



**CRI GROUP ANNUAL  
RESEARCH REPORT  
2011 / 2012**

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## 1 INTRODUCTION

Vaughan Hattingh (CEO CRI)

This document reports on the research and technical support activities of the CRI Group over the period April 2011 to March 2012. CRI's primary source of funding remained the levy on fresh citrus exports as administered by the CGA. Valuable additional funding was accessed through the CRI Group model in the form of Government funding support to the research projects conducted at Universities. Additional Government funding was accessed in the form of grant funds through the Pesticide Initiative Programme II and the Post Harvest Innovation Programme II. Some additional funds were also accessed through international research collaboration. Various forms of sales, services and royalties made up the remainder of the income pooled for use in pursuit of CRI's mission: "To maximise the long term global competitiveness of the southern Africa citrus growers through the development, support, co-ordination and provision of Research and Technical services, by combining the strengths of all CRI Group partners".

CRI continued to operate the CRI Group Alliance model whereby CRI's own in-house resources and capacity is augmented through cooperation with CRI's network of alliance partner research organisations. A Memorandum of Understanding between CRI and ARC was concluded, bringing to a close a long negotiation process. Whereas there has been ongoing cooperation between CRI and the ARC in various forms over the years, concluding this MOU opens the doors to resumption of more formal and structured collaboration.

CRI implemented restructuring of its Cultivar Development Division. As a consequence CRI terminated its involvement in the acquisition of commercialisation rights and cultivar management. This enables CRI to more effectively focus on the industry-wide cultivar evaluation project that aims to provide growers with objective, impartial, comparative assessment of cultivars, regardless of their ownership or management status. This restructuring also strengthens CRI's impartiality in relation to its management of the Citrus Improvement Scheme. Cultivar Evaluation was merged with the Crop and Fruit Quality Management research programme, now renamed the Horticulture research programme.

CRI again conducted a review of the industry's Research and Technical priorities across the industry value chain. As usual this process was undertaken by CRI Extension by obtaining inputs from growers (using the 37 regional Technology Transfer Groups), the exporters (using the Exporters Technical Panel within CRI Citrus Cold Chain Forum) and packhouses (using the five Regional Packhouse Working Groups within the CRI Citrus Cold Chain Forum). These priorities provided the platform for the compilation of the research portfolio and budget for the 2012-2013 year. CRI continued operating various subject specialist committees, each providing recommendations on the portfolio composition and funding.

An overarching focus on Sanitary and Phytosanitary Market Access was retained across CRI activities with the CRI CEO being personally responsible for co-ordination of the requisite market access and biosecurity technical inputs. These programmes require very long term strategies and ongoing maintenance. The pursuit of access for SA citrus to Thailand was finally concluded at the end of a 12 year effort. The South Korean market was opened for the export of SA lemons and grapefruit, the culmination of another lengthy process. There was again the successful detection, containment and eradication of isolated incursions of the invasive fruit fly *Bactrocera invadens* in some northern parts of South Africa. Country-wide surveillance is maintained in collaboration with other industries and DAFF to enable early detection, containment and eradication of future incursions. The distribution of greening disease and Citrus Black Spot was detailed through exhaustive field surveys, thereby enabling the amendment of regulations pertaining to the movement of citrus propagation material to protect unaffected areas.

Within the Integrated Pest Management (IPM) research programme resources were primarily focussed on False Codling Moth (FCM) and Fruit Flies. Application of the Sterile Insect Technique (SIT) for control of FCM was expanded from the Western Cape into the Eastern Cape. The efficacy of the technique was also demonstrated through trials conducted in the Limpopo Province. A wide range of new FCM control options progressed through various stages from initial feasibility testing, through to optimising of practical application, includes both microbial and chemical strategies and is focussed on both the pre- and post-harvest environment. The potential use of irradiation as a post-harvest phytosanitary treatment made encouraging progress. A conceptual systems approach to combining various incomplete control measures in a structured programme, thereby providing a cumulative level of control that can satisfy phytosanitary trade regulations was developed and aspects thereof included in field evaluation.

Research within the Disease Management programme spans a very wide range. Citrus Black Spot research focussed on optimising field control strategies, global population genetics, climate suitability modelling and

quantitative risk assessment modelling. Soil-borne pathology is breaking new ground in studies on soil health effects on tree vitality and the development of alternatives to conventional nematicides. A new post-harvest pathology researcher was appointed. Fruit and foliar disease research focussed on optimising practical disease control strategies. A great deal of support was again provided to the CIS through the Graft Transmissible Disease project and *Citrus Viroid IV* was recorded for the first time in South Africa. The Diagnostic Centre underwent successive staff changes, which was disruptive to operations, but the situation was successfully stabilised prior to the end of this report period.

In the Horticulture research programme a long term investment was made in the research direction taken with the Rind Condition project. A few years ago it was decided to focus attention on the more fundamental scientific components of the field. This strategy has started showing successes by now progressing to the development of applied handling and treatment procedures to better manage some rind disorders, such as chilling injury, rind breakdown and peteca spot. CRI unfortunately lost the services of its Crop and Fruit Quality project coordinator and this post remains vacant. Research in the field of nutrition is starting to progress to the point of producing first answers to some long standing questions such as the role of silicon and clarification of the role of humic acid soil applications is under investigation. The optimisation of cooling within shipping containers progressed with research on manipulating airflow within containers, with considerable potential energy savings. The cultivar evaluation project developed strongly with many older trials progressing to the production of fact sheets on a large bulk of the currently available cultivars and many new cultivars having been included in a greatly expanded evaluation system.

The CIS continued to be operated by CRI with guidance from the CIS Advisory Committee. An action plan to give effect to the recommendations from an international panel of reviewers was implemented. This included structural personnel changes, provision for expertise succession planning, enhancement of technical support and upgrading of facilities. These improvements will ensure that the SA CIS retains its current reputation of being one of the best schemes in the world. With the adoption of improved molecular diagnostic techniques, a huge exercise was undertaken to screen all the mother trees at the CFB for viroids. Conclusion of this process provides a platform for greater future assurance of the disease free status of propagation material supplied within the scheme.

CRI continued operating the Citrus Cold Chain Forum (CCCF) within the CRI Extension Division. The CCCF was greatly strengthened by CRI contracting the services of a CCCF coordinator, a Chairman of the CCCF's Packaging Working Group and a post-harvest extensionist. The five Regional Packhouse Working Group meetings, convened annually by CRI as part of the CCCF, have become hugely popular, with 650 attendees at the 2012 meetings. CRI Extension is to be congratulated in making such a success of this Forum that has already made a very big contribution to enhancing the industry's global competitiveness. CRI Extension again utilised the 37 Technology Transfer Groups to revise the research priorities in 2011, thereby providing every grower with an opportunity to make inputs into how CRI resources are directed at research and technical support services. The following communication channels were again made good use of to communicate technical information to the industry: the 2011 CRI Group Annual Research Report, the SA Fruit Journal, the Cutting Edge, the CRI Net, the CRI website, the CRI Production Guidelines, various specialist technical publications, Citrus Academy Learning Material, grower meetings and various scientific journals.

## **INLEIDING**

Vaughan Hattingh (HUB CRI)

Hierdie dokument doen verslag oor aktiwiteite van die CRI-Groep wat verband hou met navorsing- en tegniese ondersteuning oor die tydperk April 2011 tot Maart 2012. CRI se primêre bron van befondsing was steeds die heffing op uitvoere van vars sitrus wat deur die CGA geadministreer word. Waardevolle addisionele befondsing is deur die CRI-Groep se model gekry in die vorm van Staatsfondse ter ondersteuning van navorsingsprojekte wat by Universiteite uitgevoer word. Bykomende staatsbefondsing is verkry in die vorm van toekennings deur middel van die Plaagdoder Inisiatief Program II en die Na-oes Innoveringsprogram II. Ander addisionele fondse is ook verkry deur middel van internasionale navorsingsamewerking. Verskillende vorme van verkope, dienste en tantieme het tot die res van die inkomste bygedra wat saamgevoeg is vir aanwending in die nastrewing van CRI se missie: "Om die langtermyn globale mededingendheid van suider-Afrika se sitrusprodusente te maksimeer deur die ontwikkeling, ondersteuning, koördinering en voorsiening van Navorsing- en Tegniese dienste, deur die sterkpunte van die CRI-Groep se vennote te kombineer".

CRI het volgens die CRI-Groep Alliansie model voortgegaan waar CRI se eie hulpbronne en kapasiteit deur samewerking met CRI se netwerk van alliansie-vennoot navorsingsorganisasies versterk is. 'n Memorandum van Verstandhouding (MVV) tussen CRI en die LNR is gesluit, wat 'n einde aan 'n lang onderhandelingsproses

gebring het. Waar daar altyd voortdurend samewerking tussen die CRI en die LNR in verskeie vorme oor die jare was, het die sluiting van hierdie MVV die deure geopen tot die hervatting van meer formele en gestruktureerde samewerking.

CRI het sy Kultivar Afdeling herstruktureer. As gevolg hiervan het CRI sy betrokkenheid by die verkryging van kommersialiseringsregte en kultivarbestuur beëindig. Dit het CRI in staat gestel om meer doeltreffend op die bedryf se kultivar evaluasieprojek te fokus, wat daarop gemik is om produsente met objektiewe, onpartydige, vergelykende evaluering van kultivars te voorsien, ongeag van hul eienaarskap of bestuurstatus. Hierdie herstrukturering versterk ook CRI se onpartydigheid met betrekking tot die bestuur van die Sitrusverbeteringskema. Kultivar evaluering het met die Oes- en Vrugskaaliteitsnavorsingsprogram saamgesmelt, wat nou die Hortologie navorsingsprogram genoem word.

CRI het weer 'n hersiening van die bedryf se navorsing- en tegniese prioriteite regoor die bedryf se waardeketting gedoen. Soos gewoonlik is hierdie proses deur CRI Voorligting onderneem deur die verkryging van insette van produsente (met behulp van die 37 Tegnologie Oordragsgroepe in die streke), die uitvoerders (met behulp van die Uitvoerders se Tegniese paneel binne CRI se Sitrus Koueketting Forum) en pakhuse (met behulp van die vyf Pakhuis Werks-groepe in die streke binne die CRI Sitrus Koueketting Forum). Hierdie prioriteite het die platform verskaf vir die samestelling van die navorsingsportefeulje en begroting vir die 2012-2013 jaar. CRI het voortgegaan met die operering van verskillende vakspecialis-komitees, waarvan elkeen aanbevelings oor die samestelling van portefeuljes en befondsing verskaf.

'n Oorkoepelende fokus op Sanitêre en Fitosanitêre Marktoegang is regoor CRI se aktiwiteite behou met CRI se uitvoerende hoof wat persoonlik verantwoordelik is vir die koördinerende van die nodige tegniese insette vir marktoegang en biosekuriteit. Hierdie programme vereis baie langtermyn strategieë en deurlopende instandhouding. Die strewe om toegang vir SA se sitrus na Thailand te kry is uiteindelik aan die einde van 'n 12-jaar-poging afgesluit. Die Suid-Koreaanse mark is vir die uitvoer van SA suurlemoene en pomelo's geopen, die hoogtepunt van nog 'n lang proses. Daar was weereens suksesvolle opsporings, inperkings en uitroeiings van geïsoleerde introduksies van die indringer vrugtevlug, *Bactrocera invadens*, in van die noordelike dele van Suid-Afrika. Landswye opsporingsnetwerke word in stand gehou in samewerking met ander bedrywe en DAFF om vroeë opsporings, inperkings en uitroeiing van verdere introduksies moontlik te maak. Die verspreiding van vergroening en Sitrus Swartvlek is deur intensiewe opnames bepaal om die wysiging aan regulasies wat betrekking het op die beweging van sitrus voortplantingsmateriaal moontlik te maak, om sodoende nie-geaffekteerde gebiede te beskerm.

Binne die Geïntegreerde Plaagbestuur (GPB) navorsingsprogram is hulpbronne hoofsaaklik op Valskodlingmot (VKM) en vrugtevlug gefokus. Toepassing van die steriele-insek tegniek (SIT) vir die beheer van VKM is van die Wes-Kaap na die Oos-Kaap uitgebrei. Die effektiwiteit van die tegniek is ook gedemonstreer deur proewe wat in die Limpopo provinsie uitgevoer is. 'n Wye verskeidenheid van nuwe VKM beheeropsies het deur verskillende stadiums gevorder vanaf die aanvanklike uitvoerbaarheidstudies na die optimalisering van praktiese toepassing, wat beide mikrobiologiese- en chemiese strategieë insluit, en is op beide die voor en na-oes-omgewing gefokus. Die potensiële gebruik van bestraling as 'n na-oes fitosanitêre behandeling het bemoedigende vordering gemaak. 'n Konseptuele benadering wat op sisteme berus, waar verskeie onvolledige beheermaatreëls in 'n gestruktureerde program gekombineer is om 'n kummulatiewe vlak van beheer te verskaf, wat voldoende vir fitosanitêre handelsregulasies sal wees, is ontwikkel en aspekte daarvan is in veldproewe ingesluit.

Navorsing binne die Siektebestuursprogram strek oor 'n baie wye reeks. Sitrus Swartvlek-navorsing het op die optimalisering van beheerstrategieë in die veld, globale populasie genetica, modellering van geskikte klimaatstoestande en kwantitatiewe risiko-assessering gefokus. Grondgedraagde patologie is besig met opspraakwekkende studies oor die effek van grondgesondheid op lewensvatbaarheid van bome en die ontwikkeling van alternatiewe vir konvensionele aalwurmdoders. 'n Nuwe navorsing in Na-oes patologie is aangestel. Vrug- en blaarsiekte navorsing het op die optimalisering van praktiese strategieë vir siektebeheer gefokus. 'n Groot mate van ondersteuning is weer aan die SVS deur die Ent-Oordraagbare Siekte projek gelewer. *Sitrus Viroid IV* is vir die eerste keer in Suid-Afrika aangeteken. Die diagnostiese sentrum het opeenvolgende personeelveranderinge ondergaan, wat ontwrigtend vir die werksaamhede was, maar die situasie is suksesvol gestabiliseer voor die einde van hierdie verslagperiode.

In die Hortologie navorsingsprogram is 'n langtermyn belegging gemaak met die rigting wat navorsing in die Skilkondisie-projek geneem het. 'n Paar jaar gelede is daar besluit om aandag op die meer fundamentele wetenskaplike komponente van die veld te fokus. Hierdie strategie het begin om sukses te toon met die vordering nou tot die ontwikkeling van toegepaste hantering en behandelingsprosedures om skilprobleme soos koueskade, skilafbraak en peteka vlek beter te bestuur. CRI het ongelukkig die dienste van sy Oes en

Vrugkwaliteit projekkoördineerder verloor en die pos is steeds vakant. Navorsing op die gebied van voeding begin nou vorder tot die punt van bekendmaking van die eerste antwoorde op lank uitstaande vrae soos die rol van silikon, en die uitklaring van die rol van humiedsuur as gronddienings word ondersoek. Die optimalisering van verkoeling binne verskepingshouers het gevorder met navorsing oor die manipulering van lugvloei binne houers met aansienlike potensieële energiebesparings. Die kultivar evalueringsprojek het goed gevorder - baie van die ouer proewe het gevorder tot die beskikbaarstelling van inligtingstukke oor baie van die kultivars wat tans beskikbaar is en baie nuwe kultivars is ingesluit in 'n evaluasie-stelsel wat aansienlik uitgebrei is.

Die SVS word steeds deur CRI bedryf met leiding van die SVS se Advieskomitee. 'n Aksieplan om uitvoering te gee aan die aanbevelings van 'n internasionale paneel van beoordelaars is geïmplementeer. Dit het strukturele personeel veranderinge, voorsiening vir kundige opvolgbeplanning, verbetering van tegniese ondersteuning en opgradering van fasiliteite ingesluit. Hierdie verbeteringe sal verseker dat die SA SVS sy huidige reputasie van een van die beste skemas in die wêreld behou. Met die ingebruikneming van verbeterde molekulêre diagnostiese tegnieke, is 'n groot taak onderneem om al die moederbome by die SGB vir viroïedes te sif. Voltooiing van hierdie proses voorsien 'n platform vir 'n groter toekomstige versekering van die siektevrye status van voortplantingsmateriaal wat binne die skema voorsien word.

CRI het voortgegaan om die Sitrus Koueketting Forum (CCCF) binne die CRI Voorligtingsafdeling te bedryf. Die CCCF is versterk deur CRI se kontrak-aanstellings van 'n CCCF koördineerder, 'n voorsitter vir die CCCF se Verpakkingswerkgroep en 'n na-oes voorligter. Die vyf Pakhuis Werkswinkels in die streke wat jaarliks deur CRI as deel van die CCCF gereël word is baie gewild, met 650 deelnemers aan die 2012 vergaderings. CRI Voorligting moet gelukkig word met die suksesverhaal van hierdie forum wat reeds 'n baie groot bydrae tot die verbetering van die bedryf se globale mededingendheid gelewer het. CRI Voorligting het weer van die 37 Tegniese Oordragsgroepe gebruik gemaak om die navorsingsprioriteite in 2011 te hersien, waardeur elke produsent die geleentheid gegee is om insette te lewer oor hoe CRI se hulpbronne vir navorsing en tegniese ondersteuning aangewend moet word. Die volgende kommunikasiekanale is weer gebruik om tegniese inligting aan die bedryf te verskaf: die 2011 CRI Groep se Jaarlikse Navorsingsverslag, SA Vrugte Joernaal, Snykant, CRI-Net, CRI webwerf, CRI Produksie Riglyne, verskeie spesialis tegniese publikasies, Sitrus Akademie se opleidingsmateriaal, produsente vergaderings en verskeie wetenskaplike joernale.

## 2 MARKET ACCESS TECHNICAL COORDINATION

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

### 2.1 SUMMARY

Although shortly after the end of the report period, the highlights of this report period was confirmation that SA may commence exporting grapefruit and lemons to South Korea and fresh citrus fruit to Thailand in the 2012 season. Citrus Black Spot (CBS) phytosanitary import regulations in the EU remained problematic. The International Plant Protection Convention (IPPC) made no progress with SA's challenge to the technical justification of these EU regulations. The matter was elevated to communication with the Director General of the FAO, eliciting IPPC assurances that resources will be directed at addressing the issue. The European Plant Protection Organisation (EPPO) indicated that they have initiated a pest risk analysis (PRA) on false codling moth (FCM). In response to concerns raised by Japan, CRI undertook to repeat aspects of earlier research conducted to support a revised time temperature protocol for exports to Japan. There were various engagements with USA regarding conditions for export of citrus from SA, but none of the issues had progressed to satisfactory conclusion by the end of the report period. CRI developed a systems approach for FCM risk mitigation as an alternative to post-harvest cold treatment. This protocol was submitted to China for official evaluation for potential inclusion as an option in the China export protocol. A national surveillance network was maintained to provide for early detection of incursions by the fruit fly *Bactrocera invadens*. Several incursions were detected during the report period. By the end of the report period eradication had been successfully undertaken in all incursions sites with the exception of one site where the process had not yet been completed. CRI made inputs to SA-DAFF on the revision of several pieces of domestic regulation of importance to SA biosecurity.

### OPSOMMING

Alhoewel dit net na die einde van die verslagperiode gebeur het, is die hoogtepunte van hierdie verslagperiode die bevestiging dat SA met die uitvoer van pomelo's en suurlemoene na Suid-Korea en vars sitrusvrugte na Thailand in die 2012-seisoen kan begin. Die fitosanitêre invoervereistes vir Sitrus Swartvlek (SSV) na die EU bly steeds problematies. Geen vordering is deur die Internasionale Plantbeskermingskonvensie (IPPK) met SA se uitdaging van die tegniese regverdiging van die EU-regulasies gemaak nie. Die saak is verder geneem deur

kommunikasie wat aan die Direkteur-generaal van die FAO gerig is om hierdeur die IPPK se versekering te kry dat hulpbronne aangewend sal word om die kwessie aan te spreek. Die Europese Plantbeskermingsorganisasie (EPPO) het aangedui dat hul 'n Pes-Risiko-Analise (PRA) op valskodlingmot (VKM) gaan uitvoer. In reaksie op Japan se besorgdheid, het CRI onderneem om aspekte van vroeëre navorsing wat gedoen is ter ondersteuning van 'n hersiene tyd-temperatuur protokol vir uitvoere na Japan, te herhaal. Daar was verskeie vergaderings met die VSA omtrent die vereistes vir die uitvoer van sitrus vanaf Suid-Afrika, maar nie een van die aksies het teen die einde van die verslagperiode tot op 'n bevredigende punt gevorder nie. CRI het 'n benadering wat op sisteme gebaseer is ontwikkel om die risiko van VKM, as 'n alternatief tot die na-oes koue-behandeling, te verminder. Hierdie protokol is aan China vir amptelike evaluering vir moontlike insluiting as 'n opsie in die China uitvoerprotokol voorgelê. 'n Nasionale opsporingsnetwerk is in stand gehou om voorsiening te maak vir die vroeë opsporing van introduksies van die vrugtevlug *Bactrocera invadens*. Verskeie introduksies is gedurende die verslagperiode gevind. Teen die einde van die verslagperiode is uitroeiing suksesvol in alle gebiede waar die introduksies gevind is, uitgevoer, met die uitsondering van een plek waar die proses nog nie afgehandel is nie. CRI het insette oor die hersiening van verskeie plaaslike regulasies in belang van SA se biosekuriteit aan SA-DAFF gelewer.

## 2.2 EUROPE (EU)

The EU Food and Veterinary Office (FVO) undertook a follow up visit to South Africa in June 2011 to again evaluate the official control systems and the certification processes that are in place for citrus fruit exports to the EU. The EU Steering Committee met in May to prepare for the visit. The visit took place from 07-17 June 2011. In August 2011 a report was received that indicated that although there was a significant improvement in South Africa's official control and certification system for export of citrus fruit to the EU, the EU continues to question the adequacy of the orchard inspection process with regard to CBS. No problems were identified with the CBS area-freedom surveys conducted by SA, as well as the measures implemented to establish and maintain the CBS-Pest free areas. Feedback was provided to the EU. The CBS-RMS was also amended during this reporting period to allow for better implementation of the phytosanitary regulations. However by the end of this reporting period the number of official notifications of CBS interceptions in the EU went up from 9 notifications for the 2010 season to 33 official notifications for the 2011 season.

The International Plant Protection Convention (IPPC) did not make progress with the dispute between South Africa and the European Union regarding the EU's CBS citrus import regulations, despite meetings between SA-DAFF and the IPPC Secretariat. In order to expedite the process the CBS/IPPC Steering committee held a teleconference in December 2011. Decisions taken at this meeting included sending a letter by the SA Minister of DAFF to the DG: FAO. The letter was sent in March 2012. At the March 2012 meetings of the WTO-SPS a report by the IPPC Secretariat indicated that the phytosanitary trade dispute between South Africa and the European Union is ongoing and that further announcements will be made as information becomes available and when appropriate. Resource challenges within the IPPC Secretariat were given the reason for the slow progress in 2011 but assurances were given that this dispute will be addressed more pro-actively in 2012.

Notification was received from the European Plant Protection Organisation (EPPO) that they are in the process of conducting a pest risk analysis (PRA) on false codling moth (FCM). The CGA constituted a committee to oversee industry actions pertaining to this matter.

## 2.3 JAPAN

Two issues for access of South African citrus fruit to Japan remained pending at the end of this reporting period, despite several discussions and meetings between the NPPOS of SA and Japan: the broadening of access for soft citrus cultivars to include all other SA mandarins except Satsumas and; the adoption of a revised cold treatment condition for the export of fruit of all citrus types.

In February 2011, on request from Japan, CRI supplied a report on the experimental work conducted on the Phase 1 trials of the revised cold treatment condition, and DAFF submitted this to Japan-MAFF. In November 2011 Japan-MAFF informed SA-DAFF that problems were identified with the report on Phase II of the trials. In order to provide information to address these concerns, aspects of the earlier work need to be repeated and will be undertaken in the 2012 season.

## 2.4 USA

The most important unresolved issue for this market remained reversion of the FCM cold treatment period from 24d to 22d.

In June 2011 SA-DAFF informed CRI that feedback was received from USDA-APHIS on the discussions held in February 2011 in the USA relating to the 24 day cold treatment and CBS. This information was forwarded to CRI in July 2011 for inputs. CRI advised SA-DAFF that clarification on the proposal regarding a 1.5% approach rate is needed before SA could properly assess the implications of the proposal. By the end of the report period no response had been provided by APHIS. In an attempt to expedite the matter, Ron Campbell commissioned an external review of the export programme's rejection statistics.

Recognition of CBS pest free places of production in an area of low pest prevalence and inclusion of other Western Cape magisterial districts in the export programme were two other pending issues. In September 2011 CRI provided inputs to SA-DAFF on a response to USDA-APHIS queries regarding equivalence between USA domestic CBS regulations and USA import regulations, CBS pest free areas and CBS pest free places of production. In March 2012 APHIS provided DAFF with a proposal regarding prioritisation of dealing with outstanding issues. CRI-CGA provided DAFF with proposals for inclusion in a response to APHIS.

## 2.5 CHINA

In February 2011 it was agreed that CRI would develop alternative proposals to the mandatory FCM cold treatment to address the quality problems encountered with shipments of citrus fruit to this market. A system approach for FCM was developed and submitted to SA-DAFF in December 2012 together with a request to include the long outstanding issue of acceptance of non-containerised bulk shipping. In February 2012, after further consultation with SA-DAFF, a revised FCM Systems Approach document was finalised and provided to SA-DAFF. DAFF submitted the information to the Chinese authorities in March 2012. SA is currently awaiting feedback from the Chinese Authorities. CRI made arrangements with a sample of growers and packhouses to test certain aspects of the systems approach in the 2012 season.

## 2.6 SOUTH KOREA

At the end of the previous reporting no feedback had been received from the Korean Authorities on the acceptance to export grapefruit and lemons under the same management options as for sweet oranges although they promised to revert by June 2010. In May 2011 S Korea responded that they will accept the same management options for grapefruit and lemons as for oranges. They also requested, before finalisation of the new export protocol, an on-site visit to grapefruit and lemon producing areas as well as the ports. This visit took place from 26 June - 2 July 2011. In August 2011 the Authorities requested more information on the status of *Bactrocera invadens* in South Africa. This information, together with two scientific papers on cold disinfestation treatments for this pest, was supplied to the Authorities in September 2011. In October 2011 SA-DAFF received confirmation from the South Korean Authorities that the cold treatment processes that are in place for *Bactrocera invadens* are adequate and that no additional actions are required for continuation of citrus exports. In December 2012 a draft protocol for the importation of lemons and grapefruit was received and only minor changes to the existing protocol for sweet oranges were identified by CRI/CGA and inputs were submitted to SA-DAFF. In March 2012 the Korean Authorities requested notification if there is a need for their inspectors to stay longer in SA for this export season should the draft protocol for lemons and grapefruit be accepted. After consultation with exporters it was indicated that there is no need to extend the time of inspectors in SA. In March 2012 a revised draft protocol for lemons and grapefruit was received which included most of the previously requested changes and only a few further minor issues were identified by CRI/CGA. Feedback was submitted to SA-DAFF and shortly after the end of this report period SA was notified that SA may commence with exports of grapefruit and lemons to South Korea.

## 2.7 NEW MARKETS

### 2.7.1 Thailand

At the end of the previous reporting period SA notified Thailand of technical concerns (list of actionable pests, citrus fruit types and mandatory FCM cold treatment) with the draft import conditions received from Thailand. SA-DAFF forwarded this information to the Thai authorities in July 2011. In August 2011 Thailand reverted with a proposal to send two officials to inspect the South African citrus industry before finalisation of the import protocol. As it was already late in the season, with few grapefruit for export left, they were invited to visit during the week of 11 -17 September 2011. Despite several follow-up queries by SA-DAFF feedback was only received in December 2011, indicating that they accepted most of the proposed changes to the draft import protocol and that they wanted to visit the industry during early 2012, but they also wanted additional information on some of the listed pests. Information was submitted to SA-DAFF and arrangements were made for the

official Thai-visit scheduled for March 2012. In preparation for the bilateral meeting additional technical information was submitted to SA-DAFF, including data on the efficacy of the FCM cold treatment and the different citrus types. The bilateral meeting was a success and the draft import protocol was accepted by SA-DAFF on the basis of understanding that the protocol can be amended after the first export season, should problems be encountered. Shortly after the end of the report period, SA received confirmation that SA may commence exporting citrus to Thailand in the 2012 season.

### 2.7.2 Cambodia, Kazakhstan and Syria

In accordance with a decision of the Market Access Working Group, the Fresh Produce Exporters Forum (FPEF) was requested to provide certain data required for pursuit of official access to these markets. By end of this reporting period no feedback had been provided by FOEF despite several follow-up requests.

### 2.7.3 Australia, Lebanon and Philippines

No feedback and/or any responses were received from these countries during this reporting period, despite several follow up requests by SA-DAFF.

## 2.8 BIOSECURITY AND REGULATIONS

During the previous reporting period it was reported that SA declared the successful eradication of the exotic fruit fly, *Bactrocera invadens* after the pest was detected in SA for the first time. The surveillance network for this pest was expanded during this reporting period and surveillance became mandatory for farms to register for export of citrus to special markets. In June 2011 a third incursion of the pest was detected and another in July 2011. On both occasions the International Plant Protection Convention (IPPC) was notified accordingly. Both areas, Tshipise and Weipe are in the far northern Limpopo area that is adjacent to the Zimbabwean border. In August 2011 another pest report was sent to the IPPC to report on the fifth detection in South Africa in the Groblersbrug-area of the Limpopo province, adjacent to the Botswana border. In all these areas phytosanitary measures were implemented to control movement of fruit of host species and eradication was initiated.

In October 2011 a pest report on the sixth detection of this pest was sent to the IPPC. This time the pest was found in the Levubu area. In December 2011 another pest report was sent to the IPPC to report on the seventh detection in the Nwanedi area. All these areas are also within the Limpopo province and again phytosanitary measures were implemented to control movement of fruit of host species and eradication was initiated. In December 2011, January 2012 and March 2012 SA reported to the IPPC that the pest was successfully eradicated from the Groblersbrug, Tshipise, Weipe, Nwanedi and Levubu areas. By the end of the report period there was only one reported incursion site still under eradication, being an incursion again detected in the Groblersbrug-area, adjacent to the Botswana border.

CRI undertook a process, in collaboration with DAFF, to survey all magisterial districts within the Western Cape that have not previously been surveyed to confirm the absence of CBS and cater for their inclusion in relevant regulations pertaining to the movement of citrus propagation material and export protocols. SA-DAFF provided the official laboratory report for the CBS-survey conducted in the Western Cape magisterial district of Riversdale. The amendments to the Control Measures have been included in the revised Control Measures. The document has however not yet been forwarded to Minister for her approval. During 2011/2012 CBS surveys were also conducted in the remaining magisterial districts of the Western Cape (KAROO) province with no commercial citrus plantings. By the end of this reporting period the official report had not yet been supplied by SA-DAFF.

Regulations R110 and R1013 of the Agricultural Pests Act deals with the control of movement of plant material within SA and the importation of plant products for which no import permit is required respectively. Several problems with these two regulations were highlighted by CRI and inputs were provided to SA-DAFF to amend these regulations to safeguard agriculture within SA. By end of the reporting period, these documents had not yet been sent to the Minister for final approval, despite several meetings between CRI and SA-DAFF.

After the detection of Citrus Greening in 2008 in the greening free Eastern Cape Province, several detection and delimiting surveys were conducted. A workgroup was formed to address this important issue. Although destruction orders were issued to the relevant land owners by SA-DAFF, some of them refused to comply with the orders and some of the infected trees remain. In pursuit of maintaining the greening free status of this province, DAFF proposed establishing buffer zones with specific phytosanitary regulations. An action plan for '*Candidatus Liberibacter asiaticus*' and '*Candidatus Liberibacter americanus*' and their vector, the Asian citrus psyllid (*Diaphorina citri*) was also developed in cooperation with SA-DAFF.

In 2010 a “scab-like” disease was observed on fruit of pomegranates in orchards in the Eastern Cape. Detection surveys were conducted under the auspices of DAFF and a fungal species was isolated that could pose a serious threat to the citrus industry. Different reference strains were imported and pathogenicity tests were conducted in an official quarantine environment on a range of citrus types grown in South Africa. It was however demonstrated that this pathogen is not pathogenic to the range of citrus types grown in South Africa.

### 3 PROGRAMME: INTEGRATED PEST MANAGEMENT

#### 3.1 PROGRAMME SUMMARY

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

The Integrated Pest Management (IPM) Programme could have been called the Entomology Programme or simply the Pest Management Programme. However, it was decided years ago to name it the IPM Programme, as a reflection of CRI’s commitment to a bio-intensive holistic multi-faceted approach to pest management. It is clear that not only is market resistance to chemical usage and chemical residues increasing year by year but chemical control alone can never be sustainable. Having said this, the actual implementation of IPM in the southern African citrus industry is more challenging now that it was several years ago. The necessity to spray regularly for control of the phytosanitary disease, citrus blackspot, and the shortage of IPM-compatible effective thripicides is compromising biological control of a number of the major insect pest species. Unfortunately, this includes our most important pest, from a phytosanitary perspective, false codling moth (FCM).

In this challenging environment, research within the IPM Programme has to become even more innovative and multi-layered than in the past. This has been achieved through CRI’s ever strengthening relationships with other research facilities, particularly universities throughout South Africa. This has not only increased our research base but has incorporated specialist skills, complementing and enhancing CRI’s research efforts and outcomes. Simultaneously, a new generation of research entomologists is being raised up, with post-graduate students being the principal researcher on eight of the 35 separate studies listed within the programme.

FCM continues to be the greatest entomological and market access challenge faced not only within the IPM Programme but by the citrus industry as a whole. In total, 15 experiments (separate studies) were conducted within the project. Four of these studies focussed on post-harvest detection or control of FCM. If a breakthrough can be achieved in this area, this may well be the silver bullet that FCM management so desperately needs. Both gamma irradiation (most likely in combination with truncated cold-treatment) and carbon dioxide fumigation (most definitely in combination with cold treatment) are showing exciting potential for post-harvest disinfestation of fruit, particularly considering that cold-sterilisation would be unaffordable for fruit going to certain markets, including the EU. Equally exciting, but probably a bit further off implementable results, is the work being done on post-harvest detection. This is being done primarily through detection of fruit volatile emission profiles distinct to FCM infested fruit and X-ray detection. Of the remaining 11 studies, seven looked at various aspects of microbial control of FCM. Four of these looked at entomopathogenic nematodes (EPN), both production and control. Most excitingly, large scale field trials with EPNs are now underway, showing an 81% reduction in FCM infestation in one case. Two experiments involved the FCM granulovirus. One of these was particularly foresighted, isolating and identifying five new virus isolates, which are now available in the event that any FCM population develops resistance to the commercial products. The final microbial control study investigated entomopathogenic fungi (EPFs) for the control of soil-borne life stages of FCM. The final three pre-harvest FCM control trials investigated the efficacy of Coragen, which has since been registered for FCM control on citrus, a novel mating disruption dispenser, and differences in attractiveness of virgin female FCM from different regions to sterile males from Citrusdal. The final experiment in this project looked at morphological and molecular identification of moths associated with citrus.

Of similar importance and urgency to the work being done in the FCM Project, was the work conducted within the Fruit Fly Project. In addition to Natal fruit fly, the local citrus industry now has a second phytosanitary fruit fly to deal with, namely the African invasive fruit fly, *Bactrocera invadens* (Bi). To date, national efforts to eradicate the fly where it has been detected in the north of the country have been successful. This is to a large extent thanks to the Bi surveillance network, which formed part of the Fruit Fly Project activities. Within the Fruit Fly Project, basic and applied research studies were conducted with the main objectives of (1) improving the management of local fruit fly pests, (2) investigating the efficacy of a new post-harvest treatment, (3) determining the suitability of various areas in South Africa for establishment of Bi and (4) determining the efficacy of various methods for control of Bi. The tactics investigated for improving control of local fruit flies were a new biodegradable bait station and EPNs. Research on thermal biology of Medfly and Natal fly was completed and provided some insights into the factors that affect the distribution and abundance of the two species. In field

trials in Kenya M3 bait stations or Prolure bait sprays in combination with male annihilation technique (MAT) blocks were found to be effective in controlling Bi. As important as controlling the pest, is understanding its potential distribution in the world and in South Africa, which is being determined using the CLIMEX model. Finally, an alternative post-harvest fumigant treatment was found to be effective.

Even though phytosanitary pests receive most research attention, fruit must still look appealing to be sold, so cosmetic pests will always remain a threat to the crop. Controlling these pests without disrupting important natural enemies of other pests is also a continual challenge. In the Cosmetic Pests Project, five separate studies were pursued, covering thrips, leafhoppers, general Lepidoptera, and post-harvest intervals (PHIs) of pesticides. The planned leafhopper trials could not be initiated, as numbers remained too low. A commercially formulated experimental EPF unfortunately provided no thrips or mealybug control. Imidacloprid was shown to be able to cause repercussions of lepidopteran pests. PHIs for fenpyroximate were established and the effects of a few production and application practices on residue levels at harvest, such as using adjuvants and shade cloth, were investigated.

For years now, the industry has been calling for alternatives to organophosphates for controlling mealybug, reading the writing on the wall. Such alternatives were investigated in EPNs and inoculative releases of an *Anagyrus* sp. parasitoid, both showing significant potential. Furthermore, through spraying Navel oranges with 2,4-D in spring and thus reducing open navel ends, mealybug infestation was significantly reduced. Finally, as a post-harvest treatment, gamma radiation was shown to be effective in sterilising adult female mealybug.

Despite the critical need to control some phytosanitary and cosmetic pests with chemicals, the contribution from natural enemies in citrus IPM must be maximized in order to control several important pests in summer and autumn. This is becoming more important as permitted chemical residue levels on fruit at harvest decline. Ants disrupt biological control and therefore need to be kept out of the trees. In the Biocontrol Disruption Project, a novel ant bait, Saga, successfully controlled the brown house ant and the pugnacious ant. The bait will soon be commercialized.

The two pests affecting production that received research attention during the report period were woolly whitefly and fruit piercing moths.

During the research year in question CRI entomologists and many of the other entomologists working within this programme, participated actively in scientific meetings both locally and internationally, emphasising to the international scientific community the quality and relevance of research coming out of this team. Additionally, a number of papers were published in top international scientific peer-reviewed journals and in our local fruit journal. CRI entomologists also participated actively in carrying the important messages emanating from their research over to the grower community – this particularly through study group meetings and Cutting Edge publications. Our researchers once again look forward to presenting their findings at the biennial Citrus Research Symposium in August 2012 and interacting with the industry which they are serving.

## **PROGRAMOPSOMMING**

Die Geïntegreerde Plaagbestryding (GPB) Program kon die Entomologie Program of eenvoudig die Plaagbestryding Program genoem word, maar daar was besluit om dit die GPB (of IPM) Program te noem as 'n refleksie van CRI se toevertrouing tot 'n bio-intensiewe holistiese veelvoudige benadering tot plaagbestryding. Dit is duidelik dat daar nie net jaar na jaar 'n verhoging in markweerstand teen chemiese gebruik en chemiese residue is nie, maar ook dat chemiese gebruik alleen nooit onderhoubaar sal wees nie. Aan die ander kant is die werklike implementering van IPM in die suidelike Afrika sitrusbedryf meer uitdagend nou as wat dit verskeie jare gelede was. Die behoefte om gereeld vir die beheer van die fitosanitêre siekte, sitrus swartvlek, te spuit en die tekort aan doeltreffende IPM-verenigbare blaaspootjiedoders, is besig om biologiese beheer van 'n paar belangrike insek plaag spesies te kompromiseer. Ongelukkig sluit dit ook ons nommer een fitosanitêre plaag in, die valskodlingmot (VKM).

In hierdie uitdagende omgewing moet navorsing binne die IPM Program selfs meer innoverend en multi-dimensioneel word as in die verlede. Hierdie word wel bereik deur CRI se groeiende verhoudings met ander navorsingsfasiliteite in Suid-Afrika, veral universiteite. Hierdie het nie net ons navorsingsbasis uitgebrei nie maar het ook spesialiteitsvaardighede ingebring, wat CRI se navorsingspoging gekomplimenteer het. Gelyktydig word 'n nuwe generasie navorsingsentomoloë opgehef, met nagraadse studente wat die hoofnavorsers van agt van die 35 aparte studies in die program is.

VKM bly die belangrikste entomologiese en marktoegangsuitdaging, nie net in die IPM Program nie, maar dwarsdeur die sitrusbedryf as geheel. Altesaam 15 eksperimente (aparte studies) is in die projek uitgevoer. Vier van hierdie studies het op na-oes opsporing of beheer gefokus. As 'n deurbraak in hierdie area bereik kan word, kan dit dalk die wonder middel wees wat VKM bestuur so dringend nodig het. Albei gammabestraling (heel waarskynlik in kombinasie met verkorte koue-behandeling) en koolsuurstof beroking (beslis in kombinasie met koue-behandeling) wys opwindende belofte vir na-oes ontsmetting van vrugte, veral as 'n mens besef dat koue-sterilisering onbekostigbaar sal wees vir vrugte na sekere markte, insluitend die EU. Net so opwindend, maar waarskynlik 'n bietjie verder van toepasbaarheid, is die werk wat op na-oes opsporing gedoen word. Hierdie word hoofsaaklik gedoen deur opsporing van afskeiding van vlugtige verbindings van vrugte wat gekoppel kan wees aan VKM besmetting asook deur X-straal opsporing. Van die 11 oorblywende studies het sewe na verskeie fasette van mikrobiële beheer van VKM gekyk. Vier hiervan het gekyk na entomopatogeniese nematodes (EPNs), beide produksie en beheer. Die mees opwindendste is dat grootskaalse veldproewe met EPNs nou aan die gang is en tot 81% afname in VKM besmetting in een geval gewys is. In twee eksperimente is die VKM granulovirus verder ondersoek. Een van hierdie was veral proaktief gewees en het vyf nuwe virus isolate geïsoleer en geïdentifiseer. Hierdie is nou beskikbaar ingeval enige VKM bevolking ooit weerstandbiedendheid teen die kommersiële produkte ontwikkel. Die finale mikrobiële beheerstudie het entomopatogeniese swamme (EPS) ondersoek vir beheer van die grondgedraagde lewensstadiums van VKM. Die finale drie voor-oes VKM beheer proewe het die volgende ondersoek: die werking van Coragen wat nou geregistreer is op sitrus vir VKM beheer, ondersoek; 'n nuwe paringsontwrigting vrysteller; en verskille in aantreklikheid van ongepaarde wyfies VKM van verskillende streke vir steriele mannetjies van Citrusdal. Die finale eksperiment in hierdie projek het gekyk na morfologiese en molekuleêre identifikasie van motte wat met sitrus geïssosieer is.

Die werk wat binne die Vrugtevlug Projek uitgevoer word is van vergelykende belangrikheid en dringendheid as die werk wat binne die VKM Projek gedoen word. Saam met die Natalse vrugtevlug het die plaaslike sitrusbedryf nou 'n tweede fitosanitêre vrugtevlug om te hanteer, naamlik die Afrikaanse indringer vrugtevlug, *Bactrocera invadens* (Bi). Tot op datum is nasionale pogings om hierdie vlug uit te wis waar dit in die noorde van die land opgelet is, suksesvol. Hierdie is tot 'n groot mate te danke aan die Bi opname netwerk, wat deel gevorm het van die Vrugtevlug Projek se verantwoordelikhede. In die Vrugtevlug Projek is basiese en toegepaste navorsingstudies gedoen en die hoofdoelwitte is: (1) verbetering van die bestuur van plaaslike vrugtevlug plaë, (2) 'n ondersoek van die doeltreffendheid van 'n nuwe na-oes behandeling, (3) bepaling van die geskiktheid van verskeie areas in Suid-Afrika vir die vestiging van Bi en (4) bepaling van die doeltreffendheid van verskeie beheermaatreëls vir die beheer van Bi. Die tegnieke wat ondersoek is om beheer van die plaaslike vrugtevlug te verbeter is 'n nuwe bio-afbreekbare lokstasie en EPNs. Navorsing op termiese biologie van Medvlug en Natalse vrugtevlug is voltooi en het insig verskaf in die faktore wat verspreiding en volopheid van die twee spesies beïnvloed. In veldproewe in Kenia het M3 lokstasies of Prolure lokaasbespuitings in kombinasie met mannetjie uitwissingstegniek (MAT) blokke Bi doeltreffend beheer. Net so belangrik as beheer van hierdie plaag is om sy potensiële verspreiding albei in Suid-Afrika en in die wereld te verstaan, wat wel bepaal word deur middel van die CLIMEX model. Laastens is 'n alternatiewe na-oes berokings behandeling doeltreffend bewys.

Alhoewel fitosanitêre plaë die meeste navorsingsaandag ontvang, moet vrugte steeds mooi lyk om te verkoop, dus sal kosmetiese plaë altyd 'n bedreiging vir die oes bly. Om die plaë te beheer sonder om belangrike natuurlike vyande van ander plaë te ontwrig, is ook 'n voortdurende uitdaging. In die Kosmetiese Plaë Projek is vyf aparte studies aangepak, wat blaaspootjie, bladspringers, algemene Lepidoptera en voor-oes interalle (VOIs) vir plaagdoders gedek het. Die beplande bladspringer proewe kon nie voortgesit word nie omdat getalle te laag gebly het. 'n Kommersiële geformuleerde eksperimentele EPS het ongelukkig geen blaaspootjie of wilmuis beheer gegee nie. Dit is gewys dat imidacloprid reperfiksies van Lepidoptera plaë kan veroorsaak. VOIs vir fenpyroximate is vasgestel en die effek van 'n paar produksie en toedienings praktyke, soos bymiddels en skadu nete, op residu vlakke teen oestyd is bepaal.

Vir jare nou kom die versoek van die bedryf vir alternatiewe vir organofosfate vir beheer van wilmuis omdat die skrif op die muur al hoe duideliker word. Sulke alternatiewe is ondersoek met EPNs en inokulerende vrylatings van 'n *Anagyrus* sp. parasiet, albei met goeie belofte. Boonop het 'n lente bespuiting van 2,4-D op nawellemoene die persentasie oop nawelente verminder en dus ook wilmuis besmetting beduidend verlaag. Laastens het gammabestraling as 'n na-oes behandeling volwasse wilmuis wyfies doeltreffend gesteriliseer.

Ten spyte van die kritieke behoefte om van die fitosanitêre en kosmetiese plaë met chemiese stowwe te beheer, moet die bydrae van natuurlike vyande in sitrus IPM vermeerder word om verskeie belangrike plaë in somer en herfs te beheer. Dit raak al hoe meer belangrik soos wat toegelate chemiese residu-vlakke op vrugte teen oestyd verminder word. Miere ontwrig biologiese beheer en moet dus uit bome gehou word. In die Bio-beheer

Ontwrigting Projek het 'n nuwe mier lokaas, Saga, doeltreffend die bruinhuismier en die malmier beheer. Die lokaas sal binnekort kommersialiseer word.

Die twee plaë wat produksie beïnvloed en wat navorsings aandag gedurende die verslag tydperk ontvang het is wollerige witvlieg en vrugtesteek motte.

Gedurende die laaste navorsingsjaar het CRI entomoloë en verskeie ander entomoloë wat binne die program werk, aktief deelgeneem in plaaslike en internasionale wetenskaplike kongresse. Dit het die gehalte en relevansie van die navorsing wat uit dié navorsingsspan gekom het vir die internasionale wetenskaplike gemeenskap beklemtoon. Verder is 'n hele paar artikels in top internasionale wetenskaplike eweknie-resenseerde joernale asook in ons plaaslike vrugtejoernaal gepubliseer. CRI entomoloë het ook aktief deelgeneem in die oordraing van belangrike boodskappe wat uit hulle navorsing gekom het, aan die produsente gemeenskap. Hierdie is veral deur produsente studie groepe en Snykant publikasies gedoen. Ons navorsers sien weereens uit daarna om hulle resultate en ontdekkings by die twee-jaarlikse Sitrus Navorsings Simposium in Augustus 2012 aan te bied en na hulle interaksie met die bedryf wat hulle bedien.

### 3.2 PROJECT: FALSE CODLING MOTH

Project coordinator: Sean D Moore (CRI)

#### 3.2.1 Project summary

Although the FCM project has enjoyed the lion's share of the research funding allocated within the IPM Programme over the last several years, pressure for meaningful applied outcomes has never been greater than is the case currently. Concerns about potential future intensification of phytosanitary trade regulations pertaining to FCM make it imperative that we expedite new developments and research findings which can contribute towards improved control of FCM. This certainly is happening, as seen in the exciting outcomes from the FCM research project, many of which are new and innovative.

In total, 15 experiments (separate studies) were conducted within the project. Four of these studies focussed on post-harvest detection or control of FCM, something which has historically enjoyed little attention. One of these studies aimed to develop a technique for control of FCM in packed fruit through gamma irradiation (3.2.2). Through use of a sub-optimal dose of 40 Gy, it was established that insectary reared larvae were at least as radiotolerant as their feral counterparts. Insectary reared larvae will therefore be utilized in a Probit study, which will combine irradiation and cold-treatment.

Another post-harvest study aimed to look at amelioration of the cold treatment regime for FCM by using carbon dioxide fumigation (3.2.7). Combinations of 12 h fumigation with 60% CO<sub>2</sub> followed immediately by waxing and a 5 day cold treatment resulted in approximately 90% mortality. The resultant mortality was higher than the cold treatment alone. A treatment involving 24 h of 6% CO<sub>2</sub> at 4°C followed by recovery at 25°C for 2 h, cold exposure of 3 days at -1°C, 25°C for 2 h, then 5 days at -1°C resulted in 100% mortality.

The purpose of the final two post-harvest trials was detection, rather than control. In the first (3.2.9), FCM infestation could be detected one day after infestation of Satsumas using X-ray tomography. Microfocus radiography detected 71% of infestations after 13 days, 70% after 10 days, 50% after 3 days and 33% 1 day after infestation. However, micro-focus tomography detected 100% of infestations. Imaging algorithms are being developed to classify  $\mu$ CT images as clean or infested. The objectives of the second detection study are to develop a volatile detection system and to use this system to investigate differences in volatile emission between infested and healthy fruit (3.2.13). The application of an SPME procedure and the optimization of this method for detection of possible volatiles present in the headspace of intact fruit were evaluated.

Of the remaining 11 studies, seven looked at various aspects of microbial control of FCM: four on entomopathogenic nematodes (EPN), two virus studies and one on entomopathogenic fungi (EPF). An Imported commercial formulation of *Heterorhabditis bacteriophora* showed similar efficacy against FCM larvae to an endemic isolate of the same EPN species (3.2.4). A commercial formulation of *Steinernema feltiae*, once recycled through wax moth larvae, gave 100% control of FCM larvae at 25°C and 90% control at 14°C. The following two EPN studies looked at the *in vivo* production of *H. zealandica* and *H. bacteriophora* (3.2.10) and *in vitro* production of *H. zealandica* (3.2.11). Wax moth larvae proved to be the most productive host for EPN production, followed by mealworm larvae. Comparable production of nematodes was found with frozen mealworm larvae. *Steinernema yirgalemense* was successfully cultured in an adapted liquid medium. It was found that *S. yirgalemense* produced five times more infective juveniles (IJs) *in vitro* in liquid culture than the number of *H. zealandica* produced. The final EPN experiment consisted of large-scale field trials with *H.*

*bacteriophora* for control of FCM, fruit flies and thrips (3.2.16). In one trial FCM infestation was reduced by between 55% and 81% relative to control orchards. In another trial, naturally occurring *H. zealandica* was shown to reduce FCM infestation by 59% and fruit fly numbers in traps were reduced to 21%.

The first virus trial showed that efficacy with powdered Cryptogran and powdered molasses was comparable with that of the registered products (3.2.3). Results once again showed the importance of adding molasses to virus applications, unless Dithane is added. In the second virus study, five new CrleGV-SA isolates were recovered (3.2.6). These new isolates and the two commercial isolates were shown to be genetically distinct. Some significant difference in virulence between isolates was also shown. The importance of this study is that new virus isolates are available in the event that any FCM population develops resistance to the commercial products.

The final microbial control study investigated EPFs for the control of soil-borne life stages of FCM (3.2.14). Three fungal isolates showed the greatest potential for control of FCM. These three isolates also performed more effectively than two commercial isolates tested. An increase in mortality was observed with an increase in exposure time to the fungus. Fungal persistence trials in a citrus orchard are underway.

One experiment investigated chemical control of FCM using Coragen (3.2.8). Efficacy of Coragen ranged between 31% and 64% and was comparable with that of existing registered products. Differences in efficacy of Coragen may be related to a combination of the extent of pest pressure and adequacy of spray volume per tree size, influenced by the registered limitation of a maximum of 8500 L of spray per ha.

A mating disruption trial moved from the field back to the laboratory in order to investigate some disparity in understanding of efficacy from field trials (3.2.5). Although rate of pheromone release at constant temperature was more consistent with an experimental dispenser, the amount of pheromone released was 10 times higher from Isomate dispensers. Future trials will aim to compare the mating disruption effect of different combinations of pheromone isomers.

The final trial which addressed pre-harvest control of FCM, investigated whether there were differences in attractiveness of virgin female FCM from different regions to sterile males from Citrusdal (3.2.12). In olfactometer trials, adult males from a Citrusdal culture preferred Citrusdal females in all but one case. The exception was when given the option of a Marble Hall female. However, viable egg counts from Citrusdal females were significantly higher than for females from any of the other cultures. In field trials, significantly more sterile males were attracted to traps loaded with females from Marble Hall, closely followed by Addo, thus confirming the olfactometer trial results.

The final experiment in this project looked at morphological and molecular identification of moths associated with citrus (3.2.15). Initial results from cross-mating trials, using FCM from different regions, showed that FCM from these different populations were able to mate and produce viable offspring. Variation in genitalia can be used for species distinction and morphometrics to quantify differences in FCM males.

## **Projekopsomming**

Alhoewel die VKM projek die meerderheid van die navorsings bevondings binne die IPM Program oor die laaste paar jaar geniet het, die druk vir waardevolle praktiese uitkomstes was nooit groter as wat nou die geval is nie. Soos hierdie verslag geskryf word, is die Europese Unie besig met 'n plaag risiko analise vir VKM. Al wat duidelik is op hierdie stadium is dat EU regulasies vir die invoer van VKM-vatbare vars produkte van Afrika strenger gaan word. Dit is daarom uiters belangrik dat nuwe ontwikkelinge en navorsings resultate wat tot verbeterde beheer van VKM kan bydra, so spoedig as moontlik gegeneer word. Hierdie gebeur beslis, soos getuig deur die opwindende uitkoms van die VKM navorsings projek, waarvan baie nuut en inoverend is.

Al te saam is 15 eksperimente (aparte studies) binne die projek uitgevoer. Vier van hierdie studies het op na-oes opsporing of beheer van VKM gefokus, iets wat geskiedkundig min aandag geniet het. Die doel van een van hierdie studies was om 'n tegniek te ontwikkel vir beheer van VKM in gepakte vrugte met gebruik van gamma bestraling (3.2.2). 'n Suboptimale dosis van 40 Gy is gebruik en daar is bepaal dat insektariumgeteelde larwes minstens net so radioweerstandig as hul boordversamelde eweknieë is. Eersgenoemde sal derhalwe in 'n Probit-studie gebruik word wat bestraling en koue behandeling kombineer.

Nog 'n na-oes studie het gekyk na vermindering aan die na-oes koue behandeling behoeftes vir VKM met die gebruik van koolsuurstof (3.2.7). Kombinasies van 12 h beroking met 60% CO<sub>2</sub> onmiddelik gevolg deur 'n wasbehandeling en 'n 5 dae koue behandeling het om en by 90% mortaliteit veroorsaak. Hierdie mortaliteit was

hoër as die koue behandeling op sy eie. 'n Behandeling wat behels 24 h van 6% CO<sub>2</sub> beroking teen 4°C gevolg deur 'n herstel periode teen 25°C vir 2 h, 3 dae se blootstelling aan -1°C, 'n herstel periode van 25°C vir 2 h, waarna 5 dae se blootstelling teen -1°C gevolg het, het 100% mortaliteit veroorsaak.

Die doel van die laaste twee na-oes proewe was opsporing eerder as beheer. In die eerste (3.2.9), kon VKM besmetting in Satsumas een dag na besmetting plaasgevind het deur gebruik van X-straal tomografie opgespoor word. Mikrofokus radiografie het 71% van besmettings opgespoor 13 dae na besmetting plaasgevind het, 70% na 10 dae, 50% na 3 dae en 33% na een dag. Mikrofokus tomografie het egter 100% van besmettings opgetel. Beeld algoritmes word ontwikkel om  $\mu$ CT beelde as besmet of gesond te klassifiseer. Die doelwitte van die tweede opsporing studie is 'n vlugtige opsporingstelsel vir gebruik op sitrus vrugte te ontwikkel, en om hierdie stelsel te gebruik om verskille in vlugtige uitstorting tussen besmette en gesonde vrugte te ondersoek (3.2.13). Die toepassing van 'n SPME prosedure en die optimalisering van hierdie metode vir die opsporing van moonlike vlugtige verbindings wat teenwoordig is in lemoene is ondersoek.

Van die oorblywende 11 studies, het sewe verskeie fasette van mikrobiële beheer van VKM ondersoek: vier op entomopatogeniese nematodes (EPN), twee virus studies en een op entomopatogeniese swamme (EPS). 'n Ingevoerde kommersiële formulering van *Heterorhabditis bacteriophora* het gelyksoortige werking teen VKM larwes getoon as 'n endemiese isolaat van dieselfde EPN spesie (3.2.4). 'n Kommersiële formulering van *Steinernema feltiae*, wat deur wasmot larwes geteel is, het 100% beheer van VKM teen 25°C gegee en 90% beheer teen 14°C. Die volgende twee EPN studies het gekyk na *in vivo* produksie van *H. zealandica* en *H. bacteriophora* (3.2.10) en *in vitro* produksie van *H. zealandica* (3.2.11). Wasmot larwes was die beste gasheer vir EPN produksie, gevolg deur meelwurm larwes. Gelyksoortige EPN produksie is met gevriesde meelwurm larwes gekry. *Steinernema yirgalemense* is suksesvol aangeteel in 'n aangepaste vloeistof medium. Daar is ook gevind dat *S. yirgalemense* vyf keer meer infektiewe larwes produseer in vergelyking met *H. zealandica*. Die finale EPN proef behels grootskaalse veldproewe met *H. bacteriophora* vir beheer van VKM, vrugtevlieë en blaaspootjie (3.2.16). In een van die proewe is VKM besmetting met tussen 55% en 81% verminder in vergelyking met kontrole boorde. In nog 'n proef is dit gewys dat die natuurlike voorkoms van *H. zealandica* VKM besmetting met 59% verminder het en vrugtevlieg lokval vangstes is af tot 21%.

Die eerste virus proef het gewys dat werking met gedroogde Cryptogran en gedroogde molasse gelyksoortig was aan die werking met die geregistreerde produkte (3.2.3). Resultate het weereens die belangrikheid van molasse vir die werking van virus toedienings gewys, behalwe waar Dithane bygevoeg was. In die tweede virus studie is vyf nuwe CrleGV-SA isolate gevind (3.2.6). Dit is gewys dat hierdie nuwe isolate en die twee kommersiële isolate geneties van mekaar verskil het. Sekere beduidende verskille in virulensie tussen isolate is ook gewys. Die belangrikheid van hierdie studie is dat nuwe virus isolate beskikbaar is as enige VKM populasie weerstandbiedendheid teen die kommersiële produkte ontwikkel.

Die finale mikrobiële beheer studie het EPSe ondersoek vir beheer van grondgedraagde lewensstadia van VKM (3.2.14). Drie swam isolate het die grootste belofte getoon vir beheer van VKM. Hierdie drie isolate was ook meer doeltreffend as twee kommersiële isolate wat getoets is. 'n Vermeerdering in mortaliteit is opgelet met 'n verlenging in tydsduur van blootstelling aan die swam. Swam nawerkings proewe in 'n sitrusboord is tans aan die gang.

Net een eksperiment het chemiese beheer van VKM ondersoek, met die gebruik van Coragen (3.2.8). Werking van Coragen was tussen 31% en 64% en was gelyksoortig met die werking van bestaande geregistreerde produkte. Verskille in die werking van Coragen kan aan verskille in plaagdruk en geskiktheid van spuit volume vir boomgrootte toegeskryf word. Dit word beïnvloed deur die geregistreerde beperking van 8500 L per hektaar per bespuiting.

'n Paringsontwrigting proef het geskyf van die veld terug na die laboratorium om onduidelikhede rondom begrip van ekketiwigheid van veldproewe te ondersoek (3.2.5). Alhoewel feromoon vrylatingskoers teen konstante temperatuur meer konstant met 'n eksperimentele vrylater was, was die hoeveelheid feromoon wat vrygelaat is 10 maal hoër van Isomate vrylaters. In die toekoms sal proewe uitgevoer word om die paringsontwrigting effek van verskillende kombinasies van feromoon-isomere te vergelyk.

Die finale proef wat voor-oes beheer van VKM toegesprek het, het moontlike verskille in aanloklikheid van ongepaarde wyfies VKM van verskillende streke vir steriele mannetjies van Citrusdal ondersoek (3.2.12). In olfaktometer proewe, het volwasse mannetjies van 'n Citrusdal kultuur, Citrusdal wyfies in alle gevalle behalwe een verkies. Die uitsondering was toe hulle die opsie van 'n Marble Hall wyfie gegee is. Lewensvatbare eiertellings van Citrusdal wyfies was betekenisvol hoër as dié vir wyfies van enige van die ander kulture. In

boordproewe is betekenisvol meer steriele mannetjies gelok na lokvalle wat met wyfies van Marble Hall gelaai is, baie naby gevolg deur Addo. Dus het hierdie resultate die olfaktometer proef resultate ondersteun

Die finale eksperiment in hierdie projek het gekyk na morfologiese en molekuleêre identifikasie van motte wat met sitrus gëassosieer is (3.2.15). Aanvanklike resultate van kruisparingsproewe met VKM van verskillende streke het aangedui dat verskillende bevolkings tog in staat is om te paar en om lewensvatbare nageslag in daaropvolgende generasies te lewer. Variasie in genitalia kan vir spesie onderskeid gebruik word en morfometrie kan aangewend word om verskille in VKM mannetjies te kwantifiseer.

### 3.2.2 FINAL REPORT: Development of a technique for control of false codling moth larvae in packed fruit using gamma irradiation

Experiment 719 (2003-2011) by J H and M Hofmeyr (CRI)

#### Opsomming

Derde, 4<sup>de</sup> en 5<sup>de</sup> (volgroeide) instar valskodlingmotlarwes is vantevore in twee proewe met gammastralingdosisse van 200-400 Gy behandel om die radioweerstandigste instar te bepaal (CRI Jaarverslag vir 2010-11). Die dosisse was te hoog en die ontwikkeling van die larwes tot lewensvatbare papies is amper heeltemal verhoed. Dit was derhalwe moeilik om die larwes se relatiewe weerstandigheid vir bestraling vas te stel. Dié studie is met 'n derde proef afgesluit waarin 1<sup>ste</sup>, 3<sup>de</sup> en 5<sup>de</sup> instar larwes met 50 Gy straling behandel was. Deur maatstawwe te gebruik soos hul vermoë om in papies en motte te ontwikkel, voort te plant en te kan vlieg, is bewys dat 5<sup>de</sup> instar larwes die radioweerstandigste is en derhalwe in verdere navorsing gebruik kan word.

Belowende navorsingsresultate moet op Probit-9 vlak bekragtig word. Om insektariumgeteelde larwes te gebruik in plaas van natuurlik-besmette lemoene in boorde te pluk, is 'n proef uitgevoer om die relatiewe radioweerstandigheid van die twee larwebronne vas te stel. 'n Suboptimale dosis van 40 Gy is gebruik en daar is bepaal dat insektariumgeteelde larwes minstens net so radioweerstandig as hul boordversamelde eweknieë is. Eersgenoemde sal derhalwe in die Probit-studie gebruik word.

#### Summary

In two previous experiments (CRI Annual report for 2010-11), 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> (mature) instar false codling moth larvae were treated with gamma radiation doses ranging from 200-400 Gy to determine the most radiotolerant instar. The doses were too high and almost totally prevented development into viable pupae. It was consequently difficult to clearly resolve the larvae's relative tolerance to irradiation. The investigation was concluded with a third experiment treating 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> instar larvae with 50 Gy radiation. Using their ability to develop into pupae and moths, to reproduce and to fly as criteria, it was demonstrated that 5<sup>th</sup> instar larvae are most radiotolerant and should therefore be used in all further studies.

Promising research results have to be validated at Probit-9 level. To enable the use of insectary reared larvae instead of oranges naturally infested with larvae in orchards, an experiment was conducted to compare the relative radiotolerance of the two larval sources. A sub-optimal dose of 40 Gy was used and it was established that insectary-reared larvae were at least as radiotolerant as their feral counterparts. Insectary reared larvae will therefore be utilized in the Probit study.

#### General

Two studies with divergent aims and methodologies were conducted. They are consequently discussed separately.

#### Study A: Radiotolerance of different larval instars

##### Introduction

Larval populations in oranges are largely unsynchronized and different instars occur coincidentally during the season. It was consequently important to establish the effect of irradiation on larvae of various ages to ensure that research was conducted with the most radiotolerant instar. The first two studies to investigate this aspect were conducted in 2004 using gamma doses in the range 200-400 Gy. The results from these studies are included to allow a more comprehensive overview.

## Materials and methods

Three experiments (nos. 1-3) were conducted. Three age groups of larvae were used in each experiment, viz. 'small', 'medium' and 'large'. Eight replicates of one rearing jar each per larval age group were used per irradiation treatment in Exps. 1 and 2, and 4 replicates per irradiation treatment in Exp. 3. Similar numbers of rearing jars were used for control purposes. Forty to 50 larvae in different experiments were removed at random from a single additional, representative jar per age group to determine the instar composition with the aid of head capsule measurements.

In Exps. 1 and 2 the ability of treated larvae to develop into pupae and moths was investigated. The rearing jars with larvae were irradiated and incubated at 26°C. All pupae were collected per replicate with the aid of corrugated cardboard as pupation substrate, counted and incubated as before for moth eclosion. All moths were collected and counted.

The following factors were examined in Exp. 3:

(i) *Acute mortality*: It was not possible to count the larvae present in the diet of the rearing jars. To assess larval mortality caused by radiation the larvae in the treated and untreated rearing jars were therefore incubated until they pupated. All pupae from the available replicates were counted and then combined into one batch per treatment. From each batch 160 pupae were collected at random and placed individually into glass vials, 50 mm x 15 mm, with foam rubber stoppers. All moths were sexed as they eclosed and dead pupae were recorded.

(ii) *Reproductive potential*: The first 10 pairs of moths to eclose in the above mentioned glass vials were used to assess reproductive potential: A newly eclosed female and male were paired for egg laying in each of ten 100 ml plastic oviposition cages. Only inbreeding was assessed, i.e. normal untreated ♀ x normal untreated ♂ (N♀xN♂), and treated ♀ x treated ♂ (T♀xT♂). Ovipositing was allowed for 7 days after which most moths were dead. The females were collected, dissected and examined for the presence of spermatophores in the *bursa copulatrix* to confirm successful mating. To prevent the recording of unhatched eggs due to delayed hatching caused by irradiation, treated eggs were incubated for 7 days after all untreated, viable eggs in the control had hatched. All hatched and dead eggs per oviposition cage were recorded.

(iii) All moths eclosing from the remainder of the 160 pupae were used for flight tests. Tests were conducted out-of-doors. Moths were released individually from a height of 2.5 m above ground level. Moths that flew strongly and comparably to untreated control moths were considered to be flight capable. Moths unable to gain height and invariably descending involuntarily, or falling directly to the ground, were regarded as unable to fly.

## Results and discussion

The instar distribution in Exps. 1 and 2 was unsatisfactory as larvae in the 3 categories were older than originally estimated (Table 3.2.2.1). The smaller larvae were predominantly 4<sup>th</sup> instar, and the medium and large larvae were mostly 5<sup>th</sup> instar. The medium larvae were consequently eliminated from the test range and the small larvae were moved up one category. The medium category in Exp. 2 also consisted of mainly large (5<sup>th</sup> instar) larvae; a replacement source containing a higher percentage of 4<sup>th</sup> instar larvae was unavailable at that time and the study was continued with the available stock. The instar distribution in Exp. 3 was satisfactory.

**Table 3.2.2.1.** Instar distribution of larvae according to head capsule measurements.

Exp. no.	Larval size in rearing jars		
	Small	Medium	Large
1	-	97% 4 <sup>th</sup> instar; 3% 5 <sup>th</sup> instar	5% 4 <sup>th</sup> instar; 95% 5 <sup>th</sup> instar
2	80% 3 <sup>rd</sup> instar; 20% 4 <sup>th</sup> instar	35% 4 <sup>th</sup> instar; 65% 5 <sup>th</sup> instar	5% 4 <sup>th</sup> instar; 95% 5 <sup>th</sup> instar
3	100% 1 <sup>st</sup> instar	100% 3 <sup>rd</sup> instar	10% 4 <sup>th</sup> instar; 90% 5 <sup>th</sup> instar

(a) **Experiment 1**: A small number of treated 4<sup>th</sup> and 5<sup>th</sup> instar larvae developed into pupae in Exp. 1. A dose effect was noticeable – a decreasing number of pupae developed with increasing dose (Table 3.2.2.2). More pupae developed from treated 5<sup>th</sup> instar larvae than from 4<sup>th</sup> instar, indicative of an increased radiotolerance in the older larvae.

(b) **Experiment 2**: No larvae survived a similar dose range although a small number developed into malformed, non-viable hybrids displaying features from both larvae and pupae (Table 3.2.2.2).

**Table 3.2.2.2.** Effect of gamma treatment to larvae on sustained development into pupae and moths.

Experiment	Treatment dose (Gy)	Mean no. of pupae and moths developing from treated larvae					
		3 <sup>rd</sup> instar larvae		4 <sup>th</sup> instar larvae		5 <sup>th</sup> instar larvae	
		Pupae	Moths	Pupae	Moths	Pupae	Moths
1	0	-	-	140,5	133,0	134,1	132,6
	200	-	-	0,4	0,0	5,1	0,0
	250	-	-	0,0	0,0	0,3	0,0
	300	-	-	0,0	0,0	0,1	0,0
	350-400	-	-	0,0	0,0	0,0	0,0
2	0	104,3	97,8	173,6	165,3	125,0	123,3
	200-400	0,0	0,0	0,0	0,0	0,0	0,0

(c) **Experiment 3:** Due to the almost total mortality achieved by the high doses involved in Exps. 1 and 2 the difference in radiotolerance between the various instars could not be adequately assessed. It was also later established that these doses would in any case be far too high for commercial use due to fruit quality constraints. A third experiment was therefore conducted using a single dose of 50 Gy. Results were as follows (Tables 3.2.2.3 and 3.2.2.4):

- (i) *Acute mortality:* The mean number of pupae that developed from larvae treated in the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> instar controls varied from 688, 636 to 737 per jar respectively. From similar numbers of treated larval instars, 80.1%, 82.3% and 98.9% developed into pupae, indicating a probable increase in radiotolerance as the larvae increased in age from 1<sup>st</sup> to 5<sup>th</sup> instar (Table 3.2.2.3). Pupal mortality also decreased with an increase in larval age. Pupal malformation fluctuated from 2.6 to 3.0% in the three instars, compared to their respective controls at 0.1 to 0.4%.
- (ii) *Eclosion of moths:* Respectively 84%, 90.1% and 99.3% of the pupae developed into moths from 1<sup>st</sup> to 5<sup>th</sup> instar larvae (Table 3.2.2.3) again revealing an increased radiotolerance as the larvae matured. Due to the relatively low dose applied no obviously malformed moths were noticed. The gender ratio of all instars was affected. Although no consistent trend occurred between the instars, there was a preponderance of males throughout and varied from 65.2% to 71.4% to 68.8% between the treatments. It is highly improbable that radiation would have caused a gender change in the larvae and it is more than likely that the treatment was simply more lethal to female larvae than males. The decreased radiotolerance of female FCM compared to the males has been recorded before (Bloem *et al.*, 2003).

**Table 3.2.2.3.** Effect of 50 Gy gamma treated 1<sup>st</sup> to 5<sup>th</sup> instar larvae on the acute mortality of larvae as expressed by the production of pupae and moths.

Larval instar treated	%*		
	pupae produced	pupal mortality	moth eclosion
1 <sup>st</sup>	80,1	16,0	84,0
3 <sup>rd</sup>	82,3	9,9	90,1
5 <sup>th</sup>	98,9	0,7	99,3

\*Calculations for gamma treatments relative to respective controls.

(iii) *Reproductive potential*

- There was a progressive increase in the moths' mating ability as the treated larvae matured from 1<sup>st</sup> to 5<sup>th</sup> instar, which is commensurate with an increased radiotolerance (Table 3.2.2.4).
- Compared to similar eggs deposited by the moths from treated larvae, the number of dead, partially developed eggs in the controls is typical and can be attributed to the so-called 'egg clump' factor: In all past and present studies with untreated eggs on wax paper and fruit, natural egg mortality has consistently been greater on egg sheets than on fruit – on average 15-20% compared to 3-8%. This is ascribed to the fact that eggs are often deposited in clumps on wax paper or any other smooth surface, with many eggs overlapping each other partially or totally. The mortality of eggs in these clumps is always greater than that of eggs deposited either singly on wax paper or on oranges where they are typically laid discretely in the small indentations texturing the fruit rind.
- It had been noticed in many mating experiments prior to this project (Hofmeyr, unpub.) that a highly variable number of undeveloped eggs, from a very few to several hundred per female, (colloquially

called 'dud' eggs), were often produced by females. The production of these eggs was largely unpredictable and unrelated to any specific treatment, except that the phenomenon would probably manifest itself more often with moths that had developed from irradiated eggs or larvae. The egg shells were of poor quality and often deteriorated and shrivelled much faster than dead, partially developed eggs. Dud eggs can be recorded under the following circumstances:

- Unmated females, treated or untreated, deposit either no, or dud eggs.
- Mated, untreated females ( $N_{\text{♀}} \times N_{\text{♂}}$ ) occasionally deposit dud eggs, or on rare occasions, a combination of viable and dud eggs.
- Mated, treated females ( $T_{\text{♀}} \times T_{\text{♂}}$ ) often produce dud eggs, frequently subject to dose strength.

On average, far fewer eggs were produced in the gamma treatments compared to the respective controls. Erratic means of respectively 9.5, 66.4 and 24.0 eggs per female were produced by moths from the treated 1<sup>st</sup> to 5<sup>th</sup> instar larvae (Table 3.2.2.4). This was inconsistent with the expected trend, which should have been a progressive increase in the mean number of eggs due to increasing radiotolerance. The distorted data were the result of a few mated and unmated females from treated larvae of all age groups that produced large numbers of dud eggs. The dud eggs were therefore ignored in the calculations for fertility, which reflected the expected increase in radiotolerance.

- The progressive development of radiotolerance was evident in a gradual shift in the eggs' viability – from 100% dud eggs (1<sup>st</sup> instar) to the partial development of a few eggs (3<sup>rd</sup> instar), and finally to fully developed eggs, some of which remained unhatched and the rest yielding live larvae (5<sup>th</sup> instar) (Table 3.2.2.4, blocked in red). The fecundity of the moths therefore improved as the radiotolerance of the treated larvae increased from 1<sup>st</sup> to 3<sup>rd</sup> to 5<sup>th</sup> instar.

**Table 3.2.2.4.** Effect of 50 Gy gamma treated 1<sup>st</sup> to 5<sup>th</sup> instar larvae on the adults' fertility and fecundity (Exp. 3).

Treatment dose (Gy)	Larval instar	No. of mated ♀♀ (max. 10)	Eggs produced by moths developed from treated larvae				
			Mean no. per ♀	% dead: Undeveloped	% dead: Partially developed	% dead: Larvae developed	% hatched
0	1 <sup>st</sup>	10	446,1	0,0	11,2	2,5	86,3
	3 <sup>rd</sup>	10	458,2	0,0	9,7	2,4	87,9
	5 <sup>th</sup>	9	482,3	2,8	9,9	3,6	83,7
50	1 <sup>st</sup>	3	0,0	100,0	0,0	0,0	0,0
	3 <sup>rd</sup>	6	1,3	98,0	2,0	0,0	0,0
	5 <sup>th</sup>	9	17,2	27,1	0,8	6,3	65,8

- (iv) *Flight test:* The consistent trends substantiating increased radiotolerance with larval age, viz. pupal development, moth eclosion, and reproductive potential, were supported when comparing the ability of the moths to fly. The flight capability of moths from treated larvae increased proportionately from 1<sup>st</sup> (12.6%) to 3<sup>rd</sup> (29.8%) to 5<sup>th</sup> instar (65.9%).

## Conclusion

The results from Exps. 1 and 3 presented sufficient evidence that mature larvae were most radiotolerant and the use of 5<sup>th</sup> instar larvae in further studies was justified.

## Study B: The relative radiotolerance of larval populations developing in artificial diet and naturally infested oranges

### Introduction

For practical reasons the basic studies reported on in previous annual reports were conducted with FCM larvae mass-reared in artificial diet. It was possible that the relative radiotolerance of such populations could differ from their feral counterparts developing naturally in oranges. This could hypothetically be due to the physical properties of the diet involved, ambient conditions and/or inherited disparities in the morphological make-up of the 2 moth sources. This project has to be concluded with a Probit-9 evaluation and the most radiotolerant FCM source needs to be utilized to ensure ultimate treatment security. Consequently, the source of the larvae required for the study had to be resolved. Larvae could be obtained from:

- (a) **Artificial rearing on synthetic diet:** The procurement, number and age of the larvae are easily and effectively managed.

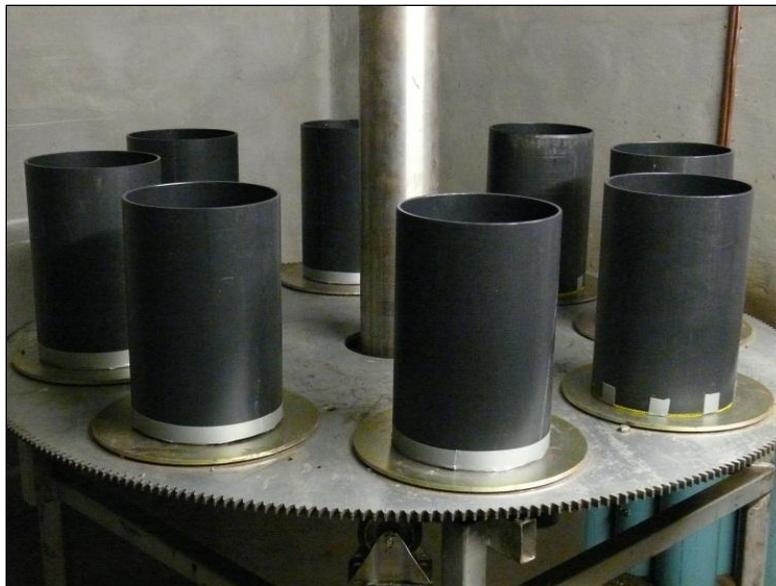
(b) **Naturally infested oranges:** It is conceivable that up to 300 000 potentially infested oranges will have to be collected in an attempt to obtain the approximately 94 000 mature larvae required for the study. Furthermore, this number of insects preferably needs to be evaluated in 3 consecutive replicates, which adds to the challenge. Factors such as availability, time restraints, degree of fruit infestation and instar composition are difficult or impossible to manipulate. From a practical point of view this source can't be exploited with any degree of confidence or reliability.

A comparative study was therefore conducted to establish the relative radiotolerance of FCM larvae in artificial diet and naturally-infested oranges.

### Materials and methods

A sub-optimal dose of 40 Gy was used to ensure that irradiated larvae develop into partially sterile moths that would produce sufficient viable eggs for comparative purposes. The following sources were exploited in the study:

(a) **Oranges:** Three consignments of 300 navel oranges each, with symptoms of possible FCM infestation were collected consecutively with 2-3 week intervals from orchards on each of 3 farms in the Citrusdal region, Western Cape. A secondary selection was carried out in the laboratory to discard fruit subsequently regarded to be uninfested. Twenty oranges were collected at random from each consignment and cut open to determine the infestation level. Head capsules of the retrieved larvae were measured to establish the instar distribution per consignment. Fifty oranges per consignment were retained as controls. The balance of the fruit, respectively 133, 156 and 98 oranges per consignment, was irradiated. Depending on fruit size, 12-15 oranges were placed into each of 8 PVC irradiation containers before being treated (Fig. 3.2.2.1).



**Fig. 3.2.2.1.** Containers used to promote radiation build-up when treating oranges naturally infested with FCM larvae.

After treatment the oranges were placed individually into 500 ml plastic containers and the pupae were collected in 20 mm lengths of plastic drinking straw supplied as pupation sites. As many matings as possible were prepared for mating and oviposition studies by collecting pairs of females and males as they eclosed. Means of 8.3 and 22 mating pairs were accumulated respectively per consignment in the control and gamma treatments. Many moths could not be used due to protracted eclosion patterns that prevented collection of mating pairs, or gender ratios which were biased either pro-female or pro-male.

(b) **Rearing jars:** For each consignment of oranges 2 batches of 6 rearing jars with larvae were used for control and irradiation treatments respectively. It was considered inevitable that the composition of instars encountered in oranges would differ from the more synchronized larval populations available from artificial rearing. Each of the batches were consequently subdivided into 2 sub-batches of 4<sup>th</sup> and 5<sup>th</sup> instar larvae respectively in an attempt to more closely simulate infestations in the oranges. Each sub-batch consisted of 3 rearing jars. This expanded instar distribution was intended to enable the interpretation of possible anomalies in

the jar/orange comparisons. Larval head capsule measurements from 30 larvae were conducted from an additional representative rearing jar per larval age group to establish the instar selection.

The pupae that developed from each sub-batch per age group treatment were combined. From each of these, 160 pupae were collected at random and placed individually into glass vials [consult Study A: Materials and Methods – (i) *Acute mortality*]. Ten pairs of moths were collected for mating tests and oviposition. The procedure for oviposition studies was described above [Study A: Materials and methods – (ii) *Reproductive potential*].

### Results and discussion

(a) **Fruit infestation:** The sub-sample of 20 fruit collected for assessment were respectively 60% (Consignment 1) and 50% (Consignments 2 and 3) infested with FCM larvae. This relatively low degree of infestation is disappointing – especially after two attempts to remove uninfested fruit – and reveals the difficulty inherent to a large scale study using oranges.

(b) **Instar composition:** Head capsule measurements showed that the instar composition of larvae in the rearing jars was representative of the required age groups (Figs. 3.2.2.2 and 3.2.2.3).

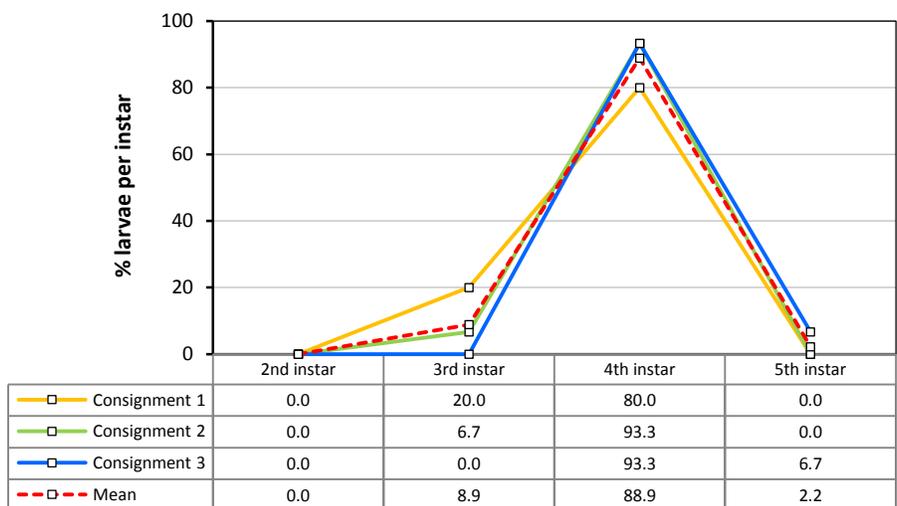


Fig. 3.2.2.2. Larval instar composition in rearing jars selected for 4<sup>th</sup> instar.

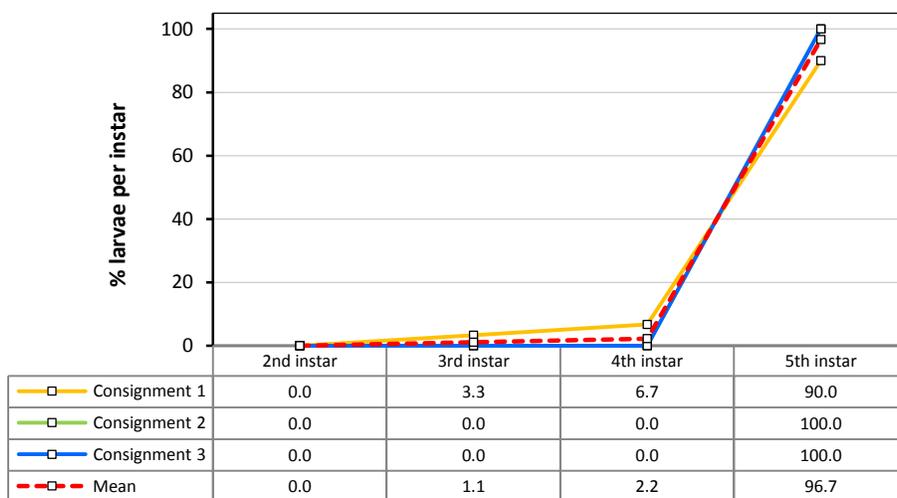
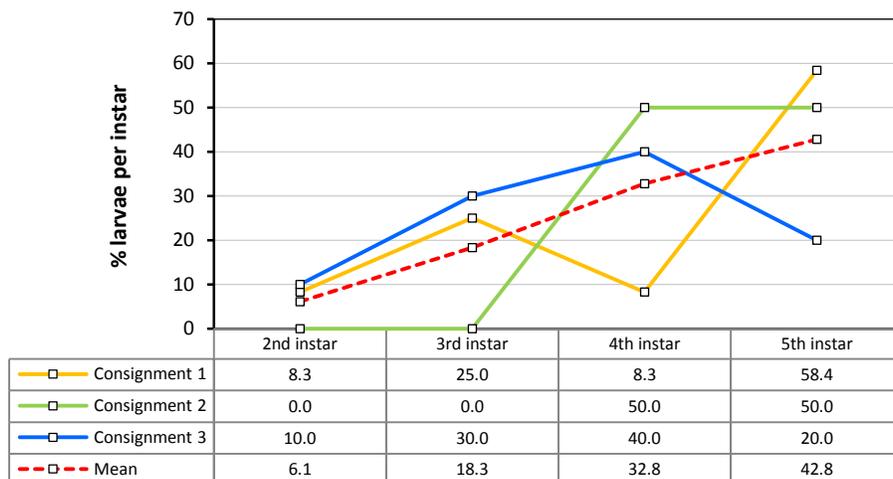


Fig. 3.2.2.3. Larval instar composition in rearing jars selected for 5<sup>th</sup> instar. The instar distribution in batches 2 and 3 was identical.

The instar composition was variable in and between the 3 consignments of oranges, comprising 0-40% of 2<sup>nd</sup> plus 3<sup>rd</sup> instars and 60-100% of 4<sup>th</sup> plus 5<sup>th</sup> instars respectively (Fig. 3.2.2.4). This disparate distribution was caused by two natural factors, viz.

- The oranges were progressively and irregularly infested by feral FCM often occurring in poorly defined generations throughout the citrus season, and
- a natural tendency for similarly aged individuals to develop at inherently different rates.

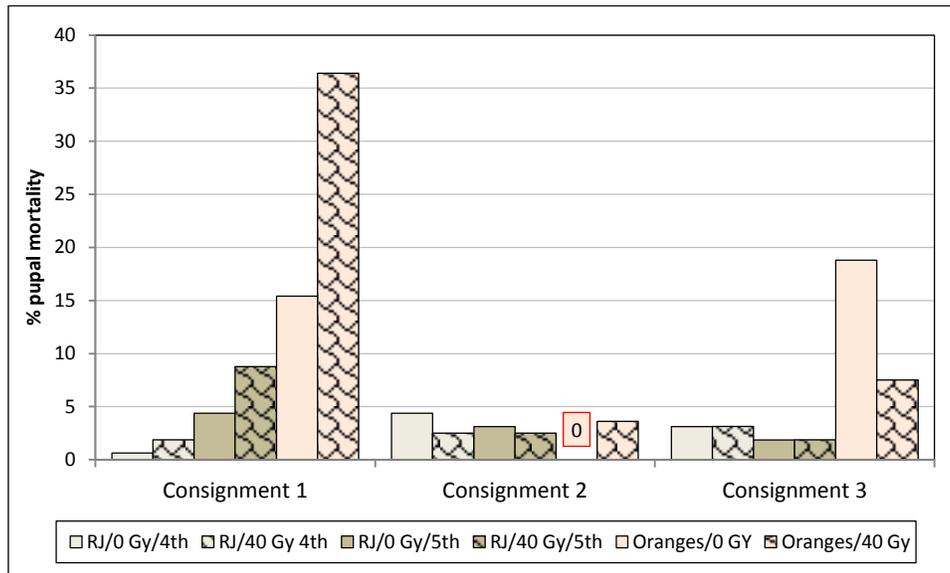


**Fig. 3.2.2.4.** Larval instar composition in 3 consignments of naturally infested oranges collected on different farms.

(c) **Acute mortality:** Respectively 2.4%, 12.8% and 9.2% fewer pupae were collected from treated than untreated oranges in the three consignments, indicating mortality of the treated larvae. This represents a relatively minor treatment effect of little practical value. The number of pupae collected from the rearing jars was not recorded. However, in an earlier experiment 5<sup>th</sup> instar larvae were treated in rearing jars with 40 Gy which reduced the number of pupae relative to the control by 9.1% (CRI Annual Report for 2010-11, Table 3.2.2.10). If the data in the 2 experiments are compared, the reduction in pupal numbers is on average 81% in oranges and 91% in rearing jars, which does not indicate a difference in radiotolerance between the larval sources.

No firm trends could be observed in pupal mortality due to data variability between consignments, for example (Fig. 3.2.2.5):

- The mortality of pupae from untreated 4<sup>th</sup> and 5<sup>th</sup> instar larvae from rearing jars in Consignment 1 should have been very similar (compare the evenly coloured light and dark brown columns).
- Pupal mortality of treated 5<sup>th</sup> instar larvae was higher than that of the 4<sup>th</sup> instars in Consignment 1, similar in Consignment 2 and less in Consignment 3 (compare the tiled light and dark brown columns in each consignment). For a difference in radiotolerance to be demonstrated for this particular attribute mortality should have been consistently less for the 5<sup>th</sup> instar larvae.
- Pupal mortality from treated larvae in oranges was higher than that of the corresponding control treatments in Consignments 1 and 2, but lower in Consignment 3 (compare the evenly coloured and tiled orange columns).

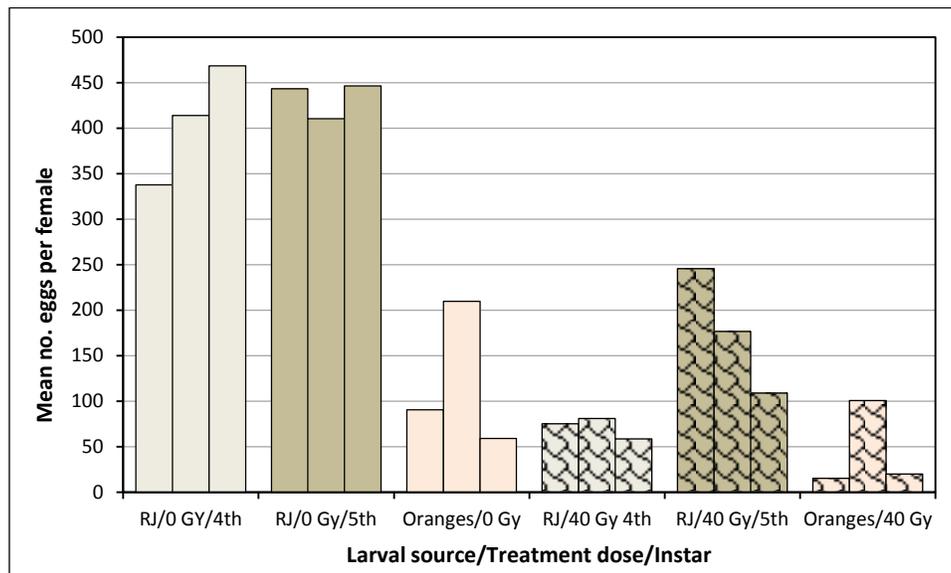


**Fig. 3.2.2.5.** Pupal mortality caused by 40 Gy irradiation indicating considerable variability between treatments and consignments.

(d) **Reproductive potential**

(i) *Fertility (Fig. 3.2.2.6)*

- *Unirradiated treatments:* More eggs were produced by moths developing from larvae in rearing jars than those from oranges (compare the evenly coloured light and dark brown columns with the evenly coloured orange columns). This difference suggests that feral FCM are less fertile than artificially reared moths. It is possible, although yet empirically untested for FCM, that in unnatural settings adaptation to, conceivably, less varying ambient conditions and/or a more nutritious diet, could enhance the reproductive capabilities of insectary moths. It is an accepted practical problem that feral FCM collected for incorporation into an existing insectary colony has to be reared through several generations before they adapt/acclimatize and their reproductive capabilities develop sufficiently to be introduced into a formal rearing environment (Honiball, Moore, Stotter, *pers. com.*).
- *Unirradiated versus irradiated treatments:* Moths from untreated larvae in rearing jars and oranges were more fertile than their treated counterparts (compare the evenly coloured brown and orange columns with their respective tiled counterparts). This is a typical consequence of radiation treatment (Study A, Table 3.2.2.4; also discussed in various experiments in Section B, CRI Annual Report for 2010-11).
- *Irradiated treatments:* More eggs were produced by females from treated 5<sup>th</sup> instar larvae than treated 4<sup>th</sup> instar larvae. This was to be expected keeping the greater radiotolerance of 5<sup>th</sup> instar larvae in mind (consult Study A). Moths from treated 4<sup>th</sup> instar larvae in Batch 2 of the rearing jars were slightly less fertile than the moths from their fruit counterpart. This could be an indication that Consignment 2 comprised a large percentage of 5<sup>th</sup> instar larvae. However, the instar assessment showed the number of 4<sup>th</sup> and 5<sup>th</sup> instar larvae divided 50:50 with no younger larvae present, which does not support the supposition. Furthermore, there were approximately 10% more 5<sup>th</sup> instar larvae in Consignment 1, which did not result in similar or higher fertility than Consignment 2. This difference may therefore have to be ascribed to a natural anomaly. Fertility of moths from the other two 4<sup>th</sup> instar batches, as well as all the 5<sup>th</sup> instar batches, were higher than that of moths from the respective fruit counterparts, indicating a strong probability of greater radiotolerance in the artificially reared larvae.

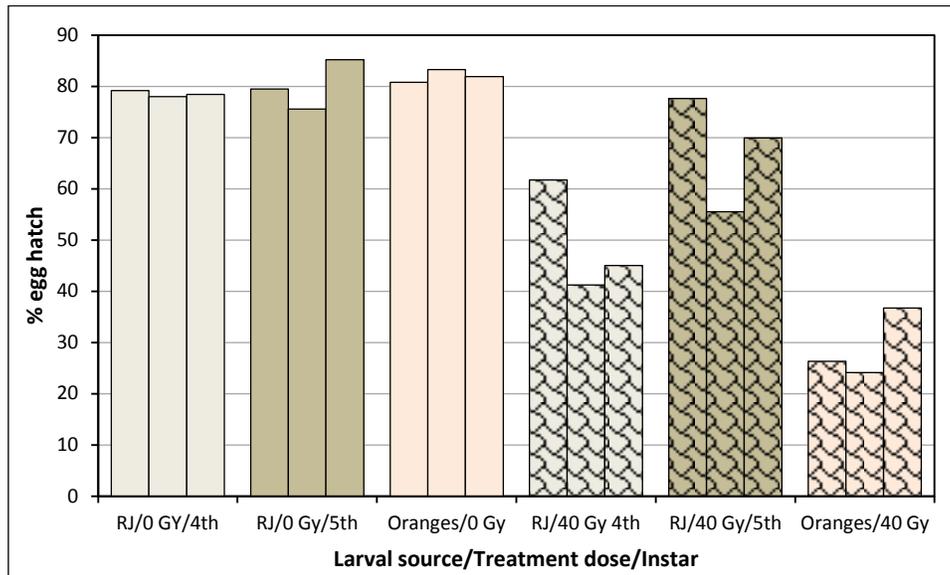


**Fig. 3.2.2.6.** Fertility of moths reared from 4<sup>th</sup> and 5<sup>th</sup> instar larvae gamma treated with 40 Gy in rearing jars (RJ) and oranges. The 3 columns in each group represent a consignment of oranges from each of 3 farms (evenly coloured and tiled orange columns), as well as the representative batches of rearing jars (evenly coloured and tiled light and dark brown columns).

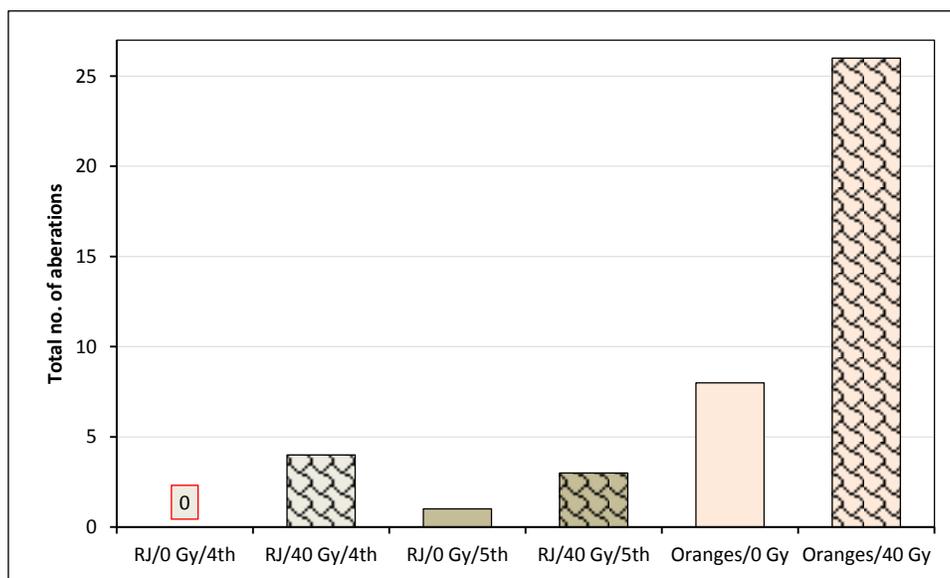
(ii) *Fecundity*

- *Unirradiated treatments (Fig. 3.2.2.7):* In paragraph (i) (*Fertility*) above, large differences in fertility between moths from untreated larvae in rearing jars and oranges were observed. The fecundity of these groups, however, was normal for the attribute and very similar between the two untreated larval sources (compare the evenly coloured columns).
- *Unirradiated versus irradiated treatments (Fig. 3.2.2.7):* Gamma treatment reduced the fecundity of all moths that developed from treated larvae in rearing jars and oranges compared to the untreated controls (compare all evenly coloured columns with their respective tiled counterparts). A similar effect to fertility, this could have been predicted from previous data (Study A, Table 3.2.2.4; also discussed in various experiments in Section B, CRI Annual Report for 2010-11).
- *Irradiated treatments (Fig. 3.2.2.7):* Moths from treated 5<sup>th</sup> instar larvae were more fecund than the moths from treated 4<sup>th</sup> instar larvae. Moths from 4<sup>th</sup> and 5<sup>th</sup> instar treated larvae from rearing jars were more fecund than the moths from oranges. This result coincides with the fertility data and indicates that the radiotolerance of the rearing jar sourced larvae was greater than that of the fruit sourced larvae.

Moth fecundity varied to a degree from batch to batch within any specific treatment (compare the 3 tiled light brown columns with each other, as well as the tiled dark brown and orange columns respectively). This is not surprising if the atypical reproductive responses from some moths of irradiated larvae recorded in the various treatments are considered: Respectively 50%, 56% and 39% of the number of confirmed matings in the three consignments presented aberrations consisting of (i) unmated females depositing no eggs, (ii) unmated females depositing dud eggs and (iii) mated females depositing dud eggs. Predictably, the recurrence of these aberrations was related to treatment (Fig. 3.2.2.8), viz. less in moths from artificially reared larvae and much more in moths from larvae in the oranges. They also occurred throughout the chronological range of matings, and were not restricted to either earlier or later matings, *i.e.* they occurred independently of any possible instar effect. This event therefore also confirms the higher radiotolerance of the artificially reared larvae.



**Fig. 3.2.2.7.** Fecundity of moths reared from larvae gamma treated with 40 Gy in rearing jars (RJ) and oranges. The 3 columns in each group represent a consignment of oranges from each of 3 farms (evenly coloured and tiled orange columns), as well as the representative batches of rearing jars (evenly coloured and tiled light and dark brown columns).



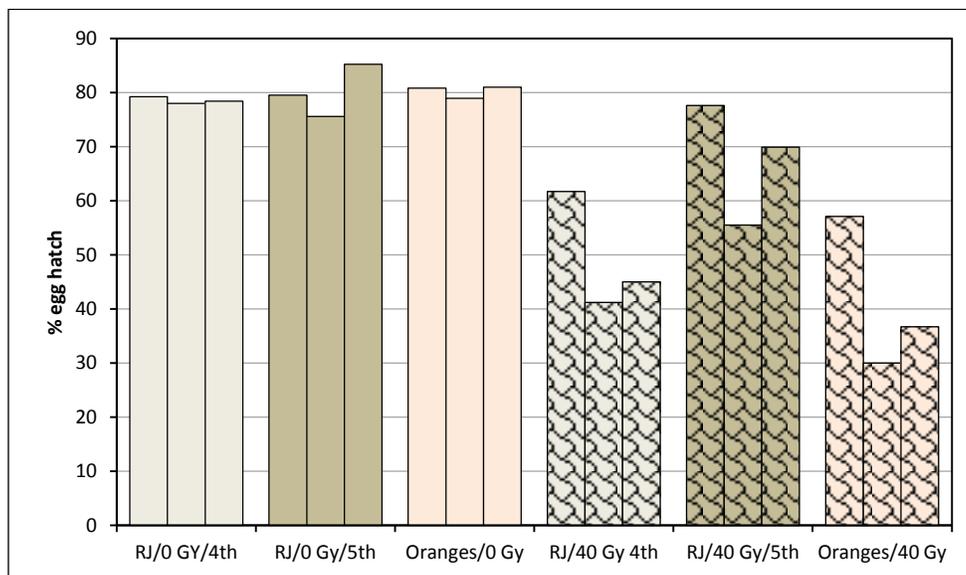
**Fig. 3.2.2.8.** Frequency of atypical mating responses occurring per treatment.

(iii) Possible contribution of the young instar component to reduced radiotolerance of FCM in oranges

It can't be accepted without reservation that the assessed increase in treatment impact on the fruit sourced feral larvae was entirely due to an inherently lower radiotolerance. The instar distribution of larvae had been verified pre-treatment by head capsule measurements; a mean of approximately 24% of the larvae in the 3 consignments consisted of 2<sup>nd</sup> and 3<sup>rd</sup> instars. Not all of these younger larvae would have survived irradiation due to acute mortality, thereby reducing the relative contribution of the younger instars' diminished reproductive performance to the overall result to an unknown extent. Of whatever size this contribution was, it is still considered highly unlikely that even the approximately 24% of the total number of treated larvae would have caused a mean reduction of 70% in fertility and 50% in fecundity. To put this aspect in perspective, certain data were recalculated as follows:

The 10 mating pairs of moths for each rearing jar treatment were collected first and within the first 4 days of moth eclosion. The larvae that they developed from would therefore have been the most developed at the time of treatment. Mating pairs from the oranges were assembled over a period of time lasting up to 19 days. The later the moths were mated during this period, the better the chance was that their larvae had

been irradiated as younger instars. These matings were consequently ignored for the recalculations by assessing only moth pairs that eclosed within the first 4 days of eclosion for fecundity calculation. In so doing, (i) it was accepted that these moths probably also originated from larvae well-developed at the time of treatment, and (ii) the number of available mating replicates were reduced from a mean of 8,3 (untreated) and 22,0 (treated) to 3.0 and 9.3 respectively. The analysis results (Fig. 3.2.2.9) showed that fecundity readings for the smaller, selected sample of fruit sourced moths had improved compared to the data supplied for all mating pairs (Fig. 3.2.2.7). However, the radiotolerancy of the moths from all 3 fruit consignments was still lower when compared with that of the artificially reared moths.



**Fig. 3.2.2.9.** Adjusted fecundity of moths reared from larvae in rearing jars (RJ) and oranges gamma treated with 40 Gy.

## Conclusion

The considerable variability in larval ages from naturally infested oranges in this study is indicative of what will inevitably be found in practice. From a practical point of view it was important to demonstrate that there was no indication that feral larvae developing naturally in oranges were measurably more radiotolerant compared to their artificially reared counterparts. What matters most is that the fertility and fecundity of moths from feral larvae treated in oranges from 3 farms did not exceed the reproductive capabilities of artificially reared larvae. It can therefore be concluded that artificially reared FCM are at least as radiotolerant as their feral counterparts and can safely be utilized in a Probit-9 study.

## Task table

Objective / Milestone	Achievement
Confirm relative radiotolerance of different larval instars	5 <sup>th</sup> instar larvae confirmed as most radiotolerant instar and decision to use these larvae in research vindicated.
Investigate relative radiotolerance of artificially (diet jars) and naturally reared (oranges) larvae	No evidence obtained that larvae developing naturally in orchard infested oranges were more radiotolerant than artificially reared larvae. Therefore possible to use the last-mentioned for Probit-9 study.
Termination of project with Probit-9 study	Comprehensive report submitted 08/01/2012 to enable management decision on continuation with Probit-9. Approved 26/02/2012. The study will therefore be conducted in 2012/13 budget year (funds approved for 2011/12),

## Conclusion to date

Up to date the overall results suggest that gamma irradiation is achievable for the phytosanitary control of FCM larvae in oranges. The envisaged radiation dose of 60 Gy is debatably acceptable with regard to fruit quality

considering Dose Uniformity Ratios of 3-4:1. This aspect may still have to be addressed by Horticulture before a final conclusion on the commercial suitability of gamma treatment can be made.

### **Technology transfer**

None.

### **Further objectives and work plan**

A new study aimed at a multidisciplinary approach involving sub-lethal cold and sub-sterilization gamma doses has been proposed and approved for 2012-13. The first unofficial steps were taken to involve USDA-APHIS in a debate on the acceptability of the tactic.

### **Reference cited**

Bloem, S., J.E. Carpenter, and J.H. Hofmeyr. 2003. Radiation biology and inherited sterility in false codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.* 96: 1724-1731.

### **3.2.3 FINAL REPORT: Investigating and improving field persistence of Cryptogran** Experiment 791 (April 2005 – March 2011) by Wayne Kirkman and Sean Moore (CRI)

### **Opsomming**

Proewe het getoon dat gedroogde melasse en dedroogde Cryptogran geskikte plaasvervangers is vir die geregistreerde formulasies, en sal die gebruik van die produkte vergemaklik. Meeste geregistreerde produkte het VKM besmetting met omtrent 50% verminder wanner hulle laat in die seisoen toegedien is. Dit beklemtoon die belangrikheid van VKM bestuur van vroeg in die seisoen. Proewe het weereens gewys dat virusse beter werk as melasse bygevoeg word. Dithane het ook goed gewerk as 'n byvoegsel. Alhoewel 2,4-D die voorkoms van oop nawelente in Nawellemoene verminder het, het dit nie VKM besmetting beduidend verminder nie. 'n Proef is ook uitgevoer om te bepaal of die toemaak van nawelente Cryptogran se werking verminder het, omdat virus nie meer binne 'n oop nawelent beskerm kon word nie. Resultate was onoortuigend omdat VKM besmetting baie laag was, en daar was geen betekenisvolle verskil tussen behandelings nie.

### **Summary**

Trials showed that powdered molasses and dried Cryptogran are suitable substitutes for the registered formulations, and would increase ease of use for growers. Most registered products gave around 50% control of FCM when applied late in the season. This highlights the importance of good FCM management from early in the season. Results once again showed the importance of adding molasses to virus applications, unless Dithane is added. Although 2,4-D reduced the occurrence of open navel ends in Navel oranges, it did not significantly reduce FCM infestation. A trial was also conducted to determine whether closing navel ends reduced Cryptogran efficacy, by removing protection of virus within an open navel end. Results were inconclusive, as FCM infestation was very low and there was no significant difference between treatments.

### **Introduction**

Field trials have been conducted with Cryptogran since the year 2000. Cryptogran is now in its eighth year of commercial use. Results from both field trials and commercial use have shown varying degrees of field persistence. A principal disadvantage of the use of baculoviruses in the field is their short residual activity due to their inactivation by UV irradiation (Huber, 1990; Shapiro, 1995). This has also been demonstrated for CrleGV (Moore, 2002), the virus in Cryptogran. A prerequisite for the success of Cryptogran as a means of controlling false codling moth (FCM) is to understand all of the factors affecting field persistence of the virus (not only UV irradiation) and to find ways to improve it. Environmental persistence can be improved by ensuring rain fastness and UV protection (Most & Quinlan, 1986). Different formulations of molasses and Cryptogran were tested. New and old chemical alternatives for FCM control were tested in a late-season application. The effect of a 2,4-D application on the efficacy of Cryptogran was also tested.

### **Objectives**

Funding for this project has been terminated, but due to the current status of FCM as a phytosanitary threat, and to aid further development, three field trials were conducted.

## Materials and methods

### Field trial 1: Late-season control options

A late season spray trial was conducted to compare the efficacy of most of the available control options for FCM. Powdered Cryptogran and powdered molasses were included in various combinations (Table 3.2.3.1). The trial was conducted on Bernol Farm in the Sundays River Valley, in an orchard of Lane Late navel orange trees. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 2005. The trial was laid out in a single-tree randomised block format, replicated 10 times. Treatments were applied with a Janisch hand-gun applicator on 4 May 2011, at an average rate of 15.0 L per tree for all treatments. Dropped fruit from each tree were collected weekly, and analysed separately. FCM infestation was determined by the presence of a larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the LSD multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 2001).

**Table 3.2.3.1.** Various treatments applied to an orchard of Lane Late Navel orange trees on Bernol Farm in the Sundays River Valley on 4 May 2011.

Treatment no.	Treatment (all doses per 100 L water)
1	Untreated control
2	Cryptogran (10 ml)
3	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)
4	Cryptogran (10 ml) + powdered molasses (225 g) + Break-Thru (5 ml)
5	Powdered Cryptogran (0.9 g) + powdered molasses (225 g) + Break-Thru (5 ml)
6	Powdered Cryptogran (0.9 g) + molasses (250 ml) + Break-Thru (5 ml)
7	Cryptex (3.3 ml)
8	Cryptex (3.3 ml) + molasses (500 ml)
9	Coragen (17.5 ml)
10	Delegate (20 g)
11	Triflumuron (20 ml)
12	Meothrin (30 ml)
13	Cypermethrin (25 ml)

### Field trial 2: Cryptogran formulations

A second field trial was conducted to compare the efficacy of powdered Cryptogran and powdered molasses, in various combinations, as well as the effect of Dithane on the efficacy of Cryptogran (Table 3.2.3.2). The trial was conducted on Bernol Farm in the Sundays River Valley, in an orchard of Palmer Navel orange trees. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 2005. The trial was laid out in a single-tree randomised block format, replicated 10 times. Treatments were applied with a Janisch hand-gun applicator on 19 December 2011, at an average rate of 24.0 L per tree for all treatments. Dropped fruit from each tree were collected weekly, and analysed separately. FCM infestation was determined by the presence of a larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the LSD multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 2001).

**Table 3.2.3.2.** Various treatments applied to an orchard of Palmer orange trees on Bernol Farm in the Sundays River Valley on 19 December 2011.

Treatment no.	Treatment (all doses per 100 L water)
1	Untreated control
2	Cryptogran (10 ml)
3	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)
4	Cryptex (3.3 ml)
5	Cryptex (3.3 ml) + molasses (500 ml)
6	Powdered Cryptogran (0.9 g) + molasses (250 ml) + Break-Thru (5 ml)
7	Powdered Cryptogran (0.9 g) + powdered molasses (225 g) + Break-Thru (5 ml)

8	Cryptogran (10 ml) + powdered molasses (225 g) + Break-Thru (5 ml)
9	Cryptogran (10 ml) + Dithane (200 g)
10	Dithane (200 g)

### Field trial 3: Cryptogran and 2,4-D

In a third field trial, Cryptogran was applied to trees which had earlier been sprayed with 2,4-D, as well as to trees which had not received the 2,4-D application. This was to test if the reduction in navel end size due to 2,4-D could negatively affect Cryptogran efficacy against FCM. The trial was conducted on Bernol Farm in the Sundays River Valley, in an orchard of Palmer Navel orange trees. The orchard, in which trees were spaced at 6 m x 3 m (rows x trees), was planted in 2003. The trial was laid out in a single-tree randomised block format, replicated 10 times. Twenty trees were sprayed with 2,4-D on 5 October 2011, at an average rate of 16 L per tree. Twenty trees were left unsprayed. Cryptogran was applied with a Janisch hand-gun applicator on 19 December 2011, at an average rate of 22.5 L per tree. Dropped fruit from each tree were collected weekly, and analysed separately. FCM infestation was determined by the presence of a larva or its frass. Mean numbers of FCM infested fruit per tree per week were compared using ANOVA and the LSD multiple range test, using Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 2001).

## **Results and discussion**

### Field trial 1: Late-season control options

Infestation in four of the treatments was not significantly different from the untreated control (Table 3.2.3.3). These were Cryptogran without molasses, Cryptex with and without molasses, and Cypermethrin. All the other treatments reduced FCM infestation significantly, with no significant difference between them (Table 3.2.3.3). This highlights the fact that there is no silver bullet for late-season FCM control.

**Table 3.2.3.3.** FCM infestation for various treatments applied to an orchard of Lane Late Navel orange trees on Bernol Farm in the Sundays River Valley on 4 May 2011, evaluated from 26 May to 17 July 2011.

Treatment no.	Treatment (all doses per 100 L water)	Mean FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	0.54a	
2	Cryptogran (10 ml)	0.39ab	27.9
3	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)	0.25b	53.5
4	Cryptogran (10 ml) + powdered molasses (225 g) + Break-Thru (5 ml)	0.23b	58.1
5	Powdered Cryptogran (0.9 g) + powdered molasses (225 g) + Break-Thru (5 ml)	0.26b	51.2
6	Powdered Cryptogran (0.9 g) + molasses (250 ml) + Break-Thru (5 ml)	0.28b	48.8
7	Cryptex (3.3 ml)	0.39ab	27.9
8	Cryptex (3.3 ml) + molasses (500 ml)	0.31ab	41.9
9	Coragen (17.5 ml)	0.26b	51.2
10	Delegate (20 g)	0.28b	48.8
11	Triflumuron (20 ml)	0.26b	51.2
12	Meothrin (30 ml)	0.3b	46.5
13	Cypermethrin (25 ml)	0.35ab	34.9

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

### Field trial 2: Cryptogran formulations

Cryptogran without molasses, Cryptogran with powdered molasses, Cryptex with and without molasses, and Dithane treatments did not reduce FCM infestation significantly (Table 3.2.3.4). All the other treatments reduced

FCM infestation significantly, with no significant difference between them (Table 3.2.3.4). Powdered Cryptogran with powdered molasses resulted in the highest FCM reduction (73.7%). However, it was not significantly different from the registered combination (treatment 3). Reduction in FCM infestation where Dithane was added to Cryptogran was statistically similar to the registered combination (treatment 3). Although not significantly, Dithane on its own did show FCM reduction. It is therefore not clear whether Dithane has a synergistic effect on Cryptogran (e.g. UV protection) or a cumulative effect with Cryptogran on FCM. Unlike previous trials, Cryptex without molasses was not inferior to Cryptex with molasses. However, efficacy of both was not significant.

**Table 3.2.3.4.** FCM infestation for various treatments applied to an orchard of Palmer Navel orange trees on Bernol Farm in the Sundays River Valley on 19 December 2011, evaluated from 11 January to 13 March 2012.

Treatment no.	Treatment (all doses per 100 L water)	Mean FCM infestation (fruit/tree/week)	Reduction in infestation (%)
1	Untreated control	0.19a	
2	Cryptogran (10 ml)	0.10ab	47.4
3	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)	0.07b	63.2
4	Cryptex (3.3 ml)	0.12ab	36.8
5	Cryptex (3.3 ml) + molasses (500 ml)	0.14ab	26.3
6	Powdered Cryptogran (0.9 g) + molasses (250 ml) + Break-Thru (5 ml)	0.06b	68.4
7	Powdered Cryptogran (0.9 g) + powdered molasses (225 g) + Break-Thru (5 ml)	0.05b	73.7
8	Cryptogran (10 ml) + powdered molasses (225 g) + Break-Thru (5 ml)	0.09ab	52.6
9	Cryptogran (10 ml) + Dithane (200 g)	0.08b	57.9
10	Dithane (200 g)	0.12ab	36.8

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

#### Field trial 3: Cryptogran and 2,4-D

Reduction in FCM infestation was similar where Cryptogran was applied to 2,4-D-treated and untreated trees (Table 3.2.3.5). The reduction in navel end size due to 2,4-D did not appear to detrimentally affect Cryptogran efficacy against FCM. Where 2,4-D was applied without any Cryptogran (treatment 2), FCM infestation was slightly lower than the control. However, there was no significant difference between any of the treatments, making the results inconclusive.

**Table 3.2.3.5.** FCM infestation for various treatments applied to an orchard of Palmer navel orange trees on Bernol Farm in the Sundays River Valley on 19 December 2011, evaluated from 11 January to 13 March 2012.

Treatment no.	Treatment (all doses per 100 L water)	Mean FCM infestation (fruit/tree/week)
1	Untreated control	0.13a
2	2,4-D (3.25 ml)	0.10a
3	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml) + 2.4D (3.25)	0.07a
4	Cryptogran (10 ml) + molasses (250 ml) + Break-Thru (5 ml)	0.08a

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

## Conclusion

Two field trials showed that powdered molasses and dried Cryptogran are suitable alternatives to the currently registered formulations, confirming findings in previous trials. These formulations could improve ease of use for growers, while not compromising efficacy.

It was also shown that there is no silver bullet for late-season FCM control, highlighting the importance of good FCM management throughout the season. Most products showed similar efficacy, reducing FCM infestation by around 50%. However, Cryptogran without molasses, Cryptex with and without molasses and cypermethrin failed to significantly reduce FCM infestation. The trials confirmed the importance of adding molasses to virus applications and showed Dithane to be a beneficial additive to Cryptogran.

## Acknowledgments

River Bioscience is thanked for the supply of Cryptogran for trials. Sunriver Citrus is thanked for making their orchards available and for assisting with the management of trial sites.

## Future research

No further research is planned. Should the study be continued at any stage, trials investigating the carry-over effect of a virus application from one season to the next should be repeated. More frequent applications at lower rates should also be revisited.

## Technology transfer

Wayne Kirkman and Sean Moore made various presentations at grower meetings. See Section 8.7 on Technology Transfer for details.

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### 3.2.4 PROGRESS REPORT: Entomopathogenic nematodes for the control of false codling moth in citrus orchards

Experiment 793 (2005/06 – 2012/13) by A P Malan (SU) and S D Moore (CRI)

## Opsomming

Ingevoerde kommersiële formulasies van *Heterorhabditis bacteriophora* en *Steinernema feltiae* is evalueer in laboratorium biotoetse vir hul effektiwiteit as bio-beheer agente teen vals-kodling mot (FCM) laaste instar larvale stadium. Daar is gebruik gemaak van lae konsentrasie van 50 infektiewe larwes (IJ) per insek en mortaliteit is bepaal na 24 h. Geformuleerde *H. bacteriophora* veroorsaak 76% mortaliteit van VKM in vergelyking met lokale *H. bacteriophora* met mortaliteit van 78%, sonder om beduidende te verskil. Geformuleerde *S. feltiae* veroorsaak 100% beheer van VKM larwes by 25°C, maar baie lae mortaliteit (<6%) by 14°C. Om te bepaal wat die LC van *S. feltiae* en *H. bacteriophora* is by 14°C is konsentrasies van 0, 25, 50, 100 en 200 IJ/insek gebruik. In die eerste eksperiment is geformuleerde *S. feltiae* en as herhaling is *S. feltiae* geteel in wasmot larwes gebruik. Die twee eksperimente vir *S. feltiae* het beduidend van mekaar verskil wat betref mortaliteit by konsentrasies van 50 en 100 IJ by 14°C, maar het geen beduidende verskil getoon by hoër konsentrasies met mortaliteit digby 100% nie. Die lae mortaliteit in die eerste eksperiment kan toegeskryf word aan ongunstige toestande tydens invoer van die *S. feltiae*. In kontras, kon geen mortaliteit verkry word met *H. bacteriophora* by 14°C nie, maar daar is wel aangetoon dat gewasde larwes wel met nematodes infekteer is, maar eers 48 uur later dood is. Die gekombineerde resultate het aangetoon dat daar by 78 en 242 IJ van *S.*

*feltiae* nodig om LC<sub>50</sub> en LC<sub>90</sub> by 14°C te verkry. Ter afsluiting kan gesê word dat die geformuleerde *H. bacteriophora* minimaal verskil van die lokale spesie. *Steinernema feltiae* gee 100% beheer by 25°C en 90% beheer by 14°C by hoër konsentrasies van ongeveer 250 IJ/insek. *Heterorhabditis bacteriophora* toon geen mortaliteit by 14°C by enige konsentrasie na 38 uur nie, maar neem langer om die vals-kodling mot larwes te dood. Hierdie eksperimente gedoen in die huidige studie is die eerste om vas te stel wat die effektiwiteit van *S. feltiae* op die beheer van VKM larwes is.

## Summary

Imported commercial formulations of *Heterorhabditis bacteriophora* and *Steinernema feltiae* were evaluated in laboratory bioassays for their efficacy as biocontrol agents against false codling moth (FCM) last-instar larvae. A low concentration of 50 infective juveniles (IJs) per insect was used and mortality determined after 48 h. Formulated *H. bacteriophora* caused 76% mortality of FCM larvae in comparison to endemic *H. bacteriophora* with mortality of 87%, but with no significant difference between them. Formulated *S. feltiae* caused 100% control of FCM larvae at 25°C, but very low mortality (<6%) at 14°C. To determine the LC of *S. feltiae* and *H. bacteriophora* at 14°C, concentrations of 0, 25, 50, 100, 200 and 400 IJs/insect were used. In the first trial, formulated *S. feltiae* was used, with, in the second trial, *S. feltiae* being recycled through wax moth larvae. The two trials differed significantly from each other in the main effect of mortality at 14°C with concentrations of 50 and 100 IJs, but with no significant difference at higher concentrations with mortality close to 100%. Low mortality in the first trial can be ascribed to unfavourable conditions for the nematodes during transport. In contrast, no mortality of FCM larvae was obtained using *H. bacteriophora* at 14°C; however, it was shown that they were indeed infected, as washed larvae died after 48 h at 25°C. The LC<sub>50</sub> and LC<sub>90</sub> for the combined concentration trials for *S. feltiae* were 78 and 242 IJs of *S. feltiae* per FCM larvae at 14°C. In conclusion, the results showed formulated *H. bacteriophora* to be marginally less infective than were local *H. bacteriophora*. *Steinernema feltiae* gave 100% control at 25°C and 90% control at 14°C, using a higher concentration of 250 IJs/insect. *Heterorhabditis bacteriophora* caused no mortality with the different concentrations used at 14°C after 48 h, but took longer to kill the FCM larvae. The trials described in the current study are the first undertaken to determine the efficacy of *S. feltiae* for the control of FCM larvae.

### 3.2.5 PROGRESS REPORT: Investigation of the potential for the development of a locally produced mating disruption system

Experiment 955 (April 2009 – March 2011) by Sean Moore and Wayne Kirkman (CRI)

## Opsomming

Die doel van hierdie studie is om 'n doeltreffende vrylater vir paringsontwrigting vir VKM te ontwikkel en om sy werking met dié van kommersieel beskikbare paringsontwrigting produkte te vergelyk. Oplettende vordering is in die laaste twee seisoene met die ontwerp en ontwikkeling van 'n vrylater gemaak. Daarna is boordproewe uitgevoer om effektiwiteit met kommersieele produkte te vergelyk. Resultate is positief, alhoewel dit het voorgekom dat Isomate se werking nog beter was, ondanks die feit dat die eksperimentele vrylater (ExpMD) dramaties meer aanloklik vir VKM was toe hulle in lokvalle gelaai is. Gevolglik is verdere laboratorium proewe uitgevoer om te bepaal of hierdie verduidelik kan word deur verskille in feromoon vrylatingskoers. Alhoewel vrylatingskoers teen konstante temperatuur meer konsekwent met ExpMD was, die hoeveelheid feromoon wat vrygelaat is, was 10 tot 15 maal hoër van twee verskillende tipes Isomate vrylaters. In die toekoms sal proewe uitgevoer word om die paringsontwrigting effek van verskillende kombinasies van feromoon-isomere te vergelyk.

