitted sub-isolates GFMS 12/7 and GFMS 12/9 exhibited good qualities for a cross-protecting isolate. They were highly transmissible, revealed simple single-strand polymorphism profiles (indication of a single strain) and had no negative effects on growth of seven grapefruit selections. Evaluations of these two isolates are being continued, singly and in combination.

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 will be bud-inoculated by two buds containing selected CTV sub-isolates GFMS 12/7 and GFMS 12/9. The two sub-isolates were also combined as a treatment and one bud of each isolate was inoculated per plant. The sub-isolates were compared to GFMS 12 (standard isolate for white grapefruit), GFMS 35 (standard isolate for red grapefruit) and uninoculated virus-free plants as controls. Each scion/CTV combination was replicated five times. After inoculation the plants were cut back at two buds above the inoculation point to force new growth. The plants 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 & 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. At 12 months the growth since inoculation was removed, measured and the bark stripped to evaluate stem pitting.

Results and discussion

The transmission rating of the isolates, the growth of the grapefruit selections, and stem pitting development in the selections are presented in Table 4.2.10.1, Table 4.2.10.2 and Table 4.2.10.3 respectively.

Transmission. The transmission to the different grapefruit selections did not differ, however, the transmissibility of the CTV isolates and sub-isolates differed significantly. The highest transmission was obtained with the two isolates. The transmissibility of sub-isolate GFMS 12/9 did not differ from GFMS 35 but both the sub-isolates were significant lower than that of GFMS 12. The lowest transmission was obtained when the two sub-isolates were combined.

Growth. None of the isolates or sub-isolates affected growth. Of the grapefruit selections, Flame had significantly better growth than Rio Red. There were no inter-actions among isolates, sub-isolates and the grapefruit selections.

Stem pitting. As a result of the high temperature at which the plants were kept, stem pitting was generally low. Overall, there was no difference in the occurrence of stem pitting between the grapefruit selections. Isolate GFMS 12 induced the most stem pitting in all the selections and was significantly higher than that of isolate GFMS 35 and the two sub-isolates.

Table 4.2.10.1. Transmission rating of CTV isolates and sub-isolates to five grapefruit selections*.

CTV	MARSH	STAR RUBY	FLAME	RIO RED	HENDERSON	MEAN
GFMS 12/7	3.4 ab	3.4 b	4.2 a	3.4 a	3.4 a	3.6 x
GFMS 12/9	4.2 ab	5.0 a	5.0 a	3.4 a	3.4 a	4.2 wx
12/7 + 12/9	2.6 bc	1.0 c	2.6 b	3.4 a	3.4 a	2.6 y
GFMS 12	5.0 a	5.0 a	5.0 a	5.0 a	5.0 a	5.0 v
GFMS 35	5.0 a	4.2 ab	5.0 a	5.0 a	5.0 a	4.8 vw
Control	1.0 c	1.0 c	1.0 c	1.0 b	1.0 b	1.0 z
MEAN	3.5 z	3.3 z	3.8 z	3.5 z	3.5 z	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.10.2. Growth (cm) of five grapefruit selections 12 months after inoculation with CTV isolates and sub-isolates*.

CTV	MARSH	STAR RUBY	FLAME	RIO RED	HENDERSON	MEAN
GFMS 12/7	102.3 a	81.7 a	121.2 a	85.8 a	86.4 a	95.5 z
GFMS 12/9	77.1 a	99.6 a	103.1 ab	101.0 a	101.7 a	96.5 z
12/7 + 12/9	82.6 a	104.9 a	103.6 ab	96.2 a	108.9 a	99.3 z
GFMS 12	98.2 a	80.8 a	97.8 ab	72.9 a	106.4 a	91.2 z
GFMS 35	94.4 a	91.8 a	92.6 b	82.9 a	86.6 a	89.7 z
Control	98.5 a	79.6 a	95.6 ab	86.3 a	105.3 a	93.1 Z
MEAN	92.2 yz	89.7 yz	102.3 y	87.5 z	99.2 yz	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Table 4.2.10.3. Stem pitting (pits/cm²) of five grapefruit selections 12 months after inoculation with CTV isolates and sub-isolates*.

CTV	MARSH	STAR RUBY	FLAME	RIO RED	HENDERSON	MEAN
GFMS 12/7	0.00 a	0.00 a	0.04 b	0.00 a	0.00 a	0.01 z
GFMS 12/9	0.00 a	0.14 a	0.06 b	0.02 a	0.08 a	0.06 z
12/7 + 12/9	0.00 a	0.00 a	0.00 b	0.00 a	0.00 a	0.00 z
GFMS 12	2.96 b	6.14 b	10.18 a	3.40 b	5.02 b	5.54 y
GFMS 35	0.48 ab	0.93 ab	0.54 b	0.62 a	0.68 a	0.64 z
Control	0.00 a	0.00 a	0.00 b	0.00 a	0.00 a	0.00 z
MEAN	0.57 z	1.21 z	1.80 z	0.67 z	0.96 z	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fisher's LSD).

Conclusion

Isolate GFMS 12 was the most severe and caused stem pitting in all the grapefruit selections in an environment of 28-32°C. It was highly transmissible and did not reduce growth of any of the selections.

Isolate GFMS 35 was less severe, inducing significantly less stem pitting than GFMS 12 and equal to the two mild sub-isolates. It was also highly transmissible and did not reduce growth of any of the grapefruit selections.

Both the two sub-isolates were mild, causing very few stem pits. While the transmissibility of GFMS 12/9 was equal to that of GFMS 35, the transmissibility of GFMS 12/7 was unacceptably low. When both were inoculated in the same plant, transmission was even lower.

The objective of this study was to construct a CTV isolate from mild sub-isolates to obtain an isolate that will be mild in all the major grapefruit selections used in the industry. Although the two sub-isolates were mild, transmissibility decreased when used in combination. This will make the trees vulnerable in the field for severe challenges. GFMS 35 proved to be milder than GFMS 12, equally mild in than the sub-isolates and suitable for all grapefruit selections.

The two sub-isolates (GFMS 12/7 and GFMS 12/9) were incorporated in three field experiments (experiments 679, 738, 742) for evaluation in Star Ruby and Marsh grapefruit in several climatic conditions. Trials at Tambuti Estates (Swaziland), Riversbend (Nkwaleni Valley) and the Orange River Valley have been established and another that will be planted at two sites is in preparation.

Future research

The objective of the experiment has been achieved and therefore it will be terminated. The plants will be transferred to Pretoria University where Prof. Gerhard Pietersen and his team will use them in experiments.

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4.2.11 The effect of CTV pre-immunization on the fruit size of Clementine and satsuma Experiment 816 by S.P. van Vuuren (CRI), J.G. Maritz & N. Combrink (ITSC)

Opsomming

Vruggrootte is 'n groot probleem by Clementines in die Oos- en Wes-Kaap. Om die invloed van pre-immunisering op vruggrootte te bepaal, word ongepre-immuniseerde en gepre-immuniseerde bome van sewe Clementine seleksies (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) en een satsuma seleksie (Miho Wase) op Addo Navorsingstasie vergelyk. Die bome is nou twee jaar oud en

nog nie in drag nie. Stam omtrekke van die bome is gemeet en alhoewel die bome nog te jonk is om gevolgtrekkings te maak, wil dit voorkom of al die seleksies nie dieselfde reageer op tristeza besmetting nie.

Introduction

All citrus propagation material is pre-immunized with a mild isolate of *Citrus tristeza virus* (CTV). Cross protection is specific with regard to the citrus type, i.e. the most effective protecting strain for a given citrus type is usually obtained from the same type (Müller & Costa, 1987). With the initiation of the Citrus Improvement Programme, all citrus, including mandarin types, was pre-immunized with an 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 approved to replace GFMS 12 as the pre-immunizing isolate for the mandarin types. The suitability of LMS 6 as a protector for Clementines has not been confirmed and evaluations are currently being done (van Vuuren & Maritz, 2002).

Fruit size of Clementine is a major problem in the Western and Eastern Cape citrus production regions. Production costs associated with fruit size improvement cultural practices are high. Since mandarins have a lower sensitivity to CTV, it may not be essential to pre-immunize mandarin cultivars to protect them against severe strains of CTV. The prospect to improve size of fruit borne on virus-free trees needs to be investigated.

Materials and methods

This trial was initiated by Prof. E. Rabe and was taken over by S.P. van Vuuren when prof. Rabe left South Africa. Virus-free and LMS 6 pre-immunized trees of seven Clementine selections (Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons) and one satsuma selection (Miho Wase) were prepared on Swingle citrumelo rootstock in a commercial nursery (rootstocks may have got infected with CTV prior to budding).

When the scions had developed they were planted at Addo Research Station according to a randomized block design in 2003. Since there was a variation in the number of trees available, they were split in three separate trials. Trial one consisted of selections Clementine late, Oronules, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated four times. Trial two consisted of selections Clementine late, Esbal, Orogrande, Guillermina, Nour, Clemenpons and each treatment was replicated five times. Trial three was Miho Wase satsuma and each treatment was replicated eight times. Growth, production and fruit size were the main criteria in the trials.

Results and discussion

The trunk circumference of the trees was measured one year after planting and is presented in Table 4.2.11.1. The trials are too young to interpret the results, however, there are indications that the different selections do not react similarly to CTV infection.

Table 4.2.11.1. Trunk circumference (mm) of virus-free (VF) and pre-immunised (PI) Clementine selections and Miho Wase satsuma one year after planting.

Cultivar or selection	Trial 1			Trial 2			Trial 3		
	VF	PI	SIGN ¹	VF	PI	SIGN ¹	VF	PI	SIGN ¹
Oronules	139	146	ns	-	-	-	-	-	-
Clementine late	143	128	*	145	127	*	-	-	-
Esbal	157	146	ns	134	138	ns	-	-	-
Orogrande	114	119	ns	157	152	ns	-	-	-
Guillermina	150	120	*	130	138	*	-	-	-
Nour	151	137	ns	154	134	*	-	-	-
Clemenpons	131	113	ns	129	133	ns	-	-	-
Miho Wase	-	-	-	-	-	-	168	147	*
MEAN	141	130	*	142	137	ns	168	147	*

¹ Significance: *Difference significant at P=0.05, ns = no significant difference.

Conclusion

No conclusion yet.

Future research

Measure tree size and harvest fruit when they start bearing.

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4.2.12 Evaluation of CTV isolates in navel

Experiment 787 by S.P.van Vuuren (CRI), J.G. Maritz & N. Combrink (ITSC)

Opsomming

Verskillende *Citrus tristeza virus* isolate (LMS 6, SM 36, SM 41, SM, 45, SS2) word in Palmer nawel op verskillende kommersiële onderstamme (Growweskil suurlemoen, Troyer citrange, Swingle citrumelo, C35 citrange) in die Oos Kaap geëvalueer. Tans presteer bome met LMS 6, die huidige kruisbeskermings-agent, die beste.

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.

The aim of this research is 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 will be determined.

Results and discussion

Tree size, production and yield efficiency are presented in Table 4.2.12.1, Table 4.2.12.2 and Table 4.2.12.3 respectively.

Tree size. Overall, trees with isolate LMS 6 (present pre-immunizing isolate) were the largest but not significantly larger than those with isolate SM 36 (Table 4.2.12.1). The rootstocks did not affect tree size.

Production. Trees on Rough lemon and C 35 produced significantly more than trees on Swingle, with the latter rootstock also with a significantly higher yield efficiency (Table 4.2.12.2; Table 4.2.12.3). Production was according to tree size except for the trees with the severe isolate that had a significantly higher yield efficiency.

Table 4.2.12.1. The effect of different CTV isolates on the growth (canopy volume = m³) of 6-year-old Palmer navel on different rootstocks¹.

CTV Isolate	Rootstock ²				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	10.2 ab	12.4 a	11.0 a	14.5 a	11.7 a
SM 36	11.1 a	9.9 ab	12.1 a	9.3 bc	10.2 ab
SM 41	7.2 ab	11.2 ab	8.3 abc	8.4 bcd	8.5 bc
SM 45	8.8 ab	8.1 ab	6.4 bc	6.4 cd	8.2 cd
SOSS 2	7.0 b	7.1 b	5.3 c	5.5 d	6.2 d
Control	9.7 ab	8.5 ab	10.2 ab	9.9 b	9.6 bc
Mean	9.0 z	9.3 z	8.9 z	9.0 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.

Table 4.2.12.2. The effect of different CTV isolates on the production (kg/tree) of 6-year-old Palmer navel on different rootstocks¹.

CTV isolate	Rootstock ²				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	48.8 a	50.7 a	11.2 a	91.9 a	53.6 a
SM 36	52.8 a	44.5 a	9.9 a	47.1 ab	49.6 ab
SM 41	41.0 a	19.6 a	8.1 a	34.1 b	33.3 c
SM 45	52.6 a	34.0 a	11.2 a	38.6 b	38.3 bc
SOSS 2	40.1 a	38.3 a	8.1 a	50.5 ab	43.3 abc
Control	43.3 a	21.2 a	8.5 a	47.4 ab	35.8 c
Mean	46.5 y	34.7 z	41.5 yz	46.6 y	

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.

Table 4.2.12.3. The effect of different CTV isolates on the yield efficiency (kg/m³) of 6-year-old Palmer navel on different rootstocks¹.

CTV Isolate	Rootstock ²				Mean
	RL	Swingle	Troyer	C 35	
LMS 6	4.9 a	5.1 a	4.8 b	4.3 b	4.8 z
SM 36	5.9 a	2.4 a	4.6 b	4.1 b	4.3 z
SM 41	6.6 a	3.2 a	4.0 b	6.7 ab	5.2 z
SM 45	5.2 a	5.8 a	4.5 b	5.0 b	5.1 z
SOSS 2	6.1 a	5.3 a	8.8 a	10.7 a	7.7 y
Control	4.4 a	2.1 a	3.4 b	4.9 b	3.7 z
Mean	5.5 yz	4.0 z	5.0 yz	5.9 y	

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

Currently trees with LMS 6, the present pre-immunising CTV isolate, are performing the best.

Future research

Measure trees, harvest and grade fruit.

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4.2.13 Identification of suitable *Citrus tristeza virus* isolates for pre-immunizing Turkey Valencia Experiment 789 by JHJ Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Daar is gevind dat Turkey Valencia meer gevoelig is vir *Citrus tristeza virus* (CTV) as ander Valencia tipes (CRI Groep Navorsings-jaarverlag, 2003). Aangesien Turkey Valencia 'n vroeë Valencia is, vorm dit 'n belangrike deel van sitrus produksie in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte CTV isolaat te vind om Turkey Valencia te pre-immuniseer. Virusvrye Turkey Valencia op Troyer citrange onderstam word in 'n glashuis voorberei en met CTV isolate (LMS 6 (standaard), SM 45, SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemonoë versamel), geïnkuleer om die beste ligte isolaat te identifiseer vir kruisbeskermings-doeleindes. Bome wat met GFMS 12 geïnkuleer is en bome wat virusvry gelaat word dien as positiewe en negatiewe kontroles respektiewelik. Die proef is nog in die glashuis stadium waar die isolate geïnkuleer word waarna preïmmunisasie bevestig sal word met ELISA en die bome in 'n boord uitgeplant sal word.

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).

Recently it was found that Turkey Valencia trees on Rough lemon and Volckameriana rootstocks developed bud-union creasing symptoms (personal observation) (Beeton, *et al.*, 2000). Various sources of Turkey Valencia had stem pitting symptoms and it appears that this cultivar is more sensitive to CTV than other Valencia cultivars (CRI Group Annual Research Report, 2003). Since Turkey Valencia is an early cultivar, it forms an important part of citrus production, and therefore the identification of a suitable CTV isolate for cross-protection remains a high priority.

The objective of this study is to evaluate CTV isolates to identify a suitable cross protecting isolate without the bud-union crease factor for Turkey Valencia.

Materials and methods

Virus-free Turkey Valencia scions on Troyer citrange rootstocks are prepared in the glasshouse according to normal nursery practices. When the scions had developed to approximately 5 mm, they were inoculated with different mild CTV isolates by budding two buds containing the required CTV isolate into the scions (Table 4.2.13.1). After three months the trees will be tested for the presence of the CTV isolates by ELISA. When pre-immunisation is confirmed, they will be planted in the field where they will be subjected to normal CTV challenge by aphids. Each treatment will be replicated five times and uninoculated virus-free trees will serve as controls. Evaluations will be on growth, production and tree health.

Table 4.2.13.1. Treatments for Turkey Valencia on Troyer citrange rootstock to identify a suitable CTV isolate for pre-immunisation.

Treatment	Origin and comments
LMS 6	Mexican lime, Tzaneen. Present pre-immunising isolate for sweet orange
SM 45	Portsgate Valencia, Hoedspruit. Show promise in current cross protecting trials
SM 46	Shamouti Midseason, Messina.
SM 47	Valencia, Grahamstown. Tree > 100 years old
SM 48	Midseason, Citrusdal. First planting of citrus in the area
SM 49	Valencia, Nelspruit. Induce greening tolerance
GFMS 12	Grapefruit, Nartia. Positive control
Control	Virus-free. Negative control

Results and discussion

Virus-free Troyer citrange rootstocks were budded with virus-free Turkey Valencia. Trees were pre-immunized with the different mild strains

Conclusion

No conclusion.

Future research

As soon as pre-immunization is confirmed by means of ELISA, they will be planted in the field and the trees will annually be evaluated for horticultural performance i.e. growth (tree size) health (stem pitting ratings) and harvest data (fruit size and kg/tree).

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4.2.14 Evaluation of CTV isolates in Valencia

Experiment 788 by S.P. van Vuuren, J.H.J. Breytenbach (CRI) & B.Q. Manicom (ITSC)

Opsomming

Die effek van verskillende ligte *Citrus tristeza virus* (CTV) isolate (LMS 6 (standard), SM 36, SM 41, SM 45 and SM 49) word in drie Valencia bostamme (McClellan, McClellan Saadloos en Delta) op Troyer citrange onderstam ge-evalueer. Boom grootte van McClellan Valencia was betekenisvol kleiner as die van Delta – en McClellan Saadloos Valencias. Oor die algemeen was produksie swak met uitermatige klein vrugte. Die rede hiervoor is onbekend maar kan as gevolg van droogte of voedingstekorte wees. Die oesdata van hierdie jaar kan egter nie gebruik word om die CTV isolate of die bostam cultivars te evalueer nie.

Introduction

Refer to section 4.2.12.

The objective of this trial is to evaluate promising CTV isolates in three Valencia scions and identify suitable cross-protecting isolates.

Materials and methods

McCleen, McCleen 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 approximately 5 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 SM 49. Trees with these isolates will be 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 4.2.14.1). Overall the canopy volumes of McCleen Valencia were significantly smaller than that of the Delta – and McCleen Seedless Valencia trees. The three Valencia selections did not differ significantly. With the CTV isolates, however, trees with the SM 45 isolate and the severe isolate were significantly smaller. Some interactions between CTV isolates and the McCleen Seedless scion occurred.

Comparisons of the effects of the CTV isolates on the growth of the three scions show that mild isolates LMS 6, SM 36, SM 49 were stable in all the scions. With the other isolates, isolate SM 34 was significantly better in McCleen Seedless Valencia than in both the other scions, SM 41 and SM 45 were significantly better in McCleen Seedless Valencia than in McCleen Valencia.

Production and yield efficiency. Overall the yield was poor and variable with the majority of fruit small (Table 4.2.14.2). The cause is unknown but could be the result of drought conditions or lack of fertilization. The yield data of this year cannot be used for assessment of the CTV isolates or scions.

Table 4.2.14.1. Tree size (canopy volume = m³) of three Valencia selections that were pre-immunized with different mild CTV isolates, a severe isolate and trees that were planted virus-free, four years after planting*.

CTV Isolate	Scion**			Mean
	McC	McC SL	Delta	
LMS 6	15.8 A	21.9 Ab	19.3 A	19.0 Xy
SM 34	18.6 A	22.4 A	19.3 A	20.1 X
SM 36	17.0 A	21.7 Ab	19.6 A	19.4 X
SM 41	18.4 A	19.0 Bc	18.2 A	18.6 Xy
SM 45	15.3 a	18.1 C	18.3 A	17.2 Y
SM 49	18.0 A	19.4 Bc	17.8 A	18.4 Xy
SOSS 3	7.2 B	11.5 d	10.7 B	9.8 Z
VF	16.6 A	18.6 C	19.1 A	18.1 xY
Mean	15.9 y	19.8 x	17.8 X	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

** Scions: McC = McCleen Valencia; McC = McCleen seedless Valencia; Delta = Delta Valencia.

Table 4.2.14.2. 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, four years after planting*.

CTV Isolate	Scion**			Mean
	McC	McC SL	Delta	
LMS 6	56.7 ab	30.0 a	20.9 ab	35.9 ab
SM 34	57.9 ab	21.1 ab	23.0 ab	34.0 abc
SM 36	43.0 abc	9.3 b	6.7 b	19.7 bcd
SM 41	66.3 a	17.6 ab	35.0 a	39.6 a
SM 45	35.7 bc	1.0 b	15.2 ab	17.3 cd
SM 49	55.5 ab	6.6 b	6.6 b	22.9 abcd
SOSS 3	20.2 c	7.6 b	2.7 b	10.2 d
VF	34.4 bc	5.4 b	23.7 ab	21.2 bcd
Mean	46.2 y	12.3 z	16.7 z	

* Figures in each column followed by the same letter do not differ significantly at the 5% level (Fishers' LSD).

** Scions: McC = McClean Valencia; McC = McClean seedless Valencia; Delta = Delta Valencia.

Conclusion

Because of the variable production and small fruit no conclusion can be made.

Future research

Harvest, size and weigh fruit. Determine tree size.

References cited

Refer to section 4.2.12.

4.2.15 The effect of different CTV isolates in Valencias on different rootstock combinations for the Orange River Valley

Experiment 739 by JHJ Breytenbach & S.P. van Vuuren (CRI)

Opsomming

Omdat *Citrus tristeza virus* gasheer en klimaat spesifiek is, is dit nodig om verskillende beskermende isolate in die verskillende sitrus produserende streke te evalueer. Ligte isolate wat oorspronklik uit soetlemoen bome versamel is (SM 45, SM 46, S 47, SM 48, SM 49), word gebruik om virusvrye Delta -, Midnight -, McClean -, McClean seedless - en Turkey Valencia op C 35 citrange onderstam te pre-immuniseer. Hierdie isolate sal vergelyk word met LMS 6 (standard vir soetlemoene) en boompies wat virusvry geplant word. Nadat pre-immunisering bevestig is deur middel van ELISA, word die boompies in die Kakamas omgewing uitgeplant en jaarliks ge-evalueer vir boomgrootte, vruggrootte, oes opbrengs, sowel as hul gesondheids-toestand.

Introduction

Citrus tristeza virus (CTV) is the causal agent of possibly the most destructive disease of citrus in the world. The disease is spread by propagation material and by various aphid species of which *Toxoptera citricida* is the most abundant. Symptoms induced by CTV range from mild with no noticeable effect on the host to severe stem pitting and decline resulting in uneconomic production. As CTV exhibits host and geographical specificity, it is necessary that mild protective isolates be evaluated in the different production areas. The only practical means of controlling CTV disease at present is by mild strain cross-protection. The objective of this experiment is to evaluate selected CTV isolates in four different Valencia selections on three different rootstocks in order to identify a suitable cross-protecting CTV isolate for specific rootstock/scion combinations. The experiment will give horticultural information on the use of the most suitable Valencia selection on a specific rootstock as well.

Materials and methods

Five virus-free Valencia scions (Delta, Midnight, McClean, McClean seedless, Turkey) were budded on C35 citrange rootstock. When the scions had developed sufficiently, each Valencia selection was bud-inoculated with five selected CTV isolates originating from sweet orange (SM45, SM46, SM47, SM48, SM49). These isolates will be compared to trees inoculated with LMS6 (standard) and trees planted virus-free. Successful pre-immunisation will be confirmed with ELISA where after the trees will be planted in the field.

Results

Virus-free rootstocks were budded with the different virus-free scions. Trees were pre-immunized with the different mild isolates.

Conclusion

This experiment is still in the phase of applying all the different treatments.

Future research

As soon as pre-immunization is confirmed by means of ELISA, they will be planted in the field experiment and the trees will be evaluated annually for horticultural performance i.e. growth (tree size) health (stem pitting and decline ratings) and harvest data (fruit size and kg/tree).

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- Van Vuuren, S.P. 2002. Effects of *Citrus tristeza virus* isolates on two tolerant commercial scions on different rootstocks in South Africa. Proc. 15th Conf. IOCV: 31 – 38.
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4.2.16 Screening of rootstocks for Citrus Blight tolerance

Experiment 32 by JHJ Breytenbach (CRI)

Opsomming

Die inokulasie van sitruskroei in Delta Valencia bome op 17 verskillende onderstamme induseer 'n afname in boom grootte en produksie 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. Die resultate van die boom volumes, produksie van klein vrugte, die water opname toets en die voorkoms van die 12-kd proteïen komplimenteer mekaar nie en maak die interpretasie van die data moeilik. Onderstamme C35 citrange, Empress mandaryn en Carrizo citrange word die meeste ge-afgete deur sitruskroei. Bome op X639, Sun Chu Sha en Cleopatra mandaryn toon die meeste toleransie teen die siekte.

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 Sitrus. The trial comprises Delta Valencia on Rough lemon, Empress mandarin, Troyer citrange, *P. trifoliata*, Volckameriana, Gou Tou, Orlando tangelo, Sampson tangelo, M&T, X639, Marsh grapefruit, Swingle citrumelo, Troyer citrange, Zhu Luan, Sweet Orange and Carrizo citrange, that were planted in 1990. In 1995 trees on Cleopatra mandarin, C35 and Sun Chu Sha were added, and during 1996 trees on Benton citrange and Sunki mandarin were included.

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 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 recorded 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

Since CB is a disease that develops mostly after eight years or more after planting, the results of the canopy size and yield are presented according to their planting dates (Tables 4.2.16.1 and 4.2.16.2).

Tree size. In the 1990 planting, trees on Swingle citrumello and Sampson tangelo rootstocks show the least effect on tree size reduction after inoculation. The tree sizes of the 1992 planting show that rootstocks X639 and Gou Tou was the least affected. Results of the 1995/96 planting indicate that tree sizes of Sun Chu Sha and Cleopatra mandarin rootstocks were the least affected. To summarize, trees on Swingle citrumello, X639, Gou Tou, Sun Chu Sha and Cleopatra mandarin rootstocks showed tolerance in a CB situation while trees on Empress mandarin, C35 citrange and Carrizo citrange rootstocks were the most susceptible.

Yield

Trees on X639 rootstock had the highest yield in all of the plantings. Fruit size was average (16.2% < count 105) and it was increased in the CB inoculated trees (27%) with almost no reduction in yield. Comparing the percentage decrease in production and the presence of small fruit in control and inoculated trees, Empress mandarin performed the worst overall.

Water uptake

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. Trees on Swingle citrumello (early planting) and Gou Tou (later planting) show the least effect and C35 citrange the most affected (Table 4.2.16.3).

CB protein

The presence of the 12-kd protein was much higher in the root-grafted trees than in the control non-grafted trees (Table 4.2.16.4). The latter can get infected by natural means. The 12-kd protein was higher in control and inoculated trees on Carrizo citrange (early planting) and C35 citrange (later planting) 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.

Generally the results are difficult to interpret since water uptake and protein tests do not complement the growth and yield data, *i.e.*, trees on X639 weren't the largest trees in the 1990 planting but the yields in the control and inoculated trees were higher with a poor water uptake and a high percentage trees with the 12-kd protein of CB.

Table 4.2.16.1. 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>Poncirus trifoliata</i>	1990	64.9	48.2	-26
Swingle citrumelo	1990	73.7	75.8	3
Empress mandarin	1990	59.1	26.1	-56
Carrizo citrange	1990	42.1	29.3	-30
Volckameriana	1990	67.3	58.1	-14
Sampson tangelo	1990	73.2	79.2	-8
Average 1990		50.7	38.8	-19.1
MxT	1992	67.4	61.5	-9
X639	1992	57.4	69.9	22
Gou Tou	1992	82.8	108.6	31
Orlando tangelo	1992	64.9	64.4	-1
Zhu Luan	1992	36.5	28.1	-23
Marsh grapefruit	1992	45.1	42.6	-6
Average 1992		41.5	42.5	2.5
Cleopatra mandarin	1995	42.3	44.1	4
Sun Chu Sha	1995	62.8	66.6	6
C35 citrange	1995	33.4	21.4	-36
Sunki mandarin	1996	29.6	21.9	-26
Benton citrange	1996	16.5	13.0	-21
Average 1995/96		36.9	33.4	-14.5
Mean		52.4	50.2	-10

Table 4.2.16.2. 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 (kg)	% Small fruit	Production (kg)	% Small fruit
<i>P. trifoliata</i>	1990	128.4	41.9	160.1	44.3
Swingle citrumelo	1990	133.5	26.9	139.7	39.3
Empress mandarin	1990	139.2	42.3	61.5	59.2
Carrizo citrange	1990	130.4	20.4	80.2	12.3
Volckameriana	1990	178.5	26.1	124.9	23.7
Sampson tangelo	1990	81.6	25.4	62.3	24.4
Average 1990		131.9	30.5	104.8	33.9
MxT	1992	108.1	30.7	103.3	33.6
X639	1992	158.1	16.2	163.2	26.9
Gou Tou	1992	81.0	33.7	126.9	34.7
Orlando tangelo	1992	64.0	21.6	108.9	34.6
Zhu Luan	1992	80.9	17.5	56.9	8.6
Marsh grapefruit	1992	64.9	25.3	74.1	26.4
Average 1992		92.8	24.1	205.6	27.5
Cleopatra mandarin	1995	28.6	11.01	27.3	19.8
Sun Chu Sha	1995	64.3	31.0	68.1	38.1
C35 citrange	1995	97.3	18.5	69.0	12.5
Sunki mandarin	1996	58.8	16.6	89.8	25.5
Benton citrange	1996	38.9	20.8	59.4	27.1
Average 1995/96		57.6	19.6	62.7	24.6
Mean		94	25	91	29

Table 4.2.16.3. Comparison of water-uptake (seconds/10 ml) through the trunk xylem of CB-inoculated and uninoculated Delta Valencia trees on different rootstocks.

Rootstock	Year Planted	Uninoculated	Inoculated	% Difference
<i>P. trifoliata</i>	1990	35.3	41.2	17
Swingle citrumelo	1990	23.2	21.3	-8
Empress mandarin	1990	27.8	24.6	-12
Carrizo citrange	1990	24.2	58.2	140
Volckameriana	1990	24.1	36.7	52
Sampson tangelo	1990	35.3	38.3	8
MxT	1992	55.0	59.2	8
X639	1992	65.0	64.5	-1
Gou Tou	1992	34.7	39.6	14
Orlando tangelo	1992	58.3	53.8	-8
Zhu Luan	1992	80.0	59.0	-26
Marsh grapefruit	1992	35.3	72.5	105
Cleopatra mandarin	1995	70.0	65.6	-6
Sun Chu Sha	1995	40.5	45.0	11
C35 citrange	1995	37.5	90.0	140
Sunki mandarin	1996	44.6	42.2	-5
Benton citrange	1996	42.5	90.0	112

Table 4.2.16.4. 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	0
Empress mandarin	1990	50	0
Carrizo citrange	1990	100	50
Volckameriana	1990	66.6	33.3
Sampson tangelo	1990	16.6	16.6
MxT	1992	33.3	33.3
X639	1992	66.6	33.3
Gou Tou	1992	16.6	16.6
Orlando tangelo	1992	16.6	16.6
Zhu Luan	1992	16.6	33.3
Marsh grapefruit	1992	33.3	0
Cleopatra mandarin	1995	33.3	33.3
Sun Chu Sha	1995	16.6	0
C35 citrange	1995	66.6	83.3
Sunki mandarin	1996	50	50
Benton citrange	1996	33.3	50

Conclusion

The results of the different tests do not compliment each other and it is therefore not possible to draw a meaningful conclusion to transfer to growers. Since some of the un-inoculated trees of the first planting are developing CB symptoms due to natural spread, they cannot be compared to the inoculated trees regarding the effect of CB on growth and production any longer. Therefore these trees were evaluated for the last time during 2005. Evaluations of trees of the other plantings will also be terminated when they reach the same age. Trees on X639, Swingle citrumelo, Gou Tou, Sun Chu Sha and Cleopatra mandarin appear to exhibit some tolerance while trees on Empress mandarin, C35 citrange and Carrizo citrange are the most sensitive.

Future research

Continue to monitor disease development, measure canopy volumes and take yield data. Final evaluations of the 1990 plantings were made in 2005, 15 years after planting. Final evaluations will be made on the other plantings accordingly.

Reference cited

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4.2.17 Genetic modification of citrus material for greening resistance – a literature survey

Experiment 817 by G. Pietersen and M.N.B. Phahladira (CRI at UP)

Opsomming

’n Literatuur oorsig is versoek om die moontlikheid van transgeniese weerstand teen vergroening te ondersoek. Die oorsig dek dus relevante aspekte van vergroening soos die veroorsakende organismes, vektore, en huidige beheer maatreels. Natuurlike weerstand word bespreek, gevolg deur vier baie breë kategorieë van transgeniese benaderings wat relevant mag wees tot vergroening, naamlik: 1) die gebruik van gasheer verdedigingsmeganismes; 2) gebruik van patogeen gene; 3) weerstand teen die vektor; 4) weerstand met nie-patogeen of vektor middels. Die stand van tegnologie om transgeniese sitrus te ontwikkel word ook baie kortweg bespreek.

Introduction

Citrus greening disease is a serious problem in South Africa, with control relying on use of insecticides, reduction of inoculum and use of clean planting material, all of which contribute towards production costs. While some natural resistance to the causal organism of greening does seem to exist in *Citrus* spp., with

differing severities to the disease being found amongst species, this has not been exploited for greening control. The possibility of using novel resistance strategies through the use of transgenic citrus has been proposed. This literature survey was requested by Dr. H. le Roux, CRI, to explore literature relevant to this possibility. Unfortunately current expertise of the authors does not include in-depth knowledge about alpha-proteobacteria, transgenic plants resistant to bacteria, and plant resistance mechanisms pertaining to bacteria. Therefore the authors have had to rely heavily on some excellent review articles for much of the information (da Graca, 1991; Bent, 1996; van Loon, *et al.*, 1998; Kuc, 2001; Tuzan, 2001; Tsolis, 2002; Martin, *et al.*, 2003; Halbert & Manjunath, 2004) and the PhD thesis of Margareta Melander, 2004, conducted at the Swedish University of Agricultural Sciences, Alnarp. Readers are invited to turn to these for greater clarity on specifics within the topics covered.

The disease, Huanglongbing

Huanglongbing (HLB, yellow dragon disease), formerly known as citrus greening, is one of the most severe diseases of citrus in Asia, Africa, the Arabian Peninsula, and the islands of Mauritius, Reunion, and Madagascar (de Graca, 1991), and has recently also been recorded in Brazil (Coletta-Filho *et al.*, 2004; Teixeira *et al.*, 2005a, b) and Florida, USA (September, 2005; http://www.aphis.usda.gov/lpa/news/2005/09/greening_ppq.html). The disease is characterised by small yellow leaves on one shoot, limb or section of the tree canopy initially (hence the name "yellow dragon disease"). The disease spreads slowly within the tree, leading progressively to yellowing of the entire canopy. Chronically infected trees are sparsely foliated and show extensive twig die-back (Martinez, 1972). The most diagnostic symptom of citrus greening is leaf mottling that often is not defined by the leaf veins (Schneider, 1968). The newest leaves may show symptoms resembling zinc deficiency, while older leaves have the characteristic greening mottle. Fruit is small, poorly colored, and/or lopsided. Fruit taste is bitter, medicinal, and acid (McClellan & Schwarz, 1970). Seeds usually abort, and fruit set is poor. Symptoms vary according to cultivar, tree maturity, time of infection, stage of disease, and other abiotic or biotic agents that affect the tree. In some areas of the world where the disease is endemic, citrus trees decline within five years of planting and then most never bear usable fruit (Lin, 1963). Such losses are significant, since profits are only attainable 8 to 10 years after planting. In countries where HLB occurs, it is the primary limiting factor for citrus production. Globally, HLB has been regarded as one of the most important threats to commercial and sustainable citrus production.

Two forms of the disease, an African and an Asian form, are recognized. These differ with the former being found in Southern African countries, including South Africa, at higher altitudes and temperatures ranging from 20-23°C (Schwarz and Green, 1970) whereas the latter, more severe form of the disease, occurs at lower altitudes and heat tolerant temperatures of 30-35°C in Asia, the Arabian Peninsula, Brazil and the islands of Mauritius, Reunion, Madagascar and now the USA (Garnier, *et al.*, 1987; Subandiyah, *et al.*, 2000, Coletta-Filho, *et al.*, 2004; Teixeira, *et al.*, 2005a, b; http://www.aphis.usda.gov/lpa/news/2005/09/greening_ppq.html).

Causal organisms

The causal agent of HLB is a fastidious, sieve tube-restricted organism related to the α -proteobacterium group, "*Candidatus*" *Liberibacter africanus* (previously "*Candidatus*" *Liberobacter* (Planet, *et al.*, 1995; Jagoueix, *et al.*, 1997; Hocquellet, *et al.*, 1999a, 1999b); Hung, *et al.*, 2004) in Africa and "*Candidatus*" *L. asiaticus* in Asia, and "*Candidates*" *L. americanus* and *L. asiaticus* in Brazil (Coletta-Filho, *et al.*, 2004; Teixeira, *et al.*, 2005a, 2005b). A subspecies of *L. africanus* namely "*Candidatus*" *L. africanus* subsp. *capensis* has been detected in a Rutaceous indigenous tree in the Western Cape, South Africa, *Calodendrum capensis* (Cape chestnut), but could not be detected in proximal citrus groves (Garnier, *et al.*, 2000). None of the species have been cultured. The HLB Liberibacters were among the first bacteria to receive "*Candidatus*" designations according to the rules established for uncultured organisms (Murray and Schleifer, 1994). Phylogenetically, the closest relatives of the liberibacters are members of the alpha subgroup of the *Proteobacteria* (Jagoueix, *et al.*, 1994). However, the liberibacters cannot be assigned to this subgroup as their 16S rRNA gene sequence has only one of the seven or more oligonucleotide signatures characteristic of the subgroup and also as they have some signatures characteristic of the beta- and gamma- subgroups (Woese, *et al.*, 1984; Jagoueix, *et al.*, 1994).

The alpha-Proteobacteria is a genetically diverse group of bacteria, many members of which interact with eukaryotes by way of parasitism or mutualism (Sallstrom & Andersson, 2005). Some are important plant pathogens or human pathogens, some establish chronic infections in animals without causing disease symptoms, whereas others are plant symbionts, soil-growing nitrogen fixers of plants or providers of other nutrients (Batut, *et al.*, 2004). Environmental genome shotgun sequencing has shown that α -proteobacterial DNA is very common in environmental samples taken from the water or the soil (Venter, *et al.*, 2004; Tringe,

et al., 2005), hence, this bacterial subdivision is of interest from a medical, agricultural and environmental perspective. The phylogenetic group of alpha-proteobacteria contains bacterial species with a wide variety of lifestyles, including obligate intracellular (*Liberibacter*, *Rickettsia*), facultative intracellular (*Bartonella*, *Brucella*), and extracellular pathogens (*Agrobacterium*), as well as symbionts of both animals and plants (*Wolbachia*, *Sinorhizobium*) (Tsolis, 2002). The members of this group live in intimate association with eukaryotes and in many cases have acquired the ability to survive and multiply within an arthropod vector.

Vectors

The HLB liberibacters are transmitted by two psyllid insects, *Trioza erytreae* (Del Guercio) in Africa and *Diaphorina citri* (Kuwayama) in Asia (Capoor, *et al.*, 1967; Chen, *et al.*, 1973; Aubert, 1987; Gottwald, *et al.*, 1989), Brazil and the USA. The two insect species are responsible for the large geographical distribution of HLB in these areas. The Mediterranean region and Australia are free of both HLB and HLB psyllid vectors. In Africa, HLB and the psyllid vector are restricted to cool areas, as both *T. erytreae* and “*Candidatus*” *L. africanus* are susceptible to temperatures higher than 25-30°C (Bové, *et al.*, 1974). Also tree flushes tend to be more prolonged in cooler areas. In South Africa, HLB was reported to be present in the eastern and western parts of the former Transvaal province (now North-West and Mpumalanga provinces) as early as 1929 (Van der Merwe & Andersen, 1937; Oberholzer, *et al.*, 1965; Aubert, *et al.*, 1988; Da Graca, 1991), where it was shown to be transmitted by the citrus psyllid *Trioza erytreae* (McLean & Oberholzer, 1965). The psyllid was also abundant in other citrus-growing areas of South Africa, but the disease was not present. Thus, strong quarantine measures were applied to prevent movement of citrus from the former Transvaal province to other citrus growing regions of the country. However, in 1995, symptoms of leaf mottle resembling those of HLB were observed on Clementine and lemon trees in the Western Cape and “*Candidatus*” *L. africanus* detected (Garnier, *et al.*, 1999). The “*Candidatus*” *L. africanus* subsp. *capensis* variant has only been detected at two sites in the Western Cape, South Africa on *C. capensis*, but this tree has a relatively wide-spread distribution in South Africa and no surveys for this liberibacter in these areas have been done.

While the “official” name for the disease caused by the various liberibacters has been reduced to Huanglongbing (HLB) (Van Vuuren, 1996), the obvious differences in the disease, the fact that they are caused by different organisms with unique vectors suggests that this should be revisited, possibly with the african form being referred to as “greening” (Pietersen, personal opinion), while the asian form is referred to as HLB. Characterisation of the disease properties caused by “*Candidatus*” *L. americanus* may result in its disease being referred to by a different name.

Control strategies

A number of strategies have been applied worldwide to combat the disease. Regulation of propagation material is used to prevent the spread of HLB to new areas, especially in cases where the vector already exists or could become established. In regions where HLB occurs, control is based on three major strategies: (1) propagation of new trees from HLB-free plants; (2) vector control by chemical or biological means; and (3) elimination of the inoculum sources, by removing infected citrus trees or branches and alternative hosts of the liberibacters. None of the control strategies is completely effective alone and must be used in an integrated manner.

Elimination of the pathogen from budwood sources within a certification scheme is applied in South Africa. Nucleus material is obtained from important clonal material following thermotherapy and shoot tip grafting. Antibiotics injected into infected citrus trees provide temporary remission of symptoms (Schwarz, *et al.*, 1974; van Vuuren, *et al.*, 1977; Su, *et al.* 1986; Buitendag & von Broembsen, 1993; Lin, *et al.*, 1990), and if associated with a reduction of the pathogen levels could be combined with vegetative propagation for pathogen elimination. Antibiotics are no longer utilized in South Africa for greening control following concerns over their large-scale release in nature. However in India it was still recommended as part of an integrated management programme in 1981 (Nariani, 1981). The pathogen can also be eliminated from infected young citrus trees by a process of solarization, where the plant is covered with a plastic sheet and subjected to the sun for a limited time interval (Schwarz & Green, 1970). In the case of plants subjected to elimination procedures it is necessary to index for the pathogen. This has been made more feasible in the past few years since the advent of PCR systems to detect the liberibacters (Jagouieix, *et al.*, 1994; Harakava, *et al.*, 2000; Hocquellet, *et al.*, 1999; Teixeira, *et al.*, 2005b).

In cases where the insect vector and the pathogen are present, it is necessary to control psyllids with pesticides (Tolley, 1990), even on apparently disease-free plants (Aubert, 1990b), and on their alternate hosts in proximity to citrus groves (Halbert & Manjunath, 2004). It is important to protect the spring flush (Aubert, 1987). To control the vector, regular application of contact insecticides are required during the flush

period, often only days apart (Gonzales & Viñas, 1981; Su, *et al.*, 1986; Roistacher, 1996). Furthermore it is recommended that farmers in a citrus production area synchronize chemical applications (Aubert, 1990b). Control of the vector by contact insecticides is difficult because of the mobility of the insect as well as its relatively wide host range, and is not always effective (van Vuuren, *et al.*, 1978). However, because psylla are sap feeders, systemic insecticides are more effective (Wortmann & Schafer, 1977). The likelihood of HLB or the vector establishing in areas treated with insecticides is reduced but repeated applications may be necessary. Trunk applications have proven useful (Aubert, 1988; Buitendag & von Broembsen 1993). Two patented applicators have been used in South Africa that adjust the dose based on the diameter of the tree (Buitendag & von Broembsen, 1993). Supriyanto and Whittle (1991) and Shivankar, *et al.* (2000), also had success with trunk applications. The best time to apply was just prior to spring flush. A computer model indicated that even with careful attention to inoculum reduction, at least 70% reduction in transmission is needed to delay the epidemic significantly (Supriyanto & Whittle 1991). Control of the disease by regular insecticide application on citrus and alternate hosts is rather costly and can lead to the selection of insecticide-resistant vectors. Furthermore more stringent regulations around the use of insecticides and residue levels make use of this control strategy increasingly difficult.

Both *D. citri* and *T. erytrae* have been effectively controlled in Reunion by the introduction of the parasitic wasps *Tamarixia (ex Tetrastichus) dryi* and *T. radiatus* (Aubert, 1987; Aubert, *et al.*, 1996). However in many other countries biological control is not effective as hyperparasites affect the biocontrol agents (Supriyanto & Whittle 1991).

To achieve a reduction of inoculum levels, infected limbs and trees should be removed as symptoms appear. The pathogen apparently moves fairly slowly within the plant after infection, so severe pruning can be helpful. For African greening specific recommendation regarding removal of trees of different ages and removal of specific branch diameters have been made (Buitendag & von Broembsen, 1993). However, as HLB has a long incubation period, and many latently infected citrus plants occur in the field, removing of infected trees and plants is not fully effective (Mc Clean, 1970; Huang, 1979). Aubert (1990), estimated that 15% to 20% of infected plants are overlooked by nursery inspectors who rely on visual inspection. Little information is available on how temperature may influence the pathogen latent period (time to expression of symptoms), which ranges from 4 months to more than a year. There is also little information on the effect of rainfall and relative humidity on symptom development and pathogen latent period.

Control through resistance

Because of the difficulties with each of the existing control strategies, control of greening through some form of resistance is considered highly desirable.

A large amount of non-host resistance exists with HLB being a disease of essentially Rutaceous plants. Citrus relatives such as *Atalantia*, *Balsamocitrus*, *Calodendrum*, *Clausena*, *Fortunella*, *Microcitrus*, *Murraya*, *Poncirus*, *Severinia*, *Swinglea*, *Toddalia*, and *Triphasia* (Halbert & Manjunath, 2004) are susceptible. There is disagreement in the literature about the status of *Murraya paniculata* as a host for citrus greening pathogens. It is a host in Brazil but not in Taiwan. The citrus greening bacterium has been experimentally transmitted, by *Cuscuta campestris* (dodder) from citrus to one non-rutaceous host *Catharanthus roseus* (Garnier & Bové, 1983).

Although there is no recorded resistance in *Citrus* spp. to HLB, some species and cultivars are somewhat tolerant. Koizumi, *et al.* (1993), did extensive field surveys showing that some cultivars were less susceptible to decline than others. Most of the sweet orange trees became infected with the pathogen and subsequently declined, while grapefruit was more tolerant. In general, sweet oranges, mandarins and tangelos are most susceptible, grapefruit and lemon are more resistant, and limes, *Poncirus trifoliata* and citranges are the most tolerant (Lee, 1996). De Lange, *et al.* (1985), initiated a breeding programme for resistance to the African form of HLB in South Africa, but this was unfortunately terminated before completion of the studies. Somatic mutations on HLB affected fruit often result in chimeras with some healthy-looking segments (van Vuuren, *per. comm.*), these may represent mutations leading to resistant or tolerant phenotypes. Studies are being conducted in South Africa to explore this (van Vuuren *per. comm.*).

In some cases, it is known that the rootstock can affect symptom expression. Five out of 23 rough lemon rootstock selections from India induced a degree of tolerance in the sweet orange scion in greenhouse trials (Cheema, *et al.*, 1982). In another study, 100% of trees of rough lemon were infected, compared to only 25% of trees on 'Blood Red' sweet orange rootstock (Kapur, *et al.*, 1984). In South Africa the percentage of greening in Valencia oranges was higher on trifoliolate orange rootstock than on Empress mandarin or Troyer citrange. While these differences may be due to relative changes in tolerance, it could also be that the trifoliolate rootstock causes an extension of the flushing period and thus extends the feeding time of the insect

vector, as suggested by van Vuuren and Moll (1985). No differences were found in a Chinese study on the effects of 13 rootstocks on symptoms in Ponkan mandarin (Lin, 1963).

Cross-protection of greening has not been well studied. It is known that plants can become infected with both Asian and African greening bacteria (Garnier, *et al.*, 1996). Naidu and Govindu (1981) doing greenhouse studies on cross-protection demonstrated that mild isolates did not provide complete cross-protection when graft-inoculated sweet orange plants were challenged with severe isolates. Moreover, isolates that were mild in sweet orange were severe in grapefruit in a subsequent host range test. In contrast, Singh, *et al.* (1994), demonstrated that plants pre-immunized with a mild strain of HLB, had better agronomic characteristics when challenged with a moderate/severe source of HLB than plants that were not subjected to pre-immunization. In South Africa it was reported that a population of several strains of *Citrus tristeza virus* were able to cross-protect citrus plants from *Liberobacter africanus* (van Vuuren, *et al.*, 2000). Glasshouse work was done to investigate the causative agent(s), present in the GXI isolate (greening cross-protecting isolate, formerly known as citrus dwarfing isolate (CD4)). Further studies are being conducted for the isolate and its aphid transmitted sub-isolates (Van Vuuren *pers. comm*). The reason for the occurrence of this cross protection is not known but may be due to the activation of some of the broad spectrum plant defence mechanisms discussed below (van Vuuren, *et al.*, 2000; Halbert, *et al.*, 2004).

No attempts to develop transgenic resistance to any of the liberibacters have been published, however, the theoretical potential of developing transgenic plants, resistant to HLB, have been discussed (Bove, 1986; Aubert, 1988). Possible approaches are discussed below, using examples of other bacteria (and fungi in some cases), especially of other members of the Alpha-proteobacter group (e.g. *Agrobacterium*).

Transgenic resistance

Four very broad categories of transgenic resistance which may potentially be applied to control of HLB are discussed, namely: 1) enhancement of existing or novel host defence responses; 2) pathogen derived resistance; 3) resistance of the host to the vectors of greening; 4) resistance obtained that is neither host or pathogen derived.

1) Transgenic resistance through host defence mechanisms.

Some background in plant defense mechanisms is presented before discussion of the exploitation thereof with transgenic plants. Plant defence responses involve the activation of multiple, coordinated and apparently complimentary defence responses, such as the production of phytoalexins or other antimicrobial compounds, the formation of physical barriers through increased cross-linking, the elicitation of the hypersensitive response, or the elevated expression of pathogenesis-related proteins. These fall onto two broad categories of defence, namely, 1) preformed defenses such as antimicrobial secondary compounds and 2) by inducing defense responses (Hammond-Kosack & Jones, 2000; Heath, 2000).

Within the preformed resistance category, 'Single gene' disease resistance, also known as gene-for-gene resistance (Flor, 1947), depends on the possession of a single resistance (R) gene by the plant, which must interact with a specific avirulence (*avr*) gene product produced by the pathogen in order for a defence response to be invoked. If a plant lacks the correct R gene to match at least one of the *avr* genes possessed by an invading pathogen, that plant will be unable to use its R genes to detect and stop the pathogen (Buell, 1999). This is one of the most convenient, inexpensive, and environmentally sound ways to control plant disease, and plant breeders make extensive use of classically defined R genes (Agris, 1988). Recent work has revealed the structure of a number of plant R genes, and a striking degree of similarity among these genes has been observed. The cloning of R genes has stimulated additional research on exploitation of the mechanisms by engineering of improved disease resistance in plants.

Also in the pre-formed defence category, multigenic resistance, also known as 'horizontal', 'quantitative' or 'polygenic' resistance, refers to plant disease resistance generated via interactions between the products of multiple plant genes, not a single R gene (Nelson, 1978; Simmonds, 1991). Multigenic resistance is considered to be non-specific in that the plant and pathogen do not require matching R and *avr* genes for a timely plant defense response to occur. Multigenic resistant plants, bred to resist a specific pathogen, tend to resist a greater variety of pathogens and pathogen races than those bred or engineered to express particular R genes (Simmonds, 1991).

The remaining category of disease resistance depends upon the induction of defenses following exposure to organisms or compounds. A variety of organisms, including virulent and avirulent pathogens (Tuzun, *et al.*, 1986, 1992; Tuzun & Kuc, 1991), mycorrhizal fungi (Borowicz, 1997) and non-pathogenic rhizobacteria (Tuzun & Kloepper, 1995; Benhamou, *et al.*, 1998) have been observed to activate plant defense responses.

Abiotic inducing agents include compounds isolated from plant pathogens (Wei & Beer, 1996; Norman, *et al.*, 1999) and a variety of chemicals (Fought & Kuc, 1996; Benhamou & Belanger, 1998). The activity of the inducing agents is not due to antimicrobial activity *per se* or their ability to be transformed into antimicrobial agents. However, antimicrobial agents can induce resistance, and they provide protection from the time of application until the resistance is expressed (Kuc, 2001). This general phenomenon is known as induced systemic resistance (ISR), and generally results in a non-specific resistance against a broad spectrum of pathogens and pests. Induced resistance is not the creation of resistance where there is none, but the activation of latent resistance mechanisms that are expressed upon subsequent, so-called "challenge" inoculation with a pathogen (van Loon, 1997). Induced resistance occurs naturally as a result of limited infection by a pathogen, particularly when the plant develops a hypersensitive reaction. Although tissue necrosis contributes to the level of induced resistance attained, activation of defense mechanisms that limit a primary infection appears sufficient to elicit induced resistance (Hammerschmidt & Kuc, 1995). Generally, induced resistance is systemic, because the defensive capacity is increased not only in the primary infected plant parts, but also in non-infected, spatially separated tissues. Because of this systemic character, induced resistance is commonly referred to as systemic acquired resistance (SAR) (Ross, 1961b; Ryals, *et al.*, 1997; Sticher, *et al.*, 1997). However, induced resistance is not always expressed systemically: Localized acquired resistance (LAR) occurs when only those tissues exposed to the primary invader become more resistant (Ross, 1961a). SAR and LAR are similar in that they are effective against various types of pathogens. A signal that propagates the enhanced defensive capacity throughout the plant in SAR appears to be lacking in LAR.

SAR is characterized by an accumulation of salicylic acid (SA) and pathogenesis-related proteins (PRs) (Kessman, *et al.*, 1994; Ryals, *et al.*, 1996; Sticher, *et al.*, 1997; Uknes, *et al.*, 1992; Ward, *et al.*, 1991). Accumulation of SA occurs both locally and, at lower levels, systemically, concomitant with the development of SAR. Exogenous application of SA also induces SAR in several plant species (Gaffney, *et al.*, 1993; Ryals, *et al.*, 1996; van Loon & Antoniw, 1982). Both pathogen- and SA-induced resistance are associated with the induction of several families of PRs. Induction of PRs is invariably linked to necrotizing infections giving rise to SAR, and has been taken as a marker of the induced state (Kessman, *et al.*, 1994; Uknes, *et al.*, 1992; Ward, *et al.*, 1991). Some of these PRs are β -1,3-glucanases and chitinases and capable of hydrolyzing fungal cell walls. Other PRs have more poorly characterized antimicrobial activities or unknown functions. The association of PRs with SAR suggests an important contribution of these proteins to the increased defensive capacity of induced tissues. Although SA may be transported from the primarily infected leaves, it does not appear to be the primary long-distance signal for systemic induction (van Loon, 1997). So far, the nature of the signal has not been established (Vernooij, *et al.*, 1994), but the level of SAR is modulated by ethylene and jasmonic acid (JA) (Knoester, 1998; Lawton, *et al.*, 1995; Sticher, *et al.*, 1997; van Loon & Antoniw, 1982; Wasternack & Parthier, 1997; Xu, *et al.*, 1994). These results suggest that induction and expression of SAR are regulated through an inter-play of several signaling compounds.

A biological system of induced systemic resistance (ISR) distinct from SAR has been described for rhizosphere bacteria in several dicots (van Loon, *et al.*, 1998). It is not dependent on SA accumulation in the plants but requires functioning jasmonic acid (JA) and ethylene (ET) responses for activation. No clear biochemical markers have been established for this biological system for the induced state, but resistance responses are more effective in induced plants against a broad spectrum of diseases. Application of higher levels of JA or ET induces the production of antimicrobial peptides (defensins), which, however, do not seem to be required for rhizobacteria-induced resistance (Pieterse, *et al.*, 1998).

While most attempts to produce transgenic plants utilizing host defence mechanisms have been done using fungal pathogens and not bacteria, they are nevertheless discussed here because of the theoretical application to the liberibacters through their broad spectrum responses.

a) Transgenic plants utilizing pathogenesis related (PR) proteins: Hydrolytic PR proteins chitinase and β -1,3-glucanase have been extensively studied in transgenic plants. In 1991 (Broglie, *et al.*, 1991) it was demonstrated that heterologous expression of a bean chitinase in oilseed rape and tobacco could reduce the susceptibility to *Rhizoctonia solani*. Since then chitinases of various classes from different plant species, as well as other sources, have been expressed in a large number of crops with effects against various fungal pathogens (*e.g.* Yamamoto, *et al.*, 2000; Oldach, *et al.*, 2001; Mora & Earle, 2001; Pappinen, *et al.*, 2002). The effects demonstrated have mainly been a reduction of fungal development and reduced number and size of lesions. In some examples it has also been shown that β -1,3-glucanases have the potential to improve resistance against fungal as well as *Oomycete* infections (Yoshikawa, *et al.*, 1993; Lusso & Kuc, 1996). When chitinase and β -1,3-glucanase enzymes are combined they can act synergistically with improved suppressive effects on fungal infection. Expression of a class 1 chitinase and β -1,3-glucanase in tomato significantly enhanced resistance to *Fusarium oxysporum* f. sp. *lycopersici* while comparable expression levels of either gene alone did not (van den Elzen, *et al.*, 1993; Jongedijk, *et al.*, 1995). Also

expression of PR proteins from other classes, such as osmotin and thaumatin-like proteins (PR-5), in transgenic plants have been shown to have negative effects on fungal and *Oomycete* infections such as delayed development of disease symptoms (Liu, *et al.*, 1994; Datta, *et al.*, 1999; Fagoaga, *et al.*, 2001). PR proteins of class 1a are expressed at high levels in response to pathogen attack, but the biochemical function is still unknown. When PR-1a was constitutively expressed in tobacco the derived plants displayed enhanced resistance to oomycetes (Alexander, *et al.*, 1993). The cysteine-rich peptides defensins (PR-12), thionins (PR-13) and lipid transfer proteins (PR-14) are thought to be involved in induced defence responses (van Loon & van Strien, 1999) and are also often found in seeds. Various such cysteine-rich peptides from different plant species have been expressed in transgenic plants where they have been shown to confer enhanced resistance to infections caused by fungi and bacteria (Epple, *et al.*, 1997; Molina & Garcia-Olmedo, 1997; Gao, *et al.*, 2000; Iwai, *et al.*, 2002).

b) Transgenic resistance through exploitation of Phytoalexins: Phytoalexins are normally synthesized via complex biochemical pathways and are thus more complicated to produce transgenically. However, expression of some enzymes involved in phytoalexin synthesis, such as resveratrol synthases and isoflavone O-methyltransferase, demonstrates the potential of phytoalexins in conferring enhanced resistance to fungal infections (Hain, *et al.*, 1993; Hipskind & Paiva, 2000; He & Dixon, 2000).

c) Transgenic plants utilizing resistance genes: The transfer of *R*-genes between related species has been shown to be a possible way to introduce disease resistance against both bacterial, *Oomycete* and fungal pathogens. The *Pto* gene of tomato and *Bs2* gene of pepper have been transferred to other *Solanaceous* species conferring resistance to the bacterial pathogens *Pseudomonas syringae* and *Xanthomonas campestris*, respectively (Rommens, *et al.*, 1995; Tai, *et al.*, 1999). Successful examples of transfer of *R*-genes have normally taken place between related species. However, for the *RPW.8* genes of *A. thaliana* it has been shown that these are functional against powdery mildew also when transferred to tobacco (Xiao, *et al.*, 2003). A possibly more broad-spectrum approach to make use of *R*-genes and the SAR-pathway has also been described. According to this approach transgenic plants are transformed with pathogen *avr*-genes under control of a heterologous infection-inducible promoter. If the generated transgenic plant carries the corresponding *R*-gene a defence response will be initiated upon infection. One example in this direction is the pathogen-inducible expression in tobacco of the elicitor cryptogein from *Phytophthora cryptogea*, a likely avirulence factor of *Phytophthora* spp. (Keller, *et al.*, 1999). Challenge infection induced both HR and defence gene activation enhancing resistance to *Phytophthora parasitica* var. *nicotianae* as well as several fungal pathogens. In another example the *Cf9* gene from tomato conferring resistance to the fungus *Cladosporium fulvum* was expressed in oilseed rape plants also expressing the corresponding fungal *Avr9* gene (Hennin, *et al.*, 2001). These oilseed rape plants were shown to have enhanced resistance to the fungus *Leptosphaeria maculans*.

d) Transgenic resistance utilizing defence signalling components: Different steps in plant defence pathways have been evaluated to get a better understanding of defence responses and potentially produce transgenic plants with improved defence systems. One step in the defence-signalling cascade that has been evaluated is the *NPR1* gene from *Arabidopsis*, which regulates SA-signalling. When *NPR1* was overexpressed in *Arabidopsis* as well as rice, plants with stronger PR protein induction and enhanced bacterial and fungal resistance were generated (Cao, *et al.*, 1998; Chern, *et al.*, 2001). When bacterial SA-generating enzymes were expressed in transgenic tobacco, SA accumulation was substantially increased and PR proteins were constitutively expressed conferring enhanced resistance to fungal, as well as viral, infections (Verberne, *et al.*, 2000). Also expression of a transcriptional regulatory protein gene, *Tsi1* from tobacco, induced constitutive expression of PR proteins and conferred broad-spectrum resistance to both bacterial and *Oomycete* pathogens as well as viruses (Shin, *et al.*, 2002). When the *Prf* gene involved in resistance to *Pseudomonas syringae* in tomato was overexpressed in tomato the transgenic plants displayed constitutively activated SAR with enhanced SA accumulation, constitutive PR protein synthesis and broad-spectrum resistance to bacterial and viral pathogens (Oldroyd & Staskawicz, 1998). Another defence pathway step that has been modified is the expression of glucose oxidases that generates H₂O₂ production (Wu, *et al.*, 1995; Murray, *et al.*, 1999). Plants transformed with glucose oxidase genes displayed enhanced tolerance to a broad spectrum of bacteria, fungi and oomycetes but also distorted plant growth. Careful regulation of the glucose oxidase expression by pathogen-inducible promoters may overcome these negative effects (Kachroo, *et al.*, 2003).

e) Transgenic plants exploiting RNA interference: Gene silencing by RNA interference has been applied as a method to confer improved tolerance against the crown gall causing bacterium *Agrobacterium tumefaciens*. Transformation of *Arabidopsis* and tomato by inverted repeats of the bacterial oncogenes resulted in plants that could still be infected by *A. tumefaciens* but where the formation of crown galls was fully prevented (Escobar, *et al.*, 2001).

2) Transgenic resistance through inactivation of pathogen produced compounds

Pathogen derived resistance (PDR) has been exploited to a large extent for the control of plant viruses. However, during plant infection fungal or bacterial pathogens can produce plant cell wall degrading enzymes, such as polygalacturonase, as well as various toxins, such as oxalic acid and mycotoxins. A possible target for transgenic resistance against fungal pathogens is the inactivation of these enzymes and toxins. The expression of a polygalacturonase inhibitor protein in tomato was able to reduce disease development by *Botrytis cinerea* (Powell, *et al.*, 2000). Expression of oxalate oxidase as well as oxalate decarboxylase has been shown to confer enhanced resistance to fungal infections (Kesarwani, *et al.*, 2000; Donaldson, *et al.*, 2001). Resistance by transgenic detoxification has also been demonstrated against bacterial infection in sugarcane by introduction of an albicidin detoxifying gene (Zhang, *et al.*, 1999). Some fungal pathogens produce mannitol to suppress reactive oxygen-mediated plant defences. The expression in plants of heterologous mannitol dehydrogenase has been shown to enhance resistance to the mannitol-secreting fungus *Alternaria alternata* (Jennings, *et al.*, 2002). A large number of bacterial *avr* genes have been cloned and sequenced, primarily from the genera *Pseudomonas* and *Xanthomonas* (Vivian & Arnold, 2001), and could possibly be exploited in transgenic resistance strategies as a fair amount of evidence exist that these genes may interact directly with a corresponding R gene of the host (Bonas & Lahaye, 2002).

3) Transgenic resistance to insects

a) Using Bt toxin: For transgenic resistance to insects the expression of *Bacillus thuringiensis* (Bt) toxins is the most well known approach, which is also used commercially on a significant number of hectares. There are also many examples of the expression of other proteins with insecticidal activities, such as proteinase inhibitors and lectins. Some more complex approaches including expression of secondary metabolites and R-genes have also been initiated. The main insecticidal activity of *B. thuringiensis* is due to insecticidal crystalline inclusions formed during sporulation. These crystalline inclusions are composed of protoxin subunits, called δ -endotoxins or Cry proteins. The Cry proteins are classified into 24 major groups and are usually specific for a limited range of species within certain insect orders, mainly Lepidoptera, Coleoptera and Diptera (Hilder & Boulter, 1999). The protoxins are solubilized in the insect midgut, where they are cleaved by gut proteases to form the active toxin. The toxin binds to receptors of epithelial cells in the midgut and then inserts into the cellular membrane. This leads to pore formation that lyses the cells and then causes death of the insect by starvation or septicaemia (Whalon & Wingerd, 2003). Cloned genes encoding Cry proteins of *B. thuringiensis* were expressed in tobacco and tomato in the late 1980s (Fischhoff, *et al.*, 1987; Vaeck, *et al.*, 1987). In the first reports published the expression levels of the introduced Cry genes were very low, probably due to the bacterial codon usage being suboptimal for plant expression and the occurrence of polyadenylation signals within the coding region. Later, attempts to express Cry proteins have made use of partially or totally synthetic genes optimised for plants resulting in considerably increased expression levels (Mazier, *et al.*, 1997). Cry genes have been transferred to a large number of different crop species and have in field trials been shown to confer resistance to various pests, mainly of the orders Lepidoptera and Coleoptera (e.g. Perlak, 1993; Tu, *et al.*, 2000; Moellenbeck, *et al.*, 2001; Kumar & Kumar, 2004). In 1995 the first insect resistant transgenic crops; corn, cotton and potato; expressing Cry proteins were approved for market release in the US (http://www.aphis.usda.gov/brs/not_reg.html; 21-Aug-2004) and in 2002 14 million ha were planted with Bt-crops globally (James, 2002). One concern with the use of Bt-crops has been the possibility of insects developing resistance to Cry proteins. Apart from resistance management strategies by mixing non-Bt- and Bt-cultivars prolonged durability may be achieved by pyramiding different Cry genes (Cao, *et al.*, 2002; Zhao, *et al.*, 2003; Estela, *et al.*, 2004) or the development of hybrid Cry proteins (Naimov, *et al.*, 2003). Other toxic proteins produced by microorganisms have also been proposed as alternatives or complements to Bt-toxins. One example is the Vip1 and Vip2 proteins from *Bacillus cereus* as well as Vip3A from *B. thuringiensis* that have activities comparable to that of Bt-toxins (Estruch *et al.*, 1997). Other examples are the insecticidal toxin complexes produced by *Photorhabdus luminescens* and *Xenorhabdus nematophilus*, which are bacteria associated with entomopathogenic nematodes (French-Constant & Bowen, 1999). A synthetic plant-codon-optimized gene encoding the toxin A protein from *P. luminescens* has been transferred to *A. thaliana* resulting in high levels of insect resistance (Liu, *et al.*, 2003).

b) Transgenic resistance to insect vectors using proteinase and α -amylase inhibitors: Animals are dependent on proteinases for their amino acid metabolism. Thus production of proteinase inhibitors (PIs) may defend plants against herbivorous pests. Insects may contain proteinases of four different classes; serine, cysteine, aspartic and metallo proteinases. Different classes of proteinases predominate depending on insect species and gut pH. In lepidopterans, with alkaline gut pH, serine proteinases normally dominate while many coleopterans with neutral to mildly acidic pH use cysteine and aspartic proteinases. The antimetabolic effect of PIs is partially explained by inhibition of the corresponding proteinases and thereby a reduction in amino acid availability. Another important effect is an induced hyperproduction of proteinases, which further

reduces the availability of essential amino acids (Reeck, *et al.*, 1997; Schuler, *et al.*, 1998; Gatehouse & Gatehouse, 1999). The first demonstration that transgenic expression of *PI* genes can confer insect resistance was when a gene from cowpea (*Vigna unguiculata*) encoding a serine PI with inhibitory activity against trypsin (CpTI) was expressed in tobacco. The transgenic tobacco plants displayed enhanced protection against a range of lepidopteran storage and field pests, typically shown as reduced larval growth and less plant damage (Hilder, *et al.*, 1987; Hoffman, *et al.*, 1992; Gatehouse, *et al.*, 1992). CpTI as well as other serine PI genes have been transformed into many different plant species conferring resistance not only to lepidopterans but also to e.g. coleopteran insects as well as nematodes (Atkinson, 1993; Duan, *et al.*, 1996; Christeller, *et al.*, 2002; Alfonso-Rubi, *et al.*, 2003). Cysteine PIs, e.g. cystatins from rice and chicken egg-white, have also been demonstrated to confer enhanced pest resistance, mainly against coleopterans and nematodes (Leplé, *et al.*, 1995; Urwin, *et al.*, 1997, 2001) but also against aphids (Rahbe, *et al.*, 2003). Some insects may overcome the effects of certain PIs by switching to production of alternative, insensitive proteinases (Zhu-Salzman, *et al.*, 2003; Brunelle, *et al.*, 2004). A strategy for more effective inhibition of insect proteolysis could be expression of linked or hybrid inhibitors active against different proteinase classes (Urwin, *et al.*, 1998; Inanaga, *et al.*, 2001). In addition to proteinase inhibitors also α -amylase inhibitors may confer insect resistance. This has been shown by expression of an α -amylase inhibitor from common bean in peas resulting in resistance to various bruchid beetles (Shade, *et al.*, 1994; Schroeder, *et al.*, 1995).

c) Transgenic resistance to insect vectors exploiting Lectins: Lectins are sugar-binding proteins that can be found in various plant tissues but often in high amounts in seeds and other storage organs. Different lectins have specificities for different mono- or oligosaccharides. It has been shown that some lectins are toxic to certain insects and it is also well known that some lectins have toxic or antinutritive effects on mammals. The exact toxicity mechanism against insects is not fully understood but there is evidence for lectins binding specifically both to epithelial cells and to the peritrophic membrane in the midgut. In addition to this, a reduced intake of food due to feeding deterrence and a restriction in uptake of nutrients due to partial blockage of pores of the peritrophic membrane have been proposed (Gatehouse & Gatehouse, 1999; Murdock & Shade, 2002). Enhanced insect resistance by transgenic expression of lectins was first shown when tobacco plants expressing pea (*Pisum sativum*) lectin displayed improved performance against tobacco budworm (*Heliothis virescens*; Boulter, *et al.*, 1990). Lectins from other plant species, such as concanavalin A (con A; from jackbean, *Canavalia ensiformis*) and snowdrop (*Galanthus nivalis*) lectin, have also been demonstrated to confer improved resistance to lepidopteran insect pests when expressed transgenically (Fitches, *et al.*, 1997; Gatehouse *et al.*, 1999). The snowdrop lectin, as well as other monocot mannose-binding lectins, has also been shown to be effective against sap-sucking insects of the order Hemiptera as shown for various aphids (Hilder, *et al.*, 1995; Yao, *et al.*, 2003; Chang, *et al.*, 2003) and planthoppers (Rao, *et al.*, 1998; Tinjuangjun, *et al.*, 2000; Wu, *et al.*, 2002). Lectins, as well as proteinase inhibitors, are not as effective as Bt-toxins for insect control. However, combinations of different insecticidal factors with different modes of action could lead to appropriate levels of insect control and possibly also reduce the risk of insects developing resistance (Boulter, *et al.*, 1990; Maqbool, *et al.*, 2001).

d) Transgenic resistance to insect vectors exploiting enzymes: Enzymes with different modes of action have also been proposed as alternative crop protection agents. Chitin is an important structural component of insects and transgenically expressed chitinase have shown some effect against insects (Ding, *et al.*, 1998). Other examples of enzymes that potentially could be of interest for protection against insects include anionic peroxidase (Dowd, *et al.*, 1998), cholesterol oxidase from *Streptomyces* (Corbin, *et al.*, 2001) and the esterase patatin from potato (Strickland *et al.*, 1995). Also the biotin-binding proteins avidin and streptavidin have been demonstrated to have deleterious effects on insects by causing biotin deficiency (Kramer, *et al.*, 2000; Burgess, *et al.*, 2002; Markwick, *et al.*, 2003).

e) Transgenic resistance to insect vectors exploiting secondary metabolites: Non-protein, secondary metabolites, such as alkaloids, glucosinolates and terpenoids, that may take part in plant defence mechanisms against insects, have been proposed as transgenic resistance factors. These compounds are normally produced via complex metabolic pathways and thus the transgenic production of such compounds is difficult. Several attempts in this direction, involving single enzymatic steps, have been made showing the potential of this strategy (Thomas, *et al.*, 1995; Mikkelsen, *et al.*, 2002; MacGregor, *et al.*, 2003; Wang, *et al.*, 2004). Additionally, an entire pathway involving three enzymatic steps for production of a cyanogenic glucoside has been transferred from *Sorghum bicolor* to *A. thaliana* conferring improved insect resistance (Tattersall, *et al.*, 2001).

f) Transgenic resistance to insects exploiting known genes for resistance: Transgenic expression of R-genes is a possible way to improve resistance. R-genes targeted against nematodes from beet, tomato and potato have been shown to confer nematode resistance when expressed transgenically in susceptible varieties (Cai, *et al.*, 1997; Milligan, *et al.*, 1998; Paal, *et al.*, 2004). The nematode resistance gene from tomato was

demonstrated to have dual actions as the transgenic plants also displayed resistance to aphids (Rossi, *et al.*, 1998; Vos, *et al.*, 1998).

4) Transgenic resistance utilizing non-host or non-pathogen genes

a) Plantibodies: As for virus resistance, expression in plants of antibodies binding to pathogen or pathogen products have been proposed as a strategy for conferring resistance to bacteria and fungi. However, so far the examples are very limited. Single-chain variable-fragments of an antibody specific to the wall-less bacteria stolbur phytoplasma expressed in tobacco resulted in symptomless shoots when inoculated by grafting onto stolbur phytoplasma-infected rootstocks (Le Gall, *et al.*, 1998). Peschen, *et al.* (2004) have expressed fusion proteins comprising chicken-derived single-chain antibody fragments against *Fusarium graminearum* linked to antifungal peptides in *Arabidopsis thaliana*. When the transgenic plants were challenged with *Fusarium oxysporum* a high level of protection was obtained, whereas expression of either antibody or antifungal peptides alone resulted in moderate levels of protection only.

b) Antimicrobial peptides and proteins: In addition to PR proteins also other peptides and proteins with antimicrobial activities have been evaluated as transgenic resistance factors. Antimicrobial peptides of animal origin have also been assayed in transgenic plants. When the sarcotoxin gene from flesh fly was expressed in transgenic tobacco (Mitsuhara, *et al.*, 2000) enhanced resistance against both bacterial and fungal pathogens was obtained. Similarly, other antimicrobial peptides of animal origin, such as magainin and temporin from frog skin, have been shown to confer enhanced resistance against bacteria, fungi and oomycetes in plants (Li, *et al.*, 2001; Chakrabarti, *et al.*, 2003; Osusky, *et al.*, 2004). In addition to naturally occurring antimicrobial peptides also synthetic or hybrid peptides have been designed and expressed transgenically in plants conferring improved resistance to bacterial, fungal and *Oomycete* pathogens (Osusky, *et al.*, 2000; Cary, *et al.*, 2000). An example of proteins with antimicrobial activities are the ribosome inactivating proteins, that have been shown to confer resistance to fungal infection by inactivating foreign ribosomes (Logemann, *et al.*, 1992). However, also a non-functional mutant of a RIP from pokeweed has been shown to confer improved fungal resistance by activating SA-independent constitutive overexpression of PR proteins (Zoubenko, *et al.*, 1997). Also antimicrobial proteins of animal origin, such as human lysozyme, have been demonstrated to improve bacterial and fungal resistance when expressed in transgenic plants (Nakajima, *et al.*, 1997; Takaichi & Oeda, 2000).

Preparation of Transgenic Citrus Plants

Conventional breeding of temperate fruit trees is constrained by their extensive reproductive cycle with long juvenile periods, complex reproductive biology, and high degree of heterozygosity. As the commercial production of transgenic annual crops becomes a reality in many parts of the world, the question remains whether genetically engineering fruit trees will find commercial application (Petri & Burgos, 2005b).

Gene transfer for fruit tree improvement has several inherent advantages. Once a useful transformant is isolated, vegetative propagation, which is often the normal method of multiplying fruit trees, provides unlimited production of the desired transgenic line. Fixation through the sexual cycle is unnecessary and, in fact, inconvenient if commercially-accepted cultivars are transformed. Since production of most fruit tree species is based on a few cultivars, the impact of transforming one of them would be significant. Currently, however, the only transgenic fruit tree commercially produced is papaya (*Carica papaya* L.) resistant to PRSV (papaya ringspot virus).

The DNA most commonly transferred to fruit trees is from disarmed and genetically engineered *Agrobacterium* strains, which drive foreign DNA into plant cells. Together with the gene of interest, the genes required for transformation are transferred, including marker genes that allow selection of transformed cells. Among the most commonly used selection genes are the neomycin phosphotransferase gene (*nptII*), which confers resistance to aminoglycoside antibiotics, and the phosphinothricin acetyl transferase gene, that confers resistance to the herbicide phosphinothricin (Miki & McHugh, 2004). However, given public concern with the introduction of antibiotic resistance genes into food, methods to eliminate selection genes from the transformed plants and strategies that avoid selection of transformed cells with antibiotics are being developed. However, these new methodologies have yet to be applied to the production of transformed fruit trees.

Several strategies utilizing *in vitro* techniques and emerging biotechnologies have been employed for citrus to develop improved scion and rootstock cultivars. These include: 1) Somaclonal variation: somaclones of Hamlin and Valencia sweet orange are being evaluated and superior clones selected for improved fruit quality or altered maturity date at the CREC, Lake Alfred, Florida (Grosser, *et al.*, 2000); 2) Somatic hybridization: the production of somatic hybrid plants from many parental combinations evaluated as

rootstock and scion improvement; 3), Embryo rescue/triploid production: information from *in vitro* embryo rescue experiments to recover triploid plants from interploid crosses in order to produce improved seedless fresh fruit cultivars; 4) Citrus transformants have been derived also from the use of protoplast-mediated transformation and green fluorescent protein (GFP) selection (Niedz, et al., <http://www.ars-grin.gov/ars/SoAtlantic/fp/hb/niedz/gfp/>). At CREC this system is being used to screen genes derived from *Citrus tristeza virus* (CTV) for their ability to block CTV replication in transformed tissue of various sweet oranges.

Much work has been published reporting improved methods using only marker genes and integration of putative beneficial genes, but without sufficient evaluation of the effect on the transformed plants. Additionally, some advances have also been reported specifically in the transformation of fruit trees (Petri & Burgos, 2005a).

Major objectives of *Citrus* transformation have been resistance to CTV mediated by pathogen-derived genes, resistance to *Phytophthora citrophthora* using antifungal proteins, and tolerance to salinity by introducing *HAL2* yeast-derived genes. In addition, *Arabidopsis* floral genes, such as *LEAFY* (*LFY*) or *APETALA1* (*AP1*), constitutively expressed in citrus seedlings from apomictic seeds, shortened the juvenile phase and promoted precocious flowering. Transgenic plants produced normal, fertile flowers that set fruits containing seeds. These traits were transmitted to the progeny, resulting in trees with a generation time of one year from seed to seed. Whereas *LFY* lines showed alterations in growth and development, *AP1* plants were adult and fully normal. Citrus plants expressing bovine lysozyme and snowdrop lectin are being evaluated in the greenhouse and in the field for their resistance to citrus canker (*Xanthomonas axonopodis* pv. *citri*) and insects, respectively (Petri & Burgos, 2005b). These transgenic plants may hold potential for the control also of liberibacters or their vectors.

Transformed seedlings from the cultivars 'Rio Red', expressing lectin, and 'Carrizo', expressing lysozyme, are currently the only *Citrus* field tests in the USA (Petri & Burgos, 2005b). *Citrus* cultivars have very long juvenile periods, and transformation of adult material would be preferable. Transformation of adult tissues of 'Pineapple' sweet orange produced plants that flower and set fruits in 14 months, whereas sweet oranges (which account for approximately 70% of world citrus production) need up to 20 years to completely lose juvenile characteristics and commence production. However, transformation efficiencies in adult *Citrus* are much lower than in juveniles. For instance, transformation efficiency in adult sweet orange 'Pineapple' is one-third of that obtained with apomictic seedlings of this cultivar (Petri & Burgos, 2005b).

Genotype is a major determinant for transformation, and procedures developed for one cultivar are often not suitable for other cultivars. This is the most serious hindrance to the application of gene transfer technologies to fruit crops. For species with cultivars that can be reliably transformed, the literature generally reveals that few genotypes of a particular species are being transformed and, in some cases, that these genotypes are not commercially important.

Meristem transformation may eliminate the need for regeneration in production of transgenic plants, allowing genetic manipulation of established cultivars. However, high explant mortality and difficulties controlling *Agrobacterium* growth have limited the development of this methodology. Recently, a reliable procedure for transformation of different grape cultivars has been developed (Mezzetti, et al., 2002). The authors generated "meristematic bulk" (MB) tissue from *in vitro* shoots by mechanical (dissection of the apical dome) and chemical (progressive increase in cytokinin concentration) treatments that abolish shoot apical dominance and also promote basal meristem proliferation. The MB tissue is a large aggregate of meristematic tissue with high regenerative competence, which can be transformed efficiently by *Agrobacterium* given the large number of dividing cells. This system seems to be easily adapted to other fruit trees, for instance, MBs have been produced from three apricot cultivars with similar regeneration efficiencies (Petri & Burgos, 2005b).

Selection of transformed regenerants is a critical step in any transformation procedure. Most commonly in fruit trees, antibiotics have been used as selection agents after integration of genes that confer antibiotic resistance. Concentration of the selective agent and timing of application must be optimized for each plant species. In *Citrus*, selection is often provided by 100 mg/L kanamycin. Selection of transformed shoots is often complicated by the inactivation of the selection agent by transformed cells and persistence of *Agrobacterium* in the explants, which permits regeneration of non-transformed shoots (escapes), sometimes at a high frequency.

Initiation and progress with the *Citrus* genome sequencing project (Forment, et al., 2005) will deliver many homologs which may be useful in one of the transgenic resistance strategies, and needs to be monitored for this.

The future of genetic transformation as a tool for breeding fruit trees requires the development of genotype-independent procedures, based on the transformation of meristematic cells with high regeneration potential and/or the use of regeneration-promoting genes. Yet another obstacle is that European law will neither allow deliberate release of plants carrying antibiotic resistance genes after 2004 nor their commercialization after 2008 (Directive 2001/18/EEC of the European Parliament and the Council of the European Union). Therefore, development of procedures to avoid the use of antibiotic selection or to allow elimination of marker genes from the transformed plant will be a research priority in the coming years.

Conclusion

While numerous possibilities exist for the exploitation of transgenic resistance to liberibacters and/or their vectors, a number of fundamental questions regarding the pathogenesis of the organisms, and specificities of the vector need to be resolved, before selecting an appropriate transgenic strategy. Increased information on the *Citrus* genome (Forment, et al., 2005) will provide numerous opportunities for transgenic strategies. Information is also needed on the variability of liberibacters and their products to ensure inclusive resistance strategies. Of personal interest is the association of gentisic acid build-up with liberibacter infection. What is the mechanism involved, is it liberibacter specific, and can this be exploited? Are their unique products associated with the vector specificity? Clearly appropriate expertise to answer some of these questions needs to be recruited, and funds made available for a number of fundamental questions prior to obtaining transgenic plants, which may or may not be resistant to the pathogen or insect. Furthermore public perceptions of transgenic plants need to be considered before the practical application of this technology.

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4.2.18 Evaluation of citrus material for greening resistance

Experiment 815 by S.P. van Vuuren (CRI)

Opsomming

Daar word gepoog om vergroenings-weerstandbiedendheid te verkry deur embryo's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Die plantjies wat genereer word, word op groeikragtige onderstamme deur middel van mikro-enting gevestig. Hierdie klone word op onderstamme vermeerder en aan sitrus bladvlouie, die vektor van vergroeningsiekte, blootgestel. Nadat die insekte vir 'n week op die plante gevoed het, word hulle verwyder en deur middel van PKR getoets om te bevestig dat hulle besmet is met vergroening en sodoende die plante blootgestel het aan die organisme. Na drie maande word die plante ge-evalueer vir die voorkoms van vergroeningsimptome. Klone wat 'n hoë persentasie simptoomlose plante het word d.m.v. PKR getoets om te bepaal of hulle vry van die vergroenings-organisme is (weerstandbiedend) of die organisme huisves sonder dat simptome ontstaan (verdraagsaamheid of toleransie). Twee klone, E2 en T2, is die afgelope jaar geïdentifiseer as simptoomloos na blootstelling aan die vektor. PKR is nog nie op die plante toegepas nie. Die klone word op onderstamme vermeerder om in die boord ge-evalueer te word.

Introduction

Citrus greening remains the most destructive disease in the cooler production areas. Despite the present control measures, the disease still invades plantings. The ultimate control measure will be the use of resistant plant material. Several new laboratory techniques became available allowing the detection and classification of the bacterium in plants and the psylla vector (Hocquellet, *et al.*, 1999; Planet, *et al.*, 1995; Villachanoux, *et al.*, 1992). These techniques will assist in the development of plants resistant to the bacterium.

The objective of the study is to screen citrus material, recovered through embryo rescue, for genetic resistance against greening disease.

Materials and methods

Fruit with greening symptoms, which display healthy chimera sectors, will be collected in different orchards in a greening area during harvest time (Fig. 4.2.18.1). The fruit will be surface sterilized in the laboratory on a flow bench by dipping them for 20 min. in a 0.5% sodium hypochlorite solution. Tween emulsifier is added at a rate of 0.1% to enhance spreading of the disinfectant. The symptomless chimera is dissected aseptically from the fruit and seed embryos in the sector removed and transferred to sterile water. The embryos are then cultured on a Murashige & Tucker (1969) culture medium. The cultures are allowed to develop for 4 weeks continuously at 30°C.

When shoots have developed to 1-3 cm, they are micro-grafted to virus-free rootstocks in the glasshouse. When the grafts have grown and sufficient material has developed, trees are made for evaluation in the

screenhouse. Each clone is replicated five times and a sensitive greening susceptible cultivar such as Ponkan mandarin is included.

Field psylla or psylla from a clean colony which have fed on an infected plant, are collected and 5 psylla placed in a small transparent cage over the growth point of each plant for 7 days. Hereafter the psylla are removed and placed in tubes for PCR testing to establish if they were infected with the greening organism. The psylla samples are stored at -20°C until they are used.

The plants that were exposed to the psylla are sprayed with a suitable insecticide to kill all the psylla eggs. They are then transferred to the glasshouse and kept at a temperature of 24°C. Greening symptoms will start developing on the sensitive Ponkan mandarin control plants after approximately 3 months. A final visual evaluation is made after 6 months and a PCR test is employed on the symptomless plants as well as the psylla that fed on the plants. Clones that show tolerance or resistance will be multiplied on rootstocks and evaluated in the field.

sector with greening symptoms
resistant clones

sector for potential

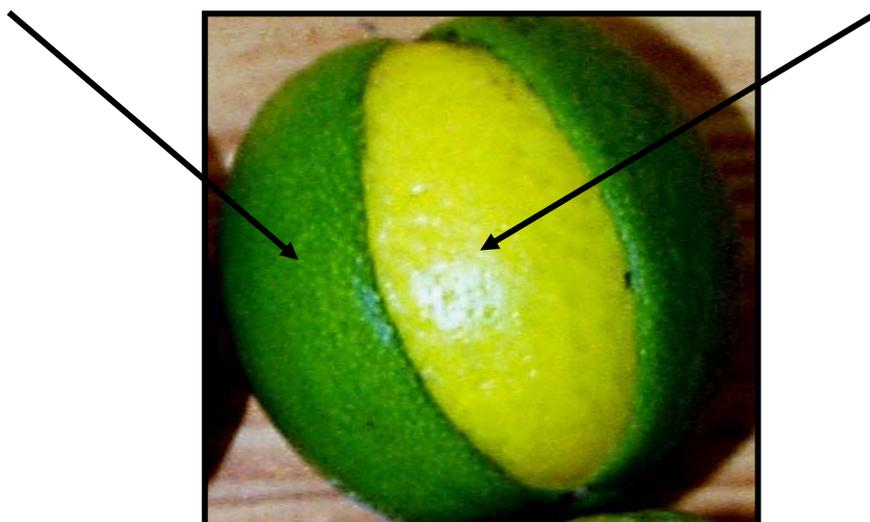


Figure 4.2.18.1 A greening infected fruit showing a healthy chimera. It is expected that tolerant or resistant plants will be obtained from the 'clean' sector (arrows).

Results and discussion

Eight embryo-rescued clones, replicated six times, were evaluated for tolerance or resistance by exposing them to field psylla. The results are given in Table 4.2.18.1. Neither the symptomless plants nor the psylla used for challenge, were tested by PCR to confirm absence or presence of the greening organism. Clones E2 and T2 showed promise and are being multiplied on Rough lemon rootstocks for a field trial.

PCR has not been employed on the symptomless plants or the psylla used for infection yet.

The plants are thorny and therefore have characteristics of seedlings and may be hybrids. In addition callus should also be derived from the rind, albedo and sap vesicals. This may overcome juvenility.

Table 4.2.18.1 The percentage healthy plants of embryo-rescued clones after exposure to field psylla.

CLONE	% HEALTHY
Ponkan mandarin (Control)	83
O6	67
O7	67
O8	50
E2	100
E3	50
E4	50
E6	50
T2	100

Conclusion

Two clones showed promise of resistance or tolerance after exposure to the psylla vector. They should be evaluated in the field for greening resistance as well as for their horticultural characteristics.

Future research

The experiment will continue and will be taken over by Ms Jacolene Meyer who will use the results as part of a PhD study. Do PCR on symptomless plants and psylla.

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4.2.19 Eradication of citrus greening infections in existing orchards

Experiment 818 by M.C. Pretorius (CRI)

Opsomming

Die gebruik van alternatiewe beheer metodes om sitrus vergroeningsiekte effektief te beheer behoort weer ondersoek te word. Veral vir gebiede waar vergroening nooit in die verlede teenwoordig was soos in gedeeltes van die Wes Kaap. Deur die vektor effektief te beheer kan die insidensie van vergroening verlaag word. Daar is geen nuwe sistemiese insekdoders wat die huidige produkte kan vervang nie en daarom behoort alle moontlike maatreëls gekombineer word sodat 'n geïntegreerde beheer program ontwikkel kan word. Die her-evaluering van metodes wat vroeër jare gebruik is en wat effektief was sal geëvalueer word. Hierdie maatreëls sluit die gebruik van antibiotikas sowel as 'n hitte behandeling deur bome te bedek vir 'n bepaalde tydperk. Die gebruik van antibiotikas ten opsigte van residue in die vrugte sal bepaal moet word. Die proef sal gedurende die 2006 seisoen uitgevoer word.

Introduction

Huanglongbing, better known in South Africa as citrus greening, has had a devastating effect on citrus production in the North-West, Limpopo, Mpumalanga and KwaZulu-Natal Provinces since it was first observed in 1928. When the disease was first described in South Africa in 1937, it was presumed to be a mineral toxicity (Van der Merwe & Andersen, 1937). At that stage the disease was known as yellow branch in the western Transvaal region (North-West) and as greening in the eastern Transvaal region (Mpumalanga) (Oberholzer, von Staden & Basson, 1965). Citrus greening disease is a major cause of crop and tree losses in many parts of Asia and Africa. Losses due to greening are not easy to assess. Sometimes only sectors of a tree are affected and losses are small, but in other cases the entire tree is infected and crop loss is total (da Graça, 1991).

An uncultured Gram-negative phloem-limited bacteria belonging to the alpha sub-division of the *Proteobacteriaceae* causes greening. The casual organism is classified as "Candidatus" sp. and is referred to as *Liberibacter africanum*, African greening, and *Liberibacter asiaticum*, Asian greening, which is not present in Africa.

Although the disease syndrome to some extent differs according to citrus varieties, common symptoms are yellowing of the veins and adjacent tissues, followed by yellowing or mottling of the entire leaf. Advanced or chronically infected trees show yellowing of the entire canopy and have sparse foliage and twig die-back. Diseased trees produce small, lopsided fruit that tend to remain mostly green in colour even when mature, have undeveloped seed, and impart an objectionable bitter-salty flavour and lower sugars. Root systems of these infected trees are poorly developed with relatively few fibrous roots, possibly because of root starvation. New root growth is suppressed and the roots often start decaying from the rootlets (Zhao, 1981).

The African species is only transmitted by the psyllid, *Trioza erythrae*, whereas the Asian species is transmitted by the psyllid, *Diaphorina citri*. It was found that the adult citrus psylla transmits the disease by feeding on infected young leaves. Nymphs can also acquire the greening agent. The number of adult psylla species in a population that carry the disease is relatively small, but under experimental conditions, a single adult species can transmit greening. Psylla acquires the organism after one day of feeding and transmits greening 7 days later, and can infect with an exposure time of less than 1 h. Long over-wintering feeding on old leaves makes adults highly infective on young flush in spring. Psylla is strongly attracted by yellow green wavelength of 550 nm, making diseased trees attractive targets and thereby increasing the proportion of disease-carrying insects (van den Berg, van Vuuren & Deacon, 1987).

The incidence of greening on individual citrus trees was evaluated over a seven-year period in certain citrus orchards in the Lowveld. The incidence of greening disease varies considerably, as does its intensity from season to season. It was found that the disease decreased over a period of time in these monitored orchards. It is common to find severely infected trees with one or more healthy branches. These healthy branches bear a larger proportion of the total crop, and thus give the illusion of recovery. Those parts of the tree affected with greening never recover from the disease, but because of low psylla populations during the period covered during the evaluation process, little of the healthy growth ever became infected from vector inoculation. The disease occurring in the infected White River and Nelspruit areas seems not to move rapidly, but instead, in the absence of the psylla vector, progress only slowly to encompass the tree. Healthy sectors of the tree then enlarge, seemingly to outgrow the disease, and increase the bearing capacity of the tree (Schwarz, Moll & van Vuuren, 1974).

These results emphasized the importance of reducing vector population numbers, therefore chemical control measures to control the psylla populations were intensified (C. Buitendag, personal communication). Systemic insecticides are the most effective because psylla are sap feeders. These applications include foliar application of insecticides such as endosulfan sprays, trunk applications with monocrotophos which is no longer used in South Africa, however, methamidophos can still be used. Soil applications of Aldicarb will also protect young leaves effectively. The use of trunk injections with antibiotics such as tetracycline hydrochloride reduces fruit symptoms. Van Vuuren, Moll, & da Graça, 1977 indicated that multiple applications of antibiotics reduced the greening fruit symptoms by more than 97%. This practice is, however, not commonly used by producers due to antibiotic presence in trees which is unacceptable for overseas markets.

Schwartz and Green (1972) indicated that greening disease was less severe in the hot, low-lying areas than in the cool, high-lying areas. There seemed to be a direct effect of heat on symptoms as well as on the build-up of psylla populations. In laboratory tests, high temperatures of $>32^{\circ}\text{C}$ killed all stages, 27°C allowed rapid development of the insect but with a 52% mortality, whereas at 21°C , 91% survived. Budwood from infected trees heated over a hot water bath at 51°C for 1 h, 49°C for 2 h and 47°C for 4 h eliminated the disease although some tissue viability was lost at higher temperatures. Infected trees covered for 2-5 months with polyethelene fibreglass sheets showed a dramatic decrease in the number of diseased fruit. However this method is impractical for large-scale use and therefore not considered as a control measure by producers (da Graça, 1991).

It was suggested that CRI should re-evaluate the possible use of antibiotics to determine whether new antibiotics are available. The use of antibiotics should be carefully considered because the existence of these products could be traced in harvested fruit. The use of alternative methods such as heat to effectively reduce the presence of greening disease in infected young trees in regions such as the Western Cape must be evaluated. It is clear from the literature that by reducing the insect vector, the incidence of the disease will decrease over time. Therefore, an integrated approach of a combination of different control measures will be effective, especially in regions where the psylla populations are still very low and the disease incidence is also low.

Materials and methods

Young citrus trees in pots, infected with greening, will be covered with plastic to increase temperatures over a period of time to determine the effectiveness and practicality of such an effort on a large scale. Recognized, effective antibiotics as well as new antibiotics will be evaluated on infected trees in pots. The residues of these antibiotics will be determined to confirm whether an antibiotic treatment could be a viable control option. PCR will be employed before and after treatments to assess the effectiveness of the treatments.

Results and Discussion

This trial will commence during 2006.

Conclusion

No conclusion yet.

Future research

Apply heat and antibiotic treatments.

Assess success of treatments by PCR.

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4.3 PROJECT: CITRUS BLACK SPOT

Project Co-ordinator: J.M. Kotzé

4.3.1 Project summary

Over the past few years considerable progress has been made with research and development. A mass of new information has been evaluated and processed to improve control measures and to determine the way forward. It is now time to compile the information in a single Master Report. This is necessary because the research projects were fragmented from the start, according to the expertise of individuals in three different research groups. This Master Report can be published as a prestige feature article in *Plant Disease*, or it may be kept in private files by CRI for future reference.

It is now possible to “fine tune” control programmes and producers should encourage communication and state their specific needs and circumstances to extract the maximum advantage from available information (4.3.2, 4.3.5).

So far, the CBS control strategies were based on well-tested chemical spray schedules (4.3.2, 4.3.6 & 4.3.7). This was underpinned by spore trap data on ascospore releases (4.3.11 & 4.3.6). We know now that the data represent ascospore counts of two *Guignardia* spp., the pathogen, *G. citricarpa* and the omnipresent endophyte, *G. mangiferae*. This leads to a conservative approach of spraying when spore traps show positive for ascospores of both species. It is risky to change this culture until molecular techniques can enable us to have a rapid identification kit to tell the differences between the spores.

It is now also possible to determine the stage of ascospore maturity in orchards within two hours in the laboratory by using the Inoculum Monitor. To determine the presence of CBS in an orchard without symptoms can be done with a PCR test, using specialised techniques. This is a most valuable contribution (4.3.9). The status of CBS in nurseries can now be done using the information and techniques which were developed through co-operation of the different groups (4.3.10).

Efforts to break the life cycle of CBS were done on relatively small areas due to possible cost of failure to the grower. The information gathered (4.3.12) enables us to make strategic decisions on CBS control in different situations of disease severity.

Progress has been made to develop a semi-selective medium for CBS (4.3.5). Disease forecasting models (DACOM) are being developed (4.3.13) using the most modern equipment. The system is already being used by growers in the Groblersdal area but refinement and greater accuracy are now in progress.

An *ad hoc* investigation was undertaken (4.3.14) to investigate the incidence, occurrence and ascospore release patterns of CBS in the Northern Limpopo and Eastern Cape. The project commenced in September 2005 and will be completed in May 2006. The work involves Inoculum Monitor surveys and PCR determinations on a regular basis.

The co-operation of the following participants is sincerely appreciated:

QMS: Dr. S.H. Swart, Ms. S. Serfontein, and their capable technicians.

CRI: Dr. G.C. Schutte with his co-workers.

University of Pretoria: Prof. Lise Korsten, Dr. Linda Meyer, Ms. Mariette Truter and Mr. Chris van Ginkel.

Projekopsomming

Daar is besonder goeie vordering gemaak oor die afgelope paar jaar. 'n Groot hoeveelheid inligting is versamel en word geëvalueer en verwerk om siektebeheer te verbeter en om die pad vorentoe te beplan. Dit het nou tyd geword dat navorsing in 'n Meester Verslag opgestel word as 'n permanente rekord.

Die bestrydingsmaatreëls kan ook nou afgerond word om die beste voordeel vir produsente te verkry. Produsente kan ook na vore kom om hulle besondere behoeftes en omstandighede aan te spreek.

Die ruggraad van Swartvlekbeheer het so ver berus op deeglik uitgetoetsde spuitprogramme (4.3.2, 4.3.6 & 4.3.7). Hierdie nuwe programme word gebaseer op die mees onlangse epidemiologiese bevindings (4.3.11 & 4.3.6).

Spoorvangers het in die verlede 'n belangrike rol gespeel, maar ons weet nou dat ons 'n bietjie mislei word deur die alomteenwoordige askospore van die endofiet, *G. mangiferae*. Om hierdie inligting meer noukeurig te kry sal 'n molekulêre studie nodig wees wat sowat 2 jaar sal neem.

Die stadium van rypheid van askospore kan met behulp van 'n Inokulum Monitor vroegtydig bepaal word. Dit is ook moontlik om 'n Swartvlek Indeks vir 'n boord of op 'n plaas te bepaal (4.3.9). Die tegnieke word voortdurend verbeter. Al is swartvlek selfs nie in 'n sigbare vorm teenwoordig in 'n omgewing nie, kan die aan of afwesigheid van *G. citricarpa* noukeurig bepaal word.

Die teenwoordigheid van swartvlek in kwekerie kan nou ook bepaal word, maar die protokol is nog nie uitgetoets en geakkrediteer nie. Warm water behandelings saam met sterool swammiddels het by Dr. Schutte se proewe belowende resultate gegee by kwekerie (4.3.10).

Pogings om die lewensiklus van swartvlek te breek was geslaagd, ten spyte van die feit dat die proewe op 'n klein oppervlakte uitgevoer is (4.3.12). Die resultate verleen vertroue om die beginsels op 'n groot skaal toe te pas. Hierdie werk moet voort gaan. Vooruitgang is gemaak met die gebruik van 'n selektiewe medium deur die samevoeging van die inligting van seksie 4.3.5.

Siektevoorspellingsmodelle (DACOM) word uitgetoets (4.3.13) waar die nuutste tegnologie toegepas word. Die sisteem word reeds met sukses in Groblersdal toegepas. Die kritieke besmettings periode vir uiteenlopende klimaatstreke word ondersoek, soos byvoorbeeld Tshipise, en koelstreke in die Oos-Kaap. Die werk word gedoen deur gebruik te maak van die nuutste tegnologie (4.3.14).

Die samewerking van die volgende navorsers word terdeë waardeer:

QMS: Dr. S.H. Swart, Me. S. Serfontein, en hulle baie bekwame tegnisi.

CRI: Die G.C. Schutte met sy medewerkers.

Universiteit van Pretoria: Prof. Lise Korsten, Dr. Linda Meyer, Me. Mariette Truter en Mnr. Chris van Ginkel.

4.3.2 Positioning of a single benomyl application in a strobilurin spray programme

Experiment 707 by G.C. Schutte (CRI)

Opsomming

Afwisselende spuitprogramme bestaande uit tenkmengsels met 'n benzimidazool, mancozeb en olie in November maand opgevolg met 'n strobilurine, koper en olie (A+B; C+D volgorde) in Januariemaand, toon groot potensiaal vir die beheer van swartvlek selfs in sitrusboorde waar die benzimidazool weerstandbiedende sitruswartvlekpopulasie tussen 87% (2003/2004 seisoen) en 63-66% (2004/2005 seisoen) beslaan. Die toekomstige gebruik van die benzimidazole sal afhang van die uitkoms van 'n carbendazim residuestudie (studienommer 05/1119) wat tans op sitrus in die RSA uitgevoer word deur die CRI en die SABS ter ondersteuning van 'n EU invoertoleransie. Indien die studie toon dat een benzimidazoolbespuiting aanvaarbare lae residue tot gevolg het, sal dit die spuitprogram behels waar die benzimidazool in Novembermaand gespuit word (A+B; C+D volgorde).

Introduction

Fungal resistance development to strobilurins is an ever-existing possibility. Therefore, anti-resistance strategies using fungicides with different modes of action, that do not result in a loss in effective control with less spray rounds, must be investigated. Recently a consultant (I.J. Bruwer, personal communication) found that a single Benlate application gave good citrus black spot (CBS) control at Lisbon Estates where resistance towards benzimidazoles was reported in the 1980s (De Wet, 1987). Since then, no benzimidazoles had been sprayed on that Estate until the end of February 2002 when a single benomyl application at a rate of 75 g/hl water was advised and applied. With this application, good CBS control was achieved. It was therefore decided to investigate how a single curative benomyl application will perform in a benzimidazole-resistant environment and the placement thereof in a strobilurin spray programme, also consisting of two applications in November and January according to the label, but where a tank mixture with mancozeb is replaced with a copper fungicide.

Materials and methods

(a) 2003 – 2004 field trial

Two orchards were selected. One was at Crocodile Valley Citrus Co. and one at Friedenheim Estates, both in the Nelspruit region. A randomised block design with 5 and 3 single-tree plots per treatment, respectively, was used. Fungicides were applied using a trailer-mounted, high-volume, high-pressure (2,500-3,000 kPa) sprayer with two hand-held spray guns. Each treatment was replicated six times in single-tree plots arranged in a randomised block design. Guard trees were assigned between plots within rows. Trees in both groves were selected for uniformity in canopy density and tree size. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. Currently, most commercial fungicide applications for CBS control in South Africa commence in mid-October, based on research findings from ascospore releases and spore trap data (Kotzé, 1963; Kellerman & Kotzé, 1977). The standard registered mancozeb treatment commenced in mid-October, depending on the climatological information required for infection during the critical infection period. The fungicides tested included mancozeb (Sancozeb 80% WP; Dow AgroSciences), benomyl (Benlate 50% WP; Du Pont), trifloxystrobin (Flint 50% WG; Bayer), carbendazim (Bavistin 50% SC; BASF), copper hydroxide (Copstar 12% SC; Ag Chem Africa) and the mineral spray oil, Citrex. They were sprayed as either A+B; C+D in November and January or as C+D; A+B also in November and January where A = Benlate, B = mancozeb, C = Flint and D = Copstar (Tables 4.3.2.1 & 4.3.2.2). At fruit maturity in July or August, CBS severity was rated on 100 fruit per tree according to a 3-point index (McOnie, 1964; Schutte, 1995) where 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportions of fruit per category were analysed by ANOVA, using Fisher's LSD test ($P = 0.05$).

(b) 2004 – 2005 field trial

The same trial sites and the same experimental procedures were used as described above. The same fungicides were also used except that trifloxystrobin (Flint, 50% WG; Bayer) was replaced with azoxystrobin (Ortiva, 25% SC; Syngenta) and copper hydroxide (Copstar, 12% SC; Ag Chem Africa) was replaced with copper oxychloride (Fynox, 85% WP; Unisun).

(c) Determination of benomyl resistance

Fruit infected with CBS were randomly sampled from all trees with visible CBS lesions throughout the orchard at Friedenheim Estates in 2004 as well as at both Crocodile Valley Citrus Co. and Friedenheim Estates during 2005. About 150 isolates were made from the fruit and plated onto PDA and incubated at 25°C under artificial light for 14 days.

An aqueous stock suspension of benomyl was made up in sterile distilled water at a rate of 5 ppm. This was added to PDA after it was autoclaved at 120°C for 15 minutes and cooled to approximately 45°C. The amended agar (20 ml) was thoroughly mixed and poured into 80 mm diameter petri dishes.

Fungal plugs (5 mm in diameter) of 14-day old *Phyllosticta citricarpa* cultures were aseptically removed and placed onto each petri dish containing amended agar and incubated at 25°C under artificial light. Radial growth was recorded after 14 days. Fungal plugs were also taken from the same isolates and plated onto oatmeal-agar to distinguish between pathogenic and non-pathogenic strains as described by Baayen *et al.*, (2002). *Guignardia citricarpa* isolates produce a distinct yellow pigment halo on the oatmeal-agar which is absent from the *G. mangiferae* isolates (Fig. 4.3.2.1).

Results and discussion

(a) 2003-2004 field trial

Results at Friedenheim Estates (Table 4.3.2.1) showed that the alternation of the tank mix combination with either trifloxystrobin + copper hydroxide + oil; benomyl + mancozeb + oil (A+B:C+D) or benomyl + mancozeb + oil; trifloxystrobin + copper hydroxide + oil (C+D:A+B) sprayed in mid-November and mid-January, only resulted in 86% clean exportable fruit that was significantly different ($P < 0.05$) from the standard mancozeb treatment. The mancozeb treatment resulted in 10% more clean, exportable fruit. However, where benomyl was replaced with its breakdown product, carbendazim, also in tank mixtures with mancozeb and oil, these treatments sprayed as either trifloxystrobin + copper hydroxide + oil; carbendazim + mancozeb + oil (A+B; C+D) or carbendazim + mancozeb + oil; trifloxystrobin + copper hydroxide + oil (C+D; A+B) resulted in more clean exportable fruit for export of 99.33%, that is 3% better than the standard mancozeb treatment. All these treatments were significantly different from the control that had only 19.33% clean exportable fruit.

The trial site at Crocodile Valley Citrus Co. showed that there were no significant differences ($P > 0.05$) between any of the treatments. However, they were all significantly different from the control that resulted in 39.60% clean exportable fruit. In both trials, the trifloxystrobin + copper hydroxide + oil; carbendazim + mancozeb + oil (A+B; C+D) sequence at Friedenheim Estates and the trifloxystrobin + copper hydroxide + oil; benomyl + mancozeb + oil (A+B; C+D) sequence at Crocodile Valley Citrus Co., was the only spray programme that resulted in fruit with no lesions in the category of four and more CBS lesions. The latter spray programme sprayed at Friedenheim Estates had 7% fruit with four and more CBS lesions. This can be attributed to the poor spraying and coverage of one particular tree.

(b) 2004-2005 field trial

Results at Friedenheim Estates (Table 4.3.2.2) showed that the alternation of the tank mix combination with either azoxystrobin + copper oxychloride + oil; benomyl + mancozeb + oil or azoxystrobin + copper oxychloride + oil; carbendazim + mancozeb + oil (A+B; C+D) or benomyl + mancozeb + oil; azoxystrobin + copper hydroxide + oil or carbendazim + mancozeb + oil; azoxystrobin + copper hydroxide + oil (C+D; A+B) sprayed in mid-November and mid-January, resulted in 92-98% clean, exportable fruit that was not significantly different ($P > 0.05$) from the standard mancozeb treatment. A similar result was evident using the other fruit infestation criterion. All these treatments were significantly different from the control that had only 31.67% clean exportable fruit.

The trial site at Crocodile Valley Citrus Co. showed that there were no significant differences ($P > 0.05$) between any of the sprayed treatments. The standard mancozeb treatment resulted in the most clean, exportable fruit of 98.2%. However, all treatments were significantly different from the control that resulted in 33.8% clean exportable fruit and the same scenario was experienced with the other criteria as well.

(c) Determination of benomyl resistance

From the initial 150 CBS isolates made in 2004 from the fruit collected at Friedenheim Estates, only 49 grew and 87% of these CBS isolates were resistant to benomyl. Furthermore, 85% of the same isolates were *G. citricarpa* and the rest were *G. mangiferae*.

From the initial 100 CBS isolates made in 2005 from the fruit, only 62.9% grew and were resistant to benomyl and 100% of all the isolates were *G. citricarpa* from the Friedenheim Estates site, while 100% of the isolates were *G. citricarpa* from the Crocodile Valley Citrus Co. orchard of which 66% were resistant to benomyl.

Conclusion

It appears from the 2003/2004 trial results that the residual action of the strobilurin, trifloxystrobin, is long enough to protect fruit for 8 weeks if mixed with copper hydroxide and spray oil. [One cannot make such extrapolations from the Restrictions Document PHIs since there may be other reasons for differences in PHI, e.g. absence of adequate supportive data or differences in tolerances] Benomyl on the other hand, although it was sprayed where the benzimidazole resistant population was 87% (2003/2004 season) and 63-66% (2004/2005 season) resistant, still maintained clean fruit and could still provide a curative action perhaps in a synergistic way with the strobilurins. The addition of copper hydroxide or copper oxychloride to a tank mixture of either trifloxystrobin or azoxystrobin and mineral spray oil, showed no phytotoxic symptoms like stippling and can play a role in the extended protection period required and could be considered as an alternative to mancozeb in such treatment mixtures. Therefore, the alteration of strobilurins with

benzimidazoles is not only effective against CBS, but can also result in less residues on fruit. The sequence of spraying will depend on the outcome of a carbendazim residue decline study (study number 05/1119) on citrus conducted by CRI and the SABS for support of EU import tolerances. If the residue trials indicate that a single carbendazim spray does not result in residues in excess of the revised EU import tolerance, then the benzimidazole + mancozeb + oil; strobilurin + copper fungicide + oil sequence (A+B; C+D) would be suitable for CBS control.

Future research

The trial work is completed and no more trials are planned in this regard.

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Fig. 4.3.2.1. Seven-day-old cultures of *Guignardia citricarpa* producing a distinct yellow pigment that is absent in cultures of *G. mangiferae*.

Table 4.3.2.1. Evaluation of spray programmes consisting of one tank mixture of trifloxystrobin (Flint), copper hydroxide (Copstar) and spray oil and another of benomyl (Benlate) or carbendazim (Bavistin) and mancozeb (Sancozeb) and mineral spray oil during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Friedenheim Estates and Crocodile Valley Citrus Co. during 2003 and 2004.

Treatments (A+B; C+D)	Rate / 100 ℓ water	Friedenheim Estates, Nelspruit ^y			Crocodile Valley Citrus Co., Nelspruit ^z		
		% Clean exportable fruit ^w	% Fruit with 1-3 CBS lesions ^w	% Fruit with four and more CBS lesions ^w	% Clean exportable fruit ^x	% Fruit with 1-3 CBS lesions ^x	% Fruit with four and more CBS lesions ^x
Carbendazim+mancozeb+oil; Trifloxystrobin+copper hydroxide+oil	55 ml + 150 g + 0.25%; 10 g + 300 ml + 0.25%	99.33 a	0.33 a	0.34 a	ND	ND	ND
Trifloxystrobin+copper hydroxide+oil; Carbendazim+mancozeb+oil	10g + 300 ml + 0.25%; 55 ml + 150 g + 0.25%	99.33 a	0.67 ab	0.00 a	ND	ND	ND
Mancozeb	200 g	96.33 a	0.66 ab	3.00 a	95.20 a	1.60 a	3.20 a
Trifloxystrobin+copper hydroxide+oil; Benomyl+mancozeb+oil	10 g + 300 ml + 0.25%; 50 g + 150 g + 0.25%	86.00 b	7.00 bc	7.00 a	100.00 a	0.00 a	0.00 a
Benomyl+mancozeb+oil; Trifloxystrobin+copper hydroxide+oil	50 g + 150 g + 0.25%; 10 g + 300 ml + 0.25%	86.00 b	8.00 c	6.00 a	95.00 a	2.20 a	2.80 a
Control		19.33c	20.33 d	60.33 b	39.60 b	14.60 b	54.20 b

^w Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 16 October 2003, 13 November 2003, 11 December 2003, 8 January 2004 for mancozeb alone and 13 November 2003 and 8 January 2004 for the other treatments sprayed in a tank mixture

^z Spray dates were 14 October 2003, 11 November 2003, 9 December 2003, 6 January 2004 for mancozeb alone and 11 November 2003 and 6 January 2004 for the other treatments sprayed in a tank mixture

ND = Not determined

Table 4.3.2.2. Evaluation of spray programmes consisting of one tank mixture of azoxystrobin (Ortiva), copper oxychloride (Fynox) and mineral spray oil and another of benomyl (Benlate) or carbendazim (Bavistin) and mancozeb (Sancozeb) and mineral spray oil during the susceptible period from October to January for citrus black spot (CBS) control on Valencia oranges at Friedenheim Estates and Crocodile Valley Citrus Co. during 2004 and 2005.

Treatments (A+B; C+D)	Rate / 100 ℓ water	Friedenheim Estates, Nelspruit ^y			Crocodile Valley Citrus Co., Nelspruit ^z		
		% Clean exportable fruit ^w	% Fruit with 1-3 CBS lesions ^w	% Fruit with four and more CBS lesions ^w	% Clean exportable fruit ^x	% Fruit with 1-3 CBS lesions ^x	% Fruit with four and more CBS lesions ^x
Azoxystrobin+copper oxychloride+oil; Carbendazim+mancozeb+oil	20 ml + 150 g + 0.25%; 55 ml + 150 ml+ 0.25%	98.33 a	1.67 a	0 a	97.0 a	1.2 a	1.8 a
Mancozeb	200 g	95.33 a	3.0 a	1.67 a	98.2 a	1.4 a	0.4 a
Azoxystrobin+copper oxychloride+oil; Benomyl+mancozeb+oil	20 ml + 150 g + 0.25%; 50 g + 150 g + 0.25%	94.67 a	3.0 a	2.33 a	95.2 a	2.8 a	2.0 a
Benomyl+mancozeb+oil; Azoxystrobin+copper oxychloride+oil	10 g + 150 g + 0.25%; 50 g + 150 g + 0.25%	94.0 a	3.33 a	2.67 a	97.2 a	1.2 a	1.6 a
Carbendazim+mancozeb+oil; Azoxystrobin+copper oxychloride+oil	55 ml + 150 g + 0.25%; 20 ml + 150 g + 0.25%	91.67 a	3.67 a	4.66 a	97.4 a	1.6 a	1.0 a
Control		31.67 b	11.67 b	56.66 b	33.8 b	19.6 b	46.6 b

^w Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^x Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

^y Spray dates were 15 October 2003, 12 November 2003, 10 December 2003, 7 January 2004 for mancozeb alone and 12 November 2003 and 7 January 2004 for the other treatments sprayed in a tank mixture

^z Spray dates were 14 October 2003, 10 November 2003, 8 December 2003, 5 January 2004 for mancozeb alone and 10 November 2003 and 5 January 2004 for the other treatments sprayed in a tank mixture.

4.3.3 Evaluation of warm water and fungicide dip treatments for the elimination of endophytic *Guignardia citricarpa* in budwood

Experiment 708 by G.C. Schutte (CRI)

Opsomming

Triazole soos difenoconazole, penconazole and propiconazole teen dosisse van 1 ml produk per litre water wat gedoop is in warm water (40°C) vir 1 uur, is effektief vir die beheer van sitruswartvlek (SSV) in okkuleerhout. Slegs penconazole teen 'n dosis van 1.6 ml produk per litre water het SSV ontwikkeling tot gevolg gehad. Warm water alleen het 'n effek op SSV gehad, omrede dit tot 8% SSV ontwikkeling tot gevolg gehad het in vergelyking met die 35% SSV ontwikkeling van die onbehandelde kontrole. Suurlemoene en Nawel lemoene en tot 'n mate, Valencia lemoene, was almal geaffekteer deur die warm water dip behandeling van 45°C omrede dit 'n effek op die vatvermoë van ogies gehad het wat op Carrizo citrange geokkuleer was.

Introduction

It is commonly known that citrus black spot (CBS) is mainly transmitted through nursery material such as budwood. The USDA requires that Western Cape propagation material may not be sourced in the CBS provinces to the north and Eastern Cape to prevent the transmission of the fungus to disease-free areas. In Brazil, a warm water dip technique was tested and introduced to curb the spread of the disease from Sao Paulo state to Brazilia state. This technique has been tested on lemon cuttings from a commercial orchard using hot water treatments of 45, 50, 55 and 60°C dipped for 15 minutes. Treatments were effective at 55°C and higher.

In apples, the uncontrolled heating of entire trees under plastic for 4 to 7 days has been shown to eradicate the fireblight pathogen (*Erwinia amylovora*) from active cankers. In most regions of Australia, bacterial spot (caused by *Xanthomonas campestris pv. pruni*) is an important disease of stone fruit. Although copper is registered for its control, copper may cause fruit russeting if applied shortly after budburst. Trials using heat treatment of 45°C for 5 hours appeared to be effective at eliminating *X. campestris pv. pruni* from >90% of cankers in Blackamber plum trees (Stephens, et al. 2001). The effect of heat treatment was only assessed on cankers derived from relatively narrow wood. Although the cost of the plastic was expensive in this study, this material could be used repeatedly or moved to adjacent tree rows. This treatment is most likely to be of practical use in stonefruit nurseries to significantly reduce infestation by *X. campestris pv. pruni* where the close planting of trees will enable the treatment to be economically viable. The same can be said for South African citrus nurseries and this is why the following work was conducted. If proven to be successful, this practise can be incorporated into protocols as a risk mitigation treatment, especially from regions which are earmarked to export to the EU and USA.

Materials and methods

Lemon cuttings from an old neglected commercial orchard at Tekwane Estates were sampled that showed a high incidence of CBS infection (Fig. 4.3.3.1). From these, ten pieces were cut into cuttings of about 10 – 15 cm lengths and tied together with a cable-tie. Triazole fungicides consisting of difenoconazole (25% EC, Ag Chem Africa), penconazole (20% SC, Ag Chem Africa) and propiconazole (25% EC, Ag Chem Africa) were selected for the trial. Each fungicide was made up at rates of 1, 10 and 50 ml product/l water. Plastic containers (2 l volume) were placed in a temperature controlled warm water bath and the water was heated to 40°C before the fungicides were added. After the expected temperature was reached, fungicides and cuttings were added and left for 1 hour (Fig. 4.3.3.2). The cuttings were removed and surface sterilized with 2% NaOCl, dissected into small pieces (50 CBS lesions per treatment) and placed onto PDA, whereafter the CBS growth, if present, was recorded. The control treatment consisted of cuttings that were placed in warm water at 40°C and another without this treatment. The experiment was repeated three times.

Two sets consisting of one hundred budwood sticks from each cultivar (as listed in Table 4.3.3.2) were either dipped for 1 hour at 40°C or untreated at the CFB in Uitenhage. Buds were budded onto Carrizo citrange and placed in glasshouses. The sustainability was determined after 25 days.

Results and discussion

Results from only one experiment are presented as the results from all three trials were similar. Difenoconazole and propiconazole at rates of 1 ml product/l water and more, dipped in warm water for 1 hour were effective in controlling CBS infected budwood. Penconazole at a rate of 1.6 ml product per litre water resulted in CBS development but was effective at higher dosages. Warm water alone was shown to

have an effect on CBS as well, because only 8% of the CBS grew from the plant material in comparison to the 35% from the untreated control (Table 4.3.3.1). Lemons and Navel oranges and to some extent, Valencia oranges, were negatively affected by the warm water dip treatment of 40°C (Table 4.3.3.2) when the buds were inoculated onto Carrizo citrange after the treatment.

It is recommended that nurseries dip budwood in difenoconazole and propiconazole (at rates of 1 ml product/l water and more) in warm water at 40°C for 1 hour for the control of CBS in budwood.

Conclusion

Difenoconazole and propiconazole at rates of 1 ml product/l water and more dipped in warm water for 1 hour, are effective in controlling CBS infected budwood. Care should be taken about the cultivars used in the hot water dip treatment as lemons and Navel oranges were affected in comparison with Valencia oranges, Clementines, Satsumas and grapefruit that were not affected by the heat treatment. Lower temperatures will not be effective and lemon, which is also the most susceptible cultivar to CBS, should be re-investigated using an alternative method.

Future research

The trial work is completed and no more trials are planned in this regard.

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Fig. 4.3.3.1. Lemon cuttings infected with citrus black spot sampled from trees from Tekwane Estates used in the study.

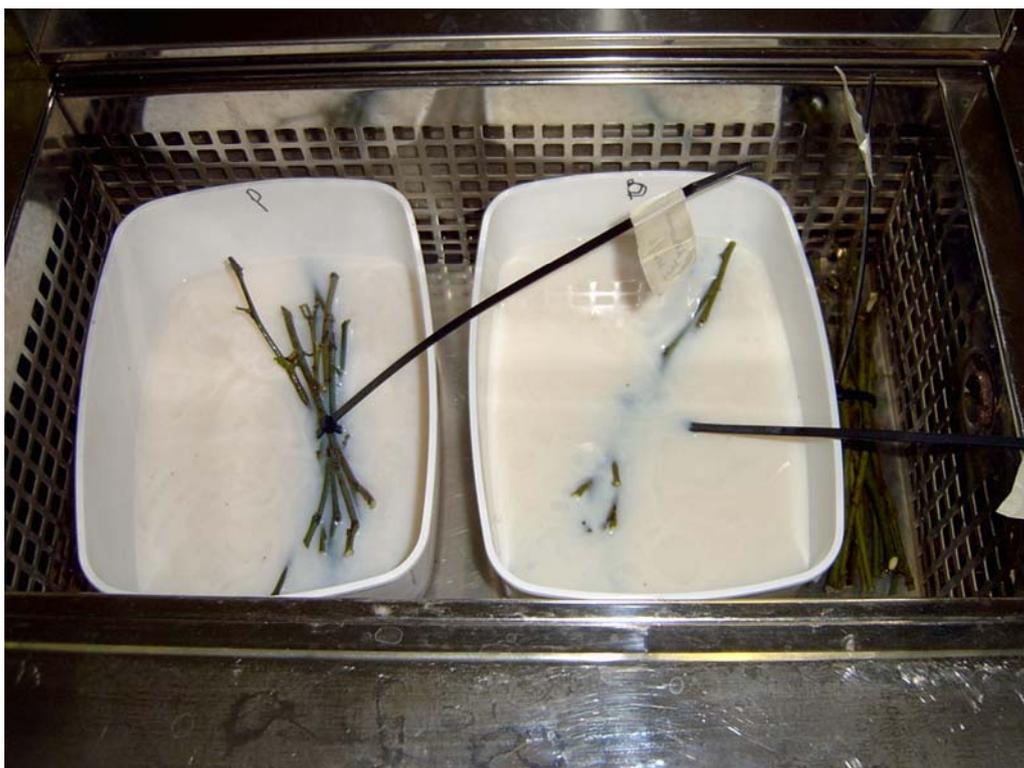


Fig. 4.3.3.2. Warm water dip evaluation at 40°C for 1 hour of different triazole fungicides for the control of citrus black spot in lemon cuttings.

Table 4.3.3.1. Warm water (40°C) dip evaluation for 1 hour of difenoconazole, propiconazole and penconazole for the control of *Guignardia citricarpa* in lemon twigs.

Fungicide	Rate per litre water	Percentage CBS growth from treated lemon twigs
Difenoconazole	1 ml	0
	10 ml	0
	50 ml	0
Penconazole	1.6 ml	4
	16 ml	0
	60 ml	0
Propiconazole	1 ml	0
	10 ml	0
	50 ml	0
Control (warm water (40°C))	-	8
Control (no treatment)	-	35

Table 4.3.3.2. Effect of warm water dip treatment (40°C) for 1 hour on the sustainability of budwood of different citrus cultivars.

Cultivar (selection/type)	Sustainability of budwood	
	Warm water (45°C) dip treatment for 1 hour	Control
Clementine (CCP)	98%	99%
Valencia orange (TV)	85%	99%
Navel orange (CN)	66%	97%
Satsuma (SEM)	97%	98%
Grapefruit (FG)	100%	92%
Lemon (ELSL)	41%	94%

4.3.4 Determining the age and decomposition rate of citrus leaves using different irrigation methods

Experiment 718 by G.C. Schutte (CRI)

Opsomming

Mikrobesproeiing het tot 40% meer gekomposteerde blare onder Valencia bome tot gevolg gehad in vergelyking met drupbesproeiing en die onbehandelde kontrole. Die natuurlike komposteringstempo is gelykstaande aan die aantal blare wat weekliks val soos bepaal is oor 'n periode van ses maande.

Introduction

Fallen dead leaves on the orchard floor are the main source of inoculum of CBS. Perithecia of *G. citricarpa* occur on fallen, decomposing leaves and are the most important source of inoculum. They develop within 40-180 days after leaf drop, depending mostly on the frequency of wetness and the prevailing temperatures (McOnie, 1963, McOnie, 1964a; Kotzé, 2000). Cultivation techniques such as mulching, physical removal or tillage of the dead leaves have been reported which assist in eliminating the primary source of infection (Schutte, 1995). Timing of these techniques is crucial.

Materials and methods

A 55-year old Valencia orange orchard at Friedenheim Estates near Nelspruit was selected for the trial. The trees are about 5x5x5 m in size. Sites were randomly selected within this orchard where wooden frames (1x1 m) were placed under the trees and leaves collected on a weekly basis. Ten sites were subjected to micro-irrigation, 10 sites were subjected to drip-irrigation and 10 sites had no irrigation and only received rain as all the other sites. Each of the 10 sites were subdivided into 5 sites each where leaves were collected on a weekly basis to be counted and 5 sites that were left until the end of the season when the total amount of fallen leaves were counted. This was done to determine the rate of natural decomposition over time under both micro- and drip irrigation. Ten rain gauges were randomly placed under selected trees in the orchard and the amount of precipitation recorded on a weekly basis.

Results and discussion

The amount of leaf drop per season in a mature Valencia orange orchard is approximately 1000 leaves per square metre over a period of 6 months, irrespective of the type of irrigation method used. The critical period for CBS infection is from the 100% drop (September) until the end of February when fruit become resistant to CBS infection. However, perithecia of CBS occur on fallen, decomposing leaves and are the most important source of inoculum. They develop within 40-180 days after leaf drop, depending mostly on the frequency of wetness and the prevailing temperatures (Kotzé, 2000). If one can enhance the decomposition of the leaves at an early stage, then it will result in less inoculum during the susceptible period from October to January.

Under South African conditions, the perithecia on leaves that fall from the trees before March apparently mature and release their spores before winter. Whether or not autumn leaf drop constitutes a danger to the following fruit crop seems to depend on the rate of leaf decomposition. At Letaba Estates, leaves abscised in May were too decayed for examination in October, but in Nelspruit it was found that leaves shed in April the previous year could still be fairly intact and have mature perithecia in January (McOnie, 1964b). However, if frequently wetted, this inoculum source can be decomposed and will not serve as a source during the latter part of the season.

In this experiment, the rate of leaf decomposition equals the amount of leaves that dropped per week under trees that were subjected to drip irrigation while micro irrigation resulted in less leaves per m² over the same period, showing that the rate of decomposition can be enhanced through this irrigation method (Table 4.3.4.1.). One shortcoming in this experiment is that the total amount of fallen leaves should also have been counted on a weekly/monthly basis and placed back in the frames. Then the total amount of decomposed leaves could have been calculated on a weekly/monthly basis. The total amount of rainfall was also higher during January than the preceding months of October, November and December, but the frequent rain spells must have contributed to the decomposition of the leaves during this period (Fig. 4.3.4.1).

Conclusion

Micro irrigation resulted in nearly 40% more decomposed leaves under Valencia orange trees than drip irrigation and control plots that did not receive any irrigation. The rate of natural leaf decomposition is also equal to the amount of leaves that drop weekly over a period of six months.

Future research

There are organic products available that have to be imported from the USA like “Compost-Aid” and “Breakdown” that can be applied through the irrigation system. They have to be tested under natural conditions to see if one can decompose leaf litter during the warm and dry period in October which is before the onset of the first summer rain. If it holds potential, this will put less pressure on fungicides and will form part of the broader control strategy.

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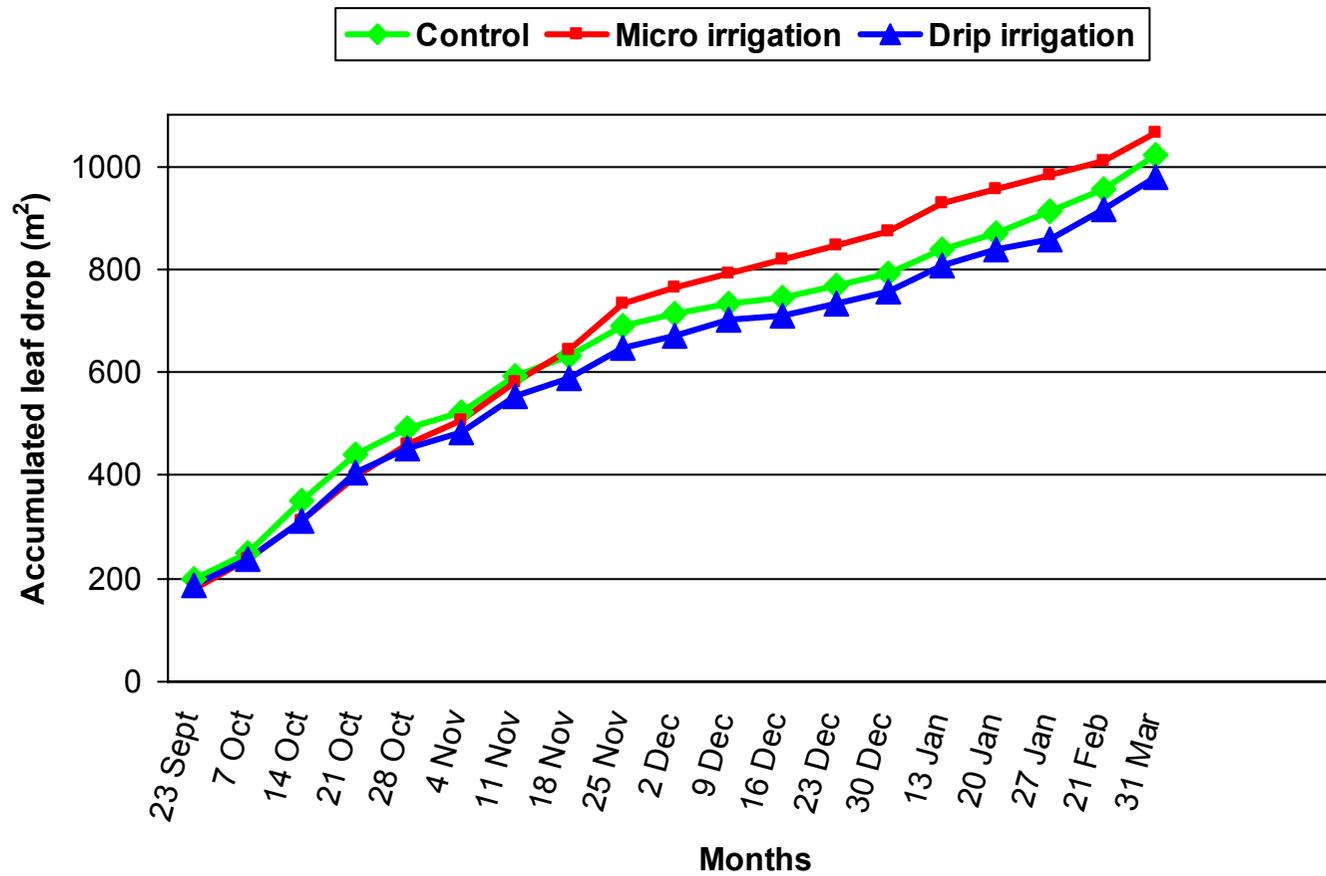


Fig. 4.3.4.1. Accumulative leaf drop (1 x 1m) under Valencia orange trees (5 x 5 x 5m) at Friedenheim Estates from 23 September 2004 to 31 March 2005 under different irrigation regimes.

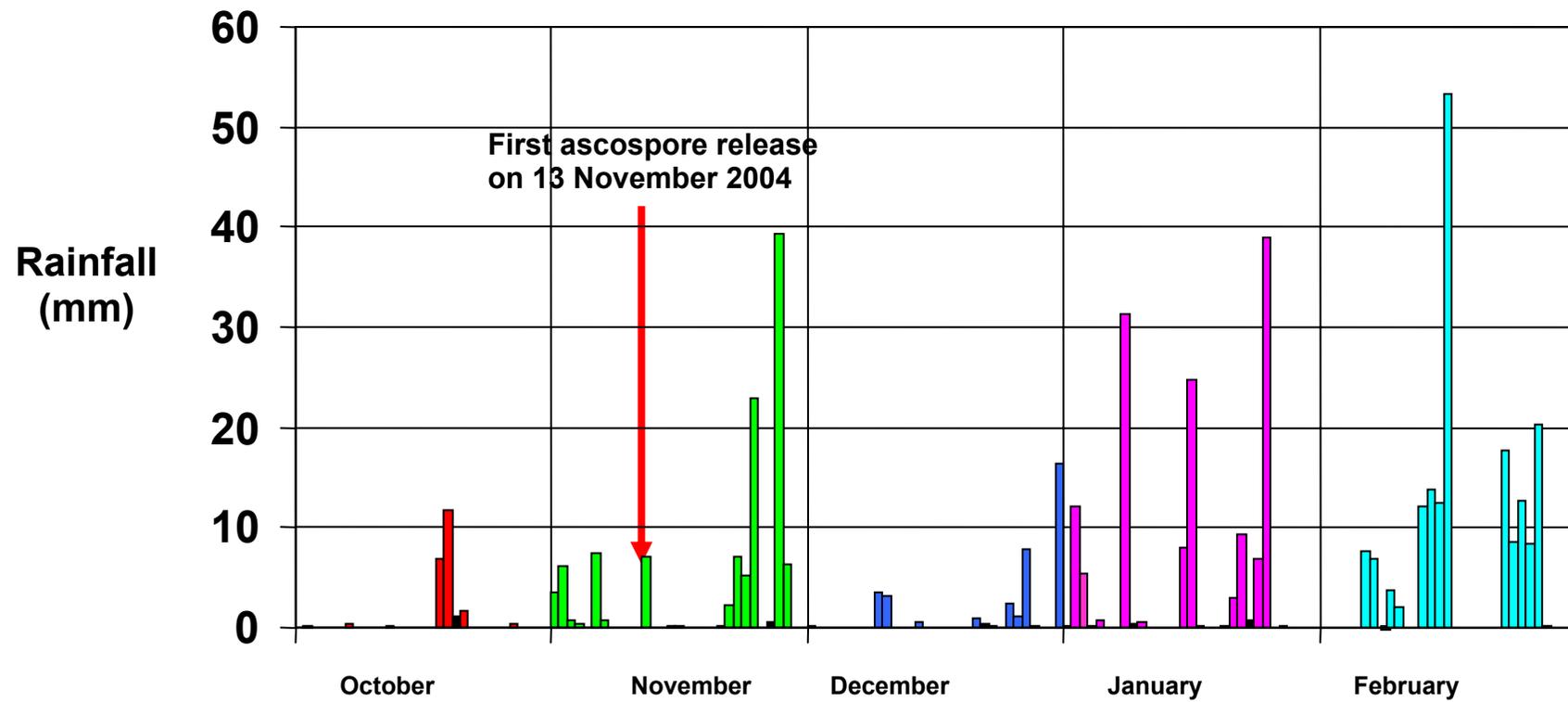


Fig. 4.3.4.2. Rainfall for the period October 2004 to February 2005 for the Nelspruit region.

Table 4.3.4.1. Total amount of fallen leaves/m²/week under Valencia orange trees subjected to different irrigation methods over a period of 6 months at Friedenheim Estates, Nelspruit.

Date counted	Total amount of leaves/m ² /irrigation method ^x		
	No irrigation	Micro irrigation	Drip irrigation
23 September 2004	163	155	192
31 March 2005	138	87	147

^x Means in a column are based on five replicates

4.3.5 Further development of spray programmes consisting of registered fungicides in tank mixtures with Sporekill for the control of citrus black spot on Valencia oranges

Experiment 799 by G.C. Schutte (CRI)

Opsomming

Behandelings bestaande uit mancozeb, koperoksichloried en koperhidroksied teen geregistreerde dosisse van 200 g/h ℓ water asook dieselfde behandelings teen gehalveerde dosisse van 100 g/h ℓ water met Sporekill teen 'n dosis van 100 ml/h ℓ water, was almal ewe effektief teen sitruswartvlek (SSV). Daar was ook 'n verskil van 12% tussen die geregistreerde dosisse van mancozeb en die gehalveerde dosis van mancozeb; 3% tussen die geregistreerd dosis van koperhidroksied en die gehalveerde dosis van koperhidroksied en 'n 24% verskil tussen die geregistreerde dosis van koperoksichloried en die gehalveerde dosis van koperoksichloried met verwysing na die getal skoon uitvoerbare vrugte in beide veldproewe. In een van die veldproewe het 'n tenkmengsel van die gehalveerde mancozeb behandeling teen 'n dosis van 100 g/h ℓ water met Sporekill wat verder verlaag is na 50 ml/h ℓ water, 'n verlaging van 20% minder skoon uitvoerbare vrugte tot gevolg gehad in vergelyking met die aanbevole dosis 100 g mancozeb en 100 ml Sporekill/h ℓ water. Koperammonium- karbonaat wat teen 'n dosis van 500 ml/h ℓ water (1x) getoets is, het 10 -14% meer skoon uitvoerbare vrugte opgelewer as die verlaagde dosis van 250 ml/h ℓ water ($\frac{1}{2}$ x) in beide veldproewe. Sporekill op sy eie teen 'n dosis van 200 ml/h ℓ water, was nie betekenisvol verskillend ($P > 0.05$) van Sporekill wat teen dosisse van 100 en 50 ml/h ℓ water getoets is nie selfs al was daar 'n verskil van 16 -18% meer skoon uitvoerbare vrugte tussen Sporekill wat teen dosisse van 200 en 50 ml/h ℓ water getoets is in beide veldproewe. Sporekill kan daarom nie op sy eie aanbeveel word nie.

Introduction

Control of citrus black spot (CBS) disease, caused by *Guignardia citricarpa* Kiely, is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in South Africa (Kotzé, 1963; McOnie, 1964; Kellerman & Kotzé, 1977, Schutte, 1995). When properly applied, fungicides containing copper afford excellent and inexpensive control and are used widely to control citrus diseases like CBS, scab, melanose, greasy spot, Phytophthora brown rot, Botrytis blight, Alternaria brown spot, mal secco, blast, and canker (Brodrick, 1970). Copper fungicides do not only have a broad spectrum of activity, but are relatively cheap. Moreover, mancozeb is not permitted on fruit destined for the USA. In countries like Canada, a 28-day pre-harvest interval is required and only packhouses with wet lines, i.e. where fruit is washed or rinsed, can be used. In all other countries a 21-day pre-harvest interval is permitted. Furthermore, copper fungicides are registered at 35-day intervals in comparison with the 24-25-day intervals for mancozeb.

There is an urgent need for new chemicals to control citrus black spot, even if they are sanitizing agents. Results from field trials the past two seasons were very promising where a sanitizing agent, Sporekill, was evaluated with mancozeb and copper fungicides and both groups were used at half their registered rates. In the two field trials conducted in the 2003/2004 season and the 2004/2005 season, the latter combinations yielded between 95 – 99% clean exportable fruit. For registration purposes, the trials need to be repeated at different sites and using different rates and combinations with other fungicides such as copper as well. The aim of this study is to see if tank mixtures of current registered contact fungicides at lower than registered rates can be sprayed to limit stippling due to frequent copper applications.

Materials and methods

Two experiments were conducted in commercial Valencia orange (*Citrus sinensis* (L.) Osbeck) groves on Rough lemon rootstock (*C. jambhiri* Lush.) at Crocodile Valley Citrus Co. and Friedenheim Estates near Nelspruit during 2004 and 2005. The trees were 38 and 59 years old, respectively and 3 to 4 m high. The rows ran directly north to south. Each treatment was replicated five and three times, respectively, in a randomized block design. 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 approximately 35 l of spray mix per tree per application. All treatments commenced in mid-October before the onset of the first summer rain (Fig. 4.3.5.1, Tables 4.3.5.1 and 4.3.5.2). 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. Proportions of fruit per category were analyzed by ANOVA, using Fisher's LSD test ($P = 0.05$). Spray dates and rates of applications are listed in Tables 4.3.5.1 and 4.3.5.2.

Results and discussion

The first ascospore release occurred on the 13 November 2004 and is exactly as recorded before in previous studies (Kellerman & Kotzé, 1977) (Fig.4.3.5.1.). Results from the field trial conducted at Crocodile Valley Citrus Co. (Table 4.3.5.1) show that there were no significant differences ($P > 0.05$) between the standard registered mancozeb, copper oxychloride and copper hydroxide treatments and all tank mixtures of reduced mancozeb, copper oxychloride and copper hydroxide rates of 100 g/hl water with Sporekill tested at a rate of 100 ml/hl water. Furthermore, although not significantly different ($P > 0.05$), there was a difference of 11.4% between the registered rate of mancozeb and the reduced rate of mancozeb; 3.4% between the registered rate of copper hydroxide and the reduced rate of copper hydroxide and 24% between the registered rate of copper oxychloride and the reduced rate of copper oxychloride with regards to the amount of clean exportable fruit. Tank mixtures of reduced mancozeb at a rate of 100 g/hl water with Sporekill tested at a rate of 50 ml/hl water, resulted in 20% less clean exportable fruit in comparison with a tank mixture of Sporekill and mancozeb at a rate of 100 ml/hl + 100 g/hl water. Although not registered for the control of citrus black spot, copper ammonium carbonate tested at a rate of 500 ml/hl water (1x), resulted in 14% more clean exportable fruit than the rate of 250 ml/hl water (½x). In this experiment there was a dosage response between all the rates of Sporekill evaluated on their own with regards to the criterion clean exportable fruit. Sporekill on its own tested at a rate of 200 ml/hl water, was not significantly different ($P > 0.05$) from Sporekill tested at rates of 100 and 50 ml/hl water even if there was a difference of 16% more clean exportable fruit between Sporekill at rates of 200 and 50 ml/hl water. All the treatments were, however, significantly different from the control. The untreated control only resulted in 34.4% clean exportable fruit. The same results were observed with the criterion 'fruit with 1 to 3 CBS lesions' where all the treatments were significantly different from the control except for the Sporekill rates of 50 and 100 ml/hl water. With regards to the criterion fruit with 4 and more CBS lesions', a significant difference ($P < 0.05$) was achieved between all the treatments and the control.

Results from the field trial conducted at Friedenheim Estates (Table 4.3.5.2) show no significant differences ($P > 0.05$) between the standard registered mancozeb, copper oxychloride and copper hydroxide treatments and all tank mixtures of reduced mancozeb, copper oxychloride and copper hydroxide rates of 100 g/hl water with Sporekill tested at a rate of 100 ml/hl water. Concomitantly, although not significantly different ($P > 0.05$), there was a difference of 10% between the registered rate of mancozeb and the reduced rate of mancozeb; 3% between the registered rate of copper hydroxide and the reduced rate of copper hydroxide and 25% between the registered rate of copper oxychloride and the reduced rate of copper oxychloride with regards to the amount of clean exportable fruit. Although not registered for the control of citrus black spot, copper ammonium carbonate tested at a rate of 500 ml/hl water (1x), resulted in 14% more clean exportable fruit than the rate of 250 ml/hl water (½x). In this experiment there was a dosage response between all the rates of Sporekill evaluated on their own with regards to the criterion clean exportable fruit. Sporekill on its own tested at a rate of 200 ml/hl water, was not significantly different ($P > 0.05$) from Sporekill tested at rates of 100 and 50 ml/hl water even if there was a difference of 18% more clean exportable fruit between Sporekill at rates of 200 and 50 ml/hl water. All the treatments were however significantly different from the control. The untreated control only resulted in 31.7% clean exportable fruit which is similar to orchard "Brits 1" at Crocodile Valley Citrus Co.. The same results were observed with the criterion 'fruit with 1 to 3 CBS

lesions' where all the treatments were significantly different from the control except for the Sporekill rates of 50 and 100 ml/hl water. With regards to the criterion 'fruit with 4 and more CBS lesions', a significant difference ($P < 0.05$) was achieved between all the treatments and the control.

Conclusion

Tank mixtures of mancozeb, copper oxychloride and copper hydroxide rates of 100 g/hl water with Sporekill at a rate of 100 ml/hl water, were equally effective for the control of CBS. Copper hydroxide should be re-investigated because coppers have become more expensive over the past years. Tank mixture of reduced mancozeb at a rate of 100 g/hl water with Sporekill at a rate of 50 ml/hl water cannot be recommended. Copper ammonium carbonate tested at a rate of 500 ml/hl water was effective against CBS but this treatment resulted in severe stippling of the fruit. Sporekill on its own tested at rates of 200 and 50 ml/hl water were not effective against CBS and can also not be recommended for CBS control on its own.

Future research

More field trials of Sporekill in combination with different fungicidal groups are planned.

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Table 4.3.5.1. Evaluation of didecyl dimethyl ammonium chloride (Sporekill) in tank mixtures with copper fungicides and mancozeb applied from October 2004 to January 2005 for the control of benzimidazole-resistant *Guignardia citricarpa* on 'Valencia' oranges at Crocodile Valley Citrus Co., Nelspruit, South Africa.

Treatment ^y	Concentration (g or ml product/ 100 litre of water)	Percentage of fruit in each class ^z		
		Lesions/fruit		
		0	1-3	≥4
Copper oxychloride + Sporekill	100 + 100	99.2 a	0.6 a	0.2 a
Copper hydroxide	200	98.4 a	1.4 a	0.2 a
Mancozeb+ Sporekill	100 + 100	97.8 a	1.8 a	0.4 a
Copper oxychloride	200	97.8 a	2.2 a	0.0 a
Copper ammonium carbonate	500	97.6 a	1.6 a	0.8 a
Mancozeb	200	97.2 a	1.2 a	1.6 a
Copper hydroxide	100	95.0 a	4.0 ab	1.0 a
Copper hydroxide + Sporekill	100 + 100	92.6 ab	4.0 ab	3.4 a

Copper ammonium carbonate+ Sporekill	250 + 100	91.4 ab	6.0 abc	2.6 a
Copper ammonium carbonate	250	87.2 ab	7.2 abc	5.6 a
Mancozeb	100	85.8 ab	6.4 abc	7.8 a
Copper oxychloride + Sporekill	100 + 50	78.6 abc	6.2 abc	15.2 ab
Copper oxychloride	100	74.2 bcd	12.4 cd	13.4 ab
Sporekill	200	73.8 bcd	10.4 bcd	15.8 ab
Sporekill	100	67.2 cd	14.2 de	18.6 ab
Sporekill	50	57.8 d	15.8 de	26.4 b
Control		34.4 e	19.2 e	46.4 c

^y Spray dates were 13 October 2004, 8 November 2004, 6 December 2004, 3 January 2005

^z Means in a column, based on 5 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

Copper oxychloride = Fynox
 Copper hydroxide = Hydrox
 Copper ammonium carbonate = Copper Count N

Table 4.3.5.2. Evaluation of didecyl dimethyl ammonium chloride (Sporekill) in tank mixtures with copper fungicides and mancozeb applied from October 2004 to January 2005 for the control of benzimidazole-resistant *Guignardia citricarpa* on 'Valencia' oranges at Friedenheim Estates, Nelspruit, South Africa.

Treatment ^y	Concentration (g or ml product/ 100 litre of water)	Percentage of fruit in each class ^z		
		Lesions/fruit		
		0	1-3	≥4
Copper oxychloride + Sporekill	100 + 100	100.0 a	0.0 a	0.0 a
Copper oxychloride	200	99.6 a	0.4 a	0.0 a
Mancozeb+ Sporekill	100 + 100	99.6 a	0.4 a	0.0 a
Copper hydroxide	200	99.3 a	0.4 a	0.3 a
Copper hydroxide + Sporekill	100 + 100	97.6 a	1.0 a	1.4 a
Mancozeb	200	96.3 a	2.3 a	1.4 a
Copper hydroxide	100	96.0 a	2.0 a	2.0 a
Copper ammonium carbonate	500	95.3 a	2.0 a	2.7 a
Copper oxychloride + Sporekill	100 + 50	92.6 a	3.6 a	3.8 a
Copper ammonium carbonate+ Sporekill	250 + 100	88.3 a	2.3 a	9.4 a
Mancozeb	100	86.6 a	2.4 a	11.0 a
Copper ammonium carbonate	250	81.3 ab	10.7 a	8.0 a
Sporekill	200	77.3 ab	10.0 a	12.7 a
Sporekill	100	75.6 ab	8.0 a	16.4 a
Copper oxychloride	100	74.3 ab	14.0 a	11.7 a
Sporekill	50	59.3 b	10.7 a	30.0 b
Control		31.7 c	13.3 a	55.0 c

^y Spray dates were 15 October 2004, 10 November 2004, 8 December 2004, 5 January 2005

^z Means in a column, based on 3 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

Copper oxychloride = Fynox
 Copper hydroxide = Hydrox
 Copper ammonium carbonate = Copper Count N

4.3.6 The evaluation of several chemical programmes for the control of Citrus Black Spot Experiment QMS 2.3 by S.H. Swart, V. Phalandwa & W. van der Pypekamp (QMS)

Opsomming

Die doel van die proef was om standaard programme te evalueer ten opsigte van hul vermoë om swartvlek te verhoed, veral onder toestande wat laat infeksie bevorder. Blare van behandelde bome is in Januarie en September 2005 gepluk en in die boord gelaat tot Maart 2005 en Januarie 2006, respektiewelik. Blare is daarna met 'n askospor monitor geëvalueer vir die teenwoordigheid van ryp askospor. Dit was 'n lae druk seisoen met slegs een ernstige infeksie periode in Desember 2004. Klimaatstoestande was nie bevorderlik vir infeksie gedurende Februarie en Maart nie. Resultate het wel gedui dat korter spuitintervalle (21 tot 23 dae) beter beheer (6% meer vrugte met geen swartvlek letsels nie) van swartvlek gegee het as intervale van 30 en 35 dae. Resultate het ook aangedui dat ten spyte van die teenwoordigheid van bensimidazole bestande populasies in die boord, het die toediening van benomyl nog steeds die persentasie vrugte sonder letsels van 85% (vir onbespuite bome) met amper 13% na 97.6% verhoog, waar benomyl bespuit is. Resultate met betrekking tot die vermoë van blare om askospor inokulum op die boord vloer te produseer was baie wisselvallig. Aanduidings is dat programme met strobilurine en benomyl, wel die produksie van askospor op dooie blare verminder.

Introduction

The control of CBS is mainly aimed at preventing fruit from getting infected. Popular belief in the Limpopo province is that fruit only needs to be protected from October until the end of January and, under certain circumstances, until the end of February. During the 2002/03 and 2003/04 seasons several late hanging fruit showed high levels of citrus black spot (CBS) symptoms in spite of programmes that should have ensured adequate protection from early October until the end of February. At the Letaba, Letsitele and Hoedspruit areas ascospore discharges normally peak between November and March. Data recorded by QMS Agri Science show that during the 2002/03 and 2003/04 seasons, high numbers of ascospores were discharged between December and March and that climatic conditions were extremely favourable for infection, especially during March of both seasons. The occurrence of CBS infected fruit in several orchards raised the question whether standard registered programmes can protect fruit adequately for markets that show zero tolerance to CBS infected fruit. The aim of this project was to evaluate the efficacy of standard chemical programmes to prevent infection of citrus fruit, especially under conditions that favour late infection and to establish whether applied fungicides had the ability to reduce inoculum production on leaf litter due to a specific mode of action.

Materials and methods

A trial was conducted at Mahela, Letsitele, in a 52-year old Valencia orchard with a history of CBS infection. Eleven different programmes were evaluated (Table 4.3.6.1), which included treatments with mancozeb (800 g/kg, WP, Dithane M45) with a contact action, applied at 21, 23, 30, and 35-day intervals (treatments 2, 3, 4, 5), strobilurin fungicides such as azoxystrobin (250 g/l, SC, Ortiva), trifloxystrobin (500 g/kg, WG, Flint) and pyraclostrobin (250 g/l, EC, Cabrio) applied according to traditional programs (treatments 6, 7, 8), and benomyl (500 g/kg, WP, Benomyl) with a systemic and curative mode of action (treatment 9). Mancozeb, pyraclostrobin and benomyl were also applied in programs to ensure protection until at least middle March (treatments 2, 10, 11). Treatments were applied to single-tree plots, replicated 5 times in a randomised block design. Fruit was evaluated for visible CBS symptoms in August 2005. Leaves of fungicide treated trees were picked in January and September 2005, left on the orchard floor, and retrieved in March 2005 and January 2006, respectively. Leaves were evaluated with an ascospore monitor (Interlock Systems, South Africa) for the presence of mature ascospores.