## Summary

The objective of this study was to develop an effective dispenser for mating disruption of FCM and to compare its efficacy with that of commercially available mating disruption products. Notable progress was made during the last two seasons with design and development of a dispenser. Thereafter, field trials were conducted to compare efficacy with commercial products. Results were positive, however, Isomate did appear to have superior efficacy, despite the experimental dispenser (ExpMD) being dramatically more attractive to FCM when loaded in traps. Consequently, further laboratory trials were conducted to determine whether this could be explained by differences in pheromone release rate. Although rate of pheromone release at constant temperature was more consistent with ExpMD, the amount of pheromone released was 10 and 15 times higher from two different Isomate dispensers. Future trials will aim to compare the mating disruption effect of different combinations of pheromone isomers.

### 3.2.6 FINAL REPORT: Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa

Experiment RU959 (2009/10 - 2011/2012) by John Opoku-Debrah (RU), Sean Moore (CRI), Martin Hill (RU) and Caroline Knox (RU)

#### Opsomming

Aangesien dit moontlik is dat sekere geografiese bevolkings van valskodlingmot (VKM) 'n verlaagde toleranssie vir Cryptogran en Cryptex kan ontwikkel, soos wat die geval met kodlingmot (KM) vir die KM virus (CpGV) in Duitsland was, is die soektog vir nuwe isolate van die VKM virus as uiters belangrik beskou. Hiermee word daar terugvoer gegee oor die suksesvolle induksie van 'n latente bakulovirus besmetting in vyf geografiese populasies van VKM en dus die herkryging van vyf nuwe CrleGV-SA isolate. Hierdie sluit in viruse van VKM kulture van Addo, Citrusdal, Marble Hall, Nelspruit en 'n gemengde kultuur. Met gebruik van restriksie ensiem analise is dit gewys dat hierdie isolate en die kommersiele isolate, Cryptogran en Cryptex, geneties van mekaar verskil. Die nuwe isolate is soos volg genoem: CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl, CrleGV-SA Nels en CrleGV-SA Mix. Sekwensie-analise van die *granulin* en *egt* genes van al die isolate het enkel-nukleotied-polimorfismes (ENP) in albei genes vertoon. ENPs in die *egt* genes van hierdie isolate het in 'n verandering in aminosuur sekwensie veroorsaak. DNA profiele van RFLPs, asook filogenetiese analise gebaseer op *granulin* en *egt* sekwensies het die teenwoordigheid van twee CrleGV-SA genoom tipes getoon. Cryptex, CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl en CrleGV-SA Mix is as lede van Groep een geplaas, en Cryptogran en CrleGV-SA Nels isolate is in Groep twee geplaas. Studies wat die biologiese aktiwiteit van die virus isolate verglyk het, het beduidende verskille tussen die relatiewe sterkte van die isolate teen die Addo en gemengde kolonie getoon.

#### Summary

Considering the possibility of some geographic populations of the false codling moth (FCM) developing a lower susceptibility to Cryptogran and Cryptex, as was the case with codling moth (CM) to the codling moth virus (CpGV) in Germany, the search for new isolates of the FCM virus becomes eminent. Here we report on the successful induction of a latent baculovirus infection in five geographic populations of FCM and the subsequent recovery of five new CrleGV-SA isolates. These include the Addo, Citrusdal, Marble Hall, Nelspruit and Mixed colony isolates. These isolates were shown to be genetically different from each other and from the commercial isolates, Cryptex and Cryptogran, using restriction enzyme analysis. The new isolates have been named CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl, CrleGV-SA Nels and CrleGV-SA Mix isolates. Sequence analysis of the *granulin* and *egt* genes of all isolates revealed single nucleotide polymorphisms (SNPs) in both genes. Significantly, SNPs in the *egt* genes of these isolates resulted in a change in amino acid sequence. DNA profiles from RFLPs, as well as phylogenetic analysis based on *granulin* and *egt* sequencing showed the presence of two CrleGV-SA genome types. Cryptex and CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl and CrleGV-SA Mix have been placed as members of Group one CrleGV-SA, and Cryptogran and CrleGV-SA Nels isolate placed into Group two CrleGV-SA. Studies on the comparative biological activity of the isolates also revealed significant differences between the relative potencies of the viral isolates against FCM from the Addo and Mixed colonies.

#### Introduction

A group of naturally occurring insect viruses, the baculoviruses, have proven to be effective in use as biopesticides in the control of most lepidopteran insects (Moscardi, 1999). In South Africa, two baculovirus products, Cryptogran® (River Bioscience, South Africa) (Moore, 2002; Moore & Kirkman, 2004) and Cryptex® (Andermatt Biocontrol, Switzerland) (Kessler & Zingg, 2008) have been registered for the control of the false codling moth (FCM), *Thaumatotibia* (= *Cryptophlebia*) *leucotreta* (Meyrick) (Lepidoptera: Tortricidae), an important economic pest. These two biopesticides both contain the baculovirus, *Cryptophlebia leucotreta* granulovirus (CrleGV) as its active ingredient.

On the other hand, despite the successful use of baculovirus-based biopesticides to control most lepidopteran insects, a few reported cases of host resistance have brought into light some potential challenges accompanied by their usage. One notable scenario was that reported for codling moth (CM), *Cydia pomonella* (L.) where field populations developed resistance to the CM virus, *Cydia pomonella* granulovirus (CpGV-M, Mexican isolate) in Europe (Eberle & Jehle, 2006). However, since baculoviruses undergo high rates of mutation leading to the occurrence of several mixed wild-type isolates, with some isolates being more virulent than others, new isolates of superior virulence can be used as substitutes to manage resistance (Cory *et al.*, 1997; Eberle *et al.*, 2008; Jehle *et al.*, 2008).

In consequence, CpGV–M based biopesticides which showed reduced efficacy in controlling FCM populations as reported by Eberle & Jehle (2006) have been replaced with those containing CpGV-R5 (Besse *et al.*, 2011; Zingg *et al.*, 2011). In view of this, it is imperative that the search for yet more new isolates of the FCM virus (CrleGV), in South Africa, be expedited.

A preliminary study in relation to novel FCM virus isolates showing genetic differences was initially conducted by Moore (2002) in South Africa. Moore (2002) speculated about the probable existence of other South African isolates (CrleGV-SA) that needed to be confirmed by future work. Only one of these variants was subsequently developed into a commercial product, Cryptogran®, as was the focus of the study (Moore, 2002). The novel CrleGV-SA isolate is currently the active ingredient of Cryptogran® (Moore, 2002; Moore *et al.*, 2004). Further studies also showed that although Cryptogran (Moore, 2002; Moore *et al.*, 2004) and Cryptex (Kessler & Zingg, 2008) are both CrleGV isolates they differ significantly in their genetic profile (Goble, 2007). In a previous study, Timm (2005a) found that FCM populations sampled from different geographic regions in South Africa showed high levels of genetic variation. The inference drawn was that FCM populations in South Africa were more or less locally adapted populations (Timm, 2005a & 2005b). Timm (2005b) speculated that these locally adapted FCM populations could vary in their response to insecticide resistance or virus susceptibility. Other studies found differences in the biological activity of Cryptogran and Cryptex against neonate FCM larvae from two geographically distinct populations in South Africa (Opoku-Debrah, 2008). These marked differences in the genotypes and biological activity of some baculovirus isolates has led to suggestions that the clonal diversity of isolates be determined and those with defined activity profiles selected for specific biocontrol programmes - in order to avoid loss in virus efficacy and yield (Cory *et al.*, 1997). It might be possible that FCM populations from different geographic regions in South Africa are more susceptible to a particular CrleGV isolate than another.

### **Stated objectives**

In view of the above, the main aims of this study were:

1. To search for new CrleGV-SA isolates to prepare for possible resistance by FCM to Cryptogran or Cryptex. This will aid in the formulation of multiple isolates of CrleGV-SA for future use, thus lowering the probability of some FCM populations developing resistance to a particular isolate.
2. To investigate the biological activity of any new isolates recovered as well as the two commercial CrleGV-SA isolates (Cryptex & Cryptogran) against the five geographically distinct laboratory populations of FCM.
3. Characterise and sequence some important baculovirus genes such as the *granulin* and *egt* genes of Cryptex, Cryptogran and any new CrleGV-SA isolates recovered and to determine any evolutionary relationships between them.

The protocol and results pertaining to this study are discussed.

### **Materials and methods**

#### Induction of a latent baculovirus in FCM laboratory populations

Most pathogens, initially appearing dormant (latent) in their host, can become infective under favourable conditions such as host stress. Such latent infections have been reported with insects (Steinhaus, 1958a & 1958b). To investigate this phenomenon, five geographically distinct laboratory colonies of FCM (Addo colony, Mixed colony, Citrusdal colony, Marble Hall colony and the Nelspruit colony) previously established by Moore (2002) and Opoku-Debrah (2008) were used as a strategic stock for the execution of latent virus induction trials. A host of virus induction protocols such as, changes in diet, temperature and overcrowding were conducted in order to induce viral infection in the aforementioned laboratory colonies.

Overcrowding: Overcrowding involves increasing the population of insects per unit area (rearing chamber) or varying the size of the rearing chamber per unit population of insects (Steinhaus, 1958b). The standard laboratory conditions for rearing FCM involve incubating approximately 300 to 400 FCM eggs on 40 g of Moore's (2002) standard FCM diet per jam jar. Therefore, for the treatments, the number of eggs was increased four-fold. At least four jam jars, each containing approximately 1200 – 1600 eggs were selected from each of the five aforementioned colonies. Two jam jars with approximately 400 – 500 eggs were used as controls. Both the treatment and controls were held under similar rearing conditions ( $25.0 \pm 2.0$  °C, 50 - 60% relative humidity, RH). Larvae were then observed periodically for the presence of baculovirus symptomatic infection. The trial was replicated at least three times, where possible (depending on the availability of eggs).

Temperature: Some researchers contend that, under laboratory conditions, manipulating the environmental temperature of the host can be a useful stressing mechanism (Kitajima, 1926; Hukuhara & Aruga, 1959; Smith, 1967). To do this, a number of 3<sup>rd</sup> instar FCM larvae (developing in jam jars with diet) were selected for these experiments. For the treatments, at least four jam jars were selected from each of the five aforementioned colonies. Two jam jars holding 3<sup>rd</sup> instar larvae were used as controls. The larvae (held in jam jars) were selected from either of the colonies, depending on the availability of larvae, as some larvae had to be reserved for the maintenance of the colonies. The treatments were then sent to an incubation room with a constant temperature of 37°C until pupation. The controls, on the other hand, were maintained at normal rearing conditions in the insectary. Each trial was replicated at least three times where possible (depending on the availability of eggs).

Changes in diet: Some authors indicate that, feeding insects with an artificial diet or any diet other than the host's natural diet, can sufficiently stress them leading to the incidence of baculovirus infections in such colonies (Ripper, 1915; Vago, 1951 & 1955; Smith, 1967). In this study, since the host (FCM) had already been reared successfully on Moore's standard artificial FCM diet (see Chapter two), another diet recipe had to be used. For the treatments, at least two jam jars were selected from each of the five aforementioned FCM colonies and filled with a new diet consisting of a food cereal mix (Morvite®) - with each holding approximately 400 – 500 eggs. For the controls, two jam jars holding approximately 400 – 500 eggs was incubated on Moore's standard FCM diet. The treatments and controls were then incubated in the insectary and kept under similar rearing conditions. Again, trials were replicated at least three times where possible (depending on the availability of eggs).

#### Occlusion body purification

Diseased larvae (CrleGV infected) as well as the biopesticides, Cryptogran and Cryptex, were subjected to a glycerol purification protocol in order to recover the virus particles (occlusion bodies, OBs). This was in order to get rid of all insect matter (from virus infected larvae) as well as formulation additives (from Cryptogran and Cryptex) and end up with a pure virus. The virus purification protocol as described by Hunter-Fujita *et al.* (1998) and Moore (2002) was employed.

#### Virus Identification

Once the OBs were obtained, electron microscopy was used to confirm that this was indeed a GV (granulovirus). A modified version of the virus identification protocol, as described by Dezianian (2010) was employed. Approximately 5 µl of purified OBs was pipetted onto a carbon grid. Afterwards the virus suspension was left for 20 seconds and then blotted out to dry using filter paper. The carbon grids (holding the virus OBs) were then placed in an electron microscope to determine their shapes and sizes.

#### DNA extraction protocol

A modified version of the CTAB DNA extraction protocol as described by Aspinall *et al.* (2002) and Goble (2007) was employed. To extract DNA from baculovirus OBs using this method, 200 µl of OBs was pipetted into sterile microcentrifuge tubes and then 90 µl of sodium carbonate, Na<sub>2</sub>CO<sub>3</sub> (1 M) was added to the suspension. More Na<sub>2</sub>CO<sub>3</sub> was added to the reaction if the suspension did not clarify. The contents were then incubated in a water bath (set at 37°C) for 30 min. After incubation, the suspension was neutralized with 120 µl of Tris-HCL (1 M, pH 6.8). Thereafter, 90 µl of SDS (10% w/v) and 50 µl of Proteinase K (25mg/ml) was then added. The contents were then incubated again for 30 min at 37°C. Afterwards, 10 µl of RNaseA (10mg/ml) was added to the contents of the tube and then incubated for a further 30 min at 37°C. The tubes were then taken out and spun at 14000 rpm for 3 min in a tabletop laboratory centrifuge (BIO-RAD, model 16K). The supernatant was transferred into a new tube and the pellets, if any, were discarded. Afterwards, 400 µl of pre-warmed (at 70°C) CTAB buffer was then added to the supernatant and incubated in a heating block (set at 70°C) for 1 h. Every 10 min the tubes were inverted several times to allow the contents to mix. Another, 400 µl of chloroform (stored at 4°C) was then added. The tubes were again inverted briefly and spun for 10 min at 10000 rpm. The upper aqueous phase was transferred into a new tube and 400 µl of cold isopropanol (stored at -20°C) was added to it. The tubes were then incubated at -20°C overnight. The next day, the tubes were centrifuged at 14000 rpm for 20 min and the supernatant was discarded. The resulting pellet was then re-suspended in 1 ml of ice cold 70% ethanol (stored at -20°C). Thereafter, the tubes were then spun again at 14000 rpm for 5 min. The ethanol was gently poured off, without discarding the DNA pellet in the process, and the pellet retained. The tubes were then incubated in a heating block (set at 50°C) until all remaining traces of ethanol had evaporated. The DNA pellet was then re-suspended overnight (at -4°C) in 20 µl of RNase free water or 10mM Tris-HCL (pH 8.0) buffer. The DNA was then stored for a few days at -4°C or for longer periods at -25°C (or in a freezer). The genomic DNA extracted could then be used for PCR and REN analysis.

## REN analysis of CrleGV genomic DNA

A restriction endonuclease analysis (REN) profile of the granulovirus isolate's genomic DNA was carried out in order to establish differences between them. Eight restriction enzymes: *Bam*H1, *Sal*I, *Xba*I, *Pst*I, *Xho*I, *Kpn*I, *Hind*III and *Eco*R1 were used to digest the genomic DNA from the five new CrleGV isolates (the Addo, Nelspruit, Citrusdal, Marble Hall and Mixed colony isolates) as well as the Cryptex and Cryptogran isolates. Differences in band patterns were used to establish differences between isolates. DNA digests were carried out at 30 volts for 16 h on 0.6% agarose gels in tris-acetate (TAE) buffer.

## Amplification of the *granulin* and *egt* genes of the CrleGV-SA isolates

The *granulin* and *egt* genes were amplified from the genomic DNA of the seven isolates using a polymerase chain reaction technique (PCR). The protocols as described by Goble (2007) were employed. Primers used for amplifying the genes were designed by Inqaba Biotechnical Industries (Pty) Ltd.

## Droplet dose and time-response bioassays

The modified version of the droplet feeding bioassay technique developed by Hughes & Wood (1981) and described by Opoku-Debrah (2011) for the bioassay of neonate FCM larvae was used. Seven-fold serial dilutions (1:7 dilution factor) ranging in concentrations of  $6.07 \times 10^2$ ,  $4.25 \times 10^3$ ,  $2.97 \times 10^4$ ,  $2.08 \times 10^5$ ,  $1.46 \times 10^6$ ,  $1.02 \times 10^7$  and  $7.14 \times 10^7$  OBs/ml were used per isolate in conducting bioassays. Bioassays were conducted in 24-cell bioassay trays. Three replicate assays of 48 larvae per treatment and control were conducted for each colony and assays evaluated for larval mortality 7 days post inoculation.

Time-response bioassays were conducted in the same manner as described by Moore (2002) and Opoku-Debrah (2011) using 30 ml plastic polypots (Evron, South Africa). For the controls, larvae were fed with a water-dye solution and for the treatments larvae were droplet-fed with a single concentration of  $1.0 \times 10^8$  OBs/ml. Five neonates were selected per colony and placed into polypots with a total of 50 larvae per treatment and control. The polypots were then covered with multiple layers of sterile paper towel and secured with their lids. After 12 hours, both treatment and control polypots were inspected for larval mortality. Thereafter, polypots were inspected every 8 hours, three times a day, at 07:00, 15:00 and 23:00 until all larvae had died. Bioassays were conducted in triplicates for each of the five colonies.

## Statistical analysis

Data from the droplet dose-response assays were analysed using PROBAN (Van Ark, 1995). Time-response relationships were determined by the Kaplan-Meier product limit estimator, using the GraphPad Prism software (version 5.04), which also takes into account control mortality. The median survival time ( $ST_{50}$ ) was determined and expressed as the time at which 50% of the treated insects are still alive. Survival times of three replicates were pooled from each of the colonies and their means compared using the Log-rank (Mantel-Cox) and significant differences established at  $P < 0.05$ .

## **Results and discussion**

### Induction of a latent baculovirus in FCM laboratory populations

FCM populations from the Addo, Nelspruit, Citrusdal, Marble Hall and the Mixed colonies that were subjected to overcrowding, as a stress induction mechanism, exhibited characteristic CrleGV symptomatic infection. The other methods, changes in diet and temperature, were not successful in this regard. The number of jam jars containing CrleGV infected larvae from the Addo, Mixed, Nelspruit, Marble Hall, and Citrusdal colonies when expressed as a percentage was recorded to be 21.43%, 26.67%, 25.93%, 14.81% and 18.52%, respectively (Table 3.2.6.1). The controls, on the other hand, did not show any baculovirus symptomatic infection. Symptoms of diseased larvae involve the development of irregular white to yellow patches below the cuticle. Infected larvae crawl upwards and hang up-side down from the cotton wool stoppers. Infected larvae in their advanced stages of infection swell and distend slightly, appearing darkish brown, with their body liquefying due to their injured epidermis (Moore, 2002). All diseased larvae showing the above symptoms were stored in Eppendorf tubes or Schott bottles and preserved at  $-20^{\circ}\text{C}$ .

**Table 3.2.6.1** Summarised data from six treatment replicates for five laboratory populations of FCM subjected to overcrowding as a virus induction protocol.

FCM population	total number of jars used	Total number responding	Percentage (%) response
Addo colony	28	6	21.43
Mixed colony	30	8	26.67
Nelspruit colony	27	7	25.93
Marble Hall colony	27	4	14.81
Citrusdal colony	27	5	18.52

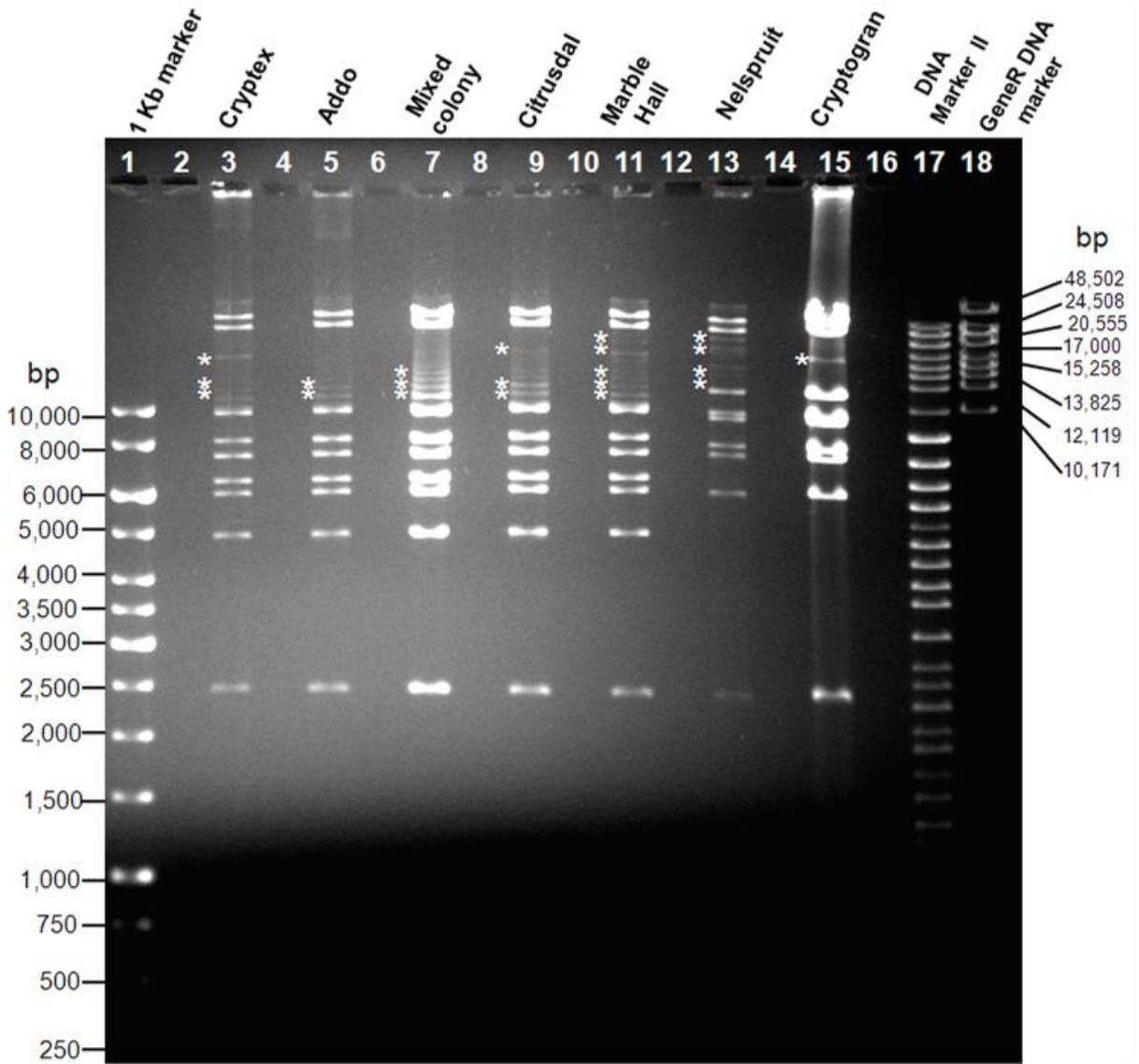
Since no incidence of a CrleGV infection in the aforementioned laboratory colonies was observed during rearing, the virus was able to maintain itself in the colonies for several generations via vertical transmission. This has been reported for a nucleopolyhedrovirus, NPV infection in the cabbage moth, *Mamestra brassicae* (L.), which was able to persist in laboratory insect populations due to a vertical transmission of the virus over several generations (Hughes *et al.*, 1993). It is possible that, once the virus was induced in some larvae, it was then transferred to other susceptible larvae via horizontal infection. This may have occurred due to susceptible individuals ingesting CrleGV on contaminated FCM diet or frass, as well as exudates from dead or diseased larvae. This is supported by the fact that, during infection, crowding increases contact between infected and susceptible individuals hence increasing the rate of viral transmission (Steinhaus, 1958b).

#### Virus Identification

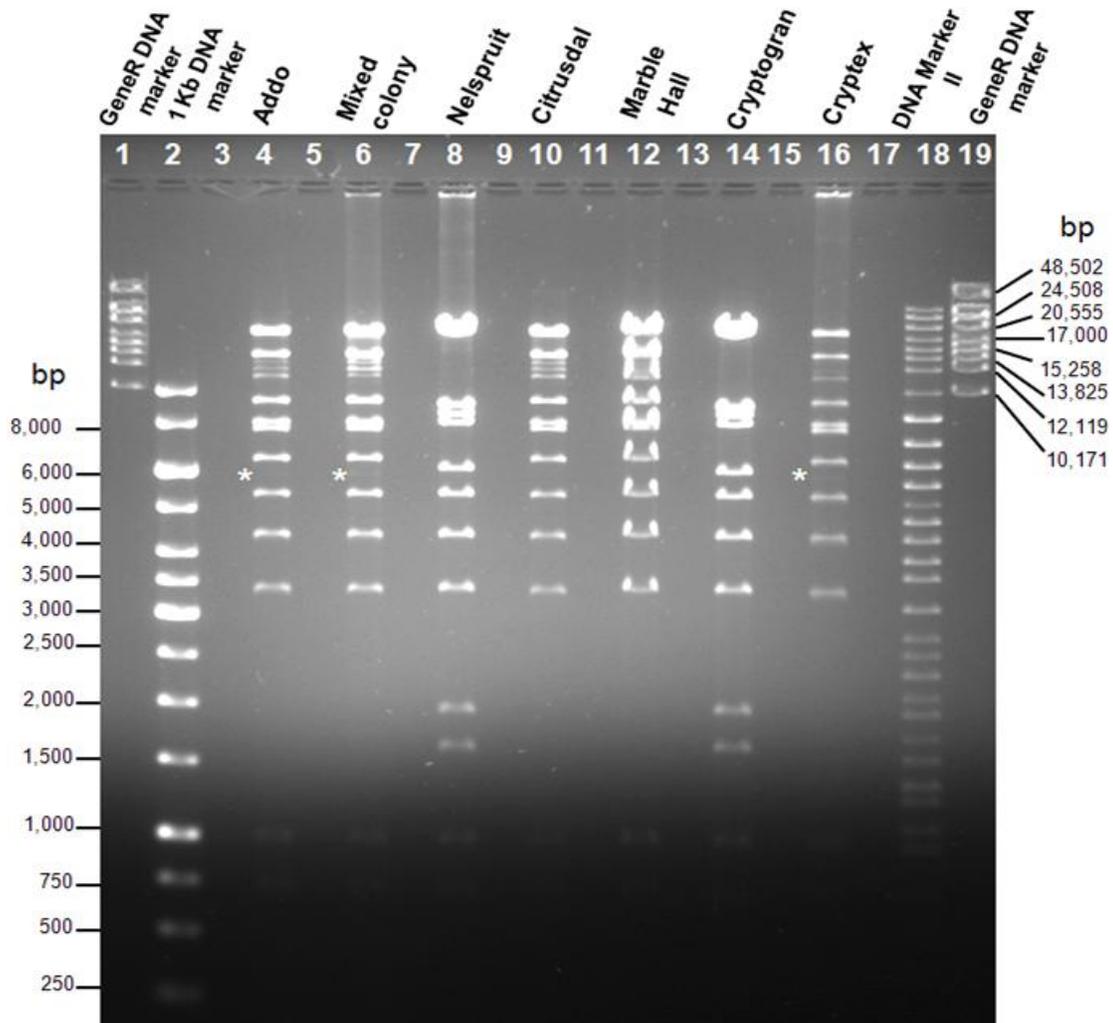
Using the known granulovirus biopesticides, Cryptex and Cryptogran as controls, similarities in shapes and sizes (using transmission electron microscopy, TEM) between the five new GV isolates could be easily drawn. The individual particle sizes (length) of the Cryptex, Cryptogran, Nelspruit, Citrusdal, Marble Hall and Mixed colony isolates, ranged from 315 nm to 395 nm. This was in accordance with that described by Hunter-Fujita *et al.* (1998) for granuloviruses. According to Hunter-Fujita *et al.* (1998) the GVs are small and ovicylindrical or granule-like, with a particle size ranging between 300 nm to 500 nm in length and may contain one or rarely two virions per occlusion body. The NPVs (nucleopolyhedroviruses), on the other hand, are much bigger and have a polyhedral shape (150 nm - 15000 nm in diameter) with numerous virions in their crystalline occlusion body (Hunter-Fujita *et al.*, 1998).

#### Restriction endonuclease digestion of CrleGV genomic DNA

By using the restriction enzymes *Bam*H1, *Sal*1, *Xba*1, *Pst*1, *Xho*1, *Kpn*1, *Hind*III and *Eco*R1 to digest CrleGV genomic DNA recovered from infected larvae from the Addo, Nelspruit, Citrusdal, Marble Hall, Mixed colonies, including Cryptex and Cryptogran isolates, differences in band patterns could be observed. Each isolate showed the presence of unique submolar REN bands indicating genetic differences between samples, with each isolate consisting of a mixture of more than one CrleGV-SA genotype. RE profiles of the genomic DNA of the seven isolates using *Bam*H1 and *Sal*1 are shown in Figure 3.2.6.1 and Figure 3.2.6.2 below. RE profiles of *Xba*1, *Pst*1, *Xho*1, *Kpn*1, *Hind*III and *Eco*R1 are not shown.



**Figure 3.2.6.1** *Bam*H1 restriction endonuclease digest profiles of Cryptex (lane 3), Addo colony isolate (lane 5), Mixed colony isolate (lane 7), Citrusdal colony isolate (lane 9), Marble Hall colony isolate (lane 11), Nelspruit colony isolate (lane 13) and Cryptogran (lane 15) samples analysed by 0.6% AGE at 30 V for 16 hours. DNA markers: 1 Kb DNA marker (lane 1), DNA marker II (lane 17) and GeneRuler High range (lane 18) were run along the outside lanes of gels. Asterisks (\*) indicate submolar bands.



**Figure 3.2.6.2** *SalI* restriction endonuclease digest profiles of Addo colony isolate (lane 4), Mixed colony isolate (lane 6), Nelspruit colony isolate (lane 8), Citrusdal colony isolate (lane 10), Marble Hall colony isolate (lane 12), Cryptogran (lane 14) and Cryptex (lane 16) samples analysed by 0.6% AGE at 30 V for 16 hours. DNA markers: GeneRuler High range (lane 1 & 19), 1 Kb DNA marker (lane 2), DNA marker II (lane 18) and were run along the outside lanes of gels. Asterisks (\*) indicate submolar bands.

The increased genetic diversity among the five new CrleGV-SA isolates (Addo, Citrusdal, Marble Hall, Nelspruit and Mixed colony isolates) may have been influenced by several factors. Firstly, the establishment of several geographically distinct laboratory colonies of the host (FCM) may have played a significant role in obtaining a high genetically diverse gene pool of CrleGV-SA. Secondly, the methodology employed for the induction and recovery of latent CrleGV-SA isolates, by pooling several insect larvae from each of the colonies, may have contributed to this increased genetic diversity (Cory & Myers, 2003; Erlandson, 2009). The origins of this genetic diversity in baculoviruses have been speculated as being caused by small mutations, sequence duplications and in some cases the acquisition of host DNA (Brown *et al.*, 1985). The Addo, Nelspruit, Marble Hall, Citrusdal and Mixed colony isolates have been named as CrleGV-SA Ado, CrleGV-SA Nels, CrleGV-SA Mbl, CrleGV-SA Cit and CrleGV-SA MixC isolates, respectively.

On a different note, genetically different baculovirus isolates can be placed into different genome groups based on the presence of prominent bands observed in their DNA profiles following RFLP analysis (Eberle *et al.*, 2009; Redman *et al.*, 2010). Similarly in this study, similarities in the REN profiles of the five new CrleGV-SA isolates, including Cryptex and Cryptogran isolates were observed. Subsequently, Cryptex and CrleGV-SA Ado, CrleGV-SA Mbl, CrleGV-SA Cit and CrleGV-SA MixC isolates were placed as members of the Group one CrleGV-SA, while Cryptogran and CrleGV-SA Nels isolate were placed into the Group two CrleGV-SA. The terms Group one and two CrleGV-SA would therefore be used through out this report.

### Amplification of the *granulin* and *egt* genes of the CrleGV-SA isolates

Sequence data for the *granulin* gene, of both Group one and two CrleGV-SA isolates showed a few changes in their nucleotide sequence. However, there was no difference or change in their amino acid sequence, indicating that this gene is highly conserved among the CrleGV isolates. The *granulin* gene is generally conserved among baculoviruses (Federici, 1997).

The *egt* genes of all seven isolates were compared with each other as well as against another Cape Verde CrleGV isolate, CrleGV-CV3 (accession number AY229987) (Lange & Jehle, 2003). CrleGV-CV3 *egt* gene sequence data was obtained from the National Centre for Biotechnology Information (NCBI) database. Sequence data for the *egt* gene, of all isolates, showed some changes in their nucleotide sequence. These differences in their nucleotide sequences translated into some significant changes in their amino acid sequences. This indicates that this gene is highly variable among all CrleGV isolates. A summary of this variation in their amino acid sequences is captured below (Table 3.2.6.2).

**Table 3.2.6.2.** Amino acid substitutions between the *egt* genes of the Groups one and two CrleGV-SA and CrleGV-CV3 (AY229987) isolate.

CrleGV isolate		Amino acids							
		Position							
		96	152	196	225	241	310	315	373
<b>CrleGV-CV3 (AY229987) isolate</b>		V	M	N	R	V	K	I	M
Group one CrleGV-SA	CrleGV-SA Ado	A	I	N	Q	L	R	F	L
	CrleGV-SA Cit	A	I	N	Q	L	R	F	L
	Cryptex	A	I	N	Q	L	R	F	L
	CrleGV-SA Mbl	A	I	N	Q	L	R	F	L
	CrleGV-SA Mix	A	I	N	Q	L	R	F	L
Group two CrleGV-SA	Cryptogran	A	M	H	Q	V	G	F	L
	CrleGV-SA Nels	A	M	H	Q	V	G	F	L

Cape Verde isolate of *Cryptophlebia leucotreta* granulovirus, CrleGV-CV3 (accession #: AY229987) (Source: [www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)). \*Columns with different letters indicate different amino acids.

Analysis of partial gene sequences of the *granulin* and *egt* genes for both the Group one and two CrleGV-SA revealed some evolutionary relationships between them. For example, phylogenetic analysis based on the SNPs observed in their *granulin* and *egt* genes indicated the presence of two *granulin* and *egt* gene types. Analysis of the Group one CrleGV-SA showed that these isolates were closely related and possess a unique CrleGV-SA *granulin* and *egt* gene which was different to the Group two CrleGV-SA. These findings correlate well with the DNA profiles and support the grouping of the seven CrleGV-SA samples into two distinct genome types. More importantly, the amino acid substitutions in their *egt* genes may have significant implications as this could affect viral protein expression and possible differences in viral virulence. Based on the amino acid sequences for the *granulin* and *egt* genes, the *granulin* gene was found to be highly conserved among CrleGV isolates and slightly variable among different GV species. On the other hand, the *egt* gene showed more variability among the Group one and two CrleGV-SA isolates and a higher variability to the CrleGV-CV3 (AY229987) isolate.

### Droplet dose and time-response bioassays

Dosage-mortality bioassays conducted with seven CrleGV-SA isolates against neonates from the Addo colony showed differences in their LC<sub>50</sub> values. The LC<sub>50</sub> values of Cryptex was 3.11-, 2.87-, 2.66-, 2.53-, and 2.26-fold lower than CrleGV-SA Mbl, CrleGV-SA Nels, CrleGV-SA Cit, Cryptogran and CrleGV-SA Ado, against neonates from the Addo colony respectively. The LC<sub>50</sub> values of CrleGV-SA MixC isolate was also 3.76-, 3.47-, 3.22-, 3.06- and 2.74-fold higher than CrleGV-SA Mbl, CrleGV-SA Nels, CrleGV-SA Cit, Cryptogran and CrleGV-SA Ado, against neonates from the Addo colony respectively. The concentration required to elicit 50% mortality in neonates from the Addo colony with Cryptex and CrleGV-SA MixC was slightly higher than the other five CrleGV-SA isolates. For each neonate larva from this colony inoculated with Cryptex or CrleGV-SA MixC, approximately 3 OBs was required to elicit 50% mortality. By contrast, 1 OB from CrleGV-SA Mbl, CrleGV-SA Nels, CrleGV-SA Cit, Cryptogran and CrleGV-SA Ado was enough to cause the same effect. There were also significant differences between the relative potencies of the viral isolates against FCM from the Addo colony (Table 3.2.6.3). For example in Table 3.2.6.3, the potency of Cryptex was lower than CrleGV-SA Mbl, CrleGV-SA Nels, CrleGV-SA Cit, Cryptogran and CrleGV-SA Ado but higher than CrleGV-SA MixC against insects from the Addo colony.

**Table 3.2.6.3** Relative potency comparisons of isolates against 1<sup>st</sup> instar FCM larvae from the Addo colony.

Line/slope	AdoTex	AdoGra	AdoAdo	AdoMix	AdoNel	AdoCit	AdoMbl
AdoTex		2.540	2.263	0.820	2.868	2.666	3.101
AdoGra	0.394		0.891	0.323	1.129	1.050	1.221
AdoAdo	0.442	1.122		0.363	1.267	1.178	1.370
AdoMix	1.219	3.095	2.758		3.495	3.249	3.779
AdoNel	0.349	0.886	0.789	0.286		0.930	1.081
AdoCit	0.375	0.953	0.849	0.308	1.076		1.163
AdoMbl	0.322	0.819	0.730	0.265	0.925	0.860	

\*If value of reference isolate (first column) is < 1.0, then the reference isolate is more potent than the test isolate (first row) and vice versa. A value of 1.0 generated for both reference and test isolate indicates similarities in potency. Treatments-subject combinations are indicated as: AdoTex (Addo colony treated with Cryptex); AdoGra (Addo colony treated with Cryptogran); AdoAdo (Addo colony treated with CrleGV-SA Ado isolate); AdoMix (Addo colony treated with CrleGV-SA MixC isolate); AdoNel (Addo colony treated with CrleGV-SA Nels isolate); AdoCit (Addo colony treated with CrleGV-SA Cit isolate); AdoMbl (Addo colony treated with CrleGV-SA Mbl isolate).

For neonate larvae from the Citrusdal, Nelspruit and Marble Hall colonies inoculated with Cryptogran, Cryptex, CrleGV-SA Nels, CrleGV-SA MixC, CrleGV-SA Ado, CrleGV-SA Mbl and CrleGV-SA Cit there was no significant difference in the number of OBs required to elicit a 50% mortality (LD<sub>50</sub>) in all test insects, as approximately 1 OB was sufficient to cause the desired effect. On the other hand, the LC<sub>50</sub> values of CrleGV-SA Ado were found to be 3.82-, 3.18-, 3.05-, 2.85-, 2.82- and 2.79-fold higher than CrleGV-SA Nels, CrleGV-SA MixC, CrleGV-SA Mbl, Cryptogran, Cryptex and CrleGV-SA Cit, against neonates from the Mixed colony respectively. Based on LC<sub>50</sub> values, the concentration required of CrleGV-SA Ado isolate to elicit 50% mortality in neonates from the Mixed colony was slightly higher than the other six CrleGV-SA. Subsequently, a single neonate larva from this colony, inoculated with CrleGV-SA Ado, required an estimated 3 OB to cause 50% mortality in insects tested, in contrast to the approximately 1 OB required by insects fed with CrleGV-SA Nels, CrleGV-SA MixC, CrleGV-SA Mbl, Cryptogran, Cryptex and CrleGV-SA Cit. There were significant differences between the relative potencies of the viral isolates against FCM from the Mixed colony (Table 3.2.6.4). For example, in Table 3.2.6.4 by comparison the potency of CrleGV-SA Ado was lower than all the other six isolates against insects from the Mixed colony.

**Table 3.2.6.4** Relative potency comparisons of isolates against 1<sup>st</sup> instar FCM larvae from Mixed colony.

Line/slope	MixTex	MixGra	MixMix	MixAdo	MixCit	MixMbl	MixNel
MixTex		1.007	1.134	0.349	0.996	1.083	1.348
MixGra	0.993		1.126	0.346	0.989	1.076	1.339
MixMix	0.882	0.888		0.307	0.878	0.955	1.189
MixAdo	2.868	2.888	3.253		2.856	3.107	3.866
MixCit	1.004	1.011	1.139	0.350		1.088	1.354
MixMbl	0.923	0.929	1.047	0.322	0.919		1.244
MixNel	0.742	0.747	0.841	0.259	0.739	0.804	

\*If value of reference isolate (first column) is < 1.0, then the reference isolate is more potent than the test isolate (first row) and vice versa. A value of 1.0 generated for both reference and test isolate indicates similarities in potency. Treatments-subject combinations are indicated as: MixTex (Mixed colony treated with Cryptex); MixGra (Mixed colony treated with Cryptogran); MixMix (Mixed colony treated with CrleGV-SA MixC); MixAdo (Mixed colony treated with CrleGV-SA Ado); MixNel (Mixed colony treated with CrleGV-SA Nels); MixCit (Mixed colony treated with CrleGV-SA Cit) and MixMbl (Mixed colony treated with CrleGV-SA Mbl).

The LC<sub>50</sub> values for the seven CrleGV-SA isolates reported in this study, ranged between 1.45 x 10<sup>5</sup> – 7.10 x 10<sup>5</sup> OBs/ml. The median survival times (ST<sub>50</sub>) for neonate FCM larvae did not differ substantially from each other and was determined to range between 80 – 88 hours (3.33 – 3.67 days), for all five colonies. The ST<sub>50</sub> values obtained in this study, were similar to that reported by Sciocco-cap *et al.* (2001) in droplet assays with EpapGV (*Epinotia aporema* GV) against neonate *Epinotia aporema* (Wals) which ranged between 85.6 – 94.4 hours (3.56 – 3.93 days).

## Conclusion

Five new CrleGV-SA isolates latent within five FCM laboratory populations were brought into an overt lethal state, using overcrowding as a stressor. Characterisation of the new CrleGV-SA isolates, including Cryptogran and Cryptex isolates were successfully achieved. REN and PCR analysis of the seven isolates showed the presence of two unique CrleGV-SA genome types for the CrleGV-SA isolate. The isolates were genetically different from each other with each isolate consisting of a mixture of several genotypes. Variation in FCM larval susceptibility to different CrleGV-SA isolates was observed in two populations.

## Technology transfer

- Oral presentation at the 17<sup>th</sup> Congress of the Entomological Society of Southern Africa (3<sup>rd</sup> – 6<sup>th</sup> July, 2011).
- Poster presentation at the 7<sup>th</sup> Citrus Research Symposium (19 – 22 August, 2012).

## Further objectives and work plan

It is recommended that future field trials be conducted on the new isolates in order to evaluate their efficacy.

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### 3.2.7 PROGRESS REPORT: Amelioration of the post-harvest cold treatment regime for FCM with the use of carbon dioxide

Experiment 965 (April 2009 – March 2012) by J Terblanche (SU), TG Grout, V Hattingh and PR Stephen (CRI)

#### Opsomming

**CRI:** Na die ontvangs van 'n gasontleider wat CO<sub>2</sub> teen hoë konsentrasies kan meet, kon die invloed van hoë konsentrasies CO<sub>2</sub> op valskodlingmot (VKM) larwes in vrugte evalueer word. Slegs vier eksperimente kon gedurende die laaste gedeelte van die seisoen gedoen word, terwyl vrugte nog beskikbaar was. Eksperimente het gewys deur gedeeltes van eiervelle op Valencia-lemoene te plaas, die vrugte net so effektief soos nawel-lemoene kunsmatig besmet kon word. Kort 5 dag kouebehandelings teen -0.5°C het 'n dodetal van tussen 40 en 71% veroorsaak in die verskillende eksperimente, terwyl 8% mortaliteit of laer voorgekom het in die eksperiment waar 30% CO<sub>2</sub> vir 12 h teen 25°C in die lug gehandhaaf is. Verder het daar 36% mortaliteit of laer voorgekom wanneer 60% CO<sub>2</sub> vir 12 h teen 25°C in die lug gehandhaaf is. Kombinasies van 12 h beroking met 30% CO<sub>2</sub>

onmiddellik gevolg deur 'n wasbehandeling en 'n 5 dag kouebehandeling het mortaliteit van ongeveer 60% veroorsaak. Die vertraging van die kouebehandeling vir 24 h na die wasbehandeling het daartoe gelei dat mortaliteit met ongeveer 35% afgekom het. Die uiteindelijke mortaliteitsvlak was laer as dié met net kouebehandeling. Kombinasies van 12 h beroking met 60% CO<sub>2</sub> onmiddellik gevolg deur 'n wasbehandeling en 'n 5 dag kouebehandeling het mortaliteit van ongeveer 90% veroorsaak, maar die vertraging van die kouebehandeling vir 24 h na die wasbehandeling het daartoe gelei dat mortaliteit met ongeveer 25% verminder het. In hierdie geval, was die uiteindelijke mortaliteit met die kouebehandelings na die onderbreking steeds hoër as die kouebehandelings alleenlik. Verdere navorsing met 60% CO<sub>2</sub> vir 24 h sal uitgevoer word met varierende intervalle tussen beroking en kouebehandeling.

**SU:** Die kritiese termiese minima (deur gebruik te maak van termo-limiet respirometrie) en die superverkouingspunt van VKM larwes is onder 'n reeks van O<sub>2</sub> konsentrasies (2.5%, 5%, 10%, 24% en 40%) ondersoek. Nie die  $KT_{min}$  of die SCP is betekenisvol geaffekteer deur die veranderde atmosfeer nie ( $KT_{min}$ :  $P > 0.38$ ; SCP:  $P > 0.9$ ), wat daarop dui dat die wyse waarop die gasbehandelings aangewend is, nie die gevolg van 'n verandering in die verdraagsaamheid van koue was nie.

Addisionele behandelings sluit in 'n kombinasie van CO<sub>2</sub> en koue voor die standaard 10 h teen -1°C behandeling, sowel as ses addisionele langtermyn behandelings (6% CO<sub>2</sub> vir 24 h teen 4°C voor 'n reeks van herstel-behandelings, gevolg deur 5 dae teen -1°C). Die ses addisionele behandelings het almal 'n betekenisvolle toename in mortaliteit, in vergelyking met die oorspronklike nege behandelings, getoon wat moontlik as gevolg van die toenemende duur van blootstelling aan koue was. Die behandeling behels 24 h van 6% CO<sub>2</sub> teen 4°C gevolg deur 'n herstel periode teen 25°C vir 2 h, 3 dae se blootstelling aan -1°C, 'n herstel periode van 25°C vir 2 h, waarna 5 dae se blootstelling teen -1°C volg en veroorsaak 100% mortaliteit. In vergelyking, 'n soortgelyke behandeling waar die larwes teen 25°C vir die middelste 3 dae gehou was (in plaas van 3 dae teen -1°C), het slegs 21% mortaliteit veroorsaak. Dit dui aan dat die verlengde tydperk wat by -1°C deurgebring is (n totaal van 8 dae) tesame met die herstel periode teen 25°C vir 2h, voor die 5 dae teen -1°C, 'n beduidende toename in larwe mortaliteit veroorsaak. Verder, is papievorming en ontpopping aangeteken en het gewys dat in gevalle waar hoë oorlewing gebeur het, het larwes gewoonlik hulle ontwikkeling tot volwassendheid voltooi.

Die ontleding van die lewensvatbaarheid van lewende/dooie selle was vir alle behandelings uitgevoer en die data is in die proses om vanaf die mikroskoopbeelde ontgin te word. 'n ELISA van HSP70 was vir alle behandelings (n = 246) gedoen. Die voorlopige resultate dui aan dat daar betekenisvolle veranderinge binne en tussen die behandelings is. Nietemin, addisionele monsters sal ondersoek word om 'n duideliker antwoord vir die rigting van hierdie veranderinge te verskaf.

## Summary

**CRI:** Trials to evaluate the effect of CO<sub>2</sub> on false codling moth (FCM) larvae in fruit could at last be initiated with receipt of a gas analyser that could measure high concentrations of CO<sub>2</sub>. Only four trials could be conducted during the latter part of the season while fruit were still available. These trials showed that Valencias could be artificially infested just as effectively as navel oranges by placing pieces of egg-sheets on the fruit. Short 5 day cold treatments at -0.5°C caused from 40 to 71% mortality in the different trials whereas 30% CO<sub>2</sub> in air for 12 h at 25°C caused 8% or less mortality and 60% CO<sub>2</sub> in air for 12 h at 25°C caused 36% or less mortality. Combinations of 12 h fumigation with 30% CO<sub>2</sub> followed immediately by waxing and a 5 day cold treatment resulted in approximately 60% mortality but delaying the cold treatment by 24 h after waxing reduced the mortality by around 35%. The resulting level of mortality was lower than with the cold treatment alone. Combinations of 12 h fumigation with 60% CO<sub>2</sub> followed immediately by waxing and a 5 day cold treatment resulted in approximately 90% mortality but delaying the cold treatment by 24 h after waxing reduced the mortality by around 25%. In this case the resultant mortality with the cold treatment after the interval was still higher than the cold treatment alone. Further research will be conducted using 60% CO<sub>2</sub> for 24 h and varying the interval between fumigation and cold treatment.

**SU:** The critical thermal minima (using thermolimit respirometry) and supercooling point of FCM larvae were investigated under a range of O<sub>2</sub> concentrations (2.5%, 5%, 10%, 21%, 40%). Neither CTmin nor SCP was significantly affected by the altered atmosphere (CTmin:  $P > 0.38$ ; SCP:  $P > 0.9$ ) indicating that the mode of action of gas treatments is likely not a result of changing the cold tolerance.

The additional treatments involving CO<sub>2</sub> and cold in combination before the standard 10h at -1°C, as well as the additional 6 longer-term treatments (24h of 6% CO<sub>2</sub> at 4°C before a range of recovery treatments, followed by 5 days at -1°C), have been completed. The additional 6 treatments all resulted in a significant increase in mortality

relative to the initial 9 treatments, likely as a result of the increased duration of the cold exposure. The treatment involving 24 h of 6% CO<sub>2</sub> at 4°C followed by recovery at 25°C for 2 h, cold exposure of 3 days at -1°C, 25°C for 2 h, then 5 days at -1°C resulted in 100% mortality. In comparison, a similar treatment that kept larvae at 25°C for the middle 3 day period (instead of 3 days at -1°C) resulted in only 21% mortality. This suggests that the increased time spent at -1°C (totalling 8 days) together with the recovery at 25°C for 2 h before the 5 days at -1°C is causing a significant increase in larval mortality. In addition, pupation and emergence were scored and showed that in cases where high survival was observed, larvae generally completed the transition into adults.

Live/dead cell viability assays have been completed for all treatments and the data are in the process of being extracted from the microscope images. ELISA of HSP70 has been completed for all treatments (n = 246). The preliminary results indicate that there are significant changes within and between treatments, however, additional samples will be analyzed to provide a more definitive answer as to the direction of these changes.

### 3.2.8 **FINAL REPORT: The efficacy of Coragen for control of false codling moth in the Eastern Cape** Experiment 973 (April 2009 – June 2011) by Sean Moore and Wayne Kirkman (CRI)

#### **Opsomming**

Vyf veldproewe is met Coragen (200 g/L SC) vir beheer van VKM op nawellemoene in die Oos-Kaap uitgevoer. Twee van die proewe is op klein bome en een op matige grote bome uitgevoer en het net een toediening behels met die hoofdoel om die tydsduur van residuele nawerking te bepaal. Die ander twee proewe is op groot bome uitgevoer en het albei enkel- en dubbel-behandelings, 'n paar weke uitmekaar, behels. In die twee klein boom proewe het Coragen VKM besmetting met 64% en 57% verminder, met doeltreffendheid vergelykbaar aan Cryptogran, Delegate en Alsystin. In die medium-boomgrote proef het Coragen VKM besmetting met 53% verminder. Omdat hierdie 'n laat seisoen proef was en die bome daarom digter was, goeie resultate teen VKM op hierdie stadium was natuurlik moeiliker. Al lyk 'n 53% afname nie baie indrukwekkend nie was Coragen saam met Cryptogran die mees doeltreffende behandeling in die proef, waarin 'n totaal van sewe geregistreerde produkte vergelyk is. In die groot boom proewe is VKM besmetting met net 31% en 32% verminder, ten spuite van die feit dat hierdie dubbel-toedings was. Hierdie swak werking is gekoppel aan onvoldoende spuit bedekking as gevolg van die verskaffers se beperking tot nie meer as 8500 L per hekaar per toediening. Hierdie hipotese kon nie ondersteun word in 'n proef wat die volgende seisoen op dieselfde bome uitgevoer is nie. Nietemin was die werking van albei beperkte en onbeperkte volumes Coragen beter in die tweede proef in vergelyking met die vorige een. VKM besmetting is met tussen 48% en 55% verminder. Verskille in die werking van Coragen mag dus dalk veroorsaak word deur 'n kombinasie van hoë plaagdruk en onvoldoende spuitvolume per boom grote wees.

#### **Summary**

Five field trials were conducted with Coragen (200 g/L SC) for control of FCM on navel oranges in the Eastern Cape. Two of the trials were conducted on small trees and one was conducted on moderately sized trees and all consisted of only one application. One of the main purposes of these trials was to determine the period of residual efficacy. The other two trials were conducted on large trees and included both single applications and double applications, spaced a few weeks apart. In the two small-tree trials, Coragen reduced FCM infestation by 64% and 57%, with comparable efficacy to Cryptogran, Delegate and Alsystin. In the medium-tree trial Coragen reduced FCM infestation by 53%. However, as this was a late season application on trees which has spent the season increasing in density, achievement of good results would be more difficult. Additionally, Coragen along with Cryptogran, was the most effective treatment in this trial, in which a total of seven registered products were compared. In the first large-tree trial, FCM infestation was reduced by only 31% and 32%, despite these being double applications. This poor efficacy was related to inadequate spray coverage due to the product suppliers restricting application to no more than 8500 L per hectare per application. This hypothesis could not be supported in a trial conducted the following season on the same trees. However, efficacy of both limited and unlimited volume Coragen applications were superior in this second trial compared to the previous one. FCM infestation was reduced by between 48% and 55%. Differences in efficacy of Coragen may therefore be related to a combination of high pest pressure and inadequate spray volume per tree size.

#### **Introduction**

Citrus Research International (CRI) was contracted to conduct efficacy trials with a new insecticide, Coragen (200 g/L SC), for the control of false codling moth (FCM) in citrus orchards. Coragen is a registered trademark of DuPont and its affiliates. This is the third season of such trials conducted in the Eastern Cape.

## **Stated objectives**

To test the efficacy of Coragen against FCM in citrus and to interpret results to assist in establishing the best possible usage of the product.

## **Materials and methods**

Sprays in all trials were applied with high pressure hand guns.

### Field trial 1

A trial was conducted on Kleinwelgemoed Farm near Kirkwood, Sundays River Valley. The main purpose of the trial was to compare various virus treatments (Kirkman et al, 2010). It was decided to include a Coragen spray, with the objective of determining the residual efficacy or point of breakdown of Coragen. An orchard of 4 year old (planted 2006) Palmer Navel orange trees was used (orchard 11). Trees were spaced at 6 m x 3 m (rows x trees), giving 555 trees per hectare. The trial was laid out in a single-tree randomised block design, replicated 10 times. Several treatments, other than Coragen, were included. These were all virus treatments, the results of which are given in full in a separate report (Kirkman et al, 2010). Coragen (17.5 ml per 100 L water) was sprayed on 9 December 2009 at an average of 18.5 L of spray mix per tree. This would extrapolate to 10 267 L of spray mix per hectare, higher than the registered permissible volume of 8 500 L per hectare (may be applied twice). The trial was evaluated for seven weeks from 5 January to 25 February 2010.

### Field trial 2

Another trial was conducted on Penhill Farm near Barkley's Bridge in the Sundays River Valley. An orchard of 26 year old (planted 1984) Palmer Navel orange trees was used for the trial. Trees were spaced at 6 m x 3 m (rows x trees), giving 555 trees per hectare. The trial was laid out in a single-tree randomised block design, replicated 10 times. There were three treatments: an untreated control; a double Coragen (17.5 ml/100 L water) on 2 February 2010 and again 6 weeks later (16 March); a double Coragen on 2 February and again 8 weeks later (2 April). The February spray was applied at an average of 15 L per tree, extrapolating to 8325 L per ha. The March and April sprays were applied at an average of 16.5 L per tree, extrapolating to 9157.5 L per ha. These two later sprays were therefore applied at a volume slightly higher than the permissible maximum limit of 8500 L per ha. The trial was evaluated for 15 weeks from 23 February until 1 June 2010.

### Field trial 3

A third trial was conducted on Far Away Farm in the middle of the Sundays River Valley. An orchard of four-year old (planted 2007) Newhall Navel orange trees (orchard 54) was used for the trial. Trees, which were still small, were spaced at 6 m x 3 m (rows x trees), giving 555 trees per hectare. The trial was laid out in a single-tree randomised block design, replicated 10 times. There were a total of 17 treatments. Most of them were virus treatments, applied for comparison in another trial and are therefore not referred to here. For the purpose of comparison within this trial, only the chemical pesticides are included i.e. Coragen, Delegate and Alsystin. Coragen was mixed at 17.5 ml per 100 L water; Delegate was mixed at 20 ml per 100 L water; and Alsystin was mixed at 20 ml per 100 L water. All treatments were sprayed only once on 7 December 2010 at an average of 10.7 L per tree. The trial was evaluated for a 9-week period from 11 January to 8 March 2011. Unlike the previous two trials, evaluation was commenced four weeks after spraying, rather than three weeks.

### Field trial 4

A fourth trial was conducted on Penhill Farm as in Field trial 2. The trial was conducted in the same orchard and similarly laid out in a single-tree randomised block format, replicated 10 times. Five treatments were applied: Coragen applied twice, four weeks apart; Coragen applied once as a full cover spray; Coragen applied once at an even higher volume; and Delegate applied twice, four weeks apart. Coragen was mixed at 17.5 ml per 100 L water and Delegate was mixed at 20 ml per 100 L water. The double Coragen was sprayed on 14 March and 12 April 2011 at 15.5 and 16.0 L per tree respectively. This extrapolated to 8602 and 8880 L per ha. The two single spray Coragen treatments were applied on 14 March at 28 L and 43 L per tree, extrapolating to 15540 and 23865 L per hectare respectively. Each Delegate spray was applied on the same dates as the double Coragen treatment, at 26 L and 28 L per tree, extrapolating to 14430 and 15540 L per hectare. The trial was evaluated for 11 weeks from 5 April to 21 June 2011.

### Field trial 5

A fifth trial was conducted on Bernol Farm (Willem Bouver) in the middle of the Sundays River Valley. An orchard of six-year old (planted 2005) Lane Late Navel orange trees (Orchard 9) was used for the trial. Trees were spaced at 6 m x 3 m (rows x trees), giving 555 trees per hectare. The trial was laid out in a single-tree randomised block design, replicated 10 times. There were a total of 13 treatments. Most of them were experimental virus treatments, applied for comparison in another trial and are therefore not referred to here. Only the registered treatments in the trial are included here for comparative purposes. The treatments were Coragen (17.5 ml/100 L water), Delegate (20 ml/100 L water), Alsystin (20 ml/100 L water), Meothrin (30 ml/100 L water), cypermethrin (25 ml/100 L water), Cryptogran (10 ml + 250 ml molasses + 5 ml Break-Thru/100 L water) and Cryptex (3.3 ml + 500 ml molasses/100 L water). All treatments were applied on 4 May at an average of 15 L per tree, extrapolating to 8325 L per hectare. The trial was evaluated for six weeks from 26 May to 28 June 2011.

### Evaluation of field trials

All trials were evaluated and analysed in the same way, unless otherwise stated. Two weeks after spraying, all fallen fruit were cleared from underneath data (sprayed and untreated) trees. A further week later (i.e. three weeks after spraying) fruit were collected from underneath each data tree. Fruit were analysed separately per tree. This was conducted each week on the same day. Fruit were then assessed for FCM infestation. A fruit was categorised as FCM infested if there was an FCM larva or its frass in the fruit.

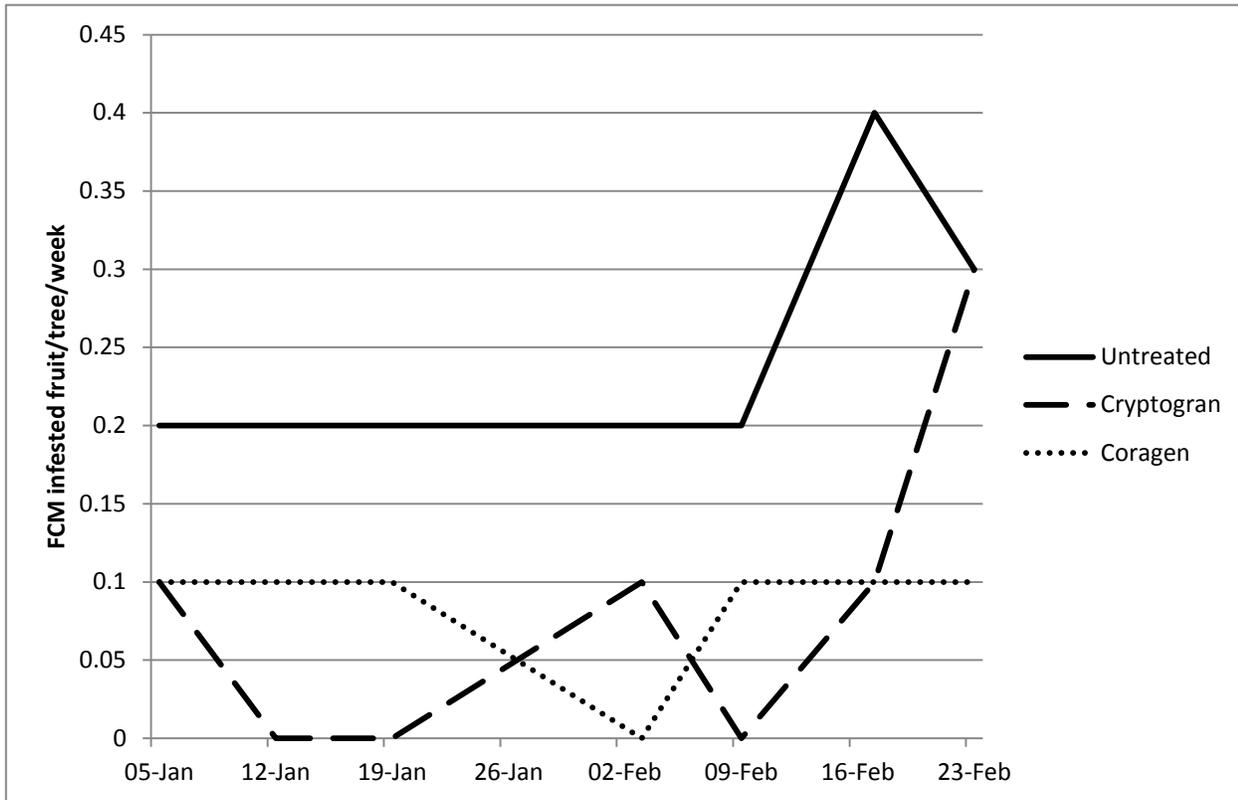
### Statistical analysis

Means of fruit infested per tree per week for all treatments were compared using an ANOVA and Bonferroni multiple range test with Statgraphics Plus for Windows Version 5.1 (Statistical Graphics Corporation 2001).

## **Results and discussion**

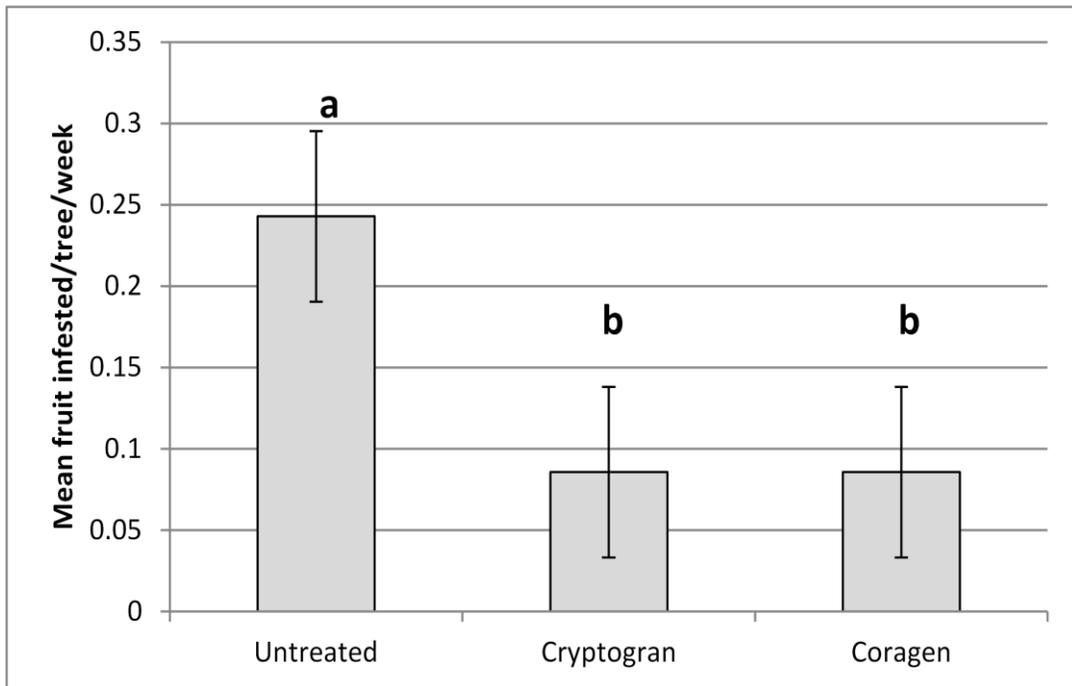
### Field trial 1

The trial was only evaluated for seven weeks, which proved inadequate for determining the point of breakdown of Coragen. When the trial was terminated, there was no sign of a decline in efficacy (Fig. 3.2.8.1). However, as the main purpose of the trial was to compare the virus treatments, there was inadequate justification to continue with the trial. Additionally, FCM infestation was very low, possibly making results less reliable. At this point, there clearly was a breakdown in the efficacy of Cryptogran. This was to be expected, as the trees were small (only four years old) and therefore did not offer adequate protection (shading) against deleterious UV-irradiation.



**Figure 3.2.8.1** FCM infestation of Navel oranges on Kleinwelgemoed Farm, treated with Coragen or Cryptogran, evaluated from 5 January to 23 February 2010.

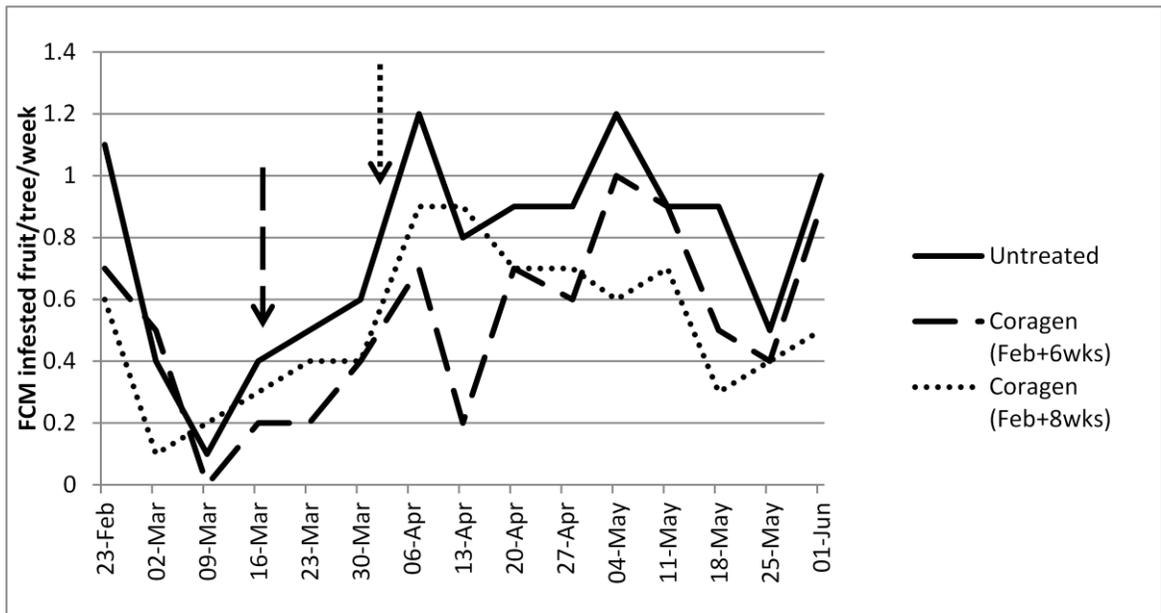
Apart from noting that the residual efficacy of Coragen was at least seven weeks, it was also interesting to note that efficacy was superior to what had been recorded in a previous trial. Previously, two double Coragen applications (the second application in each set being applied at slightly different times) resulted in reductions in FCM infestation of 32% and 41% (Moore & Kirkman, 2010). However, in this trial Coragen caused a 64.7% reduction in FCM infestation over a seven week period (Fig. 3.2.8.2). This was identical to the efficacy of Cryptogran applied at the same time. Mean FCM infestation per tree per week (in numbers of fruit) was  $0.24 \pm 0.03$  (mean  $\pm$  SE) for the control,  $0.09 \pm 0.04$  for Cryptogran and  $0.09 \pm 0.01$  for Coragen. However, Coragen did appear to have a better residual efficacy than Cryptogran on the small trees which were used. The difference in infestation between Coragen treated trees and untreated trees was significant ( $\alpha = 0.05$ ).



**Figure 3.2.8.2** Mean FCM infestation of Coragen-treated and untreated Navel oranges on Kleinwelgemoed Farm over a 7-week period (5 January to 23 February 2010). Bars with the same letters are not significantly different (Bonferroni multiple range test;  $\alpha = 0.05$ ).

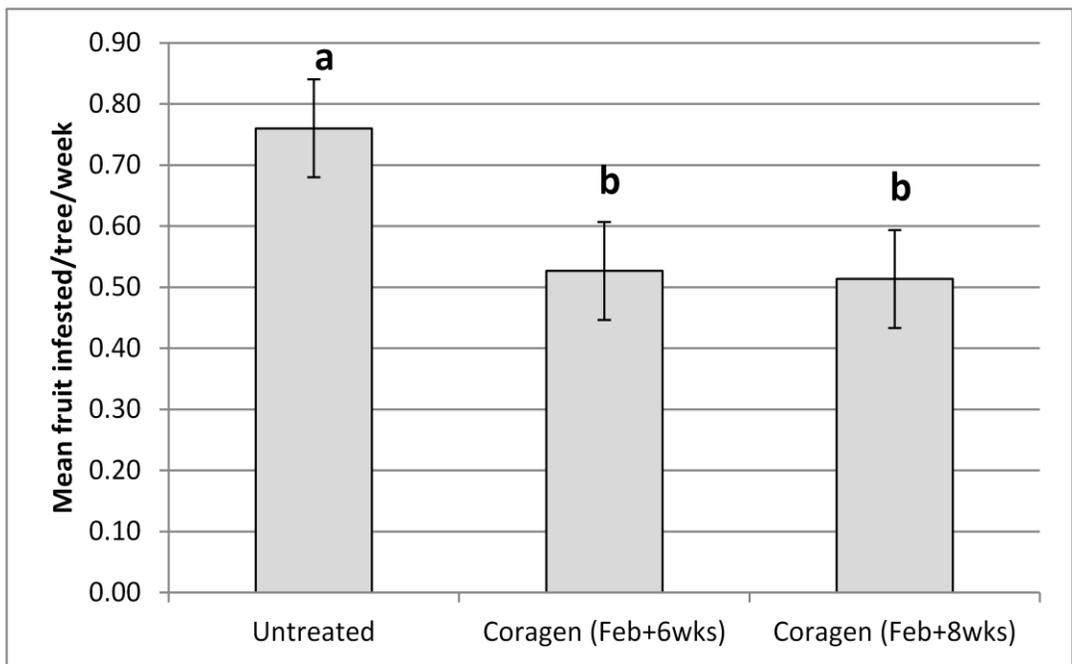
#### Field trial 2

Results with Coragen were not impressive. There was a notable reduction in infestation (Fig. 3.2.8.3), but this equated to only a 30.7% and 32.5% reduction in infestation over a 15 week period with the two double Coragen applications (Fig. 3.2.8.4). Mean FCM infestation per tree per week over this time was  $0.76 \pm 0.08$  (mean  $\pm$  SE) for the control and  $0.53 \pm 0.08$  and  $0.51 \pm 0.06$  for the two treatments respectively. However, the difference between the two treatments and the control was statistically significant ( $\alpha = 0.05$ ; LSD multiple range test). The previous trial (Field trial 1 above) indicated that there was no detectable breakdown for the full 7-week duration of the trial. There should not have been any breakdown in residual efficacy of Coragen during this time, as there was a maximum gap of 8 weeks between the second spray and the last evaluation date; and it will normally take at least three weeks for an infested fruit to drop. Therefore, according to the evaluation method used in this trial, breakdown should only be detected a minimum of three weeks after it has actually occurred.



**Figure 3.2.8.3** FCM infestation of Coragen-treated and untreated Navel oranges on Penhill Farm, evaluated from 23 February to 1 June 2010. The dashed arrow indicates the second application (on 16 March) after 6 weeks; the dotted arrow indicates the second application (on 1 April) after 8 weeks.

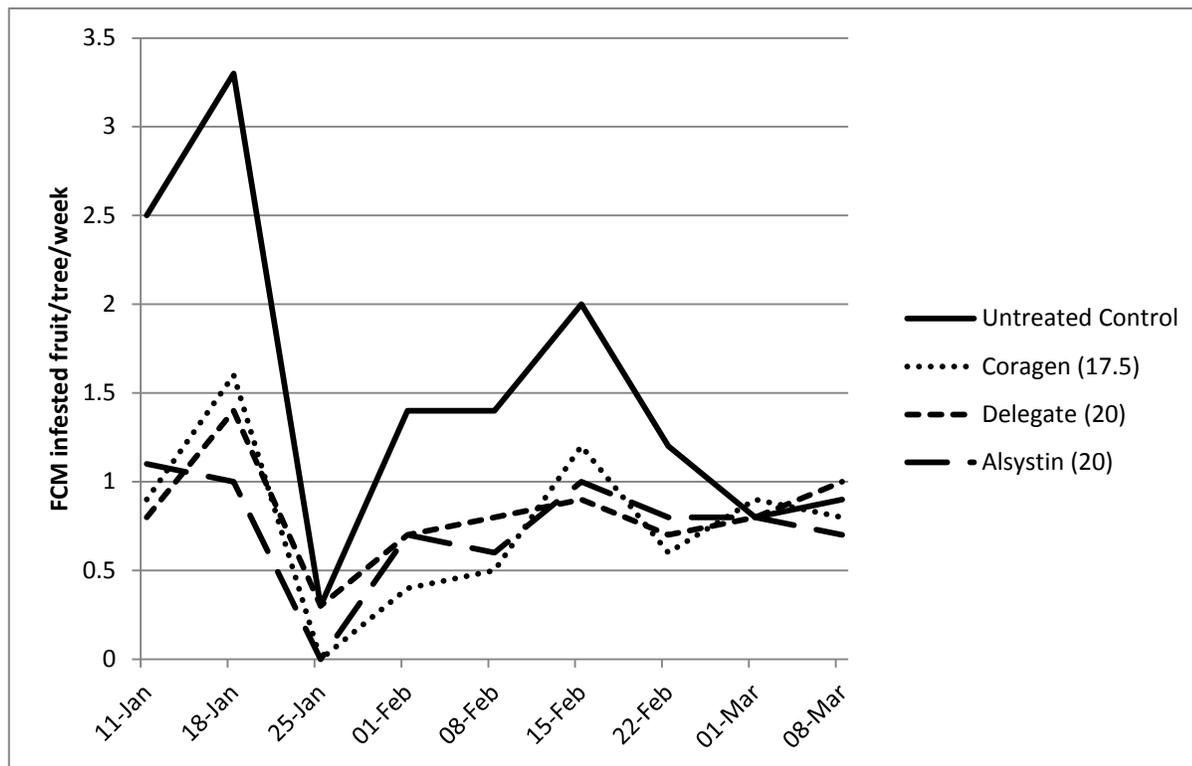
It was therefore hypothesised that the poor efficacy was a result of inadequate spray application on very large trees. A restriction of 8500 L per hectare may be fine for optimal efficacy on small or medium-sized trees but can be hopelessly inadequate on very large trees. Both Coragen trials conducted on very large trees i.e. this and a previous one (Moore & Kirkman 2010), showed poor results, whereas the one trial conducted on small trees (see results for field trial 1) showed far better results. However, another difference between the trials was the FCM pressure, which was higher in the two trials conducted on large trees (Far Away Farm and Penhill Farm) than in the trial conducted on small trees (Kleinwelgemoed Farm). Mean FCM infestation in the untreated controls in the two former trials was 0.82 (Moore & Kirkman, 2010) and 0.76, whereas FCM infestation in the untreated control of the latter trial was only 0.24 (infested fruit per tree per week).



**Figure 3.2.8.4** Mean FCM infestation of Coragen-treated and untreated Navel oranges on Penhill Farm over a 15-week period (23 February to 1 June 2010). Bars with the same value are not significantly different (LSD multiple range test;  $\alpha = 0.05$ ).

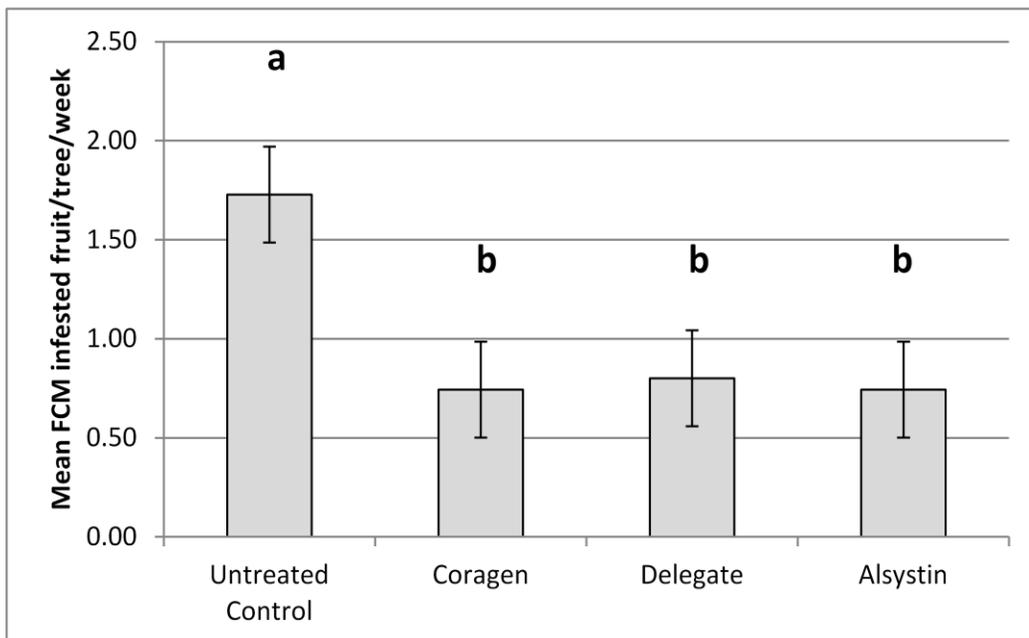
### Field trial 3

Initially, there was a dramatic difference visible in fruit infestation in the control and the three chemical treatments (Fig. 3.2.8.5). However, as infestation in the untreated control declined, so too did the difference between treatments and control. This is unlikely to have been an indication of decline in residual efficacy. By eight weeks post-treatment, the efficacy of all three treatments appeared to have broken down. The trial was evaluated for a further week, at which time there was no notable recovery by any of the treatments. As evaluation of the trial was initiated a week later than was the norm (i.e. four weeks after spraying instead of three weeks), it can be stated that the efficacy of Coragen had broken down by nine weeks after spraying. The residual efficacy of the product is therefore estimated at eight weeks.



**Figure 3.2.8.5** FCM infestation of treated and untreated Navel oranges on Far Away Farm, evaluated from 11 January to 8 March 2011.

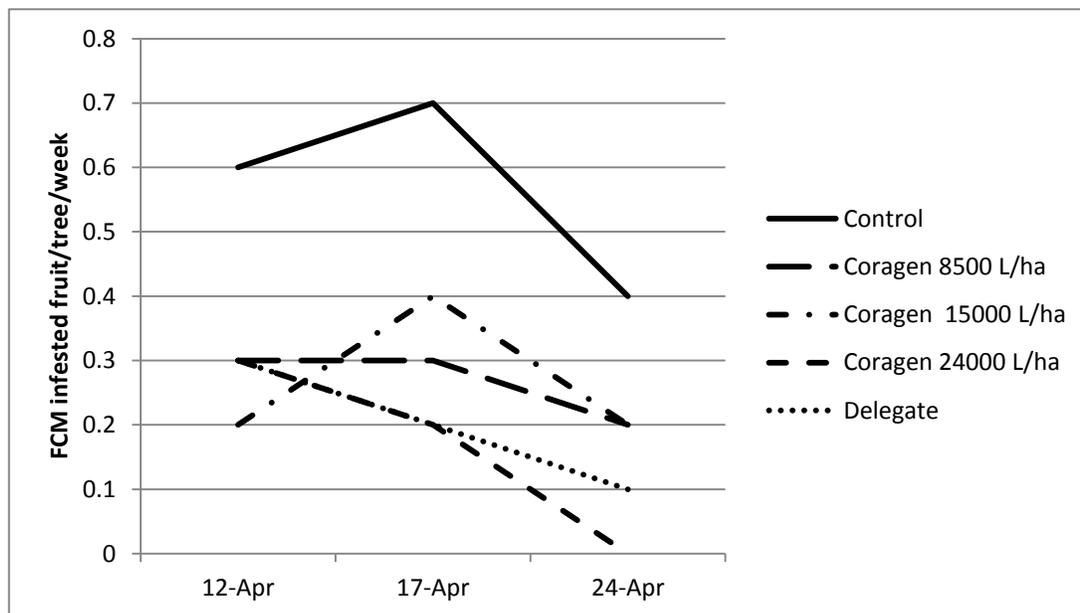
Mean FCM infestation in the untreated control for the full duration of the trial was  $1.53 \pm 0.31$  (mean  $\pm$  SE) fruit per tree per week. For Coragen, Delegate and Alsystin it was  $0.77 \pm 0.15$ ,  $0.82 \pm 0.10$  and  $0.74 \pm 0.11$ , respectively (Fig. 3.2.8.6). There was a significant difference between all three treatments and the control ( $\alpha = 0.05$ ; LSD multiple range test), but there was no difference between any of the three treatments. Considering only the first seven weeks of evaluation (11 January to 22 February), FCM infestation was reduced by 57.0%, 53.7% and 57.0% for Coragen, Delegate and Alsystin, respectively. Coragen therefore showed good efficacy even against high FCM pressure. The reason for the poor efficacy recorded on large trees in the other trials may therefore not have been due only to high FCM pressure, but may have been influenced by inadequate spray coverage. It is therefore imperative that the restriction of 8500 L per hectare limitation be reviewed by the registration holders of Coragen.



**Figure 3.2.8.6** Mean FCM infestation of treated and untreated Navel oranges on Far Away Farm over a 9-week period (11 January to 8 March 2011). Bars with the same letter are not significantly different (LSD multiple range test;  $\alpha = 0.05$ ).

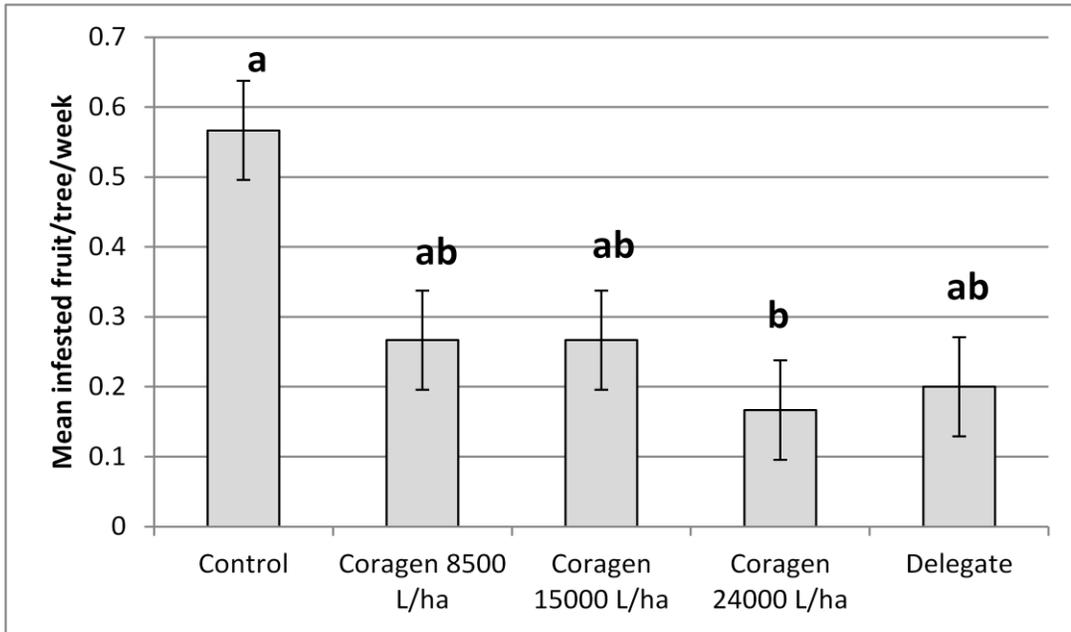
#### Field trial 4

Data from the first week of evaluation has not been included, as the effect of the treatments was not yet evident. A first analysis was conducted on the data from only the first three weeks (Fig. 3.2.8.7). This was in order to compare the efficacy of only the first sprays.



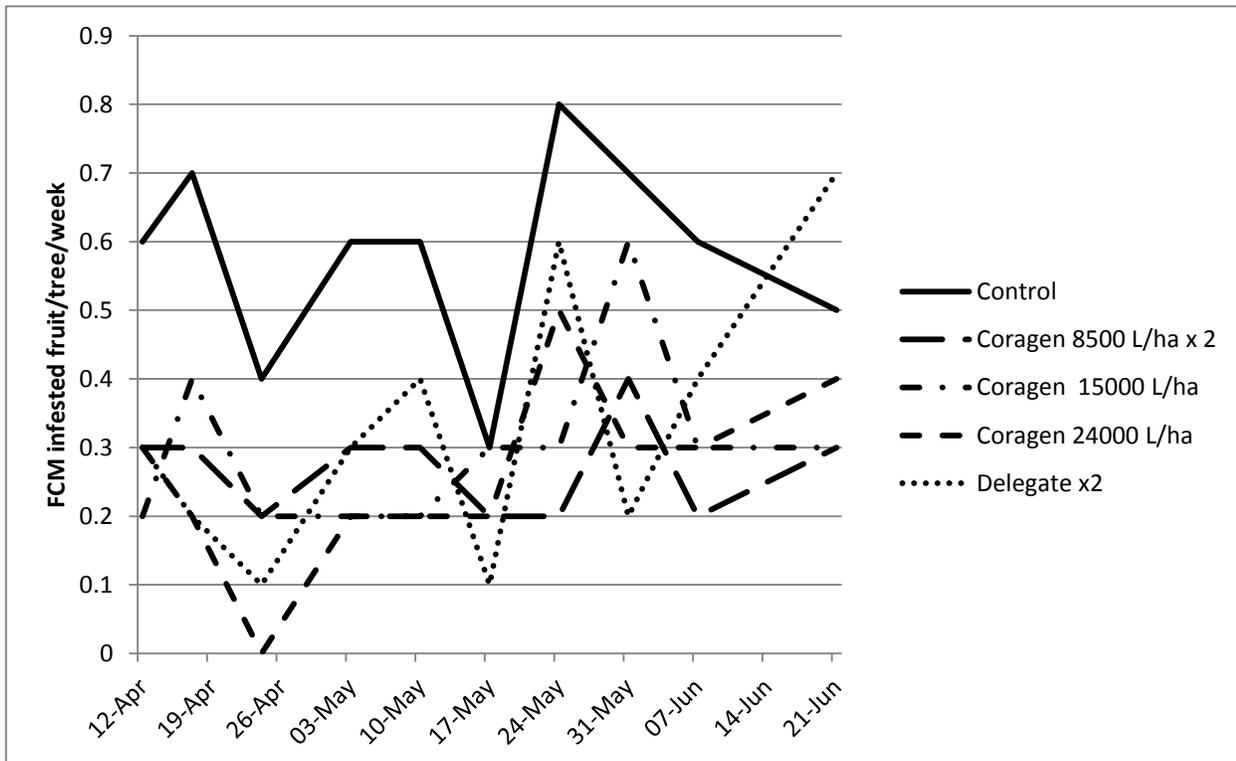
**Figure 3.2.8.7** FCM infestation of treated and untreated Navel oranges on Penhill Farm, recorded for the first three weeks of the evaluation period, from 12 to 24 April 2011.

There did not appear to be a great difference in the efficacy of treatments. However, when statistically analysed, the Coragen application was the only treatment for which significantly less FCM infestation than the untreated control was recorded (Fig. 3.2.8.8). Coragen applied at around 8500 L per hectare and Coragen applied at around 15000 L per hectare, both reduced FCM infestation by 52.9%. Coragen applied at around 24000 L per hectare reduced FCM infestation by 70.6% and Delegate at 15 000 L per hectare reduced FCM infestation by 64.7%.



**Figure 3.2.8.8** Mean FCM infestation of treated and untreated Navel oranges on Penhill Farm over the first three weeks of the evaluation period (12 to 24 April 2011). Bars with the same letter are not significantly different (LSD multiple range test;  $\alpha = 0.05$ ).

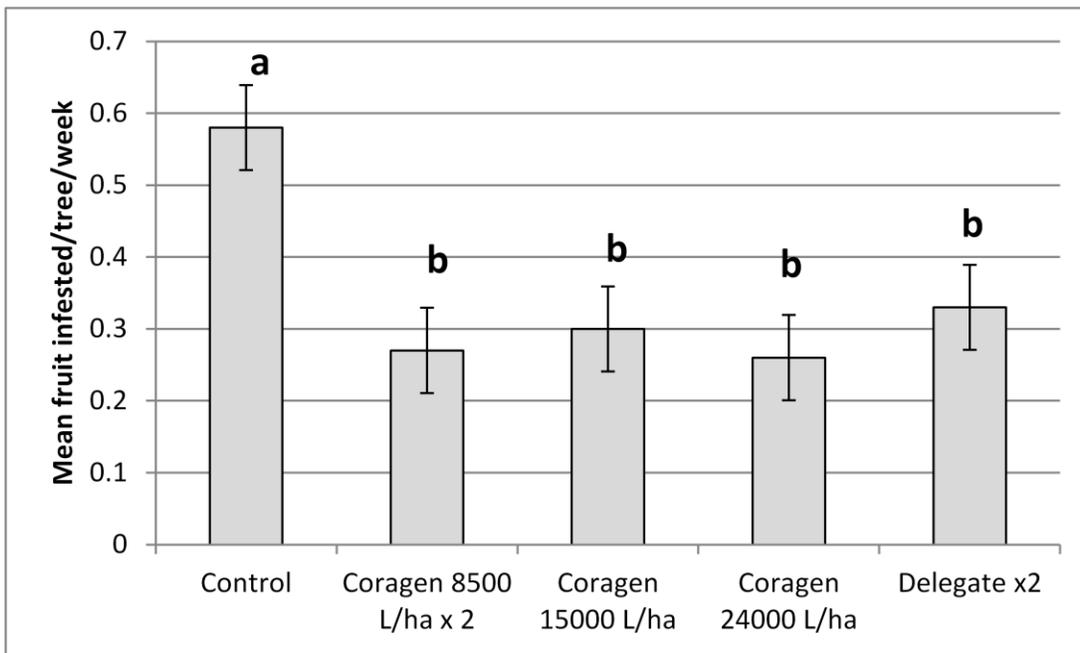
The differences between treatments recorded during the first three weeks of evaluation (i.e. those based on only a single application of each treatment) were not maintained. The very high volume Coragen was unable to retain its superiority. Both high volume applications of Coragen reduced FCM marginally by more than did the low volume application of FCM. However, this difference seemed to disappear by about 10 May (Fig. 3.2.8.9). This may have been due to the second low volume Coragen application taking effect.



**Figure 3.2.8.9** FCM infestation of treated and untreated Navel oranges on Penhill Farm, evaluated from 12 April to 21 June 2011.

Mean FCM infestation in the untreated control for the full duration of the trial was  $0.58 \pm 0.05$  (mean  $\pm$  SE) fruit per tree per week. For the double Coragen (8500 L/ha) it was  $0.27 \pm 0.02$  fruit;

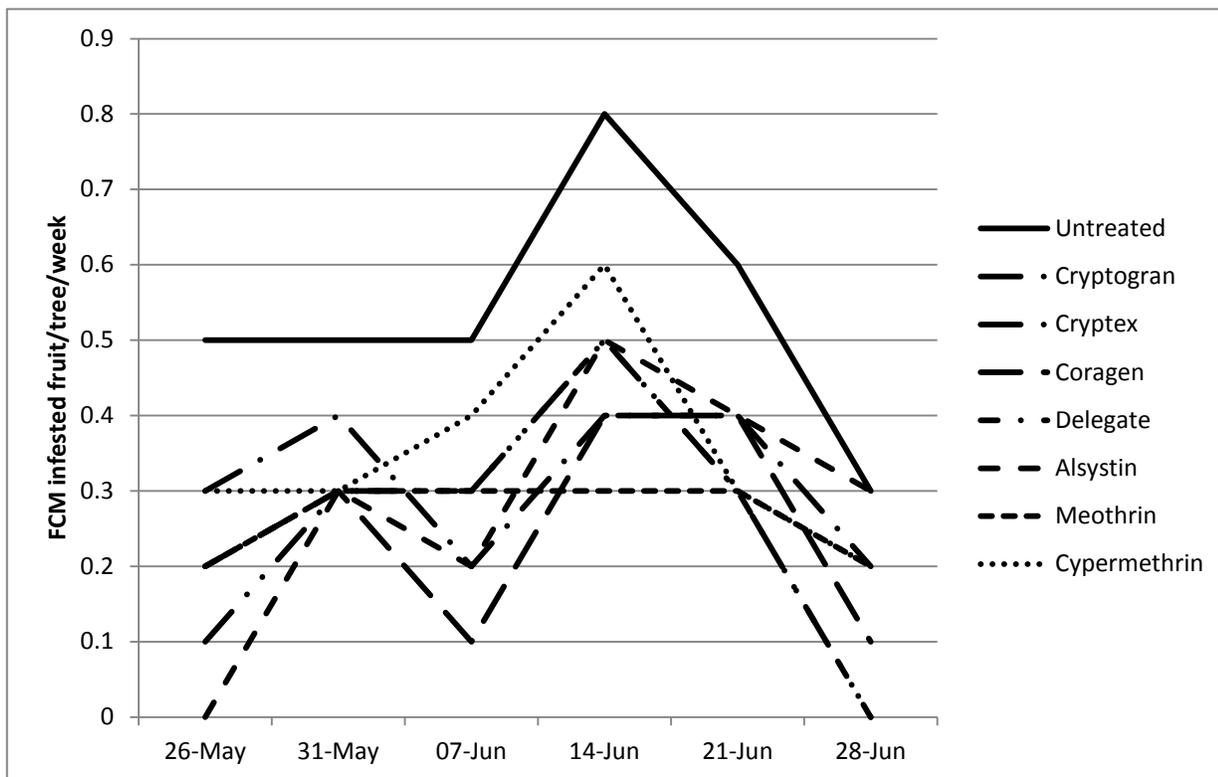
it was  $0.30 \pm 0.04$  fruit; for single Coragen at 24000 L/ha it was  $0.26 \pm 0.04$  fruit; and for Delegate it was  $0.33 \pm 0.06$  fruit (Fig. 3.2.8.10). Over the full 10 week evaluation period, there was no statistically significant difference between any of the treatments (Fig 3.2.8.10). However, they all succeeded in significantly reducing FCM infestation relative to the untreated control. The double Coragen application (at around 8500 L per hectare) reduced FCM infestation by 53.4%; the single Coragen at around 15000 L per hectare reduced FCM infestation by 48.3%; the single Coragen application at around 24000 L per hectare reduced FCM infestation by 55.2%; and the double Delegate at 15 000 L per hectare reduced FCM infestation by 43.1%. These results did not support the theory that the restricted volume of 8500 L per hectare was inadequate on large trees. It was previously mentioned that another possible reason for differences in efficacy (measured by reduction in FCM infestation) could be level of pest pressure. The data from this trial may support this theory, as FCM infestation in the untreated control in this trial averaged 0.58 fruit per tree per week, compared to 0.82 (Moore & Kirkman, 2010) and 0.76 fruit per tree per week in the large-tree trials in which Coragen performed poorly. However, efficacy of Coragen in Field trial 3 at Far Away Farm was good, despite FCM pressure being high (average of 1.53 infested fruit per tree per week in the untreated control). Poor efficacy on large trees may therefore not be a simple matter. It may be related to a combination of inadequate spray volume per tree size and high pest pressure.



**Figure 3.2.8.10** Mean FCM infestation of treated and untreated Navel oranges on Penhill Farm over a 10-week period (12 April to 21 June 2011). Bars with the same letter are not significantly different (LSD multiple range test;  $\alpha = 0.05$ ).

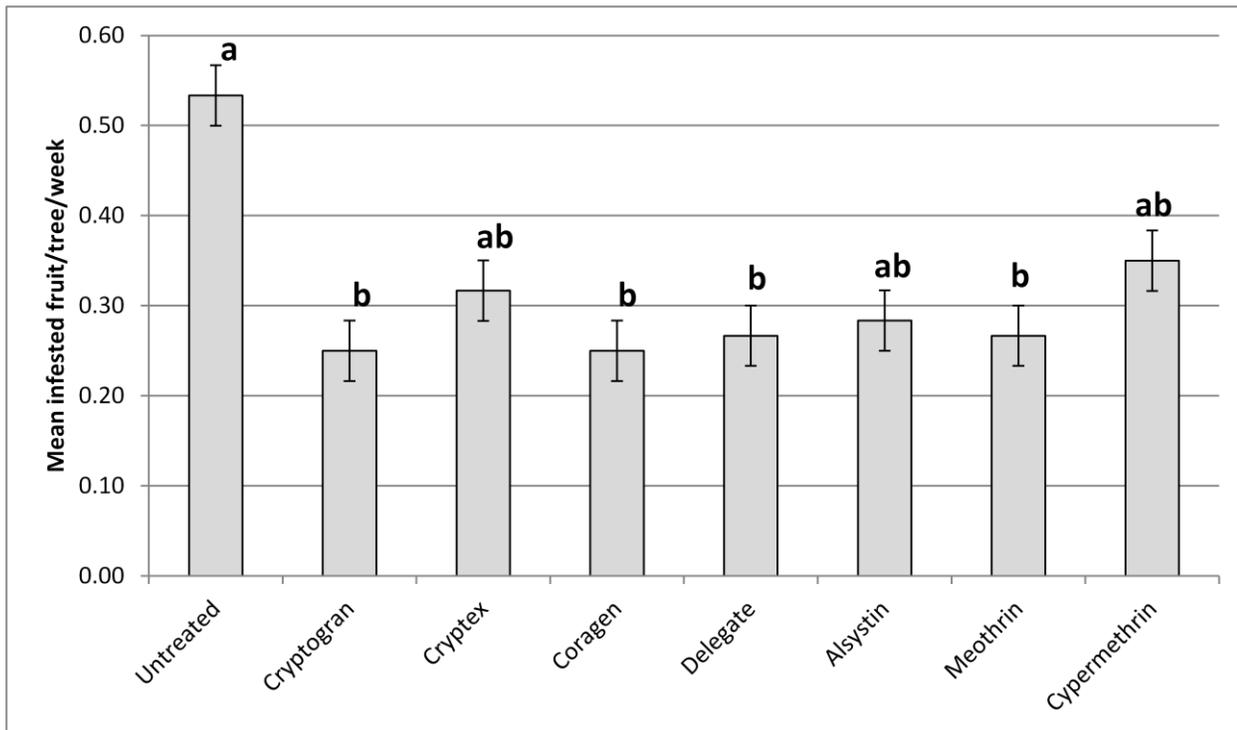
#### Field trial 5

FCM infestation in the untreated control averaged 0.53 infested fruit per tree per week in the untreated control over the full period of evaluation. This could be described as a moderate level of infestation. Not much could be deduced from inspection of the data over this period, except that infestation for all treatments was lower than that for the untreated control (Fig. 3.2.8.11). Also, efficacy with Coragen appeared to be superior to the other treatments on 7 June and the overall efficacy of cypermethrin appeared to be the worst of all treatments.



**Figure 3.2.8.11** FCM infestation of treated and untreated Navel oranges on Bernol Farm, evaluated from 26 May to 28 June 2011.

Mean FCM infestation in the untreated control for the full duration of the trial was  $0.53 \pm 0.07$  (mean  $\pm$  SE) fruit per tree per week. For Cryptogran, Cryptex, Coragen, Delegate, Alsystin, Meothrin and cypermethrin it was  $0.25 \pm 0.07$ ,  $0.32 \pm 0.04$ ,  $0.25 \pm 0.06$ ,  $0.27 \pm 0.07$ ,  $0.28 \pm 0.07$ ,  $0.27 \pm 0.02$  and  $0.35 \pm 0.06$ , respectively (Fig. 3.2.8.12). Over the full trial period, Cryptogran, Coragen, Delegate and Meothrin significantly reduced FCM infestation, whereas Cryptex, Alsystin and cypermethrin did not. Cryptogran and Coragen were the two most effective treatments, both reducing FCM infestation by 53.1%. Delegate and Meothrin reduced infestation by 50.0%. Alsystin reduced infestation by 46.9% and Cryptex and cypermethrin reduced infestation by only 40.6% and 34.4% respectively. However, there was no statistically significant difference between the efficacy of any of the treatments.



**Figure 3.2.8.12** Mean FCM infestation of treated and untreated Navel oranges on Bernol Farm over a 10-week period (26 May to 28 June 2011). Bars with the same letter are not significantly different (LSD multiple range test;  $\alpha = 0.05$ ).

Results with Coragen in all trials reported here are summarised in Table 3.2.8.1.

**Table 3.2.8.1.** Summary of results with Coragen against FCM on citrus in trials in the Eastern Cape (2009-2011).

Farm	Tree age (years)	Treatment	Application date	Evaluation period (weeks)	Average fruit infested/tree/week in the untreated control	Reduction in FCM infestation (%)
Kleinwelgemoed	4	Coragen	9 Dec 2009	7	0.24	64.7
Penhill	26	Coragen	2 Feb 2010 16 Mar 2010	15	0.76	30.7
		Coragen	2 Feb 2010 2 Apr 2010			32.5
Far Away	4	Coragen	7 Dec 2010	9	1.53	57
Penhill	27	Coragen	14 Mar 2011 12 Apr 2011	11	0.58	53.4
		Coragen	14 Mar 2011			48.3
		Coragen	14 Mar 2011			55.2
Bernol	6	Coragen	4 May 2011	6	0.53	53.1

### Conclusion

Coragen showed comparable efficacy to other registered products for control of FCM on navel oranges. Residual efficacy appeared to be in the region of eight weeks. Efficacy on small trees initially appeared superior to efficacy on large trees. This was related to inadequate spray coverage on large trees, due to the volume restriction placed on Coragen. However, a subsequent trial conducted to test this hypothesis did not manage to support it. Differences in efficacy of Coragen may therefore be related to a combination of high pest pressure

and inadequate spray volume per tree size. It is nevertheless recommended that the Coragen label be amended to provide growers with the option of two applications at no more than 8500 L per hectare or one application at no more than 17000 L per hectare.

### Future research

No further research is required as the product has now been registered.

### Technology Transfer

These results have been passed on to Du Pont, who has used them at various meetings. Once an MRL for the EU for citrus has been granted, these results will be presented by CRI at grower meetings.

### References cited

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### 3.2.9 PROGRESS REPORT: Development of mechanisms for the postharvest detection of cryptic pests in citrus fruit

Experiment 976 (April 2010 – March 2015) by Wayne Kirkman & Sean Moore (CRI)

### Opsomming

VKM skade op Satsumas is een dag na besmetting sigbaar met behulp van X-straal tomografie. Op Delta Valencias was die skade minder sigbaar, aangesien die larwes steeds in die skil of albedo teenwoordig was. 'n Deeglike ondersoek is met mikrofokus-X-straal tegnologie uitgevoer. Hierdie tegnologie lewer 'n baie hoër resolusie as gewone X-strale. Van verskeie teikens wat getoets is was silwer die mees gepas. Dit het die duidelikste beelde getoon, en die mees gedefinieerde histogrampeke. Die optimum energievlak was 150 KV, en dit het 'n Gray-waarde van 55000 gelever. Met mikrofokus-tomografie ( $\mu$ CT) was beelde vanaf 500 projeksies amper so duidelik as die met 1000 projeksies, en die proses is dubbel so vinnig. Mikrofokus-radiografie het 71% van besmetting opgespoor na 13 dae, 70% na 10 dae, 50% na 3 dae en 33% 1 dag na infestasië. Tekens van besmetting kon nie bepaal word terwyl die larwes in die skil of albedo was nie. Mikrofokus-tomografie het 100% van infestasiës opgespoor. Beeld algoritmes word deur samewerking tussen CRI en NECSA ontwikkel om automaties  $\mu$ CT beelde van besmette vrugte en gesonde vrugte te skei. Proewe het getoon dat besmette dele Gray-waardes van tussen 15482 en 17045 oplewer, terwyl natuurlike gapings binne vrugte waardes tussen 13641 en 23485 oplewer. Samewerking tussen CRI, Maf-Roda (VSA) en die Universiteit van Valencia is bewerkstellig om algoritmes te ontwikkel en om vlugtige afskeidings van besmette vrugte te ontleed deur middel van naby-infrarooi spektroskopie.

### Summary

FCM infestation could be detected one day after infestation of Satsumas using X-ray tomography. Infestation after one day was not as easily detectable in Delta Valencia oranges, as many of the larvae were still in the albedo. Microfocus X-ray technology was intensively investigated, demonstrating that image resolution was much higher than normal X-ray. Of the various targets tested, silver was the most appropriate, giving the clearest images and most defined histogram peaks. The optimum energy level was determined to be 150 KV, delivering a Gray scale value of 55 000. With microfocus computed tomography ( $\mu$ CT), images from 500 projections per scan were almost as clear as with 1000 projections and the process was twice as fast. Microfocus radiography detected 71% of infestations after 13 days, 70% after 10 days, 50% after 3 days and 33% 1 day after infestation. Infestation was not detectable while the larvae were in the rind or albedo. Micro-focus tomography detected 100% of infestations. Imaging algorithms are being developed in collaboration with NECSA, to automatically classify  $\mu$ CT images as clean or infested. Initial studies revealed Gray values of between 13641 and 23485 for natural pores in the fruit, and values between 15482 and 17045 for infested regions. Collaborative studies have been initiated with Maf-Roda (USA) and the University of Valencia to detect algorithms and the possibility of detecting volatiles from infested fruit using near infrared spectroscopy.

3.2.10 **FINAL REPORT: *In vivo* mass culture of *Heterorhabditis zealandica* and *H. bacteriophora***  
Experiment 983 (March 2010– March 2012) by Carolien van Zyl, Antoinette Malan (SU) and Sean Moore (CRI)

### Opsomming

Wasmotlarwes (*Galleria mellonella* L.) en meelwurms (*Tenebrio molitor* L.) (MW) is aangeteel op vyf verskillende diëte om vas te stel watter dieet die grootste toename in larvale gewig tot gevolg het. Die gemiddelde massa van WML op die dieet wat die swaarste larwes geteel het, was 0.19 g per larwe. Vir MW was dit gemiddeld 0.0154 g per larwe. WML het die meeste *H. zealandica* en *H. bacteriophora* per gasheergram opgelewer 1 459 205 en 1 898 512 onderskeidelik, gevolg deur MW larwes 836 690 en 414 566 onderskeidelik. Laer getalle *H. zealandica* en *H. bacteriophora* per gram kodlingmot 57 582 en 39 653 en per gram vals kodlingmot 192 867 en 97 652 is egter geproduseer. 'n Beduidende positiewe verwantskap tussen die gewig van wasmot- en meelwurmlarwes is verkry. Vergelykbare produksie van nematodes is gekry met gevriesde meelwurmlarwes. Drie inokulasietegnieke is evalueer en vergelyk deur *H. zealandica* en *H. bacteriophora* te gebruik om WML en MW gashere te inokuleer. Die hoogste persentasie infeksie is gekry deur inokulasie met 'n pipet, gevolg deur skud van gashere in 'n nematode-inokulum, en gasheerindompeling in 'n nematode-suspensie. Onderwerping van MW aan stressors en bymiddels het nie nematode-infeksie suksesvol bevorder nie. Laer infeksievlakke is opgemerk waar MW gashere in kraanwater van 70°C gedompel is en daarna met *H. bacteriophora* (12% infeksie) of *H. zealandica* (21% infeksie) geïnokuleer is, vergelyke met die kontrole. Die virulensie van nematodes het nie beduidend toegeneem deur *H. zealandica* en *H. bacteriophora* IL in 'n suspensie met  $Mn^{2+}SO_4 \cdot H_2O$  te behandel voor MW geïnokuleer is nie. 'n Ondersoek is gedoen na die vermoë van twee formulاسies, Pesta korrels en klappervesel, om biologiese aktiwiteit en virulensie van *H. zealandica* te onderhou, in vergelyking met 'n kraanwater suspensie kontrole. Die aantal lewende *H. zealandica* in Pesta korrels en klappervesel het drasties verminder na sewe dae in die formulاسie. Oorlewing van nematodes in Pesta korrels het gedaal tot 10% na 21 dae vergeleke met die kontrole, waar 80% oorleef het. Nog minder nematodes, 2%, het na 21 dae in die klappervesel oorleef vergeleke met die kontrole waar 100%+ oorleef het. Die toepassing van 'n kwaliteitsstandaardtegniek om die virulensie van geformuleerde *H. zealandica* te bepaal, het ondoeltreffend geblyk.

### Summary

Wax moth larvae (*Galleria mellonella* L.) and yellow mealworm larvae (*Tenebrio molitor* L.) were reared on five different diets respectively, to determine which diet resulted in the highest increase in insect larval weight. The average weight of wax moth larvae on the diet which produced the heaviest larvae, was 0.19 g per larva. For mealworm larvae, this was 0.0154 g. Wax moth larvae produced the highest number of *H. zealandica* and *H. bacteriophora* per gram host (1 459 205 and 1 898 512 per gram host, respectively). The mean number of EPNs produced per g codling moth and false codling moth was 57 582 and 192 867, respectively for *H. zealandica* and 39 653 and 97 652 for *H. bacteriophora*. A significant positive linear relationship existed between host weight and the number of nematodes produced for wax moth larvae and mealworms. Comparable production of nematodes was found with frozen mealworm larvae. For EPN production, three different inoculation treatments were compared, using wax moth larvae and mealworms as hosts, and *H. bacteriophora* and *H. zealandica* as the respective inoculum sources. The highest percentage infection of both insect hosts was achieved by pipetting nematodes onto hosts, followed by immersion of hosts into nematode suspensions, and, lastly, by shaking hosts together with nematode inoculum. It was found that the effects of stressor treatments on mealworm larvae did not improve infection rates. By exposing mealworm larvae to 70°C tap water prior to inoculation, infection levels of 12% for *H. bacteriophora* and 21% for *H. zealandica* were actually lower than that of the control. Pre-inoculation of infective juveniles (IJs) of both *H. zealandica* and *H. bacteriophora* with  $Mn^{2+}SO_4 \cdot H_2O$  led to no change in infection rate of mealworms. A comparative study was conducted to determine the storage stability of *H. zealandica* in modified Pesta granules and coconut fibres compared to a control, consisting of a suspension of nematodes in distilled water. Storage stability in both formulations decreased during the storage period of 21 days at 4°C. Only 9.79% *H. zealandica* IJs survived 21 days in Pesta granules, and 2.25% in coconut fibres. The percentage IJ survival in the control for both Pesta granules and coconut fibres was 79.79% and 100%+, respectively. A quality control measure implemented to determine the virulence of formulated nematodes, using the percentage mortality of mealworms as an indicator, was not effective.

### Introduction

Entomopathogenic nematodes (EPNs) are endoparasitic organisms that possess desirable pest control attributes and that are effective biological control agents against several soil-borne and foliar pests. EPNs can be produced in large numbers by *in vivo* or *in vitro* culturing techniques (Gaugler and Han 2002; Shapiro-Ilan

and Gaugler 2002). *In vivo* production is a cost-efficient method of producing nematodes on a smaller scale. The *in vivo* culturing process can be split up into five steps: host and nematode selection; inoculation of hosts; harvesting of nematodes; formulation of nematodes; and application in the target area. The efficacy of host inoculation with EPNs greatly influences the nematode yield achieved (Shapiro-Ilan and Gaugler 2002). Efficient *in vivo* production depends on a high level of host infection during inoculation, and even more so with the scaling-up of production (Gaugler 2002). Low levels of infection lead to the time-consuming and expensive task of removing naturally dead hosts to avoid further contamination of other insect hosts (Woodring and Kaya 1988). To ensure maximal infections, parameters to optimise inoculating techniques should be implemented in the production process (Lacey and Brooks 1997; Gaugler 2002; Shapiro-Ilan et al. 2002).

The efficiency of inoculation techniques can be influenced by host density, nematode concentration, and the inoculation method used (Shapiro-Ilan and Gaugler 2002; Shapiro-Ilan et al. 2002). Apart from such factors, optimally suited environmental conditions during inoculation, as well as a close natural association between the host and the nematode, further improve chances of high infectivity levels (Shapiro-Ilan et al. 2004). Inoculation methods that have been applied and tested in prior studies are: immersion; spraying; pipetting; and application of nematodes directly to the food source of target insects (Blinova and Ivanova 1987; Shapiro-Ilan and Gaugler 2002; Shapiro-Ilan et al. 2002). It has been demonstrated that significant differences can occur among inoculation methods used, with regard to time-efficiency and latent infections obtained (Blinova and Ivanova 1987; Flanders et al. 1996; Gaugler et al. 2002; Shapiro-Ilan et al. 2002).

Additional measures that can be applied to optimise latent infection levels are through applying physical and chemical stress to insect hosts and nematodes (Brown et al. 2006). Doing so could enhance susceptibility of hosts by compromising insect host defences. The measure could be beneficial when rearing costs of a highly susceptible host are more expensive compared to those of a host that is less susceptible to the specific nematode species (Gaugler and Han 2002). For instance, rearing of mealworm larvae (MW) has proven to be less expensive than has rearing of wax moth larvae (WML) (Brown et al. 2006), but MW are also less susceptible, compared to WML, to certain EPN strains used for commercial pest control strategies. Therefore, if imposing physical and chemical stress upon MW proves to enhance infection to acceptable levels, *in vivo* production process costs can be reduced when MW are used. Prior studies have proved that physical stress in the form of sublethal heat treatments, by immersing hosts in hot water (50-80°C), or exposing them for periods of time to dry heat (35-40°C), can also increase the susceptibility of host insects (Brown et al. 2006). In studies by Jaworska et al. (1997a, b) and Brown et al. (2006), the effect of chemical stress on nematode infectivity, applied by mixing nematodes with a solution of manganese and magnesium ions prior to being used for inoculation, was tested. Their findings suggested that submersion of MW into a  $Mn^{2+}SO_4 \cdot H_2O$  / nematode solution increased the MW mortality and infection levels, as a consequence of increased nematode virulence (Brown et al. 2006; Jaworska et al. 1997a, b).

The objective of the current study was to test different methods of nematode and insect host manipulation, which would lead to the optimisation of host infection levels during the *in vivo* mass culture of nematodes. Three nematode inoculation techniques were compared, as well as the efficacy of three treatments used to improve nematode infectivity through host and nematode manipulation. Two locally selected, endemic South African nematode strains of two different species of EPNs were used.

## Objectives

The objective of the current study was to test different methods of nematode and insect host manipulation, which would lead to the optimisation of host infection levels during the *in vivo* mass culture of nematodes. Three nematode inoculation techniques were compared, as well as the efficacy of three treatments used to improve nematode infectivity through host and nematode manipulation. Two locally selected, endemic South African nematode strains of two different species of EPNs were used.

## Materials and methods

### *Source of nematodes and host insects*

*Heterorhabditis bacteriophora* Poinar, 1967 (SF351) (Genbank accession number EU699436) and *H. zealandica* Poinar, 1990 (SF41) (Genbank accession number FJ455843) from the Stellenbosch University nematode collection (Malan et al. 2006) were used. Infective juveniles (IJs) were produced by *in vivo* culturing in WML. The *in vivo* production process was based on the modified White trap method (White 1927; Dutky et al. 1964; Woodring and Kaya 1988; Lindegren et al. 1993). IJs were harvested for up to a week post emergence and stored horizontally in the dark at 14°C in 50 ml distilled water, in 160-ml ventilated culture flasks. Culture flasks were shaken on a weekly basis to facilitate aeration. IJs used in all experiments conducted were not older than

four weeks.

WML and MW were obtained from laboratory cultures. WML were cultured in ventilated plastic containers on a diet consisting of 118 g wheat flour, 206 g wheat bran, 118 g milk powder, 88 g brewer's yeast, 24 g wax powder, 175 ml honey and 175 ml glycerol (Chapter 2). MW larvae were reared in wooden culture boxes on 100% wheat bran and, as an added water source, carrots were used (Chapter 2). Both cultures were reared at 25°C in the dark, as was described in Chapter 2. Last-instar larvae of both insects were used in all experiments.

#### *Post-inoculation protocol*

A total of 13 Petri dishes with 10 insect larvae each ( $n = 130$  larvae) for the three different inoculation techniques and for each host and nematode species combination were used. After inoculation, Petri dishes, containing inoculated hosts for the different treatments, were placed in sealed plastic containers (11 × 11 × 7.5 cm), fitted with moist paper towels and incubated at 25°C. After two days, the percentage mortality of the insect hosts was recorded for each treatment. Mortality was defined by lack of host movement on prodding with a pair of tweezers. Percentage host infection of each treatment was recorded after seven days, based on colour change. Infection was confirmed by means of dissection of the host with the aid of a dissecting microscope.

#### *Inoculation by pipetting*

Inoculation of insect hosts by pipetting involved placing 10 MW or WML in a 90-mm Petri dish lined with filter paper (Whatman No. 1). Each Petri dish was inoculated with 500 µl tap water containing IJs at a concentration of 200 IJs/50 µl of either *H. zealandica* or *H. bacteriophora*, by means of an Eppendorf micropipette. After inoculation, the post-inoculation protocol was followed. The experiment was repeated on a different test date.

#### *Inoculation by shaking*

For the 'inoculate-and-shake' method, 130 insect larvae of either WML or MW were placed into one small plastic container (250 ml), to which 1 ml nematode inoculum, containing 26 000 IJs (200 IJs per host) of either *H. zealandica* or *H. bacteriophora* was added. The container was sealed and shaken by hand for one minute, to ensure coverage of the surface area of the insect bodies with nematodes. Insects were then removed from the container using a pair of tweezers, and 10 insects were placed into a 90-mm filter-paper-lined Petri dish. After inoculation, the post-inoculation protocol was followed and the experiment was repeated on a different test date.

#### *Inoculation by immersion*

The immersion technique involved placing 130 mealworms in a small tea sieve (1200 µm) and attaching a plastic lid to the top of the sieve, to avoid mealworms floating out of the sieve when submerged. The sieve was then dipped for five seconds into a 500-ml plastic container, containing a 300-ml suspension of either *H. zealandica* or *H. bacteriophora*, at a concentration of 200 IJs/50 µl. The sieve was then placed for two seconds on a single sheet of paper towel, to allow absorption of excess suspension. Insect hosts were picked from the sieve with a pair of tweezers and ten hosts were placed into individual 90-mm Petri dishes, lined with moist filter paper, whereafter the post-inoculation protocol was followed. The experiment was repeated on a different test date.

#### *Post-stress treatment protocol*

After each of the stress treatments, 10 MW were placed in each of the 10 filter-paper-lined Petri dishes (90-mm diameter) and incubated at 25°C in the dark in sealed plastic containers (11 × 11 × 7.5 cm), fitted with a moist paper towel. Percentage mortality of MW for each treatment was recorded after two days. Percentage infection was recorded after seven days. Infection was evaluated, based on cadaver colour change. EPN infection was confirmed by dissection of the host larva with the aid of a dissecting microscope. The same number of MW was immersed in a suspension that contained only either *H. zealandica* or *H. bacteriophora*, and served as the untreated control.

#### *Warm-water treatment*

Treating the host with hot tap water involved the immersion of 100 MW in a sieve (1200 µm) in 300 ml of 65-70°C warm water for five seconds. The sieve was momentarily dried of excess water for two seconds by blotting it on a single paper-towel sheet, before immersing it for another five seconds into a beaker containing 250 ml of a nematode suspension containing either *H. zealandica* or *H. bacteriophora* at a concentration of 200 IJs/50 µl. Afterwards, the post-stress treatment protocol was followed. The experiment was repeated on a different test date.

#### *Manganese $Mn^{2+}SO_4 \cdot H_2O$ treatment*

Treating the IJs of the two respective EPN species with manganese involved adding 1.7mM  $Mn^{2+}SO_4 \cdot H_2O$  to a 250-ml nematode suspension, at a concentration of 200 IJs/50 µl. MW, contained in a sieve, were then immersed in the nematode/ $Mn^{2+}SO_4 \cdot H_2O$  suspension for five seconds and placed on a paper towel for two

seconds. Afterwards, the post-stress treatment protocol was followed. The experiment was repeated on a different test date.

#### Combination of hot water and $Mn^{2+}SO_4 \cdot H_2O$ treatment

The last treatment was a combination of the first and second treatments, in which 100 MW were immersed in 65-70°C warm tap water for five seconds, placed on a paper towel for two seconds and then immersed into the nematode/ $Mn^{2+}SO_4 \cdot H_2O$  suspension for five seconds. Afterwards, the post-stress treatment protocol was followed and the experiment was repeated on a different test date.

#### Data analysis

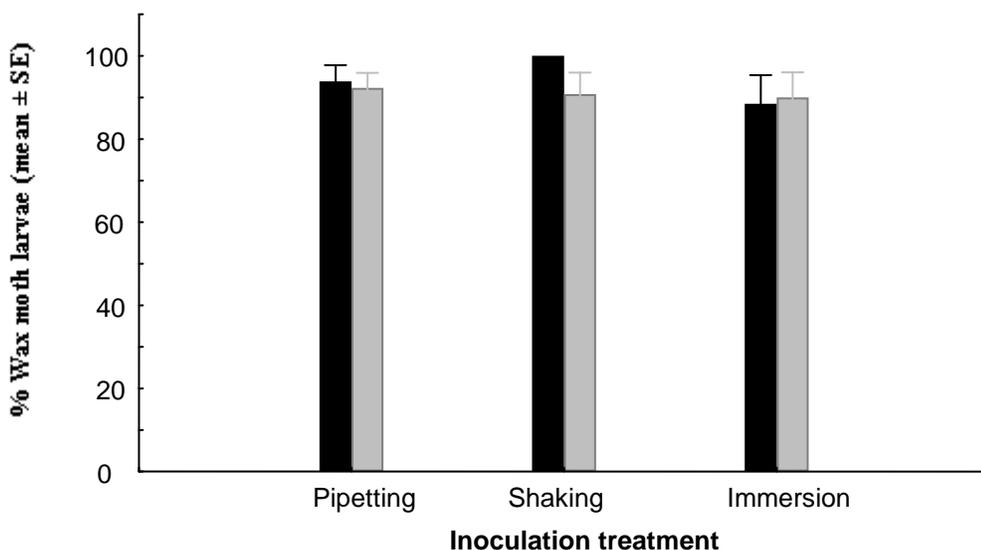
The data analysis software system Statistica version 10 (Statsoft Inc. 2011) was used to perform all statistical analysis. Percentage insect host mortality and EPN infection data were analysed by using a one-way ANOVA with post-hoc comparisons of means, using Bonferroni's method. For each experiment, data from both trials were pooled if no interaction was observed between trials. In cases where there was significant interaction among trials, the data of each trial were analysed separately.

### Results and discussion

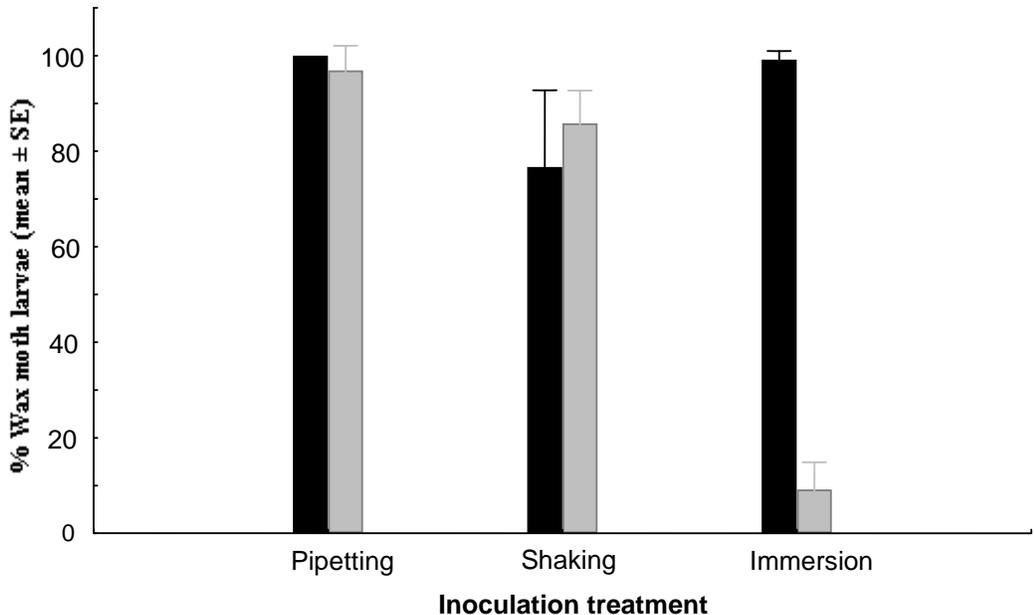
#### Effects of the three inoculation methods on mortality and infection of WML, using *H. bacteriophora* (SF351)

Significant interaction was observed between the initial (Trial 1) and repeat (Trial 2) trials, due to difference in percentage infectivity levels ( $p < 0.001$ ), and were thus analysed separately. The average mortality rate of WML for all inoculating treatments tested was above 80%. The mean percentage mortality achieved for the three inoculating treatments differed significantly among each other on both trial dates (Trial 1:  $F_{(2, 36)} = 7.511$ ,  $p < 0.05$ ; Trial 2:  $F_{(2, 36)} = 12.484$ ,  $p < 0.001$ ). The highest percentage insect mortality obtained in both trials was with pipetting. Inoculating using a pipette resulted in  $93.84\% \pm 1.80\%$  (mean  $\pm$  SE) mortality in Trial 1 and in 100% mortality in Trial 2. Using the 'inoculate-and-shake' treatment in Trial 1 resulted in a WML mortality of 100%; however, in Trial 2 it declined to  $74.61\% \pm 7.04\%$ . The mean percentage mortality recorded using immersion as inoculating treatment resulted in an  $88.46\% \pm 3.17\%$  mortality for Trial 1 and in an  $99.23\% \pm 0.80\%$  mortality for Trial 2 (Figs. 3.2.10.1 and 3.2.10.2).

Notable differences in infection rates of WML with *H. bacteriophora* among treatments was recorded for Trial 2, but not for Trial 1 (Trial 1:  $F_{(2, 36)} = 0.256$ ,  $p = 0.775$ ; Trail 2:  $F_{(2, 34)} = 310.330$ ,  $p < 0.001$ ). The lowest recorded infection rate was during Trial 2, using immersion as inoculating treatment ( $9.17\% \pm 2.60\%$ ). Contrary to that, the mean percentage infection recorded for the same treatment during Trial 1 was  $90\% \pm 2.77\%$ . The highest infection rates were obtained with pipetting in both trials (Trail 1:  $92.31\% \pm 1.66\%$ ; Trial 2:  $96.92\% \pm 2.37\%$ ). Relatively consistent infection rates between trials were obtained using the 'inoculate-and-shake' treatment with  $90.77\% \pm 2.40\%$  infection recorded in Trial 1 and  $85.83\% \pm 3.13\%$  in Trial 2 (Figs. 3.2.10.1 and 3.2.10.2).



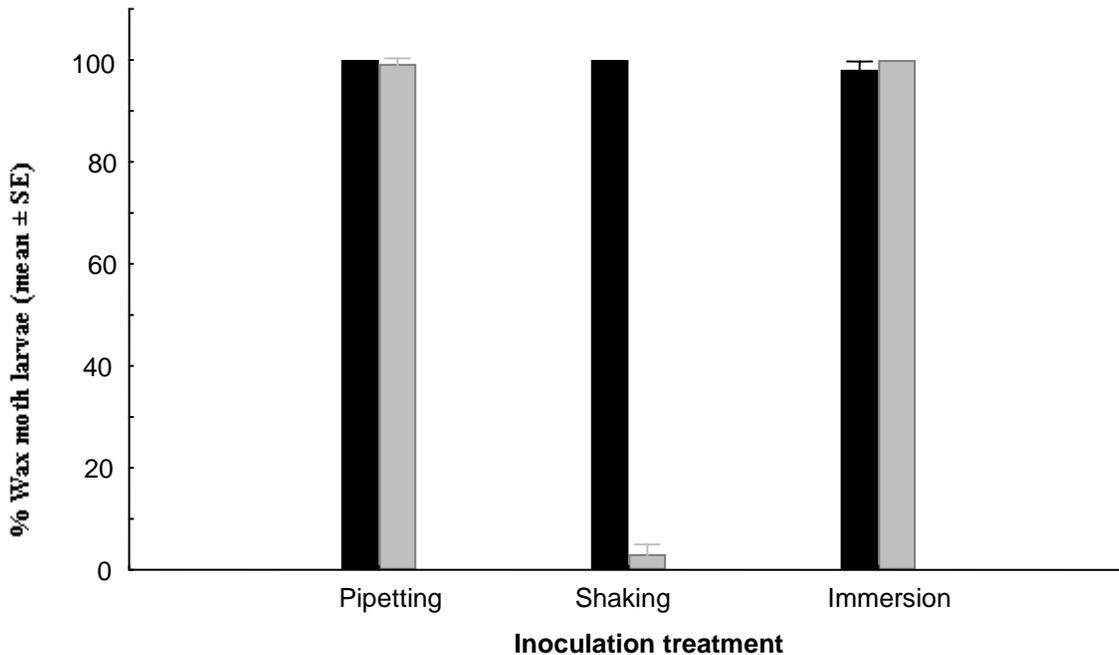
□  
**Fig. 3.2.10.1.** Trial 1 for the mean  $\pm$  SE (standard error) (95% confidence interval) wax moth larval mortality (□) and infection (■) for each of the three inoculating treatments, using *H. bacteriophora* (SF351).



**Fig. 3.2.10.2.** Trial 2 for the mean ± SE (95% confidence interval) wax moth larval mortality (■) and infection (■) for each of the three inoculating treatments, using *H. bacteriophora* (SF351).

*Effects of three inoculation methods on mortality and infection of WML, using H. zealandica (SF41)*

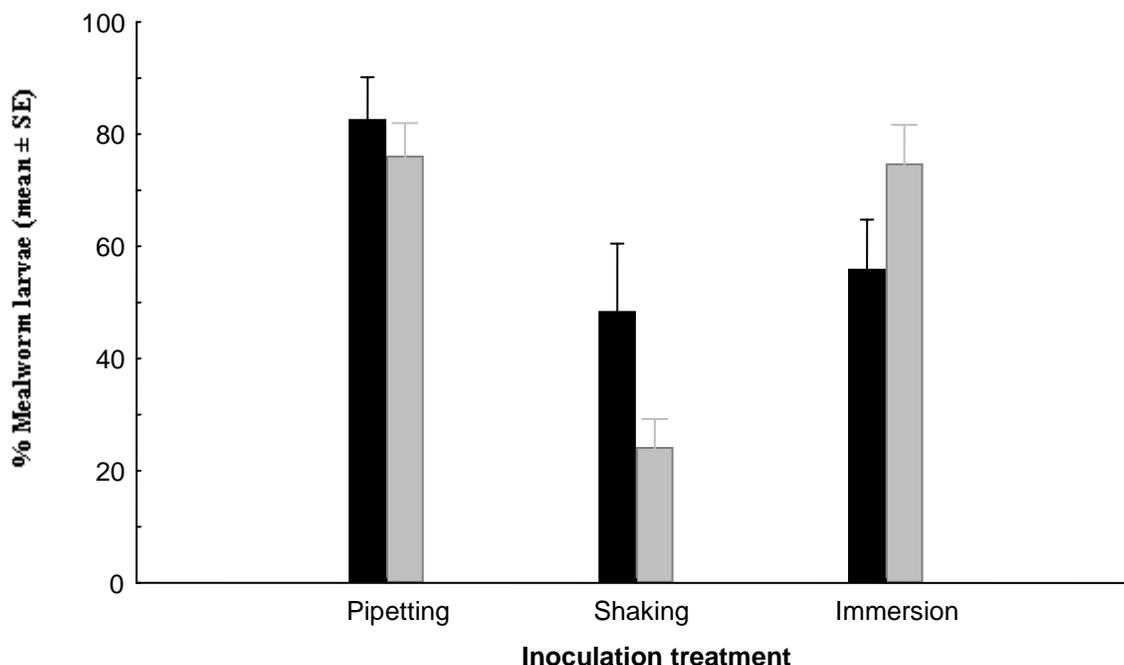
No significant interaction was observed between the results of the two trials, and the data were thus pooled before analysis. Significant differences occurred among treatments regarding mortality rates ( $F_{(2, 75)} = 6.0, p < 0.05$ ) and infection rates ( $F_{(2, 75)} = 8203.65, p < 0.001$ ). The highest mortality of WML and infection rates for *H. zealandica* were obtained with pipetting (100% mortality and 99.23% ± 0.53% infection). An extremely low infectivity rate (3.08% ± 0.92%) was achieved with 'inoculate-and-shake', even though the mortality rate was 100%. Mortality rates of 98.08% ± 0.79% and infection rates of 100% were recorded using immersion as inoculating treatment (Fig. 3.2.10.3).



**Fig. 3.2.10.3.** The mean ± SE (95% confidence interval) wax moth larval mortality (■) and infection (■) for each of the three inoculating treatments, using *H. zealandica* (SF41).

*Effects of three inoculation methods on mortality and infection of MW, using H. bacteriophora (SF351)*

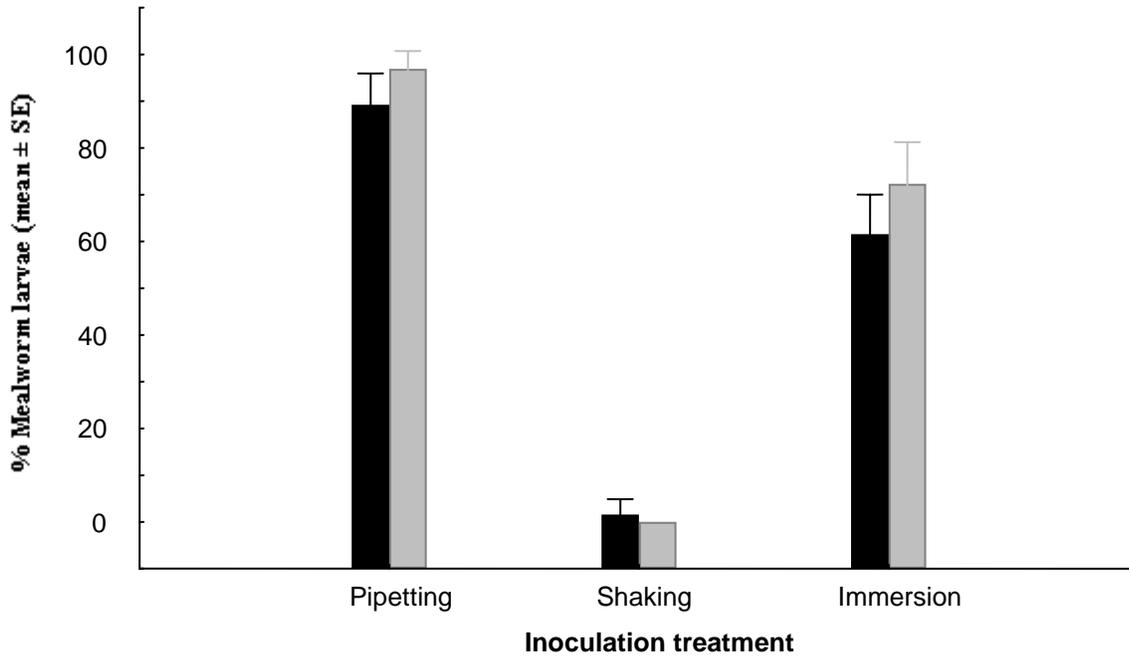
No significant interaction was observed between the results of the two trial dates, and the data were thus pooled before analysis. Inoculating treatments differed significantly from one another, with regard to percentage mortality ( $F_{(2, 72)} = 71.324$ ,  $p < 0.001$ ) and percentage infection ( $F_{(2, 69)} = 107.63$ ,  $p < 0.001$ ) recorded. Mortality of MW obtained with *H. bacteriophora* for pipetting was  $82.69\% \pm 3.62\%$ , followed by  $56\% \pm 4.24\%$ , achieved with immersion. The lowest mortality rate was recorded for 'inoculate-and-shake', at  $48.46\% \pm 5.84\%$ . A similar and consistent trend was observed for the infection rates compared to the mortality rates, with pipetting achieving the highest infection rates at  $76.15\% \pm 2.83\%$ , followed by immersion ( $74.80\% \pm 3.32\%$ ) and 'inoculate-and-shake' ( $24.23\% \pm 2.43\%$ ) (Fig. 3.2.10.4).



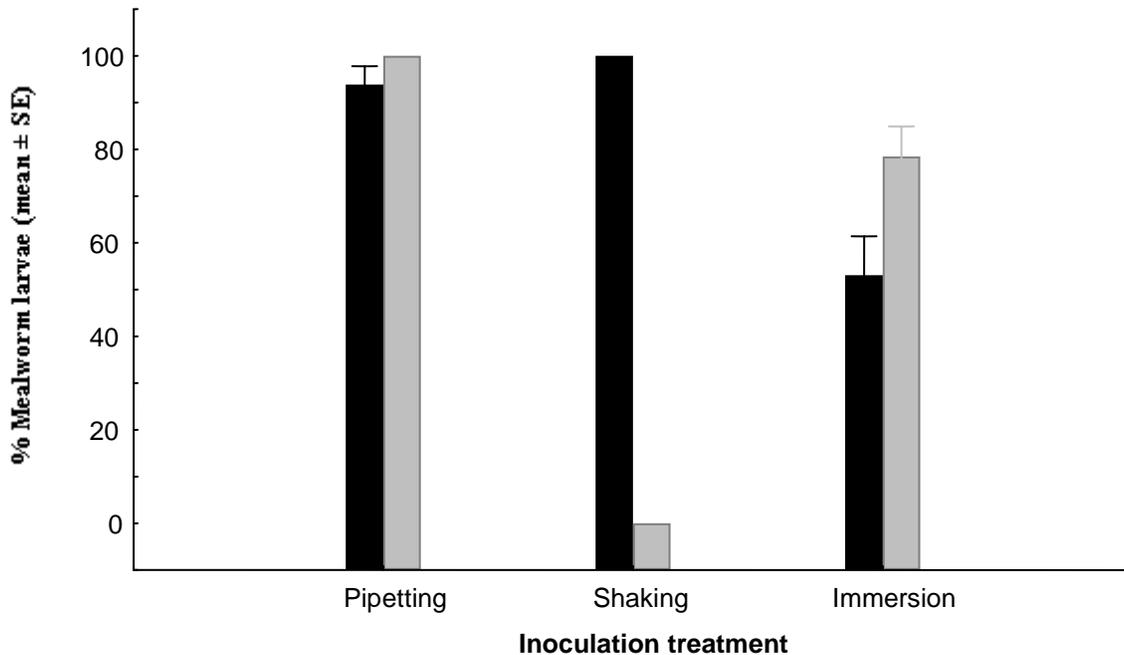
**Fig. 3.2.10.4.** The mean  $\pm$  SE (95% confidence interval) mealworm larval mortality (■) and infection (■) for each of the three inoculating treatments, using *H. bacteriophora* (SF351).

*Effects of three inoculation methods on mortality and infection of MW, using H. zealandica (SF41)*

There was significant interaction between the two trials conducted ( $p < 0.001$ ). Significant differences occurred between treatments with *H. zealandica* for mortality of MW in both trials (Trial 1:  $F_{(2, 36)} = 222.28$ ,  $p < 0.001$ . Trial 2:  $F_{(2, 36)} = 109.29$ ,  $p < 0.001$ ), as well as for infection (Trial 1:  $F_{(2, 36)} = 382.22$ ,  $p < 0.001$ . Trial 2:  $F_{(2, 36)} = 946.79$ ,  $p < 0.001$ ). Pipetting, in both trials, was the most efficient inoculating treatment, with  $96.92\% \pm 1.75\%$  and  $100\%$  infection and  $89.23\% \pm 3.09\%$  and  $93.85\% \pm 1.80\%$  mortality obtained in the respective trials. Even though a high mortality rate ( $100\%$ ) was observed in trial 2 for the 'inoculate-and-shake' treatment, no insect larvae were infected using the treatment concerned, making it the least effective treatment of all treatments employed. Mortality and infection in both trials for immersion were within the same range, and competitive infection rates were obtained using the method specified. In trials 1 and 2, the mortality rates were  $61.54\% \pm 3.90\%$  and  $53.08\% \pm 3.82\%$ , respectively. Infection rates were slightly higher compared to mortality rates, at  $72.31\% \pm 4.11\%$  and  $78.46\% \pm 2.96\%$  for trials 1 and 2, respectively (Figs. 3.2.10.5 and 3.2.10.6).



**Fig. 3.2.10.5.** Trial 1 for the mean  $\pm$  SE (95% confidence interval) mealworm larval mortality (■) and infection (■) for each of the three inoculating treatments, using *H. zealandica* (SF41).

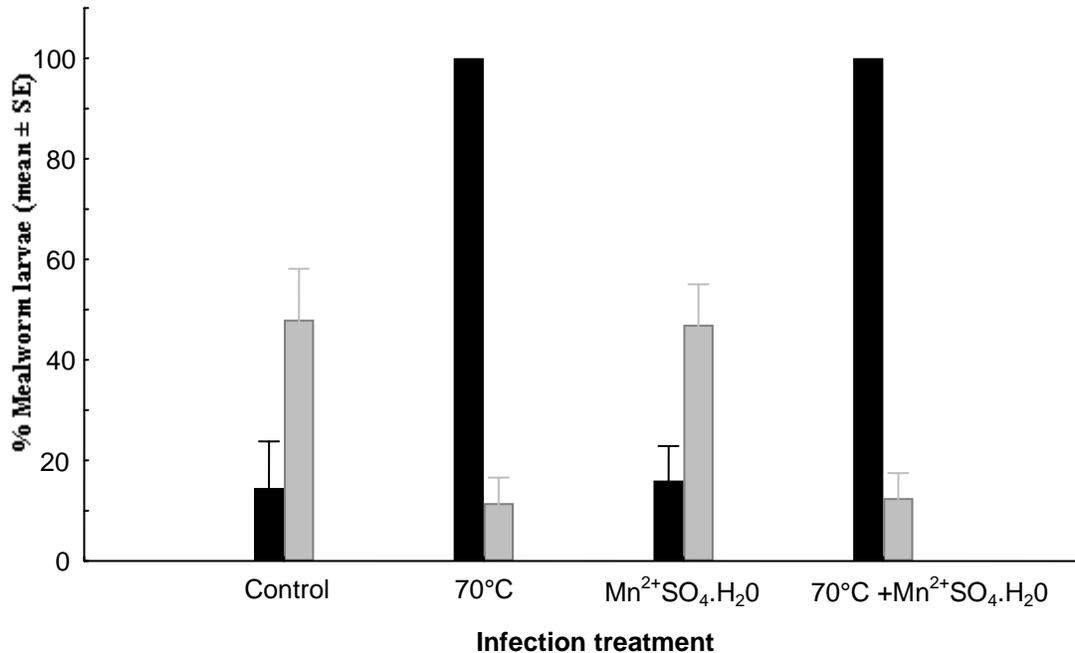


**Fig. 3.2.10.6.** Trial 2 for the mean  $\pm$  SE (95% confidence interval) mealworm larval mortality (■) and infection (■) for each of the three inoculating treatments, using *H. zealandica* (SF41).

*Effects of physical and chemical stress methods on mortality and infection of MW, using H. bacteriophora (SF351)*

There was a significant interaction between treatments regarding both MW mortality ( $F_{(3, 76)} = 314.448$ ,  $p < 0.001$ ) and infection ( $F_{(3, 76)} = 33.687$ ,  $p < 0.001$ ). The two treatments, in which MW larvae were immersed into hot water, led to 100% mortality of larvae. Only  $14.50\% \pm 4.44\%$  mortality was recorded for the control treatment,

in which larvae were immersed into a nematode-water suspension. A slight increase of 1.5% in mortality rate was achieved by immersing hosts into a suspension containing both  $Mn^{2+}SO_4.H_2O$  and nematodes. Even though the mortality rate was initially low for the control treatment, infection rates were the highest, at  $48\% \pm 4.85\%$ , compared to the other treatments. MW infection by *H. bacteriophora* recorded for the  $Mn^{2+}SO_4.H_2O$  treatment followed closely, at  $47.00\% \pm 3.85\%$ . An infection rate of  $12.50\% \pm 2.39\%$  was obtained by pre-treating MW larvae and nematodes with  $70^\circ C$  water and  $Mn^{2+}SO_4.H_2O$ . The lowest infection rate of  $11.50\% \pm 2.44\%$  was recorded when MW larvae were treated with only the  $70^\circ C$  water treatment (Fig. 3.2.10.7).

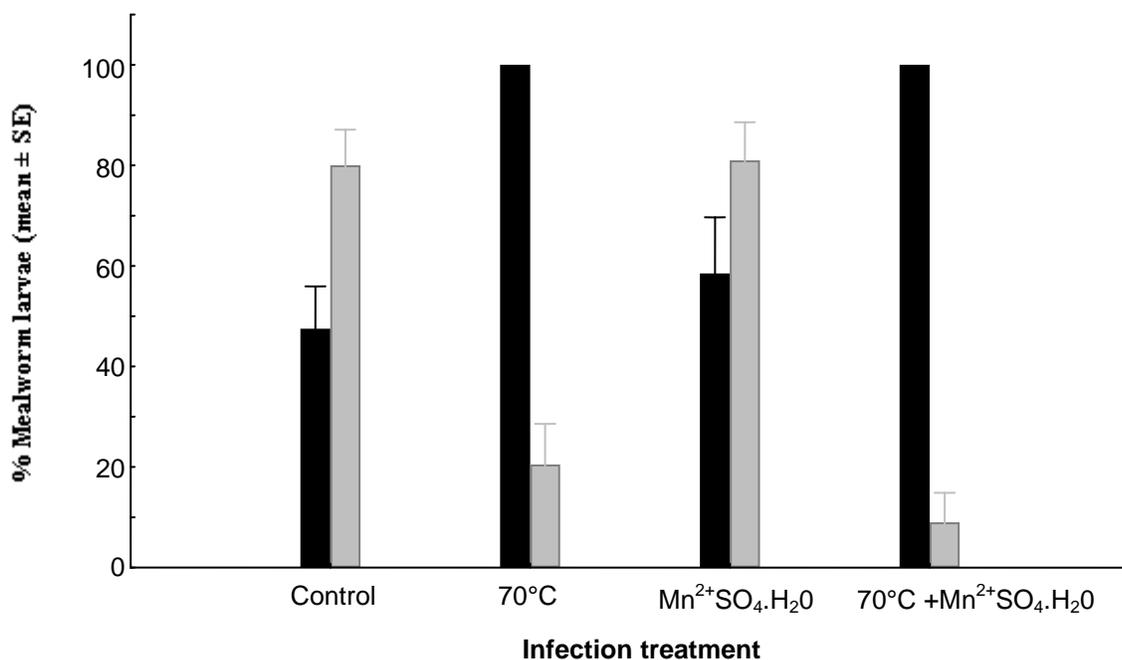


**Fig. 3.2.10.7.** The mean  $\pm$  SE (95% confidence interval) mealworm larval mortality (■) and infection (■) for each of the three applied physical and stress methods, using *H. bacteriophora* (SF351).

*Effects of physical and chemical stress methods on mortality and infection of MW, using H. zealandica (SF41)*

Significant differences occurred between treatments regarding MW mortality ( $F_{(3, 76)} = 67.521$ ,  $p < 0.001$ ) and infection ( $F_{(3, 76)} = 123.185$ ,  $p < 0.001$ ). The highest mortality rate of MW (100%) for *H. zealandica* was recorded for the  $70^\circ C$  water treatment of MW larvae and  $70^\circ C$  water plus  $Mn^{2+}SO_4.H_2O$  treatments. That was followed by  $58.50\% \pm 5.34\%$  mortality, achieved with the  $Mn^{2+}SO_4.H_2O$  method. The control produced the lowest MW mortality rate at  $47.50\% \pm 4.03\%$ .

Latent infection rates were the lowest with treatments where MW larvae were pre-treated using warm water, even though most larvae died with the application of the physical stress measure concerned. Only  $9.00\% \pm 2.80\%$  of MW larvae were infected applying the  $70^\circ C$  water plus  $Mn^{2+}SO_4.H_2O$  treatment, and  $20.50\% \pm 3.87\%$  using the  $70^\circ C$  water treatment alone. Treating nematodes with  $Mn^{2+}SO_4.H_2O$  improved infection rates slightly, achieving 1% higher infection rates compared to the rates attained with the control. With 81.00% MW infection achieved, treating nematodes with  $Mn^{2+}SO_4.H_2O$  was found to be the most effective treatment (Fig. 3.2.10.8).



**Fig. 3.2.10.8.** The mean  $\pm$  SE (95% confidence interval) mealworm larval mortality (■) and infection (■) for each of the three applied physical and stress methods, using *H. zealandica* (SF41).

Optimising infection levels during inoculation is a critical step in the development of an effective and cost-efficient *in vivo* nematode production process (Shapiro-Ilan et al. 2004). In previous studies, Jaworska et al. (1997a, b; 1999) and Brown et al. (2006) also used various techniques to increase the infection of insect larvae, by applying physical and chemical stress to hosts and nematodes. The two nematode species, *H. bacteriophora* and *H. zealandica*, that were used during this part of the study, are local strains that have been selected to provide satisfactory control of mealybug (Stokwe 2009), codling moth (De Waal 2008; 2011), false codling moth (Malan et al. 2011) and the banded fruit weevil (Ferreira 2010) on citrus and deciduous fruits in the South African agriculture industry. The hosts used in this study were commonly available, and easy and cheap to culture.

Differences in percentage mortality and infection of both WML and MW occurred among some of the trials, despite attempts to standardise the inoculation procedures. Significant interactions occurred between trials in which WML were inoculated with *H. bacteriophora* and MW with *H. zealandica*. The interaction occurring between the trials can possibly be due to virulence variation within batches of the respective nematode species. According to Griffin and Downes (1994), variation in *H. bacteriophora* batches is especially common. In addition, the general condition of the host may have influenced mortality and infectivity. Different host sizes and recency of last feeding tend to influence host susceptibility to nematodes (Kondo 1987; Flanders et al. 1996; Boff et al. 2000).

Both percentage mortality and percentage infection was recorded for each treatment. However, from the results obtained, it was concluded that percentage insect mortality cannot be used as an indication for the percentage infection, as significant variation can occur between the two parameters concerned. For example, application of the 'inoculate-and-shake' treatment to MW larvae led to a mortality rate of 49%, two days after inoculation with *H. bacteriophora*. However, the mean percentage infection after seven days was almost half, at 24%. Determining infection is labour-intensive, as hosts need to be washed and individually dissected, in order to observe developing nematodes in the haemocoel by means of a microscope. The higher mortality rate can be due to several external factors that are able to cause the natural death of the insect host. Apart from biotic and abiotic factors (Woodring and Kaya 1988; Grewal et al. 1994; Flanders et al. 1996; Shapiro-Ilan et al. 2002), physical damage of the hosts during experimental handling can also cause death of the host insect. Efficiency of methods for the optimisation of inoculation for *in vivo* production purposes should therefore be measured according to the levels of latent infections and not of mortality (Shapiro and Lewis 1999). Latent infections lead to the production of nematodes; therefore, nematode yield is directly influenced by the number of latent infections obtained (Shapiro-Ilan and Gaugler 2002).

The general procedure used for inoculating insects in the laboratory is pipetting. It also proved to be the most effective inoculating method for most treatment combinations investigated during the current study, excluding the *H. zealandica* and WML combination, in which immersion proved to produce higher infection rates. An infection rate of above 90% was maintained for all nematode–host combinations, except for MW inoculation with *H. bacteriophora*, where infection dropped to 76%. This lower infection rate could possibly have been as a result of a suboptimal nematode–host combination, compared to the other combinations. Even though pipetting produced the highest infection rates, it is impractical when nematodes are produced on a larger scale, due to its time-consuming nature (Shapiro-Ilan et al. 2002). Therefore, more suitable, alternative inoculating techniques were considered, such as the immersion of WML in an *H. zealandica* suspension, which resulted in a 100% infection. High infection rates (90%) of WML in trial 1 were also experienced with immersion, when *H. bacteriophora* was used. In contrast, the second trial produced very low levels of WML infection when immersion was used as the inoculation method. It is unclear what was specifically responsible for the result obtained, but the use of substandard-quality inoculum can possibly be due to the phenomena concerned. To obtain conclusive results, the same inoculation technique should be repeated with different batches of nematodes. Immersion of WML could be a more practical and time-efficient method of inoculation.

Infection rates obtained by applying the immersion technique to inoculate MW were much lower compared to pipetting, yet still offered the best alternative. When *H. bacteriophora* was used as the inoculum with MW, 75% infection was achieved. Infection levels within the same range were obtained when *H. zealandica* was used, where the mean percentage infection for trials 1 and 2 were, respectively, 72% and 79%. The success of the treatment was surprising, since MW are known to have an extremely smooth cuticle covering the small surface area of the body, making it difficult for nematodes to adhere to the host when they are immersed for a short period of time into a suspension (Shapiro-Ilan et al. 2002). It seems, however, that immersing MW for five seconds in a nematode suspension provided sufficient time for the nematodes to attach and adhere to the insect larva. Despite immersion being more time-efficient and delivering competitive levels of infection, it should be noted that more nematodes are required for the treatment specified, compared to the number required for the other treatments (Shapiro-Ilan et al. 2004). Shapiro-Ilan et al. (2002, 2004) also state that it appears that the immersion of MW is not a viable method for the mass production of EPNs, if infection levels of 90% and higher are required, which is said to be the standard acceptable percentage for mass-producing nematodes. The ‘inoculate-and-shake’ method performed very poorly using MW and both nematode strains respectively, with infection levels being below 25%. It is, thus, concluded that said method is not an effective inoculation method for MW.

MW has a smooth cuticle and a smaller surface area, compared to WML. Such morphological characteristics of the two respective insect hosts could impede nematode infection, making mealworms less susceptible to the nematodes. Therefore, to increase the usefulness of MW as hosts for nematodes, they were exposed to various host-stressor regimes, whereas the nematodes, in contrast, were exposed to infectivity-enhancing additives. Three treatments, plus a control treatment, were compared: hot-water treatment of MW; infective juvenile (IJ) stimulation by  $\text{Mn}^{2+}\text{SO}_4\cdot\text{H}_2\text{O}$ ; and a combination of hot-water MW treatment with IJ stimulation by  $\text{Mn}^{2+}\text{SO}_4\cdot\text{H}_2\text{O}$ . Hot-water treatment of insect larvae was selected above that of dry-heat treatment for the study, even though dry-heat treatment led to more consistent results in terms of IJ yield production in the study by Brown et al. (2006). The reason for choosing the hot-water treatment was that, under a small-scale producer’s conditions, where the funds that are available for capital investment are limited, hot-water treatment would be a more practical and cost-efficient method to implement, provided that acceptable levels of infection of insect larvae can be achieved.

Pre-inoculation heat treatments of mealworms proved to be unsuccessful in increasing EPN infection of MW. Reduced infection levels, when using *H. bacteriophora*, occurred after MW were exposed to 70°C water, compared to that which was used in the control. Only 12% and 13% infection was obtained with 70°C water treatment and the 70°C water treatment combined with the  $\text{Mn}^{2+}\text{SO}_4\cdot\text{H}_2\text{O}$ -treated IJs respectively, compared to the 48% that was obtained with the control treatment. Despite the mortality being 100% for both hot-water treatments, infection levels remained low. The same trend was observed with *H. zealandica*, with which the infection was 21% and 9% for treatments 70°C and 70°C +  $\text{Mn}^{2+}\text{SO}_4\cdot\text{H}_2\text{O}$ , respectively. A study undertaken by Brown et al. (2006) demonstrated that exposure of MW larvae to 65°C and 70°C water increased infection by *H. bacteriophora* compared to that which was attained with the control. Exposing the insect larvae to high temperatures compromises effective functioning of insect muscles responsible for closing bodily orifices, thus keeping the spiracles and anus open for periods long enough to facilitate nematode penetration. Furthermore, an anti-microbial protein, tenecin 1, which is found in MW larvae, and which has a negative influence on the bacterial symbiont of *H. bacteriophora*, is denatured when exposed to high temperatures, thus overcoming such a defence mechanism and increasing the susceptibility of MW (Brown et al. 2006). Despite the effect that hot-water treatment had on MW larval physiology, infection levels were not increased in the current study. A possible

reason for the lack of such an increase can be due to the longer period (five seconds) of MW immersion in 70°C water in the current study, compared to the one-second immersion of MW in the study by Brown et al. (2006). The longer period of immersion in hot water could have affected the nutritional value of MW larvae, making them an unattractive, suboptimal substrate for nematode penetration and proliferation.

As opposed to findings recorded in prior studies, in which manganese ions considerably increased the infectivity of *H. bacteriophora* against insect hosts (Jaworska et al. 1997; Brown et al. 2006), similar results were not obtained in the current study. *Heterorhabditis zealandica* IJ infectivity was slightly increased under application of a treatment with  $Mn^{2+}SO_4 \cdot H_2O$ , and was the most effective treatment in increasing MW infectivity, causing a 1% higher infection rate (81%) compared to that which was attained with the control (80%). Said treatment did, however, not have similar effects on *H. bacteriophora*, where infection levels were 1% lower than with the control, at 47%. According to Jaworska et al. (1999) and Brown et al. (2006), manganese and magnesium ions have a protective effect on nematodes, by pairing with other metal ions in a solution that is toxic to nematodes, that reduces nematode mortality and that also increasing its virulence towards such hosts as WML, MW and *Sitona lineatus* (L.) weevils. A possible explanation for the lack of infectivity enhancement obtained in the current study by means of treating IJs with  $Mn^{2+}SO_4 \cdot H_2O$ , is that the contact period between the ions and nematodes was too short. At no stage were the nematodes in contact with the ions for longer than one h at a time, whereas exposure lasted for up to 96 h in the experiments conducted by Jaworska et al. (1997) and Brown et al. (2006). In addition to the time factor, it has been shown that *S. carpocapsae* is much more predisposed to the positive effect that such ions have on nematode pathogenicity, compared to *H. bacteriophora* (Jaworska et al. 1997).

## Conclusion

Based on the results obtained in the current study, significant differences in the effectiveness among inoculating treatments investigated were observed. Pipetting was the treatment that caused the highest levels of infections in most of the nematode–host combinations. However, inoculation by immersion was selected as the method of choice for both WML and MW, due to its time-efficiency and ability to produce acceptable levels of infection in the case of WML. The lower levels of infection obtained with MW may, however, be acceptable in some instances, if it proves to be a cost-effective option when compared to the use of WML. Major improvement in nematode infection rates, using chemical and physical stressors in previous studies, did not resonate in the current study.

## Future research

Streamlining the protocol followed in the study by optimising such parameters as the exposure time of both nematodes to manganese ions and hosts to hot water could amplify the positive effect that such stressors have on infection rates.

## Technology transfer

1. Van Zyl, C., (2011). *In vivo* production of entomopathogenic nematodes. 20th Symposium of the Nematological Society of Southern Africa (NSSA), Spier Estate, Stellenbosch, South Africa (NSSA). (Oral)
2. Van Zyl., (2011). *In vivo* production of *Heterorhabditis zealandica* and *H. bacteriophora* for use as biopesticide against key insect pests. XVII Congress of the Entomological Society of Southern Africa, 3 - 6 July, Bloemfontein. (Oral)
3. Van Zyl, C. (2011). Department of Conservation Ecology and Entomology, Research Day. (Prize for the best oral presenter of the day. (Oral)
4. Van Zyl, C., (2012). Culturing EPNs *in vivo*. IPM meeting. February. (Oral)
5. Van Zyl, (2012). MSc defence seminar. *In vivo* production of entomopathogenic nematodes. 22 February, 13:00, Room 3028, JS Marais Building (Oral).

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### 3.2.11 PROGRESS REPORT: *In vitro* culture of *Heterorhabditis zealandica*

Experiment 984 (2010/13 – 2012/13) by T Ferreira (SU), A P Malan (SU) and S D Moore (CRI)

#### Opsomming

Die steinernematid, *Steinernema yirgalemense*, is suksesvol aangeteel in 'n aangepaste vloeistof medium. Daar is ook gevind dat *S. yirgalemense* vyf keer meer infektiewe larwes produseer in vergelyking met *Heterorhabditis zealandica*. Dit is die eerste aanmelding van die produksie van *S. yirgalemense* in 'n vloeistof medium. Groeikurwe toetse was gedoen met *Photorhabdus* (SF41 isolaat) om te bepaal wanneer die bakterieë die stasionêre fase bereik en die resultate wys dat dit plaasvind tussen 33 en 36 uur van groei by 30°C. Die simbiotiese bakterieë AM7 was geïsoleer vanuit 'n nuwe *Heterorhabditis* nematode spesie wat huidig beskryf word. Hierdie bakteriese isolaat bevat die mees algemene fenotipiese eienskappe van *Photorhabdus* spesies (gram negatief, staaf vormige selle, bioluminescent, katalase positief, reduseer nie nitraat nie en negatief vir oksidase) en is bewys om hoogs patogenies te wees vir *Galleria mellonella*. Dit gaan hand aan hand met die entomopatogeniese eienskappe van hierdie bakteriële genus. Die filogenetiese analise gedoen, is gebaseer op 'n multi-geen benadering (16S rRNA, *recA*, *gyrB*, *dnaN*, *gltX* en *infB* gene). Hierdie benadering het die klassifikasie van die isolaat AM7 in die groep spesies *P. luminescens* bevestig, en het ook bevestig dat dit naverwant is aan *P. luminescens* subsp. *caribbeanensis*, *P. luminescens* subsp. *akhurstii* en *P. luminescens* subsp. *hainanensis*. Verskeie fenotipiese eienskappe (suur produksie vanaf adonitol; sorbitol en xylitol; assimilasië van xylitol; tekort aan lipase aktiwiteit op Tween 20 en Tween 60) bewys dat daar gedifferensieer kan word tussen AM7 en die ander *P. luminescens* isolate wat behandel is in hierdie studie. Gebaseer op hierdie resultate stel ons voor dat die AM7 isolaat 'n nuwe *P. luminescens* subsp. is naamlik *Photorhabdus luminescens* subsp. *noenieputensis* sp. nov.

#### Summary

The steinernematid, *Steinernema yirgalemense*, was successfully cultured in an adapted liquid medium. It was found that *S. yirgalemense* produced five times more infective juveniles (IJs) *in vitro* in liquid culture in comparison to the number produced by *Heterorhabditis zealandica*. The current report is the first on the successful culturing of *S. yirgalemense* in liquid medium. During the study described in the report, growth curve tests were also conducted with *Photorhabdus* (strain SF41), in order to determine when bacteria reaches stationary phase. It was concluded that stationary phase is reached after 33 to 36 h. The bacterial symbiont, AM7, was isolated from a new entomopathogenic nematode species of the genus *Heterorhabditis*. The bacteria identified showed the main phenotypic traits of the genus *Photorhabdus* (Gram-negative rod-shaped cells; bioluminescent; catalase positive; non-nitrate reduction; and negative for oxydase) and was shown to be highly pathogenic for *Galleria mellonella*, in agreement with the entomopathogenic character of such a bacterial genus. The phylogenetic analysis, which was based on a multigene approach (16S rRNA, *recA*, *gyrB*, *dnaN*, *gltX* and *infB* genes), confirmed the classification of isolate AM7 within the species *Photorhabdus luminescens* and, closely related to *P. luminescens* subsp. *caribbeanensis*, *P. luminescens* subsp. *akhurstii* and *P. luminescens* subsp. *hainanensis*. Several phenotypic traits (acid production from adonitol, sorbitol and xylitol; assimilation of xylitol; lack of lipase activity on Tween 20 and Tween 60) allowed differentiation between AM7 from the other *P. luminescens* strains included in the current study. Based on the results, we propose strain AM7 as the type strain of a new *P. luminescens* subspecies, *P. luminescens* subsp. *noenieputensis* sp. nov.

### 3.2.12 FINAL REPORT: Relative attractiveness of virgin female FCM from different regions to sterile FCM males

Experiment 1019 (April 2011 – March 2012) by Dan Niland, Martin Hill, Tanya Pretorius (Rhodes University), Sean Moore and Wayne Kirkman (CRI)

#### Opsomming

Die steriele insek tegniek vir valskodlingmot (VKM) beheer is 'n paar jaar gelede ontwikkel, met die eerste kommersiële toepassing wat kort daarna, in 2007, in die Wes-Kaap gevolg het. Beide 'n SIT proef en die eerste paar jaar van kommersiële toepassing in spesifiek die Citrusdal streek het goeie resultate getoon. Gedurende die 2008/09 en 2009/10 seisoene is VKM SIT proewe in die Letsitele streek van Limpopo uitgevoer met teleurstellende resultate. Dit het gelei tot vroeë oor moontlike verskille in aanloklikheid van wyfies van streeks-spesifieke bevolkings vir steriele mannetjies, en indien wel, wat die effek van hierdie verskille op die vlak van die doeltreffendheid van die VKM SIT program sou wees. Bykomend tot 'n laboratorium kultuur van VKM, wat vir baie jare en generasies onderhou is, is vyf nuwe kulture gevestig afkomstig van verskillende streke. Ongelukkig kon 'n kultuur van Letsitele nie suksesvol gevestig word nie. In olfaktometer proewe is volwasse mannetjies van die Citrusdal-kultuur 'n keuse tussen twee opsies, 'n Citrusdal wyfie en 'n wyfie van een van die ander kulture,

gegee. In alle gevalle, behalwe een, het die meerderheid Citrusdal mannetjies Citrusdal wyfies verkies. Die uitsondering was gekry met die opsie van 'n Marble Hall wyfie. Hierdie verskille was egter nie betekenisvol nie. Waar mannetjies 'n positiewe keuse gemaak het, is wyfies behou en toegelaat om eiers te lê. Lewensvatbare eiertellings van Citrusdal wyfies was betekenisvol hoër as die vir wyfies van enige van die ander kulture. In boordproewe is betekenisvol meer steriele mannetjies gelok na lokvalle wat met wyfies van Marble Hall gelaai is, baie naby gevolg deur Addo. Dus het hierdie resultate die olfaktometer proef resultate ondersteun. Die moontlike implikasies van hierdie resultate vir die sukses van SIT in streke anders as die Wes-Kaap word bespreek.

## Summary

The sterile insect technique for false codling moth (FCM) control was developed a few years ago, with the first commercial roll out following in the Western Cape in 2007. Both an SIT trial and the first few years of commercial application in this region, Citrusdal specifically, proved to be successful. During the 2008/09 and 2009/10 seasons, FCM SIT trials were conducted in the Letsitele region of Limpopo Province with disappointing results. This raised the question of whether there is a difference in the attractiveness of females from regionally distinct FCM populations to sterile males, and if so, how this difference would affect the level of efficacy of the FCM SIT programme. In addition to a laboratory culture of FCM, which had been maintained for many years and generations, five new cultures originating from different regions, were initiated. Unfortunately, a Letsitele culture could not be successfully established. In olfactometer trials, adult males from the Citrusdal culture were given a two-way choice between a Citrusdal female and a female from one of the other cultures. In all cases but one, the majority of Citrusdal males preferred Citrusdal females. The exception was when given the option of a Marble Hall female. However, these differences in preference were not significant. From cases where positive choices were made by males, females were retained and allowed to oviposit. Viable egg counts from Citrusdal females were significantly higher than for females from any of the other cultures. In field trials, significantly more sterile males were attracted to traps loaded with females from Marble Hall, closely followed by Addo, thus confirming the olfactometer trial results. The possible implications of these findings for the success of SIT in regions other than the Western Cape are discussed.

## Introduction

False codling moth (FCM), *Thaumatotibia leucotreta*, sterile insect technique (SIT) pilot trials were conducted on Letaba Estates at Letsitele during the 2008/09 and 2009/10 seasons (Hofmeyr & Hofmeyr 2009 & 2010). Contrary to the positive results achieved with similar trials in the Western and Eastern Cape (Hofmeyr & Hofmeyr, 2006 & 2010), there were no differences in FCM infestation between release and control blocks. This led to a question about compatibility between FCM males and females from different regions. It is hypothesised that the sterile FCM males (from one production facility) may be differentially attracted to virgin females from different regions. A study conducted by Timm (2010) demonstrated that there are distinct genetic differences between FCM populations from different regions (and even within regions). It is possible that genotypic differences could sometimes relate to sexual attractiveness.

Rhodes University houses five different FCM cultures, originating from Addo, Citrusdal, Groblersdal, Nelspruit and the Baths (Citrusdal) (Opoku-Debrah, 2011). In addition, there is an old laboratory colony of uncertain origin. An unsuccessful attempt was made at establishing a sixth laboratory culture from field-collected larvae from Letsitele (specifically Letaba Estates). Comparison of the relative attractiveness of adult virgin female FCM from the different cultures to sterile male moths from the Xsit SIT production facility in Citrusdal will enable us to determine whether sexual incompatibility is the reason for the poor trial results in Letsitele and if this issue need be considered when planning any new cross-regional SIT trials or programmes. Incidentally, a third SIT pilot trial in Letsitele (on a different farm to that previously used) produced positive results (Moore, 2011).

## Objectives

The aim of this project is to determine if there is a difference in the attractiveness of regionally distinct populations of FCM females to sterile males, and if so how this difference would affect the level of efficacy of the FCM SIT programme.

## Materials and methods

There are currently five regionally distinct populations of FCM that are being maintained at Rhodes University, Eastern Cape, South Africa. These populations are originally from Marble Hall (Mpumalanga, 24° 58' 0" S, 29° 18' 0" E), Nelspruit (Mpumalanga, 25°27'57"S, 30°59'07"), Addo (Eastern Cape, 33°26'46"S, 25°44'45"E),

Citrusdal (Western Cape, 32°36'0"S, 19°1'0"E) and The Baths (Western Cape, 32°44'0"S, 19°2'0"E). The fifth colony is an old laboratory colony of unknown origin.

### Rearing virgin FCM

All cultures of FCM were kept separately (Opoku-Debrah, 2011) and were reared in the standard way, as developed by Moore (2002). Once larvae had pupated they were carefully removed from the cotton wool (standard pupation substrate, stoppering rearing jars) and sexed. The pupae were sexed by looking at their genital morphology. Once the pupae had had been sexed they were placed individually into 1.5 ml eppendorf tubes, which were plugged with a piece of cotton wool. Virgin females were used for trials as soon after eclosion as possible.

### Olfactometer trial

Glass Y-tubes were used in order to determine the attractiveness of the different populations of FCM to the Citrusdal males. The Y-tubes that were used were 20 cm in length, with the main shaft being 10 cm and each arm being 10 cm; the diameter of the tube was 8 mm. Virgin females that were 24 h - 48 h old were selected for this trial as in most lepidopteran species, pheromone production and release start a few days after eclosion (Babilis & Mazomenos 1992). Arenas were constructed for the virgin females using plastic pill vials. The vials had a hole drilled into the base which allowed for one arm of the Y-tube to fit tightly into it. Seven 1 mm holes were drilled into the lid of each of the vials to allow for air to flow through it easily. A small piece of gauze was placed over the end of each of the arms of the Y-tubes before the plastic arenas were placed onto them to prevent the females from entering the Y-tube. The Y-tubes and the arenas were soaked in ethanol before use to remove any volatiles that might interfere with the trial.

A female from the Citrusdal culture was placed into one of the arenas, and a female from another culture was placed into the other arena. A control was run by placing a Citrusdal female into one arena and nothing in the other. A male was then placed into the bottom of the main shaft and a piece of gauze was then placed over the shaft to prevent the male from escaping. The trial was conducted at a controlled temperature of 27°C, as this is the optimum temperature for FCM development (Moore pers.comm.). The female and the male FCM were placed into the Y-tube apparatus at between 18h30 and 19h30 and left overnight. The trial was left to run over night because it was shown using the corn stalk borer (*Sesamia nonagrioides* (Lef.)) that pheromone production starts two hours after night fall and peaks six hours after night fall (Babilis & Mazomenos 1992). Allowing the male FCM to be exposed to the pheromones for as long as possible would allow for a more accurate decision to be made. The Y-tubes were exposed to an air flow to allow for the pheromones to move from the female toward the male, the air flow through the Y-tubes was tested using smoke (Colazza *et al.* 1997). It is known that the FCM males locate females by following a pheromone concentration gradient (Schoeman & De Beer, 2008). The following morning the Y-tubes were checked, the male was considered to have made a decision if it had passed a decision line which was made 5cm up each of the arms. If the male had not made a decision it was not recorded. Twenty one replicates were run for each of the populations and the control. After each replicate the Y-tubes were flipped over in order to prevent any external stimuli, such as light, from affecting the results.

The Y-tube trial was analysed using a chi squared test. The preference of the Citrusdal male towards each population's pair of females was compared separately. Since this analysis only allowed for one degree of freedom, the Yates' correction for continuity was added to the chi squared test to prevent the test statistic from being too high (Flower *et al.* 1998). A Kruskal-Wallis ANOVA was used to analyse the numbers of viable eggs that were produced in the compatibility trial.

### Egg viability

A male from the Citrusdal population and one female were placed into a 50 ml glass vial plugged with cotton wool. This was replicated for all of the colonies 10 times. A Citrusdal male and a Citrusdal female were used as a control. The male and female were left for one week. This allowed for enough time for the male and female to mate, lay eggs and the eggs to change to a red colour so that their viability could be determined. The number of viable eggs was counted for each of the vials.

### Field trials

A field trial was conducted throughout the month of April, 2011, on Sommersby Farm in the Sunday's River Valley, Eastern Cape. The trial was run in three orchards that were situated parallel to each other, in a north to south direction. The orchards comprised of citrus trees of the varieties, Lane Late Navel, Cara Cara Navel and

Turkey Valencia. Each of the orchards consisted of 22 rows of trees. There were 36 delta traps erected on the south end of all three of the orchards. Two traps were placed in each of the rows; they were placed on the fourth and fifth tree from the end of the row to avoid edge effect. The traps were erected on this side to take advantage of the prevailing wind in the area. Two traps were erected on the western side on each row to ensure each trap experienced the same climatic conditions. The traps were erected in rows 3 to 20, and three trees in, this was done to avoid the edge effect.

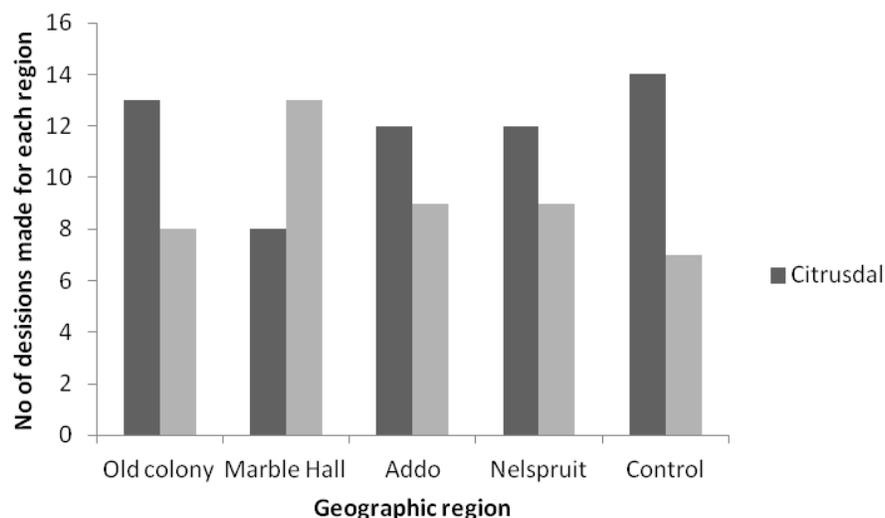
The virgin females were placed into plastic cages which were wrapped in gauze. Six of these cages, for each population along with six control cages, with no females, were secured randomly into the Delta traps in each of the orchards. Sterile moths, which had been sent up overnight from Xsit in Citrusdal, were then released. They were released in a line perpendicular to the rows, 30 – 40 m on the northern side of the traps. About 20000 sterile moths were released in each of the orchards. The traps were left for three days then collected and the number of sterile moths caught counted and analysed.

The trial was repeated three times on Endulini Farm in Sundays River Valley in 2012. This was done in a grapefruit orchard with rows running east-west. The 36 traps were positioned one per row in 36 adjacent rows, starting from the 5<sup>th</sup> row from the northern side of the orchard. Traps were hung on the south-easterly aspect of the tree. Two virgin females were placed into each trap cage and traps were positioned randomly. A total of 8000 sterile moths were released as evenly as possible in each of the 36 trap rows at 10 trees away from traps (in the case of the first replicate) and five trees away from traps in the (in the case of the second and third replicates). Trials were conducted on the mornings of 13 February, 23 March and 11 May. Traps were inspected exactly one week later and numbers of wild and sterile moths caught in each trap were counted.

## Results and discussion

### Olfactometer trial

The trial was run 21 times for each of the populations. Each time a male made a decision in the Y-tubes it was scored on either the side of the Citrusdal female or on the side of the female from the other population. The majority of the males placed into the Y-tubes made a decision, the ones that made no decision were replaced. New FCM's were used for each of the Y-tube trials undertaken.



**Fig. 3.2.12.1.** The total number of decisions made indicating the difference in attractiveness between females from the Citrusdal population and females from other population located around South Africa to males from the Citrusdal population.

The Y-tube trials were statistically tested separately, i.e. the old colony was compared with Citrusdal colony for each trial. It can be seen that the Citrusdal Population consistently received more scores than the other population in all of the trials, except the trial that compared the Marble Hall population with the Citrusdal population (Fig. 3.2.12.1.). When comparing the attractiveness of the old colony with that of the Citrusdal colony it was found that there was no significant difference between them ( $X^2 = 1.24$ ). This was true for all of the

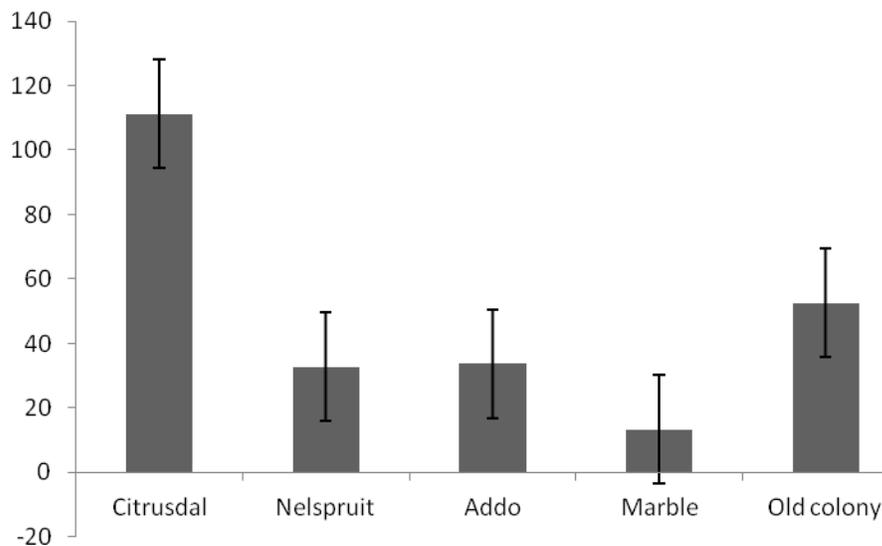
colonies that were tested. Marble Hall ( $X^2=1.24$ ), Addo ( $X^2 = 0.476$ ), Nelspruit ( $X^2 = 0.476$ ), and the control ( $X^2 = 2.38$ ). An  $X^2$  value of 3.84 or greater was needed to have a significant result at a level of significance of 0.05.

The Y-tube trial that was conducted did not reveal a statistically significant result. However it could be seen that there was a trend that was displayed by the Y-tube experiments. This result could be due to the number of trials conducted for each of the colonies. The  $n$  that is taken for a data set affects the accuracy of the data taken. If the  $n$  is large the statistical test will have a higher accuracy. However if the  $n$  is low that accuracy of the data is limited (Agresti & Franklin 2007). The result of the Y-tube trial may have changed if the number test had increased. In a study by Knight & Light (2001), a significant result was achieved by replicating their trials 50 times. The number of individuals used in this trial was limited to the number of individuals that eclosed. More FCM's for each of the colonies was not cultured as there was a time constraint on this trial which did not allow for a greater number of tests to be run.

There are a number of improvements that could be made to this trial that could have been made to this trial that also may have allowed for a better result. These trials were run in a small controlled environment room (CE room). Because this room is so small the pheromones produced by the female moths may have flooded the air in the CE room. This may have resulted in the males being confused by the mixture of pheromones and hence making an incorrect decision. This could have been easily corrected by using a purified air source (Boo *et al* 1998). One method that has been used to purify air in Y-tube experiments, is to bubble normal air through distilled water containing activated charcoal. This method serves two purposes, firstly it removes any impurities from the air that may affect the decision that may be made by the moth, and secondly it humidifies the air which allows for the pheromone to be better carried in the air (Hern & Dorn 2004).

### Egg viability

The egg viability trial was done using adults that had been used in the Y-tube trial and had scored a result. It was observed that the moths would lay eggs with in three days of being placed into the chamber with a male. There was mating observed between Citrusdal males and females. Mating between Citrusdal males and females from the other colonies was not observed, but can it be seen that mating must have occurred as there were viable eggs produced (Fig. 3.2.12.2).



**Fig. 3.2.12.2.** The mean number of eggs produced by each population of FCM when mated with a male from the Citrusdal population.

The average number of eggs produced by the each of the populations is a less that the maximum of  $\pm 240$  (Peña *et al.* 2002). However it can be seen that the number of eggs that were produced by the Citrusdal female was far higher than that produced by any of the other populations (Fig. 3.2.12.2.). This result is also statistically significant ( $P=0.0014$ ) ( $H= (4, N = 55) = 17.70197$ ). When compared to each of the other populations it was shown that the Citrusdal population was significantly different to all of the colonies except the old colony.

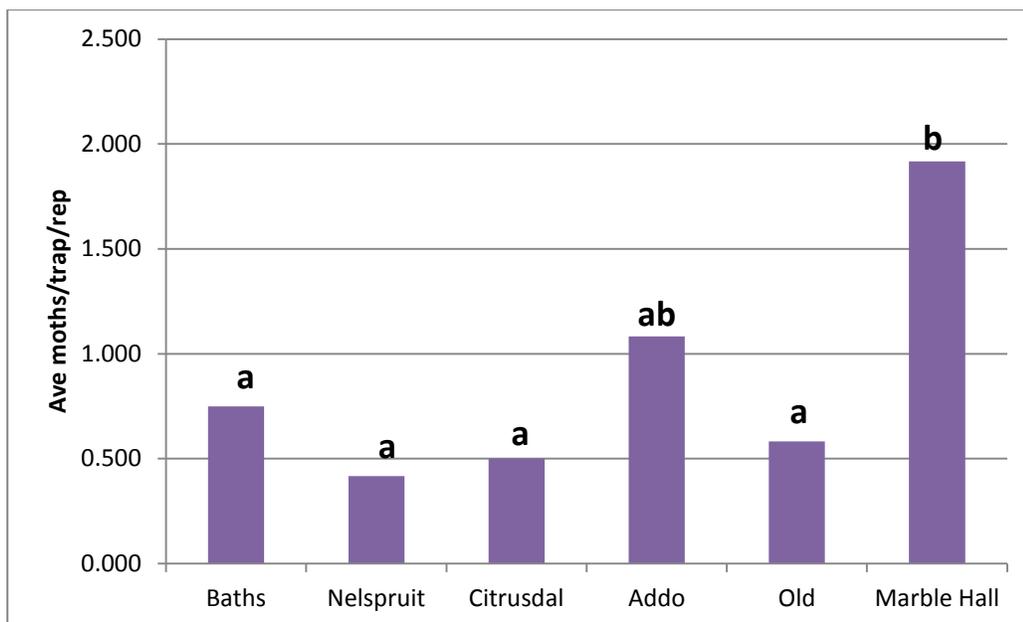
The results that were attained from the egg viability trial were shown to be significant. However the results that were attained only show that the number of viable eggs produced by the different colonies were significantly

different to those produced by the Citrusdal population and do not show a difference in mating compatibility. From this trial it is impossible to determine if the Citrusdal males were able to mate with the female FCM's from the other colonies and satisfy their urge to mate. In all of the colonies except for the Citrusdal colony, it was observed that a number of the female did not lay eggs at all. There were also a number of moths that produced an entire clutch of unviable eggs, these eggs may have been dumped because they had not been fertilised. Understanding if the Citrusdal males did mate with all of the female FCM's from the other colonies, and satisfied their urge to mate, is relevant to the control of the FCM. If it is physiologically difficult for the sterile males to mate with females from other regionally distinct colonies, it could be a disadvantage to sterile moths when used to control an outbreak. It is therefore essential to test if there is a physiological difference between the populations.

### Field trials

There were no sterile moths recaptured in any of the traps that were set up. This result was anticipated as the quality of the sterile moths that were sent up from Xsit where of very poor quality. There was also a cold snap during the time that these moths were released which would have also caused a reduction in the fitness of the moths. There were two wild males caught. But they were caught in traps containing females from different populations. The capture of these males can therefore be described as coincidence. In order to obtain a result for a trial such as this one the trial should be run in late September or early October, depending on when the mass releases of sterile moths occur.

Substantially more moths were attracted to the Marble Hall virgin traps than to any of the other traps (Fig 3.2.12.3). This was interesting in that it confirmed the olfactometer trial results. The implications of this are that there do appear to be differences in attractiveness of virgin females from different regions to laboratory reared sterile males. It is most likely the genetic difference which affects this attraction, rather than having anything to do with sterility, as although Citrusdal males were used as the benchmark in the laboratory trials, these were not sterilised.



**Figure 3.2.12.3** Average number of sterile moths caught per trap per replicate at Endulini Farm, Sundays River Valley (bars with the same letters are not significantly different;  $\alpha=0.05$ )

SIT field trials have been conducted in both Citrusdal and Addo with good results and the technique is now being used commercially with success in both regions. If this is the benchmark, then SIT should also work in Mpumalanga i.e. Nelspruit and Marble Hall – possibly better than in Citrusdal and Addo in the case of the latter.

### Conclusions

The trials that were conducted in this project were unable to determine if the sterile males from the Citrusdal population were at a disadvantage to the males from other populations that they were trying to control. Instead more questions have been raised about the possible physiological mating differences between populations of

FCM and how this may also affect the control that SIT can exhibit on the control of regionally distinct populations.

## Acknowledgements

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### 3.2.13 **PROGRESS REPORT: Identifying volatile emissions associated with false codling moth infestation of citrus fruit.**

Experiment 1022 (2011/12 – 2012/3) by Rachel van der Walt, Vaughan Oosthuizen, Ben Zeelie, Melissa Gouws (NMMU) and Sean Moore (CRI)

#### **Opsomming**

Valskodingmot is n bekende plaag van ekonomiese belang vir baie verbouings-gewasse in Suid-Afrika en in Afrika suid van die Sahara, en is veral 'n probleem op sitrus. Indien die vrugte kort voor oes besmet is, is die kans vir opsporing van tekens van besmetting baie laag. Daarom is daar 'n beduidende risiko dat hierdie besmette vrugte as gesonde vrugte gepak en uitgevoer sal word. Dit is dus n prioriteit om 'n na-oes tegniek vir die opsporing van VKM in sitrusvrugte op verskillende stadiums van besmetting te ontwikkel, om fitosanitêre risiko te verminder. Die vlugtige verbinding profiel van n spesifieke plant kan deur stres of die teenwoordigheid van die herbivoor in die plant beïnvloed word. Hierdie kan tot veranderinge in die metabolisme van 'n plant lei, met gevolg dat 'n ander vlugtige verbinding profiel genereer word, wat vir die na-oes opsporing van besmette vrugte uitgebuit kan word. Dit is noodsaaklik om n spesifieke vlugtige stof te identifiseer wat as 'n aanwyser vir n spesifieke plant en herbivoor gebruik kan word. Dit moet verskil van enige vlugtige samestelling wat as gevolg van omgewings- of voedingsstres geproduseer word. Die doelwitte van hierdie studie is 'n vlugtige opsporingstelsel vir gebruik op sitrus vrugte te ontwikkel, en om hierdie stelsel te gebruik om verskille in vlugtige uitstorting tussen besmette en gesonde vrugte te ondersoek. Die toepassing van n SPME prosedure en die optimalisering van hierdie metode vir die opsporing van moonlike vlugtige verbindinge wat teenwoordig is in lemoene is ondersoek.

#### **Summary**

False codling moth is a known pest of economic importance to many cultivated crops in South Africa and Africa south of the Sahara, and is particularly severe on citrus. If the fruit is infested just before harvest the chances of detecting signs of infestation are very low. As a result, the risk of packaging infested fruit and exporting them as healthy fruit is high. It is therefore a priority to develop a post-harvest technique for detection of FCM in citrus fruit at different levels of infestation in order to reduce phytosanitary risk. The volatile compound profile of a particular plant can be influenced by stress or the presence of herbivores in the plant parts. This can cause changes in the plant's metabolism, resulting in the production of a different volatile compound profile, which can be exploited for postharvest detection of unhealthy fruit. It is vital to correctly identify a distinct volatile compound that can act as a biomarker for a particular plant and herbivore, which will be different from a volatile compound produced due to environmental or nutrient stress. The objectives of this study are to develop a volatile detection system for use on citrus fruit, and to use this system to investigate differences in volatile emission between infested and healthy fruit. The application of an SPME procedure and the optimization of this method for detection of possible volatiles present in the headspace of intact fruit have been evaluated.

### 3.2.14 **PROGRESS REPORT: Entomopathogenic fungi for the control of soil-borne life stages of FCM**

Experiment 1024 (Jan 2011 – Dec 2012) by Candice Coombes (RU), Sean Moore (CRI), Martin Hill and Jo Dames (RU)

#### **Opsomming**

Die beheer van VKM in die sitrusbedryf is noodsaaklik vanweë die finansiële verliese wat hoofsaaklik as gevolg van die fitosanitêre status geassosieer met VKM kan gebeur. Entomopatogeniese swamme (EPS) kan moontlik as 'n addisionele biologiese beheer metode teen VKM dien, veral die grondgedraagde lewensstadie. Goble (2009) het 62 swam isolate geïsoleer waarvan 12 met goeie beheer potensiaal geïdentifiseer is. Nog navorsing volg egter. Deur die voltooiing van laboratorium biotoetse, groter pot-proewe en veld nawerkingsproewe is die doel van hierdie studie om verder die potensiele gebruik van EPS vir VKM beheer te ondersoek. Die resultate dui daarop dat drie swam isolate (G 11 3 L6, FCM Ar 23 B3 en G Ar 17 B3) die grootste potensiaal vir beheer van VKM toon. Hierdie drie isolate was ook meer doeltreffend as die twee kommersiële isolate wat getoets is. Oor die algemeen, is 'n dosis-afhankende verhouding tussen 'n toename in mortaliteit en swam konsentrasie waargeneem. Net so, is 'n verhoging in mortaliteit waargeneem met 'n verhoging in tydsduur (in dae) wat larwes aan die swam blootgestel is. Swam nawerkingsproewe is nog nie voltooi nie, maar sal teen Mei 2012 voltooi wees. Tot op datum toon die data 'n beperkte korrelasie tussen tellings van kolonie vormings eenhede (KVE) en persentasie mikose. Al het KVE tellings oorspronklik 'n afname getoon, wil dit voorkom dat hulle nou gestabiliseer het. Pot-proewe om die mees geskikte metode van toediening te bepaal is nog nie begin nie.

## Summary

The control of FCM is essential in the citrus industry due to the financial loss which can occur mainly due to the phytosanitary status associated with FCM. Entomopathogenic fungi (EPF) can potentially serve as an additional biological control method against FCM particularly the soil-borne life-stages. Goble (2009) isolated 62 fungal isolates of which 12 were identified as showing good control potential. More research however remains. Through the completion of laboratory bioassays, larger pot-trials and field persistence trials, this studies aims to further investigate the potential use of EPF for FCM control. Results indicate that three fungal isolates show the greatest potential for the control of FCM (G 11 3 L6, FCM Ar 23 B3 and G Ar 17 B3). These three isolates also performed more effectively than the two commercial isolates tested. In general a dose-dependent relationship was found with an increase in mortality associated with an increase in conidial concentration. Likewise, an increase in mortality was observed with an increase in the amount of time (in days) the larvae were exposed to the fungus. Fungal persistence trials are not yet complete, but will be completed by May 2012. To date, the data show limited correlation between colony forming unit (CFU) counts and mycosis percentage. CFU counts, although showing an initial decrease, do appear to have stabilised. Pot-trials to determine the most appropriate method of application have not yet been initiated.

### 3.2.15 PROGRESS REPORT: Morphological and molecular identification of moths associated with citrus in the Western Cape Province.

Experiment US/ENT-08-A2 (April 2011 – March 2013) by P. Addison, M. Rentel, H. Geertsema (SU) and J. Brown (USDA, Washington DC)

## Opsomming

Sewe ekonomies-belangrike tortrisied spesies, elkeen met 'n groot impak op die plaaslike vrugtebedryf, word in Suid-Afrika aangetref. Hulle is: *Cydia pomonella* (Kodlingmot), *Thaumatotibia leucotreta* (Valskodlingmot), *Grapholita molesta* (Oosterse vrugtemot), *Cryptophlebia peltastica* (Lietsjiemot), *Thaumatotibia batrachopa* (Makadamia neutboorder), *Epichoristodes acerbella* (Peerbladroller/angelierrusper) en *Lozotaenia (Tortrix) capensana* (Appelbladroller). Larwes van al hierdie spesies kan belangrike fitosanitêre probleme veroorsaak en korrekte identifikasie is dus belangrik. Vir die tortrisied spesies bestaan daar egter net 'n paar onvolledige identifikasiesleutels. Vir die rede is een van die doelwitte van die huidige studie om 'n verbeterde taksonomiese begrip van ekonomies-belangrike tortrisied spesies in Suid-Afrika op te stel. Hierdie studie, saam met die ontwikkeling van 'n interaktiewe sleutel, word tans onderneem en behoort teen Julie 2012 voltooi te wees. Vorige DNS-ontledings het aangedui dat valskodlingmot van verskillende geografiese bevolkings geneties verskil, wat 'n aanduiding van naderende spesiasie kan wees. Dit is dus van die uiterste belang om die spesies status te bepaal en of inteling tussen bevolkings kan voorkom. Kruisparingsproewe is onderneem om dit te bepaal, maar vanweë verskeie probleme het die wederkerende kruisparingsproewe misluk en is nie gekwantifiseer nie, alhoewel aanvanklike resultate aangedui het dat valskodlingmot van verskillende bevolkings tog in staat is om te paar en om lewensvatbare nageslag in daaropvolgende generasies te lewer. Genitalia van Lepidoptera word algemeen gebruik vir klassifikasie doeleindes op spesiesvlak. By ander tortrisied spesies wil dit voorkom dat genitalia van oënskynlik identiese individue tog variasie in struktuur kan vertoon. Hierdie variasie kan vir spesies onderskeid gebruik word en morfometrie kan aangewend word om verskille in VKM mannetjies te kwantifiseer. Die studie word ook tans onderneem met verwagte voltooiing in Julie 2012.

## Summary

In South Africa, there are seven major economically important tortricid species are found, each with great impact on the local fruit industry. They are: *Cydia pomonella* (Codling moth) *Thaumatotibia leucotreta* (False codling moth), *Grapholita molesta* (Oriental fruit moth), *Cryptophlebia peltastica* (Litchi moth), *Thaumatotibia batrachopa* (Macadamia nut borer), *Epichoristodes acerbella* (Pear leafroller/carnation worm) and *Lozotaenia (Tortrix) capensana* (Apple leafroller). The larvae of all these species could cause major phytosanitary problems thus the correct identification is important. However, for tortricid species only a couple of incomplete identification keys exist. Thus one aim of the present study is to develop an improved taxonomic understanding of economically important tortricid moth pests in South Africa. This study together with the development of an interactive key is currently underway and should be completed by July 2012. DNA analysis previously done showed that the false codling moth from different geographic populations were genetically distinct, which could be evidence of impending speciation. Thus it is of utmost importance to determine the species status and if interbreeding between populations could occur. Cross-mating trials were undertaken to determine this but due to various problems, the reciprocal cross-mating trial failed and was never quantified, but initial results showed that the false codling moth from different populations were still able to mate and produce viable offspring in further generations. Lepidopteran genitalia are commonly used for classification purposes at a species level. In other

tortracid species genitalia of seemingly identical individuals showed some variation in structure. This variation can be used for species distinction and morphometrics can be used to quantify differences in FCM males. This study is also currently underway with expected completion in July 2012.

### 3.2.16 PROGRESS REPORT: Large scale field trials with entomopathogenic nematodes for control of FCM, fruit fly and thrips

Experiment 1042 (Sep 2011 – March 2015) by Sean Moore (CRI), Ralf-Udo Ehlers (University Christian Albrechts), Aruna Manrakhan, Wayne Kirkman, John-Henry Daneel (CRI), Jean de Waal (Dow) and Paul De Wet (SU)

#### Opsomming

In totaal is vyf veldproewe met entomopatogeniese nematodes (EPNs) in sitrusboorde gedurende die 2011/12 seisoen uitgevoer: twee in die Oos-Kaap, twee in Mpumalanga en een in die Wes-Kaap. Vier van die proewe is ontwerp om die effektiwiteit van grond toediening met 'n kommersiele formulاسie van *Heterorhabditis bacteriophora* te toets. Een van die proewe is ontwerp om die effektiwiteit van die natuurlike teenwoordigheid van EPNs – *H. zealandica* – te toets. Nie een van die proewe in die Oos-Kaap het positiewe resultate opgelewer nie, hoofsaaklik as gevolg van 'n tekort aan vogtigheid in die grond en daarom min oorlewing van EPNs in die grond. Die Wes-Kaap (Citrusdal) proef het positiewe resultate opgelewer. Nawerking van EPNs in die grond is tot 8 weke na toediening gemeet, waarna daar geen verdere ontleding van EPN oorlewing uitgevoer is nie. VKM besmetting van vrugte is vir die tydperk van Desember 2011 tot Maart 2012 met tussen 55% en 81% verminder in vergelyking met kontrole boorde. Geen vermindering in blaaspoottjie vlakke en blaaspoottjie skade op vrugte is aangeteken nie. Die eerste Mpumalanga proef het geen resultate opgelewer nie omdat die natuurlike voorkoms van *H. zealandica* in die grond te hoog was. Hierdie het tot 'n finale proef gelei om te bepaal presies hoe doeltreffend is hierdie EPNs. Een boord is met 'n aalwurmdoder (cadusafos) behandel en die boord langsaan is onbehandeld gelaat. Cadusafos het EPN besmettings vlakke met 90% verminder, maar dit het voorgekom dat vlakke 8 weke later voliedig herstel het. Nietimin was VKM besmetting in die onbehandelde blok 59% laer as in die cadusafos-behandelde blok. Vrugtevlieg lokvalle in die onbehandelde blok het net 21% van die hoeveelheid vlieë gevang wat in die behandelde blok gevang is. Baie waardevolle lesse wat die kritiese behoeftes vir sukses met EPN toedienings is geleer. Verdere proewe sal in die herfs uitgevoer word.

#### Summary

A total of five field trials were conducted with entomopathogenic nematodes (EPNs) in citrus orchards during the 2011/12 season: two in the Eastern Cape, two in Mpumalanga and one in the Western Cape. Four of the trials were designed to test the efficacy of soil application with a commercial formulation of *Heterorhabditis bacteriophora*. One of the trials was designed to determine the effectiveness of naturally occurring EPNs – *H. zealandica*. Neither of the two trials in the Eastern Cape produced results, mainly due to a lack of moisture in the soil and therefore a lack of survival of the EPNs in the soil. The Western Cape (Citrusdal) trial produced positive results. Persistence of EPNs in the soil was measured up to 8 weeks after application, after which no further assessment of EPN survival was conducted. FCM infestation of fruit was reduced by between 55% and 81% relative to control orchards for the period December 2011 to March 2012. No reduction in thrips levels and thrips damage to fruit was recorded. The first Mpumalanga trial produced no results, due to a prohibitively high level of naturally occurring *H. zealandica* in the soil. This prompted a final trial to determine how effective these EPNs were. One orchard was treated with a nematicide (cadusafos) and the adjacent orchard left untreated. Cadusafos reduced EPN infection levels by 90%. However, levels seemed to have fully recovered by 8 weeks later. Despite this, FCM infestation in the untreated block was 59% lower than in the cadusafos-treated block. Fruit fly traps placed in the untreated block caught only 21% of the fly numbers that were caught in the treated block. Many valuable lessons pertaining to the critical requirements for success with EPN applications were learned. Further trials will be conducted in autumn.

### 3.3 PROJECT: FRUIT FLY

Project Coordinator: Aruna Manrakhan

#### 3.3.1 Project summary

Fruit flies are of economic importance in the citrus industry mainly due to the phytosanitary concern associated with these pests and direct damage caused on mature ripe fruit. The three main local fruit fly pests are *Ceratitis capitata* (Medfly), *Ceratitis rosa* (Natal fly) and *Ceratitis cosyra* (Marula fly). The fruit industry of South Africa is however also being faced with the threat of introduction of an invasive fruit fly of Asian origin- *Bactrocera invadens* - known to be in Africa since 2003 and reported in Southern Africa (Namibia, Zambia and Mozambique) since 2008. *Bactrocera invadens* was detected in areas in northern Limpopo in 2010 and 2011 and in both years, the pest was eradicated.

Within the fruit fly project, basic and applied research studies were conducted with the main objectives of (1) improving the management of local fruit fly pests, (2) investigating the efficacy of a new post-harvest treatment, (3) determining the suitability of various areas in South Africa for establishment of *B. invadens* and (4) determining the efficacy of various control methods for control of *B. invadens*. The maintenance of the *B. invadens* surveillance network also formed part of the fruit fly project activities.

Laboratory reared colonies of Medfly, Natal fly and Marula fly were maintained at CRI Nelspruit to provide materials for research studies (3.3.2).

A new biodegradable bait station was developed (3.3.3). The efficacy of this new bait station was determined in a mango orchard and compared with the M3 bait station and GF-120. Field evaluation results indicated that overall, the best treatment in terms of lowest fruit fly trap catches (all fly species) was the M3 bait station. In terms of fruit infestation however, no fruit fly infestation was recorded in the block treated with the new bait station. Fruit fly damage, although very low, was recorded in blocks treated with M3 and GF-120 and the untreated blocks.

The potential of entomopathogenic nematode (EPN) isolates for control of Medfly and Natal fly was demonstrated in laboratory assays in 2011 (3.3.4). However, no field evaluation could be conducted thereafter due to unavailability of these isolates from University of Stellenbosch.

In South Africa, Medfly and Natal fly differ in their distribution patterns and relative abundance in different areas. While Medfly has a more ubiquitous distribution across South Africa, Natal fly is mainly restricted to the north, east and coastal parts of the south/south east. Research on thermal biology of Medfly and Natal fly was completed and provided some insights into the factors that affect the distribution and abundance of the two fruit fly species (3.3.5).

The increasing interceptions of *B. invadens* in South Africa called for continuation of surveillance efforts which would enable timely detection and intervention (3.3.6). *B. invadens* was recorded in five separate areas in northern Limpopo in 2011. The pest was successfully eradicated in all areas and results were reported in the International Plant Protection portal.

The efficacy of the male annihilation technique (deployment of blocks impregnated with methyl eugenol and malathion- MAT blocks) and a bait application technique for control of *B. invadens* was evaluated over 2 seasons in a citrus field trial in Kenya (3.3.7). M3 bait stations or Prolure bait sprays in combination with MAT blocks were found to be effective in controlling *B. invadens*.

The potential distribution of *B. invadens* in the world and in South Africa is currently being determined using the CLIMEX model (3.3.8). Parameters used in the model will be based on distribution, relative abundance and seasonal occurrence of the pest in different climatic regions of Africa obtained through trapping.

An alternative post-harvest treatment for fruit flies is currently being evaluated (3.3.9). The fumigant GRASFRUM was found to provide effective control of fruit fly larvae in loose media. Research is ongoing to determine the efficacy of the fumigant for control of larvae inside fruit. A combination of fumigation and short cold treatment will also be evaluated.

#### Projekopsomming

Vrugtevlieë is van ekonomiese belang vir die sitrusbedryf hoofsaaklik as gevolg van die fitosanitêre probleem wat met hierdie plaeg geassosieër word en ook die direkte skade wat veroorsaak word aan volwasse ryp vrugte. Die

drie belangrikste vrugtevlieg plaë is *Ceratitis capitata* (Medvlieg), *Ceratitis rosa* (Natale vlieg) and *Ceratitis cosyra* (Maroela vlieg). Die vrugbedryf van Suid-Afrika staan ook 'n nuwe bedryding in die gesig, naamlik die introduksie van die indringervlieg wat van Asiatiese oorsprong is - *Bactrocera invadens* – bekend dat hy vanaf 2003 in Afrika is en in Suidelike Afrika (Namibië, Zambië en Mosambiek) vanaf 2008 gerapporteer is. *Bactrocera invadens* was in areas in die noordelike Limpopo in 2010 en 2011 opgespoor en is in beide hierdie twee jare uitgeroei.

In die vrugtevliegprojek is basiese en toegepaste navorsing studies gedoen en die hoofdoelwitte is (1) verbetering in die bestuur van plaaslike vrugtevlieg plaë, (2) 'n ondersoek op die doeltreffendheid van 'n nuwe na-oes behandeling, (3) bepaling van die geskiktheid van verskeie areas in Suid-Afrika vir die vestiging van *B. invadens* en (4) beapaling van die doeltreffendheid van verskeie beheermaatreëls vir die beheer van *B. invadens*. Die *B. invadens*-waarnemingsnetwerk se onderhoud vorm ook deel van die vrugtevlieg projek se aktiwiteite.

Die laboratorium geteelde kolonies van Medvlieg, die Natale vrugtevlieg en Maroela vrugtevlieg wat te CRI Nelspruit onderhou word, is gebruik om materiaal vir navorsingsstudies te verskaf.

'n Nuwe bio-afbreekbare lokstasie is ontwikkel (3.3.3). Die effektiwiteit van hierdie nuwe lokstasie was in 'n veselperskeboord bepaal en is met die M3-lokstasies en GF-120 bespuitings vergelyk. Boord evaluasie proewe dui daarop dat die beste behandeling in terme van laagste vrugtevlieglokval vangste (vir alle vrugtevlieg spesies) die M3-lokstasie was. In terme van vrugbesmetting, was daar egter geen besmetting in die blok wat met die nuwe lokstasie behandel was nie. Vrugtevliegskade, alhoewel dit baie laag was, was aangeteken in die blokke wat met M3 en GF-120 behandel was asook die onbehandelde blokke.

Die potensiaal van entomopatogeniese nematode isolate (EPN) om Medvlieg en die Natale vrugtevlieg te beheer, was in 2011 in laboratoriumproewe gedemonstreer (3.3.4). Geen boord evaluasies kon egter hierna gedoen word nie omdat die isolate van die Universiteit van Stellenbosch nie beskikbaar was nie.

In Suid-Afrika verskil die Medvlieg en die Natale vrugtevlieg se verspreidingspatrone en relatiewe volopheid in verskillende areas. Terwyl die Medvlieg se verspreiding alomteenwoordig is deur Suid-Afrika, is die Natale vrugtevlieg beperk tot die noorde, ooste asook die suid/suid-oostelike seekusgebiede. Navorsing op die termiese biologie van Medvlieg en die Natale vrugtevlieg was voltooi en het lig op sekere faktore wat die verspreiding en oorvoedigheid van die twee vrugtevlieg spesies beïnvloed gewerp (3.3.5).

Die toename in onderskeppings van *B. invadens* in Suid-Afrika het gelei tot die voortsetting van waarnemingpogings om sodoende tydig opsporing en intervensie te verseker. *B. invadens* was in vyf aparte areas in die noordelike Limpopo in 2011 aangeteken. Die plaag was in al die areas suksesvol uitgeroei en die resultate was op die "International Plant Protection" portaal gerapporteer.

Die effektiwiteit van die mannetjie uitwissingstegniek (ontploffing van blokke geïmpregneer met metiel-eugenol en malation- MAT-blokke) en die lokmiddel toedienings tegniek vir die beheer van *B. invadens* was vir twee weke in 'n sitrusboord in Kenia geëvalueer (3.3.7). M3-lokstasies of Prolure-lokmiddel toedienings in 'n kombinasie met MAT-blokke het *B. invadens* doeltreffend beheer.

Die potensiele verspreiding van *B. invadens* oor die wêreld en in Suid-Afrika word tans bepaal deur gebruik te maak van die CLIMEX model (3.3.8). Die parameters wat in die model gebruik was, is gebaseer op die verspreiding, relatiewe volopheid en seisoenale voorkoms van die plaag in verskillende klimaatstreke van Afrika en was deur lokvalvangstes verkry.

'n Alternatiewe na-oes behandeling vir vrugtevlieë word tans geëvalueer (3.3.9). Daar is gevind dat die berokingsmiddel GRASFRUM effektiewe beheer vir vrugtevliegglarwes in los media verskaf. Navorsing om die effektiwiteit van die berokingsmiddel vir die beheer van larwes binne-in vrugte te bepaal, is tans aan die gang. 'n Kombinasie van die berokingsmiddel en 'n kort koue behandeling sal ook geëvalueer word.

### 3.3.2 PROGRESS REPORT: Fruit fly rearing

Experiment 407 (1999/2000 – 2012/3) by A Manrakhan, J-H Daneel & R Beck (CRI)

#### Opsomming

Drie vrugtevliegspesies: *Ceratitis capitata* (Mediterreense vrugtevlieg), *C. rosa* (Natale vrugtevlieg) en *C. cosyra* (Maroela vrugtevlieg) word tans op kunsmatige voedingsmedia by CRI, te Nelspruit geteel. Vrugtevlieg

materiaal is gebruik vir navorsing op: (1) 'n Nuwe lok-en-doodmaak vrugtevlieg sisteem (Eks. 915), (2) 'n GRAS na-oes beroking vir vrugtevlieë (Eks. 913), (3) Termiese toleransies van *Ceratitis*-vlieë (navorsing is gedoen by die Universiteit van Stellenbosch onder Eks CRTT-01) en (4) die evaluasie van nuwe geformuleerde lokmiddels van BASF (Pty) Ltd. and Dow AgroSciences (kontraknavorsing). Die Maroela vrugtevliegkolonie was hernu deur marulavrugte, *Sclerocarya birrea*, in die begin van Maart 2012 te versamel. 'n Totaal van 500 nuwe uitgeboreide laboratorium geteelde volwasse wyfies was by 575 mannetjies gevoeg, wat uit *S. birrea* geteel is. Die nuwe F1 generasie en ander opeenvolgende generasies was op 'n geelwortelbasis dieet geteel.

## Summary

Three fruit fly species: *Ceratitis capitata* (Medfly), *C. rosa* (Natal fly) and *C. cosyra* (marula fly) are currently being reared on artificial diets at CRI Nelspruit. Fruit fly materials were used for research on: (1) a new attract and kill fruit fly system (Exp 915), (2) a GRAS post-harvest fumigant for fruit fly (Exp 913), (3) thermal tolerance of *Ceratitis* flies (research conducted at the University of Stellenbosch under Exp CRTT-01) and (4) evaluation of new formulated baits from BASF (Pty) Ltd. and Dow AgroSciences (contract research). The colony of Marula fly was refreshed by sampling marula fruit, *Sclerocarya birrea* at the beginning of March 2012. A total of 500 newly emerged laboratory reared adult females were added to 575 males which emerged from *S. birrea*. The new F1 generation and other successive generations were then reared on the carrot-based diet.

### 3.3.3 PROGRESS REPORT: A new bait for more effective control of all *Ceratitis* fruit flies Experiment 915 (2008/9 – 2012/3) by A Manrakhan, John-Henry Daneel & Rooikie Beck (CRI)

## Opsomming

Die hoof doel van hierdie studie was om 'n meer effektiewe lokmiddel toedienings tegniek vir vrugtevliegbeheer te kry. 'n Nuwe bio-afbreekbare lokmiddel was ontwikkel. Die nuwe lokstasie was gemaak van 'n bedekte, gebleikte pulp materiaal (SAPPI) en is ontwerp in 'n keël gevormde struktuur. Die keëllokstasie was gelaai met 'n mengsel van ammoniumasetaat, trimethylamine en Hym lure in 'n verhouding van 2:1:1 (lokmiddel komponent), en malation teen een gedeelte gifmiddel vir elke tien gedeeltes lokmiddel. 'n Boord evaluasie met die nuwe keëllokstasie was in 'n veselperskeboord uitgevoer tussen Januarie en Maart 2012 tydens die hoogtepunt van die oesseisoen vir 'n periode van 10 weke waarvan 8 weke onder behandeling was, met nog 'n week voor en na-oes.. Die nuwe lokaasstasie was met M3-lokstasie behandelde blokke, blokke behandel met GF-120 (deur die produsent toegepas) en onbehandelde blokke vergelyk. Die nuwe lokstasies was teen 200 eenhede per hektaar uitgeplaas terwyl die M3-lokstasies teen 400 eenhede per hektaar uitgeplaas was. Gedurende die behandelde weke, was die blokke wat of met die keëllokstasie of met die M3-lokstasie behandel was, aansienlik laer in manlike Medvlieg getalle in Capilure lokvalle in vergelyking met die blokke behandel is met GF-120 of die onbehandelde blokke ( $F_{3,189} = 10.00$ ,  $P=0.000$ ). Die vangste van mannetjies van die Natalse vrugtevlieg in Capilure lokvalle was aansienlik hoër in blokke wat met die nuwe keëllokstasie behandel was in vergelyking met die M3-lokstasies, maar was laer as die ander twee blokke ( $F_{3,189} = 7.07$ ,  $P=0.002$ ). Vir die Natalse vrugtevliegwyfies was daar aansienlike laer vangste in die blokke wat met enige van die twee tipe lokstasies behandel was in vergelyking met die ander blokke, met geen aansienlike verskil in vangste tussen die twee tipes lokstasies nie ( $F_{3,193} = 18.90$ ,  $P=0.000$ ). Maroela vrugtevliegwyfies se vangste was aansienlik laer in blokke wat met M3-lokstasies behandel was in vergelyking met ander blokke ( $F_{3,193} = 11.83$ ,  $P=0.000$ ). Baie min Medvliegwyfies was tydens die studie gevang en daarom is daar geen analises vir hierdie spesie/seks kategorie gedoen nie. 'n Vrugte skadebepaling was voltooi vir elke behandeling deur 20-40 vrugte wat op die grond gelê het na die oes te versamel. Die versamelde vrugmonsters was geweeg en geïnkubeer vir 7 weke om die graad van vrugtevlieg investasie te bepaal (getal vrugtevlieg papies per kg vrugte) asook die spesifieke vrugtevlieg spesies wat die vrugte geïnvesteer het vir elke behandeling. Geen vrugtevlieg investasie in die keëllokstasie behandeling was waargeneem nie. Oor die algemeen was vrugtevlieg investasie laag in die ander blokke. Slegs *C. cosyra* vlieë was uit grond versamelde vrugte geteel. Die hoogste graad van investasie was in die M3-lokstasie behandelde blok waargeneem (1.39 vlieë per kg veselperskes; 0.28 *C. cosyra* vlieë per kg veselperskes). Die investasievlakke (getal pupae per kg veselperskes) was onderskeidelik in die GF-120 behandelde blok en onbehandelde blok 0.20 en 0.28).

## Summary

The main aim of this study was to develop more efficient bait application techniques for fruit fly control. A new biodegradable bait station was developed. The new station was made of coated bleached pulp material (SAPPI) and designed into a cone shape structure. The cone station was baited with a mixture of ammonium acetate, trimethylamine and HymLure at a ratio of 2:1:1 (attractant component), and malathion at one part toxicant to ten parts attractant. A field evaluation of the new cone bait station was carried out in a mango orchard between January and March 2012, at the peak of the fruiting season for a period of 10 weeks with 8 weeks under treatment and a week each before and after harvest. The new bait station was compared to blocks treated with M3 bait station, blocks treated with GF-120 (grower applied) and to blocks left untreated. The new bait stations were set at 200 units per ha while the M3 bait stations were set at 400 units per ha. During the weeks when treatments were applied, blocks treated with either the cone bait station and the M3 bait station had significantly lower male Medfly numbers in Capilure traps compared to the blocks treated with GF-120 and the untreated blocks ( $F_{3,189} = 10.00$ ,  $P=0.000$ ). Catches of Natal fly males in Capilure traps were however significantly higher in blocks treated with the new cone station than in blocks treated with M3 bait stations but were lower than the other two blocks ( $F_{3,189} = 7.07$ ,  $P=0.002$ ). For Natal fly females, there were significantly lower catches in blocks treated with either type of bait stations compared to the other blocks, with no significant differences in catches between the two types of bait stations ( $F_{3,193} = 18.90$ ,  $P=0.000$ ). Captures of marula fly females were significantly lower in blocks treated with M3 bait stations compared to other blocks ( $F_{3,193} = 11.83$ ,  $P=0.000$ ). Very few Medfly females were captured during the study so no analysis was done for this species/sex category. A fruit damage assessment was carried out by collecting 20-40 fruit on the ground after harvest from each treatment. Collected fruit samples were weighed and incubated for 7 weeks to determine degree of fruit fly infestation (number of fruit fly pupae per kg of fruit) and fruit fly species infesting fruit for each treatment. No fruit fly infestation was recorded in the cone bait station treatment. Generally, fruit fly infestation was very low in the other blocks. Only *C. cosyra* flies were reared from ground collected mangoes. The highest degree of infestation was recorded in the M3 treated block (1.39 flies per kg of mangoes; 0.28 *C. cosyra* flies per kg of mangoes). Infestation level (number of pupae per kg of mangoes) in the block treated with GF-120 and in the untreated block was 0.20 and 0.28 respectively.

### 3.3.4 FINAL REPORT: Efficacy of formulated entomopathogenic nematodes against *Ceratitis capitata* and *Ceratitis rosa* in citrus orchards

Experiment 980 (2010/11 – 2011/2) by A Manrakhan (CRI), AP Malan (SU) & J-H Daneel (CRI)

## Opsomming

Die potensiaal van entomopatogeniese (EPN) isolate: *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica* en *Steinernema yirgalemense* vir die beheer van twee belangrike vrugtevliegplae - *Ceratitis capitata* en *Ceratitis rosa* is in laboratorium biotoetse ondersoek. Vyf konsentrasies (5, 10, 20, 40 en 80 IJ/cm<sup>2</sup>) van elke EPN-isolaat is op elke vrugtevliegspesie getoets. Vir beide spesies was papievormende larwes meer vatbaar vir infeksie in vergelyking met volwassenes. *C. capitata* was meer vatbaar vir *H. zealandica*, terwyl *C. rosa* meer vatbaar vir *S. yirgalemense* was. Alle EPN-isolate wat getoets is, was meer effektief teen die hoogste konsentrasie - 80 IJ/cm<sup>2</sup>. Die LC<sub>50</sub> en LC<sub>90</sub> waardes vir *H. zealandica* op *C. capitata* is as 22.3 en 126.5 IJ/cm<sup>2</sup> onderskeidelik bepaal. Die LC<sub>50</sub> and LC<sub>90</sub> waardes vir *S. yirgalemense* op *C. rosa* is as 17.1 en 172.8 IJ/cm<sup>2</sup> onderskeidelik bepaal. Geen boord evaluasies kon gedoen word nie as gevolg van die onbeskikbaarheid van EPN-isolate. Fondse is vanaf hierdie projek geskuif na 'n nuwe Eksperiment 1042, wat handel oor grootskaalse boord proewe vir die beheer van VKM, vrugtevlieg en blaaspootjie met kommersiële vervaardigde entomopatogeniese nematodes (E-nema).

## Summary

The potential of entomopathogenic (EPN) isolates: *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica* and *Steinernema yirgalemense* for control of two important fruit fly pests - *Ceratitis capitata* and *Ceratitis rosa* was investigated in laboratory bioassays. Five concentrations (5, 10, 20, 40 and 80 IJ/cm<sup>2</sup>) of each EPN isolate were tested on each fruit fly species. Pupariating larvae were more susceptible to infection compared to adults for both species. *C. capitata* was more susceptible to *H. zealandica* while *C. rosa* was more susceptible to *S. yirgalemense*. All EPN isolates tested were more effective at the highest concentration - 80 IJ/cm<sup>2</sup>. The LC<sub>50</sub> and LC<sub>90</sub> values of *H. zealandica* on *C. capitata* were calculated at 22.3 and 126.5 IJ/cm<sup>2</sup> respectively. The LC<sub>50</sub> and LC<sub>90</sub> values of *S. yirgalemense* on *C. rosa* were calculated at 17.1 and 172.8 IJ/cm<sup>2</sup> respectively. No field evaluation could be conducted due to unavailability of EPN isolates. Funds from the project were moved to a new Experiment 1042 on large scale field trials for control of FCM, fruit fly and thrips with commercially produced entomopathogenic nematodes (E-nema).

## Introduction

Control of fruit flies in citrus orchards in South Africa has traditionally relied on the application of poisoned baits. However increasing international pressure to lower insecticide residues on fruits and to even discontinue the use of organophosphates used in some fruit fly baits, is calling for exploration of alternative fruit fly control options such as the use of biological control agents.

Entomopathogenic nematodes (EPN) inhabit the soils throughout the world and are obligate parasites of insects, killing them with the aid of their bacterial symbiont within 48 hours. Since the late 1970's they gained status as one of the best non-chemical alternatives for control of insects (Gaugler, 2002). Nematodes have been found to be efficacious against insects in cryptic and soil habitats (Kaya & Gaugler, 1993).

Fruit flies have a natural window of contact with EPNs during their life cycle especially at their soil inhabiting stages: third instar larvae, pupae and emerging adults. Third instar larvae emerging from infested fruits burrow 1-3 cm below the soil surface and start pupating within a period of 24 hours. The pupae remain in the soil for a period of 10-14 days, and emerging adult fruit flies are in contact with the soil for a short period. In South Africa, pupariating larvae of *Ceratitis capitata* (Mediterranean fruit fly- Medfly) and *Ceratitis rosa* (Natal fly) were found to be highly susceptible to two EPN isolates: *Heterorhabditis zealandica* and *H. bacteriophora* (Malan & Manrakhan 2009).

The aim of this study was to evaluate the efficacy of three in vivo cultured EPN isolates: *H. zealandica* (SF41), *H. bacteriophora* (SF351) and *Steinernema yirgalemense* (157-C) for control of *C. capitata* and *C. rosa*.

## Stated objectives

The specific objectives were to (1) test different EPN isolates and concentrations on the two fruit fly species, (2) compare the infection rate of free moving third instar larvae with those larvae inside fruit and (3) investigate the persistence of EPN in the soil at different times after application.

In the original proposal, a field evaluation of the EPN isolates was planned. However due to unavailability of the EPN isolates following the first set of laboratory assays no further laboratory and field tests could be done.

## Materials and methods

Infection of third instar larvae of *C. capitata* and *C. rosa* by different EPN isolates was determined in laboratory assays.

### Insect and EPN supply

Third instar larvae of *C. capitata* and *C. rosa* originated from colonies maintained at CRI, Nelspruit. Third instar larvae were collected in water placed under artificial diet containers. Larvae were drained from the water after 24 hours and used for the tests.

Three EPN isolates were tested: *H. zealandica* (SF41), *H. bacteriophora* (SF351) and *S. yirgalemense* (157-C). Isolates were obtained from in vivo cultures maintained at the University of Stellenbosch.

Five nematode concentrations of each EPN isolate were tested separately on the two fruit fly species: 5, 10, 20, 40 and 50 IJs/cm<sup>2</sup>.

### Experimental set-up

Plastic cups (7 cm deep and 11 cm diameter with a surface area of 95 cm<sup>2</sup>) were used in the assay. 200 g of cooled sterilised soil collected from a citrus orchard in Mpumalanga (heated to 100°C for 2 hours) was used per cup. In each cup 29 ml of distilled water was added to keep the soil moist.

1 ml of EPN dilution was added to each cup. A control was included in each test and consisted of addition of 1 ml of distilled water only.

Twenty larvae of each species were added to each cup, 24 hours after inoculation with nematodes.

Following inoculation and addition of larvae, each cup was placed separately in an aerated closed container of 27 cm x 27.5 cm x 15 cm and kept at 25 ± 2°C and 60% RH in an incubator. Each cup was covered with an aerated lid for the first 5 days and thereafter the lids were removed so that any emerged flies could fly out of the

cup into the aerated container. Containers were kept for 14 days. For each concentration tested and fruit fly species, there were 3 cups representing 3 replicates during each test. The tests were repeated twice with different batches of flies. At the end of each test, the number of emerged flies, dead larvae and unemerged pupae were counted. All adults, larvae and pupae were dissected to determine infectivity. Mortality was calculated as the difference between number of exposed larvae and the number of uninfected emerged adults. The LC<sub>50</sub> and LC<sub>90</sub> values for the three isolates were calculated for each fruit fly species using the programme PROBAN.EXE (VAN ARK, 1992).

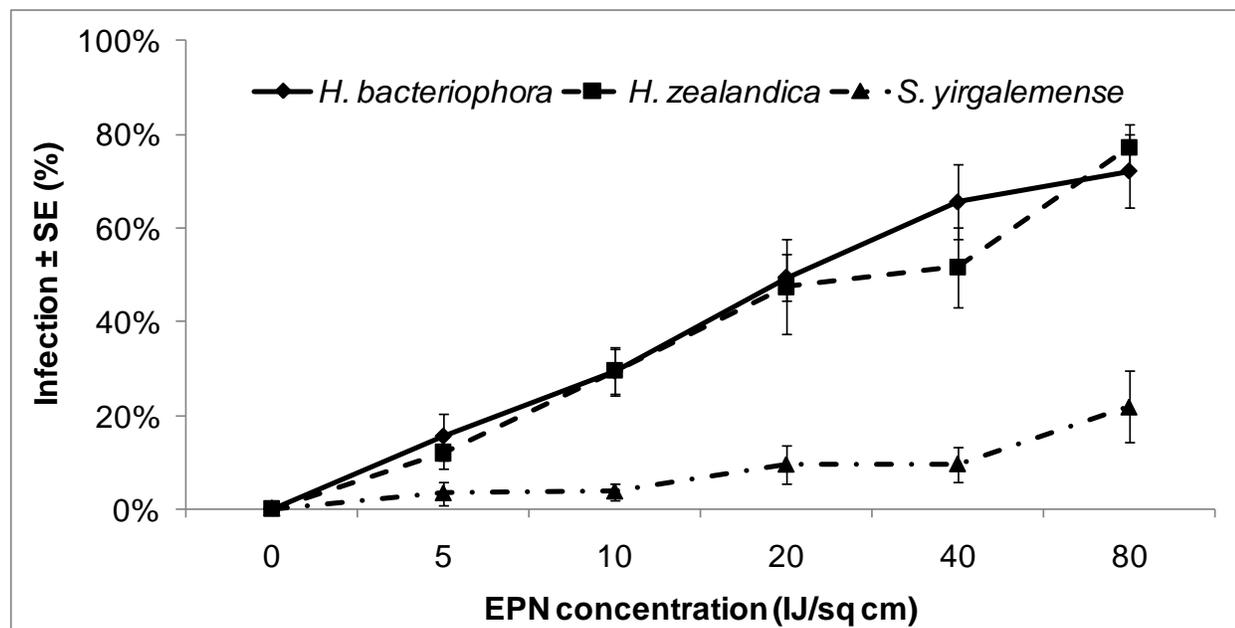
## Results and discussion

For all EPN isolates and fruit fly species tested, pupariating larvae were more susceptible to infection compared to emerging adults. Out of a total of 995 infections obtained, 92.4% were larval to pupal infection.

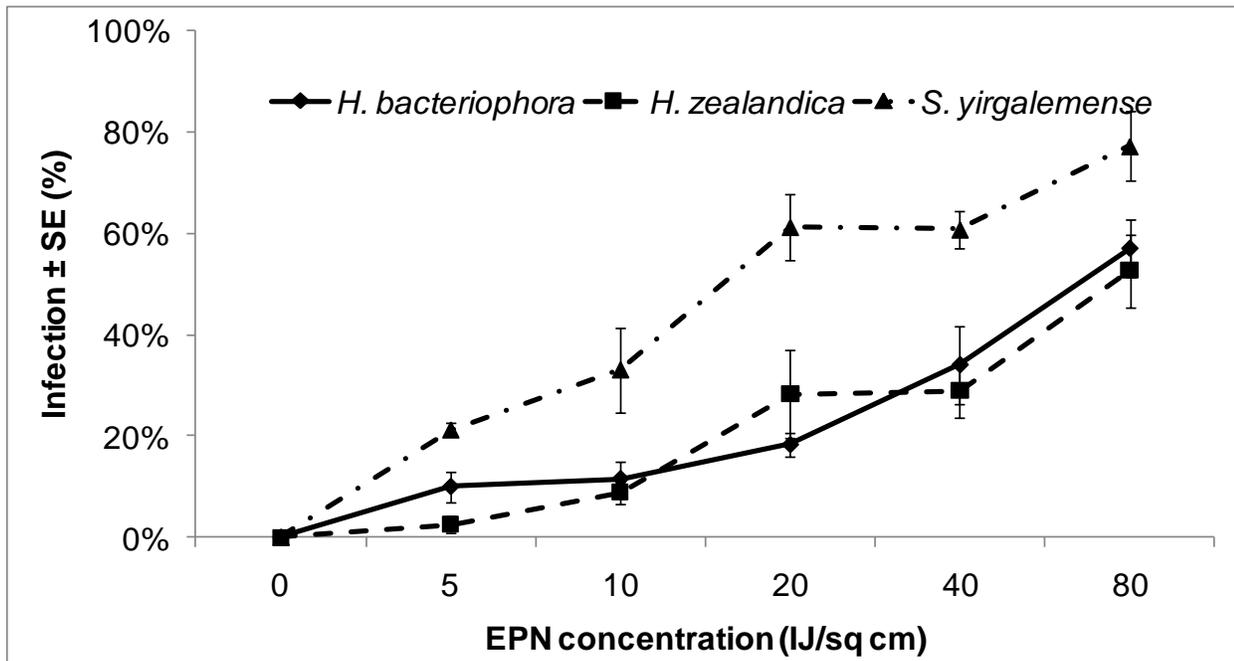
All EPN isolates were more effective at the highest concentration tested – 80 IJ/cm<sup>2</sup>. At a concentration of 80 IJ/cm<sup>2</sup>, infectivity of *C. capitata* was numerically higher with *H. zealandica* (77.2 ± 5.1 %) whilst infectivity of *C. rosa* was higher with *S. yirgalemense* (77.3 ± 6.9%) (Fig. 3.3.4.1 & 3.3.4.2). The high density of EPN required for infection is possibly due to the short pupariation time for *C. capitata* and *C. rosa* (a process occurring within less than one day). Among the soil stages for these two species, only the larval stages and adults were previously found to be susceptible to EPN (Malan & Manrakhan 2009). Fully formed pupae of *C. capitata* and *C. rosa* cannot be penetrated by infective juveniles of EPN.

The calculated LC<sub>50</sub> and LC<sub>90</sub> values for all isolates tested on the two fruit fly species are shown in Table 3.3.4.1. The LC<sub>90</sub> values especially for *S. yirgalemense* on *C. capitata* and for *H. bacteriophora* and *H. zealandica* on *C. rosa* might not be accurate since the highest average mortalities for these EPN/fruit fly combinations were below 90%.

These results indicate that a range of concentrations between 150 and 200 IJ/cm<sup>2</sup> would be optimal for both *H. zealandica* and *S. yirgalemense* targeting *C. capitata* and *C. rosa* respectively. The range of calculated optimal EPN concentration for these two fruit fly species is approximately 10 fold higher than the EPN concentration required to provide control of false codling moth, *Thaumatotibia leucotreta* (Malan et al. 2011).



**Figure 3.3.4.1.** Percent infection of *C. capitata* when pupariating larvae were exposed to isolates of *H. bacteriophora*, *H. zealandica* and *S. yirgalemense*.



**Figure 3.3.4.2.** Percent infection of *C. rosa* when pupariating larvae were exposed to isolates of *H. bacteriophora*, *H. zealandica* and *S. yirgalemense*

**Table 3.3.4.1.** LC<sub>50</sub> and LC<sub>90</sub> values of EPN isolates for *C. capitata* and *C. rosa*. LC values represent the number of infective juveniles per cm<sup>2</sup>. N is number of individuals.

Fruit fly species	EPN isolate	N (replicates)	Slope ± SE	LC <sub>50</sub>	LC <sub>90</sub>
<i>C. capitata</i>	<i>H. bacteriophora</i>	655 (6)	1.3 ± 0.1	19.2 (15.5-23.6)	198.0 (124.9-397.8)
	<i>H. zealandica</i>	598 (6)	1.7 ± 0.2	22.3 (18.8-26.7)	126.5 (90.1-205.1)
	<i>S. yirgalemense</i>	587 (6)	0.9 ± 0.2	705.2 (244.4-3007.4)	20409.4 (2442.5-8160038.0)
<i>C. rosa</i>	<i>H. bacteriophora</i>	699 (6)	1.2 ± 0.1	74.8 (55.3-116.7)	943.0 (445.1-3228.8)
	<i>H. zealandica</i>	630 (6)	1.4 ± 0.2	77.4 (57.6-119.7)	663.5 (339.9-1952.8)
	<i>S. yirgalemense</i>	538 (6)	1.3 ± 0.2	17.1 (12.9-21.7)	172.8 (106.7-387.6)

## Conclusions

These results demonstrate a potential for control of *C. capitata* and *C. rosa* using EPN. For optimal control of *C. capitata* and *C. rosa* with *H. zealandica* and *S. yirgalemense* respectively, a range of concentration between 150-200 IJ/cm<sup>2</sup> is recommended. At such high concentrations however, control of fruit flies with EPN might be too costly.

## Future research

Funds from Experiment 980 have been moved to a new Experiment (no. 1042; 2012-2013) on large scale field trials for control of FCM and fruit fly with commercially produced EPNs. Evaluation of different in vitro commercially produced EPN isolates for control of false codling moth and fruit fly will be carried out in selected citrus orchards in the Western Cape, Eastern Cape and Mpumalanga. Different concentrations of EPN isolates will be tested (10 IJ/cm<sup>2</sup> and 20 IJ/cm<sup>2</sup>- concentrations which are appropriate for FCM control) as well as different times of application (spring versus autumn) and different methods of application (ground sprays versus through irrigation systems).

## Technology transfer

A talk on Biological Control of fruit flies using entomopathogenic nematodes in South Africa- Current Status and Future Prospects was delivered at the 20<sup>th</sup> Symposium of the Nematology Society of Southern Africa (NSSA) 16-18 May 2011.

A poster on Evaluation of EPN for control of *Ceratitis capitata* and *Ceratitis rosa* (Diptera: Tephritidae) was presented at the 17<sup>th</sup> Congress of the Entomological Society of Southern Africa in July 2011 in Bloemfontein.

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### 3.3.5 FINAL REPORT: Cold tolerance of Natal fruit fly (*Ceratitis rosa*): geographic distribution and overwintering physiology

Experiment CRTT-01 (2009/10-2011/2) by J S Terblanche and C. Nyamukondiwa (Dept Conservation Ecology & Entomology, Faculty of AgriSciences, University of Stellenbosch, Private Bag X1, Matieland, 7602)

## Opsomming

*Ceratitis capitata* (Weidemann) en *Ceratitis rosa* (Karsch) (Diptera: Tephritidae) is ekonomiese belangrike plaë wat 'n wye verskeidenheid vrugte wat kommersieël verbou word in Afrika beïnvloed, insluitende sagtevrugte en sitrus. Die faktore wat egter die geografiese verspreiding sowel as volopheid van hierdie verwante spesies in Suid-Afrika bepaal, word egter steeds verkeerdelik verstaan.. In hierdie studie ondersoek ons die moontlikheid dat die variasie in die termiese biologie van die twee spesies, as 'n potensiële meganisme gebruik kan word om die verskille in die geografiese verspreiding en volopheid te kan verduidelik. Verskeie belangrike resultate was verkry. Eerstens, het ons gevind dat hoë en lae temperatuur toleransie vir aktiwiteite dramaties wissel gedurende die volwasse lewens stadium. Alhoewel die termiese toleransie van *C. capitata* en *C. rosa* soortgelyk was by lae temperature onder dinamiese toestande, was *C. capitata* egter meer tolerant teenoor hoër temperature as *C. rosa* (kritiese termiese maksima (CT<sub>max</sub>) 42.4-43.0 en 41.8-42.4°C vir *C. Capitata* en *C.rosa* onderskeidelik). Tweedens, het ons die binne-ingenerasie se reaksies in kort- en langtermyn akklimasie teen 20, 25 en 30°C ondersoek. Beide spesies het die vermoë getoon om hul termiese toleransie by nuwe temperatuur toestande, binne-in'n week aan te pas. Wanneer enige van die twee spesies by warmer toestande (30°C) gehou was het die CT<sub>max</sub> toegeneem terwyl akklimasie by laer temperature (20°C) die kritiese termiese minima (CT<sub>min</sub>) verbeter het. In sommige gevalle was daar egter beduidende interaksie tussen kort-en langtermyn akklimasie effekte, wat aandui dat daar omkeerbare en onomkeerbare komponente is in CT<sub>max</sub> en CT<sub>min</sub>. Derdens, het ons die basale en vinnige plastiese lae temperatuur oorlewingsoptrede van beide spesies ondersoek. Resultate het getoon dat daar beduidende verskille tussen die spesies was gedurende die tydsverloop van die plastiese oorlewingsoptrede tot die akute lae temperature was, maar nie in omvang nie. 'n Eenvoudige teoretiese bevolkingsmodel wat gebaseer is op die beraamde tydsverloop van plastiese verandering in oorlewing het getoon dat hierdie verskil 'n oorlewingsvoordeel aan *C. capitata* verleen na die blootstelling aan 'n habitatte waar die temperatuur gereeld daal onder 10°C. Dit stel voor dat lae temperatuur plastisiteit kan bydra tot die indringings potensiaal van *C. capitata*. Vierdens, het 'n ondersoek na die fiksheidskoste van vinnige koueverharding van *C. capitata* getoon dat daar geen metaboliese-of fekunditeitskoste is nie, maar 'n verlaging in die gemiddelde oorlewing van vlieë wat gehard is teenoor vlieë uit die kontrole groep. Dus, tenminste in *C. capitata* is direkte mortaliteit a.g.v. koue skade een van die hoof kostes van vinnige koue-verharding. Laastens, seisonale monitering van *C. capitata* in die Wes-Kaap het getoon dat vlieë gedurende die hele jaar aktief is, maar dat volopheid aansienlik afneem gedurende die winter. Mikroklimate temperatuur data vanaf hierdie habitatte het getoon dat die frekwensie en duur van suboptimale lae temperature hoog is en daarom is dit hoogs waarskynlik

dat dit volwasse aktiwiteit, paring, oviposisie en ontwikkeling onderdruk. Gebaseer op laboratorium beramings van larwe, popstadium en volwasse vlieë se laer dodelike temperature, is dit egter hoogs onwaarskynlik dat winter temperature enige individue van enige lewenstadia van hierdie spesies sal dood en dus is dit moontlik dat hierdie spesies kan oorwinter in hierdie areas. In geheel stel hierdie werk voor dat die variasie in termiese toleransie *C. capitata* kan help om te oorleef wanneer dit in 'n nuwe termiese omgewing vrygestel word en kan bydra tot die waargenome breër geografiese verspreiding van *C. capitata* relatief tot *C. rosa*. Die resultate van hierdie werk help om 'n empiriese raamwerk daar te stel om die termiese faktore wat die geografiese verspreiding van *C. capitata* en *C. rosa* beperk te verstaan. Hierdie resultate kan ook nuttig wees vir Steriele Insek Tegniek programme, na-oes beheer en sterilisasie tegnieke. Die voorspelling van potensiële indringing van hierdie twee spesies by verskillende tydskaal kan ook waardevol wees in plaag risiko-analises.

## Summary

*Ceratitis capitata* (Wiedemann) and *Ceratitis rosa* (Karsch) (Diptera: Tephritidae) are economic pests affecting numerous commercially-grown fruits in Africa including deciduous and citrus crops. However, factors limiting the geographic distribution and abundance of these two congeners in South Africa are poorly understood. Here, we explored variation in thermal biology between these two species as a potential mechanism explaining the differences in *Ceratitis* species geographic distribution and population abundances. Several key results were found. First, we showed that high and low temperature tolerance for activity varies dramatically through the adult life-stage. A comparison of thermal tolerance between *C. capitata* and *C. rosa* showed that low temperature tolerance seems similar under dynamic (ramping) conditions, while at high temperatures *C. capitata* can withstand slightly higher temperatures than *C. rosa* (critical thermal maxima ( $CT_{max}$ ) 42.4-43.0 and 41.8-42.4°C for *C. capitata* and *C. rosa* respectively). Second, we investigated within-generation responses to short- and long-term acclimation to 20, 25 and 30°C. Both species are able to adjust thermal tolerance to the new temperature conditions within a week. Holding either species at warmer conditions (30°C) increased  $CT_{max}$  while acclimation to lower temperatures (20°C) improved critical thermal minima ( $CT_{min}$ ). However, in some cases, there were significant interactions between short- and long-term acclimation effects, suggesting some reversible and irreversible components to  $CT_{max}$  and  $CT_{min}$ . Third, we explored basal and rapid plastic low temperature survival responses in both species. Results showed significant differences between species in the time-course of plastic survival responses to acute low temperature but not magnitude. A simple theoretical population model based on the estimated time-course of plastic changes in survival showed this difference may confer a survival advantage for *C. capitata* upon introduction in habitats where temperatures frequently drop below 10°C. This suggests that low temperature plasticity may contribute to the invasion potential of *C. capitata*. Fourth, an investigation into the fitness cost to rapid cold-hardening in *C. capitata* revealed no metabolic or fecundity costs, but reduction in average survival of hardened compared to control flies. Thus, at least in *C. capitata*, a major cost to rapid cold hardening is likely through direct mortality caused by chilling injury. Finally, seasonal fly monitoring data from the Western Cape showed flies are active all year round, but abundance declines markedly during winter. Microclimatic temperature data from these habitats showed that the frequency and duration of suboptimal low temperatures are high, and thus, likely to suppress adult activity, mating, oviposition and development. However, based on laboratory estimates of larval, pupal and adult lower lethal temperatures, it is unlikely winter temperatures will kill any individuals of any life-stages of these species and thus, these species may overwinter in this region. Broadly, this work suggests variation in thermal tolerance may aid *C. capitata*'s survival upon introduction to novel thermal habitats and might contribute to the observed wider geographic distribution of *C. capitata* relative to its congener *C. rosa*. The results of this work help provide an empirical framework for understanding the thermal factors limiting geographic distribution of *C. rosa* and *C. capitata*. These results may also be useful to sterile insect technique programmes, post-harvest control and sterilization techniques, prediction of the potential invasiveness of the two species at various timescales, and could also be valuable to pest risk analyses.

## Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) (Medfly), which originated from sub-Saharan East Africa (Baliraine et al. 2004), is considered one of the most invasive insect species, having spread and successfully established throughout much of the tropical-temperate parts of the world (Carey 1991; Vera et al. 2002; Malacrida et al. 2007). Medfly invasion and establishment success is probably facilitated by its highly polyphagous life-history (Malacrida et al. 2007), short generation times (Duyck et al. 2002; Grout & Stoltz 2007), and possibly rapid evolutionary adaptation (Huey et al. 2005; Malacrida et al. 2007; Leibhold & Tobin 2008). However, a major factor which contributes to the successful establishment after introduction of an insect species to a novel environment is its basal and inducible physiological tolerance to environmental stress (e.g. to temperature and water stress) (Richardson & Pysek, 2006; Huey et al. 2005; Chown et al. 2007).

Medfly is not the only tephritid with major invasion potential, and indeed, several other fruit flies (e.g. *Bactrocera*, *Dacus* spp.) have been introduced and successfully established in various locations around the world despite strict quarantine measures. In particular, the Natal fruit fly *Ceratitits rosa*, which is also a highly polyphagous congeneric species with a broad African distribution (De Meyer et al. 2008), has shown alarming invasion potential. On Reunion Island, *C. rosa* was able to rapidly outcompete and competitively exclude Medfly (White et al. 2001; Duyck et al. 2004) possibly owing to niche segregation (Duyck et al. 2006). At present the factors limiting geographic distribution and abundance of *Ceratitits* spp. in the Western Cape and indeed, South Africa as a whole, are poorly understood and represents a potentially major problem to fruitfly control and management strategies (Manrakhan & Addison 2007). However, the potential for establishing permanent populations (i.e. invasion potential) is also not well understood in these flies (De Meyer et al. 2008), hence several existing research projects to understand competition and behaviour already funded by the DFPT & CRI.

Variation in temperature tolerance might be a key mechanism explaining the differences in *Ceratitits* species geographic distribution and their potential invasiveness at global (Duyck et al. 2006; Gutierrez et al. 2008) and regional scales (De Meyer et al. 2008). Similarly, in the fruit fly *Bactrocera dorsalis*, predictions of geographic range under current and future climate scenarios suggests low temperature is a principal limiting factor in the U.S.A. (Stephens et al. 2007). By contrast, drought stress seems to be an important limiting factor in CLIMEX models, based on the Mediterranean distribution data, of global potential geographic distribution of *C. capitata* (Vera et al. 2002). Thus, different approaches to predicting distribution suggest different underlying mechanisms of the species in question. Understanding *Ceratitits* geographic distribution and the potential limiting factors in the Western Cape is, however, currently limited by a lack of basic temperature tolerance information, particularly for the two key species *C. rosa* and *C. capitata*.

Recent studies suggest physiological differences between *C. rosa* and *C. capitata* from Reunion Island (Duyck et al. 2006) which might explain the species' distinct geographic distributions in southern Africa (De Meyer et al. 2008). On the basis of experiments undertaken for Reunion Island strains, Duyck et al. (2006) argued that *C. capitata* and *C. rosa* can be separated both ecologically and geographically. *Ceratitits rosa* succeeds in cooler (22-23°C), wetter (3000-3500mm rainfall) environments while *C. capitata* tend to inhabit warmer (24-26°C) and drier (0-1000mm rainfall) regions (Duyck et al. 2006). Similar observations have been made for these species in southern Africa, in which *C. rosa*'s natural distribution is mainly limited to eastern, coastal regions with high annual rainfall, while *C. capitata* is generally more widespread (Manrakhan and Addison, 2007; and see De Meyer et al. 2008).

The studies by Duyck et al. (2006) and De Meyer et al. (2008) have provided some important insights into aspects of *Ceratitits* biology which might potentially limit the geographic distribution of *C. rosa* and *C. capitata* in the Western Cape. Modelling work predicting potential *Ceratitits* distributions in the Western Cape is presently being undertaken (CRI, M. de Villiers), and has been undertaken previously for *C. capitata* at a coarse global scale (Vera et al. 2002). However, several factors limit the application of the available data to predict the fine-scale distribution of these flies in South Africa of which four factors are perhaps most significant. First, comparable data for local populations is not available and, therefore, the degree of region-specific differences among populations, which could have been altered through local climatic adaptation in distinct ways (Hoffmann & Parsons 1997; Hoffmann & Willi 2008) is presently unknown. Such a process frequently occurs in traits of temperature tolerance in other Dipteran species (e.g. *Drosophila*, *Glossina* e.g. Hoffmann et al. 2005; Terblanche et al. 2006; reviewed in Hoffmann et al. 2003; Chown and Nicolson 2004) but its extent, and the rate at which such changes might accumulate, is unknown for Medfly and Natal fly from any region. Second, overwintering strategy, a major potential source of re-introduction of *Ceratitits* species is not known for local South African populations. Regardless, similar data are available from other parts of the world. For example, in the Judean Hills of Israel *C. capitata* is unable to overwinter (Israely et al. 2004), and instead, there is a process of re-invasion each spring from adjacent agricultural regions (Israely et al. 2005). This type of data has direct pest management implications since it assists by knowing when and where to focus control efforts. Third, Duyck et al. (2006) consider temperature tolerance from early life-stages as the temperature-dependent growth rate. However, it may be important to consider survival and limits to behavioural activity (e.g. low temperature flight activity thresholds, limits to mating), which play a role at different, often less severe, temperatures and may have subtle but distinct differences for biogeography predictions (Terblanche et al. 2006; Chown and Terblanche 2007). Moreover, temperature tolerance from the adult life-stage, which has not been given much attention to date, should also be considered since dramatic differences can occur between life-stages of various insect species (Bowler & Terblanche 2008). Knowledge of which life-stage is the most temperature-limited under natural conditions, an important component of any population dynamics or biogeography modelling, is presently unknown for *C. rosa* and *C. capitata* owing to variation in methodological approaches confounding comparisons among studies (Terblanche et al. 2008). Finally, no studies of *Ceratitits* spp. to date have considered physiological responses to temperature treatment, a major mechanism used by insects to cope with temperature

variation at daily (Meats 1976; Kelty and Lee 1999/2001; Overgaard & Sorensen 2008) and seasonal timescales (Terblanche et al. 2006; Chown and Terblanche 2007), but significantly, also upon introduction into new environments (Chown et al. 2007; Kristensen et al. 2008). For flies, much work has been undertaken on the family Drosophilidae, and most studies have focused extensively on *Drosophila melanogaster* and *D. simulans* (e.g. Hoffmann & Watson 1993; Jensen et al. 2007). By contrast, little or no work has been published on rapid heat- and cold-responses in Tephritidae for *Ceratitis capitata* or *C. rosa* (though see early work on *Dacus* by Meats (1973)). Furthermore, temperature responses can affect post-harvest control techniques, especially if fruit is temperature treated (cold sterilization) during distribution, as is presently the case for *Ceratitis*-infested fruits. Another aspect of significant concern is how global climate change might alter the rate and impact of invasive pest insects (Chown et al. 2007; see also Helmuth et al. 2005). Without some insight into the mechanisms underlying these species' thermal biology and their ability to compensate under changing weather conditions we are simply unable to make informed decisions for strategy and intervention plans. In consequence, several commercially important aspects of *Ceratitis* thermal biology require urgent investigation.