Results and discussion

Black spot infection was restricted during the 2004/05 season due to relatively dry conditions. According to data from the CBS-monitoring-system in Letsitele, supplied by QMS Agri Science, the season had only one high disease pressure infection period in December 04. Other infection periods were limited to two medium

rated periods in October 04 and in January 05 and four low to very low periods in November 04 and January, February and March 05.

Unsprayed fruit in the control programme had approximately 15% infected fruits of which 10% were in the 1 - 3 lesion, and 5% in the more than 3 lesion per fruit, categories (Table 4.3.6.3 and Figure 4.3.6.1). This was significantly less fruit without any lesions than all other treatments evaluated. Fruit sprayed on a 30-day and 35 day interval, Mancozeb 5 x and Mancozeb 4 x, (Treatments 4 and 5) also had significantly less fruit without lesions than other programmes evaluated in this trial. The best control was obtained with mancozeb applied at 21 and 23-day intervals (Treatments 2 and 3) with 99.8 and 99.9% fruit without any lesions, respectively. Results in this trial also showed that azoxystrobin, applied at 20 ml/100 ℓ, and pyraclostrobin, applied at 10 ml / 100 ℓ, were equally effective in suppressing disease with trifloxystrobin, applied at 10 g/100 ℓ, slightly, but not significantly, inferior.

Table 4.3.6.1. Treatments evaluated for their ability to control Citrus Black Spot.

Treatment no.	Programme Description	Program *	Active ingredients	Dosages g or ml / 100 l
1	Control	-	-	-
2	Manc x 7	M,M,M,M,M,M,M	mancozeb	200
3	Manc x 6	M,M,M,M,M,M	mancozeb	200
4	Manc x 5	M,M,M,M,M	mancozeb	200
5	Manc x 4	M,M,M,M	mancozeb	200
6	M A A M	M,AmO,AmO,M	azoxystrobin mancozeb oil	20 200 / 150 300
7	M T T M	M,TmO,TmO,M	trifloxystrobin mancozeb mineral oil	10 200 / 150 300
8	M P P M	M,PmO,PmO,M	pyraclostrobin mancozeb mineral oil	10 200 / 150 300
9	M B B M	M,BmO,BmO,M	benomyl mancozeb mineral oil	50 200 / 150 300
10	M B B B	M,BmO, BmO, BmO	benomyl mancozeb mineral oil	50 150 300
11	M P P P	M,PmO, PmO, PmO	pyraclostrobin mancozeb mineral oil	10 150 300

M = mancozeb 200 g / 100 ℓ, m = mancozeb 150 g / 100 ℓ, A = azoxystrobin, O = Citrex oil, T = trifloxystrobin, P = pyraclostrobin, B = benomyl

Table 4.3.6.2. Timing of fungicide application.

Treatment no.	Programme* Description	Date of application						
		-	-	-	-	-	-	-
1	Control	-	-	-	-	-	-	-
2	Manc x 7	12/10	03/11	24/11	15/12	05/01	26/01	16/02
3	Manc x 6	16/10	05/11	02/12	23/12	13/01	07/02	
4	Manc x 5	18/10	22/11		21/12	17/01		18/02
5	Manc x 4	18/10	22/11		28/12		07/02	
6	M A A M	18/10	05/11		22/12		07/02	
7	M T T M	18/10	05/11		22/12		07/02	
8	M P P M	18/10	05/11		22/12		07/02	
9	M B B M	18/10	05/11		22/12		07/02	

10	M B B B	18/10	05/11		22/12		07/02	
11	M P P P	18/10	05/11		22/12		07/02	

Manc = M = mancozeb , A = azoxystrobin, T = trifloxystrobin, P = pyraclostrobin, B = benomyl.

In this trial no additional value could be demonstrated by late fungicide applications to ensure protection until middle March, since only one infection period, rated as high, was recorded in December 2004. During February and March only infection periods rated low and very low occurred. The apparent inferior efficacy of Benomyl can be attributed to benzimidazole resistance in the *Guignardia* population present in this orchard at Mahela. Between 90% and 100% of *Guignardia* isolates, obtained from lesions on fruit sprayed with benomyl (programs 10 and 11), tested resistant to benomyl in laboratory tests. One can probably deduce from this data that it would be still beneficial to spray benomyl in this orchard, in spite of resistant populations of *G. citricarpa* occurring, since the percentage fruit with no lesions will still be improved with approximately 13%, from 85% (for unsprayed fruit) to 97.6% should benomyl be applied.

Table 4.3.6.3. Number of fruit in different infection categories of citrus black spot for different spray programmes applied during the 2004/2005 growing season.

Treatment no.	Programme description	Percentage fruit with black spot symptoms *		
		No lesions	1 to 3 lesions	More than 3 lesions
1	Control	85.2 e	9.8 e	5.0 c
2	Manc x 7	99.8 a	0.2 a	0 a
3	Manc x 6	99.9 a	0.1 a	0 a
4	Manc x 5	94.1 d	4.6 d	1.3 b
5	Manc x 4	94.3 d	4.3 d	1.4 b
6	M A A M	99.6 abc	0.4 ab	0 a
7	M T T M	97.9 bc	2.0 cd	0.1 a
8	M P P M	99.7 abc	0.1 a	0.2 a
9	M B B M	97.6 bc	2.1 bc	0.3 a
10	M B B B	97.9 bc	1.9 bc	0.2 a
11	M P P P	99.4 abc	0.4 ab	0.2 a

* Figures in the same column followed by the same letter do not differ significantly according to the LSD test at a 5% level of significance.

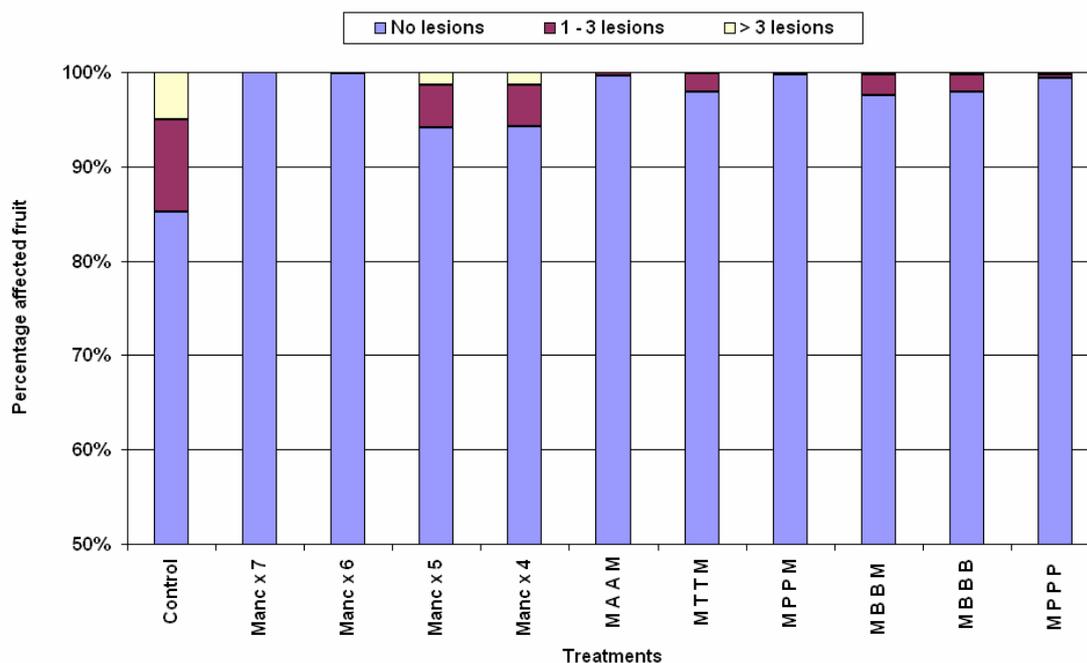


Fig. 4.3.6.1. The percentage affected fruit for different spray programmes applied at Mahela during the 2004/05 growing season.

To determine the effect of fungicide programmes on the production of ascospore inoculum on leaf litter, mature leaves from the five replicate trees of individual programmes, were picked randomly and mixed thoroughly. Approximately 80 leaves per treatment were picked in order to prepare 3 grids for each fungicide programme. These leaves were placed between two plastic grids, tied together with cable ties and placed under three trees of equal appearance, approximately 10 m from each other, on the orchard floor. Leaves were picked in January 05 and retrieved from the orchard in March 05. The process was repeated in September 05 and leaves were retrieved in January 06.

Large variation within treatments was observed (Table 4.3.6.4). It also appeared that less ascospores were produced under tree 3, than the other two trees during both assessments, in spite of the fact that these trees were in close vicinity of each other and of similar appearance regarding canopy size and density. Both high and low ascospore numbers were recorded under all three replicate trees but not necessary for the three replicates of the same programmes. Results showed that leaves picked in January 2005 from unsprayed trees allowed for very little ascospores to develop or it might also be that ascospore development and release had already taken place at the time of evaluation. Larger numbers of ascospores were produced on unsprayed leaves when picked in September 2005. Leaves obtained from trees sprayed six times with mancozeb and picked in January 2005, supported the production of extremely high numbers of ascospores, but on leaves picked in September, virtually nothing. The reason for this phenomenon cannot be explained from this trial. We suspect that a larger trial will have to be conducted with more replicates, longer time of exposure and under different climatic regimes, in order to clarify these observations.

Table 4.3.6.4. Number of fruit in different infection categories of citrus black spot for different spray programmes applied during the 2004/2005 growing season

Treatment no	Program description	Number of ascospores found on leaf samples					
		Picked Jan 2005			Picked Sep 2005		
		Total	Mean	Std Error	Total	Mean	Std Error
1	Control	3	1	0.6	355	118	114
2	Manc x 7	1920	640	252	369	123	122
3	Manc x 6	1110	370	200	1	0.3	0.3
4	Manc x 5	2160	720	640	2401	800	164
5	Manc x 4	35	11	10	210	70	70

6	M A A M	0	0	0	48	16	8
7	M T T M	165	55	39	27	9	6
8	M P P M	10	3	3	0	0	0
9	M B B M	1814	605	476	3	1	1
10	M B B B	47	15	15	9	3	3
11	M P P P	170	56	56	0	0	0

However, trends in both observations show that programmes with strobilurin and benomyl fungicides, are inclined to reduced inoculum development on leaf litter on the orchard floor. This was more apparent for leaves picked in September 05 and evaluated in January 06. We suspect that the effect of programmes evaluated were better demonstrated on leaves picked later in September 05 since these leaves were much younger and probably more prone to be influenced by the fungicide programmes at the time of application between October 04 and March 05, than the mature leaves picked in January 2005.

These are interesting results but much larger studies will have to be conducted over several seasons in order to make reliable conclusions, since it is generally accepted that leaves have a life span approximately 3 years and it might be that the current programmes did not have a direct effect on the oldest leaves on the tree at the time of application. In the past the control of citrus black spot was always focussed on protecting the fruit. However, in a zero tolerance market and with increased pressure on the allowed residue levels, other means of disease management will have to be explored. One method might be reducing infection of leaves by protection or curative mode of action in order to reduce the available inoculum in an orchard. This cannot be done within one season due to the specific phenology of the host and epidemiological characteristics of the pathogen involved. Therefore, a trial will have to be conducted over 3 to 5 seasons in order to establish the effect of fungicides programmes on leaf infection and ascospore development after leaves had dropped to the orchard floor. We propose that larger numbers of leaves, chosen more carefully regarding maturity and position in the tree, must be used in assessments and probably more replicates included (5 to 8), in order to gather reliable data.

4.3.7 Determine the effect of increasing fruit age on resistance to infection by *Guignardia citricarpa* Experiment QMS 03/68n by S.H. Swart, W. van der Pypekamp & V. Phalandwa (QMS)

Opsomming

Sitrusvrugte is hoogs vatbaar is vir infeksie deur *Guignardia citricarpa* vanaf vrugset, maar daar word beweer dat vrugte meer bestand raak teen infeksie met toenemende vrug ouderdom. Vrugte word dus normaalweg net tot die einde Januarie en in sommige gevalle tot die einde Februarie teen infeksie deur swartvlek patogene beskerm. Data, in die Letaba, Letsitele en Hoedspruit areas, toon dat groot getalle askospore tussen Desember en April geproduseer kan word en dat kondisies na Februarie nog uiters gunstig vir infeksie kan wees. Verskeie projekte is aangepak om te bepaal of vrugte nog vatbaar vir infeksie in Februarie en Maart is.

In Oktober 2003 is jong vrugte met papiersakke bedek om op verskillende tye oop te maak sodat vrugte van verskillende ouderdomme aan natuurlike infeksie periodes blootgestel kan word. Die projek het misluk omdat te veel vrugte binne 3 maande na bedekking afgespeen het. Volwasse vrugte is ook in Maart 04 met piknidiospore geïnkuleer, maar geen simptome het ontwikkel voor evaluasie in September 04 nie. Op Letaba Landgoed is 750 vrugte vroeg in Desember 04 met papiersakke bedek om maandeliks in Januarie, Februarie en Maart aan kunsmatige infeksie periodes bloot te stel deur askospoorproduserende blare reg bo vrugte vas te maak en met behulp van oorhoofse besproeiing 'n kunsmatige infeksie periode vir 120 uur te skep. Gedurende Januarie, Februarie en Maart 05 is 50 vrugte, in elk van 5 bome, aan infeksie periodes blootgestel. Gedurende dieselfde periode is ook 50 vrugte in Februarie, Maart en April kunsmatig met piknidiospore geïnkuleer. Tydens 'n voorlopige evaluasie, laat in Julie, is nog geen duidelike swartvlek letsels op vrugte waargeneem nie. Die vrugte is deur die produsent gepluk voor finale evaluasies gedoen kon word.

Op Mahela, Letsitele, is vrugte vanaf middel-Oktober 04 tot Maart 05 elke 23 dae met mancozeb gespuit. Een stel bome in elk van die 7 verskillende spuit rondtes is onbeskermd gelaat. Vrugte is in Augustus 2005 gepluk en die persentasie vrugte met swartvlek letsels is vir elke program bepaal. Resultate het getoon dat onbeskermdde bome tot 15% vrugte met letsels gehad het. Bome wat nie op 6 Desember gespuit is nie, het

9% vrugte met letsels gehad. Resultate word ondersteun deur infeksie data van die swartvlek monitoring sisteem en skale soos deur QMS Agri Science bepaal. Gedurende die 2004/05 seisoen was daar net een êrentige infeksie periode van 8 tot 10 Desember 04. Resultate dui daarop dat vrugte in Maart moontlik minder gevoelig vir infeksie was.

Die metode kan wel gebruik kan word om te bepaal tot wanner vrugte beskerm moet word en wanneer belangrike infeksie periodes plaasvind, maar ons sal egter vir 'n seisoen met hoë siekte druk in Februarie en Maart moet wag voordat sinvolle aanbevelings geaak kan word. Aangesien klimaat onbeheerbaar is, is dit uiters belangrik dat ons voortgaan om tegnieke te soek om gesimuleerde infeksies onder beheerde toestande te kan laat plaasvind sodat betroubare data meer ekonomies en vinniger gegenereer kan word.

Introduction

The control of CBS based on chemical protection of susceptible young fruit. Ascospores, produced on leaf litter, are the primary source of CBS inoculum (Kotzé, 1981). According to published data, citrus fruit is highly susceptible to infection by *G. citricarpa* from fruit set on, but become more resistant to infection as fruit mature (Kotzé, 1981; 1988, 1996). In the Letaba, Letsitele and Hoedspruit areas, data obtained with the ascospore monitor showed vast numbers of inoculum can be produced on leaf litter from September until April (QMS 2.1 report for 2006). Ascospores are mainly trapped in orchards from October until March. Climatic conditions are often favourable for infection during late February and March according to data recorded by QMS Agri Science. The occurrence of high levels of infected fruit, in spite of spray programmes to protect from early October until the end of February, raised some questions regarding acquired resistance with fruit maturity. The aim of this study was to determine whether fruit can become more resistant to infection by *G. citricarpa* with increasing maturity and whether additional protection is needed until the end of February and even March for late hanging cultivars. The design of the trial also allowed for gathering data on the most important infection periods during the growing season.

Materials and methods

a) Covering and exposing fruit during periods conducive to infection

This trial was conducted in 2004/05 in a Delta Valencia orchard. Seven hundred and fifty fruits were covered with paper bags during October 04. In January, February and March 05, 50 fruit in each of 5 replicate trees were uncovered and exposed to artificial infections. Sets of small mesh grids, containing ascospore bearing leaves, were suspended over each fruit. Leaves from the same batch were tested for the presence of mature ascospores with the ascospore monitor (Interlock Systems, South Africa). Overhead irrigation was used to simulate rain for a period of 120 hours. This was done during January, February and March on different sets of fruit. After the simulated rainy conditions, fruit was covered again with paper bags until the end of May when we assumed no additional natural infection could occur. Paper bags were removed and fruit were left to mature naturally. Fruit was supposed to be harvested in August 05.

b) Artificially inoculated fruit

In another trial in the same orchard at Letaba Estates, 50 Delta Valencia fruits were inoculated during February, March and April 04 with a suspension of artificially produced pycnidiospores obtained from 3-week-old laboratory grown *G. citricarpa* cultures on carnation-leaf-agar. Fruit was covered with plastic bags containing moisturised cotton wool for 120 hours in order to enhance infection. Thereafter bags were removed and fruit were left to develop normally. Fruit was supposed to be harvested in August 2005.

c) Protecting fruit with a contact chemical

A trial was conducted in a 52-year old Valencia orchard at Mahela, Letsitele. In this trial, chemical treatments with 200 g mancozeb / 100 ℓ water commenced middle October 04 and continued until middle March 05. The trial consisted of 7 groups of trees, which were sprayed every 23 days, but 1 group was left unprotected in each of the 7 spray rounds (Table 4.3.7.1). Each treatment was replicated 5 times in a randomised block design. Fruit was harvested middle August and rated according to 3 categories of infection (fruit with no lesions, fruit with 1 to 3 lesions and fruit with more than three lesions). The percentage fruit in each category was subjected to arcsine transformation and differences between means determined with the Least Significance Difference Test at a 5% level of significance.

Results and discussion

a) Covering and exposing fruit during periods conducive to infection

The last preliminary evaluation was done in July 05. At this stage no citrus black spot symptoms could be observed. Fruit in this trial was picked before black spot symptoms developed and no final assessment could be made on ripe fruit in August.

b) Artificially inoculated fruit

The last preliminary evaluation was done in July 05. At this stage no citrus black spot symptoms could be observed. Fruit in this trial was picked before black spot symptoms developed and no final assessment could be made on ripe fruit in August.

c) Protecting fruit with a contact chemical

Results for this trial are shown in Table 4.3.7.1 and Figure 4.3.7.1. Unsprayed trees had 15% infected fruit of which 10% was in the 1 - 3 lesion, and 5% in the more than 3 lesions per fruit, category. Data show that the omission of a single spray round with mancozeb on 6 December 04 was responsible for 9% infected fruit, of which 4% was in the 1 - 3 lesion, and 5% in the more than 3 lesions per fruit, category.

Table 4.3.7.1. Number of fruit in different infection categories of citrus black spot for different spray programs applied during the 2004/2005 growing season.

Date of mancozeb application	Black spot infection category		
	No lesions	1 to 3 lesions	More than 3 lesions
Oct 14	99.6 b*	0.3 c	0.1 b
Nov 11	99.5 b	0.5 c	0 b
Dec 6	91.1 a	4.2 b	4.7 a
Dec 29	99.4 b	0.5 c	0.1 b
Jan 25	99.6 b	0.4 c	0 b
Feb 17	99.6 b	0.3 c	0.1 b
Mar 14	99.9 b	0.1 c	0 b
None	85.2 a	9.8 a	5.0 c

* Figures in the same column followed by the same letter do not differ significantly according to the LSD test at a 5% level of significance

Very little disease was due to the omission of other spray rounds during this season. Data show that more than 99% fruit in most cases was free of black spot lesions and that infection during March was probably the least with 99.9% fruit free of any black spot lesions. Results obtained in this trial is supported by data obtained by QMS Agri Science regarding ascospore released and potential infection periods that occurred between October 04 and March 05 (Fig. 4.3.7.2). The graph shows only one infection period, rated as "high", occurred from 8 to 10 December 2004. This explains why the omission of mancozeb on 6 December resulted in 9% infected fruit. It is possible that the medium to low infection ratings for other infection periods was responsible for 0.4 to 0.6% infected fruit. The fact that only 0.1% of fruit had lesions when fungicide application was omitted in March might be an indication of acquired resistance, however we feel that the infection potential during these periods was very low in general and that definite conclusions cannot be made from this single trial.

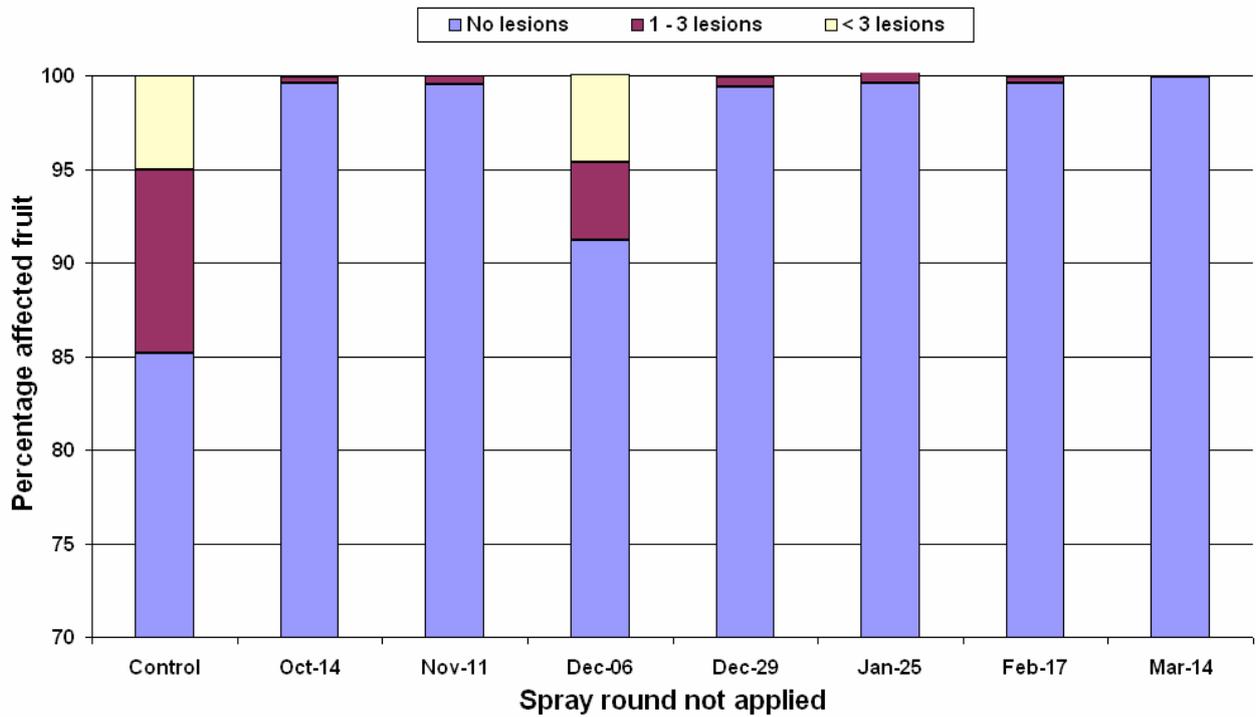


Figure 4.3.7.1. The effect of omitting a spray round with mancozeb on Valencia fruit subjected to natural infection conditions during the 2004/2005 season.

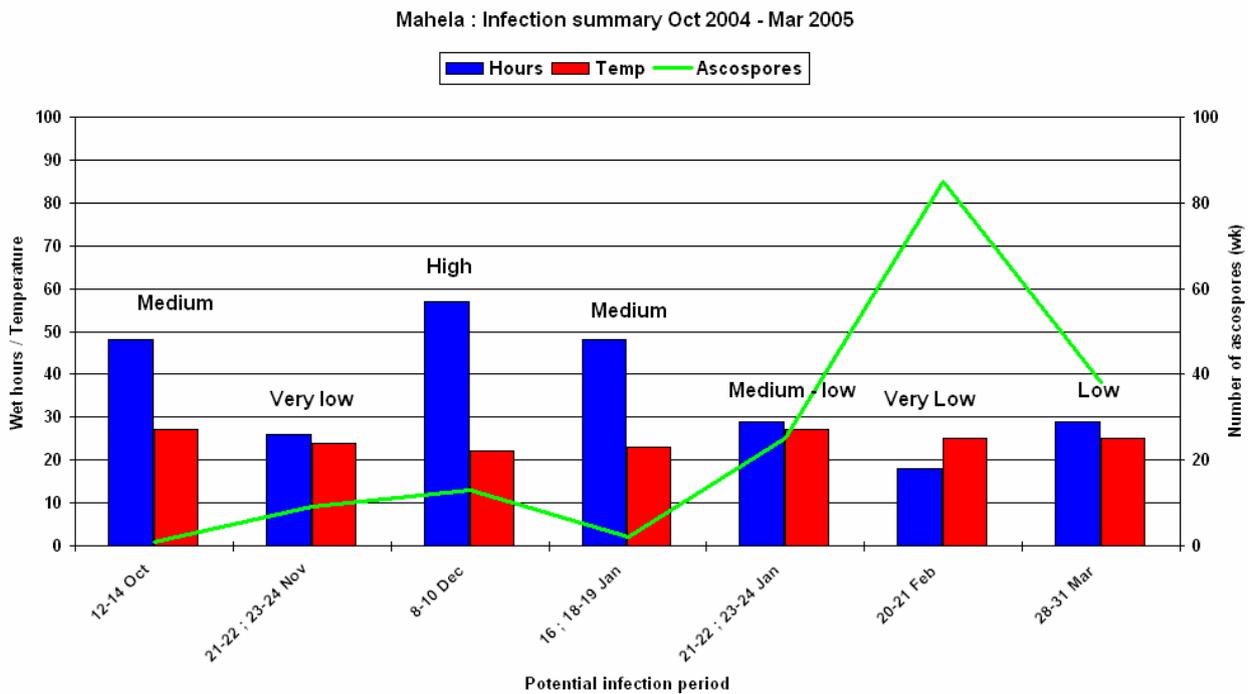


Figure 4.3.7.2. Ascospore release during potential infection periods for October 2004 and March 2005.

Results show that this is a useful, practical method to determine until when fruit should be protected and when the most important infection periods occurred. However, periods of higher disease pressure are

needed, especially late in the season, in order to make clear conclusions. Therefore, this trial will have to be repeated under conditions more conducive to infection and disease development.

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4.3.8 The correlation between leaf drop and production of ascospore inoculum in citrus orchards Experiment QMS 2.1 by S.H. Swart (QMS)

Opsomming

Die doel van die studie was om seisoenale verskille in inokulum potensiaal, dit is die aantal beskikbare askospore van *Guignardia* spp, wat op dooie sitrus blare op die boordvloer geproduseer word, te bepaal. Die teenwoordigheid van askospore is met 'n askospoormonitor (Iterlock Systems) bepaal. Die projek is in Augustus 2002 geïnisieër in 'n Navel boord op Letaba Landgoed, waar geen swamdoder bespuitings gedoen word nie. In Mei 2004 is Valencia boorde op Mahela, Letsitele, en op Richmond Landgoed, Hoedspruit, by die projek ingesluit om die invloed van klimaatsverskille in verskillende geografiese areas ook te ondersoek.

Resultate tot op datum dui op duidelike seisoenale verskille in 'n spesifieke area met betrekking tot die hoeveelheid inokulum wat geproduseer word, sowel as die tyd wanneer piek produksie plaasvind. Blare op die boordvloer kan op enige tydstip gedurende die jaar as substraat vir die produksie van askospore dien, maar gedurende hierdie ondersoek is die meeste inokulum in die periode Desember tot April geproduseer. Die meerderheid van askospore word binne 1 tot 3 maande na blaarval geproduseer tydens periodes van hoë reënval. Tydens droër, koel periodes neem die proses langer (4 tot 6 maande). Onder natuurlike toestande in 'n boord verweer blare totaal binne 6 maande op die boordvloer. Klimaat, veral reënval (aantal dae wat dit reën), en temperatuur speel 'n belangrike rol, veral met betrekking tot die tempo van die kompostering proses van blare wat moontlik 'n direkte korrelasie met die vorming van perithecia en die produksie van askospore deur *Guignardia* spp het.

Introduction

Management of plant diseases is mainly determined by the epidemiology of the causal pathogen. The sexual stage of *Guignardia citricarpa* produces perithecia on leaf litter and airborne ascospores are the major source of inoculum causing citrus black spot in South Africa (Kotzé, 1981). The aim of this study was to determine seasonal variation in inoculum potential that is the number of available ascospores produced on citrus leaf litter on the orchard floor and the effect of variable climatic conditions in geographically different areas. The trial was also designed to produce data on the most important source of inoculum, that is which leaf litter is responsible for supporting the majority of inoculum during the growing season, and how long it took to produce mature ascospores.

Depending on cultivar, the citrus production season is normally considered to be from flowering in August/September until the end of the picking period in August/September the next year. Fungicide applications, for prevention of citrus black spot, traditionally commence early October and fruit are normally protected until the end of February in "high risk" areas. In "low risk" areas spraying commences at the end of October and fruit are normally protected until the end of January.

Materials and methods

This project was initiated in August 2002 in a Navel orchard on Letaba Estates where no chemicals for the control of insects or diseases are applied. In May 2004, two Valencia orchards, one at Mahela, Letsitele, and one at Richmond Estates, Hoedspruit, were also included in the project in order to study inoculum production in different climatic production areas. All leaves used in this study were collected from naturally

infected trees in an unsprayed Navel orchard, Plot L 57, planted in 1956 on Letaba Estates, near Tzaneen. Mature leaves were picked randomly in the orchard, mixed well and placed between sets of two plastic mesh grids in order to cover approximately 700 cm². Sets of grids were secured with cable ties, and 18 sets were randomly selected and transported to three orchards representing the different production areas. The three replicate trees were approximately 10 to 15 meters from each other in each of the three orchards. Each month, six grids with leaves were placed under each of three replicate trees in each orchard. Every four weeks a set of grids, representing the preceding six consecutive months, was collected from data trees in each of the three production areas.

In the laboratory, grids were submerged in hot water (40°C ± 1°C) for 5 min, allowed to drain for 5 min and then placed in an inoculum monitor (Interlock Systems, South Africa) for two hours. In the inoculum monitor mature ascospores are actively released from ripe asci into a chamber, sucked by a vacuum pump and deposited on a Vaseline coated standard microscope slide. After two hours the slides are removed, stained with lactophenol-cotton-blue and covered with a cover slip. The number of deposited ascospores, resembling the morphology of *Guignardia sp.*, was counted under a light microscope at 400x magnification. The total number of ascospores in 4 lanes (0.5 mm x 45 mm) covering an area of approximately 90 mm² was recorded for slide. The number of ascospores produced on leaves under three replicate trees in an orchard varied considerably, therefore, the total number of ascospores from all three replicate grids were pooled and are presented as the total number of ascospores for three grid sets.

Results and discussion

Letaba Area

The total number of ascospores counted on leaf samples picked since August 2002 and evaluated until Jan 2006 were 10 163, 23 034 and 30 056 for tree 1, tree 2 and tree 3 at Letaba Estates. Therefore, under tree 1, three times less ascospores were produced on leaf litter, compared to tree 3. However, leaf samples under tree 3 did not always produce more inoculum than the other two trees. The reason for this phenomenon cannot be explained from this trial, since all leaves used in samples came from the same orchard and were always gathered according to the same protocol. These leaves were mixed, placed in grids and then grids were selected randomly for each area and for each replicate tree in each area. We can assume that the infection levels of leaves can differ for different monthly sampling and that some variance will exist amongst leaves for a specific month or period, but these large differences were unexpected. No difference in the condition, canopy structure the position or apparent micro-climate under these replicate trees could be observed. However, since ascospore numbers on leaf samples, representing a specific set of three replicates differed mostly, it was decided to use the total number of ascospores for all three replicates for comparative analysis.

Data for the total number of ascospores available in a specific month was calculated by adding the number of ascospores found on 6 leaf samples, placed on the orchard floor during the 6 preceding months. The total number of ascospores produced in each month from December 02 until November 05 is presented in Fig. 4.3.8.1. This trial was initiated in August 02 and therefore the first sets of data in December 02 and January 03 is not complete, since the total number of mature ascospores produced was calculated for only 4 and 5 months respectively instead of 6 months as for all the following months.

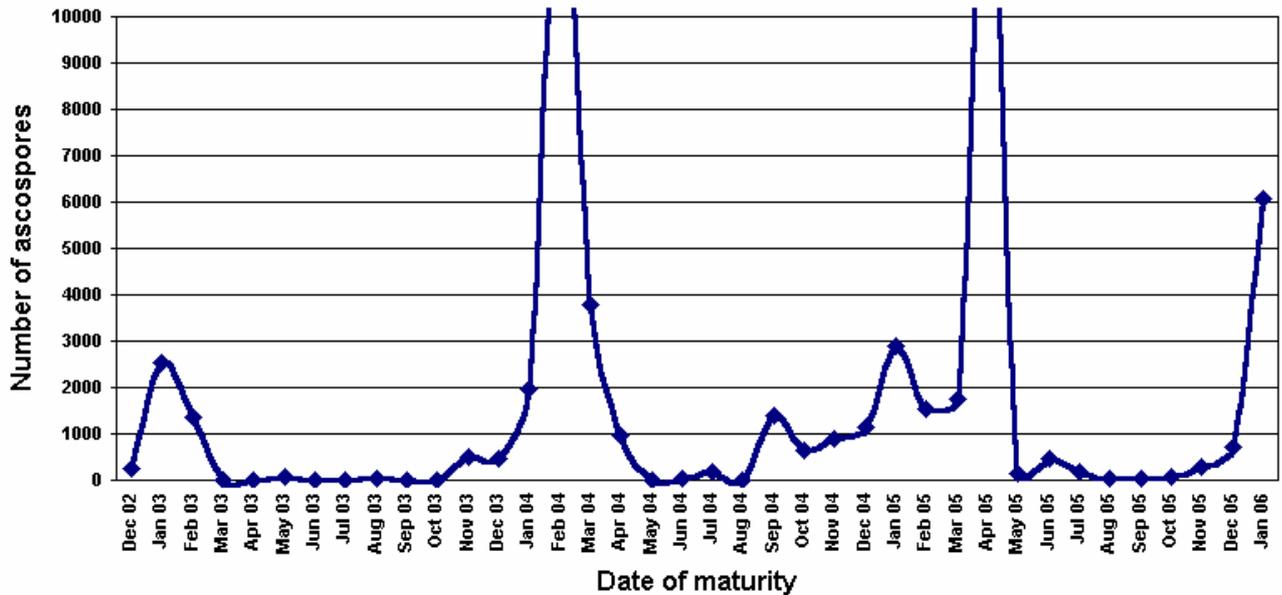


Figure 4.3.8.1. The total number of mature ascospores found monthly on leaf samples incubated at Letaba Estates representing leaf sources that dropped during the preceding 6 months.

Results clearly show seasonal differences regarding the number of ascospores produced as well as the periods when the majority of inoculum was produced. During the 2002/03 growing season the major inoculum peak was produced on leaf samples assessed in January and February 03 with 2500 and 1500 ascospores respectively. Very little ascospores were produced on leaf samples during the rest of the season. From September 03 until August 04 the first inoculum production was observed on leaves assessed in November and December 03 (approximately 500 ascospores). The available inoculum increased to 2000 ascospores in January 04 and peaked at more than 10 000 in February 04. Large numbers of ascospores were still observed in March (3750 ascospores) and April 04 (1000 ascospores) but numbers were depleted in May 04. During the 2004/05 season the first inoculum was already available in September (1500 ascospores). During January 05 ascospore numbers rose to approximately 3000 and a significant peak of more than 10 000 was available in April 05. It is clear from results that the high rainfall area at Letaba Estates support inoculum production from September until at least in April during a growing season. The number of ascospores as well as the position of a peak for inoculum production can vary from season to season Fig 4.3.8.2.

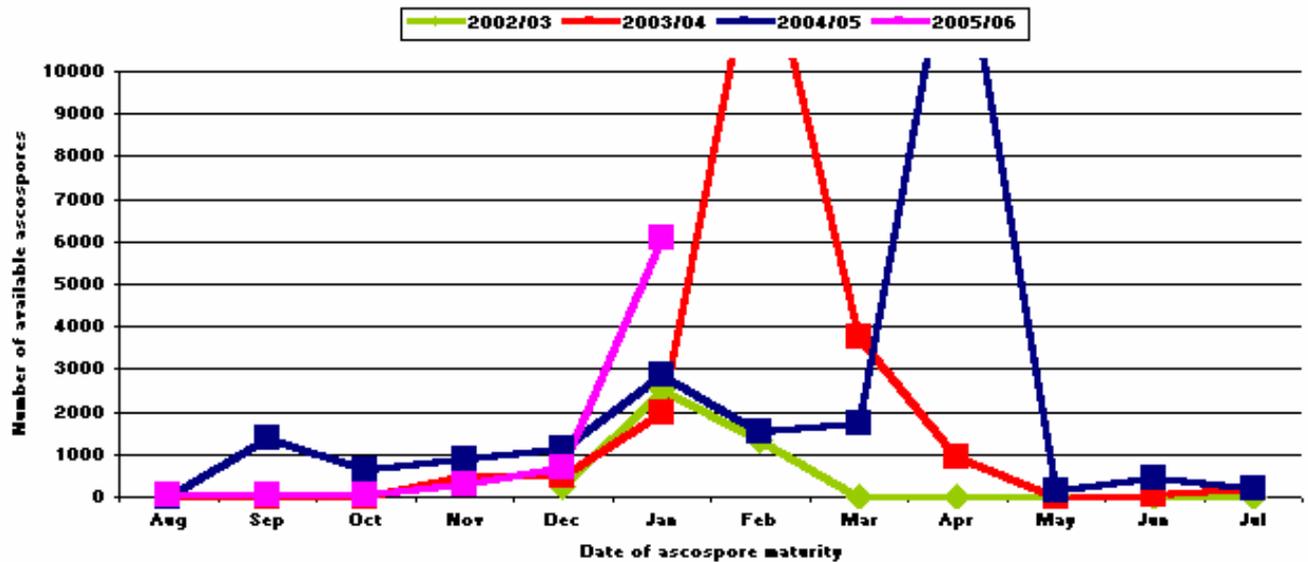
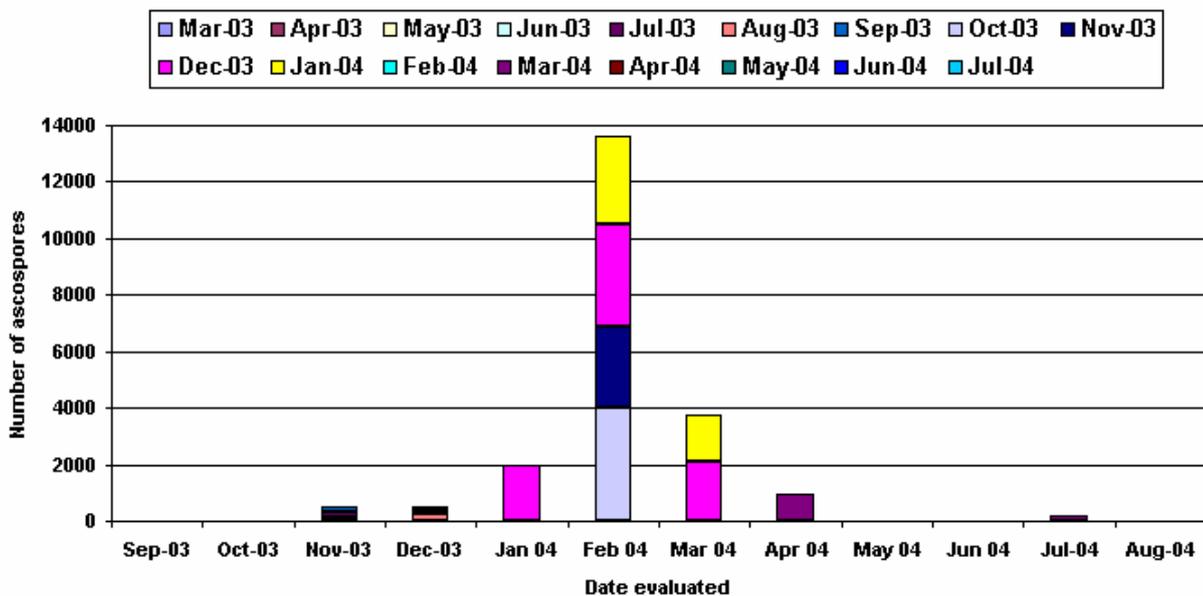


Figure 4.3.8.2. The seasonal total number of mature ascospores found monthly on leaf samples at Letaba Estates representing leaf sources that dropped during the preceding 6 months.

Data sets for 2003/04 and 2004/05 will be discussed regarding the leaves on the orchard floor responsible for the majority of inoculum production and the time needed to produce perithecia with mature asci. Results for the 2003/2004 season show that it took more than 2 - 6 months to produce mature asci on leaves in November and December (Fig. 4.3.8.3). However, leaves that were picked in December made a



significant contribution to the available ascospores in January, that is within one month.

Figure 4.3.8.3. The month in which leaves were picked contributing to inoculum production at a specific time during the 2003/2004 season at Letaba Estates (colour represent month in which leaves were picked according to legend).

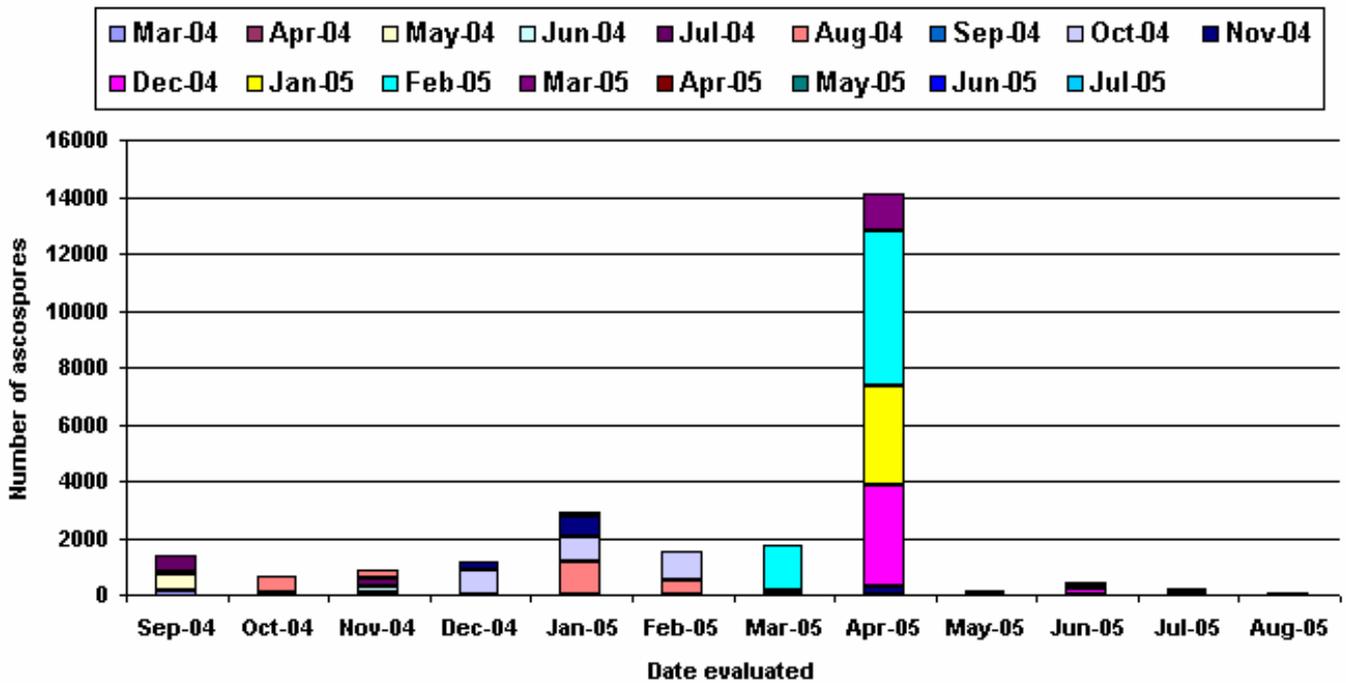


Figure 4.3.8.4. The month in which leaves were picked contributing to inoculum production at a specific time during the 2004/05 season at Letaba Estates Estates (Colour represent month in which leaves were picked according to legend).

The peak of ascospores that was produced in February 04 was on leaves that were picked in October, November, December and January. In March leaves that were picked in December and January were responsible for the main inoculum source (within 2 – 3 months) and in April leaves that were picked in March was the main source of inoculum (within 1 month). During the 2004/05 season a slightly different observation were made (Fig. 4.3.8.4). The mature ascospores that were already available in September were mainly produced on leaves picked in March, May and July, which means within 2 to 6 months. Leaves picked in August contributed to ascospore inoculum from October until February, which is over a period of 2 to 6 months. Inoculum during the January peak was mainly produced on leaves picked in August, October and November. On Letaba Estates the main peak of inoculum was produced during April 05 on leaves picked during December, January, February and March. Results indicate that the time it took to produce mature perithecia on leaves on the orchard floor varied from season to season and that the majority of inoculum was normally produced within 4 to 6 months during cool, dry periods and 1 to 3 months during hot, wet periods.

Climatic data, especially rain, appeared to be correlated with the seasonal number of ascospores produced as well as the time it took for leaves to reach a sufficient level of compostation in order to support perithecial development and ascospore production. Therefore, as a preliminary observation, the correlation between rainfall and ascospore production are shown in Figures 4.3.8.5 and 4.3.8.6. Results show similar trends between the amount of rain (mm) and the number of ascospores produced in a specific month (Fig. 4.3.8.5).

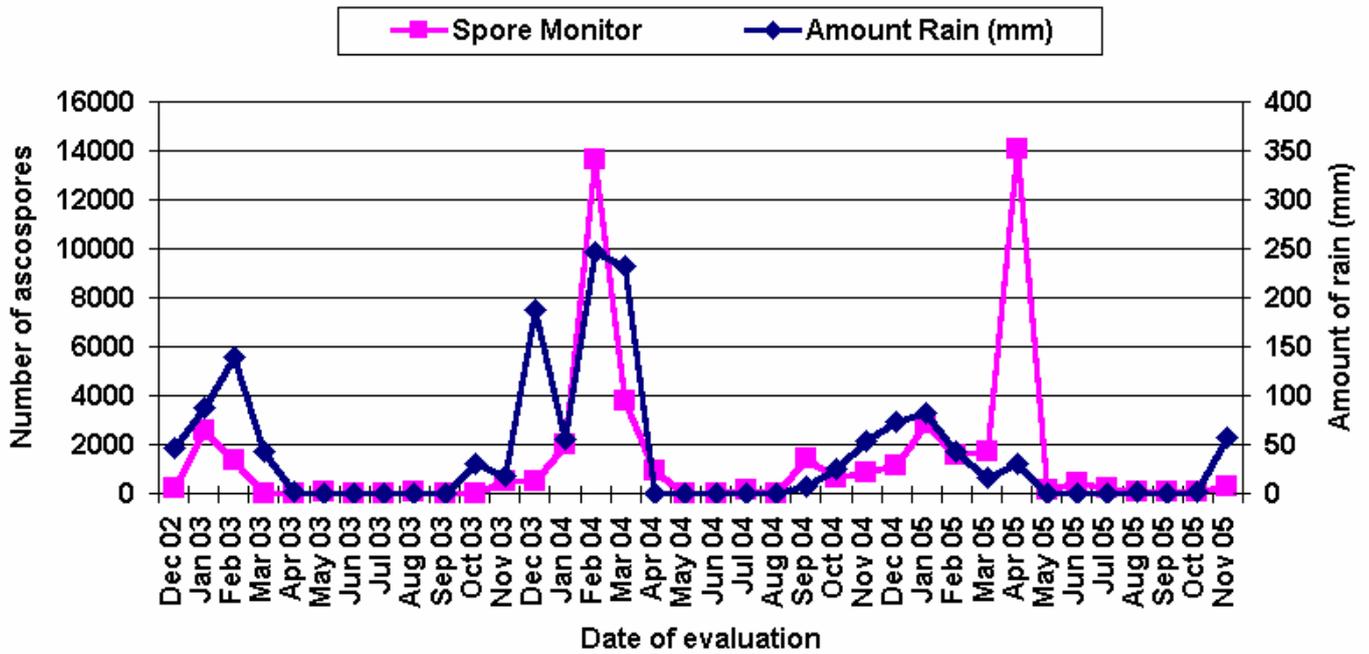


Fig. 4.3.8.5. Number of ascospores produced per month compared to amount of rain (mm) measured during that month.

The number of rainy days seem to have a better correlation with ascospore production (Fig. 4.3.8.6). Even more so the number of rainy days during the preceding three months. In setting up a model for potential ascospore production one should probably look at the number of rainy days during the previous 3-month period and in that also determine what would qualify as a rainy day.

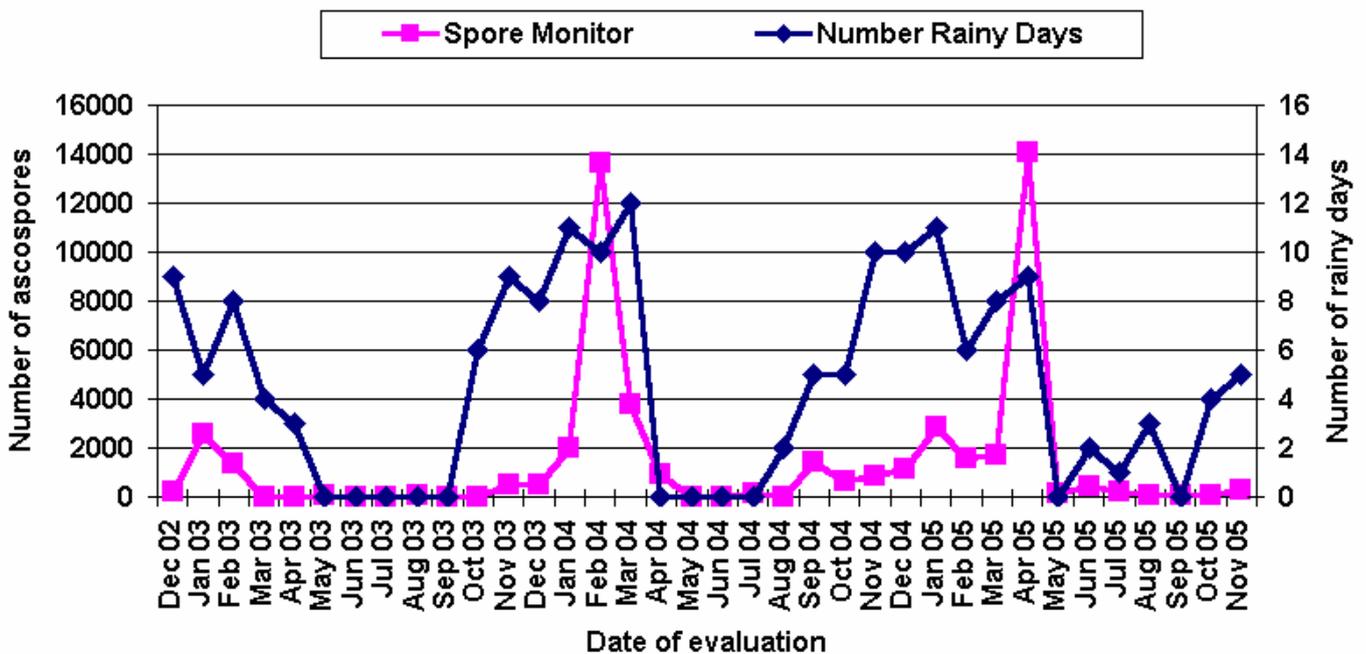


Fig. 4.3.8.6. Number of ascospores produced per month compared to the number of rainy during that month.

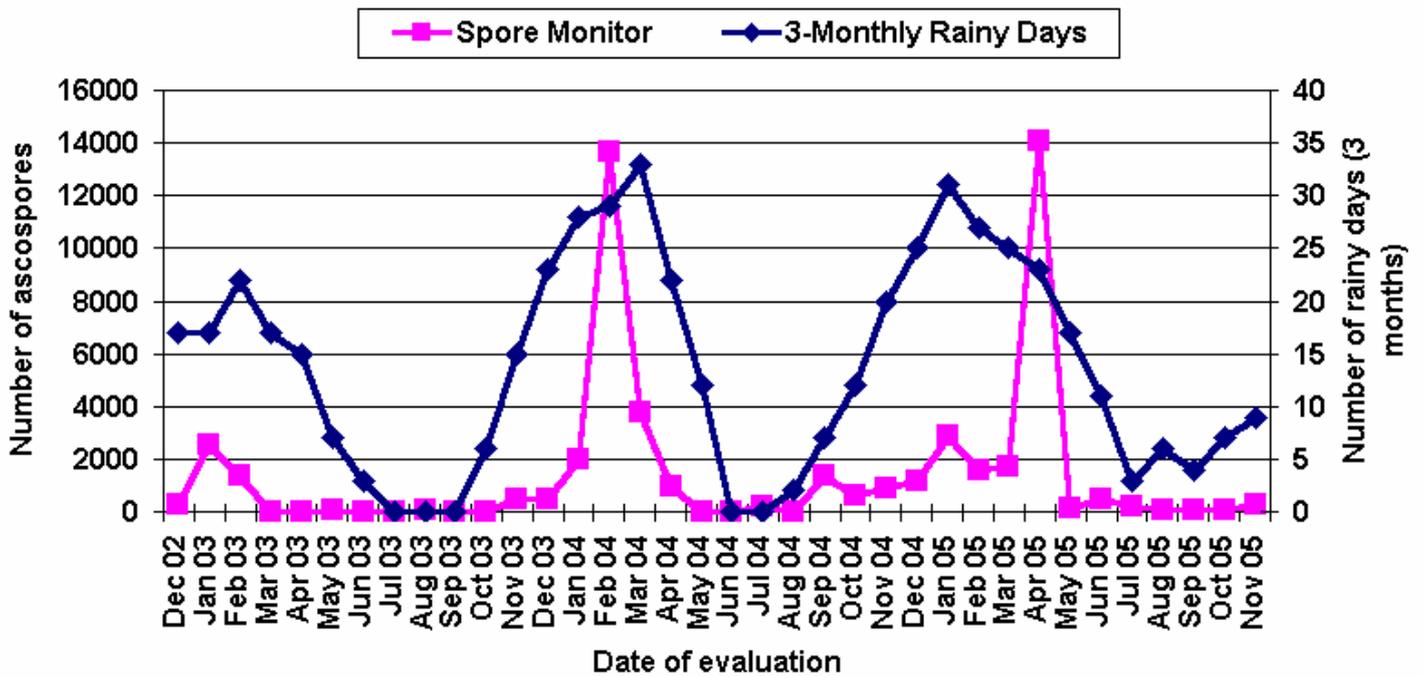


Fig. 4.3.8.7. Number of ascospores produced per month compared to the number of rainy days during the previous 3 month period.

Rain, however, is not the only parameter playing a role, and we can assume that other parameters like temperature, relative humidity, microclimate and composition and level of micro-organisms involved in the composting process, can play a significant role in the compostation process of leaves on the orchard floor. The latter may be the reason for variability amongst replicates in one orchard. From the results at Letaba Estates, depending on climatic conditions, it appears to take naturally infected detached leaves between 1 and 6 months to serve as substrate for inoculum production. During the dry, cooler winter months (May to July) it can take between 4 and 6 months to produce perithecia and ripe ascospores which normally serve as early source of inoculum during September and October. Leaves that became detached between August and March normally took between 2 and 3 months to produce perithecia and ripe ascospores. However, ascospores could also be produced within 1 month from leaves that was picked from December to March, contributing significantly to inoculum production. It generally appears that the majority of ascospores are produced within 4 months during periods when rain occurs frequently. During periods when conditions are drier, and probably cooler, it seems to take longer (4 to 6 months). In most cases leaves were totally composted after 6 months of exposure on the orchard floor.

Letsitele area

The total number of ascospores counted on leaf samples picked since May 2004 and evaluated until Jan 2006 were 5 082, 6 273 and 11 757 for tree 1, tree 2 and tree 3 at Letsitele. Therefore, approximately twice the number of ascospores were produced on leaf litter under tree 3, compared to trees 1 and 2.

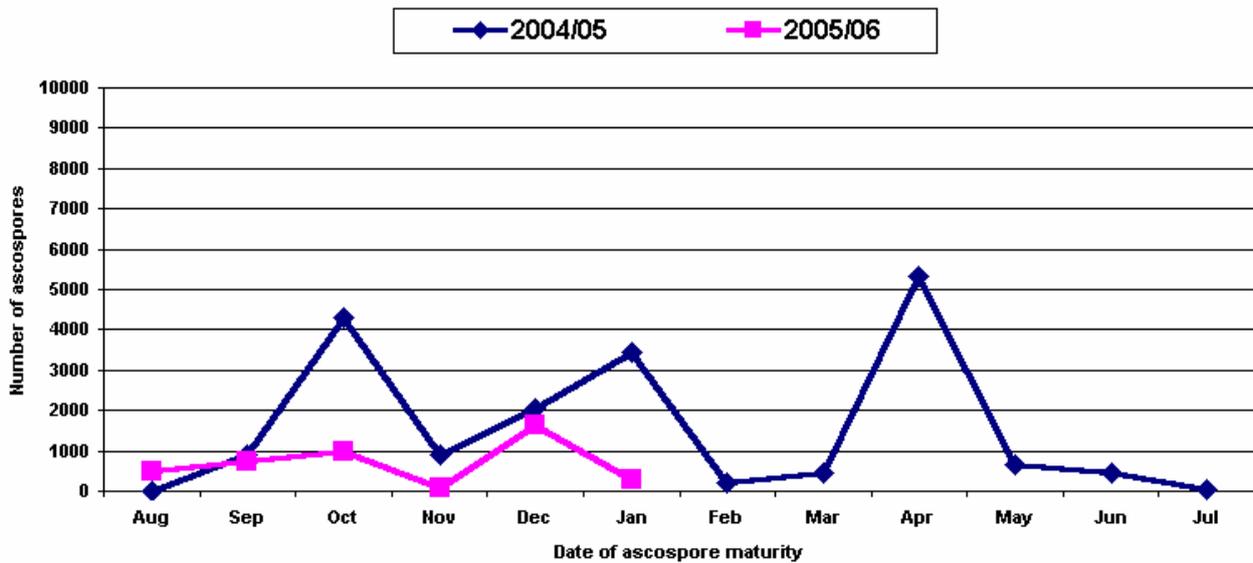


Fig. 4.3.8.8. The seasonal total number of mature ascospores found monthly on leaf samples incubated at Letsitele representing leaf sources that dropped during the preceding 6 months.

However, leaf samples under tree 3 did not always produce more inoculum than the other two trees. The first leaves were picked in May 2004 and data from August 04 until November 05 are presented. Results follow a pattern very similar to that of data obtained at Letaba Estates, with mature ascospores available from September until April (Fig. 4.3.8.8). A peak was observed in September, similar to that at Letaba Estates, but an additional high peak occurred in October (more than 4000 ascospores). From December to January there was a gradual increase in inoculum, with few spores in February and March and a large peak in April 05. Inoculum in September was produced on leaves picked in May, June and July, that is within 2 to 4 months (Fig. 4.3.8.9). The peak in October was produced on leaves from June to September, that is, within 1 to 4 months. Leaves that were picked in May and June produced mature asci after 4 to 6 months on the orchard floor. The period needed for ascospore production became shorter when climatic conditions became warmer and rainfall more frequent. Leaves that were picked in August produced spores within 2 to 5 months while leaves that were picked in February attributed to the majority of inoculum produced in April 05, that is within 2 months.

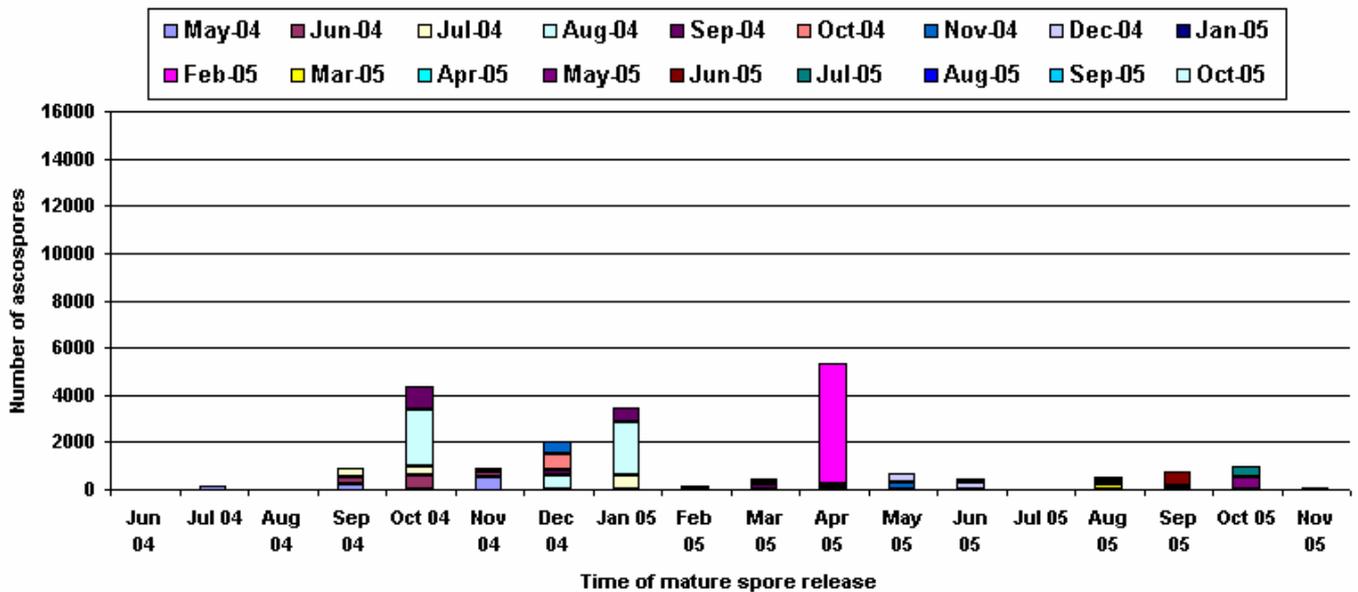


Fig. 4.3.8.9. The month in which leaves were picked contributing to inoculum production at a specific time during the 2004/05 season at Letsitele.

Hoedspruit area

The total number of ascospores counted on leaf samples picked since May 2004 and evaluated until Jan 2006 were 4 564, 2 214 and 3 454 for tree 1, tree 2 and tree 3 at Hoedspruit. The difference in total number of ascospores produced under the three replicate trees were not so pronounced compared to Letaba and Letsitele. However, variation between replicates representing a specific period was also large and inoculum production varied considerably under the three replicate trees. The first leaves were picked in May 2004 and data from August 04 until Nov 05 are presented (Fig 4.3.8.10). During both seasons of observations mature ascospores only became available in December. There was a gradual increase and significant numbers of ascospores were produced until April. However these numbers never exceeded 1500 ascospores and therefore, were much lower than the numbers observed in the Letsitele or Letaba areas. In Hoedspruit some inoculum levels, comparable to that observed in December was also produced during July. The source of inoculum production from this area is shown in Figure 4.3.8.11.

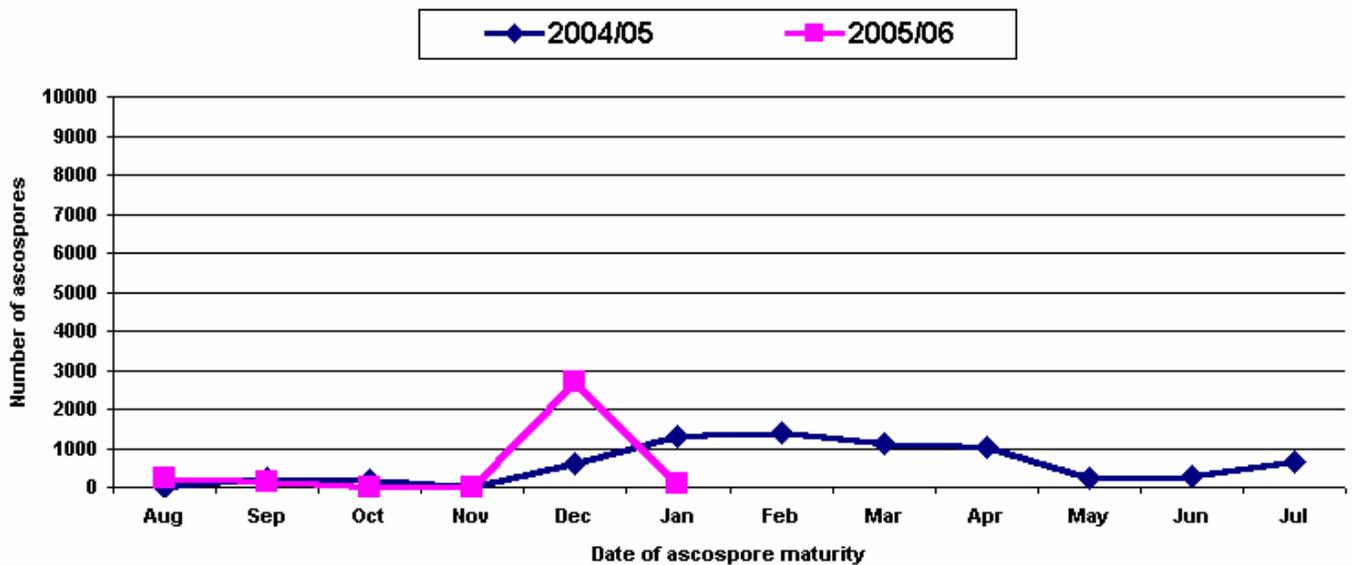


Fig. 4.3.8.10. The seasonal total number of mature ascospores found monthly on leaf samples incubated at Hoedspruit representing leaf sources that dropped during the preceding 6 months.

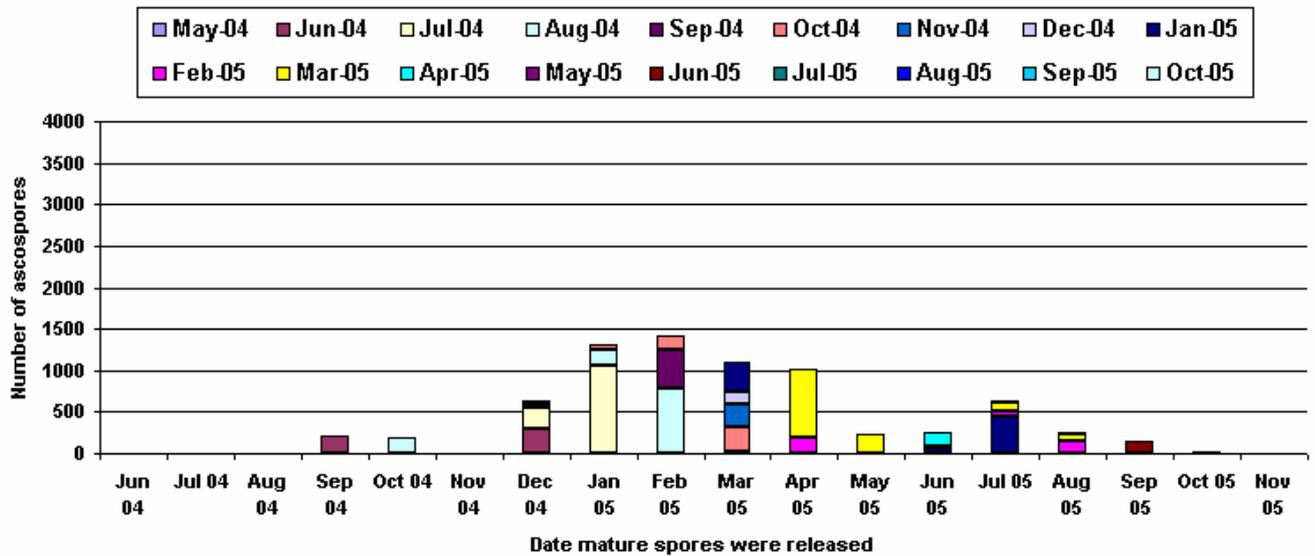


Fig. 4.3.8.11. The month in which leaves were picked contributing to inoculum production at a specific time during the 2004/05 season at Hoedspruit.

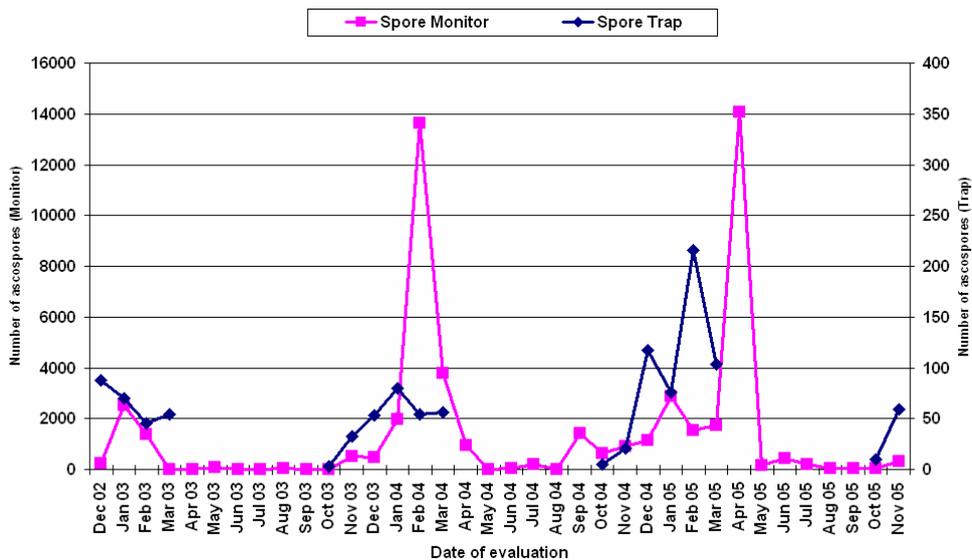


Fig. 4.3.7.12. Trends over three seasons regarding ascospore production on leaf litter on the orchard floor and airborne ascospores caught in a traditional spore trap.

The first significant inoculum levels were produced in December on leaves picked in June and July, that is within 5 to 6 months. Leaves picked in July significantly contributed to inoculum production up to January. Also leaves picked in August produced inoculum within 2 to 6 months. This period was shortened to 2 to 4 months for inoculum production in February, March and April. Inoculum produced in July was mainly on leaves pricked in January, that is within 6 months.

Conclusions

In general, it appears that ascospores can be produced on leaves that drop throughout the year. However, the major portion of inoculum is produced between September and April on leaves that drop between May and March. Leaves take longer to support ascospore production during the cool, dry winter months than the hot, wet summer months. The ability of infected leaves to produce viable ascospores primarily depends on the infection status of the leaves. However, climate, especially rainfall (number of days with rain) and temperature, play an important role, especially regarding the speed of the compostation process of leaf litter on the orchard floor. Normally most leaves were totally decomposed within 6 months after detachment. During cool dry winter months it takes 4 to 6 months to produce mature asci on leaf litter. During the early stage of the rainy season in August to October this period is shortened to 3 to 4 months. During the peak ascospore production period, January to April, it can take between 1 and 3 months to produce ascospores on leaf litter in most areas. We presume that the type of irrigation, composition of micro-organisms and microclimate in the orchard can have a significant effect on the composting process of leaf litter and ultimately production of inoculum by *Guignardia* spp.

In order to reduce disease pressure the removal of leaf litter can probably be useful early in the season (August, September) when extended periods are needed to produce inoculum due to the cooler and drier climatic conditions, especially after severe leaf drop, due to natural causes or after hail or pruning. However, during warmer, wet conditions the process of compostation on the orchard floor and inoculum production is very rapid and constant removal of the inoculum source might not be practical, especially if inoculum is produced within 1 or 2 months. It might be beneficial to look at other means of concentrating leaf drop or to accelerate the compostation process. The use of fungicides or physical barriers to contain inoculum on the orchard floor during high pressure periods should also be investigated, especially late in the season when spray residue on fruit can become risky. This is also very applicable on multi-crop cultivars such as lemons, that have small, very susceptible fruit during February and March when fungicide spraying cannot be done on the mature crop due to fungicide withholding periods that should be adhered to.

The general phenomenon of inoculum production and ascospore dissemination from November until March has been demonstrated by QMS Agri Science over a 5-year period of monitoring spore release in the Letaba, Letsitele and Hoedspruit areas. Figure 4.3.7.12 shows trends in ascospore releases as determined with the ascospore monitor on leaf samples in the laboratory and under natural conditions, with a spore trap in an orchard. Data for the spore trap and ascospore monitor show very similar trends over three seasons, however, data for the spore trap is normally gathered only from the beginning of October until the end of March. Therefore, until now, the large number of inoculum produced until April has gone undetected. The availability of inoculum until April is a point of concern since fruit are normally only protected until the end of January and under high disease pressure conditions until the end of February. Climatic data showed that infection periods can occur late in February and even until middle March. Based on this data and traditional strategies, it is customary practice to protect fruit from early fruit set, when the first climatic conditions conducive to infection, normally occur (middle October). Therefore, it is of the utmost importance to get clarity on acquired resistance of fruit to infection by *G. citricarpa*, due to maturity, in order to determine until when fruit must be protected.

This project reveals some information over 3 years for high pressure disease areas regarding ascospore production during relatively dry conditions. Unfortunately, due to the lack of funding this project cannot continue and I believe valuable information regarding ascospore production during wet seasons will be lost.

Reference cited

Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Disease* 65: 945-950.

4.3.9 *In vitro* production of *Guignardia citricarpa* ascospores Experiment QMS 2005/09 by S. Serfontein and S.H. Swart (QMS)

Opsomming

Guignardia citricarpa veroorsaak swartvlek by sitrus. Die hoof inokulum bron vir infeksies is luggedraagde askospore wat op droë blare ontwikkel. Hierdie swam groei maklik op media, maar produseer slegs piknidiospore. Die produksie van askospore deur 'n geïdentifiseerde *G. citricarpa* is nodig vir epidemiologiese studies van sitrus swartvlek en vir vinnige evaluasie van swamdoder effektiwiteit.

'n Totaal van 35 media is ge-evalueer, maar geen askospore is op enige van die media deur *G. citricarpa* geproduseer nie. Piknidia en piknidiospore is egter deur al die *G. citricarpa* isolate gevorm. Al die *G. mangiferae* isolate het askospore gevorm.

Dit is maklik om die endofietiese *G. mangiferae* van die patogeniese *G. citricarpa* op 'n medium met 0,5 % mout ekstrak, 1,5 % agar met 'n pH van 5.6 van mekaar te onderskei, aangesien die kulture onderskeidelik swart vir *G. mangiferae* en swart met 'n groen skynsel is vir *G. citricarpa* is. Beide spesies produseer groot hoeveelheid piknidiospore op hierdie medium.

Introduction

Media supporting ascospore production by *G. mangiferae* and by *G. citricarpa* will assist research considerably. Infection on fruit, according to literature, is primary caused by the airborne ascospores of *G. citricarpa*, therefore mass production of ascospores is essential for epidemiological studies and will also be useful for the value for the evaluation of fungicides. The objective of this study was to develop a medium to initiate the production of ascospores by isolates of *G. citricarpa*.

Materials and methods

Isolates: Typical isolates of *Guignardia* spp. were obtained from lemon and orange fruit showing citrus black spot (CBS) symptoms. These isolates were characterised with PCR by the Department of Microbiology and Plant Pathology, University of Pretoria. Six of the isolates were identified as *G. citricarpa* and six as *G. mangiferae* (Table 4.3.9.1).

Media preparation: Leaf extract malt agar (LEMA) as described by Lemir *et al.*, 2000 was supplemented with different concentrations of Lemon leaf extract (0, 25, 50, 100% v/v) and four concentrations of malt extract (0.5, 0,1, 0,2 and 0,5% g/v) in different combinations (Table 4.3.9.2). In total 28 different combinations were prepared. Leaf extract was prepared by boiling 30 g Lemon leaves (cv. Eureka) or 30 g of Navel leaf litter collected in a biological plot (Letaba) in 1 l of filtered water, for 20 min. After sieving, the extract was diluted in filtered water to the required.

Table 4.3.9.1. Isolates used for the evaluation of media for ascospore production.

Isolate no.	PCR Result	Origin of isolate
1	<i>G. citricarpa</i>	Letsitele, leaf
2	<i>G. mangiferae</i>	Letsitele, leaf
3	<i>G. mangiferae</i>	Fruit
4	<i>G. mangiferae</i>	Letsitele, leaf
5	<i>G. citricarpa</i>	Leaf spot
6	<i>G. citricarpa</i>	Letsitele, leaf
7	<i>G. mangiferae</i>	Letsitele, leaf
8	<i>G. citricarpa</i>	Letsitele, leaf
9	<i>G. mangiferae</i>	Letsitele, leaf
10	<i>G. citricarpa</i>	Letsitele, leaf
11	<i>G. mangiferae</i>	Letsitele, leaf
12	<i>G. citricarpa</i>	Letsitele, leaf

Concentrations. Malt extract at the different concentrations and agar (15 g/l) were added and the pH was adjusted to 5.6 with NaOH solution. The media was autoclaved and approximately 20 ml was aseptically poured in petri dishes. The different combinations of media were numbered 1-28.

Table 4.3.9.2. Ingredients used in the media tested for ascospore production.

% Organic material used	Percentage malt extract used in a medium			
	0,05	0,1	0,2	0,5
0	1*	2	3	4
25 % Navel leaf litter extract	5	6	7	8
50 % Navel leaf litter extract	9	10	11	12
100 % Navel leaf litter extract	13	14	15	16
25 % Lemon leaf extract	17	18	19	20
50 % Lemon leaf extract	21	22	23	24
100 % Lemon leaf extract	25	26	27	28

*For each medium 15 g/l agar was used and pH was adjusted to 5.6. Media numbers assigned to combinations of organic matter and malt used in a medium. (Read down and across the respective connecting columns of a specific medium.)

Different plant material, as well as autoclaved carnation leaves was placed on water agar (15 g agar/l, pH 5.6). This was numbered 29-32 (Table 4.3.9.3). Water agar with carnation leaves and the pH not adjusted, were also included (medium 33) (Table 4.3.9.3). Autoclaved pieces of Navel leaf litter and Lemon leaves were placed on medium containing malt extract (medium 4, Table 4.3.9.2) (Timossi *et al*, 2003, modified). These media were media 34-35 (Table 4.3.9.3).

Table 4.3.9.3. Solid media with two pieces of plant material, to promote ascospore production.

Autoclaved plant material	Medium used as base	Medium number
Navel leaf litter	Water agar (pH 5,6)	29
Lemon leaves	Water agar (pH 5,6)	30
Lemon twigs	Water agar (pH 5,6)	31
Navel twig litter	Water agar (pH 5,6)	32
Carnation leaves	Water agar	33
Lemon leaves	Medium 4*	34
Navel leaf litter	Medium 4	35

*Medium 4 from Table 4.3.9.2.

Inoculation of media: Plates were inoculated by placing a 4 mm² block of a 5-day-old *Guignardia* culture, grown on potato dextrose agar, in the centre of the plate. All the isolates (Table 4.3.9.1) were inoculated in triplicate on media 16, 24, 29, 30, 31, 32, 33, 34 and 35 (Tables 4.3.9.2 and 4.3.9.3). Isolates 2, 6, 8 and 12 (Table 4.3.9.1) were inoculated in triplicate on all the media (Tables 4.3.9.2 and 4.3.9.3).

Incubation: All cultures were grown at 26°C, 12 hour alternating light and dark periods for 17 days. One set of plates of medium 12 was removed after 8 days and placed in the dark at 6°C for 21 days (Lemir *et al.*, 2000).

Evaluation: After 17 days the diameter of the colonies were measured, the colour noted and the fruiting structures examined microscopically to determine the presence of either perithecia with ascospores or pycnidia with conidiospores.

Results

After 17 days all the *G. mangiferae* isolates produced superficial multi porous perithecia with ascospores on all media, except media 17 and 18 on which small colonies developed. Abundant ascospores were produced on medium 4. On medium 33, isolate 7 produced ascospores as well as pycnidiospores.

All the *G. citricarpa* isolates produced embedded pycnidia on all media. Isolates 5, 6, 8, and 10 also produced spermatia on medium 35 after 17 days of incubation. After an additional 10 days, spermatia could be found on all the media. No ascospores were produced on any of the media by these *G. citricarpa* isolates, even after an additional two weeks (41 days from inoculation) incubation.

Ascospore producing *G. mangiferae* cultures were black and fast growing compared to the pycnidiospore producing *G. citricarpa* isolate which were black with a green tinge, and slower growing.

Table 4.3.9.4. The average diameter of colonies on different media 17 days after inoculation.

Medium	Size of colonies in mm			
	<i>G. citricarpa</i> , 6	<i>G. citricarpa</i> , 8	<i>G. mangiferae</i> , 2	<i>G. mangiferae</i> , 11
1	25	25	50	50
2	32	30	55	75
3	40	58	80	80
4	80	80	80	80
5	25	45	50	58
6	30	60	55	55
7	45	70	80	75
8	60	60	80	80
9	25	25	35	40
10	26	45	45	45
11	33	32	45	84
12	51	75	80	80
13	22	25	33	42
14	32	34	45	45
15	53	35	50	60
16	73	70	80	80
17	18	5	10	8
18	15	10	0	5
19	48	60	53	58
20	55	70	80	80
21	30	40	40	48
22	50	55	65	58
23	42	65	60	65
24	53	35	50	60
25	7	11	25	20
26	7	12	34	23
27	8	12	38	23
28	11	16	43	28
29	12	11	23	25
30	8	14	12	15
31	35	35	33	30
32	8	14	12	33
33	25	25	34	31
34	55	58	60	58
35	50	54	43	42

Media 3 and 4 supported vigorous and fast growth with all cultures, the cultures had dense mycelium and were dark compared to cultures on media 1 and 2 which were slower growing and the mycelium were noticeably less dense. The addition of the leaf litter extract (media 5 – 16) seems to slow growth and the mycelium in the cultures was also less dense than that in medium 3 and 5. The addition of the leaf extract (media 17-28) also slowed the growth of the colonies but all the colonies on these media had very dense mycelium and a dark appearance (Table 4.3.9.4). When plant material was placed on the solid media, it also slowed the growth of the cultures (media 34 and 35). The spore producing structures were evenly

distributed and not confined to the plant material. *G. citricarpa* cultures on medium 12 placed in the dark, also only produced conidiospores. Colony diameter on the different media after 17 days of growth are given in Table 4.3.9.4.

Discussion

None of the media evaluated promoted *G. citricarpa* to produce ascospores. Ascospores are most probably formed when conditions are adverse for growth or when nutrients are depleted. In nature, ascospores are formed on leaf litter at a certain stage of compostation, especially under warm and wet environmental conditions (Kotzé, 1981). We aimed to use this knowledge to stimulate spore production. It can be speculated that the leaf litter used was not in the right stage of compostation to stimulate the formation of ascospores. Incubation conditions could also have played a role in that conditions might have been too conducive for optimal growth to trigger the production of ascospores.

When leaf and leaf litter extract were added to medium (media 8, 12, 16, 28, 34 and 35), the cultures grew slower than on medium with the comparable amount of malt extract (medium 4). The same tendency was observed when plant tissue was placed on solid media (media 34 and 35). It can be that substances in the plant tissue suppress the growth of the fungus and therefore, plant material must be "treated" or aged in order to reduce this toxic or suppressive substances.

The original aim of this project, the production of ascospores in media by *G. citricarpa*, was not achieved. However, the media consistently differentiated the 6 *G. citricarpa* isolates from the 6 *G. mangiferae* isolates with the later producing ascospores and *G. citricarpa* not. This differentiation takes 2 weeks but may prove to be a valuable tool to identify *G. citricarpa* in unsophisticated laboratories with no PCR facilities. The colony colour also differed with *G. citricarpa* producing green tinted colonies and *G. mangiferae* producing black colonies. A larger number of isolates of each species will have to be tested on the media before a general conclusion in this regard can be made. Of the 35 media tested, medium 4 showed the best potential as differential culture medium. Abundant conidiospores were produced by all *G. citricarpa* and ascospores by all *G. mangiferae* isolates on this medium.

It is unfortunate that this project has to be terminated at this stage due to a lack of funding. The production of ascospores by defined pathogenic strains of *G. citricarpa* remains a priority for epidemiology studies. If ascospores are available the optimum temperature, wetting period and amount of spores required for infection can be determined more accurately. In order to develop a model for citrus black spot predictions this information is crucial. Equally important is the survival of inoculum (ascospores) in such a model in order to determine how long inoculum can survive adverse conditions (dry periods, high and low temperatures). *In vitro* production of ascospores is also a crucial for a technique to screen fungicide efficiency quicker and more cost effective than the current orchard trials where disease cannot always be guaranteed.

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4.3.10 **Detection of *Guignardia* from leaf litter and symptomless leaves and fruit with CBS PCR** Experiment PPL 4 by L. Meyer and L. Korsten (UP)

Opsomming

Skoon kwekerymateriaal is van kritiese belang wanneer nuwe boorde gevestig word en vir aanplantings in swartvlek-vrye areas. Die voorkoms van CBS askospore in kwekerye word suksesvol mbv spoorvangers en inokulum monitormeters gedoen, maar onderskeid kan steeds nie getref word tussen *Guignardia citricarpa* en *G. mangiferae* nie. Die CBS-PCR tegniek is aangepas om latente infeksies in groen, simptomeelose kwekerye en

boord sitrusblare op te spoor. Dit behels 'n verrykingsproses waartydens geplukte groen blare aan daaglikse nat-droë toestande onderwerp word met kort periodes van direkte sonlig. Die ontwikkeling van die vrugliggame word op die manier gestimuleer en daaropvolgens kan die PCR proses met groter akkuraatheid gebruik word. Resultate dui op 'n besonders hoë sensitiwiteit. *G. citricarpa* en *G. mangiferae* kan gelyktydig vanuit 'n enkele blaarmonster van minder as 5 mg opgespoor word. Die protokol van monsterneming en voorbereiding is verder verfyn vir vinniger en meer koste effektiewe analisering.

Introduction

On the request of the citrus industry the focus of this phase was shifted to the detection of *Guignardia* species on leaf litter and symptomless leaves and fruit with CBS PCR. The objective of this study was to compare the most common elements of DNA extraction and purification protocols from leaves and to use the information obtained to develop a comprehensive method for obtaining GC DNA from different types of leaf samples (i.e. green, dry, litter etc.). Molecular methods were expected to give a more realistic view of the presence of the pathogen. DNA-based methods do not depend on the culturability of microorganisms and therefore they offer an attractive alternative for the study of complex fungal community structures.

Materials and methods

1. Sample preparation
Leaf samples are subjected to daily wetting and drying and short exposures to direct sunlight. 20-25 leaf punches ((Harris UNI-CORE 1.5 mm) are used per sample.
2. DNA extraction
Use standard protocol for DNA isolation (Manual: Qiagen DNeasy Plant Mini kit, p19-21). Store at –20°C.
3. PCR amplification
Standard protocol.
Positive controls: Isolate 1 (*Guignardia citricarpa*) and 2 (*G. mangiferae*).
Negative controls: Col326 (*Colletotrichum gloeosporioides*) and dH₂O.
PCR program on Eppendorf machine: citrus/general/citrcam.
4. Gel electrophoresis
Standard protocol.

Results and discussion

The maturation process of green detached leaves to enhance the production of fruiting bodies proves to be exceptional. The maturation steps consist of daily wetting and drying of the leaves and short exposures to direct sunlight. The enhanced production of fruiting bodies improves the detection probability of the PCR process. The DNeasy DNA extraction kit in conjunction with the FastPrep Instrument to extract DNA of the *Guignardia* citrus pathogen from dry and green leaves is rapid and steadfast. The procedure in its entirety is sensitive and well-defined.

Future research

No funds allocated for 2006-7.

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4.3.11 **Seasonal availability of ascospore inoculum of *Guignardia citricarpa***

Experiment PPL 12 by M Truter and L Korsten (UP)

Opsomming

Die beskikbaarheid en rypwording van *Guignardia citricarpa* askospore is ondersoek in twee Eureka lemon boorde ongeveer 10 km uitmekaar in Brits gedurende 2005. Natuurlike blaar afval is maandeliks versamel en die beskikbare ryp askospore op die materiaal gevang op a Vaseline bedekte mikroskoopplaatjie d.m.v. 'n Kotzé Inokulum Monitor. Natuurlike geïnfecteerde blaar afval het gemiddeld 0 tot >8000 askospore gelewer per 20 blare. Akkurate tellings van askospore bo 8000 was nie moontlik nie. Askospore is gevang van blaar molm wat tydens Oktober tot April versamel is. Die tydperk waartydens askospore gevang is, stem ooreen met die data wat gedurende 2004 versamel is. Die ondersoek sal voortduur tot aan die einde van die somer waarna data van September 2003 tot April 2006 opgeskryf sal word vir 'n publikasie.

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 perithecia 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 Interlock Systems. 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 Interlock Systems in collaboration with Prof J.M. Kotzé to determine the inoculum present in samples of leaf material at any given time (Truter *et al.*, 2004). Information obtained using the new Kotzé Inoculum Monitor (KIM) 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 Eureka lemon leaf litter during 2005 was assessed from two farms in Brits, ca. 10 km apart and will continue until April 2006 with the aid of a KIM. The availability of ascospores was determined by collecting natural leaf litter at a monthly interval and processing it with the KIM. Selected leaf litter was secured between two circular plastic grids (10 mm mesh size) with three replicate grids per sample and stored at room temperature until processed for spore capturing. A grid with leaf litter was submersed in water at 40°C for 5 min, placed on paper towels for 5 min to remove excess water, and placed in the KIM. A standard microscope slide, coated with a thin layer of Vaseline, was used to collect spores. After the two-hour KIM 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.

Results and discussion

Natural leaf litter contained zero up to >8 000 ascospores upon collection as determined with the KIM (Table 4.3.11.1). Ascospores were captured from leaf litter that were collected between October and April. Large variation in available ascospores occurred between replicate grids from the same sample as well as between farms in the same area on the same sampling date (results not shown).

The average ascospore numbers captured were higher on these two farms than those from Burgersfort collected previously (results not shown). This may be due to the warmer climate in Brits compared to Burgersfort. We are in the process of correlating data of ascospore capturing with climate data over the collection periods. These will be presented later in an article.

Recent investigations showed that ascospores of *Guignardia mangiferae* A.J. Roy were released during similar periods when *G. citricarpa* spores are abundant. There is no technique yet to tell the difference between ascospores of the two species, although the presence or absence of each can be determined by PCR. The results above must be seen as the presence of *Guignardia* ascospores and do not necessarily reflect the situation of *G. citricarpa*.

Table 4.3.11.1. Ascospores captured on Vaseline coated microscope slides with the Kotzé Inoculum Monitor from Eureka lemon leaf litter collected from the orchard floor at two farms ca. 10 km apart near Brits.

Collection month	Ascospores captured ^a	
	Farm 1	Farm 2
January	3253	6942
February	5965	>8000
March	3807	5890
April	853	1218
May	0	0
June	0	0
July	0	0
August	0	0
September	0	0
October	0	281
November	361	685
December	674	4230

^a Data represents the average ascospores counted from three replicate grids. Total ascospores were counted from four microscope field lanes per slide.

Future research

The 2005/2006 season was the last year of funding for PPL 12 and the study will be completed by April 2006 after which the data from September 2003 till April 2006 will be incorporated in a publication.

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4.3.12 **Breaking the life cycle of *Guignardia citricarpa*: Removal or confinement of inoculum** Experiment PPL 13-A by M Truter and L Korsten (UP)

Opsomming

Verwydering of bedekking van die oorwinterende askospor inokulum van *Guignardia citricarpa* was ondersoek in 'n >35-jarige Valencia boord naby Burgersfort. Die behandelings is vir die derde agtereenvolgende jaar toegepas in dieselfde boord vanaf 2003 tot 2005. Al die blare op die boordvloer was met die hand verwyder en verbrand middel Oktober 2004. Agt aangrensende rye van 20 bome elk was gebruik vir die behandelings en het deur die jaar geen chemiese bespuiting vir swartvlek ontvang nie. Die boordvloer oppervlak van vier uit die agt rye was bedek met 'n laag koringstrooi. Die dooie blare was by die ander vier rye weer verwyder van die totale boordvloer 'n maand later. Die omliggende boorde was drie maal met aanbevole chemiese middels gespuit vanaf Oktober 2004 tot Januarie 2005. Evaluering van die boorde tydens die opeenvolgende seisoen het 'n sitrus swartvlek insidensie op die vrugte aangedui van 0.70% in die strooi bedekte rye en 0.77% in die onbedekte rye. Die insidensie op die vrugte by die strooi bedekte bome is

laer as die vorige jaar teenoor dieselfde vir die onbedekte bome. Geen sitrus swartvlek was sigbaar op vrugte van die chemiese behandelde boorde.

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 >35-year-old Valencia orange on Rough Lemon rootstock. All the leaves on the orchard floor were manually removed and burned in October 2004. A total of eight adjacent rows of 20 trees each were used and received no chemical spray for CBS. In late October, 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 was not removed. Trees were evaluated in August 2005. 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.

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

There was a very low disease incidence and no significant differences between treatments with a CBS incidence of 0.70% in the mulched rows and 0.77% in the unmulched rows (Table 4.3.12.1). No significant differences were evident between fruit borne within the canopy and on the outside (results not shown). Differences were evident between fruit of different aspects of the tree, with CBS occurring only on the northern and eastern side of the tree (results not shown). No CBS was present on chemically sprayed fruit in the adjacent orchards at the time of assessment.

Table 4.3.12.1. Incidence of citrus black spot in a citrus orchard where leaf litter was either removed or removed together with mulching with wheat straw.

Parameter	Mulched*	Unmulched*	Chemical sprayed*
Infected trees (%)	2.46	0.78	0
Infected fruit (%)	0.04	0.05	0
CBS-index	0.70	0.77	0

* Values do not differ significantly according to Fisher's protected *t*-test least significant difference ($P \geq 0.05$).

The airborne ascospores may infect fruit 500 meters or more from the inoculum source. To lay out a field experiment along statistical guidelines, is virtually impossible and the costs will be prohibitive due to fruit loss and the logistics of transporting straw and collecting fallen leaves. The experiment was initiated to show that ascospore inoculum can be reduced which will ease chemical programmes. This investigation showed that reduction of inoculum is a viable option. In theory, if entire citrus farms can apply practices of inoculum reduction, chemical spraying may be eliminated, depending on the inoculum index and climate.

Conclusion

Good control was achieved with inoculum removal and confinement as alternative to chemical control, although less efficient than chemical control. The available newly produced ascospores were reduced in the orchard by continuous leaf litter removal and almost no ascospores could be detected from the newly formed leaf litter with a Kotzé Inoculum Monitor.

Future research

The project ended in the 2005/2006 season. The data from 2002 till 2005 will be processed for publication in 2006.

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4.3.13 Development of a Citrus Black Spot (CBS) disease forecasting model Experiment PPL15 by C.M. van Ginkel (UP)

Opsomming

Die waarskynlikheid dat sitrus-swartvlek (*Guignardia citricarpa* Kiely) infeksies sal plaasvind hang af van die beskikbaarheid van ryp askospore; die plotselinge styging in relatiewe humiditeit; temperatuur; die beskikbaarheid van vogtigheid en die periode wat hierdie kondisies voortduur op die oppervlak van die gasheer. Suksesvolle infeksie sal ook slegs plaasvind indien die oppervlak van die sitrusgasheer waarop hierdie askospoor land nie beskerm word deur 'n stof wat die askospoor dood of inhibeer nie.

Omgewingsinligting is *in situ* oor die afgelope vier seisoene (2002-2006) in die Marble Hall sitrus-distrik ingesamel en in verband gebring met werklike spoorvystellings en daaropvolgende effektiewe infeksies. Die beskikbaarheid van ryp inokulum vroeg in die seisoen is met behulp van die Kotzé Inokulum Monitor (KIM) bepaal.

In die ontwikkeling van die voorspellingsmodel word hierdie omgewingsinligting, tesame met die groeistadium van die gewas, en die beskerming deur chemiese agente verleen gekoppel aan 'n weervoorspellingsdiens gelewer deur die Nederlandse **Dacom maatskappy** via die **Plant-PLUS** rekenaarprogram.

Introduction

This study was initially started in the District of Marble Hall because the resistance of *Guignardia citricarpa* Kiely (CBS) towards the chemical active ingredient benomyl was still negligible in the area. By careful timing and the elimination of unnecessary spraying the active life of this chemical (and others) could be prolonged. A cost saving would be an additional spin-off. Through the initiative of Mr. Anton Bredell (Lowveld Agrochem, Marble Hall) and the Vallei-adviesdiens a weather station network was established in order to facilitate the

development of a Disease Forecasting Model for CBS. It was anticipated to extend this model to other citrus producing areas, once developed.

The possibility for CBS to successfully infect citrus fruit or leaves is a function of the availability of ripe ascospores; the sudden rise in relative humidity; minimum temperature; moisture; and the duration of these conditions on the surface of host tissue. Furthermore, infection will only occur if the surface of the citrus host on which the ascospore lands is not protected by a fungicide or an inhibiting agent.

Environmental data were collected *in situ* over the past four seasons (2002-2006). The study focused on the citrus district of Marble Hall. This was correlated to real-time spore-trapping and the eventual development of disease symptoms on fruit and leaves. The availability of the first ripe inoculum early in the season was confirmed by the use of the Kotzé Inoculum Monitor (KIM).

In developing the disease forecasting model the following were integrated:

- The environmental data (as measured *in situ*);
- The growth stage of the citrus crop;
- Spore-trapping as measured in tandem with the environmental data;
- Protection rendered by applied chemical agents;
- A weather forecast service by the Dutch **Dacom** Company via the **Plant-PLUS** computer program.

Citrus producers in the district of Marble Hall that acted according to the guidelines presented over the past four seasons encountered few, if any, CBS blemishes on the packing lines for export. It was also possible for these producers to control the epidemic by two chemical sprays compared to three and even four preventative sprays that used to be the norm. The ultimate aim of the Disease Forecasting Model would be to accredit partaking orchards as being "Managed free of CBS".

Materials and methods

The principal study area is the District of Marble Hall with a radius of approximately 90 km. This incorporates the areas of Loskopdam, Groblersdal, Marble Hall, Toitskraal and Nutfield. This District was covered by five ADCON Meso-climatic weather stations as well as five crop specific Disease stations; the latter incorporating Austrian leaf-wetness sensors. All stations transmit "raw" environmental data by radio telemetry to a central hub connected to a computer in Marble Hall, once every 15 minutes. Raw data include date, time, air temperature, precipitation, relative humidity, wind speed, wind direction, and solar radiation. In addition to these the government weather stations at Marble Hall and at Groblersdal serve as back-up.

This *in situ* data can be studied as a multiple climatological graph on the computer program **Advantage**. This real-time data can be compared to actual *Guignardia citricarpa* Kiely ascospores trapped *in situ* by the Quest volumetric spore trap to an accuracy of 15-30 minutes linked events. Two Quest Volumetric Spore Traps are employed. Either a two- or an eight-day (spray-vaseline covered) sampling disk is used. This disc interfaces with a standard light microscope (Nikon YS 100) for identifying and counting of the sample. Air sampling takes place at 20 litres of air per minute. The orchard chosen as benchmark for ascospore trapping is central to the District, has an established history of CBS and is therefore ideally suited as an early warning site for CBS ascospore releases. The GPS (Garmin 5) established co-ordinates for the disease station and spore-trap is: S 25° 3' 38.9" and E 29° 16' 55.7" at an elevation of 937 m.

Photographic records of actual spores trapped, as well as observable infection symptoms on fruit are kept. Infection development time on fruit artificially inoculated by pycnidiospores will be compared to natural field infected symptom development.

Weather data from **Advantage** is transmitted every 90 minutes by modem to the Dacom program, **Plant-PLUS** to generate an individualistic five day weather prediction for all five Meso-climatic weather stations. This weather prediction is supported by a United States of America government owned weather satellite and updates can be obtained by modem at 180 minute intervals. **It is essential to link this weather prediction to the Disease Forecast Model in order to have any meaningful timely decision support.** The five day forecast is simultaneously an indication of expected spraying conditions, -evapo-transpiration, and -irrigation requirement.

A KIM is utilized to confirm the presence of early inoculum in collected leaf litter as well as in chemically treated leaf samples. The possibility of integrating the parameters of the developed model into the existing Plant-PLUS disease forecasting software arsenal is being investigated.

Results and discussion

As this is a progress report, focus will be on some fresh observations that emerged from the elaborate amount of direct data as well as from related experiments that were undertaken to fill in blanks over these four seasons. The main events of the past four CBS seasons are attached as Figs. 4.3.13.1 to 4.3.13.4.

The presence of inoculum

The presence of ripe CBS perithecia on decomposing leaf litter in an orchard equals an orchard with a CBS epidemic. The KIM offered a reliable and quick way to test this. In a separate trial in an orchard 3 km downwind, CBS was completely removed as detectable disease within three years of thorough leaf-litter removal in September-October (Mr. Johan Meyer, Vaalfontein, PPL 12 experiment) and no CBS chemical spraying is necessary at present. Likewise it was found that a hail event in October, enhancing the available leaf-litter inoculum, will influence the inoculum availability twelve months later. The very early availability of ascospores in 2005 (16/09/2005 detected by KIM) could be attributed to this. The first natural ascospore release for 2005/6 was recorded on 19/10/2005. This is more in tune with the historical norm, albeit a week early.

Triggering ascospore release

The environmental conditions found to be associated with an ascospore release amounts to this:

- Temperature: Above 18°C. The minimum temperature for the day was never found to be below 17°C. This correlation is evident from Figs. 4.3.13.1-4.3.13.4. The highest temperature at which spores were trapped was 32°C.
- Relative Humidity: A sudden rise in relative humidity (typically 10% or more) triggered the actual spore release. This sudden *rise* is more important than a very high relative humidity. This could mean spore release before the advent of the actual rain event. This would enable the ascospores to utilize the typical pre-precipitation turbulent winds for dispersal. This sequence was confirmed by careful interpretation of the spore disk, linked to environmental conditions measured in real time.

The strength of an ascospore release

The reliability of a high ascospore release recording on the Quest volumetric spore trap is open to debate. However, a large amount of air is sampled by active suction (20 litres/minute) through the spore trapping apparatus. From data collected it was evident that:

- Spore releases at the start of the season were typically low or medium in strength.
- High releases could be correlated to high minimum temperatures for the day.
- At the end of the season spore release strengths would diminish as leaf-litter inoculum is decomposing. Environmental conditions are evidently influencing this.
- At the end of the season, spore catches would fade away and end as minimum day temperatures fall below 17°C. This would also signal the end of leaf infection (future inoculum) via ascospores, for the season.

The actual infection event

Specific detail is still being investigated. The time-span for infection (formation of penetration peg from appressorium) to occur is expected to be very similar to those found for infection by pycnidiospores (Shaw *et. al.* 1998). This work indicates a period of ideal conditions for infection lasting from 18-24 hours. I found this ideal infection conditions to occur more often than initially expected within the many niches that exist in an orchard citrus tree. The fact that the pycnidiospores, and most probably the ascospores too, require dryness on the substrate (a hydrophobic substrate) to bond effectively is very significant. Germination will not occur readily, if this bonding is jeopardized by either too much moisture or an anti-penetrant fungicide (e.g. Tricyclazole).

The *Guignardia citricarpa* (CBS Disease) model for Citrus

1. Susceptible part of the crop
 - i. Fruit (20 October – 31 January) = Four months
 - ii. Leaf infection (October – April) = Seven months
 - iii. Degradation and Wear-off of Chemicals
 - iv. Ascospore availability: 20 October until 30 April according to inoculum and climate
2. Infection events of the disease
 - i. Formation of ascospore producing perithecia
 - ii. Spore release: Ejection & Dispersal
 - iii. Infection conditions
3. Treatment Recommendations
 - i. Combining Susceptible Parts data (1) with Infection events of the disease (2)
4. INPUTS: Crop data
 - i. Crop emergence = Always recorded as 100% (Crop (Fruit infection: 20th October to 31st January)
 - ii. Tree Density 1-5 years/ 10 l/tree: value 3
 6-10 years/ 20l/tree: value 6
 10-25 years/35l/tree: value 10
 - iii. Crop Protection: Enter Chemical Treatments
 - iv. Fruit Growth: Flowering to set- 1-3, Intermediate growth- 4-8, Near maturity- 9-10
5. Observed disease symptoms: ID the Disease (region and/or local on site), downwind distance from disease
6. Epidemiology: Light infection in region=1, Moderate in region=2, Heavy in region=3, Light local spot=4, Medium local spot=5, Heavy local spot=6, Light local extended=7, Medium local extended=8, Heavy local extended=9, Heavy local extended and region=10
7. Additional Data: Hail events (before October of the present season), Orchard history of CBS, History of Chemical Resistance, Cultural Practices- Inoculum removal, -treatment, -covering, -testing

The data thus far collected links events from the CBS life cycle (sporulation, spore release, conditions for infection and first appearance of symptoms) very closely to climatic conditions at the exact time the event took place. Further information concerning the precise effect of temperature, relative humidity and the longevity of inoculum under different climatic regimes became apparent over these four seasons.

Knowledge thus obtained was used over four seasons to predict critical periods and to base management decisions thereon. These decisions concern chemical intervention, inoculum management and the management of infection conditions. Identified bench-marks were thus validated for reliability and usefulness at the same time over several orchards in the Marble Hall district.

The primary handicap of forecasting in general is vested in the weather forecast being only fairly reliable for 5 days into the future.

The value of this model in the protection of the citrus crop is based upon improved timing for spraying, the choice (and protection) of appropriate chemical agent, and in the reduction of the available inoculum for the next season.

Future research

Benchmarks will be transferred to the most suitable modelling framework. Monitoring will continue in order to incorporate any fresh observations or important relationships into the model and to omit any erroneous ones if/when encountered. An artificial inoculation experiment for comparison to natural infection is still to be evaluated. The Modelmaker Package (Cherwell Scientific, Oxford, UK) or similar will be obtained and utilized to test slight variations in parameters if uncertainties come to light via field observations. Further it is envisaged to evaluate the *in situ* protection rendered by the available chemical agents. Pycnidiospore inoculum will be applied artificially over time on chemically treated leaves/fruit in order to establish the actual time-span of protection that can be expected from each chemical under field conditions. Infection will be

confirmed by PCR techniques and/or by subjecting fruit to Ultraviolet light to accelerate CBS blemish development. Although this facet will strengthen the model it might eventually become a separate project.

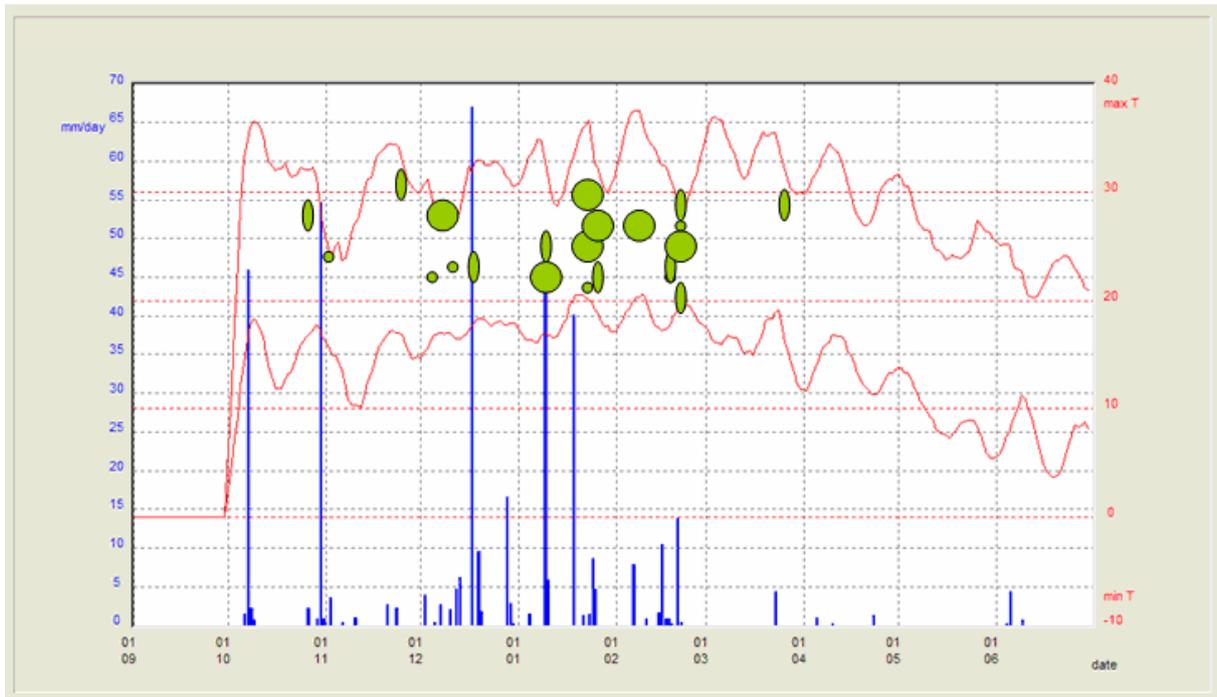


Fig. 4.3.13.1. CBS Season 2002/3. Temperature, Precipitation and Ascospore releases. The size of the ellipse gives an indication of spore release strength.

Key: Low Release (1-3 spores/225 litres of air sampled)
Medium Release (4-10 spores/225 litres of air sampled)
High Release (11+ spores/225 litres of air sampled)

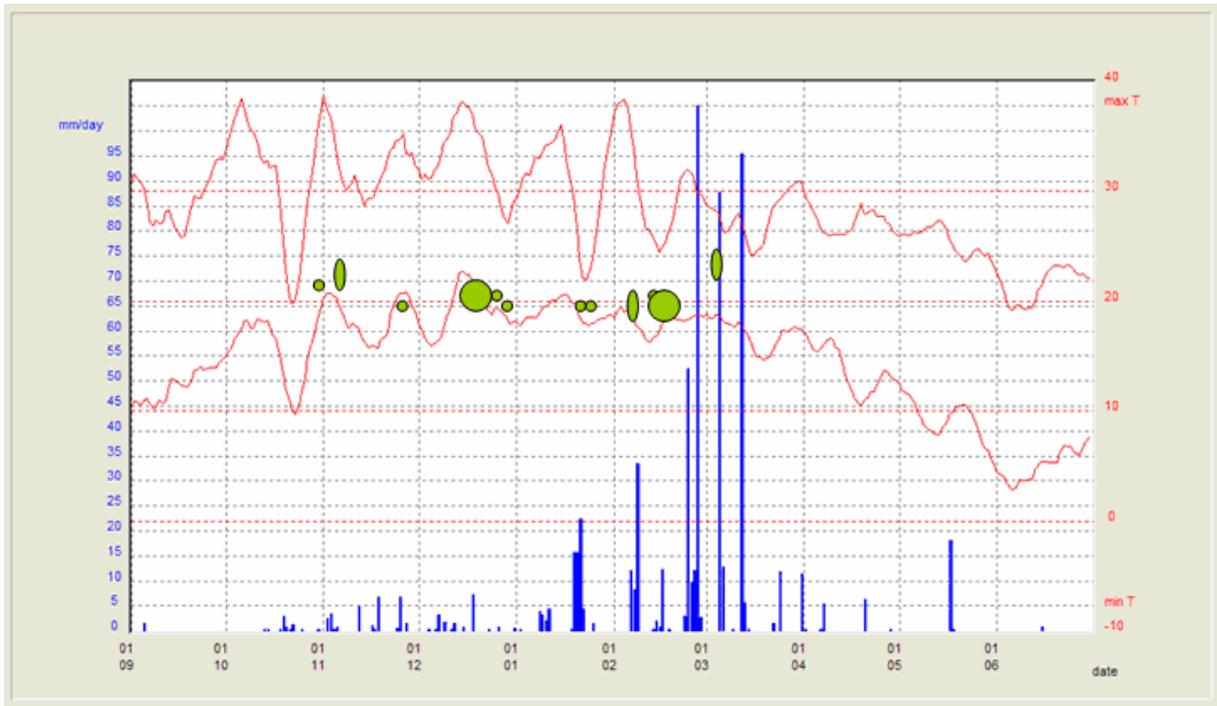


Fig. 4.3.13.2. CBS Season 2003/4. Temperature, Precipitation and Ascospore releases. The size of the ellipse gives an indication of spore release strength.

Key: Low Release (1-3 spores/225 litres of air sampled)
 Medium Release (4-10 spores/225 litres of air sampled)
 High Release (11+ spores/225 litres of air sampled)

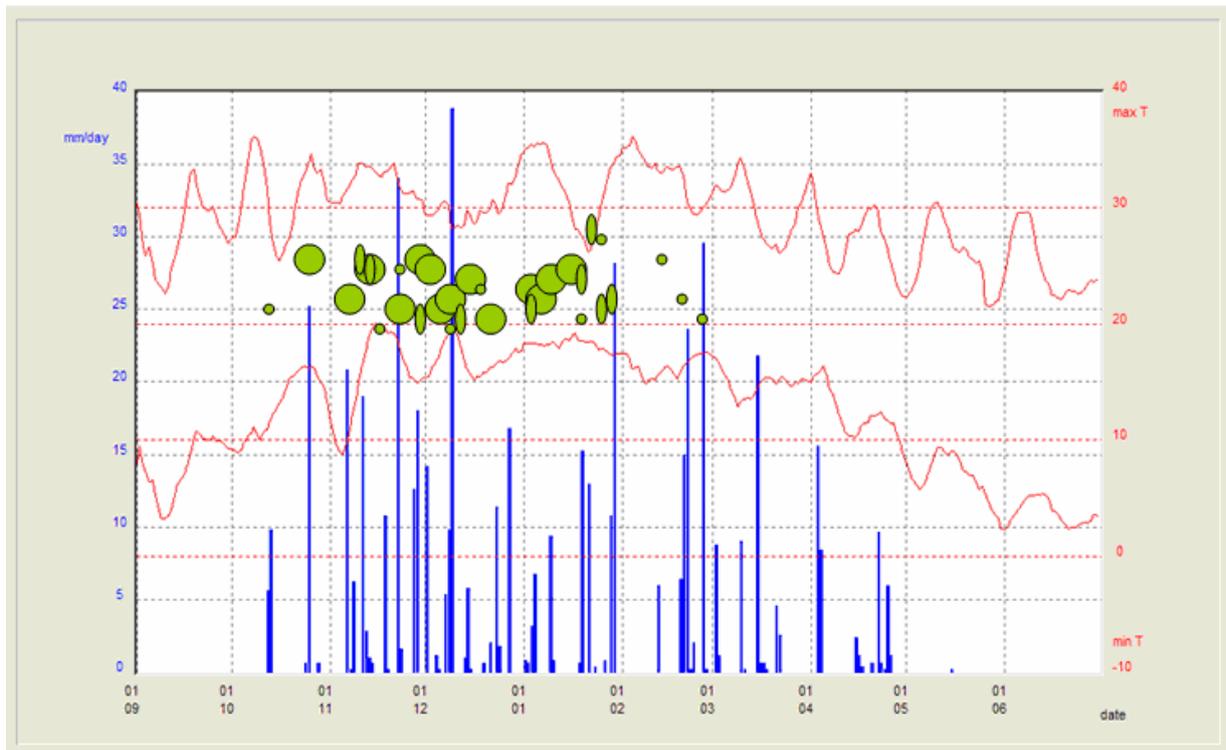


Fig. 4.3.13.3. CBS Season 2004/5. Temperature, Precipitation and Ascospore releases. The size of the ellipse gives an indication of spore release strength.

- Key:
- Low Release (1-3 spores/225 litres of air sampled)
 - Medium Release (4-10 spores/225 litres of air sampled)
 - High Release (11+ spores/225 litres of air sampled)

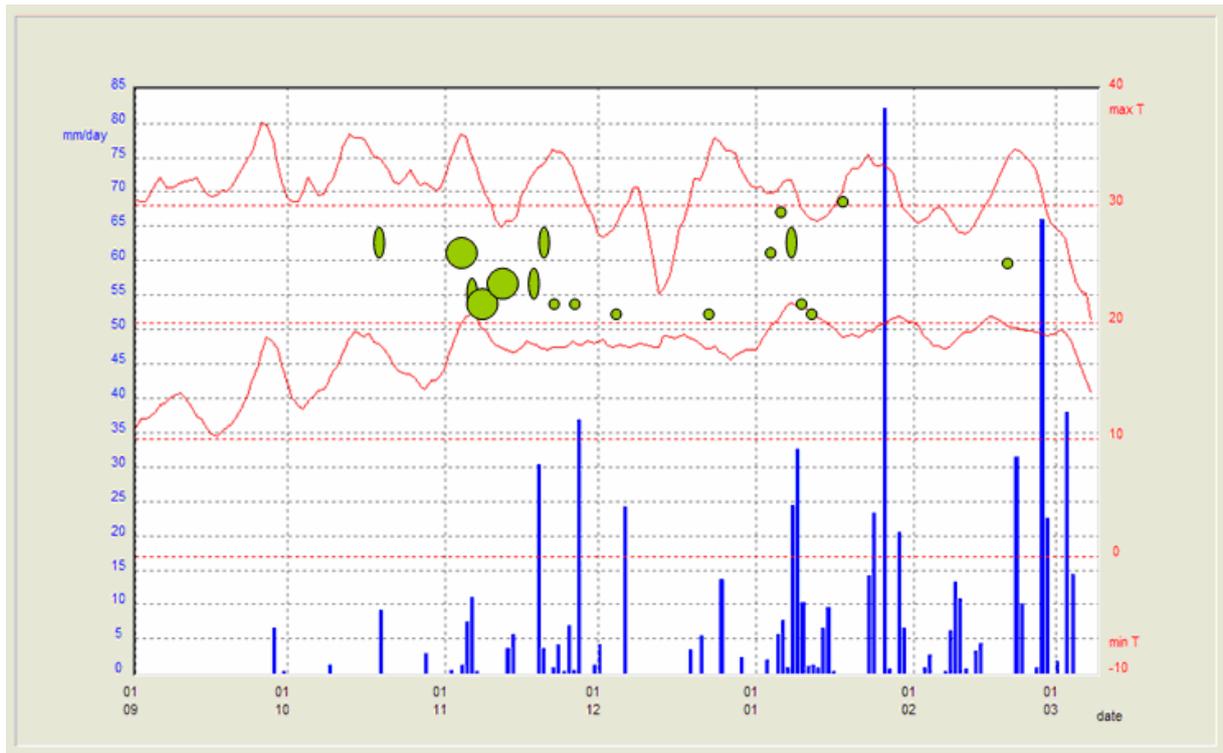


Fig. 4.3.13.4. CBS Season 2005/6. Temperature, Precipitation and Ascospore releases. The size of the ellipse gives an indication of spore release strength.

Key: Low Release (1-3 spores/225 litres of air sampled)
 Medium Release (4-10 spores/225 litres of air sampled)
 High Release (11+ spores/225 litres of air sampled)

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4.3.14 A preliminary survey of the Critical Infection Period (CIP) of Citrus Black Spot in Limpopo, Eastern Cape and Western Cape

Experiment 848 by J.M. Kotzé (CRI consultant), L. Meyer, M. Truter and L. Korsten (UP)

Opsomming

Voorlopige ondersoek toon dat die Swartvlek patogeen op 'n lae vlak teenwoordig is in die Tshipise area, maar dat die Kritieke Infeksie Periode nie voorkom nie en dat *G. citricarpa* glad nie gevind is nie in volwasse groen blare vanaf Oktober tot Maart, met die sensitiewe PKR (PCR) tegniek. *G. mangiferae* was feitlik altyd teenwoordig in die blare. 'n Swartvlek strategie, spesiaal vir hierdie gebied behoort aandag te kry. 'n Suurlemoenboord naby Franschhoek is geen *Guignardia spp* op dooie of groen blare gevind nie.

By Swellendam is hoë tellings van *G. mangiferae* askospore gevind, maar geen *G. citricarpa* kon gevind word in Valencia en Suurlemoenboorde nie. Geen CBS simptome is waargeneem nie.

Swartvlek is skynbaar goed gevestig in die Oos-Kaap en is besig om te versprei. Aanduidings dat die Kritiese Infeksie Periode later is, moet getoets en verder ondersoek word, waarna spuitprogramme aangepas moet word. 'n Opgedateerde voorspellings diens kan onnodige bespuitings uitkakel.

Vroeg in Oktober 2005 is ryp askospore in Groblersdal, Malelane en Swaziland gevind wat strydig is met standaard aanbevelings. Hierdie projek het geleentheid gebied om tegnieke aan te pas en tot protokolle te ontwikkel. Nuwe monsterneming prosedures is ontwikkel.

Introduction

The CIP was first described for Letaba Estates in 1963, but was found to be true for most parts of the Transvaal Lowveld and parts of KwaZulu-Natal. It was wrongly accepted that the Letaba Model is also true for all citrus areas. Spraying experiments already indicated that the first infections are delayed for up to 6 weeks in areas with cold winters.

It was postulated that in hot areas with relatively warm winters, like Tshipise, Limpopo, ascospores will mature earlier, before the crop sets. Furthermore it was hypothesized that the summer temperatures (and relatively dry conditions) would be too hot for infection. Spore germination is considerably reduced above 30°C and the pathogen cannot complete the life cycle. These theories had to be challenged.

Materials and methods

The PCR and Kotzé Inoculum Monitor (KIM) techniques were used at the University of Pretoria by Dr. Linda Meyer and Ms. Mariette Truter, but protocols for sampling had to be sorted out and are described as an addendum to this report.

Through the kind co-operation of members of CRI and farmers in the different areas, samples of leaf litter of Valencia and Lemon orchards were collected as we prescribed and speed posted to the PPL Laboratories in Pretoria for PCR tests and spore counts. From each locality, two leaf samples were taken from each cultivar at monthly intervals, starting September 2005 and ending in April 2006 (Fort Beaufort).

To detect ascospores of *G. citricarpa* and *G. mangiferae* the KIM was used throughout as described by Truter et al. (2004).

To differentiate between *G. citricarpa* and *G. mangiferae* and to detect CBS the PCR primer process was used as described by Meyer et al. (2000).

The test procedures were modified for the leaf samples and were carried out by the S A N A S accredited laboratory of Plant Pathology Laboratories, University of Pretoria. The sampling of green leaves and the processing of the leaves for PCR is described by J. M. Kotzé: An empirical “wet dry” technique to detect CBS in nurseries and production areas with low disease pressure (addendum to this report). Also included herewith: Recommendations to monitor for CBS in Citrus Nurseries by J. M Kotzé. The PCR techniques for research are reported in PPL4 in the latest CRI annual research report by L. Meyer and L. Korsten (4.3.10).

Participants who collected samples:

	Farm	Area	Contact	Cultivar (orchards)
1.	Alicedale Estates	Tshipise	Bennie Nicholson	Valencia Delta BC Later also A1, O2 and O6
2.	Alicedale Estates	Tshipise	Bennie Nicholson	Lemon Eureka
3.	Grondsake, Buitepos	Patensie	Ian Grieb	Valencia Midnight
4.	Gonja (Scheepers)	Patensie	Ian Grieb	Lemon Eureka
5.	Lone Tree (Bouwer)	Addo	Dave Gerber	Valencia Delta
6.	Kangela (Oranjesicht)	Addo	Dave Gerber	Lemon Eureka
7.	Beddaford (Roberts)	Fort Beaufort	Bruce Knott	Valencia Delta
8.	Baath (Mildenhal)	Fort Beaufort	Bruce Knott	Lemon Eureka
9.	Thornlands (Neethling)	Swellendam	Sarel Neethling	Valencia Late
10.	Olivedale (van Deventer)	Swellendam	Piet van Deventer	Lemon Eureka
11.	Môreson (Friedman)	Happy Valley, Franschhoek	Dr S. Kotze	Lemon Eureka

Table 4.3.14.1. Survey for the presence of CBS in a Lemon orchard, Happy Valley, Franschhoek.

Date Collected	Cultivar	Leaf litter: Ascospore Counts	Green leaves: Processed and PCT	Comments
10/10/2005	Lemon	0	Negative	Orchard is free of <i>G. citricarpa</i> and <i>G. mangiferae</i> after repeated testing. Could trees be used for nursery purposes? Or for establishing a new nursery, free of CBS? The complete absence of <i>G. mangiferae</i> is also noteworthy as it is speculated that it is universally present.
05/03/2006	Lemon	0	Negative	

Table 4.3.14.2. An ad hoc survey of *Guignardia spp* in two citrus orchards near Swellendam.

Date of collecting samples	Cultivars	Leaf litter Ascospore Counts	Mature green leaves: Processed and PCR	Comments
8/10/2005	Valencia late (Sarel Neethling, Thornlands)	0	<i>G. mangiferae</i>	The pathogen <i>C. citricarpa</i> , the cause of CBS, was not detected.
"	Eureka Lemon (Piet van Deventer, Olivedale)	244	<i>G. mangiferae</i>	The high ascospore counts in the Lemon orchard were unexpected. No symptoms observed on green leaves or fruit.
14/11/2005	Valencia Late (Thornlands)	0	<i>G. mangiferae</i>	This survey should continue parallel with in vitro and biological studies on <i>G. mangiferae</i> , especially the conditions necessary to infect citrus.
"	Lemon (Olivedale)	2685	<i>G. mangiferae</i>	Any fruit symptom that may occur on lemons in future should be submitted for identification. There is no quarantine on <i>G. mangiferae</i> .
25/01/2006	Valencia Late (Thornlands)	0	<i>G. mangiferae</i>	No ascospores of <i>Guignardia spp</i> were found in Valencia orchard. Although <i>G. mangiferae</i> appeared on processed leaves and was confirmed with PCR.
"	Lemon (Olivedale)	2830	<i>G. mangiferae</i>	

Table 4.3.14.3. Survey of ascospore release and occurrence of *G. citricarpa* in citrus leaves at Alicedale, Limpopo.

Date leaves collected	Cultivars	Leaf litter Ascospore counts	Mature green leaves: Processed and PCR	Comments
14/09/2005	Delta Valencia	>1400	<i>G. citricarpa</i> <i>G. mangiferae</i>	<i>G. citricarpa</i> was not found after 14 September 2005.
"	Lemon	48	<i>G. mangiferae</i>	
14/10/2005	Delta Valencia	>4000	<i>G. mangiferae</i>	No ascospores of <i>Guignardia spp.</i> were found between mid- October until mid-January on Lemons and in Valencia orchards until mid-January. The evidence suggest that all the ascospores which were found sporadically after 14 September were <i>G. mangiferae</i> . The theory that climatic conditions are not conducive for CBS in the Tshipise area, was confirmed. However, <i>G. citricarpa</i> was found in the past in old orchards. It was never an epidemic.
"	Lemon	0	<i>G. mangiferae</i>	
15/11/2005	Delta Valencia	0	<i>G. mangiferae</i>	
"	Lemon	0	<i>G. mangiferae</i>	
16/12/2005	Delta Valencia	0	<i>G. mangiferae</i>	
"	Lemon	0	<i>G. mangiferae</i>	
16/01/2006	Delta Valencia	22	<i>G. mangiferae</i>	
16/01/2006	Lemon	0	<i>G. mangiferae</i>	
Additional	Valencia A1	>6000	<i>G. mangiferae</i>	

samples taken on 06/01/2006	Valencia 02 Valencia 06	950 0	<i>G. mangiferae</i> <i>G. mangiferae</i>	<p>Alicedale is a candidate area to break the life cycle (on a reduced inoculum basis) and to keep it that way for extended periods of time. The commitment to it is determined by the monetary reward that may follow.</p> <p>Recommend to continue this survey for one more season and to monitor the packhouse.</p> <p>The Tshipise area is unique for CBS observation. Further studies will uncover valuable information, especially when compared with cooler regions.</p>
15/02/2006	Delta Valencia	0	<i>G. mangiferae</i>	
"	Lemon	0	<i>G. mangiferae</i>	

Table 4.3.14.4. Survey of ascospore release and the occurrence of *G. citricarpa* and *G. mangiferae* in Katriver area.

Date of sampling	Cultivars	Ascospore counts on Leaf Litter	Mature green leaves Processed on PCR <i>G. citricarpa</i> <i>G. mangiferae</i>		Comments
10/10/2005	Valencia (Roberts)	16	-	+	The spore counts and PCR results were repeated several times for each sampling date. The spore counts are averages of total counts.
"	Lemon (Mildenthal)	0	+	+	
15/11/2005	Valencia (Roberts)	7500	+	+	
"	Lemon (Mildenthal)	0	+	-	The high counts in November on Valencias were unexpected. Indications are that the November counts represented <i>G. mangiferae</i> and not the CBS pathogen.
16/12/2005	Valencia (Roberts)	185	+	+	
"	Lemon (Mildenthal)	1264	+	+	No conclusions can unfortunately be made after one season's observations. The observations should be continued. (The Inoculum Monitor will detect spores that are not caught by a conventional spore trap. The latter usually will record spores only when it rains.)
27/01/2006	Valencia (Roberts)	1251	+	-	
"	Lemon (Mildenthal)	4000			
17/02/2006	Valencia (Roberts)	99	+	-	
"	Lemon (Mildenthal)	0	+	+	
28/03/2006	Valencia (Roberts)	188	+	-	
"	Lemon (Mildenthal)	19	+	+	

Table 4.3.14.5 . Survey of early release of ascospores in two orchards near Addo 2005.

Date leaves collected	Cultivars	Dead leaves: Ascospore counts	Green leaves: Processed and PCR	Comments
06/10/2005	Delta Valencia (Bouwer)	0	<i>G. citricarpa</i> <i>G. mangiferae</i>	Ascospore release is delayed compared to Nelspruit. With more information the spray programme may commence later. <i>G. mangiferae</i> was abundant on processed leaves, suggesting that the spore counts may represent the endophyte and not CBS. Further studies are recommended to improve CBS control.
"	Lemon (Oranjesicht)	20	<i>G. citricarpa</i> <i>G. mangiferae</i>	
14/11/2005	Delta Valencia (Bouwer)	0	<i>G. citricarpa</i>	
"	Lemon (Oranjesicht)	0	<i>G. citricarpa</i>	

Table 4.3.14.6. Survey of early release of ascospores in two orchards near Patensie 2005.

Date leaves collected	Cultivars	Dead leaves: Ascospore counts	Green leaves: Processed and PCR	Comments
06/10/2005	Valencia Midnight (Buitepos)	1706	<i>G. mangiferae</i>	Ascospores earlier than expected. <i>G. mangiferae</i> very prominent on processed leaves.
"	Lemon (Gonja)	>3000	<i>G. citricarpa</i> <i>G. mangiferae</i>	
15/11/2005	Valencia Midnight (Buitepos)	281 (away from dripper) 578 (at dripper)	<i>G. mangiferae</i>	The ascospore counts at the dripper and away from dripper show no significant difference. This aspect should be studied in more detail over longer periods. Samples in December could not be taken due to holiday season.
"	Lemon (Gonja)	1952 (away from dripper) 166 (at dripper)	<i>G. citricarpa</i>	

Discussion

Mc Onie (1964) identified temperature and moisture as key factors in the CBS epidemic. In epidemiological terms this was a very simplified view, because it omitted time (t) and the rate of disease increased (r) which form important criteria in epidemiological models.

Inoculum (I) is never static. The numeric status of inoculum depends on environmental conditions, but also on the susceptibility of the plant and therefore the rate of increase of disease and inoculum. Likewise any fungicide that reduces inoculum, or inhibits or delays sporulation will influence the epidemic. This is time related.

We know that the leaf is the sole custodian of the inoculum source in South Africa. The leaf is very susceptible immediately after emergence, but remains susceptible for up to 10 months. Therefore, unless

there is chemical (fungicidal) interference or composting with antibiotic challenge, the leaf provides the mechanism that ensures the CBS epidemic.

It is a biological fact that plant material like leaves, will turn to compost-breakdown products in the soil and that *G. citricarpa* (a pathogen) cannot maintain itself among the saprophytes during compostation. When the leaf drops the CBS pathogen which is already sporadically present in the leaf, becomes a rapid coloniser of the leaf before compostation sets in. Once colonised, that portion of the leaf will be the substrate for the pathogen to complete its life cycle and will produce perithecia with ascospores. At this point moisture, temperature and time determine the economic consequences of CBS.

One can postulate that the winter temperatures in Mediterranean regions are too low for growth, that the pathogen can survive the temperature, but not when it is in severe competition with all the natural saprophytes in winter-wet soils, where and when the temperature does allow some growth, but the life cycle is delayed.

Because of the delay, ascospores must be produced long after the fruit has become resistant, but the leaves will still be susceptible and during rains infection will occur. In such a case PCR tests will detect *G. citricarpa*. But, so far it has not happened; certainly not in Franschhoek. In Swellendam the endophyte *G. mangiferae* completes its life cycle; produces ascospores in abundance and continues to occupy the leaves. It does infect the leaves. That means that conditions (moisture, temperature and time) are in place in Swellendam. Is *G. mangiferae* pioneering the way for *G. citricarpa*?

On the other side of the scale, a similar picture emerges under the extremely hot conditions of Tshipise. *G. mangiferae* is there all the time. But *G. citricarpa* finds conditions difficult to increase inoculum. One can also postulate that the pathogen prefers conditions between 18°C and 30°C. It can survive lower and higher temperatures, but cannot thrive. The absence of rain is not in its favour either. We know that alternate wetting and drying of infected mature leaves are important for the development of perithecia. It was found that 6 – 7 rainy days precede the first effective spore releases during the susceptible stage in November, in the Lowveld. But, the relative absence of the pathogen in green leaves in Tshipise, means that leaf infection does not take place and inoculum of CBS cannot build up.

We know the inoculum is very low to completely absent. We accept that conditions: moisture, temperature and the time factor are against a sudden outbreak. The situation can be monitored on an ongoing basis, using all the tools we have. Chemical sprays can guarantee clean fruit in the packhouse. It can remove uncertainty. In the end it is a commercial decision for the grower in which he should receive all the technical help available.

This study revealed that the CBS pattern of spore release during the past season was contrary to expectations of experienced consultants, who still go by the findings of 40 years ago.

It appears as if the situation has changed. Two factors have been introduced that may partially explain the situation, e.g. pruning has become a common practice just after harvest. The branches are left in the orchard where all the leaves remain to produce abundant inoculum. The leafless branches are removed at the convenience of the grower later.

The second factor is that irrigation has changed completely from basin and furrow to drip and micro sprinklers. These factors have not been evaluated yet.

And then there is climate change. Are our winters getting warmer?

It is now theoretically possible to predict the onset of the Critical Infection Period (CIP). The research in this direction is promising. With co-operation of all, we can move forward rapidly.

Throughout the survey, *G. mangiferae* was in our faces all the time on all sites, except Franschhoek. My conclusion is that we ignore the obvious. Is this endophyte as innocent as claimed? From leaf lesions we repeatedly isolated *G. mangiferae*, but mostly *G. citricarpa* and *G. mangiferae*.

This preliminary study does not include a climatic analysis of the different regions. Obviously this should be part of future research.

This investigation exposed the urgent need to differentiate between *G. citricarpa* and *G. mangiferae* at the moment when the spores are trapped. The Industry relies heavily on the trapping of ascospores in orchards during rains. We are bluffing ourselves and the growers that such data is a true reflection of the CBS pathogen. It is a psychological mobilization technique to growers. It may be entirely true or just a false alarm. Fact is, we do not know. Research on this aspect is overdue, but it requires technology that is not yet available in South Africa.

Conclusions

The most significant conclusions from this preliminary survey are:

1. **Franschhoek:**

No *Guignardia spp* were found in a mature Lemon orchard. No symptoms of CBS were found on leaves or fruit. Processed leaves, for the multiplication of DNA, produced no fruit bodies. PCR tests were negative.

2. **Swellendam:**

The endophyte, *G. mangiferae* was abundant on processed green leaves. The PCR tests were consistently negative for *G. citricarpa* but positive for *G. mangiferae*. Ascospores of *G. mangiferae* were detected on leaf litter from the Lemon orchard, but never in the Valencia orchard. Results and circumstantial evidence suggest that *G. citricarpa* cannot complete its life cycle in the Mediterranean region. The pathogen was never detected in processed green leaves.

There are, however, rumours that *G. mangiferae* can cause symptoms on fruit. It is not a quarantine disease and is rated by the Dutch workers, R.P. Baayen *et al*, as a “cosmopolitan endophyte of woody plants”. That may be so, but if CBS-like symptoms do appear, it should be handled calmly and confidentially until a proper investigation is completed. A pre-emptive study of the biology of *G. mangiferae* would be wise.

3. **Tshipise, Alicedale :**

The situation at Alicedale is fascinating in a scientific sense, but frustrating in marketing terms. The presence of *G. citricarpa* in the first sample, taken in September was discussed with Dr. Meyer, who repeated the PCR tests. She checked thoroughly and politely wrote to me on 7th February 2006. “Ek het weer na die gels van die September monsters gekyk en daar is baie ligte *G. citricarpa* bande. Ons beskou sulke ligte bande as positief, daarom die positiewe resultaat in die verslag.” That’s it! A norm is a norm. One does not mess around with it!

The Tshipise area is a special case. The results suggest that spore releases (if any) may occur, but not after fruit set. There is no CIP because there is no inoculum, because it is too hot and dry! Is this just a temperature thing? Surely not. Moisture to produce inoculum on the ground and wetness to facilitate infection are some of the other factors. The orchards are irrigated, but no positive information was obtained with spore counts. Conditions are not conducive to CBS at Alicedale.

4. **Kat River, Fort Beaufort**

Two orchards were monitored: Velencias and Lemons. The control practices differed. During my visit in October 2005 CBS symptoms were observed on fruit and green leaves. The “cosmopolitan” presence of *G. mangiferae* made it very difficult to pin point the CIP. If one considers the detection of ascospores in the Lemon orchard, the CIP seems to be later than the Letaba Model. A staggered spray experiment is at this stage proposed. Until we can separate the ascospores of *G. citricarpa* and *G. mangiferae*, this area is uncharted.

5. **Sundays River, Addo and Gamtoos: Patensie**

The disease seems to be well established but still spreading and inoculum is also building up. We monitored potential spore releases and not actual spores in the atmosphere. The “potential” tells us what will happen when it rains; the actual tells us what happens during rains. The “potential” is a

more accurate figure, because of the efficiency of the instrument used. In Addo the CIP seems to be later than the Letaba Model, but in Patensie the observations are spoiled by the ever present *G. mangiferae*.

It is interesting that in Patensie *G. citricarpa* was not detected in the Valencia orchard, but only in the Lemon orchard. This indicates that the Valencias will shortly become infected.

In the Eastern Cape, disease forecasting models cannot rely on spore-trap results.

6. Letaba Estates and Nelspruit

At Letaba Estates and Nelspruit the CIP is usually expected from the beginning of November, but early infections in October are recorded in about one in 10 years. During the past season in Groblersdal, Malelane and Swaziland, mature ascospores were found from early September. This was unexpected and is a source of concern to consultants and progressive farmers.

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- ❖ **Prof. Lize Korsten** for making the services of SANAS accredited Plant Pathology Laboratories available for this investigation.
- ❖ **Dr. Linda Meyer** for the PCR tests.
- ❖ **Ms. Mariette Truter** who analysed the spore counts.
- ❖ Then, **Bruce Knott, Dave Gerber, Ian Grieb, Bennie Nicholson and Hannes Bester** who faithfully collected samples and dispatched them to me for analysis.

Thanks for the team work.

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4.3.15 An empirical “wet-dry” technique to detect CBS in nurseries and production areas with low disease pressure

By J.M. Kotzé (CRI consultant)

Opsomming

’n Tegniek waar blare afwissellend op ’n bepaalde wyse gedroog en bevochtig word vir vier dae en dan vir ’n totaal van 10 dae word bespreek. Dit stel die operateur in staat om die inokulumvlakke in boorde ’n jaar vooruit te bepaal vir ’n bestrydingstrategie en bemerking. Die tegniek laat die swartvlek swam baie vinnig vermeerder sodat sy teenwoordigheid met groot akkuraatheid bepaal kan word. Dit onderskei *G. citricarpa* en *G. mangiferae*.

Introduction

The success of the technique is based on a proper understanding of the biological processes involved. The operator must prevent the natural processes of degradation like composting, fermentation etc., and selectively encourage rapid colonization of the detached leaf by the pathogen. During the colonization mycelial growth is very active, producing an abundance of DNA for PCR. If one fails at this early stage of the technique, start all over again.

Composting is a very strong and natural biological process, which eliminates the pathogen. *G. citricarpa* cannot compete for survival against the saprophytes and antibiotic producers in the soil environment. The two processes are not compatible for our purposes. The composting organisms include very strong antagonists, like: *Bacillus licheniformis*, *B. subtilis* and *B. macerans*.

Among the fungi, the following are known to have antibiotic activity: *Aspergillus sydowi*, *A. caespitosus*, *Gliocladium catenulatum* and *Trichoderma harzianum*. Therefore, if composting sets in, the end result will be negative – a false negative, which has far reaching consequences for decision makers.

The description below is the result of empirical research and years of experience. It is a successful technique and was used exclusively in an extensive survey of CBS in areas where the incidence of CBS is low to very low. It must be considered as the most advanced technique at the moment. In situations of high disease incidence like the Biological Block at Letaba Estates, it was 100% accurate over 3 years. It was highly effective in all situations of low disease incidence. The sampling is vital. For example, if randomized leaf collection is done, the chances of detecting CBS is much reduced. It can be missed. In fact, sampling can be done in such a way that the outcome will be negative in a heavily infected orchard. Downstream the sampling of specially prepared leaves with CBS, can be done in such a way that the PCR test is negative, no matter how careful the PCR laboratory carries out the subsequent procedures.

Components of the technique

The leaf –

In the past the infected leaf was largely ignored in control strategies. The leaf harbours the invisible inoculum (in nurseries for example), *Guignardia citricarpa* exists for most of its life as a tiny microscopic fragment of mycelium below the cuticle (not in the mesophyll) of the attached leaf on the tree, until it drops after 24 months or later. The first few days after leaf drop are critical for the survival of the pathogen and completion of the life cycle. This is when colonization takes place and mycelium occupies the mesophyll tissue inside the leaf. If the soil is wet, high in nitrogen with a C/N ratio of 7 – 10 and temperature is conducive to compostation, the breakdown process sets in and the pathogen will perish. The faster the composting process, the more complete is the destruction of *G. citricarpa*.

The sun –

Exposure to the sun plays an important role. The sun causes drying out of the leaf and raises the temperature which triggers the colonization process. Sunlight stimulates CBS but when the exposure is too long and the heat that it generates is too high, *G. citricarpa* is inhibited and finally destroyed. This stage is indicated when the leaf turns grey-white and brittle.

Water –

The water status of the leaf itself and the surrounding environment play vital roles. When leaves are processed after picking, intermittent dipping in water prevents total desiccation. Ideally the leaf must remain pliable, in a leathery condition. Under such conditions the mycelial growth is encouraged and will trigger fruiting bodies. If the leaves are left in water for too long and the water becomes brownish, fruiting bodies will not develop or the development will be delayed considerably. During the first four days of the process, the leathery condition must be maintained. After four days, brief periods of dryness encourage fruiting bodies.

Temperature –

For active mycelial growth, the temperature should be between 20° and 30°C. Short periods below and above the given temperature range stimulate fruiting bodies, but below 18°C mycelium growth slows down. At 5°C and above 35°C germination of spores and active growth, stops. Short exposures (10 minutes) between 35°C and 40°C in water were found to stimulate colonization and fructification. Storage of leaves at 5°C before processing delays colonization and fructification.

Plastic bags –

There is much misunderstanding on the use of plastic bags to enhance the process. It can delay the process or it can be most useful if the moisture and temperature requirements are in place. The main disadvantages are that plastic bags become too wet, the leaves stick to one another and creates conditions for degradation and growth of saprophytic bacteria. When this happens, the water turns brownish and soon a whitish mould will be observed. *Colletotrichum* will soon colonise under such conditions.

Another disadvantage of the plastic bag is that if it is left in the sun, the temperature increases very rapidly and creates conditions which will kill the pathogen. This is a big problem during summer months.

Operator –

There is no secret. An operator must be interested and dedicated. Training and experience are important components. The objective is not just to acquire the technique, but to follow the protocol so that surveys can be carried out. If the operator does not stick to the same protocol, the survey will be riddled with false negatives.

Rapid colonization stage

This step takes **four days** in summer and longer in winter, after the leaf samples have been collected as described. It is advisable to keep the leaves from the beginning in the standard 3 kg red/orange pocket commonly used for local marketing of fresh oranges.

On arrival at the laboratory, rinse in tap water. Remove all dirt with cotton wool. Drain all excess water and place in direct sun. It is best for the mycelium colonization that the leaves do not cling to each other and that they are exposed to the sun outside the pocket. This is risky because even a slight wind can blow the leaves and when dealing with a large number of samples (never exceed 10), confusion is unavoidable. If in doubt, repeat the process. I also used hypochlorite to rinse. This is irritating and unnecessary when good hygiene principles are followed. Do not work with dirty leaves!

Leaves should be left in the sun for 8 hours, rinsed again for 2 minutes, drained and placed back in the sun for 8 hours until the leaf colour becomes paper-brown, not white or dark-brown. The sun is not quantifiable. In winter it may be necessary to place the samples in the sun for 3 consecutive days. A transparent plastic bag can be used at this stage. The temperature should be kept above 20°C and under 35°C. The summer sun is sometimes too hot to expose for longer than 2 hours at a time. If the temperature runs too high for too long *G. citricarpa* will be killed or much reduced.

After the sun exposure, rinsing and drying, place the samples (in the orange/red pocket) in plastic bags for 8 hour periods. Inspect regularly and remove excess water which condenses in the plastic bag. Watch the temperature. Rinse and dry. If the water, during rinsing, becomes brown and the leaves become sticky and olive-brown, the wetting in the plastic bag was overdone and composting has set in. The result will almost certainly be mouldy and will be covered with *Colletotrichum* within days.

After four days of sun, rinsing and drying, the mycelium colonization would be optimum and samples can be used for PCR. Please note – no fruiting bodies are seen at this stage.

Stimulation of fruiting bodies

Continue with the process until day 8, when the first fruiting bodies (mostly pycnidia), will be seen. After day 10, the fruiting bodies will be well defined and abundant, depending on the CBS incidence and the success of colonization. The fruiting bodies of both *G. citricarpa* and *G. mangiferae* will appear. The latter will produce the first perithecia while *G. citricarpa* is very slow to produce perithecia and ripe ascospores.

For situations of low CBS it may be advisable to dissect the areas where fruiting bodies are well defined and do the PCR on the fragments which are collected in this way. It is clear that prior training is advisable for the operator. There are many ways to cut the corners and there can be a measure of success, but cutting corners will inevitably lead to false, negative results.

If this technique is applied with the necessary discipline, the spore trapping technique with the Inoculum Monitor and direct isolation of the pathogen will largely fall away, but can be used for specific objectives.

With dedication the factors that determine the required result, can be quantified in a laboratory with the view of automatisisation in future.

At the moment Mariette Truter continues my research to standardise the “Wet-Dry” process under laboratory conditions. What matters is that we have a technique that works very well in the hands of a reliable operator.

4.3.16 Draft recommendation to monitor for CBS in citrus nurseries

By J.M. Kotzé (CRI Consultant)

Opsomming

Dit is baie moeilik om swartvlek in kwekerie op simptomeelose plante op te spoor. Daarom is 'n protokol ontwikkel om sekere blare te selekteer, te versamel en daarna spesiaal te behandel om latente infeksies te vermeerder sodat die siekte-organisme met groot betroubaarheid bepaal kan word. 'n Protokol word voorgestel om swartvlek opnames by alle kwekerie te kan doen.

Introduction

Several methods can be employed to detect CBS. The first is to trap ascospores. If ascospores are found it does not imply that CBS is present because the test in itself is non-specific. It takes us only to the genus level. The absence of ascospores can also be misleading because they are not always present and lead to false negatives.

Another way is to isolate and culture from suspected lesions as described by Dr S H Swart in the CRI Group Annual Report 2004 in Experiment Report QMS 5.3. He described an atypical leaf lesion from which *G. citricarpa* was cultured at a very low frequency. PCR tests were also carried out for identification.

There is another feasible option. Detection can be much simplified if the very low level of CBS DNA can be rapidly multiplied and PCR tests can be done after preparing the leaves. I have used an empirical “wetting-drying” technique in another study (4.3.15) with great accuracy to detect CBS under conditions of extremely low CBS pressure. This technique can be part of a protocol for nurseries. It includes PCR tests.

Motivation

1. CBS infections in nurseries are low. The statistical chances of sampling infected leaves at random are extremely low.
2. Citrus leaves are susceptible for 10 months after emergence. The older the leaves, the greater the chances of sampling infected material. Intentional biased sampling is needed and only potentially infected leaves should be gathered.
3. Infections in the nursery are mostly latent and invisible. With experience, potentially infected leaves can be selected.

4. Inoculum can occur in the nursery on fallen leaves where leaves are allowed to lie around and produce spores. This is a sure sign of neglect.
5. All the tests employed in the past sometimes gave false, negative results. The proposed protocol will overcome the problem.

Inspection

1. Before leaves are sampled for PCR tests, the nursery should first be inspected. Note the distance from infected sources, wind direction and irrigation system. Wetting of leaves in the nursery should be avoided. Also note the discipline; the movement of materials into the nursery and basic good nursery practices.
2. Collect dead leaves lying around or hidden between bricks and plastic containers. Such samples should be sent for a professional opinion.
3. Collect any leaf on the plants showing typical CBS symptoms and submit for professional confirmation.

Sampling

The purpose of sampling is to ensure that if CBS is in the nursery, it must be found. It is a “**yes**” or “**no**” situation. What happens after this will depend on several factors and must be dealt with separately once the protocol is established. Therefore, the sampling will be strongly biased towards finding CBS. It is not a statistical randomized situation at this point.

Ignore the new and immature leaves. Use a hand lens and examine the older, senescent leaves of scion and rootstock. Pick suspected leaves, also those showing “small protruding lesions”.

The size and the number of samples depend on the feasibility of the sampling procedure and costs. The experience of the sampler is paramount. A decision on sample size is subject to the standardization process that follows on this report.

Collection of leaf samples

Collect only the oldest leaves from the rootstock and the scion. Select the senescent leaves, especially those showing necrotic and CBS-like symptoms. A sample of 500 leaves can be handled with ease, but the number of leaves will depend on the feasibility. The sample can be made smaller by selecting from the bigger collection. The wet-dry process can only handle 100 leaves per individual sample but after processing, the most “promising” leaves are selected to reduce the sample to 25 leaves for the PCR test. It is a continuous process of selecting for CBS.

Place the leaf sample in a 3 kg orange/red pocket which is commonly used for marketing. All the relevant information should be written on a plastic label (with pencil) and placed inside the pocket with the leaves. The samples must reach the laboratory as soon as possible for the wet-dry process. It is not advisable to place the samples under refrigeration because it delays the process.

It is proposed that the draft document be discussed by the relevant CRI personnel, Dr Swart and Prof Kotzé. The protocol can be finalized and standardized among the staff involved, and accredited.

4.3.17 **Market Access: CRI survey of Citrus Black Spot in the Western Cape districts of Knysna and Mosselbay**

By V. Hattingh, H.F. le Roux (CRI) and J.M. Kotze (CRI Consultant)

Opsomming

In die Wes Kaap is *Guignardia mangiferae* ge-isoleer vanaf dooie blare wat afkomstig is uit boorde in Leeukloof in die Mosselbaai distrik. Geen *Guignardia* spore kon ge-isoleer word van boode in Herbertsdale, ook in die Mosselbaai distrik nie. Lg. area is heelwat droeër. *G. mangiferae* is ook in die Knysna distrik ge-isoleer. Geen *G. citricarpa*, die organisme wat swartvlek veroorsaak, kon in enige van hierdie distrikte ge-isoleer word nie. Met die uitsondering van Herbertsdale is die gebied gunstig vir die ontwikkeling van

swartvlek. Aansoek moet daarom nie gedoen word vir area wye vrystelling nie. Dit moet gedoen word op 'n produsente uitvoerder kode basis, gevolg deur 'n sisteembenadering.

Introduction

Citrus Black Spot (CBS) is an economically important disease in many of the citrus producing areas of southern Africa. There are, however, areas in which the disease has never occurred on fruit and where no rejections of fruit have ever been found as a result of this disease. This is most probably as a result of the phenology of the fruit and the climatological requirement of the fungus to infect, not being synchronised. It is unlikely that the pathogen *Guignardia citricarpa* Kiely, has never been introduced into all these areas at some stage or another through the movement of planting material before this movement was prohibited by law. The Western and the Northern Cape are the only provinces in South Africa with certain districts being declared CBS free. Two magisterial districts in the Western Cape which are producing CBS-free fruit and have to date not yet been approved as CBS-free areas, are the Mosselbay and Knysna districts.

Materials and methods

The Mosselbay district was surveyed during December 2002 whereas the Knysna area was surveyed during January 2004. Both areas were visually inspected for fruit lesions on lemon trees and samples were taken from dead leaves from commercial orchards and home grown citrus trees and sent to QMS to be tested for the presence of *Guignardia*. PCRs were conducted by the University of Pretoria to determine the presence of *G. citricarpa*. Thirteen samples were taken in the Mosselbay district and 29 in the Knysna district. Samples were again taken in December 2004 and sent for PCR testing for *G. citricarpa* in January 2005.

Results

The results of the survey done in the Mosselbay are given in Table 4.3.17.1. The Herbertsdale and Hartenbosch areas were free of *Guignardia*, whereas *G. mangiferae* could be found on two of the three farms sampled in the Leeukloof, close to Ruitersbos. The Knysna results are given in Table 4.3.17.2.

Table 4.3.17.1. Blackspot survey: Mosselbay district – December 2002.

Area	Farm & Street Address	Owner	Contact no.	Cultivar	Age (yrs)	Home garden/ Comm.	°S	°E	Altitude (m)	No. of ascospores
Herbertsdale	Roodehoogte	A.C. Muller	044-6511697	Eureka lemons	5	Comm	34°03.846	21°44.350	66	0
				Midnight/Cara navels	5	Comm	As above	As above	As above	0
				Nikie HG (cultivar block)	20	HG	34°03.549	21°44.600	38	0
				Isak HG	14	HG	34°03.669	21°44.597	41	0
				Eureka lemons	30	HG	34°03.617	21°44.658	29	0
	Hemelrood	H.P.P. Muller	044-6511631	Valencia	8	HG	34°00.000	22°30.000	80	0
	Hunters Home	Oosthuizen Broers (Nic Oberholzer)	044-6511675	Valencia	20+	Comm	33°59.393	21°46.823	108	0
	Uitkyk	W. de Vos Muller (N)	044-6511632	Eureka lemons	4	Comm	34°03.	21°44.	± 38	0
Uitkyk	W. de Vos Muller (S)	044-6511632	Eureka lemons	4	Comm	34°03.	21°44.	± 38	0	
Ruitersbos (Leeukloof)	Die Koppetjie	W.D.W. Terreblanche	044-6310048	Eureka lemons	3	Comm	33°57.006	22°04.234	170	8*
	De Akker	Pieter Steyn		Navels	5	Comm	33°57.227	22°04.357	156	0
	Leeukloof	R. Oosthuizen		Valencias	± 60	HG	33°56.980	22°04.028	173	9*
Hartenbosch	Rooiheuvel	J. Robertson	044-6966707	Nules Clementines	60	Comm	34°03.422	22°08.244	21	0

* Both resampled and sent for PCR testing in January 2005. Both tested positive for *G. mangiferae*.

Table 4.3.17.2. Blackspot survey: Knysna district - 2004-5.

Area	Description of site	Surname & initials	Phone / Fax no.	Tree age (yrs)	Home garden/ Comm.	Cultivar	°S	°E	Altitude (m)	Guignardia spore counts Jan2004	Guignardia citricarpa (PCR test) Jan 2005
Rheenendal	Candlewood Farm	J. Stanwix	044-3884611	16	C	Clementines	33° 58.233	22° 58.704	222	1	Negative
				16	C	Lemons*	33° 58.100	22° 58.666	219	16	Negative
				11	C	Satsumas	33° 57.869	22° 59.140	223	0	
Rheenendal	Trewyn Park	W. Rosewall	044-3884642	12	C	Clementines	33° 57.003	22° 53.924	240	1	Negative
				15	C	Satsumas	33° 57.270	22° 59.123	224	2	Negative
Rheenendal	Spurwing Lodge	Johan Buhr	044-3884732	15	C	Clementines	33° 56.981	22° 8.861	249	0	
				12	H	Lemons*	33° 56.981	22° 58.861	249	184	Negative
Rheenendal	Woodside	R. Sandberg	044-3884774	15	C	Clementines	33° 56.433	22° 59.132	256	0	
Rheenendal	Rushmere	K. Elphick	044-3431935	15	C	Satsumas	34° 00.418	22° 49.094	22	336	Negative
				15	C	Clementines	34° 00.418	22° 49.092	22	0	
				10+	H	Lemons*	34° 00.585	22° 49.407	27	22	Negative
Rheenendal	Lancewood	J. Rubin	044-8502155	10	C	Clementines	33° 56.132	22° 45.658	263	71	Negative
				10	C	Satsumas	33° 56.086	22° 45.765	267	0	
Sedgefield	Klein Begin	H. Niehaus		10	C	Clementines	33° 56.711	22° 46.211	240	> 2282	Negative
Sedgefield	Mandalay	P. Leppan	044-8501157	10+	C	Lemons*	33° 56.249	22° 39.998	260	0	
Buffelsbaai afdraai	Ganzvlei	C. Metalerkamp	044-3830035	± 100	H	Oranges	34° 02.319	22° 55.632	20	54	Negative
N2	Essendale	V. Samuelson	044-3860132	16	C	Clementines	34° 01.765	22° 57.648	106	0	
Knysna	Catholic Church, Graham Str	St. Boniface Graham Street	044-3821391	10±	H	Lemons*	34° 02.074	23° 02.060	20	0	
Sedgefield	1 Kingfisher Street	E. Barnard	044-3431745	60±	H	Rough lemon	34° 00.918	22° 48.164	18	0	
Sedgefield	C/o Ull/Kingfisher Streets	J. Stavros	044-8770332	20+	H	Lemons*	34° 00.806	22° 48.131	17	0	
Harkerville	Forest Garden	K. Walter	044-5327705	50+	H	Rough lemon*	34° 01.938	23° 16.058	200**	0	
				6		Lemons*	4° 01.848	23° 15.870	221	>1378	Negative
Harkerville	Sunbird Nursery	J. de Jong	044-5327704	5	C	Lemons*	34° 02.023	23° 15.068	236	0	
Harkerville	Morning Glory	D.H. Mostert	044-5327642	8	C	Lemons*	4° 03.271	23° 14.758	245	19	Negative
Harkerville	Agrume	A. Schnetler	044-5327734	20+	H	Valencia	34° 02.122	23° 13.800	262	0	
Wittedrif	Stofpad	R.W. van Huysteen	044-5359852	20+	H	Lemons	33° 59.957	23° 18.448	262	0	
Knysna-bos		Diepwalle plantasie	-	50+	H	Rough lemon	33° 57.726	23° 09.518	434	0	
Prins Alfred pas		Diepkloof	-	60+	C	Valencias	33° 51.640	23° 10.374	300	0	
De Vlugt		A. van Rooyen	044-7523125	40+	H	Rough lemon	33° 48.733	23° 10.483	292	0	

* Fruit – no symptoms

** Old Forest

Discussion

Mosselbay district

When comparing Leeukloof to Herbertsdale, and looking at the natural vegetation, it is clear that Leeukloof has a much wetter micro-climate, despite the two areas being less than 50 km apart. Herbertsdale has almost a Karoo climate, which is too dry for the disease to establish, whereas Leeukloof is much greener, indicating higher rainfall. *Guignardia* spp. was absent in Herbertsdale but *G. mangiferae* could be found in Leeukloof. *G. citricarpa* was absent in both areas.

Knysna district

The Knysna district is far wetter than any of the other districts surveyed. Despite this, no *G. citricarpa* could be found. *G. mangiferae* was present in the district.

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4.4 PROJECT: SOILBORNE DISEASES

Project Co-ordinator: M.C. Pretorius (CRI)

4.4.1 Project summary

A number of species of sheath nematode, *Hemicycliophora*, have been identified from the citrus rhizosphere. *Hemicycliophora* causes root-tip swelling and stunted root or plant growth. Previous trials conducted by CRI indicated that registered post-plant nematicides do control these nematodes effectively. The purpose of this trial was to determine the effect of *Hemicycliophora* on different rootstocks currently used by the citrus industry, viz. Rough lemon, Carrizo Citrange, C35 and Swingle citrumello. The results in this trial, which was laid out in the Gamtoos River Valley, confirmed that Swingle citrumello and C35 rootstocks are tolerant rootstocks against nematode infestations and can be considered by producers when replanting a nematode-infested orchard (4.4.2).

Plant parasitic nematodes spend at least some part of their lives in the soil, one of the most complex of environments. In the past, applications of the PL+ treatments were done by hand under a micro irrigation system. The use of PL+ should therefore be evaluated under a drip irrigation system, which could ensure that conditions are more favourable for a Biological control agent to become established in the soil. No suitable trial site, with high nematode population numbers, could be found in the Nelspruit, Tzaneen and Letsitele regions. The investigation was not extended to regions in the Western or Eastern Cape because of financial constraints due to the regular application schedule required by the manufacturer. No results are therefore available (4.4.3).

The search for alternatives to soil fumigants and very toxic nematicides, is a priority at research stations and by researchers worldwide. By incorporating bio-fumigants into traditional control strategies, could reduce the usage of toxic nematicides polluting the environment. No replant action was executed during the 2005 season in the Gamtoos River Valley, on farms with suitable orchards for the trial and therefore the trial could not commence. The trial was not approved by the Disease Management Committee for the 2006 season (4.4.4).

A registration trial was executed on a contract basis for FMC Southern Africa, to establish the efficacy of a new Rugby 20CS formulation compared to the registered Rugby G and ME formulations. The new Mocap liquid formulation was also compared to the previous granular formulation. The trial was laid out at Moosrivier Citrus Estate and positive results were obtained. A final report on the work conducted was sent to FMC (4.4.5).

CRI evaluated Crop Guard, a non-organophosphate nematicide, on a contract basis for Illovo Sugar to determine the efficacy of the product for the control of the citrus nematode. Five trials were laid out at Karino, Mpumalanga (four trials) and at Citrusdal, Western Cape (one trial). A final report was handed to Illovo Sugar. CRI was requested to evaluate the product for one more season in 2006 (4.4.6).

Phytophthora is an aggressive root rot and fruit rot pathogen in citrus. 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. Rough lemon citrus seedlings from Esselen Nursery, were used for the trial in the glasshouse at CRI. A standard chemical fungicide (Ridomil), non toxic products, a product that would act as a contact fungicide (Product X), at different rates, and a biological control product (*Trichoderma*), were evaluated in this trial. Only two products proved effective by ensuring that the trees were *Phytophthora*-free. Ridomil Gold and the BASF treated treatments tested negative (4.4.7).

Potassium phosphonate (e.g. Phytex) is used worldwide for the control of Peronosporales such as *Phytophthora nicotianae*, the causal agent of citrus brown rot, collar rot and root rot. Phytotoxicity is the single worst problem due to the use of the phosphonates. There is a need to reduce the incidence of phytotoxicity. Three cultivars, viz. a Clementine-, Empress mandarin and navel orange were evaluated. No phytotoxic symptoms were visible on any of the fruit cultivars sprayed. The brown rot results clearly indicate that the Phosphonates applied on their own as well as Aliette are the most effective and economically viable option to be utilised to control *Phytophthora* brown rot on a variety of citrus cultivars. No buffer agents should be mixed with spray tank mixtures when the Phosphonates (Phytex or Fighter) or other fungicides are used to control brown rot on citrus (4.4.8).

The two most common methods of phosphonate applications on citrus are foliar sprays and painting of trunks. The long-term use of soil treatments may result in the building up of soil micro-organisms able to biodegrade potassium phosphonate. Soils with different clay contents were collected from two orchards at Letaba Estates with a history of phosphonate application via irrigation. This experiment will give more clarity on the fate of phosphonate products in soil in order to pursue this method of phosphonate application as a possible replacement for foliar and stem paint applications (4.4.9).

Projekopsomming

Hemicyclophora, die skedeaalwurm, is reeds so vroeg as 1963 in verskeie boorde in Suid Afrika geïdentifiseer. Tans is dié aalwurm teenwoordig in die Gamtoosriviervallei, in sekere boorde in die Wes-Kaap asook in sekere boorde in Mpumalange se Laeveld. Standaard na-plant chemiese aalwurmdoders, geregistreer op sitrus, sal die skedeaalwurm ook beheer. Vier van die gewildste onderstamme is tydens die evaluasie proses gemonitor, naamlik growweskijsuurlemoen, Swingle citrumelo, Carrizo citrange en C35. Swingle en die C35 onderstamme het die laagste aalwurmgetalle in vergelyking met die ander twee vatbare onderstamme gehad wat bevestig dat hierdie onderstamme oorweeg kan word in 'n herplant situasie. Die proef word getermineer, en voorgestel dat 'n addisionele proef uitgelê word waar die verskillende onderstamme direk met mekaar vergelyk word ten opsigte van prestasie en die effek wat die twee aalwurms op die onderstamme ondeskeidelik sal uitoeven (4.4.2).

PL+ se biologiesebeheerprodukt word gewoonlik per hand of deur middel van 'n mikro- besproeiingstelsels toegedien. Om die effektiwiteit van die produk moontlik te verhoog, word die toediening van die produk deur 'n drupbesproeiingstelsel voorgestel. Geen geskikte drupbesproeiingsperseel met hoë aalwurmgetalle kon gevind word in boorde wat om Nelspruit, Tzaneen sowel as in die Letsitele gebied, geleë is nie. Drupbesproeiingsboorde sal steeds gemonitor word om 'n geskikte perseel te vind (4.4.3).

Die soektog na alternatiewe beheermaatreëls teen die sitrusaalwurm is 'n prioriteit vir navorsers wêreldwyd. Die effek van Bio-beroking word tans wêreldwyd nagevors maar geen sukses verhale in die boord omgewing is bekend nie. Geen boorde in die Gamtoosrivier vallei, waar die proef tydens 2005 beplan is, was verwyder nie. Hierdie projek voorstel sal weer vir die 2007 seisoen voorgelê word (4.4.4).

'n Registrasieproef vir FMC, België, is uitgevoer om die geregistreerde Rugby formulasies met 'n nuwe Rugby 20CS formulاسie asook die nuwe Mocalp vloeibaar met die ou korrel formulاسie, op 'n kontraktbasis te vergelyk. Die proef is oor 'n tweejaar tydperk ge-evalueer op Moosrivier sitruslandgoed. 'n Finale verslag is aan FMC België gestuur (4.4.5).

CRI is deur Illovo Suiker genader om Crop Guard, 'n chemiese afvalprodukt van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te evalueer. Vyf afsonderlike proewe is gedurende die 2004 en 2005 seisoen geëvalueer. 'n Finale verslag is aan hulle gestuur (4.4.6).

Die fosfonate is tans die mees effektiefste produkte beskikbaar vir die beheer van *Phytophthora* spp. op sitrus. Dieselfde resultaat word ongelukkig nie weerspieël in die kwekery bedryf waarvan fosfonate gebruik gemaak word nie. Die beplande proef het 'n standaard Ridomil swamdoder behandeling asook 'n nie-toksiese kontak swamdoder, 'n biologiese beheer agent (*Trichoderma*), en 'n nuwe BASF kombinasie behandeling, wat teen verskillende dosisse en tye toegedien is. Nie een van die produkte behalwe die standaard Ridomil asook die nuwe BASF behandelings was effektief teen *Phytophthora* infeksies nie (4.4.7).

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is 'n uiters agressiewe grondgedraagde patogeen wat wortel-kraag en bruinvrot op sitrus kultivars veroorsaak. Die fosfonate is die mees effektiefste produkte beskikbaar vir die beheer van *Phytophthora* spp. op sitrus. Die doel van hierdie proef was om fitotoksiteit wat soms op vrugte voorkom indien van die fosfonate gebruik word te, voorkom deur produkte te kombineer met Sporekill. Drie kultivars, Clementines, Empress en nawels, is gespuit en geëvalueer. Die resultate het getoon dat die fosfonate asook Aliette die mees effektiefste bruinvrotbeheer produkte is om die siekte te beheer. Geen fitotoksiese simptome, Phytex brand, is op enige van die kultivars se vrugte waargeneem nie. Geen buffers behoort by spuitmengsels gevoeg te word indien fosfonate asook ander swamdoders gebruik word om bruinvrot te beheer nie (4.4.8).

Blaartoediening van kaliumfosfonaat om *Phytophthora* wortel- en kraaggvrot te beheer, veroorsaak soms fitotoksiese skade op vrugte. Produsente is bekommerd dat die alternatiewe, nl. grondtoedienings op die langduur 'n opbou van grondmikro-organismes kan veroorsaak wat versnelde afbraak van kaliumfosfonaat tot gevolg mag hê. In die proef is grond met verskillende klei-inhoud uit twee sitrusboorde op Letaba Landgoed versamel. Beide boorde het minstens 13 kaliumfosfonaat toedienings oor die afgelope drie jaar deur die besproeiingssisteem ontvang. Die resultaat van die ontledings wat gedoen is, is nog nie beskikbaar nie (4.4.9).

4.4.2 Rootstock evaluation against *Hemicycliophora* in the Gamtoos River Valley Experiment 676 by M.C. Pretorius (CRI)

Opsomming

Die sitrusaalwurm is steeds the 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. *Hemicycliophora*, die skedeaalwurm, is reeds so vroeg as 1963 in verskeie boorde in Suid Afrika geïdentifiseer. Tans is dié aalwurm teenwoordig in die Gamtoosriviervallei, in sekere boorde in die Wes-Kaap asook in sekere boorde in Mpumalange se Laeveld. Die skedeaalwurm kom in kombinasie met die sitrusaalwurm op wortels van sitrus voor. Die effek van die skedeaalwurm op die sitrusboom word as gevolg van die bg. kombinasie bemoelik. Drie spesies is geïdentifiseer, nl. *Hemicycliophora halophila*, *H. nortoni* en *H. typica*. Die standard na-plant chemiese aalwurmdoders, geregistreer op sitrus, sal die skedeaalwurm ook beheer, soos vasgestel in vorige proewe. Vier van die gewildste onderstamme was in 'n *Hemicycliophora*-geïnfekteerde boord gemonitor, naamlik Growweskiisuurlemoen, Swingle citrumelo, Carrizo citrange en C35. Alhoewel die bome nog jonk is, en die situasie oor 'n langer termyn gemonitor behoort te word, het die Swingle en C35 onderstamme die laagste aalwurmgetalle in vergelyking met die ander twee vatbare onderstamme gehad. Dit is bekend dat Swingle as 'n weerstandbiedende onderstam suksesvol in herplant situasies gebruik kan word. Die bome is verwyder en elke boom se wortels is individueel gebruik vir analise wat 'n meer spesifieke weergawe van die situasie weergegee het. Die *Hemicycliophora* tellings is steeds laag maar kan toegeskryf word aan die kombinasie verteenwoordiging van sitrus en skede aalwurms in die grond asook die boompies se ouderdom. Dit is uiters belangrik dat boorde wat ge-oormerk is vir herplant, gemonster word voor die verwydering van die bome, sodat die aalwurmpopulasiestatus in die grond en wortels bepaal kan word. Hierdie sal dien as 'n bestuurshulpmiddel wat deur produsente aangewend kan word in hul besluitnemingsproses tydens die beplanning van die nuwe uitbreiding. Die proef word getermineer, daar word wel voorgestel dat 'n adisionele proef uitgelê word waar die verskillende onderstamme direk met mekaar vergelyk word ten opsigte van prestasie en die effek wat die twee aalwurms op die onderstamme ondeskeidelik sal uitoefen.

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).

Hemicycliophora causes root tip swelling and stunted root or plant growth. The nematode feeds in large numbers at root tips. Root galls are formed by an increase in cell divisions (hyperplasia), giving rise to an enlarged cortex (Evans, 1993). Greenhouse evaluations indicate a 36% growth reduction in citrus and 28% in tomato plants (Franklin *et al.*, 1974). *Hemicycliophora* nudity 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). Registered post plant nematicides do control these nematodes effectively.

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 purpose of this trial was to determine the effect of *Hemicycliophora* on different rootstocks currently used by the citrus industry. The trial was laid out in the Gamtoos River Valley.

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. Unfortunately none of the four rootstocks were planted in nematode-free soils so that the direct effect of the nematodes on the rootstocks could be compared to the rootstocks that were planted in infested soils.

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 second month. These trees were fertilized by means of a liquid fertilizer mixture through the drip irrigation system by the producer. The trees were removed allowing for a more definite result as the roots from each tree could then be analysed. This was the final evaluation.

The following parameters were evaluated for the duration of this trial:

1. Soil analysis and root analysis to determine the nematode population counts.
2. Visual rating.

The trees were removed in January 2006, three years after planting. The trees were marked and the different rootstocks with roots were brought to the Diagnostic Centre in Nelspruit.

Results and discussion

It is clear from the results in Table 4.4.2.1 that citrus nematode female populations were present on all the rootstocks evaluated and that very low infestations of *Hemicycliophora* populations were recorded. Initially the citrus nematode female population counts were low but the September 2004 results indicate a female infestation of > 2000 ♀/ 10 g of roots. The presence of nematodes in the Swingle treatment was alarming because it is known that Swingle is resistant against the citrus nematode. The presence of citrus nematodes on Swingle rootstocks were only recorded once, in a specific orchard, in the Sundays River Valley, and never again in any of the other citrus producing regions in South Africa. The reason for these results could

be attributed to the fact that the young trees were planted close to the infested mature trees and when treatments were sampled it was difficult to determine if roots were collected from the mature trees or the young trees. It was therefore decided that during the final analysis these trees should be removed and individually analysed. The final data indicate that the female population counts in the Swingle and C35 rootstocks were significantly less when compared to the other two susceptible rootstocks. It is known that the Swingle rootstock is more tolerant to nematodes and these results confirm that C35 is also tolerant to citrus nematode infections. These two rootstocks could therefore be considered in a replant situation. 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 final results also indicated that the Rough lemon and Carrizo Citrange rootstocks had the highest citrus nematode female counts. These results confirm the susceptibility of these two rootstocks to nematode infestations in replant situations.

It is clear from the results obtained that the citrus nematode populations increased to alarming high levels within three years. It is very important to determine nematode population status in the soil before removing an orchard for replant purposes. This approach will assist producers in making a rootstock choice on replant soils when high nematode population numbers are present. The *Hemicycliophora* counts were very low throughout the monitoring process of the trial and no significant differences in *Hemicycliophora* female counts between the different rootstocks occurred. The reason for the delay in an increase in the *Hemicycliophora* populations can be ascribed to the presence of both the citrus and Sheath nematodes in this orchard and due to the fact that the trees are still too young and do not have well-established root systems where infection can take place. The trees were under stress due to the competition effect of the older trees and due to the removal of half of the young trees' root systems during 2004 for analysis.

Table 4.4.2.1. *Tylenchulus semipenetrans* and *Hemicycliophora* juvenile and female population counts on 4 different rootstocks.

Rootstocks	<i>Tylenchulus semipenetrans</i>								<i>Hemicycliophora</i>							
	J2/250ml soil				♀ / 20 g roots				J2/250ml soil				♀ / 20 g roots			
	Feb 2003	Dec 2003	Sept 2004	Jan 2006	Feb 2003	Dec 2003	Sept 2004	Jan 2006	Feb 2003	Dec 2003	Sept 2004	Jan 2006	Feb 2003	Dec 2003	Sept 2004	Jan 2006
Swingle citrumelo	990 a	1270a	5180ab	1910ab	320a	240a	2700a	220a	196.66a	174.5b	240a	650 b	360ab	330 a	140a	130a
Carrizo Citrange	3130a	4020b	7790b	4180bc	2020ab	2580b	3420a	2680b	60a	44a	300a	350ab	90a	250 a	200a	110a
Rough lemon	3330a	2580ab	6200ab	5580c	1940ab	2820b	3960a	5760c	193a	27.5a	60a	390ab	810b	230 a	140a	140a
C35 citrange	1790a	1610a	3790a	1020a	2660b	1460b	3740a	540a	126.5a	98.5ab	140a	160a	340ab	230 a	180a	140a

Means in a column, followed by the same letter are not significantly different ($P>0.05$) according to Fisher's least significant difference test.

Conclusion

This trial confirmed that Swingle citrumello and C35 rootstocks are tolerant rootstocks against nematode infestations and can be considered by producers when replanting a nematode infested orchard. The results in this trial indicated the importance that orchards to be removed for replant purposes should be sampled prior to the removal action to determine the nematode female population status. The result would assist producers with rootstock choices and make them aware of increased nematode population numbers if more susceptible rootstocks such as Rough lemon and the Citranges are used. It is suggested that this trial should be repeated to compare the different rootstocks with each other to determine the effect of the nematodes on the rootstocks. The control treatment trees should be replanted in nematode-free soils. This treatment should therefore include a Methylbromide application as a pre-plant application to ensure that the trial plots will be nematode free for at least six years.

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4.4.3 Biological control of the citrus nematode under a drip irrigation system Experiment 760 by M.C. Pretorius (CRI)

Opsomming

PL+ se biologiesebeheer produk word gewoonlik per hand of deur middel van 'n mikro- besproeiingstelsels toegedien. Om die effektiwiteit van die produk moontlik te verhoog, word die toediening van die produk deur 'n drupbesproeiingstelsel voorgestel. Gunstige omgewingstoestande kan d.m.v. dié benadering geskep word indien die biologiesebeheer agente toegedien word. Die produk sal oor 'n tydperk toegedien word en die aalwurmpopulasies in die grond en wortels sal gemonitor word. Geen geskikte drupbesproeiingsperseel met hoë aalwurmgetalle kon gevind word in boorde wat om Nelspruit, Tzaneen sowel as in die Letsitele gebied, geleë is nie. Die soektog sal uitgebrei word na ander sitrusproduserendegebiede. Boorde op drupstelsels sal voortdurend gemonitor word om 'n geskikte perseel te vind.

Summary

Plant parasitic nematodes spend at least some part of their lives in the soil, one of the most complex of environments. The biological component of the soil ecosystem is particularly important in limiting or stabilizing nematode populations. In the past, applications of the PL+ treatments were done by hand under a micro irrigation system. The use of this biological control product should be evaluated under a drip irrigation system, which could ensure that conditions are more favourable for a biological control agent to become established in the soil. The trial should be laid out in a drip irrigation orchard as a semi commercial trial, with nematode numbers exceeding the threshold value of 1000 females/10 g roots. Six treatments with 20 trees per row will be treated at different intervals. Soil and root samples will be taken three times during the first year and then before each follow-up treatment, the year thereafter. A standard nematicide (Rugby ME) application will be done as registered and will serve as a chemical control treatment. No suitable trial site, with high nematode population numbers, could be found in the Nelspruit, Tzaneen and Letsitele regions. Orchards sampled and analysed for nematode female population numbers did not even exceed the required threshold value of 1000 females / 10 g roots to justify the application of a nematicide or the biological control product. Orchards in other regions will however be monitored to continually seek for a suitable trial site. The investigation was not extended to regions in the Western or Eastern Cape because of financial constraints

due to the regular application schedule required by the manufacturer. No results are therefore available. CRI will continue to seek a suitable trial site.

4.4.4 To evaluate alternative nematode control products, i.e. Biofumigants, as part of an integrated nematode control approach in citrus replant situations Experiment 762 by MC Pretorius (CRI)

Opsomming

Die soektog na alternatiewe beheermaatreëls teen die sitrusaalwurm is n prioriteit vir navorsers wêreldwyd. Die gebruik van hoogs toksiese aalwurmdoders wat 'n groot effek het op die omgewing asook lewende organismes, word al meer en meer veroordeel deur verbruikers asook omgewingsbewuste groepe regoor die wêreld. 'n Ge-integreerde beheerbenadering sal in die toekoms meer aandag moet ontvang vanaf die produsente om te verseker dat die beheer van die aalwurm probleem nie agter weë gelaat word nie. In die verlede is hoofsaaklik van chemiese produkte gebruik gemaak om die probleem in 'n kits op te los. Die effek van Bio-beroking word tans wêreldwyd nagevors maar geen sukses verhaale in die boord omgewing is bekend nie. CRI het 'n reeks van voor-plant behandeling voorgestel om die effek van die produkte oor 'n lang termyn te evalueer, op herplant gronde. Die voordeel van so 'n behandeling indien effektief sal die gebruik van toksiese aalwurmdoders, wat huidiglik ook baie duur is, verminder. Geen boorde waar die proef tydens 2005 beplan is, was verwyder nie. Daar word wel beplan om boorde gedurende 2006 te verwyder en te herplant. Weens finansiële beperkinge is die proefvoorstel ongelukkig nie verlede jaar goedgekeur nie maar daar word steeds geglo dat die benadering wel belofte sal inhou as 'n lang termyn oplossing op herplant gronde.

Introduction

The search for alternatives to soil fumigants and very toxic nematicides, is a priority at research stations and by researchers worldwide. Producers will have to change their way of thinking from a one shot control strategy they have become accustomed to, to a more integrated approach such as host plant resistance, bio-fumigation, alternative chemicals and cultural practices. By focusing on alternative control strategies e.g. by incorporating bio-fumigants into traditional control strategies, one could reduce the usage of toxic nematicides polluting the environment. A combination of bio-fumigants, alternative non-toxic chemicals, biocontrol agents, rootstock choices and cultural practices should be implemented as a new approach of pest control in the soil against known soil borne pests and diseases. This trial was initiated due to the high post-plant nematicide treatment costs that have a negative impact on nematode control. Alternative measures should therefore be available to producers to keep their replant orchards nematode-free for as long as possible before a post-plant nematicide treatment is necessary, if at all.

The idea is to include pre-plant treatments of different products at different rates and times. The effect of the pre-plant treatments will then be monitored by means of soil and root analysis to determine the nematode population status. A visual evaluation will also be done on an annual basis. This trial will have to be monitored for at least eight years.

The trial layout will consist of at least five rows each representing a different treatment which includes an untreated control, Vapam, Biofumigation product A, Biofumigation product B, nematode egg stimulating product A, nematode egg stimulating product B and a non-toxic nematicide. A suitable replant site with nematode female population numbers in excess of 6000 females/10 g roots will be ideal for this trial. The ideal location to layout the trial would be in Patensie in the Gamtoos River Valley due to the high citrus nematode numbers and the presence of the sheath nematode in their soils.

Results and Conclusion

No replant action was executed during the 2005 season by any of the producers in the Valley with orchards suitable for the trial and therefore the trial could not commence. During the 2006 season suitable orchards will be removed but due to financial constraints this trial proposal was not approved by the Disease Management Research Committee. It is believed that it is still a priority and therefore the proposal shall again be presented to the Disease Management Research Committee for approval in the 2007-8 financial year.

4.4.5 Evaluation of cadusafos on citrus trees in nematode-infested soils

Experiment 684 by M.C. Pretorius (CRI)

Opsomming

'n Registrasieproef vir FMC, België, is uitgevoer om die geregistreerde Rugby formulasies met 'n nuwe Rugby 20CS formulاسie asook die nuwe Mocap vloeibaar met die ou korrel formulاسie, op 'n kontrakbasis te vergelyk. Die proef is oor 'n tweejaar tydperk ge-evalueer op Moosrivier sitruslandgoed. 'n Finale verslag is aan FMC België gestuur.

Summary

FMC Belgium approached CRI to conduct a registration trial to establish the efficacy of a new Rugby 20CS formulation compared to the registered Rugby G and ME formulations on a contract basis. The new Mocap liquid formulation was also compared to the previous granular formulation. The trial was laid out at Moosrivier Citrus Estate. The trial site was selected on 12-year-old Valencia trees with ± 8000 ♀/10 g roots. The trial was applied and sampled three times during the season and the trial was evaluated over two seasons. A final report was sent to FMC for the work conducted.

4.4.6 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 chemiese afvalprodukt van suiker, se doeltreffendheid teen die sitrusaalwurm op 'n kontrakbasis te evalueer. 'n Registrasie proef is ook uitgelê met die oog op moontlike registrasie werk. Vyf afsonderlike proewe is gedurende die 2004 en 2005 seisoen op kontrak basis vir hulle ge-evalueer. 'n Finale verslag is aan hulle gestuur. Illovo het nog 'n enkele proef aangevra vir die 2006 seisoen wat ook op 'n kontrakbasis uitgevoer sal word gedurende die 2006 seisoen.

Summary

Illovo Sugar approached CRI to evaluate Crop Guard, a non-organophosphate nematicide, to determine its efficacy in controlling the citrus nematode, *Tylenchulus semipenetrans*, on a contract basis. Crop Guard is registered as a nematicide on peanuts. Five trials were laid out at Karino, Mpumalanga (four trials) and at Citrusdal, Western Cape (one trial). This research is ongoing and Illovo Sugar requested that the information be kept confidential until the final results are available. A final report was handed to Illovo Sugar for work done during the 2005 season. CRI was requested to evaluate the product for one more season in 2006.

4.4.7 The evaluation of enhancing products for the control of *Phytophthora* root rot diseases in citrus nurseries

Experiment 761 by M.C. Pretorius (CRI)

Opsomming

Die fosfonate is tans die mees effektiefste produkte beskikbaar vir die beheer van *Phytophthora* spp. op sitrus. Dieselfde resultaat word ongelukkig nie weerspieël in die kwekery bedryf waarvan fosfonate gebruik gemaak word nie. Die beplande proef het 'n standaard Ridomil swamdoder behandeling asook 'n nie-toksiese kontak swamdoder, 'n biologiese beheer agent (*Trichoderma*), en 'n nuwe BASF kombinasie behandeling, wat teen verskillende dosisse en tye toegedien is. Hierdie behandelings is met 'n *Phytophthora*-behandelde asook onbehandelde kontrole vergelyk. Die dosisse van die nie-toksiese produk RSAN-01 het visuele fitotoksiese simptome in al die behandelings getoon. Nie een van die produkte behalwe die standaard Ridomil asook die nuwe BASF behandelings was effektief teen *Phytophthora* infeksies nie. Die BASF resultate vertoon heel belowend en die produk behoort beslis weer ge-evalueer 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.

Initial trials concerning *P. nicotianae* var. *parasitica* and *P. citrophthora* were conducted with regard to chemical control of fungal root pathogens of citrus. 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/ml. 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). However these products were contact fungicides and, when irrigated or during rainfall, were washed off the plant.

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, chlamyospores 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 in field situations (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, chlamyospores and oospores was highly sensitive to fosetyl-Al, but zoospore and chlamyospore 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 (Zentmyer & Ohr, 1978). In the plant, fosetyl-Al is degraded to H₃PO₃.

The phosphonates are currently the most effective product that can 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. This research was conducted for this reason.

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 two months 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 twice a week. The treatments and time of application are shown in Table 4.4.7.1. A standard chemical fungicide (Ridomil), Unisun plus Phytix, three contact fungicides at different rates and a biological control product (*Trichoderma*), were evaluated in this trial.

Table 4.4.7.1. The evaluation of different fungicides applied at different dosages and times of application for the control of *Phytophthora* root rot in citrus nurseries.

	Treatment	Dosage	Treatment schedule
1.	Untreated control	No <i>Phytophthora</i>	
2.	Treated control	+ <i>Phytophthora</i>	Innoculate with Phyto (4 weeks)
3.	Ridomil	0.1 ml /bag	1 x month after 1 x inoculation of Phyto
4.	RSAN-1	0.1 ml / 100 ml	Innoculate with Phyto (4 weeks) – apply every 2
5.	RSAN-1	0.25 ml / 100 ml	
6.	RSAN-1	0.50 ml / 100 ml	

7.	RSAN-1	0.75 ml / 100 ml	weeks for 3 months
8.	RSAN-1	1 ml / 100 ml	
9.	RSAN-1	1 ml / 100 ml	Inoculate with Phyto (4 weeks) then apply 1 x month (2)
10.	Tricho 1	1 g / 4ℓ	Inoculate with Phyto (4 weeks) then apply once a month
11.	Tricho 2	1 g / 4ℓ	Inoculate with Phyto (4 weeks) then apply every two weeks
12.	Tricho 1	1 g / 4ℓ	Inoculate with Phyto (4 weeks) + EcoT and then only once a month
13.	Tricho 2	1 g / 4ℓ	Inoculate with Phyto (4 weeks) + EcoT and then only once every 2 weeks
14.	Unisun + Phytex	15 ml + 0.1 ml in 10ℓ	Inoculate with Phyto (4 weeks) then apply product
15.	Unisun + Phytex	30 ml + 0.1 ml in 10ℓ	
16.	Unisun + Phytex	60 ml + 0.1 ml in 10ℓ	
17.	BASF C	8 ml / 10ℓ (2.4 ml / 3ℓ)	Inoculate with Phyto (4 weeks) then apply product
18.	BASF A	13 g / 10ℓ (3.9g / 3ℓ)	Inoculate with Phyto (4 weeks) then apply product
19.	BASF C	8 ml / 10ℓ (2.4 ml / 3ℓ)	Inoculate with Phyto (4 weeks) then apply product once a month
20.	SK1 x	3 ml / 3ℓ	Inoculate with Phyto then apply product
21.	SK 2x	6 ml / 3ℓ	Inoculate with Phyto then apply product

Trees were sampled in August, September and November before the final analysis was done. The final parameters that were evaluated were tree height; stem diameter; wet-or-dry root mass and a visual tree health rating.

Results and discussion

It is clear from the results in Table 4.4.7.2 that the *Phytophthora* infection rate was not very high and a maximum average percentage infection rate of 53.7% was recorded in the *Phytophthora*-infested treated control treatment. The reason for the low infection rate could be due to the low day temperatures obtained during the winter season while the trial was executed. The temperatures in the nursery bags therefore did not rise high enough to favour pathogen activity. Most of the *Phytophthora*-infested and fungicide-treated treatments had a percentage of the trees infested with *Phytophthora* to a certain degree, however, only two products were effective by ensuring that the treatments were *Phytophthora* free. The untreated control, Ridomil Gold and BASF treated treatments tested negative to any possible *Phytophthora* infections. Although the percentage infection in most of the nursery bags was not very high, the mere presence of this devastating pathogen in a nursery bag is not permitted in any nursery tree originating from nurseries participating in the Citrus Improvement Programme.

Table 4.4.7.2. The effect of different treatments regarding the *Phytophthora* infection rate per bag, presented as an average percentage per treatment.

% <i>Phytophthora</i> infection			
	August 2005	September 2005	November 2005
Untreated control	0	0	0
Treated control	45.8	52.7	53.7
Ridomil	0	0	0
RSAN-1	28.6	12.9	28.6
RSAN-1	15.7	8.6	22.9
RSAN-1	15.7	10	10
RSAN-1	20.1	24.2	22.6
RSAN-1	20.9	33.4	19.8
RSAN-1	16.3	15.9	20.9
Tricho 1	11.4	20	25.7
Tricho 2	30	31.4	57.0
Tricho 1	25.7	22.9	20.0
Tricho 2	48.6	25.7	45.7

Unisun + Phytex	5.7	14.3	37.1
Unisun + Phytex	20	18.6	17.1
Unisun + Phytex	4.3	4.3	28.6
BASF C	0	0	0
BASF A	2.9	1.4	0
BASF C	0	0	0
SK1 x	4.3	8.6	17.1
SK 2x	5.7	14.3	20

The results (Table 4.4.7.3) where all the other parameters evaluated are presented indicate that no significant correlation regarding the stem diameter of the different treatments could be made. However, the results clearly indicate that the RSAN-01, *Trichoderma* and two of the Unisun treatments had a negative effect on the seedlings' growing process. A visual stunting of the seedlings in the RSAN-01 treatments was visible during the final visual evaluation of the trial. A visual phytotoxic effect of the RSAN-01 treatments was visible since the second application of this product where most of the trees were smaller and had a yellow appearance. Gum was also visible on the seedling stems at all the different rates applied. The wet root mass evaluation clearly indicates that the untreated control treatment seedlings had the most healthy root system. The root systems of the RSAN-01 treatments with the higher dosages indicate a phytotoxic effect due to the poor wet root mass weight with no correlation towards the *Phytophthora* infection rate of the root systems at all (Table 4.4.7.3). The combination of a Phosphonate, Phytex, and a product called Unisun (with apparent qualities to enhance the absorption process of products) did also not perform well and could not reduce the incidence of *Phytophthora* in the nursery bags as a soil drench. The *Trichoderma*-treated seedlings also did not respond positively to the treatments. Although the SK treated trees appear visually healthy the product was not effective in controlling *Phytophthora* as a soil drench application. The dry root mass clearly indicates that the BASF treatments were the better treatments when compared to the untreated control treatment. The dry root mass evaluation process is an indication of the health condition of the root system of a plant. The average tree mass of the untreated control treatment trees was clearly the most, whereas the RSAN-01 (1 ml/100 ml water), was the least.

Table 4.4.7.3. The effect of different fungicide applications against *Phytophthora* root rot in citrus nursery trees.

Treatment		July 05	Sept 05	Nov 05	July 05	Sept 05	Nov 05	Nov 05		
		Stem diameter	Stem diameter	Stem diameter	Tree Height (m)	Tree height (m)	Tree height (m)	Wet root mass (g)	Dry root mass (g)	Tree mass (g)
1	Untreated control	8.47 abcde	10.68 def	9.36 cdef	0.7 a	0.97efgh	1.16 g	64.58 h	15.54 hi	183.0 j
2	Treated control	8.83 bcdef	11.44 g	10.36 ghij	0.7 a	0.98 efgh	1.02 de	36.92 bcd	10.02 cdef	117.2 def
3	Ridomil	9.06 cdef	11.29 fg	10.12 fghij	0.7 a	1.04 hij	1.06 efg	47.92 fg	12.58 fgh	151.2 ghi
4	RSAN-1	8.56 abcde	11.23 fg	10.01 efghij	0.7 a	0.95 defg	0.98 cde	38.14 de	9.53 bcde	127 fgh
5	RSAN-1	9.09 def	10.49 cde	9.59 defg	0.7 a	0.95 defg	0.98 cde	31.55 abcd	8.21 abcd	111.6 cdef
6	RSAN-1	8.21 ab	9.73 ab	9.17 bcde	0.7 a	0.84 bc	0.89 abc	23.90 a	6.38 a	91 abc
7	RSAN-1	8.68 bcdef	9.72 ab	8.73 abc	0.7 a	0.78 ab	0.86 ab	31.67 abcd	8.34 abcd	98.2 abcd
8	RSAN-1	8.8 bcdef	9.43 a	8.3 a	0.7 a	0.71 a	0.81 a	23.16 a	6.06 a	77.3 a
9	RSAN-1	8.33 abc	9.49 ab	8.5 ab	0.7 a	0.79 ab	0.86 ab	22.56 a	6.24 a	84.7 ab
10	Tricho 1	8.55 abcde	10.87 efg	9.8 defghi	0.7 a	0.91 cdef	0.98 cde	30.78 abcd	8.81 abcd	120.2 def
11	Tricho 2	8.67 bcdef	10.52 de	9.22 bcde	0.7 a	0.922 cdef	0.95 bcd	30.70 abcd	8.20 abcd	101.5 abcd
12	Tricho 1	8.64 bcdef	10.43 cde	9.87 defghij	0.7 a	0.90 cde	0.94 bcd	29.27 abcd	7.80 abc	118.2 def
13	Tricho 2	8.27 ab	10.08 bcd	9.13 bcde	0.7 a	0.98 efgh	1.04 def	26.59 ab	6.56 a	107.0 bcdef
14	Unisun Phytex +	8.71 bcdef	10.53 de	9.55 defg	0.7 a	1.01 ghi	1.04 def	30.45 abcd	7.73 abc	113.2 cdef
15	Unisun Phytex +	8.40 abcd	9.87 abc	9.62 defgh	0.7 a	0.88 cd	0.89 abc	27.23 ab	6.63 ab	105.5 bcde
16	Unisun Phytex +	7.83 a	9.86 abc	9.07 abcd	0.7 a	0.93 defg	0.79 a	27.91 abc	6.97 ab	103.3 bcde
17	BASF C	9.35 f	11.33 g	10.58 hij	0.7 a	1.11 j	1.15 fg	53.22 g	15.61 i	173.3 ij
18	BASF A	9.08 def	11.03 efg	10.63 ij	0.7 a	0.99 fgh	1.07 efg	45.88 efg	13.02 ghi	155.3 hi
19	BASF C	9.16 ef	10.84 efg	10.27 fghij	0.7 a	1.01 ghi	1.04 def	44.62 efg	12.31 efg	156 hi
20	SK1 x	8.5 abcde	10.44 cde	10.40 hij	0.7 a	0.99 fgh	1.02 de	34.25 bcd	8.8 abcd	131.4 fgh
21	SK 2x	8.94 bcdef	10.97 efg	10.76 j	0.7 a	1.07 ij	1.14 fg	38.47 def	11.09 defg	149.3 ghi

Means in a column, followed by the same letter are not significantly different ($P>0.05$) according to Fisher's least significant difference test.

Conclusion

The results clearly indicate that healthier more vigorous trees can be grown in the absence of *Phytophthora* root infections. *Phytophthora*-free plant material is a prerequisite for citrus nurseries participating in the Citrus Improvement Programme. RSAN-01 had a phytotoxic effect on the citrus seedlings since the first application by visually appearing yellow and stunted and having gum on the stems of the seedlings. These observations were reflected in the results obtained during the final analysis of the trial. RSAN-01 did not control the *Phytophthora* infections in the nursery bags. None of the other fungicides were effective in reducing *Phytophthora* infections in the nursery bags despite the inoculum pressure being very low. The only two products that were effective were the Ridomil and BASF products.

Future research

BASF did register the BASF C product against *Phytophthora* spp. on tobacco as a soil drench application. The potential of this product should be evaluated on its own or in combination with other fungicides as an alternative for the citrus nurseries. It is essential that new fungicides or products are evaluated to assist the nursery industry in ensuring that healthy plant material is available to the citrus producer on a continual basis.

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4.4.8 Application of the phosphonates in combination with other chemicals at lower concentrations for *Phytophthora* brown rot control Experiment 813 by MC Pretorius and G.C. Schutte (CRI)

Opsomming

Phytophthora nicotianae var. *parasitica* (Dastar) Waterhouse is 'n uiters agressiewe grondgedraagde patogeen wat wortel- en bruinvrot op sitrus kultivars veroorsaak. Die fosfonate is tans die mees effektiewe produkte beskikbaar vir die beheer van *Phytophthora* spp. op sitrus en word tans met groot sukses in die bedryf gebruik. Die doel van hierdie proef was om fitotoksiteit wat soms op vrugte voorkom te monitor wanneer fosfonate in tenkmengsels gebruik word met produkte soos Sporekill en koperswamdoders. Die effektiwiteit van hierdie kombinasie deur van die dosisse laer aan te pas is ook geëvalueer. Drie kultivars; Clementines, Empress en Navels, is gespuit en geëvalueer. Resultate toon dat die fosfonate steeds die mees effektiewe bruinvrotbeheer-produkte is om die siekte te beheer. Geen fitotoksiese simptome is op enige van die kultivars se vrugte waargeneem nie. Blaarval het wel by sekere behandelings voorgekom veral die kombinasie waar Sporekill en koper asook Sporekill met Fighter, Phytex en Aliette op hul eie aangewend is. Die kombinerings van Sporekill met die fosfonate kan nie nou al

aanbeveel word nie, en moet verder ondersoek word. Geen buffers moet by tenkmengsels gevoeg word indien fosfonate asook ander swamdoders gebruik word om bruinvrot te beheer nie. Die pH van hierdie mengsels sal fitotoksies vir die boom wees aangesien die pH van die fosfonate reeds laag is. 'n Proef om die effek van al die gewilde buffers, "Stickers" asook enige ander "adjuvant" produkte in kombinasie met bruinvrotbeheerprodukte, fosfonate asook ander swamdoders, in dieselfde spuitmengsel, sal geëvalueer 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*.

Potassium phosphonate (e.g. Phytex) is used worldwide for the control of Peronosporales such as *Phytophthora nicotianae*, the causal agent of citrus brown rot, collar rot and root rot. This product has proved itself to be the most effective control method not only in citrus but also on a wide variety of other crops including trees, shrubs (Azalea) and pastures (Clover).

The ED50 value for *in vitro* inhibition of *Phytophthora* root rot with H₃PO₃ is 30 µg/ml, and according to Afek & Szejnberg (1989) H₃PO₃ is also 6-14 times more active than fosetyl-Al (Aliette) in inhibiting mycelial growth. *Phytophthora* has decreased from being the most important citrus disease in SA in the 1970s to an almost forgotten problem in the year 2000 since the use of CIP certified trees in 1984, combined with phosphonate foliar applications and stem paints.

Phytophthora brown rot as a post harvest disease, caused by *Phytophthora* spp. is a major problem in most of the citrus production areas of South Africa. Epidemic seasons are commonly associated with periods of prolonged wetness and with temperatures in the range of 20-32°C coinciding with the maturation of early and mid-season citrus cultivars. The chances of developing new fungicides in the near future are slim. The current trend is to move towards products to stimulate the plant's own defence mechanisms, for instance the phosphonates to control *Phytophthora* on citrus (Schutte, 1990). Excellent results were obtained in trials and in the industry in controlling *Phytophthora* brown rot on citrus with the use of phosphonates. But phytotoxicity is the single worst problem with the use of the phosphonates. There is a need to evaluate phosphonates in combination with other fungicides or chemicals to reduce the incidence of phytotoxicity.

Materials and methods

Three different orchards West of Nelspruit in the Schagen area were selected for the trial. The three orchards consisted of three cultivars, viz. a Clementine, Empress mandarin and Navel orange orchard. Three trees per treatment were used and the trees received a single spray application. Fungicides were applied as a single application with a trailer-mounted, high volume, high-pressure (2500 to 3000 kPa) sprayer with two hand-held spray guns. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of runoff. Treatments as well as the rates of application are presented in Table 4.4.8.1.

Table 4.4.8.1. A single application of different fungicides and phosphonates applied on their own and in combination with a sanitizing agent at different rates to determine the efficacy of these products on three different cultivars, viz. Empress Mandarin, Clementine and Navel orange trees against *Phytophthora* brown rot.

Treatments	Rate/100ℓ water
1. Untreated Control	-
2. Phytex	1 ℓ
3. Fighter	570 ℓ
4. Sporekill	100 ml
5. Phytex + Sporekill	500 ml+100 ml
6. Fighter + Sporekill	258 ml+100 ml
7. Sporekill + Copper Oxychloride	100 ml+100 g
8. Sporekill + Copper Oxychloride	100 ml+ 200 g
9. Fighter + Sporekill	285 ml + 50 ml
10. Sporekill + Amino - Copper	100 ml +100 ml

Fruit was harvested from each cultivar 2, 3 and 4 weeks after the spray application. Twenty fruit per treatment were harvested and placed on moist soil known to be infested with *Phytophthora*. The incidence of *Phytophthora* brown rot was visually monitored on a daily basis. The fruit was removed after a week and the process was repeated when the next week's fruit was harvested. The ideal would be to monitor the same fruit once harvested and placed on infested *Phytophthora* soil to determine the long term protection that the different treatments would achieve. This was not possible because space and logistical aspects limited such an effort.

The trees and fruit were visually inspected on a weekly basis for any visual phytotoxic reaction of the products to the trees and fruit in the orchard.

The day temperature recorded when the sprays were done was 32°C. The pH of the water used for spraying was 10. The Empress and Navel trees had a very heavy crop load when the trial was conducted. No other stress factors were visible when the trial was sprayed. See Figs. 4.4.8.1 & 4.4.8.2 for fruit colour.

The pH of the spray tank mixture of the different phosphonates and fungicides with water was recorded and then after a buffer (25 l/100 l water) was added to the mixture another reading was recorded. The aim was to determine the effect of products such as a buffer to the spray tank mixture's pH when used to adjust the water pH prior to spraying.



Fig 4.4.8.1. Colour stages of the Clementine fruit, compared with the CRI colour plate, at the time of spraying.



Fig 4.4.8.2. Colour stages of Navel fruit, compared with the CRI colour plate, at the time of spraying.

Results and discussion

No phytotoxic symptoms were visible on any of the fruit of all cultivars sprayed in this trial. The fruit on the trees was monitored for up to six weeks after application. The fruit was harvested thereafter. A phytotoxic reaction that caused leaf drop was recorded on the navels only. The treatments responsible for the phytotoxic reaction were: treatment 2, Phytex (1 ℓ), treatment 6, Fighter (285 mℓ) + Sporekill (100 mℓ), treatment 10, Amino-Copper (100 mℓ) + Sporekill (100 mℓ) and treatment 12, Aliette (250 g) (Figs. 4.4.8.3, 4.4.8.4, 4.4.8.5 & 4.4.8.6).



Fig. 4.4.8.3. Leaf drop due to a phytotoxic reaction of the combination of amino-copper and Sporekill on navels.



Fig. 4.4.8.4. Leaf drop due to a phytotoxic reaction of Aliette on navels.



Fig. 4.4.8.5. Leaf drop due to a phytotoxic reaction of the combination of Fighter and Sporekill on navels.



Fig. 4.4.8.6. Leaf drop due to a phytotoxic reaction of Phytex on navels.

The results obtained in Table 4.4.8.2 clearly indicate that when a buffer is added to the spray tank mixture after the different treatments were added, the pH of the tank mixture drops to very low levels which will have a phytotoxic effect on any tree sprayed. Therefore spray mixtures should be monitored more regularly prior to spraying.

Table 4.4.8.2. The effect of a buffer (Bladbuff) on the pH of tank mixtures of phosphonates alone and in combination with Sporekill, Amino-Copper and copper oxychloride.

Treatments	Rates	Spray tank mixture (pH)	Spray tank mixture (pH) with buffer added
1. Untreated Control	-	-	-
2. Phytex	1 l	6.9	5.9
3. Fighter	570 l	6.4	5.5
4. Sporekill	100 ml	7.3	2.9
5. Phytex + Sporekill	500 ml+100 ml	6.5	5.8
6. Fighter + Sporekill	258 ml+100 ml	6.1	5.4
7. Sporekill + Copper oxychloride	100 ml+100 g	7.4	3.8
8. Sporekill + Copper oxychloride	100 ml+ 200 g	7.2	3.7
9. Fighter + Sporekill	285 ml + 50 ml	5.8	5.1
10. Sporekill + Amino-Copper	100 ml +100 ml	6.9	2.4
11. Aliette	250 g	6.2	2.9

The results from Table 4.4.8.2 indicate that no buffer should be used in a spray tank mixture when these products are sprayed. A pH of 5.5 could be regarded as the minimum pH level, whereafter any spray mixture would be phytotoxic when sprayed. These results should serve as a warning to producers and should be added to the warnings on the labels of the phosphonates and other registered products to be sprayed for *Phytophthora* brown rot. Variable results were obtained with the brown rot efficacy evaluations that were done on a daily basis at CRI in Nelspruit. The results are presented in Tables 4.4.8.3, 4.4.8.4 & 4.4.8.5.

Table 4.4.8.3. The percentage *Phytophthora* brown rot infection on Clementine fruit harvested 2, 3 and 4 weeks after spraying with different treatments as recorded in May 2005.

Treatments	% Brown rot infection on Clementine fruit 2 to 4 weeks after different fungicide applications												
	2 weeks			3 weeks					4 weeks				
	03/05	04/05	05/05	10/05	11/05	12/05	13/05	16/05	23/05	24/05	25/05	26/05	ummary
1. Untreated Control	5	5	15	50	25	35	45	60	10	40	100	100	100
2. Phytex	0	0	0	0	0	0	5	15	15	15	15	35	50
3. Fighter	0	0	0	0	0	0	0	5	5	5	5	20	30
4. Sporekill	30	30	35	5	20	30	40	50	5	20	40	60	80
5. Phytex + Sporekill	5	5	20	10	15	20	30	40	10	35	50	60	75
6. Fighter + Sporekill	5	5	20	5	10	15	15	35	40	65	70	80	80
7. Sporekill + Copper oxychloride 100 g/hℓ	20	20	25	5	5	10	25	30	45	65	75	75	100
8. Sporekill + Copper oxychloride 200 g/hℓ	0	0	0	0	0	10	20	15	0	20	65	65	85
9. Fighter + Sporekill	15	20	20	5	12	20	25	30	20	65	85	100	100
10. Sporekill + Amino-copper	40	45	45	0	5	10	10	20	40	95	100	100	100
11. Aliette	30	30	35	0	0	10	10	15	10	80	100	100	100

Table 4.4.8.4. The percentage *Phytophthora* brown rot infections on Navel orange fruit harvested 3 and 4 weeks after spraying with different treatments as recorded in May 2005.

Treatments	% Brown rot infection on Navel orange fruit 3 to 4 weeks after different fungicide applications									
	3 weeks					4 weeks				
	10/05	11/05	12/05	13/05	16/05	23/05	25/05	26/05	27/05	
1. Untreated Control	60	80	80	85	100	0	10	55	100	
2. Phytex	0	20	20	30	40	0	5	10	20	
3. Fighter	5	40	40	45	50	0	5	10	35	
4. Sporekill	55	85	90	90	90	0	5	50	75	
5. Phytex + Sporekill	0	30	30	65	80	0	10	10	15	
6. Fighter + Sporekill	5	30	30	35	35	0	20	55	70	
7. Sporekill + Copper oxychloride 100 g/hℓ	45	70	75	75	80	0	30	55	65	
8. Sporekill + Copper oxychloride 200 g/hℓ	45	65	65	65	75	0	10	80	95	
9. Fighter + Sporekill	0	10	10	20	20	10	10	10	30	
10. Sporekill + Amino- Copper	30	40	40	40	45	0	20	45	65	
11. Aliette	0	0	0	5	10	0	20	70	70	

Table 4.4.8.5. The percentage *Phytophthora* brown rot infections on Empress Mandarin fruit harvested 3 and 4 weeks after spraying with different treatments.

Treatments	% Brown rot infection on Empress Mandarin fruit 2-4 weeks after different fungicide applications										
	2 weeks			3 weeks				4 weeks			
	03/05	04/05	05/05	11/05	12/05	13/05	16/05	24/05	25/05	26/05	27/05
1. Untreated Control	50	65	65	25	50	50	100	35	50	80	100
2. Phytex	45	45	45	5	5	35	35	5	10	20	35
3. Fighter	10	10	10	5	5	35	35	0	0	0	10
4. Sporekill	60	60	65	60	75	80	100	70	95	100	100
5. Phytex + Sporekill	20	20	65	20	30	40	40	15	25	35	70
6. Fighter + Sporekill	15	15	30	25	40	55	80	5	20	20	40
7. Sporekill + Copper oxychloride 100 g/hℓ	45	45	60	30	35	35	55	30	55	55	85
8. Sporekill + Copper oxychloride 200 g/hℓ	15	20	20	10	10	25	35	5	15	40	80
9. Fighter + Sporekill	20	20	20	10	25	65	50	25	50	60	80
10. Sporekill + Amino- Copper	40	50	65	5	10	10	10	20	35	45	75
11. Aliette	0	0	0	0	15	25	25	0	5	5	15

The results in Table 4.4.8.3 indicate that the two phosphonate treatments and the Sporekill and copper oxychloride mixture treatments were the most effective treatments in restricting any brown rot development on Clementines up to 17 days after application. Brown rot developed immediately after harvest on treatments 4, 7, 9, 10 & 11. After 17 days at least 35% of the fruit in treatments 4, 10 and 11 were infested with *Phytophthora* brown rot. The final results (5 weeks after application) of the fruit harvested 4 weeks after application indicated that the Phosphonates on their own resulted in the least infected brown rot fruit.

The results in Table 4.4.8.4 indicate that treatments 11, 9 and 6 harvested 3 weeks after application, were the most effective treatments resulting in the least fruit being infected with brown rot on navel oranges. The final evaluations of the fruit harvested 4 weeks after application indicate that the Phosphonates (treatments 2 & 3), Phytex + sporekill (Treatment 5) and Fighter and Sporekill (treatment 9) had the least infected fruit.

According to Table 4.4.8.5., treatments 11, 3, 8, 9 & 6 applied on the Empress Mandarin trees were the least infected 17 days after application (Table 4.4.8.5). The final evaluations on the fruit harvested 4 weeks after application indicate that the phosphonates (treatments 2 & 3) and Aliette (treatment 11) were the most effective treatments.

Conclusion

The brown rot results clearly indicate that the phosphonates applied on their own, and Aliette, are still the most effective and economically viable options to be utilised to control *Phytophthora* brown rot on a variety of citrus cultivars. No phytotoxic symptoms were visible on any fruit although leaf drop did occur. The reason for the leaf drop in the Aliette and Phytex treatments cannot be explained. The day temperature, however, was 32°C and a heavy crop load was evident on the navel oranges. These factors could have contributed towards the phytotoxic reaction. No phytotoxic reactions were visible on the more susceptible Clementine and Empress cultivars. This reaction could not be explained. No buffer agents should be mixed with spray tank mixtures when the phosphonates (Phytex or Fighter) or other fungicides are used to control brown rot on citrus.

Future research

A trial to determine the pH of buffers, stickers and generally used adjuvants in combination with the phosphonates and other brown rot controlling fungicides should be screened to reduce any future phytotoxic reaction that might occur when utilising brown rot controlling products.

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4.4.9 *Phytophthora* root rot control - Determining the long term effect of phosphorous acid equivalent products in soil applied systems

Experiment by J.J. Serfontein, S. Serfontein, & S.H. Swart (QMS)

Opsomming

Blaartoediening van kaliumfosfonaat om *Phytophthora* wortel- en kraaggvrot te beheer, veroorsaak soms fitotoksiese skade op vrugte. Die ander algemene wyse van toediening, nl. stamverf, is duur weens die hoë arbeidsinset. Produsente is bekommerd dat die alternatiewe, nl. grondtoedienings op die langduur 'n opbou van grondmikro-organismes kan veroorsaak wat versnelde afbraak van kaliumfosfonaat tot gevolg mag hê. Daar bestaan ook 'n persepsie dat kaliumfosfonaat se effektiwiteit in klei-gronde beperk is weens moontlike adsorpsie aan klei-partikels.

In die proef is grond met verskillende klei-inhoud uit twee sitrusboorde op Letaba Landgoed tydens mid somer (Januarie) tydens die hoogste verwagte grond mikrobe aktiwiteit versamel. Beide boorde het minstens 13 kaliumfosfonaat toedienings oor die afgelope drie jaar deur die besproeiingssisteem ontvang. Twee grondmonsters is ook uit areas met dieselfde tiepe grond wat nog nooit met kaliumfosfonaat behandel is nie, versamel. Grond is by steriele water gevoeg (100 g/100 ml) wat 40 µg/l kaliumfosfonaat (Phytex) bevat, geskudinkubeer waarna steriele minimale medium wat 40 µg/l kaliumfosfonaat bevat, met die grondwater geïnkuleer is. Die media is weer geskudinkubeer waarna dit gevries is om in te dien vir kaliumfosfonaat

konsentrasie bepaling. 'n Ongeïnkuleerde minimale medium bevattende kaliumfosfonaat het as kontrole gedien. Grond met 'n hoë klei-inhoud en 'n sanderige grond is ge-outoklaveer om mikrobe aktiwiteit te inaktiveer. Die grond is daarna by steriele gedistilleerde water bevattende 40 µg/ℓ kaliumfosfonaat gevoeg, geskudinkubeer, die bostand verwyder en gevries vir kaliumfosfonaat bepaling.

Hierdie resultate behoort meer duidelikheid te gee wat die effek van grondgedraagde mikrobe en/of klei op kaliumfosfonaat in grond het.

Introduction

The use of phosphonates to control *Phytophthora* root and collar rot is common practice in South Africa. The two most common methods of phosphonate applications on citrus are foliar sprays and painting of trunks. Foliar sprays sometimes result in phytotoxicity on fruit while the intensive use of labour makes this type of application costly. Soil application of phosphonates via irrigation is becoming a popular method of application. Producers fear, however, that the long term use of this method may result in the building up of soil micro-organisms that are able to biodegrade potassium phosphonate or related products. There is also a perception that potassium phosphonate is not very effective if applied in clay soils due to possible absorption to clay particles. The aim of this study was to determine the fate of potassium phosphonate in soils with a history of application of the product as well as in soils with high clay content without taking the plants into consideration.

Material and methods

Soil with different clay content was collected from two orchards at Letaba Estates with a history of phosphonate application via irrigation. The soil, ~2 kg, was collected with a clean spade into plastic bags. The spade was cleaned with 70% ethanol between samples to prevent possible cross contamination by soil microbes. Soil of similar type as the two mentioned samples was collected from areas where no potassium phosphonate has been applied via the irrigation system. Soil was stored in the cold room (~11°C) until further processing. Sterile soft water (300 ml quantities) containing 40 µℓℓ was prepared in 500 ml Erlenmeyer flasks and inoculated with 30 g quantities of the different soil samples respectively. A flask to which no soil was added, served as control. These flasks (one control and four with soil added) were shake-incubated for ~72 h at room temperature to promote the growth of possible phosphonate degrading bacteria. Flasks containing 300 ml of a minimal medium (Parekh, *et al.* 1994, Roberts, *et al.*, 1991) amended with 40 µℓℓ potassium phosphonate were inoculated with 300 µℓ of the supernatant of each of the soil cultures. These cultures were shake incubated for 72 h at room temperature after which the medium was frozen in Ziplock bags to be submitted for chemical analysis (phosphonate presence).

In a second experiment soil with a high clay content, and sand was added to different beakers, wetted to about field capacity and autoclaved at 121° for 15 min in order to eliminate possible interfering microbes. Soil (200 ml) was added to 400 ml sterile soft water containing 40 µℓℓ potassium phosphonate in 1 000 ml Schott bottles and shaken vigorously by hand for two minutes and then shake-incubated for 20 min. The bottles were left on the table for the soil to settle (~20 to 30 min) after which the supernatant (~200 ml) was poured off into Ziplock bags and frozen for chemical analysis.

Results and discussion

The phosphonate content analysis was not done by the time of the report and the fate of potassium phosphonate in soil is therefore not known. In California the application of phosphonate to avocado trees via irrigation systems is common practice and according to Prof. Menge (*personal communication*) no problems regarding enhanced microbial degradation has been experienced after many years' applications. In his view, it is highly unlikely that the chemical will be degraded in soil at a rate that will interfere with its efficacy. In the absence of roots, due to advanced root rot, application via irrigation will be ineffective as the roots will not be able to take up the chemical.

This experiment will hopefully give more clarity on the fate of phosphonate products in soil in order to pursue this method of phosphonate application as a possible replacement for foliar and stem paint applications.

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4.5 PROJECT: POST-HARVEST PATHOLOGY

Project Co-ordinator: K.H. Lesar (CRI)

4.5.1 Project summary

In South African citrus packhouses, citrus fruits are treated with the post-harvest fungicides, imazalil, thiabendazole (TBZ) and guazatine (certain markets) to control infections caused by post-harvest pathogens. When using chemicals to control diseases, difficulties may be experienced when the pathogen becomes resistant to the pesticide or fungicide. The threat of the development of pathogen resistance to post-harvest fungicides has prompted the ongoing evaluation of new chemicals against the citrus pathogens in order to find new, safe compounds (GRAS compounds, biocontrol agents etc.), potential new chemistry fungicides and even formulated mixtures of two fungicides with different modes of action for more efficient waste control and to also prevent the build-up of resistance to one or more of the fungicides (4.5.2).

A new imazalil 500 EC formulation from ICA international, demonstrated effective control of the post-harvest citrus pathogen *P. digitatum* (green mould). The ICA imazalil 500 EC formulation was compatible with the water soluble citrus wax used in these evaluations. A new fungicide formulation, Guazalil, made up of a mixture of two post-harvest fungicides, imazalil and guazatine, effectively controlled *P. digitatum* (green mould) and *G. candidum* (sour rot). Philabuster, a mixture of two fungicides, imazalil and pyrimethanil (a new chemistry fungicide) demonstrated promising control of *P. digitatum* imazalil sensitive and imazalil resistant infections. This work is ongoing (4.5.3 & 4.5.4). Dodine, a guanidine like guazatine, demonstrated no control of infections caused by *P. digitatum*. No further work is planned with this compound (4.5.5).

The post-harvest fungicide guazatine, formulated into the CitriWax and Deccowax, controlled both *P. digitatum* (green mould) and *G. candidum* (sour rot). This demonstrated that there was no reduction in the efficacy of guazatine as the formulation aged over a period of time (4.5.6).

Two of the three sanitising agents, SteriHarvest, Aseptrol and G-cide A & B screened did not achieve the desired disinfestation of a packhouse dumptank, nor the prevention of infection of injured fruit moving through the bath compared to the control Sporekill. G-cide A shall be evaluated at higher concentrations (4.5.7).

Evaluation of Erador and Xterminator for post-harvest application for the control of Grain Chinch bug indicated that Xterminator was compatible with the fungicides but not Erador. Neither of the compounds indicated any phytotoxicity on the fruit in combination with the fungicides, nor alone (4.5.8).

Two packhouse wax trials with kaolin and lemons and kaolin and mangos were conducted at CRI. The reported breakdown of the wax formulation on the fruit was not observed because the kaolin residue on the fruit was effectively washed off on the packline, resulting in good, evenly waxed applications (4.5.9).

High imazalil residues in Japan on Star Ruby grapefruit from Neonovo (Lowveld) were reported during the 2005 season (4.5.10). It was determined that human error was the cause of the high residue. No corrective and preventative action was advised because packhouse procedures and critical control points were found to be well managed. Various trials were conducted to determine if certain variables in the application of imazalil in the packhouse contributed to the retention of high levels of imazalil residues on South African export citrus. The trials under controlled conditions and the results thereof did not reveal any imazalil residue levels higher than the 5.0 ppm MRL (4.5.11).

Twenty one *P. digitatum* fungal spore samples from the Eastern and Western Cape and Zimbabwe were screened for resistance to imazalil (4.5.12). Ten of the samples revealed different levels of resistance. These samples confirm the concern that the build up of *Penicillium* resistance to the post-harvest fungicide, imazalil, is on the increase. The screening of the *Penicillium* spore samples have shown thusfar that there are not only signs of detection of imazalil resistant spores but also indications of possible resistance to guazatine. These results would have to be correlated with the *in vivo* screenings before any resistance can be confirmed (4.5.13). A joint imazalil resistance strategy between CRI and Katco was proposed and approved during the 2005 season. The project involves the screening of *Penicillium* (green and blue mould)

fungal spore samples from all the major citrus production areas. The spore samples will be screened, *in vitro*, by Katco and the positive resistant samples will then be screened *in vivo* by CRI.

A number of plant growth regulators (PGRs) were screened, as alternatives to 2,4-D, for calyx (button) retention on citrus fruit under simulated shipping conditions. Promising results with a few of the PGRs, compared with the standard 2,4-D formulation, were recorded. These trials are ongoing (4.5.14).

Projekopsomming

Sitrusvrugte word behandel, in Suid Afrikaanse sitruspakhuse, met die na-oes swamdoders imazalil, thiabendazole (TBZ) en guazatine (sekere markte) om infeksies, veroorsaak deur na-oespatogene, te beheer. Tydens die gebruik van chemikalieë vir siektebeheer, probleme mag ondervind word sodra die patogeen bestand teen die plaagdoder of swamdoder word. Die dreiging van bestande *Penicillium* swamspoor populasies en moontlike algehele bestandheid teen die klein aantal na-oes swamdoders het die aangaande evaluering vir nuwe chemikalieë veroorsaak om nuwe, veiliger middels (GRAS chemikalieë, biologiesebeheermiddels ens.), nuwe chemie swamdoders en ook geformuleerde mengsels van twee swamdoders met verskillende werking, te ontdek, vir beter bederfbeheer en ook die vermeerdering van bestande spoor populasies te onderdruk (4.5.2).

'n Nuwe 500 EC formulاسie van imazalil, vanaf ICA Internasionaal, het goeie beheer van die na-oes sitrus patogeen *P. digitatum* (groenskimmel) gewys. Die ICA imazalil 500 EC formulاسie was verenigbaar met die wateroplosbare sitrus wakse gebruik in hierdie evaluاسies. 'n Nuwe swamdoder formulاسie van 'n mengsel van die twee na-oes swamdoders, imazalil en guazatine, Guazalil, het goeie beheer van *P. digitatum* (groenskimmel) en *G. candidum* (suurvrot) gewys. Philabuster, ook 'n mengsel van twee swamdoders, imazalil en pyrimethanil ('n nuwe chemie swammiddel), het belowende beheer van infeksies deur *P. digitatum* sensitiewe en imazalil bestande spore. Hierdie werk is aangaande (4.5.3 & 4.5.5). Dodine, 'n guanidine soos guazatine, het geen beheer gewys teen infeksies van *P. digitatum* nie. Geen verdere werk is beplan met die middel nie (4.5.5).

Die na-oes swamdoder guazatine, geformuleer in die Citriwaks en die Deccowaks, het albei *P. digitatum* (groenskimmel) en *G. candidum* (suurvrot) effektief beheer. Dit het gewys dat daar geen afname in effektiwiteit van guazatine in Citriwaks sodra die formulاسie mettertyd verouder (4.5.6).

Twee van die drie saniteermiddels, SteriHarvest, Aseptol en G-cide A & B, geevalueer, het nie die gewenste ontsmetting van 'n pakhuis dompelbad uitgevoer nie, en ook nie die besmetting van beseerde vrugte deur die bad voorkom nie, in vergelyking met die kontrole Sporekill. G-cide A sal teen 'n hoer konsentrasie geevalueer word (4.5.7).

Evaluاسie van Erador en Xterminator, aangewend na-oes vir die beheer van Chinchluis, het gewys dat Xterminator en nie Erador verenigbaar met die na-oes swam doders is. Albei middels het geen fitotoksisiteit gewys in kombinasie met die swam middels nie (4.5.8).

Twee pakhuis waks proewe met kaolien en suurlemoene en mangos is by CRI uitgevoer. Die beweerde verbrokkeling van die waks is nie op hierdie suurlemoene en mangos, na gesimuleerde verskeping, waargeneem nie omdat die kaolien residu op die vrugte op die paklyn afgewas is, en dit het goeie egalige waks aanwending veroorsaak (4.5.9).

Hoë imazalil residu in Japan op Star Ruby pomelos vanaf Neonovo (Laeveld) is tydens die 2005 seisoen geraporteer (4.5.10). Dit is bepaal dat 'n menslike fout, wat bygedra het tot die hoë residu, ondergaan is. Geen regstellende aksie is voorgestel nie, omdat al die pakhuis prosedure en kritiese beheer punte in die pakhuis deeglik bestuur word. Verskeie proewe is uitgevoer om te probeer bepaal of sekere variاسies tydens die aanwending van imazalil in die pakhuis bydra tot die behoud van hoë vlakke van imazalil residue op S. Afrikaanse uitvoer sitrus. Dië behaalde proewe en die resultate het geen onreëlmatige residu vlakke van imazalil gewys wat hoer as die MRV van 5.0 dpm was (4.5.11).

Een-en-twintig *P. digitatum* swamspoor monsters vanuit die Oos- en Wes-Kaap en Zimbabwe is ge-evalueer teen imazalil vir bestandheid (4.5.12). Tien van die monsters het verskillende vlakke van bestande spore gewys. Dié aantal positiewe monsters bevestig die kommernis dat die bestandheid teen imazalil besig is om te vermeerder.

Die evaluering van die *Penicillium* spoor monsters tot dusvêr het gewys dat daar nie net tekens van imazalil bestande spore is nie, maar wel ook aanduidings van moontlike bestandheid teen die ander belangrike na-oes swamdoder, guazatine is. Hierdie resultate sal eers met die *in vivo* evaluering gekorreleer moet word

voor die bevestiging van enige bestandheid (4.5.13). 'n Gesaamentlike imazalil weerstandbiedendheid strategie tussen CRI en Katco is voorgestel en goedgekeur tydens die 2005 sitrus seisoen. Die projek bevat die evaluering van *Penicillium* (groen en blouskimmel) swamspoormonsters, vanuit al die hoof sitrus produksie gebiede. Die spoormonsters sal deur Katco *in vitro* geëvalueer en die positiewe monsters sal dan *in vivo* deur CRI geëvalueer word.

Verskeie plantgroeireguleerders (PGR's) is na-oes geëvalueer as alternatief vir 2,4-D natrium sout (Deccomone) vir blomkelk behoud op sitrus vrugte, tydens gesimuleerde verskepings toestande. Goeie blomkelk behoud deur van die PGR's, in vergelyking met die standaard aanbevole 2,4-D (Deccomone) is waargeneem (4.5.14).

4.5.2 The evaluation of a new 500 EC formulation of imazalil against post-harvest diseases for the purpose of registration

Experiment 123 by K.H. Lesar (CRI)

Opsomming

Die imazalil 500 EC formulering, ingestuur deur ICA Internasionaal, het goeie beheer van die na-oes sitrus patogeen *P. digitatum* (groenskimmel), in vergelyking met die standaard Fungazil 800 EC formulering, gewys. Die ICA imazalil 500 EC formulering was verenigbaar met die wateroplosbare sitrus waxes gebruik in hierdie evaluasies.

Introduction

A new 500 EC formulation of imazalil (ImazaCure) was submitted to CRI by ICA International Chemicals for evaluation of efficacy against the post-harvest citrus pathogen *Penicillium digitatum* (green mould), for the purpose of registration of the product.

Materials and methods

A spore suspension of this pathogen was made up 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 Navel and Benny Valencia oranges (Crocodile Valley Citrus Company) and lemons (Larten Estates) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was then divided up into 3 replicates 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, giving a total of 40 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 relevant chemical being evaluated and the control chemical, were incorporated into a carnauba citrus wax.

The inoculated fruit was treated 4 hours after inoculation (to simulate a short delay after harvesting prior to packhouse treatments) by dipping the fruit into the fungicide-wax combination for 3 minutes. After treatment the waxed fruit was allowed to dry overnight and then 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 inhibition.

Treatments

1. Untreated control (*P. digitatum*)
2. Treated control – Fungazil 800 EC (3000 ppm) in wax
3. 1500 ppm ICA imazalil in wax (1/2x)
4. 3000 ppm ICA imazalil in wax (x)
5. 6000 ppm ICA imazalil in wax (2x)

Three trials were conducted with the ICA imazalil using Navel oranges, Benny Valencia oranges and lemons.

Please note that all concentrations designated ppm refer to the a.i. of imazalil.

Results

The imazalil 500 EC formulation submitted by ICA International demonstrated good control of the citrus pathogen, *P. digitatum*, compared to the standard, recommended Fungazil 800 EC formulation. The ICA imazalil 500 EC formulation was compatible with the water emulsion citrus wax used in these evaluations.

Table 4.5.2.1. The effect of ICA Imazalil on *P. digitatum*.

NAVELS	
Treatments	% Inhibition ^a
1. Untreated control	0.0 b
2. Treated control 3000 ppm Fungazil 8000EC	100.0 a
3. 1500 ppm ICA Imazalil	90.0 a
4. 3000 ppm ICA Imazalil	90.0 a
5. 6000 ppm ICA Imazalil	100.0 a

Table 4.5.2.2. The effect of ICA Imazalil on *P. digitatum*.

BENNY VALENCIAS	
Treatments	% Inhibition ^a
1. Untreated control	0.0 b
2. Treated control 3000 ppm Fungazil 800EC	90.0 a
3. 1500 ppm ICA Imazalil	80.0 a
4. 3000 ppm ICA Imazalil	90.0 a
5. 6000 ppm ICA Imazalil	100.0 a

Table 4.5.2.3. The effect of ICA Imazalil on *P. digitatum*.

LEMONS	
Treatments	% Inhibition ^a
1. Untreated control	0.0 b
2. Treated control 3000 ppm Fungazil 800EC	80.0 a
3. 1500 ppm ICA Imazalil	70.0 a
4. 3000 ppm ICA Imazalil	80.0 a
5. 6000 ppm ICA Imazalil	100.0 a

^a Values represent the means of 3 replicates of 20 fruit each.
Means in columns followed by the same letter are not significantly different.
(Fisher's Unprotected LSD; $P \leq 0.05$)

No phytotoxicity was evident on the fruit treated at the highest concentration (6000 ppm) of the ICA.

Conclusion

The imazalil 500 EC formulation submitted by ICA International demonstrated good control of the citrus pathogen, *P. digitatum* compared to the standard, recommended Fungazil 800 EC. These data were submitted for registration of the product.

4.5.3 The evaluation of Guazalil, a new formulation of a combination of imazalil and guazatine, for efficacy against post-harvest diseases

Opsomming

'n Nuwe swamdoder formulاسie van 'n mengsel van die twee na-oes swamdoders, imazalil en guazatine, Guazalil, het goeie beheer van *P. digitatum* (groenskimmel) en *G. candidum* (suurvrot) gewys, vergelyk met die standard imazalil en guazatine. So 'n formulاسie van imazalil en guazatine (na markte toe wat guazatine toelaat) sal ideal wees vir die onderdrukking van die ontwikkeling van *Penicillium*-bestande spore.

Introduction

A new fungicide formulation of a mixture of the two post-harvest fungicides, imazalil and guazatine, Guazalil, was submitted to CRI by ICA International Chemicals for evaluation of efficacy against the two post-harvest pathogens *Penicillium digitatum* (green mould) and *Geotrichum candidum* (sour rot). The purpose of formulating such a product is for the prevention of the build up of resistant *Penicillium* spores to these two post-harvest fungicides.

Materials and methods

Spore suspensions of these pathogens were made up in sterile deionised water containing the surfactant, or wetting agent, Tween 20. Both spore suspensions were then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good sound, untreated Delta 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 were then divided up into 3 replicates of 20 fruit per treatment and then all the fruit were washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit were 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, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette. All the treatments, with the relevant chemical being evaluated, were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated 4 hours after inoculation (to simulate a short 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 inhibition.

Treatments

1. Untreated control (*P. digitatum*)
2. Treated control – Fungazil 750 WSP 67 g/100 ℓ (500 ppm imazalil)
3. Treated control – CitriCure 210SC 480 m ℓ /100 ℓ (1000 ppm guazatine)
4. ICA Guazalil – 250 m ℓ /100 ℓ (250 ppm imazalil) and (500ppm guazatine) (1/2x)
5. ICA Guazalil – 500 m ℓ /100 ℓ (500 ppm imazalil) and (1000 ppm guazatine) (x)
6. ICA Guazalil – 1000 m ℓ /100 ℓ (1000 ppm imazalil) and (2000 ppm guazatine) (2x)

Three trials were conducted with the ICA Guazalil, i.e. two with Delta Valencia oranges for the control of *P. digitatum*, and one with Delta Valencia oranges for the control of *G. candidum*.

Results

The Guazalil SL demonstrated good control of the citrus pathogens, *P. digitatum* and *G. candidum*, compared to the standard, recommended Fungazil sulphate 750 WSP and CitriCure 210 SC.

Table 4.5.3.1. The effect of ICA Guazalil on *P. digitatum*.

DELTA VALENCIAS	
Treatments	% Inhibition ^a
1. Untreated control	0.0 b
2. Treated control Fungazil 67 g/100 ℓ	100.0 a
3. Treated control CitriCure 480 m ℓ / 100 ℓ	90.0 a
4. ICA Guazalil 250 m ℓ / 100 ℓ	90.0 a

5. ICA Guazalil 500 mℓ / 100 ℓ	100.0 a
6. ICA Guazalil 1000 mℓ / 100 ℓ	10.0 a

Table 4.5.3.2. The effect of ICA Guazalil on *P. digitatum*.

DELTA VALENCIAS	
Treatments	% Inhibition ^a
1. Untreated control	0.0 b
2. Treated control Fungazil 67 g/100 ℓ	100.0 a
3. Treated control CitriCure 480 mℓ / 100 ℓ	100.0 a
4. ICA Guazalil 250 mℓ / 100 ℓ	90.0 a
5. ICA Guazalil 500 mℓ / 100 ℓ	100.0 a
6. ICA Guazalil 1000 mℓ / 100 ℓ	100.0 a

^a Values represent the means of 3 replicates of 20 fruit each.

Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \geq 0.05$)

Table 4.5.3.3. The effect of ICA Guazalil on *G. candidum*.

DELTA VALENCIAS	
Treatments	% Inhibition ^a
1. Untreated control	6.7 c
2. Treated control Fungazil 67 g/100 ℓ	100.0 a
3. Treated control CitriCure 480 mℓ / 100 ℓ	0.0 d
4. ICA Guazalil 250 mℓ / 100 ℓ	63.3 b
5. ICA Guazalil 500 mℓ / 100 ℓ	100.0 a
6. ICA Guazalil 1000 mℓ / 100 ℓ	100.0 a

^a Values represent the means of 3 replicates of 20 fruit each.

Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \geq 0.05$).

No phytotoxicity was evident on the fruit treated at the highest concentration (2000 ppm) of the ICA Guazalil.

Conclusion

Guazalil demonstrated good control of both citrus pathogens compared to the standard imazalil and guazatine. Such a formulation of both imazalil and guazatine (to markets that permit the use of guazatine) would be ideal for use in preventing the build up of *Penicillium* resistance in spores.

4.5.4 Evaluation of "Philabuster 400 SC" from Janssen Pharmaceutica for efficacy against the post-harvest citrus pathogen, green mould

Opsomming

'n Nuwe swammiddel Philabuster vanaf Janssen Pharmaceutica, ook 'n mengsel van twee swamdoders, imazalil en pyrimethanil ('n nuwe chemie swammiddel), is geevalueer vir beheer van infeksies deur *P. digitatum* sensitiewe en imazalil bestande spore. Dié middel is spesiaal geformuleer vir die doel om bederf deur die *Penicilliums* te beheer en meer belangrik die imazalil bestande spore uit te wis. 'n Loodsproef het gewys dat daar wel 'n vermindering in bederf is met albei spore. Hierdie werk is aangaande.

Introduction

A new product, Philabuster, which consists of a combination of two fungicides, imazalil and a new generation fungicide, pyrimethanil, in a single formulation, was forwarded to CRI from Janssen Pharmaceutica for evaluation. The product was formulated for the control of the *Penicillium* moulds, *Diplodia* stem-end rot and Anthracnose, and more importantly, for the prevention of the build up of *Penicillium* resistant spores. Pyrimethanil on its own is apparently also effective in the control of the *Penicillium* moulds.

A pilot trial was conducted where the product was evaluated against infections caused by both imazalil sensitive and resistant *P. digitatum* (green mould) spores.

Materials and methods

Spore suspensions of both the imazalil-sensitive and resistant *P. digitatum* spores were made up in sterile deionised water containing the surfactant, or wetting agent, Tween 20. Both spore suspensions were then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good sound, untreated Delta 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 were then divided up into lots of 20 fruit per treatment and then all the fruit were washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit were 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, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the spores by applying $35 \mu\ell$ of spore suspension to each injury site using a micropipette. All the treatments, with the relevant chemical being evaluated, were done in a hot water bath at 40°C , thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated 4 hours after inoculation (to simulate a short 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.

Treatments

- 1(a) Untreated control (*P. digitatum* sensitive spores)
- 1(b) Untreated control (*P. digitatum* resistant spores)

- 2(a) Treated control – 500 ppm Fungazil 750 WSP (sensitive spores)
- 2(b) Treated control – 500 ppm Fungazil 750 WSP (resistant spores)

- 3(a) 250 ppm Philabuster (1/2x) }
- 3(b) 500 ppm Philabuster (x) } - sensitive spores
- 3(c) 1000 ppm Philabuster (2x) }

- 4(a) 250 ppm Philabuster (1/2x) }
- 4(b) 500 ppm Philabuster (x) } – resistant spores
- 4(c) 1000 ppm Philabuster (2x) }

Results

The results indicate that the incidence of *P. digitatum* resistance to the standard recommended imazalil at 500 ppm is 70% (Table 4.5.4.1). The same spores treated with Philabuster at 250 ppm and 500 ppm only indicate a 20% reduction in resistance and a 50% reduction at 1000 ppm, which indicates that pyrimethanil has played a role in reducing the incidence of resistance. However the formulation of Philabuster is made up of 200 g/l imazalil sulphate, compared to the standard imazalil sulphate of 750 g/l, and 200 g/l pyrimethanil. These results thus indicate that higher concentrations of Philabuster need to be used to inhibit the resistant spores 100%.

Table 4.5.4.1. The effect of Philabuster on imazalil sensitive and resistant *P. digitatum* spores.

DELTA VALENCIAS	
Treatments	% Decay
1(a) Untreated control sensitive spores	100
1(b) Untreated control resistant spores	100
2(a) Treated control sensitive spores	Nil
2(b) Treated control resistant spores	70

3(a) 250 ppm Philabuster	Nil
3(b) 500 ppm Philabuster - sensitive spores	Nil
3(c) 1000 ppm Philabuster	Nil
4(a) 250 ppm Philabuster	50
4(b) 500 ppm Philabuster - resistant spores	50
4(c) 1000 ppm Philabuster	20

No phytotoxicity was evident on the fruit treated at the highest concentration (1000 ppm) of the Philabuster.

Conclusion

These trials with Philabuster are ongoing. Larger scale trials will be conducted.

4.5.5 The evaluation of Sylitt (a.i. dodine) for efficacy against post-harvest diseases

Opsomming

Dodine, 'n guanidine soos guazatine, het geen beheer gewys teen infeksies van *P. digitatum* nie. Geen verdere werk is beplan met die middel nie.

Introduction

Guazatine is effective against the control of infections caused by *P. digitatum* (green mould) and *P. italicum* (blue mould) and is the only post-harvest fungicide registered for the control of *Geotrichum candidum* (sour rot). Dodine, like guazatine, is a guanidine, therefore dodine was evaluated for efficacy against the control of the post-harvest pathogen *P. digitatum* (green mould).

Materials and methods

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 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 randomised. The fruit were then divided up into 3 replicates of 20 fruit per treatment and all the fruit were washed in clean water and surface sterilized by dipping in a 1% NaOCl solution for a few minutes. The fruit were allowed to dry prior to inoculation.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette. All the treatments, with the relevant chemical being evaluated, were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse. The inoculated fruit was treated 4 hours after inoculation (to simulate a short 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 inhibition.

Treatments

1. Untreated control *P. digitatum*
2. Treated control 500 ppm imazalil sulphate
3. Treated control 1000 ppm guazatine
4. 250 ppm dodine* (1/2x)
5. 500 ppm dodine (x)
6. 1000 ppm dodine (2x)

*Styllit 400SC

Results

The fungicide dodine demonstrated no effect against the control of *P. digitatum*.

Table 4.5.5.1. The effect of Styllit (dodine) on *P. digitatum*.

NAVEL ORANGES	
Treatments	% Inhibition ^a
1. Untreated control	0.0 b
2. Treated control 500 ppm imazalil	100.0 a
3. Treated control 1000 ppm guazatine	100.0 a
4. 250 ppm dodine	0.0 b
5. 500 ppm dodine	0.0 b
6. 1000 ppm dodine	0.0 b

^a Values represent the means of 3 replicates of 20 fruit each.

Means in columns followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \geq 0.05$)

Conclusion

A trial to determine the effect of dodine on the control of infection caused by *G. candidum* (sour rot) will also be conducted.

4.5.6 The evaluation of the efficacy of guazatine formulated into citrus waxes (i.e. CitriWax and Deccowax)

Opsomming

Die na-oes swamdoder guazatine, geformuleer in die Citriwaks en die Deccowaks, het albei *P. digitatum* (groenskimmel) en *G. candidum* (suurvrot) effektief beheer. Dit het gewys dat daar geen afname in effektiwiteit van guazatine in Citriwaks oor 'n tydperk van ses maande of in die 11 jaar oue Deccowaks was nie.

Introduction

It was indicated to CRI, via the Spanish citrus industry that the efficacy of guazatine, formulated into citrus waxes, decreases as the guazatine-wax formulation ages over a period of time. The following research was then conducted.

Materials and methods

A recently formulated batch of Citriwax was evaluated monthly from December 2004–June 2005 for efficacy of the fungicide guazatine against the pathogens *P. digitatum* (green mould) and *G. candidum* (sour rot). An old batch of Deccowax (1994) was also evaluated against the same pathogens.

Spore suspensions of the two pathogens were made up in sterile deionised water containing the surfactant, or wetting agent, Tween 20. Both spore suspensions were then adjusted to a concentration of 1×10^6 spores/m^l spectrophotometrically.

Good sound, untreated lemons (Larten Estates) and 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. 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.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides, giving a total of 40 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 (to simulate a short delay after harvesting prior to packhouse treatments) with the relevant wax-guazatine combination being evaluated. All the treatments were done by dipping the fruit into the wax for 3 minutes.

After treatment, the waxed fruit was allowed to dry overnight and then 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.

Treatments

1. Untreated control *P. digitatum*
2. Treated control 500 ppm imazalil sulphate
3. Untreated control *G. candidum*
4. Treated control 1000 ppm guazatine
5. 3000 ppm guazatine in wax (CitriWax or Deccowax) *P. digitatum*
6. 3000 ppm guazatine in wax (Citriwax or Deccowas) *G. candidum*

Six, monthly evaluations were conducted on the Citriwax. Three evaluations were done on lemons and three on Valencias. One trial was conducted on the Deccowax, using Valencia oranges.

Results

The guazatine in the Citriwax and the Deccowax effectively inhibited infections caused by both of the citrus pathogens, indicating that there was no decrease in efficacy of the fungicide in the Citriwax over a 6 month period nor in the Deccowax that was an 11 year old formulation (Tables 4.5.6.1 & 2).

Table 4.5.6.1. The efficacy of guazatine in Citriwax against *P. digitatum* and *G. candidum*.

LEMONS	
Treatments	% Decay
1. Untreated control <i>P. digitatum</i>	100.0
2. Treated control 500 ppm imazalil	Nil
3. Untreated control <i>G. candidum</i>	100.0
4. Treated control 1000 ppm guazatine	Nil
5. 3000 ppm guazatine in Citriwax <i>P. digitatum</i>	Nil
6. 3000 ppm guazatine in Citriwax <i>G. candidum</i>	Nil

Table 4.5.6.2. The efficacy of guazatine in Deccowax against *P. digitatum* and *G. candidum*.

VALENCIAS	
Treatments	% Decay
1. Untreated control <i>P. digitatum</i>	100.0
2. Treated control 500 ppm imazalil	Nil
3. Untreated control <i>G. candidum</i>	100.0
4. Treated control 1000 ppm guazatine	Nil
5. 3000 ppm guazatine in Deccowax <i>P. digitatum</i>	Nil
6. 3000 ppm guazatine in Deccowax <i>G. candidum</i>	Nil

Conclusion

The other two evaluations with Citriwax on lemons and three on Valencias showed the same results as above. The guazatine in both formulations effectively inhibited the pathogens, indicating that there was no decrease in efficacy of the fungicide in the waxes over a long period of time. Packhouses using either of these two waxes must order sufficient wax and use up the wax over a 3-4 week production period and thus avoid the situation of storing a quantity of the wax for several months and even into the following season.

4.5.7 Evaluation of the sanitizing agents, SteriHarvest, AsepteroI and G-cide, in a simulated packhouse dumptank washing system for efficacy of sterilisation of the system and possible infection of injured fruit moving through the system

Opsomming

Twee van die drie saniteermiddels geevalueer, het nie die gewenste ontsmetting van 'n pakhuis dompelbad uitgevoer nie, en ook nie die besmetting van beseerde vrugte deur die bad voorkom nie. Die G-cide A het wel die besmetting van die vrugte beperk tot 47% in vergelyking met die kontrole Sporekill (0%). G-cide A sal teen hoër konsentrasies geevalueer word.

Introduction

Three sanitising agents, SteriHarvest (a.i. dioxo–MP 14), AsepteroI (a.i. chlorine dioxide) and G-cide, made up of two products, a gluteraldehyde–quaternary ammonium combination (G-cide A) and a gluteraldehyde (G-cide B), were evaluated in a simulated dumptank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The three products were compared with the standard recommended Sporekill (a quaternary ammonium compound). The simulated dumptank was seeded with a suspension of the post-harvest pathogen *P. digitatum* in this evaluation.

Materials and methods

A spore suspension of this pathogen 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 from Crocodile Valley Citrus Co. were obtained in bulk. Blemish free, sound fruit were 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 the treatments being conducted.

A clean water dumptank washing system in a packhouse was simulated. The dumptank water was maintained at ambient temperature for this trial. Clean, surface-sterilised Delta Valencia oranges were injured by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured three times, twice equatorially on opposite sides and once at the stylar end of the fruit, giving a total of 60 injury sites per treatment.

Each treatment (x20 fruit) was then left to stand in the dumptank for 3 minutes. The first lot of 20 injured fruit that stood in the clean water bath for 3 minutes served as the untreated control. The system was then seeded with the 1×10^6 spores/mℓ concentration of *P. digitatum*.

The seeded dumptank was then sanitised with recommended concentrations of 3 ℓ and 4 ℓ /1000 ℓ of SteriHarvest, and 30, 60 and 90 ppm of AsepteroI and 250 mℓ / 1000 ℓ for both formulations of G-cide for 3 minutes. The standard Sporekill was used as control at concentrations of 1 ℓ and 2 ℓ/1000 ℓ for 3 minutes exposure as well. After sanitising with the three compounds, injured fruit was allowed to stand for 3 min. in the “sanitised” mixtures at each concentration specified.

After treatment, the fruit was allowed to dry and then 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.

Treatments

1. Untreated control – injured fruit dipped in clean water.
2. Infected control – injured fruit dipped in water + 1×10^6 spores/ mℓ *P. digitatum*.
3. Injured fruit dipped + spores + 3 ℓ / 1000 ℓ SteriHarvest.
4. Injured fruit dipped + spores + 4 ℓ / 1000 ℓ SteriHarvest.
5. Infected control as in 2.
6. Injured fruit dipped + spores + 30 ppm AsepteroI.
7. Injured fruit dipped + spores + 60 ppm AsepteroI.
8. Infected control as in 2.
9. Injured fruit dipped + spores + 250 mℓ / 1000 ℓ G-cide A.

10. Injured fruit dipped + spores + 250 ml / 1000 l G-cide B.
11. Treated control as in 2.
12. Injured fruit dipped + spores + 1 l / 1000 l Sporekill.
13. Injured fruit dipped + spores + 2 l / 1000 l Sporekill.

Results

The results indicated that the three sanitising agents evaluated achieved a higher incidence of infected fruit than the control, Sporekill, i.e. 53 and 47% for Steri-Harvest and 87 and 73% for Asepterol and 47% for G-cide A and 100% for G-cide B, compared to the 0% for both concentrations of Sporekill.

Table 4.5.7.1. The efficacy of sanitising agents SteriHarvest, Asepterol and G-cide in a packhouse dumptank.

DELTA VALENCIAS	
Treatments	% Decay
1. Untreated control	Nil
2. Infected control	94
3. 3 l / 1000 l SteriHarvest	53
4. 4 l / 1000 l SteriHarvest	47
5. Infected control	87
6. 30 ppm Asepterol	87
7. 60 ppm Asepterol	73
8. Infected control	100
9. 250 ml / 1000 l G-cide A	47
10. 250 ml / 1000 l G-cide B	100
11. Infected control	94
12. 1 l / 1000 l Sporekill	Nil
13. 2 l / 1000 l Sporekill	Nil

Conclusion

Neither of these sanitising agents demonstrated the desired effect of disinfection of a packhouse dumptank washing system, nor preventing the infection of injured fruit moving through the system, compared to the standard recommended Sporekill.

No further work is planned on SteriHarvest nor Asepterol. However G-cide A will be screened at a higher concentration.

4.5.8 Evaluation of Erador and Xterminator for compatibility with the post-harvest fungicides in a post-harvest dip treatment for the control of the grain chinch bug

Opsomming

Van die twee middels ge-evalueer is dit bevind dat Erador nie verenigbaar met die na-oes swamdoders is nie. Erador bevat neem olie ook, en omdat die olie boontoe uit die mengsel styg, met te min roering in kombinasie met die swamdoders, kan dit dalk die rede vir die onverenigbaarheid wees. Albei middels het geen fitotoksiesiteit gewys in kombinasie met die swam middels nie.

Introduction

The compounds Xterminator a.i. pyrethrum 20 g/l, Erador (Organo-Z) a.i. pyrethrum 5.44g/l and azadirachtin (neem oil), both organic products used for grain chinch bug (GCB) control, were evaluated for compatibility with the post-harvest fungicides, to determine if the product could be applied post-harvest in either the pre-degreening drench or the hot water fungicide bath.

Materials and methods

Xterminator and Erador were evaluated on Penicillium-inoculated fruit in combination with the post-harvest fungicides imazalil and guazatine in a hot water dip treatment.

A spore suspension of *P. digitatum* (green mould) was made up 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 Delta Valencia oranges (Crocodile Valley Citrus Company) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. The fruit was divided up into lots of 20 fruit per treatment and 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.

Inoculation

The inoculation of the fruit was done by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured twice equatorially on opposite sides, giving a total of 40 inoculation sites per treatment. Each fruit was then infected with the pathogen by applying 35 $\mu\ell$ of spore suspension to each injury site using a micropipette.

All the treatments, with the relevant chemical being evaluated, were done in a hot water bath at 40°C, thus simulating a heated fungicide bath in the packhouse.

The inoculated fruit were treated 4 hours after inoculation (to simulate a short 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.

Treatments

1. Untreated control (*P. digitatum*)
2. Treated control – Fungazil WSP 67 g/100 ℓ (500 ppm imazalil)
3. Treated control – CitriCure SC 480 ml/100 ℓ (1000 ppm guazatine)
4. 0.25% Erador/Xterminator
5. 0.25% Erador/Xterminator plus 500 ppm imazalil
6. 0.25% Erador/Xterminator plus 1000 ppm guazatine
7. 0.5% Erador/Xterminator
8. 0.5% Erador/Xterminator plus 500 ppm imazalil
9. 0.5% Erador/Xterminator plus 1000 ppm guazatine

Results

Erador was found to be incompatible with the post-harvest fungicides in that the product interfered with the efficacy of the fungicides (Table 4.5.8.1).

Another drawback with using Erador in a mixture with the fungicides is that with insufficient agitation, which is the case in most fungicide baths and some drenches, the oil base product tends to settle out at the top of the mixture.

Table 4.5.8.1. Compatibility of Erador with the post-harvest fungicides imazalil and guazatine

Delta Valencias	
Treatments	% Decay
1. Untreated control (<i>P. digitatum</i>)	100
2. Treated control – 500 ppm imazalil	Nil
3. Treated control – 1000 ppm guazatine	Nil
4. 0.25% Erador	100
5. 0.25% Erador plus 500 ppm imazalil	20
6. 0.25% Erador plus 1000 ppm guazatine	10
7. 0.5% Erador	100
8. 0.5% Erador plus 500 ppm imazalil	40
9. 0.5% Erador plus 1000 ppm guazatine	10

Xterminator was found to be compatible with the post-harvest fungicides (Table 4.5.8.2).

Table 4.5.8.2. Compatibility of Xterminator with the post-harvest fungicides imazalil and guazatine

Delta Valencias	
Treatments	% Decay
1. Untreated control (<i>P. digitatum</i>)	100
2. Treated control – 500 ppm imazalil	Nil
3. Treated control – 1000 ppm guazatine	Nil
4. 0.25% Erador	100
5. 0.25% Erador plus 500 ppm imazalil	Nil
6. 0.25% Erador plus 1000 ppm guazatine	Nil
7. 0.5% Erador	90
8. 0.5% Erador plus 500 ppm imazalil	Nil
9. 0.5% Erador plus 1000 ppm guazatine	Nil

Both products were also evaluated at concentrations of 0.25% and 0.5%, in a dip application, in combination with 200 ppm available Chlorine (standard recommendation), and also a 1000 ppm (product) of the quaternary ammonium compound Sporekill (standard recommendation).

No phytotoxicity was observed with both products in combination with the two fungicides nor in combination with chlorine and Sporekill.

Conclusion

These evaluations were conducted with fully coloured Delta Valencia oranges with rinds that were not sensitive, as is the case with greener fruit and some of the more sensitive citrus cultivars, where possible phytotoxicity could occur in combination with other products. These pyrethrum products need to be screened against greener fruit as well. A separate dip application, prior to the packhouse treatments, would probably be the safest option.

4.5.9 Kaolin – citrus wax packhouse trial conducted on lemons and mangos for prevention of sunburn

Opsomming

Die beweerde verbodskeling van die waks is nie op hierdie suurlemoene en mangos, na gesimuleerde verskeping, waargeneem nie, omdat die kaolien residu op die vrugte op die paklyn afgewas is, en dit het goeie egalige waks aanwending veroorsaak. Hierdie proewe op kaolien behandelde vrugte is nou afgehandel.

Introduction

Lemon and mango trees were sprayed with different concentrations of kaolin for the prevention of sunburn. The purpose of this trial was to determine the effect of kaolin residue on the fruit, on the waxing of the fruit. It had been reported that the presence of kaolin on the fruit leads to a breakdown of the wax formulation on the fruit.

Materials and methods

Trial trees of Sensation mangos and lemons were sprayed at Bavaria Est., (Hoedspruit) with Mangocote® (kaolin) and Fruitcote® (kaolin) for the prevention of sunburn. The lemons were sprayed with four different concentrations of kaolin. These lemons together with two trials on the mangos were harvested and treated at CRI on the packhouse line on 23/02/05 and 21/03/05.

All the fruit received was washed in the high pressure spray with a suitable sanitizer (Prasin, a quaternary ammonium compound), exposed to the hot water bath at 40°C for 2-3 minutes and dried in the packline drying tunnel. The lemons and mangos were divided up into the following treatments.

Mango treatments

- 9 cartons x 10 fruit – Untreated Control
- 9 cartons x 10 fruit – Mangocote® Std
- 9 cartons x 10 fruit – Mangocote® unformulated + wetting agent

9 cartons x 10 fruit – October 2004 spray 2 x Fruitcote®
9 cartons x 10 fruit – November 2004 spray 4 x Fruitcote®
9 cartons x 10 fruit – December 2004 spray 6 x Fruitcote®
9 cartons x 10 fruit - January 2005 spray 8 x Fruitcote®

Lemon treatments

9 cartons x 10 fruit – Untreated Control
9 cartons x 10 fruit – October 2004 spray 2 x Fruitcote®
9 cartons x 10 fruit – November 2004 spray 4 x Fruitcote®
9 cartons x 10 fruit – December 2004 spray 6 x Fruitcote®
9 cartons x 10 fruit - January 2005 spray 8 x Fruitcote®

Three cartons of each treatment were then waxed with the following three citrus waxes.

Sasol Carnauba wax (Carnauba Natural)
Sasol polyethylene wax (Quick dry poly) and
Sasol Citriwax (a polyethylene wax plus the fungicide guazatine)

The waxed fruit was allowed to dry, packed into cartons and then stored under simulated shipping conditions at 8°C for 3 weeks. After simulated shipping of both trials the treatments were stored at ambient for 1 week and then evaluated.

The reported breakdown of the wax was not observed on these mangos and lemons after simulated shipping as the kaolin residue on the fruit was washed off in the packline resulting in good waxing of the fruit.

Conclusion

This trial work on kaolin treated fruit has now been completed.

4.5.10 High imazalil residue on Star Ruby grapefruit from Neonovo (Lowveld) in Japan

Opsomming

Terugvoering vanaf IPM Nishimoto in Japan het die sitrus bedryf ingelig dat pomelos vanaf Suid Afrika, tydens die 2005 sitrus seisoen, getoets is met 'n residu van 7.0 dpm vir die na-oes swamdoder imazalil. Na 'n vergadering met die betrokke pakhuis en deeglike ondersoek is bepaal dat 'n menslike fout ondergaan is. Geen regstellende aksie is voorgestel nie, omdat al die pakhuis prosedure en kritiese beheer punte in die pakhuis deeglik bestuur word.

Introduction

Correspondence received from IPM Nishimoto (Mr. Hiroshi Tsujikawa) reported on 20 June 2005 that grapefruit imported into Japan from South Africa during the 2005 citrus production season was found after testing to have a residue level for the agri-chemical imazalil of 7.0 ppm. The citrus packhouse from which the consignment of grapefruit originated was Neonovo (Lowveld). A meeting was convened to evaluate the cause of non-conformance and assist in corrective and preventative actions.

Investigation

Meeting at Neofresh Packhouse

During the meeting at Neonovo packhouse the following facts were established concerning this issue of high residue level of imazalil on the grapefruit.

- The reported high residue level of imazalil in Japan was detected in a sample of a consignment of Star Ruby grapefruit, from Neonovo.
- This consignment was made up of 30 pallets x 55 ctns (1650 ctns). Half of the cartons had already been sold and the sample of fruit that was tested for imazalil residue was taken from the balance of this consignment still in cold storage.
- An official laboratory document with the test results, indicating 7.0 ppm, was received from Japan.

4.5.11 Residue levels of the post-harvest fungicide imazalil on South African citrus fruit

Opsomming

Verskeie proewe is uitgevoer om te probeer bepaal of sekere variansies tydens die aanwending van imazalil in die pakhuis bydra tot die behoud van hoë vlakke van imazalil residu op Suid Afrikaanse uitvoer sitrus. Dié beheerde proewe en die resultate het geen onreëlmatige residu vlakke van imazalil gewys wat hoer as die MRV van 5.0 dpm was.

Introduction

During the 2005 citrus season a number of instances of high residue levels of the post-harvest fungicide imazalil (higher than the MRL of 5 ppm) were reported by the South African accredited laboratories and also on a consignment of grapefruit in Japan.

Similar reports had also been reported in 2003 and 2004.

These reports prompted the conducting of various trials to evaluate a number of variables in the application of imazalil in the packhouse to determine what effect, if any, these variables might have on the retention of high levels of imazalil on South African exported citrus fruit.

- (a) Evaluation of the effect of exposure times of fruit to imazalil in the hot water fungicide bath, on the level of imazalil residue retained on the fruit

Materials and methods

Star Ruby grapefruit, obtained from Colors Exporters was used for this trial. The fruit was dipped in the standard recommended concentration of imazalil (500 ppm) for 30 sec, 1 minute, 2 minutes, 5 minutes and 10 minutes. The treatments were conducted at a temperature of 30°C in the hot water fungicide bath on the packline at CRI, Nelspruit.

Six fruit per treatment were submitted to the accredited laboratory, Hearshaw and Kinnes, for imazalil residue analyses.

Results

<u>Exposure time</u>	<u>Imazalil residue (mg/kg)</u>
Untreated control	0.0
30 sec	0.2
1 min	0.2
2 min	0.2
5 min	0.2
10 min	0.3

Conclusion

The exposure times in the fungicide bath did not indicate any significant differences in imazalil residue levels.

- (b) Topping up of 1.5x imazalil concentration directly onto the fruit in the bath

Materials and methods

Hot water fungicide baths are topped up, in citrus packhouses on a standard basis, with full strength fungicide plus "x" grams of fungicide per ton of fruit to compensate for the stripping out of the compound by a certain tonnage of fruit throughput.

In this trial a 1.5x concentration of imazalil (i.e. 750 ppm) was dumped directly onto the grapefruit that had already been exposed to the standard concentration of imazalil in the bath for 2 minutes. The fruit was then exposed for a further 1 minute to the increased concentration of imazalil. These treatments were also conducted at a temperature of 30°C, as above.

Six fruit per treatment were submitted to Hearshaw and Kinnes for imazalil residue analyses.

Results

<u>Exposure time</u>	<u>Imazalil residue (mg/kg)</u>
Untreated control	0.0
2 + 1 minute	0.4

Conclusion

No significant increase in imazalil residue was observed, compared to the level of residue retained on exposure to the standard imazalil concentration, as indicated in (a).

(c) Evaluation of the effect of fungicide bath temperature on the level of imazalil residue retained on the fruit

Delta Valencia oranges obtained from Crocodile Valley Citrus Co. were used in this trial. The fruit was exposed to the standard recommended concentration of imazalil (500 ppm) for 3 minutes at temperatures of 30, 40 and 50°C in the hot water fungicide bath.

Six fruit per treatment were submitted to Hearshaw and Kinnes for imazalil residue analyses.

Results

<u>Bath temperature (°C)</u>	<u>Imazalil residue (mg/kg)</u>
Untreated control	0.0
30	0.4
40	0.5
50	1.2

Conclusion

A significant increase in imazalil residue, retained on the fruit, was observed at the highest temperature of 50°C.

The trials conducted above under controlled conditions and the results did not indicate any untoward residue levels to justify high imazalil residues (higher than the MRL of 5.0 ppm).

4.5.12 **The *in vivo* screening of Penicillium spore samples for resistance to the post-harvest fungicide imazalil**

Opsomming

Een-en-twintig *P. digitatum* swamspoormonsters vanuit die Oos- en Wes-Kaap en Zimbabwe is ge-evalueer teen imazalil vir bestandheid. Tien van die monsters het verskillende vlakke van bestande spore gewys. Dié aantal positiewe monsters bevestig die kommissie dat die bestandheid teen imazalil besig is om te vermeerder.

Introduction

The screening of post-harvest pathogens for resistance to the post-harvest fungicides is done randomly on an ongoing basis from season to season. Twenty-one fungal spore samples of *P. digitatum* from the Eastern and Western Cape and Zimbabwe production areas were screened for resistance to imazalil.

Materials and methods

Spore suspensions of all the samples were made up in sterile deionised water containing the surfactant, or wetting agent, Tween 20. The spore suspensions were then adjusted to a concentration of 1×10^6 spores/ml spectrophotometrically.

Good, sound, untreated Delta Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. The fruit was then divided up into lots of 10 fruit per treatment. 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.

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 infected with the spores being screened by applying 35 µl of spore suspension to each injury site using a micropipette. The inoculated fruit was treated 4 hours after inoculation. All the treatments were treated with 500 g/kg imazalil 750 WSP (imazalil sulphate). The controls consisted of an **untreated control** (fruit inoculated with imazalil sensitive spores of *P. digitatum*) and a **treated control** (fruit inoculated with imazalil sensitive spores of *P. digitatum* and treated with 500 g/kg imazalil 750 WSP). 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

Ten of the twenty-one spore samples screened indicated varying degrees of resistance to imazalil. This verifies the concern that the build-up of *Penicillium* resistance to the post-harvest fungicide, imazalil, is on the increase.

Table 4.5.12.1. *Penicillium* spore samples screened for imazalil resistance.

Treatments	% Decay
1. Untreated control SRCC Kirkwood packhouse	100 20
2. Untreated control SRCC Hermitage	100 100
3. Untreated control Citripack Mvurwi (Zimbabwe)	100 10
4. Untreated control Second sample as in 3.	100 20
5. Untreated control Goedehoop Citrusdal	100 50
6. Untreated control Third sample as in 3 & 4	100 Nil
7. Untreated control Swellenfruit Satsuma	100 Nil
8. Untreated control Imimbala 1. W.Cape	100 Nil
9. Untreated control Imimbala 2.	100 Nil
10. Untreated control Imimbala 3.	100 Nil
11. Untreated control Imimbala 4.	100 Sample contaminated
12. Untreated control Imimbala 5.	100 Nil
13. Untreated control Cedarpack Citrusal	100 Nil
14. Untreated control GPV Citrusdal	100 Nil
15. Untreated control Imimbala 6. (<i>P. italicum</i> blue mould)	100 Nil
16. Untreated control ALG Citrusdal	100 100
17. Untreated control Fruit2 U Packers Sandriver Wellington (Midnight)	90 50
18. Untreated control Citrus Select Citrusdal	100 100
19. Untreated control	100

Novo Packhouse Hectorspruit Valencias	Nil
20. Untreated control Olympus Packers Citrusdal	100 100
21. Untreated control Sun Citrus Kirkwood	100 90
22. Treated control (<i>P. digitatum</i> sensitive strain)	Nil

Conclusion

The steady increase in the detection of imazalil resistant *Penicillium* biotypes in the citrus industry, since 1999, has prompted the introduction of a major resistance strategy to screen spores from all production areas. This strategy would aid the industry in determining what the state of resistance is in the industry and thereby then introducing treatment strategies to reduce the resistant spore populations and ultimately reduce the risk of losing the efficacy of our post-harvest fungicides.

4.5.13 The screening South African isolates of *Penicillium* fungal spores from all citrus production areas for resistance to the post-harvest fungicides imazalil and guazatine

Experiment 850 by K.H.Lesar (CRI), J. Mildenhall and L. Trollope (Katco)

Opsomming

Die evaluering van die *Penicillium* spoormonsters tot dusvêr het gewys dat daar nie net tekens van imazalil bestande spore is nie, maar wel ook aanduidings van moontlike bestandheid teen die ander belangrike na-oes swamdoder, guazatine is. Hierdie resultate sal eers met die *in vivo* evaluering gekorreleer moet word voor die bevestiging van enige bestandheid.

Introduction

Blue and green mould of citrus fruit caused by *Penicillium italicum* and *Penicillium digitatum* respectively, are the major contributors to post harvest decay in South African export citrus fruit. There are currently only two fungicides registered for the control of these pathogens on export fruit, namely, imazalil and guazatine. Certain countries, however, do not permit the use of guazatine on fresh fruit, leaving imazalil as the sole fungicide available for the control of post harvest *Penicillium* infections. Development of resistance to imazalil in the wild type populations of *P. italicum* and *P. digitatum* poses a serious threat to the export of citrus fruit and resistance has already been found in the Citrusdal area. In order to pre-empt large-scale spoilage of export fruit, it is necessary to survey the incidence of imazalil resistance in orchards and packhouses in the major citrus producing areas of southern Africa.

In the year 2000 Kat River Citrus Co-operative (KATCO) began monitoring fungal levels in the tip baths and fungicide baths by conducting in-house tests in a small microbiological laboratory. The Research Department of Outspan, thereafter Capespan and thereafter CRI, randomly screened *Penicillium* spores samples, *in vivo*, from the early 80s on an ongoing basis. The first imazalil-resistant spores were detected in the Western Cape in 1999. Since then there has been a steady increase in the detection of imazalil resistant *Penicillium* biotypes in the citrus industry. This increase in resistant spores prompted the initiation of a joint strategy between CRI and Katco to assess the incidence of imazalil and guazatine resistance by *in vitro* screening of *Penicillia* spore samples collected throughout the citrus producing areas of Southern Africa. The results obtained from screening over 100 isolates for their sensitivity to imazalil and guazatine are presented in this report.

Materials and methods

Isolation and Purification of Cultures

Spore samples from decayed fruit in the field were obtained by rotating cotton buds on the sporulating surface. Each cotton bud was sealed individually in a plastic bank bag and the bags mailed to KATCO. Initial isolations were made by gently touching the cotton bud to the surface of sodium polypectate (NaPP) agar plates (NaPP 20 g; orange or lemon rind homogenate, 50 g homogenized in 100 ml distilled water. Filter through cheesecloth. Add 50 ml homogenate per litre of medium. Agar 12 g/l; water 1 litre). Plates overgrown by *Rhizopus* were discarded. The cultures obtained on the NaPP agar were relatively pure except for contamination by *Streptomyces* spp. Subsequent transfer to Potato Dextrose Agar (PDA) medium supplemented with 250mg/l chloramphenicol eliminated the *Streptomyces* contaminants. Repeated transfer

on PDA plates eliminated any other contaminating organisms. Pure cultures on PDA were transferred to PDA slants in test tubes for storage.

In vitro Screening

The imazalil (Sanazil 750 imazalil sulphate 750 g/1000 g) stock solution was prepared by dissolving 133.5 mg Sanazil 750 in 500 ml sterile distilled water. This solution contained 0.2 mg/ml a.i. One milliliter of this solution was added to 100 ml sterile distilled water at 60°C. PDA was dissolved in 900 ml distilled water and after autoclaving cooled to 60°C. The fungicide solution was added to the medium, mixed thoroughly and plates were poured immediately after mixing, giving a final concentration of 0.2 mg imazalil a.i. per litre of medium. By using different volumes of imazalil stock, PDA media containing 0.1, 0.2, 1.0 and 10 mg/litre concentrations of imazalil (a.i.) were prepared.

The guazatine stock solution was prepared by making a 1000 fold dilution of the commercial product (210 g guazatine/litre) and adding 0.7 ml/litre of PDA as described for imazalil. This volume gave a final concentration of 0.15 mg/l a.i. guazatine in the medium.

Isolates to be screened were inoculated on to PDA plates and incubated at 24°C for 7 days. Screening was conducted on four different concentrations of imazalil and one of guazatine.

The procedure outlined by the Fungicide Resistance Action Committee in Appendix 1 of the manual entitled Sensitivity Baselines in Fungicide Resistance Research and Management was used to test for resistance to various fungicides. Plates were inoculated with five millimeter agar plugs cut from the margins of 7 day old parent cultures on PDA medium. The plugs were transferred aseptically to each of 3 control plates (PDA) and 3 replicates per concentration of imazalil or guazatine. Each plug was inverted and placed in the centre of each petri-dish. Plates were incubated for 7 days at 24°C, and colony diameters were measured to the nearest millimeter.

Inversion of the agar plugs from the *P. italicum* cultures dispersed spores over the test plates resulting in numerous colonies per plate. These colonies interfered with the growth of the central inoculum, thereby distorting the colony diameter. In order to minimize spore dispersal each plug was blotted on a sterile filter paper strip soaked in sterile water before transfer to the test plate. This procedure did not entirely solve the problem.

The colony diameters on PDA and fungicide amended media were used to calculate the percentage inhibition of growth for each isolate compared to the control (PDA only) according to the formula of Holmes and Eckert (1999):

$$\% \text{ inhibition} = \frac{M - x_i}{M} \times 100$$

Where M is the mean colony diameter on non amended medium (PDA only) and x_i is the colony diameter on a single fungicide amended plate.

Results

In the initial screening, 28 isolates of *P. italicum* and 21 isolates of *P. digitatum* were evaluated for sensitivity to imazalil (0.2 µg/ml) and guazatine (0.15 µg/ml) (Table 4.5.13.1). Eight isolates of *P. italicum* were resistant to both fungicides, one was resistant to imazalil only and two were resistant to guazatine only. Two of the *P. digitatum* isolates were resistant to both fungicides and three were resistant to imazalil only. The remaining *P. digitatum* isolates were sensitive to guazatine. Resistance to imazalil and guazatine was defined as those isolates whose growth was equal to (\pm 1 mm) or greater on the fungicide-amended media than the control (PDA only).

The results obtained from screening 23 *P. italicum* and 26 *P. digitatum* isolates over a range of imazalil concentrations (0.1; 0.2; 1.0; 10 µg/ml) and one level of guazatine (0.15 µg/ml) are given in Table 4.5.13.2. All isolates of *P. italicum* and *P. digitatum* tested grew on imazalil-amended plates at 0.1 µg/ml. Ten isolates of *P. italicum* were totally resistant to 10 µg/ml. Six of these isolates were also resistant to guazatine (0.15 µg/ml). One *P. italicum* isolate was resistant to both imazalil at 1.0 µg/ml and guazatine and another isolate was sensitive to guazatine but not imazalil (10 µg/ml). All *P. digitatum* isolates tested were sensitive to

imazalil at 10 µg/ml (complete inhibition of growth). Growth of *P. digitatum* on guazatine-amended plates (0.15 µg/ml) varied from complete inhibition to approximately 50% of the control.

After initial screening of all the fungal spore samples received to date, against imazalil at 0.2 ppm (µg/ml) and guazatine at 0.15 ppm (µg/ml), logarithmic dilutions of imazalil and guazatine at 0.1, 1.0 and 10.0 ppm were made up for incorporation into the growth medium for a repeat screening of the samples and standardised screening *in vitro*, of all future spore samples, against imazalil and guazatine.

The repeat screening of the spore samples showed fungal growth by the majority of spore samples at 0.1. Therefore only the growth at the 1.0 and 10.0 ppm concentrations were recorded in the following tables. The following tables indicate the number of spore samples, per production area, that recorded positive growth at 1.0 ppm and 10.0 ppm imazalil incorporated into the growth medium used for this *in vitro* screening.

Table 4.5.13.1. *In vitro* sensitivity of *P. digitatum* to imazalil.

Production Area	No. Samples Screened	Positive growth at 1.0 ppm imazalil	Positive growth at 10.0 ppm imazalil	No. samples indicating fungal growth at both concentrations
Pongola	2			Nil
Nkwaleni	1			Nil
Richmond	2			Nil
S. Swaziland	2		1	1
N. Swaziland	8	1		1
Kat River incl. Ft. Beaufort	6	5		5
Sundays River	8	6		6
Citrusdal	4	2		2
Piketberg	3	3		3
Ashton	3	3		3
Paarl	1	1		1
Oudam	1			Nil
Grobblersdal	1			Nil
	42	21	1	22

Table 4.5.13.2. *In vitro* sensitivity of *P. italicum* to imazalil.

Production Area	No. Samples Screened	Positive growth at 1.0 ppm imazalil	Positive growth at 10.0 ppm imazalil	No. samples indicating fungal growth at both concentrations
Pongola	9			Nil
Nkwaleni	4		4	4
Richmond	12	2	5	7
S. Swaziland	4			Nil
N. Swaziland	4		3	3
Kat River incl. Ft. Beaufort	8	1	3	4
Sundays River	6	2	1	3
Citrusdal	2	1	1	2
Piketberg	1			Nil
Ashton	3			Nil
Paarl	1			Nil
Porterville	1			Nil
Wellington	1			Nil
Robertson	1		1	1
Hectorspruit	1		1	1
	58	6	19	25

Table 4.5.13.3. *In vitro* sensitivity of *P.italicum* to guazatine.

Production Area	No. Samples Screened	Positive growth at 1.0 ppm imazalil	Positive growth at 10.0 ppm imazalil	No. samples indicating fungal growth at both concentrations
Pongola	3		3	3
Nkwaleni	1		1	1
Richmond	6	1	5	6
Kat River	3	1	2	3
	13	2	11	13

Table 4.5.13.4. *In vitro* sensitivity of *P. digitatum* to guazatine.

Production Area	No. Samples Screened	Positive growth at 1.0 ppm imazalil	Positive growth at 10.0 ppm imazalil	No. samples indicating fungal growth at both concentrations
N Swaziland	5			Nil
Richmond	1			Nil
	6			Nil

NB. The results recorded in the tables above will have to be correlated with the results obtained with the *in vivo* screenings of the same spore samples at the standard concentrations of imazalil (500 ppm) and guazatine (1000 ppm) before resistance can be confirmed.

Conclusion

1. Levels of resistance to imazalil varied considerably among the isolates tested.
2. Most of the isolates resistant to both fungicides were found in *P. italicum*.
3. Guazatine (0.15 µg/ml) suppressed the growth of most isolates relative to the control but failed to totally inhibit growth.
4. Isolates showing resistance to guazatine (0.15 µg/ml) should be evaluated at higher concentrations to establish the actual level of resistance.
5. Since signs of resistance to imazalil have become evident in the industry it is necessary to expand the screening process in 2006 in order to plan strategies for areas with different levels of resistance to imazalil.
6. It is also important that the collection of spore samples is expanded to cover a wider and more comprehensive geographic distribution and also includes the collection of samples from both orchards and packhouses.
7. It is important that these samples be clearly identified so that it can be established where the major source of resistance is developing.

4.5.14 The evaluation of plant growth regulators (PGRs), applied post-harvest, as alternatives to 2,4-D sodium salt (Deccomone) for calyx retention on citrus fruit Experiment 754 by K.H.Lesar (CRI)

Opsomming

Die plantgroeireguleerders, Retain, Gibberelliensuur, Califix, ISR 2000, Agromos en Bioboost is op Valencia lemoene, in water en waks doopbehandelings, aangewend vir blomkelk behoud op vrugte na gesimuleerde verskeping. Goeie blomkelk behoud deur Retain, Califix en die fitoalexin verheffers, in vergelyking met die standaard aanbevole 2,4-D (Deccomone) is in albei aanwendingsmetodes waargeneem. Verdere proewe op ander sitruskultivars, om hierdie resultate te bevestig, sal uitgevoer word.

Introduction

Prior to the start of the 2003 citrus season notification was received from the EU MRL committee informing the citrus industry that the MRL for 2,4-D would be decreased from 2.0 mg/kg to 0.05 mg/kg. Communication between the Citrus Growers Association, Citrus Research International and the European Union residue committee during the mid-portion of the 2003 South African citrus season led to a harmonised EU MRL of 1,0 ppm for 2,4-D being considered. In the interim, the UK set a national MRL at 1,0 ppm, but the other

member states retained the 0,05 ppm MRL for the remainder of the 2003 season until a national MRL of 1,0 ppm was set. A harmonised EU MRL of 1,0 ppm was finally set at the start of the 2004 citrus season.

In the eventuality of 2,4-D being discontinued as a post-harvest treatment in the not too distant future, there is currently no alternative product registered as a post-harvest treatment on citrus for the preservation of fruit buttons (calyx). Therefore it is imperative to evaluate new safe products that could prevent calyx abscission, given the distance of the South African fruit from the markets. Button abscission on citrus fruit could possibly expose the fruit to infection by one or more of the latent citrus pathogens, viz. Anthracnose, *Diplodia* and *Alternaria*, as was evident in the 2003 production season. Button abscission also detracts from the eye-appeal of the fruit on the market. The following research was therefore conducted.

Materials and methods

Pilot trials were initially conducted with several PGRs to determine the effect on calyx (button) retention on citrus fruit. The following PGRs were evaluated.

- Retain (aminoethoxyvinylglycine)
- GA₃ (Gibberellic acid)
- Califix (2,4-D sodium salt)
- ISR 2000 (phytoalexin enhancer)
- Agromos (phytoalexin enhancer)
- Bioboost (phytoalexin enhancer)

The pilot trials demonstrated varying degrees of button retention by these PGRs on citrus fruit, prompting the conducting of larger-scale statistical trials as follows.

Good, sound, untreated Benny and Delta Valencia oranges (Crocodile Valley Citrus Co.) were obtained in bulk. For the purpose of this trial, blemish-free, sound fruit was selected and randomised. The fruit was washed and surface sterilised on the packline at CRI in a high-pressure spray using a suitable sanitising agent. The fruit was then dried in the drying tunnel on the packline. All the fruit for this trial were selected with live, firm green buttons (calyxes).

Trial 1: Benny Valencias were used in this first trial. All the fruit in treatments 1-9 were dipped in citrus wax at ambient temperature for 3 minutes. Fruit in treatments 10-20 were immersed in water solutions for 3 minutes. Each treatment consisted of 5 replicates of 8 fruit each.

Wax Dip Treatments

1. Untreated control - waxed fruit only
2. Treated control 250 ppm 2,4-D (Deccomone)
3. Treated control 500 ppm 2,4-D (Deccomone)
4. Retain 250 ppm
5. Retain 500 ppm
6. Califix 250 ppm
7. Califix 500 ppm
8. GA 50 ppm
9. GA 100 ppm

Water Dip Treatments

10. Untreated control
11. Treated control 250 ppm 2,4-D (Deccomone)
12. Treated control 500 ppm 2,4-D (Deccomone)
13. Retain 150 ppm
14. Retain 250 ppm
15. Retain 300 ppm
16. Retain 500 ppm
17. Califix 250 ppm
18. Califix 500 ppm
19. GA 50 ppm
20. GA 100 ppm

After dipping, the treated fruit was allowed to dry overnight and then all the treatments were placed into paper packets and stored under simulated shipping conditions, i.e.:

- 1 week at 20°C
- 6 weeks at 8°C
- 1 week at 20°C

After the simulated shipping period, the treatments were evaluated and the results recorded as percentage button retention. The fruit was also evaluated for any stem-end infections, caused by any of the latent pathogens.

Trial 2: The technique used for this trial was similar to the first trial but the fruit were Delta Valencias and only a water dip application was used with the following treatments.

Water Dip Treatments

1. Untreated control.
2. Treated control 250 ppm 2,4-D (Deccomone)
3. Treated control 500 ppm 2,4-D (Deccomone)
4. Retain 250 ppm
5. Retain 500 ppm
6. Agromos 2.4 *l*/100 *l*
7. Bioboost 1.2 *l*/100 *l*
8. Agromos + Bioboost 2.4 *l* + 1.2 *l*/100 *l*
9. ISR2000 1.2 *l*/100 *l*
10. Agromos 4.8 *l*/100 *l*
11. Bioboost 2.4 *l*/100 *l*
12. ISR2000 2.4 *l*/100 *l*
13. GA 100 ppm
14. GA 200 ppm

All the fruit in the above treatments were dipped in a water dip at ambient, and each treatment was immersed for 3 minutes. Each treatment consisted of 4 replicates of 5 fruit each. After drying, the treated fruit were placed into paper packets and stored under simulated shipping conditions, i.e.

- 1 week at 20°C
- 10 weeks at 8°C
- 1 week at 20°C

After simulated shipping, the treatments were evaluated and the results recorded as percentage button retention. The fruit was also evaluated for any stem-end infections, caused by any of the latent pathogens.

Results

Trial 1: Good calyx retention by Retain and Califix, compared to the standard recommended 2,4-D (Deccomone), is indicated in the results for both applications (Tables 4.5.14.1 and 4.5.14.2). No stem-end infections by any of the latent pathogens were observed on the fruit evaluated.

Table 4.5.14.1. The effect of PGRs in wax on calyx retention on Benny Valencia oranges.

Treatments	Mean Calyx Retention (%)
1. Untreated control - waxed fruit only	15.0 e
2. Treated control 250 ppm Deccomone	92.5 ab
3. Treated control 500 ppm Deccomone	100.0 a
4. Retain 250 ppm	80.0 c
5. Retain 500 ppm	92.5 ab
6. Califix 250 ppm	95.0 ab
7. Califix 500 ppm	100.0 a
8. GA 50 ppm	65.0 d
9. GA 100 ppm	85.0 bc

Means followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \geq 0.05$)

Table 4.5.14.2. The effect of PGRs in a water dip on calyx retention on Benny Valencia oranges.

Treatments	Mean Calyx Retention (%)
10. Untreated control	17.5 e
11. Treated control 250 ppm Deccomone	95.0 a
12. Treated control 500 ppm Deccomone	100.0 a
13. Retain 150 ppm	65.0 d
14. Retain 250 ppm	82.5 bc
15. Retain 300 ppm	80.0 bc
16. Retain 500 ppm	90.0 ab
17. Califix 250 ppm	95.0 a
18. Califix 500 ppm	97.5 a
19. GA 50 ppm	60.0 d
20. GA 100 ppm	77.5 c

Means followed by the same letter are not significantly different. (Fisher's Unprotected LSD; $P \geq 0.05$)

Trial 2: Good calyx retention by ISR 2000 at the standard concentration and Retain, Bioboost and ISR 2000 at the higher concentrations, compared to the standard recommended 2,4-D (Deccomone), is indicated in the results below (Table 4.5.14.3). No stem-end infections by any of the latent pathogens were observed on the fruit evaluated.

Table 4.5.14.3. The effect of PGRs in a water dip on calyx retention on Delta Valencia oranges.

Treatments	Mean Calyx Retention (%)
1. Untreated control.	15.0 e
2. Treated control 250 ppm Deccomone	70.0 ab
3. Treated control 500 ppm Deccomone	85.0 a
4. Retain 250 ppm	45.0 cd
5. Retain 500 ppm	80.0 ab
6. Agromos 2.4 l/100 l	60.0 bc
7. Bioboost 1.2 l/100 l	70.0 ab
8. Agromos + Bioboost 2.4 l + 1.2 l/100 l	75.0 ab
9. ISR2000 1.2 l/100 l	80.0 ab
10. Agromos 4.8 l/100	70.0 ab
11. Bioboost 2.4 l/100 l	85.0 a
12. ISR2000 2.4 l/100 l	85.0 a
13. GA 100 ppm	25.0 de
14. GA 200 ppm	35.0 de

Means followed by the same letter are not significantly different (Fisher's Unprotected LSD; $P \geq 0.05$)

Results and conclusion

Good calyx retention was obtained by Califix, ISR 2000, Retain and Bioboost compared to the standard recommended 2,4-D (Deccomone). Further research will be conducted to confirm these results on other citrus cultivars.

4.6 PROJECT: FRUIT AND FOLIAR DISEASES

Project Co-ordinator: G.C. Schutte (CRI)

4.6.1 Project summary

Copper hydroxide (Hydrox) spray programmes sprayed at monthly intervals in the winter rainfall regions of the Western Cape, performed well at the registered rate of 200 g/hℓ water against *Alternaria* brown spot. Where the copper fungicide rate was lowered from 200 g/hℓ water to 100 g/hℓ water but combined with Sporekill (100 mℓ/hℓ water) in a tank mix and sprayed at monthly intervals (7 applications in total), the results were just as good. Both the copper hydroxide and copper hydroxide with Sporekill resulted in 98-99% clean exportable fruit. No stippling due to copper was observed in the latter treatment with Sporekill. Only copper hydroxide sprayed at a rate of 200 g/hℓ water resulted in stippling (4.6.2).

Five applications consisting of 3 strobilurins and 2 mancozeb applications sprayed in the summer rainfall region, gave good control of *Alternaria* brown spot versus 6 of the other spray programmes that consisted mainly of contact fungicides. Both the copper oxychloride and copper hydroxide spray programmes sprayed at monthly intervals, performed well at registered rates of 200 g/hℓ water against *Alternaria* brown spot. Where both the copper fungicide rates were lowered from 200 g/hℓ water to 100 g/hℓ water but combined in a tank mix with Sporekill (100 mℓ/hℓ water) and sprayed at monthly intervals (6 applications in total) from September to February, the results were just as good. Both the copper fungicide treatments with Sporekill resulted in 98% clean exportable fruit. No stippling due to copper was observed in the latter treatments with Sporekill. In all the spray programmes, the contact fungicides (copper oxychloride and mancozeb) sprayed as a tank mixture with the strobilurins, were lowered from 150 g/hℓ water to 100 g/hℓ water, thereby lowering spray costs (4.6.3).

Phytophthora citrophthora isolations made from trunks of Clementine trees in the Eastern Cape, showed that colonies on PDA had a petaloid growth habit. Radial growth rate on PDA was 6.5 mm² at 25°C and the maximum temperature for growth was 33°C. No growth was measured at 35°C. This property is commonly used to distinguish between *Phytophthora citrophthora* and *P. nicotianae*. Using the BLASTn algorithm, all strains showed a high percentage sequence similarity (>98 %) with significant alignments of E values = 0.0 and bit scores, aligning all S.A. strains with *P. citrophthora* ITS spacer sequence data accessible from Genbank. Sequence data, sporangial shape and dimensions as well as typical symptoms of gummosis on citrus trunks, indicate that symptoms were caused by a population of *P. citrophthora*. A global sequence alignment of ITS spacer sequence data (Fig. 4.6.4.1) shows a grouping of all *P. citrophthora* strains in one cluster, with *P. citricola* strains from Genbank grouping in a separate subcluster. Other species with distinct papillate sporangia clustered separately with *P. primulae* and *P. porri* with semi-papillate sporangia forming an outgroup in this dendrogram. No results were obtained with any of the separate treatments with Ridomil Gold (2 mℓ/m²), Fighter (1 ℓ/hℓ water) and Captan (200 g/hℓ water). Some infected trees in the Fighter treatments did recover while none of the Ridomil or Captan treatments reacted to the treatments on their own (4.6.4).

Projekopsomming

Koperhidroksied (Hydrox) spuitprogramme wat met maandelikse intervalle toegedien is in Weskaap se winterreënvalgebied, het goed gevaar teen 'n geregistreerde dosis van 200 g/hℓ water teen *Alternaria* bruinvlek. Waar die koperhidroksied dosis verlaag is van 200 g/hℓ water na 100 g/hℓ water, maar in 'n tankmengsel met Sporekill (100 mℓ/hℓ water) en gespuit is met maandelikse intervalle (7 toedienings in totaal), was die resultate ook uitstekend wat beide tot 98-99% skoon uitvoerbare vrugte tot gevolg gehad het. Geen stippelvorming was met die koperhidroksied tankmengsel Sporekill waargeneem nie terwyl koperhidroksied wat teen die geregistreerde dosis van 200 g/hℓ water gespuit is, wel stippelvorming gehad het (4.6.2)

In die somerreënvalgebied het vyf bespuitings, bestaande uit 3 strobilurin en 2 mancozeb toedienings het goeie beheer van *Alternaria* bruinvlek tot gevolg gehad teenoor 6 toedienings van swamdoders wat hoofsaaklik uit kontakswamdoders bestaan het. Beide die koperoksichloried- en koperhidroksiedspuitprogramme wat met maandelikse intervalle gespuit is, het beide goeie beheer van *Alternaria* bruinvlek gegee teen geregistreerde dosisse van 200 g/hℓ water. Waar beide die koperswamdoders se dosis verlaag is van 200 g/hℓ water na 100 g/hℓ water, maar in 'n tankmengsel met Sporekill (100 mℓ/hℓ water) en gespuit is met maandelikse intervalle (6 behandelings in totaal) van September tot Februarie, was die resultate net so goed en het beide die koperswamdoders met Sporekill, 98% skoon uitvoerbare vrugte tot gevolg gehad. Geen stippelvorming weens koperbespuitings in die

tankmengsel met Sporekill is waargeneem nie. In al die spuitprogramme is die kontakswamdoders (koper oksichloried en mancozeb) wat in tankmengsels met die strobilurines gespuit is, se dosisse verlaag van 150 g/hℓ water na 100 g/hℓ water, desnieteenstaande goeie beheer van *Alternaria* bruinvlek gegee en sodoende kan die kwekers geld hierdeur spaar (4.6.3).

Phytophthora citrophthora isolasies wat van die stamme van Clementine bome in die Oos Kaap gemaak is op ADA, het 'n petaliedagtige groeivoorkoms. Radiale groeitempo op ADA was 6.5 mm² by 25°C en die maksimum temperatuur vir groei is 33°C. Geen swamgroei was waargeneem by 35°C nie. Hierdie eienskap word algemeen gebruik om te onderskei tussen *Phytophthora citrophthora* en *P. nicotianae*. Die gebruik van die BLASTn algoritme, toon dat alle rasse 'n hoë persentasie volgorde ooreenkoms (>98%) met 'n betekenisvolle groepering met waardes van E values = 0.0 en brokkie waardes, wat die Suid Afrikaanse isolate groepeer met *P. citrophthora* ITS spasieerder volgorde soos verkry van Genbank. Die volgorde data, sporangiale vorms en dimensies asook die tipiese simptome van gomafskiedings op die stamme, dui daarop dat dit deur 'n populasie van *P. citrophthora* veroorsaak word. 'n Globale volgorde groepering van die ITS spasieerder volgorde data toon die groepring van al die *P. citrophthora* rasse in een groep, met *P. citricola* rasse van Genbank wat op sy beurt groepeer in 'n aparte subgroep. Ander spesies met kenmerkende tepelvormige sporangia groepeer afsonderlik *P. primulae* en *P. porri* met semi-tepelvormige sporangia wat weer in 'n anderlike groep groepeer in die dendrogram. Geen resultate was verkry met die afsonderlike behandelings met Ridomil Gold (2 mℓ/m²), Fighter (1 ℓ/hℓ water) en Captan (200 g/hℓ water) nie. Sommige geïnfekteerde bome het op die Fighter behandeling gereageer, terwyl nie een van die Ridomil en Captan behandelde bome gereageer het op behandelings nie (4.6.4).

4.6.2 Evaluation of new spray programmes for the control of *Alternaria* brown spot in the winter rainfall region of South Africa

Experiment 749 by G.C. Schutte (CRI)

Opsomming

Koperhidroksied (Hydrox) spuitprogramme wat met maandelikse intervalle toegedien is, het goed gevaar teen 'n geregistreerde dosis van 200 g/hℓ water teen *Alternaria* bruinvlek. Waar die koperhidroksied dosis verlaag is van 200 g/hℓ water na 100 g/hℓ water, maar in 'n tankmengsel met Sporekill (100 mℓ/hℓ water) en gespuit is met maandelikse intervalle (7 toedienings in totaal), was die resultate ook uitstekend wat beide tot 98-99% skoon uitvoerbare vrugte tot gevolg gehad het. Geen stippelvorming was met die koperhidroksied tankmengsel Sporekill waargeneem nie terwyl koperhidroksied wat teen die geregistreerde dosis van 200 g/hℓ water gespuit is, wel stippelvorming gehad het.

Introduction

Brown spot disease of citrus caused by *Alternaria turkisarifra* is one of the most prevalent fungal diseases in all production areas in South Africa. Minneola tangelos, Novas, mandarins and their hybrids, tangors and grapefruit are the most susceptible cultivars. The disease can affect both fruit and foliage and is most prevalent under wet, humid conditions. The fruit lesions are very unsightly and readily reduce crop value. Toxic formation in foliar and twig infections causes defoliation.

South Africa has both winter and summer rainfall areas. In both these areas, wet, humid periods can occur which favour brown spot disease (Schutte, Beeton, Swart, Beyleveldt, & Burger; 1994). This applies particularly to the autumn in southern areas that have a Mediterranean climate. Heavy dew can also create suitable conditions for disease development and again the southern areas are more susceptible to this climatic factor. Nevertheless, due to the unpredictability of seasonal climatic trends, it is necessary to annually protect the most susceptible cultivars against the disease in all areas (Timmer, Darhower, Zitko, Peever, Ibanez & Bushong; 2000). This typically requires a multiple spray programme to cover the possible infection periods from spring to autumn. In this regard, the strategy employed by South African growers is to use the more expensive systemic fungicides during the wet, high-disease pressure periods and the less expensive contact fungicides during dry, low disease pressure periods.

The aim was to evaluate coppers in a modified brown spot spray programme to assist growers in the winter rainfall region of the Western Cape with cheaper yet more effective spray programmes resulting in more acceptable residues for export markets. The wet, high disease period runs from September or October through to April or May. Sporekill, a disinfectant or plant sanitiser, previously showed promise against citrus black spot in tank mixtures with copper fungicides and mancozeb and therefore I wanted to evaluate it for the possible control of *Alternaria* brown spot as well.

Materials and methods

A trial site was selected on the farm Sovereign Estates near Swellendam, where 'novas' were available with a high incidence of brown spot. Different cost-effective spray programmes were selected comprising copper hydroxide (Hydrox) and a disinfectant or plant sanitizer (Sporekill). A randomised row design with 25 trees per row was used per treatment and sprayed with a commercial spray machine. Buffer rows were allowed between each of the treatments. Trees were thoroughly sprayed to the point of run-off. The evaluation of brown spot (200 fruit per replicate) on the fruit rind was conducted just prior to harvesting, during mid-June. Criteria used for rating the fruit were:

- 0 = fruit with no brown spot lesions,
- 1 = fruit with one to five brown spot lesions,
- 2 = fruit with six or more brown spot lesions.

The results were expressed as percentages and the means compared using Fisher's LSD test for significance.

Results and discussion

Results from the field trial conducted at Sovereign Estates at Swellendam (Table 4.6.2.1) show that there were no significant differences ($P > 0.01$) between the standard registered copper hydroxide and the tank mixtures of reduced copper hydroxide plus Sporekill at the rate tested. Both treatments yielded 99% and 97.33% clean exportable fruit. All the other criteria (fruit with 1-5 brown spot lesions per fruit and fruit with 6 or more brown spot lesions per fruit) were also not significantly different ($P > 0.01$) from each other, but all the treatments were significantly different from the control. Stippling was only apparent on fruit that received sprays with the higher rate of copper hydroxide (Fig. 4.6.2.1).

Conclusion

A combination of copper hydroxide (Hydrox) at 100 g/hℓ water and Sporekill at 100 mℓ/hℓ water was as effective in controlling *Alternaria* brown spot as copper hydroxide alone at 200 g/hℓ with the additional advantage that the lower rate of copper did not cause stippling. This is promising as a spray programme based on this mixture will result in residues that are more acceptable in Europe than some of the existing products in use.

Future research

More spray programmes consisting of different mixtures with Sporekill will be evaluated in the new season.

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Table 4.6.2.1. Application of copper hydroxide (Hydrox) alone or in a tank mixture with Sporekill for *Alternaria* brown spot control on 'novas' at Buffeljachtsrivier, near Swellendam, S.A. during 2004 and 2005 (dosages per 100 ℓ water).

15 September 2004	16 October 2004	17 November 2004	17 December 2004	25 January 2005	1 March 2005	5 April 2005	No. of <i>Alternaria</i> brown spot lesions on fruit ²		
							No lesions (%)	1-5 lesions (%)	6 or more lesions (%)
Hydrox 200 g	Hydrox 200 g	Hydrox 200g	Hydrox 200 g	Hydrox 200 g	Hydrox 200g	Hydrox 200g	99.00 a	0.67 a	0.33 a
Hydrox + Sporekill 100 g + 100 ml	Hydrox +Sporekill 100 g+100 ml	Hydrox +Sporekill 100 g+100 ml	97.33 a	2.00 a	0.67 a				
Control							52.33 b	21.50 b	26.17 b

²Means in a column, based on 200 fruit per replicate, followed by the same letter are not significantly different ($P > 0.01$) according to Fisher's least significant difference test.



Fig. 4.6.2.1. Stippling due to frequent copper hydroxide spray applications at a rate of 200 g/100 ℓ water.

4.6.3 Positioning and evaluation of new spray programmes consisting of strobilurins for the control of *Alternaria* brown spot in the summer rainfall regions of South Africa
Experiment 750 by G.C. Schutte (CRI)

Opsomming

Vyf bespuitings, bestaande uit 3 strobilurin en 2 mancozeb toedienings het goeie beheer van *Alternaria* bruinvlek tot gevolg gehad teenoor 6 toedienings van swamdoders wat hoofsaaklik uit kontakswamdoders bestaan het. Beide die koperoksichloried- en koperhidroksiedspuitprogramme wat met maandelikse intervalle gespuit is, het beide goeie beheer van *Alternaria* bruinvlek gegee teen geregistreerde dosisse van 200 g/hℓ water. Waar beide die koperswamdoders se dosisse verlaag is van 200 g/hℓ water na 100 g/hℓ water, maar in 'n tankmengsel met Sporekill (100 ml/hℓ water) en gespuit is met maandelikse intervalle (6 behandelings in totaal) van September tot Februarie, was die resultate net so goed en het beide die koperswamdoders met Sporekill, 98% skoon uitvoerbare vrugte tot gevolg gehad. Geen stippelvorming weens koperbespuitings in die tankmengsel met Sporekill is waargeneem nie. In al die spuitprogramme is die kontakswamdoders (koper oksichloried en mancozeb) wat in tankmengsels met die strobilurines gespuit is, se dosisse verlaag van 150 g/hℓ water na 100 g/hℓ water, desnieteenstaande goeie beheer van *Alternaria* bruinvlek gegee en sodoende kan die kwekers geld hierdeur spaar.

Introduction

Alternaria brown spot is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all South African regions. The causal agent was originally described as *Alternaria citri* Ellis & N. Pierce, and later renamed *A. alternata* pv. *citri*. Simmons (1999) described 10 new species from a worldwide collection of *Alternaria* isolates from citrus. According to his study, isolates from Turkey, Israel, Australia and South Africa were the same and were renamed *A. turkisafrica*. Isolates from the USA and Colombia were morphologically different and were named *A. tangelonis* and *A. colombiana* respectively. Peever *et al.* (2002) confirmed the research of Simmons, determining the worldwide phytogeography of the brown spot pathogen using molecular markers and sequence data, as well as determining the quantitative differences in virulence among isolates from different citrus growing areas of the world. Recently, Vincent *et al.* (2000) reported that *Alternaria* brown spot (sp. unknown) was detected in Spain.

Alternaria brown spot attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. The pathogen produces a host-specific toxin that causes lesions to expand, often

resulting in leaf and fruit drop and twig dieback. On more mature fruit, lesions may vary from small necrotic spots to large, sunken pockmarks. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit that are unacceptable to the consumer. Leaves are susceptible to infection from the time of formation until they are fully expanded and hardened, and fruit are susceptible from petal fall until harvest. In the USA however, fruit are only susceptible from petal fall until they reach about 5 cm in diameter.

Cultural measures such as wider tree spacing to allow air movement and dry-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards. However, fungicide applications are essential for disease control and production of blemish-free fruit. In South Africa it is important to protect fruit and flushes of cultivars such as tangerines and their hybrids with fungicides from September to April/May, often requiring 8+ spray applications. This number of sprays and the products being used are not economically sustainable and may result in unacceptable residues on fruit. Our aim was to evaluate three strobilurins (with EU MRLs) using two and three applications during the high disease pressure period from October to January to establish whether fewer fungicides can be used and whether fungicides like the strobilurins with longer lasting residues will be effective enough for the control of brown spot. Sporekill, a disinfectant or plant sanitiser, showed promise against citrus black spot in tank mixtures with copper fungicides and mancozeb and therefore we wanted to evaluate it for the possible control of *Alternaria* brown spot as well.

Materials and methods

Ten single-tree plots per treatment were selected randomly from a 'nova' orchard at Sterkfontein near Nelspruit (25° 22.81' S 30° 31.89' E). The trees were 11 years old and planted in 2x5 m tree spacing in rows that ran from North to South. Trees were selected for uniformity in canopy density and tree size. Single untreated guard trees were located between plots within rows. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2500-3000 kPa) sprayer with two hand-held spray guns on the dates mentioned in Table 4.6.3.2.1. The weather was fine and dry on all occasions with minimal wind. Spray volumes varied according to the size and canopy density of the tree but all trees were sprayed to the point of run-off. At fruit maturity in June, *Alternaria* brown spot severity was rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no brown spot lesions; 1 = one to five brown spot lesions per fruit; and 2 = six and more brown spot lesions per fruit. Data per tree were analysed by ANOVA, using Fisher's LSD test ($\alpha = 0.05$).

Results and discussion

Results from Table 4.6.3.2 showed that there were no significant differences between any of the spray programmes evaluated. All the spray programmes were, however, significantly different from the untreated control with regards to all criteria used for evaluation. Spray programme 10 resulted in the most clean exportable fruit, followed by spray programme numbers 9, 6, 8 and 5. Of these, spray programmes 10, 8 and 6 comprised 5 spray applications (consisting of 3 strobilurins and 2 mancozeb applications) versus 6 of the other spray programmes that consisted mainly of contact fungicides.

Both the copper oxychloride and copper hydroxide spray programmes (spray programmes nos. 1 and 2) sprayed at monthly intervals, performed well at registered rates of 200 g/hℓ water against *Alternaria* brown spot. Where both the copper fungicide rates were lowered from 200 g/hℓ water to 100 g/hℓ water but in a tank mix with Sporekill (100 mℓ/hℓ water) and sprayed at monthly intervals (6 applications in total) from September to February, the results were just as good and both the copper fungicide treatments with Sporekill resulted in 98% clean exportable fruit. There were also no significant differences between the spray programmes with regards to the criteria 1 to 5 *Alternaria* brown spot lesions per fruit and 6 and more *Alternaria* brown spot lesions per fruit. However, all the treatments were significantly different from the control (Table 4.6.3.2).

Three strobilurin applications sprayed at 60 day intervals gave good control of *Alternaria* brown spot. In all the spray programmes, the contact fungicides (copper oxychloride and mancozeb) sprayed as a tank mixture with the strobilurins, were lowered from 150 g/hℓ water to 100 g/hℓ water, thereby saving the growers on spray costs. Although not quantified, the frequent copper applications as sprayed in spray programmes 1 and 2 both at a rate of 200 g/hℓ water, usually lead to serious stippling. However, the incidence of stippling in spray programmes 3 and 4, was non-existent. Sporekill in tank mixtures with copper oxychloride, copper hydroxide and mancozeb can be recommended for future use as it nullifies the secondary effect of stippling due to frequent spraying.

Conclusion

Five applications consisting of 3 strobilurins and 2 mancozeb applications gave good control of *Alternaria* brown spot versus 6 of the other spray programmes that consisted mainly of contact fungicides. This type of spray programme will save the growers one spray round if compared with the contact/preventative type of spray programme that one has to spray with monthly intervals. Concomitantly, the strobilurins do have a systemic or local systemic mode of action and their long lasting residual action plays an important role in the good fungicidal action against *Alternaria* brown spot (Häuser-Hahn, Pontzen & Baur, 2003). Furthermore, the strobilurin, Flint, has a mesostemic mode of action whereby it has a high affinity for the plant's waxy layer and is thus stored there very effectively. This results in a fungicide reservoir from which the active ingredient penetrates continuously into the deep-lying tissue of the plant. Due to this reservoir, a continuous protective effect is exerted against fungal-spore attack (Krieg, Weile & Göhlich, 2003).

Both the copper oxychloride and copper hydroxide spray programmes sprayed at monthly intervals, performed well at registered rates of 200 g/hℓ water against *Alternaria* brown spot. Where both the copper fungicide rates were lowered from 200 g/hℓ water to 100 g/hℓ water but in a tank mix with Sporekill (100 ml/hℓ water) and sprayed at monthly intervals (6 applications in total) from September to February, the results were just as good and both the copper fungicide treatments with Sporekill resulted in 98% clean exportable fruit. No stippling due to copper was observed in the latter treatments with Sporekill. In all the spray programmes, the contact fungicides (copper oxychloride and mancozeb) sprayed as a tank mixture with the strobilurins, were lowered from 150 g/hℓ water to 100 g/hℓ water, thereby saving the growers on spray costs.

Future research

More spray programmes consisting of different mixtures with Sporekill will be evaluated in the new season.

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Table 4.6.3.1. Application of copper oxychloride and copper hydroxide alone or in tank mixtures with Sporekill as well as strobilurins (Flint, Ortiva, Cabrio and Blighter) using two and three applications during the high disease pressure period from October for *Alternaria* brown spot control on Novas at Sterkfontein, near Nelspruit in S.A. during 2004 and 2005 (dosages are per 100 ℓ water).

No	27 & 28 September	25 October	8 November	22 November	6 December	20 December	6 January	18 January	21 February
1	Hydrox 200g	Hydrox 200g		Hydrox 200g		Hydrox 200g		Hydrox 200g	Hydrox 200g
2	Fynox 200g	Fynox 200g		Fynox 200g		Fynox 200g		Fynox 200g	Fynox 200g
3	Hydrox +SK 100g + 100mℓ	Hydrox +SK 100g + 100mℓ		Hydrox +SK 100g + 100mℓ		Hydrox +SK 100g + 100mℓ		Hydrox +SK 100g + 100mℓ	Hydrox +SK 100g+100mℓ
4	Fynox + SK 100g + 100mℓ	Fynox + SK 100g + 100mℓ		Fynox + SK 100g + 100mℓ		Fynox + SK 100g + 100mℓ		Fynox + SK 100g + 100mℓ	Fynox + SK 100g + 100mℓ
5	Fynox 200g	Flint+MZ+O 100g+100g+2.5%			Fynox 200g		Flint+ MZ + O 100g+100g+2.5%		Fynox 200g
6	Flint+Fynox+O 10g+100g+2.5%		Flint+Fynox+O 10g+100g+2.5%			Flint+Fynox+O 10g + 100g + 2.5%		MZ 200g	MZ 200g
7	Fynox 200g	Cabrio+MZ+O 100g+100g+2.5%			Fynox 200g		Cabrio+MZ+O 100g+100g+2.5%		Fynox 200g
8	Cabrio+Fynox+O 10g+100g+2.5%		Cabrio+Fynox+O 10g+100g+2.5%			Cabrio+Fynox+O 10g+100g+2.5%		MZ 200g	MZ 200g
9	Fynox 200g	Ortiva+MZ+O 20mℓ+100g+2.5%			Fynox 200g		Ortiva+MZ+O 20mℓ+100g+2.5%		Fynox 200g
10	Ortiva+ Fynox+O 20ml+100g+2.5%		Ortiva+Fynox+O 20ml+100g+2.5%			Ortiva+Fynox+O 20mℓ+100g+2.5%		MZ 200g	MZ 200g
11	Fynox 200g	Blighter+Ortiva+O 200g+20mℓ+2.5%			Fynox 200g		Blighter+Ortiva+O 200g+20mℓ+2.5%		Fynox 200g
12	Blighter+Ortiva+O 200g+20mℓ+2.5%		Blighter+Ortiva+O 200g+20mℓ+2.5%			Blighter+Ortiva+O 200g+20mℓ+2.5%		MZ 200g	MZ 200g
13	Control								

MZ = mancozeb SK = Sporekill O = spray oil

Table 4.6.3.2. Evaluation of copper oxychloride and copper hydroxide alone or in tank mixtures with Sporekill as well as strobilurins (Flint, Ortiva, Cabrio and Blighter) using two and three applications during the high disease pressure period from October for *Alternaria* brown spot control on Novas at Sterkfontein, near Nelspruit in S.A. during 2004 and 2005.