The results of this study will therefore provide an empirical framework for understanding the factors limiting geographic distribution of *C. rosa* and *C. capitata*, laboratory handling for SIR programmes, post-harvest control and sterilization techniques, and will directly aid in the prediction of the potential invasiveness of *C. rosa* and *C. capitata* at various timescales. This information is regarded as critical for the effective integrated management of fruit fly species in the Western Cape.

The general aims of this project were to understand the thermal physiology potentially limiting the geographic distribution of *Ceratitis rosa* relative to *C. capitata* in the Western Cape, South Africa.

### **Stated objectives**

- 1) if variation in thermal tolerance between *C. rosa* or *C. capitata* results in different levels of survival under semi-field conditions;
- 2) if plastic low temperature responses are costly from a fitness or energetic perspective in *C. capitata*;
- 3) if *C. rosa* and *C. capitata* show inducible cold tolerance at daily and seasonal time-scales under semi-field conditions;
- 4) if *C. capitata* populations have permanently or reversibly adapted to local climatic conditions in South Africa and if *C. rosa* might overwinter in South Africa.

### **Materials and methods**

Thermal tolerance were assayed using protocols established previously for critical low and high activity limits and low and high temperature survival (e.g. Terblanche et al., 2008) in the laboratory. All species were assayed for rapid temperature responses and lethal temperatures under a range of conditions. Adults were assayed as this is the life-stage which contributes directly to changes in population dynamics via reproduction and it is this stage which is released in SIT operations. Individuals were acclimated to summer- and winter-like conditions in laboratory incubators and assayed for thermal tolerance after 10 days as it has been shown previously that acclimation responses are generally fully developed within 5-7 days across several insect species (Chown & Terblanche 2007). Comparison was also made between laboratory and field-collected individuals. This will assist monitoring of quality in the laboratory-reared insects and provide screening of traits which may compromise SIT projects. Gender and age were scored in all individuals and accounted for in statistical analyses if necessary. All work were undertaken in the IPM lab at Department of Conservation Ecology and Entomology (SU).

#### Rapid Temperature Responses

Temperature tolerance were measured as survival after exposure to a constant temperature for a fixed period of time over a range of experimental test temperatures using circulating, programmable water baths (e.g. Grant LTC-12 or GD150-R4, Grant Instruments; accuracy  $\pm 0.1^\circ\text{C}$ ). If necessary, to allow for sub-zero temperature operation the water baths were filled with a solution of propylene glycol and water (1:1 ratio). In brief, individual animals are placed into plastic vials in groups ( $n=10-12$ ) and subjected to temperature treatments for a fixed time period. The animals were then removed and placed into climate chambers at normal rearing temperatures with access to food and water to aid recovery and survival is scored after 24 h. This process was replicated at least five times per temperature and time combination to fully account for variation among individuals. Once complete replicated survival curves were obtained for upper and lower lethal temperatures of a species, (i.e. fully encompassing the range of 0 to 100% mortality) a range of sub-lethal temperature exposures (pre-treatments) were used to determine if rapid cold or heat hardening can be induced following the methods outlined in Terblanche et al. (2008). Handling controls were included in the pre-treatments to confirm that if survival

improves it is a consequence of temperature treatment and not handling stress. Therefore, all statistical comparisons were made relative to handling controls, i.e. as proportion survival in the treatment group minus proportion survival in control group. During all experiments mercury thermometers and calibrated temperature loggers were used to confirm desired treatment or rearing conditions.

#### Rapid diurnal and seasonal thermal adaptation during field temperature variations

In this study, investigations were made into how thermal tolerance of *C. rosa* and *C. capitata* varies when exposed to natural variations of light and temperature under semi-field conditions. The experiment was conducted on both laboratory reared sterile and fertile fruit flies from SIT Africa (normal and irradiated males of both *C. capitata* and *C. rosa*). Therefore the treatments were (1) normal Medflies, (2) Unirradiated *tsl* Medflies, (3) irradiated *tsl* Medflies and (4) normal natal flies (5) irradiated natal flies.

In preliminary studies, investigations were made into the effects of relative cooling or heating rates on fly survival and thermal equilibration times of vials and flies body temperature. This was done by acclimating *C. capitata* flies at different temperatures (10, 15, 20, 25, 30 and 35 °C) for 2hrs before subjecting them to a cold shock (-5 °C for 2 h) and heat shock (41 °C for 2 h) and scoring survival after 24 h. Five replicates of 10 insects each (~5 days old and mixed sex, all fasted for 12 hrs) were used per temperature acclimation. For each temperature acclimation, ambient ( $T_a$ ) and insect body temperature ( $T_b$ ) inside the 60 ml plastic containers were recorded using Type T 36 SWG thermocouples connected to a Picotech TC-163 08 thermocouple interface and data acquired at 1 sec intervals using PicoLog software for Windows (Pico 164 Technology, Cambridge, UK). Thermocouples recording  $T_a$  were hung in one of the 5 replicate vials while those recording  $T_b$  were glued to the body of an insect during the cold/heat shock treatments. Rate of ambient temperature change was compared to the rate of body temperature change. Time constants to reach -5°C were compared among acclimation temperatures for their possible influence on survival results.

Following the preliminary trial, ~2000 flies per species (treatments 1-5 above) were transferred into 5 plexiglass cages (800 x 800 x 800 mm) (5 cages per treatment/species with ~400 flies each) placed in the natural environment in a shaded, rain-protected environment near to the laboratory. This was done on both fertile and sterilized/irradiated flies of similar age (~5days old). All the flies (of both species) were placed outside at a time when the natural thermal environment was almost the same as the rearing conditions of 25 °C. The first batch of flies (100 per species, for both fertile and sterilised flies) was withdrawn from the cages (20 flies/cage) 1 hour later and subsequently after every 6 hours for 24 hours. Immediately after withdrawing each of the groups of flies, these were distributed in 10 vials with 10 flies each for each species. Heat and cold shock of the two fruit fly species were measured by exposing the flies to -5 °C and 41 °C for 2 hours. Five replications of 10 insects each were used and after the cold/heat shock, insects were returned back to 25 °C for 24 hours after which mortality/survival was scored. For heat shock, high humidity was maintained by a wet cotton wool plug which was inserted into each of the vials, to ensure that any effects are due to the heat shock and not fly dehydration. Survival was defined as a coordinated response to muscle stimuli, e.g. mild prodding or normal behaviours such as flying and mating. The experiment was replicated over 3 days in winter and summer to determine seasonal effects on thermal adaptation. Outside temperatures were obtained from one of the 5 Thermocron iButtons (Dallas Semiconductors, Model DS1920), which were placed randomly in 5 of the experimental cages. The relationship between thermal tolerance and the ambient environmental temperature was analysed using regression analysis. Results were compared with those of Overgaard and Sorensen (2008).

Following Nyamukondiwa *et al.* (2010) a simple population survival model based on estimated time course of rapid cold-hardening responses was undertaken to simulate time to extinction for *C. rosa* and *C. capitata* when exposed to similar, low but thermally variable environments. The model showed that time to extinction is greater for *C. capitata* than for *C. rosa*, especially in habitats where temperatures frequently drop below 10 °C. To verify this prediction under semi-field conditions, and if it differs between fertile and sterilised flies, 5 replicate cages of 200 flies each per treatment/species (for both fertile and sterilised flies) were placed outside the laboratory again at time when the natural thermal environment was almost the same as the rearing conditions of 25 °C. Every morning, all dead flies were removed from the 5 replicate cages for the 2 species, sexed and recorded. This was continued until all the cages were exhausted. The flies had access to food and water throughout the whole experiment. Outside temperatures were recorded using Thermocron iButtons (Dallas Semiconductors, Model DS1920). A comparison of survival was made between species.

#### Energetic or fitness costs of plastic thermal responses

This work was undertaken following Marshall and Sinclair (2010) and Shreve *et al.* (2004). Briefly, we directly measured the metabolic rate (energy consumption), survival and egg production rates under fluctuating versus

stable temperature conditions over a period of 10 days in adult *C. capitata*. Here the stable temperature treatment acted as age controls for each of the time-points sampled.

### Geographic variation of thermal tolerance

Using field-collected, geo-referenced samples of infested fruit and live flies from locations ranging throughout South Africa, we initiated new laboratory cultures (or 'isofemale lines') of up to 15 geographic locations of *C. capitata* and *C. rosa*. All flies were reared under standard, common rearing conditions to eliminate effects of seasonal or thermal variation upon collection and held for 2 generations. Using the F<sub>3</sub> adult flies, we determined a range of thermal tolerance traits, as described above. If time allows, we also assessed the plasticity (acclimation responses) of these traits. Comparisons were undertaken among geographic strains to assess and quantify the degree of local climatic adaptation. Several populations were established in the laboratory and physiology assays were carried out.

### Statistical Analyses

Statistical analyses were carried out using SAS 9.0 (*proc probit*) for non-linear modeling of lethal temperature curves and custom randomization software for analyses of survival improvement in the treatment group relative to controls following methods outlined previously in Terblanche et al. (2007) and Terblanche et al. (2008). After verifying the assumptions of ANOVA have been met, critical thermal limits and chill coma recovery times were analysed in separate one-way ANOVA analyses for the effects of age, gender, acclimation temperature, geographic location or diet/rearing medium as categorical factors. The need for e.g. log<sub>10</sub> transformations was confirmed for each variable separately and is usually necessary for chill coma recovery time but not critical thermal limit data. Post-hoc analyses were used to identify statistically homogeneous groups.

## **Results and discussion**

### Variation in thermal tolerance between adult *C. rosa* and *C. capitata*

Variation in temperature tolerance might be a key mechanism explaining the differences in geographic distribution and potential invasiveness of *Ceratitis* species at global (Duyck et al. 2006) and regional scales (De Meyer et al. 2008). However, it is poorly understood which factors significantly affect temperature tolerance at the species or population level. We therefore explored the effects of age, gender and feeding status on the thermal limits of activity in adult *C. capitata* and *C. rosa* (Nyamukondiwa & Terblanche 2009). Determining the thermal limits of activity is a first step towards clarifying how temperature affects population dynamics and, thus, their geographic distribution. We measured critical thermal maximum (CT<sub>max</sub>) and critical thermal minimum (CT<sub>min</sub>) using a dynamic (ramping) method on different ages (2, 5, 9, 14, 28 days after adult eclosion) and feeding status (recently fed vs. fasted for 48 hrs) in both sexes of adult *C. capitata* and *C. rosa*. Results showed that for the adult life-stage of *C. capitata* and *C. rosa*, CT<sub>max</sub> and CT<sub>min</sub> significantly improved with age up to an extent, but significantly decreased with older age. Similar studies have shown that Hsp70 expression decreases with age in adult *Drosophila melanogaster* (Sørensen & Loeschcke 2002). It may be possible that declining thermal tolerance with ageing in *C. capitata* and *C. rosa* in this study may similarly reflect age-related changes in Hsp70 expression because Hsp70 is the primary heat shock protein in *C. capitata* (Kalosaka et al. 2009). Similarly, high temperature pre-treatment increased cold tolerance in *C. capitata* indicating heat shock proteins may be important for temperature tolerance in adult *Ceratitis* species. Preliminary comparisons of *C. capitata* and *C. rosa* thermal tolerance suggests that both species have similar basal cold tolerance but *C. capitata* has significantly higher heat tolerance than *C. rosa*. These results support observations that *C. capitata* inhabits warmer, drier geographic regions while *C. rosa* succeeds in cooler wetter environments (Duyck et al. 2006). Similar observations have been made for these species in southern Africa, in which *C. rosa*'s natural distribution is mainly limited to eastern, coastal regions with high annual rainfall, while *C. capitata* is generally more widespread (Manrakhan & Addison 2007; and see De Meyer et al. 2008). Results also showed that temperature tolerance is enhanced with recent feeding suggesting it is an active process. This finding supports previous studies that demonstrate that energy reserves, and possibly even diet quality, may play a significant role in thermal tolerance (Hoffmann et al. 2005; Colinet et al. 2006; Shreve et al. 2007; Andersen et al. 2010). These data are significant for understanding population dynamics under agro-ecosystem conditions and the potential geographic distribution of *C. capitata* and *C. rosa*.

### Effects of thermal history on critical thermal limits in adult *C. rosa* and *C. capitata*

Insect thermal tolerance shows a range of responses to thermal history depending on duration and severity of exposure. However, few studies have investigated these effects under relatively modest temperature variation or

interactions between short and longer term exposures (e.g. McDonald et al. 2000; Rako & Hoffmann 2006; Terblanche et al. 2007; Marais & Chown 2008). Using a full-factorial design, the acclimation responses in  $CT_{min}$  and  $CT_{max}$  following exposure to 20, 25 and 30 °C for a duration of 7 days and their interactions with short-term (2 h) sub-lethal temperature exposures to these same conditions (20, 25, and 30 °C) were investigated in *C. capitata* and *C. rosa* (Nyamukondiwa & Terblanche 2010). Acclimation results showed that flies generally improved heat tolerance with high temperature acclimation and resisted low temperatures better after acclimation to cooler conditions, in keeping with several studies to date (reviewed in Whitman 2009). However, in several cases significant interaction effects were evident between short- and long-term temperature treatments for CTLs suggesting considerable complexity when attempting to predict effects of transient weather patterns on traits of insect thermal tolerance (Dillon et al. 2007; Marshall & Sinclair 2010).

In addition, to better comprehend the flies' responses to natural microclimate conditions, the effects of variation in heating and cooling rates on  $CT_{max}$  and  $CT_{min}$  were explored. Few studies to date have specifically considered these responses in order to elucidate ecologically relevant estimates of thermal tolerance under laboratory conditions (but see e.g. Slabber et al. 2007; Mitchell & Hoffmann 2010). Nyamukondiwa & Terblanche (2010) revealed that under agro-ecosystems, the rate of ambient temperature increase is typically  $0.04 \pm 0.01$  °C/min while the rate of temperature decrease is  $0.03 \pm 0.01$  °C/min. Experiments conducted using these more ecologically relevant ramping rates indicated slower rates of temperature increase/decrease significantly improved CTLs. This finding suggests that both species may have the capacity to rapidly adjust their cold and heat tolerance (rapid cold- and heat-hardening) and indeed, this was true for adults of both species (Nyamukondiwa et al. 2010). Such responses have previously been reported for high temperature tolerance in *C. capitata* (Kalosaka et al. 2009), *Drosophila* (Loeschcke & Hoffmann 2006; Johnson et al. 2009) and other insect species (e.g. Huang et al. 2007). Similarly, rapid cold hardening has been documented in a range of Diptera including *Drosophila* (Kelty & Lee 2001; Overgaard et al. 2006), *Sarcophaga crassipalpis* (Rinehart et al. 2000), *Musca domestica* (Coulson & Bale 1990) and in other tephritid flies e.g. *Bactrocera tryoni* (Meats 1973), *Bactrocera oleae* (Koveos 2001), *Eurosta solidaginis* (Lee et al. 1993), *C. capitata* and *C. rosa* (Nyamukondiwa et al. 2010). The present results therefore supports the assumption that rapid cold hardening, and possibly also heat hardening, can be induced during the cooling and heating phases, respectively, of natural diurnal thermal cycles (Kelty & Lee 2001). Hence, cold and heat hardening in *C. capitata* and *C. rosa* may potentially protect these fruit flies during diurnal temperature changes that naturally occur in agro-ecosystems. Indeed, microclimatic temperatures recorded in the Western Cape (Nyamukondiwa et al. *in prep*) indicated these flies spend a significant part of their lifespan at sub-lethal low temperatures. This emphasises the ability of *Ceratitis* to track changes in ambient temperature might be critical for surviving such harsh thermal environments. Critical thermal limits estimated under conditions which most closely approximate natural diurnal temperature fluctuations (rate =  $0.06$  °C/min) indicate these species  $CT_{max}$  are  $\sim 42$  °C and  $CT_{min}$  are  $\sim 6$  °C in the wild, though some variation between these species has been found in  $CT_{max}$  previously (Nyamukondiwa & Terblanche 2009). Our acclimation results clearly support the notion that thermal pre-treatments may enhance thermal tolerance, especially over longer-timescales, and this might improve field performance of insect control using Sterile Insect Technique (SIT) (e.g. Kristensen et al. 2008; though see discussion in Overgaard et al. 2010). However, attempts at thermal conditioning of flies for SIT will need to be mindful that some of the benefits associated with acclimation may be lost relatively rapidly (e.g. Meats & Fay 1976; Fay & Meats 1987). Hence field mark recapture studies of thermally conditioned *Ceratitis* may need further exploration to compliment and optimise these laboratory results, before thermal acclimation can be incorporated into rearing protocols targeted at increasing the efficacy of SIT programs.

#### Plasticity of low temperature tolerance in adult *C. rosa* and *C. capitata*

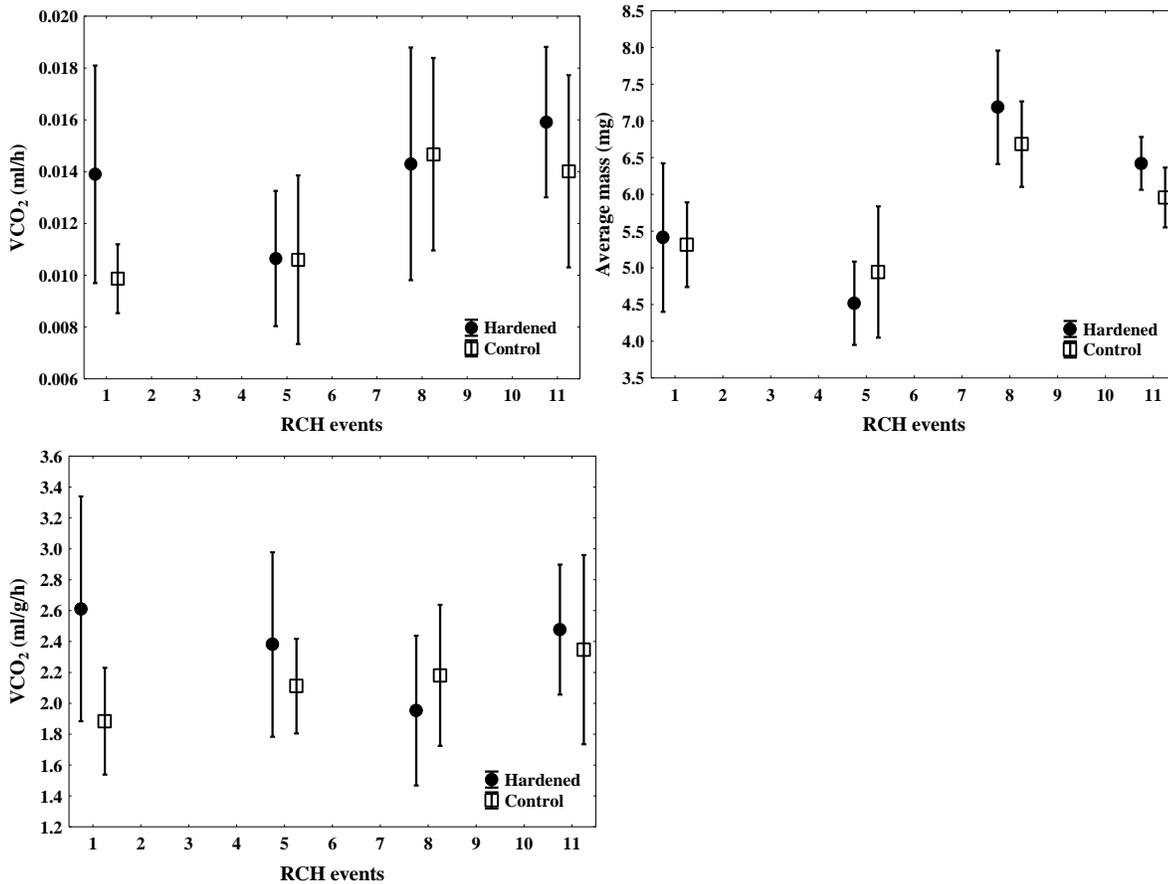
Few studies to date have investigated phenotypic plasticity of temperature tolerance in *Ceratitis* species (but see Kalosaka et al. 2009). Nevertheless, phenotypic plasticity is a major mechanism used by insects to cope with temperature variation at daily (Kelty & Lee 2001; Overgaard & Sorensen 2008) and seasonal timescales (Chown & Terblanche 2007). Moreover, such mechanisms could also be important upon introduction into new environments (Chown et al. 2007; Kristensen et al. 2008; Nyamukondiwa et al. 2010). Indeed, the invasion success of *Ceratitis capitata* probably stems from physiological, morphological and behavioural adaptations which enable them to survive when introduced into areas outside of their native range (Vera et al. 2002; Malacrida et al. 2007; DeMeyer et al. 2008). However, it is generally poorly understood if variation in acute thermal tolerance and its phenotypic plasticity might be important in facilitating survival of *C. capitata* upon introduction to novel environments (Chown & Terblanche 2007; Whitman 2009). In direct comparisons between the widely-distributed *C. capitata* and a narrowly-distributed congener, *C. rosa*, that were reared under common conditions, we found that both species have similar levels of survival to acute high and low temperature exposures. However, these species differed dramatically in the time-course of plastic responses to acute low temperature treatments (Nyamukondiwa et al. 2010), conferring a survival advantage for *C. capitata*, especially in

habitats where temperatures frequently drop below 10 °C. These results are in keeping with previous studies on plasticity of thermal tolerance to date (Meats 1973; Coulson & Bale 1990; Czajka & Lee 1990; Chen et al. 1991; reviewed in Chown & Nicolson 2004; Denlinger & Lee 2010). Similarly, in another Tephritid fruit fly, *Bactrocera dorsalis*, predictions of geographic range under current and future climate scenarios suggests low temperature tolerance may be the principal limiting factor in the U.S.A. (Stephens et al. 2007), in keeping with our results (Nyamukondiwa et al. 2010). Moreover, Nyamukondiwa et al (in prep.) suggest low temperature survival is likely more critical than high temperature survival in the wild. Thus, variation in RCH responses may translate into significant variation in survival upon introduction to novel thermal habitats for *C. capitata*, particularly in cooler and more thermally variable geographic regions, and may contribute to their ongoing invasion success relative to other, more geographically-constrained *Ceratitidis* species.

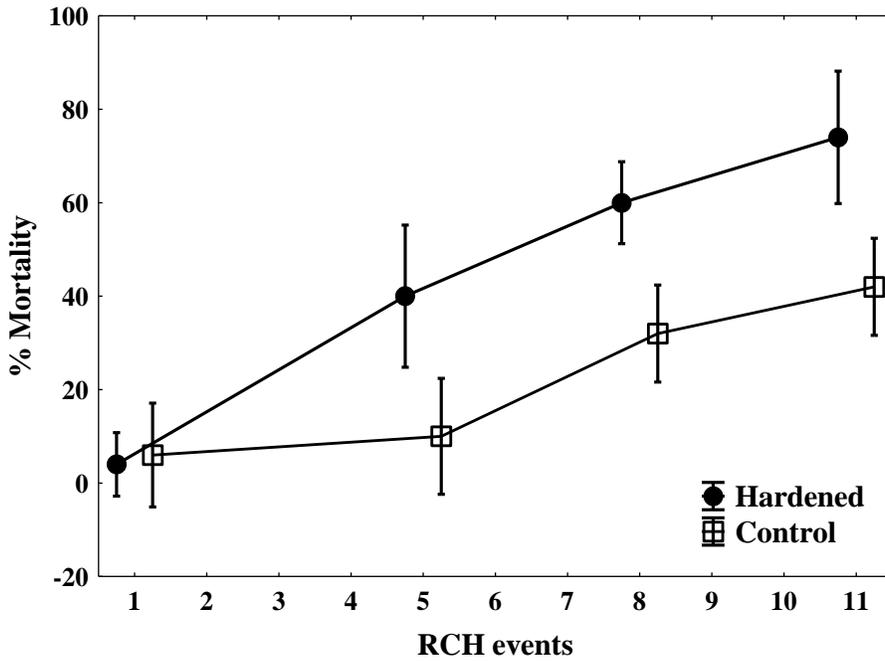
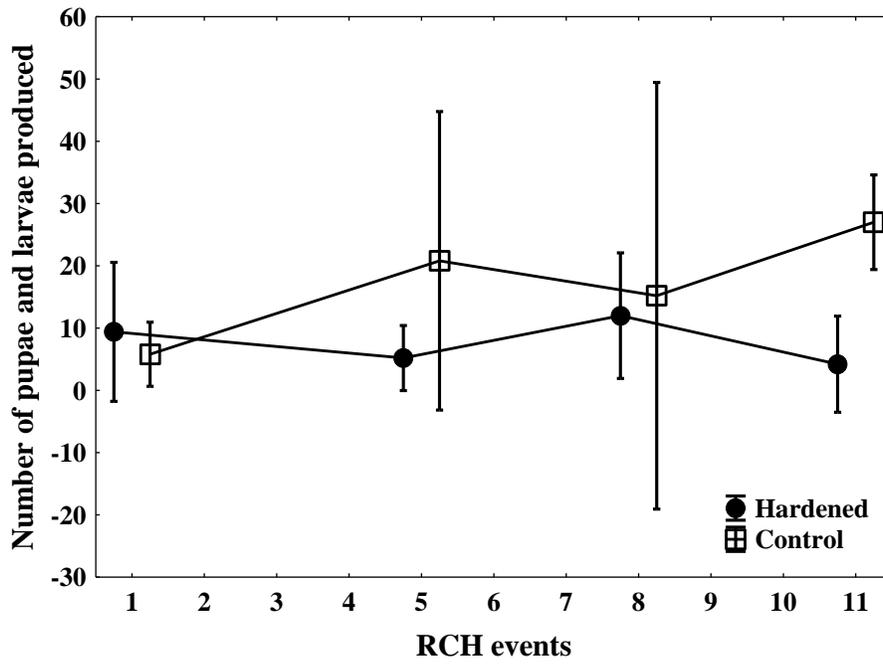
#### Fitness costs in *C. capitata*

The costs involved for insects that undergo rapid cold hardening may vary and are generally poorly established (Lee & Denlinger 2010). Since metabolic demands, but not necessarily overall whole-organismal rates, change under different thermal conditions and perhaps also depending on the number of stressful events encountered within an organisms lifetime (e.g. Nedved et al., 1998; Petavy et al., 2001; Sinclair & Chown 2005; and see Gutschick & Bassirirad 2003), this can potentially lead to trade-offs in resource allocation that affects various life-history traits or strategies (e.g. growth-rate, body size, egg-laying, survival, tissue or cell maintenance, mating or courtship rituals as well as decreased heat-tolerance), essentially affecting long-term survival of populations (e.g. Chown & Gaston, 2010; Coulson & Bale, 1990, 1992; Marshall & Sinclair, 2010; Overgaard & Sørensen, 2008; Shreve et al., 2004). However, maintaining a plastic response such as RCH which could potentially result in consumption of valuable body resources, could allow the insect to exploit resources available later on as a result of increased acute survival at low temperatures. Nevertheless, the evolution of RCH remains complex and unresolved (Sinclair et al., 2003; Strachan et al., 2011; and see Lee & Denlinger, 2010) perhaps at least partly owing to poor understanding of the relative fitness costs of such a response.

In this study, we examined whether ecologically relevant diel temperature fluctuations which induce RCH responses carry a metabolic, survival or fecundity cost in the Mediterranean fruit fly, *Ceratitidis capitata*. We predicted that any potential costs in RCH would be manifested as 1) a difference in metabolic rate, fecundity or survival in flies which have hardened versus those which have not hardened, or 2) flies which have experienced more hardening events would show greater costs than those which have hardened on fewer occasions. Flies were exposed to two treatments: one group was cooled to 10 °C for 2 h for 11 consecutive days (25-10 °C) and effectively experienced daily rapid cold-hardening (Hardened), whilst the other group was exposed to 15 °C for the same 2 h period each day (25-15 °C) and acted as a Control group. The Hardened group had significantly higher acute low temperature survival (i.e., showed RCH during thermoperiodic cycles) than the 25-15 °C group (Control) when exposed to -5 °C for 2 h directly after acclimation treatment for 1, 5, 9 and 11 days (% survival averaged across all RCH events: 69±9% vs. 44±19%,  $P<0.05$ ). Cold-hardened flies showed no metabolic (Fig. 3.3.5.1) or fecundity costs (Fig. 3.3.5.2), but these groups had reduced average survival at their rearing conditions relative to control flies (across all time-points sampled) (Fig. 3.3.5.2). Furthermore, the number of RCH events did not incur greater metabolic rate ( $P>0.05$ ) or reduced fecundity ( $P>0.05$ ), although mortality increased over time in the Hardened group ( $74 \pm 11.4\%$  vs.  $42 \pm 8.3\%$  for the Control group after 11 RCH events,  $P<0.001$ ). Thus, at least in *C. capitata*, a major cost to RCH is likely through direct mortality caused by chilling injury. Thus, a major cost to repeated low temperature exposures in *Ceratitidis capitata* is through direct mortality caused by chilling injury, although this appears not to be a direct cost of RCH (for further results and discussion see Basson et al. 2012).



**Fig. 3.3.5.1.** (A) Mean unadjusted VCO<sub>2</sub> (i.e. not mass-corrected RMR) (mlCO<sub>2</sub>/h, means ± 95% CLs) for *C. capitata* adults measured at 25 °C in the Hardened (i.e. rapidly cold-hardened) for 1, 5, 8 and 11 days by thermal fluctuations from 25 to 10 °C. The Control group was subjected to 15 °C for 2 h and the Hardened group was exposed to 10 °C for 2 h for 11 consecutive days. (B) Average mass (mg, means ± 95% CLs) of flies acclimated over 11 days for both Control and Hardened groups. (C) Mass-adjusted RMR (mlCO<sub>2</sub>/g/h, means ± 95% CLs.) for *C. capitata* adults at 25 °C after 1, 5, 8 and 11 RCH events. Mass was the only variable to significantly affect MR ( $P < 0.005$ ), whereas treatment and test temperature showed no significant effect, nor did the interaction of the two variables significantly affect RMR over 11 RCH events (see Basson et al. 2012).



**Fig. 3.3.5.2.** (A) Mean ( $\pm 95\%$  CLs) number of pupae and larvae (measures of fecundity) produced following 1, 5, 8 and 11 RCH events. (B) Mean ( $\%$ ,  $\pm 95\%$  CLs) mortality scored as  $n$  dead flies/ $n$  total flies after 1, 5, 8 and 11 RCH events in both the Control and Hardened groups.

Work on the common housefly *Musca domestica* by Coulson & Bale (1990, 1992) showed a decrease in the number of eggs oviposited by cold-hardened *M. domestica*, along with a reduced life-span of the adults and decreased emergence rate of eggs (Coulson & Bale 1992) suggesting multiple costs, both in terms of reproduction and survival. Shreve et al. (2004) found that cold-hardened *D. melanogaster* preserved reproductive behaviors, i.e. courting and mating. Similarly, our results indicate that although flies exposed to several RCH events pay some minor reproductive penalty, this is likely not a consequence of RCH *per se* but rather overall reduced average temperature in Hardened flies. This implies that diurnal temperature variation, at least for an extended period of time, perhaps comes at a marginal reproductive cost, but there is great variation among days even within the Control group. A lack of reproductive costs has been reported by Kelty & Lee (1999) in short term exposures (5 days) in *D. melanogaster*. However, the most likely explanation of the fitness cost of the difference in the variable temperatures between treatment groups in *C. capitata* is the highly significant increase in mortality at benign temperatures. This is probably a consequence of direct, chilling injury as opposed

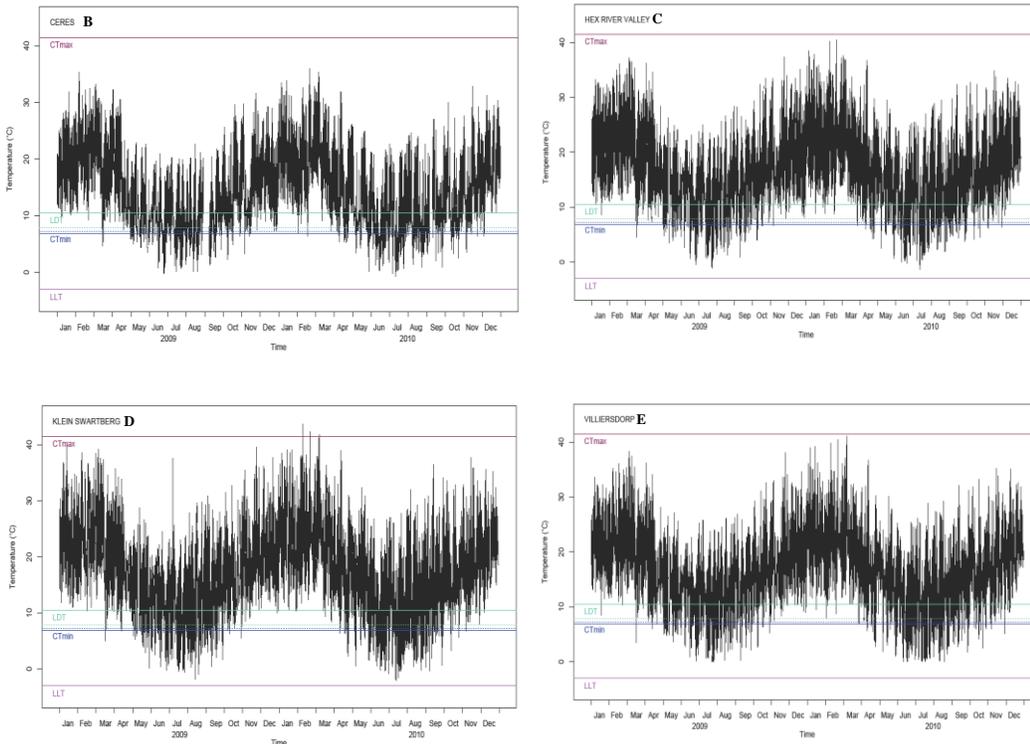
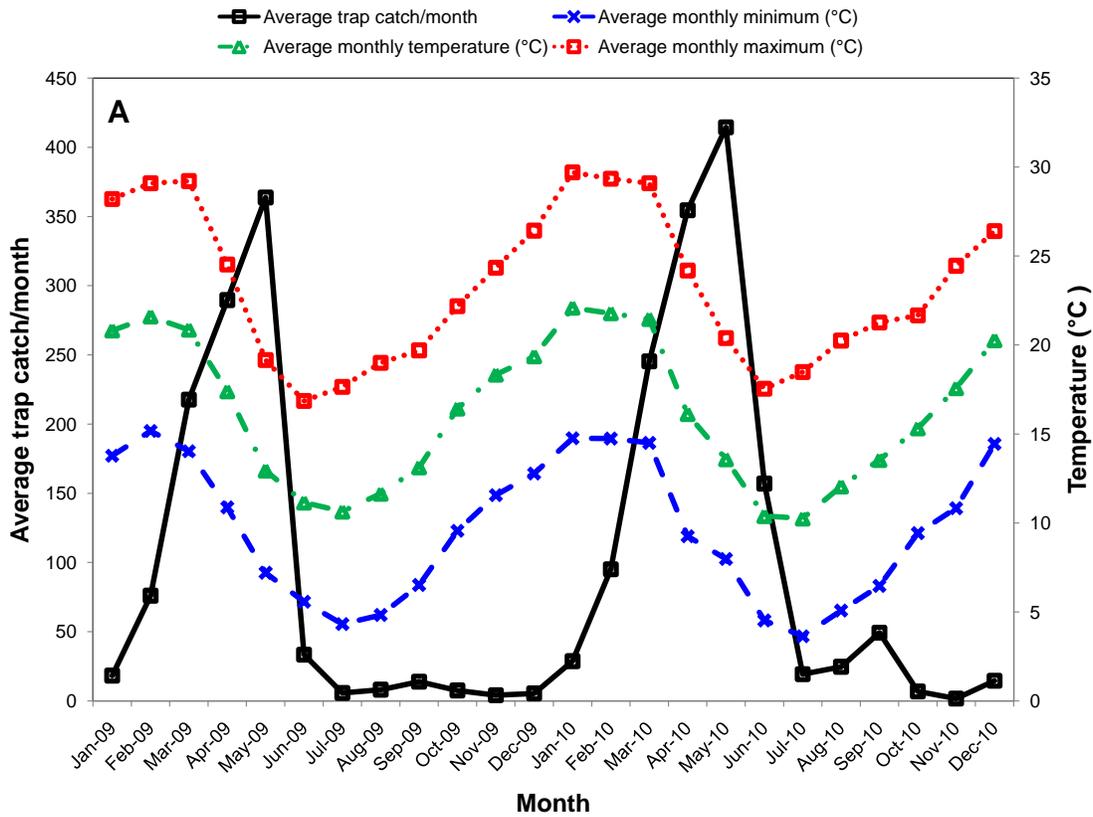
to indirect effects on reduced fecundity, given the manner in which we scored these traits. Given that mortality did not increase over time (i.e. no RCH event x treatment group effect was detected), it seems more likely that this is an effect of reduced temperatures, rather than a cost of RCH. Nevertheless, recent work has shown repeated exposure to chilling events in fluctuating thermal regimes could also result in life-history tradeoffs and, ultimately, negative population growth rates (e.g. Marshall & Sinclair 2010). *Sarcophaga crassipalpis* only shows injury from cold-shock three days after treatment due to damage to the neuromuscular system (Kelty et al. 1996) and thus our speculation of chilling damage is not unfounded (and see Yi & Lee 2003).

Phenotypic plasticity of the kind discussed here, (i.e. acute, reversible plasticity) resulting in RCH in adult *C. capitata* likely comes with both evolutionary fitness benefits and costs, as might be expected for plastic responses more broadly (see discussion in e.g. Gilchrist 1995; Hoffmann 1995; Sultan & Spencer 2002; Angilletta 2009; Kingsolver et al. 2009). Even though the flies do not expend extra metabolic energy on the process, fitness penalties are incurred. The costs involved are not necessarily acclimation or plasticity costs *per se*, but might be a result of chill injury due to exposure to sub-optimal temperatures. While the flies had hardened at 10 °C, which likely minimizes chill injury (Yi & Lee 2003), repeated exposure to fluctuating low temperatures has an obvious detrimental effect on population survival under benign conditions, an effect which was not compensated for (or if so, at least not completely) by the RCH response itself.

#### Thermal biology and population abundance of *Ceratitis capitata* and *Ceratitis rosa* under semi-field conditions

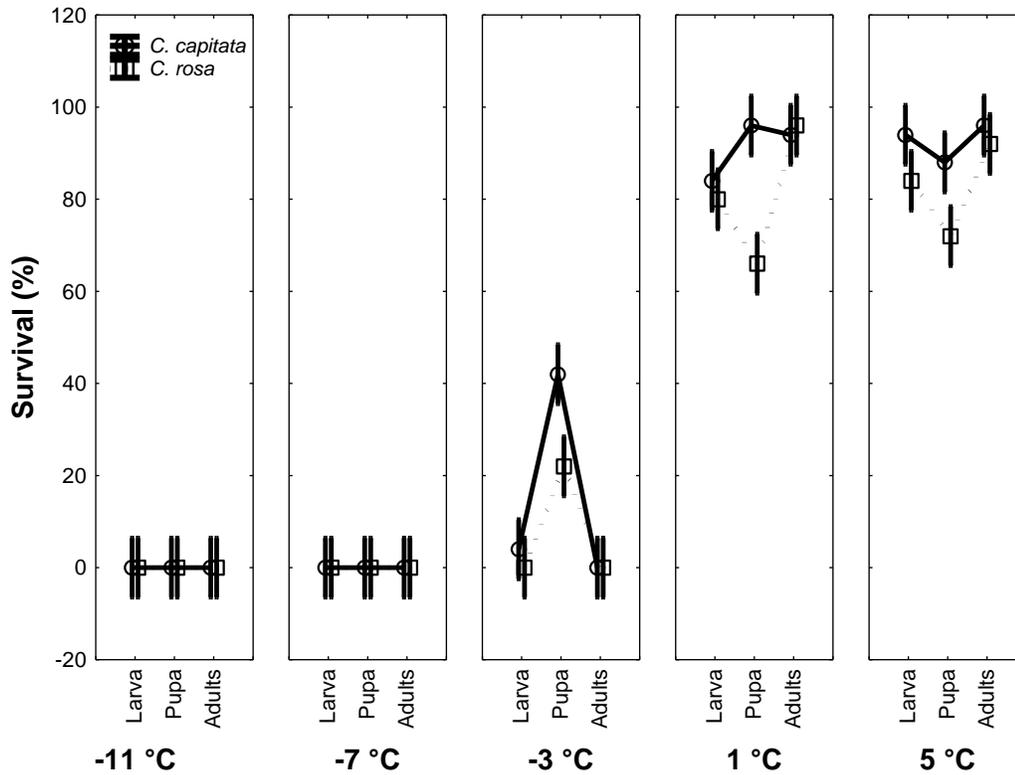
Like other insects *Ceratitis* are faced with temperature variability temporally and spatially. Hence, the ability to tolerate and adapt to novel thermal conditions over short- and long timescales may determine *Ceratitis* abundance and distribution (Chown et al. 2010; Hoffmann, 2010). The potential for sub-tropical and tropical insects to colonise temperate zones largely depends upon their ability to withstand the cold climate, either behaviorally or physiologically (Chown & Nicolson, 2004; Denlinger & Lee, 2010). Overcoming such environmental challenges forms the initial of several potential barriers that determine whether a species becomes established, naturalized and, ultimately, invasive (reviewed in Richardson & Pysek 2006). Following introduction to a novel environment, a species may be able to persist over short time-scales either by having greater resistance to climate conditions, or by mounting a rapid response to these extremes and thereby avoiding potential detrimental effects. This means phenotypic plasticity may be a critical mechanism enhancing survival of insects upon a change in their environment (reviewed in Chown & Terblanche 2007; Whitman 2009). The process may involve some genetic differentiation to achieve required physiological tolerance of the traits involved (Richardson & Pysek, 2006). Thus migration and dormancy may help species escape unfavourable environmental variability, e.g. temperature. Migration leads to escape from the unfavourable environmental variable in space while dormancy leads to escape in time (Begon et al. 1986). The overwintering strategy of a species could explain local population refugia, and hence the level of establishment of a pest species. For *Ceratitis* species it is largely unknown what happens to populations in the winter in South Africa. Some data are available from other parts of the world. For example, *C. capitata* is unable to overwinter in the Judean Hills of Israel (Israely et al. 2004). Instead, there is a process of re-invasion each spring from adjacent agricultural regions (Israely et al. 2005). Overwintering of adults has been reported in some studies (Messenger & Flitters 1954; Carante & Lemaitre 1990). Nevertheless, Papadopoulos (1998; 2002) suggested that *C. capitata* overwintered mainly as preimagos in late season apple varieties and therefore argued that adults emerge the following spring with subsequent infestation of early hosts. Thus, understanding of the overwintering strategy has direct pest management implications since it assists by knowing when and where to focus control efforts.

Seasonal monitoring of *C. capitata* showed that adult flies were active all year round although mainly *C. capitata* are trapped in the Western Cape (Nyamukondiwa et al. in prep) (Fig. 3.3.5.3). However, frequency and duration of suboptimal winter temperatures appear particularly stressful (Fig. 3.3.5.3) and therefore are likely to significantly affect adult activity, mating, oviposition and retard preimago development. Accumulated degree days were low enough to reduce *Ceratitis* development rates. However, these low temperatures are unlikely to kill fly populations (Fig. 3.3.5.4). Therefore, as long as oviposition can take place, local populations may continue to develop, albeit slowly. Estimates of larval, pupal and adult lower lethal temperatures, SCPs and day degree data suggest both species are chill-susceptible and are unlikely to be killed by winter temperatures (Nyamukondiwa et al. in prep). Moreover rapid cold hardening may be elicited during winter temperatures, indicating this process might be a significant survival mechanism during exposure to sub-lethal low winter temperatures (Nyamukondiwa et al. 2010). Thus *C. capitata* and *C. rosa* may overwinter as adults in the Western Cape Province, South Africa. Moreover, even without winter oviposition, mean adult life expectancy of both *C. capitata* (Carey et al. 2008) and *C. rosa* (Duyck et al. 2010) is probably sufficient to see them through the coldest part of the year.



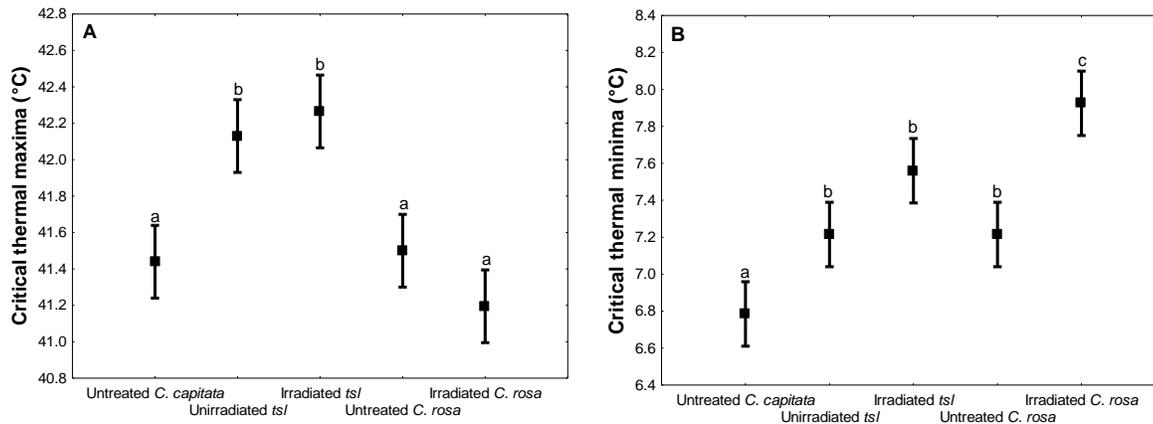
**Fig. 3.3.5.3.** (A) Seasonal population phenology of *C. capitata* across four sites in the Western Cape Province as a function of average monthly, average minimum and average maximum temperature during 2009 and 2010. *Ceratitis rosa* was not caught in any traps so the results are omitted. Traps were serviced every fortnight and average *C. capitata* caught/trap/month was calculated. Population abundance was then averaged across the four sites. Microclimate temperature was recorded from (B) Ceres, (C) Hex River Valley, (D) Klein Swartberg and (E) Villiersdorp over two years (2009-2010). Ambient temperature was recorded using Thermochron iButtons (0.5 °C accuracy; 1 h sampling frequency). LDT and LLT represents lower developmental and lethal

temperatures respectively while  $CT_{max}$  and  $CT_{min}$  represents upper and lower critical thermal limits respectively. *Ceratitis capitata* = solid and *C. rosa* = dotted lines.



**Fig. 3.3.5.4.** Mean survival ( $\pm 95\%$  CLs) of different developmental stages of *C. rosa* and *C. capitata* under different low temperature treatments for 8 h. Each data point represents an average of 5 replicates,  $N = 10$  individuals per replicate/treatment.

Several aspects of Tephritid performance and survival are influenced by irradiation and laboratory culture conditions (e.g. Dyck *et al.* 2005). To our knowledge, no study has documented irradiation effects on thermal tolerance under laboratory or field conditions in *C. capitata* or *C. rosa*. Several studies have shown that irradiation negatively affects fitness parameters including mating performance (Lux *et al.* 2002), flight ability and survivorship in *C. capitata* (Barry *et al.* 2003) and *Anastrepha obliqua* (Toledo *et al.* 2004). Similarly, irradiation also impacts negatively on starvation and desiccation resistance in *Bactrocera tryoni* (Collins *et al.* 2008). Our results showed that time to extinction was highest in *tsl C. capitata* and lowest in irradiated *C. rosa* (Nyamukondiwa *et al.* in prep.). *tsl C. capitata* have the highest  $CT_{max}$  while  $CT_{min}$  of irradiated *C. rosa* was elevated (i.e. poorer) than the other strains/treatments (Fig. 3.3.5.5). Interestingly, the rank order of time to extinction in the field matched the rank order of  $CT_{max}$  (but not for  $CT_{min}$ ) which may suggest that heat tolerance is a significant determinant of field survival, at least under the summer field conditions tested, irrespective of assay method. This result is significant in light of debates surrounding the ecological relevance of different estimates of thermal tolerance, and the link between laboratory methods and field performance (see discussions and review in Terblanche *et al.* 2011). The precise reason why *tsl C. capitata* survived better and showed increased thermal tolerance is unclear. Laboratory adaptation may alter the genetic composition of organisms perhaps affecting traits such as thermal tolerance (Jensen *et al.* 2010). Two possible explanations for this result are i) that *tsl C. capitata* are in better condition owing to high-quality diet and optimal rearing conditions or ii) that the *tsl* mutation has given the flies an overall thermal tolerance advantage. Further work is required to distinguish among these possibilities. In summary, the current results show that irradiation may negatively affect field survival times and thermal tolerance in *C. rosa* but has little or no apparent detrimental effect on *C. capitata*. This is in keeping with the literature on radiation effects more generally, in which trait- or species-specific effects may be detected (e.g. Lux *et al.* 2002; Collins *et al.* 2008; Dyck *et al.* 2005).



**Fig. 3.3.5.5.** (A) Variation in mean CT<sub>max</sub> and (B) mean CT<sub>min</sub> in different *Ceratit*s species/strains. CT<sub>max</sub> and CT<sub>min</sub> experiments started at 25 °C (ramp rate: 0.25 °C/min). (*N* = 20 per strain/treatment group). Error bars represent 95% CLs.

## Conclusion

To date we have undertaken a systematic exploration of the population level factors influencing cold and heat tolerance in adult Natal fly and Medfly (Nyamukondiwa and Terblanche, 2009 J. Therm. Biol. 34: 406-414). We have also shown that thermal history influences thermal tolerance in both species (Nyamukondiwa and Terblanche, 2010 Physiol. Entomol. 35: 255-264). Furthermore, we have shown that both species can rapidly alter thermal tolerance over a few hours but have different time-courses of plastic responses to low temperature between these two species (Nyamukondiwa et al., 2010 Ecol. Entomol.). *C. capitata* responds faster to temperature variation and the benefits of the response last longer compared to *C. rosa*. Based on these results, we therefore developed a mathematical model which predicts population extinction under variable temperatures and suggests that *C. capitata* will survive better in low temperature, variable environments than *C. rosa* (Nyamukondiwa et al., 2010 Ecol. Entomol.). Under field and semi-field conditions, similar results have been found. At high temperatures, field survival is correlated with high temperature tolerance scored in the lab, while at low temperatures *C. capitata* generally survives longer than *C. rosa*. Results for geographic variation have yet to be obtained (several geographic lines are still being reared to maturity and sufficient sample sizes).

The simple, short answer to the key objectives of this project can be answered as follows:

- 1) if variation in thermal tolerance between *C. rosa* or *C. capitata* results in different levels of survival under semi-field conditions - YES
- 2) if plastic low temperature responses are costly from a fitness or energetic perspective in *C. capitata*- YES
- 3) if *C. rosa* and *C. capitata* show inducible cold tolerance at daily and seasonal time-scales under semi-field conditions - YES
- 4) if *C. capitata* populations have permanently or reversibly adapted to local climatic conditions in South Africa - WORK ONGOING, UNKNOWN AT PRESENT - and if *C. rosa* and *C. capitata* might overwinter in South Africa - YES, CERTAINLY POSSIBLE IN WESTERN CAPE.

These results are of broad significance to understanding the evolution of rapid, reversible phenotypic plasticity in thermal tolerance traits of terrestrial ectotherms. These results should help explain the temperatures limiting geographic distribution in both species in South Africa and should be of value to understanding field performance in SIT programmes presently underway.

Future research will investigate costs and benefits of thermal acclimation for field performance using release-recapture experiments in both *C. capitata* and *C. rosa*.

## Technology transfer

### Talks or presentations:

- The effects of age, gender and feeding status on thermotolerance in *Ceratitis rosa*. Nyamukondiwa, C., Terblanche, J.S. 16<sup>th</sup> meeting of the Entomological Society of Southern Africa, Stellenbosch, South Africa. 2009. (presenting author underlined)
- Phenotypic plasticity of thermal tolerance in adult *Ceratitis capitata* and *Ceratitis rosa* (Diptera: Tephritidae). Nyamukondiwa, C., Terblanche, J.S. 8<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, Valencia, Spain 12-17 October 2010. (presenting author underlined)

### Peer reviewed publications:

- Nyamukondiwa, C. & Terblanche, J.S. (2009) Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. *Journal of Thermal Biology* 34: 406-414.
- Nyamukondiwa, C. & Terblanche, J.S. (2010) Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies, *Ceratitis capitata* (Wiedemann) and *Ceratitis rosa*, Karsch: thermal history affects short-term responses to temperature. *Physiological Entomology*, 35, 255-264.
- Nyamukondiwa, C., Terblanche, J.S., & Kleynhans, E. (2010) Phenotypic plasticity of thermal tolerance contributes to the invasion potential of Mediterranean fruit flies (*Ceratitis capitata*). *Ecological Entomology* 35: 565-575.
- Nyamukondiwa, C. & Terblanche, J.S. (2010) Rapid cold-hardening in *Zaprionus vittiger* (Coquillett) (Diptera: Drosophilidae). *CryoLetters* 31: 504-512.
- Terblanche, J.S., Nyamukondiwa, C. & Kleynhans, E. (2010) Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). *Entomologia Experimentalis et Applicata* 3: 304-315.
- Basson, C.H., Nyamukondiwa, C. & Terblanche, J.S. (2012) Fitness costs of rapid cold-hardening in *Ceratitis capitata*. *Evolution* 66: 296-304.
- Nyamukondiwa, C., Weldon, C.W., le Roux, P.C., Chown, S. L. & Terblanche, J.S. (2012). Thermal biology and population persistence of Mediterranean and Natal fruit flies, *Ceratitis capitata* and *Ceratitis rosa* (Diptera: Tephritidae), in South Africa. (In preparation).

### Non peer-reviewed publications:

- Terblanche, J.S. & C. Nyamukondiwa. 2010. An update and overview of recent progress in the thermal physiology of *Ceratitis capitata* and *C. rosa*. TEAM Newsletter December 2010, No. 10, pp. 3-7.
- Terblanche, J.S., P. Addison, C. Nyamukondiwa, A. Manrakhan. 2012. Factors influencing the distribution of Medfly and Natal fly in South Africa: Current research status. South African Fruit Journal. April/May, pp. 56-59.

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**3.3.6 PROGRESS REPORT: Surveillance of *B. invadens* in commercial citrus orchards in South Africa**  
Experiment 966 (2009/10 – 2012/3) by Aruna Manrakhan, John-Henry Daneel (CRI) & Rooikie Beck (CRI)

**Opsomming**

*Bactrocera invadens* bly 'n potensiële bedryging vir die sitrusbedryf van Suid-Afrika, hoofsaaklik as gevolg van die kwarantyn status van die plaag en die moontlike impak op mark toeganklikheid. *B. invadens* is ook bekend om skade aan sekere kultivars soos suurlemoen en pomelos te veroorsaak, wat in Suid-Afrika nie direk deur plaaslike vrugtevlug plaeg beskadig word nie. CRI se *B. invadens*-opnamenetwerk bestaan uit 'n totaal van 108 lokvalle, 63 hiervan word op 'n weekliks/maandeliks basis deur CRI personeel en medewerkers in stand gehou. Die data van hierdie CRI-opnamenetwerk word dan op 'n gereelde basis in die *Bactrocera invadens* National Surveillance Database (BINSD) ingelees, wat onder die outoriteit van die Departement van Landbou, Bosbou en Visserye staan. Tot en met Maart 2012 is daar in BINSD 'n totaal van 1414 metiel-eugenol lokvalle in Suid-Afrika vir die waarneming van *B. invadens* geregistreer. Lokvalle is deur DAFF, CRI, Fruitgro en Subtrop uitgesit en in stand gehou. In 2011, is vyf areas in die noordelike Limpopo: Groblersbrug, Weipe, Tshipise, Nwanedi-Pafuri en Levubu tot uitwissing en kwarantyn verklaar weens onderskeppings van *B. invadens*. In Januarie 2012 was uitwissing in al vyf areas as suksesvol verklaar. Daar was egter nog invalle van *B. invadens* in Limpopo en Mpumalanga gewees tussen Februarie en April 2012. In al die areas waar *B. invadens* eksemplare onderskep was, was kwarantyn maatreëls in plek gestel. In areas waar daar ten minste 2 positiewe lokvalle binne 'n afstand van 5 km was, was daar begin met uitwissing en is dit op die "International Plant Protection" portaal gerapporteer.

'n Netwerk van *B. invadens* opnamelokvalle word tans ook in die sitrus produserende areas van suidelike Zimbabwe en ook op twee sitrus plase in Swaziland instand gehou. In suidelike Zimbabwe word 34 aktiewe metiel-eugenol lokvalle deur kwekers wat ook data aan CRI verskaf op 'n weeklikse/maandelikse basis in stand gehou. In Swaziland word vier lokvalle op 'n maandelikse basis deur CRI personeel nagegaan en herlaai. Gedurende 2011 was geen *B. invadens* in sitrus produksie areas in suidelike Zimbabwe en Swaziland aangeteken nie.

## Summary

*Bactrocera invadens* remains a potential threat to the citrus industry of South Africa due mainly to the quarantine status of this pest and possible impact on market access. *B. invadens* is also known to cause damage on certain citrus cultivar such as lemon and grapefruit which, in South Africa, are not directly damaged by local fruit fly pests. The CRI *B. invadens* surveillance network consists of a total of 108 traps, 63 of which are maintained on a weekly/monthly basis by CRI personnel and collaborators. Data from the CRI surveillance network is then entered on a regular basis in the *Bactrocera invadens* National Surveillance Database (BINSD) which is managed under the authority of the Department of Agriculture, Forestry and Fisheries. In March 2012, in BINSD, a total of 1414 methyl eugenol traps were registered in South Africa for surveillance of *B. invadens*. Traps were set and maintained by DAFF, CRI, Fruitgro and Subtrop. In 2011, five areas in northern Limpopo: Groblersbrug, Weipe, Tshipise, Nwanedi-Pafuri and Levubu, were declared under eradication and quarantine due to interceptions of *B. invadens*. In January 2012, eradication was declared successful in all 5 areas. However, between February and April 2012, there were further incursions of *B. invadens* in Limpopo and Mpumalanga. In all areas where *B. invadens* specimens were intercepted, quarantine regulations were implemented. In areas where there were at least 2 positive traps within a distance of 5 km, eradication was initiated and reported on the International Plant Protection portal.

A network of *B. invadens* surveillance traps is also currently being maintained in citrus production areas of southern Zimbabwe and in two selected citrus farms in Swaziland. In Southern Zimbabwe, 34 active methyl eugenol traps are being maintained by growers who provide data to CRI on a weekly/monthly basis. In Swaziland, four traps are being checked and re-baited on a monthly basis by CRI personnel. In 2011, no *B. invadens* was recorded in citrus production areas in southern Zimbabwe and Swaziland.

### 3.3.7 PROGRESS REPORT: Field control of *Bactrocera invadens* with MAT and bait Experiment 926 (2008/9 – 2012/3) by T G Grout and P R Stephen (CRI)

## Opsomming

Vorige navorsing het getoon dat die mannetjie vernietiging tegniek (MAT) alleen, sonder die ondersteuning van 'n tipe lokaas behandeling vir die wyfies, nie kommersiële beheer kan gee nie. Waar *B. invadens* slegs gedurende die reënseisoen gevind word, soos naby Tsumeb, Namibië, is kommersiële beheer in lemoen boorde maklik verkrygbaar met MAT (Invader-B-loks) plus lokaasbespuitings of M3 lokstasies. 'n Sitrus veldproef in Kenia met 'n groter bevolking van vlieë, het gewys dat waar óf M3s plus Invader-B-loks of Prolure lokaas plus Invader-B-loks gebruik is, was die gemiddelde hoeveelheid vrugtevlieë wat skadelik vir sitrus is (insluitend *B. invadens*) minder as die helfte van die vlieë was waar Mazoferm lokaas plus Invader-B-loks of Invader-B-loks alleen gebruik is. Die vorige twee behandelings het ook kommersiële beheer verskaf. 'n Soortgelyke proef is tans by dieselfde perseel in Kenia aan die gang wat ook GF120 lokaasbespuitings plus Invader-B-loks ingesluit, maar die resultate is nog nie beskikbaar nie. Sodra hierdie navorsing opgeskryf is sal hierdie eksperiment beëindig word.

## Summary

Earlier research showed that the male annihilation technique (MAT) alone cannot provide commercial control without the support of some type of bait treatment for the females. Where *B. invadens* is only present during the rainy season such as near Tsumeb, Namibia, commercial control was easily obtained in orange orchards with MAT (Invader-B-loks) plus either bait sprays or M3 bait stations. A citrus field trial in Kenya with higher populations of flies showed that where either M3s plus Invader-B-loks or Prolure bait plus Invader-B-loks were used the mean numbers of fruit flies harmful to citrus (including *B. invadens*) were less than half as abundant as where Mazoferm bait plus Invader-B-loks were used or where Invader-B-loks alone were used. The former two treatments were also providing commercial control. A similar trial is underway at the same site in Kenya that also included GF120 bait sprays plus Invader-B-loks, but the results are not yet available. Once this research is written up it will be terminated.

### 3.3.8 **PROGRESS REPORT: Determine the potential global distribution for *Bactrocera invadens* using CLIMEX**

Experiment: SU De Villiers (2010/4 – 2013/3) by M de Villiers (CRI at Stellenbosch University), Vaughan Hattingh (CRI), Marc de Meyer (Royal Museum of Central Africa, Belgium), Jean-François Vayssieres (WAFFI, Benin), Antonio Sinzogan (WAFFI, Benin), Sunday Ekesi (ICRPE, Kenya), Maulid Mwatawala (Sokoine University of Agriculture, Tanzania), Domingos Cugala (Eduardo Mondlane University, Mozambique), Faiza Salah (University of Gezira, Sudan), Hayder Abdelgader (The Agricultural Research Corporation, Sudan)

#### **Opsomming**

Sedert die eerste verskyning van die indringer vrugtevlug, *Bactrocera invadens*, op die Afrika vasteland in 2003, het die vlieg wydverspreid oor die kontinent geword. Weens die aanpasbaarheid en wye gasheerreëks van die vlieg, is dit 'n groot bedreiging vir landbou in Afrika, en dit hou ook 'n fitosanitêre risiko in, wat internasionale vrugtehandel in verskeie lande beperk. Die doel van hierdie studie is om die potensiële globale verspreiding van *B. invadens* met CLIMEX te bepaal, gebaseer op die vlieg se verspreiding, relatiewe volopheid en seisoenale voorkoms in Afrika. Monsternemingsareas, verteenwoordigend van verskillende klimaatstreke, is in Senegal, Ghana, Benin, Niger, Soedan, Kenia, Tanzanië en Zambië gekies. Chempac "bucket" valle, met metiel-eugenol as lokmiddel, teen 'n digtheid van drie valle per area, word gebruik om die vlieë te monitor. Resultate het gewys dat *B. invadens* 'n wye reëks van klimate kan oorleef, insluitend die warm woestynklimate aangrensend tot die Sahara. Reënval en besproeiing blyk 'n belangrike rol te speel in die fenologie en volopheid van die spesie, met getalle wat oor die algemeen toeneem met die aanvangs van reën.

#### **Summary**

Since its first detection on the African mainland in 2003, the invasive fruit fly, *Bactrocera invadens*, has become widespread across the continent. Due to its adaptability and wide host range, it is a major threat to agriculture in Africa, and is also posing a phytosanitary risk, restricting international fruit trade in many countries. The objective of this study is to determine the potential global distribution of *B. invadens* with CLIMEX, based on its distribution, relative abundance and seasonal occurrence in Africa. Sampling sites, representing different climatic regions, were chosen in Senegal, Ghana, Benin, Niger, Sudan, Kenya, Tanzania and Zambia. Chempac bucket traps, baited with methyl eugenol at a density of three traps per site, are used to monitor the flies. Results showed that *B. invadens* can survive a wide range of climates, including the hot desert climates bordering the Sahara. Rainfall and irrigation seems to play an important role in the phenology and abundance of the species, with numbers generally increasing with the onset of rains.

### 3.3.9 **PROGRESS REPORT: Evaluating a GRAS post-harvest fumigant for fruit fly and other phytosanitary pests**

Experiment 913 (2011/2 – 2013/4) by T G Grout, K C Stoltz and P R Stephen (CRI)

#### **Opsomming**

Evaluering van die berokingsmiddel GRASFUM het getoon dat insekte met sagte ligame, soos witluis, vrugtevlug larwes en valskoddingmot (VKM) larwes baie vatbaar is, terwyl hulle aan die berokingsmiddel aan die buitekant van die vrug of in los media blootgestel word. Witluis eiers en VKM eiers, is egter minder vatbaar en vereis die hoogste dosis vir 'n tydperk van 24 uur om voldoende beheer uit te oefen. Hierdie tydsduur en dosis maak 'n hoë persentasie van VKM larwes binne lemoene dood, maar die vatbaarheid van vrugtevlug larwes binne natuurlik-besmette vrugte moet nog bepaal word. Kombinasies van beroking en verkorte koue-behandeling sal ook in die toekoms evalueer word as beroking alleen nie voldoende is nie.

#### **Summary**

Evaluation of the fumigant GRASFUM has shown that soft-bodied insects such as mealybugs, fruit fly larvae and false codling moth (FCM) larvae are very susceptible while they are exposed to the fumigant on the outside of the fruit or in loose media. However, mealybug eggs and FCM eggs are less susceptible and require the highest dosage for a period of 24 hours to provide adequate control. This length of time and dosage is killing a high percentage of FCM larvae inside oranges but the susceptibility of fruit fly larvae inside naturally-infested fruit must still be determined. Combinations of fumigation and short cold treatment will also be evaluated in the future if fumigation alone is inadequate.

### 3.4 PROJECT: COSMETIC PESTS

Project coordinator: Tim G Grout (CRI)

#### 3.4.1 Project summary

Even though phytosanitary pests receive most research attention, fruit must still look appealing to be sold, so cosmetic pests will always remain a threat to the crop. Controlling these pests without disrupting important natural enemies of other pests such as false codling moth (FCM), mealybugs and scale insects is also a continual challenge. Control options late in the season when preharvest intervals (PHIs) must be short, are also very limited. Fenpyroximate can now be used up to a month before harvest for most markets for all citrus but for Canada and South Korea we needed to determine what the PHI should be for non-detectable residues. The research showed that 150 days would be required (3.4.2). Nothing is registered for the control of leafhoppers which can sometimes cause a lot of fruit damage between January and harvest, however, as with the previous year, numbers remained low in most areas and no suitable trial sites could be found (3.4.3). Now that imidacloprid has been used in citrus for almost 20 years and the cost of the treatment is no longer a consideration, questions are increasingly being asked about its non-target effects. Publications on its effect on insect hormones and Lepidoptera in particular, coupled with field observations, led to research being conducted on the possibility that imidacloprid may be at least partially responsible for increased population levels of lepidopteran pests in citrus (3.4.4). Indications are that imidacloprid may be increasing populations of FCM. Maximum residue levels (MRLs) and PHIs are an important part of pest management of export citrus and there were concerns that some plant protection trends may result in higher residues at harvest. Stickers were found to have no influence and the addition of oil to plant protection products only had a slight effect in one of four cases. A low volume spray at 4X concentration also resulted in similar residues at harvest to a dilute spray. Only the use of shade cloth was cause for concern and growers with fruit under shade cloth should be cautious and use a longer PHI than that recommended (3.4.5). Thripicides that can be used late in the season without residue problems or disruption of IPM are in short supply. A trial to evaluate a commercial entomopathogenic fungus against thrips and mealybug showed that it was ineffective. An organic product Biocure caused some thrips suppression but this was less than with abamectin plus oil and in some cases it caused an increase in mealybug (3.4.6). Research on the non-target effects of imidacloprid and plant protection products that can be used for thrips and leafhopper late in the season will continue.

#### Projekopsomming

Alhoewel fitosanitiere plaë die meeste navorsingsaandag ontvang, moet vrugte steeds mooi lyk om te verkoop, dus sal kosmetiese plaë altyd 'n bedreiging vir die oes bly. Om die plaë te beheer sonder om belangrike natuurlike vyande van ander plaë soos valskodlingmot (VKM), witluise en dopluise te ontwrig, is ook 'n voortdurende uitdaging. Beheeroepsies laat in die seisoen wanneer voor-oes intervalle (VOIs) kort moet wees, is ook baie skaars. Fenpyroximate kan nou tot 'n maand voor oes vir meeste markte vir alle sitrus gebruik word, maar vir Kanada en Suid-Korea het ons nodig om te bepaal wat die VOI vir nie-opspoorbare residu moet wees. Die navorsing het getoon dat 150 dae nodig sal wees (3.4.2). Niks is vir die beheer van blaarspringers, wat soms baie vrugskade tussen Januarie en oes kan veroorsaak, geregistreer nie, en soos met die vorige jaar het getalle egter laag in die meeste areas gebly en geen geskikte proefpersele kon gevind word nie (3.4.3). Nou dat imidacloprid amper vir 20 jaar in sitrus gebruik is en die koste van die behandeling nie langer 'n oorweging is nie, word al hoe meer vrae oor sy nie-teiken effekte gevra. Publikasies oor sy effek op insek hormone en in besonder Lepidoptera, tesame met waarnemings in die veld, het tot navorsing gelei wat uitgevoer is op die moontlikheid dat imidacloprid ten minste gedeeltelik verantwoordelik kan wees vir toenames in populasie vlakke van Lepidoptera plaë in sitrus (3.4.4). Daar is aanduidings dat imidacloprid populasies van VKM kan verhoog. Maksimum residu-vlakke (MRLs) en VOIs is 'n belangrike deel van plaagbeheer van uitvoersitrus en daar was kommer dat sommige tendense in plantbeskerming mag lei tot hoër residu tydens oes. Kleefmiddels is gevind om geen invloed te hê nie en die byvoeging van olie by plantbeskermingsprodukte het slegs 'n effek in een van vier gevalle gehad. 'n Lae volume bespuiting teen 4X die konsentrasie het ook soortgelyke residu as 'n verdunde bespuiting tydens oes tot gevolg gehad. Slegs die gebruik van skadunette was rede tot kommer en produsente met vrugte onder skadunet moet versigtig wees en 'n langer VOI as wat aanbeveel word, gebruik (3.4.5). Blaaspootjiedoders wat laat in die seisoen gebruik kan word sonder residu probleme of ontwrigting van GPB is skaars. 'n Proef om 'n kommersiële entomatopatogeniese swam teen blaaspootjies en witluise te evalueer, het getoon dat dit nie effektief is nie. 'n Organiese produk, Biocure het blaaspootjie onderdruk, maar dit was minder as met abamectin plus olie, en in sommige gevalle het dit 'n toename in witluis veroorsaak (3.4.6). Navorsing op die nie-teiken effekte van imidacloprid en plantbeskermingsprodukte wat vir blaaspootjies en blaarspringers laat in die seisoen gebruik kan word, sal voortgaan.

3.4.2 **FINAL REPORT: Residue trials for fenpyroximate used for citrus bud mite control**  
Experiment 916 (2011/12) by Tim G Grout and Peter R Stephen (CRI)

**Opsomming**

In sekere lande bestaan daar nie 'n algemene MRL vir fenpyroximaat op sitrus nie, maar het MRLs vir spesifieke sitrustipes. Navorsing is om die rede in twee suurlemoenboorde en twee mandarynboorde gedoen om 'n geskikte voor-oes interval (VOI) vir nie-opspoorbare residue te bepaal. Die resultate het getoon dat die VOI 150 dae moet wees en dat bespuitings nie later as Oktober toegedien moet word nie.

**Summary**

In some countries, MRLs for fenpyroximate exist for oranges but not lemons and mandarins. Research was therefore conducted at two lemon orchards and two mandarin orchards to determine a suitable preharvest interval (PHI) for non-detectable residues. The results showed that the PHI should be 150 days and sprays should not be applied later than October.

**Introduction**

Fenpyroximate was registered for the control of bud mite during 2010 after bromopropylate residues became unacceptable on fruit. However, in some markets there is no fenpyroximate maximum residue limit (MRL) for lemons or mandarins so further residue breakdown information was therefore required to develop safe preharvest intervals (PHIs) for these citrus types.

**Stated objective**

Determine the preharvest interval required for residues of fenpyroximate to be non-detectable.

**Materials and methods**

This research was conducted in the vicinity of Nelspruit in two lemon orchards (Bakgat in Schoemanskloof and Kavalla near Karino) and two Nova mandarin orchards (Belmont at Rivulets and Larten at Karino) while the fruit were still expanding. Sprays were applied at all sites on 26 November 2010 at high pressure (20 bar) using hand guns with a 2.5 mm nozzle orifice. Fenpyroximate 50 g/L EC was applied at the registered rate of 150 ml/hl water as a medium cover spray to the point of run-off. Approximately 10 trees were used per site, depending on the size of the trees and the amount of fruit available. Fruit were picked for residue analysis after 28, 56, 90, 119, and 151 day intervals (Table 3.4.2.1) and frozen at -15°C within 6 hours of picking. Two samples were picked and bagged separately from each site on each occasion. When all fruit samples had been collected one sample from each date and each site was sent to SABS for whole-fruit residue analysis. At SABS each sample was chopped with a Stephan food cutter and split into two samples for analysis. Analysis began with the longest period after treatment and only 150, 120 and 90 days were required.

**Table 3.4.2.1.** Treatments and post-treatment sample times for fenpyroximate in Mpumalanga.

Type	Trial	Treatment	Sample	Sample number
Lemons	Trial 1	150 ml	T28	L1/T28
		150 ml	T56	L1/T56
		Control	T56	L1/T56C
		150 ml	T90	L1/T90
		Control	T90	L1/T90C
		150 ml	T120	L1/T120
		Control	T120	L1/T120C
		150 ml	T150	L1/T150
	Control	T150	L1/T150C	
	Trial 2	150 ml	T28	L2/T28
		150 ml	T56	L2/T56
		Control	T56	L2/T56C
		150 ml	T90	L2/T90
		Control	T90	L2/T90C
150 ml		T120	L2/T120	

		Control	T120	L2/T120C
		150 ml	T150	L2/T150
		Control	T150	L2/T150C
Novas	Trial 1	150 ml	T28	N1/T28
		150 ml	T56	N1/T56
		Control	T56	N1/T56C
		150 ml	T90	N1/T90
		Control	T90	N1/T90C
		150 ml	T120	N1/T120
		Control	T120	N1/T120C
		150 ml	T150	N1/T150
		Control	T150	N1/T150C
		Novas	Trial 2	150 ml
150 ml	T56			CG2/T56
Control	T56			CG2/T56C
150 ml	T90			CG2/T90
Control	T90			CG2/T90C
150 ml	T120			CG2/T120
Control	T120			CG2/T120C
150 ml	T150			CG2/T150
Control	T150	CG2/T150C		

## Results and discussion

The results (Table 3.4.2.2) showed that residues were still detectable in one lemon sample and one mandarin sample after 119 days but that no residues were detectable at 151 days. The recommended PHI has therefore been changed to 150 days and sprays not later than October for citrus types being exported to markets that do not have an MRL or recognise Codex MRLs.

**Table 3.4.2.2.** SABS results of whole-fruit analysis for fenpyroximate residues.

Date sampled	Days after application	Fenpyroximate residue content (mg/kg)			
		Bakgat lemons	Kavalla lemons	Belmont mandarins	Larten mandarins
24/02/2011	90	0.03 ; 0.03	0.01 ; 0.01	0.02 ; 0.02	0.02 ; 0.02
25/03/2011	119	0.02 ; 0.02	<LOQ ; <LOQ	0.02 ; 0.02	<LOQ ; <LOQ
26/04/2011	151	<LOQ ; <LOQ	<LOQ ; <LOQ	<LOQ ; <LOQ	<LOQ ; 0.01

Where LOQ stands for Limit of Quantitation

## Conclusion

The results of four residue trials with lemons and mandarins showed that for these cultivars the PHI should be 150 days and sprays should not be applied later than October in order for fruit to be sold in markets with no MRL for these fruit types.

## Future research

No further research is planned.

## Technology transfer

Results were included in Paul Hardman's food safety Cutting Edge 130 in January 2012.

3.4.3 **PROGRESS REPORT: Treatments for the control of leafhoppers on citrus**  
Experiment 942 (2008/9-2012/3) by Tim G Grout and Peter R Stephen (CRI)

**Opsomming**

Alhoewel blaarspringers ernstige kosmetiese plae kan wees, is hul uitbrake sporadies en geen chemiese bespuitings is vir hul beheer geregistreer nie. Mevinphos en methomyl is bekend om effektief te wees volgens vroeëre CRI proefwerk, maar meer inligting word vir registrasie doeleindes benodig. Ongelukkig was blaarspringer-besmettings in die afgelope seisoen skaars en geen proefpersele in óf Mpumalanga of die Noord-Kaap is gevind nie. Verdere proewe sal, wanneer persele beskikbaar kom, uitgevoer word.

**Summary**

Although leafhoppers can be serious cosmetic pests, their outbreaks are sporadic and no chemical sprays have been registered for their control. Mevinphos and methomyl are known to be effective from earlier CRI trial work but more data is required for registration purposes. Unfortunately, leafhopper infestations were scarce in the past season and no trial sites were found in either Mpumalanga or the Northern Cape. Further trials will be conducted when sites become available.

3.4.4 **PROGRESS REPORT: The effect of systemically-applied imidacloprid on lepidopteran pests of citrus**  
Experiment 954 (April 2010 – March 2012) by Sean Moore, Wayne Kirkman (CRI) and Rachel van der Walt (NMMU)

**Opsomming**

Hoër vlakke van valskodlingmot (VKM), *Thaumatotibia leucotreta*, op sitrus is in die laaste paar jaar aan die gebruik van sistemies toegediende imidacloprid gekoppel. Daarby is vrugskade op suurlemoene, wat aan suurlemoenboordermot (*Prays citri*) toegeskryf word, aangeteken in boorde waar imidacloprid gebruik is. Hierdie tipe skadesimptome is nie voorheen waargeneem nie. Boonop is verskeie spesies van vreemde Lepidoptera in imidacloprid-behandelde sitrusboorde aangeteken. As dit bewys kan word dat imidacloprid wel 'n effek op die aantelingsvermoë van VKM (en ander Lepidoptera) het, sal heelwat produsente dalk besluit om nie produkte wat imidacloprid bevat op hulle sitrus te gebruik nie. Twee ontledings metodes is gebruik om die fisiologiese effek van imidacloprid op monsters van volwasse VKM-wyfies te toets: massaspektrometrie vir ovarium proteïen en HPLC om jeughormoon (JH III) vlakke te kwantifiseer. Hierdie twee parameters kan aandui of imidacloprid 'n invloed op die aantelingsvermoë van die plaag kan hê. Hierdie studie het verhoogde vlakke ovarium proteïen en jeughormoon getoon in volwasse VKM wyfies wat op imidacloprid behandelde vrugte of dieet ontwikkel het. Hierdie het die vorige seisoen se proefresultate bevestig. Boonop was eierlegging van motte wat in imidacloprid behandelde vrugte ontwikkel het, betekenisvol hoër as die van motte wat in onbehandelde vrugte ontwikkel het. In 'n veldproef is VKM besmetting in die imidacloprid behandelde helfde van die boord amper dubbel die in die onbehandelde helfde van die boord. Hierdie vergelyking is betroubaar omdat daar geen ander verskille in die plaagdoder program was nie en VKM vlakke in die twee helfdes van die boord was die vorige seisoen amper identies gewees. Hierdie tendens is egter nie in 'n tweede boord herhaal nie. Dit kon nie bewys word dat imidacloprid toediening deur 'n driebesproeiing stelsel enige konsekwente of voorspelbare effek op miere in die boord of op eierparasiete gehad het nie. Finale herhalings van ovarium proteïen, jeughormoon, laboratorium eierlegging studies en studies op VKM vlakke in boorde moet uitgevoer word.

**Summary**

Higher levels of false codling moth (FCM), *Thaumatotibia leucotreta*, on citrus have anecdotally been associated with the use of systemically applied imidacloprid for a number of years. Additionally, in the last 10 years fruit damage attributed to the lemon borer moth or citrus flower moth (*Prays citri*), have been recorded in orchards where imidacloprid has been applied. These damage symptoms have not previously been recorded. Moreover, previously unrecorded presence of several Lepidoptera has been observed in orange and lemon orchards that have been treated with imidacloprid. If it can be proven that imidacloprid does have an effect on the fecundity of FCM (and other Lepidoptera) many growers may decide not to use imidacloprid products on their citrus crops. Two analytical methods were used to determine the physiological effect of imidacloprid on the adult female specimens: mass spectrometry for ovarian protein and HPLC for quantifying Juvenile Hormone (JHIII) levels. These two parameters would show if imidacloprid could be influencing the fecundity of these pests. This study showed heightened ovarian protein and juvenile hormone levels in FCM adult females which had developed from imidacloprid-treated fruit or diet, confirming results from previous trials. Additionally, fecundity of moths

developed from imidacloprid-treated fruit was significantly higher than that of moths which had developed on untreated fruit. In a field trial, FCM infestation in the imidacloprid-treated half of the orchard was almost double that in the untreated half of the orchard. This comparison was reliable, as there were no other differences in the pesticide programme and FCM levels in the two halves of the orchard had been almost identical during the previous season. This trend was, however, not repeated in a second orchard. It could not be proven that imidacloprid application through drip irrigation had any consistent or predictable effect on ants in the orchard or on egg parasitoids. Final repetitions of ovarian protein, juvenile hormone, laboratory fecundity studies and studies on field levels of FCM should be conducted.

### 3.4.5 FINAL REPORT: The effect of adjuvants, shade and increased concentration on pre-harvest intervals of insecticides

Experiment 1025 (2011/12) by Tim G Grout and Peter R Stephen (CRI)

#### Opsomming

Die gevolge van MRLs wat op uitvoersitrus oorskry word, is finansiële problematieke vir die produsent. Aangesien meeste residu-data vir sitrus in Suid-Afrika op verdunde bespuitings, sonder bymiddels gebaseer is, en omdat vrugte aan die elemente blootgestel is, het ons gedink dit sal waardevol wees om te bepaal of residue deur sekere praktyke wat toenemend geïmplementeer word, geïmpak word. Die byvoeging van 'n kleefmiddel het nie residu-vlakke van 'n oplosbare poeier (methomyl) of 'n emulgeerbare konsentraat (EC) (chlorpirifos) verhoog nie. Byvoeging van tuinboukundige minerale olie (0.3%) het slegs 'n effense toename in chlorpirifos residue op pomelo's veroorsaak, maar kan as weglaatbaar onbeduidend beskou word. Die aanwending van 'n 4x konsentrasie teen 'n kwart van die verdunde volume per hektaar het ook nie tot 'n verhoging van residue gelei wanneer 'n EC n paar weke na blomval gebruik is nie. Wanneer die boom egter 80% met skadunet bedek is, het die opspoorbare residue van methomyl egter verhoog na vlakke bo van dit wat op vrugte gevind is wat aan die son blootgestel is. In die geval van methomyl is hierdie effek steeds deur die aanbevole voor-oes interval gedek, maar produsente wat skadunet gebruik moet hul voor-oes tussenposes effens verleng om moontlike residu oorskrydings van ander plantbeskermingsprodukte te verhoed.

#### Summary

The consequences of MRLs being exceeded on export citrus are financially dire for the grower. As most residue data for citrus in South Africa is based on dilute sprays, without adjuvants and fruit exposed to the elements, we thought it valuable to determine whether residues are affected by certain practices that are increasingly being implemented. The addition of a sticker did not increase residue levels of a soluble powder (methomyl) or an emulsifiable concentrate (EC) (chlorpyrifos). Adding horticultural mineral oil (0.3%) only caused a slight increase in chlorpyrifos residues on grapefruit but this could be considered negligible. Applying a 4X concentration at one quarter of the dilute volume per hectare also did not result in increased residues when using an EC a few weeks after petal fall. However, covering the tree with 80% shade cloth did increase detectable residues of methomyl above levels found in fruit exposed to the sun. In the case of methomyl this effect was still covered by the recommended preharvest interval but growers using shade cloth should extend their preharvest intervals slightly to avoid possible residue exceedances with other plant protection products.

#### Introduction

Several plant protection products that are registered for use on citrus in South Africa have no Maximum Residue Limit (MRL) in certain markets so must be used with caution after petal fall because residues must remain below the detectable level. The addition of oil or other adjuvants may affect the uptake of the plant protection product in the fruit and should be investigated. In other cases the MRL and pre-harvest interval are fixed but a sticker may be added to the spray mixture which could slow down the breakdown period so the MRL may be inadvertently exceeded. Attempts to improve the efficiency of spray applications to citrus trees may result in farmers spraying at 4X in the future but all the residue work is based on dilute sprays. The possibility that high concentration, low volume sprays result in a more persistent residue than dilute sprays needs to be investigated. There is also a growing trend of growing high-value citrus under shade cloth and it appears that fungicide residues on fruit under shade cloth last longer than usual. If this applies to an insecticide like Lannate too, PHIs will have to be adjusted when growing citrus under shade cloth.

#### Stated objective

Determine whether 4X concentrations, the use of adjuvants or shade cloth result in higher pesticide residues at harvest.

## Materials and methods

Trials were conducted at two sites in the Mpumalanga lowveld. One site was a Valencia orchard (Town 7) at Crocodile Valley Citrus Co. (S25 28 21.9 E30 59 48.4), Nelspruit and the other was a Star Ruby grapefruit orchard on Magnesite farm (S25 30 31.0 E31 27 01.3), Malelane. Insecticides used were chlorpyrifos (Dursban 480 g/L EC manufactured 7 May 2011) and methomyl (Methomex 900 g/kg SP manufactured 15 June 2011). Other adjuvants used were Nufilm 17 sticker and BP Medium horticultural mineral oil. With the grapefruit trial, yield was variable but at least 7 trees were used per treatment to ensure sufficient fruit for testing (Fig.3.4.5.1). Treatments containing chlorpyrifos were applied on 21 October 2011 and treatments containing methomyl were applied on 15 November as film wet, outside cover sprays, except for the 4X spray which was applied at one quarter of the volume of the other sprays. All sprays were applied at 20 bar pressure using handguns. Shade cloth (80% white) was used to cover the treated trees in one of the methomyl treatments. This percentage of shade cloth is higher than would normally be used by growers but we wanted to see if we could get a difference. Dosages and treatment details are provided in Table 3.4.5.1.

In the Valencia orchard, the crop yield was better so only 6 trees were required per treatment. Treatments containing chlorpyrifos were applied on 14 November and those containing methomyl on 8 December 2011. Trial layout is shown in Fig. 3.4.5.2 and treatment details in Table 3.4.5.1.

**Table 3.4.5.1.** Treatments applied to evaluate the effect of shade, concentration and adjuvants on residues of two commonly-used insecticides.

No.	Treatment details
1	Untreated
2	Methomyl SP 100 g/hl
3	Methomyl SP 100 g/hl shaded
4	Methomyl SP 100 g/hl plus Nufilm 17 at 50 ml/hl
5	Methomyl SP 100 g/hl plus BP medium 300 ml/hl
6	Chlorpyrifos 480 EC 100 ml/hl to point of run off (volume X)
7	Chlorpyrifos 480 EC 400 ml/hl at volume X/4
8	Chlorpyrifos 480 EC 100 ml/hl plus BP medium 300 ml/hl
9	Chlorpyrifos 480 EC 100 ml/hl plus Nufilm 17 at 50 ml/hl

Residue samples were picked after 25, 50 and 100 days, frozen within hours, then sent for whole fruit residue analysis via Speed Services to Hearshaw and Kinnes for analysis. Duplicate samples were retained as back-ups.

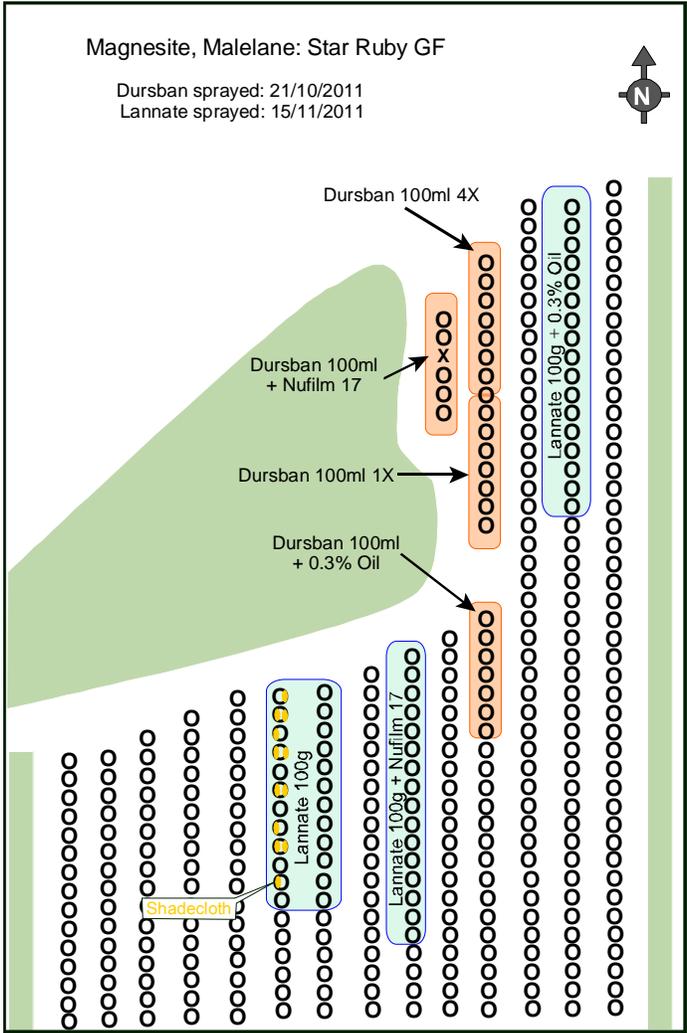


Figure 3.4.5.1. Trial layout in Star Ruby grapefruit near Malelane.

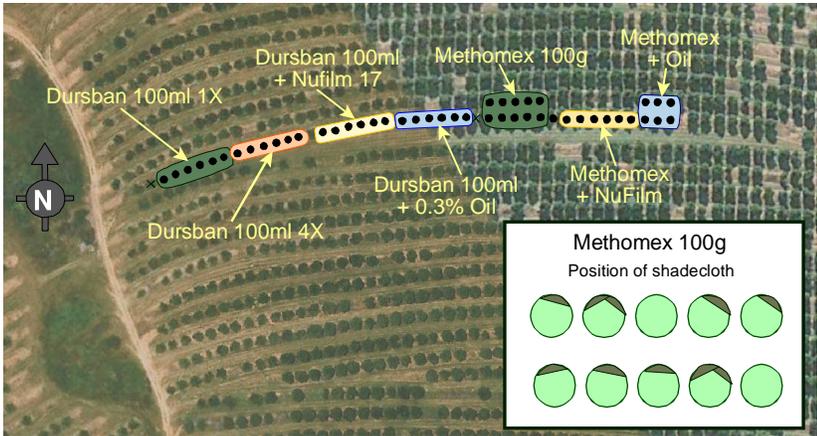


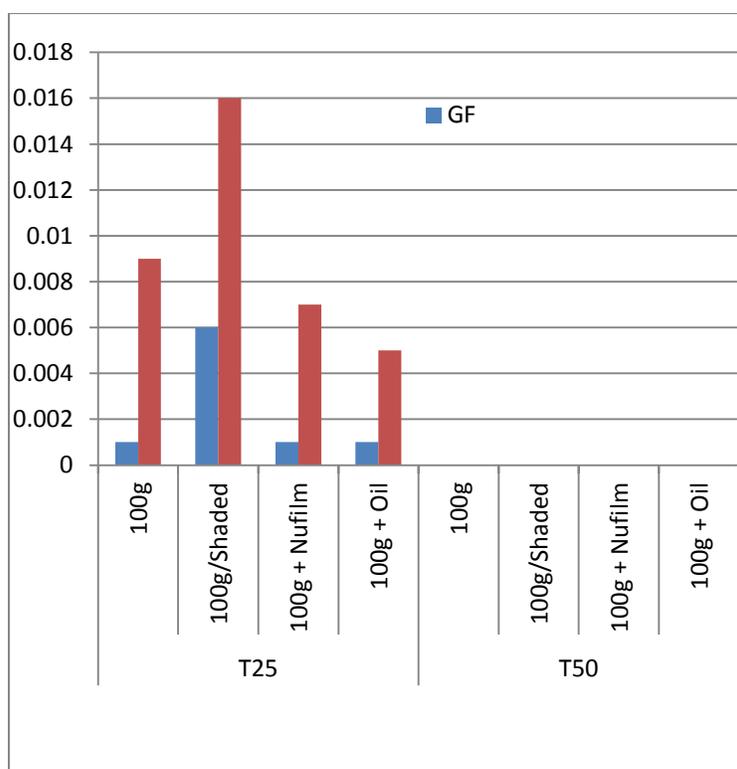
Figure 3.4.5.2. Trial layout in Valencia orchard near Nelspruit.

## Results and discussion

No detectable residues were found 50 days after the methomyl application in any of the treatments at either site so the recommended preharvest interval (PHI) (Hattingh and Hardman 2012) is adequate for any single treatments of 100 g/hl water that a grower is likely to apply under different conditions or mixtures (Table 3.4.5.3, Fig. 3.4.5.3). Methomyl combinations with oil or Nufilm had similar residues after 25 days to methomyl alone in both Valencia and grapefruit, showing that increased penetration with the oil or reduced washing with the sticker had a negligible effect. Only the shading increased the methomyl residue in both Valencia (X2) and grapefruit (X6). Although we used denser shadecloth than is the developing trend, this is an indication that growers using shadecloth should be cautious about exceeding recommended PHIs even by just a few days.

**Table 3.4.5.3.** Residue results in mg/kg for whole fruit picked 25, 50 and 100 days after treatment

Methomyl		Grapefruit Malelane	Valencia Nelspruit	Chlorpyrifos		Grapefruit Malelane	Valencia Nelspruit
T25	100 g	0.001	0.009	T50	100 ml	0.070	0.360
	100 g/Shaded	0.006	0.016		400 ml/LV	0.080	0.510
	100 g + Nufilm	0.001	0.007		100 ml + Nufilm	0.070	0.230
	100 g + Oil	0.001	0.005		100 ml + oil	0.170	0.300
T50	100 g	0.000	0.000	T100	100 ml	<0.01	0.150
	100 g/Shaded	0.000	0.000		400 ml/LV	0.040	0.130
	100 g + Nufilm	0.000	0.000		100 ml + Nufilm	0.020	0.110
	100 g + Oil	0.000	0.000		100 ml + oil	0.050	0.110



**Figure 3.4.5.3.** Methomyl residues in ppm, 25 and 50 days after treatment.

With the chlorpyrifos treatments, residues were obtained at both sampling dates and residues on Valencia fruit were often several orders of magnitude greater than on grapefruit. This difference between the cultivars

appeared to be greater with the EC formulation than had been the case with the soluble powder formulation of methomyl. This may be correlated with higher numbers of oil cells in the peel in Valencias or just the fact that the grapefruit expand more than Valencias. The 4X treatment caused a slight increase in the residues after 50 d but this was negligible by 100 d (Table 3.4.5.3, Fig. 3.4.5.4). The addition of a sticker did not cause any noticeable increase in residues. The addition of oil did cause a slight increase in chlorpyrifos residue in grapefruit but not in Valencias. However, this increase in grapefruit was small relative to the MRL for this active ingredient (0.2 or 0.3 after 60 d). Of all the treatments with both types of actives and fruit it is therefore only the shadecloth that is most likely to result in elevated residues at harvest.

**Conclusion**

The addition of a sticker did not affect detectable residue levels of two different types of active ingredients and the addition of oil only caused a slight increase in chlorpyrifos residues on grapefruit. The use of a 4X concentration sprayed at one quarter of the dilute volume per hectare also had similar residues in fruit to the dilute, high volume spray. Only trees under shadecloth had consistently higher residues than the standard treatment in both Valencias and grapefruit, but in this case of methomyl the recommended PHI was still adequate to prevent MRL exceedance. Where shadecloth is used growers should be cautious about using chemicals close to the PHI.

**Future research**

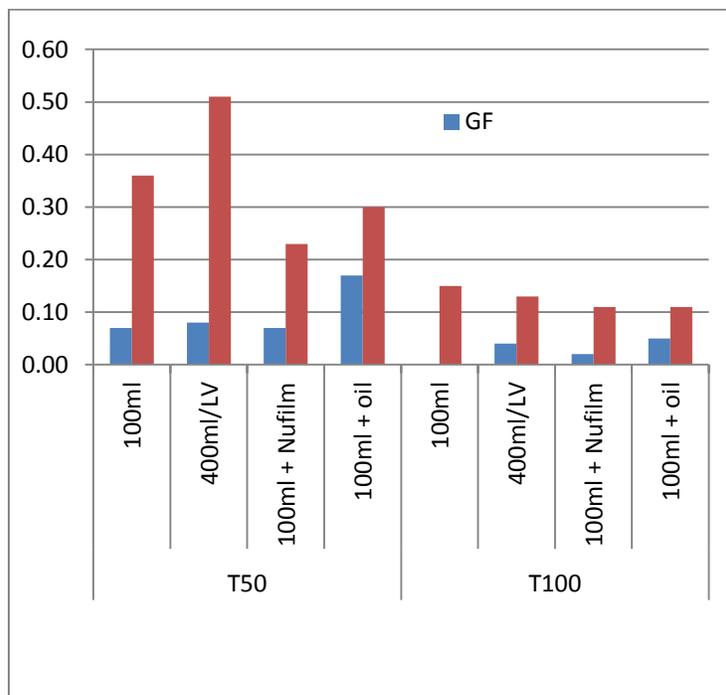
No further research is planned.

**Technology transfer**

A poster will be displayed at the Citrus Research Symposium in August 2012.

**Reference cited**

Hattingh, V. and Hardman, P. 2012. Recommended usage restrictions for plant protection products on southern African export citrus. Email periodical communication. Version March 2012.



**Figure 3.4.5.4.** Chlorpyrifos residues in ppm, 50 and 100 days after treatment.

## Opsomming

Aangesien tartar emetic nie meer vir beheer van sitrusblaaspootjie beskikbaar is nie en daar al hoe meer berigte van die veld kom van 'n toename in toleransie vir abamectin, is daar 'n dringende behoefte vir alternatiewe blaaspootjiedoders wat "IPM"-verenigbaar is. Die situasie met witluisbeheer is ooreenkomstig, met buprofezin wat die enigste laat seisoen alternatief vir chlorpyrifos is. Evaluasie van twee dosisse van 'n kommersieel geformuleerde eksperimentele entomopatogeniese swam (EPS) in twee verskillende areas van die land het teleurstellende resultate opgelewer met geen beduidende beheer van blaaspootjie of witluis nie. Die organiese produk, Biocure, het beter blaaspootjie onderdrukking in een van die streke gegee maar het nie witluis besmetting verminder nie. Verdere EPS isolate word in die laboratorium ondersoek en kan dalk in die toekoms in die veld gëevalueer word, maar hierdie huidige navorsing word nou gëeindig.

## Summary

With tartar emetic no longer being available for citrus thrips control and reports from the field of increasing tolerance to abamectin, there is an urgent need for alternative thripicides that are IPM-compatible. The situation is similar for mealybug control with buprofezin being the only alternative to chlorpyrifos that can be used late in the season. Evaluation of two dosages of a commercially formulated experimental entomopathogenic fungus (EPF) in different parts of the country gave disappointing results with the product giving no significant control of citrus thrips or mealybug. The organic product Biocure gave superior thrips suppression in one of the regions but did not reduce mealybug infestations. Further EPF isolates are being screened in the laboratory and may be evaluated in the field in the future but the current research has been terminated.

## Introduction

Over the last few years many requests have been made for research on alternatives to the diminishing number of products available for the control of citrus thrips (*Scirtothrips aurantii*) and mealybug (various spp.) that would not be disruptive to IPM. Tartar emetic is no longer available as an IPM-compatible treatment for thrips control and thrips populations are becoming increasingly tolerant to abamectin. Buprofezin is the only relatively IPM-compatible treatment that can be used for the control of mealybug after petal fall. A commercially formulated experimental entomopathogenic fungus (Exp-EPF) was included in the trials at two different dosage rates in addition to an organic product containing oxymatrine (Biocure). These were compared with other standard registered treatments in the Eastern Cape and the Mpumalanga Lowveld.

## Stated objective

To determine whether the best commercially available EPF has a significant impact on citrus thrips and mealybug infestations in citrus orchards.

## Materials and methods

Four trials were conducted, two in the Eastern Cape and two in Mpumalanga. The following treatments were evaluated against both citrus thrips and citrus mealybug.

1. Untreated control
2. Abamectin 20 ml plus medium horticultural mineral oil 300 ml/hl water
3. Delegate 10 g/hl water
4. Exp-EPF 5 ml/hl water
5. Exp-EPF 10 ml/hl water
6. Biocure 150 ml/hl water
7. Dursban WG 64 g/hl water or EC 100 ml/hl water
8. Movento 20 ml plus medium hort. oil 300 ml/hl water

### Eastern Cape

Two orchards were demarcated for trials – one Navel orange (Newhall Navels) and one Valencia. Both were on Avoca Farm near Summerville in the Sundays River Valley. Unfortunately, it was a very low thrips pressure season, and thrips levels never picked up adequately in the Valencia orchard in order to justify a trial. Two blocks in the Navel orchard were sprayed with each treatment. Each block consisted of four rows by six trees and positioning of blocks relative to one another within the trial orchard was random. Treatments were applied in the Navel orange orchard on 26 October 2011, when a precount indicated 4% fruit infestation with thrips nymphs. Exp-EPF was sprayed after 17h30, due to its UV-sensitivity. Sprays were applied using hand guns, fitted with 2 mm orifice nozzles and at a pressure of 20 bar. This resulted in an application of an average of 13.5 L per tree. The first evaluation of the trial was conducted on 2 November 2011 (7 days after treatment) and the second on 14 November 2011 (19 days after treatment). A third evaluation, only of mealybug infestation, was conducted on 25 January 2012 (67 days after treatment). Infestation for both thrips and mealybug was determined by inspecting 10 fruit (including under the calyx) on each of 8 trees per replicate (16 per treatment) and recording the number infested with thrips larvae, thrips adults and mealybug. Thus, a total of 160 fruit were inspected per treatment. On 19 April 2012, thrips damage was evaluated by inspecting 20 randomly selected fruit on each of 8 trees in the middle of each block, thus 16 trees and 320 fruit per treatment. Fruit were classified as clean, blemished or culled (pertaining specifically to thrips induced damage).

As mealybug infestation was quite high and of a similar level in several treatment blocks, it was decided to spray another trial in these blocks with some of the same products previously used. The trial was to test the efficacy against only mealybug, as it was speculated that a high pest density might be important for good efficacy with an entomopathogenic fungus. Five of the previously listed treatments were used i.e. untreated control, the two Exp-EPF rates, Biocure and Dursban. Exp-EPF was sprayed after 18h00. All treatments were mixed with 3 ml per 100 L Break-Thru. Six trees in the middle of each of two blocks were sprayed with each treatment on 6 February 2012. Sprays were applied in the same manner as previously described, at an average of 12.5 L per tree. Mealybug infestation was evaluated 22 days later on 28 February 2012.

### Mpumalanga

The first site used was a Valencia orchard at Crocodile Valley Citrus Co. at Nelspruit. Plans were made to use a grapefruit orchard at Malelane for a second site but these were abandoned due to excessive fruit drop. Eventually we had to conduct a second trial with a reduced number of treatments close to the other trial in Valencias at Nelspruit. Each trial site was divided in two and treatment blocks randomly allocated for each treatment within each half of the orchard (2 replicate blocks per treatment). Replicate blocks were 4 rows wide and at least 6 trees long so that 8 data trees could be selected from the centre 2 rows. All treatments above were applied as light cover, film-wet sprays on 3 November 2011 using hand guns at high pressure (20 bar). The thrips populations built slowly in this orchard after petal fall and the grower was concerned about psylla so the whole orchard was sprayed with mevinphos EC at 20 ml/hl water on 12 October. The first trial was evaluated 6 and 25 days after treatments were applied and the second trial was evaluated once, 19 days after treatment. Evaluations were based on fruit infestation only because there were already slight thrips scars before the treatments were applied. Infestation for both thrips and mealybug was determined by inspecting 20 fruit (including under the calyx) on each of 8 trees per replicate and recording the number infested with thrips larvae, thrips adults and mealybug.

## **Results and discussion**

### Eastern Cape

None of the treatments had any significant impact on thrips adults (Table 3.4.6.1). The possibility for detecting any differences may have been reduced by the low level of thrips presence. Differences in infestation with thrips larvae only became evident at the second evaluation, 19 days after spraying (Table 1). Although none of the treatments differed significantly from the untreated control, three of the treatments were significantly superior to the higher Exp-EPF treatment. Delegate was the most effective treatment, followed by Dursban and Biocure. Abamectin performed disappointingly, whereas the two Exp-EPF treatments appeared to have no effect on thrips at all. Entomopathogenic fungi are known to be density-dependent and thrips presence may have been too sparse.

Dursban was the only treatment which significantly reduced mealybug infestation, and this was only recorded at the first evaluation, 7 days after treatment (Table 3.4.6.1). At 19 days after treatment, mealybug infestation was notably, but not significantly, higher for the two Exp-EPF treatments and for Biocure than for the untreated control. There is no clear explanation for this. By 67 days after treatment, Movento showed its worth as a mealybug suppressant, being similar in efficacy to Dursban (Table 3.4.6.1).

**Table 3.4.6.1.** Results from Navels at Sundays River Valley, Eastern Cape.

Treatments	7 Days after treatment			19 Days after treatment			67 Days after treatment
	Fruit with thrips larvae (%)	Fruit with thrips adults (%)	Fruit with mealybug (%)	Fruit with thrips larvae (%)	Fruit with thrips adults (%)	Fruit with mealybug (%)	Fruit with mealybug (%)
Control	2.5a	1.3a	8.1ab	10.0ab	1.3a	11.3ab	41.9b
Abamectin	1.3a	0.6a	3.8ab	6.9ab	0a	13.8b	31.9b
Delegate	0.6a	1.9a	5.6ab	0.6a	1.9a	11.3ab	40.6b
Exp-EPF 5 ml	1.9a	2.5a	6.9ab	8.1ab	2.5a	16.9b	34.4b
Exp-EPF 10 ml	3.1a	0a	10.0b	15.0b	1.3a	18.1b	40.6b
Dursban	1.3a	0.6a	0a	3.1a	1.9a	2.5a	8.1a
Movento	2.5a	0.6a	4.4ab	7.5ab	0a	10.6ab	7.5a
Biocure	1.3a	2.5a	9.4b	3.1a	2.5a	18.1b	49.4b

Means in the same column followed by the same letter were not significantly different at  $\alpha=0.05$  (SNK)

As thrips infestation was not high, neither was the level of damage recorded, particularly damage which would be severe enough to lead to the fruit being culled. The only two treatments for which there was significantly less damage than the untreated control were abamectin and Delegate (Table 3.4.6.2). It is a bit surprising that this was not also the case for Dursban, as it appeared to be one of the more effective treatments in reducing thrips numbers.

**Table 3.4.6.2.** Thrips scarring recorded on Navels at Sundays River Valley, Eastern Cape, on 19 April 2012.

Treatments	Fruit with thrips scarring (%)	Fruit which would be culled for thrips (%)	Total fruit with any thrips damage (%)
Control	17.2a	7.8a	25.0a
Abamectin	7.7b	3.0a	10.7b
Delegate	8.4b	3.8a	12.2b
Exp-EPF 5 ml	12.3ab	6.7a	19.0ab
Exp-EPF 10 ml	14.6ab	5.0a	19.6ab
Dursban	12.3ab	5.7a	18.0ab
Movento	11.6ab	3.4a	15.0ab
Biocure	14.7ab	6.0a	20.7ab

Means in the same column followed by the same letter were not significantly different at  $\alpha=0.05$  (SNK)

Dursban was the only treatment which significantly reduced mealybug infestation in the second trial (Table 3.4.6.3). The other treatments seemed to have no effect at all. This was disappointing, as a trial conducted with Exp-EPF against a high level of mealybug in the previous season showed significant reduction in numbers (Wayne Kirkman, pers comm.). It was not clear why this did not happen again, as great care was taken to apply the Exp-EPF in the cool of the evening and mealybug infestation was such that it was clearly exposed to the sprays.

**Table 3.4.6.3.** Supplementary trial in Navels at Sundays River Valley, Eastern Cape.

Treatments	22 Days after treatment
	Fruit with mealybug (%)
Control	34.2b
Dursban	2.5a
Exp-EPF 5 ml	36.7b
Exp-EPF 10 ml	42.5b
Biocure	43.3b

Means in the same column followed by the same letter were not significantly different at  $\alpha=0.05$  (SNK)

## Mpumalanga

Both dosages of Exp-EPF had no significant impact on adult citrus thrips or mealybug in any of the three evaluations in the two trials (Tables 3.4.6.4 and 3.4.6.5). This fungus also did not appear to have any effect on thrips larvae 6 days after treatment (DAT) but when evaluated 19 or 25 DAT it lowered the infestation significantly ( $P < 0.05$ ), but not enough to provide control. Biocure caused no significant reduction in mealybug infestation and in the supplementary trial caused a significant increase, perhaps due to a detrimental effect on natural enemies. However, Biocure always caused a significant reduction in thrips larval infestation and in two out of three evaluations it reduced infestation by adult thrips significantly. In most cases this impact on thrips was significantly greater than that of Exp-EPF but significantly worse than abamectin against the larvae. As Biocure contains various fertilisers it is possible that it is causing an antifeedant effect in addition to some degree of direct mortality. Reports of thrips suppression from the field after spraying Movento were confirmed in the main trial and this high rate of Movento (20 ml) gave similar control to Delegate of citrus thrips in all evaluations, even 6 DAT. Movento's efficacy against mealybug was slow at first due to minimal contact toxicity but by 25 days after treatment it gave similar control to Dursban. Delegate appeared slightly superior to abamectin against citrus thrips larvae, although not significantly so. However, it resulted in significantly more mealybug than abamectin. The increased level of mealybug was not significantly more than in the control 25 DAT, but infestation levels after 6 days in the control, abamectin and Exp-EPF treatments had all declined by the 25 DAT evaluation, whereas the levels in the Delegate treatment increased from 12% to 22%. This therefore indicates that although Delegate may be used late in the season from a residue viewpoint, it is more detrimental to natural enemies than abamectin at its registered dosage of 20 ml/hl.

**Table 3.4.6.4.** Results from Valencias at Nelspruit, Mpumalanga.

Treatments	6 Days after treatment			25 Days after treatment		
	Fruit with thrips larvae (%)	Fruit with thrips adults (%)	Fruit with mealybug (%)	Fruit with thrips larvae (%)	Fruit with thrips adults (%)	Fruit with mealybug (%)
Control	35.6 a	19.1 a	20.0 a	23.4 a	10.3 a	15.0 ab
Abamectin	5.9 c	4.1 bc	14.7 a	2.5 d	2.2 b	10.6 b
Delegate	1.9 c	5.3 bc	12.2 a	0.9 d	1.3 b	21.9 a
Exp-EPF 5 ml	40.6 a	17.2 a	22.2 a	14.7 b	9.4 a	10.9 b
Exp-EPF 10 ml	30.9 a	14.4 a	14.4 a	16.9 b	9.4 a	9.4 b
Dursban	3.8 c	0.6 c	1.6 b	0.0 d	0.3 b	0.9 c
Biocure	17.5 b	3.1 bc	16.6 a	10.0 c	3.1 b	16.3 ab
Movento	5.9 c	7.2 b	10.6 a	0.9 d	2.8 b	1.9 c

Means in the same column followed by the same letter were not significantly different at  $\alpha = 0.05$  (SNK)

**Table 3.4.6.5.** Supplementary trial in Valencias at Nelspruit, Mpumalanga.

Treatments	19 Days after treatment		
	Fruit with thrips larvae (%)	Fruit with thrips adults (%)	Fruit with mealybug (%)
Control	20.0 a	6.3 a	10.6 b
Metarhizium 10 ml	10.9 b	3.8 ab	8.4 bc
Dursban	0.9 d	0.9 b	2.8 c
Biocure	5.0 c	3.4 ab	21.3 a

Means in the same column followed by the same letter were not significantly different at  $\alpha = 0.05$  (SNK)

## Conclusion

Results were disappointing for the entomopathogenic fungus for both pests but other fungal isolates will be screened for toxicity to these two pests and may be evaluated in the field if they look promising. Although thrips control from Biocure was poor, it probably still warrants further investigation because of the lack of alternatives to abamectin. The mealybug and thrips suppression achieved with Movento was encouraging.

## Future research

Screening of other entomopathogenic fungi will be conducted on a small scale to see whether there are more efficacious isolates than the one used in this trial. Further research on controlling late season infestations of thrips, leafhoppers, psylla and woolly whitefly may include Biocure.

## Technology transfer

Results will be mentioned in grower talks on the spring pest complex but a publication other than this report is not planned.

### 3.5 PROJECT: MEALYBUG AND OTHER MARKET ACCESS PESTS

Project coordinator: Sean D Moore (CRI)

#### 3.5.1 Project summary

Although this project covers more than just mealybug pests of citrus, it has been some while since research was conducted on any of these other pests eg grain chinch bug. During the past research cycle, four studies were conducted within this project, once again all were on mealybug. The first study looked at the impact of 2,4-D induced navel end size reduction on pest infestation of fruit (3.5.2). This was the third year of the study. At one site, significantly higher levels of mealybug were recorded on trees which had not been sprayed with 2,4-D. No difference in FCM infestation was recorded. Another experiment was conducted to evaluate gamma irradiation as a post-harvest treatment for mealybug (3.5.3). Gamma radiation at 150 Gy was shown to be effective in preventing pre-ovipositing citrus mealybug from reproducing and effectively sterilized ovipositing females. This dose can therefore be accepted as the lowest effective radiation dose for mealybug and can be used for validation in a final Probit-8.7 study. In the third experiment, *Steinernema yirgalemense* appeared to be more effective than *Heterorhabditis zealandica* for control of citrus mealybug under suboptimal conditions. Results of a semi-field trial showed that the addition of a water-retention agent, Zeba<sup>®</sup> and a surfactant, Nufilm-P<sup>®</sup> to nematode suspensions, increased the ability of *S. yirgalemense* to control citrus mealybug by 20%, obtaining 53% control at 10°C at night. In the final experiment within the project, the parasitoid, *Anagyrus sp. nr psdeudococci*, was released for control of mealybug. Positive results were achieved in three out of four trials. In a trial in the Eastern Cape, mealybug infestation of Navel oranges was reduced by 6%, 73% and 80%, relative to the control block, where 2500, 5000 and 30000 parasitoids per ha respectively were released. In a trial in the Western Cape, mealybug infestation was 59% and 72% lower than in the untreated control at harvest, where 2500 and 5000 parasitoids were released per ha, respectively.

## Projekopsomming

Alhoewel hierdie projek meer as net witluisplae op sitrus dek, is dit 'n redelike tyd vandat navorsing op enige van hierdie ander plae, bv graansnuitkewer, uitgevoer is. Gedurende die laaste navorsingsiklus is vyf studies binne in hierdie projek uitgevoer en weereens was hulle almal op witluis. Die eerste studie het gekyk na die impak van verkleinde navelente as gevolg van 2,4-D behandeling op pesinfestasië van vrugte (3.5.2). Hierdie was die derde jaar van die studie. By een perseel is beduidend hoër vlakke van witluis aangeteken op bome wat nie met 2,4-D gespuit is nie. Geen verskille in VKM besmetting is aangeteken nie. Nog 'n eksperiment is uitgevoer om gammabestraling as 'n na-oes behandelingsmaatreeël vir beheer van sitruswitluis te evalueer (3.5.3). Gammabestraling teen 150 Gy het volwasse, nie-eierleggende wyfies verhoed om aan te teel en het eierleggende wyfies gesteriliseer. Dié dosis kan derhalwe as die laagste doeltreffende stralingsdosis vir sitruswitluis aanvaar word en in 'n finale Probit-8.7 studie bekragtig word. In die derde eksperiment was *Steinernema yirgalemense* meer doeltreffend as *Heterorhabditis zealandica* vir beheer van sitrus witluis onder suboptimale omstandighede. Resultate van 'n semi-veldproef, met die byvoeging die water-retensie agent Zeba<sup>®</sup> en die benatter Nufilm-P<sup>®</sup> tot die nematode suspensie, het 20% tot die beheer van *P. citri* bygedra, met 53% beheer in die veld onder baie swak omgewingstoestande, insluitende lae temperature van 10°C gedurende die nag. In die finale eksperiment in die projek is die parasiet, *Anagyrus sp. nr psdeudococci*, vrygelaat vir beheer van witluis. Positiewe resultate is in drie uit vier proewe gekry. In 'n proef in die Oos-Kaap is witluis besmetting van Nawellemoene met 6%, 73% en 80% verminder, in vergelyking met die kontrole blok, waar 2500, 5000 en 30000 parasiete per ha onderskeidelik vrygelaat is. In 'n proef in die Wes-Kaap was witluis besmetting teen oestyd 59% en 72% laer waar onderskeidelik 2500 en 5000 parasite per ha vrygelaat is as in die onbehandelde kontrole.

**3.5.2 FINAL REPORT: The impact of 2,4-D induced navel end size reduction on pest infestation of fruit**  
Experiment 957 (April 2009 – July 2010) by Sean D. Moore, Wayne Kirkman and Stephan Verreyne (CRI)

### Opsomming

Proewe wat op nawellemoene in Suid-Afrika vanaf 2006 uitgevoer is, het gewys dat 2,4-D bespuitings teen volblom teen 'n konsentrasies van tussen 15 en 45 mg/L die persentasie vrugte met 'n toe nawelent betekenisvol kan vermeerder. Hierdie effek hou beduidende hortologiese en kosmetiese voordele vir die vrugte in. Omdat daar ook beweer word dat laer plaagbesmetting, skade en vrugval gekry is, is nog 'n proef aan die gang gesit om hierdie teorie te toets. Gedurende die 2009/10 seisoen is 5 boorde wat met 2,4-D gespuit vir plaag besmetting en skade evalueer. In 3 uit die 5 boorde het die 2,4-D die persentasie oop nawellente betekenisvol verminder. By een van die persele is hierdie nie gemeet nie maar daar is opletterend meer oop nawellente in die onbehandelde kontrole waargeneem. In Desember of Januarie is besmetting met VKM eiers, witluis besmetting en bolwurm besmetting evalueer. Baie min VKM eiers is waargeneem en daar was geen verskil in bolwurm skade. By 3 van die persele is daar statisties betekenisvolle verskille tussen witluis besmetting in sekere van die behandelings. Egter is daar geen oortuigende tendens nie. In Maart of Mei is blaaspootjie skade en knopmyt tipe simptome evalueer. Net by een perseel is enige betekenisvolle verskille in blaaspootjie skade tussen behandelings aangeteken. Teen verwagting is laer vlakke van skade aangeteken vir die twee behandelings met die hoogste persentasie oop nawellente. Die mees oortuigende resultate omtrent die effek van 2,4-D bespuitings op plaag vlakke of skade is vir knopmyt aangeteken. Oor die algemeen is die voorkoms van uitstop nawels hoër en die voorkoms van bul-neus laer vir onbehandelde vrugte (meer oop nawellente). Geen betekenisvolle verskille in VKM besmetting van vrugte is gekry nie. Nogtans, as gevolg van die hoë plaagstatus van VKM en witluis is die effek op hierdie twee plae in twee nuwe proewe weer ondersoek. Geen verskil in VKM besmetting is by enige van die proefpersele gekry nie. Witluis was net by een perseel teenwoordig, waar betekenisvol hoër vlakke aangeteken is op bome wat nie met 2,4-D gespuit is nie.

### Summary

Trials conducted on navel oranges in South Africa since 2006 have shown that spraying with 2,4-D at full bloom at concentrations of between 15 and 45 mg/L can significantly increase the percentage of fruit with closed navel ends. This has been shown to hold significant horticultural and cosmetic benefits for fruit. As it was asserted that lower levels of pest infestation, damage and fruit drop were also observed, a spin-off trial was initiated to test this. During the 2009/10 season 5 orchards, which were sprayed with 2,4-D in spring, were evaluated for pest infestation and damage. In 3 out of the 5 orchards, 2,4-D sprays significantly reduced the percentage of open navel ends. At one of the sites, this was not measured, but there were conspicuously more open navel ends in the untreated control. In December or January, infestation with FCM eggs, mealybug infestation and bollworm damage were evaluated. Very few FCM eggs were recorded and no difference in bollworm damaged was recorded. At three of the sites, there were statistically significant differences between mealybug infestation in some of the treatments. However, there was no compelling trend. In March or May, thrips damage and bud mite related symptoms were evaluated. Only at one site were any significant differences in thrips damage recorded between treatments. Contrary to expectations, lower levels of damage were recorded for the two treatments with the highest percentage of open navel ends. The most compelling results on the effect of 2,4-D sprays on pest levels or pest damage were recorded for bud mite. Generally, the occurrence of protruding navels was higher and the occurrence of bull-nosing was lower in untreated fruit (more open navel ends). No significant differences in FCM infestation of fruit were recorded. However, considering the high pest status of FCM and mealybug, these two pests were again monitored in two trials during 2010/11. No difference in FCM infestation was recorded at either trial site. Mealybug was only present at one site, where significantly higher levels were recorded on trees which had not been sprayed with 2,4-D.

### Introduction

Work in Chile on Lane Late navel oranges demonstrated that 2,4-D sprayed during full bloom reduced the size of the navel end. Concentrations of 5 to 20 ppm were evaluated, with 20 ppm giving the best results. This rate resulted in 49% closed navel ends compared to 3% in the untreated control and a navel size of 0.48 cm compared to 1.20 cm in the control. In the following season, 15 ppm on Lane Late navel oranges resulted in smaller navels (0.25 vs. 0.76 cm), and a greater percentage closed navel ends (82% vs 22%) with again no effect on yield or fruit size (Gardiazabal, 2006). These results were confirmed in a separate study, which also demonstrated that percentage fruit with split navel ends was reduced (Saavedra, 2006). A third study in California confirmed this trend (E. Rabe, personal communication).

During the last three seasons (2007 to 2009) similar trials were conducted on Palmer navel oranges in the Western Cape of South Africa (Verreyne, 2008; Mupambi, 2010). 2,4-D increased the percentage of closed navels and decreased average navel size of all fruit. Similar results were achieved on Robyn and Lane Late navel oranges.

In addition, less fruit drop and lower levels of pest damage were reported for the 2,4-D treated plots. As no entomologist was involved in this study, this observation was not qualified or quantified. However, there are a number of pests which are intimately associated with the navel end of navel oranges. Most importantly, this would include mealybugs, false codling moth (FCM), bud mite and bollworm.

Mealybugs tend to either pack underneath the calyx of fruit or within the navel end. Mealybug within the navel end is far more protected against parasitism than mealybug underneath the calyx (Moore et al., 1997) and is virtually inaccessible to sprays.

During the bulk of the citrus growing season, FCM will penetrate fruit through the navel end (Moore & Kirkman, 2004). A strong indication of an association between the size of the navel end and level of FCM infestation has been observed (Moore & Richards, 2003). Therefore, a reduction in the size of the navel end might reduce the attractiveness or susceptibility of the fruit to FCM. Both mealybug and FCM are phytosanitary pests and any action which can be taken to reduce their levels on fruit must be investigated as a matter of priority.

One of the symptoms of bud mite damage is enlarged and malformed (particularly protruding) navel ends (Grout, 2003). Bollworm has also been shown to cause severe damage to navel ends (Moore et al, 2007).

### Stated objectives

The objective of this study is therefore to measure and compare pest levels on 2,4-D treated and untreated navel orange trees (particularly fruit).

### Materials and methods

#### 2009-10

During the 2009/10 season six trial orchards were used (Table 3.5.2.1). The same trial sites as the previous season were used (Moore et al, 2010), except that the Navelina orchard at Karrenmelksvlei was not evaluated for pests during the 2008/09 season (even though it was treated) and in the Eastern Cape, the trial was conducted on Far Away Farm in 2008/09 and on Bernol Farm in 2009/10 (Table 3.5.2.1).

**Table 3.5.2.1** Details of 2,4-D trial sites (navel orange orchards) used for pest evaluations during the 2008/09 and 2009/10 seasons.

District	Farm	Coordinates	Orchard	Navel variety	Rootstock	Year planted	Tree spacing (rows x trees)	Row orientation
Citrusdal, W. Cape	Tienrivieren	32°30'S 19°E		Washington	Rough lemon	1984	6mx6m	East- West
Citrusdal, W. Cape	Karrenmelksvlei (ALG)	32°30'S 19°E	K08	Newhall	Rough lemon	1993	5mx2m	North-South
Citrusdal, W. Cape	Karrenmelksvlei (ALG)	32°30'S 19°E	K09	Navelina	Rough lemon	1993	5mx2m	North-South
Clanwilliam, W. Cape	Twakfontein	32°20'S 18°50'E		Robyn	Rough lemon	1987	6mx4m	North-South
Heidelberg, sthn. Cape	Kruisrivier	34°06'S 20°57'E	E1	Autumn Gold	Carrizo citrange	1999	5mx2m	East- West
Addo, E. Cape	Bernol	33°28'S 25°36'E	10	Newhall	Carrizo	2001	6mx3m	East- West

During the 2009/10 season 2,4-D Ester and 2,4-D Amine were applied at the six sites. Applications were made at 10, 15, 20 and 25 ppm at full bloom and at petal drop. Specific details for applications at each trial site are presented in the various tables under Results.

A non-ionic wetting agent (Break-Thru) with the active ingredient polyether-polymethylsiloxane-copolymer (1000 g/L) was added to the spray solution at a rate of 5 ml per 100 L of spray solution in all the experiments. Applications were made using a hand gun sprayer until run-off.

In all the experiments, each treatment consisted of eight single tree replicates in a randomized complete block design with buffer trees between treated trees. Trees were chosen for uniformity in size and only healthy trees were used. All experiments were carried out in commercial orchards under standard production practices.

At commercial harvest, a full lug box (average 80 fruit) was collected from all sectors of each replicate. Fruit diameter and navel-end size was measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) on site. This was conducted by Stephan Verreyne who reports it in full (Verreyne, 2011).

Evaluations of pest infestation and damage were conducted in December 2009 or January 2010 and again in March or May 2010. At the first evaluation period 10 fruit on each data tree were inspected for FCM eggs, mealybug infestation (both live and residues; both under the calyx and in the navel end) and bollworm damage (both on the navel end and on the sides of fruit). At the second evaluation period 10 fruit on each tree were inspected for thrips damage (differentiating between minor scars and cull damage and separating damage at the calyx end and the navel end) and bud mite associated damage (protruding navels, ridging, flattened fruit and bull-nosing i.e. elongation of fruit without protrusion of the navel). A percentage of infestation or damage was then obtained.

Additionally, FCM infestation of fruit was evaluated in the Palmer Navels at Bernol (Eastern Cape). This was done by collecting the fallen fruit underneath each tree on a fortnightly basis, from 22 January to 28 April 2010. Fruit were then dissected to determine cause of drop. Any FCM larvae or sign of FCM infestation was recorded.

Values were compared between treatments by using an ANOVA and LSD multiple range test (Statistical Graphics Corporation, 2001).

#### 2010-11

During the 2010/11 season 2,4-D Amine was applied at two sites (Table 3.5.2.2). Applications were made at only one rate, 15 ppm i.e. 3.125 ml/100 L water, at full bloom.

**Table 3.5.2.2** Details of 2,4-D trial sites (navel orange orchards) in the Sundays River Valley, Eastern Cape, used for pest evaluations during the 2010/11 season.

Farm	Coordinates	Orchard	Navel variety	Year planted	Tree spacing (rows x trees)	Application date	Mean spray volume per tree (L)
Halaron	33°29'26"S 25°40'23"E	4	Palmer	1989	5.5mx3m	28/09/2010	18
Avoca	33°28'73"S 25°35'22"E	12	Newhall	2005	5.5mx2.5m	28/09/2010	15

A non-ionic wetting agent (Break-Thru) with the active ingredient polyether-polymethylsiloxane-copolymer (1000 g/L) was added to the spray solution at a rate of 5 ml per 100 L of spray solution in both the experiments. Applications were made using a hand gun sprayer until run-off.

In both experiments, each treatment consisted of 10 single tree replicates, with buffer trees between treated trees. Trees were chosen for uniformity in size and only healthy trees were used. All experiments were carried out in commercial orchards under standard production practices.

Evaluations of FCM infestation were conducted weekly at both sites from 4 January to 12 April 2011. This was done by collecting the fallen fruit underneath each tree and dissecting the fruit to determine the cause of drop. Any FCM larvae or sign of FCM infestation was recorded.

No mealybug was present in the Halaron orchard. However, mealybug infestation was evaluated twice in the Avoca orchard – on 23 February and 2 March 2011. Inspections were conducted under fruit calyces, on the sides of the fruit and within the navel ends. In the February inspection, superficial assessments of navel end infestation were made, whereas in March, the navel ends were cut open to more accurately inspect.

Values were compared between treatments by using an ANOVA and LSD multiple range test (Statistical Graphics Corporation, 2001).

## **Results and discussion**

### 2009-10

Only in the Bernol trial was the diameter of navel ends not measured (Tables 3.5.2.7, 3.5.2.12 & 3.5.2.13). In all other trials, except the trial conducted on Autumn Gold navels at Kruisrivier (Tables 3.5.2.6 & 3.5.2.11), the 2,4-D sprays had some significant effect on navel end openings (Tables 3.5.2.3, 3.5.2.4, 3.5.2.5, 3.5.2.8, 3.5.2.9 & 3.5.2.10). Although 2,4-D did not reduce navel end size, it did reduce the percentage of fruit with open navel ends. Generally full bloom sprays were more effective than petal drop sprays. There was no apparent difference in efficacy between the ester and amine formulations. Neither was there any notable difference in efficacy between different concentrations.

No FCM eggs were recorded on fruit at most sites during the December/January evaluation. Only a few eggs were recorded on Autumn Gold navels at Kruisrivier (3.5.2.6) and on Palmer navels at Bernol (3.5.2.7). However, there were no statistical differences. This was probably more due to the low level of FCM rather than an indication that there was no influence on FCM (either attraction of moths to fruit or penetrability of fruit to larvae).

At three of the sites, there were statistically significant differences between mealybug infestation in some of the treatments. However, a trend was somewhat lacking when one compared these mealybug levels with percentage of open navel ends. In Newhall Navels at Karrenmelksvlei Farm, untreated control fruit had the lowest percentage of closed navel ends (Table 3.5.2.4) (although not significantly lower than some of the treatments), but did not have the lowest level of mealybug infestation. However, the treatment with the highest percentage of closed navel ends (15 ppm Est PD) had no signs of mealybug infestation. Admittedly, all mealybug infestation was recorded under the calyx, rather than within the navel end. It is therefore speculative that navel end size would influence mealybug infestation under the calyx. If this was so, it would be an indirect effect.

Results on Autumn Gold Navels at Kruisrivier Farm were a little more convincing. Untreated fruit had the lowest percentage of closed navel ends and the second highest level of mealybug residues and total mealybug plus residues (Table 3.5.2.6).

Results at Bernol Farm on Palmer Navels did not make sense. The only treatment with any mealybug infestation was 10 ppm Am FB+1 (Table 3.5.2.7). Although navel end size was not measured, it was clear that there were substantially more closed navels in this and the other treatment than in the untreated control. This result may therefore simply be a factor of the very low level of mealybug infestation.

There were no significant differences in bollworm damage recorded for the different treatments at any of the sites. Bollworm damage was recorded as it was previously found that the majority of bollworm damage recorded on navel oranges at harvest was inflicted on the navel end of fruit (Moore et al 2004).

Only at one of the sites were there any statistically significant differences in thrips damage between treatments. This was on Washington Navels at Tienrivieren Farm, recorded in March (Table 3.5.2.8). Damage was not recorded at the navel end of fruit (only the calyx end), as had been hoped. Additionally, results did not appear to bear much meaning. Contrary to expectations, the two treatments with the most open navel ends (untreated

control and 15 ppm Est PD) displayed two of the three lowest levels of thrips damage. The two treatments with the highest percentage closed navel ends (10 ppm Am FB & 15 ppm Est FB) had the highest levels of thrips damage. It would be premature to read anything into these results, unless they are repeated in other trials.

Significant differences in bud mite related damage were recorded in four out of the five trials (Tables 3.5.2.9, 3.5.2.10, 3.5.2.11 & 3.5.2.12). Protruding navel ends, ridging and flattening of fruit are considered as typical symptoms of bud mite damage (Grout, 2003). It was hypothesised that bull-nosing (elongation of the fruit at the bottom end, without protrusion of the navel) was also a symptom of bud mite damage, but that protrusion of the navel was prevented by closed navels as a result of spraying with 2,4-D. If this was so, then total bud mite symptoms for all fruit (treatments) should be similar, regardless of whether sprayed with 2,4-D or not. Ridging and flattening of fruit are not tabulated, but have been included in the totals for bud mite symptoms.

In all but one of the trials (Table 3.5.2.11), there were no statistically significant differences in total bud mite related symptoms between treatments. In Newhall Navels at Karrenmelksvlei Farm and in Palmer Navels at Bernol Farm, the highest level of protruding navels and the lowest level of bull-nosing was recorded for untreated control fruit, which also had the lowest percentage of closed navel ends (Tables 3.5.2.9 & 3.5.2.12). In Robyn Navels at Twakfontein Farm, the lowest level of bull-nosing was recorded for untreated fruit (which again had the lowest percentage of closed navel ends) (Table 3.5.2.10). However, there were no statistically significant differences in this case. In Autumn Gold Navels at Kruisrivier a substantially and significantly higher level of protruding navel ends was recorded for the untreated control (Table 3.5.2.11). The most compelling results on the effect of 2,4-D sprays on pest levels or pest damage were therefore recorded for bud mite. Although the actual cause of the bud mite type symptoms was not substantiated, through confirmation of the presence of bud mite, there was adequate evidence to indicate that bull-nosing was caused by the same factor as that responsible for protruding navel ends. The final question is whether a bull-nosed fruit would be more acceptable for export than a fruit with a protruding navel end. This would probably be so.

Finally, no significant differences in FCM infestation were recorded between treatments on Palmer Navels at Bernol Farm (Table 3.5.2.13).

**Table 3.5.2.3** FCM, mealybug and bollworm infestation or damage on Washington navels at Tienrivieren Farm, where 2,4-D had been applied, evaluated on 27 January 2010.

Treatment		Closed navel ends (%)	Mean pest infestation or damage (%)							
			FCM eggs	Mealybug				Bollworm damage		
		Navel end		Calyx	Residues	Total	Navel end	Side of fruit	Total	
1	Control	3.8b	0a	0a	8.75a ± 5.15	0	8.75a ± 5.15	7.50a ± 2.50	10.00a ± 5.00	17.50a ± 7.01
2	5 ppm Est FB	15.9a	0a	1.25a ± 1.25	2.50a ± 1.64	3.75a ± 2.63	7.50a ± 5.26	5.00a ± 3.78	5.00a ± 2.67	10.00a ± 5.98
3	10 ppm Est FB	21.1a	0a	1.25a ± 1.25	2.50a ± 2.50	1.25a ± 1.25	5.00a ± 2.67	5.00a ± 2.67	12.50a ± 3.66	17.50a ± 3.66
4	15 ppm Est FB	17.5a	0a	0a	2.50a ± 1.64	2.50a ± 1.64	5.00a ± 1.89	6.25a ± 2.63	7.50a ± 3.13	13.75a ± 5.32
5	20 ppm Est FB	13.7a	0a	0a	2.50a ± 1.64	1.25a ± 1.25	3.75a ± 1.83	5.00a ± 2.67	5.00a ± 1.89	10.00a ± 2.67
6	15 ppm Est PD	4.4b	0a	0a	5.00a ± 3.78	1.25a ± 1.25	6.25a ± 3.75	8.75a ± 3.50	11.25a ± 2.95	20.00a ± 5.00

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.4** FCM, mealybug and bollworm infestation or damage on Newhall navels at Karrenmelksvlei Farm, where 2,4-D had been applied, evaluated on 27 January 2010.

Treatment		Closed navel ends (%)	Mean pest infestation or damage (%)							
			FCM eggs	Mealybug				Bollworm damage		
		Navel end		Calyx	Residues	Total	Navel end	Side of fruit	Total	
1	Control	18.0c	0a	0a	2.50ab ± 1.63	0a	2.50a ± 1.64	11.25a ± 2.95	23.75a ± 4.60	35.00a ± 4.22
2	10 ppm Est FB	30.3abc	0a	0a	1.25b ± 1.25	2.50a ± 2.50	3.75a ± 2.63	5.00a ± 2.67	21.25a ± 3.50	26.25a ± 4.98
3	15 ppm Est FB	23.6c	0a	0a	6.25a ± 2.63	1.25a ± 1.25	7.50a ± 3.13	6.25a ± 3.75	17.50a ± 3.13	23.75a ± 3.75
4	15 ppm Am FB	37.8ab	0a	0a	3.75ab ± 2.63	0a	3.75a ± 2.63	10.00a ± 2.67	28.75a ± 6.39	38.75a ± 7.18
5	25 ppm Est FB	25.7bc	0a	0a	0b	1.25a ± 1.25	1.25a ± 1.25	11.25a ± 3.50	18.75a ± 4.41	30.00a ± 6.27
6	15 ppm Est PD	42.2a	0a	0a	0b	5.00a ± 3.78	5.00a ± 3.78	11.25a ± 2.95	18.75a ± 4.79	30.00a ± 7.07

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.5** FCM, mealybug and bollworm infestation or damage on Robyn navels at Twakfontein Farm, where 2,4-D had been applied, evaluated on 27 January 2010.

Treatment		Closed navel ends (%)*	Mean pest infestation or damage (%)							
			FCM eggs	Mealybug				Bollworm damage		
				Navel end	Calyx	Residues	Total	Navel end	Side of fruit	Total
1	Control	38.2b	0a	0a	1.25a ± 1.25	0a	1.25a ± 1.25	2.50a ± 1.64	0a	2.50ab ± 1.64
2	10 ppm Est FB	66.0a	0a	0a	2.75a ± 1.83	1.25a ± 1.25	5.00a ± 1.89	0a	1.25a ± 1.25	1.25ab ± 1.25
3	15 ppm Am FB	82.4a	0a	0a	2.50a ± 2.50	0a	2.50a ± 2.50	0a	0a	0a
4	15 ppm Est FB	69.7a	0a	0a	1.25a ± 1.25	1.25a ± 1.25	2.50a ± 1.64	0a	0a	0a
5	20 ppm Est FB	83.3a	0a	0a	2.50a ± 2.50	1.25a ± 1.25	3.75a ± 2.63	2.50a ± 1.64	1.25a ± 1.25	3.75b ± 1.83
6	15 ppm Est PD	64.7a	0a	0a	0a	0a	0a	0a	1.25a ± 1.25	1.25ab ± 1.25

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.6** FCM, mealybug and bollworm infestation or damage on Autumn Gold navels at Kruisrivier Farm, where 2,4-D had been applied, evaluated on 29 December 2009.

Treatment		Closed navel ends (%)	Mean pest infestation or damage (%)							
			FCM eggs	Mealybug				Bollworm damage		
				Navel end	Calyx	Residues	Total	Navel end	Side of fruit	Total
1	Control	24.2a	1.25a ± 1.25	0	3.75a ± 2.63	11.25ab ± 3.98	15.00ab ± 5.67	0a	2.50a ± 1.64	2.50a 1.64
2	10 ppm Am FB	49.5a	0a	0	5.00a ± 2.68	13.75a ± 3.24	18.75a ± 2.27	1.25a ± 1.25	2.50a ± 2.50	3.75a 3.75
3	10 ppm Est FB	38.7a	0a	0	0a	5.00ab ± 2.67	5.00b ± 2.67	0a	2.50a ± 2.50	2.50a ± 2.50
4	15 ppm Am FB	48.4a	0a	0	2.50a ± 1.64	3.75b ± 1.83	6.25b ± 3.24	0a	1.25a ± 1.25	1.25a ± 1.25
5	15 ppm Est FB	46.1a	1.25a ± 1.25	0	3.75a ± 2.63	3.75b ± 2.63	7.50ab ± 4.12	0a	1.25a ± 1.25	1.25a ± 1.25
6	15 ppm Est PD	54.1a	2.50a ± 1.64	0	2.50a ± 1.64	7.50ab ± 4.91	10.00ab ± 5.00	0a	0a	0a

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.7** FCM, mealybug and bollworm infestation or damage on Palmer navels at Bernol Farm, where 2,4-D had been applied, evaluated on 7 January 2010.

Treatment		Closed navel ends (%)	Mean pest infestation or damage (%)							
			FCM eggs	Mealybug				Bollworm damage		
				Navel end	Calyx	Residues	Total	Navel end	Side of fruit	Total
1	Control	-	3.75a ± 3.75	3.75a ± 2.63	0a	2.50a ± 1.64	6.25a ± 3.75	1.25a ± 1.25	2.50a ± 2.50	3.75a ± 3.75
2	10 ppm Am FB+1	-	0a	15.00a ± 5.98	7.50b ± 2.50	7.50a ± 1.64	30.00b ± 9.06	1.25a ± 1.25	0a	1.25a ± 1.25
3	15 ppm Am FB+1	-	0a	5.00a ± 3.27	0a	7.50a ± 3.66	12.50ab ± 6.48	1.25a ± 1.25	0a	1.25a ± 1.25

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.8** Thrips and bud mite damage on Washington navels at Tienrivieren Farm, where 2,4-D had been applied, evaluated on 24 March 2010.

Treatment		Closed navel ends (%)	Mean pest damage (%)					
			Thrips			Bud mite		
			Calyx	Navel end	Total	Protruding navel	Bull nose	Total
1	Control	3.8b	7.50ab ± 3.13	0	7.50ab ± 3.13	20.00a ± 7.07	15.00a ± 3.78	41.25a ± 5.81
2	10 ppm Am FB	15.9a	11.25b ± 2.95	0	11.25b ± 2.95	12.5a ± 4.91	18.75a ± 6.39	38.75a ± 4.41
3	10 ppm Est FB	21.1a	7.50ab ± 2.50	0	7.50ab ± 2.50	16.25a ± 5.65	28.75a ± 9.53	51.25a ± 6.39
4	15 ppm Am FB	17.5a	5.00ab ± 1.89	0	5.00ab ± 1.89	7.50a ± 2.50	28.75a ± 5.49	41.25a ± 5.81
5	15 ppm Est FB	13.7a	8.75b ± 2.27	0	8.75b ± 2.27	18.75a ± 4.41	27.50a ± 3.13	50.00a ± 7.79
6	15 ppm Est PD	4.4b	1.25a ± 1.25	0	1.25a ± 1.25	16.25a ± 2.63	21.25a ± 3.98	45.00a ± 6.55

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.9** Thrips and bud mite damage on Newhall navels at Karrenmelksvlei Farm, where 2,4-D had been applied, evaluated on 24 March 2010.

Treatment		Closed navel ends (%)	Mean pest damage (%)					
			Thrips			Bud mite		
			Calyx	Navel end	Total	Protruding navel	Bull nose	Total
1	Control	18.0c	0	0	0	17.50a ± 3.66	12.50a ± 3.13	30.00a ± 5.00
2	10 ppm Est FB	30.3abc	0	0	0	7.50b ± 3.13	36.25c ± 2.63	43.75a ± 4.98
3	15 ppm Est FB	23.6c	0	0	0	3.75b ± 2.63	36.25c ± 4.20	40.00a ± 5.34
4	15 ppm Am FB	37.8ab	0	0	0	7.50b ± 2.50	21.25ab ± 3.50	28.75a ± 5.49
5	25 ppm Est FB	25.7bc	0	0	0	6.25b ± 2.63	25.00bc ± 5.00	31.25a ± 5.81
6	15 ppm Est PD	42.2a	0	0	0	7.50b ± 2.50	23.75ab ± 5.96	36.25a ± 5.96

Different letters in the same column denote significant differences between values ( $P > 0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.10** Thrips and bud mite damage on Robyn navels at Twakfontein Farm, where 2,4-D had been applied, evaluated on 24 March 2010.

Treatment		Closed navel ends (%)	Mean pest damage (%)					
			Thrips			Bud mite		
			Calyx	Navel end	Total	Protruding navel	Bull nose	Total
1	Control	38.2b	13.75a ± 3.75	3.75a ± 1.83	17.50a ± 4.91	1.25ab ± 1.25	8.75a ± 2.95	37.50a ± 6.75
2	10 ppm Est FB	66.0a	22.50a ± 6.75	2.50a ± 1.64	25.00a ± 8.02	1.25ab ± 1.25	15.00a ± 4.23	32.50a ± 5.26
3	15 ppm Am FB	82.4a	28.75a ± 7.18	1.25a ± 1.25	30.00a ± 7.31	0a	12.50a ± 3.66	31.25a ± 8.75
4	15 ppm Est FB	69.7a	21.25a ± 6.93	2.50a ± 1.64	23.75a ± 7.54	1.25ab ± 1.25	11.25a ± 2.95	32.50a ± 5.90
5	20 ppm Est FB	83.3a	15.00a ± 5.34	1.25a ± 1.25	16.25a ± 6.25	3.75ab ± 1.83	15.00a ± 3.27	32.50a ± 3.13
6	15 ppm Est PD	64.7a	17.50a ± 2.50	3.75a ± 1.83	21.25a ± 3.98	5.00b ± 2.67	15.00a ± 5.00	47.50a ± 7.26

Different letters in the same column denote significant differences between values ( $P > 0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.11** Thrips and bud mite damage on Autumn Gold navels at Kruisrivier Farm, where 2,4-D had been applied, evaluated on 26 May 2010.

Treatment		Closed navel ends (%)	Mean pest damage (%)					
			Thrips			Bud mite		
			Calyx	Navel end	Total	Protruding navel	Bull nose	Total
1	Control	24.2a	1.25a ± 1.25	0	1.25a ± 1.25	10.00a ± 3.27	13.75ab ± 3.24	36.25ab ± 6.25
2	10 ppm Am FB	49.5a	0a	0	0a	3.75b ± 1.83	12.50ab ± 5.26	35.00ab ± 4.23
3	10 ppm Est FB	38.7a	1.25a ± 1.25	0	1.25a ± 1.25	2.50b ± 1.64	21.25bc ± 3.98	37.50ab ± 2.50
4	15 ppm Am FB	48.4a	3.75a ± 3.75	0	3.75a ± 3.75	0b	15.00ab ± 2.67	27.50a ± 2.50
5	15 ppm Est FB	46.1a	0a	0	0a	2.50b ± 1.64	8.75a ± 3.98	30.00a ± 5.67
6	15 ppm Est PD	54.1a	0a	0	0a	1.25b ± 1.25	27.50c ± 5.26	45.00b ± 7.79

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.12** Thrips and bud mite damage on Palmer navels at Bernol Farm, where 2,4-D had been applied, evaluated on 4 May 2010.

Treatment		Closed navel ends (%)	Mean pest damage (%)					
			Thrips			Bud mite		
			Calyx	Navel end	Total	Protruding navel	Bull nose	Total
1	Control	-	12.50a ± 7.26	0	12.50a ± 7.26	21.25a ± 4.79	1.25b ± 1.25	27.50a ± 6.20
2	10 ppm Am FB+1	-	10.00a ± 3.78	0	10.00a ± 3.78	5.00b ± 1.89	20.00a ± 3.78	27.50a ± 4.12
3	15 ppm Am FB+1	-	10.00a ± 2.67	0	10.00a ± 2.67	7.50b ± 3.66	8.75b ± 4.79	17.50a ± 7.01

Different letters in the same column denote significant differences between values ( $P>0.05$ , LSD multiple range test). Shaded columns indicate where statistically significant differences can be found.

**Table 3.5.2.13** FCM infestation and total dropped from Palmer navels at Bernol Farm where 2,4-D had been applied, evaluated fortnightly from 22 January to 28 April 2010.

Treatment		Closed navel ends (%)	FCM infested fruit/tree/fortnight	Total drop/tree/fortnight fruit
1	Control	-	0.21a ± 0.08	1.75a ± 0.43
2	10 ppm Am FB+1	-	0.28a ± 0.08	2.07a ± 0.45
3	15 ppm Am FB+1	-	0.30a ± 0.09	2.37a ± 0.44

#### 2010-11

At Halaron, mean fruit infestation by FCM was  $0.69 \pm 0.04$  (mean ± SE) fruit infested per tree per week in the control and  $0.77 \pm 0.11$  fruit per tree per week in the treatment. These values were not significantly different ( $\alpha=0.05$ ; students t-test).

At Avoca, mean fruit infestation by FCM was  $0.28 \pm 0.06$  (mean ± SE) fruit infested per tree per week in the control and  $0.21 \pm 0.04$  fruit per tree per week in the treatment. These values were also not significantly different ( $\alpha=0.05$ ; students t-test).

No mealybug was present in the Halaron orchard. However, mealybug infestation was evaluated twice in the Avoca orchard – on 23 February and 2 March 2011. Inspections were conducted under fruit calyces, on the sides of the fruit and within the navel ends. In the February inspection, superficial assessments of navel end infestation were made, whereas in March, the navel ends were cut open to more accurately inspect. A significantly higher percentage of fruit from untreated trees was infested with mealybug (Table 3.5.2.14).

**Table 3.5.2.14.** Mealybug infestation of navels oranges treated with 2,4-D and untreated.

Date	Treatment	Fruit infested with mealybug (%)		
		Calyx and sides	Navel end	Total
23/02/2011	Control	7.40 ± 0.58a	3.00 ± 0.61a	7.80 ± 0.55a
	2,4-D	5.00 ± 0.98b	2.40 ± 0.81a	5.48 ± 0.98b
02/03/2011	Control	6.70 ± 0.84a	3.70 ± 0.56a	7.60 ± 0.67a
	2,4-D	4.70 ± 0.84a	2.10 ± 1.00a	5.10 ± 0.84b

#### Conclusion

The hypothesis which led to this study was that closed navel ends in navel oranges should reduce pest damage and infestation, particularly by those pests which are often directly related to the navel end, such as FCM and mealybug. As a spring spray of 2,4-D can significantly reduce the percentage of open navel ends, such a spray might be a valuable pest management tool. Unfortunately, no relationship between FCM and percentage of open navel ends could be established. In the second year of evaluating mealybug, significantly less infestation was recorded on fruit from trees which had been treated with 2,4-D. The most compelling results were recorded for bud mite. Generally, the occurrence of protruding navels was higher and the occurrence of bull-nosing was lower in untreated fruit (more open navel ends). This study was continued into the 2010/11 season and will be reported in the next CRI annual report.

#### Technology Transfer

These results were briefly reported by Stephan Verreyne at the 2010 Citrus Research Symposium.

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### 3.5.3 PROGRESS REPORT: Evaluation of gamma irradiation as a post-harvest control measure for citrus mealybug, *Planococcus citri* (Risso)

IAEA 15634/RO by J H and M Hofmeyr (Citrus Research International)

#### Opsomming

Twee proewe is met sitruswitluis, *Planococcus citri*, uitgevoer om die doeltreffendheid te bevestig van 150 Gy gammastraling om die voortplanting van volwasse, nie-eierleggende wyfies te verhoed, asook om die radioverdraagsaamheid van eierleggende wyfies te bestudeer. Soos met vorige studies was die 150 Gy dosis doeltreffend teen volwasse, nie-eierleggende wyfies. Dit was ook doeltreffend om eierleggende wyfies te steriliseer wat daarop dui dat hulle nie radioverdraagsamer as nie-eierleggende wyfies is nie. Dié dosis kan derhalwe as die laagste doeltreffende stralingsdosis vir sitruswitluis aanvaar word en in 'n finale Probit-8.7 studie bekragtig word.

'n Bevolking wingerdwitluis, *Planococcus ficus*, word as 'n tweede spesie geteel om die algemene doeltreffendheid van 150 Gy gammastraling te bevestig en sodoende waarde by IAEA-projek 15634/RO te voeg.

#### Summary

Two experiments were conducted with citrus mealybug, *Planococcus citri*, to confirm the efficacy of 150 Gy gamma radiation to prevent pre-ovipositing mealybug reproduction and to study the radiotolerance of ovipositing females. Similar to previous work, the 150 Gy dose was again effective on pre-ovipositing females. It also effectively sterilized ovipositing females which indicates that they are not more radiotolerant than pre-ovipositing females. This dose can therefore be accepted as the lowest effective radiation dose for mealybug and can be used for validation in a final Probit-8.7 study.

A colony of the vine mealybug, *Planococcus ficus*, is being reared as a second species to confirm the general efficacy of 150 Gy radiation as a value-enhancer to IAEA project 15634/RO.

### 3.5.4 FINAL REPORT: The use of entomopathogenic nematodes to control *Planococcus citri* and *Paracoccus burnerae*

Experiment 985 (2010/11 – 2011/12) by S van Niekerk (SU), A P Malan (SU) and S D Moore (CRI)

#### Opsomming

*Planococcus citri*, die sitrus witluis, is 'n hoogs skadelike pes van sitrus in Suid-Afrika. Die verskuilde aard en beskermende waslaag van die insek, kan die effektiewe beheer met chemikalieë benadeel. Verder verlaag chemiese middels die populasie van natuurlike vyande wat noodsaaklik is om lae vlakke van witluis te verseker. Entomopatogeniese nematodes het bewys dat hul waardevolle bio-beheer agente teen 'n reeks van pesinsekte kan wees. Verskillende biotoetse is uitgevoer om vas te stel watter plaaslike nematode spesies die beste potensiaal vir die beheer van *P. citri* in die laboratorium toon. *Heterorhabditis zealandica* en *Steinernema yirgalemense* het die beste potensiaal getoon. Slegs in 'n tyd-blootstellings biotoets het aangedui dat *S. yirgalemense* die beter spesie is om te gebruik vir die beheer van *P. citri* onder sub-optimale toestande. Aangesien *P. citri* bo-grond voorkom, noodsaak dit die toevoeging van benatters en water-retensie middels om uitdroging te vertraag en in die proses die beweging en oorlewing van die nematodes te bevoordeel. Die drie byvoegmiddels wat getoets was, het geen nadelige effek op die nematodes self gehad nie. Die effek van bio-beheer agente en chemikalieë wat algemeen gebruik word in 'n geïntegreerde plaagbeheer program vir sitrus op die oorlewing van nematodes, is ook getoets met geen negatiewe effek op *H. zealandica* en *S. yirgalemense* nie. Resultate van simuleerde veldtoestande in die glashuis het getoon dat die byvoeging van 'n water-retensie middel, Zeba<sup>®</sup> en 'n benatter Nufilm-P<sup>®</sup> by suspensies, die vermoë van die *S. yirgalemense* verhoog om *P. citri* te beheer. Resultate van 'n semi-veldproef, met die byvoeging van Zeba<sup>®</sup> en Nufilm-P<sup>®</sup> tot die nematode suspensie, het 20% tot die beheer van *P. citri* bygedra, met 53% beheer in die veld onder baie swak omgewingstoestande, insluitende lae temperature van 10°C gedurende die nag.

#### Summary

*Planococcus citri*, the citrus mealybug, is a highly destructive pest of citrus in South Africa. The cryptic nature, protective wax covering of the insect and the possible development of resistance to chemicals, can impair the ability of insecticides to control them. Furthermore, chemicals deplete natural enemy populations that are essential in maintaining mealybugs at low levels. Entomopathogenic nematodes have proven to be valuable biocontrol agents against a range of insect pest species. Various bioassays were conducted to identify which local nematode species show great potential for the control of *P. citri* in the laboratory. *Heterorhabditis zealandica* and *Steinernema yirgalemense* showed the greatest potential. Only an exposure time bioassay showed *S. yirgalemense* to be the better species to control *P. citri* under suboptimal conditions. As mealybugs occur above ground on citrus, the addition of an adjuvant is required to retard desiccation, thus prolonging nematode mobility and survival. For this reason, the ability of three adjuvants to improve nematode control was evaluated. The compatibility of selected nematode species with biocontrol agents and agrochemicals to which they most likely will be exposed in an integrated pest management programme for citrus in South Africa was also determined. Both *H. zealandica* and *S. yirgalemense* proved to be compatible with all products tested. Experiments conducted under simulated field conditions in the glasshouse were also conducted to evaluate the ability of nematodes to control *P. citri* with and without the addition of adjuvants. Results of a semi-field trial showed the addition of a water-retention agent, Zeba<sup>®</sup> and a surfactant, Nufilm-P<sup>®</sup> to nematode suspensions, do increase the ability of *S. yirgalemense* to control *P. citri* by 20%, obtaining 53% control under unfavourable environmental conditions, including a low temperature of 10°C during the night.

#### Potential of South African isolates of entomopathogenic nematodes for control of *Planococcus citri*

##### Introduction

Mealybugs (Hemiptera: Pseudococcidae) are regarded as pests of extreme economic importance on a wide range of field crops, fruit crops and ornamentals worldwide (Bartlett & Lloyed, 1958; Franco *et al.*, 2004). Although mealybugs occur globally, they are most abundant in the tropics and subtropics (Ben-Dov *et al.*, 2010). Throughout the world, more than 60 mealybug species have been noted to develop on *Citrus* spp., of which the majority are of minor economic importance (Franco *et al.*, 2004; Ben-Dov *et al.*, 2010). Out of the twenty mealybug species considered as economically important pests of cultivated plants in South Africa (Annecke & Moran, 1982), seven are regarded as pests of economic or potential economic importance on *Citrus* spp., of which *Planococcus citri* (Risso) is the most common and destructive (Hattingh *et al.*, 1998). Mealybugs infest all parts of citrus trees, except the roots (Canhilal *et al.*, 2001). Damage caused by mealybugs on citrus includes: wilting, fruit and flower drop, fruit and leaf deformations, hyperpigmentation, fruit stippling and

sooty mould growth (Hattingh, 1993; Blumberg *et al.*, 1995; Hattingh & Tate, 1996; Hattingh *et al.*, 1998; Hattingh & Moore, 2003).

In citrus orchards, mealybug population levels are usually suppressed by a complex of natural enemies and do not reach economically damaging levels (Hattingh, 1993; Hattingh & Tate, 1996; Hattingh *et al.*, 1998; Hattingh & Moore, 2003). However, if the natural enemy complex is disrupted by the application of chemical pesticides intended for the control of other important citrus pests, such as false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), citrus thrips, *Scriptothrips aurantii* (Faure) (Thysanoptera: Thripidae) or red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), mealybug populations can rapidly increase to damaging levels (Hattingh & Tate, 1995; Hattingh *et al.*, 1998). Mealybugs are difficult to control with insecticides, as they display cryptic behaviour by hiding in spaces where chemicals cannot reach them (McKenzie, 1967; Michelakis & Hamid, 1995; Franco *et al.*, 2004). Their waxy coverings also act as a barrier against insecticides and they have the ability to develop resistance to insecticides at an alarming rate (McKenzie, 1967; Blumberg & Van Driesche, 2001; Mahfoudhi & Dhouibi, 2009). According to Ehlers (1996), the development of resistance to insecticides is one of the major driving forces for seeking alternative insect control methods.

Entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* (Rhabditida: Heterorhabditidae) and *Steinernema* (Rhabditida: Steinernematidae) have proven to control a broad range of economically important insect pest species (Grewal *et al.*, 2005) and are used as inundatively applied biological control agents (Hazir *et al.*, 2003). Both genera have a free-living non-feeding stage that can actively detect and infect hosts (Glazer & Lewis, 2000). Once inside the host's haemocoel, nematodes release symbiotic bacteria that kill hosts by means of septicaemia within 24 to 48 h (Adams & Nguyen, 2002; Dowds & Peters, 2002). These nematodes have no known negative effect on the environment, humans or other vertebrates (Akhurst & Smith, 2002). No special measures are required for application, as nematodes can be applied as an aqueous suspension, using ordinary agrochemical spray equipment (Grewal, 2002; Hussaini, 2002). Unlike most other inundatively applied biological control agents, nematodes have proven to be tolerant to short periods of exposure (2-24 h) to a wide variety of agrochemicals (Rovesti & Deseö, 1990).

To date, very few studies have been done on the susceptibility of Pseudococcidae to EPNs. With the use of sand-dish bioassays, Stuart *et al.* (1997) determined the susceptibility of *Dysmicoccus vaccinii* (Miller & Polavarapu) to four nematode strains. Mealybugs were most susceptible to *Heterorhabditis bacteriophora* Poinar, 1976, which caused 64% mortality. In sand-dish assays, both *H. bacteriophora* and *Heterorhabditis indicus* Poinar, 1992 could complete their life cycle in *D. vaccinii* and caused significant mealybug mortality. They also showed that the susceptibility of mealybugs to EPNs is not influenced by the presence or absence of their protective wax coatings. Laboratory tests aimed at determining the susceptibility of the coffee root mealybug, *Dysmicoccus texensis* (Tinsley), to various strains of *Heterorhabditis* showed that most of the strains tested were highly virulent (Alves *et al.*, 2009). Bioassays performed by Stokwe (2009) showed that the obscure mealybug, *Pseudococcus viburni* (Signoret), varies greatly in susceptibility from one nematode species to the next and was found to be generally more susceptible to *Heterorhabditis* spp. Bioassays also showed that all life stages of female *P. viburni*, from crawler to adult, were susceptible to nematodes to some degree. EPNs have also shown to have the ability to locate and to kill hosts in cryptic habitats, such as inside the calyx and ovary of apples

## Stated objectives

The objectives of this study were to:

1. to identify nematode species which are highly pathogenic to *P. citri*;
2. to observe the life cycle of a heterorhabditid and steinernematid in *P. citri*;
3. to determine the optimal nematode concentration at different humidity levels;
4. to assess the effect of the water activity ( $a_w$ ) on mortality and;
5. to establish the exposure time at various temperatures required to ensure satisfactory.

## Materials and methods

### Source of nematodes

Nematode species used in the current study (Table 3.5.4.1) were originally obtained from previous local surveys (Malan *et al.*, 2006; Malan *et al.*, 2011) and maintained in the Stellenbosch University nematode collection. IJs of the six nematode species were cultured in last larval instar of the mealworm, *Tenebrio molitor* (Linnaeus)

(Coleoptera: Tenebrionidae) or the wax moth larvae, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) at room temperature, according to the procedures described by Kaya and Stock (1997).

**Table 3.5.4.1:** *Heterorhabditis* and *Steinernema* species, strain, habitat, locality and GenBank accession number.

Species	Strain	Habitat	Locality	GenBank accession number
<i>H. zealandica</i>	SF 41	Natural	Patensie, Eastern Cape	EU699436
<i>H. bacteriophora</i>	SF 351	Disturbed	Wellington, Western Cape	FJ455843
<i>H. safricana</i>	SF 281	Disturbed	Piketberg, Western Cape	EF488006
<i>S. khoisanae</i>	SF 87	Disturbed	Villiersdorp, Western Cape	DQ314289
<i>S. citrae</i>	141-C	Disturbed	Piketberg, Western Cape	EU740970
<i>S. yirgalemense</i>	157-C	Disturbed	Friedenheim, Mpumalanga	EU625295

(Malan *et al.*, 2006; Malan *et al.*, 2011).

#### Source of insects

The identity of *P. citri* used in the current study was verified using morphological (Wakgari & Giliomee, 2005) and molecular techniques (Pieterse *et al.*, 2010). Mealybugs were reared in the laboratory on butternuts and sprouting potatoes. Cultures were kept in cages (650 mm × 350 mm × 590 mm) with substantial ventilation, allowing efficient airflow. Only adult female mealybugs were selected for use in experiments. Mealworm larvae were reared at room temperature in plastic containers on fine wheat bran. To improve humidity, potato peels or apple slices were laid over the surface of the colony. Last-instar mealworms were harvested regularly and kept at 4°C until needed. *Galleria mellonella* larvae were kept in a growth chamber at 25°C and reared on a diet consisting of baby cereal (Cerelac Nestlé™), brown bread flour, yeast, wheat germ, beeswax, glycerine and honey.

#### Bioassay protocol

*Planococcus citri* were individually exposed to IJs in multiwell bioassay plates (24 wells, flat bottom, Nunc™ Cat. No.144530). Five bioassay plates were used per treatment and five control plates for each treatment, each containing evenly distributed adult female mealybugs were prepared. Each well was lined with a circular paper disc (13-mm-diameter) before mealybugs were added. Mealybugs were then inoculated individually with the required concentration of nematodes (Navon & Ascher, 2000). Control plates received 50 µl of distilled water only. To retain insects in their individual wells, each plate was covered with a fitted piece of glass. After inoculation, plates were placed inside plastic containers lined with moistened paper towels and closed with the lid to maintain high humidity levels (RH ± 95%). Plastic containers were then incubated in a dark growth chamber at 25 ± 2°C for 48 h, after which the mortality of the mealybugs was determined by means of gentle prodding.

#### Screening

The multiwell bioassay protocol was used to test the susceptibility of adult female *P. citri* to of six indigenous EPN species under optimal conditions. Three heterorhabditids, namely *H. zealandica*, *H. bacteriophora* and *Heterorhabditis safricana* Malan, Nguyen, De Waal & Tiedt, 2008, and three steinernematids, namely *Steinernema khoisanae* Nguyen, Malan & Gozel, 2006, *Steinernema citrae* Stokwe, Malan, Nguyen, Knoetze & Tiedt, 2011 and *Steinernema yirgalemense* Tesfamariam, Gozel, Gaugler & Adams, 2005 were used. Five treatment plates and five control plates, each with twelve evenly distributed adult female mealybugs, were prepared for each species and strain tested (5 replicates; 60 insects per nematode species). Mealybugs were inoculated individually with 200 IJs/insect and, after 48 h, mortality was determined. The experiment was repeated on a separate test date. The data of both experiments were pooled for analysis. The data of both experiments were pooled for analysis

#### Biological study

In this study, the development of *H. zealandica* and *S. yirgalemense* in *P. citri* was determined. The multiwell bioassay protocol was followed. Adult female mealybugs were individually exposed to 50 IJs in multiwell plates. Thirty bioassay plates, each containing five evenly distributed mealybugs, were prepared for both *H. zealandica* and *S. yirgalemense*. Two days after inoculation, mealybugs were rinsed in distilled water to remove any remaining nematodes from their body surface and moved to clean Petri dishes (13-cm-diameter). Each new Petri dish contained a total of 50 mealybugs. To determine the developmental stage of each nematode species, 25

mealybugs were randomly selected and dissected every one to two days for ten days. To determine whether *H. zealandica* and *S. yirgalemense* could complete their life cycle in *P. citri*, five Petri dishes with ten infected mealybugs each were left undisturbed and placed in white traps. Mealybugs were also assessed for colour change, number of nematodes penetrated and quality of mealybug eggs, and the sex ratio of *S. yirgalemense* was determined.

#### Influence of nematode concentrations and humidity on *P. citri* mortality

The influence of increasing concentrations of *H. zealandica* was determined at three humidity levels (80%, 60% and 100% RH). The lethal concentration of *S. yirgalemense* was determined at 100% RH only. To achieve the required humidity levels, airtight containers with solutions of glycerol (60% RH), KNO<sub>3</sub> (80% RH) and moistened tissue paper (100% RH) were prepared (Winston & Bates, 1960). Five multiwell plates, with each containing eight evenly distributed adult female *P. citri*, were prepared (5 replicates; 40 insects) for each of the different nematode concentrations (0-, 5-, 10-, 20-, 40- and 80 IJs/mealybug) at each of the different levels of humidity (60%, 80% and 100% RH) tested. Multiwell plates were lined with filter paper discs and covered with fine netting to allow airflow while preventing mealybugs from escaping. After inoculation, mealybugs were incubated in a growth chamber with a day cycle starting at 22°C for 14 h and at 11°C for 11 h. After 72 h mealybug mortality was assessed. The experiment was repeated on a separate date. The data of both experiments were pooled for analysis.

#### Effect of water activity levels on mortality

To assess the performance of *H. zealandica* and *S. yirgalemense* against adult *P. citri* females at reduced moisture levels, the two species were tested at different water activity ( $a_w$ ) levels. The required  $a_w$  values were achieved by adding different volumes of water containing 50 IJs/insect to small Petri dishes (3-cm-diameter) lined with filter paper. Control treatments contained water only. The  $a_w$  values were measured using a Decagon Pawkit water-activity-meter (Decagon Devices Inc., Pullman, WA, USA) at a constant temperature of 25°C. Four adult mealybugs were added to each dish, covered with cling wrap and sealed with a lid to ensure an airtight seal. Dishes were incubated 22°C for 72 h, after which mealybug mortality was assessed. Five replicates were prepared for both treatments and control dishes for each nematode species and  $a_w$ -level tested (5 replicates; 20 insects per  $a_w$ -level and nematode combination). The experiment was repeated on a separate date. The data of both experiments were pooled for analysis.

#### Influence of exposure time and temperature on mortality

The activity of *H. zealandica* IJs at three different temperatures (15°C, 20°C and 25°C) was assessed, with the activity of *S. yirgalemense* being assessed at 25°C only. Five Petri dishes (13-cm- diameter) lined with filter paper, each containing six adult female mealybugs (5 replicates; 30 insects), were prepared for each of the time intervals and temperatures tested. Nematodes were applied to Petri dishes at a concentration of 80 IJs/insect in 1 ml of water and incubated at 100% RH in sealed plastic containers lined with moistened filter paper. An equal number of Petri dishes were prepared for control treatments that received 1 ml distilled water only. After inoculation, mealybugs were removed from Petri dishes after 30-, 60-, 180-, 240- and 480- min intervals, rinsed with distilled water to remove excess surface nematodes and placed in clean Petri dishes. Mealybugs were then incubated at 25°C for 48 h, after which mortality was assessed. The experiment was repeated on a separate date. The data of both experiments were pooled for analysis.

#### Data analysis

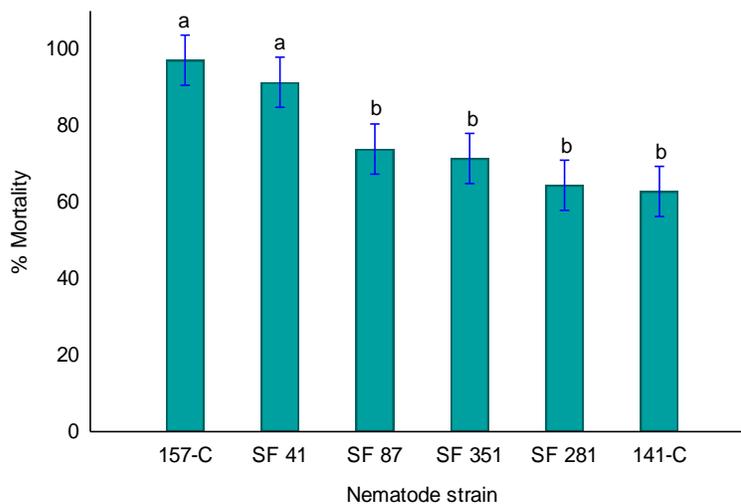
Data of all trials, apart from the lethal concentration and humidity trial, were corrected using Abbott's formula (Abbott, 1925), to compensate for mealybugs that died of natural causes prior to analysis. All statistical analyses were performed by means of Statistica 9.0 software (StatSoft Inc. 2009). Data were analysed using ANOVA, with post-hoc comparison of means using Bonferroni's method, or a bootstrap multi comparison if residuals were not evenly distributed (Efron & Tibshirani, 1993). Significant differences were determined on a 95% probability level. To determine lethal concentration (LC), water activity ( $a_w$ ) and exposure time (min), a probit analysis (Finney 1971) was conducted using Polo PC (LeOra Software 1987).

## **Results and discussion**

### Screening

The percentage mortality of *P. citri* caused by the six nematode species was analysed using a one-way ANOVA. Significant differences were obtained ( $F_{(5, 54)} = 18.91$ ;  $P < 0.01$ ) for percentage mortality between the different nematode species. Mortality (91% - 97%) caused by *H. zealandica* and *S. yirgalemense*, was significantly higher than mortality (63% - 74%) caused by the other four nematode species tested (Fig. 3.5.4.1). Although not

significant, *S. yirgalemense* caused a higher average percentage mortality of 97%, compared to mortality of 91% obtained for *H. zealandica*.



**Fig. 3.5.4.1:** Mean percentage (95% confidence interval) mortality for adult female *Planococcus citri* using *Steinernema yirgalemense* (157-C); *Heterorhabditis zealandica* SF41; *S. khoisanae* (SF87); *H. bacteriophora* (SF351); *H. safricana* (SF281); *S. citrae* (SF141) at a concentration of 200 infective juveniles/insect, after a period of 48 h in multiwell bioassay plates. Means with the same letter are not significantly different.

#### Biological studies

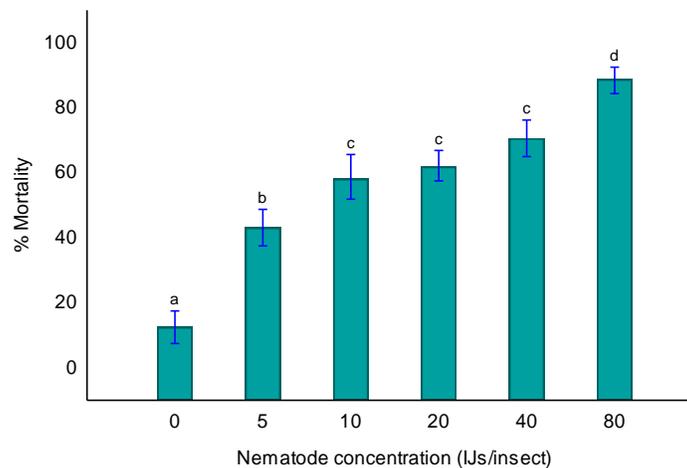
Changes in infected mealybug and nematode development were documented for ten days after exposure to IJs of *H. zealandica* and *S. yirgalemense* (Table 3.5.4.2). Mealybugs infested with *H. zealandica* became a rusty-brown colour compared to those infested with *S. yirgalemense*, which varied in colour change from none to various shades of brown. No colour changes were observed for mealybug eggs that remained viable. Hermaphrodites with eggs were observed three days after infection of mealybugs with *H. zealandica*. Larvae were observed inside hermaphrodites after four days and after eight days IJs emerged and the life cycle were completed. Mature males and females with eggs were observed three days after infecting mealybugs with *S. yirgalemense*. Females with larvae inside were observed after four days and after six days IJs emerged and the life cycle were completed. Both nematode species completed one generation before IJs emerged. Although the majority of hosts infected with *S. yirgalemense* produced IJs, in some hosts nematodes only developed up to a certain point and then died. This was generally observed when the number of nematodes that penetrated the host was too high, with no colour change being observed in such hosts. The mean number of IJs that penetrated hosts (n = 25) was five for *H. zealandica* and nine for *S. yirgalemense*. The number of male and female *S. yirgalemense* was also documented and showed that there were only slightly more females than males, with a sex ratio of 1:1.

**Table 3.5.4.2.** Nematode development in adult female *Planococcus citri* inoculated with infective juveniles of *Heterorhabditis zealandica* and *Steinernema yirgalemense*.

Nematode species	No. of days	Stage of nematode development
<i>H. zealandica</i>	2	Immature
	3	Hermaphrodites with eggs
	4	Hermaphrodites with larvae
	6	Hermaphrodites with larvae
	8	Infective juveniles
<i>S. yirgalemense</i>	10	Infective juveniles
	2	Immature
	3	Males and females with eggs
	4	Males and females with larvae
	6	Infective juveniles
	8	Infective juveniles

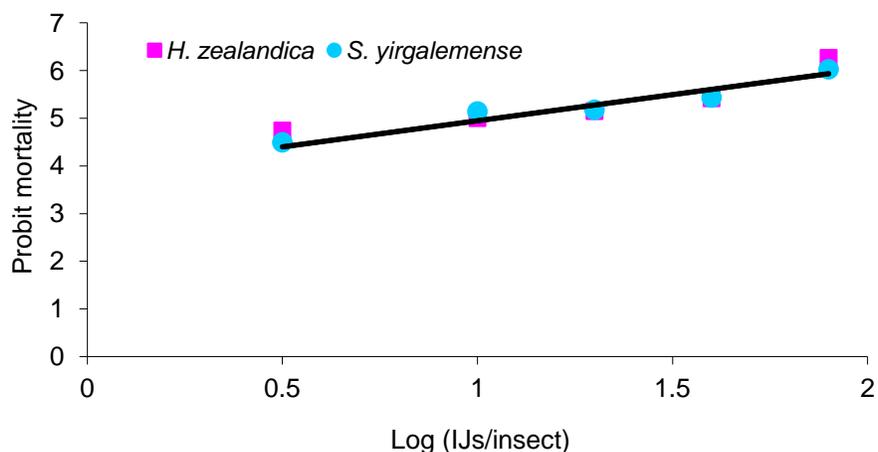
#### Influence of nematode concentration and humidity on mortality

Results were analysed using a two-way ANOVA and showed no interaction between main effects species (2 levels: *S. yirgalemense* and *H. zealandica*) and nematode concentration (6 levels: 0, 5, 10, 20, 40, 80 IJs/insect) ( $F_{(5, 108)} = 0.692$ ;  $P = 0.630$ ). Species behaved constantly at each nematode concentration level and no significant differences were observed between species at any of the nematode concentration levels. A one-way ANOVA for total mortality caused by each species further indicated that they did not differ significantly from each other ( $F_{(1, 108)} = 2.35$ ;  $P = 0.13$ ). The data of both species were thus pooled together for further analysis, using a one-way ANOVA ( $F_{(5, 108)} = 84, 734$ ;  $P < 0.01$ ) to determine the effect of increasing nematode concentrations on mealybug mortality at 100% relative humidity. The results illustrated a positive relationship between insecticidal activity and nematode concentration, with an accumulative increase in mortality as nematode concentrations increased (Fig. 3.5.4.2). At a concentration of 5 IJs/insect, 43% mortality was obtained, which was significantly higher than the control ( $P < 0.001$ ) that caused 13% mortality. Although not significant, mortality increased gradually from a concentration of 10 - 40 IJs/insect, with mortality increasing from 59% to 71%. Mealybug mortality, however, increased significantly when a concentration of 80 IJs/insect was applied, obtaining 89% control.



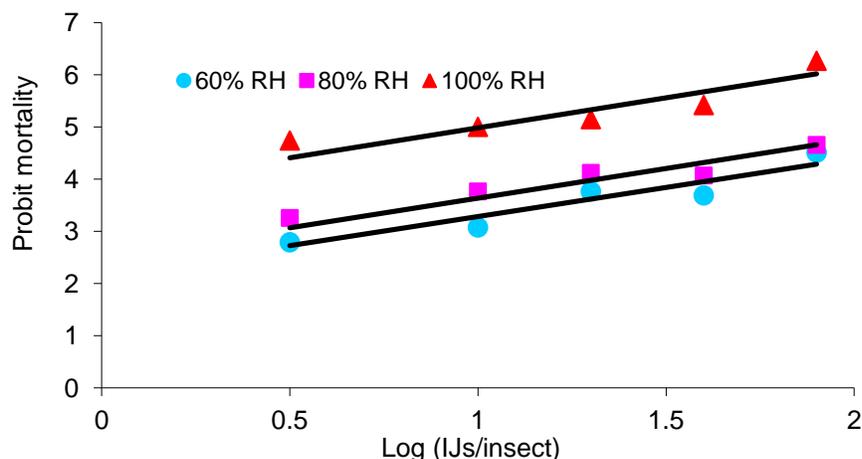
**Fig. 3.5.4.2:** Pooled mean percentage mortality (95% confidence interval) of adult female *Planococcus citri* after exposure to different concentrations of infective juveniles at 100% humidity. Different letters above vertical bars indicate significant differences.

Results of the probit analysis showed the probit regression lines for *H. zealandica* and *S. yirgalemense* to be the same ( $\chi^2 = 0.12$ ; d.f. = 2;  $P = 0.99$ ) indicating the insecticidal activity of *H. zealandica* and *S. yirgalemense* to be identical (Fig. 3.5.4.3). The common probit regression line for the two species was  $Y = 3.85 + 1.09(X)$  where  $Y$  is the probit mortality and  $X$  is  $\log(\text{nematode concentration})$ . The data fitted this model well ( $\chi^2 = 8.97$ ; d.f. = 8;  $P = 0.35$ ) indicating a positive relationship between nematode concentration and insecticidal activity of the two nematode species. The  $LC_{50}$  and  $LC_{90}$  values were 11.40 (90% fiducial limits: 8.53 – 14.34) and 107.00 (90% fiducial limits: 107.95 – 342.75) respectively.



**Fig. 3.5.4.3:** Probit mortality of *Planococcus citri* of the logarithm of the number of infective juveniles per insect at 100% humidity and 25°C.

The results obtained from the humidity and nematode concentration experiment that were analysed using a probit analysis showed the probit regression lines for 60% -, 80% - and 100% RH to differ from each other ( $\chi^2 = 204.10$ ; d.f. = 4;  $P = 0.001$ ), indicating the insecticidal activity of *H. zealandica* to differ when exposed to the various humidity levels (Fig. 3.5.4.4). The regression lines were however parallel to each other ( $\chi^2 = 1.36$ ; d.f. = 2;  $P = 0.506$ ). The probit regression lines for 60% -, 80% - and 100% RH were  $Y = 2.17 + 1.12 (X)$ ,  $Y = 2.50 + 1.12 (X)$  and  $Y = 3.83 + 1.12 (X)$  respectively where  $Y$  is the probit mortality and  $X$  is log (nematode concentration). The data fitted this model well ( $\chi^2 = 10.89$ ; d.f. = 11;  $P = 0.45$ ) indicating a positive relationship between nematode concentration and insecticidal activity of nematodes when exposed to the various humidity levels. The  $LC_{50}$  and  $LC_{90}$  values were 331.40 (90% fiducial limits: 198.05 – 671.61) and 4593.60 (90% fiducial limits: 1882.50 – 17175.0) at 60% RH, 169.33 (90% fiducial limits: 113.12 – 105.76) and 2345.60 (90% fiducial limits: 1076.80 – 7444.10) at 80% RH and 11.14 (90% fiducial limits: 8.12 – 14.51) and 154.33 (90% fiducial limits: 100.79 – 284.28) at 100% RH respectively. The relative potencies and their fiducial limits of nematodes when exposed to the different humidity levels are given in Table 3.5.4.3 which indicates nematodes to be two times more potent at 80% RH than at 60% RH, 15.19 times more potent at 100% humidity than at 80% humidity and 29.76 times more potent than at 60% humidity.



**Fig. 3.5.4.4:** Probit mortality of *Planococcus citri* of the logarithm of the number of infective juveniles per insect at 60%-, 80%- and 100% relative humidity and 25°C.

**Table 3.5.4.3:** Relative potency of nematodes when exposed to 60 - 80 and 100% relative humidity at 25°C.

Potency at	Relative to	Potency	95% Fiducial Limits
100	80	1.96	1.02 – 3.85
100	60	29.76	14.23 – 88.33
80	60	15.19	13.26 – 23.00

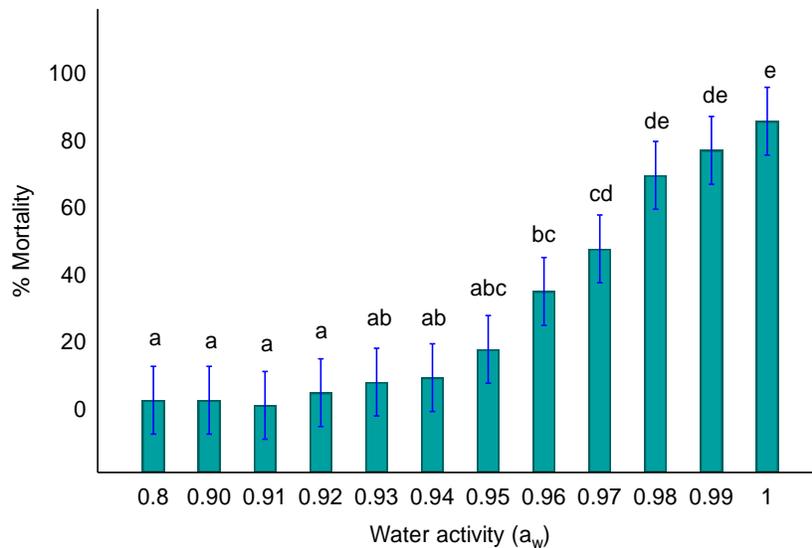
Effect of water activity levels on mortality

The volumes of water used, with and without nematodes, to attain  $a_w$  values of 0.8 to 1.0 are indicated in Table 3.5.4.4. For the lowest  $a_w$  value of 0.8, 5.5  $\mu$ l of water was used, with and without nematodes, and for the highest  $a_w$  value of 1.00, a volume of 27  $\mu$ l was used.

**Table 3.5.4.4:** The volume of water containing 50 infective juveniles per insect, or water only for control treatments, added to Petri dishes (3-cm-diameter) lined with filter paper to attain corresponding water activity ( $a_w$ ) values.

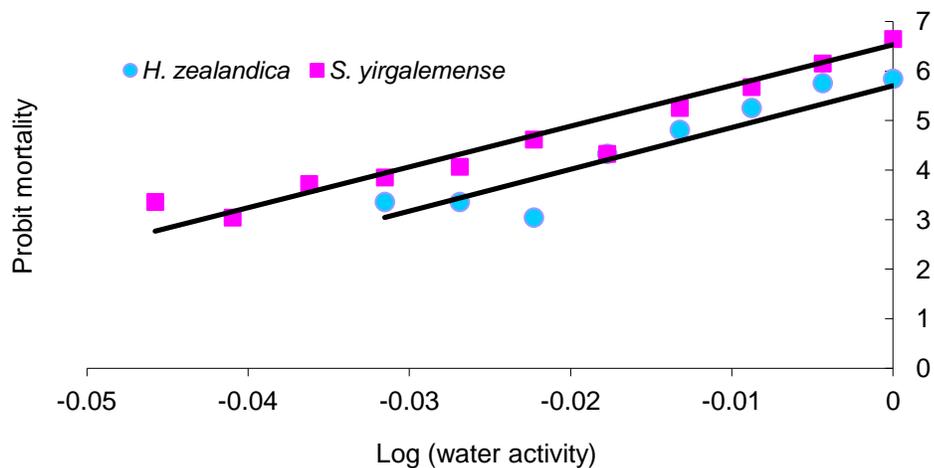
$a_w$ Value	Inoculum ( $\mu$ l)	$a_w$ Value	Inoculum ( $\mu$ l)
0.8	5.5	0.95	16
0.9	7	0.96	17.5
0.91	8	0.97	20
0.92	9	0.98	23
0.93	10.5	0.99	25.5
0.94	13	1.00	27

The data obtained on the effect of water activity ( $a_w$ ) on the two nematode species for mortality of mealybug females were analysed using a two-way ANOVA. No interaction was shown between the main effects nematode species (2 levels; *H. zealandica* and *S. yirgalemense*) and water activity level (12 levels;  $a_w = 0.8, 0.9, 0.91, \dots, 1.00$ ) ( $F_{(11, 216)} = 0.80$ ;  $P = 0.64$ ), indicating nematode species to have behaved constantly at each  $a_w$  value. Because no significant differences were observed between the two species, data were pooled for mortality and analysed using a one-way ANOVA ( $F_{(11, 216)} = 39.05$ ;  $P < 0.01$ ). The results showed that *S. yirgalemense* caused significantly higher mortality (37%) in comparison to *H. zealandica* (24%). Data for the effect of water activity ( $a_w$ ) on mortality of both species were also pooled for further analysis. A one-way ANOVA showed a significant positive effect of increasing  $a_w$  values on insecticidal activity ( $F_{(11, 216)} = 39.05$ ;  $P < 0.01$ ). Very low levels of mortality ( $\leq 20\%$ ) were observed at  $a_w$  values 0.80 to 0.94. Mortality (8%-58%) increased gradually at  $a_w$  values 0.95 to 0.97, with the highest mortality being observed at  $a_w$  values 0.98 to 1.00, causing 59% to 96% mortality (Fig. 3.5.4.5).



**Fig. 3.5.4.5.** Pooled mean percentage mortality (95% confidence interval) recorded for *Planococcus citri* after exposure to 50 infective juveniles per insect at different water activity ( $a_w$ ) levels. Different letters above vertical bars indicate significant differences.

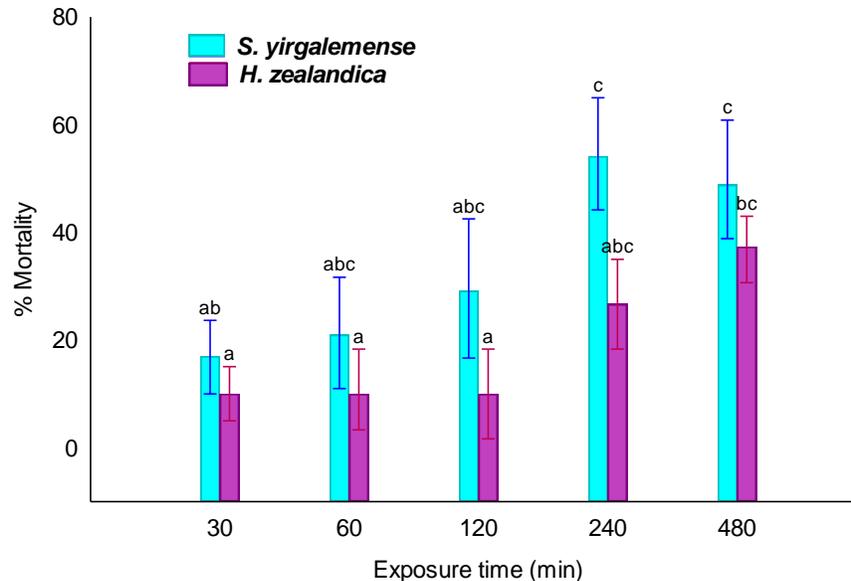
Results of the probit analysis showed the probit regression lines for *H. zealandica* and *S. yirgalemense* to differ from each other ( $\chi^2 = 26.13$ ; d.f. = 2;  $P = 0.001$ ), indicating the insecticidal activity of *H. zealandica* and *S. yirgalemense* to differ when exposed to the various water activity levels (Fig. 3.5.4.6). The regression lines were however parallel to each other ( $\chi^2 = 2.85$ ; d.f. = 1;  $P = 0.091$ ). The probit regression lines for *H. zealandica* and *S. yirgalemense* were  $Y = 6.53 + 95.98 (X)$  and  $Y = 5.94 + 95.98 (X)$  respectively where  $Y$  is the probit mortality and  $X$  is  $\log$  (nematode concentration). The data fitted this model well ( $\chi^2 = 23.13$ ; d.f. = 21;  $P = 0.66$ ) indicating a positive relationship between water activity and insecticidal activity of the two nematode species. The  $a_{w50}$  and  $a_{w90}$  values were 0.96 and 0.99 for *S. yirgalemense* and 0.98 and 1.01 for *H. zealandica* respectively. Furthermore results showed *S. yirgalemense* to be 2 times more potent than *H. zealandica*.



**Fig. 3.5.4.6.** Probit mortality of *Planococcus citri* of the logarithm of the water activity level to which *Heterorhabditis zealandica* and *Steinernema yirgalemense* were exposed.

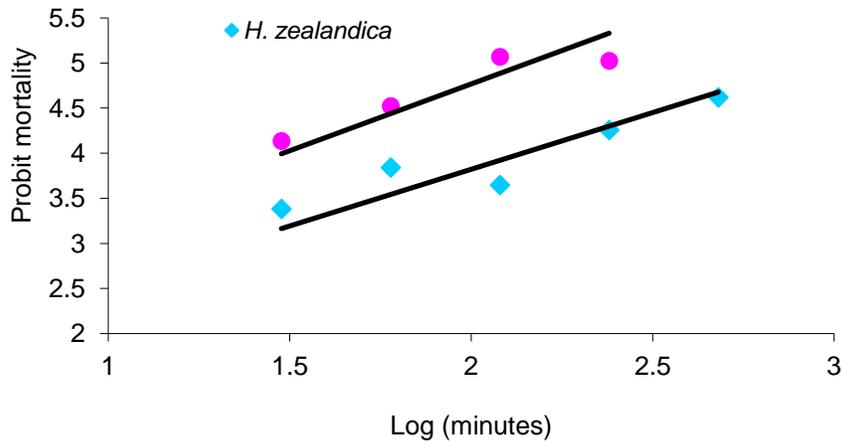
### Influence of exposure time and temperature on mortality

The two-way ANOVA showed no interaction between main effects exposure nematode species (6 levels; 0- 30- 60- 120- 240- 480 min) and nematode species (2 levels; *H. zealandica* and *S. yirgalemense*) ( $F_{(4, 90)} = 1.44$ ;  $P = 0.23$ ). Although no significant differences were observed between species at any of the exposure intervals (Fig. 3.5.4.7), *S. yirgalemense* consistently caused higher mortality than did *H. zealandica* at each exposure time. A one-way ANOVA for pooled mortality caused by each species showed that *S. yirgalemense* caused significantly higher mortality than did *H. zealandica* ( $F_{(1, 90)} = 1.44$ ;  $P = 0.001$ ). Low levels of mortality (10 - 29%) were recorded when mealybugs were exposed to nematodes for 30 to 120 min (Fig 3.5.4.6). The highest mortality levels were observed after 480 min exposure and ranged between 27% and 54%. Using a one-way ANOVA, data for both species were pooled for further analysis to illustrate that mortality (40 - 43%) obtained after 240 and 480 min exposure to nematodes was significantly higher ( $F_{(4, 135)} = 12.825$ ;  $P > 0.01$ ) than mortality (14 - 20%) after 30, 60 and 120 min.



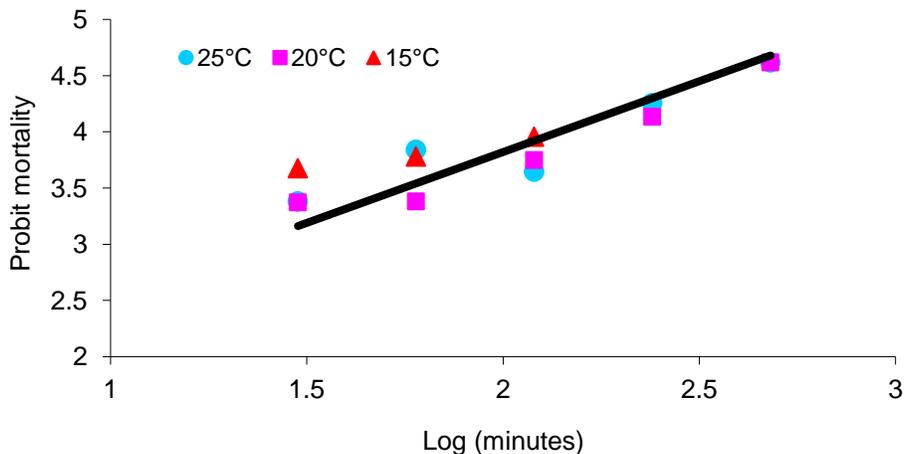
**Fig. 3.5.4.7.** Percentage mortality (95% confidence interval) recorded for *Planococcus citri* after exposure to 80 infective juveniles per insect of *Heterorhabditis zealandica* and *Steinernema yirgalemense* for different lengths. Different letters above vertical bars indicate significant differences.

Results of the probit analysis showed the probit regression lines for *H. zealandica* and *S. yirgalemense* to differ from each other ( $\chi^2 = 13.47$ ; d.f. = 2;  $P = 0.001$ ) indicating the insecticidal activity of *H. zealandica* and *S. yirgalemense* to differ when exposed to mealybugs for the various time intervals (Fig. 3.5.4.8). The regression lines were however parallel to each other ( $\chi^2 = 0.54$ ; d.f. = 1;  $P = 0.46$ ). The probit regression lines *H. zealandica* and *S. yirgalemense* where  $Y = 1.31 + 1.23 (X)$  and  $Y = 1.83 + 1.23 (X)$  respectively where Y is the probit mortality and X is log (minutes). The data fitted this model well ( $\chi^2 = 7.28$ ; d.f. = 7;  $P = 0.99$ ) indicating a positive relationship between exposure time and insecticidal activity of nematodes. The  $LC_{50}$  and  $LC_{90}$  values were 971.58 (90% fiducial limits: 595.05 – 2125.05) and 10644 (90% fiducial limits: 4086.0 – 59691.00) for *H. zealandica* and 371.43 (90% fiducial limits: 261.019 – 631.15) and 4101 (90% fiducial limits: 1847.60 – 17227) for *S. yirgalemense* respectively. Furthermore results showed *S. yirgalemense* to be 2.59 times more potent than *H. zealandica*.



**Fig. 3.5.4.8.** Probit mortality of *Planococcus citri* of the logarithm of exposure time (minutes) interval in which mealybugs were exposed to *Heterorhabditis zealandica* and *Steinernema yirgalemense* at 25°C.

The results obtained from the temperature and exposure time experiment that were analysed using a probit analysis which showed the probit regression lines for 15°C, 20°C and 25°C to be the same ( $\chi^2 = 8.21$ ; d.f. = 4;  $P = 0.84$ ) indicating the insecticidal activity of *H. zealandica* to be unaffected (Fig. 3.5.4.9). The common probit regression line for the three temperatures species was  $Y = 1.30 + 1.04 (X)$  where Y is the probit mortality and X is log (minutes). The  $\min_{50}$  and  $\min_{90}$  values were 1544.09 (90% fiducial limits: 953.00 – 3549.11) and 23080.00 (90% fiducial limits: 8013.7 – 0.15245E + 06) respectively (Fig. 3.5.4.9).



**Fig. 3.5.4.9:** Probit mortality of *Planococcus citri* of the logarithm of exposure time (minutes) to which *Heterorhabditis zealandica* and *Steinernema yirgalemense* were exposed to mealybugs at 25°C, 15°C and 20°C.

All six indigenous nematode species tested were able to cause high, but variable, levels of *P. citri* mortality (> 60%) under optimal conditions. *Heterorhabditis zealandica* and *S. yirgalemense* caused significantly higher mortality (> 91%) compared to the other four nematode species tested. Although not significant, *S. yirgalemense* caused a higher average percentage mortality of 97% in comparison to *H. zealandica*, which caused 91% mortality. The results are similar to those obtained by Stokwe (2009), which showed that both *H. zealandica* and *S. yirgalemense* were highly pathogenic to *P. viburni*. Using 24-multiwell bioassay plates, Malan *et al.* (2011) showed *S. yirgalemense* to be highly virulent to false codling moth larvae and, to a lesser degree, their pupa, with the pest being responsible for a great deal of destruction of citrus in South Africa, leading to significant financial losses. Of all the species tested, *S. citrae*, which was originally isolated from a citrus orchard near

Piketberg in the Western Cape province of South Africa (Malan *et al.*, 2011), performed the worst percentage wise. To date, *S. citrae* has only been found in South Africa. Although not significantly so, *H. bacteriophora*, a commercially produced species in Europe and the USA performed worse than did *S. khoisanae*, but better than *H. safricana* and *S. citrae*. Both *H. zealandica* and *S. yirgalemense* have shown great promise as potential biological control agents of *P. citri*, and were therefore selected for further use in laboratory studies.

In the study of the biology of the two nematode genera in *P. citri* as host, no sexually mature adults of *S. yirgalemense* were found to have developed after two days. After three days development, *S. yirgalemense* males and females were fully mature, and fertilised eggs were visible in females. The number of male and female *S. yirgalemense* was determined, with the sex ratio of males to females being found to be 1:1, with only slightly more females being present than males. Hermaphrodites of *H. zealandica* with eggs were also present after three days development. After four days, *H. zealandica* hermaphrodites with larvae and *S. yirgalemense* females with larvae inside were found to be present. The life cycle of *S. yirgalemense*, as shown by IJs emerging after six days, was found to be shorter than that of *H. zealandica*, whose IJs emerged after eight days. Both nematode species were able to complete their life cycle in *P. citri*.

Although IJs occur naturally in soil and *P. citri* is an above-ground pest of citrus, the ability of nematodes to complete their life cycle in *P. citri* could be advantageous in a citrus orchard. Infected hosts may fall onto the shaded, moist orchard floor and be recycled in the soil, potentially killing other citrus pests, such as false codling moth. IJs also have the ability to wait for favourable conditions before emerging (Brown & Gaugler, 1997). If IJs emerge after a rainy spell, or when trees are still moist from the morning dew, they could infect more mealybugs. Mealybugs tend to cluster together in protected areas on a tree, forming a microclimate that is suitable for nematodes. Crawlers that are less susceptible than adults (Stokwe, 2009) should increase in size after six to eight days, and could then possibly be infected.

The number of nematodes that managed to penetrate hosts was also determined, with the mean number of five and nine IJs being found to penetrate *H. zealandica* and *S. yirgalemense*, respectively. These results support those of Hominick and Reid (2009), who assume that nematodes with the highest invasion efficacy would also be more efficient in killing the target insect host. *Steinernema yirgalemense*, which caused higher mortality of *P. citri* than did *H. zealandica*, also had a higher mean penetration number of nine IJs, compared to *H. zealandica*, with a mean penetration number of five IJs. The general low numbers of IJs infecting mealybugs are due to the small size of adult females, which are approximately 3 mm long. Both the length of the life cycle and the penetration rate obtained indicate *S. yirgalemense* to be a better candidate than *H. zealandica* for the control of *P. citri*.

The results of this study clearly illustrate that *P. citri* has a nematode-concentration-dependant susceptibility to both *H. zealandica* and *S. yirgalemense*. Neither nematode species caused significantly higher mortality than the other. The pooled mortality of both species illustrated the highest percentage mortality (> 83%) when mealybugs were inoculated with the highest concentration of 80 IJs/insect. The LC<sub>50</sub> and LC<sub>90</sub> values for *H. zealandica* were 11 and 162 nematodes per insect, respectively. These values were considerably lower than those which were obtained by Stokwe (2009), who obtained an LC<sub>50</sub> value of 54 and LC<sub>90</sub> value of 330, which clearly indicated *P. citri* to be more susceptible to *H. zealandica* than to *P. viburni*. The LC values obtained for *S. yirgalemense* were similar to those for *H. zealandica* and their combined lethal dosage was determined. The combined LC<sub>50</sub> and LC<sub>90</sub> values were 11.40 and 170.00 respectively.

*Planococcus citri* only occur above ground on citrus trees, which is an important consideration for evaluating the potential of nematodes to control them. Lacey and Unruh (1998) found that the ambient humidity to which nematodes are exposed has a great influence on their ability to infect hosts, as nematodes proved to be active only at humidity levels of 95% or higher. Such findings were also prevalent in the concentration and humidity experiment, which demonstrated nematodes to be two times more potent at 80% RH than at 60% RH, 15 times more potent at 100% humidity than at 80% humidity and 30 times more potent than at 60% humidity.

To enable nematodes to reach the target host, a water film is required for propulsion (Blackshaw, 1987). The  $a_w$  values indicate the available free water on the surface of leaves, fruit, barks and twigs that is required to enable nematodes to move forward to locate the sought-after hosts (Koppenhöfer, 2007). The first study on the influence of water activity on nematode efficacy was conducted by Navaneethan *et al.* (2010) using *S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1998. Results of the above-mentioned study showed that *S. feltiae* could still infect codling moth larvae, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) at an  $a_w$  value as low as 0.90 with  $a_{w90} = 0.99$ . The similar results that were obtained by De Waal (2011) showed that *H. zealandica* could infect codling moth larvae, *C. pomonella*, at  $a_w$  value as low as 0.92 with  $a_{w90} = 0.96$ . When the

influence of water activity on the insecticidal ability of *H. zealandica* and *S. yirgalemense* was tested on *P. citri*, both nematode species were found capable of infecting hosts at an  $a_w$  value as low as 0.95. *Steinernema yirgalemense*, with an  $a_{w50}$  value of 0.96 and  $a_{w90}$  value of 0.99, proved to 2 times more tolerant to lower water activity levels than were *H. zealandica*, with an  $a_{w50}$  value of 0.98 and  $a_{w90}$  value of 1.0. Although nematodes have been proven to be active at lower water activity levels than expected, a positive relationship between insecticidal activity and increasing water activity levels was still found to prevail. These results indicate that the insecticidal activity of nematodes will be optimal immediately after applying nematodes to trees ( $a_w = 1.00$ ), after which insecticidal activity will gradually decrease as trees dry out and the amount of available free water on the tree surfaces decreases. However, nematodes would also be inclined to move from the exposed macro-environment to the protected micro-environment of the host, with further infection of *P. citri* benefiting from added humidity and free water. A possible method for preventing such a decrease in insecticidal activity of nematodes is to apply overhead irrigation to trees both before and after applying nematodes. Doing so is, however, most probably not feasible, as *P. citri* primarily occur on fruit and leaves that are covered by waxy coatings. The waxes covering citrus fruit and leaves impair the ability of nematodes to stick to the surfaces of such fruit and leaves. Irrigating trees before application could increase application runoff, while nematodes will most probably be washed off leaves if trees are irrigated after application. Such loss of nematodes could be compensated for by applying them during the late afternoon, which would retard desiccation and the possible chance of dew forming on trees the following morning.

As mentioned above, optimal  $a_w$  levels should prevail for as long as possible in order to ensure optimal invasion of the existing insects by nematodes. The amount of free water available on tree surfaces gradually decreases after application as water evaporates, which suggests that nematodes only have a limited period during which to locate and infect hosts before becoming desiccated. It is, therefore, important to identify and to apply a nematode species with an active host-searching ability. Factors that increase desiccation include high temperatures, low levels of relative humidity, and wind. Although higher temperatures increase the rate of desiccation, nematode activity has proved to increase from 15 - 32°C (Lacey *et al.*, 2005), and nematodes should be able to reach hosts within a shorter time, when they are exposed to higher temperatures. The exposure time and temperature experiment demonstrated that nematodes were able to infect mealybugs in an exposure time as short as 30 min at all three temperature levels tested (15°C, 20°C and 25°C), with no significant difference regarding such ability being shown among them. The influence of exposure time on both *H. zealandica* and *S. yirgalemense* was tested at 25°C, in response to which the results showed that *S. yirgalemense* caused significantly higher mortality than did *H. zealandica*. Furthermore a Probit analysis showed *S. yirgalemense* to be three times more potent than *H. zealandica* with  $hour_{50}$  and  $hour_{90}$  values of 16 h and 177 h for *H. zealandica* and 6 and 68 h for *S. yirgalemense* respectively. The results, therefore, suggest that *S. yirgalemense* may possibly perform better than *H. zealandica* in glasshouse and field trials when nematodes are exposed to suboptimal environmental conditions. The results illustrate that the first 2 h to 4 h post application is the most decisive time for establishing successful infection of mealybugs by nematodes.

## Conclusion

The overall results obtained from the different bioassays conducted during this study indicate that both *H. zealandica* and *S. yirgalemense* hold great potential for the control of *P. citri*. They performed very similarly, with only the results of the exposure time experiment and water activity experiment suggesting *S. yirgalemense* to possibly be a more effective biocontrol agent under suboptimal field conditions than *H. zealandica*.

## Evaluating the addition of adjuvants to improve control of *Planococcus citri* (Hemiptera: Pseudococcidae) using entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae)

### Introduction

As above-ground conditions are not optimal for nematode survival (Mráček, 2002; Tomalak *et al.*, 2005), the successful control of mealybugs on citrus is extremely challenging. Such abiotic factors as extreme temperatures (Lacey *et al.*, 2005), ultraviolet radiation (Gaugler and Boush, 1978; Gaugler *et al.*, 1992), wind, and low ambient humidity (Unruh and Lacey, 2001), individually and combined, limit the efficacy of EPNs above ground as a biological control agent.

Desiccation, which is accelerated by low humidity levels and high wind speed, is the most limiting factor, as nematodes require a water film to maintain mobility and ensure survival (Wright *et al.*, 2005). Schroer *et al.* (2005) evaluated the ability of various surfactant-polymer formulations to improve the ability of *Steinernema*

*carpocapsae* (Weiser, 1955) Wouts Mráček, Gerdin and Bedding, 1982 to control the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) on cabbage leaves under above-ground conditions. An application formulation containing 0.3% Rimulgan® (surfactant) and 0.3% of the polymer xanthan (antidesiccant) obtained the best control of diamondback moth, causing >90% at 80% relative humidity (RH) and >70% mortality at 60% RH. Schroer and Ehlers (2005) tested the same formulation for the control of diamondback moth on cabbage leaf bioassays under suboptimal conditions for nematode survival. Their results showed the survival time of *S. carpocapsae* applied with the formulation was found to be 22 h longer at 80% RH and 17 h longer at 60% RH than when the nematodes were applied with water only.

### Stated objectives

The objectives of this study were:

1. to evaluate the effect of adding adjuvants to aqueous suspensions of two nematode species;
2. to determine the effect of the two polymers on the sedimentation of nematodes in aqueous suspensions;
3. the mortality of *P. citri* in laboratory bioassays and;
4. to determine the effects of adjuvants on nematode deposition on citrus leaves.

### Materials and methods

#### Source of nematodes and insects

Experiments were conducted using *H. zealandica*, Poinar, 1990 (SF 41) originally isolated from soil collected in Baviaanskloof near Patensie, Eastern Cape, South Africa (Malan *et al.*, 2006) and *Steinernema yirgalemense* Tesfamariam, Gozel, Gaugler & Adams, 2005 (157-C), originally isolated from soil collected from a citrus orchard near Friedenheim, Mpumalanga (Malan *et al.*, 2011). Infective juveniles (IJs) were cultured according to the procedures described by Kaya & Stock (1997) in *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) and/or *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) larvae, at room temperature. Nematodes were harvested within the first week of emergence and stored in 150 ml of distilled water in 500-ml vented culture flasks. The flasks were stored horizontally at 14°C and shaken weekly to improve aeration. IJs were used for experiments within the first three weeks after harvest. Concentrations used in experiments were quantified by using the method developed by Navon & Ascher (2000). Mealybugs were cultured on butternuts and sprouting potatoes. The identity of *P. citri* used in this study was verified using morphological (Wakgari & Giliomee, 2005) and molecular techniques (Pieterse *et al.*, 2010).

#### Effect of polymers on nematode sedimentation

Two polymer products, Zeba® [starch-g-poly (2-propenamide-co-2-propenoic acid) potassium salt, Tongaat Hulett Starch] and Xanthan gum [polysaccharide (C<sub>35</sub>H<sub>49</sub>O<sub>29</sub>)] were evaluated in terms of their ability to retard sedimentation of *H. zealandica* in a water suspension. Zeba®, at a concentration of 0.1, 0.2 or 0.3%, and Xanthan gum, at a concentration of 0.1 or 0.2%, were added to a nematode concentration of 1 000 IJs/ml. Both polymers were compared to a control that contained nematodes in water only. Treatments were added to 25-ml measuring cylinders (of 1.5 cm diameter) and stirred thoroughly to ensure that the nematodes were evenly distributed. To estimate sedimentation time, a 50 µl sample of the suspension was collected from a depth of 2 cm after 0-, 3-, 10-, 20-, 30- and 60-min intervals from each of three cylinders prepared per treatment (n = 3) and the number of nematodes determined. The experiment was repeated on a separate date. The data of both experiments were pooled for analysis.

#### Effect of two polymers on mortality

Bioassays were conducted by using multiwell bioassay plates (24 wells, flat bottom, Nunc™ Cat. No.144530). Polymer products, Zeba® or Xanthan gum, were added to nematode suspensions containing either *H. zealandica* or *S. yirgalemense*, and mealybug mortality was determined at 60% and 80% RH. Five treatment plates and five control plates, each containing ten evenly distributed adult female mealybugs (n = 50) were prepared for each treatment. Each well was lined with a circular paper disc (of 13 mm diameter) before mealybugs were added. Mealybugs were then inoculated individually with 50 µl containing either *H. zealandica* or *S. yirgalemense* at a concentration of 80 IJs/insect for each of three treatments, which included 1) Zeba® at a concentration of 3 g/L; 2) Xanthan gum at a concentration of 2 g/L; and 3) distilled water. Each treatment received its own control, in which mealybugs were treated with 50 µl of the treatment formulation containing no nematodes. The data of control plates were used to prepare data of treatment plates with Abbott's formula before analysis, in order to compensate for mealybugs that died of causes other than nematode infection (Abbott, 1925). Multiwell plates were covered with fine netting, which allowed airflow while preventing mealybugs from escaping. To achieve the required humidity levels, airtight containers with solutions of glycerol (60% RH) and KNO<sub>3</sub> (80% RH) were

prepared (Winston & Bates, 1960). After treatment, mealybugs were placed in humidity chambers and incubated in a growth chamber with a day cycle starting at 22°C for 14 h and 11°C for 11 h. Mealybug mortality was determined after 72 h. The experiment was repeated on a separate date. The data of both experiments were pooled for analysis. The data of both experiments were pooled for analysis.

#### Effect of adjuvants on nematode deposition

Nematode suspensions containing 1) nematodes in water only; 2) Nu-Film-P® (Poly-1-P-menthene, spreader, sticker; Hydrotech) at a concentration 0.6 ml/L + nematodes; 3) Zeba® at a concentration of 0.3 g/L + nematodes; and 4) Nu-Film-P® + Zeba® + nematodes were applied to citrus trees at the Welgevallen experimental farm, Stellenbosch, Western Cape. Each treatment was applied to randomly selected leaves on individual trees, with a spacing of two untreated trees between the treated trees. Nematodes were applied at a concentration of 1 000 IJs/ml with the aid of calibrated hand-held spray applicators. Leaves were left for 3 min to allow excess fluid to run off before randomly selected leaves were removed from the application area. Two 2-cm<sup>2</sup> discs were cut out from each of the five leaves for each treatment tested (n = 10). Each leaf disc was individually rinsed off in 5 ml tap water, and the number of nematodes present in each suspension was documented. The experiment was repeated on a separate date. The data of both experiments were pooled for analysis.

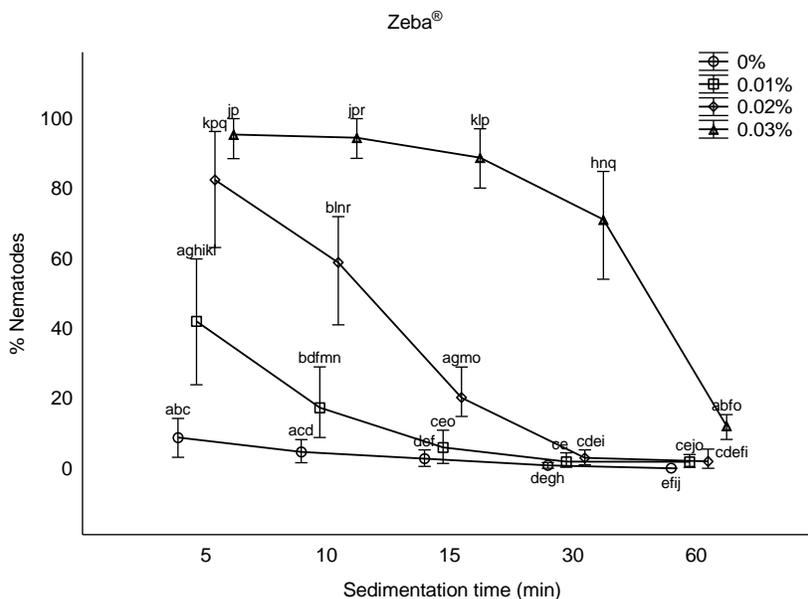
#### Data analysis

Nematode percentages for the effect of two polymers on nematode sedimentation were calculated as a percentage of the initial number of nematodes recorded directly after stirring had ceased. All statistical analyses were performed using Statistica 9.0 software (StatSoft Inc. 2009). Data were analysed using ANOVA, with post-hoc comparison of means using Bonferroni's method, or with a bootstrap multi-comparison if residuals were not normally distributed (Efron & Tibshirani, 1993). Significant differences were determined on a 95% probability level.

### Results and discussion

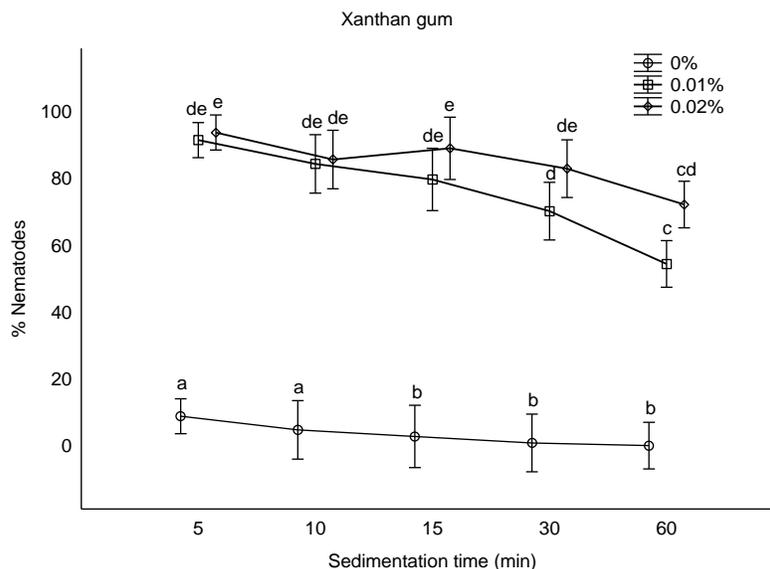
#### Effect of two polymers on nematode sedimentation

Without the addition of a polymer, 91% of nematodes were recorded beyond a depth of 2 cm after 5 min. A repeated measures ANOVA for Zeba® showed interaction between main effects concentration (4 levels; 0%, 0.01%, 0.02%, and 0.03%) and time (5 levels; 5-, 10-, 15-, 30-, and 60 min) ( $F_{(12, 80)} = 28.36$ ;  $P = 0.001$ ). Treatments did not behave consistently over time. Compared to the control, none of the polymer concentration levels tested was able to retard sedimentation significantly 1 h after stirring had ceased. Only the 0.03% Zeba® formulation was able to retard sedimentation significantly ( $P = 0.001$ ) after 30 min sedimentation with 71% of initial nematode number recorded (Fig. 3.5.4.10).



**Fig. 3.5.4.10.** Percentage nematodes recorded at a depth of 2 cm after stirring (95% confidence interval) at set time intervals for different concentrations of the polymer product Zeba®. Data points indicated with the same letters are not significantly different.

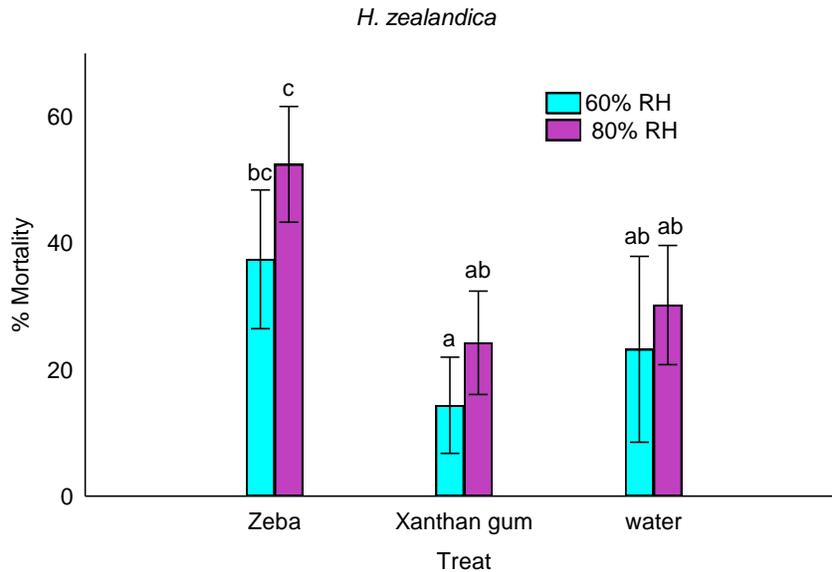
A repeated measures ANOVA for Xanthan gum showed interaction between main effects concentration (3 levels; 0%, 0.01% and 0.02%) and time (5 levels; 5-, 10-, 15-, 30-, and 60 min) ( $F_{(12, 60)} = 5.45$ ;  $P = 0.001$ ). Treatments did not behave consistently over time. Both Xanthan gum concentrations levels tested were able to retard sedimentation significantly at each time intervals recorded, compared to the control (Fig. 3.5.4.11). No significant differences between the addition of 0.01% and 0.02% Xanthan gum were observed at any of the time intervals recorded. A one-way ANOVA ( $F_{(1, 10)} = 2.95$ ;  $P > 0.01$ ) also showed the two concentration levels not to differ significantly. After 60 min sedimentation, 9%, 54% and 72% of the initial nematode number were recorded for 0%, 0.01% and 0.02% Xanthan gum, respectively.



**Fig. 3.5.4.11.** Percentage nematodes recorded at a depth of 2 cm after stirring (95% confidence interval) at set time intervals for different concentrations of Xanthan gum. Data points indicated with the same lettering are not significantly different.

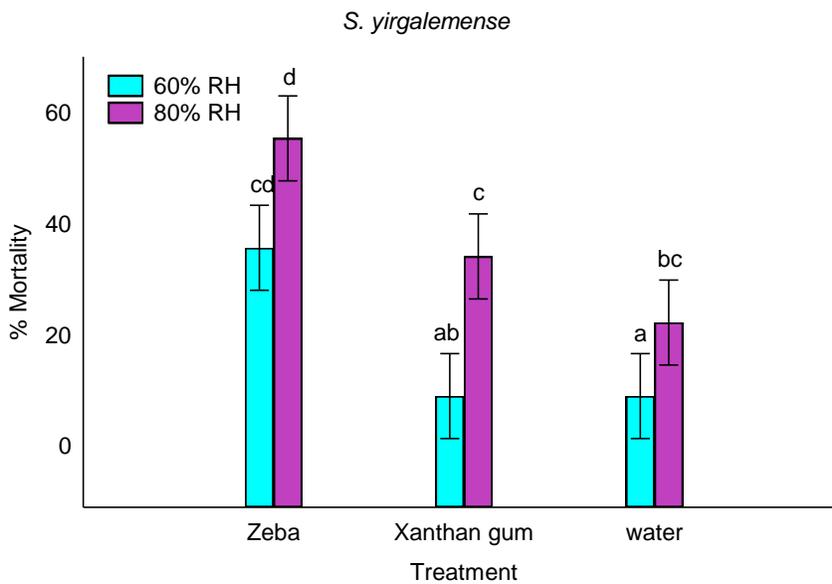
#### Effect of polymers on bioassays

Results of the effect of a suspension containing *H. zealandica* with 0.03% Zeba<sup>®</sup> or 0.02% Xanthan gum on mealybug mortality at 60% and 80% RH were analysed using a two-way ANOVA. No interaction was found between main effects humidity (2 levels; 60% and 80% RH) and adjuvants (2 levels; Zeba<sup>®</sup> and Xanthan gum) ( $F_{(2, 54)} = 0.38$ ;  $P = 0.67$ ). Treatments reacted consistently at each humidity level tested. Although not significant, compared with the water containing nematodes suspension, the Zeba<sup>®</sup> formulation increased mortality by 14% at 60% RH (Fig. 3.5.4.12). The same formulation significantly increased mortality by 22% at 80% RH ( $P = 0.001$ ). The Xanthan gum formulation performed worse than did the control, obtaining 14% control at 60% RH and 24% control at 80% RH, compared with the control that obtained 23% control at RH 60 and 30% control at RH 80. Data for both humidity levels were pooled together for further analysis using a one-way ANOVA ( $F_{(2, 57)} = 15.27$ ;  $P < 0.01$ ), which confirmed that the Zeba<sup>®</sup> formulation obtained significantly higher mortality (50%) than did either the Xanthan gum formulation (19% mortality) or the control (27% mortality).



**Fig. 3.6.4.12.** Percentage mortality (95% confidence interval) of *Planococcus citri* after exposure to 80 infective juveniles per insect of *Heterorhabditis zealandica* in a suspension of Zeba®, Xanthan gum or water only. Different letters above vertical bars indicate significant differences.

Results of the effect of a suspension containing *S. yirgalemense* and 0.03% Zeba® or 0.02% Xanthan gum on mealybug mortality at 60% and 80% RH were analysed using a two-way ANOVA that showed no interaction between main effects humidity (2 levels; 60% and 80% RH) and adjuvants (2 levels; Zeba® and Xanthan gum) ( $F_{(2, 54)} = 0.22$ ;  $P = 0.30$ ). Treatments reacted consistently at each humidity level tested. The Zeba® formulation obtained significantly higher mortality of 36% at 60% RH and of 55% at 80% RH (55% mortality) compared with the Xanthan gum formulation (9% mortality at 60% RH and 34% mortality at 80% RH) and control formulation (9% mortality at 60% RH and 22% mortality at 80% RH) (Fig. 3.5.4.13). Data for both humidity levels were pooled together for further analysis using a one-way ANOVA ( $F_{(2, 57)} = 20.68$ ;  $P < 0.01$ ), which confirmed that the Zeba® formulation obtained significantly higher mortality (46%) than did either the Xanthan gum formulation (22% mortality) or the control (16% mortality).

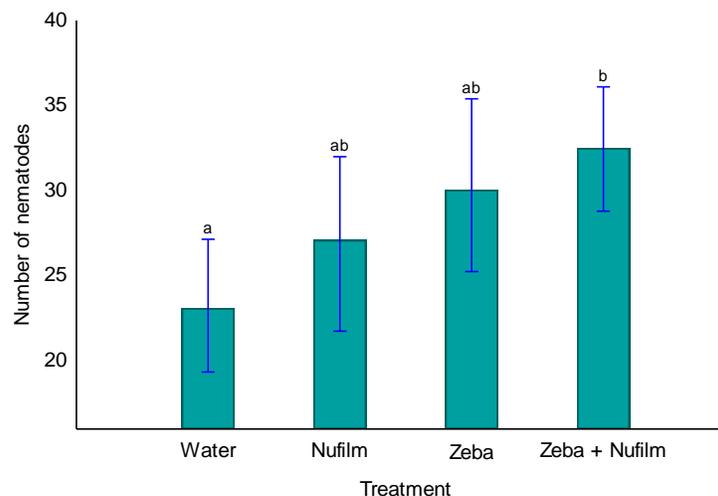


**Fig. 3.5.4.13:** Percentage mortality (95% confidence interval) of *Planococcus citri* after exposure to 80 infective juveniles/insect of *Steinernema yirgalemense* in a suspension of Zeba®, Xanthan gum or water only. Different letters above vertical bars indicate significant differences.

Data for all control plates used for Abbotts formula for corrected mortality were pooled to determine whether Zeba<sup>®</sup> or Xanthan gum had a toxic effect on mealybugs. A one-way ANOVA showed no significant differences ( $F_{(2, 117)} = 0.32$ ;  $P = 0.72$ ) for mortality of mealybugs treated with Zeba<sup>®</sup>, Xanthan gum or water. All control plates caused less than 2.5% mortality of mealybugs.

#### Effect of adjuvants on nematode deposition

Results obtained for the effect of adjuvants on nematode deposition on citrus leaves were analysed using a one-way ANOVA. Significant differences were obtained for numbers of nematodes present on leaf surfaces ( $F_{(3, 76)} = 3.03$ ;  $P < 0.05$ ). Although not significant, the addition of Nu-Film-P<sup>®</sup> and Zeba<sup>®</sup> to nematode application formulations increased the average number of nematodes deposited on 2-cm<sup>2</sup> leaf discs with four and seven nematodes respectively. Only the combined formulation of Nu-Film-P<sup>®</sup> and Zeba<sup>®</sup> significantly increased the average number of nematodes deposited on leaf discs with 10 nematodes ( $P = 0.009$ ). However, the increase observed was not significantly higher than was that observed with the other two formulations tested (Fig. 3.5.4.14).



**Fig. 3.5.4.14:** Mean number of nematodes (95% confidence interval) on 2 cm<sup>2</sup> on citrus leaf discs sprayed with a suspension containing *Heterorhabditis zealandica* infective juveniles with Zeba<sup>®</sup>, Xanthan gum or water only. Different letters above vertical bars indicate significant differences.

*Planococcus citri* is an important pest of citrus in South Africa, with the potential of infesting a high percentage of fruit under certain environmental conditions (Hattingh & Moore, 2003). Their cryptic life style and ability to develop resistance to pesticides necessitates research towards alternative control methods. EPNs, which are lethal pathogens with a wide host range, are considered as a valuable biological control method for a variety of insect pest species. The results discussed in Chapter 2 stressed the need to improve nematode application formulations in order to increase control of *P. citri* under variable environmental conditions. In the current study, sub-optimal conditions for nematode infection, with regard to nematode concentration and humidity, were maintained to simulate field conditions.

In an aqueous suspension, nematodes quickly settle to the bottom of spray tanks, causing uneven distribution. Sedimentation time is an important factor to consider, especially where larger nematode species, such as *Steinernema khoisanae* Nguyen, Malan & Gozel, 2006 with an average length of 1 064 µm (Nguyen et al., 2006), are concerned. The polymer products Zeba<sup>®</sup> and Xanthan gum were evaluated at various concentrations for their ability to retard sedimentation of IJs. Xanthan gum was not evaluated at the highest concentration of 0.03%, as the suspension became too thick to pass through spray nodules, making its use impractical. *Heterorhabditis zealandica* was used in the sedimentation experiment, because IJs of this species have an average length of 685 µm and are larger than those of *S. yirgalemense*, with an average length of 635 µm (Nguyen, 2007). Results of the sedimentation trial showed *H. zealandica* to settle quickly on the bottom of 25-ml measuring cylinders with only 9% of the initial nematode number recorded at a depth of 2 cm, 5 min after stirring. None of the Zeba<sup>®</sup> concentrations tested was able to retard sedimentation significantly after 1 h. Only the 0.03% Zeba<sup>®</sup> formulation was able to retard sedimentation significantly after 30 min, with 71% of the initial nematode number recorded. Nematode suspensions containing Xanthan gum were able to retard sedimentation significantly at both concentration levels, tested after 1 h sedimentation. The above-mentioned results are similar to those that were obtained by Schroer *et al.* (2005) that showed Xanthan gum (0.01% and 0.02%) to retard

sedimentation of *S. carpocapsae* effectively. Results also showed *S. carpocapsae* to settle quickly in water with 50% and 10% of the initial nematode number recorded at a depth of 2 cm after 5 min and 1 h sedimentation respectively. IJs of *S. carpocapsae*, being smaller in size (558 µm), settle much slower than do larger IJs (685 µm length) of *H. zealandica* (Nguyen, 2007), with 9% and 0% of the initial nematode number being recorded for *H. zealandica* after 5 min and 1 h respectively.

Although Xanthan gum performed much better than did Zeba<sup>®</sup> with regard to sedimentation, it was not the case for mortality of *P. citri* in bioassays. A nematode suspension containing 0.03% Xanthan gum performed poorly when the polymer's ability to increase the infectivity *P. citri* for both *H. zealandica* and *S. yirgalemense* was evaluated at 60% and 80% RH. The addition of Xanthan gum to nematode suspensions caused no significant increase in adult female *P. citri* mortality in any of the bioassays. Although not significant, the addition of Zeba<sup>®</sup> to *H. zealandica* application suspension increased mortality by 14% at 60% RH, and at 80% RH the same formulation significantly increased mortality with 22%. Similar results were obtained using *S. yirgalemense* and the addition of Zeba<sup>®</sup> to nematode suspensions increased mortality from 9% to 36% at 60% RH and from 34% to 55% at 80% RH. De Waal (2011) also tested Zeba<sup>®</sup> to improve control of diapausing codling moth, *Cydia pomonella* (Linnaeus) in tree trunk bioassays with *H. zealandica*, showing Zeba<sup>®</sup> to increase mortality significantly at both 60% and 80% RH, by 15% and 19% respectively.

Zeba<sup>®</sup> and Xanthan gum proved not to be toxic to mealybugs, as less than 2.5% mortality was obtained after mealybugs were treated with Zeba<sup>®</sup> or Xanthan gum, and did not obtain significantly higher mortality than was obtained with mealybugs treated with water only. Due to the poor performance of Xanthan gum, it was not evaluated further for improvement of nematode deposition on citrus leaves.

Citrus leaves and fruit have a waxy cuticle and, therefore, the ability of nematode application suspensions to stick to their surfaces is greatly impaired. The possibility of using a surfactant, Nu-Film-P<sup>®</sup>, and the polymer product, Zeba<sup>®</sup>, to stick IJs to leaf surfaces was evaluated. Results showed the individual addition of Nu-Film-P<sup>®</sup> and Zeba<sup>®</sup> to nematode application suspensions increased the average number of nematodes deposited on 2-cm<sup>2</sup> citrus leaf discs, by four and seven nematodes respectively. However, a significant increase was obtained when using the combination of Nu-Film-P<sup>®</sup> and Zeba<sup>®</sup>, increasing the average number of nematodes on leaf discs by 10 nematodes. Although nematodes will move to the same protected habitats as those occupied by *P. citri*, the additions of such adjuvants would not only increase their numbers on the leaves, but also protect them against desiccation on the exposed leaf area, which would be advantageous to movement and to the survival of the nematodes.

## Conclusion

The investigation has shown that the addition of 0.03% Zeba<sup>®</sup> to nematode suspensions effectively hinders their sedimentation, resulting in a more even distribution. The same adjuvant also increased mealybug mortality in bioassays at 60% and 80% RH by retarding desiccation, extending nematode survival and improving mobility. Furthermore, the combined addition of both Nu-Film-P<sup>®</sup> and Zeba<sup>®</sup> increased application deposits on leaf surfaces, reducing the loss of nematodes by runoff. To further determine the ability of such adjuvants to improve the control of *P. citri* on citrus by nematodes, it should be tested under glasshouse and field conditions.

## Compatibility of *Heterorhabditis zealandica* and *Steinernema yirgalemense* with agrochemicals and biological control agents

### Introduction

When nematodes are applied to the aerial parts of trees, their broad host range could also be problematic if beneficial insects present during application are also susceptible (Hazir *et al.*, 2003).

Rojht *et al.* (2009) reported high mortality of larvae of the two-spotted lady beetle, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae), and those of the lacewing species, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) when exposed to *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin and Bedding, 1998; *S. carpocapsae* (Weiser, 1955) Wouts Mráček, Gerdin and Bedding, 1982, and *Heterorhabditis bacteriophora* Pionar, 1976. In contrast, Shapiro-Ilan and Cottrell (2005) found four ladybug beetle species to be significantly less susceptible to *H. bacteriophora* and *S. carpocapsae* than was the black cutworm, *Agrotis ipsilon* (Hüfnagel) (Lepidoptera: Noctuidae), which is a known susceptible host. The above-mentioned studies show that the susceptibility of beneficial insects to nematodes varies, depending on the nematode, insect stage and combination tested. Such beneficial insects as the predatory lady beetle, *Cryptolaemus montrouzieri* (Mulsant)

(Coleoptera: Coccinellidae), and the parasitoid, *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae), are mass reared and made commercially available to citrus farmers in South Africa. Such beneficial organisms have been shown to play a vital role in IPM programmes for citrus (Hattingh & Moore, 2003). The susceptibility of such beneficial insects to a specific nematode species should first be determined, before the nematode species are applied in an IPM programme.

In contrast, when applied as part of an IPM programme, nematodes are exposed to a variety of agrochemicals and biological control formulations that could be toxic and impair nematode performance. Although some pesticides retard nematode persistence and infectivity (Zimmerman & Cranshaw, 1990; Patel *et al.*, 1997), a study conducted by Rovesti and Deseö (1990) showed nematodes to be tolerant to short periods (2–4 h) of exposure to the majority of 75 commercial pesticides tested. When applying a specific nematode species, one should also consider the fact that nematodes tend to vary in reaction to pesticides, depending on the nematode species and pesticide formulation concerned (Grewal, 2002). Nematode tolerance to agrochemicals allows the possibility of tank-mixing nematodes with other agrochemical and biopesticide formulations that will save time and labour costs and/or help achieve better control of a target pest species (Koppenhöfer & Grew, 2005).

### Stated objectives

The objective of the current study was to evaluate the compatibility of two indigenous EPN species with:

1. a commercially used biological control insect used against mealybugs;
2. two biocontrol formulations;
3. an agrochemical and;
4. two adjuvants;

to which they will most likely be exposed in an IPM programme for citrus in South Africa.

### Materials and methods

#### Source of nematodes and biocontrol agents

Infective juveniles (IJs) of *H. zealandica* Poinar, 1990 (SF 41 strain) (Malan *et al.*, 2006) and *S. yirgalemense* Tesfamariam, Gozel, Gaugler and Adams, 2005 (157-C strain) (Malan *et al.*, 2011) were produced at room temperature in last-instar *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) by using the procedures described by Kaya and Stock (1997). After being harvested, IJs were stored horizontally in 500-ml culture flasks containing 150-ml distilled water at 14°C. Culture flasks were shaken weekly to improve aeration and nematode longevity. IJ concentrations used in experiments were quantified by using the method developed by Navon and Ascher (2000). Nematodes were used within the first three weeks after harvesting.

Mealworm larvae were reared at room temperature in plastic containers on fine wheat bran. *Cryptolaemus montrouzieri* (Mulsant) (Coleoptera: Coccinellidae) larvae and adult beetles were obtained from the commercial company Du Roy IPM, Letsitele, Limpopo Province, South Africa.

#### Susceptibility of *C. montrouzieri* to nematodes

To assess the susceptibility of the biological control agent *C. montrouzieri* to *H. zealandica* and *S. yirgalemense*, adult beetles and larvae were individually exposed to IJs in multiwell plates (24 wells, flat bottom, Nunc™ Cat. No.144530). Five treatment plates and five control plates (5 replicates; 50 insects), each containing ten evenly distributed larvae or adult beetles, were prepared. Each well was lined with a circular paper disc (of 13-mm diameter) before adults and larvae were added. They were then inoculated individually with 80 IJs of either *H. zealandica* or *S. yirgalemense*. Control plates received 50 µl of distilled water only. To retain insects in their individual wells, each plate was covered with a piece of glass fitted as a lid. After inoculation, the plates were placed in plastic containers lined with moistened paper towels and closed to ensure high humidity levels (RH ± 95%). Plastic containers were then incubated in a dark growth chamber at 25 ± 2°C for 48 h, after which the mortality of mealybugs was determined by means of gentle prodding. Insects were then rinsed to remove nematodes from their body surface, transferred to clean Petri dishes (9-mm-diameter) and incubated for another 48 h. To confirm mortality due to nematode infection, each cadaver was dissected with the aid of a dissection microscope. The experiment was repeated on a separate test date. The data of both experiments were pooled for analysis.

#### Influence of a pesticide, two biopesticides and two adjuvants on EPN survival and persistence

The compatibility of *H. zealandica* and *S. yirgalemense* with an insecticide, Cyperfos 500 E.C.® [450 g/L chlorpyrifos (organophosphate), 50 g/L cypermethrin (pyrethroid)], two biopesticides, Cryptogran™ [*Cryptophlebia leucotreta* granulovirus (CrleGC-SA)] and Helicovir™ (nucleopolyhedrovirus) and two adjuvants,

Nu-Film-P® (Poly-1-P-menthene, spreader, sticker, Hydrotech) and Zeba® [starch-g-poly (2-propenamide-co-2-propenoic acid) potassium salt, Tongaat Hulett Starch], were tested. Nematode suspensions containing 4000 IJs/ml were prepared for each nematode species tested. Product formulations were then prepared at twice the recommended dose (Table 3.5.4.5). One ml of nematode suspension and one ml of product formulation was then added to Petri dishes and kept in a growth chamber at 25 ± 2°C. Five Petri dishes were prepared for each product and nematode species combination. Nematode survival was compared to five control dishes that contained nematodes in water only. To estimate nematode survival, 10 µl samples were collected until the first 50 IJs were documented as being either alive or dead. Nematode mortality was assessed directly after preparing the treatments (0 h) and again after 6, 12 and 24 h.

**Table 3.5.4.5.** Recommended dosage of substance.

Product	Active ingredient	Use	Concentration/ L
Cyberphos 500 E.C.®	Chlorpyrifos and Cypermethrin	Insecticide	1.00 ml
Cryptogran™	<i>Cryptophlebia leucotreta</i> granulovirus	FCM control	2.50 ml
Helicovir™	Nucleopolyhedrovirus	Bollworm control	0.12 ml
Nu-Film-P®	Poly-1-P-menthene	Spreader, sticker	0.60 ml
Zeba®	starch-g-poly (2-propenamide-co-2-propenoic acid) potassium salt	Antidesiccant	3.00 g

To determine whether the nematodes were still virulent after exposure to products, an additional 5 ml of each treatment was prepared and kept in the same growth chamber. After 24 h, each treatment was diluted in 1 L of distilled water. Nematodes were allowed to settle to the bottom of measuring cylinders, after which excess fluid was siphoned off to 10 ml. Five Petri dishes for each treatment and five control dishes, each containing ten mealworm larvae (5 replicates; 50), were prepared for each product tested. Treatment dishes were inoculated with a concentration of 100 IJs/insect. Control dishes received water only. After 48 h, insect mortality was assessed. This experiment in its entirety was repeated on a different test date. The data of both experiments were pooled for analysis.

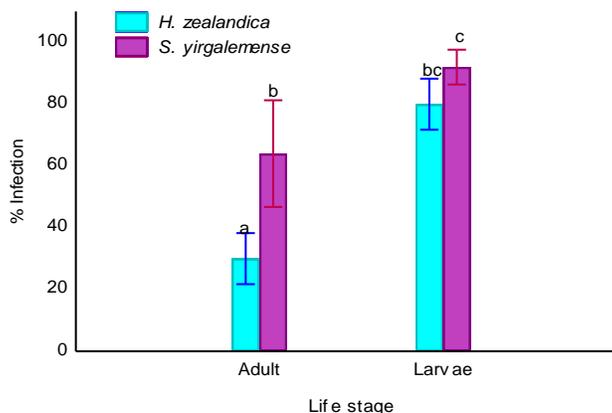
#### Data analysis

Before analysis, data for mealworm mortality were corrected using Abbott's (1925) formula, in order to compensate for those mealworms that might have died of natural causes. All statistical analyses were performed by means of Statistica 9.0 software (StatSoft Inc. 2009). Data were analysed using ANOVA, with post-hoc comparison of means using Bonferroni's method, or with a bootstrap multi comparison if residuals were not evenly distributed (Efron & Tibshirani, 1993). Significant differences were determined on a 95% probability level.

### **Results and discussion**

#### Susceptibility of *C. montrouzieri* to nematodes

Adult beetles and larvae of *C. montrouzieri* were screened for susceptibility to *H. zealandica* and *S. yirgalemense*. As the mortality of both adults and larvae was high in control plates, Abbott's formula could not be used to compensate for those mealybugs that died of natural causes (Abbott, 1925), and results were given as a percentage of nematode infection. A two-way ANOVA ( $F_{(1, 36)} = 5.31$ ;  $P = 0.03$ ) showed slight interaction between treatments and main effects could not be interpreted directly. Results showed *H. zealandica* to be significantly more virulent to beetle larvae, obtaining 80% infection ( $P = 0$ ), than to adult beetles, with only 30% infection (Fig. 3.5.4.15). Beetle larvae were also significantly more susceptible to *S. yirgalemense*, with 92% infection ( $P = 0.001$ ), than to adults, with 64% infection. *Steinernema yirgalemense* obtained significantly higher control of adult beetles compared to that attained with *H. zealandica* ( $P = 0$ ). Although not significant, *S. yirgalemense* obtained 12% higher infection of beetle larvae than did *H. zealandica*. Adult beetles in treatment plates were observed to secrete an obnoxious smelling yellowish substance compared to control plates where no such secretion was noted.

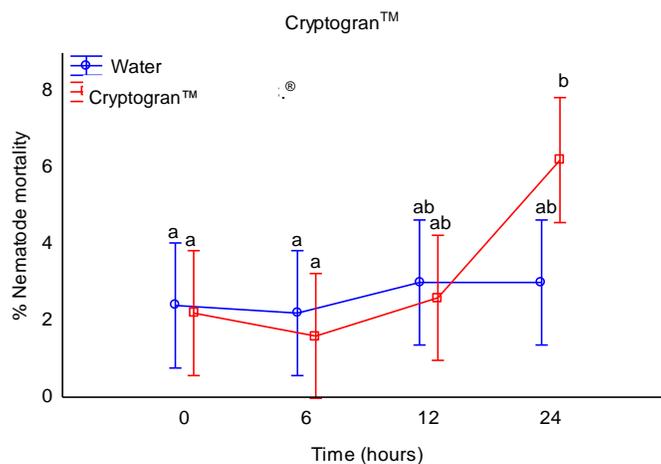


**Fig. 3.5.4.15.** Mean percentage (95% confidence interval) infection recorded for *Cryptolaemus montrouzieri* adults and larvae, 48 h after being inoculated with 80 IJs/insect in multiwell bioassay plates. Means with the same letter are not significantly different.

Influence of a pesticide, two biopesticides and two adjuvants on EPN survival and persistence

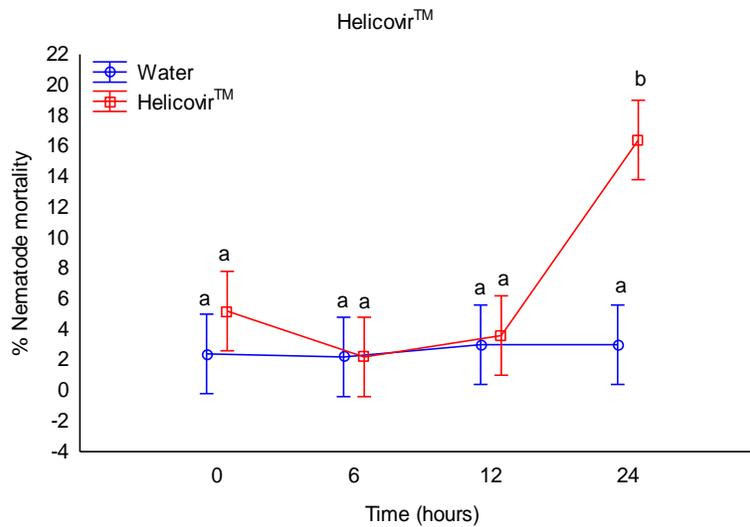
Results of nematode survival over time were analysed by using a two-way ANOVA, with treatment (2 levels; agricultural product and water) and time (4 levels; 0, 6, 12, 24) as the main effects. Each treatment was compared to their corresponding control, containing either *H. zealandica* or *S. yirgalemense* only. No significant differences in *H. zealandica* mortality were observed over time, or compared to the control for any of the treatments tested.

Zeba<sup>®</sup> also proved to have no influence on *S. yirgalemense* over time, as no significant differences in nematode mortality were observed over time when the treatment was compared to the control. A two-way ANOVA that compared nematode response to Cryptogran<sup>™</sup> over a specified length of time to their response in water only showed no interaction between main effects ( $F_{(3, 72)} = 2.72$ ;  $P = 0.07$ ), which indicated nematodes to respond consistently to both the treatment and control over time (Fig. 3.5.4.16). Mortality of *S. yirgalemense* IJs exposed to Cryptogran<sup>™</sup> increased significantly after 24 h, from 2% to 6% mortality. The increase in mortality noted was, however, not significantly higher than was the 3% mortality ( $P = 0.21$ ) recorded for the control after 24 h.



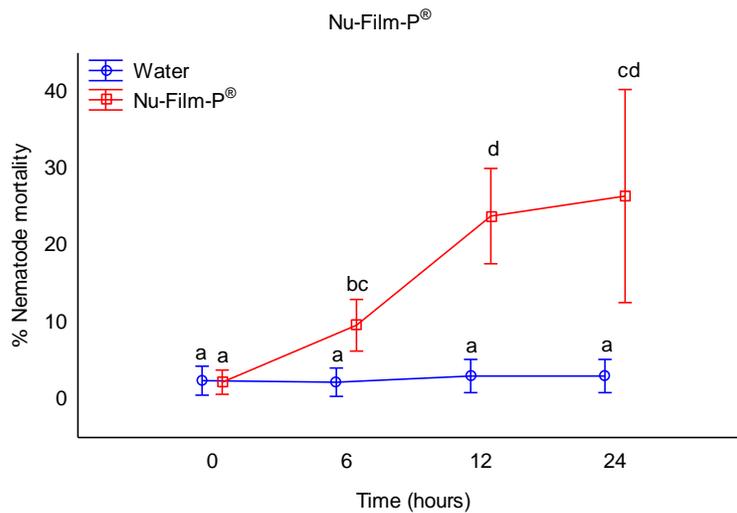
**Fig. 3.5.4.16.** Mean percentage (95% confidence interval) mortality of IJs of *Steinernema yirgalemense* after exposure to Cryptogran<sup>™</sup> over time. Means with the same letter are not significantly different.

Results for the same control treatment for *S. yirgalemense*, as compared with Cryptogran<sup>™</sup>, were also individually compared to Helicovir<sup>™</sup>, Nu-Film-P<sup>®</sup> and Cyperphos 500 E.C.<sup>®</sup>. When results for nematode mortality over time were analysed using a two-way ANOVA, they showed interaction between main effects, indicating that nematodes did not respond consistently to treatments compared to the control. A significant increase in nematode mortality from < 6% to 16% was recorded after 24 h exposure to Helicovir<sup>™</sup> (Fig. 3.5.4.17.). This increase in mortality was also significantly higher than the 3% mortality recorded for the control after 24 h ( $P = 0.001$ ).



**Fig. 3.5.4.17.** Mean percentage (95% confidence interval) mortality of IJs of *Steinernema yirgalemense* after exposure to Helicovir™ over time. Means with the same letter are not significantly different.

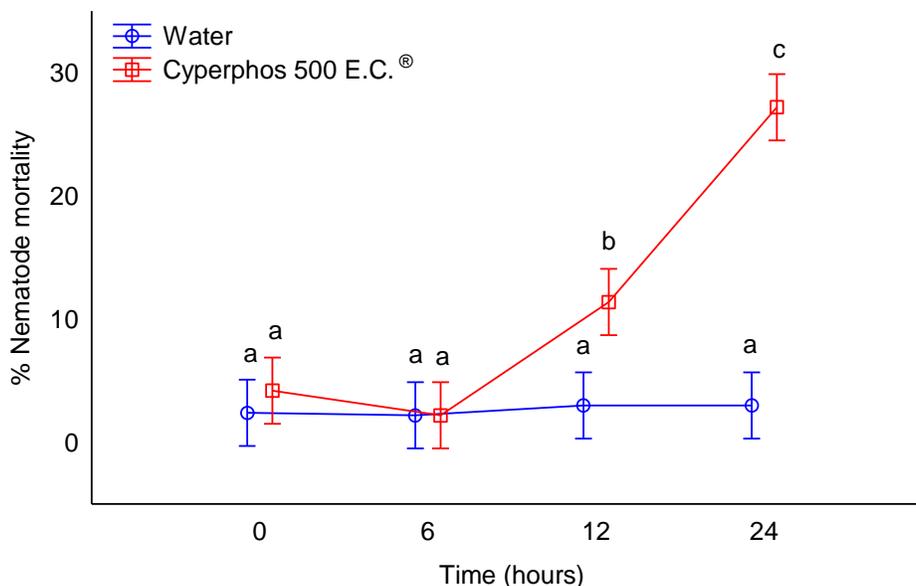
After only 6 h exposure to Nu-Film-P®, nematode mortality increased significantly ( $P = 0.001$ ) from 2% to 10% (Fig. 3.5.4.18). The increase was also significantly higher ( $P = 0.01$ ) than the 2% mortality recorded for the control after 6 h. Nematode mortality increased after further exposure, with up to 26% mortality recorded after 24 h.