Spray programme number	No. of <i>Alternaria</i> brown spot lesions on fruit ^z		
	No lesions (%)	1-5 lesions (%)	6 or more lesions (%)
10	99.8 a	0.2 a	0 a
9	99.6 a	0.4 a	0 a
6	99.6 a	0.4 a	0 a
8	99.4 a	0.2 a	0.4 a
5	99.4 a	0.6 a	0 a
7	99.4 a	0.4 a	0.2 a
2	99.2 a	0.2 a	0.6 a
11	99.2 a	0.6 a	0.2 a
1	99.0 a	0.4 a	0.6 a
3	98.0 a	1.0 a	1.0 a
4	98.0 a	1.4 a	0.6 a
12	96.6 a	2.4 a	1.0 a
13	36.9 b	24.1 b	39.0 b

^zMeans in a column, based on 10 replicates, followed by the same letter are not significantly different ($P > 0.05$) according to Fisher's least significant difference test.

4.6.4 Investigation into die-back of Clementines in the Eastern Cape Experiment 736 by G.C. Schutte (CRI) and W.J. Botha (PPRI)

Opsomming

Phytophthora citrophthora isolasies wat van die stamme van Clementine bome in die Oos Kaap gemaak is op ADA, het 'n petaliedagtige groeivoorkoms. Radiale groeitempo op ADA was 6.5 mm² by 25°C en die maksimum temperatuur vir groei is 33°C. Geen swamgroei was waargeneem by 35°C nie. Hierdie eienskap word algemeen gebruik om te onderskei tussen *Phytophthora citrophthora* en *P. nicotianae*. Die gebruik van die BLASTn algoritme, toon dat alle rasse 'n hoë persentasie volgorde ooreenkoms (>98 %) met 'n betekenisvolle groepering met waardes van E values = 0.0 en brokkie waardes, wat die Suid Afrikaanse isolate groepeer met *P. citrophthora* ITS spasieerder volgordes soos verkry van Genbank. Die volgorde data, sporangiale vorms en dimensies asook die tipiese simptome van gomafskedings op die stamme, dui daarop dat dit deur 'n populasie van *P. citrophthora* veroorsaak word. 'n Globale volgorde groepering van die ITS spasieerder volgorde data toon die groeipring van al die *P. citrophthora* rasse in een groep, met *P. citricola* rasse van Genbank wat op sy beurt groepeer in 'n aparte subgroep. Ander spesies met kenmerkende tepelvormige sporangia groepeer afsonderlik *P. primulae* en *P. porri* met semi-tepelvormige sporangia wat weer in 'n anderlike groep groepeer in die dendrogram. Geen resultate was verkry met die afsonderlike behandelings met Ridomil Gold (2 ml/m²), Fighter (1 l/hl water) en Captan (200 g/hl water) nie. Sommige geïnfecteerde bome het op die Fighter behandeling gereageer terwyl nie een van die Ridomil en Captan behandelde bome gereageer het op behandelings nie.

Introduction

Tree die-back was observed in the Knysna region of the Eastern Cape on the Nules cultivar. Other Clementine cultivars such as Marisol, SRA and Orival also seemed to be affected by this unknown disease complex. The die-back starts right on the scion (30 cm above ground level) as white fluffy mycelial growth and is accompanied by anthracnose and *Diplodia* fungal growth (both are secondary wound pathogens). Koch's postulates were successfully implemented using the same rootstock and cultivar.

Among the complex of *Phytophthora* species attacking citrus, *P. citrophthora* (R.E. Sun & E.H. Sun) Leonian 1906), is active at moderate temperatures (less than 30°C) and *P. parasitica* (*P. nicotianae*) at high temperatures (above 30°C). This explains why *P. citrophthora* is so active in the Mediterranean type of climate experienced along the Eastern and Western Cape coastline where the disease is also a problem on Nules. For the time being, the trees are being protected with different fungicides to determine the efficacy of each and to eliminate those that are not effective.

Materials and methods

Isolations

During a visit to the farm "Candlewood" near Knysna on 24 September 2003, bark samples were taken from infected trees and taken to CRI in Nelspruit. From these, *P. citrophthora* isolates were aseptically removed and placed onto PARPH-selective medium to secure a pure culture. One of the sub-cultures of each isolate was sent to the Plant Protection Research Institute in Pretoria for identification.

Cultures were purified by subculturing on PARPH-selective medium (Kannwischer and Mitchell, 1978) modified by substituting corn meal with 1% water agar. One week old colonies were subcultured on 0.7% water agar and incubated for 7-12 days at 25°C. Water cultures were prepared by pouring 2 l sterile soil extract (2%) in sterile 5 cm diameter petri dishes and floating three citrus leaf discs of 5 mm diameter per petri dish. Water cultures were incubated at 25°C for 5-8 days to observe for the formation of sporangia and the release of zoospores. Sporangia were observed and measured under a compound light microscope with Nomarski differential illumination, and photographed. The key of Stamps *et al.* (1990) was used to identify isolates. Cultures were maintained in 20 ml McCarthy bottles on potato carrot agar (PCA) slants under sterile mineral oil and in sterile distilled water with sterile wheat leaf blades at 15°C. Isolates were deposited in the National Collection of Fungi (Plant Protection Research Institute; Agricultural Research Council) and each isolate received a strain code (Table 4.6.4.1.)

Morphological studies

Isolations were made from washed diseased bark tissue on PARPH-selective medium (Masago *et al.*, 1977). Single hyphal tip cultures on soft water agar (0.7% agar) were prepared. Mycelial - and citrus leaf discs of 5 mm diameter were placed in sterile petri dishes (5 cm diameter) filled with sterile soil-extract (2%) and

incubated at 25°C for 5-7 days under white fluorescent light to stimulate sporangial formation. Sporangial papillation, dimensions, caducity and branching pattern were used for preliminary species identification according to the key of Stamps *et al.* (1990). Sporangia were photographed under a compound light microscope with Nomarski differential illumination. Cultures were maintained on 2% malt extract agar slopes under mineral oil and in sterile distilled water on colonised citrus leaf blades (*Citrus limon*). Radial growth rates were determined on 1.5% potato dextrose agar (mm^{-d}) at 25°C over 5 days.

Genomic DNA extraction and ITS sequencing

Mycelium was grown in stationary liquid cultures (1.5% potato dextrose broth) in sterile 9 cm diameter petri dishes at 25°C for 14 days. Mycelial mats were harvested by vacuum filtration and freeze dried. Genomic DNA was extracted (genomic DNA purification kit; Fermentas Life Sciences, Inc., MD) according to suppliers' instructions. The ITS 1,2 regions and 5.8 S gene regions were amplified with ITS4/5 primers (White *et al.*, 1990) in 25 µl (Fermentas Tag DNA polymerase 1.0 unit/µl; 10 mM Tris-HCl; 50 mM KCl; 1.5 mM MgCl₂; 200 µM each dNTP; pH 8.8). Thermal cycler program: hot start at 96°C for 2 min; 30 cycles of denaturation at 94°C for 30 sec; annealing at 60°C for 30 sec; elongation at 72°C for 2 min; final extension at 72°C for 5 min. PCR products were gel purified from low-melting temperature agarose and extracted using the Wizard PCR Preps DNA purification kit (Promega, Madison, WI). Purified PCR products were sequenced using the BigDye ver. 3.1 terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). ITS spacer region sequences were compared with sequenced *P. citrophthora* strains in Genbank nucleotide database (NCBI, Bethesda, MD) using the Basic Alignment Search Tool (BLASTn) algorithm function.

Analysis of ITS sequence data

ITS sequence data of South African *P. citrophthora* strains (Table 4.6.4.1), as well as selected species in Waterhouse group I and II with papillate sporangia, distorted sporangial shapes and or bipapillate sporangia (Waterhouse, 1963; Stamps *et al.*, 1990), were aligned according to global alignment parameters (gap penalty 0%; correction Kimura 2 parameter; UPGMA clustering method; Bionumerics ver. 4.0; Applied-Maths, Sint-Martins-Latem, Belgium). A pair wise distance-matrix with UPGMA as clustering algorithm was applied to prove that S.A. strains belonged to a population of *P. citrophthora*. Percentage sequence similarity between species was presented as a UPGMA dendrogram.

Fungicidal treatments

The selected orchard at Candlewood farm, west of Knysna, was sub-divided into three groups consisting of three rows each. These rows were marked and the following treatments were applied at the following rates:

- a) Ridomil Gold (2 ml/m²)
- b) Fighter (1 l/hl water)
- c) Captan (300 g/hl water)

Rows were marked and fungicidal treatments applied by means of hand-held spray guns to the trunks of the trees. Each tree was inspected and evaluated before the application of the fungicides for the presence of the disease and each tree canopy was rated on a scale from 0 – 10 (where 0 = healthy and 10 = dead). If the tree trunk was ringulated with the disease, a % scale (where 0% = healthy and 100% = totally infected) was used. Evaluations were done just before harvest the following year.

Results and discussion

Morphological studies

Colonies on PDA showed a petaloid growth habit. Radial growth rate on PDA 6.5 mm² at 25°C. Maximum temperature for growth is 33°C. No growth was measured at 35°C. For all strains investigated sporangia were papillate, non-caducous, borne on unbranched or loosely branched sporangiophores (Fig. 4.6.4.1). Sporangial shape varied from ovoid to obpyriform. Some sporangia were bipapillate with two apices. Mean length of sporangia (39 – 61 µm) and mean breadth (27 – 45 µm). Length:breadth ratio was 1.38:1. Oogonia, hyphal swellings and chlamydospores not observed.

Analysis of ITS sequence data

Using the BLASTn algorithm, all strains showed a high percentage of sequence similarity (>98 %) with significant alignments of E values = 0.0 and bit scores, aligning all S.A. strains with *P. citrophthora* ITS

spacer sequence data accessible from Genbank. Sequence data, sporangial shape and dimensions as well as typical symptoms of gummosis on citrus trunks indicate that symptoms were caused by a population of *P. citrophthora*. Strains with ENBL accession numbers are listed in Table 4.6.4.1. A global sequence alignment of ITS spacer sequence data (Fig. 4.6.4.2) shows a grouping of all *P. citrophthora* strains in one cluster, with *P. citricola* strains from Genbank grouping in a separate subcluster. Other species with distinct papillate sporangia clustered separately with *P. primulae* and *P. porri* with semi-papillate sporangia forming an outgroup in this dendrogram.

Fungicidal treatments

No results were obtained with any of the separate treatments with Ridomil Gold (2 ml/m²), Fighter (1 l/hl water) and Captan (200 g/hl water). Some infected trees in the Fighter treatments did recover while none of the Ridomil or Captan treated trees reacted to the treatments on their own.

Conclusion

Both morphological data as well as alignment of ITS spacer sequence data, indicate that strains of *Phytophthora citrophthora* isolated from stem canker tissue of infected citrus trees, belong to a small population of *P. citrophthora* strains. Typical symptoms of trunk gummosis with discolouration of bark tissue confirm the presence of *P. citrophthora* in infected bark tissue. The rapid colonisation of bark tissue and rapid decline of nursery and orchard trees, indicate that this may be a new virulent population of *P. citrophthora* infecting Clementines in the Eastern Cape province. Isolates obtained from soil and infected bark tissue indicate that the population may be wide spread. Sources of contamination may be soil, nearby rivers, nursery water and potting mixtures.

No results were obtained from the field trial at Knysna due to the infrequent fungicide applications. Future field trials should include a holistic approach consisting of a spray programme that includes different fungicide applications with different modes of action.

Future research

Reports were also received of similar problem in the Gamtoos Valley as well as certain Clementine groves in the Western Cape. These farms should be inspected to determine the extent of the problem on Clementines. Control programmes consisting of different fungicides with different modes of action should be investigated.

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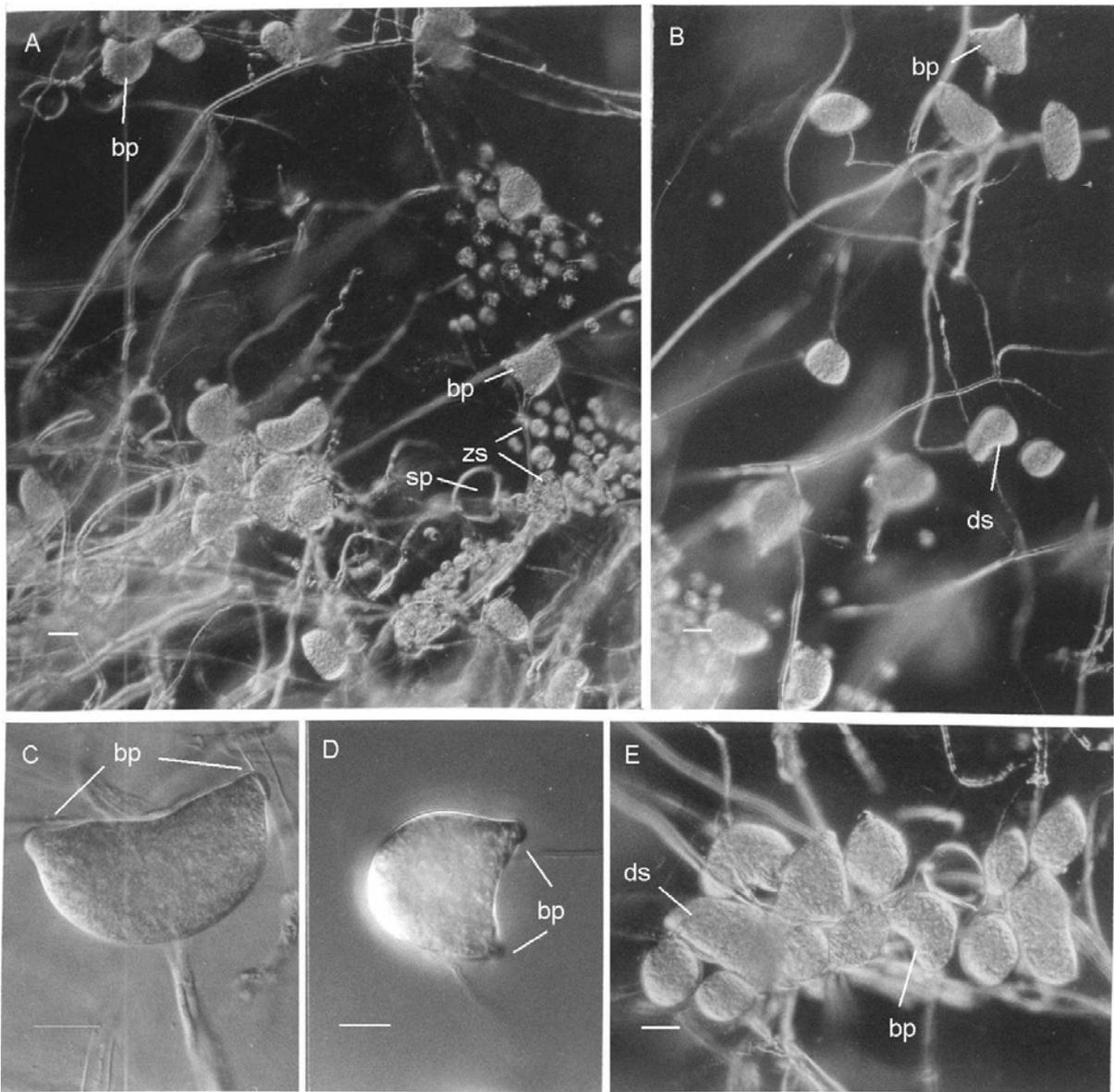


Fig. 4.6.4.1. Formation of zoosporangia and release of zoospores by *Phytophthora citrophthora* in water culture (scale bar = 10 μ m). A. Bipapillate sporangia (bp) of *Phytophthora citrophthora* showing two papillae per sporangium; release of zoospores from discharged sporangia (sp); swarming motile zoospores (zs). (B,E). Bipapillate sporangia (bp) and some sporangia with distorted shapes (ds). (C,D). Typical bipapillate sporangia (bp) with two papillae each and a sporangium attached perpendicular to sporangiophore (C).

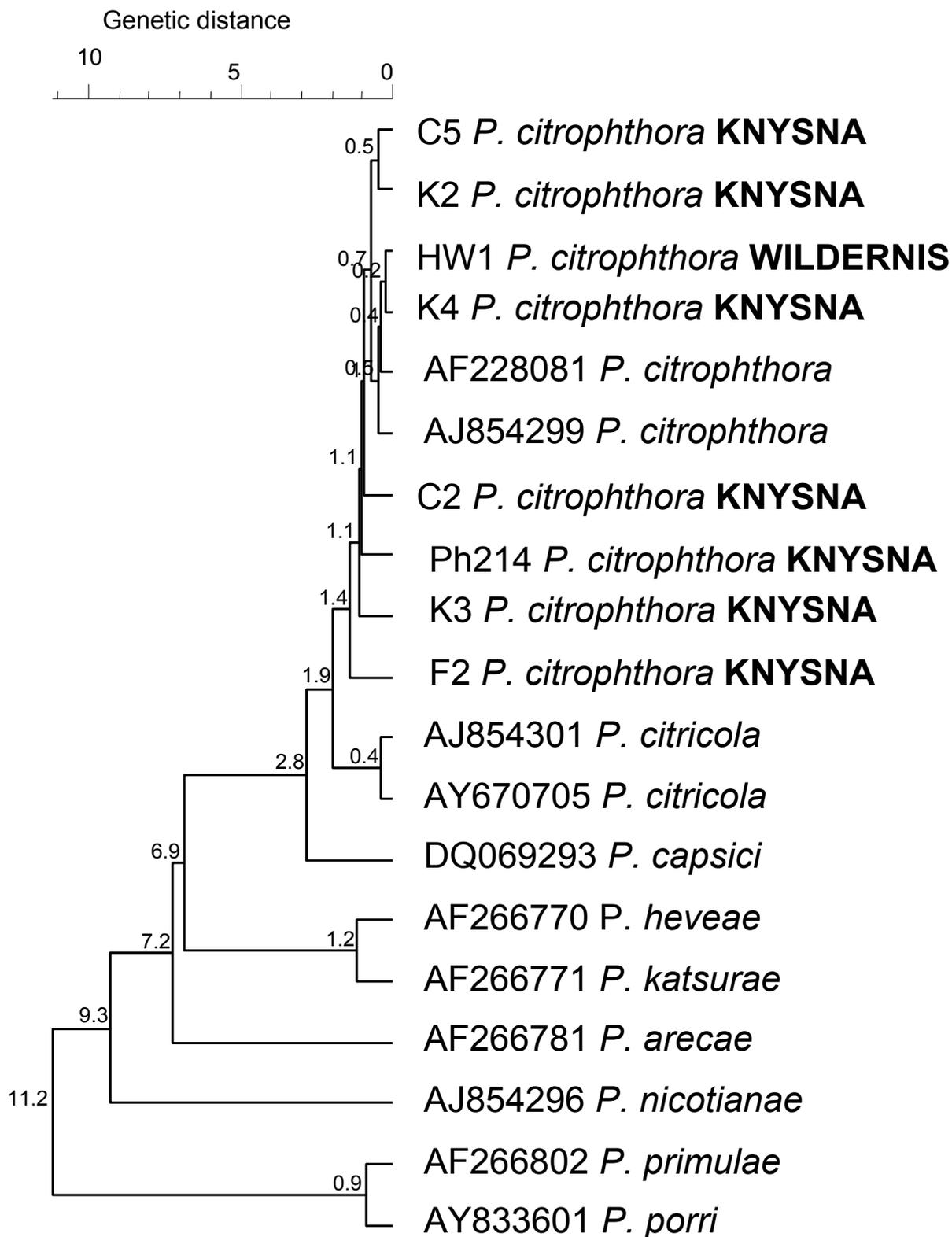


Fig. 4.6.4.2. Grouping of South African isolates of *Phytophthora citrophthora* from *Citrus reticulata* in one cluster with reference strains using a pairwise UPGMA distance algorithm, showing low genetic distance between *P. citrophthora* isolates and clustering separately from other *Phytophthora* species.

Table 4.6.4.1. List of *Phytophthora citrophthora* strains and other *Phytophthora* species included in this study.

Species	Strain code	Host	Country	Sequence data base no.
<i>P. citrophthora</i>	PPRI 7920 (C5)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7918 (C2)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7913(Ph214)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7914 (K2)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7916 (K4)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7917 (K3)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7915 (F2)	<i>Citrus reticulata</i>	South Africa, Knysna	
<i>P. citrophthora</i>	PPRI 7919 (HW1)	<i>Citrus reticulata</i>	South Africa, Wildernis	
<i>P. citrophthora</i>		Unknown	Korea	AF 228081
<i>P. citrophthora</i>		Unknown	Italy	AJ 854299
<i>P. citricola</i>	CITR-LE	<i>Citrus limon</i>	Italy	AJ 854301 *
<i>P. citricola</i>	Pc3	<i>Fragaria</i>	Poland	AY 670705 *
<i>P. capsici</i>		<i>Cucurbita</i>	United Kingdom	DQ 069293
<i>P. nicotianae</i>	33C3	<i>Piper betel</i>	India	AH 015112 *
<i>P. heveae</i>	IMI 180616	<i>Hevea brasiliensis</i>	Malaysia	AF 266770 *
<i>P. katsurae</i>	IMI 360596	<i>Cocus nucifera</i>	Ivory Coast	AF 266771 *
<i>P. arecae</i>	IMI 348342	<i>Cocus nucifera</i>	Indonesia	AF 266781 *
<i>P. primulae</i>	CBS 620.97	<i>Primula acaulis</i>	Germany	AF 266802 *
<i>P. porri</i>	UASWS0013	<i>Allium porri</i>	Switzerland	AY 833601 *

4.7 CRI DIAGNOSTIC CENTRE (Laura Huisman & Timothy Zulu: CRI)

	Citrus Nurseries	Commercial samples	Avo Nurseries	Research samples	Other crops
Nematode :Roots		282		1042	31
Soil		71		1042	191
<i>Phytophthora</i>	1439	263	38	555	290
Nursery water	71				
Black spot resistance to benzimidazole		21			
Red scale resistance to organophosphate		1			
Internal fruit quality		23			

Citrus Accredited Nurseries

There are currently 19 accredited and 2 provisionally accredited citrus nurseries. It is compulsory for the accredited nurseries to submit *Phytophthora* samples on a quarterly basis. Of the 1439 samples received, 4,2% tested positive for *Phytophthora*.

Commercial Samples

Samples were sent to the DC from most of the citrus growing areas. In 59% of the nematode samples analysed, the citrus nematode female counts per 10 grams of roots exceeded the threshold value. Forty-four percent of the *Phytophthora* soil samples tested positive. Twenty-one samples were received for black spot resistance tests against benzimidazole.

5 PROGRAMME: CROP LOAD AND FRUIT QUALITY MANAGEMENT

5.1 PROGRAMME SUMMARY

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

Although the funds allocated to this programme are a fraction of those allocated to the Disease Management or IPM programmes this does not reflect the economic value of the research because millions of Rands have been lost in the last few years to rind condition problems with mandarins and the full potential of orchards is often not achieved due to unresolved horticultural problems. In 2005, most research funding went to the ondition project that covered creasing, chilling injury, rind breakdown and Peteca spot of lemons. Creasing research involves the role of various minerals but has not yet provided any conclusive results. Research on chilling injury showed that the susceptibility of fruit to chilling injury in cold storage was more important than the method of cooling used. Intermittent warming was not effective in reducing chilling injury. Once again, Nules Clementines were shown to be more prone to rind breakdown than Oroval Clementines but the reason for this is not due to different antioxidant or carotenoid levels and requires further research. Similarly, the true cause of Peteca spot has not been resolved, although conditions in which it is more likely to occur are being defined. Losses due to sunburn on Satsuma mandarin were eliminated with kaolin sprays in the form of Surround and maturity was also advanced, but attempts to reduce acidity in Delta Valencias with MAP were not as successful as before. Preharvest applications of Ph-Ca usually improved rind colour at harvest and may allow for earlier harvesting. The role of Molybdenum and Tungsten in colour development is also under investigation. An experiment is being conducted in Zimbabwe to evaluate the partial root zone drying irrigation strategy in terms of its effect on crop yield.

PROGRAMOPSOMMING

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

Fondse wat vir hierdie programme geallokeer is, is slegs 'n fraksie van die fondse wat na die Siektebestuur - of IPB programme geallokeer is. Dit reflekteer egter nie die ekonomiese waarde van die navorsing nie. Miljoene rande is al oor die afgelope paar jare verloor as gevolg van skildefekte van mandaryne. Soms bereik boorde ook nie die volle potensiaal nie as gevolg van onopgeloste hortologiese probleme. In 2005 het die meeste van die navorsingbefondsing gegaan na die skildefekte projek wat kraakskil, koueskade, skilafbraak en Peteka van suurlemoene insluit. Die kraakskil navorsing fokus op die rolle van verskeie minerale, maar tot dusver kon geen oortuigende resultate gevind word nie. Die navorsing wat op koueskade gedoen word het getoon dat die gevoeligheid van vrugte vir koueskade tydens koue opberging belangriker is as die metode van verkoeling wat gebruik word. Dubbel temperatuur verwarming was ook nie effektief in die vermindering van koue skade nie. Dit is weereens bewys dat Nules Clementines meer gevoelig is vir skilafbraak as Oroval Clementines. Die rede hiervoor is egter nie die teenwoordigheid van 'n ander anti-oksidadant of karotenoïed vlakke nie en verdere navorsing is dus nodig. Die ware oorsaak vir die ontstaan van Peteka is ook nog nie bepaal nie, alhoewel toestande waarin dit meer geneig is om in voorkom, gedefiniëer is. Verliese as gevolg van sonbrand by Satsuma mandaryne is met kaolien bespuitings in die vorm van Surround geëlimineer en rypheid is ook bevorder. Pogings om die suurgehalte in Delta Valencias met MAP te verminder was nie so suksesvol as voorheen nie. Aanwendings van Ph-Ca voor-oes het meestal die kleur van die skil bevorder tydens oes en mag dalk moontlik lei tot vroeër oes. Die rol van Molybdenum en Tungsten in kleurontwikkeling word ook ondersoek. 'n Eksperiment word in Zimbabwe uitgevoer om die gedeeltelike uitdroging van die wortelarea as besproeiingsstrategie te evalueer, in terme van die effek op oesopbrengs.

5.2 PROJECT: RIND CONDITION

Project Co-ordinator: John Bower (UKZNP)

5.2.1 Project summary

The report covers experiments pertaining to a specific group of rind disorders, which include creasing, rind breakdown and chilling injury damage as well as Peteca Spot of lemons.

Clive Kaiser at the University of Pretoria was conducting research on the effect of timing calcium salts and micronutrients on creasing in navel oranges but this was abandoned when he left the country. Stephan Verreyne has summarised the results that were obtained in Section 5.2.2.

Stephan Verreyne's own research work on creasing (5.2.3) was initiated during the year, and thus no results are as yet available. The work has concentrated on investigating the use of mineral nutrition sprays, as well as a mixture of plant growth regulators as preventatives. The work has been conducted on navel orange trees in Groblersdal, Sundays River and Citrusdal. An ethylene inhibitor (Retain®) and De-Crease (a

mixture of Mg, Mo and Zn) have been used at various application timings from petal fall to physiological drop, and Goëmar (Mo, B, Mg, S auxins and cytokinins) to which Zn was added, was applied with and without De-Crease. Evaluation of results will be made at harvest in 2006.

A number of trials aimed at preventing or explaining the cause of rind breakdown were conducted. The role of mineral nutrition was investigated in relation to postharvest rind condition in 'nules' Clementines (5.2.4). Mineral analysis of fruit showed significant differences between inside and outside fruit, with Ca, K, and to some extent B being important, and inside fruit having higher rind breakdown. The meaning of actual element levels and seasonal changes is not yet clear, but will be further elucidated in 2006 when attempts to manipulate rind nutrient levels will be made.

Stem-end rind breakdown has been noted in 'Valencias', and linked to possible chilling injury. Forced air cooling has been suggested as a cause. This was investigated in the work outlined in section 5.2.5. Fruit was cooled to 1°C with and without forced air cooling. Once at temperature, static cooling at 1°C for 3 weeks was maintained. Considerably more damage occurred in 2005 than 2004, but there was also no difference between the cooling systems. This implies that fruit sensitivity to long periods of low temperature storage is a more important consideration than cooling method. It is essential that chilling sensitive fruit be identified, so that it is not used where cold disinfestation will be needed.

A potentially useful technique to decrease rind damage caused by low temperature storage, is that of intermittent warming. This was evaluated in the work outlined in section 5.2.6. Satsuma mandarins, 'nules' Clementines, 'Eureka' lemons, 'Valencia' and navel oranges from various sites in the Western Cape, were used. The protocol tested required a shipping temperature of -1°C, which was used as a control. The warming treatments tested were, after 3 days at -1°C, 2 days at 15°C or 6 days at 7.5°C, thereafter returning fruit to -1°C. A total of 44 days at -1°C followed. Only in the case of satsumas and 'Valencias', was any positive effect found. The technique is not considered useful on its own, and the presently used wilting technique, together with methods to identify chilling sensitivity, are believed to be more useful. Future work on the latter is needed.

Within the group of rind disorders, puffiness of satsuma mandarins, when stored at a low temperature (-0.6°C for 32 days) due possibly to high CO₂ levels in containers, can be problematical. The work in 2005 (section 5.2.7) used higher values of CO₂ than in the 2004 trial, but did not result in puffiness. Only some fruit colour loss was noted. It is envisaged that ethylene effects will be tested in 2006.

Rind breakdown has, at times been associated with the lack of anti-oxidants. The objective of the work in section 5.2.8 was to establish whether the 'Oroval' Clementine has higher anti-oxidant and carotenoid levels than 'nules', which could explain why 'nules' is more susceptible to rind breakdown. Fruit from both cultivars were degreened, and stored for 10 weeks at 7.5°C. There was no difference in the measured anti-oxidant capacity or carotenoids, but 'nules' developed significantly more rind breakdown. 'Oroval' had higher decay levels. Rind breakdown thus did not appear directly connected to any of the parameters measured, and further research will be necessary. It is recommended from this work, that 'nules' is not suited to long term storage. Where 'nules' fruit were harvested from different positions in the canopy (section 5.2.9) and stored at 7.5°C or 10°C for 12 weeks, differences were recorded in rind nutrient levels and anti-oxidants. However, this did again not correlate with rind breakdown results, and the levels at harvest could not be used for prediction purposes. When considering 2004 results together with 2005, it was concluded that only storage temperature played a role, with chilling injury occurring at low temperature (-0.5°C) and rind breakdown at higher temperatures, with the greatest at the highest temperature tested. It has thus been concluded that at present, the quality of Clementines can best be maintained through optimal storage temperature and time in storage.

Peteca Spot has created considerable problems for exporters. Various theories have been advanced as to the causes. Climatic conditions may pre-dispose lemons to the disorder, with changes from hot, dry conditions to cold and wet just prior to harvest, being problematical. From a postharvest point of view, handling, brushing, temperature of fungicide bath or drying and, most importantly, waxing, seem to play a role. Section 5.2.10 considers the effect of a range of waxes. After waxing with waxes containing solids ranging from 18% to 10%, fruit were stored for 28 days at -0.6°C. It was found that waxes with the highest solids resulted in the lowest Peteca Spot, which is contrary to present practice. It is suggested that heavier waxes resulted in less gas diffusion into and out of the fruit. It is further suggested that Peteca Spot follows the same pattern as chilling injury, where high CO₂ (which may build up in the fruit due to heavier waxes) decreases the disorder. Greener lemons also showed more damage than more coloured ones. The results of this trial indicated that there are still many unknowns, and the true cause of Peteca Spot has not yet been found. A more in-depth attempt to solve the physiological basis of the disorder is outlined in section 5.2.11. Fruit was harvested at a range of maturities, and stored at -0.5°C, 4.5°C, 7.5°C, 10°C and 20°C for 20, 40,

60 and 80 days before evaluation. Unfortunately, only low levels of Peteca Spot (<10%) occurred, making conclusions difficult. In general, earlier harvested fruit developed more disorders than later harvested fruit, suggesting a maturity effect. Chilling injury was also higher. It was concluded that storage temperature was not related to Peteca Spot, and that the disorder develops early in storage and is not progressive. It is suggested, considering the available information, that fruit water relations may play a role in development of the disorder, and that this should be the focus of future research.

Projekopsomming

Die verslag sluit eksperimente wat handel oor spesifieke skil afwykings in, en sluit kraakskil, skilafbraak en koueskade, sowel as Peteka Vlek van suurlemoene in.

Pogings is aangewend om die effek van die tyd van toediening van kalsiumsoute en mikro-elemente op die voorkoms van kraakskil te bepaal. Die voorkoms van kraakskil in die 2004-5 seisoen was egter baie laag in Patensie en geen resultate is deur die behandelings verkry (5.2.2). Verdere navorsing op kraakskil sal deur Stephan Verreyne by die Universiteit van Stellenbosch voortgesit word.

Stephan Verreyne se eie navorsing op kraakskil (5.2.3) was eers gedurende die jaar begin, en dus is geen huidige resultate beskikbaar nie. Die werk het gekonsentreer op 'n ondersoek van die gebruik van die spuit van minerale voedings elemente, sowel as 'n mengsel plant groeireguleerders as voorsog maatreëls. Die werk was in Groblersdal, Sondagsrivier en Sitrusdal gedoen. Die etileen inhibeerder Retain® en De-Crease ('n mengsel van Mg, Mo, en Zn) was gebruik met verskeie toepassings tye, van blomblaarval tot fisiologiese val, en Goëmar (Mo, B, Mg, S, auksiene en sitokiniene) saam met Zn, met en sonder De-Crease was aangewend.

Daar was 'n aantal proewe gedoen om die oorsaak van skilafbraak te verduidelik en die voorkoms daarvan te elimineer. Die rol van minerale voeding by die na-oes voorkoms van die skil toestand by 'nules' Clementines (5.2.4) is verondersoek. Die minerale analiese van die vrug skil het betekenisvolle verskille tussen binne en buite vrugte getoon, met Ca, K, Mg en tot 'n mindere mate B as belangrik beskou. Wat die vlakke van voedings elemente en seisoenale veranderinge beteken, is nog nie duidelik nie, maar nog werk sal in 2006 gedoen word, waar die manipulasie van voedingselemente sal beproef word.

Skilafbraak by 'Valencias' is as 'n moontlike deel van koueskade beskou. Geforseerde lug verkoeling is verder as 'n oorsaak beskou. Dit was in die werk by afdeling 5.2.5 ondersoek. Vrugte was tot by 1°C met en sonder geforseerde verkoeling, verkoel. Daarna, was statiese verkoeling vir 3 weke gehou. Meer skade is aangetoon as by 2004, maar daar was ook geen verskil tussen die verkoelings sisteme gevind nie. Dit dui aan dat vrugsensitiviteit by lang periode van lae temperature is meer belangrik as die metode van verkoeling. Die identifikasie van koue sensitiviteit by vrugte is noodsaaklik, en sulke vrugte moet nie gebruik word by kouesterilisasie nie.

'n Moontlike tegniek om skil skade as gevolg van laë temperature te verminder, is die gebruik van verwarming tussendeur die verkoelings proses. Die werk is in afdeling 5.2.6 aangedui. Satsuma mandaryne, 'nules' Clementines, 'Eureka' suurlemoene, 'Valencia' en nawel lemoene vanaf verskeie plekke in die Wes Kaap is gebruik. Die protokol wat getoets was, het 'n verskeping temperatuur van -1°C gebruik, wat as kontrole gedien het. Die verwarming behandelings was 3 dae by -1°C, 2 dae by 15°C of 6 dae by 7.5°C en daarna weer -1°C. 'n Totaal van 44 dae by -1°C het gevolg. Net in die geval van satsumas en 'Valencias' was enige positiewe effek gevind. Die tegniek is dus nie op sy eie bruikbaar nie, en die huidige verwelkings metode saam met metodes om die vatbaarheid van die vrugte vir koueskade sal moontlik beter wees. Toekomstige werk op die laasgenoemde word aanbeveel.

Binne die groep skil afwykings, is poferrigheid van satsumas, veral waar lae temperatuur opberging (-0.6°C vir 32 dae) moontlik as gevolg van hoë CO₂ vlakke binne houe. Die werk in 2005 (afdeling 5.2.7) het hoer vlakke CO₂ as die werk in 2004, gebruik, maar het geen resultaat gelewer nie. Net effense vrugkleur vermindering het gebeur. Dit is beoog dat etileen in 2006 getoets sal word.

Skilafbraak is soms met laë vlakke van anti-oksidante verbind. Die doel van die werk in afdeling 5.2.7 was om vas te stel of die 'Oroval' Clementine hoer vlakke het, en dat dit die rede is hoekom die kultivar minder skilafbraak toon as 'nules'. Vrugte van altwee kultivars is ontgroen, en vir 10 weke by 7.5°C opgeberg. Daar was geen verskil in die anti-oksidante gemeet nie, maar 'nules' het betekenisvol meer skilafbraak ontwikkel. 'Oroval' het meer bederf ontwikkel. Skilafbraak het dus nie goed gekorreleer met enige van die parameters wat ontleed was, en nog navorsing sal nodig wees. Dit is verder aanbeveel dat 'nules' nie teen lang opbergings tye onderwerp word. Waar 'nules' vrugte vanaf verskillende posisies in die boom gepluk was, (afdeling 5.2.9) en teen of 7.5°C of 10°C vir 12 weke opgeberg was, was verskillende voedingselemente en anti-oksidante gemeet. Weereens, was daar geen korrelasie met skilafbraak gevind nie, en vlakke kon nie vir

voorspellings doeleindes gebruik word nie. As resultate vir 2004 en 2005 in ag geneem word, word dit duidelik dat net temperatuur 'n rol gespeel het, met die laagste temperatuur van -0.5°C koueskade, en die hoër temperature skilafbraak veroorsaak het. Teen die huidige, is dit dus aanbeveel dat die gehalte van Clementines die beste sal wees as optimale opbergings temperature en tye gehandhaaf word.

Peteca vlek van suurlemoene het heewat probleme vir uitvoerders veroorsaak. Daar is verskeie teorieë vir die oorsaak daarvan. Klimaat kan dalk 'n rol speel, met veranderinge van warm droë toestande tot koud en nat, moontlik 'n faktor. Vanaf 'n na-oes oogpunt, pakhuis behandelings soos borseling, hanteering en die temperatuur van swamdoder baddens en droërs, en baie belangrik, waks, speel dalk 'n rol. Afdeling 5.2.10 ondersoek die effek van waks. Vrugte is met 'n reeks wakse vanaf 18% tot 10% soliede bestaandele behandel, en vir 28 dae teen -0.6°C opgeberg. Wakse met die hoogste soliede inhoud het die minste Peteca vlek veroorsaak, wat teen huidige aanbevelings is. Dit is moontlik dat die swaarder wakse 'n vermindering van gas wisseling in die vrugte veroorsaak, en dat die verhoging van CO_2 die vermindering van Peteco vlek veroorsaak het. Dit is dieselfde patron as kouskade, waar dit bekend is dat CO_2 vlakke die afwyking beïnvloed. Suurlemoene wat meer groen was, het meer skade as goed gekleurde vrugte getoon. Die resultate bewys dat daar nog heelwat onkunde is, wat die problem betref. 'n Meer in-diepte fisiologiese ondersoek is in afdeling 5.2.11 uitgewys. Vrugte is by 'n reeks volwassenheid gepluk, en teen -0.5°C , 4.5°C , 7.5°C , 10°C en 20°C vir 20, 40, 60 en 80 dae opgeberg. Ongelukkig, was daar min Peteca vlek ($<10\%$), wat gevolgtrekkings moeilik maak. Oor die algemeen, was daar meer Peteco vlek by die onvolwasse vrugte gewees, en die simptome het vroeg in die opbergings tydperk ontwikkel. Daar was ook meer koueskade gewees. Opbergings temperature lyk dus nie die oorsaak van Peteca vlek te wees nie, en die afwyking is nie gedurende die opbergings tydperk geneig om te vermeerde nie. Vanaf die huidige inligting, is dit moontlik dat water verhoudings binne die vrugte dalk 'n rol kan speel, en dat dit verdere navorsing handhaaf.

5.2.2 Effect of timing of calcium salts and micronutrients on creasing of navels

Experiment AH565 by Clive Kaiser (UP) (written up by Stephan Verreyne [CRI at SU])

Opsomming

Pogings is aangewend om die effek van die tyd van toediening van kalsiumsoute en mikro-elemente op die voorkoms van kraakskil te bepaal. Die voorkoms van kraakskil in die 2004-5 seisoen was egter baie laag in Patensie en geen resultate is deur die behandelings verkry. Verdere navorsing op kraakskil sal deur Stephan Verreyne by die Universiteit van Stellenbosch voortgesit word.

Introduction

Clive Kaiser at the University of Pretoria was tasked in 2004 with conducting research on the importance of the timing calcium and micronutrient applications in reducing creasing on navel oranges. The research was being conducted in Patensie with assistance from technical staff at Patensie Citrus Co-op. However, the research was abandoned when Clive Kaiser left the country in 2005. Half of the funding allocated to Clive Kaiser was then transferred to Stephan Verreyne at Stellenbosch University for use in his research on creasing. The report below was compiled by Stephan Verreyne from raw data made available by Clive Kaiser.

Materials and methods

Navel trees, 23 years of age, with a history of high creasing incidence were fertilized in two orchards in Patensie (Eastern Cape Province, latitude: 33.75258 S ; longitude: 24.81127 E ; elevation: 359 m a.s.l. ; mean annual temperature: maximum 32°C , minimum: 5°C ; mean annual rainfall: 547mm ; soil type: sandy loam) on 15 August 2004. Double supers, zinc sulphate, sodium molybdate and calcium acetate were applied in different combinations according to the factorial trial layout in Table 5.2.2.1. Both orchards were under similar management, however, Nikkaladershoek was under microjet irrigation whereas Gonnakop was under drip irrigation. Final yield, fruit size and percentage creasing were recorded for each of the paired sets of trees in the orchard. It should be noted that the 2004/5 season was considered to be an off-season with low yields, associated with a relatively low creasing percentage when compared to previous seasons when yields were much higher and creasing incidence reached levels of up to 50%.

Results

A) Nikkalandershoek (Microjet irrigation)

Yield was positively correlated using simple linear regression ($r = 0.928$; Pr. <0.017) with the percentage creasing being greatest when yields were highest (Fig. 5.2.2.1). Fruit size was negatively correlated with the percentage creasing with no fruit of diameters $> 97\text{ mm}$ showing any creasing ($r = 0.997$; Pr. <0.001).

Creasing incidence was very low (7% in control trees receiving no fertilizer treatment) (Table 5.2.2.2). Therefore, none of the treatments or treatment combinations resulted in a reduction in creasing incidence.

B) Gonnakop (Drip irrigation)

Yield was positively correlated using simple linear regression ($r = 0.962$; $Pr. < 0.031$) with the percentage creasing being greatest when yields were highest (Figure 5.2.2.2). Fruit size was negatively correlated with the percentage creasing with no fruit of diameters > 94 mm showing any creasing ($r = 0.998$; $Pr. < 0.001$). Creasing incidence was very low (8% in control trees receiving no fertilizer treatment) (Table 3). Therefore, none of the treatments or treatment combinations resulted in a reduction in creasing incidence.

Table 5.2.2.1. Treatments used in creasing investigation near Patensie

Citrus Creasing Factorial Trial	
	Treatment
Double Supers Core	300g
	None
Zinc Sulphate Core	100 g
	None
Sodium Molybdate (Broad)	40 g
	None
Calcium acetate (Broad)	100 g
	None

Discussion

The positive correlation between yield and creasing incidence was expected due to high yield resulting in smaller fruit with thinner peels and a higher potential of creasing. It should be noted that the trees in the 2 blocks were not planted on the same rootstocks and were harvested 20 days apart, making comparisons between the two irrigation blocks very difficult. Due to the off-season and low creasing incidence none of the treatments or treatment combinations showed any potential for reducing creasing incidence.

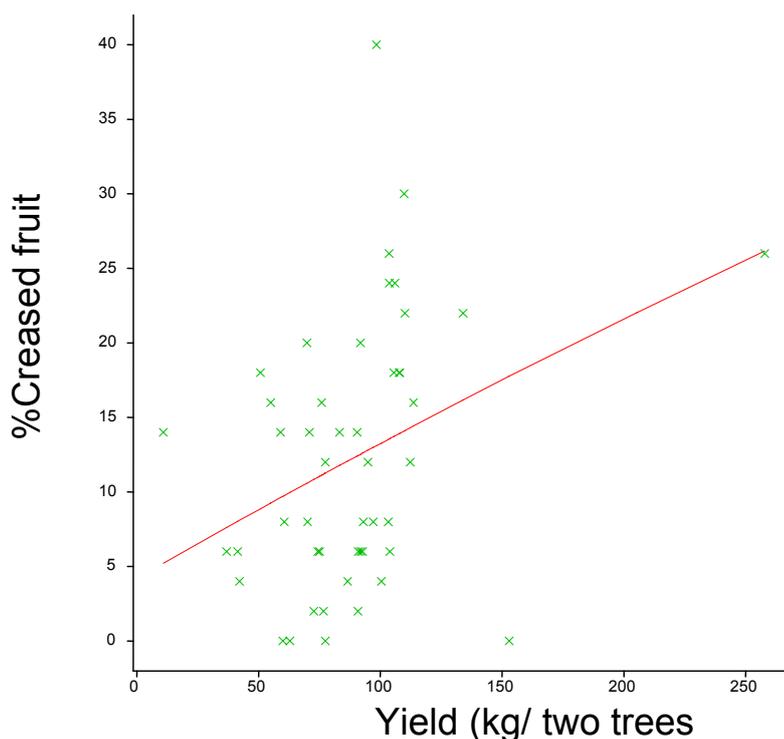


Figure 5.2.2.1. Regression plot of yield (kg/two tree plot) vs. percentage creased Navel orange fruit irrigated with microjets

Table 5.2.2.2. Creasing incidence for the different treatments under Microjet irrigation.

Double Supers	Zinc Sulphate	Sodium Molybdate	Calcium Acetate	%Creased
300g	100 g	40 g	100 g	5
300g	100 g	40 g	0	16
300g	100 g	0	100 g	9
300g	100 g	0	0	19
300g	0	40 g	100 g	23
300g	0	40 g	0	16
300g	0	0	100 g	5
300g	0	0	0	7
0	100 g	40 g	100 g	9
0	100 g	40 g	0	11
0	100 g	0	100 g	17
0	100 g	0	0	19
0	0	40 g	100 g	11
0	0	40 g	0	7
0	0	0	100 g	13
0	0	0	0	7

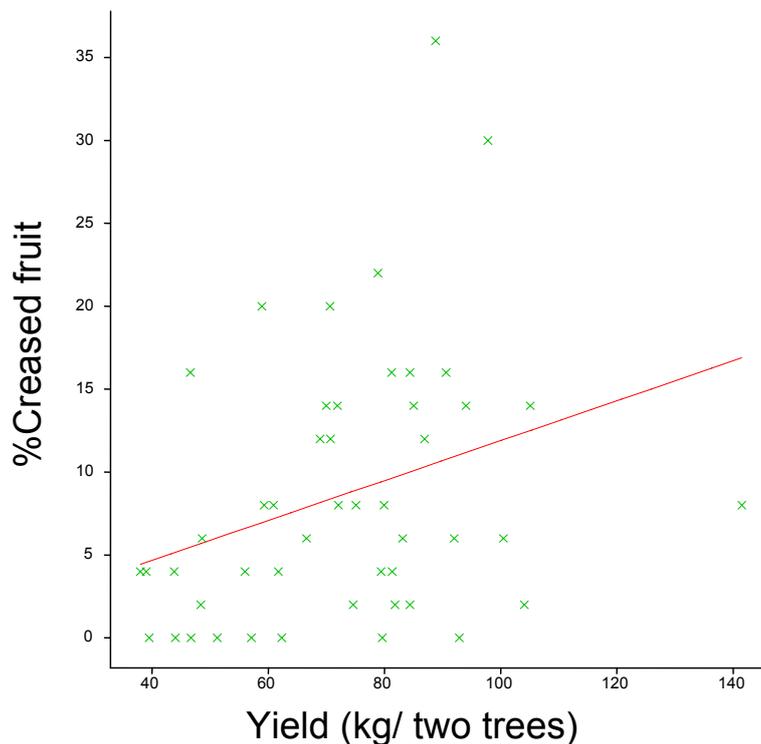


Figure 5.2.2.2. Linear regression plot of yield (kg/two tree plot) vs percentage creased Navel orange fruit irrigated with drippers

Table 5.2.2.3. Creasing incidence for the different treatments under Drip irrigation.

Double Supers	Zinc Sulphate	Sodium Molybdate	Calcium Acetate	% Creased
300g	100 g	40 g	100 g	12
300g	100 g	40 g	0	8
300g	100 g	0	100 g	1
300g	100 g	0	0	22
300g	0	40 g	100 g	3
300g	0	40 g	0	7
300g	0	0	100 g	8
300g	0	0	0	11
0	100 g	40 g	100 g	5
0	100 g	40 g	0	9
0	100 g	0	100 g	7
0	100 g	0	0	17
0	0	40 g	100 g	10
0	0	40 g	0	6
0	0	0	100 g	5
0	0	0	0	8

Conclusion

Very little creasing occurred in this season and no new knowledge was gained.

5.2.3 Evaluation of alternative means of controlling creasing (albedo breakdown) Experiment 849 by Stephan Verreyne (CRI at SU)

Opsomming

Kraaskil is 'n fisiologiese abnormaliteit wat krake in die albedo veroorsaak en lei tot kanale op die oppervlak van die vrug. Dit ontwikkel wanneer selle in die albedo van mekaar wegtrek waar selwande aanmekaar vasgeheg is. Pektien, wat die "sement" (middellamella) tussen selle van die albedo uitmaak, word afgebreek soos die vrug rypword. Baie minerale soos bv. Mo, Zn, Ca, S, B en Mg vorm komplekse met die pektien, wat dit versterk om albedo selle aanmekaar te hou. Kraaskil lei jaarliks tot noemenswaardige verliese in Navel en Valencia lemoene. Die enigste geregistreerde beheermaatreël wat sedert die aanvang van die navorsing beskikbaar is, is gibberelliensuur, wat skilveroudering vertraag en kraaskil verminder, as oes nie te lank uitgestel word nie, maar somtyds lei dit tot vertraagde kleurontwikkeling. Die doel van die studie was om alternatiewe maniere om kraaskil te verminder te evalueer. AVG (Retain®) is 'n etileen inhibeerder en voorlopige resultate uit Kalifornië toon 'n afname in die voorkoms van kraaskil. De-crease is 'n formulاسie van Mg, Mo en Zn. Goëmar is 'n formulاسie van Mo, B, Mg, S, ouksiene en sitokiniene. Blaartoediening van mikro-elemente word gewoonlik later in die seisoen gedoen en die effek van vroeër toediening op die voorkoms van kraaskil is onbekend. Mikroskopies, ontwikkel kraaskil voor fisiologiese vrugval, dus in fase I van vruggroei wanneer aktiewe seldeling plaasvind. Vroeër toediening van mikro-elemente kan heel moontlik lei tot 'n afname in kraaskil.

Proewe is gedoen op Navel bome in Groblersdal, die Sondagsriviervallei en Citrusdal om verskillende formulاسies en verskillende tye van toediening van die formulاسies te evalueer. In Groblersdal en Citrusdal, is die etileen inhibitor, AVG (Retain®) toegedien by blomblaarval of net na fisiologiese vrugval teen 125 dpm. De-crease is toegedien by blomblaarval of net na fisiologiese vrugval teen die aanbevele 50 ml/100 liter water.

In 'n aparte eksperiment in die Sondagsriviervallei en Citrusdal is De-crease toegedien by blomblaarval teen beide die aanbevele 50 ml/100 ml water en teen 100 ml/100 liter water. De-crease is ook toegedien twee weke na blomblaarval en net na fisiologiese vrugval, beide teen 50 ml/100 liter water. Goëmar, aangevul deur Zn, is toegedien teen 200 ml/100 liter water, alleen of in kombinasie met De-crease.

Vir al die bogenoemde eksperimente sal oeslading, vruggrootte en die persentasie kraaskil ge-evalueer word tydens oestyd in 2006. Resultate sal in die volgende jaarlikse verslag verskyn.

Summary

Creasing or albedo breakdown is a physiological disorder resulting in cracks in the albedo and showing channels on the surface of the fruit. It starts when cells in the albedo separate at the cell wall junctions. Pectin, that makes up the "cement" (middle lamella) between individual cells in the albedo, is broken down as fruit matures. Many minerals, namely Mo, Zn, Ca, S, B and Mg, are known to form complexes with the pectin, increasing its strength to hold individual albedo cells together. Creasing yearly causes tremendous losses in both Navels and Valencias. The only registered control measure available when the research started was gibberellic acid, which delays rind senescence and reduces creasing if harvest is not delayed, but sometimes results in delayed colour development. The objective of this study was to evaluate alternative measures to reduce creasing. AVG (Retain®) is an ethylene inhibitor and preliminary work in California resulted in a decrease in creasing. De-crease is a formulation of Mg, Mo and Zn. Goëmar is a formulation containing Mo, B, Mg, S, auxins and cytokinins. Currently, foliar application of micronutrients is being done later in the season and the effect of earlier application on creasing incidence is not known. Microscopically, creasing develops before physiological fruit drop, therefore in stage I of fruit growth when active cell division occurs. Earlier application of micronutrients may result in a reduction in creasing.

Trials were conducted on Navel orange trees in Groblersdal, the Sundays River Valley and Citrusdal to evaluate different formulations and different times of applications of these formulations. In Groblersdal and Citrusdal, the ethylene inhibitor, AVG (Retain®) was applied at petal fall or after physiological fruit drop at 125 ppm. De-crease was applied at petal fall, or after physiological fruit drop at the recommended 50 ml/100 litres water.

In separate trials in the Sundays River Valley and Citrusdal, De-crease was applied at petal fall at both the recommended 50 ml/100 m litres water as well as 100 ml/100 litres water. De-crease was also applied two weeks after petal fall and after physiological fruit drop, both at 50 ml/100l water. Goëmar, to which Zn was added, was applied at 200 ml/100l water, alone or in combination with De-crease.

For all the abovementioned trials, yield, fruit size and percentage creasing will be evaluated at harvest time in 2006. Results will be presented in the next annual progress report.

5.2.4 Preharvest conditions influencing rind conditions: determining the role of mineral nutrients on rind breakdown of 'nules Clementine' mandarin

Experiment 758 by Paul Cronjé, Graham Barry (CRI at SU) and Marius Huysamer (SU)

Opsomming

Die effek wat variërende voedingselemente samestelling van vrugte op die na-oes kwaliteit is nie goed bekend nie. In teenstelling hiermee is daar heelwat inligting beskikbaar oor die vlakke wat betrokke elemente moet wees om die verlangende opbrengste te lewer. Hierdie studie van voedingselemente fokus op wanbalanse wat gedurende die laaste vruggroefase kan plaasvind en hoe dit die flavedo sal benadeel. Spesifiek is aandag gegee aan die fisiologiese defek skilafbraak (Rind breakdown) soos gesien tydens die na-oes opberging van 'nules Clementine' mandaryne. Na volledige ontledings gedoen was van al die elemente was daar gevind dat daar betekenisvolle verskille tussen binne en buite vrugte voorkom. Die verskille was ook gevind in 'n monster waarvan die skilafbraak insidensie bekend was. Die vlakke van Ca, K en Mg blyk of dit 'n groot rol speel, asook tot 'n mindere mate B. Daar sal gedurende die 2006 seisoen gepoog word om d.m.v. manipulasies aan die boom die vlakke te verander en sodoende die hipotese te toets dat lae Ca en hoë Mg en K vlakke bydrae tot die voorkoms van skilafbraak.

Introduction

Citrus fruit consist mainly of carbon compounds and water with mineral nutrients contributing a small percentage of total weight. Nevertheless, nutrients are essential to proper metabolic functioning of the tree and in ensuring consistent commercial quality fruit. Most nutrient research in citriculture has focused on mineral nutrients and the effect on tree growth and production thereof and not directly on rind condition and postharvest quality. The determination of nutritional values and resulting fertilisation programmes use the nutrient content of leaves and not the fruit which could confound the relationships between rind disorders and nutrient contents. General relationships are known and it appears that some citrus cultivars' response to nitrogen (N), phosphorus (P) and potassium (K) are similar for oranges and grapefruit fruit quality (Ritenour et al., 2002). The importance of N, P and K in plant physiological reactions is well known. Adequate N is essential for plant and fruit growth and it is the mineral nutrient most used by plants. P forms an important component in DNA, cell membranes and the photosynthesis and respiration processes, whereas K is vital in

regulating osmotic potential of cells and activation of enzymes in the photosynthesis process (Ritenour et al., 2002). The role of calcium (Ca) in citrus postharvest quality is, unlike in apple fruit, not well documented. Ca levels are thought to be vital in the apple disorder bitter pit in warmer growing areas. Even more importantly is the cellular regulatory role Ca plays during fruit ripening and deterioration: when Ca is low, these processes proceed more rapidly leading to the fruit being “older” and less resistant to whatever stresses are producing a specific postharvest disorder (Bramlage and Weis, 2004, Watkins et al., 2004).

Although much is known about the fertilisation of citrus trees and deficiencies in relation to production, there is not much information on the effect of postharvest disorders e.g. rind breakdown and peteca spot of lemons. Certain aspects are known, such as a high N level adversely affecting rind thickness and coarseness as well as decreasing rind colour (Embelton et al., 1978), but more specific interactions are not well understood. It is therefore necessary to quantify these mineral nutrient levels in specific disorders such as rind breakdown and to try to understand the mechanism involved from basic plant physiology knowledge.

Materials and methods (General)

Fruit were sampled monthly during 2005, starting in January until harvest in May. Sampling occurred according to fruit position within the canopy. Fruit developing in full sunlight were sampled as “outside fruit” whereas fruit developing in the canopy with no direct sunlight were sampled as “inside fruit”. On 17 January 2005 the first 30 samples of fruit per treatment were picked from the ‘hules Clementine’ mandarin orchard on Welgevallen Experimental Farm, University of Stellenbosch. Eight replicates of 30 fruit each were picked for both the treatments. Fruit size was measured and the flavedo removed, frozen in liquid nitrogen and stored at -80°C . This sampling was continued every month (about every 4 weeks) until harvest.

On the determined commercial harvest date (16 May) fruit were harvested, according to their position during their development, e.g. inside or outside of the canopy. The inside fruit were somewhat smaller and less well coloured compared with the outside fruit. The fruit were picked into wooden bins and put in a 72 hour degreening treatment whereafter they were separately packed according to either the inside or outside classification. These fruit were divided into two and were stored at either 7.5°C or -0.6°C for the duration of the experiment.

The fruit were stored at these temperatures for 5 weeks before the first evaluation. On each evaluation date eight cartons with 25 fruit each were removed from storage and evaluated for occurrence of rind breakdown (RB) and chilling injury (CI). The eight carton replicates were allocated to storage duration before being put in cold rooms. After the evaluation date the flavedo of the fruit was removed, frozen in liquid nitrogen, freeze-dried and ground and stored at -80°C until analysed for sugars and pigments. A full mineral analysis of fruit sampled from January until harvest, as well as one sample that was stored and had a known RB incidence, was done at Bemlab (Somerset West).

Results

Significant differences were found between the two treatments after analysis of macro and micronutrients in the flavedo (Fig. 5.2.4.1a-k).

Nitrogen (N) and phosphorus (P) both differed significantly between treatments during specific months of stage III of citrus fruit development (Fig. 5.2.4.1a; b). The nitrogen content of inside fruit was significantly lower in January but a reversal occurred and in the last months before harvest the inside fruit had significantly more N than outside fruit. P levels showed the same pattern with inside fruit having higher content in March and April; this trend was seen during the season but not at significant levels.

Potassium (K), calcium (Ca) and magnesium (Mg) content on the other hand differed significantly between the inside and outside fruit from January until harvest in May as well as in the samples that were stored for rind breakdown (RB) incidence (Inside 17% RB and Outside 9% RB). K content of inside fruit had significantly higher levels from January until May. Conversely Ca and Mg content of outside fruit were significantly higher (Fig. 5.2.4.1 c; d; e).

Results from microelement analysis (Fig. 5.2.4.1f-k) showed occasional differences between treatments. Boron (B) levels differed significantly in March, April and May, with outside fruit having higher levels in March and May but switching in April (Fig. 5.2.4.1k). Of the other micronutrients where outside fruit had higher levels, sodium (Na) differed significantly in March, manganese (Mn) in January, March and in the postharvest sample. Zinc (Zn) content of the inside fruit was higher during fruit development but only significantly so in April.

Discussion

The negative influence of an excessive N content during colour break is known (Ritenour et al., 2002; Embelton et al., 1980). Therefore, the significantly higher N during April and May in the inside fruit could be seen as an additional factor why inside fruit developed inadequate colour (the direct exposure to sunlight is thought to be the main reason). Davies and Albrigo (1994) reported that increasing leaf P levels slightly decreased fruit size, TSS, TA, ascorbic acid, peel thickness and coarseness. The data reported do not correspond with this, as the higher P levels (Fig. 5.2.3.1b) were in the inside fruit and even though differences were not always significant, inside fruit are known to have thinner rind than outside fruit. K is known to play a vital role in fruit development and an increase in K can lead to a thicker and coarser rind while slightly decreasing the juice content and reducing the amount of creasing (Davies and Albrigo, 1994; Monselise et al., 1976). It is evident from the data that K could play a role in the sensitivity of the fruit to rind breakdown as a constant and significant difference was found from January until May (Fig. 5.2.3.1c). This significantly higher level in inside fruit was also seen in the postharvest sample. The Ca levels were directly opposite to the K, and were significantly higher in the outside fruit. It is thought that there are interactions in apple fruit between Ca and K and (K + Mg):Ca levels, which could mean that if either K or Mg levels are high the impact of low Ca will be increased (Bramlage and Weis, 2004). In apple fruit P and B also interact with Ca. P might help Ca maintain quality even at low levels whereas B is involved in movement of Ca to fruit, meaning that if B is depleted Ca movement will be low and deficiency could occur.

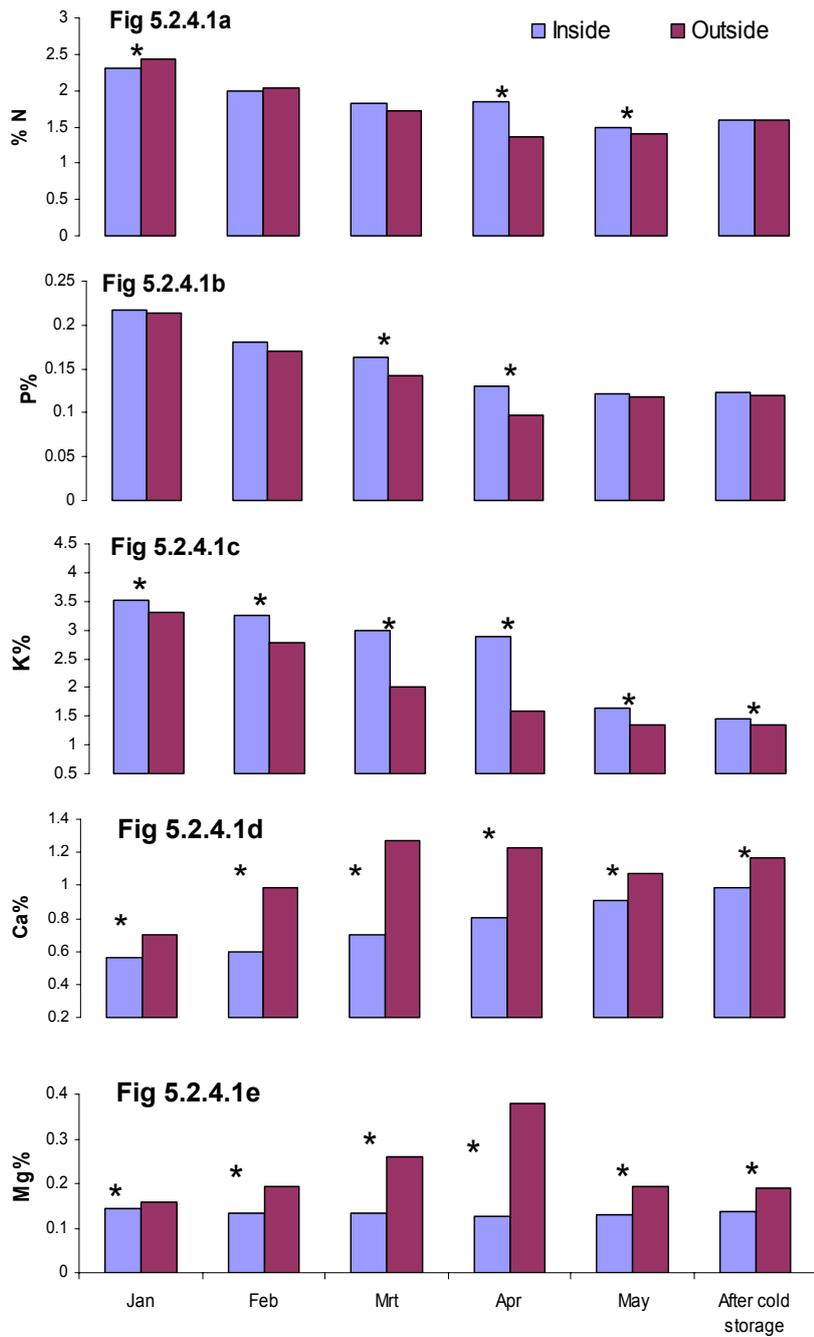
It could be hypothesised that even when nutrients are present within the fruit tree at adequate levels, certain nutrients could be a limiting factor in the citrus fruit rind and possibly cause the development of a disorder. From this hypothesis the question would arise as to why the distribution would be uneven within a tree and especially between inside and outside fruit. The model proposed by Munch in 1927 could be seen as a possible explanation. He stated that cells could be interconnected hydraulically via plasmodesmata, with some cells in the plant body engaging in solute accumulation, acting as sources whereas others are engaging in solute utilisation, acting as sinks (Fisher, 2000). This model would fit in with the assumption that a fruit positioned amongst leaves, actively engaged in the photosynthesis and transpiration processes, would be better positioned as a sink than the inside fruit. The bulk flow of water and nutrients such as Ca and Mg would thus be channelled to these outside fruit (strong sinks) during development. High levels of K in the inside fruit, unfortunately could not be explained by this argument. K is known to be readily transported to the young plant organs and plays a vital role in stomatal control and regulation of changes in distribution of other nutrients as well as being active in more than 50 plant enzymes (Kochian, 2000).

Future research

As discussed here the specific mechanism involved and the effect of macro and micronutrients on rind breakdown still remains unclear. Follow-up experiments will use the information gained to plan treatments in order to manipulate levels of mineral nutrients and study their effect on rind disorders.

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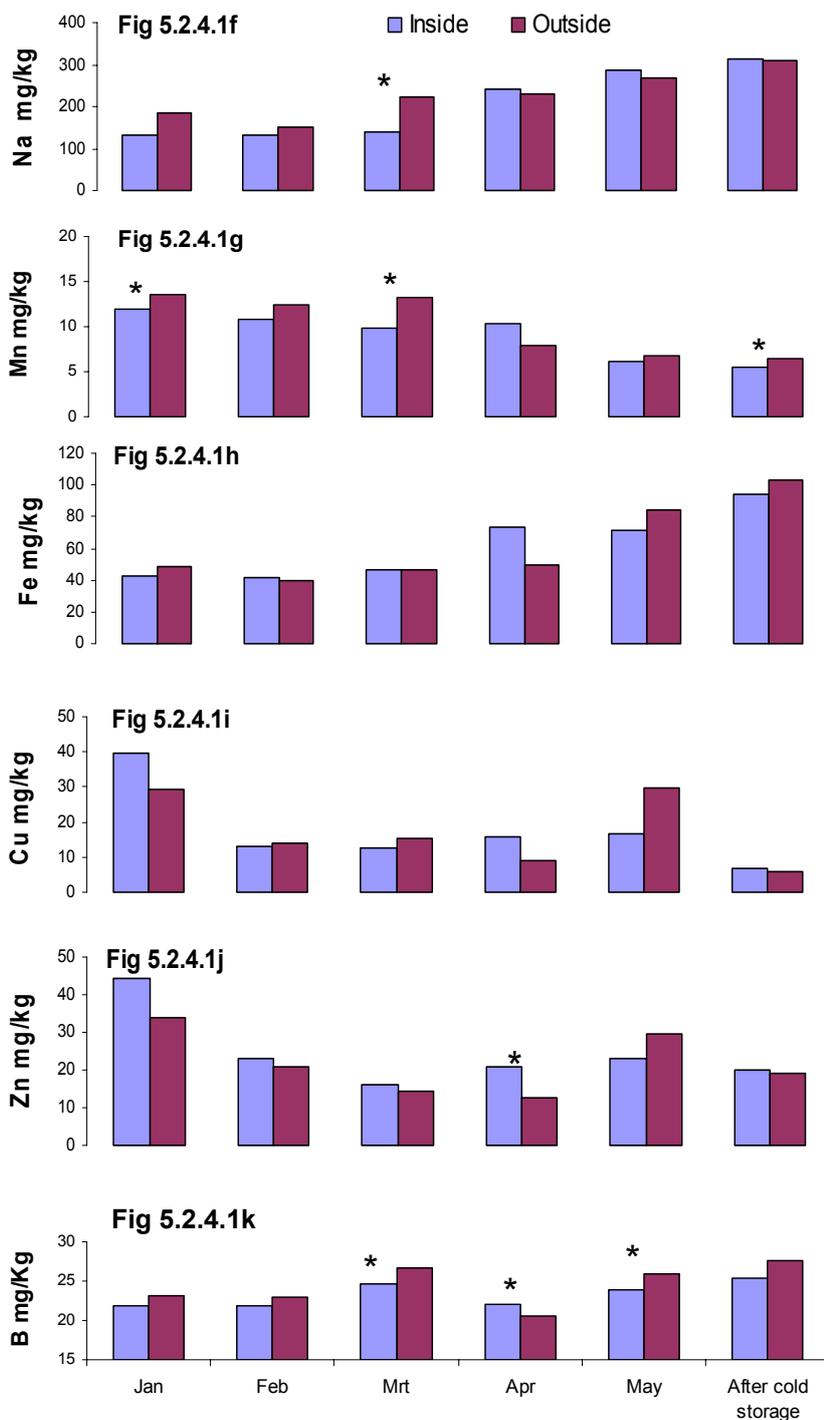


Figure 5.2.4.1a-k. Nutrient content of flavedo tissue sampled from 'hules Clementine' mandarins from January until May according to position in the canopy (Inside vs. Outside). The last data points (after cold storage) show the mineral nutrient levels of fruit with a known incidence of rind breakdown (inside fruit 17% and outside fruit 9%) after the samples were cold stored at 7.5°C. Asterisks on the specific months denote significant differences between the treatments at a 5% level.

5.2.5 Chilling injury of Valencia type oranges

Experiment 766 by Paul Cronje and Graham Barry (CRI at SU) and Marius Huysamer (DFPT at SU)

Opsomming

Na voorvalle aangemeld is waar Valencia lemoene vermoedelik koueskade opgedoen het tydens geforseerde verkoeling, is daar in 2004 en 2005 proewe gedoen om dit te ondersoek. Vir die proef is Valencias (verpak in teleskopiese kartonne) gedurende September 2005 opgeberg in 'n koelkamer gestel op 1°C. Die vrugte was in twee palette verdeel. Een pallet is onderwerp aan geforseerde verkoeling en die ander aan statiese verkoeling. Na 3 dae is die geforseerde verkoeling gestaak en die vrugte opgeberg by 1°C vir 3 weke. Daarna is die vrugte geëvalueer vir koueskade, en "stem end rind breakdown" SERB. Daar het heelwat meer koueskade voorgekom in 2005 as in 2004. Die verlengde opberging teen 1°C is vermoedelik hiervoor verantwoordelik. Daar het geen statistiese verskille tussen geforseerde en statiese verkoelde vrugte voorgekom nie.

Introduction

Most plant products contain substantially more heat at harvest than normally acceptable for subsequent handling and storage. The heat contained at harvest (field heat) largely comprises thermal energy from the environment surrounding the product. To maintain maximum storage potential of the fruit it is desirable to remove field heat as quickly as possible after harvest: the longer it is postponed the shorter the shelf life of the fruit. Postharvest cooling happens in two stages; firstly bringing down the fruit temperature to, or approaching, the desired storage temperature, and secondly maintaining the desired temperature through continued removal of respiratory heat and heat moving into the storage environment. The two techniques to reduce field heat used in the South African citrus industry are room pre-cooling and forced-air cooling. Room pre-cooling is where products are placed in a room with refrigerated air being blown horizontally through the room. The main disadvantage is the long time fruit have to stay in the rooms due to the slow rate of cooling and therefore an increase in water loss. Forced air cooling takes place in a cold room where packed fruit are cooled by creating a negative pressure across opposite faces of stacks of pallets or bins holding fruit. The cold air is forced to move around individual fruit rather than merely around the exterior of the container, as in room pre-cooling or static cooling. The danger of forced air cooling is a too sudden temperature loss that could manifest as chilling related injuries (CI), as well as increased water loss due to the increase in air velocity over the fruit. The dominant means of heat transfer is via forced convection due to the movement of air around the fruit. Because of this, air movement into the cartons is essential for rapid cooling. This is achieved using containers with ventilation holes or slots in the sides. Removal of approximately 5% of the surface area of the container side decreases the cooling time by approximately 25% (Kays and Paull, 2004).

This experiment was a follow-up on previous research comparing forced and static cooling (Cronje, Barry and Huysamer, 2004). The aim of this experiment was to study the occurrence of physiological disorders such as scalding and pitting as a result of two cooling treatments after the fruit were stored at 1°C for three weeks.

Materials and methods

Late Valencia oranges were packed at Goedehoop Sitrus packhouse on 20 September 2005 in telescopic boxes (size 88). The fruit were transported to the cold rooms of the Department of Horticultural Science, University of Stellenbosch where the boxes were divided up and packed on two pallets. Thermocouples were inserted at 8 points in each stack (static and forced cooling treatments) and distributed in all dimensions of the stack. At each point the air and pulp temperatures were measured. A forced air cooler was fixed onto one stack and set at 20 mm static pressure difference. The cold room was set at 1°C and the fruit were forced-air cooled for 3 days. Thereafter the fruit were kept in the cold room at 1°C for 3 weeks before being evaluated for disorders. Chilling injury was evaluated in two classes: *viz.* scalding and pitting. The fruit was kept at shelf life temperatures and evaluated after 1 week.

Results

The two treatments did not result in significant differences in the three evaluation variables (Fig. 5.2.4.1). However, the forced air cooling treatments did result in higher levels of physiological damage. Although no significant increase was observed after forced air cooling, in each disorder category the incidence was higher than with the static cooling treatment.

The difference in rate of cooling is very evident in Figure 5.2.5.2, and illustrates the advantage of using forced air cooling. The static cooling only reaching set point after two days, which would be a problem if large volumes of fruit had to be handled within a limited timeframe.

Conclusion

The rapid cooling of sensitive citrus fruit remains a problem, resulting in losses every year. However, the data from this experiment shows that it is not only during the initial stages of forced air cooling that CI could occur (Cronje, Barry and Huysamer, 2004), but also during prolonged storage at low temperatures.

Forced air cooling is irreplaceable in the handling of citrus fruit, especially for markets such as the USA and China that demand this treatment in their disinfestation protocol. The rate of cooling under static conditions is not sufficient to ensure satisfactory temperature in the time available before loading and shipping of the fruit.

Future research

It is advisable that cultivars and orchards be identified with a history of chilling sensitivity. Such fruit could then be excluded from cold disinfestation markets or handled more carefully. However, it is not only at producer level that fruit sensitivity should be taken into account during planning. It is critical that service providers in the postharvest chain should be aware of cultivar specifications and ensure that facilities operate within these boundaries. This will require research into chilling sensitivity prediction.

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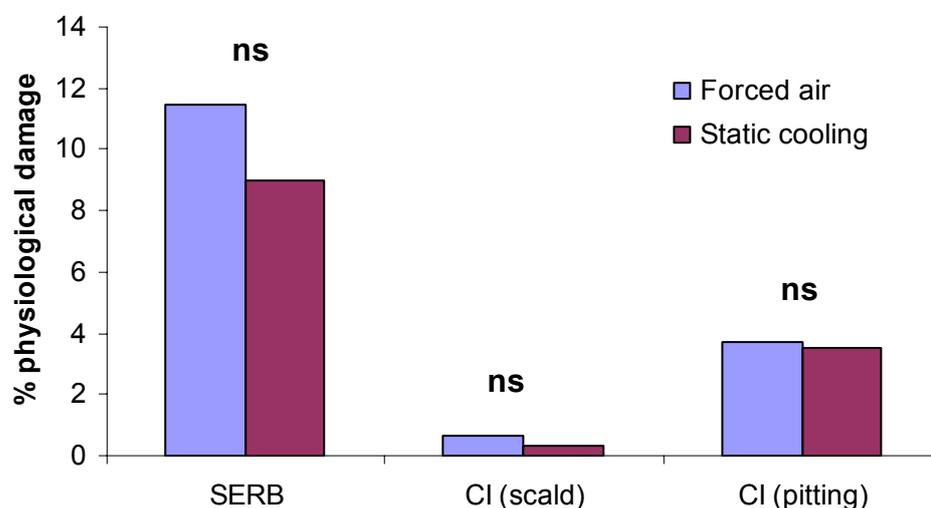


Figure 5.2.5.1: Effect of forced air and static cooling on the physiological disorders stem end rind breakdown (SERB), chilling injury (CI) recorded as scalding and pitting on late Valencia oranges from Citrusdal. Fruit was stored in a cold room set at 1°C. No significant differences between treatments were found (ns) (p=0.05).

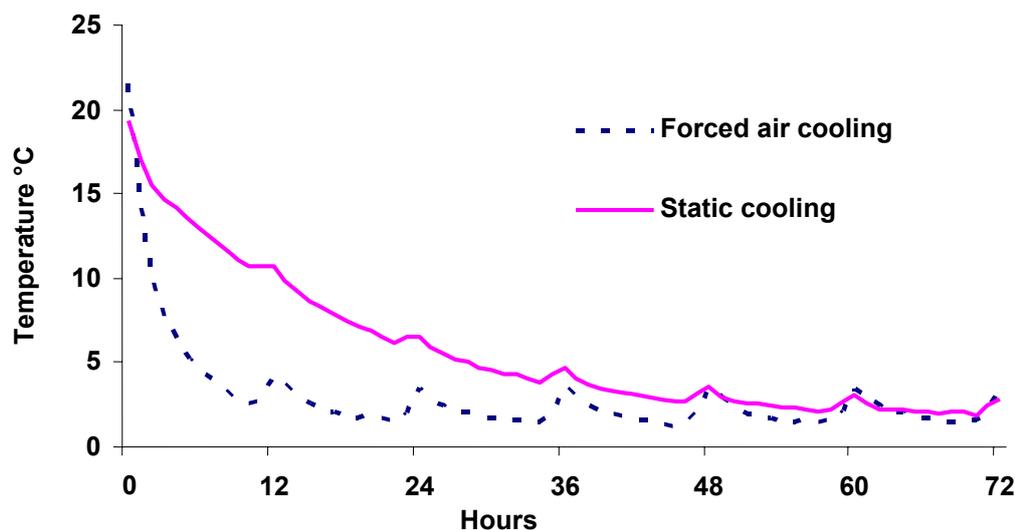


Figure 5.2.5.2: Difference in cooling rate between forced air cooling and static cooling of Valencia oranges packed in telescopic boxes. The cold room was set at 1°C.

5.2.6 Intermittent warming of citrus during storage

Experiment 832 by Paul Cronjé (CRI at SU), Mariana Jooste (DFPT at SU) and Marius Huysamer (DFPT at SU)

Opsomming

Die verlies wat jaarliks a.g.v. koueskade voorkom gedurende die uitvoer van sitrus vrugte maak die studie van maatreëls om dit te bekamp noodsaaklik. Die nuwe markte waarheen SA sitrus na uitvoer kon word, soos China en VSA, verlang die toepassing van koue disinfestasië protokol om insek spesies soos valskoddingmot en vrugtevlieg te beheer. Die protokol verlang dat vrugte by tussen -1 en 0°C vir 'n langdurende periode verskeep word. Die behandeling dra jaarliks by tot groot verliese in bykans alle kultivars. In die eksperiment is twee behandelings getoets: die lae temperatuur is afgewissel na 3 dae by -1°C en in die eerste behandeling deur 2 dae by 15°C en in die tweede behandeling vir 6 dae by 7.5°C. Die vrugte is vir 'n totaal van 44 dae opgeberg en daarna geëvalueer vir koue skade en ander fisiologies defekte. Die resultate het getoon waarom koueskade so ingewikkelde meganisme is om te manipuleer. Daar is slegs in die geval van Satsuma mandaryne en Valencia lemoene betekenisvolle afname in die voorkoms van koueskade gevind. Die behandelings sal gedurende die 2006 seisoen herhaal word en variasies sal ook getoets word.

Introduction

Plant species such as citrus evolved in the tropics or subtropics and can suffer chilling damage if exposed to temperatures in the 0 to 10°C range. Chilling injury to horticultural produce is especially important during the postharvest handling when fruit will be subjected to low temperatures to increase the storage life of the product.

The degree of chilling injury (CI) depends on temperature, exposure time at a chilling temperature and the chilling sensitivity of the fruit. The CI symptoms normally develop very slowly when under chilling conditions, but upon removal to non-chilling conditions the full effect of the damage becomes apparent. A further aspect that complicates the chilling stress is the variation in sensitivity between not only species but cultivars and physiological conditions (as a result of varying maturity, production areas etc.) (Kays and Paull, 2004). Not only does the potential for CI vary among citrus cultivars but the symptomology also differs. Some of the symptoms may manifest internally, while most of them are externally visible and vary in appearance. Commonly CI is seen as an internal discoloration and browning, the formation of surface lesions (pitting or scalding), water-soaking, abnormal ripening and increased decay (Lafuente *et al.*, 2005).

The mechanism controlling chilling injury of horticultural produce is still eluding scientists even though a considerable effort has been made to unravel the mystery. The proposed model of Lyons (1973), where changes in membrane permeability, associated with a membrane-lipid physical phase transition from a flexible liquid-crystalline to a solid-gel structure is the primary event associated with CI, still has merit. These changes would cascade into secondary events such as loss in regulatory control and metabolism imbalance,

cell autolysis and eventual cell death followed by a visible symptom such as pitting or scalding in the case of citrus fruit

Various strategies have been used and are being investigated to decrease the impact of CI on the postharvest life of citrus fruit. These manipulations such as hot water dips, curing, wilting, intermittent warming, postharvest chemical dip treatments (e.g. jasmonic acid, CaCl and fungicides), are all underpinned by a probable manipulation of the biochemical reactions in the rind ensuring more CI resistant cells (structures and organelles).

The international research focusing on CI is being done in Spain, Israel and the USA and aims to find specific physiological changes that can be manipulated. The biochemical effects being investigated focus on membrane systems and their ability to rearrange lipids in order to respond to chilling and be able to keep the membranes fluid. The involvement of oxidative stress has been proposed as one of the causes of cold-temperature damage (Sala and Lafuente, 1999; Sanchez-Ballesta et al., 2003). Purvis (1985) proposed that the alternative respiratory pathway plays a role in within-tree variation after finding a greater potential for the alternative pathway and a greater tolerance to chilling in grapefruit harvested from either the interior or exterior of the canopy. It is thought that methyl jasmonate and methyl salicylate could increase CI tolerance by increasing alternative oxidase transcription (Fung et al, 2004). The involvement of anti-oxidant species has been shown by an increase during conditioning of 'Fortune' mandarins for 3 days at 37°C which resulted in a decrease in CI (Sala and Lafuente, 1999). Considerable attention has been given to the role of polyamines due to their anti-senescent activity and radical scavenging properties but Gonzalez-Aguila et al., (2000) did not find amines a limiting factor in the chilling tolerance of citrus fruit. The research done on PAL (phenylalanine ammonia-lyase) suggests that it may serve as a biochemical marker for chilling in citrus fruit, but the pattern of change in PAL differs among cultivars and type of chilling symptom (Sala et al, 2004).

Ethylene and ABA (abscisic acid) are two plant growth regulators involved in the mechanism of CI development. Ethylene biosynthesis is known to be stimulated by chilling temperatures and it is thought to be a signal for acclimatization. However, applying ethylene at non-chilling temperatures followed by storage at chilling temperatures, did not reduce the incidence of CI (Lafuente et al., 2001b). ABA-deficient mutant 'Pinalate' which is therefore prone to dehydration is tolerant to chilling, while it's parental 'navelate' is susceptible to this disorder (Alferez et al., 1996). The levels of osmolytes glucose and fructose in grapefruit before harvest could be a defense mechanism against CI (Purvis *et al.*, 1979) but Holland et al. (2002) reported that conditioning fruit for 3 days at 37°C resulted in lowering CI and considerably reduced the glucose and fructose levels without a decrease in sucrose.

The long distances to the USA and Japan, and specifically the mandatory cold disinfestation protocols that southern African citrus fruit have to be shipped at, result in severe losses every year due to CI. Various strategies like wilting to desensitize very CI prone cultivars such as grapefruit and lemons have succeeded in limiting losses to the Japanese market. However new strategies need to be tested and old strategies revisited in order to develop protocols that would contribute to increasing the profitability of new markets such as China that would also need insect disinfestation protocols. This report outlines results on intermittent warming treatments applied to mandarins, lemons, and oranges during simulated export conditions.

Materials and methods

During the 2005 citrus season fruit were sourced from different producers and packhouses in the Stellenbosch area for the experiments. The chosen cultivars included 'Satsuma' mandarins harvested on 10 April and 'hules Clementine' mandarins on 16 May at the Welgevallen experimental farm of the University of Stellenbosch. 'Eureka' lemons were from the Simondium area and packed by the Imibala packhouse on 6 June. Navel oranges were from the SunCape packhouse, packed during 20 June. The Valencia orange were packed by Goedehoop citrus in Citrusdal on 20 September.

All of the cultivars underwent the same three treatments: Control, (49 days at -1°C and 1 week shelf life period at 15 to 20°C), intermittent warming 1 (IW1) (3 days at -1°C plus 2 days at 15°C followed by 44 days at -1°C and shelf life period) and intermittent warming 2 (IW2) (-1°C for 3 days, plus 6 days at 7.5°C and 40 days at -1°C and shelf life period). Each treatment was repeated 8 times and each replication consisted of 20 fruit. The incidence of CI was noted after the cold storage regime and once again after the shelf life period. The weight of the fruit was measured before cold storage, after cold storage and after the shelf life period.

Results

Satsuma mandarins (Fig. 5.2.6.1ab)

No marked differences were found between weight losses of the treatments. The incidence of CI in both the IW treatment differed significantly from the control (65% in the control vs. 25% in the intermittent warming). This result did not correspond with the weight data. It is thought that increase in weight loss is correlated with increase in CI (Skog, 1998). An interesting observation was the slight decrease in the CI incidence in all three treatments after 1 week shelf life. Normally an increase in CI symptom will be seen and not a reduction. No explanation can be offered for this observation.

'hules Clementine' mandarins (Fig. 5.2.6.2abc)

No significant differences were found between treatments in weight loss, CI or rind breakdown incidence and no decay developed. CI incidence was low in all three treatments.

'Eureka' lemons (Fig. 5.2.6.3abc)

No significant differences between treatments were found for weight loss and CI. The low CI incidence, 1-2%, could be explained by the fact that the fruit were harvested during June and it is known that if fruit are subjected to low temperatures on the tree they are more chilling resistant postharvest. No decay developed during cold storage and it stayed low and insignificant between treatments. The slightly higher incidence (non-significant) in the IW fruit could be an indication that temperature spikes during the postharvest handling before shipment could increase CI incidence in this cultivar.

Navel oranges (Fig. 5.2.6.4abcd)

Even though high levels of CI were found in all the treatments, no significant differences were seen. Low stem end rind breakdown (SERB) incidence was seen after cold storage (but no significant treatment differences), increasing to 1,5 to 3% during shelf life, but were still insignificant between treatments. Both the IW treatments resulted in low decay which differs significantly with the control.

Valencia oranges (Fig 5.2.6.5abcd)

There was a significant difference in weight loss between the IW1 and control after cold storage but this difference was negated after the one week shelf life. Significant CI differences occurred between IW1 and the other two treatments. However the difference was not significant after one week shelf life, even though a numerical difference of 4% remains. No significant difference in pitting of the fruit was seen after both cold storage and shelf life. The development of decay in the control was dramatic and is expressed in the nearly 10% decay vs. the 1-2% of the intermittent warming treatments.

Discussion

The known difficulties in finding a solution for a problem such as CI are illustrated by the varying results found in the experiments. The only cultivar where intermittent warming treatment did result in significantly reducing CI with Satsuma mandarin. The other cultivars showed unexpected results such as the increase in CI in the intermittent warming of lemons (although not significant) and Clementine and Navels having no treatment effect. Surprisingly Valencia oranges had a significant decrease in CI of the 15°C for two days intermittent warming but had the highest weight loss. Chilling damage and water loss are traditionally thought to go hand in hand but in this case it could be deducted that due to the increased water loss a curing effect was responsible for the decrease in CI. On both the Navels and Clementine's there were no significant treatment effects on the weight loss, CI or the SERB or RB. Interestingly the % RB and SERB showed the same trend (but not significant) in having an increased physiological disorder developing at the two intermittent warming regimes. This could be seen as support for the argument that these disorders are related to senescence and acceleration of this process at higher temperature. The lack of peteca spot development in the lemons could illustrate that CI and peteca spot are not related physiological phenomena but under certain conditions occurrence of both could develop independently resulting in a masking effect. This observation is supported by the fact that peteca spot is most often found before fruit are subjected to any chilling conditions.

Future research

The two treatments used in this experiment will not be able to decrease chilling injury on their own and follow-up research will be done during 2006. The current protocols of curing/wilting of grapefruit and lemons prior to export to the Japanese market still remain the most consistent way to decrease chilling injury.

It is advisable to identify different levels of susceptibility to chilling injury on a farm level. The results of Purvis (1985), illustrating variation in CI within the canopy, support the argument of identifying cultivar and microclimate interaction as well as a cultivar that is harvested over a prolonged period.

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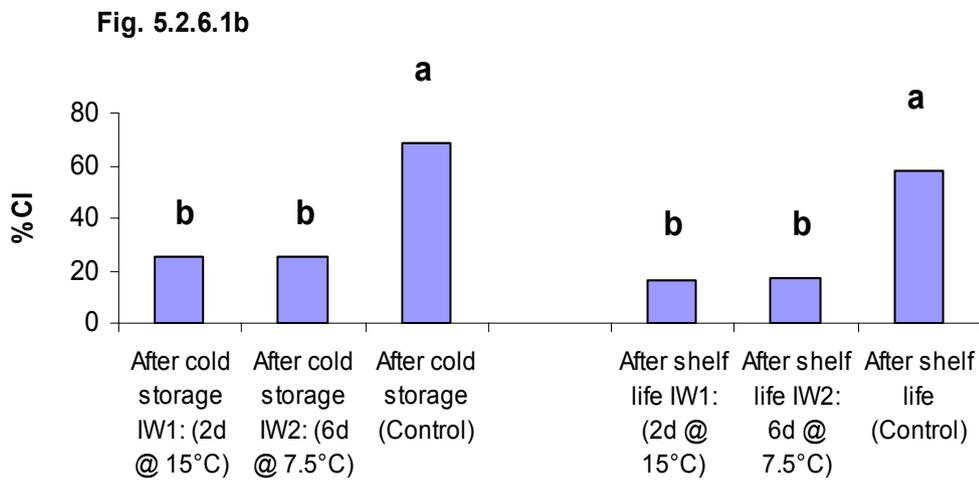
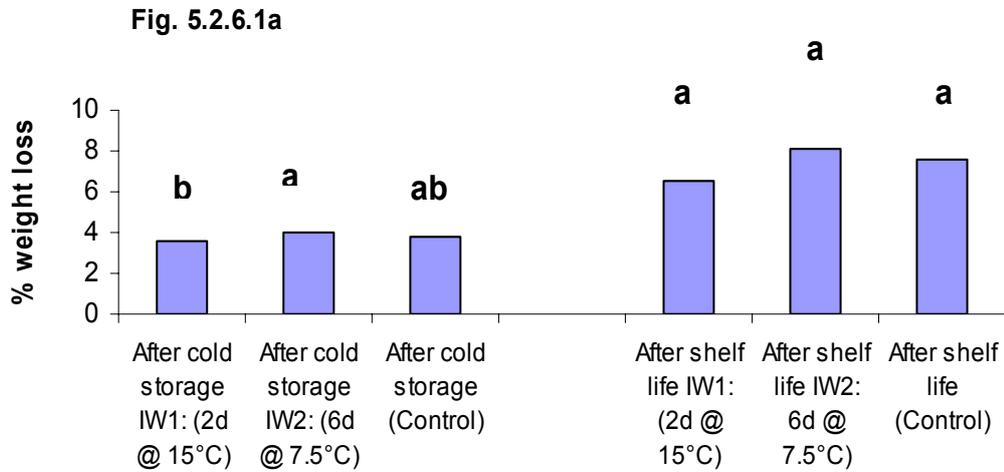


Figure 5.2.6.1a,b: Effect of two intermittent warming treatments and a control treatment on weight loss (5.2.6.1a) and chilling injury (5.2.6.1b) of 'Satsuma' mandarin. Different lower case letters on bars within evaluation periods denote significant differences between treatments ($p=0.05$).

Fig. 5.2.6.2a

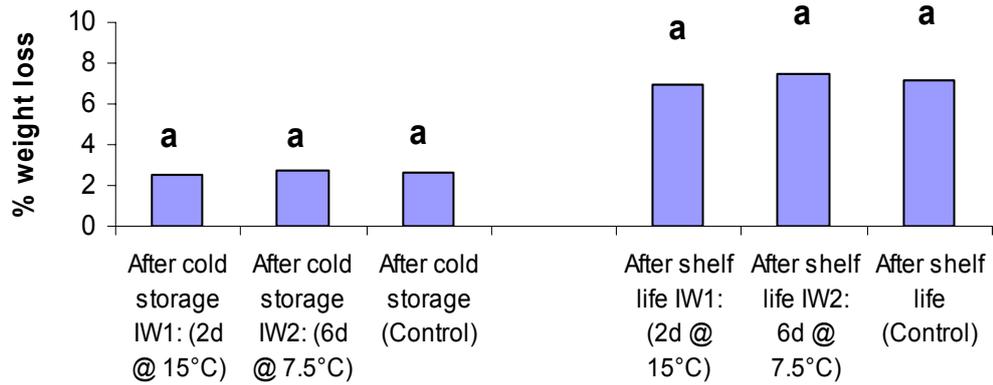


Fig. 5.2.6.2b

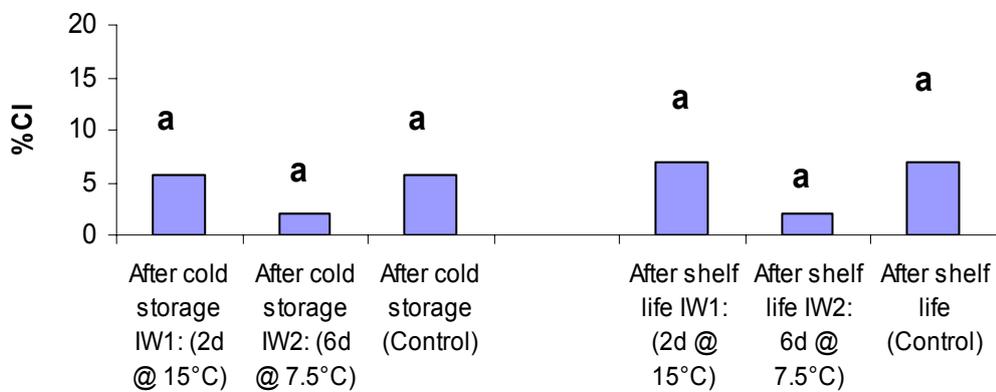


Fig. 5.2.6.2c

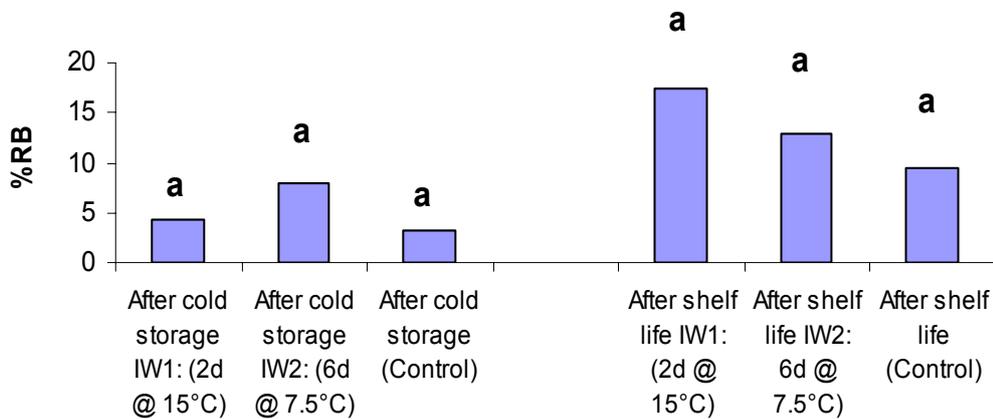


Figure 5.2.6.2 a, b, c: Effect of two intermittent warming treatments and a control treatment on weight loss (5.2.6.2 a), chilling injury (5.2.6.2 b) and rind breakdown (RB) (5.2.6.2 c) of 'hules Clementine' mandarins. Different lower case letters on bars within evaluation periods denote significant differences between treatments ($p=0.05$).

Fig. 5.2.6.3a

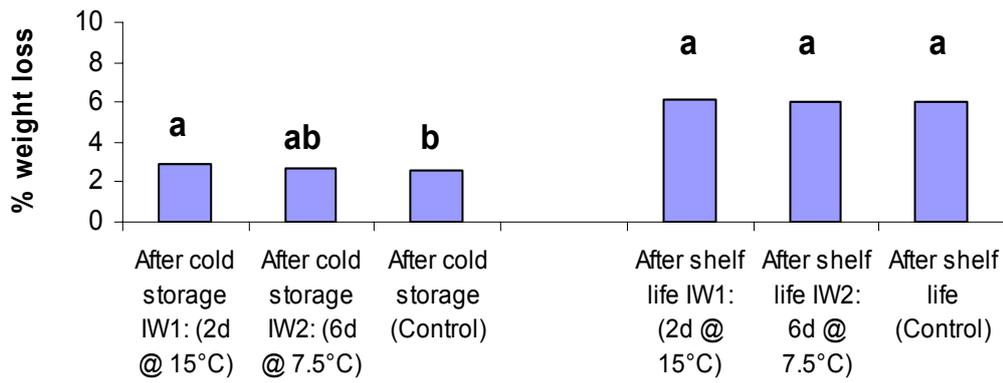


Fig. 5.2.6.3b

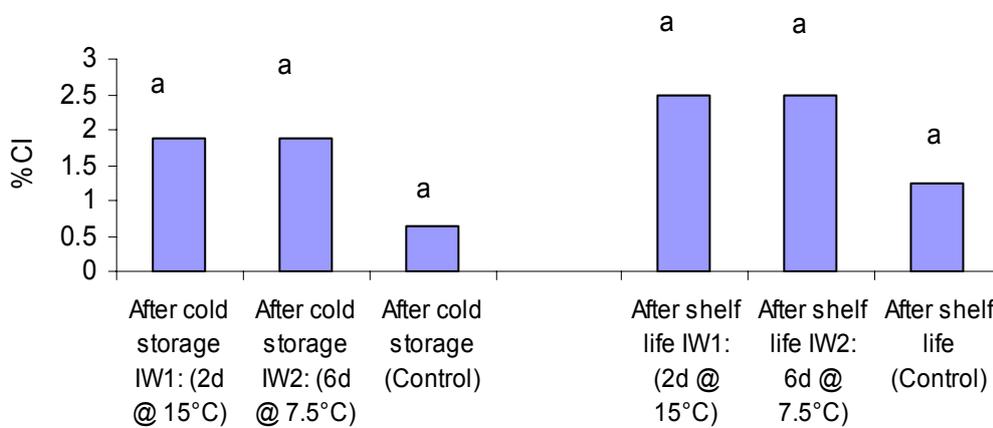


Fig. 5.2.6.3c

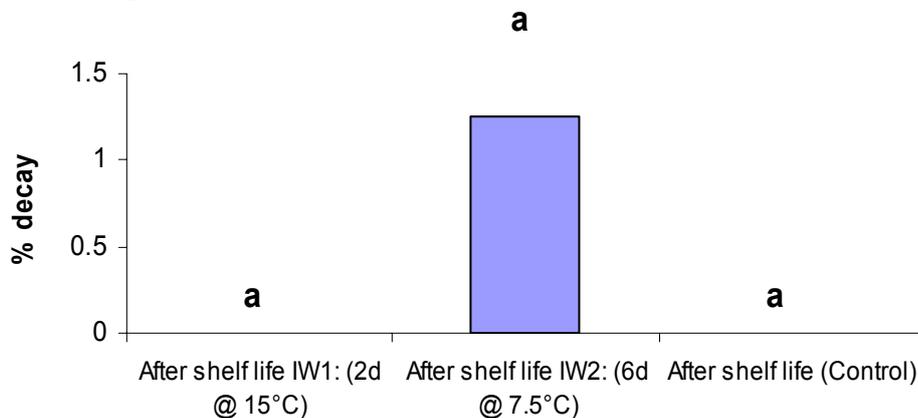


Figure 5.2.6.3 a, b, c: Effect of two intermittent warming treatments and a control treatment on weight loss (5.2.6.3 a), chilling injury (5.2.6.3 b) and decay (5.2.6.3 c) of 'Eureka' lemons. Different lower case letters on bars within evaluation periods denote significant differences between treatments ($p=0.05$).

Fig. 5.2.6.4a

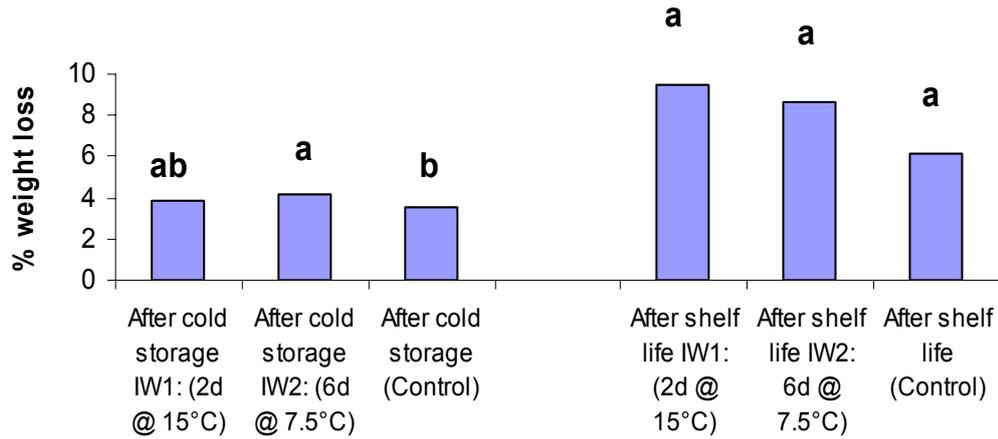


Fig. 5.2.6.4b

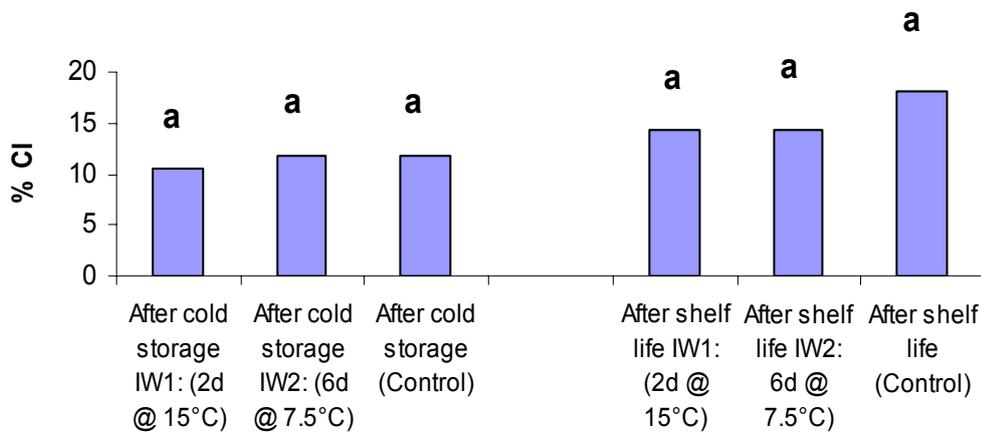


Fig. 5.2.6.4c

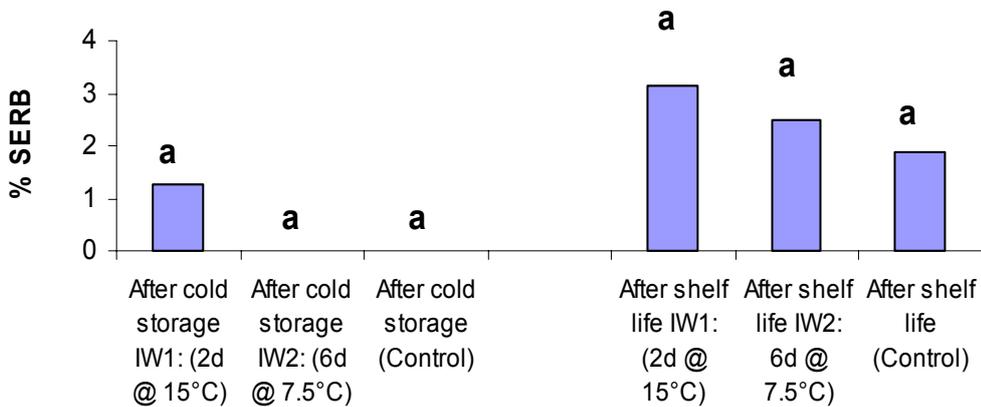


Figure 5.2.6.4a, b, c: Effect of two intermittent warming treatments and a control treatment on weight loss (5.2.6.4 a), chilling injury (5.2.6.4 b), stem end rind breakdown (SERB)(5.2.6.4 c) of Navel oranges. Different lower case letters on bars within evaluation periods denote significant differences between treatments (p=0.05).

Fig. 5.2.6.5a

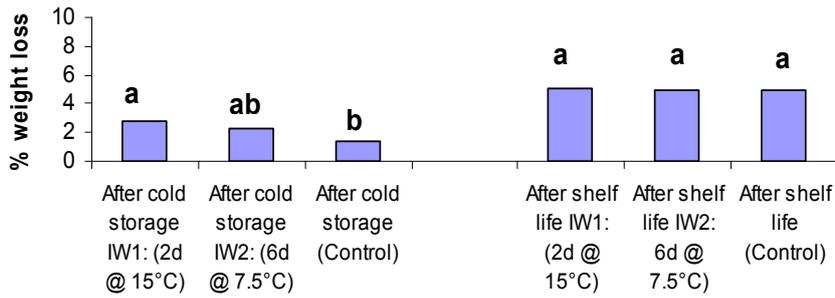


Fig. 5.2.6.5b

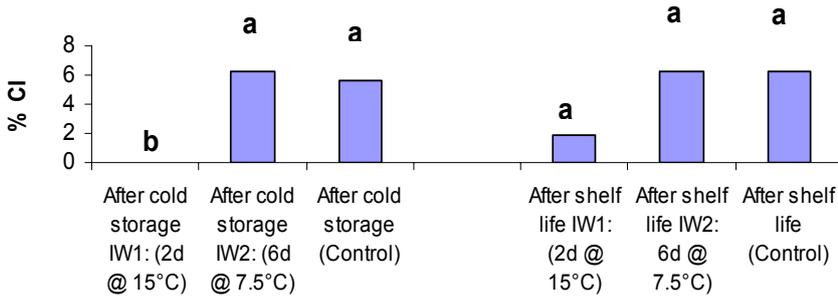


Fig. 5.2.6.5c

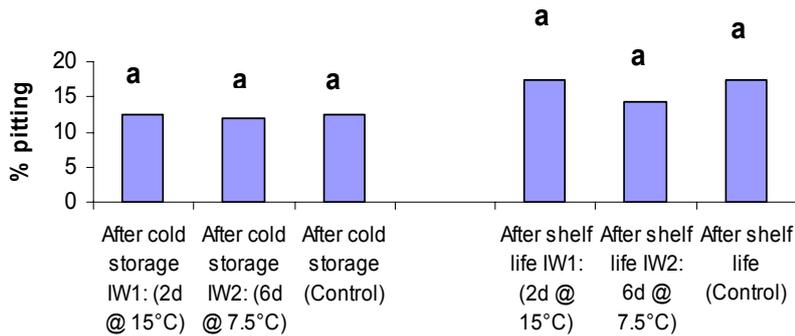


Fig. 5.2.6.5d

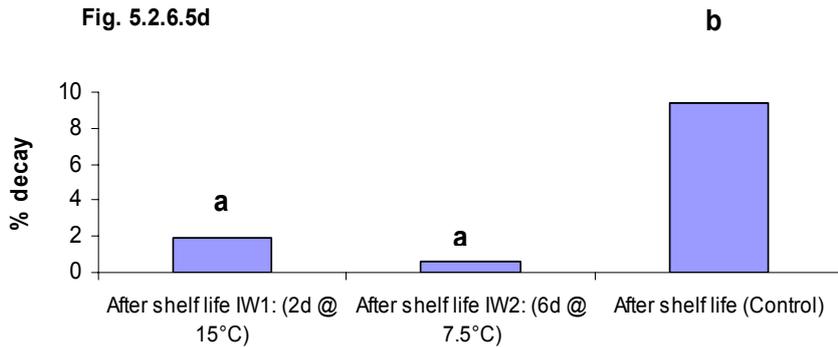


Figure 5.2.6.5 a, b, c, d: Effect of two intermittent warming treatments and a control treatment on weight loss (5.2.6.5 a), chilling injury (5.2.6.5 b) and pitting (5.2.6.5 c) and decay (5.2.6.5 d) of Valencia oranges. Different lower case letters on bars within evaluation periods denote significant differences between treatments ($p=0.05$).

5.2.7 Effect of CO₂ on rind condition of Clementine mandarin

Experiment 780 by Paul Cronjé and Graham Barry (CRI at SU) and Marius Huysamer (DFPT at SU)

Opsomming

Gedurende 2004 is 'n proef gedoen waar verhoogde CO₂ vlakke (0, 0.03, 0.3, 0.5 en 1%) toegedien is tydens opberging van 'nules Clementine' mandaryne (-0.6°C vir 32 dae). Die vrugte is ontleed vir kleurverlies en powwerigheid. Daar was egter geen betekenisvolle verskille nie. Die proef is in 2005 herhaal teen hoër CO₂ vlakke (1, 2.5 en 5%). Daar was weereens geen betekenisvolle verhoging in die powwerigheid nie maar egter ietwat van 'n kleurverlies. Die data wys dat verhoogde CO₂ konsentrasie as enkel faktor nie verantwoordelik is vir powwerigheid nie. Daar sal gedurende 2006 seisoen na etileen vlakke gekyk word as moontlike oorsaak van die fisiologiese defek.

Introduction

The loss in fruit quality during shipment of citrus fruit under a cold disinfestation protocol necessitates an investigation of the variables that could play a role. Two main problems experienced after the approximately 22 days shipment at -0.6°C are a loss in colour and the development of puffiness. Puffiness is seen as a bulging of the rind as it pulls away from the pulp.

The first factor studied was high CO₂ levels and the results were reported on by Cronje, Barry and Huysamer (2004). The effect of elevated CO₂ levels (1, 0.5 and 0.3% CO₂) on rind condition of 'nules Clementine' mandarins was not negative in either increasing the puffiness or loss of colour. However, due to the importance of CO₂, as it influences fruit respiration and quality (Kay & Paull, 2004), it was necessary to do a follow-up of the 2004 experiments. Much higher levels of CO₂ were used during the 2005 season.

Material and methods

The fruit used were 'nules Clementine' mandarins harvested on 16 May 2005 from the University of Stellenbosch experimental farm Welgevallen and were degreened and packed according to normal commercial practices.

Six replicates consisting of 20 fruit each were used per treatment. Prior to treatment, fruit were weighed and placed in a bucket with a connection to a flow board and an outlet from the cold room. Premixed gas from Afrox was used and the CO₂ treatments were as follows: 0.03% (normal air) and 1, 2.5 and 5% CO₂. The gas bottles were connected to flow boards from which tubes fed into the buckets in the cold room. In all treatments air made up the balance of the gas mixture (i.e. 21% O₂ plus nitrogen). The buckets were kept in a cold room at -1°C for 32 days (to simulate the maximum commercial period at these conditions). The flow rate of the treatment gases was high enough to prevent a build up of ethylene and CO₂ inside the bucket.

After the storage period the fruit were kept at ambient temperature (15 to 20°C) for one week, with the bucket open to prevent CO₂ and ethylene build-up. The fruit colour was evaluated with a chromameter (Minolta NR 4000, Osaka, Japan) before the treatments started, after cold storage and after one week shelf life. The symptoms of rind disorders were scored and the fruit were cut open to evaluate the degree of puffiness as well as the internal colour change of the pulp. The degree of puffiness was measured with a calliper according to the distance between the pulp and the peel and the separation of the centre of the fruit as the segments detached (photo 5.2.7.1). The data were analysed with GLM procedures of SAS 2002.

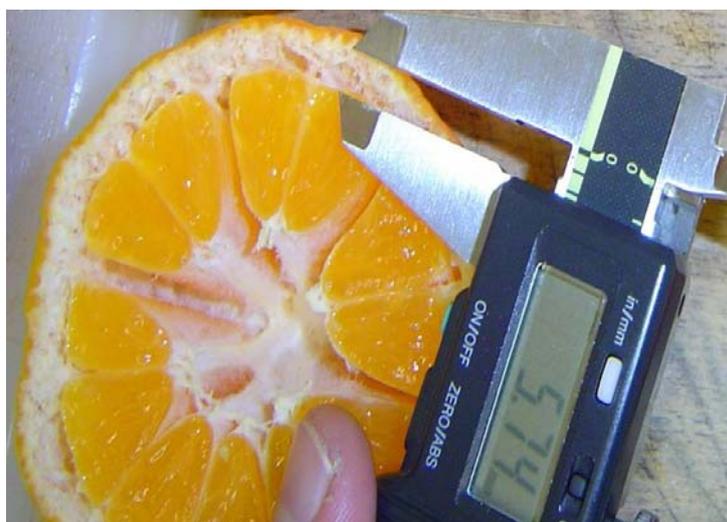


Fig. 5.2.7.1. Measurement of degree of puffiness.

Results

All the treatments (including the control) had very low levels of puffiness (Fig. 5.2.7.1a and b). The control treatment had significantly higher levels of puffiness compared to the other treatments.

The colour loss of the pulp observed in the 2004 season's experiment (Cronje, Barry and Huysamer, 2004), was not repeated in 2005 at the higher CO₂ levels (Fig. 5.2.7.2 a, b and c). There were also no significant differences between the internal quality of treatments (total juice content and TSS:acid) (Figs. 5.2.7.3a, b and c).

According to colorimeter measurements of the fruit rind, there was a significant colour decrease in the three CO₂ treatments compared with the control (Fig. 5.2.7.4a,b and c). The high CO₂ treatments resulted in significantly more yellow fruit, as measured with the colorimeter and expressed as hue°, lightness and chroma. This significant colour difference measured with the colorimeter was not visible with the naked eye. It is thought that a difference of 2-3 degrees hue° is not discernable by the human eye (G.H. Barry, personal communication). This fact is illustrated by the significant differences already evident between treatments present before the treatments were applied. The fruit were selected because of their uniform colour and randomly placed in the different treatment buckets.

Discussion

The data confirmed the 2004 report that higher CO₂ levels do not increase the incidence of puffiness of 'nules Clementine' mandarins. Therefore the cause and the mechanism of this physiological disorder are still unknown. The levels of CO₂ used (1, 2.5 and 5%) are thought to be realistic and experienced commercially and is well above limits after the data collected during experiment 759 (CRI annual report 2005) showed that the maximum CO₂ level measured in the hold of a ship was 6200 ppm (0.6%). The data suggest that CO₂, as a single factor, is therefore not responsible for puffiness.

The negative effect of low temperatures during shipment (-0.6°C) on colour of citrus fruit was illustrated by Van Wyk (2004). In this study it was found that low temperature could be responsible for the colour loss through probable degradation of carotenoids. What was more surprising was that loss of colour continued even after holding the fruit for 1 week of shelf life temperatures after shipment. This explains the results in Figure 5.2.7.4c, where the fruit rind continues to decrease in colour (higher hue° values) even at shelf life temperature. Van Wyk (2004), stresses the point that only fruit with a high level of carotenoids would be able to arrive with adequate colour after a disinfestation protocol.

The loss of colour due to the CO₂ treatments was not found in the previous experiments (CRI annual report 2005) and a possible explanation could be the lower CO₂ concentrations used in the 2004 experiments (1, 0.5 and 0.3% CO₂). No information on a comparable observation could be found in the literature.

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Fig. 5.2.7.2a

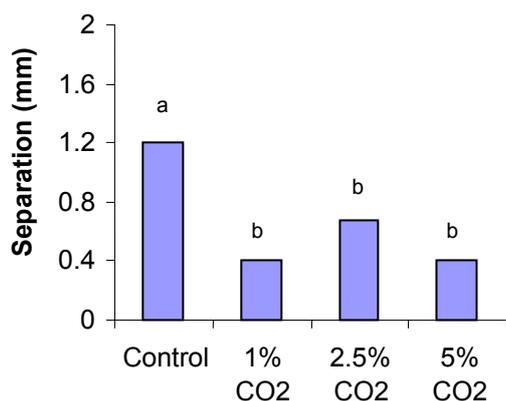
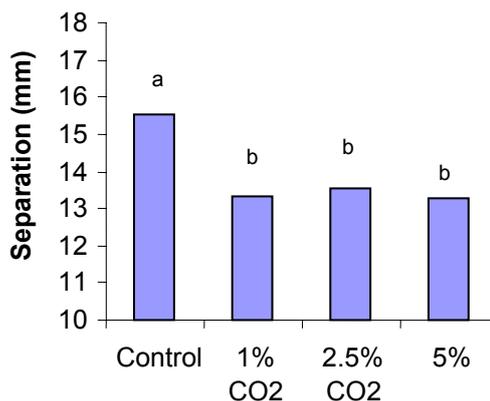


Fig. 5.2.7.2b



Figures 5.2.7.2ab: Puffiness was quantified as separation of rind from pulp (a) and separation of segments at centre of fruit (1b) were measured (mm). Significant differences between treatments are illustrated by different letters (p < 0.05).

Fig. 5.2.7.3a

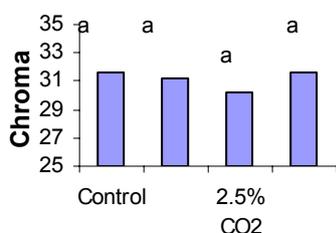


Fig. 5.2.7.3b

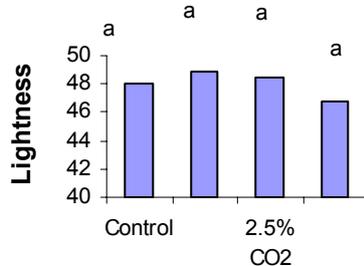
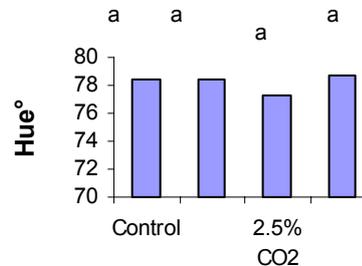


Fig. 5.2.7.3c



Figures 5.2.7.3abc: Pulp colour, expressed as chroma (a), lightness (b) and hue° (c). A high chroma value equals a more intense colour, higher lightness a whiter/lighter colour and a high hue° a more yellow colour. Significant differences between treatments are illustrated by different letters (p < 0.05).

Fig. 5.2.7.4a

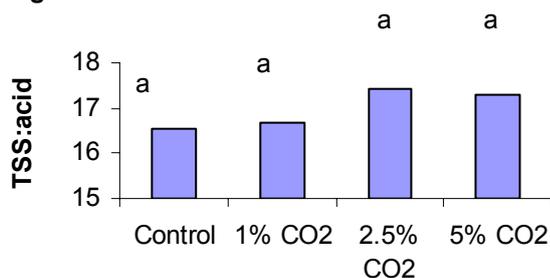
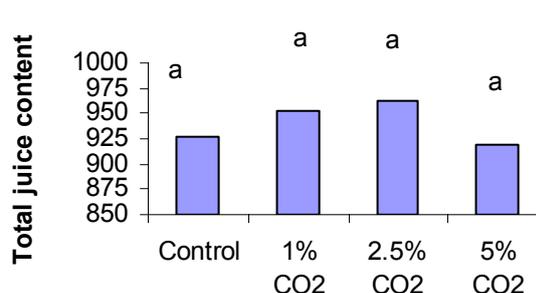


Fig. 5.2.7.4b



Figures 5.2.7.4ab: Internal quality measurements as expressed in TSS:acid (a) and total juice content (%) (b) of the pulp. No significant differences were found between treatments ($p < 0.05$).

Fig. 5.2.7.5a ■ Control ■ 1% CO₂ ■ 2.5% CO₂ ■ 5% CO₂

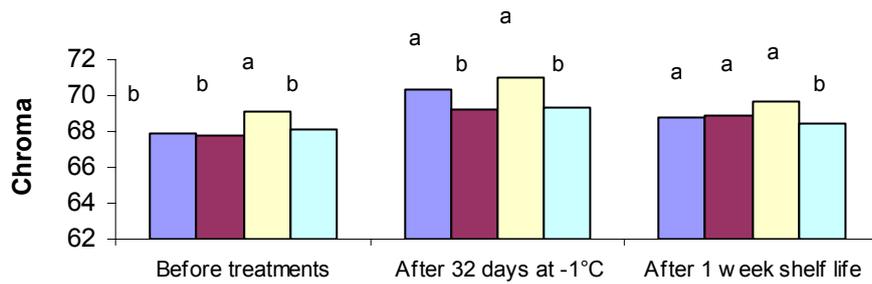


Fig. 5.2.7.5b

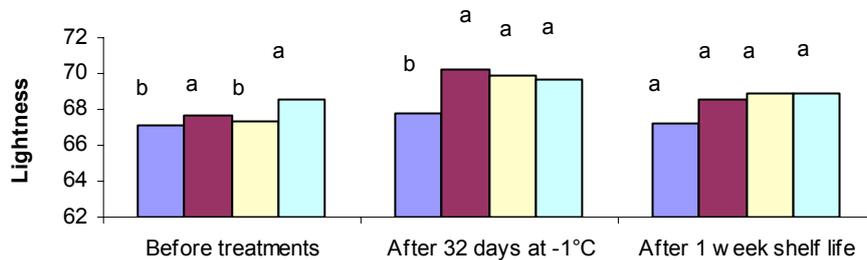
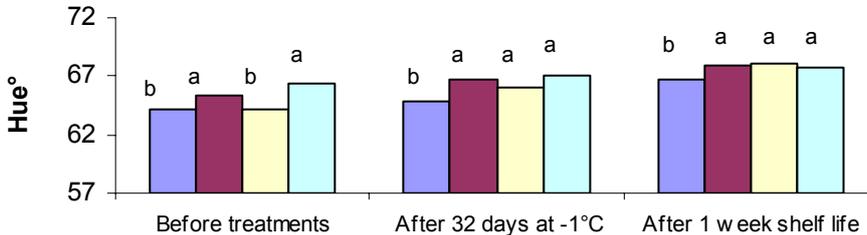


Fig. 5.2.7.5c



Figures 5.2.7.5abc: Colour measurements of fruit rind before treatment, after treatment (32 days at -1°C) and after 1 week shelf life. Colour is expressed as chroma (a), lightness (b) and hue° (c). A high chroma value equals a more intense colour, higher lightness a whiter/lighter colour and a high hue° a more yellow colour. Significant differences between treatments are illustrated by different letters ($p < 0.05$).

5.2.8 Variation in Post Storage Quality of 'nules Clementine' Mandarin and 'Oroval Clementine' Mandarin Fruit (*Citrus reticulata* Blanco) with special reference to Rind Breakdown Incidence
 Experiment CL 01-04 (a) by Parton Khumalo & Arrie de Kock, (Experico), Marius Huysamer (DFPT at SU) and Graham Barry (CRI at SU)

Opsomming

Skilafbraak kom op verskeie sitrus soorte voor, maar hoofsaaklik word dit op Clementine mandaryne gevind. Binne die Clementine groep, kry 'nules' meer probleme as 'Oroval'. Die doel van die werk was om vas te stel of 'Oroval' meer anti-oksidente het, en dus of dit die rede vir die verskille is. Vrugte van altwee kultivars is van drie areas gepluk en ontgroen, voor hulle vir 10 weke by 7.5°C opgeberg was. Daar was geen verskil in die anti-oksidente gemeet nie, maar 'nules' het betekenisvol meer skilafbraak ontwikkel. 'Oroval' het meer bederf ontwikkel. Skilafbraak het dus nie goed gekorreleer met enige van die parameters wat ontleed was, en nog navorsing sal nodig wees. Dit is verder aanbeveel dat 'nules' nie teen lang opbergings tye onderwerp word.

Introduction

Rind disorders have been reported on various citrus types, including mandarins (*Citrus reticulata* Blanco), oranges (*Citrus sinensis* (L) Osbeck), grapefruit (*Citrus paradisi* Macf.), tangelo (*Citrus reticulata* Blanco x *Citrus paradisi* Macf.) and lemons (*Citrus limon* (L) Burm) (Petracek et al., 1995; Van Rensburg and Bruwer, 2000; Ben Yehoshua, et al., 2001; Alferez et al., 2003; Van Rensburg et al., 2004). 'Clementine' mandarin is affected by rind breakdown, and 'nules Clementine' mandarin, hereafter referred to as 'nules', was identified as the selection most susceptible to this disorder (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004).

Available information on cultivar susceptibility to rind breakdown has been generated largely through commercial data obtained from exporting companies on their claims system. The objective of this experiment was to test the hypothesis that 'Oroval' fruit are more tolerant to rind breakdown than 'nules' fruit. Additional data on rind pigments and the antioxidant capacity at harvest were also generated. Our results showed that the differences in rind breakdown susceptibility between the two 'Clementine' mandarin selections was not necessarily due to differences in the antioxidant capacity and rind pigments content at harvest.

Materials and methods

Plant material and sampling

Healthy 'nules' and on 'Oroval' trees budded on Troyer citrange [*C. sinensis* Osbeck x *Poncirus trifoliata* (L.) Raf.] rootstock were used, in both seasons (2004 and 2005). Three areas, Paarl, Saron and Robertson (Western Cape Province, South Africa), were selected for the experiment and site details are summarised in Table 5.2.8.1. In the second season of research only fruit from Saron and Robertson were used. About 60 kg of fruit were harvested from the inside canopy position of ~50 randomly selected trees within each orchard. Fruit was harvested into 20 kg plastic lug boxes and immediately transported to Stellenbosch in Simondium for degreening and packing. However, in the 2005 season fruit sampled from Robertson did not require degreening.

Fruit degreening and packing

Fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (benomyl) (1 g·L⁻¹), Decamone® (2.4-D sodium salt) (5 mL·L⁻¹) and Citricure® (guazatine) (2.5 mL·L⁻¹). The fungicides were mixed in water with a wetting agent, Citowet®, added at a concentration of 0.1 mL·L⁻¹. After drenching the fruit was held at ambient temperature (~20°C) for 8 to 12 hours before being moved to a degreening chamber. Degreening was conducted for 5 days using 1 to 2 ppm ethylene at 20°C and at 90 to 95% RH. After degreening, fruit were again held at ambient temperature (~20°C) for 12 to 24 hours before packing.

Fruit were packed at a commercial packhouse. A total of eight cartons (replicates) of fruit were packed per cultivar per growing area. After packing, fruit were transported to Stellenbosch for storage.

Fruit storage

The eight cartons of fruit from each 'Clementine' mandarin selection per area were stored at 7.5°C for 10 weeks. After initial cold storage, the fruit were then subjected to a shelf life period of 1 week at 20°C before evaluations were conducted.

Data collection

Internal fruit quality and rind variables at harvest

Rind colour of 10 fruit per replicate was rated using the CRI rind colour chart set number 36. Hue angle of the rind was measured on the same 10 fruit per replicate used for colour rating. Equatorial fruit diameter (mm), soluble solids content (SSC) (°Brix), titratable citric acid content (%), juice content (% w/w). Mineral nutrient status and rind moisture of fruit was determined by obtaining rind samples from the equatorial region of five fruit per replicate and sending them for analysis at a commercial laboratory in Somerset West, South Africa.

Selected biochemical rind properties determined at harvest

The antioxidant capacity of the flavedo was determined from the rinds of five fruit per replicate per cultivar using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Rind pigments: total chlorophyll and carotenoid contents were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Lichtenthaler (1987).

Internal fruit quality and rind variables after cold storage plus a shelf-life

Rind colour, SSC, and titratable citric acid content were again determined after each cold storage period on 10 fruit per replicate as previously described.

Rind breakdown, chilling injury and decay

The incidence of this disorder was measured on ~40 fruit per replicate. Core drying was determined by cutting 10 fruit per replicate in half along the equatorial region and the disorder was recorded if there was drying, collapsed or granulated juice vesicles resulting in decreased extractable juice (Murata, 1997). Rind moisture of fruit was determined by removing ten fresh rind discs from the five fruit per replicate (two discs per fruit) using a size 12 cork borer. These discs were weighed fresh then oven-dried at 70°C for 72 hours and re-weighed after drying. The difference between the fresh weight and dry weight expressed as a percentage indicated the rind moisture content.

Statistical design and analysis

Conducting the research in a commercial orchard imposed a limitation on the experimental design as 'Clementine' selection treatments could not be randomised in the experimental plot. To allow for comparison between the 'Clementine' selections it was assumed that orchards in which each selection was planted were similar. Data collected at harvest and after storage plus shelf-life were analysed using a t-test on STATISTICA® (Tulsa, OK).

Results

Fruit characteristics at harvest

Paarl

'nules' and 'Oroval' fruit sampled from Paarl had similar rind colour ratings at harvest (Table 5.2.8.2). However, the rind of 'Oroval' fruit had a lower hue angle than that of 'nules'. 'nules' had significantly lower SSC and titratable acidity compared to 'Oroval', but the absolute differences were small, namely 0.1% for titratable acidity and 0.9 °Brix for SSC. The fruit harvested from both selections were of similar size and mass and also had similar juice contents. All the mineral nutrients (N, P, K, Ca and B) measured and rind moisture content did not differ significantly between the two 'Clementine' selections. The antioxidant capacity was slightly higher in 'Oroval' than in 'nules' ($P=0.055$). The rind pigments, chlorophylls and carotenoids, were similar for the two 'Clementine' selections.

Saron

The rind colour, determined with the CRI rind colour chart or the Minolta colour reader, was similar for both the 'Clementine' mandarin selections harvested in Saron (Table 5.2.8.3), although in the 2005 season Nules had a slightly lower rind colour rating ($P=0.056$), suggesting that this fruit was better coloured than Oroval (Table 5.2.8.4). In both seasons of trialing, 'nules' had a significantly higher SSC and lower titratable acidity than 'Oroval'. The fruit size and mass were similar in both cultivars, however, the juice content was significantly higher in 'Oroval' than in 'nules' (Table 5.2.8.3). The concentrations of P, K and Ca were similar in both 'Clementine' selections. However, the concentrations of N (only in the 2004 season) and B (in both seasons) were significantly different between the two selections, with 'Oroval' having a higher N concentration and 'nules' having a higher B concentration (Tables 5.2.8.3 and 4). Rind moisture content was significantly higher for 'Oroval' than for 'nules'. The antioxidant capacity and rind pigments were not significantly different between the 'Clementine' mandarin selections (Table 5.2.8.3).

Robertson

'Oroval' fruit had a significantly lower rind colour rating and hue angle of the rind than 'nules' (Table 5.2.8.5). However, in the 2005 season, rind colour was similar in both 'Clementine' mandarin selections (Table 5.2.8.6). In both seasons of research, 'nules' fruit had a higher SSC and lower acidity at harvest compared to 'Oroval' (Table 5.2.8.5 and 6). 'nules' fruit were significantly larger, heavier and had a higher juice content than 'Oroval' (Table 5.2.8.5). The N concentration was higher in 'Oroval' than in 'nules', whereas Ca and B were higher in 'nules' than in 'Oroval' (Table 5.2.8.5). The other mineral nutrients, P and K were of a similar concentration in both 'Clementine' selections. However, in the 2005 season, the concentration of all rind mineral nutrients was similar in both 'Clementine' mandarin selections (Table 5.2.8.6). 'Oroval' had higher rind moisture content than 'nules'. The antioxidant capacity and total carotenoid content were not significantly different between the two 'Clementine' selections, but the total chlorophyll content (in 2004) tended to be higher in 'nules' than in 'Oroval' ($P = 0.075$) [Table 5.2.8.5].

Fruit quality after cold storage plus shelf-life

Paarl

Generally, low levels (<4%) of rind breakdown were recorded in fruit from Paarl and there was no significant difference in the occurrence of rind breakdown between fruit from the two 'Clementine' mandarin selections (Table 5.2.8.7). However, the tendency was for 'nules' to develop higher levels of rind breakdown than 'Oroval' (3.7% vs. 1.4%; $P = 0.160$). The levels of decay and puffiness did not differ significantly between the two 'Clementine' selections. 'Oroval' had a significantly higher SSC content whereas the titratable acidity of the two selections was similar after the storage period. Rind colour, rated using the CRI rind colour chart or the measured with the colorimeter, and rind moisture content were similar for the two 'Clementine' mandarin selections after the storage period.

Saron

'nules' had significantly higher levels of rind breakdown than 'Oroval', which did not develop any rind breakdown (Table 5.2.8.8). However, in the 2005 season the rind breakdown levels were similar between the 'Clementine' mandarin selections (Table 5.2.8.9). 'Oroval' fruit from Saron were more susceptible to decay and puffiness (only in the 2004 season) as the levels of these disorders were significantly higher than those recorded in 'nules' fruit (Table 5.2.8.8 and 9). The SSC of the two selections was similar after storage but the acidity of 'Oroval' remained significantly higher than that of 'nules' (Table 5.2.8.8). The rind colour rating of 'Oroval' and 'nules' fruit was similar after storage (Tables 5.2.8.8 and 9). Rind moisture content of 'Oroval' was significantly higher than that of 'nules' (Table 5.2.8.8).

Robertson

In both seasons, the incidence of rind breakdown was significantly higher in 'nules' fruit than 'Oroval' (Table 5.2.8.10 and 11). 'Oroval' fruit developed higher decay levels after cold storage plus shelf-life than 'nules' fruit. The SSC was significantly higher in 'nules' fruit while acidity of this fruit was significantly lower than of 'Oroval' after the storage period (Table 5.3.8.10). Rind colour, measured with the CRI rind colour chart or colorimeter, was similar for both 'Clementine' selections, after the storage period (Table 5.2.8.10 and 11). Rind moisture content was significantly higher for 'Oroval' than for 'nules' (Table 5.2.8.10). After storage, due to the presence of rind breakdown, the rind antioxidant capacity was significantly lower in Nules than in Oroval fruit (Table 5.2.8.11). There were no chlorophylls present in the fruit rinds after storage and the carotenoid content was similar between the 'Clementine' mandarin selections.

Conclusions

In conclusion, 'nules' was more susceptible to rind breakdown than 'Oroval'. However, the difference in susceptibility to rind breakdown between the two 'Clementine' mandarin selections did not seem to be directly associated with rind pigments and antioxidant capacity at harvest. It would seem other factors may be involved in the development of the disorder.

Future research

Additional research on rind volatiles and rind moisture content may give clarity on the differences in susceptibility to the development of rind breakdown between the two 'Clementine' selections. Furthermore, these two 'Clementine' mandarin selections may present a good model to study gene regulation of rind breakdown development. Due to the decay susceptibility and the development of puffiness in 'Oroval' this selection does not have as long a storage-life as does 'nules' and therefore, should not be stored for as long as 10 weeks.

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Table 5.2.8.1. Summary of trial sites, plant material and harvest dates.

Site	Saron		Paarl		Robertson	
	'nules'	'Oroval'	'nules'	'Oroval'	'nules'	'Oroval'
Tree age	14 yrs	10 yrs	12 yrs	10 yrs	19 yrs	15 yrs
Ridging	Yes	Yes	Yes	Yes	No	No
Spacing (m)	5 x 3	5 x 3	5 x 3	5 x 3	5 x 2	5 x 2
Harvest date ¹	18/05/04 10/05/05	18/05/04 10/05/05	13/05/04	13/05/04	31/05/04 10/05/05	31/05/04 10/05/05
Degreening	Yes	Yes	Yes	Yes	Yes (2004)	Yes (2004)

¹ At each harvest date fruit were selectively harvested based on colour. Only fruit that had reached colour break or better were sampled.

Table 5.2.8.2. Characteristics at harvest of 'nules' and 'Oroval' sampled from Paarl in the 2004 season.

Response variable	Cultivar		P-value
	'nules'	'Oroval'	
Rind colour rating ¹	5.2	5.3	0.744
Hue angle of the rind	90.8	84.1	0.006
SSC (°Brix)	11.0	11.9	0.001
Titrateable citric acid (%)	1.0	1.1	0.016
Equatorial diameter (mm)	61.9	62.4	0.633
Mass (g)	109.9	111.6	0.754
Juice content (%)	51.9	50.9	0.691
N (mg/100 g fresh mass)	266	255.8	0.535
P (mg/100 g fresh mass)	31.0	28.5	0.131
K (mg/100 g fresh mass)	323.4	305.6	0.368
Ca (mg/100 g fresh mass)	217.7	207.4	0.631
B (mg/kg fresh mass)	8.2	8.3	0.779
Rind moisture content (%)	75.4	73.7	0.079
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	3.2	4.2	0.055
Total chlorophylls (µg·g ⁻¹ DW)	3.4	2.0	0.174
Total carotenoids(µg·g ⁻¹ DW)	13.0	14.0	0.441

¹ Rind colour rating determined using the CRI rind colour chart set number 36.

Table 5.2.8.3. Characteristics at harvest of 'nules' and 'Oroval' sampled from Saron in the 2004 season.

Response variable	Cultivar		P-value
	'nules'	'Oroval'	
Rind colour rating ¹	5.4	5.4	0.526
Hue angle of the rind	87.6	88.1	0.624
SSC (°Brix)	12.2	11.0	0.001
Titrateable citric acid (%)	1.05	1.27	0.001
Equatorial diameter (mm)	61.4	60.6	0.530
Mass (g)	101.2	101.2	0.997
Juice content (%)	52.4	56.8	0.001
N (mg/100 g fresh mass)	230.8	264.8	0.034
P (mg/100 g fresh mass)	29.9	30.0	0.983
K (mg/100 g fresh mass)	356.0	318.4	0.165
Ca (mg/100 g fresh mass)	140.4	128.4	0.492

B (mg/kg fresh mass)	5.92	4.84	0.001
Rind moisture content (%)	78.2	80.3	0.017
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	2.0	1.8	0.577
Total chlorophylls (µg·g ⁻¹ DW)	0.8	0.2	0.131
Total carotenoids (µg·g ⁻¹ DW)	15.2	17.0	0.181

¹ Rind colour rating determined using the CRI rind colour chart set number 36.

Table 5.2.8.4. Characteristics at harvest of 'nules' and 'Oroval' sampled from Saron in the 2005 season.

Response variable	'Clementine' mandarin selection		P-value
	'nules'	'Oroval'	
Rind colour rating ¹	4.9	5.4	0.056
SSC (°Brix)	11.9	10.3	0.004
Titrateable citric acid (%)	0.94	1.27	0.001
N (mg/100 g fresh mass)	309.6	303.8	0.681
P (mg/100 g fresh mass)	30.4	29.4	0.525
K (mg/100 g fresh mass)	333.0	317.0	0.377
Ca (mg/100 g fresh mass)	264.0	246.0	0.532
B (mg/kg fresh mass)	9.1	6.6	0.001
Rind moisture content (%)	70.5	76.3	0.001

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.5. Characteristics at harvest of 'nules' and 'Oroval' harvested from Robertson, in the 2004 season.

Response variable	Cultivar		P-value
	'nules'	'Oroval'	
Rind colour rating ¹	5.2	4.8	0.015
Hue angle of the rind	80.3	75.2	0.032
SSC (°Brix)	12.2	10.3	0.001
Titrateable citric acid (%)	1.05	1.41	0.001
Equatorial diameter (mm)	64.3	61.8	0.022
Mass (g)	120.5	99.8	0.004
Juice content (%)	57.1	51.7	0.005
N (mg/100 g fresh mass)	230.8	264.8	0.033
P (mg/100 g fresh mass)	29.9	29.9	0.983
K (mg/100 g fresh mass)	288.0	316.2	0.310
Ca (mg/100 g fresh mass)	195.0	128.9	0.007
B (mg/kg fresh mass)	6.7	4.8	0.001
Rind moisture content (%)	74.7	79.5	0.002
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	2.7	2.1	0.092
Total chlorophylls (µg·g ⁻¹ DW)	2.6	0.8	0.075
Total carotenoids (µg·g ⁻¹ DW)	19.8	17.9	0.185

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.6. Characteristics at harvest of 'nules' and 'Oroval' harvested from Robertson in the 2005 season.

Response variable	'Clementine' mandarin selection		P-value
	'nules'	'Oroval'	
Rind colour rating ¹	1.6	2.0	0.115
SSC (°Brix)	10.0	10.3	0.203
Titrateable citric acid (%)	0.93	1.12	0.039
N (mg/100 g fresh mass)	261.0	244.8	0.437
P (mg/100 g fresh mass)	24.6	27.2	0.146
K (mg/100 g fresh mass)	205.8	233.0	0.078
Ca (mg/100 g fresh mass)	66.4	75.9	0.101
B (mg/kg fresh mass)	7.1	7.0	0.795

Rind moisture content (%)	78.3	77.5	0.543
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	4.1	3.7	0.268
Total chlorophylls (µg·g ⁻¹ DW)	0.0	0.3	0.172
Total carotenoids (µg·g ⁻¹ DW)	30.7	35.4	0.085

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.7. Quality of 'nules' and 'Oroval' sampled from Paarl and stored for 10 weeks at 7.5°C plus 1 week at 20°C in the 2004 season.

Response variable	Cultivar		P-value
	'nules'	'Oroval'	
Rind breakdown (%)	3.7	1.4	0.160
Decay (%)	6.1	3.4	0.077
Puffiness(%)	0.2	2.0	0.073
SSC (°Brix)	10.9	11.8	0.001
Titrateable citric acid (%)	0.72	0.81	0.127
Rind colour rating ¹	1.2	1.0	0.067
Hue angle of the rind	53.9	54.7	0.337
Rind moisture (%)	68.2	69.2	0.489

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.8. Quality of 'nules' and 'Oroval' harvested from Saron and stored for 10 weeks at 7.5°C plus 1 week at 20°C in the 2004 season.

Response variable	Cultivar		P-value
	Nules	Oroval	
Rind breakdown (%)	14.9	0.0	0.001
Decay (%)	21.1	34.0	0.003
Puffiness(%)	0.0	7.1	0.001
SSC (°Brix)	11.5	11.2	0.154
Titrateable citric acid (%)	0.72	1.01	0.001
Rind colour rating ¹	1.1	1.1	0.681
Hue angle of the rind	54.8	53.7	0.127
Rind moisture (%)	74.0	77.8	0.001

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.9. Quality of 'nules' and 'Oroval' harvested from Saron and stored for 10 weeks at 7.5°C plus 1 week at 20°C in the 2005 season.

Response variable	'Clementine' mandarin selection		P-value
	'nules'	'Oroval'	
Rind breakdown (%)	7.0	4.8	0.373
Decay (%)	3.8	21.7	0.001
Puffiness (%)	9.4	9.3	0.970
Rind colour rating ¹	1	1	- nd

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.10. Quality of 'nules' and 'Oroval' harvested from Robertson and stored for 10 weeks at 7.5°C plus 1 week at 20°C, in the 2004 season.

Response variable	Cultivar		P-value
	'nules'	'Oroval'	
Rind breakdown (%)	17.6	0.5	0.001
Decay (%)	11.1	36.1	0.001
SSC (°Brix)	11.5	10.0	0.001
Titrateable citric acid (%)	0.74	0.96	0.001
Rind colour rating ¹	1.16	1.2	0.693
Hue angle of the rind	55.6	55.0	0.475
Rind moisture (%)	69.9	73.8	0.001

¹ Rind colour rating determined using the CRI rind colour chart set number 36

Table 5.2.8.11. Quality of 'Nules' and 'Oroval' harvested from Robertson and stored for 10 weeks at 7.5°C plus 1 week at 20°C, in the 2004 season.

Response variable	'Clementine' mandarin selection		P-value
	'Nules'	'Oroval'	
Rind breakdown (%)	30.8	5.4	0.001
Decay (%)	3.18	10.2	0.080
Puffiness (%)	17.9	29.8	0.232
Rind colour rating ¹	1	1	nd ²
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	3.1	4.1	0.037
Total chlorophylls (µg·g ⁻¹ DW)	0.0	0.0	-
Total carotenoids (µg·g ⁻¹ DW)	51.6	52.7	0.884

¹ Rind colour rating determined using the CRI rind colour chart set number 36. No statistics done because the colour rating was identical.

5.2.9 Effect of Canopy Position on Harvest and Post-Storage Quality of 'Nules Clementine' Mandarin (*Citrus reticulata* Blanco) fruit

Experiment CL 01-04(b) by Parton Khumalo, Arrie de Kock (Experico) Marius Huysamer (DFPT at SU) and Graham Barry (CRI at SU)

Opsomming

Skilafbraak by 'Nules' Clementine mandaryn het heelwat verliese vir die Suid Afrikaanse sitrus bedryf veroorsaak. Dit is deur ander werk aangedui dat vrugte vanaf die binnekant van die boom meer geneig is om skilafbraak te toon as vrugte vanaf die buitekant. Die doel van die proef was om die anti-oksidant status en pigmente van sulke vrugte te ondersoek. Waar 'Nules' vrugte vanaf verskillende posiesies in die boom gepluk was, en teen of 7.5°C of 10°C vir 12 weke opgeberg was, was verskillende voedingselemente en anti-oksidante gemeet. Daar geen korrelasie met skilafbraak gevind nie, en vlakke kon nie vir voorspellings doeleindes gebruik word nie. As resultate vir 2004 en 2005 in ag geneem word, word dit duidelik dat net temperatuur 'n rol gespeel het, met die laagste temperatuur van -0.5°C koueskade, en die hoër temperature skilafbraak veroorsaak het. Teen die huidige, is dit dus aanbeveel dat die gehalte van Clementines die beste sal wees as optimale opbergings temperature en tye gehandhaaf word.

Introduction

Rind breakdown, of 'Nules Clementine' mandarin (*Citrus reticulata* Blanco), is a physiological rind disorder that has over the years caused severe losses to the citrus industry of South Africa (Van Rensburg and Bruwer, 2000). Rind breakdown appears following leakage of oil from oil glands in the flavedo. The oil leaks into and oxidizes the albedo. Oxidized tissue appears as brown spots on the flavedo (Van Rensburg et al., 2004). It has been reported that the occurrence of rind breakdown may be affected by fruit canopy position (Van Rensburg and Bruwer, 2000; Van Rensburg et al., 2004).

The effect of canopy position on rind breakdown of 'Nules Clementine' mandarin fruit has been reported. However, the effect of canopy position on rind antioxidants at harvest on this cultivar has not been investigated. The objective of this study was to test the hypothesis that fruit originating from the inside of the tree canopy have low carotenoids and low antioxidants making them more susceptible to rind breakdown. Our results showed that inside fruit had lower carotenoids and a lower antioxidant capacity, but this fruit was not necessarily more susceptible to rind breakdown than outside fruit, which had higher carotenoids and a higher rind antioxidant capacity at harvest.

Materials and methods

Plant material and sampling detail

This experiment was conducted on 'Nules Clementine' mandarin budded on Troyer citrange (*C. sinensis* x *Poncirus trifolita*) rootstock over two seasons in Paarl (2004) and Robertson (2005) as indicated in Table 5.2.9.1.

Fruit degreening and packing

Fruit were drenched in the following fungicide mixture prior to degreening: Benlate® (benomyl) (1 g·L⁻¹), Decamone® (2.4-D sodium salt) (5 mL·L⁻¹) and Citricure® (guazatine) (2.5 mL·L⁻¹). The fungicides were mixed in water with a wetting agent, Sitowet®, added at a concentration of 0.1 mL·L⁻¹. After drenching,

the fruit was held at ambient temperature (~20°C) for 8 to 12 hours before being moved to a degreening chamber. Degreening was conducted, for 5 days, using 1 to 2 ppm ethylene at 20 °C and at an RH of 90 to 95%. After degreening fruit were again held at ambient temperature (~20°C) for 12 to 24 hours before packing. Fruit were packed at a commercial packhouse. A total of 14 cartons were packed from fruit sampled at each canopy position. After packing fruit were transported to Stellenbosch for storage.

Fruit Storage

The 14 cartons of fruit from each canopy position were divided into two groups of seven cartons (replicates) each and stored at -0.5°C or 7.5°C in the 2004 season. The storage temperatures were modified in the 2005 season to only focus on rind breakdown, therefore fruit were stored at 7.5°C or 10°C for 12 weeks. After initial cold storage, the fruit were then subjected to a shelf-life period of 1 week at 20°C before evaluations were conducted.

Data collection

Internal quality and rind variables at harvest

Rind colour was rated using 10 fruit per replicate, for each canopy position using the CRI rind colour chart set number 36. Hue angle of the rind was measured on the same 10 fruit per replicate. Equatorial fruit diameter (mm), soluble solids content (SSC) (°Brix), titratable citric acid content (%), fresh mass (g), juice content (% w/w). Mineral nutrient status and rind moisture of fruit was determined by obtaining rind samples from the equatorial region of five fruit per replicate and sending them for analysis at a commercial laboratory in Somerset West, South Africa.

Selected biochemical rind properties determined at harvest

Antioxidant capacity of the flavedo was determined from five fruit per replicate at each canopy position using the Trolox Equivalent Antioxidant Capacity (TEAC) assay as described by Re et al. (1998), with minor modifications.

Rind pigments: Total chlorophyll and carotenoid contents were determined from the rinds of five fruit per replicate. The pigments were measured using a spectrophotometer as described by Lichtenthaler (1987).

Internal quality and rind variables after cold storage plus a shelf life

Rind colour, soluble solids content, and titratable citric acid were again determined after each cold storage period on 10 fruit per replicate as previously described.

Rind breakdown, chilling injury and decay. The incidence of this disorder was measured on ~40 fruit per replicate. Core drying was determined by cutting 10 fruit per replicate in half along the equatorial region and the disorder was recorded if there was drying, collapsed or granulated juice vesicles resulting in decreased extractable juice (Murata, 1997).

Statistical design and analysis

The experiment was laid out as a completely randomised design. Data collected at harvest were analysed using a student t-test whereas data collected after cold storage and shelf-life were analysed using a two-way ANOVA on STATISTICA® (Tulsa, OK), a 2 x 2 = 4 factorial treatment set was used with Factor A being the fruit canopy position and Factor B being the storage temperature. Treatment means were compared using the LSD method.

Results and discussion

Fruit characteristics at harvest

In fruit harvested from Paarl in the 2004 season the rind colour rating was similar between fruit sampled from the inside and outside of the tree (Table 5.2.9.2). However, colour measured with the colourimeter showed that the hue angle of the rind was significantly lower in fruit originating from the outside of the tree canopy than fruit sampled from the inside of the tree canopy. In the 2005 season the rind colour rating was significantly lower in fruit sampled from the outside of the tree canopy than in fruit from the inside canopy position (Table 5.2.9.3). Internal quality measured in terms of SSC and titratable citric acid was similar between fruit originating from the different canopy positions, in the 2004 season (Table 5.2.9.2). However, in the 2005 season, inside fruit had a significantly lower SSC and higher titratable citric acid content than outside fruit (Table 5.2.9.3). Fruit from the outside of the tree canopy were significantly larger and heavier than fruit from the inside of the tree canopy (Table 5.2.9.2). No significant difference in juice content occurred between fruit from the different canopy positions. Most of the mineral nutrients, in both fruit populations, were significantly higher in fruit originating from the inside of the tree canopy than in fruit from the outside canopy position. Only the calcium and boron content in fruit were not significantly affected by canopy position. The rind water content and total chlorophylls in the rind were similar for fruit originating

from different canopy positions. The total carotenoid content was higher in fruit from the outside of a tree's canopy than in fruit originating from the inside canopy position. The antioxidant capacity was also higher in outside than in inside fruit, however, this trend was significant only in fruit sampled from Paarl in the 2004 season.

Fruit quality after cold storage plus shelf life

Rind breakdown

In both seasons the occurrence of rind breakdown was not significantly affected by fruit canopy position (Table 5.2.9.4 & 5). Storage temperature was a significant factor in the occurrence of rind breakdown in the 2004 season, with this disorder only observed in fruit stored at 7.5°C and not in fruit stored at -0.5°C (Table 5.2.9.4). However, in the 2005 season rind breakdown levels were similar in fruit stored at 7.5°C and 10°C.

Chilling injury

Chilling injury was only recorded in the 2004 season and the occurrence of this disorder was not significantly affected by fruit canopy position (Table 5.2.9.4). Storage temperature was a significant factor in the occurrence of chilling injury, with this disorder only observed in fruit stored at -0.5 °C and not in fruit stored at 7.5°C.

Decay

The occurrence of decay caused by *Penicillium digitatum* (green mould) was not significantly influenced by canopy position or storage temperature in the 2004 season (Table 5.2.9.4). However, in the 2005 season fruit storage at 10 °C resulted in higher decay levels than storage at 7.5°C (Table 5.2.9.5).

Internal quality

A significant interaction was observed between canopy position and storage temperature on the SSC and titratable citric acid content of fruit in the 2004 season (Table 5.2.9.4). At the storage temperature of -0.5°C, inside fruit had a lower SSC and titratable citric acid content than outside fruit. However, in fruit stored at 7.5°C the reverse occurred. Inside fruit had a higher SSC and titratable citric acid content than outside fruit. In the 2005 season, inside fruit had a significantly lower SSC and higher titratable acidity than fruit harvested from the outside canopy position (Table 5.2.9.5). Fruit storage at 7.5°C or at 10°C did not significantly affect the internal of fruit.