**Fig. 3.5.4.18.** Mean percentage (95% confidence interval) mortality of IJs of *Steinernema yirgalemense* after exposure to Nu-Film-P® over time. Means with the same letter are not significantly different.

A significant increase in nematode mortality from > 5% to 11% was recorded for nematodes after 12 h exposure to Cyperphos 500 E.C.® (Fig. 3.5.4.19). The increase in mortality noted was also significantly higher ( $P = 0.001$ ) than the 3% mortality recorded for the control after 12 h. Nematode mortality significantly ( $P = 0.001$ ) increased to 27% after an additional 12 h exposure to Cyperphos E.C.®.

Cyperphos 500 E.C.®



**Fig. 3.5.4.19.** Mean percentage (95% confidence interval) mortality of IJs of *Steinernema yirgalemense* after exposure to Cyperphos 500 E.C.® over time. Means with the same letter are not significantly different.

Results for mealworm mortality obtained by *H. zealandica* and *S. yirgalemense* after exposure to the above-mentioned agrochemicals were individually analysed with the use of a one-way ANOVA, which showed treatments not to differ significantly ( $P < 0.01$ ), and no significant differences were observed. Mealworm mortality of  $> 89\%$  and  $> 98\%$  were recorded, for *H. zealandica* and *S. yirgalemense* respectively, for all treatments of IJs after 24 h.

In South Africa, citrus production is plagued by a complex of major and minor insect pest species (Bedford *et al.*, 1998). In order to save time and labour costs, to achieve better control of a single pest, or to target more than one pest, it would be desirable to tank mix and apply agrochemicals with a biocontrol agent or with more than one biocontrol agent at the same time. The simultaneous application of pesticides and EPNs is possible, as some species have proven tolerant to short periods of exposure to certain pesticides (Rovesti & Deseö, 1990). Tolerance to agrochemicals is also advantageous, as nematodes can be applied within a short time interval after the application of chemicals. Above-ground insect pests in general are more susceptible to nematodes, as they did not develop resistance mechanisms over the aeons through cohabitation with nematodes (Kaya & Hara, 1981). When applying nematodes to control above-ground pests, it is important to remember that EPNs are lethal pathogens of a wide range of insect pest species (Griffin *et al.*, 2005), and could possibly also infect non-target organisms, such as the natural prey insect enemy populations.

*Planococcus citri* is a serious pest of a variety of economically important crops and ornamental plants throughout the world (Cox, 1981), and is considered the most common and destructive species of mealybug to attack citrus in South Africa (Hattingh *et al.*, 1998). *Cryptolaemus montrouzieri* is a coccinellid beetle, also called the mealybug lady beetle or the mealybug destroyer, and is considered a valuable biological control agent aimed at *P. citri* (Hattingh & Moore, 2003). Both the adult and the larvae of the beetle are voracious feeders, preying dominantly on mealybugs. In South Africa, *C. montrouzieri* is produced by a commercial company, Du Roi IPM.

Results of the susceptibility of *C. montrouzieri* larvae to *H. zealandica* and *S. yirgalemense* showed beetle larvae to be highly susceptible to both nematode species, with an infection rate of 80% for *H. zealandica* and of 92% for *S. yirgalemense*. The adult beetle was also found to be susceptible to nematode infection, with an average mortality of 30% in the case of *H. zealandica* and of 64% for *S. yirgalemense*. Unlike adult beetles in control plates, beetles exposed to nematodes were observed to excrete an abnoxious smelling yellowish liquid indicating its secretion as a defence mechanism used to prevent nematode infection. Adult beetles are also very mobile which will further impair nematode infection. These results indicate that the susceptibility of natural enemies to nematodes depends on the nematode–insect species interaction, as adults were found to be twice as susceptible to *S. yirgalemense* as to *H. zealandica*. Shapiro-Ilan and Cottrell (1995) and Rojht *et al.*, (2009) found comparable results using different beetle species.

The high susceptibility of beetle larvae and even of adult beetles to both nematode species, which is especially high for *S. yirgalemense*, should be taken into consideration when both of these biocontrol agents are to be applied in a citrus orchard. Adults of *C. montrouzieri* live up to two months, with a female producing up to 500 eggs and having the potential of being persistent (Smith *et al.*, 1997). As nematodes have only a short window period for mealybug infection after application, they should be applied before *C. montrouzieri* release. In Chapter 2, bioassays on *P. citri*, in the lethal nematode concentration trial conducted under similar conditions as were the bioassays in the current study, showed *H. zealandica* and *S. yirgalemense* to obtain up to 89% control of adult female *P. citri*, indicating *C. montrouzieri* larvae to be just as susceptible to nematodes as to the target pest species. Results also indicated that the nematode species should also not be applied when *C. montrouzieri* larvae are already present in high numbers on the trees concerned.

## Conclusion

Nematode tolerance to three agrochemicals and two biocontrol formulations, to which they are likely to be exposed in an IPM programme for citrus in South Africa, showed *H. zealandica* mortality after exposure to Zeba<sup>®</sup>, Nu-Film-P<sup>®</sup>, Cryptogran<sup>™</sup>, Helicovir<sup>™</sup> and Cyperphos 500 E.C.<sup>®</sup> over a 24-h period to be unaffected by any of the products tested. Significant increases in nematode mortality were, however, observed for *S. yirgalemense* after 12 h exposure to Cryptogran<sup>™</sup>, Helicovir<sup>™</sup> and Cyperphos 500 E.C.<sup>®</sup>. Results indicate that *S. yirgalemense* should not be tank mixed with the above-mentioned products for prolonged periods before application. A significant increase in *S. yirgalemense* mortality was also observed after 6 h exposure to Nu-Film-P<sup>®</sup>. To determine exactly how long *S. yirgalemense* is tolerant to Nu-Film-P<sup>®</sup>, the same experiment should be conducted, but nematode mortality should be recorded every hour for six h. Although *S. yirgalemense* proved to be sensitive to some of the formulations tested, results for nematode infection showed that none of the products tested influenced the ability of either nematode species tested to infect hosts after 24 h.

## Evaluating the efficacy of a polymer-surfactant formulation to improve control of *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) under simulated natural conditions

### Introduction

The family Pseudococcidae, commonly referred to as mealybugs, comprising about 2 200 species in almost 274 genera (Ben-Dov *et al.*, 2010), of which the citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae), is considered the most destructive (Cadee & Van Alphen, 1997; Blumberg & Van Driesche, 2001). *Planococcus citri* is highly polyphagous and is known to infest such commercially produced hosts as citrus, coffee, vineyards and a variety of ornamental plants (Cadee & Van Alphen, 1997; Mustu *et al.*, 2008).

In South Africa, seven mealybug species, of which *P. citri* is the most important, are regarded as economically damaging citrus pests (Hattingh *et al.*, 1998). Mealybugs feed on all parts of citrus trees, except the roots {Canhilar, 2001 73 /id}, causing both direct and indirect damage {Hattingh, 1996 195 /id}, such as wilting, premature fruit and flower drop, growth deformation and sooty mould growth {Blumberg, 1995 117 /id}. During winter, mealybugs occur throughout the tree canopy, generally residing in cracks and crevices or leaf axils (Hattingh, 1993; Smith *et al.*, 1997). First-generation nymphs emerge during spring or early summer, moving to the foliage to colonise fruitlets and young growth (Martinez-Ferrer *et al.*, 2006), settling in protected sites, such as under fruit calyxes or in between fruit clusters {Hattingh, 2003 181 /id}. Multiple overlapping generations of *P. citri* occur during a single growing season (Wakgari & Giliomee, 2003), with the highest population numbers occurring between mid- and late summer, parallel with fruit growth intensity (Franco *et al.*, 2004).

Although mealybugs are generally controlled with chemicals (Franco *et al.*, 2004), this method of control is not ideal. Mealybugs are known to develop resistance (McKenzie, 1967; Blumberg & Van Driesche, 2001; Mahfoudhi & Dhoubi, 2009) and the continuous applications of broad-spectrum pesticides has proven to be partially responsible for pest outbreaks (Michelakis & Hamid, 1995), as they disrupt natural enemies, usually keeping mealybug populations under control (Hattingh, 1993; Hattingh & Tate, 1996; Hattingh *et al.*, 1998; Hattingh & Moore, 2003). The success of chemical control is further impaired by mealybugs being covered with protective waxes and displaying cryptic behaviour, residing in protected sites where they cannot be reached by chemicals (McKenzie, 1967; Michelakis & Hamid, 1995; Franco *et al.*, 2004). Growing public awareness of detrimental environmental impact and of health risks associated with pesticides has further pressured citrus growers into trying to find alternative control methods (Hussaini, 2002).

The application of natural enemies is considered the most feasible alternative to chemical insect control (Hussaini, 2002). In citrus orchards, mealybug populations are usually controlled by means of natural enemies, if the behaviour of the latter is not disrupted by the application of pesticides (Hattingh, 1993; Hattingh & Tate, 1996; Hattingh *et al.*, 1998; Hattingh & Moore, 2003). After winter, however, natural enemy population numbers tend to increase slowly and early spring population densities are usually insufficient to prevent early feeding damage (Hattingh, 1993; Franco *et al.*, 2004).

Entomopathogenic nematodes (EPNs) in the order Rhabditida belong to the families Steinernematidae and Heterorhabditidae and are fatal pathogens of insect. Such nematodes are used as inundatively applied biological control agents against a wide variety of economically important insect pests (Grewal *et al.*, 2005). EPNs are, however, primarily applied to control the soil stages of insects (Arthurs *et al.*, 2004). Controlling foliar pests with nematodes in orchards is still a relatively new field of study, and is extremely challenging, as nematodes require a water film to maintain mobility and to ensure survival (Wright *et al.*, 2005). Above-ground conditions are not optimal for nematode survival (Mráček, 2002; Tomalak *et al.*, 2005), as nematodes are exposed to such limiting abiotic factors as ultraviolet radiation (Gaugler & Boush, 1979; Gaugler *et al.*, 1992), extreme temperatures (Lacey *et al.*, 2005), low ambient humidity and wind (Unruh & Lacey, 2001).

Nematodes are usually applied to foliage as an aqueous suspension by means of ordinary chemical- spraying equipment (Grewal, 2002; Hussaini, 2002). Water retention agents can be added to application formulations to retard desiccation (Glazer *et al.*, 1992), thus increasing the duration of nematode survival on foliage (Webster & Bronskill, 1968; Shapiro *et al.*, 1985; Glazer & Navon, 1990). According to Tomalak *et al.* (2005), the successful control of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in glasshouses is a result of the sensible use of adjuvants, which improve nematode distribution on foliage. A nematode application formulation containing 0.3% Rimulgan<sup>®</sup> (surfactant) and 0.3% of the polymer xanthan (antidesiccant) obtained more than 90% control at 80% relative humidity (RH) and > 70% control at 60% RH of the diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), on cabbage leaves (Schroer & Ehlers, 2005). The same formulation was also evaluated by Schroer and Ehlers (2005) for DBM control on cabbage-leaf disc assays, with results showing nematodes to survive 22 h longer at 80% RH and > 17 h longer at 60% RH than the control. Further field studies for DBM control on cabbage were conducted by Schroer and Ehlers (2005). The formulation significantly reduced the number of insects per plant, resulting in > 50% control after seven days. No significant effect was, however, recorded when compared to that achieved with a formulation containing nematodes only and with a surfactant, with the difference in effect being attributed to the high ambient humidity that prevailed in the experimental unit and the moist microclimate in the cabbage heads, favouring nematode survival.

### **Stated objectives**

The objective of this study was to evaluate the potential of a surfactant-polymer formulation added to nematode application suspensions to improve the ability of nematodes to control *P. citri* on citrus under simulated glasshouse conditions and semi-field trials.

### **Materials and methods**

#### Source of nematodes and insects

Infective juveniles (IJs) of *Heterorhabditis zealandica*, Poinar, 1990 (SF 41) and *Steinernema yirgalemense* Tesfamariam, Gozel, Gaugler and Adams, 2005 (157-C) were produced in last-instar mealworm larvae, *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae), at room temperature, according to the procedures described by Kaya and Stock (1997). After harvest, IJs were stored horizontally at 14°C in 500- ml vented culture flasks containing 150 ml distilled water. Flasks were shaken weekly to improve aeration and nematode survival. IJs were used within the first three weeks after emerging and harvested from white traps (White, 1927). Nematodes were kept at 22°C for 24 h prior to use in all experiments but the semi-field experiment, prior to which nematodes were kept at room temperature for 24 h. Before conducting experiments, IJ concentrations were quantified for all trials by using the method developed by Navon and Ascher (2000).

Mealybugs were laboratory-raised in ventilated cages (650 mm × 350 mm × 590 mm) on butternuts and sprouting potatoes. The identity of *P. citri* used in this study was verified using morphological (Wakgari & Giliomee, 2005) and molecular techniques (Pieterse *et al.*, 2010).

#### Growth chamber assay using leaves and fruit

To simulate glasshouse conditions, large plastic containers were filled with water and placed at the bottom of growth chambers to increase humidity. Leaves were obtained from a citrus orchard at Welgevallen experimental farm, Stellenbosch, Western Cape. Citrus fruits were obtained from a local supermarket. To eliminate other organisms, leaves and fruit were washed in a solution of water and 0.01% household bleach, rinsed thoroughly in tap water and left to dry before use. Leaves were cut to fit 13-cm-diameter Petri dishes lined with moist filter paper. Eight adult female mealybugs were transferred to each of eight leaves (8 replicates; 64 insects), for each treatment. After adding the mealybugs, the Petri dishes were covered with a lid to keep the mealybugs from escaping and the citrus leaves from drying out. Citrus fruit were cut in half, with each half being placed in a small, round plastic container (250-ml), with the open end facing to the bottom. Eight fruits with eight mealybugs each were prepared (8 replicates; 64 insects) and covered with a lid to prevent the mealybugs concerned from escaping. The mealybugs were then left for 24 h to settle on leaves and fruit before treatment.

The two adjuvants used were Zeba<sup>®</sup> [starch-g-poly (2-propenamide-co-2-propenoic acid) potassium salt, Tongaat Hulett Starch] and Nu-Film-P<sup>®</sup> (poly-1-pmenthene, spreader/sticker, Hygrotech). Treatments were: 1) water, as control; 2) *H. zealandica*; 3) *H. zealandica* + Zeba<sup>®</sup> + Nu-Film-P<sup>®</sup>; 4) *S. yirgalemense*; and 5) *S. yirgalemense* + Zeba<sup>®</sup> + Nu-Film-P<sup>®</sup>. Nematodes were applied to leaves and fruit with the aid of calibrated handheld spray applicators at a concentration of 2000 IJs/ml. Zeba<sup>®</sup>, and Nu-film-P<sup>®</sup> were used in treatments at a concentration of 0.03% and 0.06% respectively. Treatment formulations were prepared 1 h before each trial. After treatment, plastic containers with fruit were covered with fine-mesh netting to allow airflow, while preventing mealybugs from escaping. Treatments were arranged according to a randomised design in a growth chamber at 22°C and 75 ± 8% RH.

Leaves were left for three minutes after treatment to eliminate excess runoff and placed in small pockets made out of the same fine-mesh netting that covered the plastic containers with fruit. Pockets with leaves were then hung in a randomised block design in the same growth chamber as that which was used for the fruit. Leaves were hung directly above fruit containers receiving the same treatment. After 48 h, mealybugs were removed from the leaves and the fruit and mortality were assessed. The mealybugs were then washed to remove surface nematodes and placed in Petri dishes (13-cm-diameter), lined with moistened filter paper, and incubated for a further 48 h at 25°C. After further incubation, each cadaver was dissected with the aid of a dissection microscope to confirm mortality due to nematode infection. Temperature and humidity levels were monitored by Hobo<sup>®</sup> H8 Pro Series data loggers, which were placed inside the growth chambers. The experiment was repeated on a separate test date. The data of both experiments were pooled for analysis.

#### Effect of polymer-surfactant formulation on IJ infectivity

The ability of a polymer-surfactant formulation to increase the infectivity of *S. yirgalemense* under simulated gashouse conditions, as described in the growth chamber assay, was evaluated. The same procedure for preparing leaves before treatment and for determining mealybug mortality in the growth chamber assay was followed. Nematodes were applied to leaves with the aid of calibrated handheld spray applicators at a concentration of 2000 IJs/ml. A suspension of *S. yirgalemense* only was compared to a suspension containing *S. yirgalemense*, 0.03% Zeba<sup>®</sup>, 0.06% and Nu-film-P<sup>®</sup>. Five leaves were prepared for each treatment at each time interval. To estimate nematode infectivity potential after treatment application, leaves were left in a growth chamber for 0, 60, 120, 180 and 240 min, after which the leaves at each time interval, were removed and cut into smaller pieces to fit into Petri dishes (3-cm-diameter). Five mealybugs were added to each of five Petri dishes (5 replicates; 25 insects), closed with the lid and covered with cling wrap to ensure an airtight seal, and left in the growth chamber for 48 h, after which mortality was assessed, as was described in the growth chamber assay. The experiment was repeated on a separate test date. The data of both experiments were pooled for analysis.

#### Effect of a polymer-surfactant formulation on IJ survival

The ability of a polymer-surfactant formulation to retard nematode desiccation under simulated glasshouse conditions described in the growth chamber assay was evaluated. Mortality of nematodes on citrus leaves was recorded 0-, 30-, 60-, 120- and 240 min after applying nematodes to leaves. A suspension containing *S. yirgalemense* only was compared to a suspension containing *S. yirgalemense*, 0.03% Zeba<sup>®</sup>, 0.06% Nu-film-P<sup>®</sup> and *S. yirgalemense*. Nematodes were applied to leaves with the aid of calibrated handheld spray applicators at a concentration of 2000 IJs/ml. Three leaves were prepared for each treatment at each time interval. To determine percentage nematode mortality, two 2-cm<sup>2</sup> leaf discs were cut out of each leaf (3 replicates; 6 leaf discs), rinsed in 5 ml tap water and the number of live and dead nematodes recorded. Nematode mortality was determined as a percentage of the total number of nematodes recorded on each individual leaf disc. Nematodes

that did not respond to light and prodding were recorded as dead. The experiment was repeated on a separate test date. The data of both experiments were pooled for analysis.

#### Field trial

The efficiency of adjuvants to increase the ability of *S. yirgalemense* to control adult female *P. citri* under semi-field conditions was evaluated in a citrus orchard on Welgevallen experimental farm, Stellenbosch, Western Cape, South Africa. The field experiment was conducted on 6 October 2011 in the early evening during spring. The experimental layout was in a completely randomised design, with seven rows, each containing six treatment trees, except for one row that contained four treatment trees (8 replicates; 80 insects per treatment). Between the individual treatment trees stood two buffer trees, with two buffer rows separating the treatment rows from each other.

As was previously described in the growth chamber assay, pockets containing citrus leaves, each containing 10 adult *P. citri* females, were used for insect containment in the field experiment. Treatments were: 1) water only as control; 2) nematodes; 3) nematodes + Nu-Film-P<sup>®</sup>; 4) nematodes + Zeba<sup>®</sup>; and 5) nematodes + Zeba<sup>®</sup> + Nu-Film-P<sup>®</sup>. Nematodes were applied to leaves with the aid of calibrated handheld spray applicators at a concentration of 4000 IJs/ml, Zeba<sup>®</sup> at 3 g/L water and Nu-film-P<sup>®</sup> at 0.6 ml/L water. Treatment formulations were prepared 1 h before application. Pockets with leaves containing mealybugs were fastened onto the scaffold branches 1 m above ground of each of the 40 treatment trees on the day of the trial, before applying treatment applications. After 26 h, the leaves were removed from the trees and taken back to the laboratory. The mealybugs were then removed from the leaves and rinsed to remove surface nematodes, and mealybugs from each leaf were placed in individual Petri dishes (9-cm-diameter) lined with moistened filter paper. The Petri dishes were then incubated for a further 48 h at 25°C, after which the mealybug mortality was assessed. Hobo<sup>®</sup> H8 Pro Series data loggers were placed in the middle of every second treatment row to document temperature and humidity in the orchard throughout the trial period.

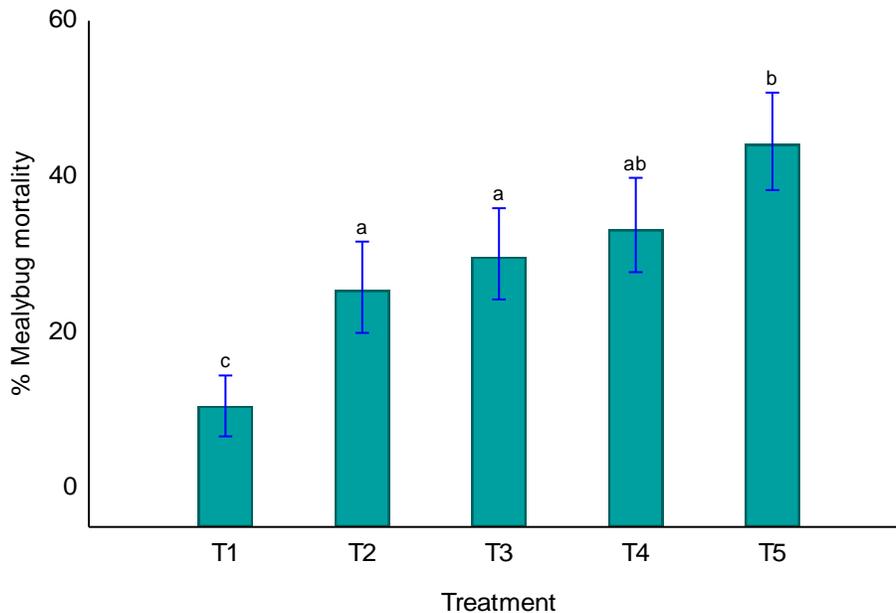
#### Data analysis

All statistical analyses were performed by means of Statistica 9.0 software (StatSoft Inc. 2009). The data were analysed using ANOVA, with post-hoc comparison of means using Bonferroni's method, or a bootstrap multi-comparison test if residuals were found not to be evenly distributed (Efron & Tibshirani, 1993). Significant differences were determined on a 95% probability level.

### **Results and discussion**

#### Growth chamber assay using leaves and fruit

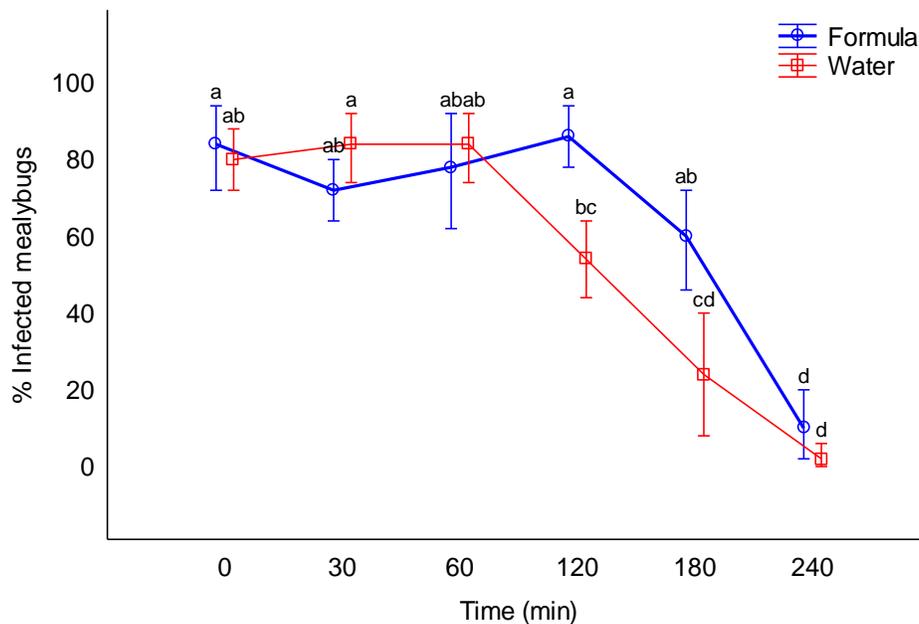
Results obtained from the growth chamber assay were analysed using a two-way ANOVA. The analysis showed no interaction between main effects part of tree (2 levels; leaves and fruit) and treatments (5 levels) ( $F_{(4, 150)} = 0.60$ ;  $P = 0.66$ ). The mortality of mealybugs on fruit and leaves was consistent with that encountered in the treatments and no significant differences were observed between the mortality of *P. citri* on leaves and fruit for any of the treatment suspensions tested. The one-way ANOVA for mortality observed separately on the fruit and leaves were pooled and showed the average percentage mortality (30%) on fruit not to be significantly higher ( $F_{(1, 150)} = 0.84$ ;  $P = 0.36$ ) than the average percentage mortality (28%) observed on leaves. For further analysis, the results of mortality obtained from treatments of fruit and leaves were pooled and analysed using a one-way ANOVA. All treatments obtained significantly higher mortality than did the control ( $F_{(4, 150)} = 16.59$ ;  $P = 0.001$ ) with an average percentage mortality of 11% (Fig. 3.5.4.20). Although not significant, the combined addition of 0.06% Nu-Film-P<sup>®</sup> and 0.03% Zeba<sup>®</sup> to *H. zealandica* (T3) suspensions increased mortality from 26% to 30%. Even without the addition of Nu-Film-P<sup>®</sup> and Zeba<sup>®</sup>, a suspension of *S. yirgalemense* only (T4) obtained higher mortality (34%) than did a suspension of Nu-Film-P<sup>®</sup>, Zeba<sup>®</sup> and *H. zealandica* (T3) (30% mortality). The highest average percentage mortality of 45% was obtained when mealybugs were treated with a suspension of *S. yirgalemense*, 0.06% Nu-Film-P<sup>®</sup> and 0.03% Zeba<sup>®</sup> (T5); the mortality obtained was significantly higher than was that of mealybugs treated with suspensions of *H. zealandica* alone.



**Fig. 3.5.4.20.** Mean percentage mortality (95% confidence interval) recorded for adult female *Planococcus citri* on leaves after exposure to different formulations of *Heterorhabditis zealandica* and *Steinernema yirgalemense* during a growth chamber assay at  $75 \pm 8\%$  RH,  $22^\circ\text{C}$  and 2000 IJs/ml. Treatments were: T1, water as control; T2, *H. zealandica*; T3, *H. zealandica* + Zeba<sup>®</sup> + Nu-Film-P<sup>®</sup>; T4, *S. yirgalemense*; and T5, *S. yirgalemense* + Zeba<sup>®</sup> + Nu-Film-P<sup>®</sup>. Different letters above vertical bars indicate significant differences.

#### Effect of a polymer-surfactant formulation on IJ infectivity

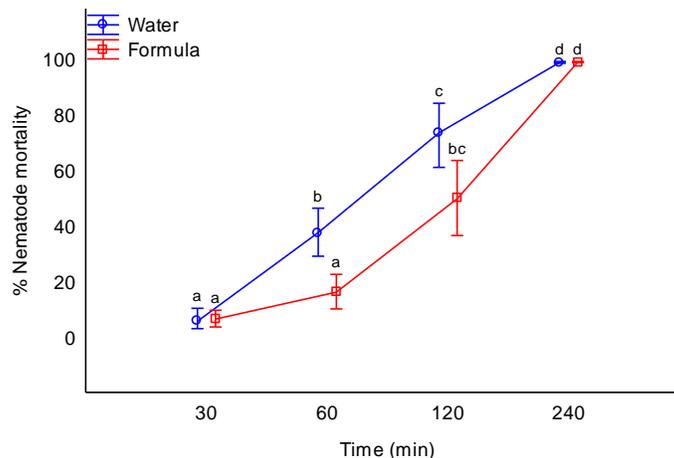
The results for *S. yirgalemense* infectivity under simulated glasshouse conditions on citrus leaves were analysed using a two-way ANOVA. The analysis showed interaction between the main effects treatment (2 levels; treatment and formula) and time (6 levels; 0, 30, 60, 120, 180, 240) ( $F_{(5, 108)} = 6.14$ ;  $P = 0.001$ ), indicating that the treatments did not behave consistently over time. No significant differences in nematode infectivity were observed during the first 60 min after applying nematodes to leaves, with mealybug mortality ranging between 72% and 84% (Fig. 3.5.4.21). Although not significant, 120 min after applying nematodes, the infectivity of nematodes applied in water only started to decrease to 54% control of mealybugs. Infectivity of nematodes applied with 0.03% Zeba<sup>®</sup> and 0.06% Nu-Film-P<sup>®</sup> was significantly higher ( $P = 0.007$ ) after 120 min than with nematodes applied in water only, and obtained high mealybug mortality of 86%. Infectivity of nematodes applied with 0.03% Zeba<sup>®</sup> and 0.06% Nu-Film-P<sup>®</sup> only started to decrease after 180 min obtaining 60% mortality; however, the percentage mortality obtained was still not significantly lower than was the mortality of 84% ( $P = 0.21$ ), obtained directly after applying nematodes. The infectivity potential of nematodes applied in water only, with 24% control after 180 min, was still significantly lower ( $P = 0.001$ ) than was that of nematodes applied with 0.03% Zeba<sup>®</sup> and 0.06% Nu-Film-P<sup>®</sup>. The lowest infectivity potential of nematodes was observed in both treatments after 240 min, obtaining < 10% control.



**Fig. 3.5.4.21.** Mean percentage (95% confidence interval) *Planococcus citri* mortality recorded after exposure to *Steinernema yirgalemense* for different time intervals during growth chamber assays at  $75 \pm 8\%$  RH,  $22^\circ\text{C}$  and 2000 IJs/ml. Data points with the same letter are not significantly different.

Effect of a polymer-surfactant formulation on IJ survival

The results for *S. yirgalemense* survival under simulated glasshouse conditions on citrus leaves were analysed using a two-way ANOVA. The analysis showed interaction between main effects treatment (2 level; formula and water) and time (4 levels; 30-, 60-, 120-, 240 min) ( $F_{(3, 88)} = 4.77$ ;  $P = 0.004$ ) indicating that the treatments concerned did not behave consistently over time. Low nematode mortality ( $< 8\%$ ) for both treatments was observed 30 min after applying nematodes to the leaves (Fig. 3.5.4.22). The mortality of nematodes applied with water only increased significantly to 38% ( $P = 0.001$ ) 60 min after application, while the mortality of nematodes applied with Zeba<sup>®</sup> and Nu-Film-P<sup>®</sup> increased only slightly, to 17%. Although not significant, after 120 min, the mortality (51%) of nematodes applied with Zeba<sup>®</sup> and Nu-Film-P<sup>®</sup> was lower than was the mortality (74%) of nematodes applied with water only. After 4 h, very few live nematodes were observed for either treatment tested.



**Fig. 3.5.4.22.** Mean percentage (95% confidence interval) mortality of *Steinernema yirgalemense* recorded on  $2\text{-cm}^2$  leaf discs at different time intervals post-treatment at  $75 \pm 8\%$  RH,  $22^\circ\text{C}$  and 2000 IJs/ml. Data points with the same letter are not significantly different.

### Field trial

Moderate temperatures, ranging between 9°C and 26°C, with an average of 11°C, were recorded throughout the trial period. The relative humidity in the orchard was average ( $\approx 52\%$  RH) at the time of application and started to increase after 1 h, as the temperature dropped after sunset (Fig. 3.5.4.22). The average temperature and humidity for the first four h post-application was 12°C and 71% RH, respectively. From approximately 03:00 h dew formed on the trees, as the ambient humidity rose to 100% and the dew point equalled the temperature ( $\pm 10^\circ\text{C}$ ). The ambient humidity remained 100% until 08:00 h. At 10.00 h, when pockets containing leaves were retrieved, the trees were still wet from the morning dew (Fig. 3.5.4.23).

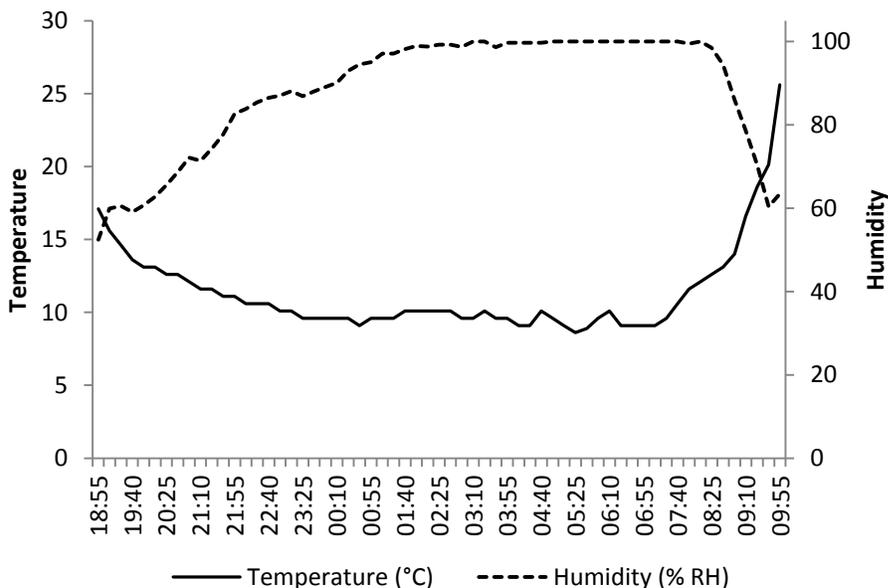


Fig. 3.5.4.22. Climatic data recorded over a 26-h period during a field experiment.

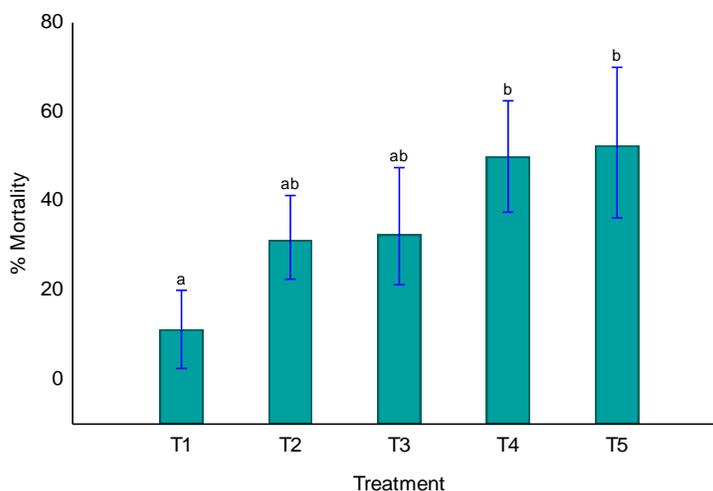


Fig. 3.5.6.23. Mean percentage mortality (95% confidence interval) recorded for adult *Planococcus citri* females after exposure to different formulations of *Steinernema yirgalemense* during a field trial conducted on 6 October 2011. Treatments were: T1, water only; T2, nematodes; T3, nematodes + Nu-Film-P<sup>®</sup>; T4, nematodes + Zeba<sup>®</sup>; and T5, nematodes + Zeba<sup>®</sup> + Nu-Film-P<sup>®</sup>. Different letters above vertical bars indicate significant differences.

Desiccation, accelerated by such abiotic factors as high temperatures, wind and low humidity, limits the effectivity of nematodes to control above-ground insect pests (Wright *et al.*, 2005). To retard desiccation, water retention agents can be added to nematode application suspensions (Glazer *et al.*, 1992). The sensible use of

adjuvants in combining water retention agents with surfactants has resulted in the successful control of western flower thrips in glasshouses (Tomalak *et al.*, 2005). In the current study, the ability of a polymer product, Zeba<sup>®</sup>, and a surfactant, Nu-Film-P<sup>®</sup>, to improve the ability of *H. zealandica* and *S. yirgalemense* to control *P. citri* on citrus was evaluated.

During a growth chamber assay, simulating glasshouse conditions of  $75 \pm 8\%$  RH and  $22^\circ\text{C}$ , both *H. zealandica* and *S. yirgalemense* were able to increase *P. citri* mortality significantly compared to the mortality attained with the control. Although not significant when using nematodes alone, the addition of 0.03% Zeba<sup>®</sup> and 0.06% Nu-Film-P<sup>®</sup> increased the control obtained with the use of *H. zealandica* from 26% to 30% and with *S. yirgalemense* from 34% to 45%. However, with the addition of adjuvants, the *S. yirgalemense* treatment was able to obtain significantly higher control than did the *H. zealandica* treatments. The general higher performance of *S. yirgalemense* correlated with the results obtained, as reflected in Chapter 2, which showed that the nematode species were able to locate and infect *P. citri* at a faster rate than they did *H. zealandica*, as well as being slightly more tolerant toward lower levels of free water.

As *S. yirgalemense* performed significantly better with the addition of adjuvants, only this species was further investigated. Under the same conditions as those mentioned above, adjuvants improved both the infectivity and the survival rate of *S. yirgalemense* 2–3 h post-application. When the ability of *S. yirgalemense* to infect mealybugs post-application was evaluated, the infection potential of nematodes in water significantly decreased 2 h post-application. The first decrease in infectivity potential of nematodes applied with the adjuvants was observed 3 h post-application, although the decrease concerned was not significant. The infectivity potential of nematodes applied with adjuvants decreased drastically 4 h post-application, obtaining only 10% control. When the mortality of nematodes was investigated, the average mortality of nematodes applied with water only increased significantly 1 h post-application, while a significant increase in the mortality of nematodes applied with adjuvants was observed 2 h post-application. Although not significant, the mortality of 51% observed for nematodes applied with adjuvants was considerably lower than was the nematode mortality of 74% observed for nematodes applied with water only, 2 h post-application. No live nematodes were recorded 4 h post-application. The death of nematodes, due to low humidity after 4 h, explained the loss of nematode infectivity observed.

The ability of the formulation to improve control of *P. citri* was further investigated during a semi-field trial. The adjuvants were added separately in order to compare their individual and combined influence on nematode performance. Results of the field experiment showed that, without the addition of Zeba<sup>®</sup>, suspensions containing *S. yirgalemense* were unable to obtain significantly higher control of *P. citri*, than that which was achieved with the control. The addition of 0.06% Nu-Film-P<sup>®</sup> to application suspensions had minimal effect on nematode performance, increasing *P. citri* control by only 3%. The highest mealybug mortality of 53% was obtained with *S. yirgalemense*, when applied together with both Zeba<sup>®</sup> and Nu-Film-P<sup>®</sup>. Similar results were obtained by Schroer and Ehlers (2005), who evaluated a formulation containing 0.3% Rimulgan<sup>®</sup> (surfactant) and 0.3% of the polymer xanthan (antidesiccant) aimed at improving the ability of *S. carpocapsae* to control DBM in a field study conducted on cabbage heads. The number of insects observed on cabbage heads declined significantly, resulting in  $\geq 50\%$  control after seven days. However, their formulation did not prove to have a significant effect, compared to that of the nematodes only, as the moist microclimate in cabbage heads, combined with high ambient humidity, favoured nematode survival.

Moderate temperatures ranging between  $9^\circ\text{C}$  and  $26^\circ\text{C}$  prevailed during the field trial, with a mean of  $11^\circ\text{C}$ . Nematodes have proven to be most active at temperatures ranging between  $15^\circ\text{C}$  and  $32^\circ\text{C}$  (Lacey *et al.*, 2005), indicating that the low temperatures of between  $10^\circ\text{C}$  and  $15^\circ\text{C}$ , which occurred during the night, suppressed the performance of *S. yirgalemense*. In Chapter 2, the exposure time experiment showed the first 2–4 h post-application to be the most important. According to the findings of Lacey and Unruh (1998), the ability of nematodes to infect hosts was found to be greatly impaired when exposed to ambient humidity lower than 95%, thus suboptimal humidity levels ranging between 52% and 87% with an average of 72% prevailing during the first 4 h post-application would have suppressed nematode infectivity even further. In spite of the suboptimal environmental conditions which occurred during the specific semi-field trial undertaken in the current research, 53% control of *P. citri* was still obtained through a suspension of *S. yirgalemense*, Zeba<sup>®</sup> and Nu-Film-P<sup>®</sup>.

The feasibility of nematodes to control *P. citri* should be investigated by applying nematodes to citrus trees that have been naturally infected with mealybugs. The insect containment method used in the field trial was very limited. Although mealybugs occur on foliage, they are cryptic in nature and usually occur in more protected sites, such as between leaves, in bud-mite-induced growth deformations or in between fruit clusters (Wakgari & Giliomee, 2003), which sites also provide a much more favourable microclimate for nematode infection than does the exposed leaf surface. Arthurs *et al.* (2004) collected data of experiments conducted over the last two

decades in order to develop a linear model for testing the efficacy of *S. carpocapsae* to control insect pests. His model showed nematode efficacy to be dependent on pest target habitat, with efficiency decreasing in the following order: firstly, bore holes; secondly, cryptic foliage; and thirdly, exposed foliage. Thus, control of *P. citri* obtained during natural conditions should theoretically be considerably higher than the control that was obtained in the current field study, in which mealybugs were much more exposed to the harsh macroenvironment.

The cost-effect efficiency of increasing nematode application concentrations should also be investigated, as the concentration of 4000 IJs/ml used in the field trial is relatively low. Also, mealybugs tend to cluster together and often to infect only a few adjacent trees, which can be treated as hot spots, with the application of a high concentration of nematodes. In Chapter 2, the feasibility of irrigating citrus trees pre- and post-application to increase humidity and to improve nematode performance was discussed, with it being concluded that, in many cases, such irrigation would, most probably, not be practical, as water is a limited natural recourse in South Africa. Furthermore, the waxy coatings that cover citrus leaves and fruit impair the ability of nematode application suspensions to stick to their surface. Irrigating trees before application could increase application runoff, while nematodes would most probably be washed off from the exposed leaf surface if trees were to be irrigated after application. It was suggested that such loss of nematodes could be compensated for by applying them during the late afternoon, as was done in the current field trial. However, if, theoretically, nematodes had been applied at 05:00 h on 7 October 2011, the nematodes would have been subjected to approximately 7 h of moisture, allowing them to detect and infect their hosts. Dew formed at 03:00 h, when the relative humidity reached 100%. The ambient humidity remained 100% until 08:00 h. At 10.00 h, when treated leaf pockets were retrieved, the trees were still wet from the morning dew. If the nematodes had been applied at 05:00 h, they would have had at least five hours of moisture before the desiccation-retarding abilities of Zeba<sup>®</sup> would have been required. Consequently, at least an additional two hours would have been added to the lifetime of the nematodes, providing them with a total of seven hours in which to locate and infect *P. citri*. During this time, the temperature would also have increase, adding to the nematode infection potential.

## Conclusion

Nematodes have been found to be best used to control above-ground pests in an IPM system (Wright *et al.*, 2005). As mealybugs tend to occur in all life stages on citrus trees throughout the year (Wakgari & Giliomee, 2003), nematodes can be applied at any time when environmental conditions are favourable. Adult mealybugs are difficult to control with chemicals, as, in addition to tending to hide in protected sites, where insecticides cannot reach them, they are also covered by protective waxes (McKenzie, 1967; Michelakis & Hamid, 1995; Franco *et al.*, 2004). Crawlers that emerge from protected hiding sites in search of food during early spring, despite being less susceptible to nematodes than are adults (Stokwe, 2009), tend to be more susceptible to chemicals (Hattingh & Moore, 2003). Although the use of chemicals is not desirable, nematodes can be applied in combination with an insecticide during early spring to target all life stages of mealybugs. Control of mealybug populations early in the growing season will tend to reduce the pesticide load that might otherwise be experienced later in the season. Furthermore, natural enemy populations recover slowly after winter, resulting in population levels that are inefficient in preventing feeding damage to young fruitlets (Hattingh, 1993; Franco *et al.*, 2004). Nematodes can, thus, be applied during this period to fill the gap left by natural enemies. If high mealybug numbers are recorded later in the growing season during late and midsummer, nematodes alone can be applied for control without problem of residues remaining on the fruit. Average temperatures will also be higher during the night at such times of the year, which will increase nematode efficiency.

## Future research

- To further determine the potential of *H. zealandica* and *S. yirgalemense* for the control of *P. citri*, glasshouse and field trials should be undertaken. As *P. citri* is an above-ground pest of citrus, this study also illustrates that desiccation is the most important hurdle to overcome before *P. citri* can be controlled successfully under suboptimal field conditions. Innovative ideas, such as the addition of anti-desiccants and surfactants to application formulations should be tested in order to determine whether desiccation could be retarded to enhance control of *P. citri*.
- Further improvement of nematode application formulations, aimed at the control of above-ground citrus pests, should be done by testing different surfactant and polymer combinations. The application of nematodes to control above-ground pests on a commercial scale is a relatively new field of study, with still much room for improvement and novel ideas with regard to application techniques and technology.
- The current study illustrates some of the factors that should be taken into consideration before nematodes are applied in commercial orchards in an IPM programme. Pest complexes that attack citrus vary from one orchard to the other and from one year to the next (Ebeling, 1959). Thus, the required

agrochemicals and biological control agents also vary. When EPNs are available as commercial biological control agents in South Africa, a database of data relating to nematode species tolerance to agrochemicals, as well as susceptibility of pests and commercially available natural enemies used in IPM programmes for citrus, should be set up, in order to aid producers or consultants in decision making with regard to nematode application. Furthermore, studies should be conducted to determine whether the simultaneous application of chemicals and biopesticides could improve control of a target pest species.

- Although the use of chemicals is not desirable, nematodes can be applied in combination with an insecticide during early spring to target all life stages of mealybugs. Control of mealybug populations early in the growing season will tend to reduce the pesticide load that might otherwise be experienced later in the season. Furthermore, natural enemy populations recover slowly after winter, resulting in population levels that are inefficient in preventing feeding damage to young fruitlets (Hattingh, 1993; Franco *et al.*, 2004). Nematodes can, thus, be applied during this period to fill the gap left by natural enemies. If high mealybug numbers are recorded later in the growing season, during late and midsummer nematodes alone can be applied for control without problem of residues remaining on the fruit. Average temperatures will also be higher during the night at such times of the year, which will increase nematode efficiency.

### Technology transfer

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5. Van Niekerk, S. (2012). The potential of entomopathogenic nematodes to control the citrus mealybug. IPM meeting, Stellenbosch, February, (presentation). 17 February, Room 3028, JS Marais Building, Stellenbosch.
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### 3.5.5 PROGRESS REPORT: Assessment of the potential of *Anagyrus* sp. as a biocontrol agent for mealybug

Experiment 1017 (April 2011 – March 2014) by Sean Moore, Wayne Kirkman (CRI), Rob Stotter (Xsit), Moshe Cohen, Rami Friedman and Shimon Steinberg (BioBee, Israel)

#### Opsomming

Aanvullingsproewe met *Anagyrus* sp. nr *psdeudococci* (Girault), afkomstig van BioBee in Israel, is by twee persele in die Oos- en Wes-Kaap in November 2011 begin. *Anagyrus* is teen drie dosisse, nl 2500, 5000 and 30000 per hektaar per seisoen vrygelaat (slegs die twee laer dosisse in die Wes-Kaap proewe). Die parasitoïedes is in twee vrylatings ingedeel, wat op 15 November en 20 Desember 2011 vrygelaat is. Witluis-infestasië en parasitisme is elke twee weke geëvalueer. In een proef in die Oos-Kaap is witluis-infestasië van Fukumoto Nawellemoene met 6%, 73% and 80% teenoor die kontrole, in die 2500, 5000 and 3000 parasitoïedes per ha respektiewelik verminder. In 'n proef in die Wes-Kaap, met Lina Nawels, was 100% van witluis ouer as 3<sup>de</sup> instar teen die middel van Maart in beide behandelde blokke geparasiteer. Teen oestyd was infestasië 59% and 72% laer as die onbehandelde kontrole vir die 2500 and 5000 per ha behandelings respektiewelik. In die ander proef op Palmer nawels in die Wes-Kaap, was witluis-infestasië 35% in die kontrole 'n paar weke na 'n Buprofezin bespuiting, teenoor 8% en 9% in die behandelde blokke. Een proef in die Oos-Kaap het geen verskil tussen behandelings getoon nie, waarskynlik omdat die behandelde blokke nie voldoende van mekaar geskei was nie. Laboratorium proewe om die biologie en gasheer voorkeur van *Anagyrus* word beplan.

#### Summary

Augmentation trials with *Anagyrus* sp. nr *psdeudococci* (Girault) were initiated in November 2011 at two sites in each of the Eastern and Western Cape, using parasitoids imported from BioBee in Israel. Three rates of *Anagyrus* were released: 2500, 5000 and 30000 per hectare per season (only the lower two in the Western Cape trials). Parasitoids were released in two instalments – on 15 November and 20 December 2011. Mealybug infestation and parasitism were monitored every two weeks. In a trial in the Eastern Cape, mealybug infestation of Fukumoto Navel oranges was reduced by 6%, 73% and 80%, relative to the control block, in the 2500, 5000 and 30000 parasitoid per ha release blocks, respectively. In a trial in an orchard of Lina Navels in the Western Cape, 100% of mealybug older than 3<sup>rd</sup> instar was parasitized by mid-March in both release blocks. By harvest, mealybug infestation was 59% and 72% lower than in the untreated control, for the 2500 and 5000 per ha treatments, respectively. In the other trial in the Western Cape on Palmer Navels, a few weeks after an application of Buprofezin, mealybug infestation in the control was 35%, compared to 8% and 9% in the treatment blocks. One trial in Eastern Cape did not show any difference between treatments, probably because there was inadequate separation between treatment blocks. Laboratory trials to investigate the biology and host preference of *Anagyrus* are planned.

### 3.6 PROJECT: BIOCONTROL DISRUPTION

Project coordinator: Tim G Grout (CRI)

#### 3.6.1 Project summary

Despite the critical need to control some phytosanitary and cosmetic pests with chemicals, the contribution from natural enemies in citrus IPM must be maximized in order to control several important pests in summer and autumn. This is becoming more important as permitted chemical residue levels on fruit at harvest decline. Ants disrupt biological control and therefore need to be kept out of the trees. Further research on the development of an ant bait has shown that Saga plus hydramethylnon has successfully controlled the brown house ant in 7 out of 7 trials and the same combination has controlled the pugnacious ant in 5 out of 5 trials (3.6.2). This bait will now be commercialized after research in 2012/3. No non-target effect bioassays were conducted for contract purposes during the report period but they will be conducted in the future when necessary.

#### Projekopsomming

Ten spyte van die kritieke behoefte om van die fitosanitêre en kosmetiese plae met chemiese stowwe te beheer, moet die bydrae van natuurlike vyande in sitrus GPB vermeerder word om verskeie belangrike plae in somer en herfs te beheer. Dit raak al hoe meer belangrik soos wat toegelate chemiese residu-vlakke op vrugte teen oestyd verminder word. Miere ontwig biologiese beheer en moet daarom uit bome gehou word. Verdere navorsing op die ontwikkeling van 'n mier-lokmiddel het getoon dat Saga plus hydramethylnon die bruin huismier

sukcesvol in 7 uit 7 proewe beheer het, en dieselfde kombinasie het ook die malmier in 5 uit 5 proewe beheer (3.6.2). Hierdie lokmiddel gaan na 2012/13 se navorsing gekommersialiseer word. Geen nie-teiken effek biootese is vir kontrakdoeleindes gedurende hierdie verslagperiode uitgevoer nie, maar sal in die toekoms, wanneer nodig, gedoen word.

### 3.6.2 **PROGRESS REPORT: Development of ant baits**

Experiment 857 (2006/7-2012/3) by Tim G Grout and Kim C Stoltz (CRI)

#### **Opsomming**

Navorsing op mier-lokmiddels is amper voltooi aangesien dit gevind is dat Saga met hydramethylon teen 0.9% effektief teen beide die bruin huismier en die malmier is in grootskaalse proewe in verskillende dele van die land. Tot op datum, het 7 uit 7 proewe getoon dat Saga met hydramethylon effektief teen die bruin huismier teen 3 kg/ha was, met effektiwiteit soortgelyk aan Seige wat aan elke boom toegedien is. Alhoewel malmier aktiwiteit meer wisselvallig is, het 5 uit 5 proewe getoon dat dieselfde mengsel van Saga en hydramethylon effektief populasies verminder het, en 'n soortgelyke effek as 'n hoë konsentrasie chlorpyrifos bespuiting op stamme gehad het. Siege is nie effektief teen die malmier nie. Daar is gevind dat Saga plus hydramethylon geen effek op *Camponotus* sp., 'n mier wat soms in sitrusboorde in Limpopo gevind word, gehad het nie. Hierdie navorsing sal in Maart 2013 beëindig word en die lokmiddel sal gekommersialiseer word.

#### **Summary**

Research on ant baits is finally drawing to a close as Saga with hydramethylon at 0.9% has been found to be effective against both brown house ant and pugnacious ant in large scale trials, in different parts of the country. To date, 7 out of 7 trials show that Saga with hydramethylon was effective against the brown house ant at 3 kg/ha with efficacy usually being similar to Seige applied to every tree. Although pugnacious ant activity is more variable, 5 out of 5 trials show that the same mixture of Saga and hydramethylon is effective in reducing populations and had a similar effect to a high concentration of chlorpyrifos sprayed on the trunks. Siege is ineffective against pugnacious ant. It was found that Saga plus hydramethylon had no effect on *Camponotus* sp., an ant that is sometimes found in citrus orchards in Limpopo. This research will terminate in March 2013 and the bait will be commercialised.

### 3.7 **PROJECT: PRODUCTION PESTS**

Project coordinator: Tim G Grout (CRI)

#### 3.7.1 **Project summary**

The two pests affecting production that received research attention during the report period were woolly whitefly and fruit piercing moths. Woolly whitefly can now be found in 7 of the 9 provinces so can be considered widespread. In towns where it has been present for a year or more the populations are relatively stable with occasional flare-ups on new foliage. However, it is also progressively becoming more common on commercial citrus farms, especially where scalcicides are not being used. No effective parasitoids have been found, although one *Encarsia* sp. appears to occur throughout the country. The release of imported parasitoids is not permitted without first proving that indigenous whitefly species will not be attacked. The most effective treatments are buprofezin, pyriproxyfen and Movento (3.7.2). Fruit piercing moths occasionally cause a great deal of damage to early-maturing varieties and the use of lights remains the only effective deterrent. However, an early warning system to determine when the lights should be used would save a lot of energy and cost. Different commercial moth attractants, fruit and molasses were evaluated in an attempt to find a suitable monitoring method and banana was found to be the best attractant. Although, the numbers of moths caught did not correlate well with the resultant fruit damage, this attractant could probably be used to determine when the moths were present and when lights would be required to repel them from the orchards (3.7.3). The research on fruit piercing moths has now been terminated but further research on woolly whitefly will be conducted for another year.

#### **Projekopsomming**

Die twee plae wat produksie raak en navorsingsaandag gedurende hierdie verslagperiode ontvang het, was wollerige witvlieg en vrugtesteekmotte. Die wollerige witvlieg kan nou in 7 van die 9 provinsies gevind word, en kan dus as wydverspreid beskou word. In dorpe waar dit vir 'n jaar of langer teenwoordig is, is die populasies relatief stabiel, met opvlammings nou en dan op nuwe blaargroei. Dit raak egter al hoe meer algemeen op kommersiële sitrusplase, veral waar dopluisdoders nie gebruik word nie. Geen effektiewe parasitoïed kon

gevind word nie, alhoewel een *Encarsia* sp. blyk om regdeur die land voor te kom. Die vrystelling van ingevoerde parasitoïedes word nie toegelaat alvorens daar bewys kan word dat inheemse witvlieë nie aangeval sal word nie. Die mees doeltreffende behandelings is buprofezin, pyriproxyfen en Movento (3.7.2). Vrugtesteekmotte veroorsaak soms baie skade aan vroeë variëteite en die gebruik van ligte bly die enigste effektiewe afskrikmiddel. 'n Vroeë waarskuwingstelsel wat bepaal wanneer die ligte gebruik moet word sal egter baie energie en kostes spaar. Verskillende kommersiële mot-lokmiddels, vrugte en molasse is geëvalueer in 'n poging om 'n geskikte moniteringsmetode te vind, en piesangs is gevind om die beste lokmiddel te wees. Alhoewel die getalle van die motte wat gevang is nie goed ooreengestem het met die gevolglike vrugskade nie, kan hierdie lokmiddel waarskynlik gebruik word om te bepaal wanneer motte teenwoordig is, en wanneer ligte benodig word om hul uit die boorde te verdryf (3.7.3). Die navorsing op vrugtesteekmotte is nou beëindig, maar verdere navorsing op wollerige witvlieg sal nog vir 'n jaar uitgevoer word.

### 3.7.2 **PROGRESS REPORT: Managing woolly whitefly, *Aleurothrix floccosus***

Experiment 975 (April 2010 – March 2013) by TG Grout, SD Moore, PR Stephen, W Kirkman (CRI), K Kruger (UP), T Pretorius, M Hill, (RU) and J Giliomee (SU)

#### **Opsomming**

Soos wat die wollerige witvlieg oor die land versprei, is dit stadig besig om meer problematies in kommersiële sitrusboorde te raak waar min dopluisdoders toegedien word. Dit word gewoonlik vir 'n jaar of twee in huistuine gevind voordat dit in kommersiële sitrusplase daar naby gevind word. Gedurende 2011 het die wollerige witvlieg noord na Mokopane versprei en is na bewering ook in Stanger, KZN gesien, maar dit het nog nie Citrusdal in die Wes-Kaap bereik nie. Intensiewe monitering van parasitisme in die Oos-Kaap het getoon dat alle parasitisme deur die wydverspreide *Encarsia* sp. is en wissel van 0 tot 56%. Die status in huistuine in dorpe waar dit vir meer as 'n jaar teenwoordig is blyk stabiel te wees, waarskynlik omdat natuurlike vyande 'n onderdrukkende rol begin speel. Resultate van 'n verdere bespuitingsproef in die Sondagsrivier Vallei van die Oos-Kaap het vorige resultate bevestig dat buprofezin 30 g/hl water plus BreakThru 5 ml/hl die mees effektiewe behandeling is, maar dit was nie betekenisvol beter as pyriproxyfen 30 ml/hl en Movento 20 ml/hl nie, beide met BreakThru 5 ml/hl water. 'n Kommersiële beskikbare isolaat van *Metarhizium anisopliae* was gedeeltelik effektief, selfs in die ongunstige mikro-klimaat van 'n nie-draende boord. Verdere identifikasie van enige belowende natuurlike vyande sal tot in Maart 2013 voortgaan.

#### **Summary**

As woolly whitefly spreads across the country it is slowly becoming more problematic in commercial citrus orchards where few scaleicides are applied. It is typically found in home gardens for a year or two before being found in commercial citrus farms nearby. During 2011 woolly whitefly spread north to Mokopane and was reported to be seen in Stanger, KZN, but has not yet reached Citrusdal in the Western Cape. Intensive monitoring of parasitism in the Eastern Cape has shown that all parasitism is by the widespread *Encarsia* sp. and ranges from 0 to 56%. The status in home gardens in cities where it has been present for more than a year seems to have stabilised, presumably as natural enemies start playing a suppressive role. Results from a further spray trial in the Sundays River Valley of the Eastern Cape confirmed previous results with buprofezin 30 g/hl water plus BreakThru 5 ml/hl being the most effective treatment, but this was not significantly better than pyriproxyfen 30 ml/hl and Movento 20 ml/hl, both with BreakThru 5 ml/hl water. A commercially available isolate of *Metarhizium anisopliae* was partially effective, even in the unsuitable microclimate of a non-bearing orchard. Further identification of any promising natural enemies will continue until March 2013.

### 3.7.3 **FINAL REPORT: Monitoring attraction of fruit-feeding moths in citrus orchards to different fruit baits in the Eastern Cape Province, South Africa**

Experiment number 1020 (Feb. 2011 - May 2012) by Craig Robinson, Tanya Pretorius (Rhodes University), Sean Moore (CRI) and Martin Hill (Rhodes University)

#### **Opsomming**

Volwasse vrugte-deurborende motte, *Serodes-partita* (Fabricius) (Lepidoptera: Noctuidae), doen skade op sagte vroeg ryp sitrus in die Sundays River Valley, Oos-Kaap, Suid-Afrika. Daar is geen doeltreffende manier om hierdie pes, wat groot skade veroorsaak tydens uitbrekings jare wat die sitrus oes drasties verminder in Suid-Afrika. Die doel van hierdie studie was om 'n lokmiddel te ontwikkel om hierdie motte te monitor, sodat 'n aard op vroeë opsporing kan bereik word. Van die vyf aas wat getoets was (Magnet®, Texas Vlughtige™, melasse, piesang en sitrus), is piesang die mees doeltreffende en kan gebruik word om vrugte deurborende motte in

sitrusboorde te monitor. Die motte was gemonitor net voor die oes vanaf 27 Februarie tot 2 Mei 2011 en was teenwoordig in lae getalle oor die hele steekproef. Die skade wat die motte veroorsaak het, is nie gekorreleer met die getalle van motte wat gevang word nie, dus is die getal van motte wat gevang was nie gebruik om die skade te voorspel nie.

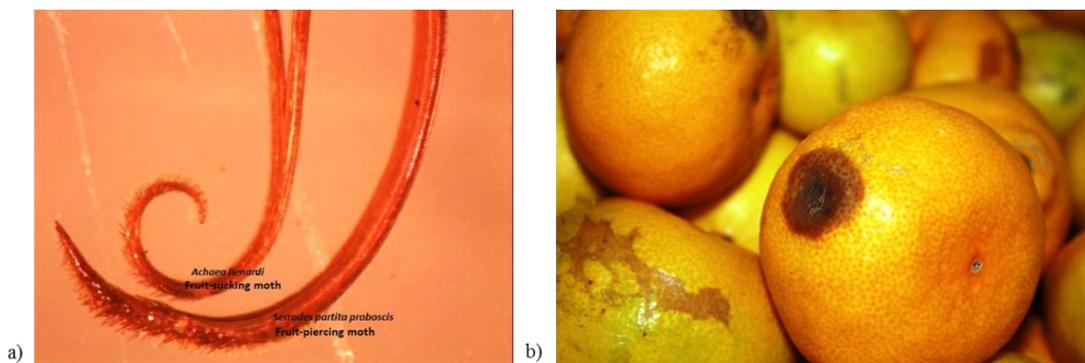
## Summary

Adult fruit-piercing moths, *Serrododes partita* (Fabricius) (Lepidoptera: Noctuidae), damage soft early maturing citrus in the Sundays River Valley, Eastern Cape, South Africa. There is no effective way to monitor these pests, which cause extensive damage during outbreak years by significantly reducing citrus crop yields and increasing post-harvest decay risk. The aim of this study was to develop an attractant to monitor these moths to enable early detection. Of the five baits tested (Magnet<sup>®</sup>, Texas Volatile<sup>™</sup>, molasses, banana and citrus) banana was the most effective and could be used to monitor fruit piercing moths in citrus orchards. The moths were monitored from just before harvesting from 27 February to 2 May 2011 and were present in low numbers over the entire sampling period. The damage the moths caused was not correlated to the numbers of moths caught, thus the number of moths caught cannot be used to predict damage.

## Introduction

Adult fruit-piercing moths are serious pests of fruit crops worldwide. Both male and female moths use a strongly sclerotised proboscis (Fig. 3.7.3.1a) to pierce the fruit for its sap (Tian et al. 2007; Reddy et al. 2007) and start feeding an hour after sundown until midnight. This makes it difficult to detect the moths presence until visible signs of damage can be seen on the fruit the following day (Fig. 3.7.3.1b). According to Johannsmeier (2001) and Hattori (1969), there are two types of fruit feeding moths recognised, based on the structure of their proboscis, namely fruit-piercing moths and fruit-sucking moths with the latter not having a strongly sclerotised proboscis (Fig. 3.7.3.1a).

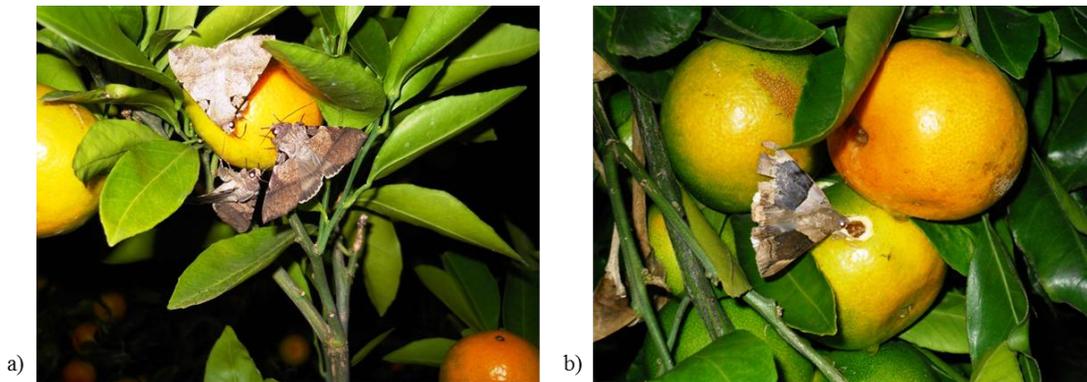
Fruit-piercing moths have historically caused considerable crop damage. In 1925 the Eastern Cape recorded 70% damage to citrus; in 1962 Limpopo Province recorded 58% damage to grapefruit and 58% damage to navel oranges (Johannsmeier 1998). More recently pre-harvest losses to Satsuma Mandarins have been recorded at 20% in Sundays River Valley, 50% near Uitenhage and 50% at Knysna (Moore 2010). There are currently only a few options farmers can implement to protect their citrus crops from damage. Light barrier systems can be erected at night or farmers can cover trees with nets. Both these methods are time consuming, expensive and thus not economically viable (Fay & Halfpapp 2006; Fay & Halfpapp 1999; Moore 2010; Johannsmeier 1998). Alternatively, insecticide can be sprayed, but due to the mobility and hardiness of the moths, and insufficient contact with the residue on the fruit, knockdown is inadequate (Fay & Halfpapp 1999; Fay & Halfpapp 2001; Moore 2010; Johannsmeier 1998). In Japan repellents have been used to deter moths from feeding using sec-butyl  $\beta$ -styryl ketone sprayed on the trees at a rate of 1.0 mg/day, which effectively deterred the moths from feeding, yet long lasting effects were not maintained due to ultraviolet breakdown of the chemical (Tian et al. 2007). A product that has some potential is a synthetic fruit-piercing moth attractant, which has been patented by Fay & Halfpapp (2003), but is not commercially available in South Africa. Fruit puree baits with a toxicant have been used successfully to attract and kill fruit-piercing moths in Australia and other countries with fruit-piercing moth problems (Reddy et al. 2007; Fay & Halfpapp 2001).



**Fig. 3.7.3.1. a)** Proboscis of *A. lienard* and *S. partita*; **b)** Feeding damage caused by *S. partita* (Moore 2011).

The fruit-piercing moth *Serrododes partita* (Fabricius) (Lepidoptera: Noctuidae) (Fig. 3.7.3.2a) is a pest of subtropical fruit in South Africa and India, and is the main fruit-piercing moth in Sundays River Valley orchards

(Johannsmeier 2001). This moth is considered the primary fruit piercer and *Achaea lienardi* (Boisduval) (Lepidoptera: Noctuidae) (Fig. 3.7.3.2b) the main secondary feeder on the holes created by *S. partita* (Moore 2010).



**Fig. 3.7.3.2. a)** *Serrodes partita* feeding on Satsuma fruit; **b)** *Achaea lienardi* feeding on damaged Satsuma fruit.

The moths do not complete their lifecycle on citrus, but are very mobile and travel great distances to find citrus fruit. The larval host plant of *S. partita* is the wild plum, *Pappea capensis* (Eckl. & Zeyh) (Sapindaceae), which is common in the Little and Great Karoo. Once the moths eclose, they are attracted to the orchards in the Eastern Cape (Rust & Myburgh 1986). When there are sufficient rains in the Karoo there is a flush of wild plum growth causing an outbreak of *S. partita* and *A. lienardi*. These outbreaks occur every five to ten years where the most recent outbreaks have been in 1999 and 2009. Outbreaks cause major yield losses especially to soft skin citrus like Satsuma as the fruit ripen early coinciding with the outbreak period (Moore 2010).

### Objectives

The aim of this study was to identify an attractant that could be used in the orchards for monitoring fruit-piercing moth populations. Once such an attractant was identified, the next aim was to monitor the presence and abundance of fruit-piercing moths in citrus orchards and determine the extent of damage they cause and seek a relationship between the two.

### Materials and methods

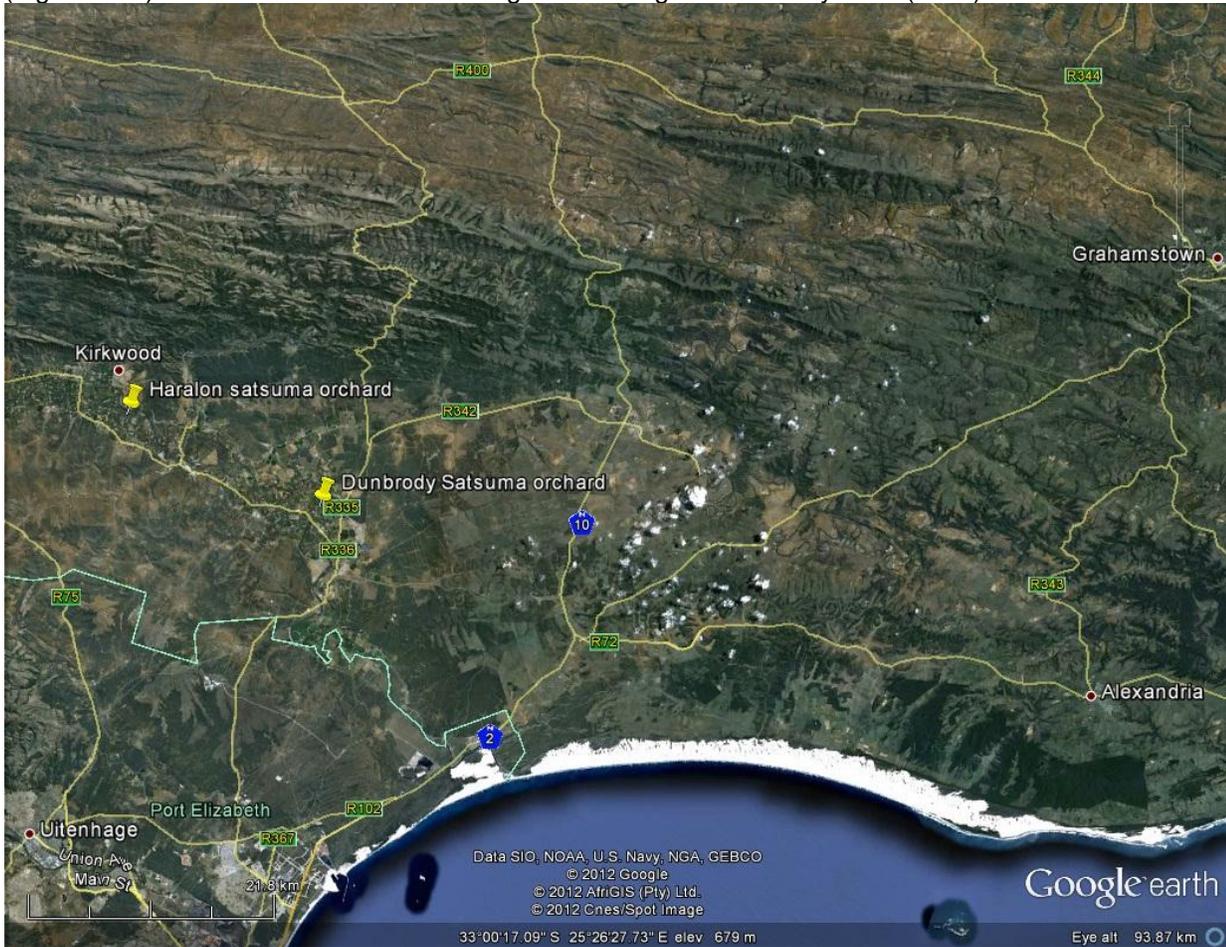
#### Study area

The study was conducted in the Sundays River Valley, Eastern Cape, South Africa on two farms namely, Haralon (33°29'27"S 25°40'25"E) and Dunbrody (33°28'57"S 25°34'27"E) (Fig. 3.7.3.3). Both farms grow the same variety of Satsuma (Miho Wase Satsuma) and these sites are in close proximity to the Little Karoo, which is where the larval host plant (*P. capensis*) of *S. partita* is located. The baits were placed in Satsuma orchards because they were the earliest ripening fruit and had previously been affected by fruit-piercing moths (Moore 2010).

#### Baits

Five baits were used to attract and monitor fruit-feeding moths on the given farms. Magnet<sup>®</sup>, Texas Volatile<sup>™</sup>, molasses, banana and citrus were the baits used. Magnet<sup>®</sup> is a commercially available product from Ag Biotech Australia and is used to monitor *Helicoverpa* spp. (Lepidoptera) in a wide range of crops (Ag Biotech Australia 2009). Texas Volatile<sup>™</sup> is a plant volatile-based product, used to trap tomato semi-looper, *Chrysodeixis acuta* (Walker) (Lepidoptera: Noctuidae), and African bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), in crops. It is commercially available and effective for monitoring general lepidopteran pests (Insect Science 2010). Molasses was used, as it is a concentrated sweet smelling substance that is easy to obtain. According to Johansmeier (2001), fruit-piercing moths are attracted to the sap of very ripe fruit to obtain energy for egg maturation. Molasses has also been used to attract other lepidopteran pests including fruit-piercing moths in other countries (Reddy et al. 2007; Landolt 1995; Kovanci & Walgenbach 2005). Banana was chosen due to work done by Reddy et al. (2007), where different fruits were tested as attractants for the fruit-piercing moth, *Eudocima (fullonia) phalonia* (L.) comb. (Lepidoptera: Noctuidae). Reddy et al. (2007) found that banana was the most attractive to fruit-feeding moths in the study. In the same study orange was the third most attractive bait following guava (Reddy et al. 2007). Satsuma was used as bait as Satsuma orchards were the

study sites for this experiment. A control bait containing water was used, demonstrating that the yellow traps (Fig. 3.7.3.4) had minimal effect in attracting fruit-feeding moths Reddy et al. (2007).



**Fig. 3.7.3.3.** Trapping sites: Haralon and Dunbrody Farms in the Sundays River Valley, are indicated by the yellow drawing pins (Google Earth 2012).



**Fig. 3.7.3.4.** Yellow bucket funnel traps used to trap fruit-feeding moths. Cage visible under trap roof from which bait filled Polytop vial was attached.

At the beginning of the study the method used by Reddy et al. (2007), using a fruit puree mixed with agar to solidify it, was attempted. Additional products such as gelatine and glycerol were also evaluated as solidifiers for the puree baits. The final conclusion was not to use a solidifying agent, but to use freshly cut banana and Satsuma pulp in the traps. The two fruit baits were perishable and needed to be changed once a week, however changing the baits every three days would have been preferable (Tian et al. 2008). A piece of banana was cut and placed into the trap whereas the citrus bait was Satsuma pulp squeezed into the trap. This was done as fresh fruit with multiple volatiles has been shown to be more attractive than a single isolated volatile (Tian et al. 2008). Molasses lasted for long periods, but needed topping up in the traps during the sampling period. The Texas Volatile™ and Magnet® baits were also non-perishable products and lasted the full ten weeks of monitoring and were thus not changed.

#### Traps

The traps that were used were yellow bucket funnel traps (Fig. 3.7.3.4) which are available from Insect Science™. Each of the baits was replicated six times, so a total of 36 traps were used. The traps were divided over two sites with three replicates of each bait at each site making a total of 18 traps at each site. Yellow traps were chosen over green traps, because yellow traps have been proven to be more effective in attracting insects (Midgley et al. 2008). Each trap had a dichlorvos block placed into the inside of the trap to kill any attracted insects without damaging them, such that they could be pinned and identified. Using wire the traps were secured to the Satsuma trees, at a height of approximately two metres above the ground. To the top of the trap a cage was attached and to this a bait-filled Polytop vial. This prevented rain from diluting the bait and attracted the insects to the mouth of the trap.

#### Experimental layout

Traps were placed on the northern side of each orchard as it is the most affected by fruit-piercing moths as the moths' larval host tree (*P. capensis*) is situated to the northern side of the orchard. The moths fly into the prevailing wind and arrive at the orchards, but may also be attracted by the fruit volatiles leading them to the orchard when they get closer (Moore 2010; Johannsmeier 1998). To avoid edge effects the traps were placed in the third row from the northern edge (Odum 1959). Spaces between traps were 12 m to ensure there was no confusion between baits, but still close enough to give the moths a choice of baits. The tree and row spacing also helped to ensure the traps were evenly spaced in both orchards. Halaron had a tree spacing of three metres with trees placed alternatively in double rows; the double rows were six metres apart. Dunbrody had rows of single trees spaced three metres apart and rows were spaced six metres apart. The positioning of each trap in the orchard was done through randomisation to reduce sampler bias (Tyre et al. 2003). To help prevent

deterioration and drying out of the baits they were placed on the southern side of the tree which is more shaded than the northern side of the tree.

The contents of the traps were collected weekly from the orchards and sample insects were pinned, identified and recorded. The number of individual fruit-feeding moths was recorded for each trap to determine which bait was the most effective in attracting these moths.

#### Damage assessment

Percentage damage caused by fruit-piercing moths in the orchard was determined by undertaking a survey of the fruit once a week. The survey started from the 27/02/2011 and ended 02/05/2011 and consisted of inspecting 20 fruit on ten trees at each site, which is similar to the method used by King & Thompson (1958) to measure fruit-piercing moth damage in citrus. The initial row and tree number was chosen by using a random number generator. From the beginning point 10 trees were sampled in a row. At each tree, fruit was sampled from around the whole tree and the fruit were chosen haphazardly.

#### Statistical analyses

A simple regression analysis was conducted on the number of *S. partita* caught and the amount of damage recorded (Fig. 3.7.3.5a) due to fruit-piercing moths. A chi-squared test was conducted on the number of moths caught per bait (Fig. 3.7.3.5b) to determine if there was a significant difference between the different baits.

### **Results and discussion**

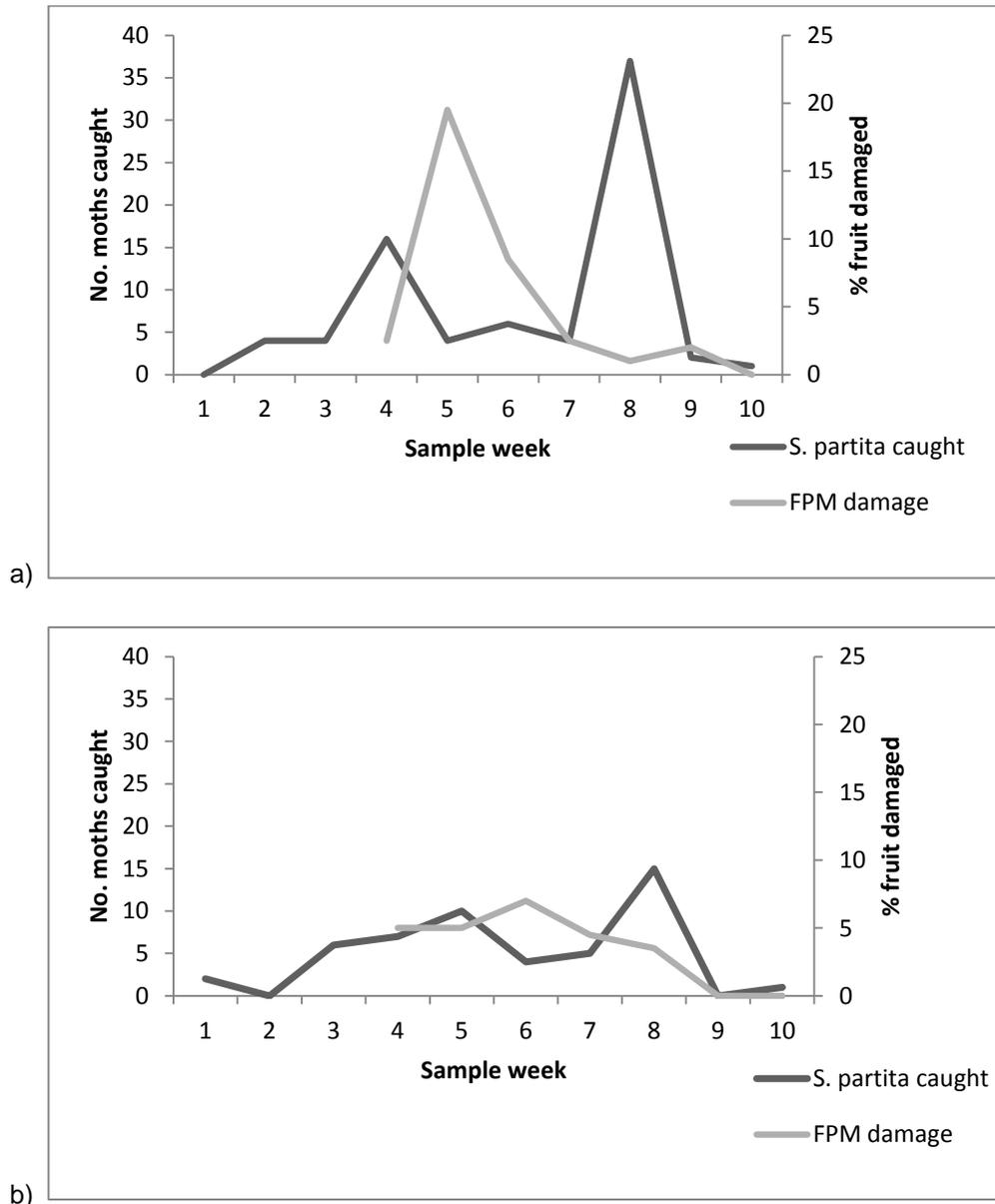
#### Orchard trapping and surveying

Catches of *S. partita* (Fig. 3.7.3.5a and 3.7.3.5b) and *A. lienardi* (Fig. 3.7.3.6) started from the beginning of the trapping period, and proceeded until catches dropped to one individual per week for two weeks. Moths of both species were present throughout the trapping period with two peaks in catches (Fig. 3.7.3.5a, 3.7.3.5b and 3.7.3.6). In the past it has been documented that fruit-piercing moths, specifically *S. partita*, have been a problem in the Eastern and Western Cape for as long as fruit has been grown there (Johannsmeier 1998; Moore 2010; Johannsmeier 2001). Currently *S. partita* is still a problem and was shown to be present in the orchards for the full ten weeks of monitoring, albeit in low numbers. Fruit damage decreased over the sampling period, but this could be attributed to harvesting which began on the 4 April 2011.

As the fruit ripened it was harvested and thus damaged fruit was removed, which prevented accurate estimates of damage (Fig. 3.7.3.5b and 3.7.3.6). Before harvesting, a relationship between moth numbers and fruit damage was noted. At Halaron, a spike in numbers of moths on 29 March was followed by a high level of damage on the 4 April (Fig. 3.7.3.5a). This was also seen at Dunbrody, but the spike in moth numbers was on 19 March and high fruit damage was recorded on 29 March (Fig. 3.7.3.5b). However, when comparing the amount of fruit damaged to the number of moths caught per week in a simple regression analysis there was no correlation, ( $R^2 = 0.09$  and  $p = 0.7$ ) and only 3% of the data explained the relationship (Fig. 3.7.3.5a and 3.7.3.5b).

It can be said that the 2011 season was not an outbreak year; numbers caught were generally low with an average of 12 *S. partita* caught per week with the highest catch of 52. This would most certainly be considered a light infestation, as Johannsmeier (1998) states that a heavy infestation of *S. partita* is 1 000 moths per 40 data trees. The last outbreak was in 2009 and outbreaks occur every five to ten years, due to good summer rains in the larval host plants' area (Johannsmeier 1998; Moore 2010).

The moths did however have two peaks in numbers which can be attributed to at least two cohorts. The first peak in numbers did result in an increase in fruit damaged the following week, but this trend could not be linked to the second peak, due to harvesting. There was no correlation between fruit damage and number of moths caught. This could be due to the fact that the baits used were very attractive and attracted moths off their alternative hosts, in the orchard and into the traps; the baits were set out in the early evening before the fruit-piercing moths could do any damage; and the damage itself was underestimated as only a small number of fruit were assessed for damage (20 fruit on 10 trees).



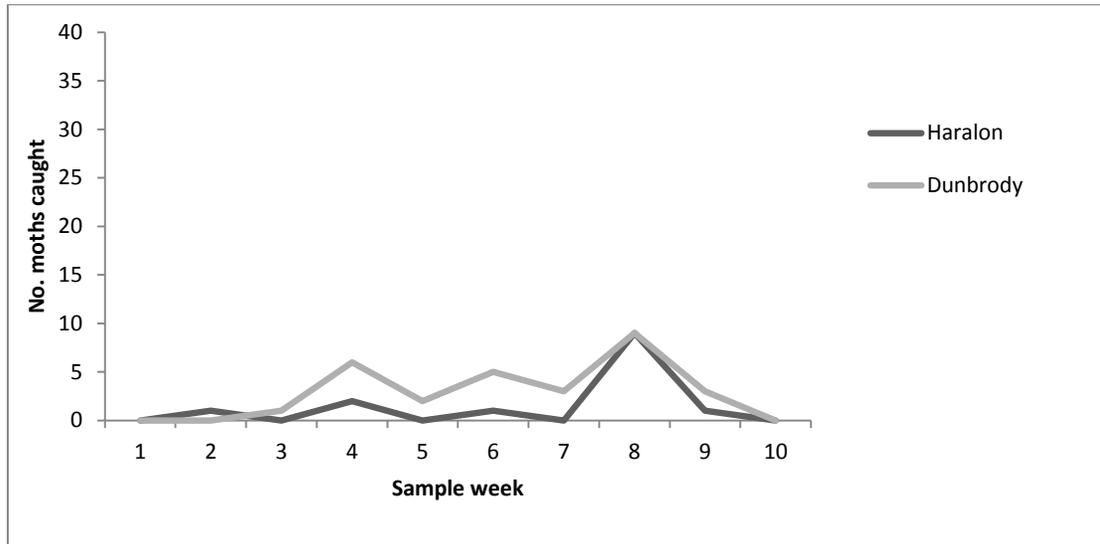
**Fig. 3.7.3.5. a)** Total *S. partita* caught compared to percentage fruit damaged at Halaron over the sampling period; **b)** Total *S. partita* caught compared to percentage fruit damaged at Dunbrody over the sampling period.

The most attractive bait to fruit-feeding moths was banana, which was significantly ( $p = 0.04$ ) more attractive than the other four baits and was most attractive to both *S. partita* and *A. lienardi* (Fig. 3.7.3.7). The second and third most attractive baits were citrus and molasses, respectively, with *Magnet*<sup>®</sup> and *Texas Volatile*<sup>™</sup> not catching any *S. partita* or *A. lienardi*. The control bait (water) caught one *S. partita* which could either be attributed to the yellow trap colour or the moth trying to find a place to rest during the day. Other literature showing the attractiveness of banana to fruit-feeding moths was thus observed in this study (Reddy *et al.* 2007; Johansmeier 1998). The commercial baits, *Magnet*<sup>®</sup>, *Texas Volatile*<sup>™</sup>, were not effective. In a study by Tian *et al.* (2008) the volatiles in peaches that attracted the fruit-piercing moth *Oraesia excavata* (Butler) (Lepidoptera: Noctuidae) were identified, but individually they were not attractive. When artificial combinations were made they attracted moths but were not as attractive as fresh peaches. Recommendation for any future monitoring of fruit-feeding moths are to use fresh banana, as volatiles in fresh fruit appear to be found in the correct ratios to effectively attract fruit-feeding moths.

More *S. partita* were caught with the best three baits than *A. lienardi*, which could be due to *S. partita* being more attracted to the baits than *A. lienardi*, or that there are more *S. partita* in the orchards than *A. lienardi*.

Currently the most effective ways to control fruit-piercing moths are to either use white light in the yellow-green spectrum (500-600 nm wavelength) as a repellent or to cover the trees with netting (Fay & Halfpapp 2006;

Johannsmeier 1998; Moore 2010). Another method that may have some merit is to harvest the fruit just before it is fully ripe and ripen it artificially using ethylene fumigation, which will reduce the amount of fruit lost to fruit-piercing moth damage. Fruit-piercing moths have been shown to only be attracted to ripe fruit and thus removing them before they are ripe could reduce damage to the fruit by the moths (Fay & Halfpapp 2006; Johannsmeier 1998).

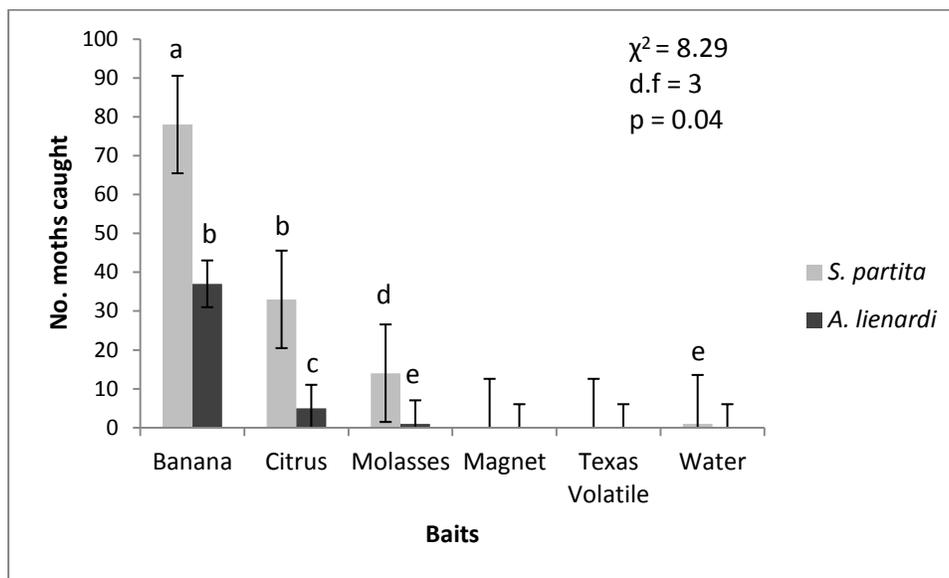


**Fig. 3.7.3.6.** Total *A. lienardi* caught at Halaron and Dunbrody over the monitoring period.

#### Night-time moth surveying

To verify what species of fruit-piercing moths were damaging the fruit, the orchard was surveyed at night on the 12 March 2011 from 19h00 to 20h00. Photographs were taken of the moths that were found to be feeding on the fruit; the main fruit-piercing moth was *S. partita* (Fig. 3.7.3.2a). This moth could be seen inserting its proboscis into the fruit, causing primary damage. *A. lienardi* was recorded feeding on already made wounds in the fruit (Fig. 3.7.3.2b).

The fruit-sucking moth *A. lienardi* showed the same population trend as *S. partita* but lower in number. Outbreaks of these moths have been recorded at the same time as *S. partita* and many farmers have thought *A. lienardi* was causing the damage to their fruit because these moths were seen feeding on the fruit during the day (Johannsmeier 1998; Moore 2010). *A. lienardi* were observed feeding in the orchards on fruit, but it is only at damaged sites caused by *S. partita*, snails or birds, as *A. lienardi* is not capable of causing primary damage to the fruit (Hattori 1969; Krenn 2010; Moraes Zenker *et al.* 2011).



**Fig. 3.7.3.7.** Attractiveness of baits is shown by the number of moths caught over 10 weeks at both sites. There was a significant difference ( $p = 0.04$ ) in the number of *S. partita* caught using banana bait compared to the other baits.

## Conclusion

Of the five baits tested (Magnet<sup>®</sup>, Texas Volatile<sup>™</sup>, molasses, banana and citrus) banana was the most attractant substance to the fruit piercing and sucking moths. Banana could thus be used to monitor fruit piercing moths in citrus orchards using the methods outlined in this study. The moths were monitored from just before harvesting from 27 February to 2 May 2011 and were present in low numbers over the entire sampling period. The damage that the moths caused was not correlated to the numbers of moths caught, thus the number of moths caught could not be used to predict damage. If the study is repeated in the future, firstly, it should be done so in an outbreak year, so that presence of moths and consequent damage is greater, and secondly, a larger fruit sample size should be used.

## Future research

In future studies it would be advisable to start monitoring earlier to determine when the first moths are active in the orchards in order to determine a date, which can be used as a starting point for monitoring fruit-piercing moths. Also, several different orchards should be used (hopefully with different levels of fruit feeding moth pressure) in order to increase the chance of determining whether there is a relationship between trap catches and fruit damage.

*Pappea capensis* trees several kilometres north-west of Kirkwood were the probable source of infestation of *S. partita* for the Sundays River Valley. If these trees are monitored during the season, possible outbreaks could be predicted and farmers can prepare mitigation measures before the moths start becoming a pest. Chemical control of *S. partita* on its natural host would not be economically or practically viable due to the large area the trees are spread over, also the land is owned by multiple landowners. Other control measures that can be used against *S. partita* populations are egg parasitizing wasps and larval parasitizing tachinid flies. The former were not found in this study but have previously been recorded (Johannsmeier 1998). However, a very high level of parasitism by an unidentified tachinid fly was recorded. Classical biological control has been used to control fruit-piercing moths in other countries (Howarth 1991). In the Pacific region two egg parasitoids, *Telenomus lucullus* (Nixon) (Hymenoptera: Scelionidae) and *Ooencyrtus papilionis* (Ashmead) (Hymenoptera: Encyrtidae), were released to try and control the numbers of the fruit-piercing moth *E. (fullonia) phalonia*, which is a major pest of fruit, but this was only marginally effective (Sands & Liebrechts 2005). The larval parasitoid *Euplectrus maternus* (Bhatnagar) (Hymenoptera: Eulophidae) was also released in Guam against *E. (fullonia) phalonia* with limited success (Muniappan et al. 2004). In South Africa biological control needs to be considered, but may not be practically or economically viable.

## Technology transfer

This research will appear in the SA Fruit Journal in the August 2012 edition.

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## 4 PROGRAMME: DISEASE MANAGEMENT

### 4.1 PROGRAMME SUMMARY

By P.H. Fourie (Manager: Disease Management Programme, CRI)

Most projects in the Disease Management programme are showing very good progress and most grower priorities are addressed in experiments designed to meet certain short-, medium- and long-term strategic objectives. The progress during the 2011-12 reporting period is briefly summarised below.

Apart from pure research experiments, the Graft Transmissible Diseases project continues to provide essential services for the Citrus Improvement Scheme (CIS) through re-indexing of foundation block trees, pathogen elimination and pre-immunisation of new entries. Virus elimination was successful in several new cultivars and these were submitted to the Citrus Foundation Block (CFB) for multiplication. Molecular techniques for detection of graft transmissible pathogens are now routinely used, which has significantly strengthened the diagnostic capacity of the CIS. All the trees in the mother block were diagnosed using conventional and molecular techniques to ensure their virus and viroid-free status. Control of *Citrus Tristeza virus* (CTV) is largely based on cross-protection, and a substantial research effort investigates the cross-protecting ability of mild CTV sources in different citrus types, and in different growing areas. The dynamics between variant groups within CTV cross-protection sources, as well as in field trees in which cross-protection has broken down, are being studied in a PhD, MSc and several Hons BSc studies at Pretoria University under guidance of Prof Gerhard Pietersen. These studies will provide valuable insight into reasons for cross-protection breakdown, but will also lay the foundation for future work aimed at the design/selection of superior cross-protection sources. Liberibacter species related to the greening pathogen "*Candidatus Liberibacter africanus*" were detected in several indigenous Rutaceae genera; at present, it seems as if each genus hosts its own unique Liberibacter species, but the greening pathogen could not be detected in any of these non-Citrus hosts. Sequencing of this species as well as the greening pathogen, "*Candidatus Liberibacter africanus*" and its *capensis* sub-species, is under way in collaboration with USA researchers. Two potential greening resistant / tolerant clones derived by rescuing embryos from healthy chimeras on greening-infected fruit are being evaluated for greening resistance in the orchard and are still greening-free.

In the Soilborne Diseases Project, several trials investigate control of nematodes with alternative, more environmentally friendly products. Promising results were again obtained with a proprietary product involving stimulation of nematode egg hatching. Outcomes from this research provide the South African citrus industry with potential alternatives, especially following the recent removal of aldicarb from the market. A trial was initiated for evaluation of several pre-plant nematicide treatments, including fumigation and biofumigation. To date, the fumigated sites remain nematode-free. With the ultimate aim of finding early indicators of tree decline, multivariate statistical analyses of large datasheets of variables comprising multi-disciplinary factors that might influence tree and root health are being conducted. An experiment evaluating the effect of compost, amended with beneficial organisms, on tree condition and general disease resistance has been concluded: compost treatments increased soil penetrability and reduced *Phytophthora* and nematode counts.

In the Citrus Black Spot project, the use of phosphonates and adjuvants to control CBS and their retention on citrus fruit and leaves are being investigated. A holistic approach aimed at CBS inoculum management through foliar sprays and accelerating leaf decomposition has been concluded, indicating no consistent effect of pre-blossom sprays, reduced ascospore loads in strobilurin-sprayed plots compared with mancozeb-plots, and increased ascospore loads where fallen leaves were treated with a decomposing agent. CBS epidemiology is being studied in the Eastern Cape Province through spore trapping and weather monitoring. Unfortunately, mechanical problems and theft of automated spore traps and weather station led to large gaps in the data. An epidemiological study predicting onset and subsequent ascospore dispersal based on climatic variables was submitted for publication and provisionally accepted. A collaborative project is also under way between SA, USA-Florida and Brazil, with the aim of developing a probabilistic model to quantitatively predict the risk of fruit as a pathway for CBS.

In the Fruit and Foliar Diseases Project, new control options for *Alternaria* brown spot (ABS) is continuously being studied. Research also focuses on improving spray application through optimal use of spray machines or adjuvants. Fluorescent pigment benchmarks indicating effective ABS control were determined and are being used to better interpret spray deposition results. Anomalous findings following adjuvant research have necessitated additional fundamental research to be conducted. To this end, Gideon van Zyl's MSc has been upgraded to a PhD study, which he will be conducting while employed by CRI as a contract researcher on spray application technology. A new experiment was initiated to study control of *Botrytis* blossom blight and fruit drop in lemons. Lemon stamens appeared to be the most important target site and benomyl and

iprodione were most effective, but spray programmes need to be studied in order to determine the optimal timing of application.

Post Harvest Diseases remain a very high priority and several experiments were directly aimed at improving post harvest disease management in packhouses. A study on the seasonal occurrence of *Penicillium digitatum* and *P. italicum*, *P. crustosum* as well as resistance frequencies against imazalil, guazatine and thiabendazole in these *Penicillium* spp. was concluded. Results show that *P. digitatum* and *P. italicum* dominated packhouse environments with inoculum load and resistance frequencies increasing as the season progressed, pointing toward the critical importance of sanitation. Imazalil, thiabendazole and pyrimethanil residue loading following application in fungicide bath and wax and subsequent bio-efficacy against sensitive and resistant strains were studied, giving valuable insight into the optimal use of these postharvest fungicides. Integration of preharvest silicon, and postharvest heat and biocontrol is also studied and to date, optimal temperatures for different citrus types were determined and the inhibition of chilling injury following silicon fertilisation was confirmed. Potential alternative fungicides and sanitisers are continuously screened, but none of the products evaluated in 2011 showed promise.

The Diagnostic Centre (DC) continues to perform a sterling service for the Citrus Improvement Scheme through routine soil and water analyses for *Phytophthora* and nematodes, as well as through these analyses in research experiments in the Soilborne Diseases project. The DC also continued providing quality control analyses for River Bioscience. In total, a staggering 8272 samples were analysed by one diagnostician, a technician and assistant. However, staff changes in DC created some unexpected problems. These were resolved by new appointments and placing DC under line management of MC Pretorius.

In general, good progress was made in Disease Management. As in previous years, 'non-research' demands on the available human resources in technical support of the industry limit the quality and quantity of tangible research outputs and divert from consolidated and focused research. In an attempt to address this concern, research alliances with universities and other research service providers are used to broaden the research capacity, while sustaining service delivery to the industry. However, this alone will not address this concern and additional capacity building for technical support as well as increased research funding should be considered.

## PROGRAMOPSOMMING

Meeste van die projekte in die Siektebestuurprogram toon baie goeie vordering en die meeste produsente-prioriteite word aangespreek in eksperimente wat ontwerp word om sekere kort-, medium- en langtermyn strategiese doelwitte te bereik. Die vordering vir die 2011-12 verslagperiode word kortliks hieronder opgesom.

Die Ent-oordraagbare Siekte projek het, afgesien van suiwer navorsingseksperimente, voortgegaan om noodsaaklike dienste aan die Sitrusverbeteringskema (SVS) te verskaf, deur her-indeksering van grondvesblokbome, groeipunt-enting en preïmmunisasie van nuwe kultivars. Virus-verwydering was in verskeie nuwe kultivars suksesvol en is by die Sitrus Grondvesblok (GVB) vir vermeerdering ingedien. Molekulêre opsporingstegnieke vir ent-oordraagbare patogene word nou as roetine gebruik, wat die diagnostiese kapasiteit van die SVS betekenisvol versterk het. Alle bome in die GVB moederblok is met konvensionele en molekulêre tegnieke gediagnoseer om hul virus- en viroïede-vrye status te bevestig. Beheer van *Sitrus Tristeza virus* (CTV) is grootliks op kruisbeskerming gebaseer, en heelwat navorsingsproewe evalueer die kruisbeskermingsvermoë van matige CTV bronne in verskillende sitrustipes en klimaatstreke. Die dinamika tussen variant groepe in CTV kruisbeskermingsbronne, asook veldbome waar kruisbeskermingsverlies waargeneem is, word as deel van 'n PhD, MSc en verskeie HonsBSc studies te Pretoria Universiteit, onder leiding van Prof Gerhard Pietersen, ondersoek. Hierdie studies en gevolglike insig omtrent kruisbeskermingsverlies sal grondlegend wees vir toekomstige studies wat sal poog om beter kruisbeskermingsbronne te ontwikkel. Liberibacter spesies na-verwant aan die vergroeningspatogeen "*Candidatus Liberibacter africanus*" is in verskeie inheemse Rutaceae genera waargeneem, maar die vergroeningspatogeen is in geen van hierdie nie-sitrus gashere waargeneem nie; tans bleik dit asof elke genus sy eie unieke Liberibacter huisves. Basispaarvolgorde-bepaling ("sequencing") van die *capensis* subspesie, asook van die vergroeningspatogeen, "*Candidatus Liberibacter africanus*", is in samewerking met V.S.A. navorsers, onderweg. Twee moontlik vergroeningsbestande klone verkry deur embrios vanaf gesonde chimeras op vergroeningsgeïnfekteerde vrugte te red, word in boorde vir vergroeningsweerstand geëvalueer en is steeds vry van vergroening.

Verskeie kontrakproewe is in die Grondgedraagde Siekte projek ondersoek die beheer van nematodes met alternatiewe, meer omgewingsvriendelike produkte. Belowende resultate is weer met 'n self-ontwikkelde produk wat uitbroei van nematode-eiers stimuleer, en verskeie ander sagter en/of biologiese beheer-opsies,

verkry. Uitkomst uit hierdie navorsing is waardevol vir die sitrusbedryf, veral na die onttrekking van aldicarb. 'n Proef is vir die evaluasie van verskeie vóór-plant nematisiedbehandelings, insluitende beroking en bio-beroking, begin. Tot dusver, is die beroekte persele steeds vry van nematodes. Met die uiteindelige doel om vroeë indikatore van boom-agteruitgang te vind, word veelvoudige veranderlike statistiese analises van verskeie faktore wat boom- en wortelgesondheid kan beïnvloed, ontleed. 'n Eksperiment wat die effek van komposbesmesting, wat met voordelige organismes verbeter is, op boomtoestand en algemene siekteweerstand bestudeer, wys interessante resultate, veral verhoogde gronddeurdringbaarheid en verminderde *Phytophthora*- en nematode-tellings.

In die Sitrus Swartvlek (SSV) projek, word die retensie van fosfonaatprodukte en hul effek op SSV beheer bestudeer. 'n Holistiese benadering gemik op SSV inokulum-bestuur deur blaartoedienings en versnelling in blaar-ontbinding, is afgehandel: geen konstante effek is met voor-blom spuite gewys, die strobilurin program het minder askospore as die mancozeb program gehad, en komposteringsprodukte het askosporvystelling verhoog. SSV-epidemiologie word in die Oos-Kaap provinsie deur spoorvangstudies en weermonitering bestudeer, maar meganiese probleme en diefstal van spoorvanger- en weerstasie-parte het tot groot gapings in die data gelei. 'n Epidemiologiese studie oor die invloed van weerstoestande op die begin en voortsetting van spoorvystelling, is gedoen en voorwaardelik vir publikasie aanvaar. 'n Samewerkingprojek is ook tussen S.A., V.S.A.-Florida en Brasilië onderweg, met die doel om 'n waarskynlikheidsmodel te ontwikkel om kwantitatief die risiko van vrugte as 'n baan vir SSV, te voorspel.

In die Vrug- en Blaarsiekte projek, is nuwe beheer-opsies van *Alternaria* bruinvlek (ABV) bestudeer. Navorsing fokus ook op die verbetering van spuittoediening deur optimale gebruik van spuitmasjiene of byvoegmiddels. Fluoresensie-pigment drempelwaardes wat effektiewe ABS-beheer toon, is bepaal, en word gebruik om spuitneerleggingsresultate beter te interpreteer. Teenstrydige resultate uit benatter-werk het daartoe gelei dat fundamentele navorsing hieroor gedoen sal moet word. Voorts is Gideon van Zyl se MSc na PhD studie opgradeer, wat hy sal doen terwyl hy as kontraknavorsers by CRI aangestel is. 'n Nuwe eksperiment is geïnisieer om beheer van *Botrytis* bloeiselsversenging en vrugval in suurlemoene, te bestudeer. Suurlemoen meeldrade bleik die mees geskikte blomdeel vir infeksie te wees, en benomyl en iprodione was mees effektief. Die optimale tyd vir bespuiting moet egter nog ondersoek word.

Na-oes siektes bly 'n baie hoë prioriteit en verskeie eksperimente is direk gerig op die verbetering van na-oes siektebestuur in pakhuis. 'n Studie oor die seisoenale voorkoms van *Penicillium digitatum*, *P. italicum* en *P. crustosum*, asook weerstandsfrekwensies teen imazalil, thiabendazool en guaziatien in hierdie drie *Penicillium* spp., is afgehandel. Resultate het aangetoon dat *P. digitatum* en *P. italicum* pakhuis-omgewings gedomineer het, met inokulum-lading en weerstandsfrekwensies wat soos die seisoen voortduur, toeneem; hierdie benadruk die belang van pakhuis sanitasie. Imazalil, thiabendazole en pyrimethanil residu-lading, volgende op funksiesbad- en wakstoedienings, en gevolglike bio-effektiwiteit teen sensitiewe en weerstandbiedende isolate, is bestudeer, en het waardevolle insig in die gebruik van ons belangrikste na-oes funksies gegee. Integrasie van voor-oes silikon, en na-oes hitte en biobeheer word bestudeer. Tot hede is die optimale temperatuur vir verskillende sitrus-tipes bepaal, en die beheer van koueskade na silikon bemesting is bevestig. Potensiële alternatiewe swamdoders of saniteerders word voortdurend ondersoek, maar geen van die produkte tydens 2011 getoets, het belowende resultate getoon.

Die Diagnostiese Sentrum (DS) lewer steeds 'n uitstekende diens aan die Sitrusverbeteringskema deur roetine grond- en waterontledings vir *Phytophthora* en aalwurms, asook ontledings vir navorsers in die Grondgedraagde Siekte-projek. Die DC doen ook gehalte-beheer vir River Bioscience. In totaal het die DC 8272 monsters analiseer, en dit met slegs een diagnostikus, een tegnikus en een assistent. Verandering is personeel het onverwags probleme geskep, wat opgelos moes word met nuwe aanstellings, en ook deur die DS onder lynbestuur van MC Pretorius te plaas.

Goeie vordering is in die algemeen in Siektebestuur gemaak. Soos tydens vorige jare, beperk 'nie-navorsing' eise op die beskikbare menslike hulpbronne in tegniese ondersteuning van die industrie die kwaliteit en kwantiteit van tasbare navorsingsuitsette en stuur weg van gekonsolideerde en gefokusde navorsing. In 'n poging om hierdie probleem aan te spreek, word navorsingsalliansies met universiteite en ander navorsingsdiensverskaffers gebruik om die navorsingskapasiteit te verbreed, terwyl dienslewering aan die industrie volgehou word. Dit alleen sal egter nie die probleem oplos nie, en addisionele kapasiteitsbou vir tegniese ondersteuning, asook verhoogde navorsingsbefondsing, moet oorweeg word.

## 4.2 PROJECT: GRAFT TRANSMISSIBLE DISEASES

Project coordinator: G. Cook (CRI)

### 4.2.1 Project summary

Within a country where *Citrus tristeza virus* (CTV) is endemic, cross-protection is implemented by the Citrus Improvement Scheme to minimise the harmful effects of this virus. Various mild sources of the virus were identified and are used to pre-immunise all citrus except lemons. This approach has mostly been successful, although the mechanism whereby cross-protection is achieved is not fully understood, necessitating further research to address the incidences of cross-protection breakdown. Research is ongoing at a national and international level to understand the interactions of various genotypes of this virus, with each other as well as with various citrus hosts. Two research approaches are followed within this program. Firstly, field sources are identified and trialed in the glasshouse and those showing potential are evaluated in the field (4.2.2, 4.2.3, 4.2.4, 4.2.5, 4.2.6, & 4.2.7). Secondly, a molecular approach of characterising these sources is taken (4.2.8). The long term expectation from this is that field data will be understandable, based on the molecular analysis which will ultimately enable implementation of better control strategies. Field analysis requires a number of years' tree health assessment and yield data, but thus far grapefruit trials support the replacement of the pre-immunising source GFMS12 with GFMS35. Indications are that other sources being trialed may show even more promise than GFMS35 (4.2.2, 4.2.3 & 4.2.5).

Research of citrus greening epidemiology is ongoing. Indigenous plants of the citrus family (*Rutaceae*) are evaluated for their ability to host "*Candidatus Liberibacter africanus*" (Laf) the African greening pathogen (4.2.9). Laf has not been detected in any indigenous host yet and current results indicate they do not play a role in the epidemiology of citrus greening. Some Rutaceous genera have now been shown to harbor Liberibacters similar, but differing from Laf and include *Zanthoxylum*, *Vepris*, *Clausena* and *Calodendrum*. Another epidemiological investigation of this disease is looking into the seasonal population fluctuation of its vector, *Trioza erythrae*, the citrus psyllid, and possible seasonal fluctuations in the infectivity of populations with Laf (4.2.10). Results from the past season show high psylla infectivity in greening infected orchards and in one instance 84% of the psylla collected were Laf infected. Population peaks correlate with previous findings of population peaks just after the spring flush and smaller population increases after the November flush.

Field evaluation continued of embryo-rescue clones, derived from sweet orange, with potential resistance/tolerance to greening. No greening was detected in these trees after the fourth year in the field. The second crop was also harvested and fruit quality was comparable to a standard commercial variety (4.2.11).

A milestone achieved this year is the near completion of the sequencing of the full genome of Laf. This will help in understanding the differences between Laf and the Asian Greening pathogen (Las) that has already been fully sequenced (4.2.9).

### Projekopsomming

Binne 'n land waar *Citrus tristeza virus* (CTV) endemies is, is kruis-beskerming deur die Sitrus Verbeteringskema geïmplementeer om die skadelike effekte van hierdie virus te beheer. Verskeie ligte bronne van die virus is geïdentifiseer en word gebruik om vooraf alle sitrus behalwe suurlemoene te pre-immuniseer. Hierdie benadering is meestal suksesvol alhoewel die meganisme waarmee dit bereik word, nie ten volle verstaan word nie en noodsaak dus verder navorsing, veral waar kruisbeskerming gevaal het. Navorsing, op 'n nasionale en internasionale vlak, ondersoek die interaksies van verskillende genotipes van die virus met mekaar sowel as hul interaksies met verskeie sitrus tipes. Twee benaderings word in hierdie navorsingsprogram gevolg. Eerstens, word veldbronne geïdentifiseer, in die glashuis getoets en die wat potensiaal toon, word verder in die veld geëvalueer (4.2.2, 4.2.3, 4.2.4, 4.2.5, 4.2.6 & 4.2.7). Tweedens, word 'n molekulêre benadering van karakterisering van hierdie bronne gevolg (4.2.8). Die langtermyn verwagting van hierdie benadering, is dat die veld resultate verstaanbaar sal wees, gebaseer op die molekulêre analise wat dan die implementering van 'n beter beheer strategieë moontlik sal maak. Veld evaluasie vereis 'n aantal jare se beoordeling van boom gesondheid en opbrengs data, maar tot dusver ondersteun huidige pomelo proewe die vervanging van die pre-immunisingsbron GFMS12 met GFMS35. Aanduidings is dat ander bronne, wat tans getoets word, selfs meer belofte as GFMS35 toon (4.2.2, 4.2.3 & 4.2.5).

Navorsing van Sitrusvergroening epidemiologie is voortgesit. Inheemse plante van die sitrus familie (*Rutaceae*), word geëvalueer vir hul vermoë om as alternatiewe gashere vir "*Candidatus Liberibacter africanus*" (Laf), die Afrika vergroening patoog te dien (4.2.9). Laf is nog nie in enige inheemse gasheer

gevind nie, en die huidige resultate dui daarop dat hulle nie 'n rol in die epidemiologie van Sitrusvergroening speel nie. Sommige genera in die *Rutaceae* familie, *Zanthoxylum*, *Vepris*, *Clausena* en *Calodendrum*, hawe wel soortgelyke Liberibacters wat van Laf verskil. Nog 'n epidemiologiese ondersoek van hierdie siekte kyk na die seisoenale bevolking fluktuasie van die insek vector, *Trioza erytreae*, die sitrus bladvlooi, en moontlike seisoensverandering in sitrusbladvlooi populasies se besmettingsvlakke met Laf (4.2.10). Resultate van die afgelope seisoen toon hoë besmettingsvlakke van bladvlooi populasies met Laf in vergroening besmette boorde. In een geval is 84% van die bladvlooi wat versamel is, met Laf besmet. Populasiepieke korreleer met vorige bevindings wat dui op bevolkingspieke net na die lente groeistuwing en kleiner bevolkingstoename na die November groeistuwing.

Veld evaluering van embryo-herwinningsklone vanaf soetlemon wat potensiële weerstand / toleransie teen vergroening toon, is voortgesit. Geen vergroening is in hierdie bome na die vierde jaar in die veld opgespoor nie. Die tweede oes vanaf hierdie bome dui dat vruggehalte vergelykbaar met 'n standaard kommersiële kultivaar is (4.2.11).

'n Mylpaal wat hierdie jaar bereik is, is die nabye voltooiing van die nukleotied volgorde-bepaling van die genoom van Laf. Dit sal help in die verstaan van die verskille tussen die Asiatiese vergroeningspatogeen (Las), waarvan volle genoomvolgorde reeds bekend is, en Laf (4.2.9).

#### 4.2.2 **PROGRESS REPORT: Cross-protection of Star Ruby grapefruit using Beltsville sub-isolates of Nartia mild strain**

Experiment 679 (2003 - 2013) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

##### **Summary**

The Nartia source (GFMS 12) was replaced with GFMS 35 as a cross protecting source for red grapefruit in 1998 and for all grapefruit in 2007. In the search for optimal cross-protection sources, 20 sub-isolates were derived from single aphid transfers (SATs) of two Nartia sources (A=GFMS 12, C=GFMS 14) and Mouton sub-isolates obtained from SATs done in Beltsville MD, USA, and imported back to South Africa. Six of these sub-isolates showed potential as cross-protecting agents in glasshouse trials and their cross-protection abilities are now being assessed for field performance. Virus-free Star Ruby and Marsh grapefruit trees were pre-immunised with the six Beltsville sub-isolates, two sub-isolates from the ITSC (GFMS 12/7, GFMS 12/9) and the mild strain sources, GFMS 12 and GFMS 35. Control trees were left virus-free. Pre-immunisation was confirmed with ELISA, which showed that two of the Beltsville sub-isolates did not comply with traits of a good cross-protecting isolate as they were poorly transmitted and translocated in the plant and were therefore excluded from further evaluation. The trial trees were planted at Tambuti Estates in Swaziland in 2003. Trees were evaluated for yield and stem pitting 8 years after planting. Currently the following observations of tree health are important: i) GFMS12 suppressed growth and was associated with severe stem pitting, ii) sub-isolate 12/7 inoculated trees also started to develop unacceptable stem pitting, iii) The current cross protector GFMS35, showed no stem pitting and is therefore superior to GFMS12 with regard to tree health. Sub isolate B390/5 has shown the best performance based on current yield data, with 74% large fruit and 26% small fruit and a good yield relative to the other treatments. The MxT rootstock has performed poorly and trees are only now producing acceptable yield data. An extension of further year's yield data is required to optimally assess the various sources.

##### **Opsomming**

Die Nartia CTV preïmmuniseringsbron (GFMS 12) is gedurende 1998 met GFMS 35 vervang as 'n CTV-kruisbeskermingsbron vir rooi pomelos en gedurende 2007 vir wit pomelos en pompelmoese. In die soeke na meer geskikte kruisbeskermingsbronne, is 20 sub-isolate deur middel van enkel plantluis oordragings vanaf twee afsonderlike Nartia bronne (A=GFMS 12, C=GFMS 14) en die Mouton bron bekom. Laasgenoemde is in Beltsville MD, VSA voorberei. Ses uit 20 sub-isolate, het potensiaal as kruisbeskermingsagente in die glashuisproewe getoon en is gebruik om hul kruisbeskermingsvermoëns in die boord te evalueer. Virus-vrye Star Ruby en Marsh pomelo boompies is met die ses Beltsville sub-isolate, twee sub-isolate van die LNR-ITSG (12/7,12/9) en die GFMS12 en GFMS35 bronne ge-preïmmuniseer. Virusvry bome is as kontroles gebruik. Preïmmunisasie is deur middel van ELISA bevestig wat ook uitgegewys het dat twee van die Beltsville sub-isolate nie voldoen aan die vereistes vir 'n goeie kruisbeskermingsbron nie; deurdad hulle 'n lae persentasie oordraagbaarheid het, asook stadig vermeerder en beweeg in die plant. Hierdie twee sub-isolate word nie verder ge-evalueer nie. Die proef is by Tambuti landgoed in Swaziland gedurende 2003 uitgeplant. Die bome se stamgleuf skade is 8 jaar na plant ge-evalueer. Tans is die volgende waarnemings betreffende boom gesondheid gemaak: i) GFMS 12 onderdruk groei en veroorsaak strawwe stamgleuf; ii) 12/7 begin ook onaanvaarbare stamgleuf veroorsaak; iii) die huidige kruisbeskermer GFMS35, presteer beter as GFMS12, die vorige beskermer deurdad geen stamgleuf

waargeneem word nie. Wat die produksie van die bome betref, het sub-isolaat B390/5 die beste presteer met 74% groot vrugte en 26% klein vrugte asook goeie oes relatief tot ander behandelings. Die MxT onderstam het tot dusvêr nie goed presteer nie en aanvaarbare oes data word nou eers verkry. 'n Verlenging van 'n addisionele jaar is nodig vir optimale evaluasie van hierdie bronne.

#### 4.2.3 **PROGRESS REPORT: Cross-protection of Star Ruby using Beltsville sub-isolates of *Nartia mild strain for the Orange River Valley***

Experiment 738 (2004 - 2014) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

##### **Summary**

Indications of a possible severe component in the *Nartia* (GFMS 12) CTV cross-protecting source necessitated the separation of the strain populations into sub-isolates by single aphid transmissions. These sub-isolates were derived from two *Nartia* sources (A=GFMS 12, C=GFMS 14) and a Mouton source. The Mouton sub-isolates were done at Beltsville, USA, and imported back to South Africa. After biological indexing, four sub-isolates showed potential for further evaluation (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). Two sub-isolates from the ITSC sources (GFMS 12/7, GFMS 12/9) were included in the trial as well as GFMS12 (previous standard cross-protector for white grapefruit) and GFMS35 (standard cross-protector for red grapefruit). Virus-free Star Ruby trees were prepared in a glasshouse and were pre-immunised with the various sources. A virus-free treatment was included as a control. After confirming pre-immunisation by ELISA, trees were planted in the Kakamas area in September 2004. This is a duplicate experiment of experiment 679, planted in 2003 in Swaziland, and the two experiments are aimed at assessing the CTV expression in different climatic conditions. In 2007 similar trials were also planted in the Malelane and Letsitele area (experiment 742). During this report period, tree sizes were measured at the Kakamas trial 7 years after planting. Trees grew much slower than in other grapefruit production areas. Stem pitting evaluations were done and trees containing sub-isolate B389/1 developed moderate stem pitting and those with GFMS 12 and 12/7 mild pitting.

##### **Opsomming**

Weens aanduidings van 'n strawwe *Citrus tristeza virus* (CTV) komponent in die *Nartia* (GFMS 12) kruisbeskermingsbron was dit nodig om die virus populasie in sub-isolate deur middel van enkel plantluis oordragings te skei. Sub-isolate is vanaf twee *Nartia* bronne (A=GFMS 12, C=GFMS 14) en 'n Mouton bron verkry. Die Mouton sub-isolate is by die kwarantyn fasiliteit in Beltsville, VSA, voorberei en terug na Suid Afrika ingevoer. Nadat die sub-isolate deur biologiese indeksering ge-evalueer is, is gevind dat slegs vier potensiaal gewys het vir verdere evaluasie (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). Twee belowende *Nartia* sub-isolate afkomstig van die LNR-ITSG (GFMS 12/7, GFMS 12/9) is in die proef ingesluit, asook GFMS12 (vorige kruisbeskermingsbron) en GFMS35 (huidige kruisbeskermingsbron). Virusvrye Star Ruby boompies is in 'n glashuis voorberei en met die verskeie bronne gepreïmmuniseer. 'n Virusvrye behandeling is as kontrole ingesluit. Hierdie proef is 'n herhaling van eksperiment 679 wat in Swaziland aangeplant is, asook gedeetelike herhaling van proewe aangeplant in Malelane en Letsitele (eksperiment 742). Die verskeie proewe dien om CTV uitdrukking in die verskillende sitrus produserende streke te evalueer. Nadat preïmunisering deur middel van ELISA bevestig is, is die boompies in die Kakamas omgewing gedurende September 2004 uitgeplant, en sal jaarliks vir boomgrootte, stamgleuf, oes opbrengs en vruggrootte ge-evalueer word. Die bome se groottes is 7 jaar na uitplant gemeet. Die bome groei heelwat stadiger as bome in die ander pomelo produserende streke. Stamgleuf evaluasies is gedoen en sub isolaat B389/1 het matige stamgleuf ontwikkel en GFMS12 en 12/7 ligte stamgleuf.

#### 4.2.4 **PROGRESS REPORT: The effect of different CTV sources in Valencias on different rootstock combinations for the Orange River Valley**

Experiment 739 (2004 - 2014) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

##### **Summary**

CTV expression differs within host cultivars and under different climatic conditions. It is therefore necessary to evaluate the various cross-protecting CTV sources in various citrus production areas. Mild CTV sources derived from sweet orange trees (SM 46, SM 47, SM 48, SM 49) were used to pre-immunise virus-free Delta, Midnight, and Turkey Valencia on C35 citrange rootstocks. These sources will be compared to LMS 6 (standard pre-immunisation source for sweet oranges) and virus-free controls. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Karsten Boerdery in the Kakamas area in September 2007. Tree size was measured 4 years after planting. Although, there are significant differences in growth between treatments, it is too early to draw any conclusions. Trees will be evaluated annually for growth, production, fruit size and tree health.

## Opsomming

Omdat *Citrus tristeza virus* (CTV) isolate verskillend reageer in verskillende sitrusgashere en onder verskillende klimaatstoestande, is dit nodig om verskillende CTV bronne in die verskillende sitrus produserende streke te evalueer. Potensiële CTV preïmmuniseringsbronne wat oorspronklik vanaf soetlemoenbome versamel is (SM 46, SM 47, SM 48, SM 49), is gebruik om virusvrye Delta-, Midknight-, en Turkey Valencia op C 35 citrange onderstam te preïmmuniseer. Hierdie bronne word met LMS 6 (die standaard preïmmuniseringsbron vir soetlemoene) vergelyk, asook met bome wat virusvry geplant is. Preïmmunisering is deur middel van ELISA bevestig waarna die boompies gedurende September 2007 by Karsten Boerdery in die Kakamas omgewing geplant is. Die boomgroottes is 4 jaar na uitplant gemeet en alhoewel daar statistiese verskille tussen die verskillende behandelinge was, is dit nog te vroeg om enige gevolgtrekkings te maak. Jaarlikse evaluasies word gedoen vir boomgrootte, vruggrootte, oesopbrengs, sowel as boomgesondheid.