Core drying

Core drying was only observed in the 2005 season, fruit sampled from the inside canopy position developed significantly higher levels of this disorder than fruit sampled from the outside position (Table 5.2.9.5). Storage temperature did not significantly affect the development of core drying.

Rind colour

Only storage temperature, in the 2004 season, was a significant factor on colour development of fruit from the different canopy positions (Table 5.2.9.4). Fruit stored at 7.5°C had a lower hue angle and rind colour rating than fruit stored at -0.5°C. In the 2005 season, fruit sampled from the different canopy positions had similar rind colour ratings after storage at either 7.5°C or 10°C.

Conclusions

Fruit harvested from the inside canopy position had lower carotenoids and a lower antioxidant capacity than fruit harvested from the periphery of the tree canopy, however, these characteristics did not accentuate rind breakdown and chilling injury after storage for 12 weeks at the respective temperatures. Fruit exposure to light is important for acceptable quality at harvest in terms of rind colour, SSC and titratable citric acid. Therefore, techniques to ensure adequate light penetration in the tree are important for harvest quality, but not necessarily for rind breakdown after storage. Fruit quality of 'hules Clementine' mandarin can best be managed through the use of an optimum storage temperature and storage duration regime.

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Table 5.2.9.1. Summary of trial sites, plant material and harvest dates.

Site	2004	2005
	Paarl	Robertson
Tree age	12 yrs	19 yrs
Ridging	Yes	No
Spacing (m)	5 x 3	5 x 2
Harvest date ¹	13 May	15 June
Degreening	Yes	No ²

¹ At each harvest date fruit were selectively harvested based on colour. Only fruit that had reached colour break or better were sampled

² Fruit from Robertson were harvested very late in the season and had attained good colour on the tree so did not require degreening

Table 5.2.9.2. Characteristics at harvest of 'hules Clementine' mandarin fruit sampled from the inside (shaded) and outside (non-shaded) canopy positions of trees in the Paarl area in 2004.

Response variable	Canopy position		P-value
	Inside fruit	Outside fruit	
Rind colour rating ¹	5.3	5.4	0.426
Hue angle of the rind	89.5	83.5	0.009
SSC (°Brix)	11.3	11.4	0.369
Titrateable citric acid (%)	1.12	1.02	0.048
Equatorial diameter (mm)	59.9	63.7	0.009
Mass (g)	98.7	115.5	0.009
Juice content (%)	53.0	54.5	0.091
N (mg/100 g fresh mass)	272.8	238.8	0.141
P (mg/100 g fresh mass)	31.2	25.1	0.034
K (mg/100 g fresh mass)	338.6	285.2	0.043
Ca (mg/100 g fresh mass)	199.7	241.0	0.148
B (mg·kg ⁻¹ fresh mass)	7.9	7.6	0.506
Rind moisture content (%)	76.1	76.8	0.304
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	5.4	6.3	0.001
Total chlorophylls (µg·g ⁻¹ DW)	1.1	1.4	0.616
Total carotenoids (µg·g ⁻¹ DW)	11.3	18.7	0.001

¹ Rind colour rating determined using the CRI rind colour chart set number 36.

Table 5.2.9.3. Characteristics at harvest of 'hules Clementine' mandarin fruit sampled from the inside (shaded) and outside (non-shaded) canopy positions of trees in the Robertson area in 2005.

Response variable	Canopy position		P-value
	Inside fruit	Outside fruit	
Rind colour rating ¹	3.6	2.4	0.001
SSC (°Brix)	9.8	10.8	0.001
Titrateable citric acid (%)	0.93	0.82	0.006
N (mg/100 g fresh mass)	285.6	250.0	0.021
P (mg/100 g fresh mass)	34.7	24.5	0.001
K (mg/100 g fresh mass)	345.0	246.6	0.001
Ca (mg/100 g fresh mass)	90.0	89.9	0.973
B (mg·kg ⁻¹ fresh mass)	11.1	10.3	0.159
Rind water content (%)	74.0	74.4	0.564
Antioxidant capacity (mM Trolox equivalents·g ⁻¹ FW)	1.4	2.0	0.255

¹ Rind colour rating determined using the CRI rind colour chart set number 36.

Table 5.2.9.4. Quality of 'hules Clementine' mandarin fruit sampled from inside (shaded) and outside (non shaded) canopy positions in the Paarl area, and stored for 10 weeks at -0.5 °C or 7.5 °C plus one week at 20°C.

Response variable	Storage temperature (°C)	Canopy position (Factor A)		Storage temperature (°C) (Factor B)		P-value ¹		
		Inside	Outside	-0.5	7.5	A	B	AxB
Rind breakdown (%)		3.2	2.0	0.0a ²	5.2b	0.256	0.001	0.256
Chilling injury (%)		10.4	14.5	24.9b	0.0a	0.268	0.001	0.268
Decay (%)		12.9	12.7	14.3	11.4	0.945	0.317	0.388
SSC (°Brix)	-0.5	10.8a	11.9c			0.055	0.233	0.001
	7.5	11.4b	10.9a					
Titratable citric acid (%)	-0.5	0.62a	0.95c			0.038	0.014	0.001
	7.5	0.77b	0.60a					
Hue angle of the rind		59.4	59.6	64.5b	53.4a	0.261	0.001	0.422
Rind colour rating ³		2.0	2.0	2.8a	1.1a	1.000	0.001	0.255

¹ Two-way ANOVA with Factor A being canopy position and Factor B being storage temperature.

² Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test.

³ Rind colour rating determined using the CRI rind colour chart set number 36.

Table 5.2.9.5. Quality of 'hules Clementine' mandarin fruit sampled from inside (shaded) and outside (non-shaded) canopy positions in the Robertson area, and stored for 10 weeks at -0.5°C or 7.5°C plus one week at 20°C.

Response variable	Canopy position	Canopy position (Factor A)		Storage temperature (°C) (Factor B)		P-value ¹		
		Inside	Outside	7.5	10	A	B	AxB
Rind breakdown (%)		10.5	12.6	9.8	13.4	0.479	0.219	0.926
Dry rind (%)		8.1	12.8	0.7a ²	20.2b	0.171	0.001	0.215
Decay (%)		3.8	3.7	1.6a	5.9b	0.984	0.048	0.837
Puffiness (%)		0.5	0.5	0.7	0.3	0.987	0.494	0.362
SSC (°Brix)		10.1a	11.0b	10.5	10.5	0.001	0.949	0.412
Titratable citric acid (%)		0.46b	0.38a	0.43	0.40	0.014	0.265	0.438
Core drying (%)		37.5b	12.0a	26.0	23.5	0.003	0.737	0.737
Rind colour rating ³		1.0	1.0	1.0	1.0	nd ⁴	nd	nd

¹ Two-way ANOVA with Factor A being canopy position and Factor B being storage temperature

² Values in the same row followed by different letters indicate significant differences (P<0.05) according to the LSD test

³ Rind colour rating determined using the CRI rind colour chart set number 36

⁴ No statistical analyses done because all fruit were deep orange and fully coloured.

5.2.10 The effect of different citrus wax applications on the development of peteca spot on lemons

Experiment 824 by K.H.Lesar (CRI) and P. Cronje (CRI at SU)

Opsomming

Petecavlek op suurlemoene het heelwat probleme vir uitvoerders veroorsaak. Daar is verskeie teorieë vir die oorsaak daarvan. Klimaat kan dalk 'n rol speel, met veranderinge van warm droë toestande tot koud en nat, moontlik 'n factor. Vanaf 'n na-oes oogpunt, pakhuis behandelings soos borseling, vrug hanteering en die temperatuur van swamdoder baddens en droërs, en baie belangrik, waksaanwending, speel dalk 'n rol. Afdeling 3.5.9 ondersoek die invloed van sitruswakse. Vrugte is met 'n reeks wakse vanaf 25% tot 10% vastestof vlakke behandel, en vir 28 dae teen -0.6°C opgeberg. Wakse met die hoogste vastestof vlakke het die minste petecavlek veroorsaak, wat teen huidige aanbevelings is. Dit is moontlik dat die swaarder wakse 'n vermindering van gas wisseling in die vrugte veroorsaak, en dat die verhoging van CO_2 die vermindering van petecavlek veroorsaak het. Dit is dieselfde patroon as kouskade, waar dit bekend is dat CO_2 vlakke die afwyking beïnvloed. Suurlemoene wat meer groen was, het meer skade as goed gekleurde vrugte getoon. Die resultate bewys dat daar nog heelwat onkunde is, wat die problem betref.

Introduction

Peteca spot is a physiological disorder that appears as deep pits on the peel surface of lemons, usually after packing. Peteca is particularly prevalent when the trees undergo water stress alternating with periods of freely available water in the period two to three months prior to harvest. Other cultural practices, that have been reported to increase the incidence of peteca spot, are late heavy pruning practices, late application of Nitrogen and late oil sprays.

Erratic environmental conditions appear to play a major role in predisposing lemons to the development of peteca. i.e. sudden changes from long periods of hot dry conditions followed by colder weather. Peteca seems to be more prevalent after the harvesting of lemons during cold, moist or wet conditions.

The rough handling of lemons, especially the more sensitive greener fruit, during picking, transport to the packhouse and operational processes in the packhouse, also seem to predispose the fruit to the development of peteca.

The operational processes in the packhouse that have triggered the development of peteca spot on lemons are the rough handling of the fruit, as already mentioned, over brushing and too high brush speeds, too high a temperature in the hot water fungicide bath and in the drying tunnel, and most importantly the waxing of the fruit.

Waxing of the fruit is one of the major critical control processes in the packhouse. Choosing the right wax for lemons, specifically peteca-prone lemons, is critical. It has been reported that the use of heavy waxes should be avoided.

Polyethylene waxes with high solid levels (18% and higher) and/or increased shellac or wood rosin levels are classified as heavy waxes. The natural waxes i.e. Carnauba waxes with lower solid levels (16% and lower) and with not too high shellac levels or without shellac are classified as lighter waxes and are reported to be the preferred waxes for peteca-prone lemons.

The application rate of the wax used for lemons is also vitally important. Even though a light wax may be used for peteca-prone lemons, the over application thereof could also induce the development of peteca. The slight under application, but a good uniform coverage of a light wax is by far the desired application of a light wax to lemons to reduce the risk of peteca development. However, the slight under application, or erratic non-uniform application of a light wax could also predispose the fruit to loss of quality and cold damage during shipping.

In this trial green and colour break lemons from Larten (Karino) and green lemons and colour break lemons from Sundays River Valley (E.Cape) were treated with Carnauba waxes with different solid levels to determine the effect of these waxes on the possible development of peteca spot after the cold disinfestation (sterilisation) treatment.

Materials and methods

Ten lug boxes of green lemons and ten lug boxes of fully coloured lemons were harvested at Larten, Karino and transported to CRI Nelspruit on 30 March 2005. Ten mixed lug boxes of green and colour break lemons were also transported from Sunday's River Valley (Eastern Cape) to Nelspruit on 30 March 2005.

The lemons from Larten were divided up into equal groups of fully coloured lemons (T1–T2) and green lemons (T6). The lemons from the E.Cape all ranged from green to colour break (T7–T5).

The lemons were treated on the packline by first washing the fruit in the high pressure spray with the quaternary ammonium compound, Prasin. The lemons were then exposed to a high temperature (45°C) in the hot water bath for 1-2 minutes and then dried in the packline drying unit.

After drying the lemons were divided up into 6 replicates x 20 fruit each per treatment.

The following FMC carnauba waxes were used for the wax treatments

1. P.C. Control Full Strength 17-18% solids
2. Base Full Strength 25% solids
3. P.C. Control 14% solids and
4. P.C. Control 10% solids

Treatments

1. Untreated control
2. Treated control - fruit washed, hot water bath and dried
3. As in 2. Waxed with wax 1
4. As in 2. Waxed with wax 2
5. As in 2. Waxed with wax 3
6. As in 2. Waxed with wax 4

The wax was then allowed to dry overnight and the fruit was then stored under simulated cold sterilisation conditions i.e. for 28 days at – 0.6°C. After simulated cold disinfestation the fruit stood at ambient (20°C) for 7 days and was then evaluated for any peteca spot symptoms.

Results and discussion

No lesion development was observed on the Sunday's River Valley (Eastern Cape) lemons after cold disinfestation. The lemons were stored for 4 weeks longer thereafter at 2°C to induce peteca spot/CI. No symptoms were observed and there are thus no results to report.

The results with the Larten lemons indicated that the two Carnauba waxes with the highest total solids levels (18 and 25%) showed the lowest incidence of peteca spot. These two waxes, with high solid levels, are classified as heavy waxes. These results are contrary to what was expected, as the lighter waxes (lower solid levels) are the preferred waxes for peteca-prone lemons (Table 5.2.10.1)

Table 5.2.10.1. The effect of citrus waxes on the development of peteca spot on lemons.

Treatments	Average number of lesions due to peteca and chilling injury ^z	
	Green fruit	Coloured fruit
1. Untreated control	19.0 c	16.5 d
2. Treated control	15.0 b	12.2 c
3. Wax Nr. 1	10.3 a	5.8 a
4. Wax Nr. 2	10.3 a	7.5 ab
5. Wax Nr. 3	16.3 b	12.7 c
6. Wax Nr. 4	15.8 b	10.2 bc

^zValues represent the means of 6 replicates of 20 fruit each. Means in columns followed by the same letter are not significantly different ($P \geq 0.05$) according to Fisher's Unprotected LSD.

Overall, the results in this trial have indicated a number of important issues concerning peteca spot on lemons.

1. Both peteca spot and chilling injury (CI) symptoms were evident on the lemons after cold disinfestation. (Figs. 5.2.10.1- 4)

2. The two waxes with the highest total solids levels (waxes 1 & 2 -heavy waxes) demonstrated the lowest incidence of lesions. The lighter waxes (lowest total solids) are currently expected to give the lowest incidence of peteca, but this is not what occurred in this trial. It has been reported by researchers in Florida (USA) that exposure of fruit to high CO₂ concentrations (e.g. 10%) reduces CI. Fruit respiration uses O₂ and gives off CO₂. Wax coatings slow the movement of O₂ into the fruit and CO₂ out of the fruit. Heavy waxes that form a stronger barrier to gas exchange (e.g. shellac and carnauba waxes with high total solids) reduce CI more than do lighter waxes that “breathe” more (e.g. carnauba waxes with no shellac or lower total solids).

Could it be that what is true for the development of CI is also true for the development of peteca, as far as citrus waxes are concerned, given that both CI and peteca lesions were seen on lemons in this trial?

3. The green lemons indicated the higher incidence of lesions compared to the coloured lemons. Lemons and Marsh grapefruit are among the most sensitive citrus cultivars. Each cultivar has a “picking window” during harvesting. It is a known fact that the picking of lemons and Marsh grapefruit at the beginning (greener fruit) and the end (fully coloured fruit) of their “picking windows” is when the fruit is the most sensitive and thus the most prone to the development of CI, peteca (lemons) and other rind conditions.



Figure 5.2.10.1. Peteca spot and chilling injury symptoms on green lemons.



Figure 5.2.10.2. Peteca spot and chilling injury symptoms on fully coloured lemons.



Figure 5.2.10.3. Peteca spot and chilling injury symptoms on green lemons.



Figure 5.2.10.4. Visible browning and typical symptoms associated with Peteca spot.

Future research

There are still far too many unknowns, both pre- and post-harvest, that lead to the development of peteca spot on lemons. The occurrence of peteca spot on lemons over the last 10 years has been very erratic and this has resulted in inconsistent research being conducted on this disorder. Nevertheless the research will continue and these trials will be repeated until the answers are found.

5.2.11 'Eureka' lemon physiological profile: Storage temperature and storage duration response curves for 'Eureka' lemon harvested at different physiological maturities, with special reference to peteca spot

By Parton Khumalo, Arrie de Kock & Jerome Davids (Experico)

Opsomming

Vrugte is by 'n reeks volwassenheid gepluk, en teen -0.5°C , 4.5°C , 7.5°C , 10°C en 20°C vir 20, 40, 60 en 80 dae opgeberg. Ongelukkig, was daar min Peteca vlek ($<10\%$), wat gevolgtrekkinge moeilik maak. Oor die algemeen, was daar meer Peteco vlek by die onvolwasse vrugte gewees, en die simptome het vroeg in die opbergings tydperk ontwikkel. Daar was ook meer koueskade gewees. Opbergings temperature lyk dus nie die oorsaak van Peteca vlek te wees nie, en die afwyking is nie gedurende die opbergings tydperk geneig om te vermeerde nie. Vanaf die huidige inligting, is dit moontlik dat water verhoudings binne die vrugte dalk 'n rol kan speel, en dat dit verdere navorsing handhaaf.

Introduction

'Eureka' lemon develops various disorders in storage, among which is peteca spot. This disorder manifests as depressions on the fruit rind with normal colour, and then oil glands begin to darken (Murata 1997). Peteca spot reduces the market value of the fruit due to rind deformation, resulting in huge economic losses to the South African citrus industry and also to the citrus industry worldwide. Therefore research aimed at reducing peteca spot is necessary.

Some research has been conducted on peteca spot and largely focused on the effect of fruit waxing. However, progress in solving the disorder has been slow, as peteca spot still occurs from time to time, suggesting that the underlying causes for peteca spot are still not understood.

To contribute to our understanding of peteca spot this experiment was set up with the objective of establishing the effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested at different maturities.

Materials and methods

Trial site detail:

Fruit were harvested from healthy 15 year old 'Eureka' lemon trees, grafted on rough lemon, in Franschoek (Imibala).

Treatments

Fruit were harvested at three maturity stages, namely early harvest (25 April 2005), mid harvest (20 May 2005) and late harvest (23 June 2005). At each harvest date ~600 kg of fruit were harvested into lug boxes. The fruit was wilted for ~24 hours in the orchard and then transported to the packhouse for waxing and packing. Fruit for this experiment was not degreened but packed immediately on arrival at the packhouse. During packing the fruit was moved through a commercial pack-line and a light natural wax was applied. Waxed fruit was then packed into the MO7T telescopic pear carton (7 kg). A total of 80 cartons were packed at each harvest date. The 80 cartons of fruit packed at each harvest date were divided into four groups of 20 cartons each and stored at -0.5, 4.5, 7.5 and 11°C. The 20 cartons of fruit at each storage temperature were further divided into 4 groups of five cartons each (replicates) corresponding to storage durations of 20, 40, 60 and 80 days. After each storage duration a shelf-life period of 7 days at 20°C was implemented. Evaluations were conducted before and after shelf-life. An additional 5 cartons of fruit from each harvest date were stored at ±20 °C and evaluated at the same times as the other cartons

Evaluation stages and parameters

At harvest (conducted on 10 fruit per replicate)

Rind colour, SSC (°brix), TA (%), mineral nutrient status, rind moisture content (%) and rind strength (N).

Before and after shelf life

Peteca spot incidence (%), chilling injury incidence (%), decay (%), rind colour and internal quality.

Statistical detail

The trial was laid out a randomised complete block design with three harvest maturities replicated 5 times. Three-tree plots was used in the orchard. Data collected after storage were analysed as a two-way ANOVA, on STATISTICA, where Factor A = storage temperature and Factor B = storage duration. Results from each harvest maturity were analysed separately.

Results and discussion

Early harvest (25 April 2005)

At harvest

Fruit harvested on the 25 April 2005 is shown in Figure 5.2.11.1. The quality parameters and mineral nutrient status of the fruit rind measured at harvest are presented in Table 5.2.11.1 and indicate a rind colour rating of 5.7 and an SSC of 7.3° Brix.

After cold storage

No peteca spot occurred on fruit when evaluated immediately after cold storage (Table 5.2.11.2). The 'Eureka' lemon fruit used in this experiment was sensitive to low temperature storage. Chilling injury occurred in fruit stored at temperatures below 10°C. The levels of this disorder increased over time in storage and reached ~100% in fruit stored for 60 days and longer at the temperatures -0.5, 4.5 and 7.5°C. Decay levels were low in fruit stored at the different temperatures for up to 60 days. Thereafter levels significantly increased in fruit stored at 7.5 and 10°C to 17.0% and 9.5%, respectively. A significant interaction was observed between storage temperature and storage duration on the colour rating of fruit. The rind colour

rating of fruit stored at -0.5 and 4.5°C did not change over time in storage. However, in fruit stored at 7.5, 10.0 and ±20°C the rind colour rating improved over time, indicating that fruit stored at these temperatures changed colour from green to yellow in storage.

After shelf-life

The levels of peteca spot were relatively high early in storage (20 days) in most of the storage temperatures. None or very low additional peteca spot developed with extended storage suggesting that the peteca spot was not progressive in storage (Table 5.2.11.3). Across temperatures, peteca spot did not show a definite trend of increasing or decreasing. The sensitivity of 'Eureka' lemon to low temperature storage was again demonstrated after the shelf-life period, with fruit stored at -0.5, 4.5 and 7.5°C developing chilling injury and the levels of this disorder increased over time in storage. Decay was low across temperatures, for up to 40 days of storage. Thereafter the levels increased in fruit stored at 7.5°C and 10°C but not at the other storage temperatures. The rind colour rating of fruit stored at -0.5 and 4.5°C did not change over time in storage. However, in fruit stored at 7.5, 10.0 and ±20°C the rind colour rating improved with time, indicating that fruit stored at these temperatures changed colour from green to yellow in storage. SSC of fruit was significantly affected by storage temperature, with fruit stored at 10°C having a statistically significant but marginally (<0.4°brix) higher SSC than fruit stored at the other temperatures. The SSC of fruit did not change significantly over time in storage. The titratable acidity was not significantly affected by storage temperature or storage duration. Fruit stored at -0.5 and 4.5°C had a stronger rind than fruit stored at 7.5 and 10°C.

Mid harvest (20 May 2005):

At harvest

The fruit quality parameters and rind mineral nutrient status measured at harvest are presented in Table 5.2.11.4, and indicate a rind colour rating of 3.1 and a SSC of 6.6 °Brix.

After cold storage

Storage temperature and storage duration did not significantly affect the development of peteca spot in fruit evaluated immediately after cold storage (Table 5.2.11.5). Chilling injury developed in fruit stored at -0.5 and 4.5°C, with low or none reported in fruit stored at higher temperatures. Chilling injury on fruit was low early in storage and levels increased with time to 100% after 80 days storage, in fruit stored at -0.5 and 4.5°C. Decay developed in fruit stored at the higher temperatures, 7.5, 10 and ±20°C. The levels of decay tended to increase over time in storage at these temperatures. A significant interaction was observed between storage temperature and storage duration on the colour rating of fruit. The rind colour rating of fruit stored at -0.5 and 4.5°C did not change over time in storage. However, fruit stored at 7.5, 10.0 and ±20°C had a lower rind colour rating which decreased over time, indicating that fruit stored at these temperatures changed colour from green to yellow in storage.

After shelf-life

Low levels of peteca spot were observed in fruit after the shelf-life period, and the occurrence of this disorder was not significantly affected by storage temperature or storage duration (Table 5.2.11.6). A significant interaction between storage temperature and storage duration was observed on the development of decay. However, only fruit stored at 4.5°C for 80 days developed significantly higher levels of decay than fruit from the other treatments. The rind colour rating of fruit stored at -0.5°C and 4.5°C showed a marginal change over time with no definite trend. However, fruit stored at 7.5°C, 10 °C and ±20°C had a lower colour rating which decreased in storage, indicating that fruit stored at these temperatures became yellow over time in storage. A significant interaction was observed between storage temperature and storage duration SSC and TA of fruit. In fruit stored at -0.5°C, 4.5°C and 10°C the SSC and TA tended to decrease over time in storage. However, in fruit stored at 7.5°C the SSC and TA increased over time. Rind strength was highest in fruit stored at -0.5°C than in fruit stored at the other temperatures. Rind strength was significantly affected by storage duration but variation over time did not show a definite trend.

Late harvest (23 June 2005):

At harvest

Fruit harvested on the 23 June 2005 is shown in Figure 5.2.11.2. Quality parameters and rind mineral nutrient status of fruit measured at harvest are shown in Table 5.2.11.4, and indicate a rind colour rating of 1.8.

After cold storage

Peteca spot was significantly higher in fruit stored at 7.5°C and in fruit stored at ±20°C than in fruit stored at the other temperatures (Table 5.2.11.8). However, the peteca spot incidence was <2% in fruit from this maturity, which is considered commercially low. Chilling injury was observed and increased significantly over

time in fruit stored at -0.5°C , with none or low levels observed in fruit stored at the other temperatures. Decay development of fruit was low early in storage (20 days), irrespective of storage temperature. The decay levels increased with extended storage at most temperatures, with the highest decay incidence occurring in fruit stored at 4.5°C . Rind colour rating decreased with increasing storage temperature and in most cases also decreased over time in storage. However, the decreasing rind colour value of fruit stored at -0.5°C and 4.5°C may have been influenced by chilling injury occurring on the fruit. Chilling injury fruit harvested at an advanced yellow colour and then stored at -0.5 or 4.5 may result in colour bleaching, which is the possible reason why rind colour rating of fruit stored at these temperatures decreased over time in storage.

After shelf-life

A significant interaction was observed between storage temperature and storage duration on the development of peteca spot in late harvested fruit (Table 5.2.10.9). However, low levels ($<5\%$) were observed in this fruit and the development of the disorder showed no definite trend. Chilling injury occurred after 20 days in fruit stored at low temperatures and increased significantly after 40 days, compared to none or lower levels that occurred in fruit stored at the other temperatures. No chilling injury was recorded in fruit after 60 days storage. This was ascribed to the fact that decay developed over the chilling injury lesions, thus masking the disorder. Decay levels were significantly higher in fruit stored for 60 days at -0.5°C and at 4.5°C compared to fruit from the other treatments. A significant interaction was observed between storage temperature and storage duration on rind colour rating of fruit. However, no definite trend could be observed on colour development as this fruit already had an advanced yellow colour at harvest. Changes over time in internal quality and rind strength of fruit stored at the different temperatures did not show a definite trend.

Most mineral nutrients and the rind strength decreased the later the harvest date and consequently with increasing harvest maturity. Differences in rind colour at harvest were also evident in fruit from the different maturities, with early harvested fruit being greener than fruit from the subsequent harvests. Results measured after storage showed that fruit harvested early in the season tended to develop more peteca spot and chilling injury than fruit harvested later. However, the reverse occurred for decay. Over time in storage, fruit harvested late in the season was more susceptible to decay. Consequently fruit harvested on the 23 June 2005 could not be evaluated after 80 days storage due to the high decay incidence in some of the treatments.

Conclusions

From the results of this experiment, it can be concluded that:

- (1) Storage temperature was not the main cause for peteca spot. Therefore, storage temperature cannot provide a solution for peteca spot in 'Eureka' lemon.
- (2) Peteca spot is a disorder that develops early during the storage life of a fruit and it is not progressive.
- (3) Early harvested fruit was most susceptible to peteca spot and chilling injury. Therefore, allowing the fruit to advance in maturity on the tree may reduce both peteca spot and chilling injury incidence. Furthermore, future experiments aimed at solving peteca spot should make use of early harvested fruit instead of the more mature population, harvested later.
- (4) Generally low levels of peteca spot ($<10\%$) were recorded in this experiment. However, the farm from which the fruit was harvested recorded medium to high levels of peteca spot in the early harvested fruit (Imibala, 2004 personal communication). The only difference between experimental and commercial fruit was in the postharvest handling. Experimental fruit was not degreened therefore did not experience fluctuations in RH during handling. Whereas, commercial fruit was degreened and therefore subjected to fluctuations in RH during handling.

Future research

This experiment has successfully identified the fruit maturity most susceptible to peteca spot and eliminated storage temperature as the possible cause for this disorder. It is therefore suggested that future experiments aimed at solving peteca spot should focus on rind water relations and fluctuations in RH during handling of 'Eureka' lemon fruit.



Figure 5.2.11.1. 'Eureka' lemon fruit harvested on 25 April 2005.



Figure 5.2.11.2. 'Eureka' lemon fruit harvested on 23 June 2005.

Table 5.2.11.1. Harvest maturity of 'Eureka' lemon fruit sampled on 25 April 2005.

Rind colour rating ¹	5.7
TSS (%)	7.3
TA ²	5.9
N (mg/100g fresh mass)	219.6
P (mg/100g fresh mass)	16.1
K (mg/100g fresh mass)	261.6
Ca (mg/100g fresh mass)	324.2
B (mg/kg fresh mass)	5.3
Rind moisture (%)	78.7
Rind strength (N)	28.4

¹ Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

² Titratable citric acid

Table 5.2.11.2. Effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested on 25 April 2005 and evaluated before shelf-life.

Response variable	Storage duration (Days) ¹	Storage temperature (°C) [Factor A]					Storage duration (Days) [Factor B]				Prob>F ³		
		-0.5	4.5	7.5	10.0	±20	20	40	60	80	A	B	AxB
Peteca Spot (%)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-
Chilling injury (%)	20	0.0a ²	0.0a	0.0a	0.0a	0.0a					***	***	***
	40	69.9d	49.0c	28.0b	0.0a	0.0a							
	60	100.0f	100.0f	100.0f	0.0a	0.0a							
	80	100.0f	100.0f	83.0e	0.0a	0.0a							
Decay (%)	20	0.0a	0.0a	0.0a	0.4a	0.0a					***	***	***
	40	0.0a	0.0a	0.5a	0.0a	0.0a							
	60	0.0a	0.0a	0.0a	0.8a	0.0a							
	80	0.0a	0.0a	17.0c	9.5b	0.0a					***	***	***
Rind rating ⁴ colour	20	5.6j	5.3hij	4.4g	3.8f	3.3e							
	40	5.2hi	5.3hij	3.6f	3.3e	2.0c							
	60	5.2hi	5.4ij	2.5d	1.1ab	1.2ab							
	80	5.1h	nd ⁵	2.1c	1.0a	1.3b							

- 1 If itemised interaction occurred between factor A and B
- 2 Values in the same row or column followed by different letters indicate significant differences (P<0.05) according to the LSD test.
- 3 Two-way ANOVA with factor A being storage temperature and factor B being storage duration
- 4 Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)
- 5 Rind colour could not be measured because of bleached rind colour due to chilling injury

Table 5.2.11.3. Effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested on 25 April 2005 and evaluated after shelf-life.

Response variable	Storage duration (Days) ¹	Storage temperature (°C) [Factor A]					Storage duration (Days) [Factor B]				Prob>F ³		
		-0.5	4.5	7.5	10.0	±20	20	40	60	80	A	B	AxB
Peteca Spot (%)	20	3.5cd	3.8d	7.6e	4.0d	0.4a					***	***	**
	40	0.0a	1.5ab	2.9bcd	1.2ab	0.0a							
	60	0.0a	0.0a	0.0a	0.0a	0.0a							
	80	0.0a	0.0a	0.0a	0.0a	0.0a							
Chilling injury (%)	20	3.1a	2.1a	0.0a	0.0a	0.0a					***	***	***
	40	65.2c	60.8c	30.8b	0.0a	0.0a							
	60	100.0f	99.6f	89.6e	0.0a	0.0a							
	80	100.0f	100.0f	83.0d	0.0a	0.0a							
Decay (%)	20	0.0a	0.0a	0.0a	0.4a	0.0a					***	***	***
	40	0.0a	0.9a	1.0a	0.5a	0.0a							
	60	0.0a	0.4a	10.5b	0.8a	0.0a							
	80	0.0a	0.0a	17.0c	9.5b	0.0a							
Rind colour rating ⁴	20	5.0g	5.1g	3.7f	3.0e	2.8e							
	40	4.9g	5.1g	2.8e	1.0a	1.2bc							
	60	4.9g	4.9g	2.3d	2.0d	1.2bc							
	80	5.1g	nd ⁵	2.1d	1.0a	1.3c							
SSC (°Brix)		7.2a	7.2a	7.1a	7.4b	nd ⁶	7.3	7.2	7.2	nd	**	NS	NS
TA (%)		5.7	5.7	5.6	6.1	Nd	5.9	5.9	5.6	nd	NS	NS	NS
Rind strength (N)		31.2b	31.8b	27.6a	26.6a	Nd	29.5	28.3	30.1	nd	**	NS	NS

¹ If itemised interaction occurred between factor A and B

² Values in the same row or column followed by different letters indicate significant differences (P<0.05) according to the LSD test

³ Two-way ANOVA with factor A being storage temperature and factor B being storage duration

⁴ Rind colour rating determined using the CRI rind colour chart set number 37, where 8 = poor coloured, dark green with no colour break, and 1= yellow and fully coloured fruit (CRI, 2004)

⁵ Result could not be measured because fruit rind was bleached due to high chilling injury

⁶ Internal quality not measured in fruit stored at ambient

⁷ Internal quality could not be measured after 80 days storage due to fruit softness because of chilling injury

Table 5.2.11.4. Harvest maturity of 'Eureka' lemon fruit sampled on 20 May 2005.

Rind colour rating ¹	3.1
TSS (%)	6.6
TA ²	6.1
N (mg/100g fresh mass)	168
P (mg/100g fresh mass)	13.6
K (mg/100g fresh mass)	210.4
Ca (mg/100g fresh mass)	77.7
B (mg/kg fresh mass)	4.3
Rind moisture (%)	83.5
Rind strength (N)	22.5

1 and 2 See Table 5.2.11.1 for definitions.

Table 5.2.11.5. Effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested on 20 May 2005 and evaluated before shelf-life.

Response variable	Storage duration (Days) ¹	Storage temperature (°C) [Factor A]					Storage duration (Days) [Factor B]				Prob>F ³		
		-0.5	4.5	7.5	10.0	±20	20	40	60	80	A	B	AxB
Peteca Spot (%)		1.0	0.1	1.5	0.7	1.7	0.7	1.1	1.3	0.9	NS	NS	NS
Chilling injury (%)	20	0.5a ²	0.5a	0.0a	0.0a	0.0a					***	***	***
	40	2.9ab	6.7b	0.6a	0.0a	0.0a							
	60	97.7d	87.1c	0.0a	0.0a	0.0a							
	80	100.0d	100.0d	0.0a	0.0a	0.0a							
Decay (%)	20	0.0a	0.0a	2.4ab	1.4ab	1.3ab					***	*	*
	40	0.0a	0.7a	9.6cd	2.9ab	2.4ab							
	60	0.0a	0.0a	6.9bc	9.7cd	3.7ab							
	80	0.0a	0.0a	5.8abc	15.4d	2.3ab							
Rind colour rating ⁴	20	4.3efg	3.7d	2.8b	3.2c	2.8b					***	***	***
	40	4.5fg	4.8g	2.4b	1.2a	1.3a							
	60	4.5efg	4.5efg	1.1a	1.3a	1.2a							
	80	4.2ef	4.1de	1.1a	1.1a	1.0a							

1-4 See Table 5.2.11.2 for definitions.

Table 5.2.11.6. Effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested on 20 May 2005 and evaluated after shelf-life.

Response variable	Storage duration (Days) ¹	Storage temperature (°C) [Factor A]					Storage duration (Days) [Factor B]				Prob>F ³		
		-0.5	4.5	7.5	10.0	±20	20	40	60	80	A	B	AxB
Peteca Spot (%)		1.3	0.3	1.8	1.8	1.8	1.9	1.6	0.6	1.4	NS	NS	NS
Chilling injury (%)	20	0.5a	0.5a	0.0a	0.0a	0.0a					***	***	***
	40	12.3b	24.1c	1.0a	0.0a	0.0a							
	60	94.5e	94.0e	0.0a	0.0a	0.0a							
	80	95.3e	77.9d	0.0a	0.0a	0.0a							
Decay (%)	20	0.0a	0.0a	2.3a	3.6a	1.2a					***	**	***
	40	1.3a	3.6a	3.1a	1.4a	0.0a							
	60	5.5a	6.0a	4.1a	0.4a	0.0a							
	80	4.7a	22.6b	1.3a	0.8a	2.3a							
Rind rating ⁴ colour	20	4.0gh	3.6f	3.2e	2.6d	2.0c					***	***	***
	40	3.7fg	4.4i	1.9c	1.2ab	1.3ab							
	60	4.0gh	4.0gh	1.5b	1.0a	1.2ab							
	80	nd ⁵	nd	1.0a	1.0a	1.0a							
SSC (°Brix)	20	6.80de	6.94e	6.58abc	6.92e	nd					***	***	**
	40	6.64cd	6.64cd	6.40a	6.88e	nd							
	60	6.54abc	6.44ab	6.64cd	6.60bc	nd							
	80	nd ⁶	nd	nd	nd	nd							
TA (%)	20	5.80bdc	5.82bcd	5.98cd	6.08de	nd					***	NS	**
	40	5.66abc	5.58ab	5.71abcd	5.98cd	nd							
	60	5.50ab	5.38a	6.36e	5.62abc	nd							
	80	nd ⁶	nd	nd	nd	nd							
Rind strength (N)		25.4b	23.8ab	22.5a	22.8a	nd	22.7a	24.6b	23.7ab	nd ⁷	**	*	NS

1 – 7 See Table 5.2.11.3 for definitions.

Table 5.2.11.7. Harvest maturity of 'Eureka' lemon fruit sampled on 23 June 2005.

Rind colour rating ¹	1.8
TSS (%)	6.1
TA ²	5.2
N (mg/100g fresh mass)	96.0
P (mg/100g fresh mass)	11.8
K (mg/100g fresh mass)	175.0
Ca (mg/100g fresh mass)	161.0
B (mg/kg fresh mass)	3.1
Rind moisture (%)	90.6
Rind strength (N)	15.3

1 and 2 See Table 5.2.11.1 for definitions.

Table 5.2.11.8. Effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested on 23 June 2005 and evaluated before shelf-life.

Response variable	Storage duration (Days) ¹	Storage temperature (°C) [Factor A]					Storage duration (Days) [Factor B]			Prob>F ³		
		-0.5	4.5	7.5	10.0	±20	20	40	60	A	B	AxB
Peteca Spot (%)		0.0a	0.2a	1.4b	0.0a	1.9b	0.7	1.0	0.5	***	NS	NS
Chilling injury (%)	20	0.9a	0.0a	0.0a	0.0a	0.0a				***	***	***
	40	4.5b	0.9a	0.0a	0.0a	0.0a						
	60	23.1c	0.0a	0.0a	0.0a	0.0a						
Decay (%)	20	0.0a	0.0a	0.0a	0.5a	3.3ab				***	***	***
	40	0.0a	0.0a	5.5abc	9.2bcd	2.2ab						
	60	12.4cd	47.7e	16.9d	12.1cd	0.0a						
Rind colour rating ⁴	20	3.0e	3.0e	2.5d	1.8c	1.0a				***	***	***
	40	2.6d	2.0c	1.1ab	1.0a	1.0a						
	60	1.1ab	1.4b	1.0a	1.0a	1.0a						

1-4 See Table 5.2.11.2 for definitions.

Table 5.2.11.9. Effect of storage temperature and storage duration on the quality of 'Eureka' lemon fruit harvested on 23 June 2005 and evaluated after shelf-life.

Response variable	Storage duration (Days) ¹	Storage temperature (°C) [Factor A]					Storage duration (Days) [Factor B]			Prob>F ³		
		-0.5	4.5	7.5	10.0	±20	20	40	60	A	B	AxB
Peteca Spot (%)	20	0.0a	0.0a	2.0ab	0.0a	1.6ab				**	NS	*
	40	0.0a	0.0a	4.4c	0.0a	1.8ab						
	60	0.0a	0.4ab	0.4ab	2.5b	1.7ab						
Chilling injury (%)	20	11.5b	1.7a	1.7a	0.0a	0.0a				***	***	***
	40	33.2c	13.4b	0.0a	0.0a	0.0a						
	60	0.0a	0.0a	0.0a	0.0a	0.0a						
Decay (%)	20	4.5a	0.6a	0.9a	1.0a	1.2a				***	***	***
	40	8.5a	1.4a	1.9a	4.0a	2.2a						
	60	100.0c	79.0b	8.3a	4.5a	5.8a						
Rind colour rating ⁴	20	1.9c	1.9c	1.1a	1.0a	1.0a				***	***	***
	40	2.3d	2.0c	1.0a	1.0a	1.0a						
	60	nd ⁵	1.7b	1.0a	1.0a	1.0a						
SSC (°Brix)	20	6.5bc	6.3b	6.8c	6.8c	nd ⁶				NS	**	***
	40	6.6bc	6.4b	6.5bc	6.5bc	nd						
	60	6.4b	6.5bc	6.4bc	5.8a	nd						
TA (%)	20	5.3bcd	5.3bc	5.8e	5.7de	nd				***	***	***
	40	5.5bcd	5.2b	5.8e	5.9e	nd						
	60	3.9a	5.7cde	5.5bcde	5.6bdce	nd						
Rind strength (N)	20	16.3ab	17.5bc	18.2c	18.2c	nd				NS	*	*
	40	17.2bc	17.8bc	17.9bc	18.1bc	nd						
	60	17.5bc	17.6bc	16.7abc	15.0a	nd						

1-4 See Table 5.2.11.2 for definitions.

5 Result could not be measured because of high chilling injury and decay.

6 Internal quality not measured in fruit stored at ambient.

5.3 **PROJECT: FRUIT QUALITY ENHANCEMENT** Project Co-ordinator: Graham H. Barry (CRI at SU)

5.3.1 **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 project is to provide Southern African citrus producers with cultural practices that assist in producing superior product quality.

Sunburn of Satsuma mandarin (*Citrus unshiu* Marc.) fruit and fruit of other sunburn-susceptible cultivars of *Citrus* can result in commercially significant crop losses. Therefore, reducing sunburn incidence and severity, thereby improving packout, will have a direct and positive effect on improving grower returns. The application of Surround® particle film as an early, double spray during mid-summer, prior to the occurrence of hot, dry weather conditions (+32 °C; <60% RH), effectively eliminated sunburn incidence on Miho Wase Satsuma mandarin fruit. Under more severe sunburn conditions than experienced in the 2004-05 season, the effect of Surround is expected to be greater than that reported here. In addition, sugar content was also higher in fruit from Surround®-treated trees. Combined with the positive effect on rind colour development, it appears as though Surround treatment advanced fruit maturity (5.3.2).

During the 2004-05 season, various concentrations of MAP applied to Delta Valencia orange did not significantly reduce acidity, although 2% MAP resulted in a numerically lower acidity. This result is contrary to results from 2002 and 2003 where 1% MAP applied 6 weeks after full bloom did reduce TA (5.3.3).

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°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. Preharvest applications of Ph-Ca improved citrus rind colour on all cultivars tested except Eureka lemon. These results were more significant immediately after harvest than after storage, with some significant difference after degreening. The differences in colour rating, colorimeter reading and pigment concentration between treated and non-treated trees could make it possible to harvest fruit earlier in the season. The critical finding from the research has been the effect of Ph-Ca on chlorophyll degradation and carotenoid synthesis (5.3.4).

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 UKZNP. Within the year 2005, pigments (chlorophylls and carotenoids) were extracted from 'Navel' and 'Turkey' oranges treated at colour-break with hot water and hydro-cooling. Skin colour changes were noted and the pigment concentration was determined spectrophotometrically. Some treated fruit accumulated significantly higher carotenoid concentrations than untreated (control) fruit. As a correlation between concentration of certain fruit micro-nutrients and the ability of fruit to synthesize carotenoids is expected, micronutrient analysis in soil and leaf samples was carried out using Inductively Coupled Plasma Emission Spectrometry (ICP-ES). Concentrations of Mo and W in soils were below the detectable limits. A significant concentration of added Mo and W, 102% and 99% respectively, was recovered from leaf samples. Mo and W at three levels (0, 0.202 and 0.404 g per tree) were foliar sprayed 19 weeks after full bloom in 2005. The analysis of samples of leaves and fruit from untreated and treated trees (with Mo and W) is already in progress (5.3.5).

Projekopsomming

Die vereiste om aan die minimum verlangde kwaliteit te voldoen en om suksesvol sitrus produkte te bemark word nou vervang deur die noodsaaklikheid om produkte van uitstekende kwaliteit, met die klem op voorkoms en eetbaarheid te produseer. Wanneer die aanbod van sitrus die aanvraag oorskry word produk differensiasie al meer belangrik om gewaarborgde verkope te handhaaf. Die doel van die vrugkwaliteit

verbeteringsprogram van die CRI is om die Suider Afrikaanse sitrus produsente met verbouings praktyke te voorsien wat kan dien as hulpmiddel in die produksie van uitstekende kwaliteit produkte.

Sonbrand van Satsuma mandaryne (*Citrus unshiu* Marc.) en ander kultivars wat sensitief vir sonbrand is, kan lei tot ernstige kommersiële oesverliese. Die vermindering van die voorkoms en graad van sonbrand, sal lei tot 'n verhoogde uitpakkersentasie, wat dan 'n direkte en positiewe effek op die produsent se inkomste sal hê. Die aanwending van 'n Surround® partikel-laag as 'n vroeë, dubbele bespuiting gedurende die mid-somer, voor warm en droë toestande heers (+32 °C; <60% RH), het sonbrand skade op Miho Wase Satsuma mandaryn vrugte ge-elimineer. Tydens meer gunstige kondisies vir sonbrand as in die 2004-2005 seisoen, word verwag dat die effek van Surround® groter sal wees op die vermindering van sonbrand as wat hier gerapporteer word. 'n Bykomende voordeel was 'n verhoging in die suikerinhoud van vrugte op Surround® behandelde bome. Addisioneel tot die positiewe effek op vrugkleurontwikkeling, lyk dit of Surround® vrugrypwording versnel het (5.3.2).

Gedurende die 2004-05 seisoen is MAP toegedien op Delta Valencia bome teen verskillende konsentrasies. Alhoewel MAP nie die suurvlaakte betekenisvol verlaag het nie, het 2% MAP gelei tot suurvlaakte wat numeries laer as die kontrole was. Die resultate is teenstrydig met die resultate van 2002 en 2003, waar 1% MAP, toegedien 6 weke na volblom, wel die suurinhoud verlaag het (5.3.3).

Skilkleur ontwikkeling van sitrus in Suid Afrika is 'n probleem, veral in die vroeër kultivars a.g.v. die herfs temperature wat te hoog is vir chlorofil afbraak en karotenoid sintese. Sitrusvrugte verlang nagtemperature van <13°C vir optimale kleur ontwikkeling. Dit is heel moontlik nie die koue nag temperature *per se* wat vir die kleurontwikkeling verantwoordelik is nie. Dit blyk egter of die lae nagtemperature vegetatiewe ontwikkeling stop. Laasgenoemde het 'n antagonistiese uitwerking op chloroplast omskakeling na chromoplast en sodoende word chlorofil afbraak en karotenoid sintese vertraag. Om hierdie rede kan tegnieke wat die oordadige vegetatiewe groei gedurende die laat somer en vroeë herfs beperk, 'n betekenisvolle rol speel in pogings om skilkleurontwikkeling te bevorder. Interne gibberellie bevorder vegetatiewe groei in plante en derhalwe kan 'n gibberellin biosintese inhibeerder die omskakeling van chloroplast na chromoplast bevorder. Vooroets toediening van prohexadione kalsium (Ph-Ca) het skilkleur verbeter op alle kultivars wat getoets is, behalwe op Eureka suurlemoen. Die resultate was meer betekenisvol net na oes as na opberging, met sommige betekenisvolle verskille na ontgroening. Die verskille in kleurkaartelling, colorimeter lesings en pigmentkonsentrasies tussen behandelde en onbehandelde bome kan dit moontlik maak om vrugte vroeër in die seisoen te oes. Die kritiese punt van die navorsing is die effek van Ph-Ca op chlorofil afbraak en karotenoid sintese (5.3.4).

Die effek van boom voedingstatus en na-oes behandelings op die konsentrasie van die belangrikste karotenoides betrokke by kleur in volwasse sitrusvrugte vorm die basis van die PhD studies van Molipa Mosoeunyane by UKZNP. In 2005 is pigmente (chlorofil en karotenoides) ge-ekstraheer van Navel en Turkey lemoene wat behandel is by kleurbreek met warm water of hidro verkoeling. Skilkleur veranderinge is waargeneem en die pigment konsentrasie is spektrofotometries bepaal. Sommige behandelde vrugte het betekenisvolle hoër karotenoid konsentrasies as onbehandelde (kontrole) vrugte gehad. Omdat 'n korrelasie tussen die konsentrasie van sekere mikro-elemente en die vermoë van vrugte om karotenoides te sintetiseer verwag word, is die mikro-element analises in die grond en blare uitgevoer deur Gekoppelde Plasma Uitstralings Spektrometrie. Mo en W konsentrasies in die grond was laer as meetbare vlakke. 'n Betekenisvolle konsentrasie van toegedienende Mo en W, 102% en 99% respektiewelik, is in blaar monsters gemeet. Mo en W teen drie vlakke (0, 0.202 en 0.404 g per boom) is toegedien as blaarbespuitings 19 weke na volblom in 2005. Die analises van blaar- en vrugmonsters van onbehandelde en behandelde bome (met Mo en W) is onderweg (5.3.5).

5.3.2 Reduction in sunburn incidence of Satsuma mandarin Graham H. Barry (CRI at SU)

Opsomming

Sonbrand van Satsuma mandaryne (*Citrus unshiu* Marc.) en ander kultivars wat sensitief vir sonbrand is, kan lei tot ernstige kommersiële oesverliese. Die vermindering van die voorkoms en graad van sonbrand, sal lei tot 'n verhoogde uitpakkersentasie, wat dan 'n direkte en positiewe effek op die produsent se inkomste sal hê. Die aanwending van 'n Surround® partikel-laag as 'n vroeë, dubbele bespuiting gedurende die mid-somer, voor warm en droë toestande heers (+32 °C; <60% RH), het sonbrand skade op Miho Wase Satsuma mandaryn vrugte ge-elimineer. Tydens meer gunstige kondisies vir sonbrand as in die 2004-2005 seisoen, word verwag dat die effek van Surround® groter sal wees op die vermindering van sonbrand as wat hier gerapporteer word. 'n Bykomende voordeel was 'n verhoging in die suikerinhoud van vrugte op

Surround® behandelde bome. Addisioneel tot die positiewe effek op vrugkleurontwikkeling, lyk dit of Surround® vrugrypwording versnel het.

Introduction

Sunburn of Satsuma mandarin (*Citrus unshiu* Marc.) fruit and fruit of other sunburn-susceptible cultivars of *Citrus* can result in commercially significant crop losses. Sunburn incidence of Satsuma mandarin in the Western Cape Province of South Africa typically results in loss of packout by about 10%. Frequently this loss is not even quantified or accounted for as fruit are removed prior to harvest or selected out at harvest and not sent to the packinghouse (pers. obs.). Reducing sunburn incidence and severity, thereby improving packout, will have a direct and positive effect on improving grower returns.

Sunburn invariably develops on fruit exposed to late afternoon sun, i.e. on the west side of trees, on the sun-facing side of fruit. The most damaging period of intense solar radiation appears to be between the summer solstice and autumnal equinox, i.e. between 22 December and 22 March in the southern hemisphere. Evidently, nonacclimated fruit or nonacclimated portions of exposed fruit are prone to sunburn damage during hot, dry summer conditions.

Glenn et al. (2002) showed that particle film technology using processed kaolin (Surround®) suppressed sunburn of apples (*Malus sylvestris* var. *domestica*) through reduced fruit temperature, possibly via increased reflection of the UV wavelengths. Various reflective substances and whitewashes have previously been tested in an attempt to reduce sunburn in Satsuma mandarin in South Africa (E. Rabe, J.P. Wahl, pers. comm.). However, these products were ineffective, or did not wash off easily in the packinghouse. Surround®, however, appears to have the right specifications for the protection of fruit against sunburn.

In the 2003 season, a preliminary trial showed that sunburn incidence could be reduced and juice Brix content increased following the application of Surround in mid-summer. In the 2004 season, these findings were confirmed (Barry, 2005) and, as a result, semi-commercial application of Surround® was recommended as a tool to reduce the incidence of sunburn in Satsuma mandarins. Therefore, the objective of the current study was to confirm the previous findings under semi-commercial conditions.

Materials and methods

Surround® was applied as a commercial treatment to a 1 hectare orchard of Miho Wase Satsuma mandarin which was planted in Nov. 1991 at Welgevallen Experimental Farm in Stellenbosch, South Africa. Surround® was applied as a double application on 13 and 27 Dec. 2004 at 3 kg/100 ℓ water, i.e. 3% solution. However, due to the relatively poor coverage, an additional application was made on 24 Jan. 2005. In all cases 25 ml Agral 90® was included in the spray mixture as an adjuvant.

Approximately 2500 ℓ/ha of spray material was applied as a medium-cover spray using a high-pressure hand-held spray gun, resulting in an application rate of 225 kg Surround® per hectare (Fig. 5.3.2.1).

Prior to maturity, on 24 Mar. 2005, and again on 5 Apr. 2005 when the final fruit harvest occurred, 10 fruit of similar size from the east side of eight trees were selected for rind colour and internal fruit quality determination. At maturity, fruit were selectively harvested according to colour development on 5 Apr. 2005 when yield (total weight and fruit number) and sunburn incidence (fruit weight and number) were determined. Ten fruit of each of the Surround and control treatments were collected from a commercial packinghouse after commercial postharvest treatment and stored at ambient temperature and RH to assess weekly weight loss changes.

Since the determination of sunburn incidence and fruit sampling for fruit quality variables was conducted in a Miho Wase Satsuma mandarin orchard that received a commercial spray application of Surround®, treatments were not randomly allocated to trees. Therefore, the statistical requirement of randomly allocating treatments to experimental units could not be met. However, site variability and tree-to-tree variability between treated and untreated plots was considered to be relatively low. Therefore, data were subjected to analysis of variance as a completely randomised design and treatment means were compared by least significant difference.

Results and discussion

Between Dec. 2004 and Mar. 2005 there were five periods of relatively high maximum air temperature (>32 °C) combined with low relative humidity (<40% RH), viz. 4 days between 16 and 24 Dec. 2004

(32.5-33.8 °C, <40% RH), 2 days during 28 Dec. 2004 to 2 Jan. 2005 (32.5-32.9 °C, <45% RH), 10 days from 9 to 18 Jan. 2005 (32-38.5 °C, 17-48% RH), 2 days during 2 and 6 Feb. 2005 (35 °C, 26-40% RH), and 3 days during 20 to 27 Mar. 2005 (33.5 °C, 37% RH). The most severe weather conditions for sunburn were during the 10-day period from 9 to 18 Jan. 2005.

Sunburn incidence of untreated control trees was moderate (>9% of total fruit yield) in the 2004-05 season, yet of commercial significance (Table 5.3.2.1). The number of sunburnt fruit on the Surround-treated trees was less than one-fourth of the sunburnt fruit on the untreated, control trees. Sunburn generally affected the larger fruit (>90 g) on the trees.

Although fruit of similar size were sampled from both untreated control and Surround-treated trees, fruit sampled from the Surround-treated trees were significantly larger (by about 4 mm) than fruit from the untreated control trees (Table 5.3.2.2). Surround did not affect juice content or titratable acidity (TA). On the first two sampling dates (29 Mar. and 5 Apr.), however, Surround treatment tended to increase Brix and the Brix:TA ratio and improved rind colour (lower rating) (Table 5.3.2.2; Fig. 5.3.2.2). On the final sampling date (11 Apr.), when some of the fruit had already been selectively harvested, there was no difference in fruit quality variables of fruit that remained on the trees (Table 5.3.2.2; Fig. 5.3.2.2).

Fruit weight (moisture) loss did not differ over 5 weeks between treatments (Fig. 5.3.2.3). Surround-treated fruit appeared to be marginally more wilted around the calyx (button) end of the fruit.

A detailed assessment of residue removal in the packinghouse was not conducted. However, fruit from trees commercially treated with Surround® (Fig. 5.3.2.1) were inspected after commercial packing, and no residue was noticeable. Furthermore, no adverse comments were received from the market.

Conclusions

The application of Surround® particle film in mid-summer, prior to the occurrence of hot, dry weather conditions (+32 °C; <60% RH), effectively eliminated sunburn incidence on Miho Wase Satsuma mandarin fruit. The quantity of sunburnt fruit on Surround-treated trees was similar to that observed during the previous two seasons, and about one-fourth that of untreated trees. Under more severe sunburn conditions the effect of Surround is expected to be greater than that reported here.

The increase in sugar content of fruit from Surround-treated trees is meaningful to both fresh and processing citrus industries, and provides an environmentally-safe way to enhance product quality. Combined with the positive effect on rind colour development, it appears as though Surround treatment advanced fruit maturity.

Future research

Further developmental work on reducing sunburn of Miho Wase Satsuma mandarin is not envisaged as semi-commercial and commercial use is now proposed.

References cited

- Barry, G.H. 2005. Reduction in sunburn incidence of Satsuma mandarin. CRI Group Annual Research Report.
- Glenn, D.M., E. Prado, A. Erez, J. McFerson, and G.J. Puterka. 2002. A reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury to apple. *J. Amer. Soc. Hort. Sci.* 127:188-193.

Table 5.3.2.1. Fruit yield and sunburn incidence of Miho Wase Satsuma mandarin fruit on 5 April 2005 of untreated control and Surround®-treated trees (n=8).

Treatment	Total yield (kg/tree)	Fruit per tree (No.)	Mean fruit weight (g)	Sunburn incidence (kg/tree)	Sunburn incidence (fruit no./tree)	Sunburn incidence (%)	Ave fruit weight (g)
Control	58.3	733	79.5	4.33	47	9.3	92.1
Surround	88.1	1204	73.2	1.19	11	1.6	108.2
<i>P</i> -values	0.1056	0.0589		<0.0001	<0.0001	0.0003	

LSD (5%)	37.0	491		0.77	8.6	3.4	
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Table 5.3.2.2. Internal fruit quality of similar size Miho Wase Satsuma mandarin fruit on 24 March, 5 April and 11 April 2005 from east canopy positions of untreated control and Surround-treated trees (n=8).

Treatment	Fruit diameter (mm)	Fruit weight (g)	Juice content (%)	Brix (°)	TA (%)	Brix:TA Ratio	Rind colour
29 March							
Control	59.3	98.7	50.7	10.1	1.29	7.9	5.3
Surround	63.2	102.5	48.4	10.8	1.28	8.5	4.9
<i>P</i> -values	0.0016	0.4439	0.1794	0.0546	0.9071	0.1174	0.2011
LSD (5%)	2.2	10.3	3.5	0.72	0.09	0.74	0.60
5 April							
Control	57.2	91.9	47.7	10.0	1.23	8.2	3.7
Surround	62.5	113.2	45.9	11.0	1.21	9.2	3.1
<i>P</i> -values	0.0171	0.0435	0.2873	0.0049	0.7181	0.0456	0.0694
LSD (5%)	4.2	20.5	3.6	0.63	0.12	0.98	0.64
11 April							
Control	55.5	77.5	45.4	12.0	1.39	8.6	4.3
Surround	59.7	96.9	46.1	11.9	1.38	8.7	3.8
<i>P</i> -values	0.0019	0.0006	0.4233	0.7687	0.8223	0.8016	0.2328
LSD (5%)	2.37	9.5	1.7	0.63	0.14	0.75	0.86



Fig. 5.3.2.1. Commercial Miho Wase Satsuma mandarin orchard after Surround® applications (3 x 3%) during March 2005.

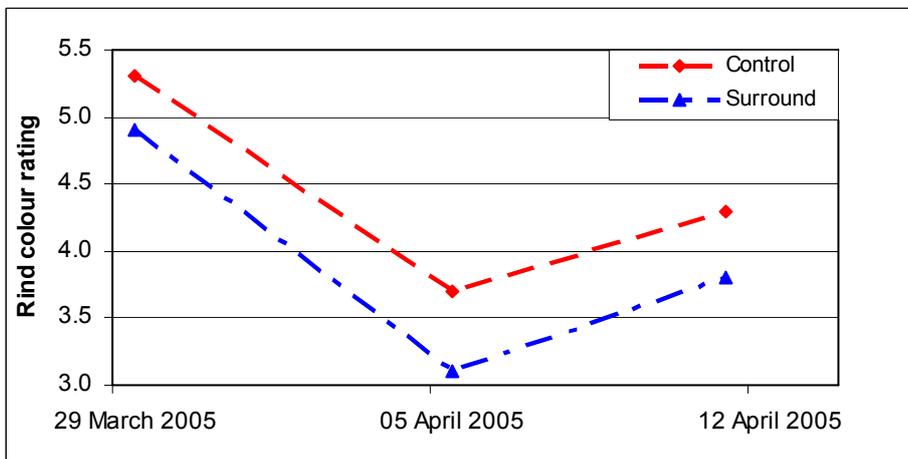
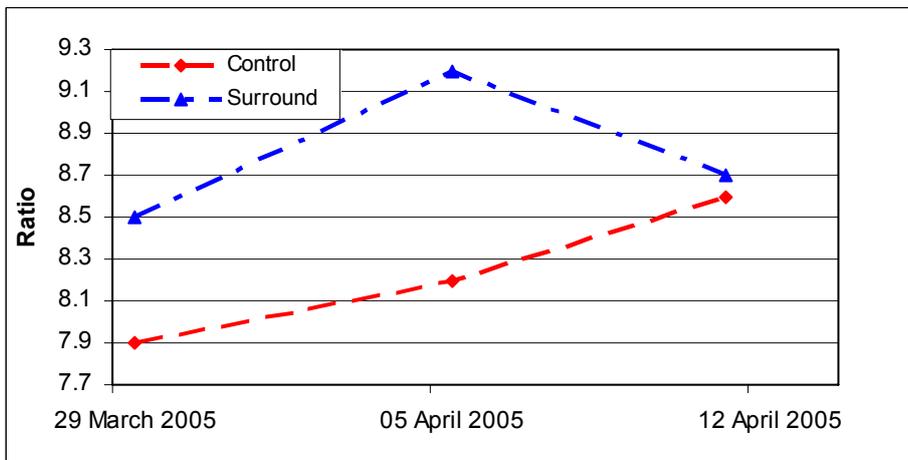
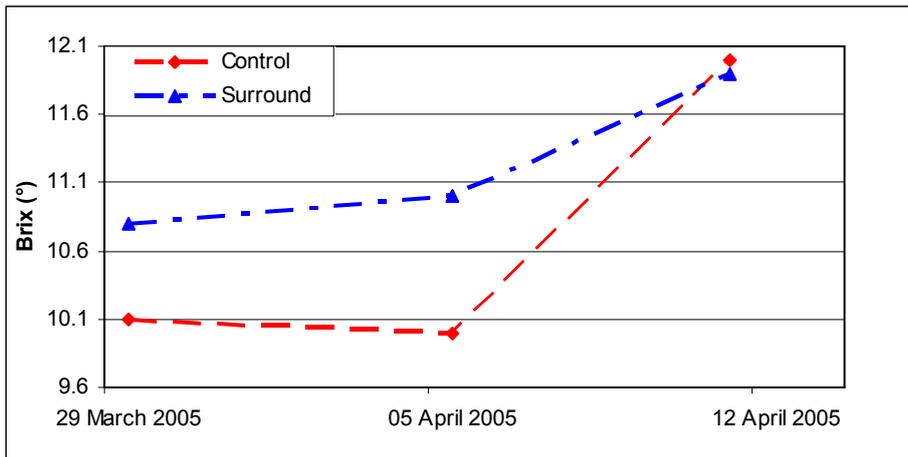


Fig. 5.3.2.2. Changes in internal fruit quality of Miho Wase Satsuma mandarin fruit during March-April 2005 of untreated control and Surround-treated trees.

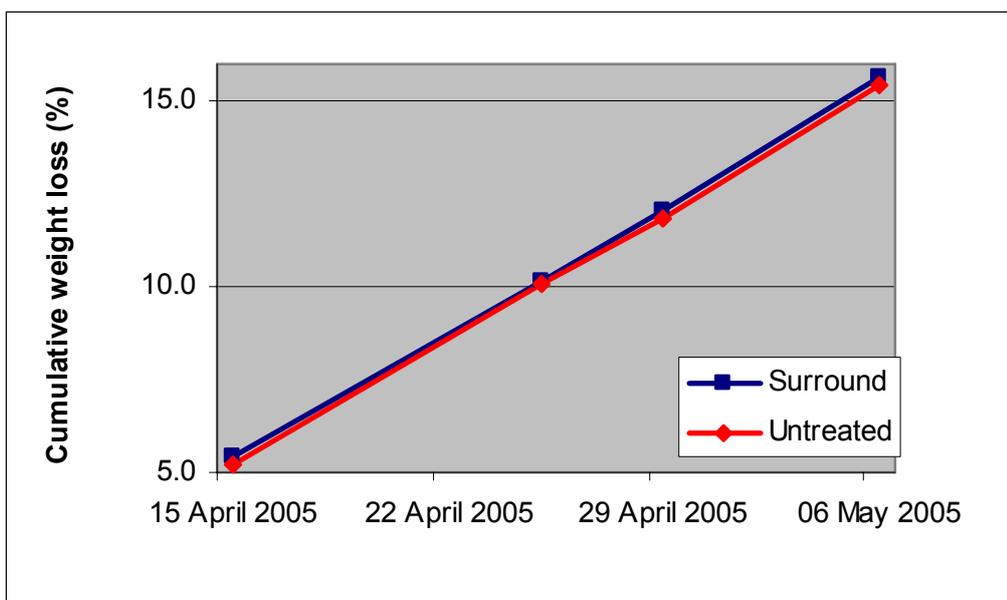


Fig. 5.3.2.3. Cumulative weight (moisture) loss of Miho Wase Satsuma mandarin fruit after commercial packing treatment from untreated control and Surround-treated trees.

5.3.3 Reduction of acidity of high acid citrus cultivars using alternatives to calcium arsenate

Graham H. Barry (CRI at SU)

Opsomming

Gedurende die 2004-05 seisoen is MAP toegedien op Delta Valencia bome teen verskillende konsentrasies. Alhoewel MAP nie die suurvlaakke betekenisvol verlaag het nie, het 2% MAP gelei tot suurvlaakke wat numeries laer as die kontrole was. Die resultate is teenstrydig met die resultate van 2002 en 2003, waar 1% MAP, toegedien 6 weke na volblom, wel die suurinhoud verlaag het.

Introduction

Following promising results in the 2002 and 2003 seasons (see 2002 and 2003 CRI Annual Research Reports) and indifferent results in the 2004 season (see 2004 CRI Annual Research Report), various concentrations of monoammonium phosphate (MAP) were tested to determine the optimal treatment concentration 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 25 November 2004 when fruit diameter was approximately 19 mm and the air temperature was 25°C on a partly cloudy day. A randomized complete block design with eight single-tree replicates was used. Treatments included 0, 2, 4 and 6% MAP and Calcium arsenate applied at commercial rates. A medium-cover spray was used to apply spray material until just before run-off. On average, 7.5 ℓ of spray material were applied per tree.

Data collection and statistical analysis. Fruit acidity, from six replicates, was determined every 8 weeks from 2 Mar. 2005 until maturity to map seasonal changes in acidity. Ten fruit of similar size were sampled from the east side of trees. At maturity on 25 Aug. 2005, eight replicates were sampled, where 10 fruit per replicate were taken for juice quality analysis. Juice content, Brix (by refractometer), titratable acidity (TA) and ratio were determined using standard procedures. Leaf samples were taken 10 weeks after spray application in Feb 2005. These samples were used for N, P and K analysis. Six replicates were sampled and analysed for the MAP treatments. Data were analysed using SAS.

Results and discussion

In this progress report, only the acidity data are presented (Fig. 5.3.3.1). Only the Ca-arsenate treatment resulted in significantly lower acidity than the untreated control ($P < 0.0001$). There was no statistical difference in acidity among MAP treatments and the control (see lower graph), but acidity was numerically lower for the 2% MAP treatment compared with the control and other MAP treatments.

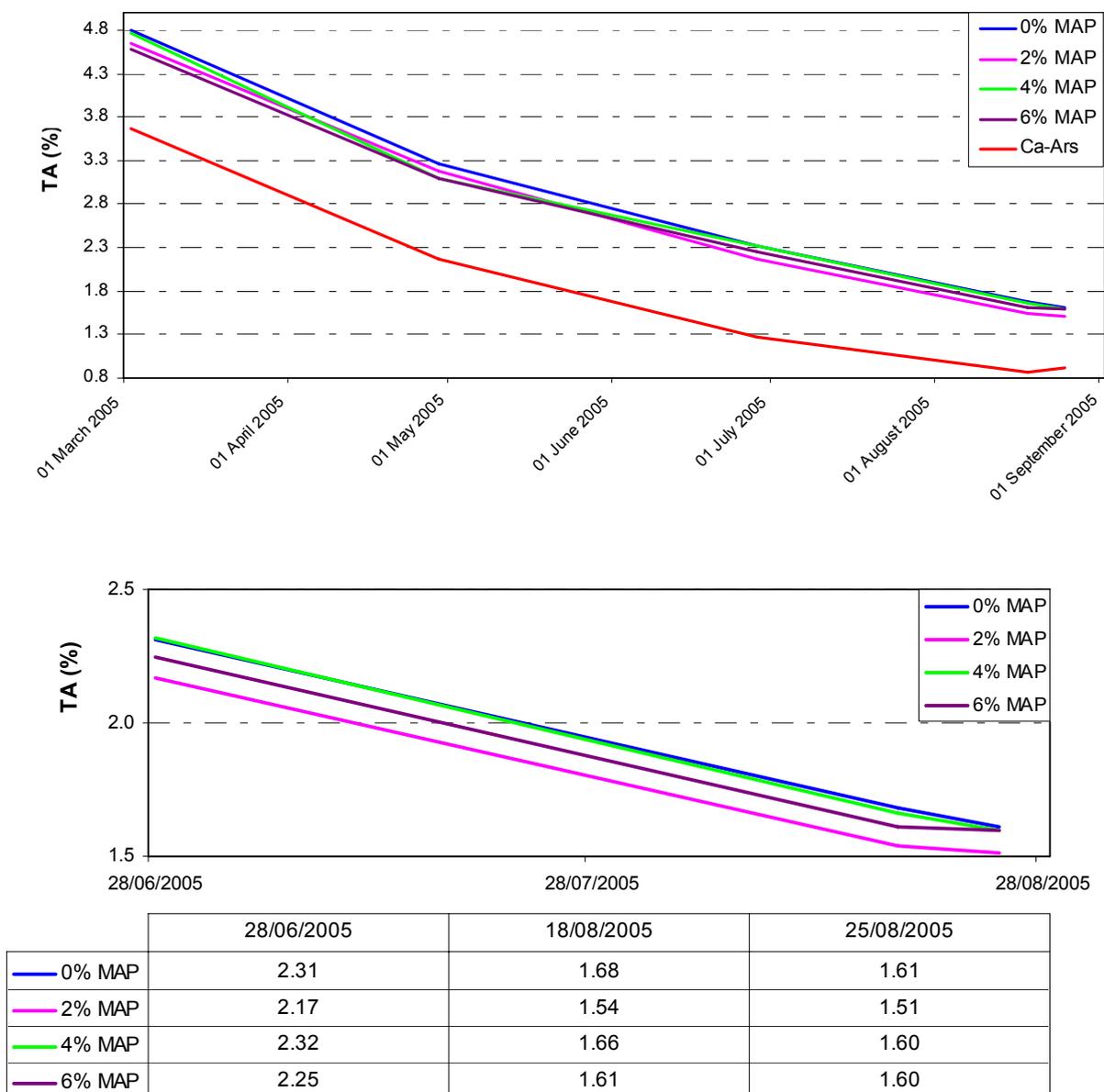


Figure 5.3.3.1. Effect of various concentrations of MAP on fruit acidity of Delta Valencia orange. Treatments were applied on 25 November 2004 and fruit were sampled eight-weekly from early March to demonstrate changes in acidity over time and to overcome issues of sampling error. The second graph shows only the last three sampling times to decrease the Y-axis scale and thereby allow the reader to more clearly see the data points ($n=6$).

Leaf analysis of N, P and K showed that MAP concentration did not affect leaf N or K levels (Table 5.3.3.1). However, P levels were increased following the application of MAP, although there was no relationship between MAP application rate and leaf P concentration. Overall, macronutrient levels were relatively low for all treatments at the time of sampling. With the relatively low leaf P level for the untreated control, a treatment response to increasing P application would be expected. Alternatively, the small increase in leaf P concentration is insufficient to translate into a meaningful response in acidity, and a larger increase in leaf P level may be required to achieve the desired reduction in acidity.

Table 5.3.3.1. Leaf analysis data (%) for Delta Valencia orange (n=6).

MAP %	N	P	K
0	2.06 NS	0.10 b	0.76 NS
2	2.10	0.12 a	1.01
4	1.96	0.13 a	0.82
6	2.04	0.12 a	0.82
P-value	0.230	0.014	0.214
LSD (5%)	0.134	0.0182	0.252
CV %	4.9	11.4	22.1
Norms	2.2-2.6	0.11-0.15	0.9-1.8

Conclusions

During the 2004-05 season, various concentrations of MAP applied to Delta Valencia orange did not significantly reduce TA, although 2% MAP resulted in a numerically lower acidity. This result is contrary to results from 2002 and 2003 where 1% MAP applied 6 weeks after full bloom did reduce TA.

Future research

Alternative sources of phosphate will be investigated as well as double applications of MAP.

5.3.4 Physiological aspects of rind colour development and enhancement of rind colour, with emphasis on early cultivars

Graham H. Barry (CRI at SU) & Smit le Roux (SU)

Opsomming

Skilkleur ontwikkeling van sitrus in Suid Afrika is 'n probleem, veral in die vroeër kultivars a.g.v. die herfs temperature wat te hoog is vir chlorofil afbraak en karotenoid sintese. Sitrusvrugte verlang nagtemperature van <13°C vir optimale kleur ontwikkeling. Dit is heel moontlik nie die koue nag temperature *per se* wat vir die kleurontwikkeling verantwoordelik is nie. Dit blyk egter of die lae nagtemperature vegetatiewe ontwikkeling stop. Laasgenoemde het 'n antagonistiese uitwerking op chloroplast omskakeling na chromoplast en sodoende word chlorofil afbraak en karotenoid sintese vertraag. Om hierdie rede kan tegnieke wat die oordadige vegetatiewe groei gedurende die laat somer en vroeë herfs beperk, 'n betekenisvolle rol speel in pogings om skilkleurontwikkeling te bevorder. Interne gibberellien bevorder vegetatiewe groei in plante en derhalwe kan 'n gibberellin biosintese inhibeerder die omskakeling van chloroplast na chromoplast bevorder. Vooroets toediening van prohexadione kalsium (Ph-Ca) het skilkleur verbeter op alle kultivars wat getoets is, behalwe op Eureka suurlemoen. Die resultate was meer betekenisvol net na oes as na opberging, met sommige betekenisvolle verskille na ontgroening. Die verskille in kleurkaartelling, colorimeter lesings en pigmentkonsentrasies tussen behandelde en onbehandelde bome kan dit moontlik maak om vrugte vroeër in die seisoen te oes. Die kritiese punt van die navorsing is die effek van Ph-Ca op chlorofil afbraak en karotenoid sintese.

Introduction

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°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.

Excessive vegetative vigour and high gibberellin and cytokinin levels are antagonistic to rind colour development in citrus. 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 purpose of this study was to increase preharvest rind colour by decreasing chlorophyll content and/or increasing carotenoids content. The objective of this research was to determine the dose response of citrus trees to Ph-Ca. An experimental orchard of Miho Wase Satsuma and Nules Clementine mandarins in Stellenbosch, and commercial orchards of Palmer Navel orange in Wellington and Eureka lemon in Gt. Drakenstein were used. Ph-Ca at 0, 100, 200, 400 and 800 ppm was applied as a foliar spray 3 months before anticipated harvest in late 2003/early 2004. The treatments did not improve rind colour (data not shown) as, it is thought that treatment timing was not optimal.

Materials and Methods

Sites and plant materials

Four cultivars at different locations were used in the experiments, viz. Nules Clementine mandarin at Welgevallen Experimental Farm (Stellenbosch), Eureka lemon at Jericho (Gt. Drakenstein), Palmer Navel orange at Landau (Wellington), and Navelina Navel orange at Hexrivier (Citrusdal). The main reason for using different sites and plant materials was to test the treatments on different cultivars and to minimise the possibility of site loss.

Treatments

Nules Clementine mandarin

Prohexadione-calcium (Ph-Ca) (Regalis®) was applied as a medium-cover spray with a hand-held spray gun with application rates of 0, 2 and 4 g·L⁻¹ on 8 and 28 Dec. 2004, 1 Feb. 2005, and 4 (8 Apr. 2005) and 2 (28 Apr. 2005) weeks before anticipated harvest (13 May 2005).

Navelina Navel orange

Ph-Ca was applied as a medium-cover spray with a hand-held spray gun with application rates of 0, 2 and 4 g·L⁻¹ applied 4 (7 Apr. 2005) and 2 (21 Apr. 2005) weeks before anticipated harvest (5 May 2005).

Palmer Navel orange

Ph-Ca was applied as a medium-cover spray with a hand-held spray gun with application rates of 0, 2 and 4 g·L on 8 and 28 Dec. 2004, 1 Feb. 2005, and 4 (22 Apr. 2005) and 2 (6 May 2005) weeks before anticipated harvest (12 May 2005).

Eureka lemon

Ph-Ca was applied as a medium-cover spray with a hand-held spray gun with application rates of 0, 2 and 4 g·L⁻¹ on 8 and 28 Dec. 2004, 1 Feb. 2005, 4 (8 Apr. 2005) and 2 (28 Apr. 2005) weeks before anticipated harvest (25 May 2005). In addition, on 4 May 2005, individual fruit and fruit plus leaves were dipped in Ph-Ca solution.

Sampling

Fruit sampling

Fruit were sampled from the outer, eastern side of trees at 1.5 to 2.0 m height. Thirty fruit were harvested from each tree for Nules Clementine and Palmer Navel of which 10 fruit were used for immediate analysis, the remaining 20 fruit were degreened. After degreening another 10 fruit were analysed and the remaining 10 were stored at 7.5°C for 2 weeks followed by 1 week at 18°C. For Navelina Navel only 20 fruit were harvested on the eastern side of the tree at a height of 1.0 to 1.5 m, 10 fruit were used for immediate analysis and 10 fruit were degreened and then analysed. Only the dipped fruit were harvested from the Eureka lemon trees as the bulk of the crop had been commercially harvested. Ten fruit per replicate were sampled for immediate analysis.

Rind sampling

Rind sampling was done by cutting the flavedo from the fruit. This was done either with a potato peeler on the Nules Clementine mandarin or with a citrus rind zester on Navelina and Palmer Navel oranges and Eureka lemon.

Measurements

Harvested fruit were colour rated with the CRI colour chart. Fruit colour was also taken with a Minolta colorimeter. These colour measurements were taken on the yellow and green sides of fruit immediately after harvest, after degreening and in some cases after storage. Photospectrometric analysis was then done on pigments present in the flavedo.

Statistical design and analysis

Experimental layout was a complete randomised block design (CRBD) consisting of eight single-tree replicates. ANOVA and LSD values were used to indicate any significant differences among treatments. Blocking was done to reduce the effect of experimental error due to within site variation.

Results

Nules Clementine mandarin

Ph-Ca significantly increased rind colour rating after harvest and after degreening, but rind colour rating was not significantly different after storage (Table 5.3.4.1). Colorimeter readings supported these findings (Table 5.3.4.2), particularly colorimeter readings on the green side of fruit (Table 5.3.4.3). Chlorophyll and carotenoids analysis (Table 5.3.4.4) also supported these findings with the most carotenoids in the 2 g·L⁻¹ application.

Navelina Navel orange

Ph-Ca significantly increased rind colour rating after harvest and after degreening (Table 5.3.4.5), colorimeter readings (Table 5.3.4.6) and pigment analysis (Table 5.3.4.7).

Palmer Navel orange

Ph-Ca significantly increased rind colour rating after harvest and after degreening, but not after storage (Table 5.3.4.8). Colorimeter readings supported these findings (Table 5.3.4.9), particularly colorimeter readings on the green side of fruit (Table 5.3.4.10). Chlorophyll and carotenoids analysis (Table 5.3.4.11) also supported these findings with the most carotenoids in the 2 g·L⁻¹ application.

Eureka lemon

Ph-Ca significantly increased rind colour rating after harvest (Table 5.3.4.12). However, colorimeter measurements (Table 5.3.4.13) and pigment analysis (Table 5.3.4.14) did not show significant improvement.

Discussion

These preliminary results indicate that preharvest applications of Ph-Ca improved citrus rind colour on all cultivars tested except Eureka lemon. These results were more significant immediately after harvest than after storage, with some significant difference after degreening. The differences in colour rating, colorimeter reading and pigment concentration between treated and non-treated trees could make it possible to harvest fruit earlier in the season. The critical finding from the research has been the effect of Ph-Ca on chlorophyll degradation and carotenoid synthesis.

Table 5.3.4.1. Rind colour rating following different prohexadione-calcium (Ph-Ca) treatments on Nules Clementine mandarin after harvest, after degreening and after 3 weeks storage (2 weeks at 7.5 °C followed by 1 week at 18°C) during the 2005 season.

Treatment	After Harvest	After Degreening	After Storage
Control	3.5 a ²	1.3 a	1.1 ns
Ph-Ca 2 g·L ⁻¹	3.2 a	1.2 b	1.1
Ph-Ca 4 g·L ⁻¹	2.6 b	1.1 b	1.0
<i>P</i> -value	0.0006	0.0006	0.2389
LSD	0.46	0.12	0.07

² Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Table 5.3.4.2. Hue angle, lightness and chroma responses following different prohexadione-calcium (Ph-Ca) treatments on Nules Clementine mandarin after harvest, after degreening, after 1, 2 and 3 weeks storage (2 weeks at 7.5°C followed by 1 week at 18°C) for yellow sides of fruit during the 2005 season.

²Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Treatment	After Harvest	After Degreening	After Week 1	After Week 2	After Week 3
Hue angle (°)					
Control	74.2a	64.5ns	64.9a	63.7ns	61.0ns
Ph-Ca 2 g·L ⁻¹	70.2b	64.0	63.4b	63.5	61.7
Ph-Ca 4 g·L ⁻¹	70.3b	64.7	63.3b	63.4	61.9
<i>P</i> -value	<0.0001	0.4553	0.0106	0.8674	0.3125
LSD	1.66	1.11	1.13	1.12	1.23
Lightness					
Control	69.9a	66.2ab	65.2ns	63.9ns	63.8ns
Ph-Ca 2 g·L ⁻¹	68.6b	65.9b	65.0	63.5	63.7
Ph-Ca 4 g·L ⁻¹	70.2a	66.8a	65.2	63.9	64.0
<i>P</i> -value	<0.0001	0.0795	0.7237	0.4441	0.7285
LSD	0.73	0.77	0.77	0.75	0.74
Chroma					
Control	69.9b	71.2a	75.6a	73.2a	68.6a
Ph-Ca 2 g·L ⁻¹	70.9ab	69.9b	73.4b	72.4a	67.4b
Ph-Ca 4 g·L ⁻¹	71.4a	70.1b	73.2b	71.6b	68.3a
<i>P</i> -value	0.0456	0.0028	<0.0001	0.0006	0.0039
LSD	1.23	0.78	1.04	0.80	0.70

Table 5.3.4.3. Hue angle, lightness and chroma responses following different prohexadione-calcium (Ph-Ca) treatments on Nules Clementine mandarin after harvest, after degreening, after 1, 2 and 3 weeks storage (2 weeks at 7.5°C followed by 1 week at 18°C) for green sides of fruit during the 2005 season
²Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Treatment	After Harvest	After Degreening	After Week 1	After Week 2	After Week 3
Hue angle (°)					
Control	83.0a ^z	67.7a	66.2a	64.9ns	62.5ns
Ph-Ca 2 g·L ⁻¹	76.4b	66.3b	65.5ab	65.0	63.2
Ph-Ca 4 g·L ⁻¹	75.8b	66.7ab	65.0b	64.8	62.7
<i>P</i> -value	<0.0001	0.0447	0.1146	0.9003	0.5606
LSD	2.23	1.13	1.16	1.12	1.3
Lightness					
Control	67.1b	68.3a	66.0ns	64.6ns	64.5ns
Ph-Ca 2 g·L ⁻¹	67.2b	67.0b	66.0	64.2	64.4
Ph-Ca 4 g·L ⁻¹	69.7a	67.5b	66.0	64.8	64.4
<i>P</i> -value	0.0006	0.0072	0.9737	0.3520	0.9502
LSD	1.47	0.79	0.77	0.76	0.73
Chroma					
Control	64.4c	71.3a	76.3a	73.6a	69.2a
Ph-Ca 2 g·L ⁻¹	67.7b	69.5b	73.4b	72.2b	67.8b
Ph-Ca 4 g·L ⁻¹	70.3a	69.9b	73.2b	71.5b	67.8b
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.07	0.80	1.10	0.86	0.69

Table 5.3.4.4. Total chlorophyll, total carotenoid, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio following different prohexadione-calcium (Ph-Ca) treatments on Nules Clementine mandarin after harvest, after degreening and after storage of fruit during the 2005 season.

Treatment	After Harvest	After Degreening	After Storage
Chlorophyll			
Control	130.0a ^z	25.4b	32.0ab
Ph-Ca 2 g·L ⁻¹	81.0ab	34.5a	35.9a
Ph-Ca 4 g·L ⁻¹	55.3b	28.9ab	28.1b
<i>P</i> -value	0.0639	0.1210	0.0890
LSD	62.94	8.87	6.89
Carotenoid			
Control	488.8b	626.0ns	961.1ns
Ph-Ca 2 g·L ⁻¹	595.3a	665.6	1011.1
Ph-Ca 4 g·L ⁻¹	503.7b	624.0	937.6
<i>P</i> -value	0.0448	0.5849	0.3754
LSD	89.19	92.92	108.85
Chlorophyll/Carotenoid Ratio			
Control	0.3a	0.042ns	0.033ns
Ph-Ca 2 g·L ⁻¹	0.1b	0.054	0.036
Ph-Ca 4 g·L ⁻¹	0.1b	0.047	0.031
<i>P</i> -value	0.0134	0.460	0.5810
LSD	0.10	0.02	0.010
Carotenoid/Chlorophyll Ratio			
Control	4.7b	28.9a	30.7ns
Ph-Ca 2 g·L ⁻¹	12.2a	20.1b	29.6
Ph-Ca 4 g·L ⁻¹	10.7ab	21.8ab	34.9
<i>P</i> -value	0.0860	0.0483	0.3581
LSD	6.9717	7.3219	7.90

^z Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Table 5.3.4.5. Rind colour rating following different prohexadione-calcium (Ph-Ca) treatments on Navelina Navel orange after harvest and after degreening during the 2005 season.

Treatment	After harvest	After degreening
Control	5.2a ^z	2.2a
Ph-Ca 2 g·L ⁻¹	4.4b	2.1a
Ph-Ca 4 g·L ⁻¹	3.9c	1.8b
<i>P</i> -Value	<0.0001	0.0001
LSD	0.29	0.19

^z Means within columns followed by a different letters are significantly different ($P \leq 0.05$).

Table 5.3.4.6. Hue angle, lightness and chroma responses following different prohexadione-calcium (Ph-Ca) treatments on Navelina Navel orange after harvest and after degreening for yellow and green sides of fruit during the 2005 season.

Treatment	Yellow Side		Green Side	
	After Harvest	After Degreening	After Harvest	After Degreening
Hue angle (°)				
Control	91.4a ^z	76.0ns	104.0a	78.4b
Ph-Ca 2 g·L ⁻¹	87.9b	76.0ns	99.9b	80.0a
Ph-Ca 4 g·L ⁻¹	84.2c	76.2ns	96.9c	79.2ab
<i>P</i> -value	<0.0001	0.9277	<0.0001	0.0398
LSD	1.89	1.02	1.95	1.19
Lightness				
Control	68.4b	70.0ab	56.9b	67.4b
Ph-Ca 2 g·L ⁻¹	69.6ab	69.7b	58.6b	66.6b
Ph-Ca 4 g·L ⁻¹	70.0a	70.7a	61.2a	68.6a
<i>P</i> -value	0.0013	0.0545	<0.0001	0.0019
LSD	1.37	0.82	1.76	1.12
Chroma				
Control	64.8c	71.8ns	52.5c	69.7ab
Ph-Ca 2 g·L ⁻¹	66.9b	71.7ns	54.9b	68.8b
Ph-Ca 4 g·L ⁻¹	69.0a	72.4ns	58.2a	70.8a
<i>P</i> -value	<0.0001	0.2839	<0.0001	0.0352
LSD	1.74	0.95	2.18	1.49

^z Means within columns followed by a different letters are significantly different ($P \leq 0.05$; ns = nonsignificant).

Table 5.3.4.7. Total chlorophyll, total carotenoid, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio following different prohexadione-calcium (Ph-Ca) treatments on Navelina Navel orange after harvest and after degreening of fruit during the 2005 season.

Treatment	After Harvest	After Degreening
Chlorophyll		
Control	211.1a ^z	22.0ns
Ph-Ca 2 g·L ⁻¹	158.7ab	25.1
Ph-Ca 4 g·L ⁻¹	124.7b	22.9
<i>P</i> -value	0.0343	0.7470
LSD	64.15	8.60
Carotenoid		
Control	193.9b	187.5b
Ph-Ca 2 g·L ⁻¹	210.0ab	283.5a
Ph-Ca 4 g·L ⁻¹	229.3a	289.5a
<i>P</i> -value	0.0462	0.0069
LSD	27.54	66.73
Chlorophyll/Carotenoid Ratio		
Control	1.1a	0.5ns
Ph-Ca 2 g·L ⁻¹	0.8b	0.1
Ph-Ca 4 g·L ⁻¹	0.6b	0.1
<i>P</i> -value	0.0105	0.3062
LSD	0.3	0.68
Carotenoid/Chlorophyll Ratio		
Control	1.1b	10.0ns
Ph-Ca 2 g·L ⁻¹	1.5ab	12.4
Ph-Ca 4 g·L ⁻¹	2.1a	13.6
<i>P</i> -value	0.0211	0.3256
LSD	0.74	4.98

^z Means within columns followed by different letters are significantly different ($P \leq 0.05$; ns = nonsignificant).

Table 5.3.4.8. Rind colour rating following different prohexadione-calcium (Ph-Ca) treatments on Palmer Navel orange after harvest, after degreening and after 3 week storage (2 weeks at 7.5°C followed by 1 week at 18°C) during the 2005 season.

Treatment	After Harvest	After Degreening	After Storage
Control	5.3a ^z	2.9a	1.9ns
Ph-Ca 2 g·L ⁻¹	4.5b	2.5b	1.8
Ph-Ca 4 g·L ⁻¹	4.5b	2.8ab	1.9
<i>P</i> -value	<0.0001	0.0129	0.3968
LSD	0.24	0.30	0.19

^z Means within column followed by a different letter are significantly different ($P \leq 0.05$; ns = non significant).

Table 5.3.4.9. Hue angle, lightness and chroma responses following different prohexadione-calcium (Ph-Ca) treatments on Palmer Navel orange after harvest, after degreening, after 1, 2 and 3 weeks storage (2 weeks at 7.5°C followed by 1 week at 18°C) for yellow sides of fruit during the 2005 season.

^zMeans within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Treatment	After Harvest	After Degreening	After Week 1	After Week 2	After Week 3
Hue angle (°)					
Control	90.7a ^z	77.1a	76.9a	75.6a	73.8a
Ph-Ca 2 g·L ⁻¹	83.8b	75.0b	74.7b	74.1b	72.8ab
Ph-Ca 4 g·L ⁻¹	84.2b	74.5b	74.0b	73.6b	72.3b
<i>P</i> -value	<0.0001	<0.0001	<0.0001	0.0007	0.0258
LSD	2.28	1.23	1.18	1.04	1.11
Lightness					
Control	67.6b	70.9ab	70.0ns	70.3a	69.2ab
Ph-Ca 2 g·L ⁻¹	70.6a	71.2a	70.2	70.2a	69.5a
Ph-Ca 4 g·L ⁻¹	70.1a	70.2b	69.7	69.4b	68.6b
<i>P</i> -value	<0.0001	0.0263	0.3603	0.0155	0.0331
LSD	1.49	0.75	0.76	0.66	0.66
Chroma					
Control	67.7b	75.8b	78.8a	76.9b	73.5a
Ph-Ca 2 g·L ⁻¹	73.3a	78.3a	78.5a	78.9a	73.8a
Ph-Ca 4 g·L ⁻¹	72.6a	76.8b	76.1b	77.6ab	72.7b
<i>P</i> -value	<0.0001	0.0016	0.0001	0.0095	0.0175
LSD	2.36	1.36	1.33	1.32	0.80

Table 5.3.4.10. Hue angle, lightness and chroma responses following different prohexadione-calcium (Ph-Ca) treatments on Palmer Navel orange after harvest, after degreening, after 1, 2 and 3 weeks storage (2 weeks at 7.5°C followed by 1 week at 18°C) for green sides of fruit during the 2005 season.

^zMeans within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Treatment	After Harvest	After Degreening	After Week 1	After Week 2	After Week 3
Hue angle (°)					
Control	105.4a ^z	82.2a	80.9a	79.6a	78.0a
Ph-Ca 2 g·L ⁻¹	95.7c	78.4c	77.4c	77.0c	75.0c
Ph-Ca 4 g·L ⁻¹	98.2b	79.9b	79.2b	78.2b	76.6b
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD	2.09	1.37	1.18	1.03	1.10
Lightness					
Control	61.6c	74.2a	73.8a	73.7a	72.7a
Ph-Ca 2 g·L ⁻¹	66.7a	73.4a	72.4b	72.2b	71.5b
Ph-Ca 4 g·L ⁻¹	63.6b	71.3b	72.2b	72.3b	71.1b
<i>P</i> -value	<0.0001	<0.0001	0.0025	0.0001	<0.0001
LSD	1.75	1.09	0.96	0.76	0.70
Chroma					
Control	56.7c	73.0b	78.0a	77.7ns	75.1a
Ph-Ca 2 g·L ⁻¹	63.3a	75.6a	77.6a	78.2	74.9a
Ph-Ca 4 g·L ⁻¹	60.5b	73.2b	75.2b	77.7	73.6b
<i>P</i> -value	<0.0001	0.0003	<0.0001	0.6016	0.0002
LSD	2.15	1.42	1.32	1.08	0.76

Table 5.3.4.11. Total chlorophyll, total carotenoid, chlorophyll/carotenoid ratio and carotenoids/chlorophyll ratio following different prohexadione-calcium treatments on Palmer Navel orange after harvest, after degreening and after storage of fruit during the 2005 season.

Treatment	After Harvest	After Degreening	After Storage
Chlorophyll			
Control	334.7a ^z	67.9ns	39.6ns
Ph-Ca 2 g·L ⁻¹	201.3b	52.2	37.5
Ph-Ca 4 g·L ⁻¹	230.1b	68.2	52.7
<i>P</i> -value	0.0247	0.3242	0.1397
LSD	97.99	24.66	16.42
Carotenoid			
Control	298.1b	256.8b	510.5b
Ph-Ca 2 g·L ⁻¹	349.3a	340.7a	605.5a
Ph-Ca 4 g·L ⁻¹	298.3b	331.9a	566.1ab
<i>P</i> -value	0.0543	<0.0001	0.0161
LSD	47.33	32.77	62.48
Chlorophyll/Carotenoid			
Control	1.1a	0.3a	0.08ns
Ph-Ca 2 g·L ⁻¹	0.6b	0.2b	0.07
Ph-Ca 4 g·L ⁻¹	0.8b	0.21ab	0.09
<i>P</i> -value	0.0036	0.0647	0.3211
LSD	0.29	0.09	0.04
Carotenoid/Chlorophyll			
Control	0.9b	4.0b	13.2b
Ph-Ca 2 g·L ⁻¹	2.3a	8.7a	20.5a
Ph-Ca 4 g·L ⁻¹	1.4b	5.5b	11.7b
<i>P</i> -value	0.0049	0.0177	0.0303
LSD	0.76	3.16	6.81

^z Means within columns followed by a different letter are significantly different ($P \leq 0.05$; ns = nonsignificant).

Table 5.3.4.12. Rind colour rating following different prohexadione-calcium (Ph-Ca) treatments on Eureka lemon after harvest during the 2005 season.

Treatment	Fruit Dipped	Fruit and Leaves Dipped
Control	5.2a ^z	4.8a
Ph-Ca 2 g·L ⁻¹	4.7b	4.9a
Ph-Ca 4 g·L ⁻¹	4.7b	4.4b
<i>P</i> -value	0.0047	0.0086
LSD	0.38	0.36

^z Means within columns followed by different letters are significantly different ($P < 0.05$).

Table 5.3.4.13. Hue angle, lightness and chroma responses following different prohexadione-calcium (Ph-Ca) treatments on Eureka lemon after harvest for yellow and green sides of fruit during the 2005 season.

Treatment	Yellow side		Green side	
	Fruit Dipped	Fruit and Leaves Dipped	Fruit Dipped	Fruit and Leaves Dipped
Hue angle (°)				
Control	105.8ns ^z	103.7ns	110.6ns	106.8ns
Ph-Ca 2 g·L ⁻¹	103.1	102.3	108.0	108.5
Ph-Ca 4 g·L ⁻¹	104.1	102.7	108.6	106.4
<i>P</i> -value	0.1611	0.7164	0.1478	0.3358
LSD	2.81	3.63	2.7	2.89
Lightness				
Control	67.8a	70.4ns	60.5ns	62.3ns
Ph-Ca 2 g·L ⁻¹	69.9a	69.2	61.8	63.2
Ph-Ca 4 g·L ⁻¹	70.0b	69.8	61.8	62.1
<i>P</i> -value	0.0413	0.5929	0.6897	0.8150
LSD	1.93	2.36	3.62	3.69
Chroma				
Control	51.8ns	52.1ns	50.0ns	52.1ns
Ph-Ca 2 g·L ⁻¹	51.8	53.7	50.2	50.3
Ph-Ca 4 g·L ⁻¹	51.0	53.2	50.7	51.988
<i>P</i> -value	0.5781	0.2579	0.7832	0.1616
LSD	1.76	2.04	1.81	2.09

^z Means within columns followed by different letters are significantly different ($P < 0.05$; ns = nonsignificant).

Table 5.3.4.14. Total chlorophyll, total carotenoid, chlorophyll/carotenoid ratio and carotenoid/chlorophyll ratio following different prohexadione-calcium (Ph-Ca) treatments on Eureka lemon after harvest of fruit during the 2005 season.

Treatment	Fruit Dipped	Fruit and Leaves Dipped
Chlorophyll		
Control	301.8ns ²	224.8ns
Ph-Ca 2 g·L ⁻¹	276.2	286.9
Ph-Ca 4 g·L ⁻¹	252.3	236.2
<i>P</i> -value	0.6549	0.3781
LSD	126.99	106.68
Carotenoid		
Control	82.8ns	81.0b
Ph-Ca 2 g·L ⁻¹	86.7	100.5a
Ph-Ca 4 g·L ⁻¹	82.5	84.5b
<i>P</i> -value	0.8997	0.0136
LSD	24.62	11.70
Chlorophyll/Carotenoid Ratio		
Control	3.6ns	2.8ns
Ph-Ca 2 g·L ⁻¹	3.1	2.8
Ph-Ca 4 g·L ⁻¹	3.0	2.8
<i>P</i> -value	0.1755	0.9745
LSD	0.72	1.24
Carotenoid/Chlorophyll Ratio		
Control	0.3ns	0.3ns
Ph-Ca 2 g·L ⁻¹	0.3	0.4
Ph-Ca 4 g·L ⁻¹	0.3	0.4
<i>P</i> -value	0.2626	0.9573
LSD	0.08	0.16

²Means within columns followed by different letters are significantly different ($P \leq 0.05$; ns = nonsignificant).

5.3.5 Effects of tree nutrition and post-harvest treatments on concentration of the most important colour-imparting carotenoids in physiologically mature citrus fruit

By Isa Bertling, John Bower, Renate Oberholster, Molipa Mosoeunyane and Tony Bruton (UKZNP)

Opsomming

Pigmente (chlorofil en karotenoides) is ge-ekstraheer van Navel en Turkey lemoene wat behandel is by kleurbreek met warm water of hidro verkoeling. Skilkleur veranderinge is waargeneem en die pigment konsentrasie is spektrofotometries bepaal. Sommige behandelde vrugte het betekenisvolle hoër karotenoid konsentrasies as onbehandelde (kontrole) vrugte gehad. Omdat 'n korrelasie tussen die konsentrasie van sekere mikro-elemente en die vermoë van vrugte om karotenoides te sintetiseer verwag word, is die mikro-element analyses in die grond en blare uitgevoer deur Gekoppelde Plasma Uitstralings Spektrometrie. Mo en W konsentrasies in die grond was laer as meetbare vlakke. 'n Betekenisvolle konsentrasie van toegediende Mo en W, 102% en 99% respektiewelik, is in blaar monsters gemeet. Mo en W teen drie vlakke (0, 0.202 en 0.404 g per boom) is toegedien as blaarbespuitings 19 weke na volblom in 2005. Die analyses van blaar- en vrugmonsters van onbehandelde en behandelde bome (met Mo en W) is onderweg.

Introduction

Several physiological and biochemical alterations occur in tropical and subtropical fruit and vegetables in response to exposure to non-freezing temperatures below 12°C (El-Hilali *et al.*, 2003). Postharvest heat treatments induce tolerance to cold temperature without impairing fruit quality (Lepeduš *et al.*, 2005). Changes in various biochemical parameters during heat treatments and cold storage have been investigated (Lepeduš *et al.*, 2005). On the other hand, cooling of citrus flavedo (4°C) followed by incubation at 22°C has been reported to positively influence the carotenoid content of 'Navel' and 'Valencia' fruit (Oberholster, 2001). The effect of hydro-cooling and hot water dipping (HWD) on carotenoid formation has not been investigated

yet. As plant nutrition is an essential component of healthy plant growth (Kaizer *et al.*, 2005) and molybdenum fertilization through foliar application can effectively supplement internal Mo deficiency and the activity of molybdoenzymes (Kaizer *et al.*, 2005) such applications could influence the colouring of citrus fruit. It has been furthermore reported that tungsten (W) can assume a similar role to Mo in organisms (Hale *et al.*, 2002). We therefore investigated if hydro-cooling / hydro-warming of oranges can affect the colour of oranges and initiated a field experiment on soil application of M and W with the intention to ultimately increase the colour expression in citrus fruit at physiological maturity.

Materials and methods

Plant material. 'Navel' and 'Turkey' citrus fruit were harvested at OrangeWood Farm, Cramond, KwaZulu-Natal in April/May 2005.

Screening of protocols for extraction of membrane-bound protein. To obtain a deeper insight into colour formation in citrus flavedo the characteristic of proteins involved in carotenoid biosynthesis (which should be membrane-bound) involved in carotenoid biosynthesis was initiated. Lyophilised citrus flavedo (fully coloured) was used for extraction of membrane associated proteins. Two protocols for extraction of proteins (Carvalho *et al.*, 2004, Mayfield and Huff, 1986) were compared. The protein concentrations were determined spectrophotometrically.

Post-harvest treatment of physiologically mature 'navel' and 'Turkey' oranges. Initially T5 and T4 Navel and Valencia orange fruit were hydro-cooled at 4°C for 1 hour followed by a brief dip in circulating hot water (40-50°C). Control fruit (untreated) and treated fruit were thereafter waxed and kept in boxes in the dark at room temperature and evaluated for colour changes and sampled for pigment analysis at various times. At each sampling time the flavedo of six selected fruit was peeled off and chopped, shock-frozen in liquid N₂ and freeze-dried. The freeze-dried material was stored at -20°C until analysis.

Spectrophotometric determination of chlorophyll and carotenoid concentration. For chlorophyll and carotenoid determination, absorbance of the extracts was recorded at different wavelengths (470, 648.6, 664.2 nm) (Lichtenthaler, 1987) using a DU[®] 800 spectrophotometer. Pigment concentration was expressed in $\mu\text{g mg}^{-1}$ dry flavedo.

Micro-nutrient extraction and analysis. *Microwave assisted acid digestion of soil samples.* Soil micronutrient concentration was analysed according to the EPA 3051H method (United States Environmental Protection Agency). One gram of soil sample was weighed in CEM digestion vessel and suspended in 10 ml of concentrated HNO₃ and microwaved (15 minutes). The digest was filtered and the resultant filtrate stored.

Microwave assisted acid digestion of plant tissue. Similarly, milled leaf tissue (500 mg) was digested according to the EPA 3052H method. After microwaving the samples were diluted with ultra-pure water and stored in sample bottles at room temperature until ICP-ES analysis.

Foliar application of micro-nutrients

The Mo concentration in foliar sprays of citrus trees was adapted from Longbottom and Dry (0 g, 0.101 g and 0.202 g per tree; unpublished). Five hundred millilitres of molybdate or tungstate (at the rate of 0, 0.202 and 0.404 g Mo or W per tree) were sprayed on a fine day, 19 weeks after full bloom in 2005. A drift retardant (Control Mist[®]) and a wetting and sticker agent (Nu-film-P[®]) were added to each solution according to manufacturer's instructions.

Results

Spectrophotometric analysis revealed that the protocol according to Mayfield and Huff (1986) effectively isolates chromoplasts and extracts chromoplast-bound proteins. Hydro-cooling followed by hot water dipping of citrus fruit resulted in a significant reduction in total green colour of the peel relative to the control (Figs. 5.3.5.1 and 5.3.5.2). After treatment, citrus fruit showed a reduction in total chlorophyll concentration and an increase in carotenoid concentration relative to the untreated fruit. However, there was no visual difference in fruit colour after 16 days storage at room temperature. Similarly, spectrophotometric results indicated a significant increase in carotenoids in treated fruit compared to the control (untreated fruit), particularly in 'Turkey' orange (Table 5.3.5.1). There was also a significant reduction in total chlorophyll concentration in treated fruit compared to the control. These results can be explained with previous findings by Bouvier *et al.* (1998), in which they revealed that stress can trigger carotenoid biosynthesis.

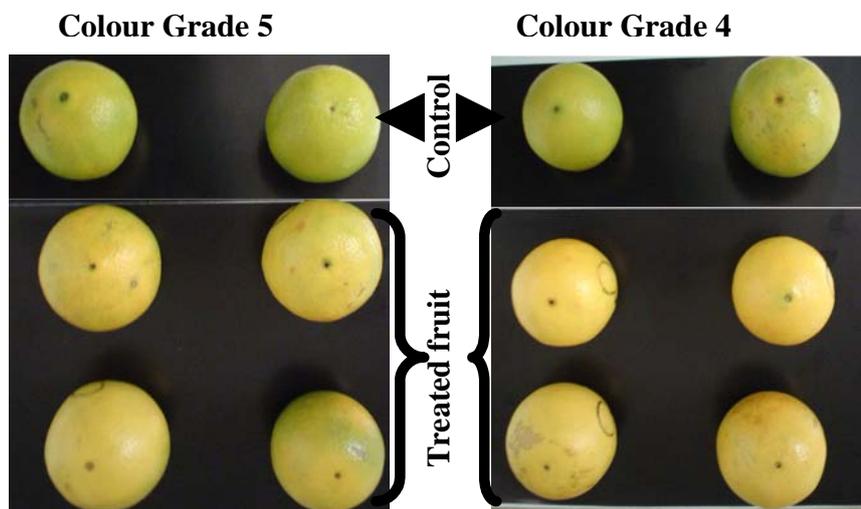


Fig. 5.3.5.1 'Navel' colour change due to post-harvest hydro-cooling followed by a brief dip in hot water. Control fruit were not treated. The picture was taken 16 days after treatment.



Fig. 5.3.5.2. 'Turkey' colour change due to post-harvest hydro-cooling followed by a brief dip in hot water. Control fruit were not treated. The picture was taken 16 days after treatment.

Table 5.3.5.1 Pigment concentration of Navel and Turkey Valencia 10 days after postharvest treatment. Fruit were hydro-cooled and dipped in hot water.

Treatment	Chlorophyll $\mu\text{g (100 mg dry flavedo)}^{-1}$		Carotenoids $\mu\text{g (100 mg dry flavedo)}^{-1}$	
	'Navel'	'Turkey'	'Navel'	'Turkey'
¹ Control	31.9 a	27.4 a	124.0 a	128.8 b
² Treated	15.0 b	12.1 b	124.8 a	149.5 a

¹Control fruit were waxed and kept at room temperature in the dark.

²Treated fruit were hydro-cooled and dipped in hot water, waxed and kept under similar conditions as the control.

Significant differences of values within a column are denoted by different letters (P=0.05).

Measurement of Mo and W concentration in soils with an ICP-ES was not successful, possibly due to the fact that their concentration was below the detectable limits ($\pm 1.5 \mu\text{g}$). Contrary to that, a significant concentration of added Mo and W, 2.5 and 12.4 μg respectively, was recovered from leaf samples. Plant tissue samples spiked with 12.4 and 2.5 μg of Mo and W, showed a high percentage recovery (Table 5.3.5.2).

Table 5.3.5.2 Concentration of molybdenum recovered from leaf samples. The Mo amount added as 'spike' was added before digestion with HNO_3 . Minerals were quantified using an ICP-ES.

Leaf Sample number	Sample mass (g)	Molybdenum		
		Mass added (μg)	Mass recovered (μg)	% recovery
1	0.5	Control	0	0
2	0.5	Control	0	0
3	0.5	2.5	2.2	89
4	0.5	2.5	2.2	89
5	0.5	12.4	12.6	102
6	0.5	12.4	12.7	103

Control: no standard was added to leaf sample.

Table 5.3.5.3. Concentration of tungsten recovered from leaf samples. Amount was added before being digested with HNO_3 . Minerals were quantified using an Inductively Coupled Plasma emission spectrometer.

Leaf Sample number	Sample mass (g)	Tungsten		
		Mass added (μg)	Mass recovered (μg)	% recovery
1	0.5	Control	0	0
2	0.5	Control	0	0
3	0.5	Control	0	0
4	0.5	2.8	2.2	77
5	0.5	2.8	2.8	100
6	0.5	13.9	13.6	97
7	0.5	13.9	14.1	101

Key: Control: no standard was added to leaf sample.

Conclusion

A significant change in peel colour and pigment concentration was observed in most treated compared to untreated fruit. As the heat/cold treatment applied did increase the carotenoid and decrease the chlorophyll concentration, means to affect these mineral levels are currently being investigated. As Mo and W could play an important role in such alterations, the methodology for determining Mo and W concentration in leaf tissue and soil using ICP-ES needs to be refined.

Future research

Citrus leaves and fruit of untreated and treated (foliar sprayed) trees were sampled in February 2006. Laboratory analysis for a variety of parameters is already in progress. Treated and untreated fruit at colour break will be subjected to hydro-cooling as well as hot water treatment in April/May 2006. The effects of treatments will be evaluated from June 2006 starting with colour parameters followed by protein profile analysis. A full report will be submitted on completion of the project.

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5.4 PROJECT: CROP LOAD MANAGEMENT

Project Co-ordinator: Graham H. Barry (CRI at SU)

5.4.1 Project summary

Various approaches have been taken to enhance fruit set and productivity of citrus trees. One approach has been to reduce heat stress and increase natural plant defence mechanisms of seedless citrus cultivars under relatively stressful fruit set conditions using particle film technology and the enhancement of plant defence mechanisms, respectively. Treatments were applied on 18 October and 18 November 2004 and ecophysiological measurements were taken on 29 November to 2 December 2004. Kaolin treatment increased photosynthetic capacity and enhanced water use efficiency through decreased leaf temperature and vapour pressure deficit. However, we were not able to demonstrate increased production efficiency due to severe pest infestation in the orchard where the experiment was conducted (5.4.2).

A new study has recently been initiated to determine the number of fruit or crop load per tree (canopy volume) for different cultivars that would give both optimal fruit size and crop load. Delta Valencia orange trees in Citrusdal and Nules Clementine mandarin trees in Wellington are being used for the study. The number of fruit and leaves per frame will be counted (0.5 x 0.5 x 0.5 m) (similar to leaf:fruit ratio) at specific positions in the canopy of trees bearing different crop loads in the same orchard. The frame counts per tree will be related to actual yields and fruit size per tree which will be determined at harvest. This study will commence in February 2006 and results will be presented in the next annual progress report (5.4.3).

An experiment has been set up at Mazowe Citrus Estate, Zimbabwe to evaluate the potential application of the partial root zone drying irrigation strategy for commercial citrus irrigation in the semi-arid tropics. Three partial root zone drying treatments and one regulated deficit irrigation treatment are being evaluated against a well-watered control treatment. The effect of the alternating partial stress in the root zone is being evaluated in terms of changes in the leaf/xylem water potential of the trees and on yield. The trial is currently in progress and detailed results will be available at the end of the growing season. However, some aspects of the ongoing trial are presented in this report (5.4.4).

Projekopsomming

Verskeie benaderings is gevolg om vrugset en produktiwiteit van sitrusbome te verbeter. Een benadering was om hittestres te verminder en die natuurlike plant verdedigingsmeganismes van saadlose sitrus kultivars onder redelike stres toestande tydens vrugset te verbeter. Partikel film tegnologie (kaolin) en die verbetering van plant verdedigingstegnologie is gebruik. Behandelings is toegedien op 18 Oktober en 18 November 2004 en ekofisiologiese metings is gedoen op 29 November en 2 Desember 2004. Kaolin behandelings het fotosintetiese kapasiteit verhoog en waterverbruiks effektiwiteit verbeter deur verlaagde blaartemperatuur en dampdruktekort. Verhoogde produksie effektiwiteit kon egter nie getoon word nie, weens 'n hoë insek druk in die boord waar die eksperiment uitgevoer is.

'n Nuwe studie is onlangs begin om die hoeveelheid vrugte of oeslading per boom (boomvolume) wat sal lei tot beide optimale vruggrootte en oeslading per boom vir 'n spesifieke kultivar te bepaal. Delta Valencia

bome in Citrusdal en Nules Clementine bome in Wellington sal gebruik word. Die hoeveelheid vrugte en blare per raamvolume sal getel word (0.5 x 0.5 x 0.5 m) (dieselfde as blaar:vrugverhouding) op spesifieke posisies in bome met verskillende oeslading binne dieselfde boord. Die vrugraamtellings per boom sal in verband gebring word met oeslading en vruggrootte per boom wat tydens oestyd bepaal sal word. Die studie sal in Februarie 2006 begin en resultate sal in die volgende jaarlikse verslag verskyn.

’n Eksperiment is uitgevoer by Mazowe Citrus Estate, Zimbabwe om die potensiële toepassing van die gedeeltelike wortelone uitdroging strategie vir kommersiële sitrus besproeiing in die semi-droë trope te evalueer. Drie gedeeltelike wortelone uitdroging behandelings en een beheerde stres besproeiings behandeling word geëvalueer teenoor ’n goed besproeide kontrole behandeling. Die effek van die alternerende gedeeltelike stres in die wortelone word geëvalueer in terme van die veranderinge in die blaar/xileem waterpotensiaal van die bome en op oeslading. Die eksperiment is huidiglik onderweg en meer gedetailleerde resultate sal in die einde van die seisoen beskikbaar wees. Sekere aspekte van die eksperiment word wel in hierdie verslag uiteengesit.

5.4.2 Fruit set enhancement of seedless citrus cultivars by reducing heat stress and increasing natural plant defence mechanisms

Experiment by Graham H. Barry (CRI at SU)

Opsomming

Verskeie benaderings is gevolg om vrugset en produktiwiteit van sitrusbome te verbeter. Een benadering was om hittestres te verminder en die natuurlike plant verdedigingsmeganismes van saadlose sitrus kultivars onder redelike stres toestande tydens vrugset te verbeter. Partikel film tegnologie (kaolin) en die verbetering van plant verdedigingstegnologie is gebruik. Behandelings is toegedien op 18 Oktober en 18 November 2004 en ekofisiologiese metings is gedoen op 29 November en 2 Desember 2004. Kaolin behandelings het fotosintetiese kapasiteit verhoog en waterverbruiks effektiwiteit verbeter deur verlaagde blaartemperatuur en dampdrukkort. Verhoogde produksie effektiwiteit kon egter nie getoon word nie, weens ’n hoë insek druk in die boord waar die eksperiment uitgevoer is.

Introduction

Clementine mandarin (*Citrus reticulata* Blanco) is sexually self-incompatible. Fruit set parthenocarpically when grown commercially in orchards isolated from cross-pollinating cultivars. However, under stressful fruit setting conditions of high temperature (>28 °C) coupled with high evaporative demand due to low relative humidity during spring, excessive fruitlet drop may occur resulting in low fruit yield. As a result, gibberellic acid (GA₃) is applied after anthesis to increase fruit set. Nevertheless, under heat wave conditions or marginal growing conditions in terms of relatively high maximum temperature in spring, GA₃ application may increase initial fruit set, but late fruit drop may still occur towards the end of the fruit set period. Other seedless citrus cultivars are also adversely affected by extreme heat during fruit set, but there are no registered fruit set treatments to ameliorate such conditions.

Glenn et al. (2001; 2002) showed that particle film technology using processed kaolin (Surround®) reduced leaf and canopy temperature of apple (*Malus sylvestris* var. *domestica*), possibly via increased reflection of the UV wavelengths. Jifon and Syvertsen (2003) reported a 3°C reduction in midday leaf temperature on grapefruit (*C. paradisi* Macf.). Concomitant with reduced leaf temperature was increased net CO₂ assimilation rate, particularly around midday on warm sunny days. Messenger®, a harpin protein, elicits a plant’s natural defence mechanisms resulting in, *inter alia*, improved stress tolerance (Eden Bioscience Corporation, 2000). The adverse affects of heat stress on accelerating fruitlet drop of a sexually self-incompatible cultivar that sets fruit parthenocarpically could be mitigated by reducing plant stress and/or increasing natural plant defence mechanisms.

The objective of this research was to increase fruit set of seedless citrus cultivars under relatively stressful fruit set conditions using particle film technology and the enhancement of plant defence mechanisms.

Materials and methods

Plant material and treatments. Ten-year-old Bahianinha Navel orange and Midnight Valencia orange trees at Addo Research Station, Addo, were used. The trees used were selected for uniformity in tree size and health. Treatments were applied in dry, windless weather on the mornings of 18 October and 18 November 2004. A randomized complete block design with ten single-tree replicates was used. Treatments included Surround® applied at 6% on the first application date followed by 3% on the second application date, plus an

untreated control. A medium-cover spray was used to apply spray material until just before run-off. On average, 5 L of spray material was applied per tree.

Data collection. Ecophysiological measurements were taken between 29 November and 2 December 2004 on a clear, sunny day when the air temperature ranged from 30 to 33°C between 09:00 and 14:00. Yield, fruit size distribution and fruit quality variables were measured at fruit maturity on 7 September 2005.

Results and discussion

Kaolin treatment increased photosynthetic capacity and enhanced water use efficiency through decreased leaf temperature and vapour pressure deficit (Fig. 5.4.2.1). Maximum photosynthetic capacity (A_{max} in $\mu\text{mol m}^{-2} \text{s}^{-1}$) for untreated Bahianinha Navel orange leaves was 5.4 ± 0.8 compared to the kaolin-treated leaves which were 6.8 ± 0.9 . For Midnight Valencia orange leaves A_{max} for the untreated leaves was 4.6 ± 0.6 compared to 5.2 ± 0.2 for the kaolin-treated leaves.

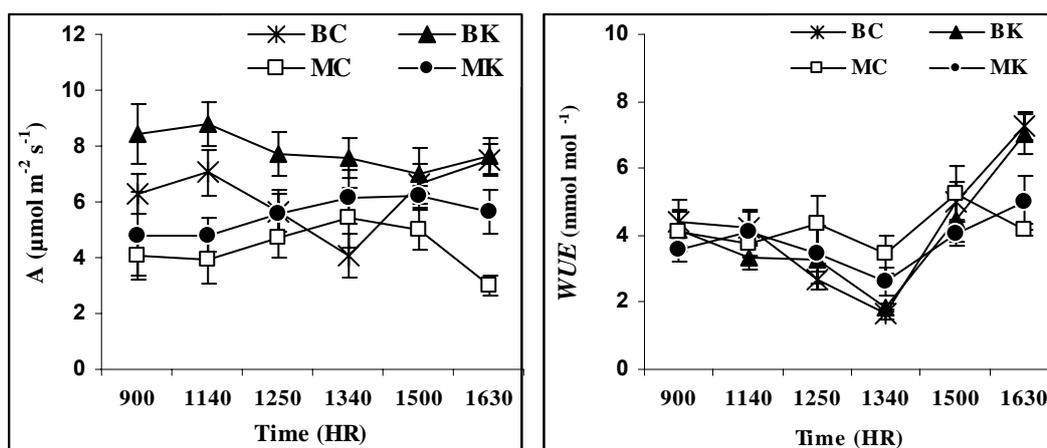
These differences in ecophysiological measurements indicate that kaolin application reduced plant stress and increased photosynthetic capacity. However, we were not able to demonstrate increased production efficiency due to severe pest infestation in the orchard where the experiment was conducted, but increased sugar content (11.0 vs. 10.7° Brix) for fruit from kaolin-treated vs. control trees and increased maturity index (10.6 vs. 9.8 Brix-to-acidity ratio) suggests that kaolin treatment advanced fruit maturity.

Future research

Further studies are required to harness the potential provided by kaolin application in reducing plant stress and increasing photosynthetic capacity.

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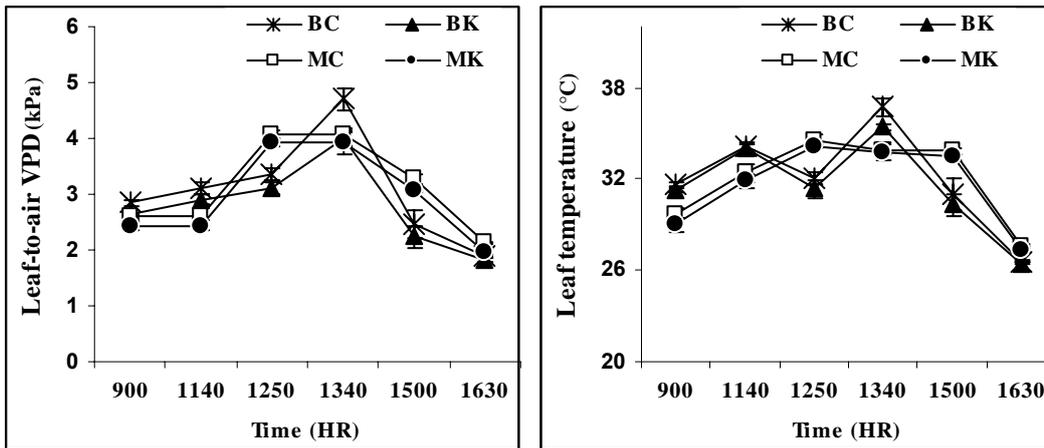


Fig. 5.4.2.1. Diurnal gas exchange of kaolin treated and untreated Midknight and Bahianinha Navel oranges under field conditions in Addo, Eastern Cape, South Africa measured under ambient conditions of temperature, radiation and humidity on 30 November 2004. (BC=Bahianinha Control; BK=Bahianinha Kaolin; MC=Midknight Control; MK=Midknight Kaolin; A=photosynthetic rate; WUE=water use efficiency; VPD=vapour pressure deficit).

5.4.3 Determining the number of fruit per tree (crop load) that would give optimal yield and fruit size
 Experiment 865 by Stephan Verreyne (CRI at SU)

Opsomming

Vruggrootte is 'n baie belangrike faktor wat die bemarkbare oeslading op 'n boom affekteer. Oeslading affekteer vruggrootte, met 'n swaar oes wat gewoonlik tot kleiner vrugte lei. Die hoeveelheid vrugte per boom of die oeslading wat sal lei tot beide 'n optimale oeslading en vruggrootte is moeilik om te behaal. Die doel van die studie is om die hoeveelheid vrugte (oeslading) per boom (boomvolume) wat sal lei tot beide optimale vruggrootte en oeslading per boom vir 'n spesifieke kultivar te bepaal. Delta Valencia bome in Citrusdal en Nules Clementine bome in Wellington sal gebruik word. Die hoeveelheid vrugte en blare per raamvolume sal getel word (0.5 x 0.5 x 0.5 m) (dieselfde as blaar:vrugverhouding) op spesifieke posisies in bome met verskillende oeslading binne dieselfde boord. Die vrugraamtellings per boom sal in verband gebring word met oeslading en vruggrootte per boom wat tydens oestyd bepaal sal word. Die studie sal in Februarie 2006 begin en resultate sal in die volgende jaarlikse verslag verskyn.

Summary

Fruit size is very important in determining marketable yield. Crop load affects fruit size, with heavy crops generally resulting in smaller fruit. However, the number of fruit per tree or crop load that would give both optimal yield and fruit size is difficult to achieve. The objective of this study is to determine the number of fruit or crop load per tree (canopy volume) for different cultivars that would give both optimal fruit size and crop load. Delta Valencia orange trees in Citrusdal and Nules Clementine mandarin trees in Wellington will be used for the study. The number of fruit and leaves per frame will be counted (0.5 x 0.5 x 0.5 m) (similar to leaf:fruit ratio) at specific positions in the canopy of trees bearing different crop loads in the same orchard. The frame counts per tree will be related to actual yields and fruit size per tree which will be determined at harvest. This study will commence in February 2006 and results will be presented in the next annual progress report.

5.4.4 Water use efficiency of Navel orange trees under partial root zone drying irrigation

By S Dzikiti¹, B Chipindu¹, R Lemeur², K Steppe², JR Milford¹, E Mashonjowa¹

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(2) Laboratory of Plant Ecology, University of Ghent, Belgium

Opsomming

’n Eksperiment is uitgevoer by Mazowe Citrus Estate, Zimbabwe om die potensiele toepassing van die gedeeltelike wortelsone uitdroging strategie vir kommersiële sitrus besproeiing in die semi-droë trope te evalueer. Drie gedeeltelike wortelsone uitdroging behandelings en een beheerde stres besproeiings behandeling word geëvalueer teenoor ’n goed besproeide kontrole behandeling. Die effek van die alternerende gedeeltelike stres in die wortelsone word geëvalueer in terme van die veranderinge in die blaar/xileem waterpotensiaal van die bome en op oeslading. Die eksperiment is huidiglik onderweg en meer gedetailleerde resultate sal in die einde van die seisoen beskikbaar wees. Sekere aspekte van die eksperiment word wel in hierdie verslag uiteengesit.

Introduction

The partial root zone drying (PRD) irrigation strategy in drip irrigated orchards employs two drip lines with one line either side of the tree row. Irrigation is applied using one line at a time by switching from one line to the other approximately every two weeks. The dry side of the root zone generates stress signals (e.g. ABA or changes in the sap pH) that are transported to the leaves via the xylem vessels. These chemical signals then optimize the stomatal aperture such that transpirational water losses are reduced. Because water is always available on one half of the root zone and stomatal conductance is minimal, no deleterious reductions in leaf/xylem water potential and hence in yield are expected. While significant improvements in water use efficiency and fruit quality have been observed in grapes (Dry *et al.*, 1996; Loveys *et al.*, 1998) and this technique has been rapidly adopted by the Australian viticultural industry, not much information exists on the commercial application of the PRD method on citrus. The irrigation experiment described in this report seeks to investigate the response of the Navel orange trees to the PRD irrigation strategy under typical semi-arid tropical conditions.

Materials and methods

Trials are in progress in a 2 ha commercial orchard with five-year-old Navel orange trees at Mazowe Citrus Estate, Zimbabwe under drip irrigation. The soil, uniformly in excess of 1 m depth, belongs to the Banket 5E.2 series, being dark red in colour with a high clay percentage. Emitter spacing along the drip lines is approx. 75 cm. The well-watered control is subjected to daily irrigation for approx. 3 h to keep the soil close to field capacity (45% v/v) using two drip lines, one either side of the tree row. The first partial root zone drying treatment (PRD50) comprises two drip lines running parallel to the tree row with each line about 1.1 m either side of the row. The trees receive 50% less water than in the control treatment but applied to half of the root zone alternated every fortnight. The second PRD treatment uses only one drip line per tree row. The local growers use this setup, so this fixed partial root zone drying (FPRD) treatment serves as the commercial control. The third partial root zone drying treatment (PRD25) is similar to the PRD50, but with half the number of emitters along the drip line blocked. Twenty-five percent irrigation is thus applied alternately using the two drip lines. Switching of the wet and dry sides is done also at 2 week intervals. The regulated deficit irrigation treatment (RDI25) comprises one drip line but with half the number of emitters blocked such that the total volume of water delivered is also 25% of the control. Each treatment comprises a row of ten trees grown on ridges to facilitate drainage. Irrigation frequency is being implemented according to the practice of the local growers (i.e. daily).

One model tree was selected in each treatment and instrumented with sap flow gauges to measure transpiration rates and dendrometers to measure fruit growth in each treatment. Soil water content is monitored in the root zone (25 cm depth) and beyond (60 cm) in the FPRD treatment-using theta probes (Delta-T Co. UK) connected to data loggers (CR23 X, Campbell Sci. Ltd, UK) and averaged every 5 min. The microclimate at the trial site is being monitored using an automatic weather station measuring solar radiation, net all wave radiation, wind speed and direction, air temperature and relative humidity and rainfall at 5 min intervals.

Results and discussion

A typical setup of the soil moisture probes in the FPRD treatment (commercial control) is shown in Fig. 5.4.4.1a while Fig. 5.4.4.1b shows a typical layout of the drip lines in a PRD treatment (PRD25). The outputs of the probes in the FPRD are illustrated in Fig. 5.4.4.1c where soil water content at the beginning of the

rainy season from 30 October (day of year; DOY 303) until 26 November 2005 (DOY 330) is shown. No deep percolation seemed to be occurring as a result of the current irrigation practice with the probe at 60 cm depth showing low readings consistently except on days with high rainfall, e.g. DOY 309 and 311 in Fig. 5.4.4.1c. However, a common feature of the current practice is the frequent occurrence of water stress (assuming a 33% depletion level at the fruit set stage) and some of it quite severe as shown in Fig. 5.4.4.1c. These data, together with the climatic data shown in Table 5.4.4.1, show that given the current levels of water application, daily irrigation is crucial. While most of the severe stress was inevitable due to pump breakdown or persistent power cuts, there is a general need for some decision support tool, e.g. soil moisture monitoring, use of the class A evaporation pan or ETo estimates from climatic data to manage the irrigation.

Table 5.4.4.1. Monthly summary of the orchard microclimate during the growing season from October 2005 to January 2006.

	OCT	NOV	DEC	JAN
Solar radiation (MJ m ⁻²)	757.2	665.0	591.6	621.3
T _{max} (°C)	36.4	35.8	32.2	30.4
T _{mean} (°C)	21.9	22.3	21.0	21.2
T _{min} (°C)	6.4	6.6	13.0	15.7
Daily mean ETo (mm)	5.9	5.1	4.3	5.2
Total rainfall (mm)	1.4	100.8	250.2	324.4
Total ET (mm)*	107.2	94.7	80.8	97.0

*The monthly total ET is calculated assuming a crop coefficient of 0.60.

In the absence of at least 5 mm of rainfall per day during October and November, irrigation should be applied daily as shown by the course of the daily mean reference crop evapotranspiration (ETo) in Table 5.4.4.1 above.

Future research

Initial results of the partial root zone drying irrigation experiment are expected after harvest in June 2006. The trial is expected to continue in future growing seasons with intensive assessments of the plant water relations and yield as a result of the PRD irrigation method.

In addition, an irrigation decision support system based on the cumulative difference between rainfall and evapotranspiration obtained from an automatic weather station is being developed to minimize incidences of water stress.

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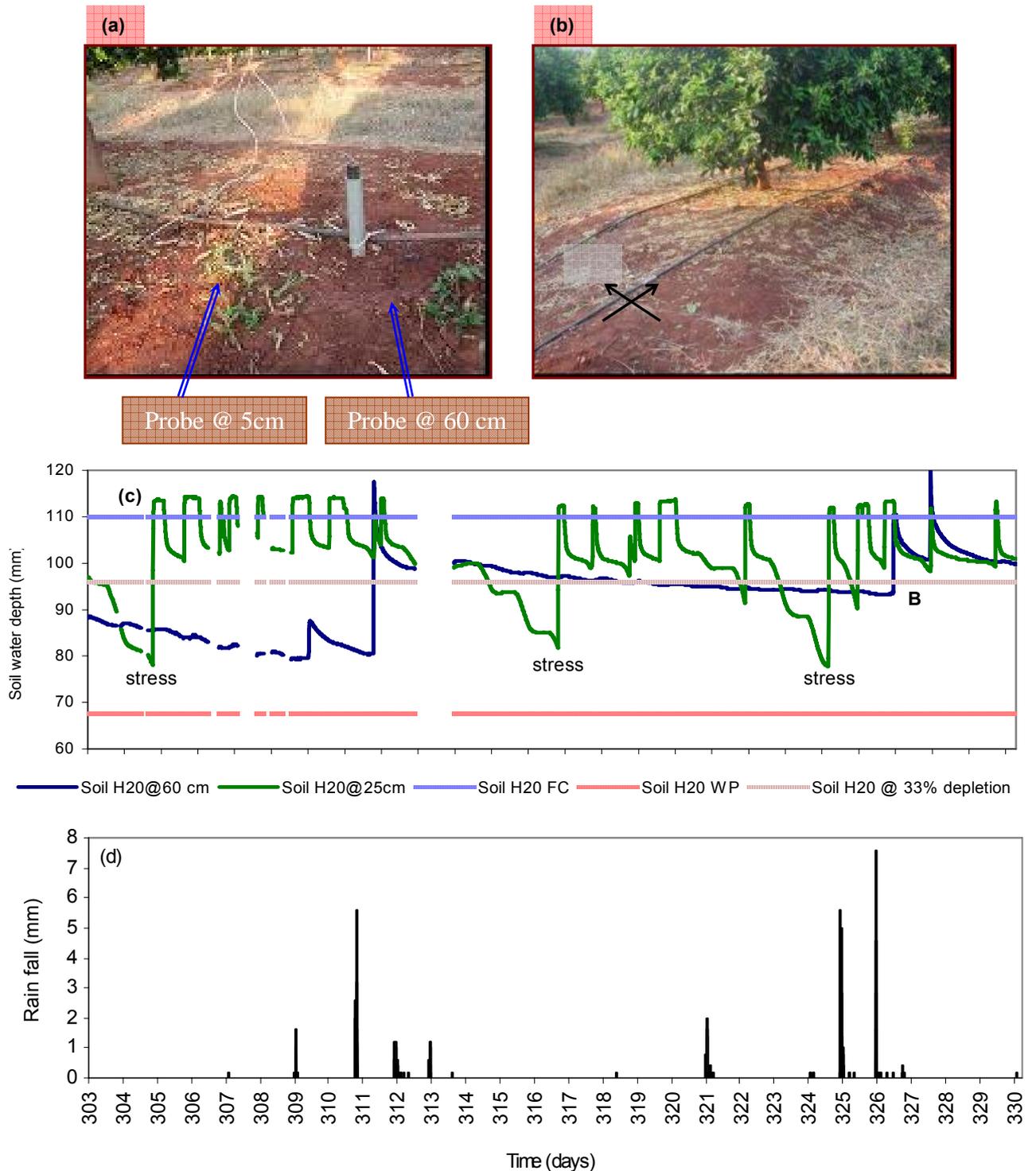


Fig. 5.4.4.1.

- (a) Theta probes installed in the root zone (25 cm depth) and beyond (60 cm) in the fixed partial root zone drying (commercial control) treatment in an orchard with 5-year-old Navel orange trees.
- (b) Navel orange trees irrigated under the partial root zone drying (PRD) system.
- (c) Soil water content in the root zone and at 60 cm depth from 30 October until 26 November 2005 (day of year; DOY 303 - 330). Gaps in the figure are due to mains power cuts.
- (d) Rainfall at the trial site at the start of the growing season (DOY 303 - 330).

6 PROGRAMME: CULTIVAR AND ROOTSTOCK EVALUATION

6.1 PROGRAMME SUMMARY

By Graham H. Barry (Manager: Cultivar Development, CRI)

Maximising the long-term global competitiveness of the Southern African citrus producer requires on-going innovation at various levels in the fruit value-chain. Product differentiation, through the commercialisation of new citrus cultivars, provides a principal means by which to achieve this goal.

CRI's Cultivar Development division aims to facilitate the rapid access to growers of new citrus cultivars to meet the changing requirements of the markets and to provide independent and objective information on all citrus cultivars to enhance grower decision-making.

To this end, the cultivar evaluation component of CRI's Cultivar Development division will be actively involved in the establishment and field evaluation of all citrus cultivars.

Programopsomming

CRI se Kultivarontwikkelingsdivisie mik daarna om die beskikbaarheid van nuwe sitruskultivars te versnel en sodoende aan die markbehoefte te voorsien. Verder wil ons onafhanklike inligting beskikbaar stel om besluitneming van produsente te verbeter. Om dié doelwit te bereik sal CRI se Kultivarontwikkelingsdivisie betrokke wees by die evaluasie van alle sitruskultivars.

6.2 PROGRAMME INTRODUCTION

The need for independent and objective cultivar information

Citrus production is a long-term, capital-intensive endeavour with a relatively long interval between the start of orchard preparation and when the economic break-even point is achieved. As a primary producer, the citrus grower is the ultimate risk-taker in the production and marketing value-chain. Therefore, the correct choice of cultivar is one of the most important pre-planting decisions that a grower must make. To make such decisions requires credible information.

The introduction and commercialisation of new citrus cultivars in South Africa during the 1980s and 1990s was dynamic and aggressive and was driven by the citrus industry. Numerous cultivars were introduced and commercialised during this era, and contributed to South Africa's position as the southern hemisphere leader in citrus supply. Yet to remain competitive, there is still an acute need to seek out, evaluate and commercialise new citrus cultivars.

The de-regulation of the citrus industry in the late-1990s and re-structuring of research and technical support services to the industry between 2002 and 2004, led to uncertainty in the role of industry-driven cultivar evaluation. In addition, this era also witnessed the advent of cultivar management companies and an increase in the number of privately-managed citrus cultivars. In 2004, the citrus industry decided to become actively involved in cultivar development, and established a Cultivar Development division within CRI in 2005.

Recognising the need of citrus growers to have access to impartial, credible, decision-making information related to cultivar planting options, the Cultivar Development division will, among other things, address the need to provide independent and objective information on all citrus cultivars through coordinated field evaluation of privately-managed and so-called "open" citrus cultivars. The ultimate purpose of this endeavour will be to maximise competitiveness of South African citrus growers through enhancing their access to new citrus cultivars and related information.

Overall objectives of cultivar evaluation

The evaluation of new citrus cultivars serves three principle purposes, namely, to

1. describe a cultivar's characteristics,
2. determine broad climatic suitability of a cultivar, and ultimately to
3. determine commercial potential in the market.

Planting decisions remain the responsibility of the grower and should be made in consultation with marketers, private cultivar agents (where appropriate), and other cultivar specialists. Commercial decisions and processes of privately-managed cultivars remain the right of private cultivar agents.

Cultivars cannot be fully evaluated under all possible conditions within a relatively short timespan. Therefore, latent defects cannot be accounted for and a degree of risk in cultivar choice will probably always exist. Therefore, the role of cultivar evaluation is to describe a cultivar's characteristics and to determine the broad climatic suitability of a cultivar, and thereby minimise the potential commercial risks involved in their commercial use. Longer-term production and postharvest information will be generated from semi-commercial or commercial plantings.

Cultivar evaluation guidelines

Guiding principles for cultivar evaluations

1. *Evaluation objectives*

1.1. The ultimate objective of cultivar evaluation is to provide citrus growers with impartial, credible information upon which they can make planting decisions.

- To achieve this objective, statistically-sound experiments and publishable scientific findings are not required. Also, exhaustive evaluations of all aspects of a cultivar's production in all regions and postharvest responses cannot be conducted. In the short-term, therefore, general cultivar characteristics will be described and broad climatic suitability of a cultivar will be determined. Longer-term, semi-commercial or commercial plantings will be used to "fill in the gaps" with respect to detailed observations.
- Furthermore, it is not the intention of the cultivar evaluation component of Cultivar Development to slow down the process of commercialisation of new citrus cultivars, but rather to facilitate the flow of cultivars through the system, including direct involvement in cultivar evaluation.

1.2. "Fast-track" or initial evaluations will be conducted to determine the general characteristics of a cultivar as opposed to the long-term performance potential. Therefore, there will be an element of risk to growers in an endeavour to avoid unnecessary delays in planting new cultivars.

2. *Site selection*

2.1. Since cultivar characteristics are climate-dependent, evaluations will be conducted in climatically suitable growing regions for each cultivar group.

- New cultivars will be established at sites according to a "cultivar group x climatic region matrix" in the major citrus-producing regions.
- Where appropriate, information generated from these evaluations will, by necessity, be used to determine cultivar suitability in other similar sites.

2.2. Suitable nursery and grower co-operators are essential.

- Relevant non-propagation agreements must be signed by nursery and grower co-operators.
- The evaluation site should preferably be within a commercial orchard and must consist of uniform and suitable soil type, and not be near a windbreak.

3. *Trial design*

3.1. The main commercial cultivar in the cultivar group will serve as the control for comparison with new cultivars in the same cultivar group.

3.2. Budded trees of the same (or similar) age on a standard rootstock should preferably be used. However, in some cases topworked trees will be evaluated. In such cases, tree condition must be taken into account when evaluation results are interpreted.

3.3. A minimum of five trees per cultivar are to be planted or topworked, and randomisation of cultivars within the test plot is not required.

3.4. Factorial type experiment designs to be avoided.

4. *Evaluation criteria*

4.1. Cultivar development strategy by cultivar group

- Navel oranges: To select cultivars with improved fruit set (and yield), packout (wind blemish, creasing, oleo, mealybug, *Alternaria* core rot), rind colour and internal fruit quality (juice content, granulation, acidity), and extended harvest period (especially earlier maturity).
 - Early maturity, deep orange rind colour.
- Midseason oranges: To select suitable midseason orange cultivars which compliment Navel and Valencia supply or provides a niche product. Specific requirements include acceptable fruit size, rind colour and texture, peelability, flavour and pigmentation (in the case of blood oranges).
 - Lower priority; acceptable size, consistent pigmentation and flavour.
- Valencia oranges: To select cultivars with improved and consistent productivity, fruit size, rind colour, peelability and internal fruit quality (seedlessness, ratio), and extended harvest period (both earlier and later maturity).

- Large, seedless, early and late maturity.
- Grapefruit: To select cultivars with improved fruit size and eating quality (reduced bitterness, higher ratio) and extended harvest period (both earlier and later).
 - Lower priority; reduced bitterness, improved flavour, large fruit size, red.
- Shaddocks: Low priority; acceptable size (smaller), thinner rinds, uniform segmentation, higher juice content and acceptable flavour.
- Lemons: To select cultivars with elongated fruit shape, high juice content and seedless cultivars with improved productivity. Trees should be thornless and compatible with a wide range of rootstocks.
- Satsuma mandarins: To select cultivars with acceptable fruit size, improved rind colour and internal fruit quality (Brix and acidity, ratio), and extended harvest period (both earlier and later maturity).
 - Early and late maturity with acceptable size, colour and flavour.
- Clementine mandarins: To select larger fruited cultivars with acceptable rind colour and flavour, and extended harvest period (especially earlier, but also later maturity).
 - Large-fruited, early maturity.
- Mandarins: To select late maturing, seedless mandarins with acceptable fruit size, good peelability, rind colour and flavour.
 - Late maturing, seedless, easy-peeling mandarin with acceptable size, colour and flavour.

6.3 PROJECT: CULTIVAR EVALUATION

Project Co-ordinator: Graham H. Barry (CRI)

6.3.1 Project summary: Cape areas

Satsumas: Primosole is early maturing. Ohtsu, Aoshima and Ueno are late maturing but had poor quality. The commercial Dobashi Beni orchard was not yet in production. Evaluation of Dobashi Beni and later selections to continue. A new trial, including all the late maturing selections planted together has been established. The ITSC Satsuma x Nova selection must be established in other areas once material is released.

Clementines: Clemenpons is early maturing with acceptable quality but has galls on and above the bud union and variable tree vigour. The long term effect of the galls needs to be established. Esbal production and quality were good, colour slightly ahead of Nules. Fruit size was good after chemical manipulation. Fruit size could be problematic in cooler areas. There was no fruit on Tardif de Janvier I and too limited information on Tardif de Janvier II to make any recommendations. The Sidi Aissa and Ain Toujdate appear to have similar characteristics, slightly later maturing than Nules and good quality. Both selections have consistently produced only mediocre fruit size and are not recommended. The CELL had variable production and fruit size, good quality and retarded colour with green stylar ends. Clementarde had poor production and fruit size, good quality, late maturing and delayed rind colour. The selection does not look promising. Hernandina had smallish fruit size, late maturing with no outstanding features. Tardivo had variable production and fruit size with good quality, good to lowish acid. Stylar ends were green and it is the latest maturing Clementine selection worthy of further evaluation. Nour production was not good and variable fruit size; maturity varied between sites. Rind colour was retarded and does not look promising. Further evaluations are necessary on some of the selections.

Mandarins: Nova SL is similar to Nova but not completely seedless in mixed blocks, although less seedy than Nova. Valley Gold looks promising but tends to have high acid and some fruit split. African Sunset looks promising, is seedless, has lower acid than Valley Gold but fruit size can be too large with a flat shape. M37 had good quality and soft rag and a slight delay in colour, maturing in June. H25 matures in May, quality poor with poor flavour and does not look promising. H36 had good quality and soft rag with slightly retarded rind colour, maturing in mid June which may clash with other mandarins. K33 although virtually seedless, does not look promising as it is unattractive. Roma had good quality, but an unattractive naartje-like appearance. Or 4 had good quality in late July; Or 2 had fair production with good fruit size, maturing late July. Mor 22 had variable production between sites and medium size with a good test. Nadorcott had good production of medium to also large fruit size, acid on eating. Murcott x Clem fruit were eaten by baboons and the semi-commercial block is not yet in production. Bay Gold had good production and fruit size but does not look promising in the cooler production areas due to high acid. Hadas had good production and fruit size but consistently high acid. Winola had good production with medium fruit size and very good colour but excessive acid. Cami had good production and fruit size, is attractive, maturing after mid June. Empress mandarin had fair production with medium fruit size. Some selections need to be evaluated further.

Navel: Atwood had similar to slightly later maturity than the Lina/Newhall. Fukumoto is early maturing, similar, to slightly earlier than Lina/Newhall, developing a deep orange/red rind colour. Local observations show that there may be incompatibility on Swingle citrumelo and to a lesser extent on Koethan citrange. More clarity is needed on rootstock choice. Maturity of Letaba Early appears to be similar to later than Lina/Newhall. Dream had smaller but acceptable fruit size, maturing one to two weeks later than Lina/Newhall. Cliff Early is early and further trials should be established. Fenix, Krajewski and Sundays River Early do not appear to be early maturing, but it is still early days. Washington (CFB material) performed well with good fruit size, quality varying between sites. Cambria (CFB material) had good fruit size, round to elongated fruit shape and good quality but acid can tend to get low. A comparison between the Autumn Gold, Powell, Chislett and Californian Lane Late showed little difference between the selections. Powell is possibly slightly earlier maturing, Chislett and Californian Lane Late slightly later. Juice percentage can be low on rough lemon/Rangpur lime rootstocks on sandy soil. Renken Late, Coetzee Late and Mouton Late 1 and 2 had poor production, Renken attractive. Glenora Late just came into production with a few fruit. Eating quality was poor, but tests good. Further evaluations of all the selections are necessary as some of the trees are still young.

Midseasons: Sanguinello at Fort Beaufort had fairly good production but fruit size tended to be on the small side. Tarocco production and fruit size was overall good. Quality was generally good but can be masked by high acid. Tarocco Gallo and 57/1E/1 both had good production, fruit size and quality but can be quite acid. Gallo colour was behind Tarocco. Blush and flesh pigmentation was variable. All three Tarocco selections have large thorns when young and on vigorous branches but appear to lessen with ageing. There were only slight differences between the various Maltese selections, Maltese Half II and Maltese Line G having the smallest, unacceptable fruit size. None of the selections were outstanding in any way. Raratonga had acceptable production and fruit size with good quality but large thorns. Further evaluations on the Tarocco selections, Raratonga and commercial Maltese are necessary.

Valencias: Mouton Early looks promising as an early maturing selection with acceptable production, good size and quality. Evaluations to be discontinued until virus-free material becomes available. Turkey can be planted as an early maturing selection although there are some reservations in terms of internal quality. The compatibility of Turkey on Rangpur lime is questionable and other rootstocks should rather be used. Various new selections were evaluated at the CFB. Rietspruit had poor production and quality but good fruit size and seedless. Portsgate had fair production and fruit size, good to acceptable quality and the occasional seed. Bend 8A2 production and quality was poor with acceptable fruit size (trees in psylla house). G5 had good quality and colour even though it is in the psylla house. McClean Seedless had good production and medium fruit size, fair to good quality and seedless. Delicia had good production and fruit size and good eating quality, although not meeting standards. Kleinhans had excessive acid as it is planted next to a windbreak and is seedless to virtually so. Further evaluations are necessary before recommendations can be made.

Knysna area: The purpose of the trial is to find suitable, high quality, especially late maturing soft citrus cultivars for the Knysna area. Ueno Satsuma had fairly large fruit size, meeting export standards but with relatively high acid levels, maturing much later than Miho Wase. Mor, Or, Nadorcott and Nectar were probably under stress conditions resulting in small fruit size and high acid. Bay Gold does not look promising due to high acid levels even when overmature. Aoshima Satsuma bore their first fruit, which were of poor quality. Sweet Spring performed well but has a pale rind colour. The flavour showed an improvement over previous seasons. Nouvelle had too few fruit to draw any conclusions and may be susceptible to *Alternaria brown spot*. Thoro Temple is not outstanding in any way and not recommended. The late maturing CELL, Clementarde and Clemlate are similar and have certain production drawbacks, including low acid levels. Evaluations to continue for at least another season.

Projekopsomming: Kaapstreek

Satsumas: Ohtsu, Aoshima en Ueno is laat maar gehalte swak en die kommersieële Dobashi Beni nog nie in produksie nie. Evaluasies van Dobashi Beni en die later seleksies moet nog voortgaan. 'n Nuwe proef met al die laat seleksies is onlangs gevestig wat saam gevalueer kan word. Die ITSG Satsuma x Nova seleksie moet in ander areas gevestig word sodra materiaal vrygestel word. Die Primosole word vroeg ryp.

Clementines: Clemenpons word vroeg ryp met aanvaarbare gehalte, maar het galle op die entlas en stam. Die uitwerking van die galle in die langtermyn moet vasgestel word. Boomgroeikrag varieer. Esbal produksie en gehalte was goed, kleur effens voor Nules. Vruggrootte was goed na chemiese manipulasie. Klein vruggrootte kan dalk probleme, veral in die koeler gebiede, skep. Tardif de Janvier I het geen vrugte

gedra en daar is te min inligting oor die Tardif de Janvier II om aanbevelings te maak nie. Sidi Aissa en Ain Toujdate is midseisoen seleksies met soortgelyke eienskappe. Vruggrootte is gereeld aan die kleiner kant en word nie aanbeveel nie. CELL het wisselvallige produksie en vruggrootte, goeie gehalte met groen blomente en vertraagde vrugkleur. Clementarde het swak produksie en vruggrootte gehad, met goeie gehalte en word laat ryp met vertraagde vrugkleur. Lyk nie belowend nie. Hernandina het relatief klein vruggrootte, word laat ryp met geen uitstaande eienskappe nie. Tardivo het wisselvallige produksie en vruggrootte gehad met goeie gehalte en goed tot relatief lae suur. Blomente is groen en is die laatste Clementine seleksie om ryp te word. Evaluasies moet voortgaan. Nour produksie was nie goed met wisselvallige vruggrootte. Rypwording het gewissel tussen persele. Vrugkleur is vertraag en die seleksie lyk nie belowend nie. Verder evaluasies is nodig met sommige van die seleksies.

Mandaryne: Nova SL is soortgelyk aan Nova maar nie heeltemal saadloos in gemengde blokke nie, maar het wel minder saad as Nova. Valley Gold lyk belowend maar is geneig om hoër suur te hê en effense vrugsplut. African Sunset is saadloos, met minder suur as Valley Gold maar vrugte kan te groot wees met 'n plat vrugvorm. M37 het goeie gehalte en sagte vesel, word in Junie ryp met effens vertraagde kleur. H25 word in Mei ryp, met swak geur en gehalte en lyk nie belowend nie. H36 het goeie gehalte met sagte vesel en effens vertraagde kleur, word in Junie ryp wat dalk met ander mandaryne mag bots. K33 is feitlik saadloos maar lyk nie belowend nie weens sy swak voorkoms. Roma het goeie gehalte met 'n swak naartjie tipe voorkoms. Or 4 het goeie gehalte in laat Julie gehad. Or 2 het redelike produksie met goeie vruggrootte gehad, en word in laat Julie ryp. Produksie van Mor 22 het gewissel tussen persele met medium vruggrootte en 'n goeie toets. Nadorcott het goeie produksie met medium asook groot vruggrootte gehad, maar suur. Murcott x Clem was deur bobbejane opgevrete en die semi-kommersieële boord is nog nie in drag nie. Bay Gold het goeie produksie en vruggrootte gehad maar lyk nie belowend in die koeler gebiede nie weens hoër suur. Hadas het goeie produksie en vruggrootte gehad maar gereelde hoër suur. Winola het goeie produksie met medium vruggrootte en goeie vrugkleur gehad maar baie hoër suur. Cami het goeie produksie en vruggrootte gehad, is aantreklik en word in mid Junie ryp. Empress mandaryn het redelike produksie met medium vruggrootte gehad. Van die seleksies moet verder evalueer word.

Nawels: Atwood het soortgelyke tot effens later rypwording as Lina/Newhall. Fukumoto word vroeg ryp, dieselfde tyd tot effens voor Lina/Newhall en ontwikkel 'n diep oranje-rooi skilkleur. Plaaslike waarnemings dui aan dat daar moontlike onverenigbaarheid op Swingle citrumelo en tot 'n mindere mate Koethan citrange is. Meer duidelikheid word benodig oor onderstam keuse. Letaba Early word dieselfde tyd tot later as Lina/Newhall ryp. Dream het kleiner, maar aanvaarbare vruggrootte en word een tot twee weke later as Lina/Newhall ryp. Cliff Early is vroeg en proewe moet uitgebrei word. Fenix, Krajewski en Sundays River Early lyk nie asof hulle vroeg ryp word nie, maar meer evaluasies is nodig. Washington (SGB materiaal) het goed gevaar, met goeie vruggrootte maar gehalte het tussen persele gewissel. Cambria (SGB materiaal) het goeie vruggrootte gehad, beide ronde en langwerpige vrugte met goeie gehalte maar suurvlaeke kan neig om laag te wees. 'n Vergelyking tussen Autumn Gold, Powell, Chislett en Californian Lane Late het min verskille tussen hulle getoon. Powell word dalk effens vroeër ryp, Chislett en Californian Lane Late effens later. Sap vlakke op growweskielsuurlemoen en Rangpur lemmetjie onderstamme op sanderige grond kan laag wees. Renken Late, Coetzee Late en Mouton Late 1 en 2 het min gedra. Renken lyk aantreklik. Glenora Late het begin dra met enkele vrugte. Eetgehalte was swak maar interne gehalte toetse goed. Verdere evaluasies van al die seleksies is nodig aangesien van die seleksies nog jonk is.

Midseisoene: Sanguinello op Fort Beaufort het redelike goeie produksie gehad maar vruggrootte is geneig om aan die klein kant te wees. Tarocco produksie, vruggrootte en gehalte was oor die algemeen goed maar die suur kan neig om die geur te verbloem. Tarocco Gallo en 57/1E/1 het albei goeie produksie, vruggrootte en gehalte gehad, maar kan neig tot hoër suur. Gallo vrugkleur was later as Tarocco. Skilblos en vleiskleur het gewissel. Al drie Tarocco seleksies het lang dorings op jong bome en groeikragtige takke gehad maar word minder sodra die boom verouder. Daar was min verskille tussen die verskillende Maltaise seleksies. Maltaise Half II en Maltaise Line G het die kleinste, onaanvaarbare vruggrootte gehad. Nie een van die Maltaise seleksies het uitblink nie. Raratonga het aanvaarbare produksie en vruggrootte gehad en goeie gehalte maar lang dorings. Verdere evaluasies op die Tarocco seleksies, Raratonga en kommersieële Maltaise is nodig.