### 4.2.5 PROGRESS REPORT: Cross-protection of Marsh and Star Ruby by using the best field isolates collected in the different grapefruit production areas of southern Africa Experiment 742 (2004 - 2014) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

## Summary

Budwood was obtained from the different grapefruit production areas of southern Africa from 108 superior grapefruit trees that harbour possible mild CTV sources. After the CTV sources were established in the glasshouse, material was inoculated to virus-free Mexican lime indicator plants to evaluate the severity of the CTV sources. After the first biological test, 19 were selected for further evaluation. These 19 sources were inoculated again to virus-free Mexican lime plants and compared to GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9, and the four best Beltsville sub-isolates (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5). The Mexican lime plants were evaluated for growth and stem pitting. Virus titre was determined by ELISA. The most promising of 19 field sources (Tabankulu 1 – derived from Star Ruby in Swaziland; New Venture 41/2 – derived from Star Ruby in the Nkwale Valley; ORE 8 – derived from Marsh in the Hoedspruit area; Tshipise 19/5 – derived from Marsh in Tshipise), indexed free for citrus viroids and are being evaluated as pre-immunising agents for Marsh and Star Ruby trees. These sources are compared to GFMS 12 (standard for white grapefruit), GFMS 35 (standard for red grapefruit), as well as the four best Beltsville sub-isolates (B389-1, B389-4, B390-3, B390-5) and the ITSC sub-isolates (GFMS 12/7, GFMS 12/9). Pre-immunisation was confirmed by means of ELISA and the Star Ruby trees were planted at Bosveld Citrus Farm in the Letsitele area in February 2007, while the Marsh trees were planted at Riverside in the Malelane area in March 2007. The trees were evaluated for growth and health 4 years after planting. In these early stages, both the Marsh and Star Ruby trees containing GFMS12 had developed unacceptably high stem pitting and resulting suppressed tree growth. Although differences were observed, it is still too early to draw further conclusions. The trees will be evaluated annually for growth, production, fruit size and tree health.

## Opsomming

Enthout is vanaf 108 uitstaande pomelo bome, wat gesondheid en produksie betref, in die verskillende pomelo gebiede in suider Afrika versamel. Die bronne is op virusvrye onderstamme in die glashuis by CRI gevestig. Hierna is die verskillende bronne afsonderlik op Meksikaanse lemmetjie geïnkuleer (biologiese indeksering) om te bepaal of die bome moontlik ligte rasse van *Citrus tristeza virus* (CTV) huisves wat as kruisbeskerdingsbronne kan dien. Na die eerste biologiese indeksering van 6 maande het slegs 19 bronne potensiaal getoon en is vir verdere evaluering gebruik. Hierdie 19 bronne is 'n tweede keer op Meksikaanse lemmetjie geïnkuleer en met bekende bronne GFMS 12, GFMS 35, GFMS 12/7, GFMS 12/9 en die vier beste Beltsville sub-isolate (GFMS14: B389-1, B389-4; Mouton: B390-3, B390-5) vergelyk. Na 'n tydperk van 6 maande is die geïnkuleerde plante vir groei en voorkoms van stamgleuf asook die virus titer d.m.v. ELISA ge-evalueer. Die 4 mees belowende bronne, wat vry is van viroïede, is Tabankulu 1 (versamel vanaf Star Ruby in Swaziland), New Venture 41/2 (versamel vanaf Star Ruby in die Nkwale Vallei), ORE 8 (versamel vanaf Marsh in die Hoedspruit gebied) en Tshipise 19/5 (versamel vanaf Marsh in Tshipise). Hierdie bronne is verder gebruik om virus-vrye Marsh en Star Ruby boompies vir boord evaluasie te preïmmuniseer. Die bronne word met GFMS 12 (vorige standaard vir pomelos), GFMS 35 (huidige standaard vir pomelos), asook die vier beste Beltsville sub-isolate (B389-1, B389-4, B390-3, B390-5) en LNR-ITSG sub-isolate (GFMS 12/7, GFMS 12/9) vergelyk. Preïmmunisering is deur middel van ELISA bevestig voordat bome geplant is. Die Star Ruby boompies is gedurende Februarie 2007 op Bosveld Sitrus Plaas in die Letsitele omgewing geplant en die Marsh boompies is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is die vierde jaar na uitplant vir groei en stamgleuf ge-evalueer. In die vroeë stadium het bome met GFMS12 in beide Marsh en Star Ruby onaanvaarbare hoë stamgleuf ontwikkel wat ook sodoende die

groeï belemmer het. Alhoewel daar verskille is, is dit nog te vroeg om enige verdere gevolgtrekkings te maak. Die bome sal jaarliks vir groei, produksie, vrugsgrootheid en algemene boom gesondheid ge-evalueer word.

#### 4.2.6 **PROGRESS REPORT: Identification of suitable *Citrus tristeza virus* sources for pre-immunising Turkey Valencia**

Experiment 789 (2005 - 2015) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

##### **Summary**

Turkey Valencia appears to be more sensitive to CTV than other Valencia types. Since Turkey Valencia is an early Valencia type, it is an important component of the citrus industry and it is therefore important to identify a suitable CTV pre-immunising source for this cultivar. Virus-free Turkey Valencia on Troyer citrange rootstocks were prepared in the glasshouse and inoculated with different CTV sources; LMS 6 (standard), SM 46, SM 47, SM 48, SM 49 (all obtained from sweet orange) to identify the best source for cross-protection purposes. Trees inoculated with GFMS12 and virus-free trees will serve as positive and negative controls, respectively. Pre-immunisation was confirmed by means of ELISA. The trees were planted at Riverside in the Malelane area in March 2007. The tree growth was measured 4 years after planting. Differences between the treatments were observed, but it is still too early to draw any conclusions. The trees will be evaluated annually for growth, production, fruit size, as well as tree health.

##### **Opsomming**

Daar is gevind dat Turkey Valencia meer gevoelig vir *Citrus tristeza virus* (CTV) as ander Valencia tipes is. Aangesien Turkey Valencia 'n vroeë Valencia is, is dit 'n belangrike kultivar in die bedryf en daarom is dit 'n hoë prioriteit om 'n geskikte CTV pre-immunisasie bron vir Turkey Valencia te vind. Virusvrye Turkey Valencia op Troyer citrange onderstam is in 'n glashuis voorberei en met verskeie CTV bronne, LMS 6 (standaard), SM 46, SM 47, SM 48, SM 49 (almal vanaf soetlemoene versamel), geïnkuleer om die beste ligte CTV bron vir kruisbeskermingsdoeleindes te identifiseer. Bome wat met die GFMS 12 bron geïnkuleer is en bome wat virusvry gelaat is, dien onderskeidelik as positiewe en negatiewe kontroles. Pre-immunisasie is deur middel van ELISA bevestig en die bome is gedurende Maart 2007 by Riverside in die Malelane omgewing geplant. Die boompies is 4 jaar na uitplant vir groei ge-evalueer. Daar was verskille, maar dit is nog te vroeg om enige gevolgtrekkings te maak. Die bome sal jaarliks vir groei, produksie, vrugsgrootheid en algemene boom gesondheid ge-evalueer word.

#### 4.2.7 **PROGRESS REPORT: Searching for a *Citrus tristeza virus* source suitable for cross-protecting soft citrus**

Experiment 968 (2009 - 2019) by J.H.J. Breytenbach and S.P. van Vuuren (CRI)

##### **Summary**

During re-indexing of the Citrus Foundation Block mother trees in 2003 it was found that a many Clementine and mandarin trees did not contain CTV. This caused concern as the budwood that was multiplied from these mother trees and supplied to the commercial nurseries, were virus-free, rendering the trees unprotected against natural CTV infection with severe strains introduced by aphids. A change to another CTV source compatible with mandarin types was required. The GFMS12 CTV source will be used for pre-immunisation in the interim until a suitable CTV pre-immunising source for soft citrus is identified. A glasshouse trial was conducted in 2006 to evaluate additional CTV sources in four different cultivars. This current trial is an extension of the glasshouse trial. Two Clementine selections (Clemenluz, Esbal) and two mandarin selections (Valley Gold, Morr 22) on Troyer citrange rootstock have been grown and pre-immunised with different CTV sources; *i.e.* SM 46, SM 47, SM 48 and SM 49. Trees with these sources will be compared to trees that were pre-immunised with GFMS12 (standard) and trees planted virus-free. Pre-immunisation was confirmed by means of ELISA and the trees were planted during 2010/11 at two localities within different climatic regions suitable for the production of soft citrus, *i.e.* Groblersdal in Mpumalanga and Citrusdal in the Western Cape. Trees at both sites were evaluated for growth 1 year after planting but both trial sites need to be re-planted due to frost and water logging damage.

##### **Opsomming**

Tydens die her-indeksering van die Grondvesblok se moederbome gedurende 2003, is gevind dat 'n groot aantal Clementine en mandaryn bome geen CTV bevat het nie. Dit het kommer gewek as gevolg van die feit dat enthout wat aan die kommersiële kwekerye verskaf word, virusvry is en nie bekerming bied teen natuurlike CTV rasse wat in die veld deur plantluise oorgedra word nie. Die CTV bekermingsbron is

noodgedwonge na GFMS12 verander totdat 'n geskikte bron vir sagte sitrus gevind is. 'n Glashuis proef is gedurende 2006 gedoen om CTV bronne in vier verskillende kultivars te evalueer. Hierdie veldproef is dus 'n uitbreiding van die glashuis proef. Twee Clementine seleksies (Clemenluz, Esbal) en twee mandaryn seleksies (Valley Gold, Morr 22) is op Troyer citrange onderstamme ge-okuleer en gepreïmmuniseer met verkillende CTV bronne, nl. SM 46, SM 47, SM 48 en SM 49. Bome met hierdie bronne sal met GFMS12 (standaard) en bome wat virus-vry geplant is, vergelyk word. Nadat preïmmunisering deur middel van ELISA bevestig is, is die bome gedurende 2010/11 in twee verskillende klimaatstreke geskik vir sagte sitrus geplant (Groblersdal in Mpumalanga en Citrusdal in die Wes-Kaap). Boompies by al twee die proefpersele is ge-evalueer vir groei 'n jaar na uitplant, maar moet oorgeplant word as gevolg van dreineringsprobleem en koue-skade.

#### 4.2.8 **PROGRESS REPORT: Dynamics of citrus tristeza virus mild and severe strains in mild strain cross-protection strategies**

Experiment 885 (April 2007 – April 2015) by Gerhard Pietersen (ARC-PPRI and UP), D. Read (UP), O. Zablocki (UP) and J. Lubbe (UP)

##### **Summary**

A recent landmark study demonstrated that hyper-exclusion of CTV genotypes is very specific between genotypes, i.e. a given genotype in a tree only prevents infection of a similar genotype in the tree. This implies that for efficient CTV mild strain cross protection to be exploited in South Africa it is important to: 1) determine the genotypes of CTV circulating in the Southern African citrus industry, primarily in grapefruit, especially those overcoming current pre-immunising sources, 2) to isolate these genotypes in pure form, 3) to characterise and confirm that these isolates are pure, 4) to test their pathogenicity on a range of citrus hosts, 5) to test the relative competitiveness and ability of mild sources to cross protect against more severe variants of the same and different genotypes, 6) to prepare artificial mixed populations of mild strains of each genotype to serve as pre-immunising sources, and 7) to assess the performance of the mixed mild strain population as a pre-immunising source. These are very long term goals. Currently initial phases of this goal are being addressed. During the report period progress has been made towards this objective by: 1) characterisation of the CTV population present in candidate pre-immunisation sources Tabankulu 1 and New Venture 41/2; 2) characterisation of the SM49 pre-immunizing source of CTV in Sweet Orange and in Mexican Lime hosts, as well as characterisation of single aphid transmission (SAT) sub-isolates of SM49; 3) further characterisation of the CTV genotype composition of the GFMS12 pre-immunising source SAT sub-isolates; 4) Preparation of an infectious clone of a South African VT genotype; 5) assessment of next generation sequencing for the genotype identification of CTV, whole genome characterisation, confirmation of viral homogeneity, and assessment of genotype components of mixed CTV populations; 6) isolation of pure CTV genotype sources; and 7) analysis of the CTV population of grapefruit cultivars in greenhouses and in the field.

##### **Opsomming**

'n Onlangse, insiggewende publikasie het gewys dat infeksie deur 'n spesifieke CTV genotype slegs beskerm teen infeksie van ander variante van daardie genotipe. Hierdie impliseer dat dat, vir die doeltreffende beheer van CTV in Suid-Afrika, sal ons matige bronne van elke genotipe wat sirkuleer, moet kry. Vir kruisbeskermingsdoelwitte, sal hierdie matige bronne saam in 'n kunsmatige mengsel van rasse gebruik moet word. Tydens die huidige verslagperiode is vordering met hierdie langtermyn doelwit gemaak deur: 1) die karakterisering die CTV populasie in die kandidaat pre-immuniserings bronne Tabankulu 1 en New Venture 41/2; 2) karakterisering van die SM49 pre-immuniseringsbron in Soet Lemoen en Meksikaanse Lemmetjie gashere, sowel as enkelplantluise oordragings isolaat (SAT's) bronne; 3) verdere karakterisering van die GFMS12 SAT sub-isolaat; 4) die maak van 'n infektiewe kloon van die Suid-Afrikaanse VT-genotipe; 5) evaluasie van volgende-geslag nukleotiedvolgorde-bepaling vir die genotipe bepaling van CTV heel-genoom karakterisering, bevestiging van homogenisiteit en bepaling van die genotipe samestelling van gemengde CTV populasies; 6) isolering van CTV genotipes; en 7) analise van die CTV genotipe samestelling van pomelos in glashuise en die veld.

#### 4.2.9 **PROGRESS REPORT: Epidemiology of greening disease – alternate hosts and spread**

Experiment 886 (April 2007 – April 2014) by Gerhard Pietersen (ARC-PPRI and UP) and Ronel Viljoen (UP)

##### **Summary**

Citrus greening disease has been reduced to economically acceptable levels in South Africa through stringent vector control strategies and the removal of inoculum in the form of infected branches and trees,

but remains a problem in cooler citrus production areas of South Africa. The perpetuation of the disease may be due to the presence of hosts other than citrus. Determining potential alternate hosts to citrus of "*Candidatus Liberibacter africanus*" (Laf), associated with greening in South Africa amongst the indigenous plants of South Africa, will aid in the development of an integrated control strategy for the greening disease by making disease pressure reduction more efficient. In this study, indigenous plants mainly of the citrus family (*Rutaceae*) are evaluated for their ability to host the pathogen and to assess their role in the epidemiology of citrus greening. During the report period, large numbers of *Vepris*, *Zanthoxylum* and *Clausena* have been collected, mainly from the eastern coastal regions, and analysed for the presence of Liberibacters. Laf *sensu stricto* has not been detected in any indigenous members of the *Rutaceae* thus far and based on current results they do not seem to play a role in the epidemiology of citrus greening. However, Liberibacters are found in all genera of the *Rutaceae* analysed thus far. Based on analysis thus far of only a small part of the genome of Liberibacters, each Rutaceous genus appears to harbour different, specific Laf-like Liberibacters. Those found in *Zanthoxylum* appear to be most closely related to Laf from citrus. *Vepris* and *Clausena* harbour Liberibacters more closely related to the LafC subspecies found in *Calodendrum*. It is important to do controlled transmission tests to test the possibility that these bacteria may infect Citrus. The full genome sequence determination of Laf, done in collaboration with Dr. Hong Lin of the USDA-ARS in California, is almost complete and will aid in understanding the differences between Laf and the Asian Greening strain (Las), which has already been fully sequenced.

## Opsomming

Sitrus vergroening is in Suid-Afrika tot op ekonomies aanvaarbare vlakke beheer deur die streng beheer van die vektor en ook die stelselmatige verwydering van inokulum deur die verwydering van geïnfecteerde takke en bome. Die siekte duur egter voort en dit mag wees as gevolg van gashere anders as Sitrus wat as bronne vir die patogeen "*Candidatus Liberibacter africanus*" (Laf) dien. Die bepaling van alternatiewe gashere vir Laf onder die inheemse lede van die Sitrus-familie (*Rutaceae*) in Suid-Afrika, kan dien om meer doeltreffende siektedruk beheer daar te stel. In hierdie studie word inheemse plante, hoofsaaklik van die *Rutaceae* ge-evalueer vir hul vermoëns om as gasheer vir die patogeen op te tree en of hulle 'n rol in die epidemiologie van sitrus vergroening speel. Tydens die verslagperiode is groot getalle *Vepris*, *Zanthoxylum* en *Clausena* monsters, hoofsaaklik teen die Ooskus, versamel en vir die teenwoordigheid van Liberibacters geanaliseer. Laf, in streng gebruik van die naam, is nog nie in enige van die inheemse lede van die *Rutaceae* gevind nie, en dit wil dusver voorkom asof hierdie bome nie 'n rol in die epidemiologie van sitrus vergroening speel nie. Liberibacters, soortgelyk aan Laf, is egter in alle genera van die *Rutaceae* tot dusver gevind. Gebaseer op die nukleotiedvolgorde van slegs 'n klein gedeelte van die genoom dusver, wil dit voorkom asof verskillende genera van die *Rutaceae* verskillende Laf-agtige Liberibacters huisves. Liberibacters van *Zanthoxylum* blyk die mees verwant te wees and Laf van sitrus, terwyl die op *Vepris* en *Clausena* groter verwantskap aan die LafC subspecies van *Calodendrum* toon. Dit is belangrik op gekontroleerde proewe te doen om te bepaal of enige van die Liberibacters sitrus mag infekteer. Die nukleotiedvolgorde bepaling van die genoom van Laf is byna voltooi. Hierdie werk is in samewerking met Dr. Hong Lin van USDA-ARS in Kalifornië gedoen. Hierdie kennis sal help om die verskille tussen Laf en die Asiese Vergroening ras (Las), wie se genoom reeds volledig ontleed is, te verstaan.

### 4.2.10 PROGRESS REPORT: Investigation into the seasonal population fluctuation of *Trioza erytreae* and infection with the greening organism, *Candidatus Liberibacter africanus*. Experiment 988 (2010 - 2013) by G. Cook, Zama Maqutu and S.P. van Vuuren (CRI)

## Summary

This is an investigation into the possible seasonal fluctuation of the infectivity of citrus psyllid (*Trioza erytreae*) populations with the greening pathogen, "*Candidatus Liberibacter africanus*" (Laf). Individual psyllids caught on yellow sticky traps are tested by PCR for the pathogen. The project approach was changed this reporting season as psyllid populations were very low the previous season and trap plants are no longer used because of minimal transmission. Yellow sticky traps were changed weekly in a sour orange orchard, Hilltop 458JT, in the Nelspruit district, which is entirely infected with greening and where no psyllid control is done. The psyllid population in this orchard peaked in September, correlating with previous findings of population peaks just after the spring flush. A lesser population increase was also seen in December after the November flush. In September to October, 67 and 68% of individual psyllids tested were positive for Laf and thereafter infectivity dropped to 15-20% for 2 months, followed by another peak of 68% infectivity in January 2012. In 2011, very few psyllids were trapped from March to June and the population started escalating from July. A similar trend was found in 2012 with few psyllids trapped from February to April thus far. Since the number of individuals trapped in late summer to mid winter is so low, it is not possible to accurately determine the percentage infectivity, but positive individuals were occasionally detected over this period. A psyllid outbreak was observed in a Crocodile Valley Citrus Co. orchard and psyllids were collected

here from September to October in 2011. This orchard is highly infected with greening and symptoms were visible on most trees. Psyllids collected at this site also showed a high Laf infectivity which peaked at 84% in the second week of October. Psyllid populations were also monitored at the CRI premises in Nelspruit, where populations were considerably higher, with a population peak in September. Various citrus trial plants are hosts at this site for the psyllids, but there are fewer infected citrus plants on site. The Laf infectivity of psyllids at this site peaked at 8.8%. In this report period a total of 1700 individual psyllids were trapped and tested for Laf of which 22% were infected.

## Opsomming

Hierdie eksperiment ondersoek die maandelike seisoenale fluktuasie van besmetting van die sitrusbladvlooi (*Trioza erytreae*) met die vergroeningspatogeen, "*Candidatus Liberibacter africanus*" (Laf). Bladvlooi word gevang met geel, taai lokvalle en individuele bladvlooi word met PKR getoets. Die projek benadering het verander in hierdie verslagperiode deurdat lokval-plante nie meer gebruik word nie. Geel, taai lokvalle word nou weekliks in 'n bitterlemoen boord by Hilltop 458JT in die Nelspruit omgewing geplaas. Die boord is volkome met vergroening besmet en geen bladvlooi beheer word toegepas nie. Die bladvlooi bevolking in die boord het in September gepeik en dit korreleer met vorige bevindinge van populasie pieke kort na die lente groeistuwing. 'n Kleinere bevolking toename is in Desember na die November groeistuwing gesien. In September tot Oktober was 67 tot 68% van bladvlooi wat getoets is, positief vir Laf. Daarna het die besmetting vir die opvolgende twee maande tot 15-20% gedaal en weer tot 68% in Januarie 2012 gepeik. Vanaf Maart tot Junie 2011 was min bladvlooi gevang, maar die bevolking het van Julie begin styg. 'n Soortgelyke neiging is in 2012 waargeneem met min bladvlooi vanaf Februarie tot April gevang. Aangesien die aantal bladvlooi gevang in die laat-somer tot mid-winter so laag was, was dit nie moontlik om die persentasie bladvlooi-besmetting akkuraat te bepaal nie, maar positiewe bladvlooi was wel soms in hierdie tydperk gevind. 'n Bladvlooi uitbraak was in 'n "Crocodile Valley Citrus" Kooperasie boord waargeneem en bladvlooi is hier vanaf September tot Oktober 2011 versamel. Hierdie boord het 'n hoë vergroeningsbesmetting en simptome is op meeste bome waarneembaar. Bladvlooi vanaf hierdie boord het 'n hoë Laf besmetting gewys en die besmetting het by 84% in die tweede week van Oktober gepeik. Bladvlooi bevolking is ook op die CRI terrein in Nelspruit, waar die getalle aansienlik hoër was, gemonitor en het ook in September 'n piek bereik. Verskeie sitrusproefbome, wat as gasheer vir die bladvlooi dien, is hier te vinde, maar 'n klein aantal bome is besmet met vergroening. Die Laf besmetting van die bladvlooi hier, het by 8.8% gepeik. In hierdie verslagperiode is 'n totaal van 1700 individuele bladvlooi getoets waarvan 22% met Laf besmet was.

### 4.2.11 PROGRESS REPORT: Evaluation of citrus material for greening resistance Experiment 815 (2006 - 2015) by S.P. van Vuuren (CRI)

#### Summary

Attempts are being made to obtain greening resistance by rescuing embryos from healthy chimeras on greening infected fruit and growing them on artificial medium. The little plants that are generated are micro-grafted on vigorous rootstocks. These clones are multiplied on healthy rootstocks and exposed to field psylla. After the insects have fed for 7 days on the plants, they are removed and tested by polymerase chain reaction (PCR) to establish if they were infectious. After 3 months the plants are evaluated for the presence of greening by visual inspections and tested by PCR. In this manner it is established whether plants show resistance, tolerance or susceptibility. Symptomless plants that were exposed to positive psylla insects and remained free of the organism are deemed resistant and plants containing the organism without symptom expression are regarded as tolerant. Two embryo rescue clones, GTC-E2 and GTC-T2 were identified symptomless in 2006 after exposure to the vector. PCR confirmed that they were free of the greening organism. It was also confirmed that some batches of psylla that were used for challenging the plants were infected with the greening organism. These two clones have been multiplied on virus-free rootstocks and separately pre-immunised with two CTV sources whereafter they were planted during 2007 in an orchard for field evaluations. After 4 years no greening symptoms were observed on the trees and PCR tests were also negative. The second crop was harvested from the trees and fruit quality compared favourably with Midknight Valencia. Six clones that were obtained in 2007 and six clones obtained in 2009 were multiplied on rootstocks for exposure to the vector. Due to low populations of psylla insects, these clones could not be challenged previously. They were challenged during September to November 2011 but none of them showed resistance to the greening *Liberibacter*. No fruit with suitable chimeras were found during 2011 and therefore no new clones were generated during 2011.

## Opsomming

Daar word gepoog om vergroening weerstandbiedendheid te verkry deur embryo's uit gesonde chimeras van vergroende vrugte te verwyder en op kunsmatige medium te kweek. Die plantjies wat genereer word, word op groeikragtige onderstamme deur middel van mikro-enting gevestig. Hierdie klone word op onderstamme vermeerder en aan sitrus bladvlooië, die vektor van vergroeningsiekte, blootgestel. Nadat die insekte vir 'n week op die plante gevoed het, word hulle verwyder en deur middel van polimerase ketting reaksie (PKR) getoets om te bevestig dat hulle besmet was met die vergroeningspatogeen en sodoende die plante blootgestel het aan die bakterie. Na 3 maande word die plante vir die voorkoms van vergroeningsimptome ge-evalueer en getoets vir die teenwoordigheid van die vergroeningsorganisme d.m.v. PKR. Daar word sodoende bepaal of simptoomlose plante wat aan positiewe sitrus bladvlooië blootgestel was, vry van die vergroeningsorganisme is (weerstandbiedend) of die organisme huisves sonder dat simptome ontstaan (verdraagsaamheid of toleransie). Twee embryo-herwinningsklone, GTC-E2 en GTC-T2, is in 2006 as simptoomloos na blootstelling aan besmette insekte geïdentifiseer. PKR het getoon dat hulle vry van die organisme is. Die twee klone is op onderstamme vermeerder en afsonderlik met twee *Citrus tristeza virus* bronne gepreïmmuniseer en gedurende 2007 in 'n boord vir verdere evaluasie uitgeplant. Na 4 jaar is nog geen vergroeningsimptome waargeneem nie en PKR toetse was ook negatief. Die tweede oes is van die bome verkry en vruggehalte vergelyk goed met Midnight Valencia. Ses klone wat in 2007 verkry is en ses wat in 2009 verkry is, is op onderstamme vermeerder vir blootstelling aan sitrus bladvlooië. As gevolg van lae bevolkings van die insek vektor kon blootstellings van die klone nie voorheen gedoen word nie. Hulle is gedurende September tot November 2011 blootgestel en geen van hulle het weerstandbiedendheid teen die Liberibakter getoon nie. Geen vrugte met geskikte chimeras is gedurende 2011 gevind nie en is daar geen nuwe klone gedurende 2011 genereer nie.

### 4.3 PROJECT: FRUIT AND FOLIAR DISEASES

Project coordinator: G.C. Schutte (CRI)

#### 4.3.1 Project summary

Results from field trials that were sprayed for the control of *Alternaria* brown spot on 'Nova' mandarins using new systemic and contact fungicides alone or in combination with registered fungicides, showed that copper oxychloride performed the best, irrespective of the huge gap between the fourth and fifth application (4.3.2). Due to serious flooding experienced in the Lowveld during January 2011, the fifth spray round could only be applied after 9 weeks after the water subsided and the orchard became accessible. Tank mixtures of Fighter and Sporekill as well as Fighter on its own, if alternated with copper hydroxide, showed promise. Lack of citrus fruit and foliar disease control is regularly attributed to insufficient fungicide spray deposition on target surfaces. A deposition assessment protocol using fluorometry, photomacrography and digital image analysis was developed to study improvement of spray application (4.3.3). It was demonstrated that SARDI Yellow Fluorescent Pigment is an effective tracer for copper oxychloride deposition through their similar particle concentration and size. Deposition benchmarks were modelled to be used to evaluate spray technology research in 18 laboratory trials as used for control of *Alternaria* Brown Spot. High spray volumes (>10 000 L/ha) did not result in better spray deposition on leaves. Similar and even improved spray deposition could be obtained at lower spray volumes. Anomalous results were found when the benchmarks were used to interpret deposition data following laboratory and orchard spray trials for the evaluation of spray adjuvants. It can be attributed to the effects of adjuvants on deposition parameters, on pathogen development, and synergistic effects between adjuvant and fungicide. Additional fundamental research is therefore required to explain these anomalies and to improve the benchmark model. The saprophytic *Botrytis cinerea* Pers., the causal pathogen of blossom blight on lemons, can survive on a variety of organic matter with a low pH. The pH, Brix and mycelium growth of *Botrytis* on a petal- and stamen-extract media of different citrus cultivars were determined. Lemon petals and stamens had lower pH levels when compared with other citrus cultivars. Lemon stamens resulted in more mycelial growth when incorporated in a selective medium if compared with the other cultivars. *In vitro* and *in vivo* results showed that benomyl and iprodione performed the best in controlling the disease (4.3.4).

#### Projekopsomming

Resultate uit veldproewe, waar nuwe sistemiese en kontak swamdoders op hul eie asook in kombinasies met geregistreerde swamdoders vir die beheer van *Alternaria*-bruinvlek op 'Nova' mandaryne gespuit is, toon dat koperoksichloried die beste gevaar het, ondanks die groot gaping tussen die vierde en vyfde bespuitings. Weens vloede in die Laeveld in Januarie 2011 kon die vyfde bespuiting eers na 9 weke uitgevoer word omrede die boord oorstrom was. Tenklangsels van Fighter en Sporekill, asook Fighter op sy eie wat afgewissel word met koperhidroksied, toon groot belofte (4.3.2). Die gebrek aan beheer van sitrusblaar- en vrugsiektes kan toegeskryf word aan onvoldoende spuitbedekking op teikenoppervlaktes. Die gebruik van

fluorometrie, digitale makrofotografie, beeld-analise en gevolglike statistiese analiese is ontwikkel as metode om spuittoedienings te verbeter (4.3.3). "SARDI Yellow Fluorescent Pigment" as maatstaf vir koperoksichloried verspreiding is gedemonstreer deurdat dit dieselfde partikelkonsentrasie en -groottes gehad het. Verspreidingsmaatstawwe vir effektiewe beheer van *Alternaria* bruinvlek is uit 18 laboratorium spuitproewe gemodelleer en sal gebruik word om spuittegnologiesnavorsing te evalueer. Hoë spuitvolumes (>10 000 L/ha) het nie tot beter spuitbedekking op blare gelei nie. Soortgelyke en selfs verbeterde bedekking is verkry met laer spuitvolumes. Met die evaluasie van bymiddels is afwykende resultate met die bedekkingsmaatstawwe verkry in laboratorium- en boordbespuitingsproewe gevind. Die afwykings kan moontlik aan die effek wat bymiddels op bedekkingsparameters, patoëen ontwikkeling en sinergistiese effekte tussen bymiddels en swamdoders toegeskryf word. Die saprofitiese *Botrytis cinerea* Pers., wat bloeiselversenging op suurlemoene veroorsaak, kan op organiese materiaal met 'n lae pH oorleef en kan sodoende blomme infekteer. Die pH, Brix en swamgroei van *Botrytis* is op 'n blomblaar- en stuifmeeldraad-ekstrak-medium van verskillende sitruskultivars bepaal. Suurlemoene se blomblare en stuifmeeldrade se pH vlakke is laer in vergelyking met ander sitruskultivars. Stuifmeeldrade van suurlemoene het meer swamgroei op die selektiewe medium in vergelyking met ander kultivars tot gevolg gehad. *In vitro* en *in vivo* het benomyl en iprodione goeie beheer van *Botrytis* bloeiselversenging tot gevolg gehad (4.3.4).

#### 4.3.2 **PROGRESS REPORT: Evaluation of new spray programmes for the control of *Alternaria* brown spot in the summer rainfall regions of South Africa** Experiment 750 (September 2004 – June 2010) by G.C. Schutte and C. Kotze (CRI)

##### **Summary**

New systemic and contact fungicides alone or in combination with registered fungicides were tested on 'Nova' mandarins for the control of *Alternaria* brown spot. Serious flooding was experienced in the Lowveld during January 2011 after only 4 spray rounds were completed. The fifth spray round could only be applied after 9 weeks. Results showed that copper oxychloride performed the best irrespective of the huge gap between the fourth and fifth application and still resulted in 91.6% clean exportable fruit. The explanation for the phenomenon is that enough copper accumulated after the first four applications, which still had an effect in controlling the disease. The three applications that followed still had an effect on fruit colour and also resulted in copper stippling as expected. Tank mixtures of Fighter and Sporekill as well as Fighter on its own if alternated with copper hydroxide, show promise and should be investigated further.

##### **Opsomming**

Nuwe sistemiese en kontak swamdoders op hul eie asook in kombinasies met geregistreerde swamdoders is vir die beheer van *Alternaria* bruinvlek op 'nova' mandaryne getoets. In Januarie 2011 na slegs 4 spuitronde voltooi is, het ons vloed in die Laeveld ondervind en kon die vyfde spuitronde eers na 9 weke i.p.v. 4 weke toegedien word. Resultate toon dat koperoksichloried die beste gevaar het, ondanks die groot gaping tussen die vierde en vyfde bespuitings en steeds 91.6% skoon uitvoerbare vrugte opgelewer het. Die verklaring hiervoor is dat die opeenvolgende maandelikse bespuitings tot op daardie stadium, genoeg koper op die vrugte opgebou het en, ongeag die baie reën, die siekte steeds beheer kon word. Soos te wagte, het die 3 bespuitings wat gevolg het, steeds 'n invloed op vrugkleur gehad het en koperstippelvorming steeds aan die orde van die dag. Tenkengsels van Fighter en Sporekill asook Fighter op sy eie wat afgewissel word met koperhidroksied, toon groot belofte en moet verder ondersoek word.

##### **Introduction**

*Alternaria* brown spot (ABS) is a serious disease of tangerines (*Citrus reticulata*) and their hybrids in all citrus producing regions of South Africa. Susceptibility to ABS is a dominant trait that is transferred from 'Dancy' mandarin to its progeny (Dalkilic *et al.*, 2005). Dancy mandarin hybrids and some cultivars of unknown origin, such as 'Murcott', 'Emperor' and 'Ponkan', are affected by the disease. The presence of ABS in South Africa is still a serious problem on all cultivars derived from crosses with Dancy tangerine such as the 'Nova', 'Minneola' and 'Mor'.

The causal agent of ABS was designated originally as *Alternaria citri* Ellis & N. Pierce (Pegg, 1966) and later renamed *A. alternata* (Fr.:Fr.) Keissl. pv. *citri*, based on the production of a toxin specific to mandarin fruit (Solel, 1991). Later, eight species were described among *Alternaria* isolates pathogenic to mandarins based on morphological and biochemical traits (Andersen *et al.*, 2005; Simmons, 1999). However, all small-spored *Alternaria* spp. from citrus are closely related by molecular analysis and they have been placed into a single phylogenetic species, *A. alternata* (Peever *et al.*, 2002, 2004 & 2005).

ABS attacks young leaves, twigs and fruit, causing small black necrotic spots after a 24 to 36 h incubation period. ABS sporulates most abundantly on lesions on mature leaves remaining in the canopy (Reis *et al.*, 2006) The pathogen produces a host-specific toxin that causes lesions to expand, often resulting in leaf and fruit drop and twig dieback. On more mature fruit, lesions may vary from small necrotic spots to large, sunken pockmarks. Leaves are susceptible until they are fully expanded and hardened. Thus, this disease may affect tree growth, cause considerable crop loss, and produce blemishes on fruit that are unacceptable to the consumer. Leaves are susceptible to infection from the time of formation until they are fully expanded and hardened, and fruit are susceptible from petal fall until harvest. In the USA, however, fruit are only susceptible from petal fall until they reach about 5 cm in diameter.

Cultural measures, such as wider tree spacing and pruning to allow air movement and dry-off of trees, the elimination of overhead irrigation and avoidance of excess nitrogen fertilizer, can assist in reducing disease severity in some orchards. However, fungicide applications are essential for disease control and production of blemish-free fruit. In South Africa, it is important to protect fruit and flushes of cultivars such as tangerines and their hybrids with fungicides from September to April/May, often requiring 8+ spray applications. This number of sprays and the products being used are not economically sustainable and may result in unacceptable residues on fruit.

### Objectives

New spray programmes containing different fungicides with a broad spectrum of activity will be evaluated. We are also looking for alternative fungicides to be used in alternation with copper fungicides that can serve as an alternative for mancozeb.

### Materials and methods

Ten single-tree plots per treatment were randomly selected in a 'Nova' orchard at Belmont 50 km west of Nelspruit. The trees were 17 years old and planted in 2x5 m tree spacing in rows that ran from North to South. Trees were selected for uniformity in canopy density and tree size. Guard trees were located between plots within rows. Only two rounds of fungicidal sprays were applied with a trailer-mounted, high-volume, high-pressure (2500-3000 kPa) sprayer with two hand-held spray guns on the dates mentioned in Table 4.3.2.1. 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.

### Results and discussion

Objective / Milestone	Achievement
A. Evaluation of new fungicides	
A.1. Spraying field trial	All treatments were applied according to the protocol and the trial was successfully completed

Results from the field trial conducted at Belmont (Table 4.3.2.4) were influenced by the heavy rain experienced during early part of January 2011. Serious flooding of the access roads prevented us from crossing the Crocodile river and the orchard was also flooded, preventing us from applying the January application. Due to this, we could only apply the next application after 9 weeks instead of the planned 4 weeks and this also resulted in one less spray application. Nonetheless, results showed that there was a significant difference ( $P < 0.05$ ) between the standard registered copper oxychloride treatment and all the other treatments. Copper oxychloride resulted in 91.6% clean exportable fruit with the other standard, mancozeb and Fighter resulting in 62.6% and 63.0% clean exportable fruit respectively.

This was quite surprising because in treatments 3 and 6 where every second Fighter treatment was altered with copper hydroxide (Copstar) and the rate of Fighter was lowered in treatment 6 to 400ml/h $\ell$  water instead of 570ml/h $\ell$  water (which is the recommended rate for root rot control) they only resulted in 46% and 47% clean exportable fruit. This poor performance shows that a single copper application (a SC formulation with less metallic copper than the WP formulations) do not have the legs to prevent infection, whereas copper oxychloride had 4 top-up applications up to 8 December 2011 and still had enough copper residues left on the fruit surface to prevent *Alternaria* brown spot from developing on the fruit. However, copper stippling still remained a problem with the frequent applications of copper oxychloride (Fig. 4.3.2.1).

Where rate of Fighter was lowered from 570ml/h $\ell$  water in treatment 4 to 400ml/h $\ell$  water in a tank mix with Sporekill in treatment 5, treatment 5 resulted in 17% less clean exportable fruit; not significant different from

each other. In similar spray programmes (treatments 3 and 6), but with the exclusion of Sporekill from the tank mixture with Fighter, showed that they were equally effective in the control of Alternaria brown spot, even where the rate of Fighter was reduced to 400 ml/hl water (treatment 6).

For the criterion, fruit with 1 to 5 lesions per fruit, copper oxychloride, the untreated control and Fighter (400 ml/hl water) in tank mixtures with Sporekill alternated with copper hydroxide were not significant different from each other. On the other hand, the untreated control resulted in 92.4% fruit with 6 and more Alternaria brown spot lesions, which was significant different from all the treatments, demonstrating the high incidence of the disease during this extremely wet season (Table 4.3.2.2.).

### **Conclusion to date**

New fungicides were evaluated for the control of Alternaria brown spot during the previous seasons. None have been reported to be registered to date yet.

### **Technology transfer**

This research will be included in the annual research report to be distributed to citrus growers and will be included in various talks to citrus growers. Certain detail of the work cannot be presented as the programmes and fungicides are not registered.

### **Future objectives and work plan**

Any new fungicides in spray programmes of different modes of action have to be evaluated depending on the MRL requirements for each and every market. Boscalid for instance, is being tested in the USA for control of the disease.

**Table 4.3.2.1.** Evaluation of different fungicidal spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' mandarins at Belmont, Schagen during 2010 and 2011.

Treatment	15 September 2009	13 October 2009	10 November 2009	8 December 2009	9 February 2010	8 March 2010	5 April 2010
1	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g	Mancozeb 200g
2	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g	Demildex 200g
3	Fighter 570ml	Copstar 350 ml	Fighter 570ml	Copstar 350ml	Fighter 570ml	Copstar 350ml	Mancozeb 200g
4	Fighter + Sporekill 570ml +100ml	Copstar 350 ml	Fighter + Sporekill 570ml+100ml	Copstar 350ml	Fighter +Sporekill 570ml+100ml	Copstar 350ml	Mancozeb 200g
5	Fighter + Sporekill 400ml +100ml	Copstar 350ml	Fighter + Sporekill 400ml+100ml	Copstar 350ml	Fighter +Sporekill 400 ml+100ml	Copstar 350ml	Mancozeb 200g
6	Fighter 400ml	Copstar 350ml	Fighter 400ml	Copstar 350ml	Fighter 400ml	Copstar 350ml	Mancozeb 200g
7	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml	Sporekill 100ml
8	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml	Fighter 570ml
9	Control						

**Table 4.3.2.2.** Results of various spray programmes applied during the high disease pressure period from September to April for the control of *Alternaria* brown spot control on 'Novas' at Belmont, Schagen.

Treatment	Percentage of fruit in each class <sup>z</sup>		
	Lesions/fruit		
	0	1-5	≥6
1	62.6 b	15.0 ab	22.4 de
2	91.6 a	3.2 c	5.2 e
3	46.0 bc	14.4 ab	39.6 bcd
4	52.8 bc	19.8 a	27.4 cde
5	35.8 c	9.0 bc	55.2 b
6	47.0 bc	16.4 ab	36.6 bcd
7	37.6 c	12.4 ab	50.0 bc
8	63.0 b	16.6 ab	20.4 de
9	4.8 d	2.8 c	92.4 a

<sup>z</sup> Means in a column, based on 5 replicates, followed by the same letter are not significantly different ( $P > 0.05$ ) according to Fisher's least significant difference test.



**Fig. 4.3.2.1.** 'Nova' mandarin fruit samples taken at harvest after 7 field applications with the standard mancozeb (A) and copper oxychloride (B) treatments; 3 x Fighter (@ 400 ml (C) and 570 ml/hL water (D)) in tank mixtures with Sporekill alternated with copper hydroxide (3 x applications) and 3 x Fighter (@ 400 ml (E) and 570 ml/hL water (F)) without Sporekill alternated with copper hydroxide (3 x applications).

Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2013 and Jan-Mar 2014

Apr-Jun:

- Evaluate previous season's field trial
- Annual progress report

Jul-Sep:

- Collect the fungicides from the different chemical companies earmarked for the trial in August.

- Layout of trial in a susceptible 'Nova' orchard.
- First applications will commence with the onset of the first spring flush

Oct-Dec:

- Continue at pre-determined intervals as registered/recommended

Jan-Mar:

- Continue at pre-determined intervals as registered/recommended

Apr-Jun:

- Evaluate current field trial

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### 4.3.3 PROGRESS REPORT: Optimisation of fungicide spray applications in citrus orchards Experiment PPL 891 (April 2007 - March 2010) by Paul Fourie (CRI-SU)

#### Summary

In South Africa, fungicide spray application at medium cover ( $\pm 9\ 000$  L/ha) is recommended to control fruit and foliar diseases. A large proportion of excessive spray volume is, however, lost to drift, which results in considerable environmental pollution of soils and air and reduced spray efficiency. This study described a novel spray deposition assessment protocol, for better interpretation of spray deposition results and determined deposition benchmarks indicative of the biological effectiveness of depositions. Suitability of the SARDI Yellow Fluorescent Pigment as tracer for copper oxychloride deposition was demonstrated through their similar particle concentration and size. Spray deposition assessment of spray targets, which were sprayed with a mixture that included the fluorescent pigment, involved photomacrography of whole leaf or fruit surfaces, followed by digital image analyses. This protocol proved to be very accurate in determining the quantity and quality of deposition. In 18 laboratory spray trials, deposition benchmarks were modelled to be used to evaluate spray technology research, specifically for control of *Alternaria* Brown Spot and similar citrus fruit and foliar diseases. From various spray trials using various machines at spray volumes from 1 000 to 24 000 L/ha, it was clear that excessively high spray volumes (>10 000 L/ha) did not result in better spray deposition on leaves. Similar and even improved spray deposition quantity and uniformity at better spray efficiency could be obtained at lower spray volumes through optimal use of equipment or through the use of more efficient sprayers. In the evaluation of spray adjuvants, anomalous results were found when the benchmarks were used to interpret deposition data following laboratory and orchard spray trials. The anomalous results can possibly be attributed to the effects of adjuvants on deposition parameters (most notably deposition quality), on pathogen development (for example, host recognition following structural changes to the epicuticular wax layer), and synergistic effects between adjuvant and fungicide. Additional fundamental research is therefore required to explain these anomalies and to improve the benchmark model.

## Opsomming

In Suid-Afrika word swamdoders teen medium-dek bespuitings ( $\pm 9\ 000\ \text{L/ha}$ ) aanbeveel om vrugte en blaarsiektes te beheer. 'n Groot deel van oormatige spuitvolumes gaan egter verlore a.g.v afloop en drif wat tot aansienlike omgewingsbesoedeling van die grond en lug asook verminderde spuit doeltreffendheid lei. Hierdie studie beskryf 'n unieke spuit neerslag-assesseringsprotokol vir beter interpretasie van spuit deponeringsresultate en bepaal drempelwaardes vir die aanduiding van biologiese doeltreffendheid van spesifieke deponeringshoeveelhede. Die geskiktheid van SARDI Geel Fluoreserende Pigment as spoorder vir koperoksichloried deponering is met hul soortgelyke partikel konsentrasie en grootte gedemonstreer. Spuit deponering assessering van spuit teikens wat met spuitstof bevattende SARDI geel fluoreserende pigment gespuit is, is gedoen d.m.v fotomakrografie van heel blaar- of vrugoppervlaktes, gevolg deur digitale beeld ontledings. Die protokol is bewys om baie akkuraat in die bepaling van die hoeveelheid en gehalte van spuit deponering te wees. Agtien laboratorium spuitproewe se neerslagdata is gebruik om neerslag drempelwaardes te modelleer vir die doel om spuit tegnologie navorsing te evalueer, spesifiek vir die beheer van *Alternaria* bruinvlek en soortgelyke sitrus vrug- en blaarsiektes. Na verskeie spuitproewe met verskillende masjiene by spuitvolumes vanaf 1 000 tot 24 000 L/ha, was dit duidelik dat die uitermate hoë spuitvolumes ( $> 10\ 000\ \text{L/ha}$ ), nie beter spuit neerslag op blare realiseer het nie. Soortgelyke en selfs beter spuit deponering, eenvormigheid en beter spuit doeltreffendheidsvlakke kan d.m.v laer volume toedienings tesame met optimale gebruik van toerusting of deur die gebruik van meer doeltreffende spuit-tegnologie verkry word. In die evaluasie van spuit bymiddels is onreëlmatige resultate gevind toe spuit drempelwaardes gebruik is om deponeringsdata na aanleiding van laboratorium- en boord spuitproewe te interpreteer. Hierdie resultate kan moontlik toegeskryf word aan die effek van bymiddels op deponeringsparameters (veral neerslag kwaliteit), patogeen ontwikkeling (bv. gasheer-erkenning a.g.v strukturele veranderings aan die epikutikulêre waslaag), en/of sinergistiese effekte tussen die bymiddel en swamdoder. Verdere navorsing is dus nodig is om hierdie afwykings te verduidelik en om die drempelwaarde-model te verbeter.

### 4.3.4 PROGRESS REPORT: Control of *Botrytis cinerea* Pers. on lemons

Experiment 1015 (April 2011 - March 2014) by GC Schutte, Charl Kotze and PH Fourie (CRI)

#### Summary

*Botrytis cinerea* Pers. has long been known as the causal pathogen of blossom blight on lemons. The saprophytic nature of the pathogen allows it to survive on a variety of organic matter with a low pH. To determine which stadium of blossom development is most suitable for infection and when they should be sprayed for the control of Botrytis, the pH, Brix and mycelium growth rates of Botrytis on a petal- and stamen-extract media of different citrus cultivars were compared. Results showed that lemon petals and stamen had significantly different pH levels of 4.95 and 5.15, respectively, and also less if compared with other citrus cultivars. Lemon petals and especially stamen also resulted in significant more mycelial growth when it was incorporated in a selective medium. Although the Brix of lemon stamen was the highest of all the cultivars, it was, however, not significantly different from Valencias and Clementines, but significant different from the petals. Stamen appears therefore as the ideal growth medium for Botrytis. Because it is our aim to protect lemon blossoms against Botrytis, certain fungicides were selected for *in vitro* and *in vivo* evaluation. *In vitro* results showed that Botrytis was most sensitive to benomyl, followed by iprodione and Sporekill. A Metos weather station was installed next to the orchard that was earmarked for the field trial to determine the infection periods during the experimental period. Only one infection period occurred during September. Blossoms were also pre-inoculated with Botrytis spores before the fungicides were applied to ensure sufficient inoculum. Although no blossom infections were observed on the untreated trees, blossoms were harvested after they were treated and plated out on a selective medium. Results showed that benomyl and iprodione performed the best in controlling the disease.

#### Opsomming

*Botrytis cinerea* Pers. is welbekend as die veroorsakende organisme wat bloeiselversenging op suurlemoene veroorsaak. Die saprofitiese aard van die patogeen lei daartoe dat dit op organiese materiaal met 'n lae pH te kan oorleef en van hier af het die patogeen die vermoë om blomme te infekteer. Om te bepaal watter blomdeel en -stadium meer vir swamgroeï geskik is en dus geteiken moet word vir die effektiewe beheer van die siekte, is die pH, Brix en swamgroeï op 'n blomblaar- en stuifmeeldraad-ekstrak-medium van verskillende sitrus kultivars gemeet. Suurlemoene se blomblare en stuifmeeldrade se pH vlakke was onderskeidelik 4.95 en 5.15, wat statisties betekenisvol laer is van ander sitruskultivars. Die blomblare en veral die stuifmeeldrade van suurlemoene het ook, in vergelyking met ander kultivars, tot betekenisvol meer groei op die selektiewe medium gelei. Alhoewel die Brix van suurlemoene se meeldrade die hoogste was, was dit nie betekenisvol verskillend van die Valencias en Clementines nie, maar wel betekenisvol verskillend van blomblare. Dus was meeldrade die mees gunstige deel van die blom waarop Botrytis sal groei. Aangesien ons hoofdoel is om suurlemoenblomme teen Botrytis te beskerm, is spesifieke swamdoders vir *in vitro* en *in*

*vivo* evaluasie geselekteer. *In vitro* resultate toon dat *Botrytis* mees sensitief was teen benomyl, gevolg deur iprodione en Sporekill. 'n Metos weerstasie is op die proefperseel in Witrivier geïnstalleer om die infeksieperiodes tydens die proeftydperk te bepaal. Hieruit kon ons vasstel dat daar net een infeksieperiode in September was. Blomme is op 1 Oktober 2011 met *Botrytis* spore geïnkuleer alvorens die swamdoders toegedien is om te verseker dat daar voldoende inokulum teenwoordig is. Alhoewel daar geen blominfeksies op die kontroles waargeneem is nie, is blomme geoes nadat hulle gespuit was en op 'n selektiewe medium uitgeplaat. Resultate toon dat benomyl en iprodione goeie beheer van die siekte tot gevolg gehad het.

#### 4.4 PROJECT: SOILBORNE DISEASES

##### 4.4.1 Project summary

Increased demands to limit the use of toxic chemicals, including nematicides, are a concern to all role players in agriculture worldwide. An integrated approach towards soilborne disease management is therefore essential rather than relying only on a single control method. The evaluation of fumigants on replant soils was initiated. Fumigants from various companies were included in a trial at Crocodile Valley Citrus Co. Two years after the trees were planted the nematode numbers in the fumigated plots are still zero and a significant increase in tree height and stem diameter is visible. Data collection is still in the early stages but the potential of the fumigants is already visible (4.4.3). Recently the use of aldicarb (Temik) was banned in South Africa and the question asked is which product will next be withdrawn from the market? Developing alternatives to chemical nematicides is therefore essential and a number of products have been selected for evaluation. The original trial had to be terminated prematurely, but was re-established and two sets of data were collected during the past season. Some activity was shown against citrus nematode populations; however, the last set of data is required before conclusions can be drawn. A tunnel trial where a number of products have been evaluated for the control of *Phytophthora* spp. was initiated because of the limited options for control of *Phytophthora* in citrus. A new superior product from Bayer was able to control *Phytophthora* infection in planting bags by 100%. Re-evaluations will include the Bayer product and various other products and if the Bayer product proves to be successful again, field trials will be planned (4.4.4). A research strategy was initiated to study soil, root and citrus plant health. Firstly, the factors, interactions and other potential causal and/or diagnostic factors associated with citrus tree decline had to be determined. Two declined (root rot related) orchards were identified and data with regards to physical, chemical and biological parameters collected. The ADE4 multi-variate statistical model was used to analyse a large quantity and variety of data. Initial analyses indicated that the tree condition categories selected in the different trials were correctly chosen. However, some trees seem to be placed visually in a wrong class according to the environmental non-subjective analysis, and/or certain factors might be non-diagnostic. More time will have to be spent analysing the available data with the multi-variate statistical model. The project will form part of a PhD study (4.4.2). The use of compost fertilisers is becoming more and more popular but the effect on soil and tree health and yield is not known. This project was therefore initiated and the effects of compost / compost derivative programmes were evaluated. The amendment of soil with compost resulted in significantly higher yields. Stem circumference of the young trees increased more rapidly and soil conditions were improved by increasing penetration depth. Lower *Phytophthora* and nematode counts were also recorded (4.4.QMS). Rootstocks play an important role as part of an integrated pest management approach of soilborne diseases on citrus. The biochemical mechanisms involved in rootstock resistance is being investigated. The relative susceptibility or tolerance of 13 citrus rootstocks to *Phytophthora* root rot was determined. Phytochemical profiles of root samples were determined by means of thin layer chromatography (TLC) and HPLC-MS. TLC analyses revealed two categories of fluorescing phytochemical compounds appearing in some rootstocks, viz. induced compounds and constitutive compounds. No consistent pattern has emerged indicating a strong correlation between a specific compound and rootstock resistance (4.4.6). Contract research evaluating two non-toxic nematicides was done for two international companies in Belgium and Israel (4.4.7, 4.4.8) as well as a glasshouse trial on citrus greening for a company in Germany (4.4.9).

#### Projekopsomming

Druk neem wêreldwyd toe om die gebruik van toksiese middels, wat nematisiede insluit, te verminder. Verskeie strategië wat bestuursaspekte, alternatiewe beheer en geïntegreerde beheermaatreëls insluit word tans ondersoek. Aalwurmdoders is in die verlede hoofsaaklik gebruik om probleme in boorde in 'n kits op te los. Voor-plant berokingsbehandelings is geïdentifiseer as 'n alternatief maar die langtermyn effek moet bepaal word. Produkte van verskeie maatskappye is ingesluit en word tans op 'n herplant boord ge-evalueer. Aalwurm wyfietellings is steeds negatief in die berokingsbehandelings maar is wel in die na-plant en kontrole behandelings gevind. *Phytophthora* is in meeste van die behandelings teenwoordig. Stamdeursnee en boomhoogte resultate is betekenisvol beter as die onbehandelde kontrole en na-plant behandelings. Visuele verskille in die boord is tussen die beroekte en nie-beroekte behandelings sigbaar. Oesopbrengraste sal ook gemonitor word indien die bome begin vrugte dra (4.4.3). *Tylenchulus semipenetrans*, die sitrusaalwurm, kan ekonomiese verliese in sitrusboorde veroorsaak. Aldicarb (Temik) se gebruik is onlangs in Suid Afrika

verbied wat dui waarom dit belangrik is dat CRI voortgaan om na alternatiewe te soek. So 'n aalwurmproof met verskeie moontlike alternatiewe is getermineer en heruitgelê. Slegs twee stelle data is beskikbaar en aanvanklike resultate dui aan dat meeste produkte wel 'n invloed op aalwurmpopulasies het. Die finale stel data is egter nodig om gevolgtrekkings te kan maak. Die toedienings word herhaal en oesopbrengrste sal in 2012 bepaal word. Beperkte *Phytophthora* beheer produkte het ook die belangrikheid bevestig om na alternatiewe te soek en daarom word verskeie produkte tans in 'n tunnel by CRI ge-evalueer. Meeste van die produkte het swak resultate opgelewer, maar 'n produk van Bayer het uitstekende resultate gelewer deur *Phytophthora* 100% te beheer. Die proef word herhaal met adisionele produkte en indien die Bayer produk weer positiewe resultate lewer word veldproewe beplan (4.4.4). 'n Nuwe navorsingstrategie gefokus op grond-, wortel- en boomgesondheid is begin. Eerstens, moes die faktore, interaksies en ander oorsake en/of diagnostiese indikatore van sitrus-agteruitgang bepaal word. Die ADE4 "Multi-variate" statistiese program is gebruik om groot datastelle van chemiese, fisiese en biologiese faktore, wat versamel is vanaf boorde waar bome tekens van agteruitgang toon, te ontleed. Aanvanklike resultate toon dat die visuele keuse van bome wat in verskillende kategorië van agteruitgang geselekteer is, korrek was en dat die twee persele wat gemonitor word duidelik van mekaar verskil. Hierdie program het die vermoë om 'n groot verskeidenheid data visueel te kan ontleed wat interpretasie vergemaklik. Twee jaar se data word tans ontleed en die proef sal as deel van 'n PhD studie gebruik word (4.4.2). Die gebruik van komposte raak al gewilder maar die werklike impak op boom-, grondgesondheid en oesopbrengrste is nie bekend nie en daarom is 'n projek gelooft waar verskillende komposprogramme en kompos-verwante programme in die Letsitele area ge-evalueer is. Boorde wat jong en ouer bome insluit is met 5 verskillende kompos kombinasies behandel. Resultate toon dat oesopbrengrste in die kompos behandelings hoër was as in die kontrole blokke. Grondkondisie en -penetrasie, het verbeter, *Phytophthora* en aalwurm getalle het afgeneem in die kompos behandelde blokke. Stamdeursnee op jonger bome het vinniger toegeneem as die kontrole bome (4.4.5) Onderstamme speel 'n belangrike rol as deel van 'n geïntegreerde beheerprogram teen grondgedraagde siektes. Biochemiese meganismes betrokke by onderstambestandheid word ondersoek. Die relatiewe vatbaarheid van 13 sitrus onderstamme t.o.v. *Phytophthora* wortelvrot is bepaal. Fitochemiese profiele van wortelmonsters is d.m.v. dunlaag-chromatografie (DLC) en HPLC-MS bepaal. DLC analyses het twee kategorieë fluoresserende verbindings in sekere onderstamme getoon, nl. geïnduseerde- en voorafbestaande verbindings. Geen konsekwente patroon wat 'n sterk korrelasie tussen 'n spesifieke verbinding en onderstambestandheid aantoon, kon nog gevind word nie (4.4.6). Kontraknavorsing met twee alternatiewe, nie-toksiese aalwurmdoders is vir 'n Belgiese en Israeliese maatskappy gedoen (4.4.7, 4.4.8) asook 'n vergroenings-glashuis proef vir 'n Duitse maatskappy (4.4.9).

#### 4.4.2 **PROGRESS REPORT: Investigation into edaphic factors and their interactions on citrus tree decline**

Experiment 910 (2008 – 2012) by MC Pretorius & C Kotze (CRI)

##### **Summary**

Market requirements, the change in climatical conditions and poor management decisions put farming units under pressure to sustain high yields and quality products. Improper growth conditions will stress trees, increasing the risk of secondary problems, consequently resulting in a reduction in yield, fruit size and eventually tree losses. The latest buzzword is soil, root and plant health and it was decided to investigate this concept for the citrus industry. The aim of the project is to identify the edaphic factors and their interactions that lead to the tree decline by using a specific multi-parameter approach, therewith enabling the early identification of citrus decline problems. Two declined (root rot related) orchards were identified and data with regards to physical, chemical and biological parameters were collected. The following parameters are under investigation: soil and leaf samples for nutritional status, leaf size, chlorophyll content in the leaves, starch content in the roots, *Phytophthora* and nematode status in the soil and roots, soil compaction, yield and Blight test (water uptake and Zn accumulation in Xylem). The ADE4 multi-variate statistical model has been researched and implemented to analyse this large quantity and variety of data. Initial results indicate that the disease categories allocated to trees in the different trial sites were visually correctly chosen. However, some overlapping occurred, indicating that some trees seem to be placed visually in a wrong class according to the environmental non-subjective analysis, or that certain factors might be non-diagnostic. It is clear that trials 1 and 2 are two different sites and that they should be studied separately. Further analyses of both years' data are ongoing and a tunnel trial is planned to generate supporting data. The project will form part of a PhD study.

## Opsomming

Markvereistes, veranderende klimaatsomstandighede asook swak bestuurspraktyke plaas boerdery eenhede onder geweldige druk om volhoubaar hoë opbrengste en goeie kwaliteit produkte te lewer. Sitrusbome wat in 'n toestand van agteruitgang is, lei groot verliese tov vruggroottes en opbrengste wat ook kan lei tot boom vrektes. Grond-, boom- en wortelgesondheid is die nuuste, algemene mode woord wat veronderstel is om bogenoemde stellings te ondersteun. Die doel van hierdie projek is om edafiese grond faktore en hul interaksies te identifiseer wat moontlik kan lei tot sitrusagteruitgang en die "multi-parameter" model te gebruik om voortydig sitrusagteruitgang simptome te identifiseer. Die parameters wat ondersoek word en waarvan data versamel en ontleed is met die ADE4 program is: grond- en blaarontledings vir bemestingsdoeleindes, blaargrootte, chlorofil inhoud van blare, stysel inhoud in wortels, *Phytophthora* en aalwurm status in die grond en wortels, grondkompaksie, oesopbrengste asook Blight toetse (wateropname toets in die stam en Zn akumulasie in xileem). Die "multi-variate" statistiese program word tans toegepas op data wat versamel is van twee boorde met bome wat in 'n stadium van agteruitgang is. Aanvanklike ontledings resultate toon dat die visuele keuse van die verskillende kategorie bome wel korrek was maar die program het wel aangedui dat sekere kategorie bome eerder geassosieer kan word in een van die ander kategorië. Die program waarby al die parameters gesamentlik ontleed is wys die resultaat dat die twee proefpersele van mekaar verskil en dus nie met mekaar vergelyk kan word nie. Die ADE4 model bied verskeie variasies om data visueel te kan voorstel en een van die grootste voordele van die model is dat groot hoeveelhede/verskeidenheid data ontleed kan word en die interpretasie daarvan vergemaklik. Meer tyd word benodig om die model te bestudeer waarna twee jaar se versamelde data ontleed sal word. 'n Tonnel proef word beplan sodra sinvolle afleidings van die data gemaak is. Die projek sal deel vorm van 'n PhD studie.

### 4.4.3 PROGRESS REPORT: The evaluation of different pre-plant products for the control of the citrus nematode, as part of an integrated nematode control approach in citrus replant situations

Experiment 762 (2007 – 2014) by MC Pretorius & C Kotze (CRI)

## Summary

The search for alternative control methods for the effective control of nematodes is a priority with all researchers worldwide. The aim of this study was to investigate an effective and economical pre-plant alternative control option to ensure that replant orchards are nematode-free for as long as possible before a post-plant nematicide treatment becomes necessary. Pre-plant fumigation and post-plant treatments were done at Crocodile Valley Citrus Co. during November 2009. The following products were applied: 50% methyl iodide and 50% chloropicrin (Midas 50:50 Arysta LifeScience); methyl bromide (Methyl Bromide, Arysta LifeScience); Metam-natrium (Metham-sodium 510 SL, Villa Crop Protection); 1.3 dichloropropene (Telone, Dow AgroSciences), 1.3 dichloropropene/chloropicrin (nitromethane) (850g/465 g/l) (Telopic, Dow AgroSciences); Furfural (Biomass, Illovo Sugar); Nematode egg stimulant + nematicide (Product X, Citrus Research International) and Cadusafos (Rugby GR, FMC South Africa (Pty) Ltd. The results indicated that the female nematode counts in the fumigated treatments are significantly lower; zero, compared to the untreated control and post-plant treatments. The tree height and stem diameter of the fumigated treatments increased significantly compared to the unfumigated treatments and untreated control. *Phytophthora* was present in most of the treatments. The trees are in a good condition but visual differences are evident between the fumigated and unfumigated treatments. The initial results indicate the potential of the fumigants; however, the trial is ongoing and the long term effect with regard to efficacy and economic impact still needs to be determined.

## Opsomming

Die soektog na alternatiewe beheermaatreëls teen die sitrusaalwurm is wêreldwyd 'n prioriteit. Die gebruik van hoogs toksiese chemikalië wat aalwurmdoders insluit kom al meer onder druk. Die doel van hierdie studie is om die effektiwiteit en ekonomiese impak van voor plant alternatiewe beheer opsies te bepaal deur te verseker dat herplant gronde aalwurmvry sal bly vir so lank moontlik om die gebruik van aalwurmdoder toedienings te beperk. In die verlede is hoofsaaklik van nematosiedes gebruik gemaak om aalwurm probleme in 'n kits op te los. 'n Reeks voor-plant berokingsbehandelings is in 'n boord op Crocodile Valley Citrus Co. toegedien: 50% methyl iodide + 50% chloropicrin (Midas 50:50, Arysta LifeScience); methyl bromide (Methyl Bromide, Arysta LifeScience); Metam-natrium (Metham-sodium 510 SL, Villa Crop Protection); 1.3 dichloropropene (Telone, Dow AgroSciences); 1.3 dichloropropene/chloropicrin (nitromethane; 850g/465 g/l; Telopic, Dow AgroSciences); Furfural (Biomass, Illovo Sugar); Nematode egg stimulant + nematicide (Product X, Citrus Research International) en Cadusafos (Rugby GR, FMC South Africa (Pty) Ltd. Resultate toon dat aalwurm wyfietellings negatief is in die berokingsbehandelings en dus betekenisvol verskil van die onbehandelde kontrole asook die na plant handelings. Die larf telling in meeste van die handelings het begin styg maar is steeds baie laag. Die boomhoogte en stamdeursnee

resultaat is ook betekenisvol beter in beide die na-plant en onbehandelde kontrole behandelings. *Phytophthora* is in meeste van die behandelings gevind. Die algehele voorkoms van die bome is goed en visuele verskille tussen die berookte en onberookte behandelings is sigbaar. Die aanvanklike resultate toon dat die berokings behandelings uiters effektief was en heelwat potensiaal het vir toekomstige gebruik op herplant gronde maar die lang termyn effektiwiteit van die behandelings asook die ekonomiese impak moet nog bepaal word.

#### 4.4.4 **PROGRESS REPORT: Evaluation of alternative products for control of citrus nematode and *Phytophthora* spp. in citrus**

Experiment 1030 (2008 – 2010) by MC Pretorius & C Kotze (CRI)

##### **Summary**

*Tylenchulus semipenetrans*, the citrus nematode, infects citrus worldwide and is the most abundant and frequent plant-parasitic nematode in citrus orchards. The use of toxic compounds becomes more and more under pressure internationally and locally. Recently the use of Aldicarb (Temik) was banned and the question asked is which product will next be withdrawn from the market? Developing alternatives to chemical nematicides is therefore essential. The following products were evaluated: Nontox–Silica (silica), Biolan (ZZ2, Product), CRI nematode egg stimulating product, a combination of a nematode egg stimulating product with a nematicide, standard nematicide application (cadusaphos), Foodprint and Diatomied. The trial was laid out at Crocodile Valley Citrus Co. but had to be terminated due to unforeseen irrigation problems. The trial was re-established on another site and initial results obtained from the first two sets of samples indicated that a number of products showed activity against the citrus nematode. However, the final set of data is necessary to draw conclusions. Not many options are available for the control of *Phytophthora* spp. in citrus. A tunnel trial was therefore laid out to evaluate various products against *Phytophthora* spp. Results indicated that a new Bayer product controlled *Phytophthora* in the planting bags by 100%. Poor results were obtained with the other products, including Ridomil. Various other alternative products including the Bayer product will again be evaluated. If the Bayer product proves once again to be effective, a field trial will be established.

##### **Opsomming**

*Tylenchulus semipenetrans*, die sitrusaalwurm, is die mees algemene aalwurm wat ekonomiese verliese in sitrusboorde veroorsaak. Internasionaal en plaaslik word al meer druk op die beperking/ verwydering van die gebruik van toksiese middels geplaas. Aldicarb (Temik) se gebruik is onlangs in Suid Afrika verbied en die vraag is watter produk is volgende? CRI het 'n pro-aktiewe benadering en 'n verskeidenheid van alternatiewe produkte word tans ge-evalueer om hul effektiwiteit vir die beheer van sitrusaalwurm te bepaal. Produkte wat tans ge-evalueer word: Nontox – Silica (silica), Biolan (ZZ2, Produk), CRI aalwurmeier stimulant, 'n eierstimulant in kombinasie met 'n aalwurmdoder, 'n standard aalwurmdoder (cadusaphos), Foodprint en Diatomied. Proef wat uitgelê is op Crocodile Valley Citrus Co. is getermineer weens onvoorsiene besproeiingsprobleme. 'n Opvolg proef op 'n nuwe perseel is uitgelê. Slegs die eerste twee monsternemings resultate is beskikbaar wat aandui dat sekere produkte wel 'n effek op die aalwurmpopulasies gehad het; die finale stel resultate is nodig om sinvolle afleidings te kan maak. Weens beperkte opsies vir die beheer van *Phytophthora* spp. word 'n verskeidenheid alternatiewe en chemiese produkte tans in die tunnel by CRI ge-evalueer. Resultate toon dat 'n nuwe Bayer produk *Phytophthora* 100% beheer het. Nie een van die ander produkte, Ridomil ingesluit, het 'n aanvaarbare resultaat gelewer nie. 'n Opvolgproef met ander alternatiewe produkte is beplan waarby die Bayer produk ook weer ingesluit is. Indien die produk weer positiewe resultate lewer sal veldproewe beplan word.

#### 4.4.5 **FINAL REPORT: The effect of compost, amended with beneficial organisms, applied as soil treatments, on tree condition and general disease resistance**

Experiment 08QMS WvdP 01 (2008 – 2012) by A Fourie (QMS Agri Science)

##### **Summary**

Chemical fertilizers can cause unfavourable soil conditions for soil microbes that can lead to unhealthy roots and plants. Unhealthy plants can be more prone to attack by pests and susceptible to infection. The aim of this study was to determine the effects of compost / compost derivative programmes on overall soil conditions, plant health, disease or pest resistance and yield. Five different compost programmes were applied to a young, as well as an older citrus orchard, and their effects evaluated. The amendment of soil with compost resulted in significantly higher yields. Stem circumference of the young trees increased more rapidly, compared to the untreated control trees. The addition of compost improved soil conditions by increasing the penetration depth, and more importantly, resulted in lower *Phytophthora* and nematode counts.

## Oplossing

Chemiese bemestingsprodukte kan ongunstige grondtoestande veroorsaak, wat kan lei tot ongesonde wortels en plante. Ongesonde plante kan meer vatbaar wees vir infeksies en aanvalle deur insekte. Die doel van die navorsingsprojek was om die effek van verskillende komposprogramme / kompos-verwante programme op grondkondisies, plant gesondheid, opbrengs en die plant se vermoë om siektes te beveg, te bepaal. Vyf verskillende kompos behandelings is toegedien op 'n jong, sowel as 'n ouer sitrusboord. Die toevoeging van kompos op die sitrusbome het 'n hoër opbrengs tot gevolg gehad. Die stamomtrek het vinniger by die jonger bome in vergelyking met die onbehandelde kontrole bome toegeneem. Die kompos toedienings het grondkondisie verbeter, deur die penetrasie diepte te verhoog en het ook 'n merkbare verlaging in *Phytophthora* en nematode getalle tot gevolg gehad.

## Introduction

Plants are more prone to fungal and bacterial infection when stressed. Their ability to resist infection by microbial organisms can subsequently be enhanced by improving general plant health. The use of compost amendments as a nutrient source for plants, as well as to improve soil condition is a general practice by many industries throughout the world. These amendments do not only provide nutrients for the plant, but also for their associated soil microbial communities. Several studies have shown that the microbial activity of the compost microflora can result in a general suppression of plant diseases (Noble, 2011; Termorshuizen *et al.*, 2006; Veeken *et al.*, 2005). Tree condition or plant health is mostly influenced by root health, which can be affected by a multitude of factors e.g. soil compaction, organic content, microbial diversity, irrigation, nutrition, etc. Improving some of these factors such as soil condition should improve general tree condition in the long run, as well as their ability to ward off diseases. Noble (2011), however, showed that soils amended with composts is generally neutral to beneficial in terms of suppression of diseases, but that the beneficial effects might be unpredictable. This study was undertaken to determine if soil properties, plant health and the ability of citrus trees to resist disease, could be improved by compost amendments.

## Stated objectives

- The main objective of this project was to determine if general soil condition and microbial diversity could be improved by re-introducing beneficial organisms and organic matter in the form of compost, which will stimulate root growth, tree condition and enhance resistance to diseases.
- To do soil and leaf analysis at the end of each growing season, to enable fertilizer companies to devise treatment programmes for the next growing season.
- To evaluate the effect of treatments on tree and soil condition.
- To determine the yield, fruit size, and internal fruit quality at harvest.
- To determine the effects of treatments on the general soil microflora, as well as the effects on pathogens such as *Phytophthora* and citrus nematodes.

## Materials and methods

The study was initiated in 2008 and was conducted in 2 commercial citrus orchards, one on young Valencia trees (Block J-16, planted in 2006), and one on older Valencia trees (Block D-25, planted in 1996). Both orchards were located on the farm Jasie (Laeveld Sitrus) in the Letsitele area (GPS coordinates Block J16: S23°44'11.52", E30°32'23.91"; Block D25: S23°44'54.39", E30°32'30.21'). The orchards were under normal production practices, including scheduled watering via micro irrigation, pest and disease control applications, as well as conventional chemical fertilization.

Treatments included compost programmes from Agrilbrium, International Carbon Fertilizer, local compost and Agron, which were compared to a control that did not receive any compost, only conventional chemical fertilization. At the end of each growing season, leaf and soil samples were collected and elemental content analysed by Agrilab (Tzaneen). These results were used by the various fertiliser companies to formulate their treatment programmes for the next season. A basic layout of the treatment programmes are given in Table 1, as their composition, recommended volumes and timing of applications varied considerably between the different manufacturers and growing seasons. Each treatment consisted of 6 tree plots, randomly replicated 8 times throughout each experimental block.

The effects of the treatments on the yield were determined at harvest in July 2009, 2010 and 2011. For each of the 8 replicates, 1 of the middle data trees within the 6 tree plots was randomly selected and the fruit stripped and weighed separately. A sub-sample consisting of approximately 20 kg of fruit was taken that were randomly picked throughout the profile of every data tree, and sized with a commercial "rope-and-roller" sizer.

Twelve fruit were sampled from each replicate and evaluated for internal fruit quality, including the percentage juice, Brix and acid content, according to the CRI internal quality protocol.

Tree trunk circumference was determined using a measuring tape and soil penetration was measured to determine if the treatments had any positive effect on the physical soil properties. Soil penetration measurements were taken, shortly after good rainfall, in the middle of 2 replicate trees using a Dickey-John-Soil-Compaction-Tester. This rendered similar moisture content throughout the experimental site, which minimized variation between measurements.

To evaluate the effect of treatments on the general soil microbial activity, soil samples were taken in April 2012 from 4 replicates per treatment, where sub-samples of each of the 6 trees within a replicate were combined to constitute a sample. The soil samples were submitted to ZZ2-laboratories (University of Limpopo, Polokwane) for analysis, where the "Active Carbon" and "Potentially Mineralizable Nitrogen (PMN)" content were measured. These 2 parameters form part of a series of indicators used in the Cornell Soil Health Test, where the Active Carbon content is a measure of the fraction of soil organic matter that is available as a carbon source for the soil microbial community, and PMN the amount of nitrogen that is converted from an organic to an inorganic form by the soil microbial community (Gugino *et al.*, 2009).

Soil samples were taken regularly throughout the growing seasons for *Phytophthora* and nematode counts. The standard citrus leaf baiting and selective plating procedure was used to determine *Phytophthora* level/incidences, which were expressed as the number of leaf discs out of 10 discs that showed development of *Phytophthora* colonies. The standard staining and counting procedure for female citrus nematodes in roots was used.

The area under the disease pressure curve (AUDPC) was calculated, in order to attain a better understanding of the effects of treatments on *Phytophthora* incidence, which was assessed throughout the trial period. The AUDPC was determined based on the logistic method (van der Plank, 1963), using the equation from Shaner and Finney (1977). The AUPDC values were normalised by dividing it with the total number of days from the start to the end of the evaluation period, thus providing a new value range from 0 to 100 %. This was done to simplify comparisons between treatments over years of evaluation.

The natural variation in nematode numbers, as a result of changing environmental conditions throughout the growing seasons, made it necessary to use different methods to get a better visual comparison of the effects of treatments. Apart from the nematode counts during the trial period, the overall average numbers were calculated, the percentage increase or decrease in numbers determined, as well as the effect on the AUDPC. The percentage increase or decrease was calculated using the Henderson-Tilton's formula (Henderson & Tilton, 1955), where each previous assessment was regarded as the before-treatment, and the current assessment as the after-treatment. The AUDPC was calculated based on a method by Lamondia *et al.* (1999) that makes use of ratios, where the ratio of nematode counts to the maximum number of nematodes counted, were integrated over the length of the evaluation period, and expressed as the AUDPC.

At the end of each growing season, fruit were evaluated for the presence of citrus black spot (CBS) symptoms. However, the absence of CBS disease pressure within the 2 experimental orchards made assessments impossible.

Data was analysed using Statistica 8.0 by Statsoft Inc. Where percentages were used, arcsin-transformations were done, an analysis of variance performed and differences between means of treatments determined with Fisher's t-test at a 5% level of significance.

**Table 4.4.5.1.** Soil-compost amendment programmes evaluated.

Treatment	Description
1. Control	Conventional chemical fertilization
2. Agrilibrum	Local compost + QCM 360 (effective micro-organisms, active organic matter, hormones for root stimulation, vitamins, nutrients) + Byocarb (bio active fulvic acid carbon concentrate) + Activator
3. ICF	Organic carbon fertilizers e.g. NPK 10-1-8, CAL 2 and CAL 3
4. Local compost	Compost from Bosveld Sitrus, Letsitele
5. Agron	Bio Soil Blend (carbon fertilizers with micro-organisms) + e.g. NPK 4-1-10, CAAN+B+Mo, and CalMagN

## Results and discussion

### Yield

The effect of compost amendment programmes on yield of the young citrus trees in block J-16 are depicted in Table 4.4.5.2, and the older citrus block D-25 in Table 4.4.5.3. At the beginning of the trial period (2008/2009) the young trees of block J-16 produced very little fruit, but this drastically increased the following season (2009/2010), with significantly higher yields when treated with locally produced compost (74.4 kg), compared to that of the control treatment (56.8 kg) (Table 4.4.5.2). With the final season of evaluation (2010/2011), the addition of local compost again resulted in significantly higher yields (104.1 kg), followed by the Agrilibrum (100 kg) and ICF (99.4 kg) treatments. However, the Agron compost amendment programme resulted in lower yields (80.9 kg) compared to the control (88.5 kg).

The older trees in block D-25 treated with local compost, also displayed higher yields compared to most of the treatments, during the first (2008/2009) and last (2011/2011) seasons of evaluations (Table 4.4.5.3). Similarly as on the young trees, the Agron compost amendments resulted in lower yields in block D-25 during the 2010/2011 growing season, compared to the control. However, for this study, the Agron products were formulated for trees to be healthy, with vigorous growth and to be able to withstand disease, rather than to obtain maximum yields. In block J-16, all the treatments showed an increase in yield between the different growing seasons, but not in block D-25 where the older trees are in a steady state of production.

**Table 4.4.5.2.** Average yield per tree, determined during each growing season for Valencia citrus trees in block J-16, planted in 2006.

Treatment	Average weight per tree (kg), per growing season*		
	2008/2009	2009/2010	2010/2011
1. Control	<b>10.7 a</b>	56.8 a	88.5 ab
2. Agrilibrum	8.9 a	56.1 a	100 b
3. ICF	9.2 a	64.4 ab	99.4 ab
4. Local compost	9.5 a	<b>74.4 b</b>	<b>104.1 b</b>
5. Agron	10.4 a	55.5 a	80.9 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

**Table 4.4.5.3.** Average yield per tree, determined during each growing season for Valencia citrus trees in block D-25, planted in 1996.

Treatment	Average weight per tree (kg), per growing season*		
	2008/2009	2009/2010	2010/2011
1. Control	114.3 a	175.9 a	129.4 ab
2. Agrilibrum	129.4 b	156.0 a	134.4 b
3. ICF	117.0 ab	<b>198.3 a</b>	124.9 ab
4. Local compost	<b>183.0 c</b>	162.2 a	<b>143.2 b</b>
5. Agron	173.1 c	185.3 a	100.0 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

### Fruit size

To allow for easier comparisons between treatments, some fruit size classes were grouped together to provide 3 size groups, namely, the small fruit (sizes 125, 105, and 88), the optimal fruit size group (72, 64, and 56), and the large fruit size group (48, and 40). In both block J-16 (Table 4.4.5.4) and block D-25 (Table 4.4.5.5) there were significant differences within some of the fruit size classes; however, these differences varied a lot between seasons and treatments, and no definite trends could be established.

**Table 4.4.5.4.** Average percentage distribution of Valencia citrus fruit from block J-16 (planted 2006), sorted as either small, optimal, or large sized fruit.

Treatment	Small fruit size* (class 125, 105, 88)			Optimal fruit size (class 72, 64, 56)			Large fruit size (class 48, 40)		
	2008/ 2009	2009/ 2010	2010/ 2011	2008/ 2009	2009/ 2010	2010/ 2011	2008/ 2009	2009/ 2010	2010/ 2011
1. Control	1.0 a	2.2 a	1.6 a	57.1 a	83.0 b	87.3 a	<b>41.9 a</b>	14.7 ab	11.1 a
2. Agrilibrum	<b>2.8 a</b>	<b>3.2 a</b>	1.1 a	62.0 a	<b>84.0 b</b>	80.8 a	35.2 a	12.8 a	18.2 a
3. ICF	1.1 a	2.3 a	<b>3.0 a</b>	<b>65.3 a</b>	79.0 ab	<b>88.4 a</b>	33.7 a	18.8 abc	8.5 a
4. Local compost	0.9 a	0.4 a	1.7 a	57.6 a	75.0 ab	72.2 a	41.5 a	24.6 bc	<b>26.0 a</b>
5. Agron	2.3 a	0.8 a	2.6 a	63.5 a	65.2 a	86.6 a	34.2 a	<b>34.0 c</b>	10.9 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

**Table 4.4.5.5.** Average percentage distribution of Valencia citrus fruit from block D-25 (planted 1996), sorted as either small, optimal, or large sized fruit.

Treatment	Small fruit size* (class 125, 105, 88)			Optimal fruit size (class 72, 64, 56)			Large fruit size (class 48, 40)		
	2008/ 2009	2009/ 2010	2010/ 2011	2008/ 2009	2009/ 2010	2010/ 2011	2008/ 2009	2009/ 2010	2010/ 2011
1. Control	<b>39.7 b</b>	4.3 ab	11.4 a	59.7 a	88.0 a	<b>81.0 a</b>	0.6 a	7.7 a	<b>7.5 b</b>
2. Agrilibrum	32.9 b	7.7 ab	20.9 ab	64.9 a	81.6 a	78.7 a	2.2 a	<b>10.7 a</b>	0.4 a
3. ICF	22.8 ab	5.5 ab	<b>27.3 b</b>	<b>73.4 a</b>	88.9 a	70.9 a	3.8 a	5.6 a	1.8 a
4. Local compost	<b>39.7 b</b>	3.4 a	19.3 ab	59.4 a	<b>92.1 a</b>	77.4 a	1.0 a	4.5 a	3.3 ab
5. Agron	13.9 a	<b>17.3 b</b>	19.3 ab	72.5 a	81.4 a	79.4 a	<b>13.6 b</b>	1.4 a	1.3 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

#### Internal fruit quality

Internal fruit quality results for 2 growing seasons for fruit from block J-16 are depicted in Table 4.4.5.6, and for block D-25 in Table 4.4.5.7. As with the fruit size, there were some statistically significant differences within some of the parameters evaluated, but there were no distinct trends over time to separate the compost amendment programmes on individual performance from each other, or from the control. In general, the Agrilibrum and Agron treatments resulted in slightly better fruit quality in block J-16, while the ICF treatment performed marginally better in block D-25. This was, however, not always significantly different from the control.

#### Trunk circumference

In block J-16 (Table 4.4.5.8), the control trees had the biggest trunk circumference when measured during the first growing season (2009/2010), although not significantly. With the final measurements in 2012, almost all the treatments showed bigger trunk circumference compared to the control. The effects of the compost amendment programmes are even more prominent upon examination of the overall percentage increase, with the ICF programme resulting in the biggest increase of trunk circumference of 34.6%, which was also significant when compared to a 17.7% increase for the control treatment. The sharp increase in trunk circumference, as seen with the younger trees in block J-16, was not as noticeable in the older citrus block D-25 (Table 4.4.5.9) with no significant difference in overall increase.

**Table 4.4.5.6.** Internal fruit quality for Valencia citrus fruit in block J-16 (planted 2006).

Treatment	% Juice*		BRIX		TSS		Acid		TSS/Acid	
	2009/ 2010	2010/ 2011								
1. Control	<b>42 a</b>	<b>40 a</b>	10.3 c	10.8 bc	10.8 c	11.3 bc	1.1 b	1.1 a	10.6 a	10.7 a
2. Agrilibrum	41 a	37 a	<b>11.0 d</b>	10.0 ab	<b>11.8 d</b>	10.5 ab	<b>1.4 b</b>	1.1 a	9.2 a	8.5 a

3. ICF	40 a	38 a	9.8 b	9.9 a	10.3 b	10.3 a	1.2 b	1.1 a	9.9 a	9.4 a
4. Local compost	41 a	35 a	9.0 a	10.0 ab	9.5 a	10.4 ab	0.6 a	1.0 a	10.4 a	17.3 a
5. Agron	<b>42 a</b>	37 a	10.0 bc	<b>11.0 c</b>	10.6 bc	<b>11.4 c</b>	1.2 b	1.1 a	10.2 a	9.4 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

**Table 4.4.5.7.** Internal fruit quality for Valencia citrus fruit in block D-25 (planted 1996).

Treatment	% Juice*		BRIX		TSS		Acid		TSS/Acid	
	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011	2009/2010	2010/2011
1. Control	45 ab	41 a	10.4 a	<b>10.7 b</b>	11.0 a	<b>11.2 b</b>	1.4 a	1.4 a	8.2 a	8.1 a
2. Agrilibrum	48 ab	<b>43 a</b>	10.8 a	10.0 a	11.4 a	10.4 a	<b>1.5 a</b>	1.3 a	8.0 a	7.8 a
3. ICF	<b>48 b</b>	36 a	<b>11.0 a</b>	10.3 ab	<b>11.7 a</b>	10.7 ab	1.4 a	1.4 a	8.7 a	7.9 a
4. Local compost	48 ab	39 a	10.0 a	9.9 a	10.7 a	10.3 a	1.2 a	1.4 a	9.7 a	7.7 a
5. Agron	44 a	37 a	10.5 a	9.9 a	11.1 a	10.3 a	1.3 a	1.2 a	8.8 a	8.3 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

**Table 4.4.5.8.** Trunk circumference for Valencia citrus trees in block J-16 (planted 2006), as well as the overall percentage increase in circumference from the first measurement.

Treatment	Trunk circumference (cm), per growing season*			Overall increase %
	2009/2010	2010/2011	2011/2012	
1. Control	<b>37.5 a</b>	41.5 a	44.1 ab	17.7 a
2. Agrilibrum	33.9 a	40.2 a	43.4 a	28.1 ab
3. ICF	33.5 a	41.4 a	45.1 ab	<b>34.6 b</b>
4. Local compost	36.2 a	40.6 a	<b>46.8 b</b>	29.1 ab
5. Agron	35.8 a	<b>42.1 a</b>	45.0 ab	25.9 ab

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

**Table 4.4.5.9.** Trunk circumference for Valencia citrus trees in block D-25 (planted 1996), as well as the overall percentage increase in circumference from the first measurement.

Treatment	Trunk circumference (cm), per growing season*			Overall increase %
	2009/2010	2010/2011	2011/2012	
1. Control	39.6 a	42.2 a	43.6 a	<b>10.2 a</b>
2. Agrilibrum	40.7 ab	<b>43.7 a</b>	<b>44.6 a</b>	9.6 a
3. ICF	<b>42.1 b</b>	43.0 a	44.2 a	4.8 a
4. Local compost	40.6 a	43.4 a	44.2 a	8.7 a
5. Agron	40.8 ab	42.9 a	43.7 a	7.2 a

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

#### Soil penetration depth

In block J-16, soil penetration was slightly better for the control treatment at the beginning of the trial period (Table 4.4.5.10). However, most of the treatments showed some improvement during subsequent evaluations, with ICF having the best soil penetration (31.9 cm) during 2010/2011, and Agron (10.5 cm) the best during the 2011/2012 growing season. These effects were not as prolific in the older citrus block D-25 (Table 4.4.5.11). A steady increase in soil penetration over the trial period could have been expected, but

even though care was taken to ensure that soil penetration was measured under similar conditions, soil characteristics such as the moisture content still varied.

**Table 4.4.5.10.** Effect of treatments on soil penetration depth, measured in block J-16 (planted 2006).

Treatment	Soil penetration depth (cm), per growing season*		
	2009/2010	2010/2011	2011/2012
1. Control	<b>14.5 b</b>	26.2 ab	7.4 a
2. Agrilibrum	11.0 ab	23.7 a	8.5 a
3. ICF	14.2 b	<b>31.9 c</b>	8.8 a
4. Local compost	6.2 a	24.4 a	8.1 a
5. Agron	14.3 b	31.1 bc	<b>10.5 a</b>

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

**Table 4.4.5.11.** Effect of treatments on soil penetration depth, measured in block D-25 (planted 1996).

Treatment	Soil penetration depth (cm), per growing season*		
	2009/2010	2010/2011	2011/2012
1. Control	<b>37.3 b</b>	33.3 b	<b>23.0 a</b>
2. Agrilibrum	34.2 ab	30.5 ab	16.7 a
3. ICF	28.9 a	<b>35.7 b</b>	15.4 a
4. Local compost	36.3 ab	34.9 b	14.0 a
5. Agron	36.5 ab	25.1 a	14.6 a

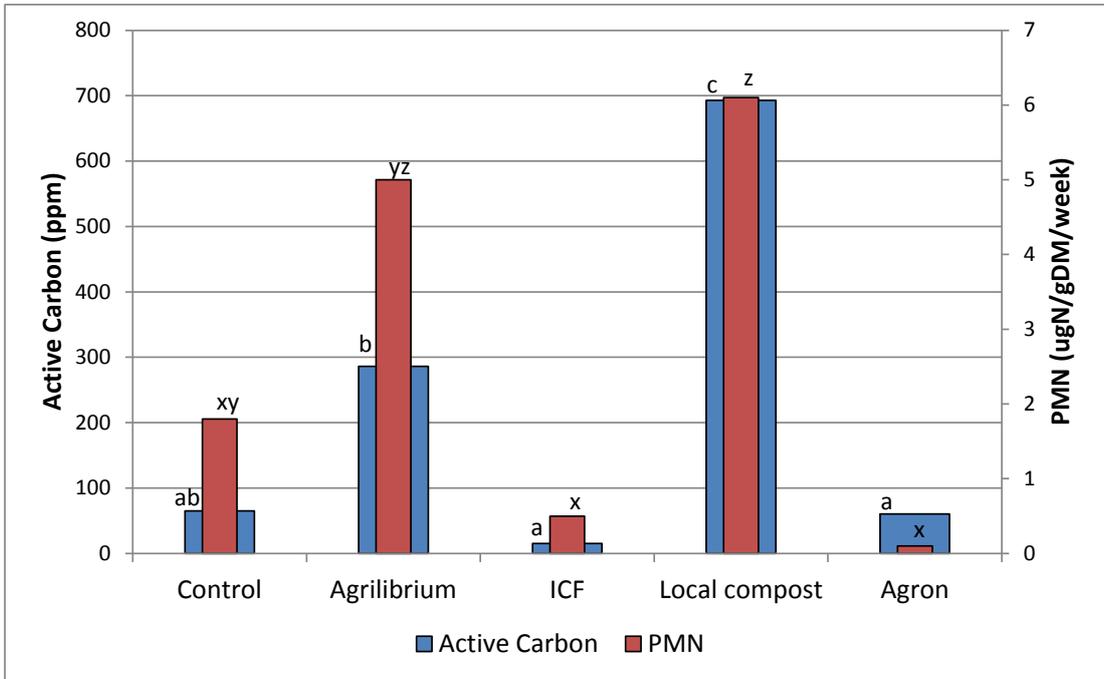
\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

#### Soil microbial activity

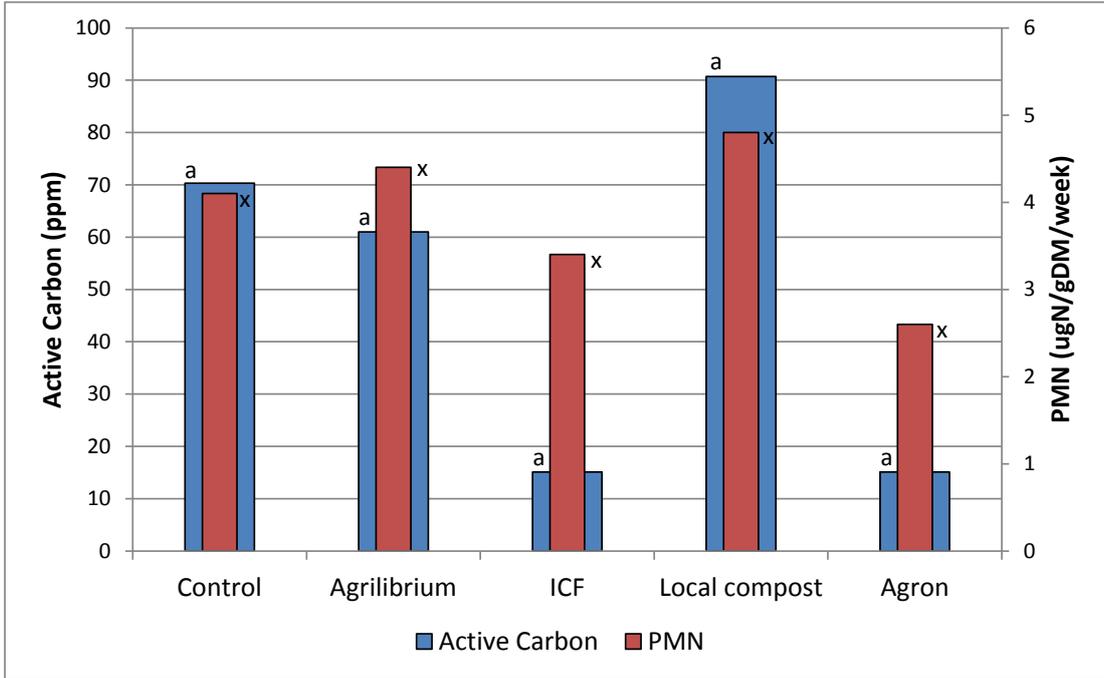
The Active Carbon and PMN content was analyzed to determine the influence of compost amendments on soil microbial activity in block J-16 (Figure 4.4.5.1), and block D-25 (Figure 4.4.5.2). In block J-16, the measured active carbon content for the local compost treatment (690 ppm) was the only treatment which fell within the optimal range of the normal distribution curve of the Cornell soil health test (Gugino *et al.*, 2009) and which differed significantly from the control treatment (ppm). All the other treatments had significantly lower values compared to the local compost treatment, indicating low biological activity within these soils. The Agrilibrum treatment had the second highest active carbon content, followed by the control, Agron and ICF treatments, none of which differed significantly from the control treatment. The PMN content for the local compost treatment differed significantly from the control, however, all the treatments in block J-16 fell below the Cornell soil health standards set for healthy soils with regard to PMN content. Once more the Agrilibrum treatment had the second highest PMN value, followed by the control, ICF and Agron treatments.

Similarly in block D-25, the local compost treatment had the highest active carbon content with a maximum value of 90.7 ppm, which was much lower than observed for the same treatment in block J-16 (692.7 ppm). The control treatment had the second highest active carbon and PMN content, followed by the Agrilibrum, ICF and Agron treatments. Both the active carbon and PMN values for all the treatments in block D-25 were lower than the Cornell soil health standards for health soils. Trends were similar to those observed in block J-16, but differences were not statistically significant.

From these tests, application of compost amendments did have an effect on the soil microbial activity, with the local compost treatment showing the best increase in biological activity, especially in the young citrus block. This increased microbial activity might contribute to the ability of soil to suppress diseases, but conversely it might be possible that the compost amendments also result in higher pathogen activity.



**Figure 4.4.5.1.** Effect of compost treatments on soil microbial activity of block J-16 (planted 2006), expressed as the Active Carbon and Potentially Mineralizable Nitrogen content. Values across series followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).



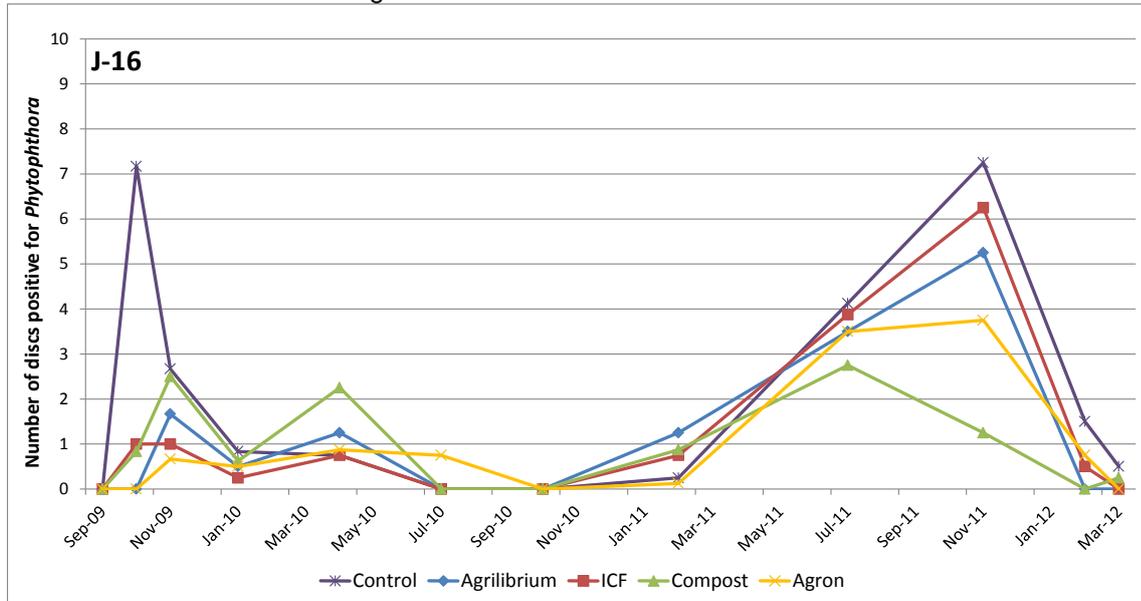
**Figure 4.4.5.2.** Effect of compost treatments on soil microbial activity of block D-25 (planted 1996), expressed as the Active Carbon and Potentially Mineralizable Nitrogen content. Values across series followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

Phytophthora counts

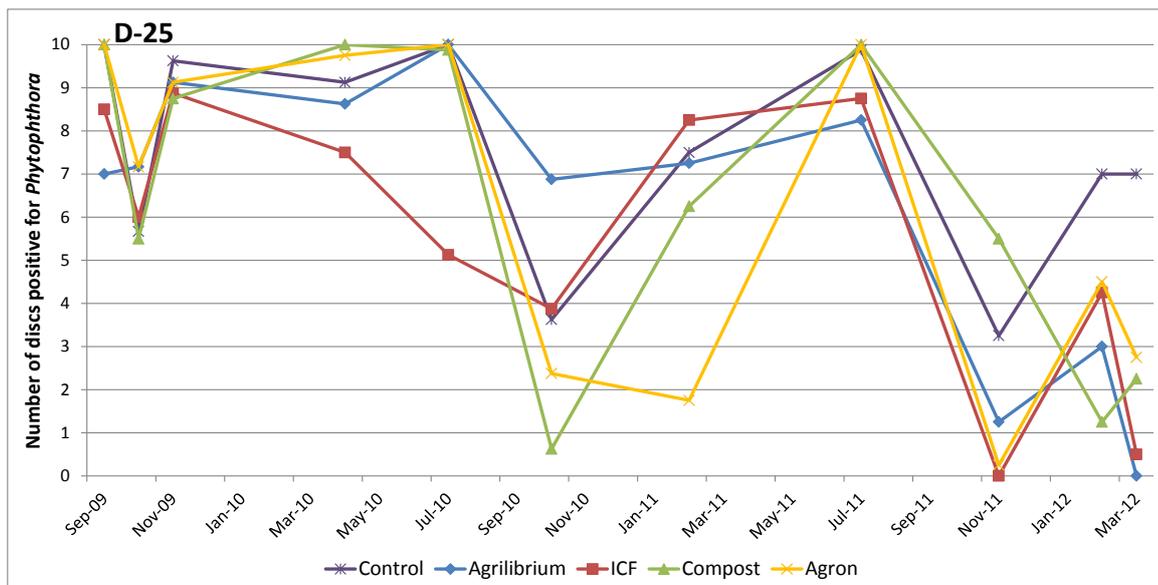
The effect of compost amendments on disease suppression was evaluated by doing *Phytophthora* and nematode evaluations throughout the trial period. A summary for the 3 seasons of *Phytophthora* counts can be seen in Figure 4.4.5.3 for block J-16, and in Figure 4.4.5.4 for block D-25. The *Phytophthora* levels were much lower for the younger trees in block J-16, compared to the older citrus block D-25. There was also noticeable natural variation in the *Phytophthora* levels, mostly as a result of changing environmental conditions.

In general, the *Phytophthora* levels were lower for the compost treatments compared to that of the untreated control, in both the experimental blocks during most of the evaluation intervals. The area under the disease progress curve (AUDPC) was calculated, to enable more effective comparison of treatments and their effects (Table 4.4.5.12). In both block J-16 and D-25, all treatments reduced the overall level of disease with corresponding lower AUDPC values, compared to that of the control treatment. In block J-16, the AUDPC values for the local compost (12.1), Agron (13.4) and Agrilibrum (16.8) treatments differed significantly from the control (23.2), followed by the ICF treatment (17.5) which did not differ significantly from the control. In block D-25, the Agron treatment had the lowest AUDPC value (54.5) followed by the ICF (56.1) and local compost (63.3) treatments that also differed significantly from the control (70.6) treatment.

The exact reason for the lower *Phytophthora* levels observed for the compost treatments is unknown, as none of the products have any direct activity against *Phytophthora*. It is most likely a combination of factors such as a slight improvement in the physical soil properties, making conditions less favourable for *Phytophthora* survival, as well as an increase in the general ability of the soil to suppress diseases with an increase in natural microbial antagonists in the soil.



**Figure 4.4.5.3.** *Phytophthora* counts for block J-16 (planted 2006), expressed as the number of leaf baits out of 10 that tested positive for *Phytophthora* throughout the trial period.



**Figure 4.4.5.4.** *Phytophthora* counts for block D-25 (planted 1996), expressed as the number of leaf baits out of 10 that tested positive for *Phytophthora* throughout the trial period.

**Table 4.4.5.12.** Area under the disease progress curve (AUDPC), calculated for experimental blocks J-16 and D-25, based on individual *Phytophthora* assessments during the period September 2009 to March 2012.

Treatment	AUDPC*	
	Block J-16	Block D-25
1. Control	23.2 c	70.6 c
2. Agrilibrum	16.8 b	63.9 bc
3. ICF	17.5 bc	56.1 ab
4. Local compost	<b>12.1 a</b>	63.3 b
5. Agron	13.4 ab	<b>54.5 a</b>

\* Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test ( $p = 0.05$ ).

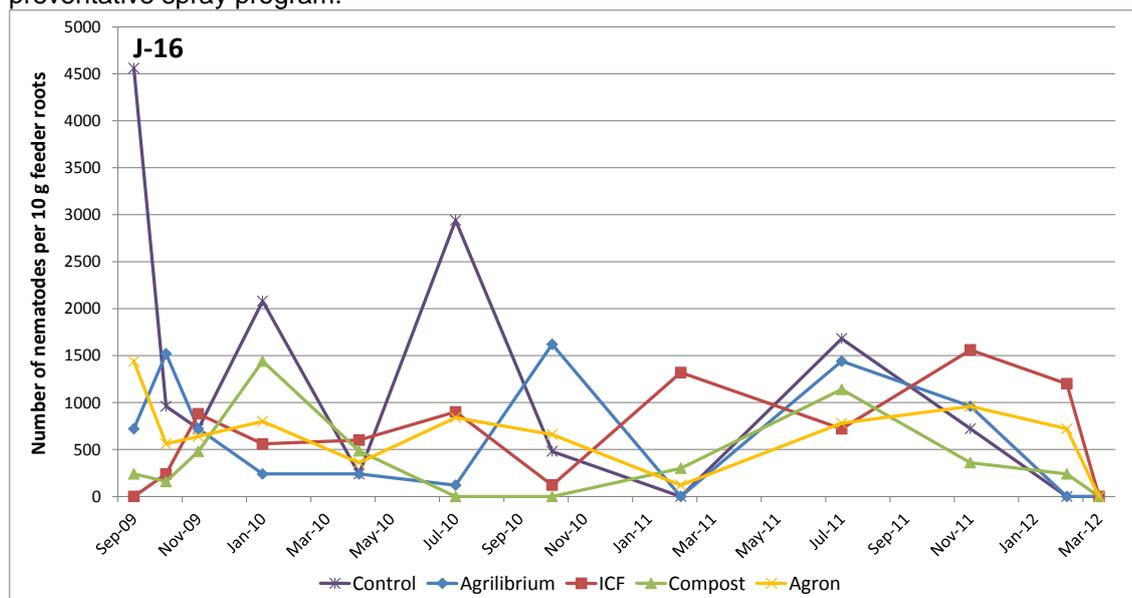
#### Nematode counts

Similar to the *Phytophthora* results, there was some natural variation in the nematode counts during the growing seasons, with lower numbers in the young citrus block J-16 (Figure 4.4.5.5), than in the older citrus block D-25 (Figure 4.4.5.6). All compost treatments had a positive effect by reducing nematode numbers in both the experimental blocks, but the variation in numbers between evaluation intervals made it difficult to effectively compare the effect of treatments. The average nematode counts, percentage increase or decrease in numbers, and the AUDPC was subsequently calculated for block J-16 (Table 4.4.5.13), and block D-25 (Table 4.4.5.14).

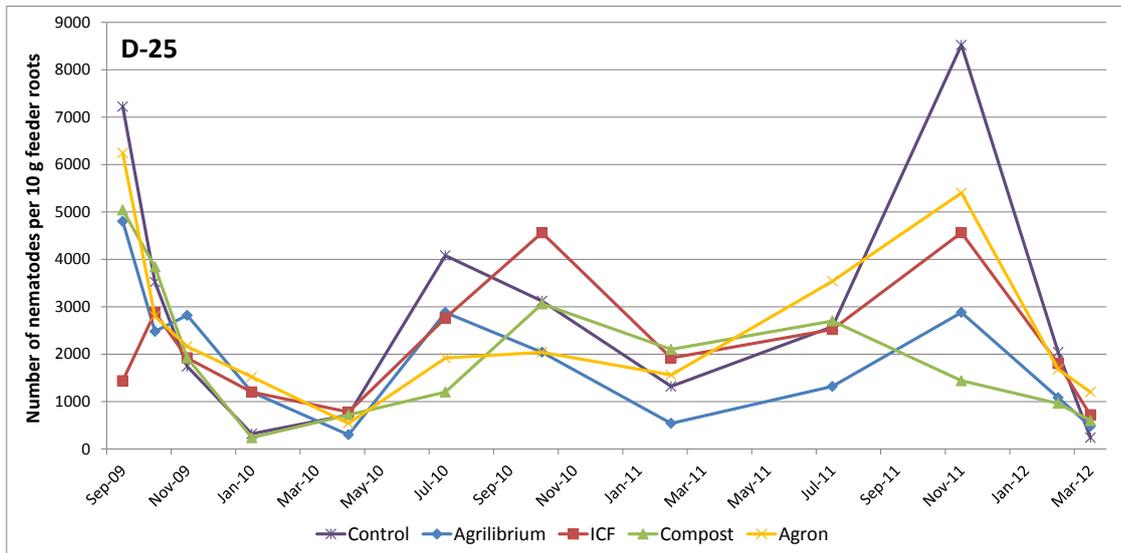
The average number of nematodes counted per evaluation interval was lower for all the treatments in both the experimental blocks. The local compost treatment in block J-16 had statistically lower nematode counts (400.4), compared to the control (1092.3). The corrected percentage increase or decrease in nematode numbers, as calculated by the Henderson-Tilton's formula, showed that all treatments reduced the number of nematodes. In block J-16, the Agrilibrum treatment had the highest reduction of 125% in nematode numbers, and in block D-25 the Agron treatment resulted in 68% lower nematode numbers. Similarly, all the treatments had lower AUDPC values compared to the untreated control in both block J-16 and D-25, although not statistically so.

#### Citrus black spot

Trees from all the treatments in both block J-16 and D-25 were free from any citrus black spot symptoms. This is mainly due to the location of the farm in the drier, northern parts of the Letsitele area, where there is very little citrus black spot disease pressure. The trees were also sprayed for citrus black spot with a preventative spray program.



**Figure 4.4.5.5.** Average number of female citrus nematodes per 10 g feeder roots, counted for treatments in block J-16 (planted 2006), throughout the trial period.



**Figure 4.4.5.6.** Average number of female citrus nematodes per 10 g feeder roots, counted for treatments in block D-25 (planted 1996), throughout the trial period.

**Table 4.4.5.13.** The effect of treatments on the average number of nematodes counted in block J-16, the percentage increase or decrease in nematode numbers, and the effects on the AUDPC.

Treatment	Average female nematode counts*	% Increase or decrease**	AUDPC
1. Control	1092.3 b	-	13.0 a
2. Agrilibrum	569.2 ab	<b>-125.3 a</b>	9.2 a
3. ICF	624.6 ab	-103.2 a	10.5 a
4. Local compost	<b>400.4 a</b>	-32.2 a	<b>6.4 a</b>
5. Agron	612.3 ab	-54.8 a	7.7 a

\* Values are the average number of nematodes counted per evaluation interval, throughout the trial period. Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test (p = 0.05).

\*\* The percentage decrease in nematode numbers are the average corrected percentages as calculated using the Henderson-Tilton's formula.

**Table 4.4.5.14.** The effect of treatments on the average number of nematodes counted in block D-25, the percentage increase or decrease in nematode numbers, and the effects on the AUDPC.

Treatment	Average female nematode counts*	% Increase or decrease**	AUDPC
1. Control	2853.8 a	-	12.3 a
2. Agrilibrum	2013.8 a	-38.5 a	<b>7.5 a</b>
3. ICF	2266.2 a	-61.1 a	11.9 a
4. Local compost	<b>1906.2 a</b>	-66.5 a	9.2 a
5. Agron	2483.1 a	<b>-67.9 a</b>	10.9 a

\* Values are the average number of nematodes counted per evaluation interval, throughout the trial period. Values in columns followed by the same alphabetical letter do not differ significantly according to Fisher's t-test (p = 0.05).

\*\* The percentage decrease in nematode numbers are the average corrected percentages, as calculated using the Henderson-Tilton's formula.

## Conclusion

Results from the study indicates that the amendment of soil with composts have a beneficial effect on general tree condition or plant health. Some of the compost treatments resulted in more vigorous tree growth with a significant increase in trunk circumference and higher yields. For some of the compost amendments soil condition was improved by increased soil penetration and higher soil microbial activity. Importantly, *Phytophthora* and nematode levels, in both the young and older Valencia citrus block, were lower for all the compost treatments, compared to the untreated control. In accordance to similar investigations, it was found that the effects may be somewhat unpredictable and manifest slowly, but that there is a gradual or cumulative improvement of soil and plant health. Disease suppression, as a result of the compost amendments, cannot be compared to traditional chemical control measures, but the lowering effect on *Phytophthora* and nematode levels in a relatively short period is truly encouraging.

## Future research

This study showed promising results in that compost amendments can improve general soil and tree conditions and its ability to reduce *Phytophthora* and nematode levels. However, these effects manifest slowly and it is important that future studies will be conducted over an extended period. This will show if compost amendments are able to ensure that trees stay in a good state of health, especially when orchards become older and disease pressure much higher.

## Technology transfer

Results from the previous seasons were presented by means of a poster presentation at the citrus research symposium in the Drakensberg during August 2010. This is the final report, which includes all the results from the beginning of the study.

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- 4.4.6 **PROGRESS: Rootstock resistance against *Phytophthora nicotianae* root rot**  
Experiment UP\_CRR1-09 by Prof. N. Labuschagne, Prof. Z. Apostolides & Mr. M. Sakupwanya (UP)

## Summary

Citrus root rot caused by *Phytophthora nicotianae* remains an important disease causing chronic losses in citrus yields globally. One of the most effective and economical ways to combat the disease is the use of tolerant / resistant rootstocks. In the current study, the biochemical mechanisms involved in rootstock resistance is being investigated. The relative susceptibility of 13 citrus rootstocks to *Phytophthora* root rot was determined in a number of greenhouse experiments. Seedlings were inoculated with *P. nicotianae* and disease severity was assessed after 2 months. Phytochemical profiles of root samples were determined by means of thin layer chromatography and later HPLC-MS. Rootstocks that consistently showed tolerance were Australian Trifoliolate, Benton citrange and C35. Rootstocks that consistently showed susceptibility included Cairn Rough Lemon, Volckameriana, and X639. Carrizo citrange, Minneola X Trifoliolate, Sunki X

Benecke, Swingle citrange, Terrabella citrumelo, Troyer citrange and Yuma citrange showed intermediate reaction in one experiment and tolerance in others. TLC analyses revealed two categories of fluorescing phytochemical compounds appearing in some rootstocks, namely a) induced compounds, which appeared in pathogen inoculated plants but not in uninfected control plants, and b) constitutive compounds which appeared in both inoculated and control plants, but at higher concentrations in the former. As yet no consistent pattern has emerged indicating a strong correlation between a specific compound and rootstock resistance.

## Oplossing

Sitrus wortelvrot veroorsaak deur *Phytophthora nicotianae* bly steeds 'n belangrike siekte wat wêreldwyd kroniese verliese in sitrus oeste veroorsaak. Een van die mees effektiewe en ekonomiese maniere om die siekte te bestry, is die gebruik van bestande / tolerante onderstamme. In die huidige studie word die biochemiese meganismes betrokke by onderstambestandheid ondersoek. Die relatiewe *Phytophthora* wortelvrot vatbaarheid van 13 sitrus onderstamme is bepaal in 'n aantal glashuiseksperimente. Saailinge is geïnkuleer met *P. nicotianae* en siektegraad is na 2 maande bepaal. Fitochemiese profiele van wortelmonsters is d.m.v. dunlaag-chromatografie (DLC) en HPLC-MS bepaal. Onderstamme wat konsekwent toleransie getoon het, was Australiese Trifoliaat, Benton citrange and C35. Onderstamme wat deurgaans vatbaarheid getoon het was Cairn growweskil suurlemoen, Volckameriana en X639. Carrizo citrange, Minneola X Trifoliaat, Sunki X Benecke, Swingle citrange, Terrabella citrumelo, Troyer citrange en Yuma citrange het intermedieër in een eksperiment en tolerant in ander gereageer. DLC ontledings het twee kategorieë fluoreserende verbindings in sekere onderstamme getoon, nl. a) geïnduseerde verbindings, wat in patogeen geïnkuleerde plante maar nie in ongeïnfekteerde plante voorgekom het nie, en b) voorafbestaande verbindings wat in beide geïnkuleerde en kontrole plante voorgekom het, maar teen verhoogde konsentrasies in eersgenoemde. Tot op hede is geen konsekwente patroon wat 'n sterk korrelasie tussen 'n spesifieke verbinding en onderstambestandheid aantoon, waargeneem nie.

### 4.4.7 **CONTRACT RESEARCH: Evaluation of a new nematicide for the control of the citrus nematode**

Experiment 950 by M.C. Pretorius & C Kotze (CRI)

#### Summary

A contract trial for Makhteshim, Israel, was laid out at Crocodile Valley Citrus Co., Karino and in the Western Cape. The final report with data collected over 2 years was sent to the company.

#### Opsomming

'n Evaluasieproef is vir Makhteshim, Israel, met 'n nuwe nematisied uitgevoer. Drie proef persele is uitgelê; een op Croc Valley, een in die Karino area en een in die Wes-Kaap. 'n Finale verslag met data van al drie persele oor 'n 2-jaar periode is aan die maatskappy gestuur.

### 4.4.8 **CONTRACT RESEARCH: Evaluation of a new safer nematicide for the control of the citrus nematode**

Experiment 951 by M.C. Pretorius (CRI)

#### Summary

DevGem, a Belgium based company, approached CRI to conduct registration trials to establish the efficacy of a new softer nematicide formulation for control of the citrus nematode on a contract basis. Two trials were monitored during the 2011 season; one at Croc Valley Citrus Co. and the second in Citrusdal. The product was applied and the sites were sampled three times during the season. A progress report was sent to DevGem for the work conducted during the 2010 season.

#### Opsomming

Registrasie proewe is vir 'n Belgiese maatskappy, DevGen, op Croc Valley en 'n tweede proef in die Wes-Kaap is gedurende die 2011 seisoen hertoegedien. Drie stelle monsternemings het plaasgevind. 'n Vorderingsverslag is aan DevGem gestuur.

#### 4.4.9 **CONTRACT RESEARCH: Evaluation of a product with possible SAR characteristics for the control of citrus greening bacteria in citrus trees** Experiment 971 by MC Pretorius (CRI)

##### **Summary**

A glasshouse trial is being conducted to determine the effect of a product with possible SAR characteristics for the control of citrus greening disease on citrus. Seedlings were obtained and the trees were inoculated with greening. This is a contract trial for Bayer CropScience, Germany.

##### **Opsomming**

'n Glashuisproef word gedoen om die effek van 'n sistemiese produk, wat moontlike plant weerstandsaktiverings-eienskappe het, teen sitrusvergroenings-siekte te evalueer. Saailinge is met vergroening-besmette plantmateriaal geïnokuleer. Die proef word op 'n kontrakbasis vir Bayer CropScience, Duitsland gedoen.

#### 4.5 **PROJECT: POST-HARVEST PATHOLOGY** Project coordinator: Arno Erasmus (CRI)

##### 4.5.1 **Project summary**

In the postharvest pathology project, five experiments are being funded. Exe123 (4.5.6) provides an industry service to strategically evaluate products as potential alternatives for sanitisers or fungicides. Several products were evaluated in 2011, but none showed potential to be safely used. An alternative approach to postharvest disease and chilling injury control is being studied at University of KwaZulu-Natal where silicon fertilisation is integrated with postharvest heat and biocontrol agent treatment. To date, optimal temperatures for induced fruit resistance without damaging the fruit was determined for different citrus types, and the beneficial effect of silicon fertilisation on chilling injury control was confirmed (4.5.4). At Stellenbosch University (Exe936) (4.5.2), residue loading and protective and curative green mould control following dip and wax applications with imazalil (IMZ), thiabendazole (TBZ) and pyrimethanil (PYR) were studied. Residue benchmarks for effective control were determined, which showed excellent curative control of the fungicide sensitive strains with all fungicides following dip treatment, but relatively poor protective control with PYR and TBZ. An isolate resistant to IMZ and TBZ could not be adequately controlled by these fungicides, but could still be controlled by PYR. Protective control of IMZ and TBZ improved when applied in wax coatings, and double IMZ application in dip and wax provided excellent curative and protective control. In Exe1034 (4.5.5) at CRI-Nelspruit, the IMZ sensitivity of several *Penicillium digitatum* and *P. italicum* isolates was determined, as well as residue benchmarks for protective and curative control. Preliminary results confirm findings from Exe936, where only one resistant isolate of *P. digitatum* was used, that control failure occurs in cases of IMZ resistance for both mould-causing species. At Pretoria University, research on the occurrence of IMZ, TBZ and guazatine resistance in *P. digitatum*, *P. italicum* and *P. crustosum* populations in packhouses in different citrus producing regions was concluded. Fungicide resistance against these fungicides occurred in all regions, but TBZ resistance was more prevalent in areas with a history of using benzimidazoles for Citrus Black Spot control. *P. crustosum* occurred at lower frequencies but appeared to be more tolerant to all fungicides. In packhouses, resistant isolates were mostly obtained around waste bins, indicating the critical importance of sanitation in resistance management. As can be expected, especially when sanitation is poor, fungicide resistance frequencies increased as the packing season progressed (4.5.6).

##### **Projekopsomming**

Vyf eksperimente word in die na-oespatologie projek befonds. Exe123 (4.5.6) voorsien 'n industrie-diens om produkte strategies as potensiële alternatiewe vir saniteerders of swamdoders te evalueer. Verskeie produkte is in 2011 geëvalueer, maar geeneen het die potensiaal getoon om veilig gebruik te word nie. 'n Alternatiewe benadering tot na-oes siekte- en koueskade-beheer, word by die Universiteit van KwaZulu-Natal bestudeer, waar silikonbemesting met na-oes hitte- en biobeheeragent-behandeling geïntegreer word. Tot op datum is optimale temperature vir geïnduseerde vrugweerstand, sonder beskadiging van die vrugte, vir verskillende sitrustipes bepaal, en die voordelige effek van silikonbemesting op koueskade-beheer, is bevestig (4.5.4). By Stellenbosch Universiteit (Exe936) (4.5.2) is residulading en beskermende en genesende groenskimmelbeheer, volgende op doop- en wakstoedienings met imazalil (IMZ), thiabendasool (TBZ) en pyrimethanil (PYR), bestudeer. Residu drempelwaardes vir effektiewe beheer is bepaal, wat uitstekende genesende beheer van die fungisiedsensitiewe isolate na doopbehandelings met al die fungisiedes getoon het, maar relatief swak beskermende beheer met PYR en TBZ. 'n Isolaat wat weerstandbiedend teen IMZ en TBZ is, kon nie voldoende met hierdie fungisiedes beheer word nie, maar

kon steeds met PYR beheer word. Beskermende beheer van IMZ en TBZ het verbeter na toedienings in waks, en dubbele IMZ toedienings in doop- en wakstoedienings het uitstekende genesende en beskermende beheer verskaf. In Exe1034 (4.5.5) by CRI-Nelspruit is die IMZ sensitiviteit van verskeie *Penicillium digitatum* en *P. italicum* isolate bepaal, asook residu drempelwaardes vir beskermende en genesende beheer. Voorlopige resultate het bevindinge van Exe936 (4.5.2), waar slegs een weerstandbiedende isolaat van *P. digitatum* getoets is, bevestig, naamlik dat mislukte beheer in die geval van IMZ weerstand vir beide skimmel-veroorsakende spesies voorkom. By Pretoria Universiteit is navorsing oor die voorkoms van IMZ, TBZ en guazatine weerstand in *P. digitatum*, *P. italicum* en *P. crustosum* populasies in pakhuis in verskillende sitrusproduserende areas afgesluit. Fungisiedweerstand teen hierdie fungisiedes het in al die areas voorgekom, maar TBZ weerstand was meer algemeen in areas met 'n geskiedenis van gebruik van bensimidazole vir Sitrus Swartvlek beheer. *P. crustosum* het teen laer frekwensies voorgekom, maar blyk meer bestand teen al die fungisiedes te wees. Weerstandbiedende isolate is in pakhuis meestal rondom afvaldromme verkry, wat op die kritieke belang van sanitasie in weerstandsbestuur dui. Soos wat verwag word, veral waar sanitasie swak is, neem fungisied-weerstandsfrekwensies toe soos wat die pakseisoen vorder (4.5.6).

#### 4.5.2 PROGRESS REPORT: Optimisation of fungicide application in citrus packhouses

Experiment 936 (April 2008 – March 2014) by Paul Fourie (CRI – SU)

##### Opsomming

Swak beheer van groenskimmel, veroorsaak deur *Penicillium digitatum*, is meestal die gevolg van onvoldoende fungisied residulading en/of fungisied weerstand. Die doel van hierdie studie is om fungisied toediening te optimaliseer deur aanwendingsmetodes, konsentrasie, blootstellingstyd, oplossingstemperatuur en -pH, sowel as genesende en beskermende beheer van *P. digitatum*, te ondersoek. Fungisied residulading is ondersoek en drempelwaarde residuwaardes vir groenskimmelbeheer, volgende op doopbehandelings met imazalil (IMZ), pyrimethanil (PYR) en thiabendazole (TBZ), is bepaal. Vir IMZ, is 'n drempelwaarde residuvlak vir 95% beheer van 'n sensitiewe en weerstandbiedende isolaat op 0.81 en 2.64  $\mu\text{g}\cdot\text{g}^{-1}$ , onderskeidelik, voorspel. Beheer van die IMZ weerstandbiedende isolaat is oor die algemeen swak, beskermende beheer is verloor, en sporulasie is nie geïnhibeer nie. Die diepgaande effek van pH op IMZ residulading is bestudeer en aanbevelings is aan die industrie gemaak. IMZ aanwending by pH c. 3 het lae residuvlakke gelaai, maar die MRL is nie ná 9 min blootstelling oorskry nie; by hoër pH vlakke, het residulading met toenemende blootstellingstyd toegeneem, wat beperk moet word ten einde oorskryding van die MRL te voorkom; en pH vlakke >6.5 moet vermy word. Beskermende beheer met IMZ was beter na toediening in waks, terwyl dubbele aanwending in doop- en waksbehandelings uitstekende genesende asook beskermende beheer gegee het. Vir TBZ, is drempelwaarde residuvlakke van 0.06 tot 0.22  $\mu\text{g}\cdot\text{g}^{-1}$  vir 75% genesende beheer voorspel; beskermende beheer was relatief swak en geen beheer van die TBZ-weerstandbiedende isolaat kon verkry word nie. TBZ in waks het beter beskermende beheer getoon en koue-skade op Valencia lemoene verminder. PYR het uitstekende genesende beheer van sensitiewe en IMZ+TBZ-weerstandbiedende isolate gegee, maar relatief swak beskermende beheer. Gemiddelde PYR residu drempelwaardes vir 50% en 75% genesende beheer was 0.268 en 0.905  $\mu\text{g}\cdot\text{g}^{-1}$ , onderskeidelik, terwyl 50% beskermende beheer by 1.181  $\mu\text{g}\cdot\text{g}^{-1}$  voorspel is. Dit blyk, uit die werk tot op hede, dat fungisiedweerstand tot mislukte beheer lei. Fungisiedes moet dus optimaal gebruik word, afgewissel word, en weerstandsfrekwensies in pakhuis moet gemonitor word. In die ontwikkeling van 'n weerstandstoets, was blootgestelde plaattoets met 'n semi-selektiewe media nie-spesifiek en onbetroubaar, en 'n kwantitatiewe "real-time" PKR metode word tans ontwikkel.