Valencias: Mouton Early lyk belowend as 'n vroeër seleksie met aanvaarbare produksie, goeie vruggrootte en gehalte. Evaluasies word gestaak totdat virusvrye materiaal beskikbaar gestel word. Turkey kan as 'n vroeër seleksie geplant word, maar die interne gehalte is 'n kwessie. Die verenigbaarheid op Rangpur lemmetjie is twyfelagtig en ander onderstamme moet oorweeg word. Verskeie nuwe seleksies was by die SGB geevalueer. Rietspruit het swak produksie en gehalte gehad maar goeie vruggrootte en saadloos. Portsgate het redelike produksie en vruggrootte gehad, goeie tot aanvaarbare gehalte en af en toe 'n pit. Bend 8A2 se produksie en gehalte was swak maar aanvaarbare vruggrootte (psylla huis). G5 se gehalte en

vrugkleur in die psylla huis was goed gewees. McClean Seedless het goeie produksie en matige vruggrootte gehad, matig tot goeie gehalte en saadloos. Delicia het goeie produksie en vruggrootte gehad met goeie eetgehalte maar nie die toetse gemaak nie. Kleinhans het buitensporige hoë suur gehad omdat dit langs 'n windbreek geplant is. Dit het geen of net enkele saad gehad. Evaluasies moet voortgaan.

Knysna area: Die doel van die proef is om geskikte, hoër gehalte, veral laat mandaryn kultivars te vind vir die Knysna area. Ueno Satsuma het taamlikke groot vrugte gedra wat uitvoer standarde behaal het, al was die suurvlaakte relatief hoog. Hulle word later as Miho Wase ryp. Mor, Or, Nadorcott en Nectar was moontlik onder stres met kleinvruggrootte en hoër suur. Bay Gold lyk nie belowend nie weens hoër suur al is die vrugte oorryp. Aoshima Satsuma het sy eerste vrugte gedra met swak gehalte. Behalwe vir die bleek skilkleur het Sweet Spring goed gepresteer met beter geur as voorheen. Nouvelle het min gedra en mag vatbaar vir *Alternariabruinvlek* wees. Thoro Temple het geen uitstaande eienskappe vertoon en word nie aanbeveel nie. Die laat CELL, Clementarde en Clemlate Clementine seleksies is eenders en het sekere produksie nadele, insluitend lae suurvlaakte gehad. Evaluasies moet vir nog minstens een jaar voortgaan.

6.3.2 Project summary: Inland areas

Clementines: The objectives of this trial was to ascertain whether certain Clementine mandarins could be produced commercially for export in the intermediate and cool inland citrus production areas of the country. The first selection to reach maturity was Ain Toujdate (middle to end of April) with the internal quality not complying with export standards. Sidi Aissa was the second selection to mature by the end of April. Unfortunately the internal quality was below the export minimum standards. Nour's internal quality was acceptable and ready to harvest by the end of May. The red scale problem seemed under control and 90% of the fruit were clean. The number of seeds per fruit was less this year due to less cross-pollination.

Mandarins: The objectives of this trial is to find suitable mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap. Primasole was the earliest selection to mature this season. Unfortunately this selection seems to develop major problems in the cooler areas (Burgersfort) – granulation, large fruit size, coarse rinds and low juice content. M26 (ARC) and Roma look promising with good internal quality and with small to medium fruit size. Proper irrigation scheduling was possible this season due to sufficient water availability.

Navels: At Burgersfort, Atwood appears to be promising and complied with internal export standards. Cara Cara remains promising, however the correct marketing is essential – the unique internal colour of the fruit plays a major role. Rootstock incompatibility with Fukumoto has not yet determined, although bud sprouts developed close to the budunion (which were not present in the 2004 season). The trees bore more fruit this season, but internal quality did not comply with the export standards. At Marble Hall, Cara Cara performed well, internal colour was dark red and uniformly distributed throughout the fruit. The internal quality and colour was better than in the Burgersfort area. Atwood did not perform well compared to Burgersfort and the juice content barely complied with the minimum export standards. Chislett performed poorly and will be removed from the trial. The trees did not produce fruit and the internal quality was below the export standards. Dream on the other hand produced medium to large fruit. This season Fukumoto produced a good yield, although the fruit did not comply with the export standards.

Valencias (Onderberg): Glen Ora Late seems promising and produced a good yield, internal quality and acceptable fruit size. McClean Seedless has low rag content with excellent fruit set. Internal quality complied with the export requirements and most of the fruit were seedless. Ruby Valencia (irradiated) with its uniform red internal colour and seedless fruit looks very promising for the future. The water supply in the Malelane area was sufficient and the trees were irrigated optimally.

Lemons: This season Villafranca produced less seed per fruit compared with Verna. The remaining selections produced high numbers of seeds per fruit. Yield of all the selections was good. Eureka SL (ARC) remains the best seedless lemon selection available.

Projekopsomming: Binnelandsestreek

Clementines: 'n Proef is saamgestel om te bepaal of sekere Clementine mandaryne kommersieel vir uitvoer in die intermediêre en koel binnelandse sitrusproduserende streke van die land met betrekking geproduseer kan word. Ain Toujdate was die vroegste (middel tot einde April) gereed vir oes, maar het nie aan die uitvoer standarde voldoen nie. Sidi Aissa was tweede gereed vir oes einde April, maar kon ook nie gepak word vir uitvoer nie. Nour het aan alle uitvoer standarde voldoen en was middel Mei gereed vir oes. Clementarde het 'n uitstekende interne kwaliteit geproduseer en was einde Mei gereed vir uitvoer. Die rooidopluis

probleem was onder beheer gewees en 90% van die vrugte het visueel skoon voorgekom. Die hoeveelheid sade per vrug het ook effens verminder, a.g.v. laer kruisbestuiving.

Mandaryne: Geskikte mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul. Primosole was die vroegste gereed vir oes, maar die seleksie ondervind heelwat probleme in hierdie area – granulasie, groot vruggrootte, baie growwe vrugte en lae sap volume was van die ergste probleme. M26 (ARC) en Roma lyk die belowendste van die seleksies met goeie interne kwaliteit, maar klein tot medium vruggrootte. Hierdie seisoen was die watervoorraad voldoende en die besproeiings skedulering in plek.

Nawels: By Burgersfort, Atwood lyk baie belowend en voldoen aan die uitvoer standaard. Cara Cara bly steeds een van die seleksies met baie potensiaal wanneer dit korrek bemark gaan word – die vrugte se interne kleur speel 'n masiewe rol. In hierdie areas is die interne kleur universeel versprei deur die vrugte. Fukumoto toets nog steeds negatief vir onverenigbaarheid by die entlas, maar die knopperige groen punte op die onderstam het vergroot. Die bome dra meer vrugte, maar die interne kwaliteit voldoen nie aan die uitvoer standaard nie. By Marble Hall, Cara Cara presteer vir hierdie area baie goed. Die interne kleur is baie univorm versprei en die interne kwaliteit lyk belowend. Die smaak en kleur is beter as in die Burgersfort area. Atwood presteer hier nie so goed soos in die Burgersfort area nie en die sap% voldoen beswaarlik aan die minimum vereistes. Chislett presteer werklik swak en gaan uit die proef verwyder word. Die bome set nie vrugte nie en voldoen ook nie aan die uitvoer standaard nie. Dream het medium to groot vrugte geproduseer. Fukumoto het hierdie seisoen 'n goeie oes geset, maar die interne kwaliteit het nie aan die vereistes vir uitvoere voldoen nie.

Valencias (Onderberg): Glen Ora Late lyk belowend met 'n goeie produksie, goeie interne kwaliteit met groot vruggrootte. McClean SL het ook 'n goeie oes geproduseer met 'n lae veselinhoud in die vrugte. Die interne kwaliteit voldoen aan die uitvoer standaard en minimale sade was in elke vrug teenwoordig. Ruby Valencia (bestraald) met sy univorm rooi interne kleur en saadlose vrugte hou goeie potensiaal in. Die watervoorraad in die Malelane area was voldoende en die bome is optimaal besproei.

Suurlemoene: Hierdie seisoen het Villafranca minder saad per vrug geproduseer as Verna. Al die ander seleksies produseer steeds heelwat sade per vrug. Die drag op al die seleksies was baie belowend gewees. Eureka saadloos (LNR) bly steeds die beste saadlose suurlemoen seleksies tans beskikbaar.

6.3.3 Evaluation of Satsuma mandarins and Primosole in the Cape areas

Experiment 57 by C J Alexander (Private Contractor)

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. Ohtsu, Aoshima en Ueno is laat maar gehalte swak en die kommersieële Dobashi Beni nog nie in produksie nie. Evaluasies van Dobashi Beni en die later seleksies moet nog voortgaan. 'n Nuwe proef met al die laat seleksies is onlangs gevestig wat saam geëvalueer kan word. Die ITSG Satsuma x Nova seleksie moet in ander areas gevestig word sodra materiaal vrygestel word. Die Primosole word vroeg ryp.

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, Ueno and Dobashi Beni Satsumas and Primosole were evaluated.

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.

Fruit quality was compared with the following standards previously considered most acceptable by the market place, based on visual and organoleptic tests: 48% juice; 8.5% Brix or 9.0% TSS; 0.7 – 1.5% acid;

7.5:1 ratio; colour T3 of set 36 of CRI blemish standards; 0 seeds per fruit. A list of the selections and sites evaluated is given in Table 6.3.3.1 and internal quality tests in Table 6.3.3.2.

Table 6.3.3.1. Satsuma trial sites evaluated during 2005.

Selection	Area	Site	Plant Date	Root stock	No of trees
Satsuma x Nova	Addo	ITSC	2000	CC	3
Dobashi Beni	Uitenhage	CFB Block 7	1994	TC	2
Dobashi Beni	Buffeljagsrivier	Sovereign	1994	CM	4 topwork
Ohtsu	Uitenhage	CFB Block 8	1997	SC	6
Ohtsu	Grabouw	Whitehall	1997	SC	5
Aoshima	Uitenhage	CFB Block 7	1994	TC	2
Aoshima	Grabouw	Whitehall	1997	SC	5
Ueno	Uitenhage	CFB Block 8	1997	TC/SC	3/3
Primosole	Stellenbosch	Slaley	1999	CC	3
Primosole	Citrusdal	Brakfontein	2001	CC	2
Miyagawa Wase (control)	Uitenhage	CFB Block 7	1994	TC	2
Okitsu Wase (control)	Grabouw	Whitehall	1997	SC	5
Owari (control)	Uitenhage	CFB	1991	SC	semi comm

Results and discussion

A discussion of each selection follows with a comparison of the selections and internal quality results presented in Table 6.3.3.2 & 3 which need to be referred to when reading the text.

ITSC Satsuma x Nova. During the 3rd week of April the fruit colour was T6, with a flat shape, not necessarily earlier than Miho Wase, but firm in spite of heavy rains.

Dobashi Beni. Trees at the CFB had slightly larger fruit size than the Owari and similar colour transparency on 26 April. However some fruit had a deep red colour and were one week earlier maturing. Eating quality was poor but the test acceptable, although not as good as Owari with much less acid (also different rootstocks).

At Sovereign both Dobashi Beni and Owari had been partly picked by 22 April. Most aspects were similar although Dobashi Beni had a slightly better test and was more tart. This is contrary to the CFB. Both have a smooth rind and flat shape with open cores at both sites. The fruit is seedless in a solid Owari block.

The semi commercial topworked orchard in the Western Cape has grown poorly and it is doubtful whether there will be fruit next season.

Ohtsu. Yield and fruit size at the CFB (next to a windbreak) was good, ahead of Aoshima and Ueno on colour, but behind Owari on 26 April. Ohtsu had borderline quality, Aoshima better and Ueno a higher acid. Owari had far higher sugars and acid. Maturity estimated early May, although the fruit was already puffy.

At Grabouw the yield was acceptable, better than Aoshima with larger fruit size. Colour was fractionally ahead of Aoshima, with better but unacceptable internal quality. Maturity appeared to be ahead of Aoshima by about a week, more puffy than Aoshima but less than Miyagawa Wase. Due to the poor quality of the three selections at Grabouw, it is difficult to estimate maturity with accuracy. Probably matures at the end of April, Aoshima early May and Miyagawa Wase around mid April. The Ohtsu and Aoshima both have a smooth rind and flat shape with open cores at both sites. Fruit were seedless at the CFB, odd seed at Grabouw. Tree vigour at Grabouw was similar to Aoshima, average.

Aoshima. At the CFB, yield and fruit size were good, behind Owari, Ohtsu and Ueno on colour on 26 April. Eating quality was fair with an acceptable test. The seed count was exceptionally high which is inexplicable (2005 – 9.9 seeds/fruit, 2004 - 1.2, 2003 - 0 and 2002 - 0.6).

Trees at Grabouw had a poor crop of large size, fractionally behind Ohtsu on colour on 5 May. Fruit quality was poor, appeared later maturing than Ohtsu and not as puffy. The fruit was flat and smooth with open cores at both sites. There were odd seed, similar to Ohtsu.

Ueno. The yield and fruit size at the CFB was good. Fruit colour was between Ohtsu and Aoshima on 26 April. Fruit quality was just acceptable. The acid was higher than Ohtsu and Aoshima. Maturity is estimated to be about the same as Aoshima, around early May. The fruit was flat and smooth with open cores and odd seed in a mixed block.

Primosole. Fruit size at Stellenbosch was fairly large. Adjacent Clemenpons and older Marisol both had overall smaller, variable fruit size. Fruit colour on 14 April was T3 – 5, Clemenpons and Marisol both mainly T6-7 with some earlier coloured fruit. Fruit was easily peeled with thin, oily rinds, soft fruit but slightly raggy, open cores and some dry fruit.

Yield and fruit size at Citrusdal was good, colour T5-6 and 6 on 30 March, while adjacent older Oronules and Esbal had smaller fruit size and later colour, T7 and T6-7 respectively. Eating quality was fair, slightly dry, the other selections sweeter. No tests were done.

The fruit had smooth rinds, roundish shape at Citrusdal, but flatter at Stellenbosch.

Conclusions

Satsuma x Nova. Looks promising and needs to be established in other areas as a potential early Satsuma.

Dobashi Beni. Production was good with good fruit size, similar colour to Owari, but rind showing a deep red colour at the CFB. Quality tests were acceptable, with differences between the sites. Maturity is around 3rd – 4th week of April. Wait until semi commercial orchard comes into production. Dobashi Beni can be tried on a semi commercial scale, with the advantage of the fruit developing a deeper red rind colour.

Ohtsu. The yields and fruit size were acceptable to good. Internal quality was poor even though an acceptable test at one site. Due to the poor quality it is difficult to estimate maturity accurately but it is later than Miyagawa Wase and Owari and just before Aoshima, probably late April, Aoshima a week later. There is too little information available to make recommendations.

Aoshima. The yield was variable between sites with good to large fruit size, fair to poor quality. Due to the poor quality it is difficult to estimate maturity, but it is estimated to be slightly later than Ohtsu, around early May. There were seed at both sites, an exceptional amount at the CFB. There is too little information available to make recommendations.

Ueno. Production and fruit size was good and fruit quality fair to poor. There were odd seed. There is too little information available to make recommendations.

Primosole. The fruit had good to too large size, probably acceptable yield, fair quality but slightly dry, early maturing. There is too little information available to make recommendations. The selection is protected.

Future evaluations

Establish the ITSC Satsuma x Nova as an early maturing selection in other areas once budwood becomes available. Evaluate Dobashi Beni when semi commercial block comes into production. A new site with the various Satsuma selections has been established in the Paarl area which must be evaluated once in production.

Table 6.3.3.2. Comparison of the production, colour, quality, maturity, comments and seed of various Satsuma selections at different sites in the Cape areas during 2005.

Selection	Date	Site	Root stock	Yield	Fruit Size	Colour	Taste	Test	Maturity	Comment	Ave seed
Dobashi Beni	26/4	CFB	SC	Good	Med	4-5 deep red	Poor quality, low sugars and fair acid	Acceptable	Mature		0.2
Ohtsu	26/4	CFB	SC	Good	Medium large	5	Poor quality, low sugars and acid	Just acceptable, borderline sugars, low acid	2 weeks to go	Already puffy	0
Aoshima	26/4	CFB	TC	Good	Med large	6	Fair quality, low sugars and acid	Acceptable	2 weeks to go		9.9
Ueno	26/4	CFB	SC	Good	Med large	5-6	Low sugars and acid	Just acceptable. Borderline sugars	2 weeks to go		0.3
Owari (control)	26/4	CFB	SC	Good	Med small	4-5	Better than Ohtsu and Ueno. Fair sugars, high acid	Good but acid high	1 week to go		0.7
Ohtsu	5/5	Vuki	SC	Fair good	Med large - large	5 odd 4, some 7-8 (later set?)	Poor. Variable. No to low sugars, no to reasonable acid	Unacceptable. Too low juice and sugars	1-2 weeks past?	Slightly puffy, less than Miyagawa. Light sunburn	1.2
Aoshima	5/5	Vuki	SC	Poor	Med large	5-6, some 7-8 (later set?)	Poor. No sugars, reasonable acid.	Unacceptable. Borderline juice and sugars too low	Peak? Appear later than Ohtsu	Light sunburn. Odd slightly puffy, but less than others	1.1
Miyagawa Wase (control)	5/5	Vuki	SC	Fair good	Med large - extra large	Some 2, mainly 3-4	Poor, no sugars, little to some acid	Unacceptable. Sugars too low,	Overmature by 3 weeks?	Puffy	0
Dobashi Beni	22/4	Sovereign	CM	Partly picked, probably good	Med-large	4 - 6	Fair quality to quite tart	Acceptable, fairly acid, high juice	Peak to 1 week to go	Partly picked	0
Owari (control)	22/4	Sovereign	CM	Partly picked, probably good	Med-large	4 - 6	Fair to slightly tart	Acceptable. Slightly lower juice, sugar and acid than Dobashi	As for Dobashi Beni	Partly picked	0
Primosole	14/4	Slaley	CC	Partly picked	Med large - large (1X - 1XX 73.1mm)	3 - 5	Lacks flavour, reasonable sugars, slightly acidic	Very good, higher TSS and slightly higher acid than Clemenpons	Not quite at peak.		0
Primosole	30/3	Brakfontein	CC	Fair good	Medium large	5-6, 6	Fair, slightly dry				

A lot of the selections have odd seed. These have been ignored for export acceptability or not. The CFB trees are in mixed blocks, Vuki in a Miho Wase block and Sovereign in an Owari block.

Table 6.3.3.3. Internal fruit quality data for Satsuma mandarins for the Eastern and Western Cape areas tested during the 2005 season.

Selection	Rootstock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Dobashi Beni	SC	CFB	26/04	4-5	1X	55.1	9.6	0.98	9.8	0.2
Dobashi Beni	CM	Sovereign	22/04	5-6	1	64.0	9.9	1.20	8.3	0
Ohtsu	SC	CFB	26/04	5	1XX	49.5	9.1	0.74	12.3	0
Ohtsu	SC	Vuki Farming	05/05	4-5	1X	46.2	7.9	0.81	9.8	1.2
Aoshima	TC	CFB	26/04	6	1XX	55.6	9.7	0.78	12.4	9.9
Aoshima	SC	Vuki Farming	05/05	5, 5-6	1X	48.1	7.1	0.89	8.0	1.1
Ueno	SC	CFB	26/04	5-6	1XX	52.4	9.0	0.87	10.3	0.3
Miyagawa Wase	SC	Vuki Farming	05/05	3	1	49.4	7.0	0.76	9.2	0
Owari	SC	CFB	26/04	4-5	2	55.6	11.3	1.41	8.0	0.7
Owari	CM	Sovereign	22/04	5-6	1	61.6	9.3	1.14	8.2	0
Primosole	CC	Slaley	14/04	3	1XX	59.0	11.5	1.12	10.3	0
Clemenpons	CC	Slaley	14/04	6	2	60.0	11.1	1.01	11.0	0
Marisol	CC	Slaley	14/04	5-6	3	62.8	10.4	1.33	7.8	0.1

6.3.4 **Evaluation of Clementine mandarins in the Cape areas**
Experiment 63 by C J Alexander (Private Contractor)

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. Clemenpons word vroeg ryp met aanvaarbare gehalte, maar het galle op die entlas en stam. Die uitwerking van die galle in die langtermyn moet vasgestel word. Boomgroei krag varieer. Esbal produksie en gehalte was goed, kleur effens voor Nules. Vruggrootte was goed na chemiese manipulasie. Klein vruggrootte kan dalk probleme, veral in die koeler gebiede skep. Tardif de Janvier I het geen vrugte gedra en daar is te min inligting oor die Tardif de Janvier II om aanbevelings te maak nie. Die Sidi Aissa en Ain Toujdate is midseisoen seleksies met soortgelyke eienskappe. Vruggrootte is gereeld aan die kleiner kant en word nie aanbeveel nie. Die CELL het wisselvallige produksie en vruggrootte, goeie gehalte met groen blomente en vertraagde vrugkleur. Clementarde het swak produksie en vruggrootte gehad, het goeie gehalte en word laat ryp met vertraagde vrugkleur. Lyk nie belowend nie. Die Hernandina het relatief klein vruggrootte, word laat ryp met geen uitstaande eienskappe nie. Die Tardivo het wisselvallige produksie en vruggrootte gehad met goeie gehalte en goed tot relatief lae suur. Blomente is groen en is die laatste Clementine seleksie om ryp te word. Evaluasies moet voortgaan. Nour produksie was nie goed met wisselvallige vruggrootte. Rypwording het gewissel tussen persele. Vrugkleur is vertraag en die seleksie lyk nie belowend nie. Verder evaluasies is nodig met sommige van die 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. 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. A list of selections and sites evaluated is given in Table 6.3.4.1.

Table 6.3.4.1. Clementine trial sites evaluated during 2005.

Selection	Area	Site	Plant Date	Root stock	No of trees
Clemenpons	Uitenhage	CFB	1999	RL	2
Clemenpons	Patensie	Tierhok			comm
Clemenpons	Stellenbosch	Slaley	1999	CC	4
Esbal	Clanwilliam	Twaktuin	1998	CC	com topw
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
AinToujdate	Uitenhage	CFB Block 8	1997	CC	6
AinToujdate	Buffeljagsrivier	Sovereign	1999	SC	7 topwork
CELL	Uitenhage	CFB Block 7	1995	TC	2
CELL	Buffeljagsrivier	Sovereign	1999	SC	5 topwork
CELL	Citrusdal	Brakfontein	1998	CC	5 topwork
Clementarde	Uitenhage	CFB Block 8	1997	CC	6
Clementarde	Buffeljagsrivier	Sovereign	1999	SC	8 topwork
Hernandina	Buffeljagsrivier	Sovereign	1999	SC	8 topwork
Tardivo	Uitenhage	CFB		TC	1
Tardivo	Buffeljagsrivier	Sovereign	1999	SC	10 topwork
Tardivo	Citrusdal	Brakfontein	1998	CC	5 topwork

Nour	Uitenhage	CFB	Block 8	1999	SC	2
Nour	Addo	Summersby		1999	CC	3
Nour	Buffeljagsrivier	Sovereign		1999	SC	7 topwork
Nour	Citrusdal	Brakfontein		1988	CC	5 topwork
Marisol (control)	Stellenbosch	L'Avenir		1992	CC	commercial
Nules (control)	Uitenhage	CFB	Block 4	2001	SC	3
Nules (control)	Buffeljagsrivier	Sovereign		1990	TC	commercial
Nules (control)	Clanwilliam	Twaktuin		1992	TC	commercial
Clemlate (control)	Buffeljagsrivier	Sovereign		1999	SC	3 topwork

Results and discussion

A discussion of each selection follows with rootstock, production, fruit size, fruit colour, maturity, comment and average seed is presented in Table 6.3.4.2 for early maturing selections and Table 6.3.4.3 for later selections and internal quality results in Table 6.3.4.4 which need to be referred to when reading the text.

Clemenpons. Yields and fruit size varied between sites. Fruit shape was fairly round and fairly smooth, an occasional nipple at Stellenbosch. Fairly easily peeled and oily. Paler orange flesh colour, openness of cores variable between sites. Slightly raggy at Stellenbosch, thin rinds, some fruit with coarse/dryish flesh, tiny aborted seeds and juice colour paler than Marisol. The test at the CFB was just acceptable (low acid) better acid at Patensie and over 1.0% at Stellenbosch, but tested earlier (Marisol much higher acid). Sugars were good and juice percentages high. There were odd seed at Patensie. Trees at Patensie and Stellenbosch have variable vigour and knobs on bud unions and stems (not noted for CFB).

Eskal. Good yield and fruit size, but fruit chemically thinned. Round fruit shape, slightly pebbly rind, fairly easily peeled with peel breaking up slightly, cores slightly more open than Nules. Slightly pale flesh colour, to slightly watery, Nules slightly drier flesh, some watery looking and thicker rinds. Nules suffered some drought stress. Excellent internal quality test.

Tardif de Janvier I. There was no fruit at the CFB nor Buffeljagsrivier. The stem is dark at Buffeljagsrivier.

Tardif de Janvier II. The T de Jan II had poor eating quality. The test had good sugars but acid too low and seedy (mixed block). Fruit shape is fairly flat with a roughish texture, oily on peeling, orange internal colour and open cores.

Sidi Aissa. Yields at both CFB and Buffeljagsrivier were good, but mediocre fruit size. Maturity was earlier at the CFB, mid to end May, but early to mid June at Buffeljagsrivier, 1-2 weeks later than Nules. Both had good eating quality and excellent tests.

Fruit shape is round, a slight nipple at Buffeljagsrivier, slightly coarse rind, fairly easily peeled and oily with good orange flesh. There was some seed (mixed block).

Ain Toujdate. Yields were fairly good to good with medium fruit size. Maturity was earlier at the CFB - end May, early to mid June at Buffeljagsrivier, 1-2 weeks later than Nules. Both had good quality, Sidi Aissa slightly better and excellent tests, similar to Sidi Aissa at each site.

Fruit shape is round, a slight nipple at Buffeljagsrivier, slightly coarse rind, fairly easily peeled and oily with good orange flesh. There was some seed (mixed block).

CELL. The CFB fruit had a fair taste and mature by 17 June, with a good test and adequate acid. The Buffeljagsrivier trees only bore a few fruit, too few to test.

The Citrusdal trees had good eating quality on 11 and 26 May and even up to 8 June, with sufficient acid, similar to Tardivo. The tests were good, retaining a good acid level into June.

Fruit shape is round, sometimes a slight nipple at Citrusdal, smooth rind at the CFB, pebbly to coarse rind at all sites. Peelability varied between sites but generally oily to very oily. Some fruit tend to be slightly dry or have a coarse flesh. There were some green styler ends. The trees are dense with a black stem. The fruit is virtually seedless under no cross pollination. Maturity was considerably earlier in Citrusdal, late May, while mature 3rd week of June at the other two sites.

Clementarde. There was a lot of out of season fruit at the CFB. The test was good on 22 June, but acid could be slightly higher.

The Buffeljagsrivier fruit had good eating quality with very high sugars and fairly high acid, still dark green styler ends in June and the fruit slightly raggy. The test was very good. The trees had out of season blossom. Clemlate on the same date had better colour and much lower acid and slightly less green styler ends.

The Clementarde are round with a slight nipple at Buffeljagsrivier, slightly pebbly rind, fairly easily peeled and oily, odd to some seed in mixed blocks. The trees are smaller, flat topped, squat and dense trees with a black stem. Maturity appears to be mid to late June to early July. Colour is not well advanced.

Hernandina. Trees were only evaluated at Buffeljagsrivier. The fruit was firm and not fully mature on 17 June, although acid was below 0.90%, lower than Clemlate. Clemlate colour was slightly ahead.

Fruit shape is round with a slight nipple, slightly pebbly rind, firm, fairly easy to difficult to peel and very oily. Stems are dark and some seed (mixed block).

Tardivo. Yield and fruit size varied between the sites. Fruit at the CFB had a good test although the acid was getting low. Fruit in Citrusdal maintained a good acid level. The fruit was slightly raggy. The fruit is virtually seedless under no cross pollination. Maturity is around late May to early June.

Fruit shape is round with some nipples in Citrusdal, fairly smooth to slightly pebbly rind, fairly easily peeled and oily. The trees are flat, squat and dense with a very dark stem.

Nour. The trees at the CFB had good eating quality on 22 June and a good test, also good quality and test at Buffeljagsrivier. The test was similar to Clemlate, except Clemlate had too low juice. Citrusdal tests were good except for June when the juice dropped too low.

The young, semi commercial trees at Addo have been topworked except for three trees which bore no fruit, possibly due to a water shortage.

The fruit is generally round with a nipple, ring at the styler end, pebbly rind, orange flesh, basically seedless when not pollinated, fairly easily peeled and oily. Fruit is sometimes slightly raggy and sometimes a little dry. The styler ends tend to be green. The trees are vigorous and dense with a dark stem.

A summarised comparison of the various selections at Buffeljagsrivier revealed the following (the orchard tends to get waterlogged during heavy rains):

- Colour – Nules, Sidi Aissa and Ain Toujdate the most advanced (T1); followed by Nour, Clemlate and CELL (T3-4) and the greenest Hernandina and Tardif de Jan II (T4-5).
- Eating quality – Nules was excellent; Sidi Aissa, Ain Toujdate and Clementarde good; CELL, Hernandina, Nour and Clemlate fairly good.
- Internal quality tests – all excellent tests except Clemlate which had too low juice; Ain Toujdate, Sidi Aissa and Clementarde had the highest TSS and acid exceeding 1.0%; Nules the lowest acid (0.83%). Ratios were all high and average seed counts varied between 0.6 – 3.2 seeds per fruit (cross pollinators in the row).
- Estimated maturity – All matured in June, Nules the earliest (beginning June), Sidi Aissa 2nd week; Ain Toujdate 2nd week – mid June; Clementarde mid – 3rd week; CELL, Nour and Clemlate 3rd – 4th week and Hernandina 4th week of June.
- Yield – only Sidi Aissa, Nules, Ain Toujdate and Hernandina had good to acceptable crops and all had poor fruit size.
- Tree size – Nour, Nules and Tardivo had the largest tree size, Clementarde and Tardivo flat and squat and also the densest trees. All except Nules, Sidi Aissa and Ain Toujdate had black stems.

A summarised comparison of the CELL, Tardivo and Nour over the three evaluation dates in Citrusdal revealed the following:

- Colour – CELL the earliest followed by Nour (most green styler ends) and Tardivo (latest).
- Eating quality – CELL (the best - good), then Tardivo and Nour the poorest, lacking flavour. Except for the Nour's lacking flavour, none were inferior. Tardivo was slightly raggy.
- Internal quality test (average of three tests) – CELL the best with the highest TSS while Nour had the lowest juice and TSS. Acid levels of all three were similar.

- Estimated maturity – there appeared to be some variation. The earliest to mature was Nour, (beginning to 3rd week of May); CELL (1st to 4th week May) and Tardivo 3rd – 4th week of May). All looking overmature in June. All selections easily met the tests on 11 May (first test), while the colour was still poor. Some dryness showing in CELL and Nour on 26 May and in all three selections in June as well as open to wide open cores.

Conclusions

Clemenpons. Variable yields and fruit size. Fruit is early maturing around mid to end April. When harvested at the right time, quality should be quite acceptable, although acid levels should be monitored. Of concern is the variation in tree size and growth on the bud union/stems. Further evaluations are necessary and also to establish the long term effect of the galls (knobs) on the trees. Clemenpons is a protected selection.

Esbal. Production and fruit size was good (chemically thinned). Fruit colour ahead to later than Nules, maturity around mid to end April. Internal quality was excellent. The Esbal looks a good selection, slightly ahead of Nules, but fruit size may be problematic, especially in cooler areas if not manipulated.

Tardif de Janvier I. No fruit. Evaluations to continue.

Tardif de Janvier II. The fruit had low acid, probably maturing early to mid June. Due to the limited information, no recommendations can be made. Evaluations to continue.

Sidi Aissa. Good production but mediocre fruit size. Maturity between mid May to mid June depending on area. Slightly later maturing than Nules and good quality. The selection has consistently produced mediocre fruit size. Characteristics are similar to Ain Toujdate. Not recommended as apart from maturing slightly later than Nules, the fruit size is not large enough.

Ain Toujdate. Fairly good to good yields but only medium fruit size. Maturity between end May to mid June depending on area, slightly later maturing than Nules and good quality. The selection has consistently produced mediocre fruit size. Characteristics are similar to Sidi Aissa. Not recommended as apart from maturing slightly later than Nules, the fruit size is not large enough.

CELL. Production and fruit size varied between sites. Eating quality was generally good, sometimes raggy with good tests. Colour tends to be retarded with some green stylar ends. Maturity around the end of May to 3rd week of June. Evaluations to continue.

Clementarde. Production was poor with medium small fruit size. Maturity is late, from mid June onwards, but rind colour delayed and green stylar ends. Internal quality was very good. The selection does not look promising as a late maturing selection.

Hernandina. The yield was just acceptable and fruit size on the smaller side. The fruit is late maturing with no outstanding features.

Tardivo. Production and fruit size varied between sites. The fruit had good quality, lowish to good acid, maturity late May around early June. Green stylar ends are prevalent and it is the latest Clementine selection to mature.

Nour. Production was generally poor - fair, fruit size variable between medium small through to medium large. The fruit quality was generally good with good tests and good acid levels. Maturity varied between sites starting in early May in Citrusdal to late June although the colour may be retarded. There were a lot of green stylar ends. The trees are vigorous and dense. The Nour does not look a promising late Clementine selection.

Future evaluations

Continue evaluations on most selections.

Table 6.3.4.2. Comparison of the production, fruit size, rind colour, maturity, comment and average seed of various early maturing Clementine selections at the different trial sites on different dates in the Cape areas during 2005.

Area	Selection	Root stock	Production	Fruit Size	Colour transparency - date	Maturity	Comment	Ave Seed
Uitenhage	Clemenpons	RL	Poor	Medium	19 April 3	Overmature on 19/4	Poor, low TSS and acid	4.0
Patensie	Clemenpons	SC?	Fair – good – excellent, odd fruit picked	Variable, med – med small, odd med large	19 April 5-6, odd 4	Peak to 1 week past	Fair, fairly sweet, lowish to borderline acid. Knobs at bud union	0
Stellenbosch	Clemenpons	CC	Fair	Variable, small – med large – large. Mainly calibre 2 (61.5mm)	14 April Mainly 6-7, odd 4-5	Firmer and better taste than Marisol. Mature in 1-2 weeks	Fair quality, good sugars, low acid. Knobbly stems	0
	Marisol	CC	Poor	Small – medium – med large	14 April Mainly 6-7, some 4-5	Mature in 1-2 weeks	Softer fruit, low sugars, fairly acid	
Clanwilliam	Esbal	CC	Excellent	Good, med large but chemically thinned	26 April 3-6. Deep rind colour, some green stylar ends	Peak to 1 - 2 weeks past	Good quality, good sugars and acid	0
	Nules	TC	Good to excellent	Medium – med large	26 April 5. Green stylar ends	Peak to 1 week past	Good quality, slightly higher sugars and acid than Esbal. Trees were stressed.	0

Table 6.3.4.3. Comparison of the production, fruit size, rind colour, maturity, comment and average seed of various mid and late maturing clementine selections at the different trial sites on different dates in the Cape areas during 2005.

Area	Selection	Root stock	Production	Fruit Size	Colour transparency - date		Maturity	Comment (ave seed)
					11,19, 26 May	8, 17, 23 June		
Uitenhage	Tardif de Janvier I	TC	Zero					
	Tardif de Janvier II	CC	Good	Med large		17 1-3 23 1-2	Probably peak 17 June or earlier	Poor, low sugars and acid (1)
	Sidi Aissa	TC	Good	Medium	19 5	17 1	Mature to 1 week past peak in May, overmature in June	Good quality, high sugars and fair acid in May (2)
	Ain Toujdate	CC	Good	Medium	19 2-3	17 1	Mature in May, overmature by 1-2 weeks in June	Good quality, fair TSS and acid (2)
	CELL	RL	Excellent	Medium small		23 1-2, some green stylar ends a week earlier.	Mature	Fair quality, sugars and acid (4)
	Clementarde	CC	Poor, also out of season fruit	Medium small		17 5, very green stylar ends. 23 4-5	Mature, but peak in 2-3 weeks	Fair quality, raggy (2)
	Tardivo	TC	Fair – good	Medium small		17 3, deep orange red stem end, green stylar end 23 1-2	Past peak by 1-2 weeks on 16 June. Looks slightly overmature	Good quality, fair sugars and acid (2)
	Nour	CC	Fair	Medium small		23 1-3, red stem ends, green stylar ends	Mature in June	Good quality, high sugar and good acid (2)

	Nules	SC	Poor	Large		23 1-3	Overmature by 17 June	Poor quality with low sugars and acid, but good test (2)
* Buffelja gsrivier	Tardif de Janvier I	SC	Zero			17		
	Sidi Aissa	SC	Good	Medium – med large. Calibre 2-4		17 1, deep orange red	Peak maturity to 1 week past	Good, juicy, extremely sweet, sufficient acid (0-1)
	Ain Toujdate	SC	Fair – good	Medium, variable Calibre 2-5, mainly 2-3		17 1, deep orange red, look overmature	Peak maturity to 1 week past	Good quality, sugars not quite as high as Sidi Aissa (0)
	CELL	SC	Odd fruit	Medium large		17 4, green stylar ends	1-2 weeks short of peak maturity. Too few to test	Good, but sugars could be higher and acceptable acid (0-1)
	Clementarde	SC	Poor – zero	Medium – med small Calibre 2-5, mainly 3		17 5, later set? Deep orange, green stylar ends	Peak to 1 week to go	Good, very high sugars, good acid (0)
	Hernandina	SC	Fair – good	Medium. Calibre 1-2		17 4-5, green stylar ends	Estimated maturity around beginning of July	Good, good sugars and sufficient acid, but slightly dry, juice not free (0-odd)
	Tardivo	SC	Zero			17		
	Nour	SC	Poor – fair	Medium-med large Calibre 1-3, mainly 2-3		17 3, good orange red stem end, green stylar ends	Mature mid to end June	Good, good sugars and sufficient acid, slightly dry (0)
	Clemlate	SC	Poor	Medium Calibre 1-3, mainly 2-3		17 3, some green stylar ends	Not quite mature, mature towards end of June	Good eating quality but juice too low. Some fruit dryish (0-1)
	Nules	SC	Good	Med – med large calibre 1-3		17 1, not red	Up to 2 weeks past peak maturity	Excellent quality, good test (0)
Citrus-dal	CELL	CC	Fair – mainly good	Medium, mainly med large, good Calibre 2-1X in June	11 5-6 26 3-5, mainly 4-5	8 1-4, mainly 2, some green stylar ends	Peak to 1 week past on 26 May, but look old and not juicy	Good quality, sufficient acid on 11 May. Good sugars and acid in June (0)

	Tardivo	CC	Good	Medium large Calibre 2-1X in June	11 5-6 26 4-6, mainly 5	8 3-5, mainly 3-4 lots of green stylar ends	Mature around end May. Later than CELL and not as dry	Fair to good quality in early May, very good in June (0)
	Nour	CC	Variable, poor	Medium-med large Calibre 2-1X, mainly 1-2 in June	11 6, 6-7 26 4-6 mainly 5	8 1-4, some green stylar ends	Past peak in early May	Good sugars and sufficient acid in early May, but lacks flavour, occasionally slightly dry (0-1)

* Note – the site at Buffeljagsrivier tends to get waterlogged during heavy rains.

Table 6.3.4.4. Internal fruit quality data of the various Clementine selections for the Eastern and Western Cape during the 2005 season.

Selection	Rootstock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Clemenpons	RL	CFB	26/04	3-4	2	60.1	10.9	0.73	14.9	0
Clemenpons	SC	Tierhok	19/04	5-6	1	62.3	10.7	0.84	12.7	0.1
Clemenpons	CC	Slaley	14/04	6	2	60.0	11.1	1.01	11.0	0
Esbal	CC	Twaktuin	26/04	4	1	63.0	12.5	0.96	13.0	0
Tardif de Jan II	CC	CFB	23/06	1-2	1	53.0	11.3	0.63	17.9	4.0
Sidi Aissa	TC	CFB	19/05	5	2	61.1	12.9	0.95	13.6	2.6
Sidi Aissa	SC	Sovereign	17/06	1	2-3	57.8	15.9	1.01	15.7	3.2
AinToujdate	CC	CFB	19/05	2	2	57.3	12.9	0.97	13.3	4.0
AinToujdate	SC	Sovereign	17/06	1	3	56.1	16.1	1.11	14.5	2.4
CELL	RL	CFB	23/06	1-2	3	54.7	11.4	0.90	12.7	9.5
CELL	CC	Brakfontein	11/05	5-6	2	56.9	14.0	1.12	12.5	1.2
CELL	CC	Brakfontein	26/05	4-5	1	50.3	14.5	1.07	13.6	0.2
CELL	CC	Brakfontein	08/06	2	1	52.0	15.1	1.06	14.2	0
Clementarde	CC	CFB	22/06	4-5	3	59.3	13.5	0.89	15.2	1.8
Clementarde	SC	Sovereign	17/06	5-6	3	55.6	15.8	1.22	13.0	1.4
Hermandina	SC	Sovereign	17/06	4-5	2	54.6	14.3	0.89	16.1	1.7
Tardivo	TC	CFB	22/06	1-2	2	58.9	13.0	0.76	17.1	3.5
Tardivo	CC	Brakfontein	11/05	5-6	2	58.2	12.9	1.23	10.5	0.3
Tardivo	CC	Brakfontein	26/05	4-5	2	57.1	13.2	0.98	13.5	0.3
Tardivo	CC	Brakfontein	08/06	4	1	55.2	13.4	1.01	13.3	0.2
Nour	CC	CFB	22/06	123	3	53.8	14.6	1.00	14.6	1.9
Nour	SC	Sovereign	17/06	2-3	2	60.9	14.6	0.92	15.9	1.3
Nour	CC	Brakfontein	11/05	6	2	54.8	12.0	1.16	10.3	0
Nour	CC	Brakfontein	26/05	4-5	1	54.7	12.5	1.00	12.5	0
Nour	CC	Brakfontein	08/06	1-3	1	47.2	13.4	0.98	13.7	1.6
Marisol	CC	Slaley	14/04	5-6	3	62.8	10.4	1.33	7.8	0.1
Nules	SC	CFB	23/06	1-2-3	1X	53.4	11.4	0.88	13.0	1.0
Nules	SC	Sovereign	17/06	1	2	60.0	14.8	0.83	17.8	0.9
Nules	TC	Twaktuin	26/04	5	2	55.8	15.1	1.22	12.4	0
Clemlate	SC	Sovereign	17/06	3-4	2	44.7	14.0	0.97	14.4	0.6

6.3.5 **Evaluation of Mandarin hybrids in the Cape areas**
Experiment 73 by C J Alexander (Private Contractor)

Opsomming

Die doel van die mandaryn projek is om hoër gehalte, goed gekleurde, saadlose mandaryn seleksies te ondersoek. Hulle moet ook goeie produksie en vruggrootte hê en die sagte sitrus seisoen kan versprei, beide vroeër en veral later. Nova SL is soortgelyk aan Nova maar nie heeltemal saadloos in gemengde blokke nie, maar het wel minder saad as Nova. Valley Gold lyk belowend maar is geneig om hoër suur te hê en effense vrugsplit; African Sunset is saadloos, met minder suur as Valley Gold maar vrugte kan te groot wees met 'n plat vrugvorm. M37 het goeie gehalte en sagte vesel, word in Junie ryp met effens vertraagde kleur. H25 word in Mei ryp, met swak geur en gehalte en lyk nie belowend nie; H36 het goeie gehalte met sagte vesel en effens vertraagde kleur, word in Junie ryp wat dalk met ander mandaryne mag bots; K33 is feitlik saadloos maar lyk nie belowend nie weens sy swak voorkoms; Roma het goeie gehalte met 'n swak naartjie tipe voorkoms; Or 4 het goeie gehalte in laat Julie gehad; Or 2 het redelike produksie met goeie vruggrootte gehad, en word in laat Julie ryp. Produisie van Mor 22 het gewissel tussen persele met medium vruggrootte en 'n goeie toets. Nadorcott het goeie produksie met medium asook groot vruggrootte gehad, maar suur. Murcott x Clem was deur bobbejane opgevrete en die semi kommersieële boord is nog nie in drag nie. Bay Gold het goeie produksie en vruggrootte gehad maar lyk nie belowend in die koeler gebiede nie weens hoër suur. Hadas het goeie produksie en vruggrootte gehad maar gereelde hoër suur. Winola het goeie produksie met medium vruggrootte en goeie vrugkleur gehad maar baie hoër suur. Cami het goeie produksie en vruggrootte gehad, is aantreklik en word in mid Junie ryp. Empress mandaryn het redelike produksie met medium vruggrootte gehad. Van die seleksies met verder evalueer word.

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 standards for Mandarins: 50% juice; 8.5% Brix; 0.7 – 1.5% acid; 7.5:1 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 6.3.5.1.

Table 6.3.5.1. Mandarin hybrid trial sites evaluated during 2005.

Selection	Area	Site	Plant Date	Root stock	No of trees
Nova Seedless	Uitenhage	CFB Block 8	1999	RL,CC,SC	6
Nova Seedless	Patensie	Paksaam	1999	SC	semi com
Nova Seedless	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
Valley Gold (B17)	Addo	Dunbrody	1998	CC	semi com t/w
Valley Gold (B17)	Fort Beaufort	Baddaford	1998	TC	3
Valley Gold (B17)	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
Valley Gold (B17)	Citrusdal	ALG	1999	BN/RL	5
African Sunset (B24)	Addo	Dunbrody	1998	CC	semi com t/w
African Sunset (B24)	Fort Beaufort	Baddaford	1998	TC	1
African Sunset (B24)	Citrusdal	Ebenhaeser	2000	RPL	semi com t/w
African Sunset (B24)	Citrusdal	ALG	1999	BN/RL	5
ITSC M37	Fort Beaufort	Baddaford	1998	TC	4
ITSC M37	Citrusdal	ALG	1999	BN/RL	5
ITSC H36	Citrusdal	ALG	1999	BN/RL	5
ITSC K33	Citrusdal	Hexrivier	1997	RPL	1
Roma	Citrusdal	ALG	1998	BN/RL	5
Or 4	Uitenhage	CFB Block 8	1999	TC	2

Or 2	Citrusdal	ALG	1998	BN/RL	5
Mor 22	Uitenhage	CFB Block 8	1999	TC	2
Mor 22	Citrusdal	ALG	1998	BN/RL	5
Nadorcott	Uitenhage	CFB Block 7	1999		2
Nadorcott	Citrusdal	ALG	1998	BN/RL	5
Murcott x Clem	Uitenhage	CFB Block 8	1999	TC	2
Murcott x Clem	Kirkwood	Kirkwood	2003	CC	semi com
Bay Gold	Uitenhage	CFB Block 8	1999	TC	2
Bay Gold	Clanwilliam	Jansekraal	2001	CC	48 semi com
Hadas	Uitenhage	CFB Block 8	1999	TC	2
Winola	Uitenhage	CFB Block 8	1997	TC	2
Cami	Uitenhage	CFB Block 8	1999	SC	2
Empress	Uitenhage	CFB Block 8	1997	TC	3
Nules	Uitenhage	CFB Block 4	2001	SC	3
Nova	Patensie	Paksaam	1999	SC	commercial

Results and discussion

A discussion of each selection follows. Various tables also need to be referred to when reading the text. Tables 6.3.5.2, 3 & 4 are a comparison of the various selections at different sites. Table 6.3.5.5 summarises observations of new mandarin hybrid selections at the ITSC-Addo, and internal quality data is summarised in table 6.3.5.6.

Nova Seedless (SL)

The CFB trees bore no fruit this season. At Patensie on 19 April Nova had a fair crop with good, medium to medium large fruit size, Nova SL a slightly poorer crop but good, medium large fruit size. Fruit colour was fairly similar, T5, Nova with some T5-6. Some *Alternaria* was evident on both selections. Internal quality was similar with fair sugars and sufficient acid, Nova SL slightly better sugars. Both had some slightly “dry” fruit, Nova slightly worse. Both selections had a good test, Nova SL superior. Both 1-2 weeks short of full maturity. Nova SL had odd seed, less than Nova, as indicated below:

Selection	Percentage seed per fruit			Average seed/fruit
	0 seed	1 seed/fruit	2 seeds/fruit	
Nova SL	96	4		0.04
Nova	90	6	4	0.12

The Nova SL at Citrusdal had been harvested. Odd fruit left on 11 May had good colour, T1 and good quality – excellent sugars and a good acid level (trees have been subjected to stress).

Valley Gold (ITSC B17)

Valley Gold was evaluated at four sites: Eastern Cape results appear in Table 6.3.5.2 and Western Cape results appear in Table 6.3.5.4.

Table 6.3.5.2. Comparison of production, fruit size and colour, quality and maturity of Valley Gold and African Sunset at Addo and Fort Beaufort in the Eastern Cape during mid June, 2005.

Selection	Production	Fruit size	Colour transparency	Comment/maturity	Ave seed/test
Dunbrody, Addo. 15 June.					
Valley Gold	Good – excellent	Medium, acceptable	T1, orange/red, deep on east side	T2-3 Good quality, good sugars and acid not too high, raggy. Peak. Excellent test, high acid.	0 - 1 0.7
African Sunset	Poor – fair	Large – extra large	T1, T3-4 on east side	Good quality, good sugars and acid, both slightly lower than Valley Gold, a good, “milder” taste, raggy. Peak. Good test.	0/0
Baddaford, Fort Beaufort. 14 June. * Severe hail damage in December 2004.					
Valley Gold	Poor *	Medium, uniform, acceptable	T1, excellent deep orange/red	Excellent quality. Excellent sugars, acid a little high. Peak in 1-2 weeks depending on acid, but looks slightly overmature. Excellent test.	0 - some/ 0.8
African	Poor *	Medium	T1-3	Excellent to slightly tart. Excellent	- /0.6

Sunset		large to large and larger		sugars but slightly more tart than Valley Gold? Peak in 1-2 weeks depending on acid. Excellent test.	
ITSC M37	Zero – fair *	Medium	T3-5 Slightly green stem ends. Also a later set, T5-6	Good flavour, sugars could be higher, good balance, juicy, messy. Peak to just past. Excellent test.	Seedy /9.6

The semi commercial topworked trees at Citrusdal had been harvested by 11 May, probably a poor crop. The trees had been stressed and leaves pale and small. There had been fruit drop with a lot of split fruit (not the stylar end). Odd fruit left had a good taste.

Fruit shape is flattish with some furrowing at the stem end and occasional slight neck in Citrusdal, smooth but pebbly in Citrusdal, fairly easily peeled, sometimes skin breaking up with a thin, oily rind, deep orange internal colour and open cores, mainly seedless to odd seed. There was some fruit split resulting in dropped fruit at Addo.

African Sunset (ITSC B24)

African Sunset was evaluated at four sites: Eastern Cape results appear in Table 6.3.5.2 and Western Cape results appear in Table 6.3.5.4.

The semi commercial topworked trees at Citrusdal had been harvested by 11 May, probably a poor crop. Some trees odd pale yellow branches, but overall tree colour was better than Valley Gold (Nova SL trees had the best leaf colour). Odd fruit left had good quality.

Fruit shape is flat with a smooth to pebbly rind and occasional ribbing at the stem end and occasional closed protruding navel ends. Internal colour was good with slightly open to open cores, thinnish rinds, very thin at the stylar end, fairly easily peeled and oily. The flesh was slightly coarse, sometimes with a halo around the core and sometimes raggy. Except for some seed at Fort Beaufort the fruit is seedless. Generally the fruit is less tart than Valley Gold with a milder flavour with good sugar and acid levels, low juice in Citrusdal. There was occasional fruit drop at Addo.

ITSC M37

ITSC M37 was evaluated at two sites (see Tables 6.3.5.2 & 4).

Fruit shape is round with a smooth rind to slightly pebbly at Citrusdal. Internal colour was orange with closed to slightly open cores, soft rag and juicy and messy to eat. Looks like an orange internally. It is fairly easily peeled and oily. There was occasional creasing at Citrusdal and plugging during picking. No signs of *Alternaria* seen in Citrusdal. Seedy in a cross pollinated site. The tests were good except for low juice at Citrusdal.

ITSC H25

Fruit shape is roundish with a nipple (30-40% pronounced), attractive with a smooth rind. Internal colour is orange, soft and juicy with almost closed cores, fairly easily peeled and very oily, albedo adhering to fruit. Odd fruit have adhering styles. Fruit quality was poor and the test unacceptable (low sugars and acid). There was some leaf drop in May.

ITSC H36

The fruit has a round to slightly elongated shape, pebbly rind, smoother inside the tree and sometimes very coarse stylar end. Some fruit have very small internal navels. The fruit is slightly soft and very juicy, fairly easily peeled and oily. Rind thickness is good, also at the stylar end except when there are internal navels, which then has a thin stylar end rind. Internal colour is good with a slightly pink albedo, cores slightly open. Although the test was satisfactory in June, the flavour was good, while in July the fruit was tasteless, lacking flavour and the test unacceptable (low juice and acid). Fruit colour is slightly retarded in June. There was some plugging during sampling and odd out of season fruit. Trees are vigorous.

ITSC K33

Production was acceptable with good fruit size, fruit quality good but high acid, the test exceeding specifications. Maturity was around 3rd – 4th week of July. The fruit are round with a green nipple, a coarse, relatively thick rind with a ring round the stylar end. The fruit is fairly easily peeled but breaks up and is oily. Internal colour is deep orange and with coarse flesh, virtually seedless.

Roma

The fruit had good quality in early June, not outstanding and not as good as previously. The test was good, but juice too low. The fruit has a naartje like appearance, slightly overmature with rind just starting to pull away from flesh. Fruit shape is flattish with a slight nipple, pebbly rind and nearly half the fruit with small navel ends. Peelability was fairly easy to difficult with a thin rind, breaking up and oily. The internal colour was good, juicy with open cores and seedy.

Or 4

The fruit had good quality with very high TSS and acid level over 1.2% the 3rd week of July. Fruit shape is round with a smooth rind. There was some seed in a mixed block.

Or 2

The fruit had high sugars and a good acid level at the beginning of August. Fruit shape is slightly flat with a slightly pebbly rind and slight fluting at the stem end and the occasional navel. Easily peeled and oily. Internal colour was good with slightly open cores and slightly coarse flesh. Rind thickness was good, the skin just starting to part from the flesh, slightly overmature. There was some seed (mixed block). Maturity estimated around 3rd week of July.

Mor 22

The fruit had fair quality at the CFB with a high sugar test and acid >1.2%. Citrusdal fruit also had high sugars and acid >1.1% but low juice (although juicy on eating) and flavour not outstanding. Fruit shape is round to flattish with a smooth to slightly pebbly rind and ribbing. The fruit is easily peeled with a thin rind and slightly oily with a fine, deep orange flesh colour, slightly open to open cores and some seed. Maturity varies between sites from mid July to mid August.

Nadorcott

Fruit at the CFB had excellent rind colour. Acid exceeded 1.1% in both June and July affecting the taste. In Citrusdal the fruit was slightly soft and became puffy in August (overmature). TSS levels were acceptable to good and acid around 1.0%, resulting in a fairly good taste but acid always coming through.

Fruit shape is flat with a shiny, smooth rind, although some pebbly fruit on vigorous branches. Peelability was fairly easy and sometimes oily. Flesh colour is deep orange with an open core and variable seed (pollinated blocks). The rind develops an excellent orange red colour. Some fruit had slight ribbing at the stem end and there was odd sunburn.

Murcott x Clementine

Most of the fruit at the CFB were stolen by baboons. The semi commercial block at Kirkwood is still too young to be in production and the trees also suffered frost damage.

Bay Gold

Most of the fruit at the CFB had been stolen by baboons, the remaining fruit poor quality and overmature 3rd week of June. The test was acceptable, juice too low and acid >1.2%. The trees at Grabouw have been removed.

The Clanwilliam trees had a heavy blossom, especially on the southern side. The final crop was fair to good with slightly variable but good, mainly medium large fruit size. The average fruit size was 74.2mm, 70% counts 1XX -1XXX; 16% 1X and 14% 1-2. Fruit colour on 8 June was mainly T1-2, deeper orange red on the southern side. Odd fruit had slightly green tinges on the cheeks or stem end. The fruit had a poor taste although sugars and acid were high. The TSS test was high but acid excessive (2.01%). The fruit was overmature, picking fairly easily, rind pulling away from the flesh and some slightly ricey fruit. Eighty percent of the fruit evaluated had internal navels. In some instances the rind was extremely thin where there was an internal navel. There was some sunburn and stylar end split. Maturity is estimated at around end May/early June, but the acid level still excessively high.

Fruit shape is obovoid (almost Minneola-like). Eighty six percent of the fruit at Clanwilliam had a rating of 2-4 for high shoulders (CRI blemish set no. 40). The rind was smooth at the CFB but roughish at Clanwilliam, worse at the stem end. The fruit is fairly easily peeled and oily, orange flesh and open cores. Seed counts varied from zero to 11 seeds/fruit.

Hadas

The fruit has a very good rind colour. Eating quality was fair, test had high TSS and acid 1.43% probably masking the sugars, meeting standards except for the high seed count (cross pollinated). The fruit shape is flattish with a very smooth rind, peelable, oily and open cores. The fruit was mature in 3rd week of July.

Winola

The fruit had excellent colour and poor taste due to excessively high acid in July (1.75%) although the TSS was also high. The fruit shape is round with a smooth rind, peelable, oily and slightly open cores and virtually seedless in a mixed block. The fruit was mature in July.

Cami

Cami is a Clementine hybrid x tangelo from Italy. Fruit colour can be pale. Eating quality was good with a good test but fruit seedy in a mixed block (reported to be seedless). The fruit shape is round with a very smooth rind and attractive. The fruit is oily on peeling with orange flesh and open cores during 3rd week of June. There was some splitting during June when mature.

Empress mandarin

Trees are tall and narrow. The eating quality was not so good but test excellent. Fruit shape is round but nose-y with a smooth rind, oily on peeling with an open core and seedy in a mixed block. The fruit was mature during the 3rd week of July.

Table 6.3.5.3. Comparison of production, fruit size, colour, maturity, comment and average seed of various mandarin hybrids evaluated at the CFB, Uitenhage during 2005.

Selection	Production	Fruit size	Colour transparency		Maturity	Comment	Ave seed /test*
			22 June	20 July			
Or 4	Poor	Medium		1	Around 3 rd week July	Good. Good sugars and fair acid. Excellent test	± 2 /1.1/
Mor 22	Poor	Medium		1	Mature	Fair, fair sugars and acid	± 2 /1.9
Nadorcott	Good	Medium		1	Pickable from late June	June – fair taste, high sugars and acid July – fair, fair sugars, high acid. Good tests	seedy /10.3
Murcott x Clem	Eaten by baboons, odd fruit left	Medium large – large	16 June 1			Fair to good quality	
Bay Gold	Eaten by baboons, some fruit left	Medium large	1		Over-mature	Poor quality, low sugars high acid. Acceptable test except juice too low	± 2 /4.7
Hadas	Good	Medium large	5	1, good colour	Mature	July - fair, fair sugars and acid. Good test, high acid	/8.1
Winola	Good	Medium	1-2 excellent colour	1	Mature	Not a good taste, fair sugars, high acid. Acid test excessive	0 /0.3
Cami	Good	Medium large	1		Mature	Quality was good with fair sugars and acid and good test	seedy /11.6
Empress mandarin	Fair	Medium	5-6	1	Mature	Quality not so good in July with high sugars but low acid. Excellent test	seedy /6.1
Nules (control)	Poor	Large	1-3		Over-mature by 17 June	Poor quality with low sugars and acid, but good test	2.0 /1.0

* Average seed counts from internal quality tests.

Table 6.3.5.4. Comparison of production, fruit size, colour, maturity, comment and average seed of various mandarin hybrids topworked on Bahianinha/rough lemon, evaluated at ALG, Citrusdal during 2005.

Selection	Production	Fruit Size	Colour transparency			Maturity	Comment	Ave Seed/ test*
			8 June	5 July	1 Aug			
Valley Gold ITSC B17	Poor - fair	Medium large – large (1xx-1xxx)	T3, good orange red but green stylar ends			Peak to just past	Good quality. Juice too low, otherwise good test	0 /0.6
African Sunset ITSC B24	Poor - Fair	Extra Large (≥ 1xxx)	T1-3, deeper than Valley Gold. Slightly green navels			Peak to 1-2 weeks past	Good quality, milder than Valley Gold. Good test, lower acid, juice too low	0 /0
ITSC M37	Variable, zero – excellent	Medium-medium large (1-1x)		1-3		3 rd – 4 th week of June	Good sugars and acid but lacks flavour, very soft, juicy, messy	seedy /9.9
ITSC H25	Fair	Medium large (1-1xx)	11 May 5,5-6 25 June 1-3 8 June 1-2			Mid May	Poor, lacks flavour, sugars ?, acid low. Unacceptable test	0 to odd /0.7
ITSC H36	Fair - excellent	Medium large – large (mainly 1xxx)	3-4, some 6	1-3		2 nd – 3 rd week of June	June - Good quality, good sugar and acid balance. Acceptable test. July – past peak, lacks flavour	0 to seedy /9.4
ITSC K33	Fair	Medium large (1xx)		3 green nipples		3 rd - 4 th week of July	Good but tart, acid too high. Raggy	0 /0.6
Roma	Fair – good	Medium medium large (1-1xx)	1-3, odd 4. Good orange red colour			End May/ Early June	Good quality, good sugars and sufficient acid. Look slightly overmature. Good test but low juice. Naartje like appearance	seedy /17.1
Or 2	Poor-good	Medium medium large (1-1xx)			1	3 rd week of July	Good, consistent quality with good sugars and slightly high acid. Excellent test. Soft rag	Odd /0.8
Mor 22	Good - excellent	Medium, even size (2-1x)			1-3, mainly 1 Good orange rind	Early-mid August	Good quality, high sugars, high acid	Some /2.1
Nadorcott	Excellent	Medium – medium large – large (2-1xxx)	3-6	1, 3-4	1, odd 4	Mid – late July	Fairly good taste in July, not too sweet. August good, variable sugars still slightly tart. Tests were acceptable to good but low juice	0 – some /3.5

* Average seed counts from internal quality tests. The trees are topworked next to each other in a navel block.

Table 6.3.5.5. Comparison of production, fruit size, colour and comment of various mandarin hybrids evaluated at ITSC, Addo on 20 April 2005. These are not discussed further.

Selection	Production	Fruit Size	Colour	Comment
WH B1-2		Small	6	Clementine like. Good taste, very raggy. Still too early
WH B2-3		Very large	7	Poorman grapefruit x Nules. Flat/round
K3 B2-36	Poor	Extra large	6	Quality dropped since rain. Firm. Normally better crop with smaller fruit.
K3 J5	Good	Medium large	6-7	Good orange like taste. Firm, roundish with a smooth rind, seed. Reasonably peeled.
K3 J15	Fair	Good	8	Better than J5. Flat/round, slight splitting, seed.
J8 O7	Odd	Medium large	4	Orange like flavour, raggy. Will compete with clementine, as it is firm with excellent colour but lacks flavour. Smooth and flattish with nipple, seed.
J8 C27	Odd		6-7	Low sugars and acid, no rag. Flat/round and fairly smooth, large oil cells, thinnish rind, seed.
J8 B18	Poor		7	Clementine x Ellendale. Orange like flavour. Round, slight nipple, variable rind texture. Firm. Should improve.
K8 P42	Poor	Medium, medium large	5	Fairchild x Midnight. Unusual orange like taste. Round, smooth. Firm, not an easy peeler. Seed.

Note: the area had a lot of rain recently which could affect the internal quality. The evaluations were also done too early in the season and some of the trees are still young.

Conclusions

Nova Seedless

There were only slight differences between Nova SL and Nova. Nova SL is not totally seedless, but has less seed than Nova. The two selections appear suitable for planting commercially, Nova SL having less seed. The selection is protected. Evaluations to be discontinued.

Valley Gold (ITSC B17)

Production varied between sites from poor to excellent, fruit size quite acceptable and generally good, attractive fruit colour. Fruit sugars are high while the acid also tends to be high. Maturity from beginning to late June, depending on the acid level. The selection looks promising, is virtually seedless but has the drawback of slight fruit split and fairly high but acceptable acid. The selection should also be evaluated in hotter areas where acid levels may be lower. The selection is protected. Evaluations to be discontinued.

African Sunset (ITSC B24)

Production at the various sites was low, fruit size acceptable (large) to very large and flat shape. Fruit quality was good with good tests, but low juice at Citrusdal, good rind and flesh colour and normally seedless. Fruit sugars are high, good acid levels and a good ratio with a milder flavour than Valley Gold. Maturity from beginning to mid June. The selection looks promising as a later maturing mandarin but has the drawback of large fruit size and flat shape. The selection is protected. Evaluations to be discontinued.

ITSC M37

Production varied between the trees, fruit size medium to larger. Fruit quality was good with soft rag, resulting in messy eating. Colour in Citrusdal was delayed and seedy in mixed blocks. Maturity around 2nd to 4th week of June. Seediness in the absence of cross pollination should be investigated to help determine market potential. Evaluations to be discontinued.

ITSC H25

The yield was fair with good fruit size. Maturity is around mid May. The quality was poor and fruit lacking flavour. The selection does not look promising and evaluations to be discontinued.

ITSC H36

Production was good with large fruit size and seedy. Maturity is around 2nd – 3rd week of mid June with slightly retarded rind colour. The fruit is soft and quality good in June (only acceptable test). Except for a

good fruit size it is doubtful whether this selection is superior to others during this time slot. The seed status in the absence of cross pollination is unknown. Evaluations to be discontinued.

ITSC K33

Production was acceptable with good fruit size, good quality but high acid, peak maturity around 3rd – 4th week of July. The fruit are not attractive with green stem ends. Except for its virtual seedlessness and late maturity, the selection does not look promising. Evaluations to be discontinued.

Roma

Production was fair to good with good fruit size and a naartje like appearance. Quality was good, not outstanding but low juice and seedy, maturing late May to early June. The seed status in non pollinated blocks is not known. Although this selection has in the past had good eating quality, it is not attractive nor superior to other selections during this time slot. The selection is protected. Evaluations to be discontinued.

Or 4

The fruit had good quality in late July with some seed in a mixed block. The selection is protected. Evaluations to be discontinued.

Or 2

Production was fair with good fruit size. Quality was good with very high TSS. Maturity around 3rd week of July. There was some seed in a mixed block. The selection is protected. As the Or is planted commercially, evaluations will be discontinued.

Mor 22

Production was variable between sites with medium fruit size. Eating quality was fair to good with good tests, acid exceeding 1%. Maturity varied between sites from about mid July to mid August. There was some seed in mixed blocks. The selection is protected. As the Mor is planted commercially, evaluations will be discontinued.

Nadorcott

Production was good to excellent with a medium to also large fruit size. Quality was good but not outstanding and acid always coming through. Maturity varies from late June to late July depending on the area. The selection is protected. As Nadorcott is planted commercially, evaluations will be discontinued.

Murcott x Clementine

Fruit at the CFB were eaten by baboons. The semi commercial block at Kirkwood is still young and the trees suffered some frost damage. Evaluations to continue.

Bay Gold

Production was good with good fruit size. Quality was poor due to high acid levels and seed counts varied from seedless to seedy. There was odd fruit split and sunburn. Maturity estimated around end May/early June. The selection does not look promising due to the high acid levels and further evaluations are necessary. It should be tried out in hotter areas in an attempt to reduce acid levels. The selection is protected.

Hadas

Production was good with good fruit size. The quality was fair but test good, acid levels high. The fruit has consistently had high acid. Mature in 3rd week of July and seedy in a mixed block. The selection is protected. Evaluations to be discontinued.

Winola

Production was good with medium fruit size and very good colour. The fruit was mature in late July but acid levels excessively high. The fruit was virtually seedless in a mixed block. The selection is protected. Further evaluations are necessary.

Cami

Production was good with good fruit size and the fruit attractive but seedy in a mixed block. The test was good during 3rd week of June and the fruit mature. Further evaluations are necessary.

Empress mandarin

Production was fair with medium fruit size. Eating quality was not so good when mature in July, but test excellent. The fruit is seedy in a mixed block. Evaluations to continue.

Future evaluations

Continue evaluating Bay Gold, Murcott x Clem, Cami, Empress mandarin and Winola.

Table 6.3.5.6. Internal fruit quality data of mandarin hybrid selections for the Eastern and Western Cape during the 2005 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Nova SL	SC	Paksaam	19/04	5	1	60.2	11.4	0.99	11.5	0.1
Nova	SC	Paksaam	19/04		1	56.9	10.9	0.91	12.0	0.3
Valley Gold	CC	Dunbrody	15/06	1	1X	60.4	13.1	1.20	10.9	0.7
Valley Gold	TC	Baddaford	14/06	1	1	60.8	12.7	1.01	12.6	0.8
Valley Gold	BN/RL	ALG	08/06			45.0	10.9 B	1.20	9.0	0.6
African Sunset	CC	Dunbrody	15/06	1	1XXX	58.8	11.8	0.87	13.6	0
African Sunset	TC	Baddaford	14/06	1-2	1XXX	57.3	13.4	1.13	11.9	0.6
African Sunset	BN/RL	ALG	08/06			45.0	10.5 B	0.90	11.6	0
ITSC M37	TC	Baddaford	14/06	4	1	63.8	12.4	0.86	14.4	9.6
ITSC M37	BN/RL	ALG	05/07			47.0	12.1 B	0.92	13.2	
ITSC H25	BN/RL	ALG	27/05	1-3		52.0	8.0 B	0.64	12.6	
ITSC H36	BN/RL	ALG	08/06			50.0	9.6 B	0.74	13.1	
ITSC H36	BN/RL	ALG	05/07			47.0	10.3 B	0.61	16.9	
ITSC K33	RPL	Boontjieskraal	04/07	3	1xx	58.6	13.3	1.53	8.7	0.6
Roma	BN/RL	ALG	08/06			46.0	11.5 B	0.90	12.8	
Or 4	TC	CFB	20/07	1	1X		15.0	1.23	12.2	1.1
Or	BN/RL	ALG	01/08			54.0	15.0 B	0.96	15.7	
Mor 22	TC	CFB	20/07	1	1X	58.6	14.1	1.25	11.3	1.9
Mor	BN/RL	ALG	01/08			47.0	14.2 B	1.13	12.6	
Nadorcott	CC	CFB	22/06	1	1X	60.4	11.4	1.21	9.4	9.4
Nadorcott	CC	CFB	20/07	1	1X	54.7	11.9	1.11	10.7	11.2
Nadorcott	BN/RL	ALG	08/06			45.0	9.7 B	1.08	9.0	5.1
Nadorcott	BN/RL	ALG	05/07			44.0	10.5 B	1.00	10.5	2.8
Nadorcott	BN/RL	ALG	01/08			52.0	10.0 B	1.01	9.9	3.3
Bay Gold	TC	CFB	22/06	1	1XXX	47.1	10.8	1.24	8.7	4.7
Bay Gold	CC	Jansekraal	08/06	1-2	1xx	48.9	13.3	2.01	6.6	3.4
Hadas	TC	CFB	20/07	1	1X	62.6	12.7	1.43	8.9	8.1
Winola	TC	CFB	20/07		2	54.4	13.2	1.75	7.5	0.3
Cami	SC	CFB	22/06	1	1X	54.7	11.5	1.09	10.6	11.6
Empress M	TC	CFB	20/07	1	1	55.3	13.2	0.98	13.5	6.1
Nules	SC	CFB	23/06	1-2-3	1X	53.4	11.4	0.88	13.0	1.0

6.3.6 Evaluation of navel oranges in the Cape areas Experiment 74 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om kultivars te bekom om die seisoen vroeër en later te versprei en produksie pieke te verminder, asook om seleksies te vind met meer gevorderde vrugkleur, veral aan die begin van die seisoen en vrugte te kry met verbeterde vrugset wat binne die gesogte vruggrootte reeks val. Atwood het soortgelyke tot effens later rypwording as Lina/Newhall. Fukumoto word vroeg ryp, dieselfde tyd tot effens voor Lina/Newhall en ontwikkel 'n diep oranje-rooi skilkleur. Plaaslike waarnemings dui aan dat daar moontlike onverenigbaarheid op Swingle citrumelo en tot 'n mindere mate Koethan citrange is. Meer duidelikheid word benodig oor onderstam keuse. Letaba Early word dieselfde tyd tot later as Lina/Newhall ryp. Dream het kleiner, maar aanvaarbare vruggrootte en word een tot twee weke later as Lina/Newhall ryp. Cliff Early is vroeg en proewe moet uitgebrei word. Fenix, Krajewski en Sundays River Early lyk nie asof

hulle vroeg ryp word nie, maar meer evaluasies is nodig. Washington (SGB materiaal) het goed gevaar, met goeie vruggrootte maar gehalte het tussen persele gewissel. Cambria (SGB materiaal) het goeie vruggrootte gehad, beide ronde en langwerpige vrugte met goeie gehalte maar suurvlakke kan neig om laag te wees. 'n Vergelyking tussen Autumn Gold, Powell, Chislett en Californian Lane Late het min verskille tussen hulle getoon. Powell word dalk effens vroeër ryp, Chislett en Californian Lane Late effens later. Sap vlakke op growweskiisuurlemoen en Rangpur lemmetjie onderstamme op sanderige grond kan laag wees. Renken Late, Coetzee Late en Mouton Late 1 en 2 het min gedra. Renken lyk aantreklik. Glenora Late het begin dra met enkele vrugte. Eetgehalte was swak maar interne gehalte toetse goed. Verdere evaluasies van al die seleksies is nodig aangesien van die seleksies nog jonk is.

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. Tuligold, Lina, Newhall, Palmer and Lane Late 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 acceptable by the market place (higher standard for Navelates in brackets): 48% juice; 8.5% (10.0%) TSS; 0.6 – 1.5% (0.8 – 1.50%) acid; 7.5:1 (9.0:1) ratio; colour T3 (T2) of set 34; 0 seeds per fruit.

A list of selections and sites evaluated during 2005 is given in Table 6.3.6.1.

Table 6.3.6.1. Navel trial sites evaluated during 2005.

Selection	Area	Site/ Orchard	Plant Date	Root Stock	No of trees
Atwood	Uitenhage	CFB Block 7	1999	RL	2
Atwood	Sunland	Woodridge *	2000	CC	2 topwork
Atwood	Heidelberg	Kruisrivier	2001	C35	5
Fukumoto	Uitenhage	CFB Block 4	2001	SC	2
Fukumoto	Sunland	Woodridge *	2000	CC	2 topwork
Fukumoto	Heidelberg	Kruisrivier	2001	C35	4
Fukumoto	Citrusdal	ALG	2002	RL	20 topwork
Letaba Early	Sunland	Woodridge *	2000	CC	2 topwork
Letaba Early	Heidelberg	Kruisrivier	2001	C35	7
Dream	Uitenhage	CFB Block 7	1999	CC	2
Dream	Sunland	Woodridge *	2000	CC	3 topwork
Dream	Heidelberg	Kruisrivier	2001	C35	5
Cliff Early	Fort Beaufort	Riverside	1996	TC	4
Fenix Early	Addo	ITSC	1999	SC	2 topwork
Krajewski Early	Addo	ITSC	1999	CM	2 topwork
Mistkraal Early	Addo	ITSC	2000	MxT	2 topwork
Sundays River Early	Addo	ITSC	1999	VA	2 topwork
Washington	Uitenhage	CFB Block 4	2001	RL	2
Washington	Addo	Willowtree	1998	CC	commercial
Washington	Patensie	Ripplehill	1999	RL	commercial
Washington	Fort Beaufort	Riverside			commercial
Santa Catarina 1 & 3	Addo	ITSC			2
Cambria	Uitenhage	CFB Block 7	1999	CC	2
Cambria	Uitenhage	CFB Psylla	1999	TC	1
Cambria	Patensie	Patensie Acht	2000	SC **	commercial
Autumn Gold	Uitenhage	CFB	2001	TC	4
Autumn Gold	Uitenhage	CFB Block 7	1994	SC	6
Autumn Gold	Fort Beaufort	Baddaford	1998	TC	10
Autumn Gold	Citrusdal	ALG	1995	RL	Semi com

Autumn Gold	Citrusdal	Hexrivier	1997	RPL	semi comm
Powell	Uitenhage	CFB Block 7	1995	TC	2
Powell	Fort Beaufort	Baddaford	1998	TC	5
Powell	Citrusdal	ALG	1995	RL	Semi com
Powell	Citrusdal	Hexrivier	1997	RPL	semi comm
Chislett	Uitenhage	CFB Block 8	1997	TC	1
Chislett	Fort Beaufort	Baddaford	1998	TC	4
Chislett	Citrusdal	ALG	1995	RPL	5
Chislett	Citrusdal	Hexrivier	1997	RPL	semi comm
Glenora Late	Uitenhage	CFB Block 4	2001	CC	2
Glenora Late	Clanwilliam	Jansekraal	2002	Val/RL	9 topwork
Renken Late Navel	Citrusdal	Hexrivier	1997	RPL	8 topwork
Coetzee Late Navel	Citrusdal	Hexrivier	1997	RPL	9 topwork
Mouton Late Navel 1	Citrusdal	Hexrivier	1997	RPL	4 topwork
Mouton Late Navel 2	Citrusdal	Hexrivier	1997	RPL	2 topwork
Witkrans B13	Clanwilliam	Jansekraal	2002	Val/RL	8 topwork
Tuligold	Citrusdal	ALG	1998	RL	17
Lina (control)	Uitenhage	CFB Block 4	2001	CC	2
Lina (control)	Heidelberg	Kruisrivier	2001	C35	5
Lina (control)	Citrusdal	ALG	1997	RL	commercial
Newhall (control)	Sunland	Woodridge *	1999	CC	commercial
Newhall (control)	Citrusdal	ALG	1997	RL	commercial
Californian Lane Late (control)	Uitenhage	CFB Block 4	2001	CC	2
Californian Lane Late (control)	Citrusdal	Hexrivier	1997	RPL	semi comm
Californian Lane Late (control)	Clanwilliam	Jansekraal	2002	Val/RL	semi co top
Royal Late	Uitenhage	CFB Block 4	2001	RL	2
Royal Late	Uitenhage	CFB Psylla	2001	CC	1
Royal Late (control)	Clanwilliam	Jansekraal	2002	Val/RL	semi co top

* Woodridge Farm previously called Paterson. ** SC because on replant soil.

Results and discussion

A discussion of each selection follows with internal quality results presented in Table 6.3.6.15 that needs to be referred to when reading the text. A comparison of the various early navel selections is presented in the following tables.

Table 6.3.6.2. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at the CFB, Uitenhage during 2005.

Selection	Yield	Fruit Size	Colour		Taste	Test	Estimated maturity
			19 May	23 June			
Atwood	Poor. Next to wind-break	Large	1		Poor, low sugars, high acid	Navel standards	Mature, but acid high in May (stress)
Fukumoto	Poor – zero	Medium large	1-2		Fair, fair sugars and acid	Too few fruit to test	Mature in May
Dream	Fair-good	Medium large	2-3	1	Good quality with fair sugars and acid in May and June	Navelate standards except for odd seed	Mature in May, slightly over-mature in June with odd creasing
Lina	Poor	Large-extra large	2		Poor quality, low sugars and acid	Navel standards	Mature in May
Washington	Poor	Medium-large		25 July 1	Poor quality, low sugars and acid	Fails standards as juice just too low. Borderline low acid	Mature in July

The young Fukumoto trees at the CFB had too few fruit to test. There was a lot of sucker growth on Swingle rootstock, some on rough lemon and none observed on Carrizo. Similar aged Linas on Carrizo had poorer quality in May and fractionally later colour, Washington maturing later.

Comparison of early navel selections on Carrizo citrange at Woodridge, Addo on 20 April and 5 May.

Table 6.3.6.3. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at Woodridge, Sunland (Addo) during 2005.

Selection	Yield	Fruit Size	Colour		Taste	Test	Estimated maturity
			20 April	5 May			
Atwood	Fair	Large	7	5	Fair quality, fair sugar and acid in April and May	Navel standards in April and May except colour	Mid May
Fukumoto	Fair	Large	5-6	2-3	Sweet, good sugars and acid in April, fair in May	Navelate standards in April and May except colour in April	Mature in early May, earlier than the others.
Letaba Early	Fair	Large	6	4-5	Fair sugar and acid in April, poor in May	Navel standards except colour	Mid May
Dream	Fair	Medium large	7	5-6	Fair sugar and acid in April, poor in May	Navel standards except colour	Mid May
Newhall	Good	Medium large	6-7	5	Fair, slightly sweet and lowish acid in April, fair in May	Navelate standards except colour	Mid May

Fukumoto and Newhall had good sugars, (above 10%), followed by Atwood, 9.6%; Dream and Letaba Early above 9%. Newhall had by far the highest acid, >1.1%, Dream and Atwood similar (0.95%); Fukumoto (0.93%) and Letaba Early low at 0.84%. All had good juice percentages. Except for colour, only Fukumoto and Lina met Navelate standards. Fukumoto had the best tasting fruit in May, followed by Newhall and Atwood (fair), Letaba Early and Dream the poorest. Fuklumoto was the earliest to mature and also ahead on colour, Letaba Early, Atwood and Newhall fairly similar colour and Dream the latest in May. In May, all selections had orange flesh colour, oily on peeling, Fukumoto and Atwood open cores, the others slightly open in May.

Comparison of early navel selections on citrange C35 at Kruisrivier, Heidelberg on 22 April and 19 May.

Table 6.3.6.4. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at Kruisrivier, Heidelberg during 2005.

Selection	Yield	Fruit Size	Colour		Taste	Test	Estimated maturity
			22 April	19 May			
Atwood	Poor-fair	Medium large-large	7-8	5-6,6	Fair-good quality, sufficient sugars, more acid than Lina	Navel standards except colour in May	End May
Fukumoto	Zero-good	Large	5-7	2-3, deep orange/red	Fair-good, sufficient sugars and acid	Navelate standards, except 1 seed	Mid May
Letaba	Zero-	Medium	7	5-6,6	Fair, similar to	Navel	Mid-end May

Early	odd	large-large			poorer than Atwood. Tender	standards, except colour and seed. Poorest test	
Dream	Poor-fair	Medium large-large	7-8	5-6-7	Fair, similar to poorer than Atwood	Navel standards, except colour and seed. Poor test	End May-early June
Lina	Fair	Medium-medium large	6-7	5-6, green heads	Fair-good, sufficient sugars and acid	Navel standards, except colour and seed	Mid –3 rd week May

Table 6.3.6.5. Percentage fruit per count of various early navel selections measured at Kruisrivier, Heidelberg on 19 May, 2005.

Selection	Average fruit size (mm)	Approx. count	Percentage fruit per count						% fruit in counts 56-72
			40	48	56	64	72	88	
Lina	78.6	64		4	12	44	36	4	92
Dream	82.2	56		12	44	44			88
Atwood	83.6	56	4	28	40	20	8		68
Letaba Early	84.5	56	20	20	35	20	5		60
Fukumoto	86.4	48	20	48	20	12			32

The trees blossomed well in spring 2004 but the whole orchard was later flooded in December and also a lot of rain in April 2005. Lina had the most fruit and smallest fruit size and Fukumoto by far the largest fruit size. Fukumoto had by far the best and deepest rind colour.

Malformed navels – all were exportable, Fukumoto having the least, closely followed by Lina; Letaba Early a fair amount, Dream and Atwood the most.

Protruding navels – Dream and Atwood (4% not exportable) had the least, Fukumoto the most with 4% not exportable, followed by Lina and Letaba Early with some.

Fukumoto had the highest sugars (10.3%), Atwood, Dream and Lina 9.1-9.4% and Letaba Early below 9.0%. Atwood, Dream and Letaba Early had similar acid, 0.97-0.98%; Fukumoto and Lina the lowest at 0.89-0.90%. Except for the odd seed, only Fukumoto met Navelate standards. Dream had a very slight bud union bench, Fukumoto also but a lot of sucker growth just above the bud union. Letaba Early had fairly small, dense trees with a slightly benched bud union.

It is interesting to note the presence of seed in some of the selections. There are some mandarins in an adjacent row. Only the Dream had some seed at the CFB (pollinators in the orchard) but no seed at Woodridge where the trial trees are in a commercial orchard.

Comparison of early navel selections on rough lemon at ALG, Citrusdal on 11 and 25 May, 2005.

Table 6.3.6.6. Comparison of production, fruit size, colour, quality and maturity of early navel selections evaluated at ALG, Citrusdal during 2005.

Selection	Yield	Fruit Size	Colour		Taste	Test	Estimated maturity
			11 May	25 May			
Fukumoto	Poor-fair	Large	4	2-4 green heads	Fair, probably sufficient sugars and acid. Raggy	Navel standards except low juice	Peak to past on 25 May
Tuligold	Good	Medium large-large	4 green heads	3 green heads	Variable, tasteless to good sugars and acid on 25 May.	Navel standards except low juice and	Peak on 25 May

					Tender	borderline sugars	
Lina	Fair	Medium large	4-5 green heads	3 slightly green heads	Variable, tasteless to very good, better than Tuligold on 25 May. Tender	Navel standards on 11 May, thereafter sugars too low and juice too low	Peak on 25 May
Newhall	Fair	Large	3-5	2-3 slightly green heads	Consistent, fair-good quality, good sugars and acid on 25 May. Slightly raggy	Navel standards except low juice	Peak on 25 May

There was little difference in fruit size between the selections, all having a large percentage of the fruit larger than count 56 (70 –90%). Tuligold had the smallest fruit size. Fukumoto had 25% count 56 and none smaller. Fukumoto, Lina and Newhall had deep orange red rind colour with some green stem ends and Tuligold had good, deep orange colour and no green stem ends. Only Tuligold was attractive (except for the large navel ends) with fairly round fruit. Fukumoto and Newhall had pebbly rinds and ribbing.

Malformed navels – Newhall had the least and Tuligold the most. Fukumoto and Lina both had 5% not exportable fruit and Tuligold 20%.

Protruding navels – Tuligold had the least and Newhall the most. Tuligold and Lina both had 5% not exportable.

Ribbing – Fukumoto and Newhall had 85-90%, Tuligold and Lina none.

The internal quality tests on 11 and 27 May were not too good. The juice percentages were all too low, Newhall the lowest. Average Brix values were not high although all acceptable, Newhall the highest at 9.6%, Fukumoto 9.2% and Lina the poorest at 8.5%. Acid values were also relatively low, but acceptable, Tuligold and Lina the highest, Fukumoto the lowest at 0.76%. All acid levels were fairly constant over the 16 day test period. Fukumoto matured about one week ahead of the other selections, colour similar to Newhall to half a colour transparency earlier than Tuligold and Lina in late May. Fukumoto sometimes has large internal navels.

A summary of the comparison of various early navel selections at various sites during 2005.

Note that these are generalisations and do not take tree age, rootstock, etc. into account except internal quality where the summary was done as comparatively as possible. Fruit colour was not taken into account. Refer Table 6.3.6.7 for average internal quality test results.

Atwood – The yield was poor–fair with mainly medium large–large fruit size. Taste was generally fair, meeting navel standards. Navel ends were Palmer navel-like with some malformed navel ends. Fruit shape is round and fruit good looking.

Fukumoto – The yield was variable, averaging fair to poor and large fruit size. Taste was fair to good, some of the tests meeting Navelate standards. Fruit had malformed and protruding navel ends, sometimes a large internal navel. Fruit shape varied and navel ends sometimes flat. The rind at the styler ends is sometimes thin. Generally, a slightly more open core than the other selections.

Letaba Early – Yields varied from zero-fair, fruit size between medium large through to large. The taste was fair, fruit meeting navel standards. There were variable protruding and malformed navel ends, generally round shape sometimes with shoulders.

Dream – Yields were fair with medium large fruit size. The taste was fair, meeting navel and sometimes Navelate standards. Navel ends were smallish, malformed, Palmer like size and round fruit shape.

Lina – Yields were poor-fair with mainly medium large to large fruit size, poor–good taste and navel quality. The navel ends are closed to protruding and elongated fruit shape.

Newhall – Yields were fair-good with medium large-large fruit size. The taste was fair-good and consistent, meeting navel and Navelate standards. Navel ends were closed to protruding and with occasional malformed navels and fruit with a slightly elongated shape.

Tuligold – The yield was good with medium large-large fruit size. The taste poor-good, meeting navel standards (except juice). Generally large malformed navel ends with a round fruit shape.

Table 6.3.6.7. Averages of test results of various early navel selections tested during May 2005.

Selection	Juice %	TSS %	Acid %	Ratio
Atwood	51.4	9.8	1.06	9.2
Fukumoto	48.8	10.2	0.87	11.7
Letaba Early	50.2	9.2	0.92	10.0
Dream	52.8	9.9	1.05	9.4
Lina	51.5	9.2	0.85	10.8
Newhall	48.0	10.3	0.95	10.8
Tuligold	46.5	9.3	0.83	11.2

Overall, Fukumoto was 1-2 weeks earlier maturing than Atwood, and half to one week earlier than Lina/Newhall. Dream was 1-2 weeks later than Atwood. Letaba Early was variable. Fukumoto generally had the earliest fruit colour and Dream the latest. Fukumoto and Lina had deeper rind colour than the other selections. Fukumoto tended to have slightly thicker rinds and Dream thinner compared to the other selections.

Observation of Fukumoto on various rootstocks at ALG Boerdery, Citrusdal.

Trees were planted in December, 1997 and Fukumoto navel budded to rootstock shoots during 2002.

Some cuts were made over the bud union on some of the trees on 23 September to see if there was a brown ring on the wood. Results and observations of the evaluations are given in Tables 6.3.6.8 & 9.

Table 6.3.6.8. Comparison of tree size, yield, fruit size, colour and fruit quality of Fukumoto navel on different rootstocks at ALG Boerdery, Citrusdal on 11 May, 2005.

Rootstock	Tree size	Yield	Fruit size	Colour	Quality/test
Swingle citumelo	Medium, variable	Zero-fair	Medium large-large	2-3, most uniform good colour	Good Navelate test except low juice
Benton citrange	Medium	Poor-good	Medium large-large	3-5	Good Navelate test except juice just too low
Rough lemon	Large, vigorous	Fair-good	Medium large-large	5	Acceptable navel test, except low juice
Koethen citrange	Small, medium-medium large and large	Poor-fair	Medium-medium large	3-4, some 5	Good Navelate test except very low juice
Citrangle C35	Small	Poor-fair	Medium large	2-3 and 4-5	Extremely high sugars, Navelate test except low juice. Overmature taste

Swingle surprisingly has the best, deep orange colour and rough lemon the poorest. Rough lemon had the thickest rind. Due to the generally low yield, the fruit was generally on the large side with not too much difference between rootstocks.

Table 6.3.6.9. Evaluation of bud unions of Fukumoto navel on different rootstocks at ALG Boerdery, Citrusdal on 23 September, 2005.

Rootstock	Bud union	Appearance of bud union under bark	Comment
Swingle citumelo	Typical bud union, quite benched	One tree clear brown ring, one blotch of brown at bud union, one yellow/beige scion and brown rootstock. One with no real ring but a 5mm wide brown band. One tree clear	One tree has tiny green knobs on bud union.
Benton		White wood (scion and	Nothing unusual.

citrange		rootstock)	
Rough lemon	Bud union looks normal	White wood (scion and rootstock)	Some small green outgrowths on cambium above bud union. Odd small nodules on rootstock below bud union and odd suckers.
Koethen citrange	Not quite smooth	Three trees white wood and one tree yellow/beige wood	Very small outgrowths above bud union and occasional sucker below bud union
Citrange C35	Slight bench	White wood (scion and rootstock)	Trees paler than others and don't look as good.

All trees were in a state of flush.

Based on the appearance of a brown ring on the cambium of the tree at the bud union, some trees on Swingle show possible signs of incompatibility and Koethen citrange to a lesser extent.

Cliff Early

Some overmature fruit were evaluated on 14 June. Fruit size was between counts 56 – 72, a good deep orange red colour (T1) with a good, overmature taste and still sufficient acid. The fruit had a smooth, thin rind and signs of creasing. Estimated timing based on previous evaluations is around mid to late April.

Fenix Early

This budsport was selected in the Addo area for its early maturity. The young topwork trees bore a few fruit of fair quality, colour T7 on 20 April. The fruit had a thin rind and was not yet mature and does not look like an early maturing navel.

Krajewski Early

This budsport was selected in the Kirkwood area for its early maturity. The young topwork trees are fairly vigorous bearing a few odd large fruit, colour T8 on 20 April. The fruit shape was fairly round with variable navel ends. The acid was still high and flesh pale. It does not appear early maturing.

Mistkraal Early

This budsport was selected in the Kirkwood area for its early maturity. The young topwork trees bore odd fruit, colour T7-8 on 20 April. Eating quality was fair with good flesh colour. Although rind colour was poor, it could be early maturing.

Sundays River Early

This budsport was selected in the Kirkwood area for its early maturity. The young topwork trees had a poor crop of large to extra large fruit, colour T7 on 20 April. The fruit had variable navel ends, some with a flat shape. The fruit had lowish sugars (Volckameriana rootstock) and fair acid. It does not appear early maturing.

Washington

The trees at Addo were picked by 14 June.

Table 6.3.6.10. Packhouse data for yield, percentage per class and count distribution of class 1 fruit for Washington navels on Carrizo citrange planted in 1998 (5.5 x 2.5m) at Willowtree Farm, Addo.

Year - Age	Tons /ha	% export grade 1&2	% grade 1	Over size	% fruit per count in grade 1							Local fruit	Factory fruit
					36	40	48	56	64	72	88		
2002 -4	10.5	66	44	0	5	16	25	25	14	12	3	29	4
2003 -5	24.9	42	26	1	12	24	24	20	12	8		42	15
2004 -6	27.0	57	38	1	11	13	24	18	18	13	3	29	13

2004 cull factors due mainly to wind blemish (lack of good windbreaks), some pests and overmaturity. With additional irrigation lines the production could have been increased.

The trees at Patensie bore an even, good-excellent crop of good medium large-large fruit size (between counts 48-72, mainly 56/64). There was variation in colour between the sides of the tree, west side T2 and east side T3-5 on 16 June. Eating quality was fair, with acceptable sugars and acid, the test just meeting Navel standards (borderline juice and acid), around peak maturity. Navel ends varied from small to large, 96% with malformed navel ends, 4% not exportable. The fruit was round and firm with a pebbly rind, closed cores sometimes with a pale halo and slightly raggy.

Trees at Fort Beaufort had a good crop of good fruit size (around count 72), colour mainly T1 on 14 June. The quality was excellent, meeting Navelate standards. Fruit shape was round, occasionally slightly elongated with a smooth to finely pebbled rind and attractive. Navel ends varied with some malformed navel ends.

Santa Catarina 1 and 3

The trees of both selections are large and vigorous with a few fruit of large to extra large fruit size. Fruit shape is generally round. Fruit colour was T8 on 20 April and the fruit still immature.

Cambria

Two lots of trees were evaluated at the CFB. Six year old trees on Carrizo had a poor crop of medium fruit size, colour T1 on 20 July (T4-5 on 16 June). Fruit quality was good and mature. Except for odd seed (mixed block) the test was excellent. Younger Royal Late trees on rough lemon had a poor crop, medium large fruit size and colour T1 on 25 July (T6 on 16 June). Fruit quality was poor and the test just meeting navel standards.

Cambria trees in the insect-proof shade house had a fair crop of large to extra large fruit size, colour T1 on 25 July. Eating quality was poor, just meeting navel standards and the fruit mature. Slightly younger Royal Late trees had a poor crop of medium large fruit size, colour T1 on 25 July and some split fruit. The quality was good and fruit mature, just missing Navelate standards due to slightly low acid.

Fruit shape was similar with both selections having elongated and also round fruit in the case of Cambria. Rinds of both were smooth and mostly open cores, peelable but oily.

The young trees at Patensie had a fair-good crop of good, medium large-large fruit size, T1-3 on 25 July. The eating quality was good, acid a little low. The test was good, easily meeting navel standards, acid too low for Navelate. The fruit was about at peak maturity. Fruit shape was rounder than last season, with both round and elongated fruit. Most fruit had closed, protruding navels and a lot of high shoulders. Rinds are fairly smooth and the fruit firm. Cores are closed with a fine, orange flesh.

On quality-inducing rootstocks, the sugars are good but the acid levels can be on the low, but acceptable side.

Comparison of late navel selections in the Eastern Cape during 2005.

Table 6.3.6.11. Comparison of production, fruit size, colour and quality of mainly Australian Late navel selections evaluated in the Eastern Cape at the CFB, Uitenhage and Baddaford, Fort Beaufort during 2005.

Selection	Rootstock Year planted	Yield	Fruit Size	Colour	Taste	Test
Uitenhage – 20 or 25 July						
Autumn Gold	TC 2001	Poor	Medium large	1	Good, high sugars, fair acid	Good, navel quality.
Autumn Gold	SC 1994	Fair	Medium	1	Good, high sugars, fair acid	Very good, Navelate quality
Powell	TC 1995	Fair	Medium large	1	Fair, acid a bit high	Very good, acid a little high, Navelate quality
Chislett	TC 1997	Poor	Medium large	1-2	Fair	Very good, Navelate quality
Glenora Late	CC 2001	Poor	Medium large	1	Poor, low sugars and acid	Good, Navelate quality but odd seed.
Californian Lane Late	CC 2001	Poor	Medium large	1	Fair	Acceptable, navel quality
Fort Beaufort – 14 June						
Autumn Gold	TC	Poor (shaded)	Medium-medium large	4-5		Navelate quality
Powell	TC	Poor	Medium large-large	3-4-5		Navel quality
Chislett	TC	Poor-	Medium	5-6		Navelate quality

		fair	large			
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At the CFB, all selections were mature by 20 or 25 July. All fruit were round with a smooth rind and peelable (Chislett more difficult) but oily, orange coloured flesh and slightly open to open cores. Powell and Chislett had some split fruit. Autumn Gold on Troyer had a poor appearance. Glenora Late had some malformed navel ends.

At Fort Beaufort, all selections were round, Autumn Gold rind smooth, Powell smooth to slightly pebbly and Chislett in between. Navel ends were variable, Autumn Gold small to closed protruding, Powell closed protruding and Chislett malformed.

Comparison of Australian Late navel selections in the Western Cape during 2005.

Table 6.3.6.12. Comparison of production, fruit size, colour and quality of Australian Late navel selections evaluated in the Western Cape at ALG and Hexrivier, Citrusdal during 2005.

Selection /rootstock	Yield	Fruit Size	Colour		Taste	Test
			4 July	1 August		
ALG						
Autumn Gold Rough lemon	Good	Medium large	2-4 green heads	1-3, most green heads	Fair-good. Not as raggy. Maturity 3 rd week July	Navel standards
Powell Rough lemon	Good	Medium large	2-4 green heads	1-2, slight green heads, deeper red	Fair-good. Very raggy. Maturity 3 rd week July	Navel/Navelate standards
Chislett Rangpur lime	Good	Medium large	1-5 slight green heads	1-3 some green heads	Fair-good. Very raggy. Maturity 3 rd week July	Navelate/navel standards
Hexrivier						
Autumn Gold Rangpur lime	Fair-good	Medium large-large	1-3 more mature		Good. Raggy. About peak maturity	Navelate quality
Powell Rangpur lime	Fair-good	Medium large	1-2		Good. Raggy. About peak maturity	Navelate quality
Chislett Rangpur lime	Fair	Medium large-extra large	1-3		Good. Raggy. About peak maturity	Navelate quality except juice too low
Caifornian Lane Late Rangpur lime	Fair-good	Medium large-large	1-4		Good. Raggy. About peak maturity	Navelate quality

At Hexrivier, all the fruit tasted similar. There was some rootstock growth and nodules on the rootstock to varying degrees.

A summary of a comparison of various Australian Late navel selections at four sites during 2005.

The differences, if any, between the various selections at the four sites are highlighted. In instances there was quite some variation of the different selections between the sites. Californian Lane Late is only at two sites. The average internal quality is used for ALG (two tests done).

- Tree size: Chislett the largest, Powell smallest.
- Yield: Powell slightly better (fair-good); Chislett fair and the others variable, poor-good between sites.
- Fruit size: Size overall tended to be on the large side, to too large but differences not huge. At Citrusdal Powell was the most consistent between the two sites with 56-60% falling within counts 56-72, the balance larger. The other selections varied between the sites.
- Colour: There was variation within the sites, but Powell possibly slightly earlier, Chislett and Californian Lane Late later.
- Fruit shape: Little difference, all generally round, Lane Late the least round.

- Rind texture: Rinds tend to be coarser in Citrusdal. There was little difference among the sites. Autumn Gold and Lane Late slightly smoother.
- Navel ends (Citrusdal): Californian Lane Late had the most malformed navel ends (Hexrivier only) and Powell the most for both sites. Lane Late had by far the least protruding navel ends, followed by Powell. Autumn Gold and Chislett were similar. With malformed and protruding navel ends, there were odd non exportable fruit.
- Split fruit: The following were observed on odd occasions at a site - Powell and Chislett odd split fruit, Chislett and Lane Late some ribbing and Chislett some creasing.
- Rind thickness: Acceptable but all thicker at Citrusdal.
- Internal colour and cores: All good to excellent colour, some halos with Autumn Gold, all cores slightly open.
- Flesh texture: The flesh was coarser at Citrusdal, Powell, Chislet and Lane Late coarse.
- Taste: Generally good, all raggy in Citrusdal.
- Internal quality: Except for odd instances of low juice, they all met navel or Navelate standards. At Citrusdal the various selections were fairly similar between and within the two sites. Chislett had low juice at both sites, Powell slightly lower acid and Lane Late (one site only) lower, but acceptable sugars. In the Eastern Cape, results varied between the sites and also within the sites, with no pattern. Lane Late had lower sugars compared to the others at the CFB. Juice levels of all the selections were somewhat higher in the Eastern Cape, but better quality-inducing rootstocks were used.
- Maturity: Around mid July, Chislett and Lane Late possibly slightly later.

Evaluation of late maturing Coetzee Late, Renken Late, Mouton Late 1 and Mouton Late 2 navels at Hexrivier, Citrusdal.

The results must be treated with caution due to the small sample size. Trees are planted in poor, sandy soil. Adjacent Californian Lane Late navels were used for comparative purposes. Comments on the evaluations are presented in the table below.

Table 6.3.6.13. Evaluation of tree size, yield, fruit size, colour and fruit characteristics of various late maturing navel selections on Rangpur lime rootstock at Hexrivier, Citrusdal on 4 July, 2005.

Selection	Tree size	Yield	Fruit size	Colour	Fruit characteristics
Renken Late	Small	Zero-fair	Medium large, mainly ct 48/56	1-2	Round, slightly pebbly, thinnish rind. The fruit had odd malformed navels and 40% protruding navel ends
Coetzee Late	Small	Poor-fair	Large, mainly ct 40/48 & 64	2-4	Round and coarse. The fruit had 50% malformed and protruding navel ends. There was some sunburn
Mouton Late 1		0–odd	Large-extra large	1	Round-slightly elongated, coarse, thick rinds
Mouton Late 2		No fruit			
Californian Lane Late	Medium large	Fair-good	Medium large-large	1-4	Fairly round, slightly pebbly. Few protruding navels, the majority with malformed navel ends

Renken Late

The eating quality was fairly good, tests easily meeting navel standards, around peak maturity, earlier colour than Californian Lane Late. Internal colour was deep orange with coarse flesh and closed cores. Fairly attractive fruit.

Coetzee Late

The eating quality was fair-good, easily meeting Navelate standards, with higher sugars and acid than Californian Lane Late, at to just past peak maturity. Internal colour was a good orange with coarse flesh and closed cores.

Mouton Late 1

The eating quality was fair with sufficient sugars and acid and tender. There were too fruit to test. Past peak maturity by 1-2 weeks. Internal colour was orange with closed cores. Overall poor fruit.

Mouton Late 2

No fruit.

Californian Lane Late (control)

The eating quality was good, meeting Navelate standards, but raggy. Around peak maturity. Internal colour was excellent, coarse flesh and closed cores.

Comparison of Glenora Late, Witkrans, Royal Late and Californian Lane Late in the Western Cape during 2005.

The topworked trees in the Clanwilliam area produced their first fruit. All are growing vigorously. Results therefore to be read with caution.

Table 6.3.6.14. Comparison of production, fruit size, colour and quality of late navel selections evaluated at Jansekraal, Clanwilliam on 1 August, 2005.

Selection	Yield	Fruit Size	Colour	Taste/Test	Comment
Glenora Late	O-odd	Extra large	1-2	Variable poor to fair quality. Too few to test. Peak to just past	Round to slightly elongated shape. Coarse flesh. Large, vigorous tree with thorns
Witkrans B13 (old selection)	Poor-fair	Medium-medium large. 76% ct 56-72	1-3	Variable poor to fair quality. Good test, navel standards. Peak Raggy	Slightly elongated shape. A lot of protruding and virtually no malformed navel ends. Fine flesh
Royal Late	Poor-fair	Medium-medium large. 64% ct 56-72	2-3 Some slightly green heads	Poor quality, lacks sweetness. Good test, inconsistent with taste. Peak	Elongated shape. Most protruding and no malformed navel ends. Fairly fine flesh. Firmest of all
Californian Lane Late	Fair	Medium large-large. 4% ct 56-72	1-3 redder colour	Variable poor to fair quality. Peak to just past	Fairly round. Some protruding and few malformed navel ends. Slightly thicker rinds. Slightly coarse flesh and occasional halo. Odd ribbing. Looks most mature.

Witkrans is the older selection, superseded by a new selection. Unfortunately there were too few fruit of Glenora Late to evaluate properly as it is the first few fruit. Because of the vigour the eating quality is still poor. However Royal Late had surprising high sugars. Californian Lane Late had the roundest fruit shape and earliest maturity. Glenora Late at the CFB are also still young trees with a poor crop and of poor eating quality but a good, Navelate test. There were odd seed (mixed block).

Conclusions

Atwood

On average over the various sites, the yield was poor to fair with mainly medium large to large fruit size. The taste was generally fair, meeting navel standards, except colour. It is a good looking fruit. Maturity is similar to Lina/Newhall to one week later. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Fukumoto

The average yield over the various trial sites was variable, averaging fair to poor and large fruit size. Taste was fair to good with some tests meeting Navelate standards. Maturity is half to one week earlier than Lina/Newhall and generally the earliest colour, which develops to a deep orange/red. Observations of trees in Citrusdal indicate that there may be incompatibility of trees on Swingle citrumelo and to a lesser extent on Koethen citrange. More clarity is needed as to what rootstock to use. As the trees are still young and vigorous further evaluations are necessary before any recommendations can be made.

Letaba Early

The yield on average at the various sites varied from zero-fair, medium large through to large fruit size. The taste was fair meeting navel standards, except colour. Maturity varies from around Lina/Newhall time to later. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Dream

The yields on average at the various sites were fair with medium large fruit size, smaller than the other early selections but quite acceptable. The taste was fair meeting navel and sometimes Navelate standards, except colour. Maturity was one, up to two weeks later than Lina/Newhall. This selection could be considered to be more a mid maturing navel. As the trees are still young, further evaluations are necessary before any recommendations can be made.

Cliff Early (Painter Early II)

The selection is early maturing, and was evaluated 6-8 weeks too late. It still had good quality. Further trial plantings need to be established and further evaluations needed.

Fenix Early

This selection does not appear to be early maturing. Further evaluations are necessary.

Krajewski Early

This selection does not appear to be early maturing. Evaluations will be continued on virus free trees when they are in production.

Mistkraal Early

There is too little data available to draw any conclusions. Further evaluations are necessary.

Sundays River Early

This selection does not appear to be early maturing. Further evaluations are necessary.

Washington

Production was good, with medium large to large fruit size. Colour was acceptable to good at maturity, which is around 2nd-3rd week June. Eating quality varied between sites from acceptable to excellent meeting navel to Navelate standards. Although the trees are still fairly young, 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.

Santa Catarina 1 and 3

The trees of both selections are large and vigorous and bore a few large to extra large fruit. The fruit was still immature in mid/late April. Further evaluations are necessary.

Cambria

Production in the commercial block was fair to good with good fruit size, good quality and tests, although acid levels tend to get low. Fruit at the CFB had poor to good quality. The fruit is firm with an elongated and round fruit shape, maturing around mid to late July. Further evaluations of trees from Foundation Block material are necessary before recommendations can be made. The selection is protected.

Comparison of various Australian Late navel selections at four sites.

There were no major differences between the selections. Powell had overall a slightly better yield. Fruit size of all the selections is on the large side, Powell the most consistent size in Citrusdal. All had good internal quality, meeting navel or Navelate standards except in the odd instance where there was low juice in Citrusdal, especially Chislett. A good quality-inducing rootstock should minimise this problem. The fruit quality between the selections and sites in Citrusdal was similar, but no patterns in the Eastern Cape. Maturity is around mid July, Powell possibly slightly earlier, Chislett and Californian Lane Late slightly later. These Australian selections should be suitable for planting in the Eastern and Western Cape areas, but attention should be paid to excessive fruit size and low juice levels. Autumn Gold, Powell and Chislett are protected. Further evaluations are necessary to confirm the results presented here.

Renken Late Navel

Production was very poor with medium large fruit size. Quality was good, earlier than Californian Lane Late and the fruit attractive. Further evaluations are necessary.

Coetzee Late Navel

Production was poor to fair with large fruit size and good quality. Further evaluations are necessary.

Mouton Late Navel 1

Production was very poor with large to extra large fruit size and fair. The fruit was unattractive. Further evaluations are necessary.

Mouton Late Navel 2

There was no fruit. Further evaluations are necessary.

Glenora Late, Witkrans, Royal Late and Californian Lane Late

The trees at Clanwilliam are still young and vigorous and only produced their first fruit. Eating quality was poor but tests acceptable to good. Californian Lane Late had the roundest fruit shape and earliest maturity. Glenora Late at the CFB also had a poor crop (young trees) of poor eating quality but good test and timing comparable with Australian Late navels. Further evaluations are necessary. Glenora Late, Witkrans and Royal Late are protected.

Future evaluations

Evaluate all sites and selections, as some of the trees are still young and only just come into production.

Table 6.3.6.15. Internal fruit quality data for navel orange selections for the Eastern and Western Cape areas during the 2005 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	* TSS %	Acid %	Ratio	Ave. Seed
Atwood	RL	CFB	19/05	1	48	51.4	10.4	1.30	8.0	0
Atwood	CC	Woodridge	20/04	7	64	51.9	9.5	0.99	9.6	0
Atwood	CC	Woodridge	05/05	5	56	52.1	9.7	0.91	10.7	0
Atwood	C35	Kruisrivier	19/05	6	56	50.6	9.4	0.98	9.6	0
Fukumoto	CC	Woodridge	20/04	5-6	48	52.3	10.9	0.90	12.1	0
Fukumoto	CC	Woodridge	05/05	2-3	56	51.0	10.7	0.96	11.1	0
Fukumoto	C35	Kruisrivier	19/05	2-3	48	50.0	10.3	0.90	11.4	0.1
Fukumoto	RL	ALG	11/05			45.0	9.0 B	0.76	11.8	
Fukumoto	SC	ALG	11/05			46.0	10.2 B	0.84	12.1	
Fukumoto	C35	ALG	11/05			46.0	14.6 B	0.83	17.6	
Fukumoto	KC	ALG	11/05			41.0	10.2 B	0.85	12.0	
Fukumoto	BC	ALG	11/05			47.0	12.4 B	0.82	15.1	
Fukumoto	RL	ALG	27/05			46.0	9.4 B	0.75	12.5	
Letaba Early	CC	Woodridge	20/04	6	56	51.0	9.0	0.81	11.1	0
Letaba Early	CC	Woodridge	05/05	4-5	48	50.7	9.4	0.87	10.8	0
Letaba Early	C35	Kruisrivier	19/05	5-6	48	49.6	8.9	0.97	9.4	0.1
Dream	CC	CFB	19/05	2-3	56	52.5	11.7	1.22	9.6	0
Dream	CC	CFB	23/06	1	48	53.2	12.5	1.12	11.2	0.4
Dream	CC	Woodridge	20/04	7	56	53.8	9.7	0.99	9.8	0
Dream	CC	Woodridge	05/05	5-6	56	52.5	9.0	0.94	9.6	0
Dream	C35	Kruisrivier	19/05	6	56	53.4	9.1	0.98	9.3	2.0
Tuligold	RL	ALG	11/05			46.0	8.7 B	0.81	10.7	
Tuligold	RL	ALG	27/05			47.0	8.8 B	0.85	10.4	
Lina	CC	CFB	19/05	2	48	54.0	9.3	0.84	11.1	0
Lina	C35	Kruisrivier	19/05	5	64	54.5	9.4	0.89	10.6	0.3
Lina	RL	ALG	11/05			45.0	9.1 B	0.80	11.4	
Lina	RL	ALG	27/05			47.0	7.8 B	0.81	9.6	
Newhall	CC	Woodridge	20/04	6-7	56	52.8	10.8	1.18	9.2	0
Newhall	CC	Woodridge	05/05	5	56	52.9	10.4	1.11	9.4	0
Newhall	RL	ALG	11/05			44.0	9.7 B	0.80	12.1	
Newhall	RL	ALG	27/05			42.0	9.5 B	0.76	12.6	
Washington	RL	CFB	25/07	1	56	45.7	9.5	0.61	15.6	0
Washington	RL	Ripplehill	16/06	3-4	56	48.3	10.0	0.68	14.7	0
Washington	CC	Riverside	14/06	1	56	49.4	10.9	0.85	12.8	
Cambria	CC	CFB	20/07	1	64	54.9	12.6	0.97	13.0	0.3

Cambria	TC	CFB	20/07	1	48	51.9	10.1	0.62	16.3	0
Cambria	SC	PatensieAcht	25/07	1-3	56	50.8	11.2	0.74	15.1	0
Autumn Gold	SC	CFB	20/07	1	56	56.4	12.6	1.04	12.1	0
Autumn Gold	TC	CFB	25/07	1	56	52.5	10.7	0.70	15.3	0
Autumn Gold	TC	Baddaford	14/06	4-5	64	52.2	11.9	1.07	11.1	0
Autumn Gold	RL	ALG	05/07			48.0	9.7 B	1.03	9.4	
Autumn Gold	RL	ALG	01/08			46.0	10.8 B	0.84	12.8	
Autumn Gold	RPL	Hexrivier	04/07	2-3	56	49.0	10.7	0.81	13.2	0
Powell	TC	CFB	20/07	1	56	57.8	13.1	1.26	10.4	0
Powell	TC	Baddaford	14/06	5	48	50.1	9.8	0.80	12.3	0
Powell	RL	ALG	05/07			47.0	10.0 B	0.96	10.4	
Powell	RL	ALG	01/08			49.0	10.9 B	0.81	13.5	
Powell	RPL	Hexrivier	04/07	1-2	56	48.6	10.4	0.80	13.0	0
Chislet	TC	CFB	20/07	1	56	50.9	13.1	1.08	12.1	0
Chislett	TC	Baddaford	14/06	5	48	50.4	10.8	0.84	12.9	0
Chislet	RPL	ALG	05/07			48.0	10.3 B	1.01	10.2	
Chislet	RPL	ALG	01/08			44.0	10.6 B	0.85	12.5	
Chislet	RPL	Hexrivier	04/07	1-3	48	45.0	10.5	0.81	13.0	0
Coetzee Late	RPL	Hexrivier	04/07	2-3	48	50.9	11.6	0.97	12.0	0
Renken	RPL	Hexrivier	04/07	1-2	56	48.8	11.0	0.73	15.1	0
Glenora Late	CC	CFB	20/07	1	48	50.5	10.2	0.86	11.9	0.2
Witkrans	LV/RL	Jansekraal	01/08			50.0	10.1 B	0.78	12.9	
Cal Lane Late	CC	CFB	25/07	1	56	50.7	9.8	0.70	14.0	0
Cal.Lane Late	RPL	Hexrivier	04/07	2-3	48	49.1	10.2	0.83	12.3	0
Cal.Lane Late	LV/RL	Jansekraal	01/08			50.0	9.9 B	0.83	12.0	
Royal Late	RL	CFB	25/07	1	56	51.0	9.4	0.64	14.7	0
Royal Late	CC	CFB	25/07	1	56	57.5	12.0	0.78	15.4	0
Royal Late	LV/RL	Jansekraal	01/08			53.0	11.3 B	0.78	14.6	

* TSS %. Values are recorded in % Total Soluble Solids (TSS). Figures with a "B" are % Brix values.

6.3.7 Evaluation of Midseason oranges in the Cape areas

Experiment 77 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om midseisoen seleksies wat beter in die koeler streke sal aard in terme van vruggrootte, gepigmenteerde vleis en saadloosheid, te vind. Sanguinello op Fort Beaufort het redelike goeie produksie gehad maar vruggrootte is geneig om aan die klein kant te wees. Tarocco produksie, vruggrootte en gehalte was oor die algemeen goed maar die suur kan neig om die geur te verbloem. Tarocco Gallo en 57/1E/1 het albei goeie produksie, vruggrootte en gehalte gehad, maar kan neig tot hoër suur. Gallo vrugkleur was later as Tarocco. Skilblos en vleiskleur het gewissel. Al drie Tarocco seleksies het lang dorings op jong bome en groeikragtige takke gehad maar word minder sodra die boom verouder. Daar was min verskille tussen die verskillende Maltaise seleksies. Maltaise Half II en Maltaise Line G het die kleinste, onaanvaarbare vruggrootte gehad. Nie een van die Maltaise seleksies het uitblink nie. Raratonga het aanvaarbare produksie en vruggrootte gehad en goeie gehalte maar lang dorings. Verdere evaluasies op die Tarocco seleksies, Raratonga en kommersieële Maltaise is nodig.

Introduction

The aim is to find midseason selections suitable to the colder areas with larger fruit size, pigmented flesh and seedless.

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 based on subjective tasting. Fruit quality was compared with the following standards: 48% juice; 8.5% Brix; 0.6 – 1.5% acid; 7.0:1 ratio; colour T3 of set 34; seed maximum average 6.0 seeds per fruit.

A list of selections and sites evaluated is given in Table 6.3.7.1.

Table 6.3.7.1. Midseason orange trial sites evaluated during 2005.

Selection	Area	Site/Orchard	Plant Date	Rootstock	No. of Trees
Salustiana	Fort Beaufort	Baddaford	1996	TC	10
Sanguinello	Fort Beaufort	Baddaford	1996	TC	10
Tarocco	Uitenhage	CFB Block 7	1994	TC	2
Tarocco	Fort Beaufort	Baddaford	1996	TC	10
Tarocco	Adelaide	Saxfold Park	1996	TC	commercial
Tarocco	Cookhouse	J&B Citrus	2002	CC	commercial
Tarocco	Ashton	Excelsior	2002	CC	T/w comm
Tarocco Gallo	Uitenhage	CFB Block 7	1999	CC	2
Tarocco 57/1E/1	Uitenhage	CFB Block 7	1999	CC	2
Tarocco 57/1E/1	Adelaide	Saxfold Park	1999	CC/SC	4
Maltaise 3 Blocks	Sunland	Junkyard	1998	SC	commercial
Maltaise Half	Uitenhage	CFB Block 8	1997	TC	2
Maltaise Half II	Uitenhage	CFB Block 8	1997	TC	2
Maltaise Line G	Uitenhage	CFB Block 8	1997	TC	3
Maltaise Line G	Fort Beaufort	Baddaford	1998	TC	5
Maltaise Barlerin	Uitenhage	CFB Block 8	1997	TC	2
Maltaise Bokhobza	Uitenhage	CFB Block 8	1997	TC	2
Raratonga	Uitenhage	CFB Psylla	1999	TC	2

Results and discussion

A discussion of each selection follows with yield, fruit size, colour, estimated maturity and internal quality results for the various selections presented in Tables 6.3.7.2 to 4 that need to be referred to when reading the text.

Salustiana

This selection was used as a control at Baddaford. Production was good with variable fruit size and good quality. Fruit shape is round with a reasonably smooth rind. The flesh is soft, although pale with a good texture and juicy with zero to odd seed. Maturity 3rd week of June.

Sanguinello

Fruit size is slightly smaller than Salustiana, later colour and slightly later maturing than Salustiana, towards the end of June. Fruit quality at Fort Beaufort was good, slightly tart, developing some flesh pigmentation. Fruit shape is round with a smoothish rind and occasional ribbing. There was the occasional seed (mixed block) and trees smaller than Salustiana and Tarocco.

Tarocco

Production at the various sites (except for the very young trees) was fair to excellent with good, medium large fruit size. Production of commercial plantings in the East Cape Midlands exceeded 50t/ha (800 trees/ha) after 3-4 years production, thereafter averaging around 60t/ha with a 63% export packout. Fifty three percent of the fruit fell into counts 56 – 72; 13% ≥ count 48 and 34% count 88 and smaller. At all sites rind colour was T1 by mid June except at Fort Beaufort where the fruit was T2 and pale, some deep blush developing at Ashton. Eating quality can be masked by high acid, otherwise good except at Adelaide where the sugars were lower, but nevertheless a good test. Juice percentages were at least 50% at all sites, sugars between 10.4 – 12.0% and acid all exceeding 1.00%. Maturity appears to be around early to mid June.

Fruit shape is generally round, sometimes with a neck and rind average thickness, smooth to sometimes slightly pebbly resulting in an attractive fruit. Creasing was evident at most sites. Fruit is soft and juicy on eating, mostly closed cores in June and odd seed. Flesh colour is a good orange with slight signs of pigmentation when the fruit is still hanging on the tree. There was some frost damaged fruit at Adelaide.

The trees grow vigorously with large thorns on the trunks and vigorous shoots but thorns becoming less to non existent on the smaller twigs.

Tarocco Gallo

Trees were evaluated at the CFB and a young commercial orchard in the East Cape Midlands. Commercial yields and fruit size were good, the fruit hanging in the lower portion of the tree. Colour in mid June was slightly behind Tarocco at both sites. Eating quality at the CFB was better than the Tarocco and 57/1E/1 and similar to slightly more acidic than the Tarocco in the Midlands. Fruit picked fairly easily at the CFB. The tests were good, lower sugars at the CFB compared to Tarocco and similar to Tarocco in the Midlands. Flesh colour was orange sometimes with flecking, improving after cold storage. Maturity around mid to 3rd week of June.

Fruit shape is round with some shoulders, a smooth to slightly pebbly rind. Fruit is soft and juicy, the occasional seed at Cookhouse. Trees are vigorous with similar to slightly less thorns than Tarocco.

Tarocco 57/1E/1

Trees were evaluated at the CFB and a young commercial orchard in the East Cape Midlands. Commercial yields and fruit size were good, colour T4-5 in mid June (younger trees), later colour than Tarocco and Tarocco Gallo. The CFB had good yields but smaller fruit size. After refrigeration the rind had variable blush at the CFB, virtually none at Adelaide. Flesh pigmentation at the CFB after refrigeration was Tarocco-like to paler, and only some blood flecks at Adelaide. Eating quality was better at the CFB with higher sugars than the Midlands, but acid too high, looking overmature. Maturity appears to be earlier at the CFB, early to mid June, but later than Tarocco in the Midlands, late June, although the trees are younger and on a later colouring rootstock.

Fruit shape is round with some shoulders and a smooth rind. Fruit is soft and juicy, the occasional seed at the CFB. There was creasing at the CFB and some frost damaged fruit at Adelaide.

Maltaise Line G

Production at Fort Beaufort was acceptable, fruit a little on the small size, but better than Salustiana and Sanguinello (Maltaise trees are slightly younger). Fruit colour was behind the other selections, at peak maturity in mid June, slightly ahead of the others and not as tart. Although the test was good (similar to Salustiana), the taste was fair to good and not tart. Fruit shape was slightly elongated with a shoulder and slightly pebbly rind. Internal colour was orange with no pigmentation, closed cores and tender with a thickish rind. There were odd seed.

Comparison of the Maltaise Half, Half II, Line G, Barlerin and Bokhobza at the CFB

Production was generally good, Maltaise Line G the best and Maltaise Half the poorest, but acceptable. Maltaise Half and Line G had the smallest fruit size, Barlerin only slightly better. All were colour T1 on 23 June, Line G slightly later colour in mid June. Line G and Bokhobza had some elongated fruit and the others round and all smooth rinds. Barlerin and Line G had the thinnest and thickest rinds respectively. Only Bokhobza had some blood flecks on sampling, developing some pigmentation and blush on refrigeration and Barlerin only some blood flecks and blush on refrigeration. Maltaise Line G cores opened slightly ahead of the others and were most overmature (although not by much) of all on 23 June, followed by Bokhobza. All had zero to odd seed. Barlerin flavour was slightly tart and Bokhobza had a unique flavour. Maltaise Half and Barlerin had extremely tender flesh and also the firmest to fairly firm fruit respectively. On testing sugars were similar except for Barlerin which was lower and which also has the highest (unacceptable) acid. Maltaise Half and Half II both had similar high acid, Bokhobza and Line G the lowest, all exceeding 1.0% on 22 June. Bokhobza had by far the highest ratio but also some creasing.

Comparison of Maltaise selections planted commercially at Sunland

There are three orchards, Blocks A, B and C, each of a different selection, which still has to be ascertained. Production was generally poor to fair, block C a slightly better crop although variable per tree. Fruit size varied between medium and medium large, Block C the largest and Block A the smallest fruit size, but fruit size is crop dependant – trees with a better crop bearing smaller fruit. Fruit colour in mid June was T1, yellow orange for Block A and B, Block C T1 and 3. Block C fruit had rounder fruit shape and rinds slightly pebbly, Block A the smoothest and thinnest rinds, also having some navels and fruit not soft like the others. Block A had some leaf drop and Block C odd fruit drop. Flesh colour of all was orange, only odd blood flecks seen pre- and post-refrigeration and occasional slight blush after refrigeration. Eating quality was good, Block B having lower sugars and acid, which is also borne out in the tests. Block B had somewhat higher juice levels, all three good sugars, Block A slightly lower and B slightly higher. Block B and C exceeded the permissible acid levels of 1.5%, Block A having the best ratio. All were around peak maturity on 15 June. There were occasional seed in Block A and B.

Raratonga

This selection originated as seed from New Zealand and was evaluated at the CFB. As it is in the psylla house the results could be influenced by the different growing conditions. Yield was fair with large fruit size and mature by 3rd week of June. Fruit shape is round with a coarse, thick rind, open cores and orange flesh. Eating quality was fair with a good test and seedless. The tree had large thorns.

Table 6.3.7.2. Yield, fruit size, fruit colour, estimated maturity, quality, average seed and comments of various midseason oranges evaluated during 2005.

Selection (year planted)	Yield	Fruit size	Colour transparency		Estimated maturity	Quality/ average seed/ Comment
			14-17 June	22-23 June		
CFB						
Tarocco ⁹⁴	Fair	Medium large	1	1	Mature by 23 June	Poor-good, high acid. Good test. 0.7 seed. Some pigmentation
Tarocco Gallo ⁹⁹	Fair	Medium large	2, pale	1	Mature by 23 June.	Fair-good, better than Tarocco and 57/1E/1. Good test, no seed nor pigmentation
Tarocco 57/1E/1 ⁹⁹	Good	Medium small	1	1	Overmature by 23 June.	Fair-good. Poor, fair sugars, high acid. Test acid too high. 0.2 seed. No pigmentation
Maltaise Half ⁹⁷	Fair	Medium large	1	1	Mature by 23 June	Fair-good. Test acid high. 1.1 seeds
Maltaise Half II ⁹⁷	Good	Medium small		1	Mature by 23 June	Fair to good. Test acid high. 2.4 seeds
Maltaise Line G ⁹⁷	Ex- cellent	Medium small		1	Mature by 23 June	Good. Good test. 1.6 seeds
Maltaise Barlerin ⁹⁷	Good	Medium	1-2, pale	1	Mature by 23 June	Poor-good. Slightly tart. Test acid too high. 1.8 seeds
Maltaise Bokhobza ⁹⁷	Good	Medium large	1-3	1	Mature by 23 June	Fair-good. Good test. 0.9 seed
Raratonga ⁹⁹	Fair	Large		1	Mature by 23 June	Fair. Good test, lowest acid. No seed
Sunland						
Maltaise ⁹⁸	Poor-fair	Medium-medium large	1		Mature by 14 June	Good. Average test just acceptable (some too high acid). 0.1 seed
Fort Beaufort						
Salustiana (control) ⁹⁶	Good	Medium small-medium large	1		One week short of peak maturity	Good. Good test. No seed
Sanguinello ⁹⁶	Fair-good	Medium small-medium+	1-3		1-2 weeks short of full maturity	Good, slightly tart. Good test, higher acid than Salustiana. 0.4 seed
Tarocco ⁹⁶	Fair-good	Medium-large	2, pale		One week short of peak maturity	Good, slightly tart. Good test. 0.4 seed
Maltaise Line G ⁹⁸	Fair-good	Medium-medium large	2-4		Peak maturity	Fair-good, not as tart as others. Good test. 0.3 seed
Adelaide						
Tarocco Upper	Ex- cellent	Medium large ?	1			Acceptable, lacks sugars
Tarocco Lower	Ex- cellent	Medium large, good	1			Acceptable, lacks sugars
Tarocco 57/1E/1	Good-excelle	Medium large,	4-5			Acceptable, lacks sugars

	nt	even				
Cookhouse						
Tarocco Gallo	Good-excellent	Medium-medium large	1-2		One week short of full maturity	Good, could have higher sugars, fairly acid
Ashton						
Tarocco	Zero-good	Medium large-large, even	19 May 3-5 17 June 1		Past peak by 1-2 weeks in June ?	Good, good sugars, acid dropped. Average rind thickness

The CFB and Baddaford trees are in a mixed block.

Ashton fruit size mainly between counts 48-72, peaking at 56/64, average 82.0mm.

Table 6.3.7.3. Rind blush and pigmentation of various midseason oranges evaluated before and after cold storage during 2005.

Selection	Kept at ambient		Cold stored	
	External blush	Internal pigmentation	External blush	Internal pigmentation
CFB. Picked 23 June, evaluated after four and a half weeks, kept at $\pm 10^{\circ}\text{C}$				
Tarocco			None	Pale to deep Moro like
Tarocco Gallo			None to slight	Mainly flecked to none
Tarocco 57/1E/1			None to slight to good	Tarocco like to paler but Moro like colour
Maltaise Barlerin			None to slight	None to blood flecks
Maltaise Bokhobza			Mostly slight to good	Tarocco like to paler, some with Moro colour
Sunland. Picked 15 June, evaluated after four weeks, kept at $\pm 5^{\circ}\text{C}$				
Maltaise		Occasional flecks, mainly none	Occasional very slight blush	Occasional flecks
Fort Beaufort. Picked 14 June, evaluated after four weeks, kept at $\pm 5^{\circ}\text{C}$				
Salustiana		None		None
Sanguinello	None	Variable, Tarocco like and poorer.	Slight	Pale Moro like (right hand sided photo)
Tarocco		Tarocco like and poorer	Slight	Tarocco like
Maltaise Line G	None. Coarser rind than others	Good orange flesh colour	Slight	Deep orange, no pigmentation
Adelaide. Picked 14 June, evaluated after four weeks, kept at $\pm 5^{\circ}\text{C}$				
Tarocco Upper	Pale rind at picking.	Variable, Tarocco like	None	Variable, blood flecks to Tarocco like
Tarocco Lower	None	Blood flecks to Tarocco like	None	Variable, blood flecks to Tarocco like
Tarocco 57/1E/1	None. Greenest rind at picking	Zero to odd blood specks.	Mostly none	Deep orange flesh, some blood flecks
Cookhouse. Picked 14 June, evaluated after four weeks, kept at $\pm 5^{\circ}\text{C}$				
Tarocco Gallo		Mainly blood specks	None	None to blood flecks to better
Excelsior. Picked 19 May, evaluated after two weeks, kept at $\pm 5^{\circ}\text{C}$				
			Some slight red blush	Variable from zero through to pale Moro.
Ashton. Picked 17 June, evaluated after four an a half weeks, kept at $\pm 5^{\circ}\text{C}$				
Tarocco	Some deep red blush	Zero to blood flecks to Tarocco like.	Slight to deep red blush	Slightly variable, Tarocco like

These are observations only with a small sample size. Colour reference is based on: Saunt, J.E. 1990. Citrus Varieties of the World. An illustrated Guide. Sinclair International. In the case of the different Tarocco selections at the CFB, most of the pigment is between the segment walls.

Conclusions

Sanguinello

Production was fairly good but fruit size tends to be a bit small. The test was good but fruit slightly tart, maturing slightly later than Salustiana. There was some flesh pigmentation improving with refrigeration. Commercial plantings are not recommended due to the probability of small fruit size. Evaluations to be discontinued.

Tarocco

Production was overall good with good fruit size. Quality is generally good but flavour can be masked by high acid. External blush on the fruit was not prominent and internal pigmentation slightly variable but enhanced with cold storage. Thorns can be problematic especially when the trees are young. Production of the Tarocco looks good in suitable areas where adequate internal pigmentation can be produced. Commercial orchards to be evaluated in the Eastern and Western Cape next season.

Tarocco Gallo

Commercial production and fruit size were good. Colour was slightly behind Tarocco but having better quality to more acidic. There was little flesh pigmentation. Maturity is around mid to 3rd week of June. Trees are vigorous. Further evaluations are necessary.

Tarocco 57/1E/1

Commercial production and fruit size were good, with good to acceptable quality, good tests to too high acid. There was some external blush at one site and variable flesh pigmentation between the sites. There was a difference in maturity between sites, between early to late June. Further evaluations are necessary.

Comparison of the Maltese Half, Half II, Line G, Barlerin and Bokhobza at the CFB

There were only slight differences between the selections. Maltese Half II and Line G had the smallest, unacceptable fruit size. Maltese Line G was slightly earlier maturing. Barlerin had the highest acid and Bokhobza the best flavour. Of the selections refrigerated, the intensity of blush and pigmentation varied. Based on current and past evaluations, fruit size is not large, Maltese Half the largest fruit size, Half II and Line G the smallest. None of the selections excelled in any way, including Line G evaluated at Fort Beaufort and are not recommended for planting.

Comparison of Maltese selections planted commercially at Sunland

There were only slight differences between the selections. The main difference noted was the internal quality where the one block had lower acid and a better ratio. Evaluations to continue and the actual selection per block to be ascertained.

Raratonga

Production and fruit size were acceptable with good quality, but rinds coarse and thick. The tree has large thorns. Further evaluations are necessary.

Future evaluations

Continue evaluations of all the Tarocco selections, Raratonga and commercial Maltese planting.

Table 6.3.7.4. Internal fruit quality data of the various midseason orange selections for the Eastern and Western Cape during the 2005 season.

Selection	Root stock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Salustiana	TC	Baddaford	14/06	1	72	57.5	10.5	0.93	11.3	0
Sanguinello	TC	Baddaford	14/06	2	105	50.3	10.9	1.25	8.7	0.4
Tarocco	TC	CFB	23/06	1	56	55.0	12.0	1.25	9.6	0.7
Tarocco	TC	Baddaford	14/06	2	64	55.8	11.4	1.05	10.9	0.4
Tarocco Upper	CC	Saxfold Park	14/06	1	72	57.3	10.4	1.38	7.5	0
Tarocco Lower	CC	Saxfold Park	14/06	1	72	57.8	10.3	1.38	7.5	0
Tarocco	CC	Excelsior	19/05	4	56	57.6	11.7	1.30	9.0	0.6
Tarocco	TC	Excelsior	17/06	2	56	55.5	12.4	1.10	11.3	0.3
Tarocco Gallo	CC	CFB	23/06	1	56	53.0	10.5	1.23	8.5	0

Tarocco Gallo	CC	J&B Citrus	14/06	1-2	72	58.6	10.6	1.32	8.0	0.4
Tarocco 57/1E/1	CC	CFB	23/06	1	64	58.5	11.9	1.51	7.9	0.2
Tarocco 57/1E/1	SC	Saxfold Park	14/06	4-5	64	50.9	10.1	1.21	8.3	0
Maltese Half	TC	CFB	23/06	1	64	58.1	11.8	1.41	8.4	1.1
Maltese Half II	TC	CFB	23/06	1	88	58.6	11.4	1.40	8.1	2.4
Maltese Line G	TC	CFB	23/06	1	72	55.0	11.3	1.14	9.9	1.6
Maltese Line G	TC	Baddaford	14/06	2	72	51.4	10.3	0.93	11.1	0.3
Maltese A	SC	Junkyard	15/06	1	56	51.8	11.5	1.31	8.8	0
Maltese B	SC	Junkyard	15/06	1	56	62.5	12.1	1.53	7.9	0.2
Maltese C	SC	Junkyard	15/06	1	64	54.8	11.9	1.58	7.5	0
Maltese Barlerin	TC	CFB	23/06		88	50.5	10.9	1.55	7.0	1.8
Maltaise Bokhobza	TC	CFB	23/06	1	64	58.8	11.7	1.08	10.8	0.9
Raratonga	TC	CFB	23/06	1	48	50.7	11.2	0.87	12.9	0

6.3.8 Evaluation of Valencia oranges in the Cape areas Experiment 77 by C J Alexander (Private Contractor)

Opsomming

Die doel van die Valencia proef is om vroeër, mid en laat seleksies met groot vruggrootte, saadloos en verbeterde vrugset as alternatiewe vir die huidige seleksies te soek, en die verenigbaarheid van Turkey op Rangpur lemmetjie onderstam te ondersoek. Mouton Early lyk belowend as 'n vroeër seleksie met aanvaarbare produksie, goeie vruggrootte en gehalte. Evaluasies word gestaak totdat virusvrye materiaal beskikbaar gestel word. Turkey kan as 'n vroeër seleksie geplant word, maar die interne gehalte is 'n kwessie. Die verenigbaarheid op Rangpur lemmetjie is twyfelagtig en ander onderstamme moet oorweeg word. Verskeie nuwe seleksies was by die SGB geevalueer. Rietspruit het swak produksie en gehalte gehad maar goeie vruggrootte en saadloos. Portsgate het redelike produksie en vruggrootte gehad, goeie tot aanvaarbare gehalte en af en toe 'n pit. Bend 8A2 se produksie en gehalte was swak maar aanvaarbare vruggrootte (psylla huis). G5 se gehalte en vrugkleur in die psylla huis was goed gewees. McClean Seedless het goeie produksie en matige vruggrootte gehad, matig tot goeie gehalte en saadloos. Delicia het goeie produksie en vruggrootte gehad met goeie eetgehalte maar nie die toetse gemaak nie. Kleinhans het buitensporige hoë suur gehad omdat dit langs 'n windbreek geplant is. Dit het geen of net enkele saad gehad. Evaluasies moet voortgaan.

Introduction

The aim is to evaluate early, mid and late maturing valencia selections in terms of their maturity, rootstock compatibility, colour, fruit size and seediness.

Materials and methods

The trees were either planted or topworked within commercial orchards, established on a semi commercial scale where possible or evaluated at the CFB. Field evaluations and laboratory analyses were conducted in the Eastern and 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 acceptable by the market (Midnight in brackets): 50% (50%) juice; 9.5% (10.0%) TSS; 0.8 – 1.5% (0.8– 1.5%) acid; 8.5:1 (9.0:1) ratio; colour T3 of set 34; seed maximum average 9.0 seeds per fruit (Midnight 1.0).

A list of selections and sites evaluated during 2005 is given in Table 6.3.8.1.

Table 6.3.8.1. Valencia orange trial sites evaluated during 2005.

Selection	Area	Site/Orchard	Plant Date	Root Stock	No of trees
Mouton Early Valencia	Citrusdal	Sewe Oliene	1997	RL	5 topwork
Midnight (control)	Citrusdal	Sewe Oliene	1986	RL	1
Valencia Late (control)	Citrusdal	Sewe Oliene	1986	RL	commercial
Turkey	Citrusdal	Hexrivier	1995	RPL	commercial
Rietspruit	Uitenhage	CFB Block 4	2001	2	RL
Portsgate	Uitenhage	CFB Block 7	1999	2	TC
Bend 8A2	Uitenhage	CFB Psylla house	1999	1	TC
G5	Uitenhage	CFB Psylla house	1999	3	TC
McClellan Seedless SL	Uitenhage	CFB Block 7	1999	2	CC
Delicia	Uitenhage	CFB Block 7	1994	2	TC
Klein hans	Uitenhage	CFB Block 8	1997	2	TC
Valencia Late (control)	Uitenhage	CFB Psylla house	1999	2	TC
Valencia Late (control)	Uitenhage	CFB Block 4	2001	2	RL

Results and discussion

A discussion of each selection follows with internal quality results presented in Table 6.3.8.4 that need to be referred to when reading the text.

Mouton Early. Trees were evaluated in a Valencia (and Midnight) orchard (upper). Results are presented in Table 6.3.8.2.

Table 6.3.8.2. Yield, fruit size, colour transparency, taste, test comments and estimated maturity of three valencia selections on rough lemon rootstock at Sewe Oliene, Citrusdal, evaluated on 4 July 2005.

Selection/ Age	Yield	Fruit size	Colour	Taste	Test	Estimated maturity
Mouton Early 1997	Fair-good	Medium-medium large	T1	Fair-good. Fair-good sugars, sufficient acid, tender flesh	Good test, easily meets Midnight standards. 0.7 seeds	Late June
Midnight 1986	Fair	Medium large – large	T1, pale	Fair-good quality, slightly tart, raggy	Not meeting Midnight standards due to slightly high acid. 0.8 seeds	Late July
Valencia Late 1986	Good	Medium +	T1-2	Good quality, high sugars but high acid	Not meeting valencia standards due to high acid. 5.4 seeds	End July, beginning August

Fruit shape is round, with a slightly pebbly rind, not as attractive as the other selections, coarser than Midnight and Valencia. Flesh colour in early July was orange (Midnight paler and Valencia Late deeper colour), with fine flesh, slightly open cores (others closed) and slightly thick rinds. The fruit picked fairly easily and looked slightly overmature, some calyxes removed on picking. There were odd seed (average 0.47/fruit – 64% seedless; 26% with 1 seed; 9% with 2 seeds and 1% with 3 seeds/fruit). There were some sunburnt fruit. Fruit size (average 76.3mm) was larger than Valencia Late and smaller than Midnight (refer count distribution table below).

Mouton Early Valencia: Percentage fruit per count						
Count	48	56	64	72	88	105
Percentage	1	8	33	42	10	6

Turkey. Evaluated at one site to determine its suitability to the Citrusdal area and compatibility on Rangpur lime rootstock. The crop was poor with medium large-large-extra large fruit size. The colour was T1 on 4 July and the fruit slightly soft. Eating quality was acceptable with sufficient sugars but fairly high acid, soft rag, fine flesh, pale flesh colour and closed to slightly open cores. The rinds were not as thick as in the past. The fruit was at about peak maturity, although the acid could drop further. Except for the average seed content just exceeding the maximum permitted (1.1 versus 1.0), the test met Midnight standards.

Some trees had suckers and or nodules growing on the rootstocks. There were no signs of rootstock incompatibility evident under the bark.

Various selections evaluated at the CFB (see Table 6.3.8.3).

Rietspruit. The fruit had poor quality and low juice with thick rinds. Trees are vigorous with some thorns.

Portsgate. Fruit quality dropped (TSS) between 20 July to 17 August with only a slight drop in acid, which was fairly high.

Bend 8A2. The trees are vigorous, the fruit having poor internal quality and not meeting Valencia standards. This could be due to the growing conditions in the psylla screen house.

G5. The fruit had good quality especially as it is in the psylla house, with a good test in July, acid dropped just too low in August. The rind colour was good.

McCleane Seedless. The fruit had fair quality and a good test, although acid a little high with virtually no change over four weeks. Similar acid to Portsgate.

Delicia. The yield and fruit size were good. Although the tests did not meet standards (ratio slightly too low), the eating quality was better than the other selections (the oldest trees). Most fruit were seedless.

Kleinhans. The trees are planted next to a windbreak. The evaluation is typical for trees under stress and the results should be interpreted with caution. The fruit had zero to odd seed.

Conclusions

Mouton Early. Production was acceptable with good fruit size, good quality and virtually seedless. Colour and maturity is early, around late June, about a month ahead of Midnight. The selection is early maturing and should rather be planted on a heavier soil or citrange rootstock to induce a smoother rind. The selection is protected. There has been a delay in the release of virus free material and no material should be propagated until clean material is made available. Commercial plantings are not yet recommended and evaluations to be discontinued until virus free material becomes available.

Turkey. Production was good to excellent with good fruit size. Fruit quality was poor and did not meet export standards. The selection can be planted commercially but quality aspects must be taken into account and a better quality-inducing rootstock be considered, although acid levels may then be too high. The compatibility of Turkey on Rangpur lime seems to be questionable as there is sucker and nodule growth on the rootstock. Other rootstocks should rather be considered. Evaluations to be discontinued.

Rietspruit. Production was poor with good fruit size, poor quality and low juice. Seedless. Further evaluations are necessary.

Portsgate. Production was fair with medium fruit size, good to acceptable quality, acid over 1.0%. There were zero to odd seed. Further evaluations are necessary.

Bend 8A2. Production was poor with medium large fruit size, poor quality and seedless. Further evaluations are necessary.

G5. Production was fair with medium large fruit size, good quality and seedless. Further evaluations are necessary.

McCleane Seedless. Production was good with medium fruit size, fair quality, a good test and seedless. Further evaluations are necessary.

Delicia. Production and fruit size was good with good eating quality, just not meeting standards. The characteristics are typical of the selection and virtually seedless.

Kleinhans. As the trees are planted next to a windbreak, the results are indicative of trees under stress and not too much emphasis should be laid on them. The fruit had zero to odd seed.

Future evaluations

Evaluate all selections at the CFB and continue evaluation of Mouton Early Valencia only once clean material is available and new trees established.

Table 6.3.8.3. Yield, fruit size, colour, quality, maturity, fruit characteristics and average seed of various Valencia selections evaluated at the CFB during 2005.

Selection/ Orchard/ Rootstock/ Age	Yield	Fruit size	Fruit colour			Taste	Test	Estimated maturity	Fruit characteristics	Ave. Seed
			16 June	20/25 July	17 Aug					
<u>Rietspruit</u> Block 4 RL 01	Poor	Large	5-6		1 good	Poor, low sugars, high acid	Juice and TSS too low	Mature in August	Round, coarse and thick rind	0
<u>Portsgate</u> Block 7 TC 99	Fair	Medium	4-5 yellow	1	1	Fair quality, raggy. Fair sugars and acid in July and August	Good test, in July, acceptable in August	Mature in July but acid could be lower	Round with a smooth rind, orange flesh and slightly open cores in July	Zero- some seed
<u>Bend 8A2</u> Psylla house TC 99	Poor	Medium large		1		Poor, low sugars and acid	Unacceptable. TSS and acid too low	Mature in July	Round, rough rind, orange flesh and slightly open cores	0
<u>G5</u> Psylla house TC 99	Fair	Medium large		1 good	1	Good, fair sugars and acid	Good test, meets Midnight standards in July, acid just too low in August	Mature in July	Round, orange flesh, open core, some splitting	0
<u>McClean SL</u> Block 7 CC 99	Good	Medium	4	1	1	Fair, fair sugars and acid, raggy	Meets Midnight standards	Mature in July but acid could be lower	Round, smooth rind, orange flesh, slightly open cores	0
<u>Delicia</u> Block 7 TC 94	Good	Medium large	7	2-3	1-2	Good, better than other valencias with fair sugars and acid	Tests not acceptable in July and August due to a low ratio	Mature in July but acid could be lower	Round, smooth rind, orange flesh, slightly open cores	Zero- odd
<u>Kleinhans</u> Block 8 TC 97	Poor. Next to a wind- break	Medium small	5	1	1	Poor, low sugars, high acid	Acid excessively high, probably stressed	Mature in July	Elongated, smooth rind, orange flesh, closed cores in July, slightly open in August. Splitting	0.7
<u>Val Late</u> (control) Psylla house TC 99	Good	Medium large		1		Good, fair sugars and acid	Meets Midnight standards	Mature in July	Good looking fruit. Round, smooth rind, soft, orange flesh and open cores.	0.6
<u>Val Late</u> (control) Block 4 RL 01	Fair	Medium large	6							

Table 6.3.8.4. Internal fruit quality data for Valencia orange selections for the Eastern and Western Cape during the 2005 season.

Selection	Rootstock	Site	Date harvest	Colour	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed
Mouton Early Val	RL	Sewe Oliene	04/07	1	64	51.0	12.0	1.05	11.4	0.7
Midnight	RL	Sewe Oliene	04/07	1	64	55.0	11.2	1.51	7.4	0.8
Valencia Late	RL	Sewe Oliene	04/07	1-2	72	50.8	11.1	1.67	6.6	5.4
Turkey	RPL	Hexrivier	04/07	1	48	52.9	10.9	1.21	9.0	1.1
Rietspruit	RL	CFB	17/08	1	56	43.8	9.1	1.14	8.0	0
Portsgate	TC	CFB	20/07	1	64	53.2	10.3	1.25	8.2	1.0
Portsgate	TC	CFB	17/08	1	64	54.6	9.7	1.10	8.8	0
Bend 8A2	TC	CFB	20/07	1	48	52.7	8.2	0.73	11.2	0
G5	TC	CFB	25/07	1	56	58.5	11.6	1.01	11.5	0
G5	TC	CFB	17/08	1	56	52.9	11.4	0.78	14.6	0
McClellan SL	CC	CFB	20/07	1	72	54.0	11.6	1.29	9.0	0
McClellan SL	CC	CFB	17/08	1	72	58.3	11.7	1.23	9.5	0
Delicia	TC	CFB	20/07	2-3	56	59.4	9.8	1.21	8.1	0
Delicia	TC	CFB	17/08	1	56	59.3	10.1	1.20	8.4	0.1
Kleinhans	TC	CFB	20/07	1	105	50.7	12.1	2.02	6.0	0.6
Kleinhans	TC	CFB	17/08	1	88	50.8	12.2	1.85	6.5	0.8
Valencia Late	TC	CFB	25/07	1	56	56.2	12.5	0.93	13.4	0.6

6.3.9 **Evaluation of existing cultivars at Lancewood, Knysna area**
Experiment CJA-1 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om geskikte, hoër gehalte, veral laat mandaryn kultivars te vind vir die Knysna area. Ueno Satsuma het taamlikke groot vrugte gedra wat uitvoer standaarde behaal het, al was die suurvlaakte relatief hoog. Hulle word later as Miho Wase ryp. Mor, Or, Nadorcott en Nectar was moontlik onder stres met kleinvruggrootte en hoër suur. Bay Gold lyk nie belowend nie weens hoër suur al is die vrugte oorryp. Aoshima Satsuma het sy eerste vrugte gedra met swak gehalte. Behalwe vir die bleek skilkleur het Sweet Spring goed gepreesteer met beter geur as voorheen. Nouvelle het min gedra en mag vatbaar vir *Alternariabruinvlek* wees. Thoro Temple het geen uitstaande eienskappe vertoon en word nie aanbeveel nie. Die laat CELL, Clementarde en Clemlate Clementine seleksies is eenders en het sekere produksie nadele, insluitend lae suurvlaakte gehad. Evaluasies moet vir nog minstens een jaar voortgaan.

Introduction

The Knysna area produces mandarins for export, but due to climatic constraints the cultivar range is limited. The aim is to find suitable, high quality, especially late maturing soft citrus cultivars for the area.

Materials and methods

The trial trees are topworked or planted in the Karatara, Rheenendal and Ruigtevlei areas. Field evaluations were conducted on the trees and fruit maturity based on subjective tasting. Fruit quality was compared with the following standards: Satsumas, Clementines and mandarins (differences between cultivars in brackets) – 48% juice (mandarins 50%); 8.5% Brix (Clementines 9.5%); 0.7-1.5% acid; 7.5:1 ratio (Clementines 8.0:1); colour set 3 of plate 36; seed maximum average 3.0 seeds per fruit (Satsumas 0). The selections and sites evaluated are presented in Table 6.3.9.1 and internal quality tests in Table 6.3.9.2.

Results and discussion

Except for Ueno Satsuma, the trees were evaluated on 12/13 July. The trees at Lancewood are in a mixed block and planted next to a windbreak, which may impose stress on the trees. The area had less rain this past season compared to high rainfall in late summer last year.

Table 6.3.9.1. Cultivar trial sites evaluated during 2005.

Selection	Area	Site	Plant Date	Rootstock	No of trees
Ueno	Karatara	Lancewood	1998	TC	2 topwork
Miho Wase (control)	Karatara	Lancewood	1996	TC	commercial
Mor 22	Karatara	Lancewood	1998	TC	4 topwork
Or	Karatara	Lancewood	1998	TC	4 topwork
Nadorcott B64	Karatara	Lancewood	1998	TC	2 topwork
Bay Gold	Karatara	Lancewood	1998	TC	4 topwork
Nectar	Karatara	Lancewood	1998	TC	5 topwork
Aoshima	Rheenendal	Candlewood	2003		5
Bay Gold	Rheenendal	Candlewood	2003	TC	5
Sweet Spring	Rheenendal	Candlewood	1995	TC	9
Nouvelle	Rheenendal	Candlewood	2001	SC	7 topwork
Thoro Temple	Rheenendal	Candlewood		SC	2
CELL	Ruigtevlei	Rushmere	1996	X639	5
Clementarde	Ruigtevlei	Rushmere	1996	X639	4
Clemlate	Ruigtevlei	Rushmere	1988	X639	4
Thoro Temple	Ruigtevlei	Rushmere	1996	RL	15

Ueno

The trees had good, large fruit size, colour T5 on 17 May, meeting export standards, but acid still relatively high (1.24%). Adjacent Miho Wase had an excellent crop, main picking 11-15 April.

Mor 22

The trees are erect and tall with a variable, zero to fair crop. Fruit size was around medium small and fruit pale, T1 and T1-2 in July. The fruit had very high sugars and high acid with a good flavour except for the

high acid. The test had exceptionally high sugars and excessively high acid. Peelability was difficult and oily with an extremely thin rind, breaking up on peeling. The internal colour was a deep orange, sometimes with a green halo. The fruit was firm with closed cores and zero to odd seed, varying between 1- 6 seeds/fruit (mixed block). The fruit had a fairly flat shape and very smooth rind with little ribbing. The fruit was 2-4 weeks short of full maturity.

Or

The trees are large, erect and pale with a zero to poor crop. Fruit size was very small and fruit T1-2, yellow orange on 12 July. The acid was extremely high. No tests were done. Peelability was difficult and very oily with an extremely thin rind, breaking up on peeling. The rinds were very smooth. The internal colour was orange with a green halo and odd seed. The fruit was about 4 weeks short of full maturity although difficult to determine accurately.

Nadorcott

There was a fair crop of small to very small fruit size. Rind colour was deep orange/red, T1 on 12 July. The fruit had high sugars and acid, with an excellent test although the acid levels were high, but acceptable. The fruit was difficult to peel and oily with a very thin skin. Cores were closed and flesh a deep orange colour and juicy. There were odd seed, varying from zero to 7 seeds/fruit. Fruit shape was flat with very smooth rinds and fruit slightly soft. The fruit was about 4 weeks short of full maturity although difficult to determine accurately.

Bay Gold

The trees at Lancewood had a poor to fair crop of small to medium to medium large fruit size, T1 on 12 July, developing a deep orange colour. The fruit had high sugars but very high acid, also evident in the test. The fruit was fairly easily peeled, not oily but some albedo retained on the segments. The internal colour was good, cores open and some fruit slightly dry with signs of the peel parting from the segments. There were some seed, varying between 0 and 10 seeds/fruit. Fruit shape is flattish to obovoid, with a very smooth rind and odd closed, protruding navel end. The fruit picked easily and looked overmature. There was some creasing and no "water spots" as observed before. The fruit was probably overmature by a week or so but acid still too high.

The young trees at Candlewood had a poor to fair crop of variable, small to large fruit size. Rind colour on 12 July was good, T1, becoming deep orange red. Fruit shape was flatter and smoother than other areas. There was some sunburn and no "water spots". Sugars were good but acid still high, although lower than other areas (young trees) with a good test. Cores were open with good flesh colour to ricey, some starting to dry out. Rinds were very thin and fruit soft, looking overmature. The fruit was past peak maturity by 1-2 weeks and picked very easily. There were 0-2 seeds/fruit.

Nectar

The trees had a zero to excellent crop of small to very small fruit size, colour T1 on 12 July. There were quite a lot of out of season fruit. Eating quality was good to excellent with high sugars and fairly high acid – still slightly tart. Peelability was difficult with very oily, thin rinds. The cores were closed and no seed. Fruit shape was flat and rinds smooth. No test was done. It is difficult to estimate maturity as the trees were probably suffering from stress.

Aoshima

The trees had their first small crop of medium to extra large fruit size with a flat shape. The fruit had been picked ten days earlier and this fruit had poor eating quality, lacking sugars.

Sweet Spring

The trees had a good crop, with medium large to large fruit size. The fruit colour on 12 July was T1-3 and pale. The eating quality was good with good sugars and sufficient acid, a good test and about at peak maturity. The fruit shape is fairly flat with a smooth to slightly pebbly rind and clean. Peelability was fair and difficult to start with orange flesh and closed cores. The rind was reasonably thin. The fruit was seedy (8.4 seeds/fruit).

Nouvelle

The young topwork trees had odd fruit of medium to medium large fruit size, colour T4 on 12 July. Eating quality was good, juicy and tart, although the acid test was too low. Fruit shape is round, a thick rind and variable rind texture. Flesh colour was deep, closed to slightly open core and fairly coarse flesh. There was zero to odd seed. The rind had brown, possibly *Alternaria* spots.

Thoro Temple

The trees at Candlewood had a good to excellent crop of good, medium and larger fruit size. The colour on 12 July was T4-5 on the northern side of the tree and a redder T4 on the other side. The quality was fair, fairly acid and lacking flavour, although the test was good. The fruit picked easily and was at or just past peak maturity. The fruit shape was fairly flat to round with a smoothish to pebbly rind and occasionally a navel end. Peelability was difficult and oily. The flesh colour was good with cores closed to slightly open and a slightly coarse flesh with undeveloped seeds. Rinds were fairly thin.

Trees are on rough lemon and Swingle rootstocks at Rushmere. Rough lemon had a fair to good crop with medium to medium large fruit size. Swingle trees had a poor crop and were not sampled. Rough lemon fruit colour on 13 July was T3, paler on the northern side and T1 and deep orange on the southern side. Eating quality was poor, lacking flavour and sugars with acceptable to high acid and slightly raggy. The fruit just met the standards (borderline sugars). Swingle had slightly better flavour and higher acid. The fruit picked fairly easily and looked slightly overmature and past peak maturity by 1-2 weeks. Peelability was fair to difficult with the rind (fairly thin) breaking up and extremely oily. Flesh colour was pale orange, slightly coarse with closed to slightly open cores and odd seed. Fruit shape is fairly flat and rind varying from smooth to pebbly and an occasional navel. There were odd out of season fruit.

CELL, Clementarde and Clemlate

The trees are on a sandy well drained slope and bore a better crop than last season, which could not be evaluated due to a lack of fruit and out of season fruit. All three selections had poor crops with medium to medium small fruit size and some out of season fruit. Overall there was not much difference between the three selections, Clementarde with the most differences. Fruit colour on 13 July was T3 for Clementarde and Clemlate, T3-4 for CELL. Clementarde had a redder rind colour followed by Clemlate, all with green styler ends. Clementarde had more dark green styler ends and CELL the least. Eating quality was similarly good but not outstanding, Clemlate slightly lower acid, Clementarde juicier and slightly more insipid. The tests differed only slightly, Clemlate the highest sugars (good) and CELL the lowest acid. The acid in all cases was too low. All were at peak maturity to just past. Clementarde had rounder fruit with a slight nipple, CELL slightly more pebbled fruit. All were fairly easily peeled and rind breaking up. Clementarde had an "older", fractionally thicker rind and juicier. All had good flesh colour with open to slightly open cores. CELL and Clemlate had sooty mould and Clementarde the densest trees. Clementarde had tiny, aborted seeds.

Conclusions

Ueno

The Ueno is much later maturing than adjacent Miho Wases, probably maturing late May, early June. Further evaluations are necessary.

Mor 22

The small fruit size, high sugars and acid may be the result of stress. Therefore the results must be read with caution. Further evaluations are necessary.

Or 2

It appeared as though the trees were under severe stress, resulting in small, extremely high acid fruit. Further evaluations are necessary.

Nadorcott

There was a fair crop of small fruit size with high sugars and acid. This could be stress related. Further evaluations are necessary.

Bay Gold

The crops were poor to acceptable with variable fruit size. The fruit were overmature while the acid levels were still too high resulting in tart fruit, which is consistent with other evaluations in other areas. The selection does not look promising and evaluations to continue for one more year.

Nectar

It appeared as though the trees were under severe stress, resulting in small, high sugar and fairly acid fruit. Further evaluations are necessary.

Aoshima

The trees bore their first few fruit, which were of poor quality. Further evaluations are necessary.

Sweet Spring

The trees carried a good crop of good fruit size but pale rind colour. Eating quality was good, whereas there was previously a lack of flavour. Although the fruit has some good characteristics the previous lack of flavour and poor colour are probably not widely acceptable to the overseas market.

Nouvelle

The young trees had a few fruit with possibly *Alternaria* spots. There were too few fruit to draw any conclusions. Further evaluations are necessary.

Thoro Temple

The crops were acceptable to good with acceptable fruit size. There was a marked difference in fruit colour between the northern (paler) and southern sides of the tree. Eating quality was not so good, sugars borderline on rough lemon. The rinds varied in terms of smoothness. The cultivar does not exhibit any outstanding attributes and not recommended for planting.

CELL, Clementarde and Clemlate

None of the selections had outstanding performance in any way and were similar in most respects, i.e. production, fruit size, colour, quality and maturity. Clemlate had the greenest styler ends and Clementarde a deeper colour fruit and denser trees. Acid levels of all three were too low. Should commercial planting be considered, low acidity and a dense growth habit with low production and small fruit size can be limiting factors.

Future evaluations

Evaluate cultivars for one more season and reconsider thereafter.

Table 6.3.9.2. Internal fruit quality data for various Satsuma, Clementine and mandarin selections for the Knysna area during 2005.

Selection	Root Stock	Site	Date harvest	Colour	Count	Juice %	TSS % *	Acid %	Ratio	Ave. Seed
Ueno	TC	Lancewood	9/05	5	1-4	49.0	9.8 B	1.24	7.9	
Mor	TC	Lancewood	12/07	1	3	57.8	16.0	2.18	7.3	2.5
Nadorcott	TC	Lancewood	12/07	1-2	4	64.3	14.0	1.45	9.7	2.3
Bay Gold	TC	Lancewood	12/07	1	1x	54.0	13.5	1.55	8.7	2.4
Bay Gold	TC	Candlewood	12/07	1	1xx	54.4	11.2	1.07	10.5	1.0
Sweet Spring	TC	Candlewood	12/07	1-2	1xx	54.1	12.0	0.94	12.8	8.4
Nouvelle	SC	Candlewood	12/07	3-4-5	1xx	55.6	10.2	0.68	15.0	0.8
Thoro Temple	SC	Candlewood	12/07		1	61.5	11.4	1.23	9.3	2.3
CELL	X639	Rushmere	13/07	3-4	2	56.9	11.7	0.59	19.8	2.3
Clementarde	X639	Rushmere	13/07	3	2	56.7	12.0	0.68	17.6	0
Clemlate	X639	Rushmere	13/07	3	3	56.4	12.4	0.67	18.5	5.8
Thoro Temple	RL	Rushmere	13/07	1	1xx	57.5	9.6	1.08	8.9	2.8

* B=Brix

6.3.10 Evaluation of Clementine mandarins in the cool inland areas

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 met betrekking tot markbehoefte, geproduseer kan word. Daar word gesoek na uitstaande seleksies met uitstekende interne vruggehalte, eksterne vrugkleur en vruggrootte verspreiding. Ain Toujdate was die vroegste (middel tot einde April) gereed vir oes, maar het nie aan die uitvoer standarde voldoen nie. Sidi Aissa was tweede gereed vir oes einde April, maar kon ook nie gepak word vir uitvoer nie. Nour het aan alle uitvoer standarde voldoen en was middel Mei gereed vir oes. Clementarde het 'n uitstekende interne kwaliteit geproduseer en was einde Mei gereed vir uitvoer. Die rooidopluis probleem was onder beheer gewees en 90% van die vrugte het

visueel skoon voorgekom. Die hoeveelheid sade per vrug het ook effens verminder, a.g.v. laer kruisbestuiwing.

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, as well as finding 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 was compared during the 2005 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 6.3.10.1. List of Clementine trial sites evaluated at Zalo Sitrus, Burgersfort area, during the 2005 season.

Selection	Rootstock	Tree Age	No. of trees
Ain Toujdate	CC, SC	1999	5 each
Clementarde	CC, SC	1999	5 each
LL	CC, SC	1999	5 each
Nour	SC	1999	5 each
Sidi Aissa	CC, SC	1999	5 each

Results and discussion

Ain Toujdate

Juice content was below 48% (46.8% to 47.9%) on both Carrizo citrange and Swingle citrumelo by the time of harvest. Tree size on Carrizo citrange seems larger in comparison to Swingle citrumelo. Yield varied from average to good, with medium to large fruit size on the trees (larger fruit on Swingle rootstock). TSS% of the fruit on both rootstocks complied with the export standards between 11.20% and 12.69%. The fruit peeled fairly easy and the internal colour remained deep orange. Maturity of the fruit was middle to end of April.

Clementarde

The fruit was ready to harvest approximately 2 weeks later in comparison to Ain Toujdate. The tree and fruit size on both Carrizo citrange and Swingle citrumelo were on the smaller side (count 1-5). Clementarde produced the highest juice content of all the selections evaluated in this trial (54.2% - 58.1%). TSS% varied from 10.8% to 14.0%, the third highest % in this evaluation. There was a lower yield on Carrizo if compared with Swingle. Clementarde produced slightly high Acid% in the beginning of the season, but by harvest time it complied with the export standards. Fruit were ready to harvest internally by the end of May, with a delay in external colour (degreening possibility).

L.L.

Yield produced on Carrizo and Swingle varied from average to good. The internal quality of the fruit evaluated complied with the export standards. LL peels easy and contains high oil quantities in the rind. The fruit picks fairly easy from the tree and the external texture was slightly coarse. The number of seeds per fruit varied from 3.1 to 15.8. Maturity was estimated between the third and last week of May.

Nour

Nour produced the highest TSS% of all the selections evaluated in this trial (14.66% - 15.67%). Fruit size was small and might be a problem (count 2- 5). This reason for the small fruit size might be the high yield. Maturity was estimated to be the middle to end of May.

Sidi Aissa

When the fruit were ready to harvest, the juice content on Carrizo and Swingle was still below the 48% export requirements (44.6% and 47.1%). The Acid% tested low (between 0.78% and 0.87%), but still above the 0.8% minimum standard for exports. This selection was also ready to harvest early in the season at the end of April with good external colour (T1-2). Fruit size was large (between 1XX – 1XXX) and the internal colour of the fruit deep orange.

Conclusions and recommendations

The red scale problem was treated with Confidor the 2005 season to prevent additional fruit waste on the trees. Ain Toujdate and Sidi Aissa were the first two cultivars ready for harvesting, followed by Nour, Clementarde and LL.

Table 6.3.10.2. Internal fruit quality data for Clementine mandarin selections at Zalo Citrus (cool inland area) during the 2005 season.

Selection	Root-stock	Date Harvested	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Ain Toujdate	CC	12/04	1XX-2	52.9	11.20	0.80	14.00	2.9	T4-6
Ain Toujdate	CC	05/05	1XX-2	46.8	12.69	0.89	14.26	3.7	T2-3
Ain Toujdate	SC	12/04	1XX-2	52.1	12.10	0.91	13.30	4.7	T5
Ain Toujdate	SC	05/05	1XXX-1	47.9	12.49	0.96	13.01	1.3	T2-3
Clementarde	CC	12/04	2-4	55.7	10.80	1.04	10.38	1.3	T8
Clementarde	CC	05/05	1-3	55.9	11.79	0.86	13.71	0.4	T6-7
Clementarde	CC	18/05	1-3	54.2	12.43	0.80	15.54	1.9	T4-5
Clementarde	SC	12/04	2-5	58.1	11.90	1.11	10.72	0.4	T7-8
Clementarde	SC	05/05	2-4	57.0	12.91	1.05	12.30	0.0	T6-7
Clementarde	SC	18/05	1-3	57.7	14.00	0.95	14.74	0.0	T5-6
LL	CC	12/04	1XX-2	50.9	11.10	0.80	13.88	11.0	T7
LL	CC	05/05	1XX-2	53.2	11.99	0.78	15.37	8.0	T5
LL	CC	18/05	1XX-2	53.4	13.05	0.82	15.91	3.2	T3-4
LL	SC	12/04	1XX-1	48.1	11.20	0.75	14.93	15.8	T6-7
LL	SC	05/05	1XX-2	47.2	11.89	0.76	15.64	8.4	T5
LL	SC	18/05	1X-2	50.7	12.43	0.72	17.26	3.1	T2-4
Nour	SC	12/04	2-5	53.9	13.72	1.11	12.36	3.2	T7-8
Nour	SC	05/05	4-5	51.1	14.66	1.15	12.75	3.1	T4-5
Nour	SC	18/05	2-4	52.1	15.67	1.13	13.87	2.4	T2-5
Sidi Aissa	CC	12/04	1XX-2	53.9	10.90	0.80	13.63	7.2	T4-6
Sidi Aissa	CC	05/05	1XX-2	47.1	11.79	0.78	15.12	1.7	T1-2
Sidi Aissa	SC	12/04	1XXX-2	50.3	11.40	0.87	13.10	7.9	T4-6
Sidi Aissa	SC	05/05	1XXX-2	44.6	12.29	0.87	14.13	6.4	T1-2

6.3.11 Evaluation of Mandarin hybrids in the cool inland areas Experiment 73 by J.Joubert (CRI)

Opsomming

Gesikite Mandaryn seleksies vir die warm, intermediêre en koel binnelandse sitrusproduserende streke moet gevind word om die vroeë-, middel- en laatseisoen leemtes te vul. Primasole was die vroegste gereed vir oes, maar die seleksie ondervind heelwat probleme in hierdie area. Granulasie, groot vruggrootte, baie growwe vrugte en lae sap volume was van die ergste probleme wat hier opgeduik het. M26 (ARC) en Roma lyk die belowendste van die seleksies met goeie interne kwaliteit, maar klein tot medium vruggrootte. Hierdie seisoen was die watervoorraad voldoende en die besproeiings skedulering in plek.

Introduction

To find suitable Mandarin selections for the hot, intermediate and cool inland citrus production areas to fill the early, mid and late season gap.

Materials and methods

Field evaluations were conducted. Internal fruit analysis was conducted for Burgersfort and Marble Hall area during the 2005 season.

Table 6.3.11.1. List of mandarin selections evaluated during the 2005 season.

Selection	Site	Rootstock	Tree age	No. of trees
A25	Moosriver Estate	CC	2001	10
Bay Gold	Moosriver Estate	CC	2001	9
Cami	Moosriver Estate	CC	2001	5
C27	Moosriver Estate	CC	2001	2
Hadas	Moosriver Estate	CC	2001	9
M26	Moosriver Estate	CC	2001	10
Primasole	Moosriver Estate	CC	2001	9
Roma	Moosriver Estate	CC	2001	10
A25	Zalo Citrus	CC	2001	5
Bay Gold	Zalo Citrus	CC	2001	5
Cami	Zalo Citrus	CC	2001	3
C27	Zalo Citrus	CC	2001	4
Hadas	Zalo Citrus	CC	2001	5
M26	Zalo Citrus	CC	2001	5
Primasole	Zalo Citrus	CC	2001	5

Results and discussion

A25

At Zalo Citrus (Burgersfort) the production was too low for evaluation purposes.

Bay Gold

Trees were evaluated at Moosriver Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2005 season. Bay Gold fruit shape is very similar to that of Minneola. The Acid% tested high throughout the season, but decreased slightly by harvest time (1.47% -> 1.37%). Fruit production on the trees varied from good to excellent with medium to large fruit size (count 1X – 1XXX). The fruit had a thin rind and picking them from the tree was fairly easy without damage. Internal fruit quality complied with the export standards. Maturity will be middle to end of May.

C27

At Zalo Citrus (Burgersfort) yield was poor, but there was enough fruit on the trees to evaluate them for the first time. Internal fruit quality seems to be good with high juice content and large fruit size. Keep in mind that the large fruit size might be the result of lighter fruit set on the trees. Picking the fruit was fairly difficult; you have to use picking scissors. The juice had a watery taste and the rind of the fruit was on the thin side. Maturity seems to be end of April.

Cami

Trees were evaluated at Moosriver Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2005 season. There was an alternative bearing pattern between the trees and the yield varied from poor to average. The trees bear most of the fruit on the end of the branches, causing the branches to hang close to the ground with some of the fruit touching the ground. When cutting the fruit for evaluation purposes, there was a sort of lime smell present. Internal quality complied with the export standards, with the TSS% at Moosriver Estate being nearly 2% higher than at Zalo Citrus. Maturity seems to be at the end of May.

Hadas

Trees were evaluated at Moosriver Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2005 season. Hadas seems to require warmer temperatures to produce better internal quality with a lower Acid%, for example in the Swaziland area. Juice content and TSS% met the export criteria, but the very high Acid% (1.97% - 2.79%) causes problems. Fruit size varied from medium to large (count 2 – 1XXX).

M26

Trees were evaluated at Moosriver Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2005 season. Internal fruit quality met the export requirements with high juice content (between 56.0% and 60.1%). The deep internal orange colour of the fruit might be one of the very positive assets of this selection. Fruit size varies from small to large and a thin peel. Maturity middle May.

Primasole

Trees were evaluated at Moosriver Estate (Marble Hall) and Zalo Citrus (Burgersfort) in Mpumalanga during the 2005 season. Primasole apparently requires a cooler area, e.g. Orighstad. The fruit had a very coarse

rind, with 80% granulation and fruit size varied from large to very large. External colour of individual fruit was uneven (green and orange spots). Maturity end of March to the beginning of April (early).

Roma

At Moosrivier Estate (Marble Hall) tree shape compares in some ways to that of grapefruit with lower branches hanging on the ground. Roma produced an average yield and the fruit peels slightly difficult due to the rather thin peel. Fruit size varied from small to medium. The number of seeds per fruit was the second highest (13.4-20.5 seeds/fruit) counted in this trial, with Bay Gold being the highest (4.5-21.4 seeds/fruit). Maturity middle to the end of May.

Conclusions

Hadas needs a warmer production area to produce fruit with better internal quality and lower Acid%. Primosole might do better in a cooler production area with less granulation and coarse rind problems. More evaluations must be conducted before final recommendations can be made.

Table 6.3.11.2. Internal fruit quality data for Mandarin selections for the cool inland areas during the 2005 season.

Selection	Root-stock	Date harvested	Site	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Bay Gold	CC	14/04	Moosriver	1X-1XXX	65.2	11.13	1.45	7.7	21.4	T1-7
Bay Gold	CC	04/05	Moosriver	1XX-1XXX	46.7	11.83	1.37	8.6	8.7	T3-4
Bay Gold	CC	12/04	Zalo Citrus	1XX-1XXX	48.7	10.53	1.41	7.5	10.4	T6-7
Bay Gold	CC	05/05	Zalo Citrus	1X-1XXX	49.4	11.29	1.47	7.7	4.5	T3
Bay Gold	CC	18/05	Zalo Citrus	1X-1XXX	48.9	11.83	1.41	8.4	6.2	T1-2
C27	CC	12/04	Zalo Citrus	1X-1XXX	56.6	9.34	0.78	12.0	10.4	T7-8
C27	CC	05/05	Zalo Citrus	1X-1XXX	55.3	10.79	0.75	14.4	15.2	T1-4
Cami	CC	14/04	Moosriver	2-1XX	51.1	11.43	1.33	8.6	15.0	T7-8
Cami	CC	04/05	Moosriver	1-1XXX	47.9	12.53	1.28	9.8	12.8	T5-6
Cami	CC	17/05	Moosriver	1X-1XXX	56.0	12.33	1.21	10.2	14.8	T4-5
Cami	CC	12/04	Zalo Citrus	2-1XX	79.2	10.80	1.09	9.9	8.8	T6-7
Cami	CC	05/05	Zalo Citrus	1-1XXX	55.5	10.89	1.02	10.7	11.1	T5
Hadas	CC	14/04	Moosriver	2-1XX	51.2	9.50	2.79	3.4	12.9	T7-8
Hadas	CC	04/05	Moosriver	2-1XXX	52.9	11.03	2.51	4.4	11.3	T6-7
Hadas	CC	17/05	Moosriver	2-1XXX	54.8	10.72	2.15	5.0	11.8	T2-6
Hadas	CC	12/04	Zalo Citrus	2-1XX	54.8	9.44	2.26	4.2	12.8	T7
Hadas	CC	05/05	Zalo Citrus	1X-1XX	54.1	9.86	1.97	5.0	13.1	T5-6
Hadas	CC	18/05	Zalo Citrus	1-1XXX	55.4	10.30	2.00	5.2	14.3	T3-4
M26	CC	14/04	Moosriver	1-1XXX	58.1	11.53	1.23	9.4	7.7	T7-8
M26	CC	04/05	Moosriver	1X-1XXX	56.0	12.43	1.24	10.0	10.6	T4-5
M26	CC	17/05	Moosriver	2-1XXX	60.1	12.93	1.20	10.8	4.3	T2-6
M26	CC	12/04	Zalo Citrus	1-1XXX	58.7	10.80	1.19	9.1	10.6	T7
M26	CC	05/05	Zalo Citrus	1X-1XXX	59.1	11.09	1.14	9.7	9.7	T4-6
Primosole	CC	29/03	Zalo Citrus	1XX-1XXX	46.4	9.83	0.46	21.37	0.0	T4-5
Primosole	CC	12/04	Zalo Citrus	1XX-1XXX	39.9	8.05	0.36	22.36	0.0	T4-6
Roma	CC	14/04	Moosriver	3-1XX	46.4	11.53	1.12	10.3	20.5	T7-8
Roma	CC	04/05	Moosriver	1-1XX	43.6	12.53	0.97	12.9	14.7	T4-5
Roma	CC	17/05	Moosriver	1-1XX	49.7	12.83	0.93	13.8	13.4	T2-5

6.3.12 Evaluation of Navels in the cool inland areas

Experiment 74 A by J. Joubert (CRI)

Opsomming

Winsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleo weerstand, kleiner nawelente om wiluis 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 (vroeë-, middel- an laatrypwordende seleksies) te verleng. Atwood lyk baie belowend en voldoen aan die uitvoer standaarde. Cara Cara bly steeds een van die seleksies met baie potensiaal wanneer dit korrek bemark gaan word. Die vrugte se interne kleur speel 'n masiewe rol en moet reg benader word. In hierdie areas is die interne kleur universeel versprei deur die vrugte. Fukumoto toets nog steeds negatief vir onverenigbaarheid by die entlas, maar die knopperige groen punte op die onderstam het vergroot. Die bome dra meer vrugte, maar die interne kwaliteit voldoen nie aan die uitvoer standaarde nie.

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, Bahianinha, Cara Cara, Dream, Fukumoto, Powel Summer and Tule Gold selections at Zalo Citrus (Burgersfort), a site in the cool inland production area.

Internal quality data were compared with the minimum average export requirements for navels: 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

Table 6.3.12.1. List of Navel selections evaluated at Zalo Citrus (Burgersfort) during the 2005 season.

Selection	Rootstock	Tree age	No. of trees
Atwood		2001	5
Autumn Gold	CC, SC	1999	10
Bahianinha	SC	2001	4
Cara Cara		2001	8
Dream		2001	9
Fukumoto		2001	3
Powel Summer	CC, SC	1999	10
Tule Gold		2001	9

Results and discussion

Atwood

At harvest time the internal quality complied with the export standards. Fruit size varied from large to very large fruit (count 40 – 72) with average yield on the trees. Fruit were seedless. Atwood looks very promising in comparison to the other selections evaluated in this trial. Maturity middle of May.

Autumn Gold (Late maturing Navel)

The TSS% of the fruit on Carrizo citrange was lower in comparison to Swingle citrumelo, but the opposite was true with the Acid% of the fruit been lower on Carrizo compared to Swingle. The fruit produced on Carrizo (T3-4) had a colour advantage to the fruit on Swingle (T3-5). Fruit were seedless. Maturity seems to be in June.

Bahianinha

Bahianinha produced medium to large fruit with fairly soft rag and high juice content. Fruit size varied from count 40 to 88, and internal quality complied with the export standards. Maturity might be middle to end of May.

Cara Cara

Cara Cara produced an average yield on the trees. The TSS% (8.37% - 9.5%) did not meet the export standards and the Acid% was on the low side (0.72%). This might be related to the drought of the previous season. The internal colour was uniformly red and remains one of the best qualities of this selection. The internal colour pigmentation was well distributed with an even deep red colour. Cara Cara needs heat units to improve the uniform red colour pigmentation inside the fruit. Maturity middle May.

Dream

Dream produced medium to large fruit on the trees (count 40-88). Juice content varied between 51.1% and 54.5%. TSS% was on the low side (9.8%), but still complied with the minimum export standards. Fruit were seedless and there was a slight delay in the external colour. Maturity end of May.

Fukumoto

Yield was poor, but there was enough fruit for two evaluations. Production of large fruit seems to be the norm on most of the trees evaluated. At the time of harvest, TSS% (9.17%) and Acid% (0.66%) were below the specifications for export. Maturity end of April.

Powel Summer (Late maturing Navel)

Internal fruit quality looks promising and complies with all the export specifications. The TSS% and Acid% on Carrizo citrange was higher in comparison to Swingle citrumello. There was an external colour advantage on Carrizo of approximately two weeks earlier compared to Swingle. The fibre content in the fruit was high and maturity seems to be in the beginning of June.

Tule Gold

Tule Gold produced fruit with a high juice content (50.7% -53.5%) and TSS% (10.2%). This was one of the highest values in this trial. The rind on the fruit was thick with large fruit size on most of the fruit. Maturity middle of May.

Conclusions

Cara Cara seems to colour internally less uniformly (red pigment) compared with the warmer inland areas. Fukumoto is still an experimental selection and bear in mind the incompatibility scenario before considering this cultivar for new plantings. Atwood looks very promising, but the large navel on the fruit might cause some problems. Evaluations will continue.

Table 6.3.12.2. Internal fruit quality data for Navel selections at Zalo Citrus (Burgersfort), a cool inland production area, during 2005.

Selection	Root-stock	Date harvested	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Atwood	CC	12/04	40-72	49.7	9.17	0.92	9.97	0.0	T6
Atwood	CC	05/05	40-64	48.0	10.73	0.85	12.62	0.0	T3-4
Atwood	CC	18/05	40-64	53.8	10.83	0.73	14.84	0.0	T1-3
Autum Gold	CC	05/05	48-72	46.3	9.70	0.98	9.90	0.0	T6
Autum Gold	CC	18/05	40-72	52.1	10.30	0.92	11.20	0.0	T4-6
Autum Gold	CC	06/06	64-72	51.0	10.75	0.84	12.80	0.0	T3-4
Autum Gold	SC	05/05	40-72	46.0	10.73	1.17	9.17	0.9	T6-7
Autum Gold	SC	18/05	40-72	51.3	11.43	1.16	9.85	0.0	T4-5
Autum Gold	SC	06/06	48-72	51.3	11.74	1.08	10.87	0.0	T3-5
Bahianinha	SC	12/04	48-88	52.7	10.10	0.94	10.74	0.2	T6-7
Bahianinha	SC	05/05	40-64	49.1	10.40	0.84	12.38	0.0	T4-6
CaraCara	CC	12/04	40-72	49.7	8.37	0.82	10.21	0.0	T2-3
CaraCara	CC	05/05	40-72	52.2	8.97	0.73	12.29	0.0	T1-2
CaraCara	CC	18/05	40-56	53.8	9.50	0.72	13.19	0.0	T1-2
Dream	CC	12/04	40-72	51.1	8.67	1.01	8.58	0.0	T7-8
Dream	CC	05/05	48-88	51.8	9.27	0.89	10.42	0.0	T5-6
Dream	CC	18/05	48-72	54.5	9.80	0.76	12.89	0.0	T4-5
Fukumoto	CC	12/04	36-88	48.9	8.87	0.75	11.83	0.0	T5-6
Fukumoto	CC	05/05	36-64	48.8	9.17	0.66	13.89	0.0	T4-5
Powel Summer	CC	05/05	64-88	46.6	12.53	1.30	9.64	0.4	T6
Powel Summer	CC	18/05	56-88	47.1	12.75	1.31	9.73	0.0	T4-5
Powel Summer	CC	06/06	48-72	49.3	13.60	1.15	11.83	0.0	T4-5
Powel Summer	SC	05/05	48-72	46.5	10.63	1.00	10.63	0.0	T6
Powel Summer	SC	18/05	40-64	49.3	11.23	0.90	12.48	0.0	T4-5
Powel Summer	SC	06/06	56-72	49.7	11.44	0.87	13.15	0.0	T4-6

Tule Gold	CC	12/04	40-64	51.1	8.37	0.85	9.85	0.0	T6-7
Tule Gold	CC	05/05	48-64	50.7	8.97	0.76	11.80	0.0	T4-5
Tule Gold	CC	18/05	40-72	53.5	10.20	0.77	13.25	0.0	T2-3

6.3.13 Evaluation of Navels in the intermediate inland area

Experiment 74 B by J. Joubert (CRI)

Opsomming

Wingsgewendheid moet verhoog word deur boomproduksie (oes- en vruggrootte), pakpersentasie (kraakskil en oleo weerstand, kleiner nawelente om wiluis 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 presteer vir hierdie area baie goed. Die interne kleur is baie univorm versprei en die interne kwaliteit lyk belowend. Die smaak en kleur is beter as in die Burgersfort area. Atwood presteer hier nie so goed soos in die Burgersfort area nie en die sap% voldoen beswaarlik aan die minimum vereistes. Chislett presteer werklik swak en gaan uit die proef verwyder word. Die bome set nie vrugte nie en voldoen ook nie aan die uitvoer standaarde nie. Dream het medium to groot vrugte geproduseer. Fukumoto het hierdie seisoen 'n goeie oes geset, maar die interne kwaliteit het nie aan die vereistes vir uitvoere voldoen nie.

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 and Tule Gold selections at Moosrivier Estate (Marble Hall), an intermediate production area.

Internal quality data were compared with the minimum average export requirements for navels: 48% Juice; 9.7% TSS; 1.5% (Max) - 0.68% (Min) % Acid; 8:1 Ratio; Colour set 34 no.T3 shipped at 11°C.

Table 6.3.13.1. List of Navel selections evaluated at Moosrivier Estate (Marbel Hall) during 2005.

Selection	Rootstock	Tree age	No. of trees
Atwood	CC	2001	5
Autumn Gold	CC	1997	6
Cara Cara	CC	1997	Semi-Com.
Chislett	CC	1997	6
Dream	CC	2001	9
Fukumoto	CC	2001	3
Tule Gold	CC	2001	9

Results and discussion

Atwood

Juice content was low and barely met the export standards (48%). Most of the fruit evaluated had seeds (0.3-0.8 seeds/fruit) with a high fibre content and thick rind. The fruit peels fairly easy and contains high oil quantities. Maturity estimated by the end of May.

Autumn Gold (Late maturing Navel)

Yield varied from poor to average, with medium to large fruit (count 40-64). Fibre content was high and internal colour was yellow. All the requirements for export standards were met with high juice content and TSS%. Maturity end of May.

Cara Cara

A semi-commercial orchard was evaluated at Moosriver Estates (Marble Hall) in Mpumalanga during the 2005 season. The fruit had good internal quality with the Acid% on the low side and with the final evaluation below the export standard. This might be the best tasting navel of all the selections evaluated in the trials. The internal colour pigmentation was well distributed with an even deep red colour. Cara Cara needs heat units to improve the uniform red colour pigmentation inside the fruit and the warmer inland area seems ideal. The fruit started colouring early in the season and matured by middle May.

Chislett

Chislett produced a very poor yield for the 2005 season. This selection did not meet the export standards for minimum juice content, but complied with the standards for TSS% and Acid%. Granulation seems to be a major problem and evaluations will not continue on this selection.

Dream

Fruit were seedless and the internal quality looks promising. Fruit size varied from medium to large (count 40-64) with a good yield on the trees. There were some fruit with minor sunburn damage, but not too serious. The internal colour was dark yellow and some fruit had a fairly large navel. Maturity middle of May.

Fukumoto

This season there was a considerable increase in yield on the trees. Medium to large fruit (count 40-64) was produced, but unfortunately the internal analysis did not comply with the export standards. This selection was ready to harvest by the middle of April and is indeed early in the season. In California incompatibility on citrange rootstock was detected and at Zalo Citrus similar symptoms occur. Trees might have been under stress conditions causing the incompatibility symptoms.

Tule Gold

All the internal quality values comply with the export standards. Fruit size varied from medium to large (count 48-64) with large open navel ends. By the time we harvest the fruit, the internal colour developed from yellow to light orange. Maturity middle May.

Conclusions

Moosriver Estate received ample rains during the season and normal irrigation practices were possible. The production and internal quality improved in comparison with the 2004 season, but some selections did not comply with the export specifications. Fukumoto is still an experimental selection and bear in mind the incompatibility scenario before considering this cultivar for new plantings.

Evaluations will continue.

Table 6.3.13.2. Internal fruit quality data for Navel selections in the intermediate inland area (Moosriver Estate, Marble Hall) during the 2005 season.

Selection	Root-stock	Date harvested	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Atwood	CC	04/04	40-56	45.7	10.20	0.75	13.60	0.0	T6
Atwood	CC	14/04	40-64	44.6	9.70	0.75	12.93	0.8	T6-7
Atwood	CC	17/05	36-56	48.1	10.68	0.74	14.43	0.3	T5-7
Autum Gold	CC	04/05	40-56	50.0	10.83	0.79	13.71	0.3	T4-5
Autum Gold	CC	17/05	36-64	53.7	11.28	0.77	14.65	0.0	T4-6
Bay Gold	CC	17/05	56-88	51.1	12.38	1.26	9.83	15.8	T3-5
CaraCara	CC	14/04	40-64	30.9	9.50	0.63	15.08	0.0	T1
CaraCara	CC	04/05	36-56	49.2	10.20	0.68	15.00	0.0	T1
CaraCara	CC	17/05	40-56	51.4	10.88	0.57	19.09	0.0	T1-2
Chislett	CC	04/05	40-72	45.9	10.20	0.67	15.22	0.0	T4-5
Chislett	CC	17/05	36-64	47.8	10.35	0.64	16.17	0.0	T5-6
Dream	CC	14/04	40-64	44.2	9.80	0.78	12.56	0.0	T6-7
Dream	CC	04/05	40-64	48.2	10.30	0.83	12.41	0.0	T5-6
Dream	CC	17/05	40-56	48.9	10.58	0.79	13.39	0.0	T5-7
Fukumoto	CC	14/04	40-64	44.5	9.70	0.61	15.90	0.0	T5-6
Fukumoto	CC	04/05	40-64	47.1	10.63	0.67	15.87	0.3	T3-4

Tule Gold	CC	14/04	48-72	49.8	9.80	0.76	12.89	0.0	T5-6
Tule Gold	CC	04/05	40-72	48.7	10.40	0.72	14.44	0.0	T4-5
Tule Gold	CC	17/05	48-64	50.0	10.68	0.73	14.63	0.0	T2-3

6.3.14 Evaluation of Valencia selections in the inland areas (Onderberg)

Experiment 75 A by J. Joubert (CRI)

Opsomming

Wingsgewendheid moet verhoog word deur interne gehalte (saadloosheid, veselsterkte, sappehalte) te verbeter; beter boomproduksie en 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). Glen Ora Late lyk belowend met 'n goeie produksie, goeie interne kwaliteit met groot vruggrootte. McClean SL het ook 'n goeie oes geproduseer met 'n lae veselinhoud in die vrugte. Die interne kwaliteit voldoen aan die uitvoer standaarde en minimale sade was in elke vrug teenwoordig. Ruby Valencia (bestraald) met sy univorme rooi interne kleur en saadlose vrugte hou goeie potensiaal in. Die watervoorraad in die Malelane area was voldoende en die bome is optimaal besproei.

Introduction

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), Delicia, EEL-T, Glen Ora Late, McClean SL, Midnight, Portsgate, Ruby Valencia and Turkey (control) at Esselen Nursery, Malelane.

Table 6.3.14.1. Internal fruit quality data was compared with the minimum export requirements for Valencia types.

Variety	% Juice	% TSS	Min % Acid	Max % Acids	Ratio	Colour
Valencia	48	9.75	0.68	1.6%	8.5:1	Colour plate 3 of set no. 34
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 6.3.14.2. List of Valencia selections evaluated at Esselen Nursery (Malelane) during 2005.

Selection	Rootstock	Tree Age	No. of trees
Alpha (Rietspruit)	CC	1996	1
Delicia (Delport)	CC	1996/2003(Top)	1
EEL-T	Troyer	1998	1
Glen Ora	CC	2000(Top)	3
McClean SL	TB		1
Midnight	C35	1998/2003(Top)	1
Ruby Val	CC		1
Turkey	C35	1998	1

Results and discussion

Alpha

Internal quality was good with high juice content (60%) and TSS% (12.23%). Fruit size was excellent (between count 56 and 88). Yield was good and external colour at harvest time T1-2. Maturity middle of June.

Delicia

Delicia produced a poor to average yield with fairly large fruit size (count 36-72). Internal quality complied with the export standards with exceptional high juice content (62.2%). There was a slight delay in rind colour relative to internal quality. Fruit had a high fibre content and maturity seems to be end of June.

EEL-T

The number of seeds per fruit varied from 6.5 to 7.3 and is relatively high. Internal quality was acceptable with fairly high TSS% (11.57% - 11.83%). EEL-T produced an excellent yield with medium to large fruit size (count 64-88), promising size when considering the number of fruit. Matures middle of June.

Glen Ora Late

Fibre content was low and juice content was high (59.9%-60.5%). Yield was good and fruit size varied from medium to large (count 56-88). Rind texture was slightly coarse, and TSS% was relatively high (12.43%). Maturity end of June.

Mc Clean SL

Yield was good with small, medium and large fruit size (count 64-105). Internal quality complied with the export standards and fibre content was low. In some of the fruit there was a minimal number of seeds counted (0.3-1.0). Maturity end of June.

Midnight

In comparison to the previous season, internal quality (including TSS%) complied with the export standards. Fruit size was fairly large (count 40-72) and yield was good. Maturity end of June.

Ruby

Ruby Valencia trees evaluated are the original trees planted on rough lemon before it was irradiated. In future the irradiated trees will be evaluated (no. of seeds counted).

Yield was good with small to medium fruit size (count 64-125). TSS% complied with the export standards in comparison with the previous season where the values were too low. Internally the fruit produced a uniform red colour and excellent taste with high juice content. Maturity middle of June.

Turkey

There were no incompatibility signs visible at the bud union between Turkey and Carrizo citrange. Fruit had low rag content with high juice content. Maturity end of June.

Conclusions

Turkey did not show any budunion incompatibility symptoms on Carrizo citrange. Fruit quality was good and rag content was low. Midnight had a high yield and acceptable internal quality. Ruby Valencia had good fruit size and was seedless.

Table 6.3.14.3. Internal fruit quality data for Valencia orange selections at Esselen Nursery (Malelane) during the 2005 season.

Selection	Root-stock	Date harvested	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Alpha	CC	02/06	56-72	59.0	11.57	1.21	9.6	0.8	T2-4
Alpha	CC	24/06	56-88	60.0	12.23	1.07	11.4	0.5	T1-2
Delicia	CC	02/06	48-72	61.3	10.48	1.06	9.9	1.4	T6-7
Delicia	CC	24/06	36-72	62.2	10.83	0.91	11.9	1.3	T5-6
EEL-T	Troyer	02/06	64-72	57.0	11.57	1.23	9.4	7.3	T4-6
EEL-T	Troyer	24/06	64-88	58.0	11.83	1.18	10.0	6.5	T3-4
Glenora Late	CC	02/06	56-72	60.5	11.38	1.48	7.7	1.5	T5-6

Glenora Late	CC	24/06	56-88	59.9	12.43	1.31	9.5	1.8	T4-5
McCleane SL	TB	02/06	64-88	56.3	11.57	0.98	11.8	0.3	T5-6
McCleane SL	TB	24/06	64-105	57.8	12.13	0.91	13.3	1.0	T2-3
Midnight	C35	02/06	48-72	59.0	10.58	1.00	10.6	0.4	T3-5
Midnight	C35	24/06	40-56	58.9	10.53	0.96	11.0	0.2	T1-2
Portsgate	CC	02/06	56-88	59.0	10.88	0.97	11.2	0.1	T4-5
Portsgate	CC	24/06	64-105	59.0	11.23	0.87	12.9	0.2	T1-2
Ruby	RL	02/06	64-88	57.0	12.17	1.10	11.1	1.4	T1-2
Ruby	RL	24/06	64-125	59.2	12.64	1.09	11.6	0.4	T2
Turkey	CC	02/06	56-72	56.9	12.69	1.06	12.0	8.3	T1-3
Turkey	CC	24/06	56-88	57.2	14.09	0.95	14.8	8.2	T1-2

6.3.15 Evaluation of Lemon selections in the inland areas

Experiment 79 by J. Joubert (CRI)

Opsomming

Kouegeharde, doring- en saadlose suurlemoenseleksies (met aanvaarbare vruggrootte), wat met 'n wye reeks onderstamme verenigbaar is, moet ontwikkel word. Die oesseisoen moet verleng word om aaneenlopende produksie van Maart tot September te verseker, aangesien dit vroeë en laatrypwordende seleksies insluit. Probleme wat met lang blomtyd ge-assosieer kan word, moet verminder word. Goeie vrugkwaliteit (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 voldoen. Hierdie seisoen het Villafranca minder saad per vrug geproduseer as Verna. Al die ander seleksies produseer steeds heelwat sade per vrug. Die drag op al die seleksies was baie belowend gewees. Eureka saadloos (LNR) bly steeds die beste saadlose suurlemoen seleksies tans beskikbaar.

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).

Materials and methods

Field evaluations were conducted on Eureka SL (ARC) as control, Eureka SL (Israel), Fino 49 & 95, Genoa, Limoneira 8A, Lisbon (control), Verna and Villafranca SL on various rootstocks.

Table 6.3.15.1. List of lemon cultivars evaluated at Tekwane (Karino area) during the 2005 season.

Selection	Rootstock	Tree Age	No. of trees
Eureka SL (ARC) *	RL	2000	1
Eureka SL (Israel)	RL	1998	4
Fino 49	RL	1998	3
Fino 95	RL	1998	4
Genoa	RL	1998	4
Limoneira 8A	RL	1998	2
Lisbon	RL,SO	1998	2,2
Verna	RL	1998	4
Villafranca	RL	1998	2

* Esselen Nursery, Malelane

Results and discussion

Eureka SL (ARC)

Good production of seedless fruit at Esselen Nursery.

Tekwane Estates

Villafranca fruit had 2.2 seeds per fruit, Verna had 2.3 seeds per fruit. Juice content of Villafranca was the highest (42.3%), followed by Fino 49 & 95, Genoa, and Lisbon all with 36%. All the other selections had high numbers of seed per fruit.

Conclusions

Villafranca and Verna had relatively low seed content compared with the other lemon cultivars.

Table 6.3.15.2. Internal fruit quality data for Lemons from Tekwane Estate (Karino) on 30 March 2005.

Selection	Root-stock	Juice %	Average Seed
Eureka SL (Israel)	RL	32.9	11.8
Fino 49	RL	36.3	14.9
Fino 95	RL	36.4	16.5
Genoa	RL	34.2	17.3
Lisbon	RL	33.9	17.7
Lisbon	SO	36.4	14.0
Limoneira	RL	32.3	17.8
Verna	RL	33.5	2.3
Villafranca	RL	42.3	2.2

6.4 PROJECT: ROOTSTOCK EVALUATION

6.4.1 Project summary

Commercial rootstock choice is relatively limited, and the best available rootstock option is seldom ideal in addressing all the site limitations and production and marketing requirements. The development of a new rootstock is inherently a long and involved process, and it is unlikely that any new rootstock will have all the desirable attributes.

One of the prime objectives of rootstock evaluation is to find reliable size-controlling rootstocks coupled with attributes such as good yield of marketable fruit size and internal fruit quality, pest and disease tolerance or resistance, and adaptability to a wide range of scion cultivars and soil types.

The rootstock research efforts of the 1980s and 1990s led to considerable changes in rootstock use from almost exclusively being rough lemon to Carrizo and Troyer citranges and Swingle citrumelo rootstocks. Yet, there still remains an acute need to seek out, evaluate and commercialise new generation rootstocks.

Projekopsomming

Die oogmerk van die onderstamprojek is om 'n bron van sitrusonderstamme te evalueer i.t.v. die produksie van hoë kwaliteit vrugte, asook siektebestand- en verenigbaarheid met verskillende grond- en klimaatstoestande regoor Suidelike Afrika. Oesopbrengs per hektaar moet geoptimaliseer word deur van bostam/onderstamverenigbaarheid, tuinboukundige prestasie te verbeter en beter interne kwaliteit, vruggrootte 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.

Rootstock evaluation guidelines

Overall evaluation objectives: Rootstock selection and evaluation serves two principle purposes, namely,

1. to minimise the effects of site limitations (soil type, irrigation water quality, disease presence), and
2. to enhance yield, fruit size and fruit quality.

Rootstock choice should aim to capitalise on any favourable soil or environmental factor and/or offset the effects of any limiting factors.

However, not all rootstocks can or will be evaluated under all possible conditions, and a degree of risk in rootstock choice will probably always exist. Therefore, the role of rootstock evaluation is to screen new rootstocks to identify their strengths and weaknesses, and thereby minimise the potential commercial risks involved in their commercial use.

Once initial screening has been conducted, citrus producers will be encouraged to further evaluate the performance of the most promising rootstocks on a semi-commercial basis.

Guiding principles for rootstock evaluations

1. Rootstock evaluations will be conducted in the major citrus-producing regions for each cultivar group using a mainline commercial cultivar (since rootstock evaluation is largely not dependent on climate). Information generated from such evaluations will, by necessity, be extrapolated to other regions or sites and to other similar cultivars.
2. Screening-type trials will be conducted with the emphasis on potential performance in terms of scion compatibility, canopy development, productivity (annual and cumulative yield potential, production efficiency), product quality (fruit size distribution, rind colour and texture, internal fruit quality), general susceptibility to rind disorders, pest and disease susceptibility, and general soil suitability.
3. A single, mainline scion cultivar on numerous rootstocks will be used, and factorial type experiment designs will be avoided. The commercial or standard scion-rootstock combination will serve as the control for comparison purposes.
4. Suitable nursery and grower cooperators are essential.
5. Use budded trees of the same (or similar) age.
6. Replicate 6 to 10 times.
7. Randomisation is not required in a screening type trial.
8. Site selection: uniform soil type, not near a windbreak, but preferably within a commercial orchard.
9. Conduct soil and site analysis, including chemical, physical and disease status of soil.
10. When the trial is discontinued, then a motivation is needed for further evaluation, e.g. to evaluate a few of the most promising rootstocks every two years, and orchard maps must be retained for subsequent evaluation and long-term performance and evaluation of disease tolerance.

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	C32 citrange (trifoliolate orange x Ruby sweet orange)
11. C35	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	F80/3 citrumelo
16. F80/9	F80/8 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	Natsudaikai
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	Rubidoux 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	Volckameriana
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	McCarthy grapefruit x <i>P. trifoliata</i>
54.	79 AC 6/2	Cleo x Swingle citrumelo

6.4.2 Summary: Cape and Inland areas

Turkey Valencia in Citrusdal: The trees were burnt during a veld fire and as a result the trial could not be evaluated. Trees will be assessed in 2006 to decide whether there is merit in continuing the evaluations.

Genoa lemon in Citrusdal: The objective of the trial is to evaluate the performance of the Genoa lemon in the Citrusdal areas well as the performance of Genoa on different rootstocks. This was the fourth year of production, with a great increase in yield over last season. Japanese sitroen, rough lemon, Swingle and commercial Genoa had the best yields. All the others had good yields except for Carrizo, which was poor. Unfortunately the trees are variable in size and only the best trees were used for evaluation purposes. Volckameriana, M x T and Carrizo had the largest tree size, Benton, Swingle and Tri x Sweet the smallest. Rough lemon and Volckameriana had the largest fruit size, Benton, Tri x Sweet, Lisbon and Carrizo falling in the most desired fruit size range. All rootstocks except Swingle (lowest, but acceptable) easily met the minimum juice export standards. Swingle, M x T and rough lemon had slightly earlier fruit colour, Rangpur

and Benton the poorest. M x T has had slightly earlier colour over the past three seasons. There were some small differences in high shoulders, the vigorous rootstocks the least, Rangpur, Benton, Tri x Sweet, Tri X and Japanese sitroen with some non exportable fruit. There was little oleocellosis, Japanese sitroen with some non exportable fruit. Benton had the highest incidence of wind scars, rough lemon, Tri x Sweet and commercial Genoa with a few non exportable fruit. Rough lemon, Volckameriana and commercial Genoa had a few coarse fruit. Rangpur and Japanese sitroen had nodules and sucker growth on the rootstock and Volckameriana some nodules. Evaluations to continue.

Delta Valencia in Marble Hall: 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. This season there was a sufficient water supply for scheduled irrigation cycles. The opposite scenario was true for the 2005 production results (compared with 2004) with most of the combinations producing a good crop. The table containing the summarised data for the 2003, 2004 and 2005 seasons emphasises the recovery time needed for normal production after a drought period. Only two rootstocks had lower production in the 2005 season (RT and SFS).

Midnight en Delta Valencias in Letaba: To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Midnight Valencia and Delta Valencia. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system. The Delta Valencia combinations produced a better yield in comparison to the 2004 season. Internal fruit quality improved, except for SC, MxT and RL-C which did not comply with the export standards. The largest fruit size was produced on CC and C35. BC produced the highest crop of 83.7 kg per tree. The Midnight Valencia combinations performed very well and the average fruit size peaked between count 72 and 88. The highest crop was produced on SC with 83.8 kg per tree. There remained some water shortage throughout the season. The problem was not that critical in comparison to the 2004 season, however production was influenced negatively.

Star Ruby grapefruit at Letaba: To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Star Ruby grapefruit. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system. The production on the Star Ruby trees decreased in comparison to the 2004 season. The trees used their reserves during the drought period in 2004 and this might explain the drop in yield. Next season should be very promising. The trees were pruned (windows) and treated with Confidor for the Waxy scale problems. The best production was on SC, but fruit did not meet the internal quality requirements for export. The fruit size decreased and the TSS% increased drastically for 2005. Therefore, more of the rootstocks produced fruit suitable for export during 2005.

Navel and Valencias in Vaalharts: The performance of Navel and Valencia selections on different rootstocks is being investigated to determine the best rootstock for the Vaalharts area. The trial site in Vaalharts experienced severe cold damage late in August 2003 with temperatures as low as - 7 °C. The Navelina trees were severely damaged and a number of trees died. The remaining trees did not produce any crop. The Bahianinha and Royal Late navel trees were less damaged by the cold and bore fruit of small size. Delta Valencia was more tolerant to the cold damage and produced a low crop. Midnight Valencia was damaged by the cold and produced small fruit. Evaluations will continue for 2006.

Valencias in Malelane: The performance of Midnight and Delta Valencias on new, imported rootstocks on replant soils was investigated. The production this season increased by more than 50% and all the combinations complied with the export standards. There was sufficient water for scheduled irrigation throughout the 2005 season.

Grapefruit in Swaziland: The performance of grapefruit cultivars on new, imported rootstocks on heavy, replant soils is being investigated. Star Ruby and Marsh produced a good crop after being transplanted from Tabankulu Estates. The average fruit size peaked at count 48 for Star Ruby and count 40 for Marsh. The internal quality improved from the previous season.

Valencias in Komaitpoort: Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks and determine superior rootstock combinations for these new selections. During 2005 the trial was harvested for the first time and the trees are still young. There was an interesting variation in yield between the different selections. Certain selections set more fruit on the young trees in comparison to other selections. This precocity aspect of the different selections should bring in an early return on investment.

Opsomming: Kaap- en Binnelandse streke

Turkey Valencia te Citrusdal: Die bome was deur 'n veldbrand erg beskadig en kon nie geëvalueer word nie. Gedurende 2006 sal weer na die bome gekyk word om te sien of dit die moeite werd is om voort te gaan met die proef of nie.

Genoa suurlemoen te Citrusdal: Die doel van die proef is om die prestasie van Genoa suurlemoen in die Citrusdal area te beproef, asook die prestasie op tien verskillende onderstamme. Dit was die vierde jaar van produksie met 'n groot verhoging in drag oor verlede seisoen. Japanse sitroen, growweskiisuurlemoen, Swingle en kommersieële Genoa het die beste drag gehad. Die ander, behalwe Carrizo wat swak was, was goed. Boomgrootte was ongelukkig tydens uitplant en net die beste bome is vir evaluasie doeleindes gebruik. Volckameriana, M x T en Carrizo het die grootste boomgrootte, Benton, Swingle en Tri x Sweet die kleinste. Growweskiisuurlemoen en Volckameriana het die grootste vruggrootte gehad, Benton, Tri x Sweet, Lisbon en Carrizo met die mees gewenste vruggrootte. Al die onderstamme (behalwe Swingle – aanvaarbaar) het maklik die sap uitvoer standaard behaal. Swingle, M x T en growweskiisuurlemoen het effens vroeë vrugkleur gehad, Rangpur en Benton die swakste. M x T het effens vroeër skilkleur oor die laaste drie seisoene gehad. Daar was klein verskille in hoër skouers, minder met die groeikragtigste onderstamme. Rangpur, Benton, Tri x Sweet, Tri X en Japanse sitroen het enkele vrugte wat nie uitvoer standaard behaal het nie. Daar was min oleo, Japanse sitroen met 'n paar nie uitvoerbare vrugte. Benton het die meeste windletsels gehad, growweskiisuurlemoen, Tri x Sweet en kommersieële Genoa het 'n paar nie uitvoerbare vrugte. Growweskiisuurlemoen en kommersieële Genoa het enkele skurwe vrugte. Rangpur en Japanse Sitroen het knoppies en suiers op die onderstamme gehad en knoppies op Volckameriana. Evaluasies moet voortgaan.

Delta Valencia te Marble Hall: Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde, moet oor 'n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word. Hierdie seisoen was daar voldoende water vir die effektiewe besproeiing van die bome beskikbaar gewees. Die 2005 seisoen se produksie is presies die omgekeerde scenario. Meeste van die kombinasies het 'n goeie oes geproduseer. Die tabel wat die opsomming verduidelik en vergelyk tussen 2003, 2004 en 2005 toon die herstel tempo tussen die verskillende onderstamme duidelik aan. Vir die 2005 seisoen is daar slegs twee onderstamme wat minder geproduseer het as in 2004 (RT en SFS).

Midnight en Delta Valencias te Letaba: Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediaire sitrusproduksie gebied geëvalueer. Die Delta Valencia kombinasies het 'n beter oes geproduseer as die 2004 seisoen. Oor die algemeen het die interne kwaliteit ook verbeter met slegs SC, MxT en RL-C wat nie uitvoer standaard behaal het nie. Die grootste vrugte is op CC en C35 geproduseer. BC het die hoogste oes geproduseer van 83.7 kg/boom. Die Midnight Valencia kombinasies het baie goed gepresteer en die gemiddelde vruggrootte het op telling 72/88 gepiek. Die hoogste produksie was op SC met 83.8 kg/boom gewees. In hierdie area was daar nog steeds met tye water tekorte gewees. Dit was nie so krities soos die 2004 seisoen gewees nie, maar het nog steeds 'n invloed op die produksie gehad.

Star Ruby pomelo te Letaba: Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediaire sitrusproduksie gebied geëvalueer. Star Ruby bome se oes het effens gedaal in vergelyking met die 2004 seisoen. 'n Moontlike rede hiervoor mag wees dat die bome met die droogte in 2004 baie reserwes gebruik het vir produksie. Die volgende seisoen behoort baie belowend te wees. Die bome is vensters in gesnoei en Confidor toegedien vir Was-doplious probleme. Die beste produksie was op SC gewees, maar die interne kwaliteit het nie aan al die uitvoer vereistes voldoen nie. Die vruggrootte oor die algemeen was aan die kleiner kant. Die TOV% het toegeneem vir 2005, en maak baie meer van die kombinasies geskik vir uitvoer.

Navel en Valencias te Vaalharts: Die prestasie van Navel en Valencia variëteite op verskillende onderstamme ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Die perseël in Vaalharts waar die proef aangeplant is het ernstige koue laat in Augustus maand ervaar met temperature so laag as -7 °C. Die Navelina bome is die meeste deur die koue beskadig en enkele bome is selfs dood. Meeste van die oorblywende bome het feitlik geen oes geproduseer nie. Die Bahianinha en Royal Late navel het minder skade opgedoen, maar die bome het 'n swak oes geproduseer met klein vruggrootte. Delta Valencia het die minste koue skade opgedoen en van die seleksies het selfs 'n ligte oes geproduseer. Midnight Valencia het in 'n mindere mate skade opgedoen, maar klein vrugte geproduseer. Evaluasies sal weer vir die 2006 seisoen gedoen word.

Valencias te Malelane: Die prestasie van Midnight en Delta Valencias op nuwe, ingevoerde onderstamme op herplant grondtipes moet ondersoek word. Die produksie het hierdie seisoen met meer as 50%

toegeneem en al die betrokke seleksies het voldoen aan die uitvoer standaard. Vir die 2005 produksie jaar was daar voldoende water vir besproeiing beskikbaar gewees, wat 'n groot bydrae gelewer het.

Pomelos te Swaziland: Die prestasie van pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplant grondtipes moet ondersoek word. Star Ruby en Marsh het 'n beter produksie gelewer (vestig goed) nadat die bome herplant is vanaf Tambankulu Estate. Die gemiddelde vruggrootte het op telling 48 vir Star Ruby, en telling 40 vir Marsh gestabiliseer, en die interne kwaliteit het verbeter. Evaluasies word verder gedoen.

Valencias te Komaitpoort: Evalueer en bepaal die tuinboukundige potensiaal en vermoë van verskillende Valencia variëteite op verskillende onderstamme en bepaal die beste onderstam kombinasie vir hierdie nuwe variëteite. Hierdie onderstam proef is vir die eerste keer ge-oes en die bome is nog jonk. Daar is interessante verskille in drag geproduseer tussen die verskillende kombinasie. Van die onderskeie bo-stam seleksies kom verseker vroeër in drag op 'n jonger ouderdom. Dit kan ook beteken dat die bome gouer in vol produksie kan kom en begin inkomste genereer vir die produsent. Hierdie tipe eienskappe kan 'n groot rol speel met die keuse van watter bo-onderstam kombinasie aangeplant moet word.

6.4.3 Evaluation of Turkey Valencia on different rootstocks Experiment CJA - 4 by C J Alexander (Private Contractor)

Opsomming

Die bome was deur 'n veldbrand erg beskagig en kon nie gevalueer word nie. Gedurende 2006 sal weer na die bome gekyk word om te sien of dit die mooiste werd is om voort te gaan met die proef of nie.

Introduction

The aim is to evaluate the early maturing Turkey Valencia on different rootstocks to establish which is the most suitable rootstock for Turkey in the Citrusdal area as incompatibility problems have been encountered with rough lemon.

Materials and methods

The trees were planted in a block on seven 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 6.4.3.1 below.

Table 6.4.3.1. Rootstocks (selections) and number of trees per selection of Turkey Valencia on seven different rootstocks planted at Sewe Oliene, Citrusdal in September, 1999.

Rootstock	Selection	No of trees
Cairn rough lemon	163	25
Carrizo citrange	608	26
Citrango C32		10
Citrumelo F 80.0		6
Rangpur lime	184	26
Swingle citrumelo	715	13
1209		22

Results and discussion

Unfortunately the trees were severely burnt during a veld fire and it is doubtful whether they will adequately recover. It is recommended that the trial be terminated but to review the situation in a year's time to see whether enough trees survived and if worthwhile to again continue with the trial.

Conclusions

The trees were burnt so the trial could not be evaluated.

Future evaluations

Review the situation of the trees during 2006 and decide on future use of the trial.

6.4.4 **Evaluation of Genoa Lemon on various rootstocks in Citrusdal**
Experiment 588 by C J Alexander (Private Contractor)

Opsomming

Die doel van die proef is om die prestasie van Genoa suurlemoen in die Citrusdal area te beproef, asook die prestasie op tien verskillende onderstamme. Dit was die vierde jaar van produksie met 'n groot verhoging in drag oor verlede seisoen. Japanse sitroen, growweskiisuurlemoen, Swingle en kommersieële Genoa het die beste drag gehad. Die ander, behalwe Carrizo wat swak was, was goed. Boomgrootte was ongelyk tydens uitplant en net die beste bome is vir evaluasie doeleindes gebruik. Volckameriana, M x T en Carrizo het die grootste boomgrootte, Benton, Swingle en Tri x Sweet die kleinste. Growweskiisuurlemoen en Volckameriana het die grootste vruggrootte gehad, Benton, Tri x Sweet, Lisbon en Carrizo met die mees gewenste vruggrootte. Al die onderstamme (behalwe Swingle – aanvaarbaar) het maklik die sap uitvoer standaard behaal. Swingle, M x T en growweskiisuurlemoen het effens vroeë vrugkleur gehad, Rangpur en Benton die swakste. M x T het effense vroeër skilkleur oor die laaste drie seisoene gehad. Daar was klein verskille in hoër skouers, minder met die groeikragtigste onderstamme. Rangpur, Benton, Tri x Sweet, Tri X en Japanse sitroen het enkele vrugte wat nie uitvoer standaard behaal het nie. Daar was min oleo, Japanse sitroen met 'n paar nie uitvoerbare vrugte. Benton het die meeste windletsels gehad, growweskiisuurlemoen, Tri x Sweet en kommersieële Genoa het 'n paar nie uitvoerbare vrugte. Growweskiisuurlemoen en kommersieële Genoa het enkele skurwe vrugte. Rangpur en Japanse Sitroen het knoppies en suiers op die onderstamme gehad en knoppies op Volckameriana. Evaluasies moet voortgaan.

Introduction

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 different rootstock options.

Materials and methods

Genoa lemon was budded to ten different rootstocks and planted at Hexrivier, Citrusdal in January 2000 in adjacent rows. The trees were evaluated according to certain criteria, including production, sets, tree size, compatibility, fruit size, juice percentage, average seed, rind thickness, high shoulders, oleocellosis and wind scars. Commercial Genoa and Lisbon lemons on rough lemon that form part of the orchard are included in the evaluations. The list of rootstocks and number of trees evaluated is presented in table 6.4.6.1.

Results and discussion

The trees were evaluated on 26 April 2005 and tree height was measured on 24 August 2005. There is some variation in the tree size and therefore only the better/larger trees have been used for evaluation purposes – refer Table 6.4.4.1. Overall the production was good, and 50 fruit were taken per rootstock combination for evaluation purposes.

Table 6.4.4.1. List of rootstocks, rootstock selection, number of trees evaluated and average tree height and diameter with Genoa lemon as scion and commercial Genoa and Lisbon lemons at Hexrivier, Citrusdal during 2005.

Rootstock	Selection	No of trees evaluated	Ave. tree height (m)	Ave. tree * diameter (m)
Cairn rough lemon	163	16	3.9	2.8
Volckameriana	575	13	3.8	3.1
Trifoliate X	1242	8	3.6	2.7
Benton citrange	980	5	3.3	2.8
Trifoliate x Sweet orange	1287	14	3.4	2.6
Japanse Sitroen	184	13	3.4	3.0
Minneola x Trifoliate	1238	13	3.5	3.1
Swingle citrumelo	715	9	3.3	2.8
Rangpur lime	225	4	3.3	3.2
Carrizo citrange	608	4	3.5	3.3
Commercial Genoa/rough lemon		20	3.6	2.9
Commercial Lisbon/rough lemon		16	3.7	3.2

* Trees are pruned between the rows to allow tractors to pass through.

Table 6.4.4.2. Visual evaluation of tree production, number of sets and appearance of bud union and rootstock. Rootstocks arranged in order of planting.

Rootstock	Production	No. of sets	Bud union and rootstock appearance
Rough lemon	Good - excellent	One	Clean, smooth
Volckameriana	Fair – good – excellent. Large trees	One	Smooth with occasional, odd nodules
Trifoliata X	Fair – good. Variable tree size	One	Odd fluting, occasional slight bench
Benton citrange	Fair & good – excellent. Variable tree size	One	
Trifoliata x Sweet orange	Good, variable	One	Slightly inverted bench
Japanese Citroen	Excellent	One	Paler rootstock, smooth, nodules, odd sucker growth, fluting
Minneola x Trifoliata	Fair – good, inside fruit?	One	Fairly smooth to very slight bench, slight fluting
Swingle citrumelo	Good – excellent	One, & very small earlier set	Slight bench, odd fluting
Rangpur lime	Good	One	Paler rootstock, lots of nodules, smoothish, rootstock shoots
Carrizo citrange	Poor	One	Clean, odd fluting. Smooth to almost inverted?
Commercial Genoa/RL	Good - excellent	One	Smooth, some fluting
Commercial Lisbon/RL	Fair – good. Vigorous, spreading trees	One	Smooth? Slightly fluted

The largest trees were on Volckameriana, M x T and Carrizo, and the smallest were on Benton, Swingle and Tri x Sweet.

All trees were girdled at full bloom and some long, inside branches bent over to sprout. Odd branches and large water shoots were cut out about a month prior to evaluations. All trees had some leaf drop. There were some slight differences in leaf colour. Rough lemon had the greenest leaves; Volckameriana, Rangpur, Japanese Citroen (variable) and Lisbon not as green; Swingle, Tri x Sweet, Carrizo, M x T, Tri X and commercial Genoa greenish to slightly paler leaf colour and the palest Benton. Volckameriana and Tri x Sweet some slightly elongated fruit, Carrizo elongated. Tri x Sweet had softer fruit. Stem and scion diameters were not measured as the trees were allowed to branch just above the bud union in the nursery that also makes it difficult to always see the bud union.

Table 6.4.4.3. 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 2005							% in counts 216-113
		≤189	162	138	113	100	88	≥75	
Volckameriana	61.6		4	12	42	32	10		58
Rough lemon	61.5	4		18	38	20	18	2	60
Trifoliata X	60.4		8	26	32	32	2		66
Japanese Citroen	60.3	2	2	26	48	16	2	4	78
Rangpur lime	59.9	8	10	20	34	16	10	2	72
Swingle citrumelo	59.3	8	8	28	32	22	2		76
Carrizo citrange	58.9	10	12	26	34	16	2		82
Minneola x Trifoliata	58.8	14	12	28	22	14	8	2	76
Benton citrange	58.6	2	16	32	38	12			88
Tri x Sweet orange	58.4	10	22	12	40	10	6		84

Comm Genoa/RL	60.0	2	12	26	36	10	12	2	76
Comm Lisbon/RL	59.5		16	24	44	12	4		84

Some of the rootstocks had a high percentage of fruit not desired by the export markets, i.e. larger than count 113. These included Volckameriana, rough lemon and Tri X. Those exceeding 80% in the popular counts are Benton, Tri x Sweet orange, Lisbon and Carrizo.

Table 6.4.4.4. Average fruit size of Genoa lemon on various rootstocks over the past four seasons and colour transparency recorded on 26 April 2005. Rootstocks arranged according to descending fruit size.

Rootstock	Average fruit size (mm)				Colour transparency (2005)		
	2002	2003	2004	2005	T4	T5	T6
Volckameriana	71.8	63.1	65.1	61.6		56	44
Rough lemon	72.4	62.9	63.4	61.5	2	56	42
Trifoliolate X	64.6	61.0	60.9	60.4		36	64
Japanese Citroen	70.8	63.7	61.6	60.3		34	66
Rangpur sime	71.6	63.1	63.9	59.9		22	78
Swingle citrumelo	64.6	57.5	59.5	59.3		74	26
Carrizo citrange	64.0		59.1	58.9		32	68
M x T	63.2	57.1	57.8	58.8		64	36
Benton citrange	69.8	61.9	60.5	58.6		22	78
Tri x Sweet orange	67.6	58.8	59.1	58.4		46	54
Comm Genoa/RL			64.9	60.0		32	68
Comm Lisbon/RL			61.5	59.2		22	78

The average fruit size was not too dissimilar to 2004. The only major differences were Rangpur and Benton with a size increase and Tri X a slight decrease. Those with earlier colour were Swingle, M x T and rough lemon. Later colour includes Rangpur, Benton, and Lisbon, followed by Carrizo, commercial rough lemon, Japanese Citroen and Tri X.

Table 6.4.4.5. Test sample size and colour, juice percentage (tested 24 June 2002, 13 May 2003, 12 May 2004 and 26 April 2005), average seed counts and rind thickness of the Genoa lemon on various rootstocks. Rootstocks arranged according to descending juice percentage in 2005.

Rootstock	Sample Count	Sample colour	Juice %				Average seed per fruit	Rind thickness (mm)
			2002	2003	2004	2005		
Cairn rough lem	138	6	46.0	45.2	45.6	53.7	7.8	3.5
Rangpur lime	138	5-6	46.7	47.0	48.3	52.6	9.1	3.7
Tri x Sweet oran	138	5-6	46.3	46.6	46.0	51.4	10.7	3.9
Carrizo citrange	138	6	44.4		47.3	51.1	8.8	3.6
Japanese Citroen	138	5-6	46.6	36.7	47.5	50.7	10.9	4.1
Trifoliolate X	138	5-6	42.9	44.3	47.9	50.4	11.9	3.6
Minneola x trifol	138		45.0	43.0	47.3	49.3	12.0	3.8
Volckameriana	113	6	45.2	43.0	47.6	49.0	8.7	3.9
Benton citrange	162	5-6	47.4	45.4	49.0	48.6	9.8	3.6
Swingle citrumel	138	5-6	45.0	43.8	46.5	41.8	8.2	3.7
Genoa/RL comm	138	5-6			44.9	47.9	9.9	3.9
Lisbon/RL comm	138				46.0	45.5	13.4	3.9

All rootstocks except for Swingle (the lowest but acceptable), easily met the minimum juice export standards of 40%. Except for Benton and Swingle there is an overall improvement on last season juice percentages. The rind thickness does not necessarily correspond with the juice percentages. There was a dramatic improvement in juice percentages with rough lemon, Rangpur, Tri x Sweet and Carrizo. Surprisingly rough lemon had the highest juice (it was the lowest last season). Benton was the highest last season, second lowest this season. Rangpur has been consistently high.

Table 6.4.4.6. Analyses of high shoulders, oleocellosis and wind scars of Genoa lemons on various rootstocks. Rootstocks arranged from least to most high shoulders.

Rootstock	High shoulders					Oleocellosis					Wind scars					
	0	1	2	3	4	0	1	2	3	5	0	1	2	3	4	5
Rough lemon	46	42	6	6		86	14				62	24	8	4		2
Volckamerian	54	32	8	6		94	6				52	30	8	4	6	
M x T	32	52	14	2		96	4				74	20	4	2		
Carrizo citran	38	40	16	6		100					62	18	16	4		
Rangpur lim	50	28	10	10	2	94	4		2		52	36	10		2	
Benton citran	50	28	12	8	2	96	4				46	40	10	2	2	
Swingle citru	38	36	16	10		94	4	2			60	24	0	4	2	
Tri x Sweet	32	42	16	8	2	96	4				70	20	8			2
Trifoliata X	34	40	18	6	2	100					66	18	10	4	2	
Japanese Sitr	48	26	16	2	8	84	10	4		2	64	22	6	2	6	
Co Genoa/RL	56	20	18	6		94	6				68	20	10			2
Co Lisbon/RL	56	38	4	2		90	10				74	20	6			

There were some small differences in high shoulders, the vigorous rootstocks having the least, Rangpur, Benton, Tri x Sweet, Tri X and Japanese sitroen with some non exportable fruit. There was a little oleocellosis on some of the rootstocks, Japanese sitroen with some non exportable fruit. Benton had the highest incidence of wind scars, rough lemon, Tri x Sweet and commercial Genoa with a few non exportable fruit.

Evaluations are based on CRI Colour Prints for Blemish and Appearance standards. High Shoulders (Set 39), Oleocellosis (Set 28), prints 0 - 3 are exportable: Wind Scars (Set 8), prints 0 - 4 exportable.

Most fruit were all smooth with a few coarse fruit on rough lemon, Volckameriana and commercial Genoa. Trifoliata x Sweet had slightly finer flesh with a less yellow flesh colour (the others yellow except for commercial Genoa which had a slightly green flesh colour). All had closed cores. The Lisbon fruit shape appeared slightly shorter.

Conclusions

This was the fourth year of production, with a great increase in yield over last season. Japanese sitroen, rough lemon, Swingle and commercial Genoa had the best yields. All the others had good yields except for Carrizo that was poor. Unfortunately the trees are variable in size and only the best used for evaluation purposes. Volckameriana, M x T and Carrizo have the largest tree size, Benton, Swingle and Tri x Sweet the smallest. Rough lemon and Volckameriana have the largest fruit size, Benton, Tri x Sweet, Lisbon and Carrizo fruit falling in the most desired fruit size range.

All rootstocks except Swingle (lowest, but acceptable) easily met the minimum juice export standards. Swingle, M x T and rough lemon had slightly earlier fruit colour, Rangpur and Benton the poorest. M x T has had slightly earlier colour over the past three seasons.

There were some small differences in high shoulders, the vigorous rootstocks the least, Rangpur, Benton, Tri x Sweet, Tri X and Japanese sitroen with some non exportable fruit. There was not much oleocellosis, Japanese sitroen with some non exportable fruit. Benton had the highest incidence of wind scars, rough lemon, Tri x Sweet and commercial Genoa with a few non exportable fruit.

Rough lemon, Volckameriana and commercial Genoa had a few coarse fruit. Rangpur and Japanese Sitroen had nodules and sucker growth on the rootstock, Volckameriana had some nodules. Evaluations to continue.

Future evaluations

Continue evaluations for another season.

6.4.5 Evaluation of Delta Valencia rootstock trial at Moosrivier Estates Experiment 94 by J. Joubert (CRI)

Opsomming

Die prestasie van Delta Valencia op 42 verskillende onderstamme in herplant gronde, moet oor 'n agt jaar produksietyd in die Marble Hall omgewing geëvalueer word. Hierdie seisoen was daar voldoende water vir die effektiewe besproeiing van die bome beskikbaar gewees. Die 2005 seisoen se produksie is presies die omgekeerde scenario. Meeste van die kombinasies het 'n goeie oes geproduseer. Die table wat die opsomming verduidelik en vergelyk tussen 2003, 2004 en 2005 toon die herstel tempo tussen die verskillende onderstamme duidelik aan. Vir die 2005 seisoen is daar slegs twee onderstamme wat minder geproduseer het as in 2004 (RT en SFS).

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 design comprising of 22 rootstocks of two replicates of five trees each, the other 20 rootstocks were planted in a non-randomised design comprising of 10 trees per rootstock. 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. The trees were planted in 1998. Trees were evaluated at Moosriver Estates (Marble Hall), in Mpumalanga during the 2005 season.

Results and discussion

Internal fruit quality analysis (Table 6.4.5.1)

- Juice%: Sunki 1113 rootstock produced the highest Juice% (58.7%) followed by RC (58.1%) and F80/8 (57.5%). The Juice% on all the rootstocks complied with the packing specifications with rootstock ChM and C measuring 52.2% (lowest).
- TSS%: The highest TSS% was produced by SM (12.17%) followed by CC (12.07%), ChM (11.97%) and PT (11.87%). Ten of the rootstocks evaluated (K, CM, ML, OT, RL-S, RP, RxT, CO, TB, JC, Volk) produced lower than 10.5% TSS% value and did not comply to the packing specifications, with Volk the lowest (8.62%).
- Acid%: CA rootstock provided the highest Acid% (1.38%). CLM produced the second highest Acid% (1.35%) followed by SCS (1.32%) and C35 (1.31%). The lowest Acid% measured 0.92% (RL-S) and complied with the minimum export specifications of 0.85%.

Fruit size distribution (Table 6.4.5.2)

- The fruit size evaluation shows the largest peak at count 105/125 on 39 of the 42 rootstocks. The next highest count in fruit size was count 72 with 11 rootstocks. The third highest count evaluated in fruit size was count 88 with 25 rootstocks. Considering that count 105/125, followed by count 72 and then count 88 was ranked from the highest percentage fruit per rootstock to the lowest percentage.

Production per tree (Table 6.4.5.3)

- RP rootstock produced the best yield per tree (79.8 kg) in comparison with the other 42 rootstocks. BC was the second highest producer with 66.5 kg per tree, followed by F80/3 (53.8kg) and RL-S, TC (52.7kg). CLM produced the lowest yield for the 2005 season with 12.7 kg per tree.
- There is an increase in yield production between the 2004 and 2005 season. The trend shows the severe impact of the water problems that occurred in this area on the different rootstocks. The 2005 season had a better rainfall and there were sufficient water available for proper irrigation.
- The table shows the variation in yield for 2003, 2004 and 2005 between the different rootstocks. The analysis points out that for the 2004 season there was a decrease in yield. It is very important to

compare the recovery rate for the rootstocks from 2003 to 2005. Please bear in mind that during the 2005 season there was enough water for normal irrigation practices.

Conclusions

The average TSS% increased considerably in comparison to the 2004 season. The average Acid% decreased in comparison to the 2004 season. The fruit size on most of the rootstocks decreased because of the higher number of fruit produced on the trees. The yield increased from 51 kg/tree to 79.8 kg/tree on the rootstock with the highest yield for 2004 (RP). Benton citrange remains the second highest producer of fruit and produced double the kg/tree in comparison to the previous season.

Table 6.4.5.1. Internal fruit quality of Delta Valencia on different rootstocks at Moosriver Estate (Marble Hall) on 11 July 2005.

Rootstock	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
F80/8	64-88	57.5	11.18	1.22	9.16	0.0	T1
PT	64-105	55.9	11.87	1.12	10.60	0.0	T1
C32	64-88	53.4	11.08	1.24	8.94	0.0	T1-3
AT	56-88	55.4	11.77	1.07	11.00	0.0	T1-2
HRS 812	56-88	54.7	11.18	1.19	9.39	0.7	T1
K	64-88	55.7	9.75	1.04	9.38	0.0	T1-3
CM	56-88	55.6	10.05	0.97	10.36	0.0	T1-3
C35	56-105	52.9	11.18	1.31	8.53	0.0	T1-4
C	72-105	52.2	11.38	1.77	6.43	0.0	T1-2
SCS	72-88	55.8	12.37	1.32	9.37	0.0	T1-2
X639	64-105	56.8	10.58	1.02	10.37	0.0	T1-3
GT	64-105	56.5	10.88	1.07	10.17	0.0	T1
ML	72-88	54.6	9.85	0.97	10.15	0.0	T1-2
OT	56-88	55.6	9.95	1.12	8.88	0.0	T1-3
CA	72-105	55.6	11.38	1.38	8.25	0.0	T1-4
RC	64-125	58.1	11.97	1.21	9.89	0.0	T1-3
JT	64-88	56.4	10.58	0.98	10.80	0.0	T1
RL-S	56-88	54.2	9.55	0.92	10.38	0.0	T1-3
SC	64-105	55.6	11.57	1.11	10.42	0.0	T1
RP	72-105	55.6	10.05	1.21	8.31	0.0	T1-3
SM	64-105	54.9	12.17	1.23	9.89	0.0	T1-2
ChM	64-88	52.2	11.97	1.03	11.62	0.0	T1-3
N	72-88	57.1	11.67	1.27	9.19	0.0	T1-2
RxT	64-88	55.0	10.05	1.13	8.89	0.0	T1-3
RL-C	64-125	55.1	10.88	1.06	10.26	0.0	T1-2
CLM	64-125	52.9	11.38	1.35	8.43	0.0	T1-3
Sunki 113	72-125	58.7	10.78	1.24	8.69	0.0	T1-3
CO	64-88	57.3	10.48	1.03	10.17	0.0	T1-3
CC	56-88	54.2	12.07	1.10	10.97	0.0	T1-2
TC	72-88	58.1	10.58	1.11	9.53	0.0	T1-2
Volk	56-88	55.2	8.62	0.96	8.98	0.0	T1-3
KC	72-125	55.9	11.77	1.14	10.32	0.0	T1-3
TB	64-88	53.2	10.15	1.04	9.76	0.0	T1-3
RT	72-88	56.7	11.28	1.00	11.28	0.0	T1
JC	72-105	54.9	10.15	0.99	10.25	0.0	T1
BC	64-88	56.2	10.68	1.05	10.17	0.0	T1-3
F80/3	64-88	55.7	10.78	1.14	9.46	0.0	T1
Sunki 112	72-105	54.9	11.77	1.16	10.15	0.0	T1-2
ST	72-88	53.1	11.38	1.35	8.43	0.0	T1-3
SFS	72-125	56.4	11.18	1.11	10.07	0.0	T1-3

Sunki 116	72-105	56.1	11.08	1.06	10.45	0.0	T1-3
RL-W	56-88	53.5	10.88	1.23	8.85	0.0	T1-2

Table 6.4.5.2. Fruit size distribution of Delta Valencia on different rootstocks at Moosriver Estate (Marble Hall) during the 2005 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/8	48	3.13	ChM	48	1.15	Sunki 112	48	0.45
F80/8	56	11.96	ChM	56	5.05	Sunki 112	56	7.42
F80/8	72	23.06	ChM	72	8.49	Sunki 112	72	13.61
F80/8	88	23.38	ChM	88	15.83	Sunki 112	88	20.81
F80/8	105/125	30.71	ChM	105/125	43.35	Sunki 112	105/125	41.17
F80/8	144	7.76	ChM	144	26.15	Sunki 112	144	16.54
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
PT	48	1.37	N	48	0.17	ST	48	1.71
PT	56	12.01	N	56	7.23	ST	56	8.90
PT	72	25.57	N	72	16.78	ST	72	17.12
PT	88	24.01	N	88	19.54	ST	88	20.38
PT	105/125	30.25	N	105/125	38.98	ST	105/125	39.38
PT	144	6.78	N	144	17.30	ST	144	12.50
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
C32	48	4.01	RxT	48	2.86	SFS	48	0.00
C32	56	13.70	RxT	56	13.11	SFS	56	5.55
C32	72	22.55	RxT	72	22.80	SFS	72	11.00
C32	88	21.02	RxT	88	24.01	SFS	88	15.56
C32	105/125	29.16	RxT	105/125	27.42	SFS	105/125	43.41
C32	144	9.56	RxT	144	9.80	SFS	144	24.48
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
AT	48	2.69	RL-C	48	0.45	Sunki 116	48	0.47
AT	56	14.35	RL-C	56	3.18	Sunki 116	56	4.51
AT	72	25.78	RL-C	72	12.18	Sunki 116	72	13.25
AT	88	20.85	RL-C	88	17.64	Sunki 116	88	15.23
AT	105/125	29.15	RL-C	105/125	42.91	Sunki 116	105/125	40.66
AT	144	7.17	RL-C	144	23.64	Sunki 116	144	25.66
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
HRS812	48	6.55	CLM	48	2.50	RL-W	48	6.50
HRS812	56	18.16	CLM	56	10.00	RL-W	56	24.60
HRS812	72	21.16	CLM	72	21.43	RL-W	72	25.88
HRS812	88	20.97	CLM	88	19.64	RL-W	88	18.21
HRS812	105/125	26.59	CLM	105/125	35.00	RL-W	105/125	23.30
HRS812	144	6.55	CLM	144	11.43	RL-W	144	3.51
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
K	48	2.19	Sunki 1113	48	2.28	JT	48	0.79
K	56	13.04	Sunki 1113	56	12.90	JT	56	10.79
K	72	18.23	Sunki 1113	72	22.20	JT	72	21.84
K	88	14.68	Sunki 1113	88	20.97	JT	88	17.89
K	105/125	32.09	Sunki 1113	105/125	32.16	JT	105/125	32.11
K	144	19.78	Sunki 1113	144	9.49	JT	144	16.58

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
CM	48	4.52	CO	48	0.00	RL-S	48	0.95
CM	56	31.95	CO	56	3.92	RL-S	56	6.38
CM	72	28.72	CO	72	14.69	RL-S	72	17.49
CM	88	17.36	CO	88	18.61	RL-S	88	17.65
CM	105/125	14.22	CO	105/125	40.81	RL-S	105/125	37.12
CM	144	3.23	CO	144	21.98	RL-S	144	20.41
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
C35	48	1.66	CC	48	1.96	SC	48	1.14
C35	56	11.41	CC	56	16.68	SC	56	7.71
C35	72	17.39	CC	72	29.56	SC	72	15.29
C35	88	19.05	CC	88	21.42	SC	88	15.29
C35	105/125	38.65	CC	105/125	26.16	SC	105/125	34.29
C35	144	11.85	CC	144	4.22	SC	144	26.29
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
C	48	1.34	TC	48	1.04	RP	48	1.40
C	56	6.98	TC	56	10.99	RP	56	13.41
C	72	15.60	TC	72	16.64	RP	72	21.73
C	88	19.17	TC	88	17.83	RP	88	18.04
C	105/125	37.44	TC	105/125	37.42	RP	105/125	34.30
C	144	19.47	TC	144	16.08	RP	144	11.12
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
SCS	48	1.79	Volk	48	2.86	SM	48	0.87
SCS	56	9.23	Volk	56	14.41	SM	56	5.40
SCS	72	13.59	Volk	72	22.25	SM	72	16.90
SCS	88	20.51	Volk	88	20.44	SM	88	22.13
SCS	105/125	36.92	Volk	105/125	29.66	SM	105/125	36.41
SCS	144	17.95	Volk	144	10.38	SM	144	18.29
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
X639	48	0.38	KC	48	1.00	BC	48	0.60
X639	56	2.20	KC	56	6.77	BC	56	10.21
X639	72	9.27	KC	72	20.22	BC	72	18.82
X639	88	13.91	KC	88	20.89	BC	88	23.19
X639	105/125	41.72	KC	105/125	37.51	BC	105/125	36.45
X639	144	32.52	KC	144	13.62	BC	144	10.74
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
GT	48	2.12	TB	48	0.98	F80/3	48	0.44
GT	56	11.32	TB	56	13.73	F80/3	56	5.70
GT	72	17.82	TB	72	24.01	F80/3	72	14.94
GT	88	17.26	TB	88	20.11	F80/3	88	18.86
GT	105/125	32.81	TB	105/125	29.93	F80/3	105/125	39.05
GT	144	18.67	TB	144	11.25	F80/3	144	21.01
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
ML	48	0.98	RT	48	1.43	CA	48	2.47
ML	56	6.19	RT	56	6.86	CA	56	10.54
ML	72	12.49	RT	72	14.71	CA	72	12.85
ML	88	16.03	RT	88	18.57	CA	88	15.49
ML	105/125	38.45	RT	105/125	40.00	CA	105/125	36.41

ML	144	25.86	RT	144	18.43	CA	144	22.24
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
OT	48	0.94	JC	48	4.95	RC	48	5.61
OT	56	7.23	JC	56	10.13	RC	56	15.89
OT	72	13.99	JC	72	19.54	RC	72	17.29
OT	88	18.55	JC	88	22.32	RC	88	19.86
OT	105/125	40.09	JC	105/125	34.26	RC	105/125	30.61
OT	144	19.18	JC	144	8.81	RC	144	10.75

Table 6.4.5.3. Production of Delta Valencia on different rootstocks at Moosriver Estate (Marble Hall) during the 2005 season.

Rootstock Selection	Kg/tree 2003	Kg/tree 2004	Kg/tree 2005	Variation in kg (2003-2004)	Variation in kg (2003-2005)	Variation in kg (2004-2005)
JT	26.3	30.8	43.0	4.5	16.7	12.2
RT	36.8	35.7	30.0	-1.1	-6.8	-5.7
CM	57.4	51.0	56.5	-6.4	-0.9	5.5
KC	39.0	32.7	50.8	-6.3	11.8	18.1
Sunki 1116	32.5	25.8	43.1	-6.7	10.6	17.3
SFS	51.4	40.1	37.8	-11.3	-13.6	-2.3
BC	61.7	47.7	66.5	-14.0	4.8	18.8
RP	59.4	45.7	79.8	-13.7	20.4	34.1
PT	43.1	32.5	47.6	-10.6	4.5	15.1
RC	50.1	37.4	40.1	-12.7	-10.0	2.7
F80/8	48.4	33.4	49.4	-15.0	1.0	16.0
RxT	36.2	22.5	41.6	-13.7	5.4	19.1
CC	37.0	22.0	45.2	-15.0	8.2	23.2
Sunki 1113	48.4	28.7	45.8	-19.7	-2.6	17.1
ML	60.5	35.4	42.3	-25.1	-18.2	6.9
ST	18.5	10.6	25.4	-7.9	6.9	14.8
X639	47.2	26.3	48.1	-20.9	0.9	21.8
F80/3	49.9	27.1	53.8	-22.8	3.9	26.7
CA	21.1	11.2	26.0	-9.9	4.9	14.8
Sunki 1112	43.9	22.9	37.4	-21.0	-6.5	14.5
JC	23.4	11.9	38.0	-11.5	14.6	26.1
Volk	56.7	28.1	42.8	-28.6	-13.9	14.7
RL-C	55.7	26.8	43.7	-28.9	-12.0	16.9
HRS 812	50.7	24.0	25.4	-26.7	-25.3	1.4
C35	58.4	27.4	39.6	-31.0	-18.8	12.2
AT	25.3	11.8	20.6	-13.5	-4.7	8.8
C32	54.9	25.4	38.6	-29.5	-16.3	13.2
RL-S	54.3	25.1	52.0	-29.2	-2.3	26.9
SM	28.8	13.3	23.9	-15.5	-4.9	10.6
RL-W	34.8	14.2	46.1	-20.6	11.3	31.9
CO	31.1	11.3	36.5	-19.8	5.4	25.2
TB	49.5	16.9	50.0	-32.6	0.5	33.1
CLM	31.7	9.6	12.7	-22.1	-19.0	3.1
OT	23.4	6.9	26.4	-16.5	3.0	19.5
SC	40.0	10.9	27.3	-29.1	-12.7	16.4
ChM	34.9	9.0	16.4	-25.9	-18.5	7.4
N	46.2	11.3	49.4	-34.9	3.2	38.1
GT	26.3	5.8	29.8	-20.5	3.5	24.0

TC	50.6	11.1	52.7	-39.5	2.1	41.6
C	18.7	3.7	26.8	-15.0	8.1	23.1
SCS	34.9	5.5	16.4	-29.4	-18.5	10.9
K	25.1	3.7	46.5	-21.4	21.4	42.8

6.4.6 Evaluation of Midnight and Delta Valencia rootstock trial at Letaba Estates Experiment 137 A by J. Joubert (CRI)

Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer. Die Delta Valencia kombinasies het 'n beter oes geproduseer as die 2004 seisoen. Oor die algemeen het die interne kwaliteit ook verbeter met slegs SC, MxT en RL-C wat nie uitvoer standarde behaal het nie. Die grootste vrugte is op CC en C35 geproduseer. BC het die hoogste oes geproduseer van 83.7 kg/boom. Die Midnight Valencia kombinasies het baie goed gepresteer en die gemiddelde vruggrootte het op telling 72/88 gepiek. Die hoogste produksie was op SC met 83.8 kg/boom gewees. In hierdie area was daar nog steeds met tye water tekorte gewees. Dit was nie so krities soos die 2004 seisoen gewees nie, maar het nog steeds 'n invloed op die produksie gehad.

Introduction

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Midnight Valencia and Delta Valencia. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system.

Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Midnight Valencia and Delta Valencia trees were budded onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates near Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per replicate (total of 20 trees per rootstock).

Table 6.4.6.1. List of cultivar x rootstock combinations planted at Letaba Estates.

Selection	Rootstock	No. of trees
Delta Valencia	F80/9	10
Delta Valencia	SC	10
Delta Valencia	CC	10
Delta Valencia	F80/3	10
Delta Valencia	C35	10
Delta Valencia	KC	10
Delta Valencia	MxT	10
Delta Valencia	BC	10
Delta Valencia	X639	10
Delta Valencia	RL-C	8
Midnight Valencia	RL-C	10
Midnight Valencia	F80/9	6
Midnight Valencia	BC	9
Midnight Valencia	MxT	10
Midnight Valencia	SC	10
Midnight Valencia	F80/3	10
Midnight Valencia	CC	9
Midnight Valencia	C35	8
Midnight Valencia	KC	10
Midnight Valencia	X639	10
Midnight Valencia	61AA 3	10
Midnight Valencia	79AC 6/2	10
Midnight Valencia	75AB 12/3	7

Results and discussion

Delta Valencia

Internal fruit quality was higher than in the 2004 season (Table 6.4.6.1.a). SC, MxT and RL-C were the only rootstocks that did not comply with the export specifications, because their Acid% was too low. The TSS% improved with values between 11.13% and 13.79%.

The larger fruit size in this trial was produced on CC (count 56-72) and C35 (count 56-72) (Table 6.4.6.1.b). The rest of the combinations produced small to medium fruit size (count 88-144).

BC produced the highest yield per tree (83.7 kg), followed by MxT and RL-C with 82 kg/tree and KC with 70.6 kg/tree (Table 6.4.6.1.c). C35 produced the lowest yield on the trees of 21 kg/tree.

Midnight Valencia

Internal fruit quality was acceptable in terms of juice content and TSS% (Table 6.4.6.2.a). SC, MxT and RL-C produced high Acid%, causing problems with meeting the export standards.

The optimal fruit size for Valentias is between count 72 and 88 (Table 6.4.6.2.b). All the combinations peaked at count 72 – 88, except for 61AA 3, 79AC 6/2 and 75AB 12/3.

Midnight Valencia on Swingle citrumello produced the highest yield (83.8 kg/tree) (Table 6.4.6.2.c). F80/3 produced 78.8 kg/tree, followed by BC with 77.7 kg/tree and MxT with 76.4 kg/tree. RL-C produced the lowest yield in this trial evaluated (52.9 kg/tree).

Conclusions

Delta and Midnight Valencia selections produced fruit with either too low or high Acid%, all the other internal quality factors complied with the specifications. The TSS% on the Deltas was impressively high in comparison with the 2004 season.

On CC and C35, Delta fruit size was between count 56 and 72. The Midnights peaked at count 72 – 88. The smaller size is likely due to the higher yields.

The trees at the trial site was pruned, treated with Confidor and fertilised for optimum production the next season.

Table 6.4.6.1.a. Internal fruit quality data of Delta Valencia on different rootstocks at Letaba Estates (Letsitele) on 20 July 2005.

Rootstock	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
F80/9	59.7	12.89	1.21	10.65	0.3	T1-2
SC	61.4	12.84	1.67	7.69	0.0	T1
CC	61.0	12.22	1.19	10.27	0.0	T1
F80/3	59.9	12.02	1.31	9.18	0.0	T1-2
C-35	59.5	11.43	1.29	8.86	0.0	T1
KC	60.4	12.42	1.22	10.18	0.0	T1-2
MxT	61.8	13.79	1.71	8.06	0.0	T1
BC	60.9	12.42	1.34	9.27	0.5	T1-2
X639	58.7	12.22	1.26	9.70	0.0	T1-2
RL-C	57.8	11.13	1.51	7.37	5.0	T2-3
AT	58.7	13.14	1.30	10.11	0.0	T1

Table 6.4.6.1.b. Fruit size distribution of Delta Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2005 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/9	48	0.97	BC	48	3.05
F80/9	56	7.93	BC	56	16.74
F80/9	72	15.35	BC	72	22.00
F80/9	88	17.29	BC	88	21.94
F80/9	105/125	38.41	BC	105/125	28.50
F80/9	144	20.03	BC	144	7.78
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
SC	48	1.45	X639	48	0.24
SC	56	11.83	X639	56	7.18
SC	72	24.29	X639	72	18.82
SC	88	21.13	X639	88	23.76
SC	105/125	30.55	X639	105/125	37.88
SC	144	10.75	X639	144	12.12
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
CC	48	4.06	RL-C	48	0.00
CC	56	24.22	RL-C	56	0.31
CC	72	30.17	RL-C	72	3.82
CC	88	19.58	RL-C	88	12.33
CC	105/125	18.64	RL-C	105/125	53.55
CC	144	3.34	RL-C	144	29.98
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/3	48	3.14	AT	48	0.20
F80/3	56	16.89	AT	56	3.12
F80/3	72	22.85	AT	72	11.41
F80/3	88	18.23	AT	88	17.11
F80/3	105/125	26.83	AT	105/125	33.94
F80/3	144	12.06	AT	144	8.51
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
C35	48	15.57	MxT	48	0.12
C35	56	44.61	MxT	56	2.71
C35	72	24.25	MxT	72	8.90
C35	88	8.83	MxT	88	17.21
C35	105/125	5.69	MxT	105/125	41.75
C35	144	1.05	MxT	144	29.30
Rootstock	Size	% Fruit			
KC	48	0.42			
KC	56	8.13			
KC	72	19.58			
KC	88	22.56			
KC	105/125	37.34			
KC	144	11.97			

Table 6.4.6.1.c. Production per tree of Delta Valencia trees on different rootstocks at Letaba Estates (Letsitele) during the 2005 season.

Rootstock	Kg/tree
F80/9	34.9
SC	36.0
CC	34.9
F80/3	66.8
C35	21.0

KC	70.6
MxT	82.0
BC	83.7
X639	37.6
RL-C	82.1
AT	58.9

Table 6.4.6.2.a. Internal fruit quality data of Midnight Valencia on different rootstocks at Letaba Estates (Letsitele) on 4 July 2005.

Rootstock	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
RL-C	48-72	57.7	10.69	1.47	7.27	0.3	T1-2
F80/9	48-88	61.4	11.09	1.41	7.87	0.0	T2-3
BC	64-88	60.0	11.58	1.27	9.12	0.2	T1-2
MxT	72-105	58.9	12.65	1.92	6.59	0.6	T1-3
SC	64-105	61.0	11.68	1.37	8.53	0.0	T2-4
F80/3	64-88	59.0	10.89	1.27	8.57	0.8	T1-3
CC	48-88	59.2	11.48	1.44	7.97	0.2	T1-3
C35	36-88	57.9	10.59	1.29	8.21	0.4	T2-4
KC	40-88	58.4	11.09	1.08	10.27	0.2	T1-2
X639	64-88	60.0	10.39	1.39	7.47	0.3	T2-3
61AA 3	72-105	54.7	12.28	1.61	7.63	0.1	T4-5
79AC 6/2	56-125	58.4	11.09	1.13	9.81	0.0	T4-5
75AB 12/3	56-105	57.3	11.68	1.26	9.27	0.0	T2-4

Table 6.4.6.2.b. Fruit size distribution of Midnight Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2005 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
RL-C	48	2.26	X639	48	0.43
RL-C	56	22.06	X639	56	11.83
RL-C	72	31.78	X639	72	31.00
RL-C	88	23.28	X639	88	32.99
RL-C	105/125	17.63	X639	105/125	21.02
RL-C	144	2.98	X639	144	2.73
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/9	48	0.88	61AA 3	48	0.30
F80/9	56	14.30	61AA 3	56	1.91
F80/9	72	26.95	61AA 3	72	7.12
F80/9	88	24.26	61AA 3	88	16.02
F80/9	105/125	28.16	61AA 3	105/125	46.09
F80/9	144	5.45	61AA 3	144	28.56
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
BC	48	0.47	79AC 6/2	48	0.17
BC	56	15.19	79AC 6/2	56	4.86
BC	72	37.70	79AC 6/2	72	15.44
BC	88	28.31	79AC 6/2	88	24.15
BC	105/125	16.53	79AC 6/2	105/125	41.61
BC	144	1.80	79AC 6/2	144	13.77
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
MxT	48	0.48	75AB 12/3	48	0.04
MxT	56	4.75	75AB 12/3	56	1.53
MxT	72	20.93	75AB 12/3	72	10.85
MxT	88	6.34	75AB 12/3	88	23.78

MxT	105/125	55.02	75AB 12/3	105/125	50.23
MxT	144	12.49	75AB 12/3	144	13.57
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
SC	48	1.58	C35	48	5.09
SC	56	16.14	C35	56	27.96
SC	72	31.81	C35	72	25.77
SC	88	27.33	C35	88	33.19
SC	105/125	20.32	C35	105/125	6.76
SC	144	2.82	C35	144	1.23
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/3	48	1.20	KC	48	3.48
F80/3	56	12.49	KC	56	36.61
F80/3	72	28.19	KC	72	36.61
F80/3	88	26.81	KC	88	16.32
F80/3	105/125	26.10	KC	105/125	6.04
F80/3	144	5.21	KC	144	0.93
	Rootstock	Size	% Fruit		
	CC	48	2.57		
	CC	56	17.90		
	CC	72	32.11		
	CC	88	25.82		
	CC	105/125	18.68		
	CC	144	2.92		

Table 6.4.6.2.c. Production of Midnight Valencia on different rootstocks at Letaba Estates (Letsitele) during the 2005 season.

Rootstock	Kg/tree
RL-C	52.9
F80/9	68.2
BC	77.7
MxT	76.4
SC	83.8
F80/3	78.8
CC	72.7
C35	58.8
KC	61.8
X639	66.6
61AA 3	71.4
79AC 6/2	58.3
75AB 12/3	62.2

6.4.7 Evaluation of Star Ruby rootstock trial at Letaba Estates Experiment 137 B by J. Joubert (CRI)

Opsomming

Die prestasie van verskillende onderstamme op herplant grond is in 'n intermediêre sitrusproduksie gebied geëvalueer. Star Ruby bome se oes het effens gedaal in vergelyking met die 2004 seisoen. 'n Moontlike rede hiervoor mag wees dat die bome met die droogte in 2004 baie reserwes gebruik het vir produksie. Die volgende seisoen behoort baie belowend te wees. Die bome is vensters in gesnoei en Confidor toegedien vir Was-dopluis probleme. Die beste produksie was op SC gewees, maar die interne kwaliteit het nie aan al die uitvoer vereistes voldoen nie. Die vruggrootte oor die algemeen was aan die kleiner kant. Die TOV% het toegeneem vir 2005, en maak baie meer van die kombinasies geskik vir uitvoer.

Introduction

To evaluate the performance of different rootstocks on replant soils in an intermediate citrus production area. Commercially grown cultivars in the area were used as scion, viz. Star Ruby grapefruit. The trial was established in February 1997. Soils are deep clay and irrigated with a micro irrigation system.

Materials and methods

Rootstock seeds were collected locally and abroad. Seeds were propagated by an accredited nursery using normal practices. Buds of Star Ruby grapefruit were budded onto 30 different rootstocks. The trees were planted in February 1997 at Letaba Estates near Letsitele, Limpopo Province. The trial layout is a randomised block design comprising two replicates of 10 trees per replicate (total of 20 trees per rootstock).

Results and discussion

Internal fruit quality looks promising, with the exception of SC producing low TSS% (8.72%) (Table 6.4.7.1). MxT produced the highest TSS% (12.48%), followed by F80/3 with 11.08%. All the other internal contents met the export specifications.

Fruit size on the different rootstocks remained more or less the same and peaked at count 48, followed by count 64 and 40 (Table 6.4.7.2).

The production on the trees decreased compared to the 2004 season (Table 6.4.7.3). Once again SC produced the highest yield (140.5 kg/tree) followed by C35 and F80/9 (120 kg/tree). RL-C produced the third highest yield (116.1 kg/tree) in comparison to last seasons lowest. MxT producing the lowest yield for this season (21.7 kg/tree).

Conclusions

The water shortage problems remained throughout the season. Production on most of the combinations was constant, except for MxT with a major decrease in yield.

Swingle produced the highest yield on the trees (140.5 kg/tree) and peaked at count 48. Unfortunately the TSS% was below the export minimum.

The trees were pruned properly (windows) and treated with Confidor for Waxy scale. There will be a major improvement in production, colour and fruit size on the trees for the next season.

Table 6.4.7.1. Internal fruit quality of Star Ruby grapefruit on different rootstocks at Letaba Estates (Letsitele) on 10 May 2005.

Rootstock	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
RL-C	40-64	54.2	10.88	1.69	6.44	0.3	T2-3
CC	23-64	54.2	9.45	1.39	6.80	0.1	T1-2
SC	32-64	57.0	8.72	1.57	5.55	0.0	T1-3
F80/9	32-64	58.0	10.98	1.55	7.08	0.2	T1-2
MxT	27-64	53.3	12.48	1.95	6.40	0.3	T1-2
BC	27-56	56.2	10.35	1.54	6.72	0.3	T1-2
X 639	32-56	56.1	10.05	1.47	6.84	0.0	T1-2
KC	23-48	56.0	10.78	1.44	7.49	0.2	T1-2
C-35	27-64	58.4	9.65	1.48	6.52	0.1	T1-2
F80/3	27-56	56.2	11.08	1.68	6.60	0.2	T1-2

Table 6.4.7.2. Fruit size distribution of Star Ruby grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2005 season.

Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
RL-C	27	2.87	BC	27	12.17
RL-C	32	1.95	BC	32	6.65
RL-C	36	6.43	BC	36	16.20
RL-C	40	9.90	BC	40	19.77
RL-C	48	27.75	BC	48	30.39
RL-C	64	51.10	BC	64	14.82
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
SC	27	2.19	MxT	27	12.82
SC	32	2.70	MxT	32	6.13
SC	36	7.46	MxT	36	16.04
SC	40	15.97	MxT	40	19.43
SC	48	37.16	MxT	48	28.50
SC	64	34.52	MxT	64	17.09
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
F80/3	27	6.12	C35	27	1.87
F80/3	32	3.83	C35	32	1.98
F80/3	36	9.89	C35	36	6.29
F80/3	40	14.15	C35	40	11.64
F80/3	48	27.46	C35	48	38.19
F80/3	64	38.55	C35	64	40.03
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
CC	27	15.43	X639	27	1.43
CC	32	6.07	X639	32	1.54
CC	36	14.25	X639	36	5.24
CC	40	14.99	X639	40	12.09
CC	48	25.12	X639	48	45.61
CC	64	24.14	X639	64	34.09
Rootstock	Size	% Fruit	Rootstock	Size	% Fruit
KC	27	7.29	F80/9	27	1.26
KC	32	4.99	F80/9	32	1.06
KC	36	11.68	F80/9	36	5.38
KC	40	16.48	F80/9	40	16.11
KC	48	33.74	F80/9	48	47.94
KC	64	25.82	F80/9	64	28.25

Table 6.4.7.3. Production of Star Ruby Grapefruit on different rootstocks at Letaba Estates (Letsitele) during the 2005 season.

Rootstock	Kg/tree
RL-C	116.1
SC	140.5
F80/3	102.8
CC	84.9
KC	84.4
BC	110.7
MxT	21.7
C35	120.3
X639	115.6
F80/9	120.7

6.4.8 Evaluation of Navel orange rootstock trial at Vaalharts Experiment 146 A by J.Joubert (CRI)

Opsomming

Die prestasie van Navel variëteite op verskillende onderstamme ondersoek en die beste onderstam vir die betrokke seleksie te bepaal in die Vaalharts omgewing. Die beste produksie, vrugsgrootte, interne kwaliteit en eksterne kleur moet verkry word vir uitvoere na bv. die VSA markte. Die perseël in Vaalharts waar die proef aangeplant is het ernstige koue laat in Augustus maand ervaar met temperature so laag as -7°C . Die Navelina bome is die meeste deur die koue beskadig en enkele bome is selfs dood. Meeste van die oorblywende bome het feitlik geen oes geproduseer nie. Die Bahianinha en Royal Late nawels het minder skade opgedoen, maar die bome het 'n swak oes geproduseer met klein vrugsgrootte. Die proef sal weer in 2006 ge-evalueer word.

Introduction

The progress of Navel selections on different rootstocks must be investigated to determine the best rootstock for 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 Navelina on the following rootstocks: CC, C35, RxT and Royal Late on the following rootstocks: BC, C32, C35, CC, GT, RC. The trees were planted in 1998.

Table 6.4.8.1. Number of trees per rootstock in the Bahianinha navel trial at Vaalharts.

Rootstock	No. of trees
C32	13
C35	12
F80	14
IRL	9
KC	12
MxT	36
RL-W	3
RxT	13
SFS	5
TB	19
X639	11
C35	19
CC	19
RxT	8
BC	16
C32	14
C35	15
CC	15
GT	17
RC	17

Results and discussion

No results due to frost.

Conclusions

This area received severe cold weather late in August 2003. The Navelina selections were not tolerant to the severe cold temperatures and trees were badly damaged. Bahianinha and Royal Late produced low numbers of fruit with small fruit size. All the dead wood was removed; the trees received proper fertiliser applications and scheduled irrigation. Evaluations will continue and selections harvested for the 2006 season.

6.4.9 Evaluation of Valencia orange rootstock trial at Vaalharts Experiment 146 B by J.Joubert (CRI)

Opsomming

Die prestasie van Valencia variëteite op verskillende onderstamme word 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. Die perseel in Vaalharts waar die proef aangeplant is het ernstige koue laat in Augustus maand ervaar met temperature so laag as -7 °C. Delta Valencia het die minste koue skade opgedoen en van die seleksies het selfs 'n ligte oes geproduseer. Midnight Valencia het in 'n mindere mate skade opgedoen, maar klein vrugte geproduseer. Evaluasies sal weer vir die 2006 seisoen gedoen word.

Introduction

The performance of Valencia selections on different rootstocks must be investigated to determine the best rootstock combination for 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 Delta Valencia on the following rootstocks: BT, C35, CC, F80, SFS, X639 and Midnight Valencia on the following rootstocks: C35, CC, X639. The trees were planted in 1998.

Table 6.4.9.1. Number of trees per rootstock in the Delta and Midnight Valencia trial at Vaalharts.

Selection	Rootstock	No. of trees
Delta Valencia	BC	17
Delta Valencia	C35	12
Delta Valencia	CC	10
Delta Valencia	F80	13
Delta Valencia	SFS	8
Delta Valencia	X639	10
Midnight	C35	7
Midnight	CC	9
Midnight	X639	8

Results and discussion

No results due to frost.

Conclusions

This area received severe cold weather late in August 2003. All the Delta x rootstock combinations seem tolerant to the cold weather and all the selections were bearing some fruit. The fruit size was too small for evaluation purposes. There was less cold damage on the trees in comparison to Midnight. Midnight Valencia bore some fruit, but of small size. The trees were recovering from the cold damage of the 2003 season. The trees were pruned to remove all the dead branches and fertilised to optimise the recovery process.

6.4.10 Evaluation of Valencias on new imported rootstocks in the Malelane area Experiment 416 A by J.Joubert (CRI)

Opsomming

Die prestasie van Midnight en Delta Valencias op nuwe, ingevoerde onderstamme op herplant grondtipes moet ondersoek word. Die produksie, interne gehalte en skilkleur moet verbeter word, terwyl vruggrootte moet toeneem. Die produksie het hierdie seisoen met meer as 50% toegeneem en al die betrokke seleksies het voldoen aan die uitvoer standaarde. Vir die 2005 produksie jaar was daar voldoende water vir besproeiing beskikbaar gewees, wat 'n groot bydrae gelewer het.

Introduction

The performance of Midnight and Delta Valencias on new, imported rootstocks on replant soils must be investigated. The production, internal quality and rind colour must be improved and at the same time fruit size must be increased.

Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 was imported and propagated in 1996 by Esselen Nursery, an accredited nursery in Malelane.

Delta Valencia was budded onto the following three newly imported rootstock hybrids at Esselen nursery in 1997: Sunki x Beneke HRS 802, Sunki x Beneke HRS 812 and Sunki x MTO trifoliolate orange (FF6). Midnight Valencia was budded onto Sunki x Beneke HRS 812. The trees were planted at Esselen Nursery in March 1999.

Table 6.4.10.1. Number of trees per rootstock in the Delta and Midnight Valencia trial at Malelane.

Selection	Rootstock	No. of trees
Midnight	Sunki 812	4
Delta	Sunki 812	5
Delta	Sunki 802	5
Delta	FF-6	5

Results and discussions

Midnight Valencia

Internal fruit quality complied with the export specifications (Table 6.4.10.2). TSS% of 11.44% was higher than the 2004 season and the juice content was very high. Production increased from 17.3 kg/tree (2004) to 64.9 kg/tree for the 2005 season (Table 6.4.10.4). Fruit size peaked at count 56, followed by count 72 and count 48 (Table 6.4.10.3).

Delta Valencia

The production on the trees increased to between 70.8 kg/tree and 79.7 kg/tree (Table 6.4.10.4). Internal fruit quality complied with all the export specifications with high juice content and TSS% (Table 6.4.10.2). All the fruit evaluated were seedless with good external colour. Fruit size was on the small side and peaked at count 105/125, followed by count 144 and count 88, which may be a result of the higher production (Table 6.4.10.3).

Conclusions

The production increased compared with 2004 and internal fruit quality was good. Fruit size of Delta was smaller, but overall satisfactory. Compared to the 2004 season, the improvement in internal quality, production and size was remarkable.

Table 6.4.10.2. Internal fruit quality of Midnight and Delta Valencias on different rootstocks at Esselen Nursery (Malelane) on 15 July 2005.

Selection	Root-Stock	Count	Juice %	TSS %	Acid %	Ratio	Ave. Seed	Colour
Midnight	Sunki 812	40-56	61.1	11.44	1.20	9.53	1.3	T1
Delta	Sunki 812	56-72	57.1	12.14	1.11	10.94	0.0	T1-2
Delta	Sunki 802	72-105	57.9	12.96	1.18	10.98	0.0	T1-2
Delta	FF-6	72-105	59.3	12.04	1.02	11.80	0.0	T1-2

Table 6.4.10.3. Fruit size distribution at Esselen nursery during the 2005 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	Sunki 812	48	18.01	Delta	Sunki 812	48	0.85
Midknight	Sunki 812	56	35.67	Delta	Sunki 812	56	7.63
Midknight	Sunki 812	72	29.01	Delta	Sunki 812	72	14.19
Midknight	Sunki 812	88	10.64	Delta	Sunki 812	88	19.04
Midknight	Sunki 812	105/125	5.85	Delta	Sunki 812	105/125	41.81
Midknight	Sunki 812	144	0.82	Delta	Sunki 812	144	16.48
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Delta	Sunki 802	48	0.00	Delta	FF-6	48	0.00
Delta	Sunki 802	56	0.41	Delta	FF-6	56	0.52
Delta	Sunki 802	72	3.92	Delta	FF-6	72	4.32
Delta	Sunki 802	88	11.65	Delta	FF-6	88	12.08
Delta	Sunki 802	105/125	48.75	Delta	FF-6	105/125	51.22
Delta	Sunki 802	144	35.27	Delta	FF-6	144	31.86

Table 6.4.10.4. Production per tree of Midknight and Delta Valencia trees on different rootstocks at Esselen Nursery (Malelane) during the 2005 season.

Cultivar	Rootstock	Kg/tree
Midknight	Sunki 812	64.9
Delta	Sunki 812	79.7
Delta	Sunki 802	70.8
Delta	FF-6	78.7

6.4.11 Evaluation of Grapefruit varieties on new imported rootstocks in the Swaziland area Experiment 416 B by J.Joubert (CRI)

Opsomming

Die prestasie van pomelo variëteite op nuwe, ingevoerde onderstamme op swaar, herplant grondtipes moet ondersoek word. Die produksie, vruggrootte, interne gehalte en skilkleur moet verbeter word. Star Ruby en Marsh het 'n beter produksie gelewer (vestig goed) nadat die bome herplant is vanaf Tambankulu Estate. Die gemiddelde vruggrootte het op telling 48 vir Star Ruby, en telling 40 vir Marsh gestabiliseer, en die interne kwaliteit het verbeter. Evaluasies word verder gedoen.

Introduction

The performance of grapefruit cultivars on new, imported rootstocks on heavy, replant soils must be investigated. The production, fruit size, internal quality and rind colour must be improved.

Materials and methods

Seed of HRS 802, HRS 812, HRS 809 and C61 was imported and propagated in 1996 by Esselen Nursery, an accredited nursery in Malelane.

Star Ruby grapefruit was budded onto the four rootstock hybrids, Marsh grapefruit onto three rootstocks, and Oroblanco onto one rootstock in 1997. The newly imported rootstock hybrids include: Pummelo x trifoliolate orange HRS 802, Changsa x English large flowered trifoliolate orange HRS 809, Sunki x Beneke HRS 812 and Sunki x macrophylla C61. The trees were planted at Tambankulu Estates, Swaziland, in 1999. Experimental trees at Tambankulu Estates were transplanted at Tambuti Estates in Swaziland during November 2000 as certain orchards were to be removed from Tambankulu Estates. The trees were cut back and painted with white PVA. Making use of an excavator, the trees were uprooted and transplanted immediately at the new site. The trees were well watered and in good condition at the time of transplanting.

Table 6.4.11.1. Number of trees per rootstock in the grapefruit trial at Tambuti, Swaziland.

Selection	Rootstock	No.of trees
Marsh	812	1
Marsh	809	2
Marsh	C61	4
Star Ruby	C32	4
Star Ruby	802	2
Star Ruby	809	1
Star Ruby	812	2
Star Ruby	C61	5
Star Ruby	C35	4
Star Ruby	SC	8

Results and discussions

Star Ruby

Star Ruby on SC, Sunki 802, Sunki 809 and C61 produced fruit with a TSS% below 9% (Japan export specification 9%) (Table 6.4.11.2). C32, Sunki 812 and C 35 produced fruit with a TSS% between 10.0% and 10.4%. The juice content was high and varied between 57.6% and 61.6%. Fruit size of all the rootstocks peaked at count 48, followed by count 40 (Table 6.4.11.3). The highest production of 160.4 kg/tree was on C32, followed by C35 with 148.9 kg/tree and Sunki 802 with 136 kg/tree (Table 6.4.11.4).

Marsh

TSS% varied between 8.57% and 9.07% (Table 6.4.11.2). Marsh on Sunki 812 had the highest juice content (57.7%). Fruit size peaked at higher counts compared with Star Ruby with count 40 (Table 6.4.11.3). Sunki 802 produced 124.8 kg/tree, followed by C61 with 114.4 kg/tree and Sunki 812 with 106.5 kg/tree (Table 6.4.11.4).

Conclusions

The Star Ruby and Marsh trees were evaluated for the third season and there were a promising increase in production this season. TSS values increased slightly, but keep in mind the drought and water problems. Evaluations will continue.

Table 6.4.11.2. Internal fruit quality data of grapefruit on different rootstocks at Tambuti Estates on 19 May 2005.

Selection	Rootstock	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Marsh	812	27-48	57.7	9.07	1.11	8.17	3.8	T2-4
Marsh	809	23-48	55.9	8.77	1.15	7.63	2.8	T3-4
Marsh	C61	32-56	54.7	8.57	1.17	7.32	3.7	T2-4
Star Ruby	SC-C1	27-40	57.6	8.97	1.19	7.54	0.1	T1
Star Ruby	SC-C2	27-40	60.2	8.87	1.19	7.45	0.1	T1-2
Star Ruby	C32	36-64	59.6	10.40	1.17	8.89	0.4	T1-2
Star Ruby	802	23-48	58.0	8.77	1.21	7.25	0.1	T1
Star Ruby	809	32-56	59.9	8.05	1.14	7.06	0.3	T1-2
Star Ruby	812	36-48	61.6	10.00	1.18	8.47	0.3	T1-2
Star Ruby	C61	27-56	57.7	8.37	1.20	6.98	0.2	T1
Star Ruby	C35	27-64	60.2	10.20	1.14	8.95	0.4	T1-2

Table 6.4.11.3. Fruit size distribution per rootstock at Tambuti Estate during the 2005 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 61	27	1.13	Marsh	C 61	27	1.31
Star Ruby	C 61	32	1.13	Marsh	C 61	32	1.79
Star Ruby	C 61	36	7.77	Marsh	C 61	36	9.18
Star Ruby	C 61	40	20.77	Marsh	C 61	40	25.40
Star Ruby	C 61	48	53.24	Marsh	C 61	48	49.41
Star Ruby	C 61	64	15.98	Marsh	C 61	64	12.91
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 812	27	1.13	Marsh	Sunki 812	27	1.22
Star Ruby	Sunki 812	32	1.13	Marsh	Sunki 812	32	3.27
Star Ruby	Sunki 812	36	7.77	Marsh	Sunki 812	36	13.88
Star Ruby	Sunki 812	40	20.77	Marsh	Sunki 812	40	39.18
Star Ruby	Sunki 812	48	53.24	Marsh	Sunki 812	48	38.37
Star Ruby	Sunki 812	64	15.98	Marsh	Sunki 812	64	4.08
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 809	27	0.70	Marsh	Sunki 809	27	4.31
Star Ruby	Sunki 809	32	1.39	Marsh	Sunki 809	32	8.19
Star Ruby	Sunki 809	36	6.26	Marsh	Sunki 809	36	25.65
Star Ruby	Sunki 809	40	26.68	Marsh	Sunki 809	40	36.64
Star Ruby	Sunki 809	48	51.28	Marsh	Sunki 809	48	22.63
Star Ruby	Sunki 809	64	13.69	Marsh	Sunki 809	64	2.59
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	Sunki 802	27	0.98	Star Ruby	SC-C1	27	3.59
Star Ruby	Sunki 802	32	1.71	Star Ruby	SC-C1	32	5.39
Star Ruby	Sunki 802	36	8.30	Star Ruby	SC-C1	36	23.36
Star Ruby	Sunki 802	40	23.20	Star Ruby	SC-C1	40	34.08
Star Ruby	Sunki 802	48	51.77	Star Ruby	SC-C1	48	29.04
Star Ruby	Sunki 802	64	14.04	Star Ruby	SC-C1	64	4.53
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Star Ruby	C 32	27	0.59	Star Ruby	SC-C2	27	2.64
Star Ruby	C 32	32	0.64	Star Ruby	SC-C2	32	3.58
Star Ruby	C 32	36	7.14	Star Ruby	SC-C2	36	19.86
Star Ruby	C 32	40	23.29	Star Ruby	SC-C2	40	34.53
Star Ruby	C 32	48	54.36	Star Ruby	SC-C2	48	35.07
Star Ruby	C 32	64	13.97	Star Ruby	SC-C2	64	4.32
Cultivar	Rootstock	Size	% Fruit				
Star Ruby	C 35	27	0.54				
Star Ruby	C 35	32	0.86				
Star Ruby	C 35	36	6.34				
Star Ruby	C 35	40	17.72				
Star Ruby	C 35	48	47.69				
Star Ruby	C 35	64	26.85				

Table 6.4.11.3. Production per tree of grapefruit on different rootstocks at Tambuti Estates during 2005.

Cultivar	Rootstock	Kg/tree
Star Ruby	C 61	107.3
Star Ruby	Sunki 812	108.5
Star Ruby	Sunki 809	132.5
Star Ruby	Sunki 802	136.0
Star Ruby	SC-C1	123.3
Star Ruby	SC-C2	133.3

Star Ruby	C 32	160.4
Star Ruby	C 35	148.9
Marsh	C 61	114.4
Marsh	Sunki 812	106.5
Marsh	Sunki 809	124.8

6.4.12 Evaluation of various Valencia selections on different rootstocks in the Komatipoort area Experiment 590 B by J.Joubert (CRI)

Opsomming

Evalueer en bepaal die tuinboukundige potensiaal en vermoë van verskillende Valencia variëteite op verskillende onderstamme. Bepaal die beste onderstam kombinasie vir hierdie nuwe variëteite. Maak betekenisvolle kommersiele aanbevelings vir die produsente. Hierdie onderstam proef is vir die eerste keer ge-oes en die bome is nog jonk. Daar is interessante verskille in drag geproduseer tussen die verskillende kombinasie. Van die onderskeie bo-stam seleksies kom verseker vroeër in drag op 'n jonger ouderdom. Dit kan ook beteken dat die bome gouer in vol produksie kan kom en begin inkomste genereer vir die produsent. Hierdie tipe eienskappe kan 'n groot rol speel met die keuse van watter bo-onderstam kombinasie aangeplant moet word.

Introduction

Evaluate and assess the horticultural performance and capability of various new Valencia selections on different rootstocks. Determine the superior rootstock combinations for these new selections. Be able to make credible commercial recommendations.

Materials and methods

Five trees of each cultivar x rootstock combination were planted in 1998.

Evaluate visually to determine production per tree, trueness to type and compatibility with scion and harvest each tree with the sizer to determine production per tree as well as fruit size distribution per tree. Samples will be taken and internal quality tested and analysed. Fruit colour will be evaluated and analysed.

Table 6.4.12.1. List of cultivar x rootstock combinations in the Valencia trial at TSB Hectorspruit in the Komatipoort area.

Selection	Rootstock
Delta (Control)	C35
Delta (Control)	CC
Delta (Control)	KC
Delta (Control)	MxT
Delta (Control)	SC
Delta (Control)	Terrabella
Delta (Control)	X639
McClean SL	C35
McClean SL	CC
McClean SL	KC
McClean SL	MxT
McClean SL	SC
McClean SL	Terrabella
McClean SL	X639
Midnight	C35
Midnight	CC
Midnight	KC
Midnight	MxT
Midnight	SC
Midnight	Terrabella
Midnight	X639
Portsgate	C35
Portsgate	CC

Portsgate	KC
Portsgate	MxT
Portsgate	SC
Portsgate	Terrabella
Portsgate	X639

Results and Discussion

Internal fruit quality of most of the combinations did not comply with minimum export standards (Table 6.4.12.2). This trial was established in 2002 and the trees were 3 years old at the time of this evaluation. Fruit size on young trees varied considerably because of the number of fruit set. Take note of the different selections bearing the highest yield on the young trees (Table 6.4.14.4). Midnight on SC bore 2.8 kg/tree, Portsgate on SC bore 32.7 kg/tree, Delta on MxT bore 24.9 kg/tree and McClean SL bore 36.5 kg/tree. Interesting to note is that Midnight produced very low yields on the young trees in comparison to the other selections. The precocity of the other selections is important as these trees might be in full production at a younger age, generating a quicker return in investment.

Conclusions

The trial looks promising at this stage. It will be very valuable to evaluate the production increase on the young trees. Over the long term this will give an indication of the precocity of the combinations.

Table 6.4.12.2. Internal fruit quality data for Valencias on different rootstocks at TSB Hectorspruit on 6 September 2005.

Selection	Rootstock	Count	Juice %	TSS %	Acid %	Ratio	Ave. seed	Colour
Midnight	C35	40-72	57.6	10.69	0.52	20.56	0.0	T3-4
Midnight	CC	36-72	58.3	10.46	0.64	16.34	0.0	T3-5
Midnight	KC	36-88	54.9	9.60	0.80	12.00	3.8	T3-7
Midnight	MxT	48-72	59.0	10.69	0.71	15.06	0.0	T3-5
Midnight	SC	40-64	56.6	9.96	0.66	15.09	0.0	T4
Midnight	TB	36-88	60.5	10.16	0.71	14.31	0.0	T3-4
Midnight	X639	56-88	67.1	12.49	0.61	20.48	0.0	T3-4
Portsgate	C35	40-72	54.3	10.89	0.57	19.11	0.0	T4-5
Portsgate	CC	40-105	54.7	11.39	0.53	21.49	0.0	T3-5
Portsgate	KC	40-72	54.6	10.36	0.48	21.58	0.0	T4-5
Portsgate	MxT	40-72	52.9	10.16	0.63	16.13	0.0	T3-5
Portsgate	SC	48-72	53.7	10.40	0.61	17.05	0.5	T3-6
Portsgate	TB	40-88	55.1	10.00	0.57	17.54	0.0	T3-4
Portsgate	X639	56-88	54.9	10.10	0.53	19.06	0.0	T3-6
Delta	C35	48-72	39.6	9.70	0.55	17.64	1.5	T4-5
Delta	CC	64-125	61.0	11.09	0.57	19.46	0.0	T4-5
Delta	KC	72-105	55.1	11.09	0.56	19.80	0.0	T3-4
Delta	MxT	56-125	54.1	10.16	0.62	16.39	0.0	T5-6
Delta	SC	48-72	50.8	9.64	0.61	15.80	0.0	T3-6
Delta	TB	72-125	57.7	10.46	0.61	17.15	0.0	T4-5
Delta	X639	64-125	54.6	9.90	0.55	18.00	0.0	T4-6
McClean SL	C35	48-88	55.5	10.46	0.54	19.37	0.0	T3-4
McClean SL	CC	40-105	53.9	10.83	0.60	18.05	0.0	T3-4
McClean SL	KC	40-105	54.8	10.89	0.57	19.11	0.0	T3-4
McClean SL	MxT	36-88	53.6	10.93	0.59	18.53	0.0	T1-4
McClean SL	SC	40-125	55.7	9.60	0.65	14.77	0.1	T5-6
McClean SL	TB	40-105	54.3	9.22	0.57	16.18	0.3	T3-5
McClean SL	X639	40-72	75.5	10.69	0.56	19.09	0.0	T4-5

Table 6.4.12.3. Fruit size distribution per rootstock at TSB Hectorspruit during the 2005 season.

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	C35	48	44.44	Delta	C35	48	0.78
Midnight	C35	56	37.04	Delta	C35	56	4.65
Midnight	C35	72	11.11	Delta	C35	72	14.73
Midnight	C35	88	7.41	Delta	C35	88	18.60
Midnight	C35	105/125	0.00	Delta	C35	105/125	37.98
Midnight	C35	144	0.00	Delta	C35	144	23.26
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	CC	48	12.90	Delta	CC	48	0.00
Midnight	CC	56	32.26	Delta	CC	56	1.75
Midnight	CC	72	35.48	Delta	CC	72	5.00
Midnight	CC	88	12.90	Delta	CC	88	10.25
Midnight	CC	105/125	6.45	Delta	CC	105/125	37.50
Midnight	CC	144	0.00	Delta	CC	144	45.50
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	KC	48	16.67	Delta	KC	48	1.15
Midnight	KC	56	33.33	Delta	KC	56	1.15
Midnight	KC	72	33.33	Delta	KC	72	6.90
Midnight	KC	88	0.00	Delta	KC	88	8.05
Midnight	KC	105/125	16.67	Delta	KC	105/125	36.78
Midnight	KC	144	0.00	Delta	KC	144	45.98
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	MxT	48	4.76	Delta	MxT	48	0.34
Midnight	MxT	56	14.29	Delta	MxT	56	3.89
Midnight	MxT	72	38.10	Delta	MxT	72	8.12
Midnight	MxT	88	28.57	Delta	MxT	88	10.83
Midnight	MxT	105/125	14.29	Delta	MxT	105/125	41.79
Midnight	MxT	144	0.00	Delta	MxT	144	35.03
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	SC	48	20.93	Delta	SC	48	0.59
Midnight	SC	56	37.21	Delta	SC	56	4.88
Midnight	SC	72	25.58	Delta	SC	72	13.09
Midnight	SC	88	11.63	Delta	SC	88	14.65
Midnight	SC	105/125	4.65	Delta	SC	105/125	38.87
Midnight	SC	144	0.00	Delta	SC	144	27.93
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	TB	48	55.56	Delta	TB	48	0.00
Midnight	TB	56	29.63	Delta	TB	56	0.43
Midnight	TB	72	3.70	Delta	TB	72	2.60
Midnight	TB	88	3.70	Delta	TB	88	5.63
Midnight	TB	105/125	7.41	Delta	TB	105/125	27.27
Midnight	TB	144	0.00	Delta	TB	144	64.07
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midnight	X639	48	0.00	Delta	X639	48	0.38
Midnight	X639	56	7.69	Delta	X639	56	4.92
Midnight	X639	72	30.77	Delta	X639	72	7.95

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Midknight	X639	88	23.08	Delta	X639	88	8.71
Midknight	X639	105/125	38.46	Delta	X639	105/125	39.77
Midknight	X639	144	0.00	Delta	X639	144	38.26
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	C35	48	5.93	McClellan SL	C35	48	5.80
Portsgate	C35	56	28.06	McClellan SL	C35	56	26.20
Portsgate	C35	72	31.62	McClellan SL	C35	72	32.00
Portsgate	C35	88	16.21	McClellan SL	C35	88	20.40
Portsgate	C35	105/125	14.62	McClellan SL	C35	105/125	13.60
Portsgate	C35	144	3.56	McClellan SL	C35	144	2.00
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	CC	48	10.38	McClellan SL	CC	48	8.91
Portsgate	CC	56	17.92	McClellan SL	CC	56	22.56
Portsgate	CC	72	24.53	McClellan SL	CC	72	21.73
Portsgate	CC	88	16.04	McClellan SL	CC	88	18.66
Portsgate	CC	105/125	24.53	McClellan SL	CC	105/125	19.78
Portsgate	CC	144	6.60	McClellan SL	CC	144	8.36
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	KC	48	3.77	McClellan SL	KC	48	8.61
Portsgate	KC	56	23.97	McClellan SL	KC	56	24.93
Portsgate	KC	72	19.86	McClellan SL	KC	72	21.07
Portsgate	KC	88	15.07	McClellan SL	KC	88	18.69
Portsgate	KC	105/125	20.89	McClellan SL	KC	105/125	22.85
Portsgate	KC	144	16.44	McClellan SL	KC	144	3.86
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	MxT	48	2.86	McClellan SL	MxT	48	11.21
Portsgate	MxT	56	12.70	McClellan SL	MxT	56	15.52
Portsgate	MxT	72	18.10	McClellan SL	MxT	72	34.48
Portsgate	MxT	88	16.19	McClellan SL	MxT	88	16.38
Portsgate	MxT	105/125	34.29	McClellan SL	MxT	105/125	20.69
Portsgate	MxT	144	15.87	McClellan SL	MxT	144	1.72
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	SC	48	4.25	McClellan SL	SC	48	5.53
Portsgate	SC	56	17.45	McClellan SL	SC	56	20.00
Portsgate	SC	72	24.05	McClellan SL	SC	72	19.61
Portsgate	SC	88	18.04	McClellan SL	SC	88	16.18
Portsgate	SC	105/125	27.57	McClellan SL	SC	105/125	27.50
Portsgate	SC	144	8.65	McClellan SL	SC	144	11.18
Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	TB	48	2.49	McClellan SL	TB	48	7.95
Portsgate	TB	56	12.47	McClellan SL	TB	56	25.83
Portsgate	TB	72	22.19	McClellan SL	TB	72	26.49
Portsgate	TB	88	14.46	McClellan SL	TB	88	13.58
Portsgate	TB	105/125	34.41	McClellan SL	TB	105/125	20.53
Portsgate	TB	144	13.97	McClellan SL	TB	144	5.63
Portsgate	X639	48	2.07	McClellan SL	X639	48	3.96
Portsgate	X639	56	14.21	McClellan SL	X639	56	20.05

Cultivar	Rootstock	Size	% Fruit	Cultivar	Rootstock	Size	% Fruit
Portsgate	X639	72	22.74	McClellan SL	X639	72	27.48
Portsgate	X639	88	17.83	McClellan SL	X639	88	21.29
Portsgate	X639	105/125	34.37	McClellan SL	X639	105/125	24.50
Portsgate	X639	144	8.79	McClellan SL	X639	144	2.72

Table 6.4.12.3. Production per tree of Valencia selections on different rootstocks at TSB Hectorspruit during the 2005 season.

Cultivar	Rootstock	Kg/tree
Midknight	C35	2.0
Midknight	CC	1.9
Midknight	KC	0.6
Midknight	MxT	1.3
Midknight	SC	2.8
Midknight	TB	2.1
Midknight	X639	0.8
Portsgate	C35	13.4
Portsgate	CC	5.4
Portsgate	KC	14.0
Portsgate	MxT	14.2
Portsgate	SC	32.7
Portsgate	TB	18.7
Portsgate	X639	17.9
Delta	C35	5.3
Delta	CC	14.8
Delta	KC	3.4
Delta	MxT	24.9
Delta	SC	21.3
Delta	TB	7.3
Delta	X639	10.1
McClellan SL	C35	26.7
McClellan SL	CC	18.2
McClellan SL	KC	17.2
McClellan SL	MxT	6.3
McClellan SL	SC	36.5
McClellan SL	TB	15.1
McClellan SL	X639	20.9

7 CITRUS IMPROVEMENT PROGRAMME 2005

By Thys du Toit and Louise Jackson (CRI)

7.1 SUMMARY

Citrus Foundation Block: A total of 2 627 528 buds were supplied by the Citrus Foundation Block during 2005, which exceeds supply for 2004 by 312 798. The two most popular cultivars in 2005 were Star Ruby (20.3% of total supplied) and Midnight (12.9%). The drastic reduction in the demand for citrus seed – 2 353 litres in 2005 compared to 4 033 litres in 2004 - can be attributed to a shrink in demand from export markets. A surplus of 1 420 litres of processed seed is still available. Seven new cultivars were received from the ITSC for establishment, evaluation and increase, compared to one cultivar in 2004. The new insect-proof house was filled with 30 000 increase trees from category 1 and 2 cultivars. Currently 4 new bays are being constructed for a further 15 600 increase trees.

Nursery Accreditation: 22 nurseries were audited in May and November 2005, of which 19 were accredited, two provisionally accredited and one accredited as a farm nursery for own use. In general the nurseries are maintaining a good standard.

Tree Certification: The demand increased to 1 866 176 trees in 2005, compared to 1 198 373 trees in 2004.

Statutory Improvement Programme: The document in support of the application to have the Citrus Improvement Scheme registered will be submitted to the Department of Agriculture towards the end of April 2006.

Protected zone around the CFB: The final document to establish a citrus-free area around the Citrus Foundation Block is in the process of being submitted to the Department of Agriculture.

Shoot-tip grafting and Gene bank: The CRI's Virological Department in Nelspruit has established 213 cultivars to date in a gene bank in its glasshouse. Applications have been submitted for shoot tip grafting of 28 cultivars.

An overview of operations in the CIP follows.

OPSOMMING

Sitrus Grondvesblok: 'n Totaal van 2 627 528 okuleerhout is deur die Sitrus Grondvesblok verskaf wat 312 798 meer is as in 2004 met Star Ruby op 20.3% en Midnight op 12.9% van die totaal die twee mees populêre kultivars. 'n Drastiese afname in die verkope van saad van 2 353 liter in 2005 in vergelyking met 4 033 liter in 2004 wat toegeskryf word aan die krimpemde uitvoer aanvraag. 'n Surplus van 1 420 liter verwerkte saad is nog in voorraad. Vanaf die ITSG is 7 nuwe kultivars ontvang vir vestiging, evaluering en vermeerdering in vergelyking met een in 2004. Die nuwe insekbeheerde kweekhuis is gevul met 30 000 vermeerderingsblokbome wat bestaan uit kategorie 1 en 2 kultivars. Tans word nog vier koepels voorberei vir 'n verdere 15 600 vermeerderingsblokbome.

Kwekery Akkreditasie: 22 Kwekerye is in Mei en November besoek waarvan 19 geakkrediteer is, twee voorwaardelik en een as 'n plaas kwekery vir slegs eie gebruik. Oor die algemeen is die standaard goed.

Boomsertifisering: Die aanvraag het gestyg na 1 866 176 bome in 2005 in vergelyking met 1 198 373 bome in 2004.

Statutêre Verbeteringsprogram: Die dokument ter voorlegging aan die Departement van Landbou om die Suid-Afrikaanse Sitrusverbeteringskema te registreer sal teen einde April 2006 ingedien word.

Beskermdede sone rondom die SGB: Die finale dokument vir 'n sitrusvrye area rondom die SGB is in die proses om aan die Departement van Landbou voorgelê te word.

Groeipuntenting en Genebron: Die CRI se Virologie Departement in Nelspruit het tot op datum 213 kultivars gevestig as 'n genebron in 'n glashuis by die departement. Aansoeke vir Groeipuntenting is ingedien vir 28 kultivars.

Citrus Foundation Block

Budwood supply during 2005 compared to the 2 preceding years, of the 10 most popular cultivar selections.

2005			2004			2003		
Selection	Buds	%	Selection	Buds	%	Selection	Buds	%
TOTAL	2627528		TOTAL	2314730		TOTAL	2379385	
Star Ruby	533913	20.3%	Midnight	284815	12.3%	Star Ruby	212450	9.2%
Midnight	337970	12.9%	Star Ruby	275550	11.9%	Bahianinha	210220	9.1%
Bahianinha	229710	8.7%	Bahianinha	183180	7.9%	Turkey	203200	8.8%
Delta	156165	5.9%	Du Roi	122870	5.3%	Palmer	173730	7.5%
Turkey	138150	5.3%	Eureka	116350	5.0%	Nadorcott 1	169500	7.3%
Palmer	126696	4.8%	Palmer	113680	4.9%	Midnight	127450	5.5%
Du Roi	84610	3.2%	Delta	109140	4.7%	Eureka	117450	5.1%
Autumn Gold	83200	3.2%	Turkey	102440	4.4%	Newhall	102460	4.4%
Cal.Lane Late	77500	2.9%	Nadorcott 1	67070	2.9%	Miho Wase	102100	4.4%
Washington	73410	2.8%	Eureka SL	59432	2.6%	Lina	82050	3.5%

Budwood supply per receiving area during 2005 compared to the 2 preceding years.

Area	2005	%	2004	%	2003	%
Eastern Cape	478079	18.2%	427080	18.5%	546660	23.0%
Western Cape	383089	14.6%	368255	15.9%	365665	15.4%
Northern Cape	46850	1.8%	68720	3.0%	116990	4.9%
Kwazulu-Natal	15500	0.6%	16500	0.7%	36030	1.5%
Limpopo	1252503	47.7%	1090435	47.1%	930650	39.1%
Mpumalanga	312552	11.9%	228750	9.9%	244590	10.3%
North West	82780	3.2%	86990	3.8%	101100	4.2%
Mozambique	12250	0.5%	0	0.0%	5400	0.2%
Swaziland	42225	1.6%	0	0.0%	10600	0.4%
Zimbabwe	1700	0.1%	28000	1.2%	21700	0.9%
Total	2627528		2314730		2379385	

Seed supplied per rootstock selection, local and export during 2005.

Area Name	C35	CC	CM	MXT	RL	RLS	SC	TC	VA	X639	YC	Total	Local	Export
Australia/NZ							8	30				38	0	38
Carribbean							7					7	0	7
Eastern Cape	43	101			24		16	40	9	13		246	246	0
Europe		100										100	0	100
Far East	20	65	30	20			70			150		355	0	355
KwaZulu Natal				2			8	4	2	2		18	18	0
Limpopo	47	265		25	84		311	110	2	38	4	886	886	0
Mpumalanga	3	20			8		16	9		2		58	58	0
North West Province	6	6			12		4		6	4		38	38	0
Northern Cape										16		16	16	0
Other African States		2	2				50	7	1			62	0	62

USA		200										200	0	200
Western Cape	50	155			74.5		20	10		20		329.5	329.5	0
TOTAL	169	914	32	47	203	0	510	210	20	245	4	2353.5	1591.5	762

Seed supplied, local and export 2003-2005.

	2005	2004	2003
South Africa	1591.5	2020	1240.5
Export	762	2013	1365.5
Total	2353.5	4033	2606

Nursery Accreditation

In total 22 citrus nurseries applied for accreditation and were audited in May and November 2005. Of these, 19 were accredited, two were provisionally accredited and one registered as a farm nursery for own use. In general the nurseries are maintaining a good standard. Growers should visit nurseries and ensure that they are satisfied with the standard in the nursery before selecting which nursery to order from. Any problems with nursery trees should first be discussed with the nursery in an effort to resolve the conflict. If no satisfaction is obtained through this route then contact the Citrus Improvement Programme Manager, Thys du Toit at: Tel: 041-9925366 Fax: 041-9227416 Cell: 0828892363 E-mail: tdt@cri.co.za

The following nurseries were accredited during 2005.

Nursery	Address	Telephone	Accreditation
Apapanzi	P O Box 147, Kirkwood, 6120	042 2300790	Full
B F Joubert	P O Box 193, Kirkwood, 6120	042 2300309	Full
Casmar	P O Box 3 Mooinooi, 0325	0145 743152	Full
Du Roi	P O Box 66, Letsitele, 0885	015 3451650	Full
Esselen	P O Box 100, Malelane, 1320	013 7900160	Full
H J Joubert	P O Box 207, Montagu, 6720	0236 142237	Full
La Rhyn	P O Box 111, Citrusdal, 7340	022 9213541	Full
Letsitele	P O Box 1, Letsitele, 0885	015 3451600	Full
Mistkraal	P O Box 16, Kirkwood, 6120	042 2301461	Full
Ngwenya	P O Box 36, Malelane, 1320	013 7903004	Full
Nucellar	P O Box 69, Simondium, 7670	021 8741033	Full
Paksaam	P O Box 16, Patensie, 6335	042 2830201	Full
Sondagsrivier	P O Box 304, Kirkwood, 6120	042 2300349	Full
Stargrow	P O Box 12536, Die Boord, 7613	021 9212232	Full
Tweeling	P O Box 190, Kirkwood, 6120	042 230 1408	Full
Vaalharts	P O Box 317, Hartswater, 8570	053 4740565	Full
Waterfall	P O Box 339, Adelaide, 5760	046 6840738	Full
Westfalia	P O Box 14, Duiwelskloof, 0835	015 309 0026	Full
Witkrans	P O Box 17, Boshoeck, 0301	014 5733036	Full

New Cultivars

During the past 12 months 7 new cultivars were received from the ITSC, compared to 1 during 2004. Five of these cultivars are under the control of private cultivar agents and information pertaining to these cultivars is treated as confidential. The 2 open cultivars are Yen Ben Lisbon lemon and Bergamot (for oil production used in perfume and tea).

New Developments

The new insect-controlled greenhouse was filled with 30 000 increase trees in 10 litre plant bags, consisting of category 1 and 2 cultivars, of which two-thirds are in full production. Currently 4 new bays are being constructed to accommodate a further 15 000 increase trees. The expired increase trees in Shade-house 1 and 2 have been

disposed of, and it is planned to replace these shade-houses with the next phase of an insect-controlled greenhouse in 2007.

Tree Certification

Area	2005		2004		2003	
	Trees	%	Trees	%	Trees	%
Botswana	270	0.0%	-		-	
Eastern Cape	401207	21.5%	530587	44.3%	504974	34.7%
Gauteng	23955	1.3%	-		-	
KwaZulu-Natal	54601	2.9%	-		-	
Limpopo	432375	23.2%	310968	25.9%	591012	40.6%
Mozambique	600	0.0%	-		-	
Mpumalanga	666730	35.7%	181501	15.1%	54259	3.7%
North-West Province	30616	1.6%	5250	0.4%	4835	0.3%
Northern Cape	-		-		167552	11.5%
Swaziland	39329	2.1%	-		-	
Western Cape	197493	10.6%	170067	14.2%	132364	9.1%
Zimbabwe	19000	1.0%	-		-	
Total	1866176		1198373		1454996	

The demand for tree certification increased to 1 866 176 in 2005 compared to 1 198 373 in 2004. The actual number of certificates issued decreased from 554 to 449 because more information is given on the certificate to conserve costs. The annual number of trees certified is still significantly lower than the average annual supply of budwood. Tree certification is viewed by EurepGAP as a “minor must” for accreditation, and this requirement’s priority cannot easily be changed due to the international scope of EurepGAP. Tree certification is nevertheless important in the EurepGAP context as a grower must score 95% in total on all the “minor must” categories.

Statutory Improvement Programme

After a long search a jurispudent with the necessary experience was found and commissioned to write a document describing the South African Citrus Improvement Programme in a legal format suitable for submission to the Department of Agriculture. He was supplied with the necessary documentation describing the programme and several meetings were held with him and various stakeholders. A concept document describing the South African Citrus Improvement Scheme was prepared and circulated to the various industry stakeholders to give them an opportunity to submit their contributions, so that changes could be made to the document where necessary. SACNA and SANA have already agreed that submission of the document may proceed. The final document will be translated into Afrikaans and submitted by the end of April 2006 to the Registrar, Plant Improvement Act, National Department of Agriculture, for approval.

Protected zone around the CFB

The final document to declare a 5 km radius citrus-free zone around the Citrus Foundation Block has been submitted to the Department of Agriculture for approval.

Shoot Tip Grafting and Gene bank

The CRI’s Virological Department in Nelspruit established the required laboratory to conduct shoot tip grafting, virus indexing and pre-immunisation of all locally selected cultivars. Kobus Breytenbach is responsible for this function and he is being advised by Dr Faan van Vuuren. To date 28 new selections have been received from clients for shoot tip grafting and indexing. A back-up of the virus-free gene bank at the ARC-ITSC has been

established at the CRI and a total of 213 cultivars and selections have been established in the glass-house. This department's full report is given under 4.2.4.

8 INTERNATIONAL VISITS

8.1 H.F. LE ROUX

8.1.1 CHINA VISIT – APRIL 2005

China was visited during April 2005 after a request for technical assistance by the Chongqing Ganges Citrus Project was received. This was in line with the negotiations between CGA/CRI and China during 2003/4 prior to allowing South African citrus into China.

Citrus is planted across 19 provinces in China. The provinces of Hunan, Jiangxi, Sichuan, Fujian, Zhejiang, Guangxi, Hubei, Guangdong, and Chongqing produce 95% of the citrus. The hectareage of plantings increased from 342 000 ha in 1978 to 1.27 m ha in 2000. Production increased from 380 000 to 8.78 m tons during the same time. In 2005 China surpassed the USA as the second largest producer of citrus in the world. The average yields for 2004 were, however, a mere 7,5tons/ha.

Mandarins (60%) dominate the Chinese citrus production followed by sweet oranges (30%). Seventy-five percent of China's citrus production peaks in November and December and the market is flooded for a three-month period after which there is a shortage in supply.

Two provinces were visited, viz. the Chongqing province in the Sichuan basin and the Fengjie county in the neighbouring Hunan province. The completion of the Three Gorges Dam Project on the Yangtze River has resulted in the relocation of thousands of farmers and navel production is seen as one of the key means of job creation for resettled communities. The Chongqing Ganges Fruit Company has been set up to develop and manage 1 m tons of citrus by 2013. Two million navel trees have already been planted and by 2008 4.5 m trees will be planted. The trees are supplied by their two nurseries, one at Bei Bei and the other at Junquin (Juilong). The main aim for the writer's visit was to inspect these nurseries and to determine the *Phytophthora* status thereof. See report attached.

Itinerary

Date	Day	Activity	Overnight
7 April	Th	Fly Jhb-Hong Kong Travel to Shenzen Airport. (Mainland China) Fly Shenzen-Chongqing. Meet Mr Allan Chuu (Ops Director) & Mr William Chu (Project Technical Manager)	Chongqing
8 April	Fr	Depart Jiangjin for Chongxian County. Visit Bei Bei nursery. Sample nursery for <i>Phytophthora</i> . Visit demonstration farm. Visit Changshou. Visit new plantings and Kowloon nursery. Dinner with Chongqing Vice-Mayor, Chan Guang Guo and the Chief of the Agricultural Department, Mr. Wang Yue.	Chongqing
9 April	Sa	Visit Jiulong Nursery in Jiangjin. Sample nursery for <i>Phytophthora</i> . Visit demonstration farms and You Xi orchard and Bai Sha orchards and pack-house. Visit the Chongqing Fruit Research Institute Process <i>Phytophthora</i> samples. Dinner with Chongqing Party Secretary.	Chongqing
10 April	So	Depart for Changshou. Inspect industrial site. Inspect new planting area. Visit Zhong County to see Valencia orchards planted for processing plant.	Wan Zhou
11 April	Mo	Depart for Fengjie (Hunan Province) by ferry on Yangtze river. Visit newly established and old navel orchards.	Fengjie
12 April	Tu	Inspect existing navel orchards. Dinner with the Director of Agriculture of the Hunan province and with the Chairman of the Fengjie Citrus Growers association.	Fengjie

13 April	We	Return to Chongqing. Further processing of <i>Phytophthora</i> samples. Visit the Chongqing University. Visit the Chinese Research Institute. Discussions with the Director dr Zhou Changyong and Prof Chen Zhusheng.	Chongqing
14 April	Th	<i>Phytophthora</i> analysis. Final meeting. Fly Chongqing – Hong Kong	
15 April	Fr	Fly Hong Kong – Johannesburg. Have <i>Phytophthora</i> genus identified by ARC at Roodeplaat.	

General impressions

- 1) No citrus foliar diseases could be found in any of the areas visited in the Chongqing and Hunan provinces. This differs from the provinces to the south where citrus canker, CBS and Huanglongbin are present.
- 2) *Phytophthora* was abundant in all the old orchards and in both the nurseries visited. This can be eliminated to a large extent by ensuring *Phytophthora*-free irrigation water in the nurseries and by budding the trees at a height of 25 cm instead of 10 cm.
- 3) Most of the soils especially those at the demonstration farms visited were unacceptable for citrus cultivation. Only the soils in the Zhong County where Valencias were planted for processing were suitable for citrus. This single factor explains much of their low yields. Other factors involved are a high incidence of *Phytophthora*, the wrong rootstocks for the different soils and poor irrigation.
- 4) Most of the orchards visited were without irrigation. The Chinese plan to install irrigation in some of these orchards. *Phytophthora* problems could increase and, in fact, be counter productive if proper care is not taken to manage the irrigation properly.
- 5) In the past most of the navels planted in China have been the Washington navel. Presently they have some of their own selections and they have also imported the Powell, Barnfield and Chislett from Australia.
- 6) There is huge government involvement in establishing the Chongqing and Fengjie areas as major navel production areas for the future. The Chongqing Ganges Fruit Company is the major role player in this. Their director Leo Chiu is a highly influential person and Mr. Gerd Born, the managing director of Medspan, has well-established ties with them through a joint venture.
- 7) Medspan uses Citrigold to assist them with technical inputs when required.
- 8) Gerd Born has also established strong relations with Korea and, if needed, is willing to assist the South African authorities both in China and Korea to enable them to understand the way in which these cultures operate.
- 9) Dr Zhou Changyong from CRI (China) will be the link with the southern hemisphere countries during the next ICC to be held in China in 2008. It is important that they be informed that Prof Vaughan Hattingh will succeed Dr Sarel du Plessis as the next representative for South Africa.
- 10) The CRI in China has good facilities and consists of 230 personnel, but they lack funds for research. The Chongqing Ganges Fruit Project will hopefully influence the Chinese government in solving this problem.
- 11) The Chinese view is that it was as a result of visits by Drs. G. Barry (March 2005) and H. le Roux (April 2005), that talks were established between CRI South Africa and CRI China, resulting in official ties being established between the two organisations. CRI South Africa should use the contacts they have with Citrosan through the Ganges Fruit Project to strengthen ties with CRI China.
- 12) The Chinese expressed on more than one occasion their eagerness to acquire the Eureka Seedless from South Africa. If this is achieved and they do acquire the Eureka Seedless, it will be extremely difficult to protect its patent rights in China. However, this situation may change in future and China may become more stringent with regard to patents and the protection thereof. This factor was mentioned by the vice Mayor of Chongqing during a conversation.



Fig. 8.1.1. Picture that appeared in the Peoples Daily in China on the 8 April 2005. It shows the Vice-mayor of the Chongqing province with the South African technical delegation, and members of the Chongqing Ganges Fruit Project.

8.2 M.M.N. DU TOIT

8.2.1 INTERNATIONAL SOCIETY OF CITRUS NURSERYMEN – ISCN - 7TH CONGRESS HELD IN EGYPT 17 – 21 SEPTEMBER 2005

1. Introduction

The scheduling of this Congress was under threat for a time as the previous ISCN President retired in Brazil before doing the necessary succession planning. Thanks to the support of certain role players the Congress nevertheless did take place in Egypt under the leadership of the newly elected President, Mr Hassan Marei of Egypt. Essential measures to ensure the continued existence of the ISCN were discussed at the management meeting during the Congress. Mr Ian Tolley of Australia played a key role in this matter. More researchers than nursery representatives attended the congress. According to information currently available, 68 international delegates attended the congress from 15 different countries. Graham Barry, Bryan Offer, Louis von Broembsen and Thys du Toit represented South Africa. In general the Congress was well organized, although everything did not always happen on time.

2. The Citrus Industry in Egypt

Citrus is considered to be the most important fruit industry with 146 986 hectares of citrus plantings yielding 2.748 million tons in 2004 of which 525 000 tons were exported. Seventy three percent of the crop comes from the delta region while 27% is produced in the desert regions. Navels, Valencias, Mandarins and Mexican Limes are grown, mainly on Sour orange (Bitter Seville) rootstock, although Volckameriana is gaining popularity, and other, quality enhancing rootstocks are starting to be used experimentally. Production per hectare is very low at an average of 15–17 tons per hectare compared to a world average of 30-60 tons per hectare.

In 1996 nursery practices were improved with the help of international consultants to help combat threats to the industry from viruses and associated diseases such as *Tristeza*, *Psorosis*, *Exocortis*, *Cachexia*, *Concave gum*, *Impietratura*, *Gummy bark*, *Stubborn* and *Wood pocket*. A comprehensive certification programme was developed in 1999. An indexing and increase facility was established to supply virus-free budwood to the nursery industry bi-annually in March and September for re-multiplication. The Congress

delegates visited this facility in Bahteem. It is anticipated that more than 100 000 buds will be supplied in September this year. New cultivars are imported mainly from California. *Phytophthora* is still a big problem both in nurseries and the orchards. Nurseries are moving from open ground production to producing trees in plant bags on recommendations from consultants.

The delegates visited the two best nurseries. No information was supplied as to how many other nurseries there were. The first visit was to Magrabi Agriculture (MAFA) 150 km outside Cairo in the desert. This business consists of 4 farms totalling 3 100 hectares, of which 2 300 hectares are planted to citrus. The nursery was established 12 years ago to produce trees for own use, but over the past 4 years nursery trees were also sold to other citrus growers. The trees are grown in plant bags in plastic tunnels and then moved to shadehouses to harden off. The nursery produces 450 000 budded trees annually. Mother trees are established in two planthouses and budwood is cut from these trees for re-multiplication only after the trees have borne true-to-type fruit. Increase blocks are housed in insect-proof planthouses with the necessary double doors. They have their own indexing facility and laboratory where regular ELISA tests are done as well as tests for *Phytophthora*, *Pythium* and nematodes. Recently a PCR facility was put in place to search for the presence of viroids. The nursery is being upgraded with improvements such as lifting the plant bags up onto bricks, instead of standing them on plastic on the ground. The irrigation is also being improved from overhead sprays and hand held hoses to drip irrigation. The growing media used in the plant bags consists mainly of sand, with a small quantity of peat moss, which promotes healthy root development. In general the standard of the trees is good. I am convinced that this nursery can develop into a very good facility with the assistance of international consultants such as Mr Brian Beyleveld, formerly of South Africa, and the capital investment available to them.

The second nursery visited was Marei Nursery & Orchards in the Giza delta region. This business belongs to Mr Hassan Marei and he produces +/- 500 000 trees annually in 3 litre plant bags. Use is made of various structures including plastic tunnels, planthouses with insect-proof netting and shadehouses. Coarse sand mixed with peat moss is used which promotes a good healthy root system. Many of the techniques used in the nursery such as seed germination, the growing of seedlings in seed trays, and many other nursery practices were the same as those used in Du Roi Nursery in South Africa because Mr Ngama Munduku has been used as a consultant in the nursery.

3. Congress Papers

In general the papers delivered were interesting and mostly in English so that little translation was required. As in previous years there were some papers, which did not address matters specific to the citrus nursery industry, and there were too few papers addressing practical problems in the nursery. This weakness was discussed at the management meeting and in future a stricter selection criteria will be imposed on proposed papers. It was also proposed that the Internet site be upgraded so that experts in the various fields can respond to questions posed on problems in the nursery. Each delegate received a summary of the papers presented at the Congress and a CD will be supplied later, which will contain a full report. This information will be made available to nurseries and researchers upon request.

Here is my summary of the most important points of interest to our nursery industry:

- In Turkey, Star Ruby is used as an interstock in various lengths for Kütüden on sour orange to promote increased fruit production of better quality fruit. More research should be done on the use of interstocks for example where incompatibility problems arise, such as the Fukumoto on citranges.
- Since 2000 the volume of annual nursery tree production in Florida has shrunk from 6 million to 3 million as a result of a worldwide over-production of fruit coupled with lower prices. Planting of nursery trees are restricted to replacing of old orchards, with very few new plantings taking place. Their voluntary budwood program started in 1953 with a few registered cultivars. Since 1997 a compulsory budwood programme has been in place where all commercial plantings are subjected to testing for CTV.
- Ian Tolley of Australia described the use of a mobile workbench, which facilitates faster budding of trees by using teamwork. Milk crates are used to group the trees for easier handling. Colour coding is cheaper than using labels. Many nurseries in South Africa are already making use of colour coding on the tree stems but a standard colour coding has not yet been developed. Seedling selection to exclude off-types remains one practice not successfully carried out in many nurseries, and attention will have to be given to this matter. Nurserymen must keep themselves well informed on all levels as they fulfill a key role in advising citrus growers on good practices.

- A trial in Australia emphasized the importance of ground covers in open ground nurseries and orchards, and compared the use of plastic sheets to cotton trash, using bare ground as a control. The cotton trash delivered the best results with better root development, uptake of nutrition, water economy and biological activity to improve the soil structure.
- Rootstock shoots that sprout after budding a seedling remain a big problem. A trial where Natal sweet orange on Swingle Citrumelo was treated with Naphthaleneacetic acid (NNA) at 400 ppm one day after budding delivered good results in this regard.
- *Poncirus trifoliata* is a very slow grower in the nursery. A trial in Brazil showed that spraying "Gibberellic acid" (GA3), 7 and 15 after planting with 200 ppm, produced bigger and thicker seedlings that could be budded 60 days earlier. With these results the use of *Poncirus trifoliata* as a rootstock can be re-evaluated.
- The Moroccan citrus industry consists of 76 000 hectares and produces 1.4 million tons of fruit annually, of which 50% is exported. There are 5 registered nurseries that produce 2 million certified trees in plastic tunnels annually. A National certification scheme falls under the jurisdiction of the Department of Plant Protection in the Ministry of Agriculture. This national programme includes 12 Clementine selections, 5 Mandarin hybrids, 13 orange selections, 4 grapefruit selections and 11 rootstocks. All trees must be free from the following viruses: - Tristeza, Exocortis, Cachexia, Stubborn and Psorosis. Sour orange (Bitter Seville) remains the most important rootstock, but the threat that virulent strains of Tristeza can be brought into the country by the Brown Citrus Aphid continually increases the use of Carrizo. Clementines represent 60% of certified trees with Nules and Sidi Aissa being the major selections. Washington, Lane Late navels and Valencia nucellar (selection not specified) are the most important orange selections. Afourer (Nadorcott) is the most important mandarin selection.
- Trials were carried out in Italy to find alternatives for chemical control of *Phytophthora*. Five monthly leaching with Chitosan ("from crab shells - Sigma Chemical co") delivered the same results as chemical control. The use of *Trichoderma harzianum* also showed good results. In another trial the use of combined formulations of *Clonostachys rosea* and *Trichoderma harzianum* also delivered good results. These components should be tested under South African conditions and the information will be forwarded to our researchers.
- Various papers and posters were dedicated to production of organic citrus nursery trees, covering media preparation, pest control through natural enemies and seed treatment with non-chemical components. This is an area where there are still many un-answered questions and the necessity of our nursery industry becoming involved in organic nursery tree production is still under question.
- Sour orange (Bitter Seville) was the main rootstock in the Spanish citrus industry until *Tristeza* broke out in 1958, after which the industry made use of *Tristeza*-tolerant rootstocks such as the citranges. Indexing proved that other viruses were also present and in 1975 the Citrus Variety Improvement Program (CVIPS) was founded in Spain with the main objective being that only pathogen-free trees should be used in new plantings. They claim it was the first programme based entirely on tissue culture technology, by making use of shoot-tip grafting (STG). The programme consisted of applying STG to local and imported cultivars under quarantine conditions, maintaining a healthy gene bank and releasing budwood to citrus nurseries. Three different programmes are included, namely the sanitary programme by IVIA Research Institution, the quarantine programme by the Plant Protection Service and the nursery certification programme by the Nursery and Seed Services. The technical responsibilities of all three programmes are coordinated by IVIA. This programme made a major impact on the continued existence of the Spanish citrus industry.

The following papers were contributed by delegates from South Africa:-

- "Cultivar innovation: Effective sourcing and commercialization of citrus cultivars in Southern Africa" by Graham Barry.
- "The challenge of commercializing protected / patented citrus cultivars". by Louis von Broembsen

Poster contributions:-

- "South African Citrus Improvement Programme" by Thys du Toit.

8.3 T.G. GROUT

8.3.1 PARTICIPATION IN INTERNATIONAL CONFERENCES IN WALES AND CALIFORNIA, 4 – 16 SEPTEMBER 2005

IX European Workshop on insect parasitoids in Cardiff, Wales

The reason for this overseas trip was to present a paper at a Thysanopteran conference in California but as I was flying to California via London I could attend the above conference in Wales at little extra cost. Although a few of the sessions were on physiology and mathematical modelling and were of little applied value, the vast majority of sessions were very valuable and showed how far science has evolved in this field since the days of trial and error parasitoid releases 20 years ago.

Systematics and Evolution

Several research groups are trying to combine molecular and morphological taxonomical approaches to understand parasitoid phylogeny. Difficulties arise due to convergent evolution of similar morphological adaptations with different genetic backgrounds. Certain genes are better suited to separating species than others. Most research groups have found that the 28S-D2 region of ribosomal DNA most closely agrees with morphological species differences. The CO1 region was not of any value for separating chalcidoids. John Heraty (john.heraty@ucr.edu) at Riverside, California, has compared several different genes for identification of chalcidoids and I am sure would be willing to look at our *Aphytis* spp. The PhD researcher (Wogi) that did look at some *Aphytis* specimens from South Africa should publish the work shortly. The molecular taxonomy has revealed some unusual situations such as two sympatric, morphologically identical species of *Cales noacki* that can occur together on the same tree but have different genetic sequences. These two species were imported from different parts of the world and have somehow retained their original identity. Perhaps the *Comperiella bifasciata* 'strain' that was first imported to South Africa and was ineffective against red scale was a different species. It will be worthwhile trying to unravel the confusion over what we call *Aphytis linganensis* and whether the *A. linganensis* that insectaries rear is the same species as the *A. linganensis* found naturally.

Behaviour and host recognition

Based on behaviour, some morphologically identical 'strains' of a species have been found to be two species, e.g. within the lacewing species *Chrysoperla carnea* are populations that use different vibrational cues for mating recognition. Some parasitoids use wing fanning for species recognition. A lot of research has been conducted on host recognition and some of this may be of relevance to host-plant recognition in citrus thrips. The wax on whitefly immature life stages is used by *Encarsia formosa* for host recognition and species without wax largely escape parasitism. Travel costs, host patch quality and host patch danger will determine the efficiency of parasitism. A spray of formic acid simulating the presence of ants will encourage parasitoids to leave a patch. Some ichneumonids use vibrational sounding techniques to detect hosts far beneath the plant surface. Silvia Dorn at ETH Zurich (silvia.dorn@ipw.agrl.ethz.ch) heads a research group on host selection and often works on codling moth. She has found that preimaginal learning is important in *Hyssopus pallidus* recognising codling moth as a host. This has implications for mass rearing of parasitoids for biocontrol. Parasitoids that had been reared on their host grown on artificial diet had only 53% success in finding their host in fruit whereas those that had been reared on a host with fruit cues were 100% successful in finding their host in fruit. This ability was not inherited and could be learnt in the preimaginal stage after previous generations had not been exposed to fruit cues. Plant synomones are often produced when plants are attacked by herbivores but sometimes the combination of these and the eggs of the herbivore are necessary to attract parasitoids, e.g. *Nezara viridula*. These synomones often involve salicylic acid and its precursor jasmonic acid but in the case of *Nezara* involves β -caryophyllene that is also a fruit fly attractant.

Apostolos Kapranas (apostolos.kapranas@email.ucr.edu) is a PhD student of Bob Luck's at Riverside, California, and is studying parasitoids of soft scales. He is rearing soft scales on yucca leaves and placing these in the orchard to determine parasitism. He has found that male parasitoid eggs are more often encapsulated by scale insects than female eggs.

Some species of Encyrtidae can produce thousands of embryos from a single egg. Furthermore, some of these polyembryonic species have a caste system where some of the embryos develop into soldiers within the host insect that kill reproductive embryos from other parasitoids.

Symbionts and parasites of parasitoids

Wolbachia are now known to occur in 16-76% of arthropods based on various publications. *Cardinium* is another bacterium known to occur in 6-7% of arthropods. Effects of these bacteria on parasitoid sex ratios are well known but additional effects on the host have also been found. One example being where eggs from *Wolbachia*-infected parasitoids are more likely to be encapsulated by *Drosophila* than uninfected eggs. In braconids and ichneumonids, viruses known as bracoviruses and ichnoviruses are transmitted to the host with oviposition and these usually assist survival of the parasitoid and may prevent encapsulation or the secretion of interferon.

Physiology

Several papers were concerned with the importance of carbohydrates in the diet of adult parasitoids. Nectar or honey was better for maintaining sustained flight than sucrose alone. Females do more flying than males. Nectaries in plants must be accessible to the parasitoids to be of benefit. If sugars are available, less host-feeding occurs and adult longevity and therefore oviposition is improved. The most important sugars in honeydew are sucrose, fructose, glucose, maltose and melezitose. The survival of parasitoids in cold storage at 4°C was dramatically improved by periodically raising their temperatures to 20°C for 2 hours.

Biological control and ecology

Although it is generally assumed that an increase in habitat diversity improves IPM, this is not always the case. Some of the extra plants can become weeds and sometimes the fourth trophic level can be increased. One example is an increase in parasitoids of lacewing predators. Nectar-producing plants should be carefully chosen and of proven benefit. Many nations now require host specificity tests before natural enemies may be imported. When these are based on small-scale laboratory experiments the results are usually reliable for strictly monophagous or polyphagous species but oligophagous species would probably be rejected, even though some of these have been very successful in the field. The braconid parasitoid *Fopius arisanus* is one of the most promising natural enemies of fruit flies and parasitises more than 20 spp. of tephritids. These parasitoids are attracted to kairomones given off by egg masses in fruit. These kairomones are stronger for *Bactrocera zonata* than for *Ceratitidis* spp. in Reunion (pascal.rousse@cirad.fr). In Hawaii, *F. arisanus*, which is an egg parasitoid, can be very effective against *Bactrocera dorsalis* and out-competes the larval parasitoid *Diachasmimorpha tryoni*. The latter species has been forced to parasitise a tephritid that causes galls in *Lantana* and is therefore considered detrimental to *Lantana* biocontrol. Russell Messing (messing@hawaii.edu) believes we should consider importing *F. arisanus* but we will first need to learn more about our own tephritid parasitoids.

Egypt introduced 12 species of natural enemies for the control of whiteflies between 1996 and 1999. Only a few have become established.

Although some papers emphasise the importance of eggs maturing faster in larger parasitoids that have access to nectar and stress the fact that larger parasitoids fly more and are better at finding hosts, in reality, parasitoids may only find one to three hosts in the field in their lifetime (martijn.bezemer@wur.nl).

Leigh Pilkington, a post-doc at Riverside, has shown with developmental and reproductive biology studies that *Gonatocerus ashmeadi*, a parasitoid of sharpshooter leafhoppers, is not well suited to the Californian climate. This explains it being much less effective there than in tropical climates. Pilkington (leigh.pilkington@ucr.edu) used a modified Logan model to estimate the upper developmental threshold.

Multitrophic interactions have been found to occur between roots and above-ground parts of the same plant. When root feeders attack the plant, secondary plant products such as sinigrin will influence above-ground parts and vice-versa (see papers by Bezemer et al. 2003 Oikos and 2004 Chem. Ecol.). Perhaps nematode feeding may reduce the effect of foliar lepidopteran pests or thrips or even affect their parasitoids.

Since the 1970s, many different models have been applied to parasitoid behavioural ecology but more comparative approaches are required as everyone measures different parameters. Not much progress has been made since the landmark book by Godfray in 1994.

Several bethylids are known to attack the coffee berry borer and two of these are from Africa. The most effective is *Cephalonomia stephanoderis* and may attack our coffee bean weevil that sometimes infests citrus. This parasitoid produces skatole that induces dispersion.

Bob Luck from Riverside gave a review of the effect of climate on red scale biocontrol by *Aphytis melinus* in the San Joaquin Valley of California. Causes of red scale mortality are: natural (no natural enemies) (40%), predation (20%), *Aphytis* (35%) and *Comperiella* (5%). A long-term project demonstrated that bio-intensive IPM could reduce costs by 40% compared with chemical programmes but most of these systems have collapsed now due to the need to spray glossy-winged sharpshooters with imidacloprid. About 17% of the red scale life cycle is available to *Aphytis* and *A. melinus* will lay female eggs and multiple eggs on larger scales and male eggs on smaller scales. The larger the parasitoid, the more eggs it lays, the longer it lives and the further it walks. However, the higher the temperature, the smaller the red scale and the lower the risk from parasitism. At 20°C there is twice as much opportunity for parasitism as at 32°C. Scale size will also vary due to the location in the tree. *Aphytis* has also been found to only search between 10h00 and 16h00. *Aphytis* in California currently cost about \$1.50 per 1000. Jacques van Alphen also mentioned that many parasitoids are inactive at night but braconids and ichneumonids are probably an exception as they are caught in light traps. Adult parasitoid mortality rates are also likely to increase with latitude in Mediterranean climates due to the lowering of relative humidity.

Due to synomones and kairomones, solitary infested plants attract fewer parasitoids than a patch of infested plants, as attraction depends on the concentration of kairomone per unit area. Parasitoids usually prefer to search on the same type of plant that their host was on. In some cases the plant type can actually affect the genetic structure of the parasitoid.

VIII International symposium on Thysanoptera and Tospoviruses

This international conference is held every four years but I have not attended one before. Most of the well-known thrips researchers were there so it was good to finally meet people that I have been corresponding with for years. The conference covered tospoviruses in addition to thrips as many of the known pest thrips species transmit viruses that can be much more devastating than the physical damage caused directly by thrips feeding. None of these viruses have been found in citrus yet but *Scirtothrips dorsalis*, which is a pest of citrus in Asia and can be found on *Ricinus communis* in South Africa, transmits viruses to peanuts. Several concurrent sessions were held so that those interested in viruses did not need to listen to talks that dealt with thrips alone, and vice versa.

Many papers involved the relationship between thrips and their plant hosts and as different populations of an individual species may have different biological characteristics, Lawrence Mound (Australia) encouraged researchers to think broadly when seeking funding for research on interactions with plants.

Taxonomy

Much of thrips taxonomy has been based on differences rather than similarities. Many families have been created with only one or two genera each because they have been based on certain unusual characteristics. Molecular taxonomy is showing that these shouldn't be separate families. It also shows that Phlaeothripidae is a sister group of Thripidae and should not be in a different suborder. Based on the 28S genome, Panchaelothripinae, within which the greenhouse thrips, *Heliothrips haemorrhoidalis* is placed, is possibly an ancestral group for thrips. Gerald Moritz (Germany) has now produced a molecular key to the major thrips pests that is supported by a morphological key. Four *Scirtothrips* species are included in the molecular key. Mark Hoddle (Riverside, California) has increased the number of *Scirtothrips* covered in the molecular key to 15 but this will only be published in 2006. According to this molecular work, *S. dorsalis* from South Africa seems very similar to *S. dorsalis* in Asia where it was described from *Ricinus communis* in 1919 but only became an agricultural pest in the 1950s. Strains that are adapted to certain host plants seem to be common within *Scirtothrips* whereas other thrips such as Western Flower thrips, *Frankliniella occidentalis*, appear to move readily between many host plants. Another example is *F. bispinosa* that moves from blueberries to citrus in Florida. The movement of *S. aurantii* between hosts in South Africa needs more investigation and more needs to be learnt about our *S. dorsalis* before it becomes a pest.

Chemical communication

The subjects of synomones and kairomones being produced by plants and various pheromones being produced by thrips were covered in several talks. There appears to be plenty of scope for research on host-attractants. Examples of these chemicals are p-anisaldehyde and ethyl nicotinate. We have evaluated the latter compound on sticky traps for our citrus thrips and citrus psylla. Acetates with longer chains or double bonds can act as ant repellents. High concentrations of β -myrcene repel cycad thrips. Geraniol was used by one researcher to increase trap attraction to thrips but the results were not clear. Thrips seem to respond to both olfactory and visual symptoms.

Host plants

Heliothrips haemorrhoidalis has become a pest of Persimmon in Brazil so may attack our persimmons if grown in suitable climatic areas for the thrips. *H. haemorrhoidalis* on citrus in southern Italy has been displaced by *Pezothrips kellyanus* which is the major citrus pest in S. Australia. We should be on the lookout for this black thrips on lemons in cool, coastal areas.

Joe Funderburk (Florida) said it was critical to distinguish between food host plants and reproductive hosts. Thrips may go to many different flowers on plants to feed on pollen but these plants may not support thrips larvae. Joe Funderburk said that the impact from *Orius* is often underestimated. It can suppress thrips at a ratio of 1:217 and control thrips at 1:51. Spinosad has little detrimental effect on *Orius*. *Orius* first takes the thrips larvae out of the population.

Microbial control and parasites

Joe Funderburk said he had no evidence that fungi played a role in suppressing *Frankliniella* spp. in Florida where it is humid. However, the free-living nematode, *Thripinema fuscum* that parasitizes *F. fusca* on the foliage of plants seemed to have some affect but required high humidity.

Michael Parrella (Davis, California) said that *Beauveria bassiana* was partially successful in tunnels. In India, *Fusarium semitectum* was found to infect over 80% of thrips in some cases (G. Mikuntham, India). The use of microbial pathogens could be enhanced by applying *B. bassiana* when thrips are mobile (to pick up more spores) and by applying nematodes when thrips are stationary. Western Flower thrips is most active in the morning and just before dark. Karin Schmidt (New Zealand) suggested that *Botrytis* infections may be assisted by thrips providing infection points and carrying spores.

Work with endosymbionts such as *Wolbachia* is being conducted and the PCR allows for easy identification of these bacteria. *Wolbachia* may be having a controlling influence on genetic sequences apart from influencing the sex of the thrips.

The use of the entomopathogenic nematode, *Steinernema feltiae* in the soil is being investigated in glasshouses in the UK (Jude Bennison). If the nematodes are sprayed on foliage they only survive 2 hours but if sprayed on the ground they can persist for 8 weeks. The commercial formulation, Nemasys, seems to be working against Western Flower thrips.

I presented a paper entitled: Biorational Control Strategies for *Scirtothrips aurantii* that minimize non-target effects on arboreal and edaphic predatory mites.

San Joaquin Valley, California

The pest status of citrus thrips has declined in the SJV due to satisfactory control with spinosad. Consultants think that pyriproxyfen may be having some impact on thrips but perhaps it is due to less usage of OPs. Beth Grafton-Cardwell and Joe Morse will be trying to see whether they can create high thrips populations by spraying OPs.

Katydid used to be a sporadic pest much like our leafrollers or bollworm but since OP usage stopped it has become a regular spring pest. Citricola scale has similarly become a major pest as OPs are no longer used and it is not being controlled by pyriproxyfen or imidacloprid. It only has one generation per year so biocontrol is also difficult. Due to honeydew production, ants have reached pest status and research is being conducted on a suitable bait station containing sugar water. Work has already been done with several toxicants such as pyriproxyfen, imidacloprid and fipronil but the chemical companies need to produce a bait station that will contain a large volume of liquid that will not evaporate. It will also have to be child-proof and coyote-proof.

Jocelyn Millar has identified the citrus leafminer pheromone and it is being used to trap large numbers of moths. This pest has finally arrived in the southern and coastal parts of California but has not yet reached the SJV.

Red scale is being controlled effectively by pyriproxyfen sprays every alternate year (16 fl oz/acre). Volumes of water being sprayed per hectare have come down over the years and are now close to 7000 ℓ/ha, although this is barely adequate. Oil sprays would be applied at 10000-14000 ℓ/ha. Control of red scale with imidacloprid is not considered adequate. In the case of sprays, this may be due to insufficient penetration to

control scale on the framework. Chemigation applications also result in branches without scale control as in South Africa but this is not tolerated as it is in South Africa. Most imidacloprid and acetamiprid sprays are being used for glassy-winged sharpshooter control where full cover sprays are not necessary. These sprays therefore result in scale repercussions on the wood and give imidacloprid bad publicity. Fortunately these leafhoppers are only problematic in certain areas.

The 'new' pest that is receiving a lot of research attention is the citrus peel miner, *Marmara gulosa*. This used to be an extremely sporadic pest many years ago much as we used to have citrus leafminer, but more recently a new strain (Tulare strain) has arrived that is much more problematic on many citrus varieties. It has an extremely broad host range and is reared in the lab on zucchini squash. Grapes and cotton are also hosts as well as oleander, eggplant and peppers. The most susceptible citrus varieties are pummelos and grapefruit and the navels: Fukumoto, Atwood, TI and Barnfield. It is rarely found on leaves but mostly in fruit and stems.

In California, the detailed knowledge of entomological pests is much higher than ours due to the manpower available and resources at hand. For example, we will determine that a product is effective in controlling a pest whereas they will know that it is more effective at a certain time of the year or against a certain life stage. Most of their extension material is available on the internet and self-teaching courses are available on CDROM. Pest Control Advisors must take courses periodically to keep themselves up to date.

I visited Mulholland Citrus, near Orange Cove, which is one of the few farms that has an insectary and is producing and using their own *Aphytis*. Last year they produced 400 million *Aphytis* using the large banana squash, oleander scale and De Bach cabinets. They sell *Aphytis* to other farms as well as releasing on their own farm. They grow their own trees and are still using some sour orange rootstock in addition to C35, Carrizo and Volckameriana. They are hoping to start producing the new seedless mandarin, Tango, soon. The nursery uses a mixture of fumigated soil and sawdust in bags but the weight is a limiting factor. The bags are placed on the ground on top of several irrigation pipes through which warm water is pumped in winter to keep the roots warm.

Recommendations and conclusions

- Collaborate with John Heraty at Riverside, California, to get molecular identification of our *Aphytis* spp.
- Find out more about our fruit fly parasitoids, then consider importing *Fopius arisanus*.
- Poor red scale biocontrol at high temperatures may be due to the production of smaller scales that are unsuitable for parasitism.
- More should be learnt about *Scirtothrips dorsalis* in South Africa and whether it is a host for *Goetheana incerta* as it may be easier to rear than *S. aurantii*.
- We must keep up to date on the development of an ant bait station in California for liquid baits.

8.4 **G.C. SCHUTTE**

8.4.1 DU PONT (ARGENTINA) SPONSORED TOUR TO TUCUMAN, ARGENTINA TO PRESENT A TALK ON CITRUS BLACK SPOT – 21-24 AUGUST 2005

Summary

I was one of three guests invited by Du Pont in Argentina to present talks on citrus diseases. Du Pont is concentrating on the launch of new formulations of copper hydroxide for the control of citrus black spot and citrus canker. To promote the usage of copper, they have invited myself, Pete Timmer (USA), Pedro Yamamoto (Brazil) and Jacqueline Ramalo (Argentina) to present talks on the following (in Spanish):

- Fungicidas cupricos en citrus deteccion de *Guignardia citricarpa* – dr. Pete Timmer
- Detección de *Guignardia citricarpa* é efecto de tratamientos sobre el desarrollo de síntomas en poscosecha – dr. Pete Timmer
- Control de mancha negra en citrus – dr. Tian Schutte
- Greening: experiencias manejo Brasil alerta productores Argentino – dr. Pedro Yamamoto
- Evaluacion productos quimicos para control de canrosis limonero – Jacqueline Ramalo

All the talks were in Spanish (except for mine – I had to make use of Pete Timmer as an interpreter) but they were kind enough to make me copies of the speeches to study later. The second day we went to visit all the important lemon growers in and around Tucuman to listen to their problems and how they are experiencing

copper applications (pros and cons). Their biggest problem to date was that they still have not finished harvest (20-30% needed to be harvested) and trees are already blossoming. The last day I had an interesting visit to the Experimental station hosted by dr. Daniël Plopper and his team working on CBS. Here they showed me how the non-pathogenic strain, *Guignardia mangiferae*, was able cause to infections and was able to fulfill Koch's postulates to show it is a parasite.

Itinerary

Sunday 21 August	Travel: Johannesburg – Cape Town – Buenos Aires
Monday 22 August	Travel : Buenos Aires – Tucuman; present talk at 16h00
Tuesday 23 August	Visit Argentinini Lemon, San Miguel, Citrusvil and Citromax the Experimental station "Obispo Colombres" in Tucuman and experimental trial sites
Wednesday 24 August	Return to South Africa

Greening in Brazil

Highlights from the talk entitled: "Greening: experiencias manejo Brasil alerta productores Argentino" by Pedro Yamamoto.

The causal organism is a bacterium belonging to the Liberibacter group by the name of *Candidatus L. asiaticus*. The symptoms are similar to those caused by *L. africanum* in South Africa (Fig. 8.4.1.). However, young trees can die within two years after the first symptoms have appeared. Furthermore, the whole tree has to be removed and burned as the bacterium is omnipresent in the tree. Alternative hosts such as Murta: *Murraya paniculata* has been identified. Fruit have abortive seed and the acid is up to 52% higher than normal fruit. U.S. Department of Agriculture and the Florida Department of Agriculture confirmed the detection of citrus greening on the 2 September 2005 in the USA.



Fig. 8.4.1. A young tree with typical greening symptoms on a branch (left) and the causal organism, *Candidatus L. asiaticus* (right).

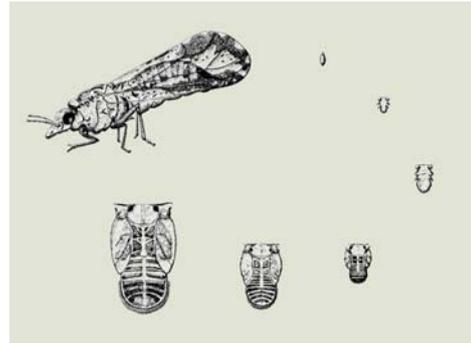


Fig. 8.4.2. The vector, *Diaphorina citri*, measuring 2-3 mm (left) with its different instars (right).



Fig. 8.4.3. A specially constructed tower with two inspectors for the search and marking of greening trees in large trees at a rate of 4000 per day (left) and manual inspections of small trees (right) at a rate of 400 trees per man per day.

Control of the vector

Control measures are done with systemic insecticides such as trunk applications with Winner (200 SL) and Convence (200 SL); soil applications of Temik (150 G) and Actara (10 G) and drenching with Confidor (700 GrDA) and Actara (250 WG)

Contact insecticides reistered in Brazil are: Neonicotinoids: Actara, Confidor, Convence and Provado; Organophosphates: Acefato/cefanol/Orthene, Dimethoate, Ethion, Malathion, Lorsban/Chlorpyrifos, Supracide/Suprathion; Pyrethroids: Decis, Karate, Danimen/Meothrin; Carbamate: Marshal and others: Vertimec, Trebom.

Control of various citrus diseases

Pete Timmer gave an overview in his first talk of the control of various citrus foliar diseases with copper fungicides. He presented results of field trials on the use of copper hydroxide for the control of Alternaria brown spot, melanose, citrus canker and greasy spot. Results are similar to ours in South Africa and they

also have a registration of strobilurins in tank mixtures with copper hydroxide. In South Africa, Ortiva is currently the only strobilurin that is registered in a tank mixture with copper oxychloride. Pete Timmer also referred the growers to a website (<http://infotech.ifas.ufl.edu/disc/download>) where copper models can be downloaded.

In his second talk entitled “Detección de *Guignardia citricarpa* é efecto de tratamientos sobre el desarrollo de síntomas en poscosecha”, he presented results from post harvest CBS control work done by a Brazilian, Natalia Peres, showing that there are no post harvest chemicals such as fludioxonil, pyrimethanil, pyrimethanil+imazalil, imazalil+thiabendazole that are able to control CBS.

Recommendations

The visit to Tucuman was as valuable as previous visits as I was introduced to over 350 people at the lecture and travelled as far north of Tucuman such as Jujuy and Salta provinces. My previous visits were all in May/June at the beginning of harvest, while this visit was in August and closer to blossom. As about 30% of their crop was still hanging on the trees, more disease expression was evident and I could familiarize myself more with the canker and CBS problems at that stage of the growing season. Meetings with the growers and the researchers were also fruitful and I hope to duplicate some of the research findings here in South Africa, especially investigating the possible pathogenic status of *Guignardia mangiferae*. The talk on greening as experienced in Brazil was also interesting and some new control measures were mentioned that can be tested in South Africa.

8.5 V. HATTINGH

8.5.1 EMERGENCY VISIT TO USA IN CONNECTION WITH A REPORTED INTERCEPTION OF LIVE FCM LARVAE IN SA CITRUS EXPORTED TO USA - 02-10 JULY 2005

1. Itinerary

02 & 03 July 2005 (Saturday & Sunday)	Transit: SA – USA Philadelphia
04 July 2005 (Monday)	Philadelphia – USA Public Holiday
05 July 2005 (Tuesday)	Philadelphia – discussions at and around Port facilities
06 July 2005 (Wednesday)	Washington/Riverdale – discussions with APHIS
07 July 2005 (Thursday)	Sacramento – discussions with California Department of Food and Agriculture
08 July 2005 (Friday)	Transit: Sacramento – Philadelphia
09 & 10 July (Saturday & Sunday)	Transit: USA – SA

2. Background

USDA APHIS reported to SA DoA that a live FCM larva had been intercepted in SA citrus in California on 17 June 2005. The SA citrus export programme to USA was consequently put on hold, pending the outcome of further investigation by APHIS. The outcome of the subsequent investigation, and negotiations between SA DoA and APHIS, resulted in a resumption of the programme on 29 June 2005. Nonetheless, the USA Alliance (producers and exporters supplying the USA market) requested Vaughan Hattingh to urgently undertake a trip to the USA, with the following objectives:

- (a) Have an SA citrus entomologist present in USA during the first week after lifting the pause on exports from SA.
- (b) Vaughan Hattingh to familiarise himself with some of the pertinent operational procedures of post-arrival inspections in Philadelphia, and meet with key contact persons in Philadelphia.
- (c) To meet some of the individuals that have been dealing with the matter, including the relevant scientific advisers, to discuss ways of avoiding recurrences of recent events. Since Vaughan Hattingh had not SA governmental mandate, these would be unofficial (non-governmental) discussions.
- (d) To initiate a process of communication with relevant Californian parties, with the objective of providing assurances about: (1) the scientific integrity of systems designed to provide phytosanitary security from the threat of FCM introduction into USA; (2) and the commitment of the SA citrus industry to ensuring continuous improvement of the phytosanitary security of its exports, through ongoing and relevant research.

3. Tuesday, 05 July 2005

Discussions were held with Marc Solomon and Tom Verbitski regarding Philadelphia port operations and quarantine inspection procedures. This was followed by an on-site visit to the quay-side facilities. A meeting was then held with George Sibley, Senior VP of Barthco International, a company that facilitates customs clearances. George facilitated a meeting with the local APHIS office. Discussions were held with David Farmer, the APHIS PPQ Officer in charge, and Frank Salantri the Area Identifier – Entomology.

Whereas the on-arrival sampling and inspection of fruit used to be conducted by APHIS, this function is now fulfilled by Customs and Border Protection (CBP), under the auspices of Homeland Security. The CBP officials that now do inspections, remain the same individuals who did it previously, but now under a different authority. There appears to be no sensitivity about an industry representative observing the inspection process. When larvae are found in the inspection sample, they are kept in a vial or petri dish and observed for signs of movement. If the CBP inspector suspects that the larva is alive, the APHIS area identifier (an entomologist) will be called in, to verify the specimen's live status. The office of the APHIS identifier is no more than 10 minutes drive from the Port facility.

The APHIS identifier is clearly very professional and familiar with FCM as found during fruit inspections.

Furthermore, the equipment and facilities available are of a high standard. The identifier was clearly familiar with the discolouration typical of most larvae that have been dead for some time, and described how he evaluates whether a larva is alive. Any indication of independent movement, including microscopically observed leg or mouth part movement, is taken as adequate evidence that a live specimen has been intercepted. In light of this, the value of video recording of such observations was questioned. Conversely the appropriateness of microscopic movement as an adequate indication of treatment failure, was raised, but this was clearly an issue for discussion at a policy making level.

4. Wednesday, 06 July 2005

Ron Campbell arranged a meeting with a group of APHIS officials in Riverdale (outside Washington) and the SA Agric Attaché to Washington, Dr Mkhize joined the meeting. The following individuals from APHIS were present: Jane Levy (Associate Executive Director Plant Health & Quarantine), Karen Ackerman (Director Plant Protection & Quarantine), Dr Shaharra Usnick (Risk mitigation specialist), Mark Knez (Associate Director Plant Health, International Services), Julie Aliaga (State Operations Support Officer – Plant Protection & Quarantine), Sharon Porsche (Import specialist – Plant Protection & Quarantine), and on telephone conference Dr Scott Wood (Director – Treatment Quality Assessment).

The objectives of the meeting were agreed upon as being: (1) to seek a better understanding of the current status of the programme and how the recent problems arose, (2) to seek means of reducing the risks of a recurrence of recent events, (3) to identify issues where further research and information provision could assist in the future.

4.1 *Current status and possible causes of recent events*

The status of the programme at present, as generally understood by the SA industry, was confirmed. In terms of possible explanations for the occurrence of recent problems, the following was discussed.

There is scientific evidence supporting the efficacy of the 22d treatment. The extension by 2d was accepted by the SA industry in the interests of providing USA parties with additional assurances and lifting the pause on the programme. It was explained that this is not an indication from SA scientists that the 22d treatment is not effective. It was explained that the research reviewed by APHIS entailed treatment of pupae, that are the most cold-tolerant life stage, but do not occur in association with fruit exports, since larvae pupate in the soil. The reports also included some data for cold treatment of larvae in rearing medium, whereas there is evidence of a trend for larvae to be slightly more cold tolerant in medium than in fruit. Reference was made to the efficacy of the 22d treatment having been confirmed through the successful execution of a probit 9 confirmatory trial in SA, in the presence of Korean Quarantine officials.

Although a high level of fruit infestation does increase the probability of isolated incidents of larval survival, the infestation levels evident in the programme, probably do not constitute a material increase in the probability of post-treatment interception of live larvae. Nonetheless, any mechanism that reduces exclusive reliance on the quarantine treatment, would provide added assurance of the programme's phytosanitary

security. It was noted that the implementation of the GAP is an appropriate mechanism to pursue this objective. Likewise, it was suggested that increased vigilance in fruit cutting during harvesting and packing, could also be of considerable value.

The possibility of hot spots within the ship's hold was raised by Scott Wood, and the current temperature mapping study, being undertaken by APHIS, was discussed. The significance of packaging, as it affects air movement and the ability to ensure uniform application of the cold treatment, was discussed. The importance of this issue was emphasized and Mark Knez and Scott Wood offered to provide advice from their specialists, if SA would provide samples of the packaging being used.

The possibility of mistaken determination of the living status of an intercepted larva was discussed. It emerged that there are two approaches to be looked at. Firstly, on the short term, provision by SA of a proposed guideline on how to handle and evaluate an intercepted larvae, would be valuable. This would include description of the typical physical condition of larvae after exposure to cold treatment and this may be the subject of a research project.

On the longer term, the generation of data on the probability for larvae, that survive an incompletely effective treatment (for example a 10 day cold treatment), to develop into the next life stage, would be valuable. This information would have two-fold potential application. Firstly, to provide added assurance of the extremely low risks posed by the programme in its current form. Secondly, this would provide a scientific basis for reviewing the appropriateness of the criteria currently being used for evaluating the risk status of larvae found during post-treatment inspection. The relevance of microscopic mouth-part movement, as an indication of larval viability in terms of the Phytosanitary risk of pest introduction and establishment, could be reviewed on the basis of such data.

4.2 *Means to reduce the risks of recurrence of recent events*

The following options have been discussed above: (a) reduce pest load; (b) implement GAP, (c) intensify pre-export culls (d) provide guideline for evaluating status of larvae found during post-treatment inspection, (e) generate data that may support amending the criteria being used to evaluate the risk status of larvae found during post-treatment inspections. The following points were also discussed.

A means of visually recording the observations of the larvae's status during post-treatment inspections was discussed. It was noted that the Philadelphia inspection point would have video recording equipment. However, it was stated that this may not be particularly useful, when the current criterion being used, is the observation of microscopic mouth-part movements. Furthermore, the inability of APHIS to enforce adoption of video recording by inspections at State level, was noted, and that this is further compounded by CBP (Homeland Security) doing inspections.

It was evident that the programme remains at high risk. The pursuit of a means to mitigate against the risks of the programme's future being jeopardised, through potentially inaccurate or inappropriate determination of treatment failure, should be a high priority for SA to address through its NPPO.

4.3 *Research and information provision, towards reducing risks of recurrences*

The following points have been discussed above: (a) a guideline for the handling and evaluation of larvae found during post-treatment inspection, (b) finalisation of the GAP guideline, (c) viability of larvae surviving incomplete treatments, (d) data describing the post-treatment physical condition of larvae, (e) mapping of temperature distribution in ships' holds, (f) impact of packaging on cooling ability. Additionally, it was noted that it would be most valuable to publish the results of the cold treatment validation trials that have been conducted in collaboration with other countries.

5. Thursday, 07 July 2005

Michael Wootton (Vice President, Corporate Relations Sunkist Growers) arranged a meeting with a delegation from California Department of Food and Agriculture in Sacramento. The following were present: AJ Yates (Under-Secretary for Agriculture, State of California), Pat Minayrd (Director Plant Health and Pest Prevention Services Division), Aurelia Posados (Assistant Director – Plant Health and Pest Prevention Services), Bill Sandige (Branch Chief: Pest Exclusion Branch), Dorthea Zadig (Programme Supervisor: Plant Health and Pest Prevention Services), Kevin Hoffman (Primary State Entomologist – Supervisor), Marc Epstein (Associate Insect Biosystematist – Lepidoptera), Ray Bingham (Area Manager – Pest Detection/Emergency Projects), Gary Leslie (Program Supervisor Border Stations, Plant Health and Pest

Prevention Services), Larry Prinzbach (APHIS Operations Support Officer, California). The objectives of the discussion were to (1) provide the Californians with assurances about the scientific foundation, both for pest management in SA, and the cold disinfestation treatment; (2) investigate plausible explanations for the recent events; (3) establish contacts and open communication channels. The majority of the discussions revolved around the technical nature of FCM pest management in SA and the export programme. There was much interest in the current research projects that would provide means to reduce FCM pest populations, thereby reducing pressure on the post-harvest disinfestation treatment. In particular, the SIT project and use of the granulovirus, were discussed with enthusiasm. Attention was drawn to the long history of collaborative interaction between the SA and Californian citrus industries.

Marc Epstein requested us to provide him with FCM larval specimens, to assist in improving their FCM identification services. SA scientists were also requested to provide advice on the best FCM pheromone trapping procedures to be used in surveillance monitoring programmes in California.

There was considerable interest in hearing how the SA citrus industry has organised itself and how it operates research and technical support services. It was apparent that there was much appreciation for SA sending somebody to Sacramento to discuss such technical matters.

The Pest Exclusion Branch provided the reports of the recent interception. The criterion used for determining the "live" status of intercepted larvae was discussed. Assurances were given that the larvae were motile (capable of motion and actively crawling). The usefulness of having a video record of such observations in future, was discussed. The absence of obvious explanations for how such an incident may have occurred, in light of the highly effective cold disinfestation treatment having been applied, was discussed.

CDFA advised that they had taken a decision to re-open California to SA citrus. However, they did caution that they would continue with diligent inspection and scrutiny of fruit entering, and within, the State

6. Conclusion

It was apparent that all parties are strongly committed to taking all reasonable steps towards ensuring that the programme remains open, on condition that the requisite phytosanitary security is maintained. There was general consensus that attention must be focussed on achieving a pre-harvest reduction in pest pressure.

The invitation for SA to provide a guideline on the handling of larvae found during post-disinfestation inspection, should receive priority attention. A study on the survival of FCM after a reduced-exposure treatment, should be undertaken as another priority. The finalisation of a FCM GAP Protocol, aimed at ensuring strict implementation of pre-harvest control practices, is important. All research projects that may result in reduced FCM population levels, should be pursued as a high priority, in the interest of securing the future of this market, and avoiding the dire consequences that its closure would have on most other SA citrus export markets.

7. Acknowledgement

The CGA is acknowledged for funding the trip. Fisher-Capespan provided valuable organisational and logistical support. Ron Campbell (who is contracted by CGA to facilitate the programme in USA) is thanked for most valuable arrangements and introductions. Discussions with the Californian parties were made both possible and productive, through the most valuable arrangements and introduction provided by Mike Wootton from Sunkist.

8.5.2 REPORT ON ATTENDANCE OF THE 2005 ANNUAL GENERAL ASSEMBLY OF C.L.A.M IN MOROCCO – 8-16 OCTOBER 2005

1. Itinerary

Saturday, 08 October Depart Cape Town – London

Sunday, 09 October Transfer London – Marrakech, join CLAM delegation

Monday, 10 October Meetings of the CLAM Committees

Tuesday, 11 October CLAM General Assembly, orchard visits, transfer to London

Wednesday, 12 October to

Sunday, 16 October

London & York: - discussions with retailers and other parties, regarding pesticide residues (see separate report).

2. Summary

The South African citrus industry, represented by the Citrus Growers Association, was invited in 2001 to again commence participation in the CLAM events, as had been the case prior to the industry deregulation. Vaughan Hattingh has subsequently participated in CLAM activities as the CGA-nominated industry representative.

At the 2005 meeting particularly significant principle support was obtained for stronger co-ordination between the Southern African and Mediterranean citrus industries. A landmark decision was made by CLAM to formally accept Southern Africa as an Associate CLAM member. It was proposed by CLAM that closer co-operation be initiated through a Southern African industry visit by the CLAM Technical and Commercial Committees in May 2006.

3. Background and context

CLAM (Co-ordination Committee for Mediterranean Citrus Production and Export) is a good example of the value of information sharing and co-ordination between parties that share common markets. CLAM members represent 6 of the world's 11 million tons of annual citrus exports. By the nature of its size and membership, CLAM is obviously highly influential in the EU on all issues of relevance to citrus, within the 25 member state union. Continued association with CLAM clearly holds significant potential value for the Southern African citrus industry.

The General Assembly meetings provide a valuable opportunity to fulfil the industry's objectives behind CLAM participation. These include the exchange of production and export statistics, and an opportunity to gain CLAM member support for EU issues of concern to both the Southern African and Mediterranean citrus industries. It also provides an opportunity to build relationships with individuals and parties of influence in the Mediterranean citrus industries.

The Southern African citrus industry has participated in the CLAM Annual General Assemblies since 2002. This has been on an invitational basis, and Vaughan Hattingh has represented the industry because of pre-existing relationships with CLAM and other EU countries, and the SPS value of strengthening ties with these parties.

Prior to the 2004 CLAM General Assembly, the CGA requested formal associate CLAM membership. It was announced at the 2005 meeting that the CLAM Board of Directors had decided to accept the Southern African membership application. This is particularly significant considering that Egypt and Portugal, both Mediterranean citrus industries, have not as yet been granted CLAM membership. Likewise, the Russian application for CLAM membership has not as yet been accepted.

The long standing CLAM General Secretary, Luis Calabozo, resigned from CLAM in 2005. It was announced at the 2005 General Assembly that Octavio Ramón will fill the position for the next year.

4. Synopsis of industry and market information

The most significant factors having affected the market over the past year, were surely the adverse weather conditions in Spain and Morocco, preceding the 2005 Southern African export season. The effect was twofold, firstly freeze-damage, shortly after the soft citrus harvest in both Spain and Morocco. The second effect entailed hail damage to the Spanish lemon crop. The external hail damage to Spanish lemons provided Southern African lemon exporters with a market opportunity. However, this advantage was greatly out-weighed by the adverse effects of the freeze on the internal quality of Spanish and Moroccan oranges. This had a negative effect on consumer demand for oranges and, despite the reduced orange volumes supplied by Spain and Morocco, the first effect was to stall sale rates of Spanish, Moroccan and Egyptian oranges in the EU market. This had a knock-on effect of over-supplying the Russian market and increased supply to the Middle Eastern market, as alternative outlets for the Mediterranean oranges. The implications for Southern African navel export campaign, compounded with variable quality, were severe.

The lesson to be learnt is that a poor preceding export campaign can be highly detrimental to the following

counter-seasonal exports. This served to accentuate the potential value of improved future information exchange and co-ordination between the Southern African and Mediterranean citrus industries.

The forecasts provided for the next (2005/6) Mediterranean season were varied. Spain forecast a continued reduction in exportable volumes, due to the effects of the freeze, but the impact on the market for Southern Africa should be less detrimental, since the principal effect on Spanish production will be in terms of volume instead of internal quality. The drought in Morocco has continued unabated, resulting in a continued decline in forecasted exports from Morocco. In contrast to the above, Turkey is expecting a massive increase in Grapefruit exports, due both to new plantings coming into production and a particularly heavy yield.

Copies of the documents relating to last year's CLAM volumes and the 2005/6 forecasts, have been deposited at the CRI library in Nelspruit and a copy lodged with the CGA.

In terms of longer-term forecasts from the Mediterranean industries, significantly increased production volumes are anticipated in general.

5. Action and recommendations

- 5.1 Finalise details of membership with the new CLAM General Secretary, Octavio Ramón.
- 5.2 Ensure that CGA, CMF and CLAM discuss the proposed South African visit of the CLAM Technical and Commercial Committees in May 2006.



8.5.3 EU VIST TO ENGAGE RETAILERS AND CONSUMER GROUPS ON THE CONTINUED USE OF SAFE AND PERMISSABLE PESTICDES IN SA

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BACKGROUND INFORMATION

There is growing concern among SA fruit and vegetable industries that retailers within the European Union (EU) are applying additional restrictions on the use of plant protection products (PPPs) over and above the EU pesticide regulations. Typically these retailers require a grower commitment prior to the production period to use a predetermined and reduced range of pesticides. Of further concern, these lists appear not to necessarily take into account the legal status of the PPP under EU Maximum Residue Level (MRL) or SA usage legislation. Consumer groups in the EU have also put pressure on growers to use fewer pesticides.

At grower level, this has caused considerable confusion, as various retailers have approached individual growers to get different pesticide usage declarations from them. Typically these growers turn to the Citrus Growers Association of Southern Africa (CGA) or Citrus Research International (CRI) to provide practical advice on which pesticides they can and should use. As the need for help has escalated, a coordination desk for communications between growers and retailers was requested and approved at the June 2006 meeting of the Citrus Marketing Forum (CMF - jointly represents citrus exporters and growers).

To address these concerns raised at the CMF, and to demonstrate the high level of importance placed on consumer, environmental and worker protection by fruit and vegetable producers in SA, some direct contact with international buyers was deemed appropriate.

Paul Hardman (Industry Affairs Manager; CGA) and Prof Vaughan Hattingh (CEO; CRI) visited the United Kingdom between the 12th - 14th October. Thereafter Paul Hardman continued on to the EUREPGAP^a conference in Paris where further informal discussions were held with retailers and consumer groups. This report reflects the most important aspects of the discussions with stakeholders, while a full itinerary can be found in Annex I showing the organizations and individuals that were engaged.

AN URGENT REVIEW OF CERTAIN EU MRLS

While visiting the Pesticide Safety Directorate (PSD) in the UK the SA representatives became aware of a process that was very recently initiated within the EU, wherein the MRLs of several chemicals were being urgently reviewed in light of new information pertaining to methodologies used in establishing residue tolerance levels (MRLs).

Given the serious potential consequences for the SA fruit and vegetable industry and the need for urgent action, the SA representatives were keen to make the best use of their time in Europe to resolve the unknowns. Slight changes to the programme resulted in some distraction from the original intentions of the trip. However, since the very issue related to the continued trade of fruit and vegetables into the EU through compliance with the appropriate pesticide legislation, which falls within the scope and objective of the SA PIP, Annex 2 has been included as a case study to document the events that occurred. Besides documenting the activity of the CGA and CRI, should a similar situation arise in future this case study could guide the approach to get to a solution.

A ZERO DETECTABLE RESIDUE APPROACH

Without exception, the meetings with retailers, retailer groups and consumer groups indeed confirmed an increasing trend among UK retailers to introduce additional restrictions on the use of certain pesticides. UK retailers are seeing food safety as a competitive issue, despite their official denial, with the intention of having *less residue detections* (counts) than their rival retailers. Some retailers are aiming for zero detectable residues within the next 15 years.

This approach clearly diverges from the legal parameters *and* food safety principle governing the usage of pesticides outside and within the EU. Key reasons by retailers for wanting to reduce the number of pesticide residue detections include:

^a 6th Global Conference, Paris, 17th-19th October 2005. "Toward Global Harmonization 2005"



- ✓ High percentage of own label (retailer branded) products in store than elsewhere in the world therefore a strong incentive to protect brand reputation. For example M&S have 100% own branded products in store.
- ✓ “Name and shame” publications by the Pesticide Safety Directorate (PSD) show the relative “performance” of retailers based on the number of products with a detectable residue. Apparently this report is difficult to interpret and creates consumer confusion causing retailers to prefer a zero detectable residue policy. Consumer groups such as *Friends of the Earth* and the Food Standards Agency, also use this information to put pressure on retailers to reduce perceived food safety risks.
- ✓ Accordingly, retailer’s opinions are that the chief food safety concern among consumers is pesticide residues in food.

The SA representatives were able to highlight inherent flaws and difficulties with managing a zero detectable residues policy, but the impression created during the discussion suggested that the corporate decision-makers, and not food safety experts, were driving the food safety policy. The following problems were highlighted to the stakeholders:

- ✓ There is no scientific justification for taking such a position; in fact the science shows that residues at or below the MRL present no short or long-term risk to human health.
- ✓ Abandoning permissible pesticides without scientific justification conflicts with the principles of Integrated Pest Management (IPM), which aims to reduce the impact of pesticides on the environment.
- ✓ Abandoning permissible pesticides without scientific justification also exposes the fruit and a vegetable industry to pesticide resistance build up, and therefore increases the need for chemical treatments.
- ✓ Fruit receiving with post-harvest treatments would be especially problematic since detectable residues would normally be found on such fruit. In response, retailers indicated that they would be phasing in this policy that would be concluded with the post-harvest treatments.
- ✓ Finally, managing public/consumer expectations becomes more difficult, since a single detection of a residue can then cast doubt on the credibility of the entire policy, and without scientific backing this can be very difficult to defend.

The next section briefly considers the role retailers could play to manage consumer perceptions.

MANAGING CONSUMER FOOD SAFETY PERCEPTIONS

It was noted that retailers collectively or individually do very little pesticide risk communication, and there was no mention of initiatives to educate consumers (except on a case-by-case basis) about the concept of an MRL or how different MRLs are to genuine health hazards. Creating negative fruit/pesticides associations in the minds of buyers is possibly one explanation for this lack of communication, but clearly retailers are taking a reactive stance to consumer complaints.

Yet the results of a recent survey conducted by Food Standards Agency, used to evaluate their own performance, indicates consumers rely more on the retailers for food hygiene and safety information than any other institution in the UK^a. The same study shows that while there is a high level of concern about food safety in general among UK consumers, fruit and vegetables were not mentioned in the list of concerning food types. Only five percent of consumers highlighted pesticides or chemicals as a concern when initially asked, but this increased to 46 percent when prompted. Unprompted, salt in food was also highlighted by five percent as a concern, but after prompting was selected as the most significant concern among UK consumers – 56 percent indicated they were concerned about it. Overall, the perception of food safety is that it has neither got better nor worse over the last 12 months.

These statistics challenge the legitimacy of using Food Safety as a competitive issue because they show that consumers tend to be more concerned about Food Safety when alerted (prompted) to it. There is no question that genuinely unsafe food should be dealt with immediately and comprehensively, but a vague,



unscientific and subjective definition of “safety”, as is the case when phasing in a zero detectable residue policy, is not helpful for consumers or retailers.

A CREDIBLE FOOD SAFETY RISK MANAGEMENT MODEL

The pressure on all participants in the value chain to sell “safe” food was entrenched in the UK legal system during the early 1990s through the introduction of the due diligence concept – that is, all reasonable steps to ensure food is suitable for human consumption have been taken. Retailers initially responded by increasing their human and technical capacity, at relatively high company cost. Some retailers even invested in private testing laboratories.

Toward the late 1990s a new approach emerged with the introduction of upstream food safety and quality assurance and accreditation schemes, such as Nature’s Choice from Tesco and EUREPGAP. These schemes provided retailers with the opportunity to demonstrate appropriate due diligence on the one hand, and shift the significant costs of large administration and technical teams on the other. Much like taking out insurance, retailers adopted outside institutions to manage the perceived risks - pesticide usage is covered in the compliance criteria of all the “standards” to the authors’ knowledge.

With smaller technical teams and arm-length assurance schemes in place, retailers have become less able or willing to deal with pesticide issues, and defend this in light of the complexity of EU pesticide and MRL legislation, whose outcomes are often uncertain. Growers seeking continued access to the retail market have been forced to either “tow the line” and reduce the range of pesticides they use, or take full responsibility to ensure they are compliant with all the legal requirements by applying an appropriate Good Agricultural Practices policy.

Yet, direct engagement with these stakeholders showed that UK retailers with a better understanding of the EU MRL legislative process and its implications for third countries exporting to the EU, were generally more pragmatic in dealing with pesticide risk management. These retailers tended also to be more willing to listen to points raised by the SA representatives and to cooperate to reduce the risk of a pesticide non-conformance for mutual benefit.

In fact, many retailers acknowledged the proactive approach by the southern African citrus industry to manage their own food safety risk through the compilation and distribution of the *Recommended Usage Restrictions For Plant Protection Products On Southern African Citrus*. This is a quarterly (sometimes more frequent) publication reflecting the most recent MRL changes in key markets, **along with appropriate USAGE RESTRICTIONS that help growers to meet the MRL targets**. The *Recommended Usage Restrictions For Plant Protection Products On Southern African Citrus (RUR)* is the sum of close monitoring of the EU MRLs, plus the findings of residue breakdown research by the CRI group, over the last 15 years.

Backed up by credible science, and in line with the legal MRL principles and policies of major trading partners, the *RUR* is firstly defensible before consumers – it meets all the food safety requirements - and secondly, is less prescriptive on what growers can and cannot use to manage pests and diseases in their own situation. It reduces the risk of a non-conformance and addresses many of the concerns highlighted by retailers as reasons for employing a zero detectable residue policy.

As a model to promote sustainable and responsible agriculture and to offer retailers the appropriate assurances (“*all reasonable steps to ensure food is suitable for human consumption have been taken*”) this approach by growers was well received by retailers. It also “fits” with commercial accreditation schemes already in place such as EUREPGAP. For example, Tesco have worked closely with the CGA and CRI in their latest version of the RAG Plant Protection Products List (PPPL) and the final draft now includes previously “objectionable” PPPs, taking into account the appropriate usage restrictions for these PPPs.



CONCLUSIONS AND RECOMMENDATIONS

Concern among SA fruit and vegetable industries that retailers within the EU are applying additional restrictions on the use of PPPs over and above the EU pesticide regulations are justified based on the unofficial confirmation by these stakeholders.

Essentially UK retailers are seeking ways to reduce their exposure to losses resulting from a Food Safety incident, particularly around pesticide exceedances as they perceive this to be their greatest risk. The current strategy is to aim for zero detectable residues over the next 8-15 years, effectively precluding the usage of PPPs in future.

As a practical alternative the SA representatives were able to demonstrate the model used in the southern African Citrus industry, through the compilation of the *Recommended Usage Restrictions For Plant Protection Products On Southern African Citrus*, which can scientifically provide consumers and retailers with the appropriate level of assurance while optimising the number of instruments/tools to manage pests and disease according to Good Agricultural Practices.

The first recommendation, therefore, is that this approach needs to be further promoted among EU retailers. Each fruit type will need to consider how best to achieve this, particularly if additional resources are required in SA to compile a usage restrictions document.

Secondly, on-going interaction with retailers and consumer groups is necessary to remain in touch with their current thinking on food safety issues. To improve communication with these stakeholders at least one of two alternatives should be used:

1. Using local expertise from SA: One trip overseas per year is recommended, supported by quarterly conference calls. Local knowledge is critical when engaging with these stakeholders.
2. Employing a consultant in the EU to keep in touch, who then provides appropriate feedback to the SA fruit and vegetable industries.

ACKNOWLEDGMENTS

As documented in the Carbendazim Case study, this trip proved highly significant for the future sustainability of the southern African Citrus industry. Relationships with key stakeholders within the EU were also strengthened significantly. Our thanks go to the SA PIP for co-funding the trip, and for all those that made the time and effort to engage with the SA representatives.



ANNEX 1: CGA/CRI ITINERARY UK AND EUROPE

CITRUS GROWERS ASSOCIATION of SOUTHERN AFRICA

23 Plantations Rd, Hillcrest

P O Box 461, Hillcrest 3650
Tel: (031) 7652514/ Fax: (031) 7658029
Reg. No. 2000/010147/08¹

CGA / CRI Itinerary UK and Europe: 12 – 19th October 2005-10-05

Wednesday, 12th October 2005

AM

PH arrives in London
VH arrives in London

PM

14H00 – 16H00: Food Standards Agency

UK Headquarters
Food Standards Agency
Aviation House
125 Kingsway
London
WC2B 6NH

Tel: 020 7276 8000

Alistair Edwards, Import Food Division, 020 7276 8459
Trevor Denham, EU & International Strategy Branch, 020 7276 8634
Raymond Benson,

16H30 – 17H30: British Retail Consortium

[Contact: Bridget La Marca, Administrator]

British Retail Consortium
Second Floor
21 Dartmouth Street
London SW1H 9BP
Tel: 020 7854 8933
Fax: 020 7854 8901

Kevin Swoffer, Head of Technical Services, email: Kevin.swoffer@brc.org.uk



Thursday, 13th October 2005

10H00 – 13H00: Pesticide Safety Directorate

Pesticides Safety Directorate
Consumer Safety and European Policy Branch
Mallard House, Peasholme Green
York,
YO1 7PX.
United Kingdom

Tel: (+44) (0)1904 455759
Fax: (+44) (0)1904 455733

Dr Mark Hawkins,
Russell Wedgbury

The remainder of Thursday spent investigating the proposal to revoke the Carbendazim MRL on citrus.

Friday, 14th October 2005

09H30-11H00: Sainsbury and supplier to Sainsbury (Chingford).

33 Holboron Road
Central London
EC1N 2HT

Dr Theresa Huxley, Quality Manager, Sainsbury, [Contact: Mobile 07785 700073; Tel: 020 76958453]
Simon Thirkell, Chingford, [Contact: Mobile 07785700074; Tel: 441322429777]

10H30-12H00: Munoz-Mehadrin (UK) Ltd, Supplier to M&S

Rob Brown, Technical Manager [Contact: +44 (0) 1354 –697600]

14H30: Tesco

Tony Palmer, Trading Law & Technical Manager (Citrus), **Tesco**, [Contact: 044 1707 678745]

Sunday, 16th October 2005

VH returns to SA
PH flies to Paris to attend EurepGAP conference.

Monday 17th October 2005 – Wednesday 19th October 2005

EurepGAP conference: Towards Global Harmonisation 2005, “Achieving a Common Understanding of Safe and Sustainable Agriculture”, Paris

Informal discussions with retailers and consumer groups at the EurepGAP conference.



Organization	Name	Position	Contact No.
ASOEX	Ronald Bown	Chairman of the Board	+56 2 4724700
Bayer Cropscience	Dr Alfons Sagenmuller	Portfolio Management/ISM Global ICM Governance	+49 21 7338 5757
CropLife International	Keith Jones	Manager, Stewardship and Sustainable Agriculture	+32 2 542 0410
Dow AgroScience	Peter Watson	Product Registration Specialist EU Department	+44 (0) 1235 437920
European Crop Protection Association	Stuart Rutherford	Environment & Food Policy Senior Manager	+32 2 663 1550
Sainsbury's	Jon Roe	Supplier Performance Manager	020 7695 7061
Freshfel	Tom Lyall & Philippe Barnard		+ 32 2 777 1580
Tesco & Global Food Safety Initiative	Chris Anstey	Product Integrity Manager, Trading Law & Technical	01707 678 772
The World Bank	Steven Jaffee	Senior Economist, International Trade Development	+ 202 458 1696
World Wildlife Fund	Jason Clay	Vice President Center for Conservation Innovation	(202) 778 9691



ANNEX 2: CASE STUDY: AVERTING THE IMPACT OF A POTENTIAL REVOCATION OF THE EU CARBENDAZIM HARMONIZED MRL ON CITRUS

CITRUS GROWERS ASSOCIATION of SOUTHERN AFRICA

23 Plantations Rd, Hillcrest
P O Box 461, Hillcrest 3650
Tel: (031) 7652514/ Fax: (031) 7658029
Reg. No. 2000/010147/08¹

PREAMBLE

During a scheduled meeting between the UK Pesticides Safety Directorate (PSD), CGA (Paul Hardman) and CRI (Prof. Vaughan Hattingh) on the 13 October 2005, the southern African citrus industry became aware that the future status of the EU carbendazim Maximum Residue Level (MRL) on citrus was under urgent review. The relevant Rapporteur's (Germany) recommendation to the EU Pesticides Residue Committee, was to revoke the carbendazim MRL for citrus. Various fungicide sprays that result in carbendazim residues are routinely applied to citrus produced in southern Africa, for the control of the fungal disease Citrus Blackspot.

The estimated value of citrus exported annually to the EU is between R1 - R1.2 billion, of which 60 percent might well have been sprayed with Carbendazim products in 2005. This case study briefly describes the urgent course of action taken by CGA and CRI to avert the ensuing crisis.

CASE STUDY:
AVERTING THE IMPACT OF A POTENTIAL REVOCATION OF THE EU CARBENDAZIM HARMONIZED
MRL ON CITRUS

13 October 2005: Meeting with PSD – southern African citrus industry learns of MRL review process and possible changes to carbendazim MRL.

One of the objectives of the meeting with PSD was to investigate concerns regarding the future status of EU MRLs for some chemical treatments of strategic value to the industry. These investigations provided access to a process that was very recently initiated within the EU, wherein the MRLs of several chemicals are being urgently reviewed in light of new information pertaining to methodologies used in establishing residue tolerance levels (MRLs).

13 October 2005: A call was immediately placed through to Tian Schutte (CRI, fungicide specialist), to enquire whether sprays had already been applied in South Africa. It was indicated sprays are only recommended for mid-November, three weeks away!

The urgent EU review process was still in a pre-regulatory phase, and no parties outside of the relevant EU structures had yet been notified. In fact, it was unlikely any communications through the normal channels, such as the WTO notifications system, would have taken place until a final decision had been made. Without personal contact with the PSD the citrus growers in southern Africa would have had no way of knowing of the imminent changes.



13 & 14 October 2005: Vaughan Hattingh and Paul Hardman urgently engaged in consultation with the relevant parties in the European Commission (Dr Bas Drukker, DG SANCO, Pesticide Residue Committee) and Rapporteur Member State (Dr Karsten Hogardt, Germany) to confirm our understanding of the situation was correct.

Prof Vaughan Hattingh was able to ascertain that Carbendazim products are not widely used among EU citrus producing countries by consulting with members of CLAM (Association of Mediterranean Citrus Producers). It was thought that Spain used Carbendazim as a post-harvest treatment, and therefore the potential revocation of the MRL strange in light of their presence on the EU Pesticide Residue Committee.

Nonetheless, Carbendazim had been identified as one chemical treatment of strategic importance to the southern African Citrus industry at the outset of the SA PIP program (June 2004) and the first year's research had already been completed during 2005. The project was allowed to run the full first year, at cost of approximately R141 000 (2 trials), but Year 2 trial work (6 trials) was terminated at the request of industry, based on the setting of an EU Harmonized MRL for Carbendazim on citrus (5.0ppm).

17 October 2005: As an emergency measure, a data package on carbendazim was put together by CRI and submitted (on behalf of the CGA) to the relevant parties in the EU for deliberation before the next Pesticide Residue Committee meeting on 21 October 2005. Available CRI data, recent trail work under SA PIP, and work conducted by SABS over time was included in this package.

At this point the CGA Executive were asked to consider whether citrus growers should be informed of the potential change to the Carbendazim MRL in light of the prevailing uncertainty. Key considerations were:

1. Cost implications and the availability of replacement products, and avoiding panic amount growers should there be little stocks available.
2. Growers applying Carbendazim sprays during the waiting period (although this would be against standard recommendations)
3. The slim possibility that the PRC could set a new positive MRL for carbendazim on citrus, with a immaterial difference to current practices.

18 October 2005: CGA Executive decided to delay communication to growers until the EU Pesticide Residue Committee could provide more direction regarding the revocation of MRL. Sprays were not due for at least two weeks.

21 October 2005: Submitted data were considered at a voting of the EU Pesticide Residues Committee. A vote on the proposal to revoke the MRL was deferred to 09 December at the latest, to enable more comprehensive evaluation of relevant data.

Without conclusive direction from the PRC, growers were still faced with the possibility of an 0.01mg/kg Carbendazim MRL on citrus effective sometime during the 2006 export season.



22 October 2005: Cautionary notice sent out to all southern African citrus growers, effective immediately, to refrain from spraying carbendazim on citrus destined for export to the EU market in 2006.

The Rapporteur Member State consistently maintained that a 5mg/kg Carbendazim MRL on Citrus was unacceptable, but would consider setting a new MRL based on Good Agricultural Practices should there be sufficient supportive data generated under Good Laboratory Practices. The deadline for submissions of such data was set at 3rd November 2005.

22 October 2005 – 3rd November 2005: CGA then made attempts to source supportive and appropriate data from:

- SA Plant Protection Product suppliers.
- SHAFFE (Freshfel)
- COLEACP PIP
- Florida Citrus Industry
- CODEX

Contact was made with all Plant Protection Product suppliers with current Benomyl, Carbendazim or Thiophate-Methyl registrations in South Africa requesting data, but none able to supply Good Agricultural Practice data. Most had relied on the original registration data by BASF in 1970's and only had efficacy data and evidence of equivalent formulation of the product.

COLEACP PIP: Engaged Roland Levy in Brussels. Roland could confirm his knowledge of the review process and expressed frustration at the lack of communications with 3rd country stakeholders. No work had been conducted by COLEACP PIP on Carbendazim on citrus (only on paw paws)

Florida Citrus Industry: Contacted Dr. Pete Timmer (Citrus Research & Education Center, Lake Alfred Florida, USA) regarding recent registrations of Carbendazim products in Florida. Dr Timmer agreed to make contact with the (emergency) registration holder of the product. Later feedback indicated this was a Japanese company, and every effort was being made to source data despite the obvious time and distance constraints. No data received. (Contact number +9 18639561267, email address lwimmer@mail.ifas.ufl.edu.

CODEX: A search of CODEX Alimentarius data showed evidence of recent toxicological reviews of data, but no recent studies on the MRLs for Benomyl, Carbendazim or Thiophanate-Methyl. In fact, the CODEX MRL was largely based on information provided by South Africa during the 1970's.

SHAFFE: Freshfel, the Secretariat for the Southern Hemisphere Associations for Fresh Fruit Exports (SHAFFE), was notified and asked to send a call to all members (Argentina, Brazil, Uruguay, Australia, New Zealand and Chile) to submit appropriate data directly to the Rapporteur Member State. To our knowledge only Uruguay and Brazil provided any feedback, in the form of residue monitoring data collected over a large proportion of their citrus crop. The residue readings were significantly lower than the 5mg/kg level, but as monitoring data the PRC would not be in a position to use this data in their deliberations.

3rd November 2005: Deadline to submit GAP data passes. Letter written to DG SANCO to request the new MRL be set as a Provisional MRL, thus creating the opportunity to generate data for future consideration.



An appeal to the PRC to set the new Carbendazim MRL on citrus as a **provisional MRL** was made through a letter to Dr Drukker. Furthermore, a request for conformation of the specifications of an adequate data package (to apply for an Import Tolerance) was made, so research could begin in South Africa before mid-November (according to the Good Agricultural Practices for Carbendazim on Citrus).

4th November 2005: Meeting held between Swaziland Region CGA Board Member and EU Commissioner to South Africa.

The CGA drafted a letter outlining the serious practical and trade consequences of a revocation of the EU Carbendazim MRL on citrus, which was handed to the SADC regional representative from the EU during a meeting between Per Noddeboe, Swaziland representative on the CGA Board. The particular concern expressed was the lack of proper appropriate communication to 3rd countries during the early discussions of the documents.

9. TECHNOLOGY TRANSFER (H.F. le Roux)

9.1 EXTENSION REPORT (H.F. le Roux & J.J. Bester)

9.1.1 Research Priorities for 2006

The most important function of the Extension Division is to ensure that the research priorities of citrus producers are defined and conveyed to the Extension Division, and thereafter solutions to problems made known to the growers as soon as possible. All citrus producing areas are therefore visited in August each year to determine the research priorities.

The research priorities in the different citrus producing areas in southern Africa were established during August 2005 by Hennie le Roux and Hannes Bester. Hannes was appointed during 2005 as Area Extension Manager for the Eastern, Western and Northern Cape, as well as a portion of KwaZulu-Natal. Graham Barry established the research priorities in Zimbabwe. Certain adjustments in research priority status were made, viz. *Rhizopus* on Satsumas in the Western Cape. This featured for the first time as a priority. FCM priorities remain the highest as a result of problems encountered during the past year with exports to the USA, and as a result of the strong demand for exports to China. As far as Citrus Black Spot is concerned, feedback is still awaited from the European Union. Until this pathogen loses its phytosanitary status in Europe, it will continue to remain the highest priority in the Letsitele and Onderberg areas. Rind defects remain the most important priority within the Horticulture section, and Peteca is now also one of the highest research priorities and is found in more than one area. The incidence of Rind Breakdown has also dramatically increased inland.

As in the past, Tables 1-3 of the research priorities for the different areas, and which are detailed in the Annual Research Report, operate on a scale of 0-3. Priorities where a 0 rating has been allocated require no further research, while a rating of 3 requires the highest priority of research in that area. Each research aspect's priorities for 2005 for the different areas are shown for easy reference, the highest research priority is also highlighted. Researchers are reminded that although the weighted aspects are shown, this can be misleading as there are certain priorities, such as *Phaeoramularia angolensis*, which is shown as a low weighting, but is in fact a high priority in Zimbabwe.

In the Disease Management programme, the emphasis is still placed on research on Citrus Black Spot, Post-Harvest Pathology, Greening and Tristeza. The status of *Alternaria* has increased as a result of the occurrence of the disease on certain Late Mandarin cultivars. False Codling Moth and Fruit Fly (especially Natal Fly) feature as high priority aspects in the Integrated Pest Management programme. Grey mite has been identified by two citrus producing areas as their highest priority, while white waxy scale is becoming a problem in certain lowveld regions. The Grain Chinch bug is also becoming a threat in the Western Cape although it can be referred to as a hitchhiker. Peteca, Rind Breakdown, Rind Pitting and Fruit Size manipulation are the main priorities in the Crop Load and Fruit Quality Management programme. Citrus producers have expressed their approval for the appointment of the new Manager of Cultivar Development and look forward in anticipation to results emanating from this appointment.

The updating of the Production Guidelines remains a priority for the industry, especially the section on Fertilization and Irrigation.

9.1.2 PROGRAM: SIEKTEBESTUUR

9.1.2.1 Projek: Swartvlek

In die geval van Weipe, Tshipise en die Benede Oranje rivier is die hoogste prioriteit toegang tot die VSA. Hierdie prioriteite is streng gesproke Marktoegang aangeleenthede maar word genoem omdat dit die aspekte is waarop CRI se waardetoevoeging vir hierdie gebiede gemeet sal word. Daar word aanbeveel dat die huidige Swartvlekprojek sal voortgaan met al die verskillende fasette wat nog nie afgehandel is nie.

1. *Piknidiospore as moontlike bron van inokulum:*

Indien piknidiospore in sekere kultivars met meervoudige vrugsette soos suurlemoene as bron van inokulum kan optree, wat is die klimaatsvereistes wat nodig is vir infeksie om plaas te vind? Op watter stadium is die blare vatbaar vir piknidiospore infeksie?

2. *Epidemiologie:*
 - Evaluering van verskillende biologiese agente om die dooie blare op die grond vinniger af te breek.
 - Evaluering van die stofsuiwer wat in die Oos Kaap ontwikkel is om dooie blare te verwyder.
 - Evaluering van grondbewerking (disc) om blare wat in die rye in gevee is effektief te bedek.
3. *Na-oesbeheer:*
 - 'n Studie om vas te stel wat gedoen kan word om die ontwikkeling van latente infeksies na verpakking in transito te onderdruk. Dit sluit in 'n oudit van wat huidiglik met vrugte gebeur vanaf die pakhuis onderweg na die hawe, by die hawens voordat dit verkoel word, tydens die laaiproses en tydens verskeping. (Kyk na Paul Cronjé se data vir vrugtemperatuur van vrugte wat vanaf Weipe na die hawe vervoer is).
 - Effek van warmwaterbehandelings op simptomeontwikkeling.
 - Chemiese / biologiese onderdrukking van simptomeontwikkeling.
 - Metodes om simptomeontwikkeling in boorde te stimuleer sodat latente infeksies kan wys voordat vrugte verpak word.
 - Metode om vrugte op die paklyn te kan "scan" vir latente infeksies.
4. *Kwekerie:*
 - Warmwaterbehandeling om swartvlekvrugte materiaal te verseker.
 - Finalisering van die protokol om sitruskwekerie te monitor vir die teenwoordigheid van swartvlek in plantmateriaal.
 - Swartvlekbeheerprogramme wat in die kwekerie gebruik kan word wat nie 'n gevaar inhou vir die ontwikkeling van weerstand teen swamdoders wat in kommersiële boorde gebruik word nie.
5. *Chemiese beheer:*
 - Evaluering van nuwe chemiese middels asook beheerstrategieë wat meer koste-effektief is, wat Dithane kan vervang en wat die gevaar van weerstand sal verminder. Sporekill program moet aan produsente bekend gemaak word.
 - Hervervaluing van die terugwerkende aksie van strobilurienes (1, 2 dalk 3 dae). Rol van die tweede strobilurienes op die genesende effek van swartvlek.
 - Registrasie van strobilurienes op suurlemoene.
 - Ondersoek om die werklike verspreiding van Benlate weerstandbiedendheid te bepaal en te onderskei tussen *G. citricarpa* en *G. mangiferae*.
6. *Weerstandbiedendheid:*
'n Toets om *Guignardia* weerstandbiedendheid teen die strobilurienes te bepaal soos wat tans vir die benzimidazole gedoen word.
7. *Nuwe PCRs en 'n Diagnostiese "kit":*
 - Ontwikkeling van PCRs om die teenwoordigheid van CBS op simptomelose blare en enthout te bepaal.
 - Die ontwikkeling van 'n vinnige toetsapparaat wat deur produsente/pakhuis /PPECB gebruik kan word om te onderskei tussen *G. citricarpa* en *G. mangiferae*.
8. *Selektiewe medium:*
'n Selektiewe medium om *Guignardia* op te kweek is dringend nodig.
9. *Genetiese weerstand:*
Ondersoek die moontlikhede om geneties gemodifiseerde weerstand in sitrusplantmateriaal te bewerkstellig.
10. *Voorspellingsmodel:*
 - Voorspellingsmodel wat kan voorspel wanneer die klimatologiese toestande gunstig sal wees vir spoorontkieming en infeksie vir inokulumbestuur (Katrivier)
 - Outomatisering van spoortellings.

9.1.2.2 **Projek: Entoordraagbare Siektes**

Vergroening

1. Vektorbeheer: Ontwikkeling van alternatiewe middels vir stamaanwending om monokrotofos en metamidofos te vervang. Ontwikkeling van lok- en dood middels om bladvlooi op 'n soortgelyke wyse te beheer as wat die M3 vrugtevlug beheer. Ontwikkeling van alternatiewe beheerpraktyke (Predatore/parasiete/paringsontwrigting.)
2. Genetiese weerstand: Weerstandsteling deur gebruik te maak van chimeras met vergroeningsverdraagsame sektore. Die werk wat die ITSG hierop doen moet ge-evalueer word deur Prof. Pietersen.
3. Weerstandsteling d.m.v hoëvlak biotegnologie en geneties gemanipuleerde weerstand. (Vergelyk met die werk wat in Bordeaux gedoen is teen *Spiroplasma citri*.)
4. Korrektiewe middels: Alle moontlike middels moet hier ge-evalueer word ongeag aanvaarbaarheid vir die markte. Dit sluit in antibiotikas, middels wat plantweerstand (SAR) verhoog en middels wat vergroeningsimptome onderdruk.
5. Hittebehandelings: Die gebruik van plastiese koepels om d.m.v. solarisasie temperature so te verhoog dat dit die bakterië binne die plant kan vernietig. Ontwikkel 'n model wat aantoon hoe die bakterie titer afneem namate sekere Temperatuur/Tyd behandelings toegepas word.
6. Bewusmakingsveldtog: Opgradering van Produksieriglyne en werkswinkels.
7. Goedkoop tegniek om kwekerybome te toets vir vergroening (UV lamp).
8. Wetgewing om die verwydering van vergroeningsbesmette bome in die Kaap af te dwing.

Tristeza

1. Onderzoek die teorie dat die saamstel van 'n super CTV kruisbeskermingsras nie nodig is nie, maar slegs die gedeelte van 'n kruisbeskermingsras se DNA wat die sein vir kruisbeskerming gee.
2. Evaluering van verbeterde kruisbeskermingsrasse vir elk van die verskillende pomeloproduiserende areas (Tshipise, Letsitele, Hoedspruit, Malelane, Komatipoort/Swaziland, Nkweleni, Benede-Oranje). Veral Letsitele voel sterk hieroor.
3. Verdere evaluering van verskillende CTV populasies op TSR. Maak seker dat die kruisbeskermingsras wat huidiglik by die Grondvesblok gebruik word wel die regte een is. Swaziland: Herevaluering van moontlike bronne met kruisbeskermingsrasse voordat boorde verwyder word. Hierdie versoek van IYSIS moet dringend aandag ontvang. Dit is nie na hulle vorige jaar se versoek gedoen nie.
4. Tuinboukundige aspekte wat die insidensie van Tristeza agteruitgang kan vertraag.
5. Alle bestaande proewe wat Faan van Vuuren voortgaan.
6. Instandhouding van groeipuntenting, kruisbeskerming van nuwe plantmateriaal en monitering van Grondvesblok.

Onverenigbaarheid

Onderzoek die rol wat "Citrus leave blotch" speel in bo-stam / onderstamonverenigbaarheid.

Ander

- Onderzoek die galvorming op die Clemenpons en maak seker dat hierdie kultivar vry is van ongewensde siektes. Al die sogenaamde "Bulbome" is vry van galle terwyl die wat die sogenaamde regte Clemenpons bome is, almal galle het. Hierdie bome is duidelik gestres wat die vroeër rypwording verklaar. Die bedryf wil die versekering hê dat daar nie 'n risiko bestaan dat die siekte wat die galle op die Clemenpons veroorsaak na ander kultivars kan versprei nie.
- Onderzoek die Lina nawels in die Katrivier en in die Grondvesblok en stel vas of *Impietratura* nie dalk deur die groeipuntentingsproses geglip het nie.

9.1.2.3 **Projek: Na-oes patologie**

1. Evaluering van nuwe wakse, oppervlak steriliseerders en swammiddels soos wat dit beskikbaar raak.
2. Ontwikkeling van strategië om alternatiewe in plek te hê indien weerstand opbou teen die bestaande na-oes swammiddels (GRAS chemikalië, Fisiese behandelings soos osoon en warmwaterstrategië).
3. Na-pak berokings om bederf en fitosanitêre plae te elimineer.
4. Evaluering van bederf wat voorkom tydens kommersiële ontgroenings.
5. Na-oes strategië vir organies geproduseerde sitrus. Kan Sporekill hier Imazalil en Guazatien vervang al is dit nie so effektief nie?

6. Verklaar hoekom die VSA vrugte se wakse in Japan soveel beter vertoon as vrugte wat in SA gewaks is.
7. Monitering (landswyd) van die insidensie en regstelling van imaziliel- weerstandbiedendheid in pakhuse (Wes Kaap).
8. Effek van humiditeitsbeheer op na-oespatogene in die ontgroeningsproses.
9. Voorkomende beheer van suurvrot. Monitering van produkte wat tans in die handel gebruik word vir suurvrot beheer. SABS resultate wys dat van hierdie produkte nie die gespesifiseerde hoeveelheid aktief bevat nie.
10. Alternatiewe beheer deur gebruik te maak van antagonistiese en ander biologiese beheer agente. Die bedryf is van mening dat die kapasiteit binne na-oespatologie verhoog moet word.
11. Evalueer die beste beheerstrategie vir **Rhizopus op Satumas** en in CHEP kratte.

9.1.2.4 **Projek: Grondgedraagde siektes**

Sitrusaalwurm

1. Evaluering van biologiese beheeragente, veral *Paecilomyces lilanicus* onder drup.
2. Stimulering van aalwurmeiertjies om uit te broei sodat eenmalige aalwurmdoertoedienings die aalwurm se lewenssiklus meer effektief kan onderbreek.
3. Onderstamevaluering.
4. Voorplantbehandeling van herplantgronde. (Beide gronde met 'n relatiewe hoë klei-inhoud en sanderige grond). Hier moet gekyk word na alternatiewe vir metielbromied soos Vapam, bio-beroking, en die kombinasie van bio-beroking en solarisasie.
5. Effek van aalwurms indien enige in OHS boorde.

Skede-aalwurm (*Hemicycliophora*)

1. Onderstamevaluering.
2. Effek op groeikragtigheid van kwekeryboompies.

Phytophthora

1. Evaluering en registrering van die gebruik van fosfonate deur drupstelsels.
2. Effektiewe beheer van *P. citrophthora*.
3. Faktore wat vrugte meer gevoelig maak vir koper en fosfonaat "stippeling" (water pH?)
4. Evaluering van nuwe chemiese middels wat op ander gewasse teen *Phytophthora* ontwikkel is in die afsienbare verlede, met die klem op kwekerye.
5. Ontwikkeling van 'n merker om onderstamme vinniger te kan toets vir *Phytophthora* verdraagsaamheid.
6. Evaluering van verskillende fosfonaat formulasies deur Agri Inspect of die DC.

Fusarium/Blight

1. Die rol van *Fusarium* op wortelvrot waar lae koolhidraatvlakke voorkom en alternatiewe drag veroorsaak, soos wat tans met die laat mandaryne ondervind word, moet ondersoek word.
2. Onderstam-evalueringstoele in Letsitele teen Blight moet gemonitor word.
3. Nuwe onderstamme se gevoeligheid vir isomartisien (*Fusarium*-toksien) moet bepaal word. Hoe vergelyk C35 met die huidige kommersiële kultivars?

Armillaria

1. Chemiese beheermaatreëls (dit wil voorkom of die Phytex wat by IYSIS in Swaziland gebruik word effektief is.)
2. Alternatiewe gashere in Patensie.

9.1.2.5 **Projek: Vrug en blaarsiektes**

Pseudocercospora angolensis (P.a.)

1. Ondersoek na huidige status van siekte sodra daar weer politieke stabiliteit in Zimbabwe is.
2. Residu ontledings van chemikalië wat vir die laaste bespuiting in Februarie/Maart aanbeveel word

- (werk kan in SA gedoen word).
3. Klimatologiese kartering van *P.a.*

Alternaria

1. Evaluering van nuwe kultivars teen *Alternaria* soos wat hulle in die land ingebring word.
2. Evaluering van meer koste-effektiewe spuitprogramme en nuwe chemiese produkte a.g.v. nuwe *Alternaria*.
3. Voorspellingsmodel vir die sitrus-industrie.

Botrytis

1. Evaluering van chemiese beheer op suurlemoene.
2. Bepaling van toedieningstye.

Cercospora

Geen navorsing maar monitor die situasie voortdurend.

9.1.3 PROGRAMME: INTEGRATED PEST MANAGEMENT

9.1.3.1 Project: False Codling Moth (FCM)

1. Evaluation of new chemicals.
2. Evaluation of granuloviruses for FCM control on a commercial scale in all the citrus production areas.
3. Evaluation of *Trichogrammatoidea cryptophlebiae* for FCM control in more areas.
4. Evaluation of the development of SIT on a commercial scale.
5. Evaluation of repellents for FCM.
6. Evaluation of biocontrol of FCM larvae.
7. Evaluation of mating disruption to control FCM.
8. Country-wide assessment of alternative host plants.
9. Re-evaluation of the Lorelei and other traps and the meaningfulness of the threshold levels. Determine threshold levels for traps other than the Lorelei.
10. Effect of Alsystin and Nomolt on red spider repercussions.
11. Importance of sanitation of November drop on FCM control.
12. Effect of higher cold storage temperatures vs. time (every raised 0.5°C cold storage temperature has a major effect on colour and rind integrity of certain cultivars).
13. Evaluation of post-packing fumigation treatments.
14. Determine whether Meothrin susceptibility exists in Patensie.
15. Evaluation of parasite releases.
16. Cultural practices that can change the severity of FCM, such as discing.
17. Packhouse line detection of infected fruit (IR?).
18. Irradiation of fruit prior to export as an alternative to cold steri.
19. Revise the intervention thresholds of FCM monitoring (all registered traps) in light of its phytosanitary status.

9.1.3.2 Project: Fruit fly

1. Registration of M3 for organic farming.
2. A model to reduce the number of M3 traps from the outside to the inside of larger orchards to reduce cost. Article in SA Fruit journal on this aspect.
3. Evaluation of insecticides used in the M3 to last longer.
4. Evaluation of grape varieties for susceptibility in order to be able to concentrate on these vineyards to eliminate fruit fly in areas where both citrus and grapes are grown. Refer to DFPT.
5. Cold sterilization of different varieties for Natal fruit fly.
6. Creation of areas which is for all practical purposes fruit fly free in Patensie and Vaalharts.
7. Evaluation of chemicals to be used in cost-effective bait spray programmes when the use of the OP's are terminated.
8. Alternative chemicals for aerial applications.
9. Alternative fumigation options to control fruit fly post packaging.
10. Re-evaluate threshold values of Sensus traps in comparison to the Delta trap.

11. Other fruit fly aspects which need research according to Tony Ware are:
 - Survey for exotic fruit fly.
 - Cold sterilization of marula fruit fly in oranges.
 - Determine the prevalence of marula fruit fly in citrus.
 - Urban testing of the M3.

9.1.3.3 **Project: Mealybug**

1. Evaluation of biocontrol of mealybug in the different citrus producing areas.
2. Determine the natural populations of predators and parasites in the different areas to establish whether the release of biocontrol agents will increase the levels of parasitism.
3. Seek acceptance of the DNA probe developed in SA by the USDA. Implement the PCR for mealybugs at the DC in Nelspruit.
4. Evaluate alternative chemicals to replace the OPs.

9.1.3.4 **Project: Cosmetic pests**

Thrips

1. Evaluation of alternative chemicals.
2. Breeding and testing of the predatory thrips collected in the Lower Orange River.

Grain Chinch bug (GCB)

1. Evaluate pre-harvest chemical control options to the point of being registered.
2. Evaluate the residue status of the above chemicals in parallel to avoid wasting time on actives that have no potential from a residue perspective.
3. Evaluate post harvest packhouse controls using pyrethrin and pyrethrin + azadirachtin products.
4. Determine GCB control in areas around the packhouse.
5. Isolate GCB attractants for monitoring and possible control purposes.
6. Determine the timing of the movement of GCB into the orchards and the risk of fruit being infested post packing.

Leafhopper

1. Thresholds on thrips traps.
2. Host range
3. Effect of intercropping.
4. Timing of control sprays.

9.1.3.5 **Project: Production pests**

Psylla

1. Alternative chemicals to replace OP stem treatments.
2. Develop biological control options.
3. Develop alternative control strategies especially in older trees. (Dimethoate?)

Ants

1. Develop strategies/chemicals to keep ants out of the trees but in the orchard.
2. Donor with alternative active ingredient.

Red scale

1. Determine the effect of oil sprays on colour, yield (effect of 1% summer oil followed by a dry January/February on yield), internal fruit quality, seedless cultivars.
2. Determine the impact of *Aphytis* releases to establish if the positive results after these releases are in fact because of the releases or as a result of the producers abstaining from chemical sprays allowing the natural occurring *Aphytis* to increase.
3. List of alternative hosts to establish predator and parasite colonies.
4. Timing of the release of *Aphytis* based on scientific principles (monitoring of male flights?)

5. Timing of chemical sprays.
6. Registration of a generic imidacloprid.
7. Rearing of *Aphis africanus*.

Soft Green scale

1. Investigate biocontrol complex.
2. Chemical control options.
3. Host range.
4. Why more prominent in IPM orchards?

Grey mite

1. Effective control strategies.
2. Determine the possible role of the spiroplasma-like organism found by UP several years ago with the concentric ring blotch caused by grey-mite.
This mite is the no. 1 priority for both the Rustenburg and Ohrigstad study groups.

Budmite

1. IPM programme for the Vaalharts area.
2. Alternative for Acarol.
3. Late miticide especially on lemons.

Lemon moth

1. Alternative control options.

Snails

Effective, affordable control measures.

Bollworm

Alternative control strategies.

9.1.3.6 **Project: Biocontrol disruption**

1. Keep data-base of non-target effects of the different chemicals updated.
2. Determine which of the OP's are the first to be removed and ensure that alternative control strategies/chemicals are available when needed.
3. Accreditation of insectaries.

Other

1. Times required by the different chemicals to dry before being effective.
2. Effect of oil on the leaves on subsequent chemical sprays. Do these chemicals then need a longer period to dry?

9.1.4 **PROGRAM: OESOPBRENGS EN VRUGKWALITEITSBESTUUR**

9.1.4.1 **Projek: Oesopbrengs**

1. Evaluering van bestaande Oop Hidroponiese Stelsels (OHS) in die verskillende areas vir verskillende kultivars.
2. Riglyne vir die bestuur van besproeiing en bemesting deur OHS om maksimum suikers en kleurontwikkeling te verseker.
3. Evaluering van puls-besproeiing teenoor minder gereelde drup.
4. Evaluering van boomgrootte beheer en ligbestuur in hoë digtheid aanplantings.
5. Formulering van 'n vrugset-strategie vir nawels in die Oos-Kaap waar groot temperatuur skommelings veroorsaak dat die vrugte afspeen.
6. Vrugsetstrategie vir saadlose suurlemoene.
7. Snoei van suurlemoene in warm areas (Pongola).

8. Verhoogde vrugset op Minneolas om vruggrootte te verbeter en kleiner vrugte te kry (Marble Hall).

9.1.4.2 **Projek: Vruggelhalte**

Vruggrootte

1. Finalisering van vruggrootte model.
2. Verfyning van die gebruik van Corasil en Maxim op vruguitdunning van Ou Kloon Valencias en Pomelos.
3. Oeskattingsmetode vir vruguitdunning.
4. Evaluering van fulviensuur, humiensuur, TopGroeï en ander soortgelyke produkte wat tans in die handel verkrygbaar is vir oes en vruggrootte verbeterings.
5. Effek van somerolie gevolg deur warm temperature op vruggrootte.
6. Onderstamevaluering vir onderstamme wat groter vrugte gee op swaarder gronde.
7. Effek van handuitdunning en snoei verskillende tye van die jaar. Soek 'n produk wat hergroeï kan onderdruk wat meer gebruikers vriendelik is as Planofix.
8. Is die bemestingsnorme wat in die sewentigerjare op lermoene op growweskiilsuurlemoene ontwikkel is steeds relefant vir vandag se kultivars op 'n wye verskeidenheid onderstamme in verskillende klimaatstreke?

Interne vrugkwaliteit

1. Verlagings van suur in sekere areas. Chemies sowel as bestuurspraktyke.
2. Ontwikkeling van 'n plaasvervanger van kalsiumarsenaat om sure te verminder.
3. Metodes om vastestowwe (TSS) te verhoog.
4. Effek van ringelering op interne kwaliteit.
5. Turkey Valencia het probleme in die mark deurdad dit pap word en druk. Kan hierdie probleem d.m.v. bemesting reggestel word?

Oesbestuur

1. Som strategieï vir vrugset op Deltas en Midnights op.
2. Snoeïstrategieï – som bestaande kennis op.

Eksterne vrugkwaliteit

1. Ondersoek die fisiologie van "stippeling" wat sekere tye van die jaar veroorsaak word deur bespuitings van bv. Koper, fosfonate en vrugtevlieglokase. Stel die verband tussen hierdie verskynsel (necrotoma), antraknose en "Swazi-spot" vas. Speel water pH dalk 'n rol? Wat van nagtemperature?
2. Opkleur van suurlemoene vir vroeër vrugte na Japan.
3. Kleurverbetering vir vrugte na die VSA (Gibbereliene, "PGR's, OHS)
4. Indusering van langer vrugte by suurlemoene.
5. Effek van osoon op rakkleefyd van vrugte.

Voorspellingsmodelle

Ontwikkel 'n toets om oorrypheid van vrugte te bepaal.

9.1.4.3 **Projek: Skilkondisie**

Peteca

1. Bepaal die bydraende oorsake.
2. Formuleer strategieï om dit te voorkom.
3. Voorspellingsmodel
4. Effek van wakse en waksaanwending.
5. Effek van ontgroening.
6. Enige na-oes behandelings?
7. Effek van warmwaterbaddens.
8. Effek van hoë stikstofvlakke
9. Effek van die spoed en intensie waarmee vrugte geborsel word.

Skilafbraak

1. Bepaal die faktore wat 'n bydrae lewer.
2. Formuleer strategië om dit te voorkom (bestuur die fisiologie van skilafbraak.)
3. Na-oesbehandelings om skilafbraak te voorkom.
4. Die rol wat die koueketting speel in skilafbraak. Die temperatuur/tyd protokolle van die verskillende kultivars moet opgegradeer word.
5. Bepaal die effek van snoei-intensiteit en die oophou van hergroei op skilprobleme (karotenoides se rol).
6. Rol wat ontgroening speel in skilafbraak (daar kom snaakse goed uit die ontgroeningskamers. 'n Hoër uitpakpersentasie is nodig na ontgroening.)

Gepokte skil (Rind pitting)

1. Bepaal die bydraende faktore.
2. Formuleer strategië om dit te voorkom.
3. Na-oesbehandelings om rindpitting te voorkom

Kraakskil

1. Bepaal die faktore wat aanleiding gee tot kraakskil. (Na al die jare se navorsing is die probleem steeds nie opgelos nie en lei Sondagsrivier tot R15milj. se verliese per jaar.)
2. Ontwikkel 'n betroubare model om kraakskil te voorspel.
3. Evalueer die bestaande boorde onder die OHS in gebiede waar kraakskil voorkom en stel vas of daar 'n afname in kraakskil in hierdie boorde is.
4. Is die verskil in kraakskil in boorde op growweskijsuurlemoen-onderstamme wel soveel laer as op die citranges dat dit 'n terugbeweeg na hierdie onderstam met sy laer interne kwaliteit regverdig?
5. Kan olie-stres 'n bydraende rol speel?
6. Bepaal watter variëteite/seleksies kry dit nie.

Oleo

1. Bepaal weereens al die faktore wat 'n rol speel.
2. Voorspellingsmodel.
3. Fisiologiese oorsake.
4. Voorkomingstrategie moet weer 'n slag gepubliseer word.

Rysterigheid

1. Bepaal die faktore wat dit veroorsaak.
2. Stel die fisiologie van die verskynsel vas.
3. Formuleer strategië om dit te voorkom.

"Checker board" effek

Stel vas of ontgroende vrugte nie weer kan vergoen nie. Daar word vrugte gekry waar die skilkleur wissel van oranje na 'n veel ligter geel.

Vrugbars

1. Faktore wat 'n rol speel.
2. Strategie om dit te voorkom (Letsitele).

Koueskade

1. Ontwikkel verskeppingsprotokolle vir nuwe kultivars soos die laat mandaryne.

9.1.5 PROGRAMME: CULTIVAR AND ROOTSTOCK EVALUATION

9.1.5.1 Project: Cultivar and Rootstock evaluations

Cultivars

1. Investigate the seediness of the late mandarins in the Citrusdal and Swellendam areas which are supposed to be seedless.
2. Investigate the alternative bearing of the Late mandarins.
3. Evaluate the Late mandarins in the Knysna area.
4. Determine the stability of the Cambria fruit. If it is not stable ask the Registrar to remove its plant breeders rights.
5. Evaluation of Satsumas, Early and Late navels in Ohrigstad.
6. Current status of the Fukomoto.
7. Letsitele Study group requested that Late navels, Late mandarins a large Late Valencia and sweeter grapefruit to be included.
8. The Groblersdal area is also looking for a large late Valencia to be picked in August and September and an early maturing navel.
9. Weipe is looking for an early Valencia that ripens before the Bennie for the Chinese market.
10. Vaalharts requested that the trials planted in that area which were not evaluated during the past two years should again be evaluated (this could have taken place in 2005).
11. Swaziland would like to have the Late navels evaluated at Ngonini.
12. The Richmond area in Natal request that CRI evaluates existing late navel trials.
13. All new cultivar trials should also be evaluated for pests and diseases as well as its susceptibility for rind problems e.g. Oleo.

Rootstocks

Evaluation of rootstocks for drought tolerance.

9.1.5.2 Project: Breeding

The industry would like the breeding programme to continue as in the past. It would also like to see the CGA and the ARC resolve their deadlock on the ownership of the cultivars emerging from the programme.

Biotechnology

1. Selecting seedless high quality oranges.
2. Cultivar improvement using chimeras and *in vitro* ovule rescue.
3. Greening disease resistance through the use of ovule rescuing of disease free chimeras in infected fruit. The industry is of the opinion that the progress with this research is too slow and that Prof. Gerard Pietersen should get involved.

Other

1. Ensure the highest standards and protocols where the shoot tip grafting and pre-immunisation is conducted at the ITSC and the CRI.
2. Ensure that the citrus gene pool facility at Nelspruit is running and erect more glasshouse facilities if necessary.

9.1.6 SITRUSVERBETERINGSKEMA

9.1.6.1 Projek: Kultivars

1. Die regte Robyn nawel moet geselekteer word en deur groeipuntenting geneem word om dit wat huidiglik by die Grondvesblok is te vervang (Patensie versoek).
2. Daar moet verseker word dat die Grondvesblokstandaarde op dieselfde vlak gehou word as wat dit deur al die jare was.

It is however not only the producer's research needs which are catered for. The Citrus Exporters Forum (CEF) is also consulted. Herewith the list of priorities as determined by a CEF technical

committee:

Prioritizing for research topics were grouped by:

1. Waste
2. Rind disorder/physiological
3. Marketability
4. Market Access
5. Shipping conditions
6. New Technology
7. Technology transfer

(The different topics were rated by 0-3: 3 = Highest priority)

	<u>Rating</u>
1. Waste/Decay	
Penicillium	3
Wet waste (suurvrot)	3
FCM	3
Fruit Fly	1
Survey on resistance to imazalil (National)	3
Replacement to imazalil	3
Manuals for decay control	2
Optimum shipping temperature to control waste	2
Reduce industry decay below 3% / no. of cartons (at arrival)	Challenge
Determination of over-maturity (puffiness)	2
2. Rind disorders/physiological	
Rind pitting	3
Peteca	3
RBD	2
Cubing/Puffiness	2
Creasing	3
3. Marketability	
Cultivar development	2
Adaptability of existing cultivars	2
4. Market Access	
FCM	3
Fruit fly (Natal most important)	3
Mealybug (WC), USA / Korea	2
Chinch bug (USA)	2
Other hitchhikers	1
CBS	3
5. Shipping conditions	
Temp profile	2
Temp regimes	2
CO ₂ /CH ₄ /Humidity	2
New Container technology	2
Negative effect of low temperature on colour/quality	2
6. New Technology	
Irradiation, etc... replacement of cold steri	3
Packaging and ventilation	2
New waxes	2
Ozone for decay control	3
7. Technology transfer	
Interpretation of research results	3
Manuals for decay control	3

**RESEARCH PRIORITIES
2005/2006**

DISEASE MANAGEMENT						Table 1																									
Citrus Area	CBS		Alternaria		Melanose		Diplodia		P. ango- lensis		Rhizopus		Botrytis		CTV		Greening		Phytoph- thora		Fusarium		Armilla- ria		Tylen- chulus		Skede aalw.		Post Harvest		
	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	
Citrusdal	0	0	1	3	0	0	0	0	0	0	0	3	0	0	0	0	2	1	2	2	0	0	0	0	2	2	0	0	3	3	
Stellenbosch	0	0	2	2	0	0	0	0	0	0	0	3	0	0	0	0	3	3	1	1	0	0	0	0	1	1	0	0	3	3	
Swellendam	2	2	1	3	0	0	0	0	0	0	0	0	0	0	0	0	3	*3	1	3	0	0	0	0	1	0	0	0	2	2	
Knysna	2	2	1	1	2	2	3	3	0	0	0	0	0	0	0	0	1	1	3	3	0	0	0	0	0	0	0	0	2	2	
Patensie	3	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	0	0	3	3	2	2	1	1	3	3	
Sondagsrivier	3	2	1	2	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0	1	2	0	0	3	3	
Katrivier	2	2	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	3	3	
Kakamas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	1	0	0	0	0	0	0	0	0	2	2	
Vaalharts	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	2	2	
Richmond	3	1	0	1	2	1	0	0	0	0	0	0	2	1	0	0	2	2	1	2	0	0	0	0	2	2	0	0	3	3	
Nkwaleni	3	2	1	1	1	2	0	0	0	0	0	0	0	0	3	3	2	2	2	3	0	0	0	0	1	2	1	2	2	2	
Pongola	3	3	1	1	1	1	0	1	0	0	0	0	0	0	3	3	1	2	2	2	0	0	0	0	0	0	0	0	2	3	
Swaziland	3	*3	1	1	1	1	0	0	0	0	0	0	0	0	3	3	2	2	1	1	0	0	1	1	1	1	0	0	2	2	
Komatipoort	3	3	1	1	1	1	0	0	0	0	0	0	0	0	3	3	0	0	1	1	0	0	0	0	1	1	1	0	2	3	
Malelane	3	3	1	0	1	0	0	0	0	0	0	0	0	0	3	3	0	0	1	1	1	0	0	0	1	1	1	0	2	2	
Nelspruit	3	3	1	1	1	0	0	0	0	0	1	0	1	1	2	1	3	*3	1	1	0	0	0	0	1	1	0	0	2	3	
Burgersfort	3	3	2	*3	1	1	0	0	0	0	0	0	0	0	0	0	3	3	1	3	0	0	0	0	0	2	0	0	2	3	
Groblersdal	3	3	1	2	1	1	0	0	0	0	0	0	0	1	0	0	3	3	2	2	1	0	0	0	1	2	0	0	2	2	
Ohrigstad	0	3	0	2		0	0	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0	0	0	0	1	0	0	0	3	
Hoedspruit	3	3	1	1	1	0	0	0	0	0	0	0	0	0	3	3	2	3	1	3	0	0	0	0	1	2	0	0	2	3	
Letsitele	3	*3	1	1	0	0	0	0	0	0	0	0	0	0	2	2	3	3	2	2	2	1	0	0	2	2	1	0	3	3	
Limpopo	2	*3	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	1	1	0	0	0	2	2	0	0	2	2	
Rustenburg	3	3	2	2	0	1	0	0	0	0	0	0	0	1	0	0	3	3	1	1	2	2	0	0	2	2	0	0	2	3	
Weipe	0	*3	0	1		0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	
Zimbabwe	3	3	2	0	3	3	0	0	3	3	0	0	1	1	3	0	3	3	2	1	1	1	0	0	2	1	0	0	2	3	
Weight	53	54	23	34	16	14	3	4	3	3	1	8	4	6	29	26	38	41	32	43	8	4	4	4	4	4	3	53	65		
Average	2.12	2.16	.92	1.36	.64	.56	.12	.16	.12	.12	0.04	.32	.16	.24	1.1	1.04	1.52	1.64	1.28	1.72	.32	.16	.16	.16	.16	1.0	1.2	.16	.12	2.1	2.6

* Highest Priority

Citrus Area	FCM		Fruit Fly		Thrips		Red scale		Ants		Grey mite		Chinch bug		Mealy-bug		Psylla		Leaf-hopper		OP replacement		Rust mite		Bud-mite		Boll-worm		Waxy scale		Lemoth		
	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	
Citrusdal	3	*3	3	2	2	1	2	2	2	1	0	0	3	3	3	3	1	0	0	1	3	3	1	2	0	2	0	2	0	0	0	0	
Stellenbosch	3	*3	3	3	1	1	1	1	1	1	0	0	3	3	3	3	3	3	0	1	3	3	0	0	0	2	0	2	0	0	0	0	
Swellendam	3	3	3	3	1	1	1	1	1	3	0	0	0	0	1	3	3	3	0	0	3	3	0	0	0	0	0	0	0	2	0	0	
Knysna	3	3	3	3	1	1	1	1	1	1	0	0	0	0	1	1	2	2	0	2	3	3	3	3	0	0	0	0	0	0	0	0	
Patensie	3	*3	3	2	2	2	1	1	1	1	0	0	0	0	2	2	2	2	1	1	3	3	1	1	0	2	0	0	0	0	0	2	
Sondagsrivier	3	3	3	3	2	2	1	1	1	1	0	0	0	0	2	2	2	0	1	1	3	3	1	1	0	2	0	0	0	0	0	1	
Katrivier	3	3	3	3	2	2	1	1	1	3	0	0	0	0	2	2	2	0	1	2	3	3	1	1	0	2	0	1	0	0	0	2	
Kakamas	3	3	3	3	0	0	3	3	1	1	0	0	0	0	1	1	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	
Vaalharts	3	3	3	3	1	1	2	2	1	1	0	0	0	0	1	*3	0	0	0	2	0	0	2	2	0	1	0	1	0	0	0	0	
Richmond	3	3	3	3	2	2	1	1	1	1	0	0	0	0	2	2	1	1	3	2	3	3	1	1	0	2	0	2	0	0	0	2	
Nkwaleni	3	*3	3	3	2	3	1	2	1	2	0	0	0	0	2	3	1	1	0	2	3	3	1	1	0	1	0	1	0	1	0	0	
Pongola	3	*3	3	3	1	2	1	2	1	3	0	0	0	0	1	3	1	2	0	0	3	3	1	1	0	1	0	1	0	2	0	1	
Swaziland	3	3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	2	2	0	0	3	3	1	1	0	2	0	0	0	0	0	0	
Komatipoort	3	3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	0	0	0	0	3	3	1	1	0	0	0	0	0	3	0	0	
Malelane	3	3	3	3	2	2	1	1	1	2	0	0	0	0	1	1	0	0	0	0	3	3	1	1	0	0	0	0	0	2	0	0	
Nelspruit	3	3	3	3	2	2	1	1	1	1	0	0	0	0	1	1	3	3	0	1	3	3	1	2	0	1	0	0	0	0	0	2	
Burgersfort	3	3	3	3	2	2	1	1	1	1	2	1	0	0	1	1	3	3	0	1	3	3	1	1	0	1	0	1	0	0	0	0	
Groblersdal	3	*3	3	3	2	2	1	1	1	1	3	3	0	0	0	2	2	3	0	0	3	3	1	1	0	1	0	1	0	0	0	2	
Ohrigstad	0	3	0	3	0	2	0	2	0	1	0	3	0	0	0	2	0	3	0	0	0	0	0	0	0	0	3	0	1	0	0	0	
Hoedspruit	3	*3	3	3	2	2	1	1	1	1	0	0	0	0	0	0	3	3	1	1	3	3	1	1	0	2	0	2	0	1	0	0	
Letsitele	3	3	3	3	2	2	1	1	2	2	0	0	0	0	2	2	3	3	0	0	1	3	1	1	0	0	0	1	0	2	0	0	
Limpopo	3	3	3	3	3	3	1	1	1	1	0	0	0	0	2	2	0	0	0	0	1	3	1	0	0	0	0	0	0	0	0	0	
Rustenburg	3	3	3	3	2	2	1	2	1	1	3	*3	0	0	1	1	3	3	0	0	3	3	1	0	0	1	0	1	0	0	0	0	
Weipe	0	3	0	3	0	1	0	2	0	0	0	0	0	0	1		0	0	2	0	3	0	2	0	0	0	0	0	0	0	0	0	
Zimbabwe	3	3	3	3	1	1	1	1	1	0	3	*3	0	0	1	0	3	3	0	0	3	2	1	0	0	0	0	0	0	0	0	0	0
Weight	69	75	69	73	39	43	27	34	25	32	11	13	6	6	32	43	40	40	7	19	59	65	25	26	0	26	0	17	0	13	0	1	
Average	2.76	3.06	2.7	2.92	6	1.72	1.08	1.36	1	1.28	.44	.52	.24	.24	1.28	1.72	1.6	1.6	0.28	0.76	2.36	2.6	1	1.04	0	1.0	0	.68	0	.52	0	.	

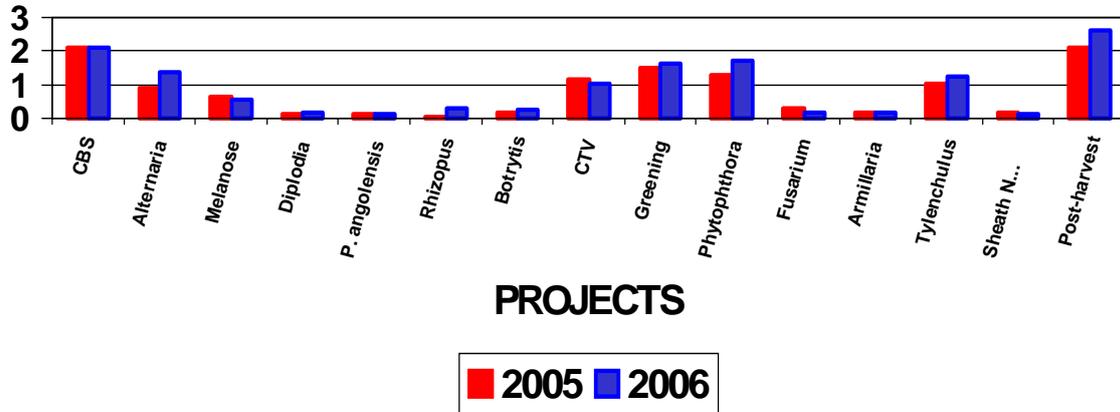
* Highest Priority

Citrus Area	Flower Manip		Fruit set		Fruit size		Internal Quality		Colour		Creasing		Rind Pitting		Rind breakdown		Peteca		Pruning		Girdling		Shelf life		Replacem of Ca-ars.		Cultivar & Rootstock Development		
	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	05	06	
Citrusdal	0	0	0	2	0	0	0	0	0	0	3	3	3	3	3	2	1	1	1	1	0	0	2	2	3	3	3	3	
Stellenbosch	0	2	0	2	0	2	0	0	0	0	3	3	3	3	3	3	2	3	1	0	0	0	2	2	0	0	3	3	
Swellendam	0	0	0	3	0	0	0	0	0	0	3	3	2	2	2	2	0	3	0	0	0	1	1	3	0	0	3	3	
Knysna	0	0	0	0	3	3	0	0	0	0	0	0	1	0	1	0	0	0	1	2	0	0	0	0	0	0	3	*3	
Patensie	0	0	0	0	3	2	2	2	0	0	3	3	1	2	1	2	0	3	0	0	0	1	0	2	0	0	3	3	
Sondagsrivier	0	0	0	2	2	2	0	0	1	1	3	3	3	2	3	2	3	*3	0	0	0	0	0	0	0	1	1	3	3
Katrivier	0	0	0	1	0	0	0	0	1	1	3	2	2	3	1	*3	2	3	0	0	0	0	0	0	0	0	0	3	3
Kakamas	3	3	3	*3	3	3	0	0	0	0	1	1	1	1	1	1	3	3	3	3	2	2	2	2	0	0	0	0	
Vaalharts	0	0	0	0	0	1	0	0	0	0	2	2	1	1	1	1	0	3	0	1	1	1	0	0	0	2	3	3	
Richmond	0	0	0	*3	3	3	0	1	0	0	2	2	1	1	1	1	2	2	1	1	0	0	0	0	0	0	1	3	
Nkwaleni	0	0	0	2	3	2	0	2	1	1	0	0	0	0	1	2	0	2	2	2	0	1	0	1	1	3	1	3	
Pongola	0	0	0	3	3	3	0	1	1	1	0	2	0	0	0	1	2	1	2	2	0	0	0	0	0	1	2	2	
Swaziland	0	2	0	2	3	3	0	0	0	0	0	0	1	1	0	2	1	1	1	1	0	0	0	0	0	2	1	2	
Komatipoort	0	0	0	2	3	2	0	2	0	2	0	*3	0	3	0	3	0	1	0	0	0	0	0	0	0	1	1	2	2
Malelane	0	0	0	0	3	2	0	3	0	0	0	*3	0	3	0	3	0	1	0	0	0	0	0	2	1	0	2	2	
Nelspruit	0	0	0	3	1	1	0	0	0	2	0	1	1	3	0	0	3	3	0	0	0	0	0	0	0	0	3	3	3
Burgersfort	0	0	0	1	1	1	0	0	0	0	1	3	0	0	0	0	0	0	1	1	0	1	0	0	0	0	3	3	
Groblersdal	0	0	0	0	1	1	0	2	0	1	1	3	0	1	0	0	2	3	1	1	0	1	0	3	0	1	2	2	
Ohrigstad	0	1		1	0	0	0	1	0	0	0	*3	0	3	0	3	0	0	0	0	0	1	0	0	0	3	0	3	
Hoedspruit	0	1	0	3	1	2	0	1	0	3	0	3	1	1	1	0	3	3	0	1	0	0	0	0	0	1	2	2	
Letsitele	0	0	0	0	0	3	0	2	0	0	0	3	1	3	1	3	0	0	0	0	0	0	0	0	0	1	3	3	
Limpopo	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	2	0	0	0	0	0	0	0	0	0	0	3	3	
Rustenburg	0	1	2	2	1	1	2	1	0	0	1	1	1	1	0	0	2	2	1	1	0	1	0	0	1	0	2	2	
Weipe	0	3	0	3	0	3	0	1	0	3	0	2	0	2	0	2	0	0	0	1	0	0	0	2	0	1	0	0	
Zimbabwe	0	0	0	2	1	1	0	0	1	3	0	0	0	0	0	3	0	2	1	0	0	2	0	0	0	0	2	0	
Weight	3	13	5	40	35	41	4	19	5	18	26	49	24	41	21	41	26	43	16	18	3	12	7	19	8	23	56	59	
Average	.12	.52	0.2	1.60	1.4	1.64	.16	.76	.2	.72	1.04	1.96	.96	1.64	.84	1.64	1.04	1.72	.64	.72	.12	.48	.28	.76	.32	.92	2.12	2.36	

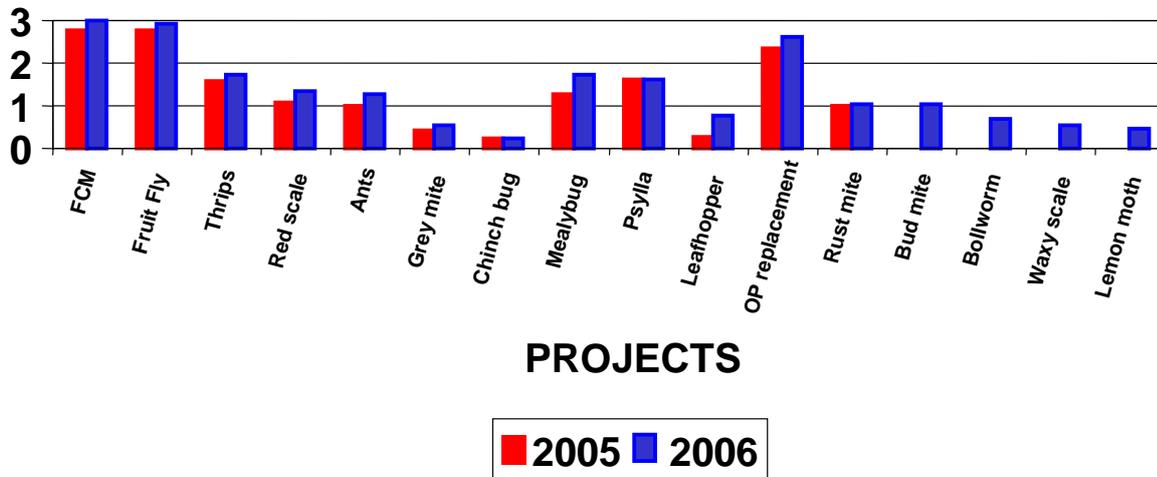
*Highest

Priority

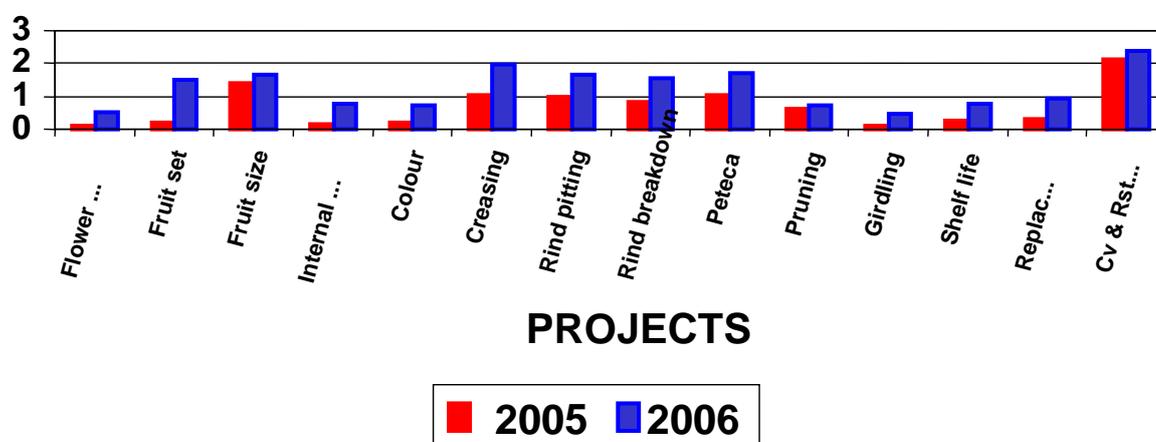
RESEARCH PRIORITIES - AVERAGES FOR DISEASE MANAGEMENT



RESEARCH PRIORITIES - AVERAGES FOR INTEGRATED PEST MANAGEMENT



RESEARCH PRIORITIES - AVERAGES FOR CROP LOAD & FRUIT QUALITY MANAGEMENT & CULTIVAR & ROOTSTOCK DEVELOPMENT



9.2 TECHNOLOGY TRANSFER GROUPS (TTG's)

During the past two years southern Africa has been divided into a North and a South region on the 28°S line with regard to Extension. Hennie le Roux manages the northern areas whereas Hannes Bester has been appointed Area Extension Manager (AEM) for the southern areas. Contracts were also given to members of SASSCON to help administrate certain of the Technology Transfer (Study) Groups. An additional study group has been established in the Breede River Valley to accommodate growers who previously did not belong to a study group because of distance, the Paarl/Stellenbosch study group being the closest to them. Dr Izak Bruwer has been contracted by CRI to administer this group. The structure of the Technology Transfer Groups is as follows:

Province	Responsible person AEM/EM	Technology Transfer Group	Responsible person	Chairman
Western Cape	AEM*	Citrusdal (including Clanwilliam & Piketberg)	Hannes Bester (083 325 8379)	Dirk Visser (082 550 0158)
	AEM	Paarl/Stellenbosch	Hannes Bester	Corrie Muller (083 631 7727)
	AEM	Breede Rivier	Dr I Bruwer (083 2262540)	
	AEM	Swellendam	Hannes Bester	Sarel Neetling (082 551 2357)
	AEM	Knysna	Hannes Bester	John Stanwix (082 789 5051)
Eastern Cape	AEM	Patensie	Hannes Bester	Ian Grieb (082 823 3960)
	AEM	Sundays River Valley	Hannes Bester	Dave Gerber (072 292 2151)
	AEM	Kat Rivier Valley	Hannes Bester	Bruce Knott (082 877 1164)
Northern Cape	AEM	Vaalharts	Hannes Bester	Tom Fouche (082 783 4842)
	AEM	Benede Oranje	Hannes Bester	Francois Reyneke (082 771 6758)
Kwa-Zulu Natal	AEM	Natal Midlands	Hannes Bester	

	AEM	Nkwaleni Valley	Hannes Bester	Bester Snyman (082 896 2856)
	EM**	Pongola	Chris Kellerman (083 2654220)	Andre Barnard (083 229 8539)
Swaziland	EM	Swaziland	Chris Kellerman	Gert Hoppner (09268 323 23110)
Mpumalanga	EM	Komatipoort	Chris Kellerman	Dirk Horn (083 259 3359)
	EM	Malelane	Chris Kellerman	Leon Esselen (013 7900160)
	EM	Nelspruit (including Hazyview)	Hennie le Roux (082 447 1537)	Graham Piner (083 610 6095)
	EM	Burgersfort/Ohrigstad	Clive Pountney	Elbert de Kock (013 231 7757)
Limpopo	EM	Tshipise (including Weipe & Beit Bridge)	Hennie le Roux	Bennie Nicolson (083 306 0552)
	EM	Letsitele/Constantia	Hennie le Roux	Pieter Vermaak (082 491 7743)
	EM	Marble Hall/Groblersdal	Hennie le Roux	Tini Engelbrecht (082 524 8925)
	EM	Hoedspruit	Tom vd Meulen (083 629 3806)	Michael Podges (082 873 2006)
North West	EM	Rustenburg	Hennie le Roux	Johan-Chris Grobler (0829221579)

AEM*: Area Extension Manager
EM** : Extension Manager

During August each year, it remains the responsibility of each of the Technology Transfer Groups to ensure that their research priorities are established and prioritised. The Zimbabwean citrus producers were visited by only Keith Lesar and Paul Cronjé during 2005 regarding post-harvest, packhouse and rind condition problems. This situation will be remedied in future.

9.3 THE RELATIVE FUNDING SUPPORT FOR RESEARCH PROGRAMMES AND PROJECTS FOR 2005

By H.F. le Roux & Tim G. Grout (CRI)

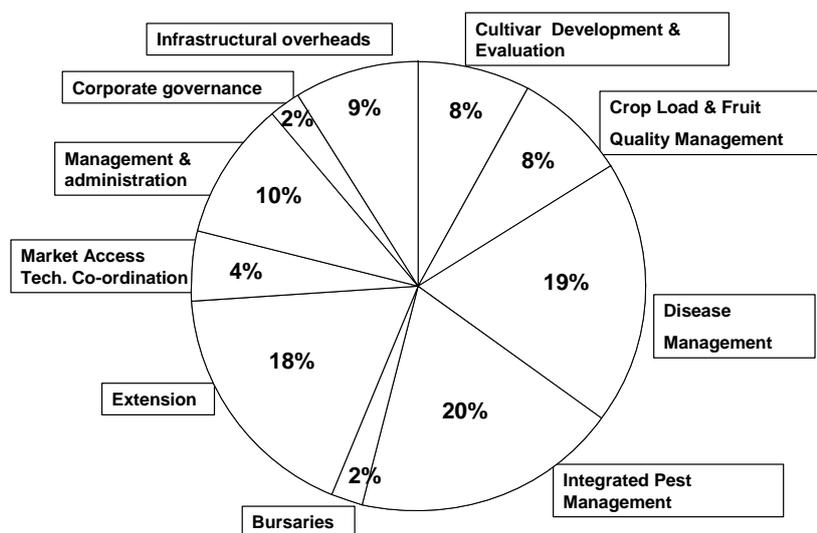


Fig. 9.3.1. Percentage funding in each CRI programme.

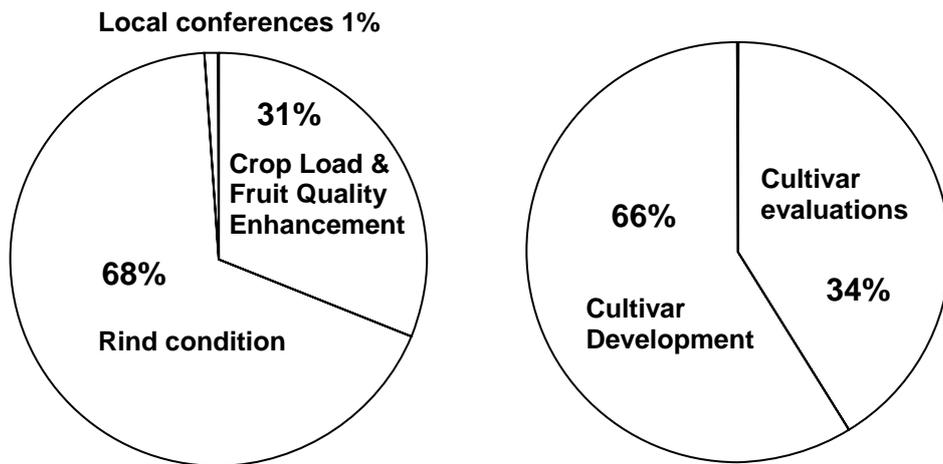


Fig. 9.3.2. Percentage funding in CRI Research Projects – Crop Load and Fruit Quality Management (left) and Cultivar and Rootstock Development (right).

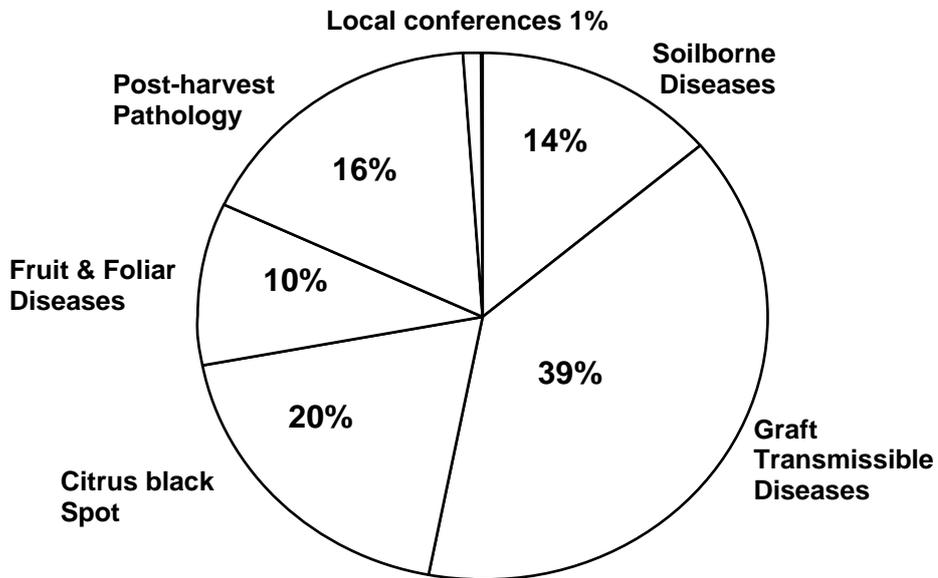


Fig. 9.3.3. Percentage funding in each CRI Research Projects – Disease Management.

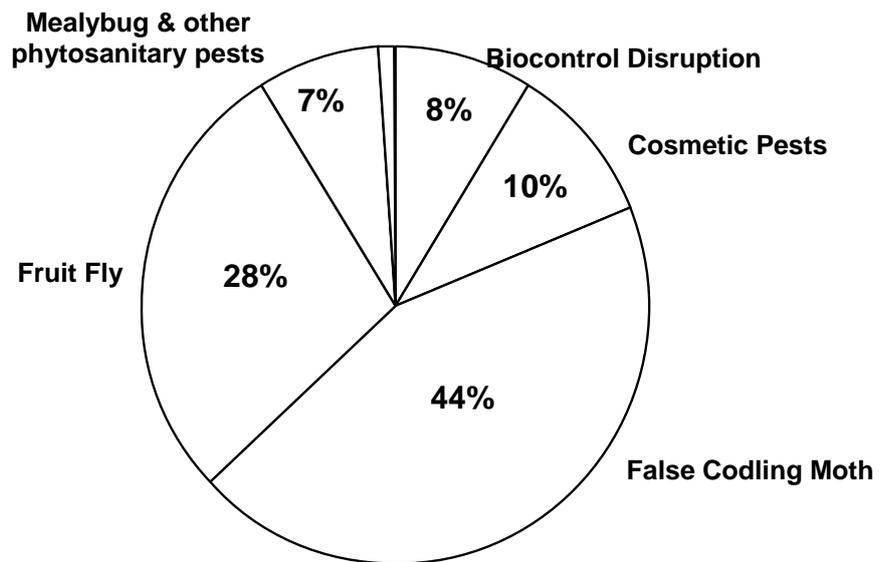


Fig. 9.3.4. Percentage funding in each CRI Research Project – Integrated Pest Management.

9.4 EXTENSION PRESENTATIONS BY CRI GROUP RESEARCHERS IN 2005

RESEARCH			
Barry, Graham H. (CRI)	12/08/2005	Zimbabwe Citrus Study Group, Chegutu	How can I optimise fruit quality in the market? Some promising citrus cultivars
	09/03/2005	Colors Cultivar Day	Fruit Quality Management: How can I optimise fruit quality in the market? Cultivar Options
	31/05/2005	Cape Pomological Association Technical Symposium, Stellenbosch	Antidotes for snake oils
	04/10/2005	CRI Citrus Technical Association Growers' Day, Stellenbosch	Rind colour enhancement
	03/11/2005	Boland Study Group, Wellington	Chemical thinning options: Corasil.E® and Maxim®
	01/12/2005	Fort Beaufort Citrus Study Group	Cultivar Options
Cronjé, P.J.R. (CRI)	02/08/2005	Western Cape	Effect of cold sterilisation on fruit quality
	04/10/2005	Western Cape	Postharvest handling: temperature and RH management
	21-24/02/2005	Zimbabwe Citrus Study Group	Postharvest handling
Grout, T.G. (CRI)	02/08/2005	Nkwaleri	Managing citrus pests in spring
	18/10/2005	Pongola	Managing citrus pests in spring
	01/11/2005	Malelane/Komatipoort	Ant control
	28/06/2005	Nelspruit – Winterveld group	Overview on the citrus industry and research
Moore, S.D. (CRI)	27/07/2005	FCM, Vaalharts	Grower meeting
	21/09/2005	Cryptogran for FCM control, Patensie	
	04/10/2005	FCM management, Stellenbosch	Grower meeting (Pomological Society)
	05/10/2005	FCM management, Swellendam	Grower meeting
	05/10/2005	FCM management, Robertson	Grower meeting
	01/11/2005	FCM management, Malelane	Grower meeting
	01/11/2005	FCM management, Constantia/Letsitele	Grower meeting
	02/11/2005	FCM management, Marble Hall	Grower meeting
	03/11/2005	FCM management, Nelspruit	Grower meeting
	04/11/2005	FCM management, Burgersfort	Grower meeting
MC Pretorius (CRI)	13/10/2005	Hoedspruit Studiegroep – Avello toergroep	Wortelgesondheid - Aalwurms en <i>Phytophthora</i>
	03/11/2005	Nelspruit Studiegroep	Wortelgesondheid - Aalwurms en <i>Phytophthora</i>
	03/11/2005	Burgersfort Studiegroep	Wortelgesondheid - Aalwurms en <i>Phytophthora</i>
	03/12/2005	Universiteit van Stellenbosch	Epidemiology control of <i>Pseudocercospora angolensis</i> fruit and leaf spot disease on citrus in Zimbabwe
G.C. Schutte (CRI)	08/03/2005	Marble Hall	CBS
	10/03/2005	Nelspruit	CBS + <i>Alternaria</i> brown spot
	17/03/2005	Nelspruit CGA	CBS
	21/06/2005	Novon	CBS + <i>Alternaria</i> brown spot
	28/07/2005	Burgersfort	CBS
	03/08/2005	Suurlemoen studiegroep - Nelspruit	CBS
	22/09/2005	Patensie	CBS + <i>Alternaria</i> brown spot
	28/09/2005	Swellendam	<i>Alternaria</i> brown spot + <i>Phytophthora</i>
	13/10/2005	Hoedspruit	CBS
	18/10/2005	Pongola	CBS
	01/11/2005	Malelane	CBS
	02/11/2005	Marble Hall	CBS
	03/11/2005	Nelspruit	CBS
	03/11/2005	Burgersfort	CBS

Verreynne, J.S. (CRI)	07/09/2005	Hectorspruit	Creasing workshop
	08/09/2005	Nelspruit (Karino)	Creasing workshop
	08/09/2005	Burgersfort	Creasing workshop
	04/10/2005	CRI / CTA Citrus Farmer's Day, Infruitec, Stellenbosch	Alternate bearing in citrus
	07/11/2005	Kakamas	Fruit size improvement
	08/11/2005	Citrusdal	Fruit size improvement
	16/11/2005	Swellendam	Fruit size improvement
	18/11/2005	Breërivier (Robertson)	Fruit size improvement
EXTENSION			
Le Roux, H.F.	31/03/2005	Citrusdal	Navorsingsprioriteitsbepaling
	14/07/2005		Areavoortligtingsbestuurder (J. Bester)
	01/02/2005	Paarl/Stellenbosch	Verpakkingswerkswinkel
	02/02/2005		Vrugtevliegbeheerwerkswinkel
	04/10/2005		Vrugkleur
	05/10/2005	Breederivier	Vergroeningsbestuur
	18/07/2005	Swellendam	Navorsingsprioriteitsbepalings
	05/10/2005		Vergroeningsbestuur
	30/03/2005	Knysna	Areavoortligtingsbestuurder (J. Bester)
	19/07/2005		Navorsingsprioriteitsbepalings
	29/03/2005	Patensie	Areavoortligtingsbestuurder (J. Bester)
	19/07/2005		Navorsingsprioriteitsbepalings
	28/03/2005	Sondagsrivier	Areavoortligtingsbestuurder (J. Bester)
	20/07/2005		Navorsingsprioriteitsbepalings
	21/07/2005	Katrivier	Navorsingsprioriteitsbepalings
	06/05/2005	Vaalharts	USDA Valskodlingmot
	27/07/2005		Navorsingsprioriteitsbepalings
	06/05/2005	Oranjerivier	USDA Valskodlingmot
	04/08/2005	Suid-Natal	Navorsingsprioriteitsbepalings
	05/08/2005	Nkwaleni	Navorsingsprioriteitsbepalings
	08/08/2005	Swaziland	Navorsingsprioriteitsbepalings
	11/08/2005	Komatipoort	Navorsingsprioriteitsbepalings
	11/08/2005	Malelane	Navorsingsprioriteitsbepalings
	12/08/2005	Nelspruit	Navorsingsprioriteitsbepalings
	10/08/2005	Burgersfort	Navorsingsprioriteitsbepalings
	10/08/2005	Ohrigstad	Navorsingsprioriteitsbepalings
	15/08/2005	Hoedspruit	Navorsingsprioriteitsbepalings
	19/04/2005	Constantia/Letsitele	Maxim ondersoek
	17/08/2005	Tshipise	Navorsingsprioriteitsbepalings
	17/08/2005	Weipe	Navorsingsprioriteitsbepalings
	18/08/2005	Marble Hall	Navorsingsprioriteitsbepalings
			Vrugtevlieg
	22/08/2005	Rustenburg	Navorsingsprioriteitsbepalings
Bester, J.J.	22/08/2005	Rustenburg	Navorsingsprioriteitsbepalings
	02/02/2005	Paarl/Stellenbosch	Vrugtevliegbeheerwerkswinkel
	05/10/2005	Breederivier	Navorsingsterugvoer
	18/11/2005		VSA GAP
	18/07/2005	Swellendam	Navorsingsprioriteitsbepalings
	16/11/2005		VSA GAP
	19/07/2005	Knysna	Navorsingsprioriteitsbepalings
		Patensie	Navorsingsprioriteitsbepalings
	20/07/2005	Sondagsrivier	Navorsingsprioriteitsbepalings
	21/07/2005	Katrivier	Navorsingsprioriteitsbepalings
	27/07/2005	Vaalharts	Navorsingsprioriteitsbepalings
	26/07/2005	Oranjerivier	Navorsingsprioriteitsbepalings
			Snoeiwerkswinkel
	04/08/2005	Suid-Natal	Navorsingsprioriteitsbepalings
	05/08/2005	Nkwaleni	Navorsingsprioriteitsbepalings
06/07/2005	Pongola	Snoeiwerkswinkel	

9.5 OTHER MEANS OF TECHNOLOGY TRANSFER

By Hennie le Roux & Tim Grout (CRI)

9.5.1 S.A. Fruit Journal

The SA Fruit Journal remains the most reliable means of getting a good quality article to every citrus grower who is paying the levy on export citrus because the subscription is paid out of the levy funds. Bimonthly Extension Briefs are provided as reminders for growers and in-depth research articles are also provided. Some examples of the latter included articles on peteca spot, citrus black spot control, citrus canker and factors causing fruit drop in navel oranges. There is a lag time of two months between submission of the articles and circulation of the journal so urgent information is circulated in the Cutting Edge via CRInet or emails to the technology transfer groups.

Table 9.5.1.1. S.A. Fruit Journal articles during 2005.

2005 Issue	Article	Author
Dec/Jan	CGA's application for statutory measures approved	J. Chadwick
	Transformasie in die Sitrusbedryf	
	Die Nkwaleni Vallei	
	Cryptogran. A virus for the biological control of false codling moth	S. Moore, W. Kirkman & P. Stephen
	Evaluation of Phosphonate for the post harvest control of <i>Phytophthora</i> brown rot on citrus fruit.	K. Lesar
Feb/March	CGA Board unanimous on approach to Fruit Information and Traceability Systems	P. Hardman
	The Pongola Valley	A. Rouilliard
Apr/May	Golden Frontiers verskuif grense	H. Nel
	Karino Sitrus Kooperasie	J. Warrington
	Cultivar Innovation	G.H. Barry
	The Birds and Fruit Flies	A.B. Ware
	Peteca spot of lemons	P. Cronje
	Citrus Packaging Forum – A priority	V. Hattingh
June/July	Phyosanitary Security- Are we covered	J. Chadwick
	CGA establish Citrus Academy	J. de Klerk
	Sitruskanker	H. le Roux
Aug/Sept	Tariffs: The playing fields is still not level	J. Chadwick
	ALG & Cedar Citrus	F. Meintjies
	<i>In vitro</i> & <i>in vivo</i> evaluation of maneb and mancozeb against <i>Guignardia citricarpa</i>	G.C. Schutte
	Citrus Industry changes to refractometers for sugar determination	G.H. Barry
	Waarom moet slegs gesertifiseerde sitrus kwekerybome geplant word?	M.N. du Toit
Oct/Nov	Groot uitdagings le voor vir Senwes sitrusarea	D. Eksteen
	United States backing for South African citrus exports	F. Meintjies
Dec/Jan	New trees for Tekwane	
	The Lowveld loses a pioneer	I. Solomon
	Huldeblyk aan dr Eric Bedford	E.A. de Villiers
	Factors causing fruit drop in navel oranges	S.D. Moore, G.C. Schutte & G.H. Barry

9.5.2 CRInet

There were fewer messages circulated on CRInet during 2005 than in 2004, but more than in 2003 (Table 9.5.2.1). This is probably reflective of fewer crises or situations requiring discussion. During the past year there was an increase in the number of email providers rejecting CRInet messages due to the use of Spam filters, but in most cases these were resolved with the provider.

Table 9.5.2.1. Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2005	14	11	3	3	3	14	8	3	23	5	11	5	103
2004	7	26	13	28	27	26	12	9	15	12	12	0	187
2003	1	4	6	14	22	4	3	6	5	6	11	3	85

9.5.3 Cutting Edge

During 2005, issues 24 to 35 were circulated via email and made available on the CRI website. Many of the issues dealt with urgent topics such as carbendazim residues and fruit fly control.

Table 9.5.3.1. Cutting Edge issues during 2005.

No.	Title	Issue	Author
24	Citrus Canker	January	Le Roux, H.F.
25	Deccowax and Citriwax	February	Lesar, K.H.
26	Monitoring Fruit flies in 2005	February	Grout, T.G. & A.B. Ware
27	Fruit Fly Control Recommendations	April	Ware, A.B. & T.G. Grout
28	Peteca Spot on lemons: Checklist and guidelines	April	Cronjé, P. & K. Lesar
29	Citrus industry changes to refractometers for sugar determination	May	Barry, G.H.
30	Carbendazim – Cautionary note	October	Hattingh, V. & P. Hardman
31	Uncertified plant material poses a risk	November	Bester, J.J.
32	Fruit size improvement	November	Verreynne, J.S. & G.H. Barry
33	EU Carbendazim MRL set at limit of determination (0.01 mg/kg)	December	Hardman, P.
34	The control of Postharvest diseases on SA export citrus by the postharvest fungicide Thiabendazole	December	Lesar, K.H.
35	Carbendazim – Cautionary notice	December	Hattingh, V. & P. Hardman

9.5.4 CRI Website

The website was updated this year with the new format catering for the four company divisions. Activity increased steadily towards the end of the year with the most active month being December 2005 (Fig. 9.5.4.1.). Since the inception of monitoring on the website in December 2003, the monthly average number of pages sent has been 3 525. In December 2005, 4 869 pages were sent. Currently there are approximately 300 members who are permitted to download files from the website. However, only about half of these are citrus growers, which represents approximately one tenth of the citrus growers in southern Africa. The Integrated Pest and Disease Management Production Guidelines will become available on the website to members during 2006.

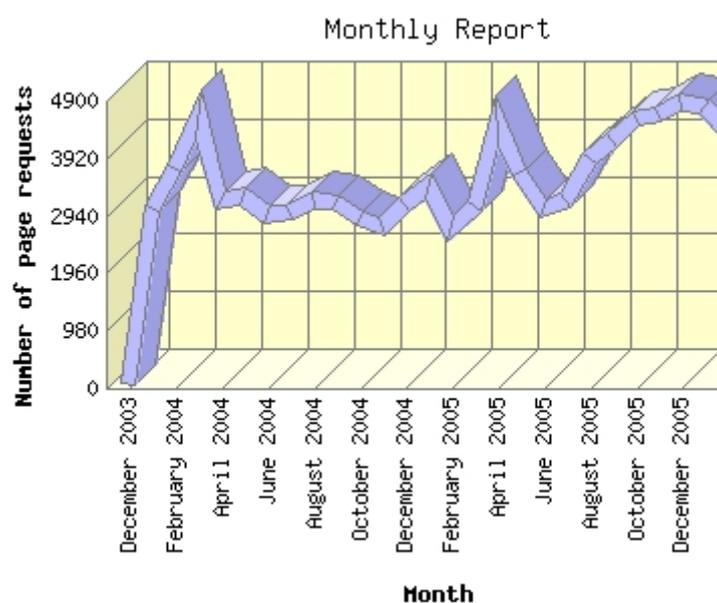


Fig. 9.5.4.1. Monthly page requests since December 2003.

9.5.5 Citrus Research Symposium

As the Citrus Research Symposium is held every two years, no symposium was held during 2005. A suitable venue has, however, been found in the Eastern Cape for CRI's 4th Citrus Research Symposium in 2006. The venue is the Tsitsikama Hall in the Boardwalk Casino, Port Elizabeth. Symposium delegates have the option of staying in the Marine and Beach Hotels in PE at a discounted price, and comfortable guesthouses are available in the vicinity. The symposium dates are 20-23 August 2006. This symposium will be the first Citrus Research Symposium where guest speakers from abroad will be invited as keynote speakers.

9.6 TRANSFORMATION

The CGA published an impressive article entitled "Our Citrus Transforms" by Louise Brodie. In the article, the author conveys success stories regarding reformation in the citrus industry to the reader. The author succeeded in achieving this and the book carries a very positive and successful image of the South African citrus industry's reformation process to the outside world. The truth of the matter at present is that these so-called success stories are presently being faced with serious financial problems and it is unlikely that a follow-on book will be written regarding this reformation.

The true facts regarding citrus cultivation in South Africa are that times are extremely difficult for most commercial citrus growers. A worldwide over-production of citrus exists, the South African Rand is strong compared to foreign exchange in countries SA exports to, and the transformation process in the agricultural sector is gripped in a tug-of-war where the government and the growers blame each other for the unsuccessful handling of the transformation process and the handing-over of white-owned citrus farms.

The situation regarding the cash flow of most emergent black citrus producers is still as critical as was mentioned in last year's annual report. The process of handing over the farms has to date not yet taken place. No emergent farmers have moved onto the farms and the growers (sellers) of the property have not yet been paid for their property. The farm Tsholofelo, near Zeerust, will most likely become abandoned as the white farmer hiring the property decided to retract and there has been no intervention from the North West government whose Department of Agriculture is non-existent. The problem regarding land reform does not lie with the emergent farmers, but with the government's incapability to manage transformation.

Extension Division has to date fully supported transformation in the citrus industry. All telephonic and/or electronic requests regarding citrus production have been dealt with. Where necessary, emergent farmers have been advised against planting citrus in areas which are climatically unsuitable for citrus. In other instances farmers have been advised to contact experts in their disciplines regarding certain problems,

e.g. the Winterveldt project where Dr. Hannes Coetzee was approached to assist.

With the exclusion of the Winterveldt project, not one project where CRI technical inputs have been provided has proved successful. The projects are unable to compile business plans and have no access to project funding. In the case of the Winterveldt project, a prominent businessman, Dr. Sam Motsuenyane, has assisted in obtaining support from organisations such as Pick & Pay, Magalies Citrus and the Indian community in Ladium. In projects such as the seedless lemon project in Elandshoek, neither the government nor the municipality have provided funding and emergent farmers therefore rely on their mentor to provide financial support without the mentor having any form of security for this funding.

Projects visited during 2005 include the Winterveldt Project, Tsofelo, Cairn Trust, Elandshoek, Indulini, Gillemburg, Bakone Community Development Trust, the Richtersveld and the Rust-de-Winter projects.

To obtain successful transformation in the citrus industry, it is imperative that the Citrus Growers' Association liaise closely with the government. The only advantage the present transformation process presents is that it curbs the overproduction of citrus.

9.7 LIAISON WITH EXPORT AGENTS

CRI's function from a technical aspect is to support the entire southern African citrus industry. A system has therefore been implemented whereby regular contact is maintained with CEF and CMF through various channels. In this manner their requirements can continually be addressed and CRI can remain informed on all changes in market conditions and requirements.

Regular attendance at the CEF and CMF meetings is maintained and this serves as a platform to accomplish communication between CRI and the export agents. Research feedback from the last two years regarding projects funded by the agents is conveyed to the agents, as well as their research priorities are established at a Research Priority meeting for Exporters, whereafter these are then relayed to the researchers.

An Exporters' Technical Panel consisting of technical representatives from the Export Agents is in the process of being formed. The primary goal is to ensure that the Export Agents' requirements are integrated with CRI's research and extension activities and that communication during the harvesting and marketing season is ongoing. This eliminates problems, and any problems that do exist can be addressed immediately. It is expected that the Exporters' Technical Panel will be functioning with the commencement of the 2006 season.

10 PUBLICATIONS IN 2005

10.1 Refereed publications (or ISI ranked journals)

Grout, T.G. & Stephen, P.R. 2005. Use of an inexpensive technique to compare systemic insecticides applied through drip irrigation systems in citrus. *African Entomology* 13(2): 353-358.

Hofmeyr, J.H., Carpenter, J.E. & Bloem, S. 2005. Developing the sterile insect technique for *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae): Influence of radiation dose and release ratio on fruit damage and population growth in field cages. *J. Econ. Entomol.* 98(6): 1924-1929.

Meyer, L., Sanders, G.M., Jacobs, R & Korsten, L., 2006. A one-day sensitive method to detect and distinguish between the citrus black spot pathogen *Guignardia citricarpa* and the endophyte *Guignardia mangiferae*. *Plant Disease* 90:97-101.

Paul, I., van Jaarsveld, A.S., Korsten, L. & Hattingh, V. 2005. The potential global geographical distribution of citrus black spot caused by *Guignardia citricarpa* (Kiely): likelihood of disease establishment in the European Union. *Crop Protection* 24: 297-308.

10.2 Semi-scientific publications

Barry, G.H. Cultivar Innovation. *SA Fruit Journal*. April/May 2005, p. 23.

Cronjé, P.J.R. Peteca spot of lemons. *SA Fruit Journal*. April/May 2005, 26-28.

Hattingh, V. Citrus Packaging Forum – A priority. *SA Fruit Journal*. April/May 2005, p. 36.

Le Roux, H.F. Sitruskanker. *SA Vrugtejoernaal*. Junie/Julie 2005, 32-33.

Lesar, K.H. Evaluation of Phosphonate for the post harvest control of *Phytophthora* brown rot on citrus fruit. SA Fruit Journal. December/January 2005, 43-47.

Moore, S.D., Kirkman, W. & P. Stephen. Cryptogran. A virus for the biological control of false codling moth. SA Fruit Journal. December/January 2005, 35-39.

Moore, S.D., Schutte, G.C. & Barry, G.H. 2005. Factors causing fruit drop in navel oranges. SA Fruit Journal. December/January 2005, 34-38.

Schutte, G.C. *In vitro* and *in vivo* evaluation of maneb and mancozeb against *Guignardia citricarpa*. SA Fruit Journal. August/September 2005, 37-41.

Ware, A.B. The Birds and Fruit Flies. SA Fruit Journal. April/May 2005, 24-25.

11 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

Barry, G.H. Reduction of acidity of high and acid citrus cultivars using alternatives to calcium arsenate. SA Society for Horticultural Science Conference, Potchefstroom, 10-13 January 2005.

Barry, G.H. Postharvest conditions affect rind breakdown of Nules Clementine Mandarin. SA Society for Horticultural Science Conference, Potchefstroom, 10-13 January 2005.

Barry, G.H.. Ecophysiological responses of citrus trees and suger accumulation of fruit in response to altered plant water relations. SA Society for Horticultural Science Conference, Potchefstroom, 10-13 January 2005.

Barry, G.H.. Factors affecting rind oil content in lemons. SA Society for Horticultural Science Conference, Potchefstroom, 10-13 January 2005.

Barry, G.H. Cultivar Innovation: Effective sourcing and commercialisation of citrus cultivars in southern Africa. International Society of Citrus Nurserymen, Cairo, Egypt, 17-20 September 2005.

Barry, G.H. Promising citrus cultivars in South Africa. Poupart International Citrus Conference, Franschhoek, 29 April 2005.

Cronjé, P.J.R.. Rind Breakdown of Nules Clementine Mandarins: Effect of postharvest conditions on its development, 10-14 January 2005.

Cronjé, P.J.R.. Postharvest handling. Zimbabwe Citrus Study Group, 21-24 February 2005.

Kirkman, W & Moore, S.D.. The residual efficacy of the *Cryptophlebia leucotreta* granulovirus on citrus. Proceedings Entomological Society of southern Africa 15th congress, 10-13 July 2005, Grahamstown.

Moore, S.D. Field persistence of FCM granulovirus. Society for Invertebrate Pathology Congress, Alaska, 7-11 August 2005.

Pretorius, M.C. Stimulation of egg hatching of *Tylenchulus semipenetrans*. Nematological Society of Southern Africa, Phalaborwa, May 2005.

Grout, T.G., Stephen, P.R. & Stoltz, K.C. 2005. Biorational control strategies for citrus thrips that minimise non-target effects on arboreal and edaphic predatory mites. Proceedings Entomological Society of southern Africa 15th congress, 10-13 July 2005. p. 29. Grahamstown

Truter, M. A sampler to determine the available *Guignardia citricarpa* inoculum on citrus leaf litter. 43rd SASPP Congress, Hartenbos, 23-26 January 2005.



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