##### Summary

Poor control of green mould, caused by *Penicillium digitatum*, is often the result of insufficient fungicide residue loading and/or fungicide resistance. The aim of this study is to optimise fungicide application by investigating application methods, concentration, exposure time, solution temperature and pH, as well as curative and protective control of *P. digitatum*. Fungicide residue loading was studied and benchmark residue values for green mould control following dip treatments with imazalil (IMZ), pyrimethanil (PYR) and thiabendazole (TBZ) were determined. For IMZ, benchmark residue level for 95% control of a sensitive and resistant isolate was predicted to be 0.81 and 2.64  $\mu\text{g}\cdot\text{g}^{-1}$ , respectively. In general, control of the IMZ resistant isolate was substantially reduced and protective control was lost, nor was sporulation inhibited. The profound effect of pH on IMZ residue loading was studied and recommendation made to industry: IMZ application at pH c. 3 loaded low residue levels, but the MRL was not exceeded after 9 min exposure; at higher pH levels residue loading increased with increasing exposure time, which should be limited to prevent exceedance of the MRL; and pH levels >6.5 should be avoided. Protective green mould control with IMZ was improved following application in the wax coating, and double IMZ application in dip and wax provided excellent curative and protective control. For TBZ, benchmark residue levels of 0.06 to 0.22  $\mu\text{g}\cdot\text{g}^{-1}$  were predicted for 75% curative control; protective control was relatively poor and no control of the TBZ-resistant

isolate could be obtained. TBZ applied in wax improved protective control and reduced chilling injury on Valencia oranges. PYR provided excellent curative control of sensitive and IMZ+TBZ-resistant isolates, but relatively poor protective control. Mean benchmark PYR residue values for 50% and 75% curative control were 0.268 and 0.905  $\mu\text{g}\cdot\text{g}^{-1}$ , respectively, while 50% protective control was predicted at 1.181  $\mu\text{g}\cdot\text{g}^{-1}$ . From the work to date, it appears that fungicide resistance leads to control failure. Fungicides should therefore be optimally used, alternated and resistance frequencies monitored in packhouses. In developing a resistance assay, exposed-plate assays using semi-selective media were non-specific and unreliable, and a quantitative real-time PCR method is being developed.

#### 4.5.3 FINAL REPORT: Screening of South African *Penicillium* isolates from citrus producing regions for resistance to the postharvest fungicides imazalil, guazatine and thiabendazole Experiment PPL 24 (April 2010 – March 2012) by R Jacobs and L Korsten (UP)

### Opsomming

Dit raak meer algemeen deesdae in pakhuisse wêreld-wyd om swamdoder weerstandbiedende *Penicillium* isolate vanuit vars vrugte te isoleer. Daar moet ekstra sorg geneem word om te verseker dat die weerstandbiedende inokulum in die pakhuis sover moontlik beperk word. Hierdie studie was daarop gerig om te bepaal watter *Penicillium* spesies voorkom en watter spesies in sitrus pakhuisse dominant is. Addisioneel was dit nodig om te kyk na wat die sensitiwiteitsfrekwensie is teenoor die huidige gebruikte swamdoders imazalil, thiabendazole en guazatine. Vier sitrus pakhuisse in geografies verskillende streke is geselekteer waar monsters geneem is van oppervlaktes en lug omgewings in pakhuisse om *Penicillium* isolate te bekom. Monsters is ook vanaf verrottende vrugte aan bome in boorde wat naby aan die pakhuisse geleë is, verkry. Drie *Penicillium* spesies met bekende patogenisiteit teenoor sitrus naamlik *P. digitatum*, *P. italicum* en *P. crustosum* is in afnemende orde van dominansie geïsoleer. 'n *Penicillium* populasie dominansie verskuiwing is verkry na gelang van die verloop van die seisoen. 'n Sensitiwiteitsverskuiwing teenoor al drie die swamdoders is in sommige isolate van *P. digitatum* en *P. italicum* wat vanaf die pakhuisse verkry is, opgemerk. *Penicillium crustosum* is teen 'n laer frekwensie geïsoleer, maar dit was oor die algemeen nie-sensitief of weerstandbiedend teenoor thiabendazole en guazatine by verskeie pakhuisse wat getoets is. Pakhuis sanitasie programme kan geïmplimenteer word om die risiko van die vestiging en vermeerdering van weerstandbiedende of minder sensitiewe populasies te beperk.

### Summary

In recent years, it has become more common to isolate fungicide resistant strains from packhouses globally. Great care should be taken to ensure that the fungicide resistant population in the packhouses are reduced as far as possible. The current study was aimed at determining the *Penicillium* species presence and dominance in citrus packhouses. In addition, the sensitivity towards the currently used fungicides imazalil, thiabendazole and guazatine was determined. Four citrus packhouses in geographically distinct areas have been selected where surfaces and aerial environments within and around the packhouses have been sampled to obtain *Penicillium* isolates. Samples have also been obtained from decaying fruit in orchards in close proximity to the surveyed packhouses. Three citrus pathogenic *Penicillium* spp. namely *P. digitatum*, *P. italicum* and *P. crustosum* were isolated in ascending order of dominance. A *Penicillium* population dominance shift became apparent as the season progressed. A possible shift in sensitivity to all three fungicides was observed in certain strains of *P. digitatum* and *P. italicum* from most packhouses, especially at waste bins and on orchard fruit amongst other areas. *Penicillium crustosum* was isolated from fruit at a lower frequency; however, it was generally observed to be non-sensitive or resistant to thiabendazole and guazatine at all packhouses tested. Packhouse sanitation programmes could be implemented to reduce the risk of establishment and increase of resistant or non-sensitive isolate populations

### Introduction

Citrus is one of the most important fruit crops produced in South Africa with a significant economical value due to export. However, some of the most detrimental threats to the industry is postharvest green and blue mould. These diseases are caused by *Penicillium digitatum* (Pers.:Fr.) Sacc. and *Penicillium italicum* Wehmer, respectively (Cohen, 1989; Eckert and Eaks, 1989; Holmes and Eckert, 1999; Hamada and Fujita, 2002; Zhu *et al.*, 2006; Boubaker *et al.*, 2009).

Airborne conidia of *Penicillium* species occur naturally in the environment; however, it can easily be formed on decaying fruit from which it is conveyed by air currents, water dumps and fruit handling equipment, to healthy fruit in the packhouse (Gardner *et al.*, 1986). For this reason, *Penicillium* spores are often found abundantly in citrus production areas and fruit handling environments such as packing houses and cold storage facilities (Prusky and Ben-Arie, 1985; Boubaker *et al.*, 2009). Primary infection is initiated through a fresh wound in the peel of the citrus fruit (Gardner *et al.*, 1986). Citrus peel extracts enhanced the

germination potential of *Penicillium* species and the inoculum spread is enhanced exponentially (Droby *et al.*, 2008). Any source of conidial inoculum should therefore be removed and destroyed as soon as it is detected in the packhouse and orchard.

With the abundance of *Penicillium* inoculum in the packhouse and the continued use of chemical fungicides throughout the season, there is a constant threat of the development of fungicide resistant *Penicillium* strains. Many chemical fungicides have been used commercially through decades to control citrus blue and green mould globally. However in most instances resistant strains populations have emerged under severe selection pressure (Gardner *et al.*, 1986; Holmes and Eckert, 1999; Kinay *et al.*, 2007; Boubaker *et al.*, 2009). The possible build-up of a fungicide resistant population in citrus packhouses could inevitably lead to excessive fruit decay, which in turn will lead to substantial economical losses to the industry (Gardner *et al.*, 1986). A functional disease control method is therefore lost and with the lack of available alternative control measures. This should be avoided as far as possible.

The optimal and controlled use of fungicides is inevitable in the attempt to prolong the use of the currently available fungicides. This should be integrated with various packhouse sanitation and management strategies, which include the careful handling of fruit during and after harvest to reduce the wounding risks, optimal and regular sanitation of the packhouse and disinfection of packhouse and fruit handling equipment (Gardner *et al.*, 1986; Schirra *et al.*, 2008). We have therefore proposed a study to determine the presence and dominance of *Penicillium* species in various citrus packhouses in South Africa and evaluate strains of these species for its sensitivity frequency towards the commercially applied fungicides imazalil, thiabendazole and guazatine. By analysing this data and the critical areas where resistant strains are detected, critical packhouse management strategy recommendations could be made to reduce the impact of the citrus pathogens on the citrus industry of South Africa, thereby extending the use of the commercial fungicides as far as possible.

#### **Stated objectives**

- Determine *Penicillium* inoculum levels, especially *P. digitatum*, *P. italicum* and *P. crustosum*.
- Determine the resistance frequency and variation within a species in a production region and compare that between production regions.
- Determine methods to routinely monitor for future resistance development and draw conclusions regarding inhibition of resistance development as confirmed by data generated.

#### **Materials and methods**

##### Sampling and fungal isolate isolation

During the 2010 and 2011 citrus production seasons, we have been able to sample four citrus packhouses. The packhouses in Marikana (Northwest province), Nelspruit (Mpumalanga), Marble Hall (Limpopo) and Sunland (Eastern Cape) were sampled during early and late packing season to determine the shift in population dynamics. The latter two packhouses mentioned were only sampled once due to unforeseen circumstances. Sampling was focused on the walls and floors of the packhouse, packlines and specific areas in the packline such as brushes, rollers, conveyor belts etc., as well as on waste bins inside and outside the packhouse. To determine if resistant *Penicillium* strains can be detected in orchards, we have sampled decaying fruit on and around ten trees in the citrus orchard closest to the packhouse.

Sampling consisted of active and passive air sampling on malt extract agar (MEA) (Merck, Johannesburg) and Transswab (Progen, Johannesburg) sampling of environments and surfaces in and around the packhouse. Air sample plates were incubated at 25°C for 4 to 7 days after which total colony counts were performed. *Penicillium* isolates were aseptically transferred to Petri dishes containing MEA and were incubated for 7 days to obtain pure cultures. All the swab samples received were processed by dilution plating of the 10<sup>-3</sup> to 10<sup>-4</sup> swab dilutions containing Ringer's solution (Merck) onto MEA to ensure that all possible *Penicillium* isolates could be detected and isolated. Dilution plates were incubated at 25°C for 4 to 7 days after which total colony counts were performed. *Penicillium* isolates were aseptically transferred to Petri dishes containing MEA and were incubated for 7 days to obtain pure cultures.

##### Penicillium species identification

*Morphological identification.* All *Penicillium* isolates were grouped into morphological groups based on identical cultural characteristics. Morphological identification of representative isolates was done by comparing cultural growth characteristics at different incubation temperatures (Pitt, 1991; Samson and Pitt, 2000). Only isolates that were identified morphologically as either *P. digitatum*, *P. italicum* and *P. crustosum* was retained for identification confirmation. Each morphological group represent a *Penicillium* species with similar morphological characteristics. All isolates from a specific morphological group was organised so that each morphological group was split to represent the four different regions sampled. An isolate from each sub-group was retained for further molecular species identity confirmation. All isolates within selected,

identified morphological groups that represent the three species mentioned above were later used for fungicide sensitivity trials. Representative isolates in each group were purified and preserved in sterile water. For long term storage at -70°C, isolates were inoculated into a glycerol solution.

**Molecular identification.** Representative isolates from morphological sub-groups were used for molecular identification purposes. DNA was extracted using the DNeasy Plant Mini DNA Extraction kit (Qiagen, USA). During the extraction process, fungal mycelia were macerated in a bead beater (Thermo, IEP SA), using micro silicone beads of varying sizes. Amplification was performed on the  $\beta$ -tubulin gene region using primers Bt2A and BT2B (Glass and Donaldson, 1995; Samson *et al.*, 2004). PCR reactions were performed in 50  $\mu$ l reaction mixtures containing 0.6  $\mu$ l DNA (10 ng/ml), 5  $\mu$ l reaction buffer, 37.4  $\mu$ l sterile double distilled water (ddH<sub>2</sub>O), 1  $\mu$ l dNTPs (10 mM), 0.5  $\mu$ l of each primer (100 pmol/  $\mu$ l) (either ITS or  $\beta$ -tubulin) and 0.5  $\mu$ l Taq DNA polymerase (2.5 U/  $\mu$ l) (Bioline, Ireland). Amplification was performed in an Eppendorf Mastercycler EP (Merck) which was programmed for 30 s at 95°C, then 35 cycles of 94°C for 1 min, followed by primer annealing at 58°C for 90 s and extension at 72°C for 2 min. A final extension of 7 min followed, after which the samples were kept at 4°C until samples were used further or stored at -20°C.

PCR amplicons were also sequenced in an ABI Prism 3700 Genetic Analyser (AB Applied Biosystems) after pre- and post-cleaning methodology of the PCR amplicons. The cycle sequencing PCR reaction constituted a final volume of 10  $\mu$ l and contained 1  $\mu$ l of template DNA, 4  $\mu$ l Dye Terminator Ready Reaction Mix, 4  $\mu$ l ddH<sub>2</sub>O and 1  $\mu$ l primer (4 pmol/  $\mu$ l) (either ITS or  $\beta$ -tubulin). Contigs were assembled and isolate identity was confirmed using sequence homology with the program NCBI Blast (Zhang *et al.* 2000).

#### Fungicide sensitivity screening of imazalil, guazatine and thiabendazole

Isolates obtained at all packhouses that were identified as *P. digitatum*, *P. italicum* or *P. crustosum* were included in this study to determine fungicide sensitivity of each species and various strains within a species. Cultures produced on MEA were used from which a 5 mm mycelial plug was taken at the edge of the 7-day-old actively growing culture. The mycelial plug was inoculated onto the centre of a Petri dish containing either imazalil, guazatine or thiabendazole at the following concentrations: 1.5  $\mu$ g/ml imazalil; 0.5  $\mu$ g/ml guazatine and 10  $\mu$ g/ml thiabendazole based on doses determined in a previous study and literature (Wild, 1994; Kinay *et al.*, 2007; Schmidt *et al.*, 2006; Amiri *et al.*, 2008; Wild, 2008; Cabanas *et al.*, 2009). Each isolate was inoculated onto the fungicide amended media in triplicate. For each isolate tested, control MEA plates were also inoculated with the culture plug method as mentioned above in triplicate. The isolates that are sensitive to the fungicide tested should sustain no or very little culture growth (rated as less than a maximum of 13mm in diameter) and isolates that are capable of producing larger colony growth were considered moderately to highly resistant in comparison to the control plates. All fungicide amended media or control MEA plates were inoculated within 1 to 2 days from date of preparation due to the change in fungicide concentration over time during storage (Cabanas *et al.*, 2009). Cultures were incubated for 7 days at 25°C and culture growth diameter measurements were taken in duplicate on each Petri dish. The fungicide screening trials were repeated once for each representative isolate and each fungicide concentration tested.

#### Data analysis

Fungicide sensitivity trials were analysed using culture growth inhibition at 25°C after precisely 7 days of incubation. The culture growth on each Petri dish was determined by measuring the colony growth diameter at two perpendicular regions. Isolates that produced a colony size smaller than 13 mm in diameter was regarded as sensitive and larger would be regarded as less sensitive or moderately to highly resistant to the fungicide tested in comparison to the colony growth of the control plate. Please note that in the sensitive case fungal growth on the amended media, if any, was always far less than the selected 13 mm maximum indicated above (this figure was based on assessing all plates and then recognising a similar pattern). Isolates had either none or slight growth (less than 13 mm) and was classed as 'sensitive', or produced large growth patterns and were considered 'resistant'. Almost all growth patterns fell into either one of these categories.

### **Results and discussion**

The most important of the postharvest diseases of citrus are blue and green mould, which is caused by *P. italicum* and *P. digitatum*, respectively (Droby *et al.*, 2008). Great efforts have been made over many years to reduce the impact of these pathogenic species on the citrus industry. It is important to focus on disease prevention; therefore citrus fruit is usually treated with chemical fungicides postharvest to reduce the risk of disease development (Droby *et al.*, 2008). Proper packhouse sanitation as part of the packhouse management system is also essential since *Penicillium* survival structures bridges the gap between seasons, making inoculum available for the start of the next season (Smilanick and Mansour, 2007). If these survival structures are resistant to the fungicides used in the packhouse, resistant populations can be established in the packhouse as was found for *P. digitatum* in California (Smilanick and Mansour, 2007), and this should be prevented in the South African citrus industry. This survey was therefore conducted to determine the

*Penicillium* species dominance and fungicide resistance in South Africa and make recommendations for the improvement of the packhouse management systems.

**Table 4.5.3.1.** Research objectives table.

OBJECTIVES	MILESTONES ACHIEVED
Determine <i>Penicillium</i> inoculum levels, especially <i>P. digitatum</i> , <i>P. italicum</i> and <i>P. crustosum</i>	<i>P. digitatum</i> was determined to dominate the citrus packhouse environment followed by <i>P. italicum</i> and lastly <i>P. crustosum</i> .
Determine the resistance frequency and variation within a species in a production region and compare between production regions	Resistance frequency within a species differed in relation to critical areas in specific packhouses, such as orchards and waste bins that seem to house more resistant strains.
Determine methods to routinely monitor for future resistance development and draw conclusions regarding inhibition of resistance development as confirmed by data generated	Packhouse managers have been informed of problems and possible solutions to the packhouse problems. The passive plate method will be evaluated as a routine means of determining inoculum and resistance in the packhouse.

#### Isolation and identification

During the current study, it was clear that the citrus pathogenic *Penicillium* species are commonly and dominantly isolated from air and surfaces in and around the packhouses with 311 and 965 isolates obtained in 2010 and 2011, respectively. Population dynamics and dominance were very much the same for both seasons, which could be seen as an annual trend.

In 2010, the *Penicillium* spp. dominance in the tested South African citrus packhouses was found to be *P. digitatum*, representing 70.2% of the total population, *P. italicum* representing 23.6% and *P. crustosum* representing 6.1% of the total *Penicillium* population obtained overall at all packhouses. During 2011, the species dominance remained the same with *P. digitatum* dominating at 64.7%, followed by *P. italicum* at 25.9% and *P. crustosum* at 9.4%. *Penicillium digitatum* was by far the most dominantly isolated species at all four geographically distinct packhouses surveyed during both seasons. The first published report of *Penicillium* spp. in South Africa was in 1911 by Pole Evans (Pole Evans, 1911 in Schutte, 1992; Smilanick *et al.*, 2006) when the *Penicillium* species isolated from decaying citrus fruit was identified as *P. digitatum*. Ever since, *P. digitatum* has been commonly associated as the dominant causal agent of citrus green mould. Table 2 represents the species dominance at the four packhouses sampled during one or two sampling trips, respectively. Very few isolates belonging to the species *P. glabrum*, *P. brevicompactum* and others were isolated; however, these were disregarded in this study since focus was only placed on possible citrus pathogenic species.

**Table 4.5.3.2.** Percentage *Penicillium* species dominance at four citrus packhouses sampled either once or twice during the 2010 and 2011 citrus seasons.

EARLY TO MID SEASON SAMPLING 2010*			
Packhouse region	<i>P. digitatum</i>	<i>P. italicum</i>	<i>P. crustosum</i>
Marikana (North-West)	87.0	9.9	3.1
Marble Hall (Mpumapanga)	73.9	13.0	13.0
Nelspruit (Mpumalanga)	57.1	21.4	21.4
Sunland (Eastern-Cape)	52.2	42.5	5.0
LATE SEASON SAMPLING 2010			
Packhouse region	<i>P. digitatum</i>	<i>P. italicum</i>	<i>P. crustosum</i>
Marikana (North-West)	53.7	38.9	7.4
Nelspruit (Mpumalanga)	62.5	37.5	0.0
<b>TOTAL</b> (Species sampling dominance 2010)	<b>70.2</b>	<b>23.6</b>	<b>6.1</b>
EARLY SEASON SAMPLING 2011			
Packhouse region	<i>P. digitatum</i>	<i>P. italicum</i>	<i>P. crustosum</i>
Marikana (North-West)	81.5	7.4	11.1
Marble Hall (Mpumapanga)	93	0	6.2

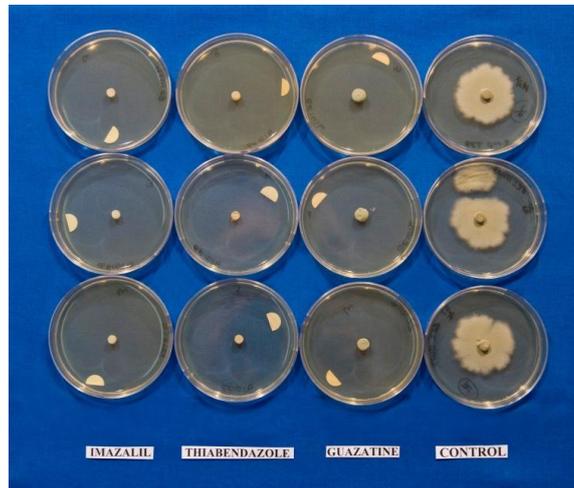
Nelspruit (Mpumalanga)	93	6.7	0
Sunland (Eastern-Cape)	75	0	25
<b>LATE SEASON SAMPLING 2011</b>			
<b>Packhouse region</b>	<b><i>P. digitatum</i></b>	<b><i>P. italicum</i></b>	<b><i>P. crustosum</i></b>
Marikana (North-West)	54.7	29.7	15.6
Marble Hall (Mpumapanga)	82.5	1.6	15.8
Nelspruit (Mpumalanga)	54.1	42.4	3.5
Sunland (Eastern-Cape)	53.1	41.7	5.2
<b>TOTAL (Species sampling dominance 2011)</b>	<b>64.7</b>	<b>25.9</b>	<b>9.4</b>

\*All table values indicate occurrence of only the three *Penicillium* spp. focused on during this study as a percentage of the total number of these isolates obtained per packhouse sampling.

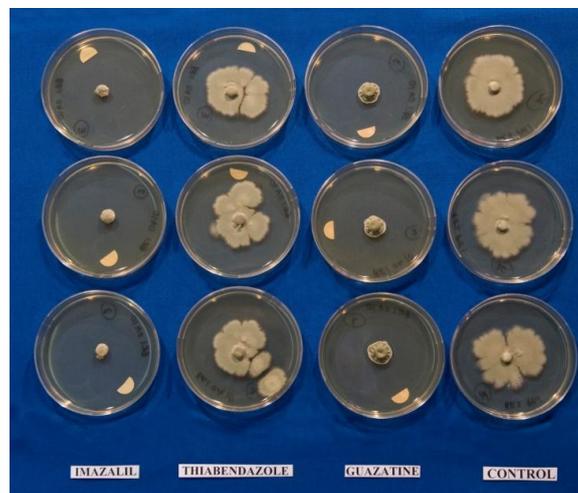
Only two packhouses were sampled twice during the 2010 season; however sampling for all four packhouses were repeated in the 2011 season. When analysing the data, it is evident that the species dynamics changed from early to late season. An increase in the number of isolates in general was detected in the later season sampling for all packhouses (Appendix A). When looking at the 2010 data, as well as the 2011 data where all sampling was repeated, it was evident that *P. italicum* occurrence were proportionally very low as the season commences and the *P. digitatum* occurrence high. As the season progressed, *P. italicum* became far more dominant and almost equally dominant with *P. digitatum*. The reason could be that *P. italicum* does not proliferate adequately in the absence of the citrus host in the packhouse during the off season. *Penicillium digitatum* on the other hand seems to have adapted to a lifestyle of survival in the absence of the host in the packhouse. Great care should therefore be taken to disinfect packhouses before the season commences. Diseased fruit should also be kept away from the packhouse as far as possible to reduce the increase of *P. italicum* as the season progresses. With proper and regular packhouse sanitation practices, levels of *Penicillium* could be reduced significantly.

#### Fungicide sensitivity trials with imazalil, thiabendazole and guazatine

Several organic and synthetic fungicides have been used in citrus industry for the control of postharvest decay caused by *Penicillium* species. However, in a number of instances, resistant strains of the pathogenic species have emerged under severe selection pressure of the fungicide compounds and these strains are annually responsible for the reduced efficacy of the commercial treatments (Gardner *et al.*, 1986; Smilanick *et al.*, 2006). Resistant strains have been reported from many packhouses that used the fungicides; however, resistance was rarely observed in orchards. The build-up of resistance in populations is of grave importance for the long term use of fungicides in packhouses (Gubbins and Gilligan, 1999). Fungicide resistance development in *Penicillium* species towards thiabendazole has been reported on extensively within a few years after incorporating the product in citrus packhouses (Davé *et al.*, 1980; Baraldi *et al.*, 2003). Imazalil or guazatine resistant *Penicillium* strains have also been identified and various reports of benzimidazole and thiabendazole resistance have been produced for decades from citrus sectors globally (Dave *et al.*, 1980; Cohen, 1989; Zhu *et al.*, 2006; Ghosop, *et al.*, 2007). We have therefore aimed to determine the level of resistance to the chemical fungicides currently used in the citrus industry. During the current study, 311 isolates in 2010 and 382 isolates in 2011 were tested for its sensitivity towards the fungicides imazalil, thiabendazole and guazatine. Mycelium growth was determined by two perpendicular colony measurements on fungicide-amended and un-amended media as observed in figures 1 to 3. Tables A1 to A14 in Appendix A indicate the areas at each packhouse where the three *Penicillium* species were isolated as well as the sensitivity of each tested isolate towards the fungicides tested.



**Figure 4.5.3.1.** *Penicillium digitatum* plug inoculation of imazalil, thiabendazole or guazatine amended fungicide media where the isolate tested was sensitive to all fungicides tested. All Petri dishes in a column represent repeats of the specific fungicide tested or repeats of the control.



**Figure 4.5.3.2.** *Penicillium digitatum* plug inoculation of imazalil, thiabendazole or guazatine amended fungicide media where the isolate tested was sensitive to imazalil, sensitive to guazatine, and resistant towards thiabendazole. All Petri dishes in a column represent repeats of the specific fungicide tested or repeats of the control.



**Figure 4.5.3.3.** Plug inoculation of different *Penicillium* isolates onto imazalil, thiabendazole or guazatine amended fungicide media indicating a variation in fungicide sensitivity between different isolates. All four Petri dishes in a row represent one isolate inoculated on different fungicide amended media with its control. All Petri dishes in a column represent different isolates treated with the same fungicide. If measurable culture growth is observed, the isolate is generally regarded as more resistant to the fungicide tested. No growth indicated fungicide sensitivity.

#### *Marikana packhouse*

Isolates obtained at this packhouse and the areas of isolation are specified in tables A1 – A4 in Appendix A.

- Early season 2010:

*Penicillium digitatum* (114 isolates), *P. italicum* (13 isolates) and *P. crustosum* (4 isolates) were isolated from the packhouse in Marikana. The resistance frequency of *P. digitatum* is summarised as follows: 10.5% imazalil, 10.5% thiabendazole and 17.3% guazatine resistance. The resistance frequency of *P. italicum* is: 0% imazalil, 0% thiabendazole and 61.5% guazatine resistance. The resistance frequency of *P. crustosum* is 100% for imazalil, thiabendazole and guazatine respectively during this trial.

- Early season 2011:

In 2011, *Penicillium digitatum* (22 isolates), *P. italicum* (2 isolates) and *P. crustosum* (3 isolates) were isolated from the packhouse in Marikana. The resistance frequency of *P. digitatum* was 4.5% imazalil, 4.5% thiabendazole and 45.5% guazatine resistance. The resistance frequency of *P. italicum* is 0% for imazalil, 0% thiabendazole and 100% guazatine respectively during this trial. The resistance frequency of *P. crustosum* is 100% for imazalil, thiabendazole and guazatine during this trial.

- Late season 2010:

*Penicillium digitatum* (29 isolates), *Penicillium italicum* (20 isolates) *Penicillium crustosum* (4 isolates) was isolated from the packhouse in Marikana. The resistance frequency of *P. digitatum* is summarised as follows: 20.7% imazalil, 20.7% thiabendazole and 24.1% guazatine resistance. The resistance frequency of *P. italicum* is: 0% imazalil, 0% thiabendazole and 90% guazatine resistance. The resistance frequency of *P. crustosum* is 100% for imazalil, thiabendazole and guazatine during this trial.

- Late season 2011:

*Penicillium digitatum* (35 isolates), *P. italicum* (19 isolate) and *P. crustosum* (10 isolate) were isolated from the packhouse in Marikana. The resistance frequency of *P. digitatum* was 11.4% imazalil, 8.6% thiabendazole and 14.3% guazatine resistance. The resistance frequency for *P. italicum* was 0% imazalil,

10.5% thiabendazole and 63% guazatine. The resistance frequency of *P. crustosum* is 100% for imazalil, 70% thiabendazole and 100% guazatine during this trial.

At Marikana packhouse, *P. digitatum* was very dominantly isolated during both seasons. A relatively low level of resistance was detected from isolates in this species in general. *Penicillium italicum* was isolated during all sampling intervals, however a very low number of isolates were resistant to imazalil and thiabendazole. A very high level of resistance to guazatine was detected in strains of this species at the packhouse in Marikana and it is suggested that guazatine is not to be used here due to the build up of the resistant population towards the chemical. A factor of concern is the observation that all the decaying fruit found in the packhouse at the time of sampling housed *P. digitatum* and/or *P. crustosum* isolates that were highly resistant simultaneously to all three fungicides tested. These inoculum sources should be removed immediately from the packhouse to avoid the build-up of the resistant population in the packhouse. High resistance frequencies were also detected from the aerial environments surrounding the waste bins in the packhouse. Improved hygiene could reduce the inoculum substantially which could have a correlation to decay incidence (Gardner *et al.*, 1986).

#### *Nelspruit packhouse*

Isolates obtained at this packhouse and the areas of isolation are specified in tables A5 – A8 in Appendix A.

- Early season 2010:

*Penicillium digitatum* (16 isolates), *Penicillium italicum* (6 isolates) and *Penicillium crustosum* (6 isolates) was isolated from the packhouse. The resistance frequency of *P. digitatum* isolates was 0% imazalil, 0% thiabendazole and 6.3% guazatine resistance. The resistance frequency of *P. italicum* isolates is: 16.7% imazalil, 66.7% thiabendazole and 83% guazatine resistance. The resistance frequency of *P. crustosum* isolates is: 100% imazalil, 0% thiabendazole and 100% guazatine resistance.

- Early season 2011:

*Penicillium digitatum* (14 isolates) and *Penicillium italicum* (1 isolate) was isolated from the packhouse. The resistance frequency of *P. digitatum* isolates were 28.6% imazalil, 0% thiabendazole and 21.4% guazatine resistance. The resistance frequency of *P. italicum* isolates was 100% imazalil, 0% thiabendazole and 100% guazatine resistance.

- Late season 2010:

*Penicillium digitatum* (20 isolates) and *Penicillium italicum* (12 isolates) were obtained during this study. The resistance frequency of *P. digitatum* isolates was 0% imazalil, 0% thiabendazole and 0% guazatine resistance. The resistance frequency of *P. italicum* isolates was 8.3% imazalil, 100% thiabendazole and 33.3% guazatine resistance.

- Late season 2011:

*Penicillium digitatum* (46 isolates), *P. italicum* (36 isolates) and *P. crustosum* (3 isolates) were isolated from the packhouse. The resistance frequency of *P. digitatum* isolates were 0% imazalil, 37% thiabendazole and 10.9% guazatine resistance. The resistance frequency of *P. italicum* isolates was 2.8% imazalil, 58.3% thiabendazole and 55.6% guazatine resistance. The resistance frequency of *P. crustosum* isolates was 66.7% imazalil, 33.3% thiabendazole and 100% guazatine resistance.

The isolation frequency at this packhouse was relatively low which could be an indication of good hygiene practices being enforced. Most isolates of *P. digitatum* obtained from the packhouse in Nelspruit displayed a high sensitivity towards all three fungicides tested. Although resistant strains were detected at different times during different seasons, this seems to be at a manageable level possibly due to proper sanitation practices being adhered to. Thiabendazole and/or guazatine resistant isolates were detected from orchard trees, waste bins inside the packhouse and air around the waste bins as well as the pre-wash and waxing areas in the packhouse respectively. Although *P. crustosum* was isolated at a low frequency compared to *P. digitatum* and *P. italicum*, the isolates tested displayed a high resistance frequency toward imazalil and guazatine. All isolates were obtained from the aerial environment in the packhouse. *Penicillium crustosum* could therefore be a common contaminant of packhouse aerial environments or ventilation systems with the ability to cause postharvest decay on citrus fruit under optimal conditions. The responsible use of imazalil and guazatine in the packhouse is suggested to limit the build up of the resistant population which is currently still at a manageable level. These results correspond well with that of a previous study (Anonymous, 2010).

#### *Marble Hall packhouse*

Isolates obtained at this packhouse and the areas of isolation are specified in tables A9 – A11 in Appendix A.

- Mid-season 2010:

*Penicillium digitatum* (17 isolates), *Penicillium italicum* (3 isolates) *P. crustosum* (3 isolates) was isolated from the packhouse. The resistance frequency of *P. digitatum* isolates was 0% imazalil, 94.1% thiabendazole and 23.5% guazatine resistance. The resistance frequency for *P. italicum* isolates was 33.3% imazalil, 66.6% thiabendazole and 33.3% guazatine. The resistance frequency of *P. crustosum* isolates was 100% imazalil, 0% thiabendazole and 100% guazatine.

- Early season 2011:

In 2011, *Penicillium digitatum* (15 isolates) and *P. crustosum* (1 isolate) were isolated from the packhouse in Marble Hall. The resistance frequency of *P. digitatum* was 0% imazalil, 73% thiabendazole and 0% guazatine resistance. The resistance frequency of *P. crustosum* is 0% for imazalil, 100% thiabendazole and 0% guazatine during this trial.

- Late season 2011:

*Penicillium digitatum* (52 isolates), *P. italicum* (1 isolate) and *P. crustosum* (10 isolate) were isolated from the packhouse in Marble Hall. The resistance frequency of *P. digitatum* was 26.9% imazalil, 42.3% thiabendazole and 28.8% guazatine resistance. The resistance frequency for *P. italicum* was 0% imazalil, 100% thiabendazole and 0% guazatine. The resistance frequency of *P. crustosum* is 90% for imazalil, 40% thiabendazole and 100% guazatine during this trial.

Although the sampling procedure was only performed once during the middle of the season in 2010 and twice in 2011, it is clear from the data that some level of loss in fungicide sensitivity was detected for all three *Penicillium* species isolated and tested. Many *P. digitatum* isolates obtained during this study in both seasons were from the decaying fruit on the citrus trees, orchard and packhouse waste fruit. Almost all isolates of *P. digitatum* displayed a high resistance frequency towards thiabendazole; however a high sensitivity was seen for imazalil. The fact that thiabendazole resistance is very high in the orchards could be contributed to the earlier use of benzimidazoles (same fungicide group as thiabendazole) in Citrus Black Spot spray programs. Some isolates did also show resistance towards guazatine. *Penicillium italicum* was isolated at a very low frequency during both seasons, although imazalil and guazatine resistance was detected in the packhouse and thiabendazole resistance from the orchard trees and orchard waste.

Thiabendazole resistance is therefore a factor of concern in the orchards with both well know citrus pathogenic *Penicillium* species. This poses a problem if fruit is treated with thiabendazole in the packhouse, since fruit could enter the packhouse with resistant conidia that are disseminated further in the packhouse and the resistant population could increase. The low isolation frequency of *P. italicum*, which tended to increase over time in the packhouses in other packhouses, could prove that proper sanitation practices prevents the drastic increase of *P. italicum* inoculum in the packhouse. This packhouse was rated the most sanitary based on observations and with questionnaires during this study.

*Penicillium crustosum* was also isolated, but at a higher frequency than *P. italicum*. Most isolates were also obtained from the aerial environments sampled. This species is therefore a common inhabitant of packhouse air, especially around the waste fruit areas. A number of isolates displayed a high resistance frequency toward imazalil and guazatine, with a high sensitivity to thiabendazole overall.

#### Sunland packhouse

Isolates obtained at this packhouse and the areas of isolation are specified in tables A12 – A14 in Appendix A.

There was no early season sampling in 2010 at this packhouse.

- Sampling early season 2011:

In 2011, *Penicillium digitatum* (12 isolates) and *P. crustosum* (14 isolate) were isolated from the packhouse. The resistance frequency of *P. digitatum* was 25% imazalil, 8.3% thiabendazole and 41.7% guazatine. The resistance frequency of *P. crustosum* was 100% for imazalil, 0% thiabendazole and 100% guazatine during this trial.

- Sampling late season 2010:

*Penicillium digitatum*, *Penicillium italicum* and *P. crustosum* (2 isolates) were isolated from the packhouse. The resistance frequency of *P. digitatum* isolates was 38.1% imazalil, 33.3% thiabendazole and 38.1% guazatine resistance. The resistance frequency of *P. italicum* isolates was 33.3% imazalil, 76.4% thiabendazole and 52.9% guazatine resistance. The resistance frequency of *P. crustosum* isolates was 100% for imazalil and guazatine, however no resistance was found towards thiabendazole.

- Sampling late season 2011:

*Penicillium digitatum* (51 isolates), *P. italicum* (40 isolate) and *P. crustosum* (5 isolate) were isolated from the packhouse. The resistance frequency of *P. digitatum* was 15.7% imazalil, 58.8% thiabendazole and 47.1% guazatine resistance. The resistance frequency for *P. italicum* was 2.5% imazalil, 77.5% thiabendazole and 30% guazatine. The resistance frequency of *P. crustosum* is 80% for imazalil, 60% thiabendazole and 100% guazatine.

Sampling at Sunland packhouse was only performed once during the end of the 2010 citrus season, but repeated during the early and late season in 2011. A level of loss in fungicide sensitivity was found for all three *Penicillium* species isolated. Various isolates displayed limited sensitivity towards all three fungicides tested. *P. digitatum* overall displayed a relatively low resistance frequency towards imazalil. It was evident that when an isolate displayed fungicide resistance in this species, it was often resistant to all three fungicides tested at the same time. These highly-resistant isolates should be eradicated to ensure the continued use of the currently available fungicides.

*Penicillium italicum* was not detected early in the season from this packhouse, but a drastic increase in isolate numbers was detected later in the season. This shown the limited potential for this species to survive in the absence of the host, and the potential that it has to increase exponentially in level of inoculum in the packhouse as the season progresses. The inoculum could therefore be brought into the packhouse during the season and care should be taken with sanitation practices to minimise this. Regular sanitation of all surfaces and the immediate removal of decaying fruit waste should be strictly enforced. The resistance frequency was quite low in the presence of imazalil; however resistant isolates were detected from the packhouse air and waste bins inside the packhouse. Thiabendazole resistant isolates were detected from waste bins inside the packhouse and decaying fruit on orchard trees. Guazatine resistant isolates were generally found from the degreening room, packhouse air and waste bins inside the packhouse. Resistance is therefore already in the orchard possibly due to the previous use of related fungicides in orchard spray programmes, or by the practice of disposing packhouse waste fruit in orchards, which should not be advised.

*Penicillium crustosum* was isolated at a low frequency; both isolates tested displayed a high resistance frequency toward imazalil and guazatine as was found at all the other packhouses tested. Most of these isolates were obtained from aerial environments in and around the packhouse.

#### General findings from all four packhouses surveyed:

According to literature, *Penicillium* spp. dominance may fluctuate slightly with different seasonal stages and inoculum load build-up due to postharvest treatments in the packhouse (Rosenberger *et al.*, 1991; Medrela-Kuder, 2003). During our study, it was evident that the dominance of *P. digitatum* was still present from early to late seasonal sampling. However, the percentage dominance dropped significantly during the late season sampling. *Penicillium italicum* levels are extremely low at the start of the season, but the inoculum builds up exponentially and this species becomes more of a decay risk as the season progresses. This could be due to the fact that this species does not survive effectively in the absence of a citrus host in the packhouse. As fruit enter the packhouse with conidia from the orchard or harvesting equipment, the inoculum builds up in the packhouse as the season progresses. More *P. italicum* related disease symptoms might be experienced later in the season as physiologically mature fruit move through the system.

*Penicillium crustosum* is a well-documented pathogen of pome and selected other fruit crops (Prusky and Ben-Arie, 1985). The presence of this species was clearly noted from all citrus packhouses during the current survey but at a much lower frequency than *P. italicum* or *P. digitatum*. The presence thereof in the South African citrus industry has been noted in a previous study (Anonymous, 2010). This species' occurrence was quite low, but its role in packhouse fruit decay should not be underestimated since this species has proven to be resistant to the fungicides imazalil and guazatine in almost all packhouses during early and late season sampling. Most isolates tested were highly resistant to both imazalil and guazatine at the same time. Similar results were obtained by Prusky and Ben-Arie (1985) when *P. crustosum* on pome fruit was proven to be resistant to the fungicide imazalil. The only promising fungicide which has been able to reduce or inhibit the development of *P. crustosum* colonies during the fungicide sensitivity trials was thiabendazole. *Penicillium crustosum* strains that have been identified as resistant to thiabendazole, imazalil and guazatine were detected simultaneously at low frequency. The role of this species in the citrus packhouse has now been determined during this study. *Penicillium crustosum* is a common inhabitant or contaminant of aerial environments in the packhouse. A limited number of isolates were obtained from the packlines and waste bins inside the packhouse as well. The presence of this species in the citrus industry can be considered low and does not pose a current threat to the industry. The isolation frequency of this species and the resistance potential thereof should, however, be monitored to determine if a resistant population is not increasing over time.

*Penicillium* isolates were commonly isolated from decaying fruit hanging from orchard trees in this study. With the analysis of these isolates, it was found that the greater majority of these isolates belong to the *P. digitatum* species. A few isolates were identified as *P. italicum* and none as *P. crustosum*; however *P. crustosum* was isolated from the orchard air. Results from previous studies have shown that *P. digitatum* and *P. italicum* strains resistant to imazalil or thiabendazole are rare to absent from orchards since fruit are normally not exposed to these fungicides in the orchards. Natural populations are encountered here; however, in citrus packinghouses, the selection for resistance could be extremely high and fungicide resistant strains are readily encountered (Holmes and Eckert, 1999; Kinay *et al.*, 2007; Boubaker *et al.*, 2009). A factor of concern during the current study is that various *P. digitatum* and *P. italicum* isolates obtained from orchards were highly resistant to thiabendazole with a few isolates also resistant to imazalil and guazatine. This was especially the case at the Marble Hall and Nelspruit packhouse orchards where many *P. digitatum* isolates were not sensitive to thiabendazole at all. This indicates that resistant strains are probably brought in from the orchard and if thiabendazole and other fungicides to which these strains are resistant are used in the packhouse as a postharvest treatment, resistant inoculum could build up in the packhouse. This could increase the risk for decay development throughout the season. Favourable environmental conditions in packinghouses and the abundant sporulation and easy attachment of *Penicillium* conidia, coupled with the fact that fungicides are used as a common daily practice, are factors contributing to the rapid development of fungicide resistant populations (Gardner *et al.*, 1986; Boubaker *et al.*, 2009). Improved orchard and packhouse sanitation and use of fungicides in the packhouse should be evaluated closely to limit the further development of resistant strains in the packhouse.

With the high *Penicillium* spp. isolation frequency detected during this study, and the exponential increase of *P. italicum* numbers as the season progresses, it is evident that improved packhouse sanitation could be vital in an attempt to reduce the risk of postharvest disease development. Improved packhouse sanitation has repeatedly been shown to reduce the inoculum load with a direct correlation to disease development (Gardner *et al.*, 1986; Scholberg, 2004). Orchard waste and specifically packhouse waste should be very regularly disposed of to ensure that the resistant strains are not constantly circulated throughout the packhouse. At the Marikana packhouse for instance, decaying fruit that was left on the packhouse floor for hours at the time of sampling were all highly-resistant to all three fungicides tested. This serves as a source of resistant inoculum in the packhouse and decaying fruit should be removed from the packhouse immediately as far as possible. Waste bins (wooden and plastic) used in, or in close proximity to the packhouse should be cleaned and sanitised regularly since it was proven from this and previous studies that these bins serve as the reservoir for resistant inoculum, which is transported between the packhouse and the orchard (Scholberg, 2004). Wooden or plastic bins should be steam cleaned for approximately 5-10 sec, or sodium hypochlorite sanitizing can be used; the latter was found to be more effective on plastic than wooden bins (Scholberg, 2004). Care should be taken to reduce the presence of resistant strains to ensure the continued use of currently applied fungicides in the packhouse by possibly improving packhouse hygiene and sanitation. This will involve the informed use of the fungicides at optimal levels and enhanced packinghouse management strategies of fungicide waste management and the fungicide application processes practices along with delicate handling of fruit to prevent wounding and ultimately decay.

## Conclusion

From the current study, it was deduced that three postharvest pathogenic *Penicillium* species were isolated from citrus decaying fruit. *Penicillium digitatum* and to a lesser extent *P. italicum* was dominantly isolated at all packhouses. Resistance to all three fungicides were detected in different areas of the packhouse and orchard. The inoculum within the packhouse could contribute to long term resistance development, which will affect the future use of these fungicides in the packhouse. Good hygiene practices could inhibit the multiplication and spread of resistant strains to a manageable level.

Improved packhouse strategies and practices that reduce the pathogen inoculum at several critical locations in the packhouse have sustainability and economically important implications for the citrus industry (Gardner *et al.*, 1986). The words of Gardner and co-workers (1986) have captured the essence of packhouse hygiene and sanitation in relation to fungicide resistance development as follows: "By restricting the movement of *Penicillium* spores, the development of a fungicide-resistance problem is delayed and the useful life of thiabendazole and other fungicides can be extended". By merely reducing the *Penicillium* inoculum build-up and circulation in the packhouse and between the packhouse and orchards, the risk of resistant strain establishment is significantly reduced. Thiabendazole, imazalil and guazatine resistant strains have been detected from various areas in and around the packhouse. In this study, we have observed critical areas in the packhouse management system that could be improved such as the waste bin sanitation and discarding rates of decaying fruit in the packhouse since highly resistant isolates have been isolated from these areas. Great care should be taken to reduce the impact that thiabendazole and other fungicide resistance from the orchard. Waste bin sanitation and the removal of decaying fruit as part of the orchard management system therefore become essential. An integrated approach to packhouse and orchard fungicide application and

management could significantly contribute to the inhibition of resistant strain development and spread in the South African citrus industry. Sanitation systems includes factors such as careful handling of fruit to reduce wounding; daily sanitation and decontamination of packhouse and fruit handling equipment and environments and; weekly assaying of *Penicillium* inoculum to be managed by the packhouse managers proactively throughout the season (Gardner *et al.*, 1986).

### Technology transfer

This work is written up as progress reports. At least one peer reviewed publication will result from this work.

### Further research

Routine packhouse inoculum screening methodologies should be evaluated for weekly analysis by packhouse managers to implement proactive packhouse sanitation strategies when required.

### References cited

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**APPENDIX A:** Isolates obtained in specific areas of packhouses during early and/or late season sampling in 2010 and 2011.

**Table A1.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marikana (Early season sampling 2010).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard tree	0	30	0	30	3	27
Orchard air	0	4	1	3	2	2
Orchard waste (waste fruit)	1	35	0	36	3	33
Decaying fruit in packhouse	11	0	11	0	11	0
Waste area air	0	11	0	11	0	11
Waste bins	0	10	0	10	1	9
Floor around waste bins	0	12	0	12	0	12
<b>TOTAL ISOLATES</b>	<b>12</b>	<b>102</b>	<b>12</b>	<b>102</b>	<b>20</b>	<b>94</b>
<b><i>PENICILLIUM ITALICUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Orchard trees	0	1	0	1	0	1
Waste bin air	0	3	0	3	2	1
Waste bins	0	5	0	5	2	3
Floor around waste bins	0	3	0	3	3	0
Packhouse air	0	1	0	1	0	1
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>13</b>	<b>0</b>	<b>13</b>	<b>7</b>	<b>6</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Waste area air	3	0	3	0	3	0
Packhouse air	1	0	1	0	1	0
<b>TOTAL ISOLATES</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A2.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marikana (Early season sampling 2011).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Packhouse rotten fruit	0	8	0	8	0	8
Waste bins	0	1	0	1	0	1
Floor around waste bins	0	6	0	6	0	6
Packhouse floors	0	1	0	1	0	1
Packline air	1	0	1	0	1	0
Packhouse waste	0	3	0	3	0	3
Orchard trees	0	1	0	1	0	1
Orchard waste	0	1	0	1	0	1
<b>TOTAL ISOLATES</b>	<b>1</b>	<b>21</b>	<b>1</b>	<b>21</b>	<b>1</b>	<b>21</b>
<b><i>PENICILLIUM ITALICUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Orchard air	0	1	0	1	1	0
Orchard tree	0	1	0	1	1	0
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>0</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Packhouse air	1	0	1	0	1	0
Packline air	1	0	1	0	1	0
Waste area air	1	0	1	0	1	0
<b>TOTAL ISOLATES</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A3.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marikana (Late season sampling 2010).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard tree	0	1	0	1	1	0
Orchard waste (decaying fruit)	0	10	0	10	0	10
Decaying fruit in packhouse	0	6	0	6	0	6
Waste area air	5	2	5	2	5	2
Floor around waste bins	0	3	0	3	0	3
Packhouse walls	1	0	1	0	1	0
Packhouse floors	0	1	0	1	0	1
<b>TOTAL ISOLATES</b>	<b>6</b>	<b>23</b>	<b>6</b>	<b>23</b>	<b>7</b>	<b>22</b>
<b><i>PENICILLIUM ITALICUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Orchard trees	0	2	0	2	2	0
Orchard waste (decaying fruit)	0	4	0	4	3	1
Waste area air	0	12	0	12	11	1
Waste bins	0	1	0	1	1	0
Decaying fruit in packhouse	0	1	0	1	1	0
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>20</b>	<b>0</b>	<b>20</b>	<b>18</b>	<b>2</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Waste area air	0	1	0	1	0	1
Decaying fruit in packhouse	1	0	1	0	1	0
Floor around waste bins	2	0	0	2	2	0
<b>TOTAL ISOLATES</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>1</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested.

**Table A4.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marikana (Late season sampling 2011).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard air	0	1	0	1	0	1
Orchard waste	1	15	0	16	1	15
Packhouse air	1	1	1	1	1	1
Packhouse rotten fruit	0	3	0	3	0	3
Waste area air	1	10	1	10	1	10
Waste bins	0	1	0	1	1	0
Floor around waste bins	1	0	1	0	1	0
<b>TOTAL ISOLATES</b>	<b>4</b>	<b>31</b>	<b>3</b>	<b>32</b>	<b>5</b>	<b>30</b>
<b><i>PENICILLIUM ITALICUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Orchard air	0	1	1	0	1	0
Packhouse air	0	1	0	1	1	0
Packhouse floor	0	1	0	1	1	0
Waste area air	0	9	0	9	5	4
Floor around waste area	0	7	1	6	4	3
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>19</b>	<b>2</b>	<b>17</b>	<b>12</b>	<b>7</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Packhouse air	2	0	0	2	2	0
Packhouse floor	1	0	1	0	1	0
Packhouse rotten fruit	1	0	0	1	1	0
Floor area around waste bins	4	0	4	0	4	0
Waste area air	2	0	2	0	2	0
<b>TOTAL ISOLATES</b>	<b>10</b>	<b>0</b>	<b>7</b>	<b>3</b>	<b>10</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A5.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Nelspruit (Early season sampling 2010).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard tree	0	10	0	10	1	9
Waste bins	0	6	0	6	0	6
TOTAL ISOLATES	<b>0</b>	<b>16</b>	<b>0</b>	<b>16</b>	<b>1</b>	<b>15</b>
<b><i>PENICILLIUM ITALICUM</i></b>						
Orchard trees	1	2	2	1	2	1
Waste bin air	0	3	2	1	3	0
TOTAL ISOLATES	<b>1</b>	<b>5</b>	<b>4</b>	<b>2</b>	<b>5</b>	<b>1</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>						
Packhouse air	6	0	0	6	6	0
TOTAL ISOLATES	<b>6</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>6</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A6.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Nelspruit (Early season sampling 2011).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard waste	0	3	0	3	0	3
Packhouse air	2	2	0	4	0	4
Packline air	1	1	0	2	2	0
Packhouse waste	1	0	0	1	1	0
Orchard air	0	1	0	1	0	1
Orchard fruit	0	3	0	3	0	3
TOTAL ISOLATES	<b>4</b>	<b>10</b>	<b>0</b>	<b>14</b>	<b>3</b>	<b>11</b>
<b><i>PENICILLIUM ITALICUM</i></b>						
Packhouse air	1	0	0	1	1	0
TOTAL ISOLATES						

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A7.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Nelspruit (Late season sampling 2010).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard tree	0	18	0	18	0	18
Waste area bins	0	1	0	1	0	1
Packhouse floors	0	1	0	1	0	1
TOTAL ISOLATES	<b>0</b>	<b>20</b>	<b>0</b>	<b>20</b>	<b>0</b>	<b>20</b>
<b><i>PENICILLIUM ITALICUM</i></b>						
Orchard trees	0	9	9	0	1	8
Waste bins	0	2	2	0	2	0
Packhouse prewash & waxing	1	0	1	0	1	0
TOTAL ISOLATES	<b>1</b>	<b>11</b>	<b>12</b>	<b>0</b>	<b>4</b>	<b>8</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A8.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Nelspruit (Late season sampling 2011).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard waste	0	22	5	17	0	22
Packhouse air	0	3	2	1	2	1
Packline air	0	1	1	0	1	0

Packhouse rotten fruit	0	1	1	0	1	0
Waste area air	0	4	1	3	0	4
Waste bins	0	14	7	7	1	13
Packhouse floor	0	1	0	1	0	1
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>46</b>	<b>17</b>	<b>29</b>	<b>5</b>	<b>41</b>
<b><i>PENICILLIUM ITALICUM</i></b>						
Orchard air	0	5	0	5	2	3
Orchard waste	0	8	1	7	2	6
Packhouse air	0	9	8	1	8	1
Packline air	1	3	2	2	3	1
Waste area air	0	9	9	0	4	5
Waste bins	0	1	1	0	1	0
<b>TOTAL ISOLATES</b>	<b>1</b>	<b>35</b>	<b>21</b>	<b>15</b>	<b>20</b>	<b>16</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>						
Packline air	1	0	0	1	1	0
Waste area air	1	1	1	1	2	0
<b>TOTAL ISOLATES</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A9.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marble Hall (Mid-season sampling 2010).

<b>ISOLATE ORIGIN</b>	<b>IMAZALIL *</b>		<b>THIABENDAZOLE *</b>		<b>GUAZATINE *</b>	
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard trees	0	17	16	1	4	13
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>17</b>	<b>16</b>	<b>1</b>	<b>4</b>	<b>13</b>
<b><i>PENICILLIUM ITALICUM</i></b>						
Packhouse floor	1	0	0	1	1	0
Orchard trees	0	2	2	0	0	2
<b>TOTAL ISOLATES</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>2</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>						
Packline	1	0	0	1	1	0
Orchard air	1	0	0	1	1	0
Waste areas	1	0	0	1	1	0
<b>TOTAL ISOLATES</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>3</b>	<b>3</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A10.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marble-Hall (Early season sampling 2011).

<b>ISOLATE ORIGIN</b>	<b>IMAZALIL *</b>		<b>THIABENDAZOLE *</b>		<b>GUAZATINE *</b>	
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard tree	0	8	7	1	0	8
Packhouse rotten fruit	0	2	1	1	1	1
Packhouse floor	0	3	1	2	0	3
Packline	0	1	1	0	0	1
Packline air	0	1	1	0	0	1
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>15</b>	<b>11</b>	<b>4</b>	<b>1</b>	<b>14</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>						
Packhouse air	0	1	1	0	0	1
<b>TOTAL ISOLATES</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A11.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Marble Hall (Late sampling 2011).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard waste	0	25	20	5	0	25
Packhouse floor	0	1	0	1	0	1
Packhouse waste	0	2	2	0	1	1
Packhouse rotten fruit	14	10	22	2	15	9
TOTAL ISOLATES	<b>14</b>	<b>38</b>	<b>44</b>	<b>8</b>	<b>16</b>	<b>36</b>
<b><i>PENICILLIUM ITALICUM</i></b>	R	S	R	S	R	S
Orchard waste	0	1	1	0	0	1
TOTAL ISOLATES	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	R	S	R	S	R	S
Packhouse air	0	1	0	1	1	0
Packhouse floor	2	0	2	0	2	0
Packline air	2	0	0	2	2	0
Packhouse waste	1	0	0	1	1	0
Packhouse rotten fruit	2	0	2	0	2	0
Waste area air	2	0	0	2	2	0
TOTAL ISOLATES	<b>9</b>	<b>1</b>	<b>4</b>	<b>6</b>	<b>10</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A12.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Sunland (Late season sampling 2010).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Degreening room air	3	5	2	6	3	5
Packhouse air	1	0	1	0	1	0
Packhouse walls	0	3	0	3	0	3
Waste bins inside	3	1	3	1	3	1
Orchard trees	1	4	1	4	1	4
TOTAL ISOLATES	<b>8</b>	<b>13</b>	<b>7</b>	<b>14</b>	<b>8</b>	<b>13</b>
<b><i>PENICILLIUM ITALICUM</i></b>	R	S	R	S	R	S
Degreening room air	0	1	0	1	1	0
Packline	0	1	0	1	0	1
Packhouse air	1	3	2	2	2	2
Waste bins inside	6	3	8	1	6	3
Orchard trees	0	2	2	0	0	2
TOTAL ISOLATES	<b>7</b>	<b>10</b>	<b>12</b>	<b>5</b>	<b>9</b>	<b>8</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	R	S	R	S	R	S
Packline	1	0	0	1	1	0
Packhouse air	1	0	0	1	1	0
TOTAL ISOLATES	<b>2</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested

**Table A13.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Sunland (Early season sampling 2011).

ISOLATE ORIGIN	IMAZALIL *		THIABENDAZOLE *		GUAZATINE *	
	R	S	R	S	R	S
<b><i>PENICILLIUM DIGITATUM</i></b>						
Orchard trees	0	6	1	5	2	4
Waste bins inside	0	3	0	3	0	3
Degreening room air	1	0	0	1	1	0
Packhouse air	2	0	0	2	2	0
TOTAL ISOLATES	<b>3</b>	<b>9</b>	<b>1</b>	<b>11</b>	<b>5</b>	<b>7</b>

<b><i>PENICILLIUM CRUSTOSUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Degreening room walls	2	0	0	2	2	0
Waste bins inside	2	0	0	2	2	0
<b>TOTAL ISOLATES</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>4</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested.

**Table A14.** Fungicide sensitivity evaluation of three *Penicillium* species isolated from selected areas in and around the packhouse in Sunland (Late season sampling 2011).

<b>ISOLATE ORIGIN</b>	<b>IMAZALIL *</b>		<b>THIABENDAZOLE *</b>		<b>GUAZATINE *</b>	
<b><i>PENICILLIUM DIGITATUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Cold room air	0	2	2	0	2	0
Degreening room air	0	5	4	1	1	4
Orchard trees	0	1	0	1	0	1
Packhouse air	0	4	2	2	1	3
Packline	1	0	1	0	1	0
Waste area air	3	16	9	10	8	11
Waste bins	0	1	1	0	0	1
Waste bins inside	3	14	10	7	10	7
Packhouse floors	1	0	1	0	1	0
<b>TOTAL ISOLATES</b>	<b>8</b>	<b>43</b>	<b>30</b>	<b>21</b>	<b>24</b>	<b>27</b>
<b><i>PENICILLIUM ITALICUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Degreening room air	0	1	1	0	1	0
Orchard air	1	7	6	2	3	5
Orchard trees	0	11	7	4	3	8
Packhouse air	0	2	2	0	0	2
Waste area air	0	12	12	0	3	9
Waste bins inside	0	6	3	3	2	4
<b>TOTAL ISOLATES</b>	<b>1</b>	<b>39</b>	<b>31</b>	<b>9</b>	<b>12</b>	<b>28</b>
<b><i>PENICILLIUM CRUSTOSUM</i></b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>	<b>R</b>	<b>S</b>
Cold room air	1	0	1	0	1	0
Packhouse air	0	0	1	0	0	0
Waste area air	1	1	0	2	2	0
Packline air	1	0	1	0	1	0
<b>TOTAL ISOLATES</b>	<b>3</b>	<b>1</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>0</b>

\* Isolate numbers sensitive (S) or resistant (R) to the fungicide tested.

#### 4.5.4 PROGRESS REPORT: Use of potassium silicate and biocontrol agents to reduce postharvest disease and chilling injury in citrus fruit

Experiment number UKZN1 (2010/10-2011/4) by Mark Laing and Nicolette du Rand (UKZN)

#### Opsomming

Die doel van die eksperiment was om die integrasie van kalium silikaat bemesting, warm water behandeling en biologiese beheeragente vir bekamping van na-oes siektes sowel as die effek op koue skade te bestudeer. Doelwitte was om (1) die optimale temperature en tyd kombinasies vir die verskei sitrus kultivars in die warm bad te bepaal, (2) warm water behandeling gekombineer word met 'n gis biobeheer agent te optimaliseer, en (3) die buffer gevolge van die voor-oes toedienings van kalium silikaat bemesting op die voorkoming van koue skade in suurlemoene te bestudeer. Gedurende die afgelope seisoen, is warm water behandelings op vier verkillende soorte sitrus tipes (suurlemone, nawels, Valencias en Minneolas) uitgevoer. Die optimale temperature en tyd kombinasies vir die warm water behandeling van suurlemoene en Minneolas was 53°C vir 90s. Die optimale tyd en temperatuur vir warm water behandeling van nawels was onduidelik en sal herhaal moet word. Warm water tyd en temperatuur kombinasies vir die behandelings vir Valencias moet nog bepaal word. Die eerste koue stoor proewe is in Januarie 2012 gedoen. Die resultate wat verkry is, dui daarop dat die suurlemoene van silikon behandelde bome minder koue skade opgedoen het as vrugte van die bome wat nie met silikon behandel was nie. Koue proewe sal in Mei 2012 herhaal word om meer inligting te verkry.

## Summary

The aim of this experiment was to study the integration of potassium silicate fertilisation, hot water treatment and biocontrol agents for postharvest disease and chilling injury control. Objectives were to (1) determine the optimal treatment temperatures and duration for hot water baths for various citrus cultivars, (2) optimise the preventative effects of the yeast biocontrol agent B13, combined with the best curative hot water treatment, and (3) evaluation of the buffering effects of pre-harvest applications of potassium silicate to citrus trees on the chilling injuries suffered by lemons. During the past season, hot water treatments were conducted on four different types of citrus (lemons, navels, Valencias and minneolas). The optimal temperatures and time combinations for the hot water treatment of lemons and minneolas have been found to be at 53°C for 90s. The optimal time and temperature for the hot water treatment of navels was found to be unclear and this trial may need to be repeated. Hot water treatments for the Valencias still need to be determined. The first cold storage trials have been conducted in January 2012. The results that were obtained indicate that the fruit of silicon treated trees does have lower rates of chilling injury than fruit from trees that have not been treated with silicon. Cold trials are to be repeated in May 2012 to obtain more data.

### 4.5.5 PROGRESS REPORT: Practical impact of fungicide resistance on control of postharvest citrus green and blue mould

Experiment 1034 (Junie 2011 - March 2012) by Arno Erasmus (CRI-Nelspruit) and Paul Fourie (CRI-USPP)

## Opsomming

Nege isolate van *Penicillium digitatum* (PD; groenskimmel) en 5 van *P. italicum* (PI; blouskimmel) met variërende vlakke van imazalil (IMZ) weerstandbiedende is uitgesoek om *in vivo* proewe mee uit te voer. 'n Reeks van IMZ residu vlakke is op sitrus vrugte gelaai waarna dit met PD en PI geïnfecteer is om so IMZ residu-drempelwaardes vir groen- en blouskimmelbeheer te bepaal. Vrugte was gedoop in oplossings van 5, 10, 20, 40, 80, 160, 320, 640, 1280 of 2560  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ. Data verwerking om die drempelwaardes te bepaal is onderweg. Sensitiewe PD isolate kon goed (> 90%) beheer word met behandelings in so laag as 160  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ. Sommige weerstandbiedende PD isolate kon beheer word, maar slegs in die 2560  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ oplossing, terwyl ander nie deur hierdie hoë konsentrasie beheer kon word nie. Al die PI isolate was gesien as sensitief omdat >90% beheer van almal bereik kon word in behandelings van 160 of 320  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ. Imazalil EC50 en EC95 bepaling is huidiglik aan die gang en geregistreerde IMZ alternatiewe swammiddels gaan geëvalueer word vir beheer van die onderskeie isolate. Bevindinge uit hierdie projek kan gebruik word vir die verbetering van siektebestuur in pakhuisse en ook om die minimum residu beperking vir siekte beheer beter te verstaan teenoor die maksimum residu beperking (MRL).

## Summary

Nine isolates of *Penicillium digitatum* (PD; green mould) and 5 of *P. italicum* (PI; blue mould) with various levels of imazalil (IMZ) resistance were selected to conduct *in vivo* trials. A series of IMZ residue levels were loaded on citrus fruit which was subsequently inoculated with the PD and PI isolates to determine the IMZ residue benchmark levels for green and blue mould control. Fruit were dipped in solution of 5, 10, 20, 40, 80, 160, 320, 640, 1280 or 2560  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ. Data analyses for the benchmark values are in progress. Sensitive PD isolates could be controlled well (> 90%) in treatments of as low as 160  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ. Some resistant PD isolates could be controlled, but only with the 2560  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ, others could not be control using this high concentration. All PI isolates were regarded as sensitive, due to the fact that >90% control could be reached with all in treatments of 160 or 320  $\mu\text{g}\cdot\text{mL}^{-1}$  IMZ. Determination of IMZ EC50 and EC95 values is in process, while registered IMZ alternative fungicides will be evaluated for control of the various isolates. Findings from this trial can be used to improve disease management in packhouses and also to understand the minimum residue limit for disease management relative to the maximum residue limit (MRL).

### 4.5.6 PROGRESS REPORT: Provision of an industry service whereby new packhouse treatments are comparatively evaluated, fungicide resistance is monitored and standardised recommendations are provided

Experiment 123 (Ongoing) by Arno Erasmus and Paul H Fourie (CRI)

## Opsomming

Verskeie loodsproewe is in hierdie eksperiment hanteer. As gevolg van die tydelike uitsluiting van produkte met kwaternêre ammonium verbindings op die CRI se aanbevelings, in afwagting vir 'n EU MRV (maksimum residu vlak), is alternatiewe produkte, naamlik Kannar OrganoKare, Oxypure, Oxyclave and Foodprint, ondersoek. Geen van die produkte was geskik vir die effektiewe en veilige gebruik in sitrus pakhuisse.

Propolis is vir die beheer teen die hoof naoes patogene op sitrus geëvalueer, maar was gevind om nie effektief te wees nie.

## Summary

Various pilot trials were conducted in this experiment. Due to the temporary exclusion of quaternary ammonium compound products from the CRI recommendation in anticipation of an EU MRL, alternatives, namely Kannar OrganoKare, Oxypure, Oxyclave and Foodprint were investigated. None that would be suitable for effective and safe use in citrus packhouses were found. The compound propolis was evaluated for control against the major postharvest diseases on citrus and was found to be not effective

## Pilot trials conducted

Five different pilot trials were conducted during the season of 2011.

1. The evaluation of Kannar OrganoKare in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease
2. The evaluation of Oxypure in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease.
3. The evaluation of Oxyclave in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease.
4. The evaluation of Foodprint in a citrus packhouse dump tank washing system as sanitising agents against postharvest disease
5. Biobalsam
  - a. The *in vitro* screening of the compound Biobalsam against *Penicillium digitatum*, *P. italicum* and *Geotrichum citri-aurantii* postharvest disease causing pathogens on citrus fruit and the *in vivo* testing of Biobalsam as fungicide to control green mould
  - b. The *in vivo* screening of the fungicidal ability of the compound Biobalsam to control citrus green mould
  - c. The evaluation of Biobalsam in a citrus packhouse dumptank washing system as sanitising agents against post-harvest disease.
  - d. The *in vivo* screening for the ability of the compound Biobalsam to protect citrus fruit against secondary infection from neighbouring infected fruit
  - e. The evaluation of Biobalsam+ in a citrus packhouse dump tank washing system as sanitising agents against *Pencillium digitatum*

## Trial 1

### The evaluation of Kannar OrganoKare in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease

#### Introduction

Kannar OrganoKare UK (Batch no 1706) was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

#### Materials and methods

A spore suspension of *P. digitatum* was made up by suspending spores in sterile deionised water containing the surfactant, Tween 20. The spore suspension was spectrophotometrically adjusted to a concentration of  $1 \times 10^6$  spores/ml. Good, sound, untreated Navel oranges from Crocodile Valley Citrus Co. were obtained in bulk. All the fruit was surface sterilised by washing on the CRI experimental packline in the recirculating spray on brush washing system with a suitable quaternary ammonium compound for 2 minutes. Thereafter the fruit was dried in the packline drying tunnel prior to treatment. The fruit was divided into lots of 5 fruit per treatment.

A clean water dump tank washing system in a packhouse was simulated. The dump tank water was maintained at ambient temperature for this trial. The clean, surface sterilised Valencia oranges were injured by means of a rubber bung with two protruding nails (2 mm long). Each fruit was prick-injured three times, twice equatorially on opposite sides and once at the stylar end of the fruit, giving a total of 15 injury sites per treatment.

The 5 fruit of the untreated control treatment were dipped in water in the clean dump tank (10 L volume) for 3 minutes. The 10 L washing system was then seeded with the  $1 \times 10^6$  spores/mL concentration, giving a final concentration of  $1 \times 10^4$  spores of *P. digitatum*. Untreated, injured fruit dipped in the contaminated water for 3 minutes (untreated, inoculated control). Untreated injured controls were prepared for the treatment of Kannar OrganoKare and Sporekill. The contaminated dump tank was then sanitised with concentrations of 5, 10 and 20 mL/L Kannar OrganoKare. Injured fruit was dipped in each of the “sanitised” systems for 3 minutes. In a similar manner, injured fruit was also exposed to the quaternary ammonium compound Sporekill at concentrations of 1 L and 2 L/1000 L for 3 minutes.

To summarise, treatments involved the following:

1. Untreated control - injured fruit dipped in clean water
2. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL
3. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 5 mL/L Kannar OrganoKare
4. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 10 mL/L Kannar OrganoKare
5. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 20 mL/L Kannar OrganoKare
6. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 1 L/1000 L Sporekill
7. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 2 L/1000 L Sporekill

All the treated fruit were placed in paper packets and incubated for 7-10 days at 23°C. After incubation, the fruit was evaluated for decay and the results were recorded as percentage decay (calculated from the number of infected wounds).

## Results and discussion

Fruit dipped in the Kannar OrganoKare treated dump tank were not protected from green mould infection by any of the concentrations. The quaternary ammonium compound Sporekill at 1 L and 2 L/1000 L inhibited the incidence of green mould decay (Table 4.5.6.1).

**Table 4.5.6.1.** The percentage decay incidence recorded on **Navel oranges** that were injured and dip-treated in a simulated dump tank system with clean water, or water with  $1 \times 10^4$  *P. digitatum* spores that had been treated with different concentrations of Kannar OrganoKare and Sporekill.

Treatments	% Decay
1. Untreated control	13.3
<b>2. Inoculated control</b>	100.0
3. 5 mL/L Kannar OrganoKare	100.0
4. 10 mL/L Kannar OrganoKare	100.0
5. 20 mL/L Kannar OrganoKare	100.0
6. Sporekill 1.0 L / 1000 L	6.7
7. Sporekill 2.0 L / 1000 L	0.0

## Conclusion

**Kannar OrganoKare** was not able to sanitise a simulated citrus packhouse dump tank washing system at any of the treated concentrations. It is not comparable to the quaternary ammonium compound Sporekill in its efficacy to inhibit green mould infection from contaminated water.

## Trial 2

### The evaluation of Oxypure in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease

#### Introduction

Oxypure was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

#### Materials and methods

Similar methodology as for Trial 1 was used. To summarise, treatments involved the following:

1. Untreated control - injured fruit dipped in clean water

2. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL
3. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 2.5 mL/L Oxypure
4. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 5 mL/L Oxypure
5. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 10 mL/L Oxypure
6. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 1 mL/L Sporekill
7. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 2 mL/L Sporekill

## Results and discussion

Fruit dipped in the Oxypure treated dump tank were only moderately protected from green mould infection by the higher concentrations with infection levels of 26.7 and 66.7% for 5% and 10 mL/L, respectively (Table 4.5.6.2). The quaternary ammonium compound Sporekill at 1 and 2 mL/L inhibited the incidence of green mould decay.

**Table 4.5.6.2.** The percentage decay incidence recorded on **Navel oranges** that were injured and dip-treated in a simulated dump tank system with clean water, or water with  $1 \times 10^4$  *P. digitatum* spores that had been treated with different concentrations of Oxypure and Sporekill.

Treatments	% Decay
1. Untreated control	13.3
2. <b>Inoculated control</b>	100.0
3. 2.5 mL/L Oxypure	100.0
4. 5 mL/L Oxypure	26.7
5. 10 mL/L Oxypure	66.7
6. Sporekill 1 mL/L	0.0
7. Sporekill 2 mL/L	6.7

## Conclusion

**Oxypure** was not able to completely sanitise a simulated citrus packhouse dump tank washing system at any of the treated concentrations. Although it shows potential it was not comparable to the quaternary ammonium compound Sporekill in its efficacy to prevent green mould infection from contaminated water.

## Trial 3

### The evaluation of Oxyclave in a citrus packhouse dumptank washing system as a sanitising agent against post-harvest disease

#### Introduction

Oxyclave was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

#### Materials and methods

Similar methodology as for Trial 1 was used. To summarise, treatments involved the following:

1. Untreated control - injured fruit dipped in clean water
2. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL
3. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 20 g/L Oxyclave
4. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 25 g/L Oxyclave
5. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 30 g/L Oxyclave
6. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 1 mL/L Sporekill
7. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 2 mL/L Sporekill

## Results and discussion

Fruit dipped in the Oxyclave treated dump tank were only moderately protected from green mould infection by all the concentrations with infection levels of 33.3, 26.7 and 40.0% for 20, 25 and 30 g/L, respectively (Table 4.5.6.3). The quaternary ammonium compound Sporekill at 1 and 2 mL/L prevented the incidence of green mould decay.

**Table 4.5.6.3.** The percentage decay incidence recorded on **Navel oranges** that were injured and dip-treated in a simulated dump tank system with clean water, or water with  $1 \times 10^4$  *P. digitatum* spores that had been treated with different concentrations of Oxyclave and Sporekill.

Treatments	% Decay
1. Untreated control	13.3
2. Inoculated control	100.0
3. 20 g/L Oxyclave	33.3
4. 25 g/L Oxyclave	26.7
5. 30 g/L Oxyclave	40.0
6. Sporekill 1 mL/L	0.0
7. Sporekill 2 mL/L	6.7

## Conclusion

**Oxyclave** was not able to completely sanitise a simulated citrus packhouse dump tank washing system at any of the treated concentrations. Although it shows potential it was not comparable to the quaternary ammonium compound Sporekill in its efficacy to prevent green mould infection from contaminated water.

## Trial 4

### The evaluation of Foodprint in a citrus packhouse dump tank washing system as sanitising agents against postharvest disease

#### Introduction

Foodprint (active ingredient: glutaraldehyde; 6% m/v) was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared with Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

#### Materials and methods

Similar methodology as for Trial 1 was used. To summarise, treatments involved the following:

1. Untreated control - injured fruit dipped in clean water
2. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup>
3. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 0.5 mL.L<sup>-1</sup> Foodprint
4. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 1.0 mL.L<sup>-1</sup> Foodprint
5. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 2.0 mL.L<sup>-1</sup> Foodprint
6. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 5.0 mL.L<sup>-1</sup> Foodprint
7. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 10.0 mL.L<sup>-1</sup> Foodprint
8. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 20.0 mL.L<sup>-1</sup> Foodprint
9. Untreated control - injured fruit dipped in clean water
10. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup>
11. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 1 mL.L<sup>-1</sup> Sporekill
12. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 2 mL.L<sup>-1</sup> Sporekill

#### Results and discussion

Green mould infection was inhibited on fruit dipped in 10 and 20 mL.L<sup>-1</sup> (i.e. 1 and 2%) Foodprint. At the higher concentration, it was comparable to the Sporekill treatments (Table 4.5.6.4).

**Table 4.5.6.4.** The percentage decay incidence recorded on Valencia oranges that were injured and dip-treated in a simulated dump tank system with clean water, or water with  $1 \times 10^4$  *P. digitatum* spores that had been treated with different concentrations of Foodprint and Sporekill.

Treatments	Infection (%)
1. Untreated control	0.0
<b>2. Inoculated control</b>	86.7
3. 0.5 mL.L <sup>-1</sup> Foodprint	93.3
4. 1.0 mL.L <sup>-1</sup> Foodprint	100.0
5. 2.0 mL.L <sup>-1</sup> Foodprint	100.0
6. 5.0 mL.L <sup>-1</sup> Foodprint	66.7
7. 10.0 mL.L <sup>-1</sup> Foodprint	6.7
8. 20.0 mL.L <sup>-1</sup> Foodprint	0.0
9. Untreated control	0.0
<b>10. Inoculated control</b>	100.0
11. 1.0 mL.L <sup>-1</sup> Sporekill	0.0
12. 2.0 mL.L <sup>-1</sup> Sporekill	0.0

### Conclusion and recommendations

Foodprint at the concentration of 2% showed a similar sanitation action compared to 0.1 and 0.2% Sporekill. It can therefore be investigated further as a possible alternative sanitation agent if the following requirements can be adhered to:

1. Foodprint at the concentration of 2% needs to be economical viable for use in wash tanks with volumes of >1000 L water
2. Foodprint needs to be able to stay active after several tons of fruit went through the dip tank bringing with it dirt and soil
3. A practical and user friendly test kit is required for concentration management at packhouse level (not dip sticks)
4. The effect of Foodprint in terms of phytotoxicity on all the varieties of citrus fruits needs to be assessed
5. The human-safety aspect of Foodprint needs to be assessed and verified
6. Analysis needs to be done for possible residues on the fruit

Foodprint was also evaluated as possible fungicide against *P. digitatum*, but no positive effect was found (results not shown).

These pilot trial results were communicated to the company, with the disclaimer that the pilot results and recommendations from this trial cannot be used in any way for the promotion of Foodprint to be used on citrus. Although conducted according to good laboratory practices, the pilot trial was not adequately replicated and only served as a screening exercise. Should they be able to comply with the requirements mentioned above, the company was invited to contact CRI to conduct further work under contract for the development and assessment of Foodprint as possible alternative sanitation product for use in South African citrus packhouses.

### Trial 5

#### Contractual evaluation of Biobalsam for River Bioscience and Beonics

Biobalsam (active ingredient: propolis) was evaluated under a product development (postharvest sanitiser and/or fungicide) and potential registration contract through River Bioscience.

#### Trial 5a

**The *in vitro* screening of the compound Biobalsam against *Penicillium digitatum*, *P. italicum* and *Geotrichum citri-aurantii* postharvest disease causing pathogens on citrus fruit and the *in vivo* testing of Biobalsam as fungicide to control green mould**

#### Introduction

Two samples containing 2 different formulations of Biobalsam were submitted to Citrus Research International (CRI) for evaluation of efficacy against postharvest infection of citrus. Formulation 1 (BB1) was extracted in 70% ethanol and formulation 2 (BB2) was a water compatible formulation. *In vitro* screening

was done for the fungicidal action of BB1 against *Penicillium digitatum* (PD; green mould), *P. italicum* (PI; blue mould) and *Geotrichum citri-aurantii* (GS; sour rot). The fungicidal action of BB1 and BB2 was screened *in vivo* against the post-harvest citrus pathogen *Penicillium digitatum* (green mould) to determine the curative and protective efficacy of the product in inhibiting infection caused by this pathogen. This screening was done as a pilot trial to determine if Biobalsam demonstrated any fungicidal properties,

## Materials and methods

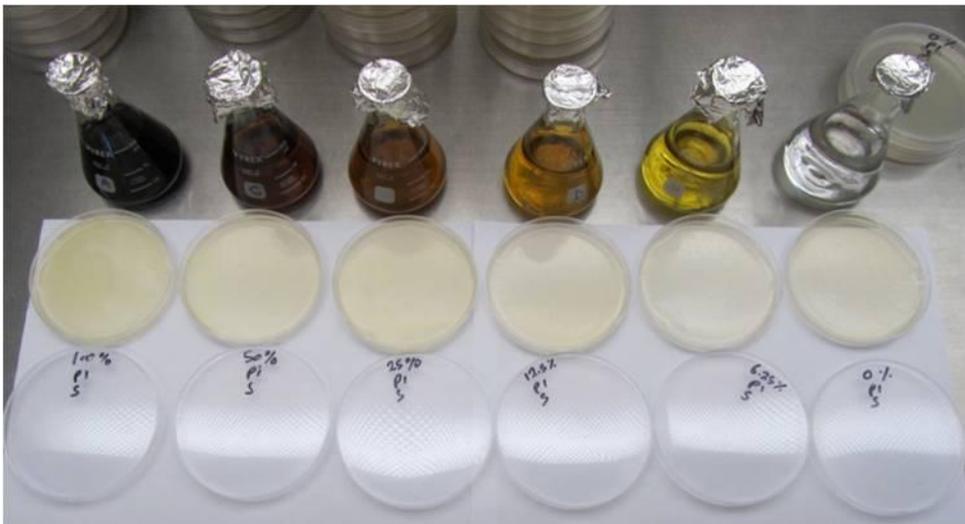
### *In vitro* screening of BB1

#### Method 1

A drop containing 0.1 mL of 100% BB1 was placed on one side of a PDA plate (a petri dish containing PDA (Potato dextrose agar)). The PDA plates were left open for several hours in a laminar flow to allow the ethanol to evaporate. PDA plugs with PD, PI and GS was placed approximately 2 cm from the BB1 drop, incubated at 25°C for 7 days and evaluated for an inhibition zone around the BB1 drop.

#### Method 2a

A volume of 0.1 mL of a specific concentration of BB1 was placed and spread over a PDA plate (Figure 1). A concentration series ranging from 0.00, 6.25, 12.50, 25.00, 50.00 and 100.00% was spread over different plates, after which it was left open in a laminar flow to allow ethanol evaporation. A volume of 0.1 mL spore suspension with a concentration of  $1 \times 10^6$  spores.mL<sup>-1</sup> of PD, PI and GS was spread over the BB1 amended PDA plates, incubated for 7 days at 20°C and evaluated for fungal growth.



**Figure 4.5.6.1.** The concentration series of BB1 spread over a PDA plate.

#### Method 2b

PDA plates were amended with BB1 as described in Method 2a. PDA plugs with PD, PI or GS was placed in the middle of a plate, incubated for 7 days at 20°C and evaluated for fungal growth. The diameter of each fungal colony was measured twice perpendicularly. The average of the two measurements was calculated for each treatment. The percentage inhibition was calculated by subtracting the measurement of a specific treatment from the control, then divided by the control and multiplied by 100.

### *In vivo* screening of BB1 and BB2

Trials were conducted on untreated export quality Valencia oranges from Crocodile Valley, Nelspruit. Treatments are laid out in Table 4.5.6.5. For testing the curative action fruit was inoculated 24 h prior to treatment and for protective action fruit was inoculated 2 to 4 h after treatment.

**Table 4.5.6.5.** Different concentrations of BB1 and BB2 applied curatively and protectively to PD inoculated Valencia oranges.

Product	Concentration	Action
BB1	6.25%	Curative and Protective
BB1	12.50%	Curative and Protective
BB1	25.00%	Curative and Protective
BB1	50.00%	Curative and Protective
BB1	100.00%	Curative and Protective
BB2	0.05%	Curative
BB2	0.50%	Curative
BB2	5.00%	Curative
Ethanol control	70% ethanol	Curative and Protective
Water control	Water	Curative and Protective
Imazalil control	500 µg.mL <sup>-1</sup>	Curative and Protective
Untreated control		Curative and Protective

The fruit was divided into lots of 5 fruit per treatment for the dip treatments. Spore suspensions of PD were made up by suspending the spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were adjusted spectrophotometrically to a concentration of  $1 \times 10^6$  spores.mL<sup>-1</sup>.

The inoculation of the fruit was done by dipping a stainless steel rod with a sharpened tip in the spore suspension prior to wounding the fruit. Four inoculation wounds were induced around the stem end at equal distances from each other. All the treatments, with the relevant chemical being evaluated, were done in a water bath at 20°C. The BB1 concentration solutions were prepared by diluting the product with 70% ethanol and not water. BB2 was diluted with water. All treated fruit were incubated for 4 days at 20°C and then evaluated for infection by means of a Labino UV light. Percentage infected wounds per treatment were calculated from the evaluation data.

## Results and discussion

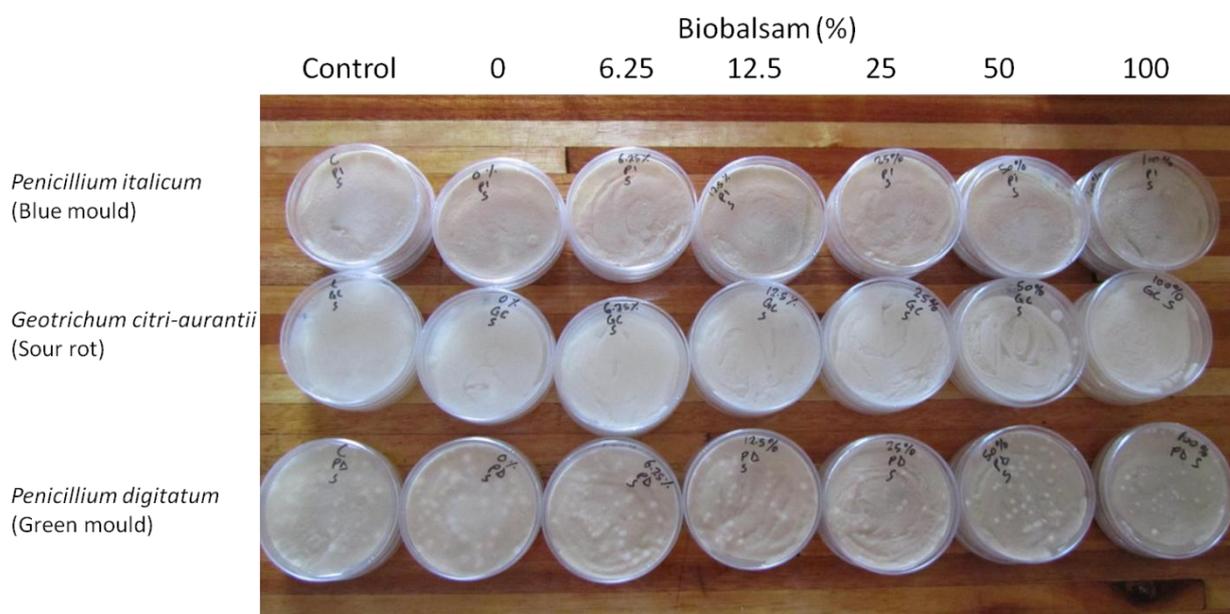
### *In vitro* screening of BB1

#### Method 1

No inhibition zone could be observed for all pathogens screened.

#### Method 2a

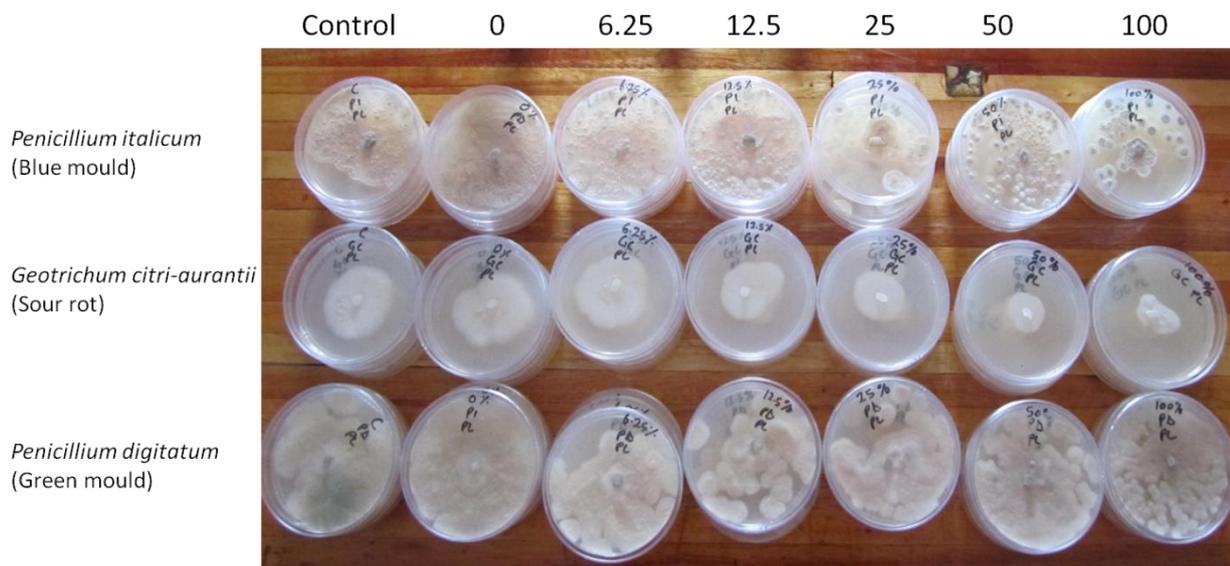
No clear difference was measurable in fungal growth in all treatments (Figure 4.5.6.2).



**Figure 4.5.6.2.** Spore suspensions of PD, PI and GS spread over various concentrations of BB1.

## Method 2b

Inhibition of growth could be clearly observed for GS cultures from 12.50% BB1 with 0.81% inhibition to 100% BB1 showing the highest level of inhibition with 24.80% (Figure 4.5.6.3 and Table 4.5.6.6). The inhibition for PD and PI was not observable or measurable.



**Figure 4.5.6.3.** PDA plugs placed on PDA plates amended with various concentrations of BB1 after 4 day of incubation at 20°C.

**Table 4.5.6.6.** Percentage inhibition of fungal growth from *Geotrichum citri-aurantii* PDA plugs placed on BB1 amended PDA plates.

BB1 concentration (%)	Inhibition (%)
0 (70% ethanol)	0.00
6.25	0.00
12.50	0.81
25.00	4.88
50.00	10.00
100.00	24.80
Control	0.00

## In vivo screening of BB1 and BB2

The results in Table 4.5.6.6 indicate that BB1 and BB2 did not sufficiently inhibit infections by the pathogen *P. digitatum* at any of the concentrations compared to the standard recommended imazalil. This demonstrates that Biobalsam did not exhibit strong fungicidal properties.

**Table 4.5.6.7.** Percentage green mould infection on Valencia oranges that were wounded and inoculated with *P. digitatum* 24 hours before or 2 – 4 hours after dip-treatment in Biobalsam and Fungazil 750 WG.

Product	Concentration	Action	Infection (%)
BB1	6.25%	Curative	20.0
BB1	12.50%	Curative	30.0
BB1	25.00%	Curative	25.0
BB1	50.00%	Curative	10.0
BB1	100.00%	Curative	30.0
Ethanol control	70% ethanol	Curative	35.0
BB2	0.05%	Curative	100.0
BB2	0.50%	Curative	95.0
BB2	5.00%	Curative	80.0
Water control	Water	Curative	95.0
Untreated control		Curative	85.0
Imazalil control	500 µg.mL <sup>-1</sup>	Curative	0.0
BB1	6.25%	Protective	85.0

BB1	12.50%	Protective	90.0
BB1	25.00%	Protective	60.0
BB1	50.00%	Protective	85.0
BB1	100.00%	Protective	100.0
Ethanol control	70% ethanol	Protective	85.0
Water control	Water	Protective	60.0
Imazalil control	500 µg.mL <sup>-1</sup>	Protective	35.0

## Conclusion

BB1 showed some *in vitro* inhibition properties on the growth of GS and possibly PD and PI, but this is not sufficient enough. There could be argued that the layer of BB1 spread over the PDA plate could be thickened to inhibit the growth further, but this would be unpractical if related to fruit. A very thick layer of BB1 would be needed to inhibit fungal growth on fruit and this could interfere with other packhouse treatments. BB1 and BB2 showed very poor to no fungicidal action against PD treated in the *in vivo* trials with the specific concentrations and formulations tested. It could be considered to increase the concentration of BB2 if this is financially viable. The final recommendation was to terminate any trials on the BB1 formulation especially due to the impracticality of ethanol in the formulation.

## Trial 5b

### The *in vivo* screening of the fungicidal ability of the compound Biobalsam to control citrus green mould

#### Introduction

A sample of Biobalsam (active ingredient: propolis) was submitted for evaluation of efficacy against postharvest infection of citrus. Biobalsam was screened against the post-harvest citrus pathogen *Penicillium digitatum* (green mould) to determine the efficacy of the product in inhibiting infection caused by these pathogens.

#### Materials and methods

An *in vivo* evaluation was conducted on Navel oranges. For the treated control imazalil (Fungazil sulphate 750 WG; Janssen Pharmaceutica) at the recommended commercial rate of 67 g/100L (giving a treatment concentration of 500 ppm imazalil) was used. The untreated control consisted of inoculated fruit dipped in water. Three concentrations of Biobalsam were applied (indicated below).

Untreated navel oranges (Crocodile Valley Citrus Company) were obtained in bulk. For the purpose of inoculation, blemish free, sound fruit was selected and randomised. All the fruit was surface sterilised by washing on the CRI experimental packline in the recirculating spray-on brush washing system with a suitable quaternary ammonium compound for 2 minutes. Thereafter the fruit was dried in the packline drying tunnel prior to inoculation.

The fruit was divided into lots of 5 fruit per treatment for the dip treatments. Spore suspensions of *P. digitatum* were made up by suspending the spores in sterile deionised water containing the surfactant, Tween 20. The spore suspensions were adjusted spectrophotometrically to a concentration of  $1 \times 10^6$  spores/mL.

#### 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 10 injury sites per treatment. Each fruit was then infected with the pathogen by applying 35 µℓ of spore suspension to each injury site using a micropipette. All the treatments, with the relevant chemical being evaluated, were done in a water bath at 20°C.

The inoculated fruit was incubated for 4 hours at ±23°C (to simulate a 4-hour delay after harvest before packhouse treatments) before treatment with the chemical compound being evaluated. Each treatment was immersed in the water bath for 3 minutes.

## Treatments

1. Untreated control (*P. digitatum*) – water dip
2. Treated control – Fungazil WG (500 ppm)
3. Biobalsam 0.5 g/L
4. Biobalsam 1.0 g/L
5. Biobalsam 2.0 g/L

After the treatments, the fruit was incubated in paper packets at  $\pm 23^{\circ}\text{C}$  for 7 days or until such time as the untreated controls exhibited sufficient green mould. The treatments were then evaluated by counting the number of infected wounds per treatment and the results recorded as percentage decay.

## Results and discussion

The results in Table 4.5.6.5 indicate that **Biobalsam** did not inhibit infections by the pathogen *P. digitatum* at the concentrations of 500, 1000 and 2000 ppm compared to the standard recommended imazalil. This demonstrates that **Biobalsam** did not exhibit any fungicidal properties.

**Table 4.5.6.8.** Percentage green mould on navel oranges that were wounded and inoculated with *P. digitatum* 4 hours before dip-treatment in water ( $20^{\circ}\text{C}$ ) and water with Biobalsam and Fungazil 750 WG.

Treatments	% Decay
1. Untreated control	100.0
2. Treated control- Fungazil (500 ppm)	0.0
3. 0.5 g/L Biobalsam	100.0
4. 1.0 g/L Biobalsam	100.0
5. 2.0 g/L Biobalsam	100.0



1. Untreated control (water)



2. Biobalsam (0.5 g/L)



3. Biobalsam (1.0 g/L)



4. Biobalsam (2.0 g/L)



5. Treated control (IMZ 500 ppm)

**Figures 4.5.6.1 to 4.5.6.5.** Green mould infection on navel oranges that were wounded and inoculated with *P. digitatum* 4 hours before dip-treatment in water ( $20^{\circ}\text{C}$ ; 1) and water with Biobalsam(2, 3 and 4) and Fungazil 750 WG (5).

## Trial 5c

### The evaluation of Biobalsam in a citrus packhouse dumptank washing system as sanitising agents against post-harvest disease

#### Introduction

Biobalsam was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. The product was compared to Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

#### Materials and methods

Similar methodology as for Trial 1 was used. To summarise, treatments involved the following:

1. Untreated control - injured fruit dipped in clean water
2. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL
3. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 0.06 g/L Biobalsam
4. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 0.12 g/L Biobalsam
5. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 0.21 g/L Biobalsam
6. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 0.43 g/L Biobalsam
7. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 1.00 g/L Biobalsam
8. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 1 L/1000 L Sporekill
9. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores / mL + 2 L/1000 L Sporekill

#### Results and discussion

Fruit dipped in the Biobalsam treated dump tank were not inhibited from green mould infection by any of the concentrations. The quaternary ammonium compound Sporekill at 1 L and 2 L/1000 L inhibited the incidence of green mould decay (Table 1).

**Table 4.5.6.9.** The percentage decay incidence recorded on **Navel oranges** that were injured and dip-treated in a simulated dump tank system with clean water, or water with  $1 \times 10^4$  *P. digitatum* spores that had been treated with different concentrations of Biobalsam and Sporekill.

Treatments	% Decay
1. Untreated control	13.3
2. Inoculated control	100.0
3. 0.06 g/L Biobalsam	100.0
4. 0.12 g/L Biobalsam	100.0
5. 0.21 g/L Biobalsam	100.0
6. 0.43 g/L Biobalsam	100.0
7. 1.00 g/L Biobalsam	100.0
8. Sporekill 1.0 L / 1000 L	6.7
9. Sporekill 2.0 L / 1000 L	0.0

#### Conclusion

Biobalsam was not able to sanitise a simulated citrus packhouse dump tank washing system at any of the treated concentrations. It is not comparable to the quaternary ammonium compound Sporekill in its efficacy to prevent green mould infection from contaminated water.

## Trial 5d

### The *in vivo* screening for the ability of the compound Biobalsam to protect citrus fruit against secondary infection from neighbouring infected fruit

#### Introduction

A sample of Biobalsam was submitted to Citrus Research International (CRI) for evaluation of its efficacy to protect citrus fruit from secondary infection of 3 major citrus pathogens, namely blue mould (*Penicillium italicum*), sour rot (*Geotrichum citri-aurantii*) and Phytophthora brown rot (*Phytophthora nicotianae*). These pathogens have the ability to spread from an infected fruit to an intact fruit without the necessity of an entry

port like a wound. This screening was done as a pilot trial to determine if Biobalsam has any potential as a protective agent against the mentioned diseases.

## Materials and methods

An *in vivo* evaluation was conducted on Valencia oranges. Fruit were inoculated with the different pathogens and placed in between 8 intact fruit that have been treated with Biobalsam or a commercial product registered for each disease. Special care was taken to ensure that each intact fruit was touching the inoculated fruit. The different products used for the specific pathogens can be seen in Table 1.

**Table 4.5.6.10.** Treatments and concentrations applied to Valencia oranges for protection against each specific pathogen.

Pathogen	Treatment	Concentration
<i>Penicillium italicum</i>	Control	
	Biobalsam	50%
	Biobalsam	100%
	Imazalil	500 ppm
<i>Phytophthora nicotianae</i>	Control	
	Biobalsam	50%
	Biobalsam	100%
	Phosphonate (Fighter)	570mL/100L
<i>Geotrichum citri-aurantii</i>	Control	
	Biobalsam	50%
	Biobalsam	100%
	Guazatine	1000ppm

The treated fruit was incubated for 21 days at ambient in brown paper bags within plastic bags. Fruit were placed on top of wet tissue paper to keep the atmosphere moist around the fruit. Intact fruit were evaluated for infection; the data were then converted to percentage control.

## Results and discussion

The results in Tables 2 indicate that **Biobalsam** did not protect intact fruit from secondary infections from neighbouring fruit infected with any one of the three pathogens tested.

**Table 4.5.6.11.** Percentage control on intact Valencia oranges placed next to an blue mould, brown rot or sour rot infected fruit after treatment with Biobalsam, imazalil, a phosphonate or guazatine and incubated for 21 days at ambient.

Pathogen	Treatment	Control (%)
<i>Penicillium italicum</i> (Blue mould)	Biobalsam (50%)	0.0
	Biobalsam (100%)	0.0
	Imazalil	69.1
<i>Phytophthora nicotianae</i> (Phytophthora brown rot)	Biobalsam (50%)	0.0
	Biobalsam (100%)	0.0
	Phosphonate	42.9
<i>Geotrichum citri-aurantii</i> (Sour rot)	Biobalsam (50%)	0.0
	Biobalsam (100%)	0.0
	Guazatine	100.0

## Conclusion

These and previous trials demonstrated that Biobalsam has no antifungal action against the major postharvest pathogens of citrus. No further fungicide evaluation work was recommended on Biobalsam.

## Trial 5e

**The evaluation of Biobalsam+ in a citrus packhouse dump tank washing system as sanitising agents against *Penicillium digitatum***

## Introduction

Biobalsam+ (active ingredient: propolis) was evaluated in a simulated dump tank washing system for efficacy of sanitising the system and preventing possible infection of injured fruit moving through the system. Biobalsam tested in 5a to d consist of propolis extracted in ethanol, whereas Biobalsam+ is a different formulation of propolis designed to be more water compatible. The product was compared to Sporekill (a quaternary ammonium compound). The simulated dump tank was seeded with a suspension of the post-harvest pathogen *Penicillium digitatum* (green mould) in this evaluation.

## Materials and methods

Similar methodology as for Trial 1 was used. Treatments involved the following:

1. Untreated control - injured fruit dipped in clean water
2. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup>
3. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 0.05% Biobalsam+
4. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 0.5% Biobalsam+
5. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 5% Biobalsam+
6. Untreated control - injured fruit dipped in clean water
7. Untreated, inoculated control – injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup>
8. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 1 mL.L<sup>-1</sup> Sporekill
9. Injured fruit dipped in water +  $1 \times 10^4$  *P. digitatum* spores.mL<sup>-1</sup> + 2 mL.L<sup>-1</sup> Sporekill

## Results and discussion

Green mould infection was not inhibited by any of the Biobalsam+ treatments. Sporekill totally inhibited green mould infection in both treatments (Table 4.5.6.10).

**Table 4.5.6.12.** The percentage decay incidence recorded on Valencia oranges that were injured and dip-treated in a simulated dump tank system with clean water, or water with  $1 \times 10^4$  *P. digitatum* spores that had been treated with different concentrations of Biobalsam+ and Sporekill.

Treatments	Infection (%)
1. Untreated control	0.0
2. Inoculated control	93.3
3. 0.05% Biobalsam+	100.0
4. 0.50% Biobalsam+	100.0
5. 5.00% Biobalsam+	100.0
6. Untreated control	0.0
7. Inoculated control	100.0
8. 1.0 mL.L <sup>-1</sup> Sporekill	0.0
9. 2.0 mL.L <sup>-1</sup> Sporekill	0.0

## Conclusion and recommendations

Biobalsam+ was unable to prevent green mould infection at concentrations as high as 5%. Biobalsam+ is therefore not suitable for the use as a sanitation agent in citrus packhouses.

## Future research

Various products from Syngenta will be evaluated against *Phytophthora* brown rot at sour rot. Alternative products for imazalil, guazatine and quaternary ammonium compounds will be searched for and evaluated. The effect of pyrimethanil against the latent pathogen will be evaluated in follow up trials from previous work in this experiment.

## Technology transfer

Information on grower talks and presentations at conferences where data from CRI-funded research were presented.

## Further objectives and work plan

New potential products will be tested as sanitation agents and/or fungicides. New products will be combined for testing at several pilot trial sessions in the season of 2013/14. Pilot trials will be kept as basic as possible to prevent loss of valuable research time. All products showing potential will be evaluated further under

contract. Potential products for the control of brown and sour rot will be actively pursued during the season with the aim to facilitate the registration of effective products against these two diseases. Ongoing focus will be to find an alternative for 2,4-D and alternatives for the control of green mould. Further work will also be done on the implementation of the use of GRAS chemicals in citrus packhouses.

#### Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2013 and Jan-Mar 2014

Apr-Jun:	Objective A: Meet with clients and collect products Objective B: Test products for the control of brown and sour rot Objective C: Conduct first session of pilot trials (May and Jun) Objective D: Prepare and analyse data
Jul-Sep:	Objective A: Meet with clients and collect products Objective B: Test products for the control of brown and sour rot Objective C: Conduct second session of pilot trials (Jul, Aug and Sep) Objective D: Prepare and analyse data
Oct-Dec:	Objective A: Finalise and send reports Objective B: Meet with clients
Jan-Mar:	Objective A: Meet with clients Objective B: Collect products Objective C: Combine results for CRI annual report Objective D: Present selected results at Packhouse workshop

#### 4.6 **PROJEK: SWARTVLEK**

Projekkoördineerder: G C Schutte (CRI)

##### 4.6.1 **Projekopsomming**

Met beperkte data tot ons beskikking van weerstasies en twee spoorvangers wat in suurlemoenboorde te Hermitage en Kirkwood geïnstalleer is om *Guignardia* askospor vrystelling te monitor, toon dat twee askospor gebeurtenisse aan die einde van November en Desember in die Kirkwood area plaasgevind het. Geen data is van Addo ontvang nie. Filterpapier spoorvangers is ook in dieselfde persele geïnstalleer (4.6.2). Geen resultate is van 'n veldproef waar verskeie nuwe sistemiese en kontakswamdoders asook bymiddels in kombinasie met geregistreeerde swamdoders getoets is vir die beheer van swartvlek op 'Valencia' lemoene versamel nie. Dit is omrede Crocodile Valley 'n kommersiële bespuiting oor die hele proef gedurende Januarie 2011 toegedien het (4.6.3). In die studie waar daar gefokus word op die globale populasie-struktuur en reprodktiewe biologie van *Guignardia citricarpa*, het die werk op verkryging van *G. citricarpa* populasies uit lande waar die siekte teenwoordig is, gefokus. Populasies is vanaf Australië, Brasilië, VSA en China gekry, en ook van KZN provinsie versamel. Aangesien nog geen populasie genetica studies op *G. citricarpa* uitgevoer is nie, is data van DNA volgordes vir molekule merkers vir hierdie populasie studie ondersoek. Vier DNA loci van 15 isolate wat verskillende lande verteenwoordig, is geëvalueer. Baie lae vlakke van "sequence" verskilte is by die vier loci gevind wat nie bruikbaar vir hierdie genetiese studies was nie. Daarom word "simple sequence repeat" (SSR) merkers nou deur middel van "next-generation DNA sequencing techniques" ontwikkel (4.6.4). Twee fosfonate is op hulle eie en in kombinasies met twee bymiddels (A en B) met behulp van fluorometrie getoets om die retensie van die fosfonate in laboratorium- en veldproewe te bepaal. Laboratoriumproewe toon dat hulle op hulle eie goeie retensie en kwantitatiewe verspreiding van neerslag tot gevolg gehad het. Bymiddel A het die swakste in laboratorium en veldproewe gevaar. Bymiddels blyk nie nodig te wees om fosfonate beter op blare te laat kleef nie (4.6.5). Navorsing is ook begin wat marktoegang sal ondersteun. Hierdie projek fokus op die ontwikkeling en verbetering van 'n model vir *Guignardia pseudothecium* rypwording en spoorvrystelling gebasseer op meso- en mikroklimaatdata. Aanvanklike modellering is afgehandel en 'n artikel voorlopig vir publikasie aanvaar. 'n Samewerkingsprojek, wat deur die Florida sitrusbedryf in VSA befonds word, het ten doel om 'n kwantitatiewe pes risiko analiese vir *Guignardia citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Twee werkswinkels is in Florida gehou, en vordering is gemaak om die stappe in die model te kwantifiseer, en gapings in die beskikbare kennis te identifiseer. Navorsing is reeds aan die gang om nuwe data ter verbetering van die modelle in te samel en om tussen die swartvlek patogene en 'n endofitiese *Guignardia* sp. te onderskei, nuwe modellering-strategie te beproef, asook ondersoek om die sukses van swartvlek beheer in boorde en pakhuis te kwantifiseer (4.6.7). 'n Holistiese beheerstrategie is bestudeer om inokulumvlakke te verlaag en voorkoms van sitrus swartvlek in boorde te verminder. Die effek van bespuitings met benomyl of urea voor blom, die behandeling van blaarafval met 'n komposterings-aktiveerder en swamdoderprogramme bestaande uit ses mankoseb bespuitings of drie strobilurien bespuitings om infeksie van vrugte te verhoed, is in 'n kommersiële sitrusboord vergelyk. Resultate was nie konstant nie, maar het getoon dat spoorvrystelling in die mancozeb program meer was as

in die strobilurien program. Voor-blom bespuitings het geen statistiese of herhaalbare effekte gehad, maar dit bleik asof kompostering van blaarafval spoorvrystelling bevorder het (4.6.6).

## Project summary

Limited data from weather stations and two spore traps installed in lemon orchards in Hermitage and Kirkwood to monitor *Guignardia* ascospore releases showed that two ascospore release events occurred at the end of November and the end of December 2011 in the Kirkwood area. Filter paper spore traps were also installed at the same sites (4.6.2). No results could be obtained from a field trial where various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on 'Valencia' oranges for the control of black spot. This was because the grower applied a commercial spray over the whole trial site during January 2011 (4.6.3). In a study that focused on the global population structure and reproductive biology of the fungal pathogen, *Guignardia citricarpa*, the work during this period concentrated on the acquisition of *G. citricarpa* populations from countries where this disease is present. Populations were received from Australia, Brazil, USA and China and populations were also collected from the KZN province. Since no population genetic studies have been conducted on *G. citricarpa*, sequence data were investigated to obtain molecular markers for the population studies. Four DNA loci were evaluated on 15 isolates that represented several different countries. All of the loci only showed very low levels of sequence polymorphisms, which would not be useful for population level genetic studies. Therefore, simple sequence repeat (SSR) markers are in the process of being developed using next-generation DNA sequencing techniques (4.6.4). Retention of two phosphonates was determined on their own as well as in combination with two adjuvants in laboratory and field trials, using fluorometry. Laboratory trials showed that the retention and quantitative deposition of these phosphonates were good if sprayed on their own. Adjuvant A performed the worst of the two adjuvants tested in laboratory and field trials. Phosphonates do not need adjuvants to improve their deposition on leaves (4.6.5). Research has started that will support market access and will focus on improving a model for *G. citricarpa* pseudothecium maturation and ascospore dispersal based on meso- and micro-climatic weather data. Initial modelling research was completed and a paper submitted for publication. A research project funded by the Florida citrus industry in USA to develop a quantitative pest risk assessment of *Guignardia citricarpa*, with special emphasis on the fresh fruit pathway, is underway. Two workshops were held in Florida in this regard and progress was made by identifying the various steps in the model, assigning probabilities to these steps and identifying research gaps. Data collection to improve the models and research to distinguish between the CBS pathogen and endophytic *Guignardia* sp. to assist with new modelling approaches and surveys to determine efficacy of CBS orchard and packhouse control measures are also underway (4.6.7). A holistic control approach aimed at the reduction of inoculum levels to reduce citrus black spot disease in citrus orchards studied the effect of pre-blossom benomyl or urea applications, treatment of leaf litter with a compost activator and fungicide spray programmes consisting of either six times mancozeb or three times strobilurin applications on ascospore inoculum levels and severity of citrus black spot on fruit. No consistent effect was seen, but overall results showed the effect of spray programmes on ascospore production with the mancozeb programmes resulting in much higher production than the strobilurin programmes. Pre-blossom sprays did not have any consistent effect, but leaf litter treatment promoted spore dispersal (4.6.6).

### 4.6.2 PROGRESS REPORT: Monitoring ascospore releases in the Eastern Cape to determine the critical period for CBS infection

Experiment 919 (September 2008 – June 2011) by G C Schutte (CRI) and S Serfontein (QMS)

#### Summary

Weather stations and two spore traps, installed in lemon orchards in Hermitage and Kirkwood to monitor *Guignardia* ascospore releases, experienced technical problems during the critical periods when ascospore releases took place. The weather station at Hermitage was also stolen. Two new Adcon weather stations were installed on 17 February 2012 at Summerville and Kirkwood. Therefore, data from another weather station had to be used. With all the cloudy days experienced, the batteries occasionally ran flat and a lot of data was lost. However, with the data to our disposal, it showed that Kirkwood had two ascospore releases that occurred at the end of November and the end of December 2011. This showed that the spray programmes that were recommended and applied were correct and protected the fruit in those critical periods.

#### Opsomming

Weerstasies en twee spoorvangers wat in suurlemoenboorde te Hermitage en Kirkwood geïnstalleer is en vir die monitoring van *Guignardia* askospore gebruik is, het tegniese probleme ondervind in die kritiese tye toe askosporvystellings moontlik plaasgevind het. Die weerstasie by Hermitage is gesteel en twee nuwe Adcon weerstasies is eers op 17 Februarie 2012 by Summerville en Kirkwood geïnstalleer. Daar moes toe

noodgedwonge van 'n ander weerstasie gebruik gemaak word om data te bekom. Met die baie bewolkte dae tydens reënweer, het die batterye ook pap geword en baie data is so verloor. Met die data tot ons beskikking kon ons aflei dat daar twee askosporvystellings einde November en einde Desember 2011 by Kirkwood plaasgevind het, wat alhoewel dit minder was as die vorige seisoen, tog ooreengestem het met die vorige seisoen. Dit beklemtoon dat daar wel askosporvystelling in mid-somer plaasgevind het en dat die spuitprogramme wat voorgestel en gespuit is was korrek en sou vrugte in daardie kritiese periodes beskerm het.

#### 4.6.3 **PROGRESS REPORT: Development of new spray programmes for the control of citrus black spot**

Experiment 970 (April 2009 – June 2012) by G C Schutte and C Kotze (CRI)

##### **Summary**

Various new systemic and contact fungicides as well as adjuvants in combination with registered fungicides were tested on 'Valencia' oranges for the control of citrus black spot according to predetermined protocols from the various companies. No results could be obtained because Crocodile Valley did a commercial spray application over the whole trial site. The trial will be repeated.

##### **Opsomming**

Verskeie nuwe sistemiese- en kontakswamdoders asook adjuvante in kombinasies met geregistreerde swamdoders is op 'Valencia' lemoene beproef vir die beheer van swartvlek volgens vooropgestelde protokolle van die onderskeie maatskappye. Geen proefresultate is verkry nie omrede Crocodile Valley onwetend 'n kommersiële bespuiting in Januarie 2011 oor die hele proefperseel toegedien het. Die proef word herhaal.

##### **Introduction**

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa), affects all commercial citrus cultivars only in the summer rainfall regions of the world. Control of the disease is entirely dependent on the application of fungicidal sprays during the critical period of infection from October to January in the southern hemisphere. The most important inoculum source of CBS is airborne ascospores. Pseudothecia of the fungus develop on dead infected leaves on the orchard floor within 40-180 days after leaf drop, depending on the temperature and frequency of wetting. Once mature, ascospores are discharged during rain spells. Ascospores are dependent on converging currents and favorable environmental conditions to reach a suitable host substrate, since the maximum vertical distance of ascospore ejection from a pseudothecium is 10-12 mm and the horizontal disease dispersion occurs at distances below 24.7 m. When protective fungicides such as copper and dithiocarbamates are used to control CBS, spray applications have to be carefully timed to coincide with the critical infection period. Spore trapping with an Interlock volumetric spore trap® and sampler is used to determine the first onset of ascospore release in South Africa.

A four-spray programme of copper fungicides used for CBS control can result in rind stippling and darkening of blemishes. However, alternating copper fungicides with mancozeb in a four-spray programme, solved this problem. Protective fungicides became less popular after the release of post-infection benzimidazole fungicides such as benomyl. In 1971, the introduction of a single benomyl application in a tank mixture with mancozeb and mineral spray oil came as a breakthrough as it replaced copper and dithiocarbamates that must be applied in a four-spray protective schedule (9). Since the detection of *G. citricarpa* resistance to benomyl in South Africa in 1981, emphasis has shifted back to the use of contact fungicides for disease control. Field evaluations using strobilurins for the control of CBS in 1993 also came as a breakthrough. Two applications of kresoxim-methyl and azoxystrobin at respective rates of 0.10 and 0.075 g a.i./liter in tank mixtures with mancozeb (1.2 g a.i./liter) and mineral oil (0.5% [vol/vol]/liter of water) were initially recommended. The possibility that CBS may develop resistance to the strobilurins, justifies the incorporation of two additional mancozeb before and after the strobilurin applications in October and January.

Since the registration of strobilurins in South Africa in 1999, no new fungicides have been registered for use against CBS. Testing of novel control measures against CBS is therefore regarded as a priority even if it includes tank mixtures with current registered fungicides.

##### **Objectives**

The aim is to evaluate any new potential fungicides for the control of CBS.

## Materials and methods

A 'Delta' Valencia orchard was selected at Croc Valley Citrus Co. to do the evaluations. A randomised design with 5 single-tree plots per treatment was used. Fungicides were applied with a trailer-mounted, high-volume, high-pressure (2,500 - 3,000 kPa) sprayer with two hand-held spray guns. Spray volumes will vary according to the size and canopy density of the tree but all trees will be sprayed to the point of run-off. Certain treatments will commence in mid-October as previously recommended, depending on the climatological information required for infection during the critical infection period. Trees were selected for uniformity in canopy density and tree size. Currently, all commercial fungicide applications for CBS control in South Africa begin in mid-October, based on research findings from ascospore releases and spore trap data.

At fruit maturity in July or August, CBS severity will be rated on 100 fruits per tree according to a 3-point index: 0 = clean fruit with no CBS lesions; 1 = one to three CBS lesions per fruit; and 2 = four or more CBS lesions per fruit. Proportional data will be analysed by ANOVA, using Fisher's LSD test ( $P = 0.05$ ).

## Results and discussion

No results were obtained because the whole trial site received a commercial application of Cabrio and mancozeb.

## Conclusion

Nothing to report.

## Future research

The trial will be repeated. There is, however, a constant need to evaluate new and old fungicide formulations as well as fungicides that may possess activity against citrus black spot (CBS). Chemical companies frequently modify and upgrade their old products to possess new characteristics such as rain fastness and particle size and they need to be re-evaluated for efficacy. Searching for new fungicides or fungicides with new characteristics as well as some new ideas how we can alter aspects of old fungicide spray programmes to be included in effective spray programmes and to cope with fungal resistance strategies at the same time. Searching for and experimenting with cheaper and more effective fungicides will contribute a lot to reducing production costs and be more environmentally friendly and sustainable with regard to resistance development.

## Technology transfer

Talks at study groups. Results will be presented on the bi-annual CRI Symposium in August 2010.

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**4.6.4 PROGRESS REPORT: The global population structure and reproductive biology of the fungal pathogen, *Guignardia citricarpa* Kiely**  
Experiment 977 (2010/11 – 2013/14) by E Carstens (CRI)

**Summary**

A population genetics study is being conducted on the Citrus Black Spot pathogen, *Guignardia citricarpa* in order to elucidate several key questions regarding its epidemiology and reproductive biology. An important aspect of any population genetics studies is the acquisition of appropriate populations from various international and national locations, which should include populations from specific orchards (15 to 30 isolates per orchard). *Guignardia citricarpa* populations were obtained from Australia, Brazil, USA and China, and populations from Argentina are expected. Populations were also collected from four South African provinces where the disease is present. Since no population genetic studies have been conducted on *G. citricarpa*, data of DNA sequences were investigated for molecular markers for this population genetics studies. Four DNA loci were evaluated on 15 isolates that represented several different countries. All of the loci only showed very low levels of sequence polymorphisms, which would not be useful for population level genetic studies. Therefore, simple sequence repeat (SSR) markers are in the process of being developed using benchtop next generation sequencing (Ion Torrent Personal Genome Machine).

**Opsomming**

'n Populasie genetica studie word op *Guignardia citricarpa*, die patogeen verantwoordelik vir sitrus swartvlek, uitgevoer om verskeie vrae omtrent die epidemiologie en reprodutiewe biologie te beantwoord. 'n Belangrike aspek van enige populasie genetica studie is die verkryging van geskikte populasies van verskeie internasionale en nasionale bronne, wat populasies van spesifieke boorde (15 tot 30 isolate per boord) moet insluit. *Guignardia citricarpa* populasies is van Australië, Brasilië, die VSA en China ontvang en populasies van Argentinië word binnekort verwag. Populasies is ook van die vier Suid-Afrikaanse provinsies, waar die siekte voorkom, versamel. Aangesien geen populasie genetica studies op *G. citricarpa* uitgevoer is nie, is data van DNA volgordes vir molekulêre merkers vir hierdie populasie genetica studie ondersoek. Vier DNA loci van 15 isolate wat verskillende lande verteenwoordig, is geëvalueer. Baie lae vlakke van "sequence" polimorfismes is by die vier loci gevind wat nie bruikbaar vir hierdie genetiese studies was nie. Daarom word "Simple sequence repeat" (SSR) merkers nou ontwikkel deur middel van "benchtop next generation sequencing (Ion Torrent Personal Genome Machine)".

**4.6.5 PROGRESS REPORT: Improving the retention of suspension liquid phosphonate fungicides on citrus fruit and leaves**  
Experiment 1012 (April 2011 - March 2014) by G C Schutte and C Kotze (CRI)

**Summary**

In order to determine the retention of the phosphonates in laboratory and field trials, two phosphonates were tested on their own and in combinations with two adjuvants (A and B; coded as trials have not been finalised) using fluorometry. Results from laboratory trials, where both phosphonates were sprayed at registered rates on upper and lower sides of static orange leaves, showed that the retention and quantitative deposition of these phosphonates were good if they were sprayed on their own. On the other hand, registered rates of adjuvant B resulted in the best quantitative deposition of phosphonate X on the lower side of orange leaves. It seems that the run-off point for both adjuvants A and B seems to be between 4-6 ml/leaf if both sides are sprayed. None of the adjuvants could result in an increase in quantitative deposition of phosphonate Y and adjuvant A performed the worst of the two adjuvants tested. Leaf and fruit samples from a field trial showed that the water-only treatment resulted in higher deposition quantities of upper and lower leaves than phosphonates sprayed on their own. Where adjuvant A was added in the tank mixture with phosphonate Y, it

resulted in significant less spray deposition on both leaves and fruit. Phosphonates X and Y resulted in significantly more spray deposition on the upper side of fruit than the lower side of the same fruit. The exception was adjuvant B with phosphonate Y, which showed that this product gave good coverage of the whole fruit. Thus far it seems that phosphonates do not need adjuvants to improve their deposition on leaves. More trials are needed to see if this applicable to fruit after inoculation with *Phytophthora brown rot*.

## Opsomming

Twee fosfonate is op hulle eie en in kombinasies met twee bymiddels (A en B) met behulp van fluorometrie getoets om die retensie van die fosfonate in laboratorium- en veldproewe te bepaal. In laboratoriumproewe waar beide fosfonate teen geregistreerde dosisse op voor- en agterkante van statiese blare gespuit is, toon die resultate dat hulle op hulle eie goeie retensie en kwantitatiewe verspreiding van neerslag tot gevolg gehad het. Andersins het bymiddel B teen geregistreerde dosisse die beste kwantitatiewe verspreiding van fosfonaat X aan die onderkante van blare tot gevolg gehad. Dit lyk asof die aflooppunt vir beide bymiddels tussen 4-6 ml/blaar is indien beide kante bespuit word. Geeneen van die bymiddels kon egter die kwantitatiewe neerslag van fosfonaat Y verhoog nie en van die twee het bymiddel A die swakste gevaar. In 'n veldproef waar dieselfde behandelings toegedien is en waar blaar- en vrugmonsters kort na die bespuitings getrek is, toon dat die waterbehandeling meer neerslag tot gevolg gehad het as die fosfonate op hulle eie. Waar bymiddel A gemeng is met fosfonaat Y, is betekenisvol minder verspreiding van spuitneerslag op blare waargeneem. Op hulle eie asook in kombinasies met bymiddels A en B het fosfonate X en Y in al die gevalle meer spuitneerslag op die boonste helfte van vrugte as die onderste helfte tot gevolg gehad. Die uitsondering was bymiddel B met fosfonaat Y, wat toon dat hierdie produk goeie bedekking oor die hele vrug gegee het in hierdie uitsonderlike geval. Tot dusver blyk dit dat bymiddels nie nodig is om fosfonate beter op blare te laat kleef nie, maar verdere studies is nodig om te bepaal of dit van toepassing is op vrugte nadat hulle met *Phytophthora bruinvrot* geïnokuleer is.

### 4.6.6 FINAL REPORT: A holistic approach to the control of citrus black spot with the emphasis on the reduction of *Guignardia citricarpa* ascospore inoculum

Project 08 CRI FS2 (March 2008 – 2012) by SH Swart, JJ Serfontein, A Fourie (QMS Agri Science) & PH Fourie (CRI)

## Summary

This study focussed on activities aimed at the reduction of inoculum levels as part of a holistic approach to reduce citrus black spot disease in citrus orchards. This is different from standard practices to protect citrus fruit from infections with fungicide spray programmes. The effect of pre-blossom benomyl or urea applications, treatment of leaf litter with a compost activator and fungicide spray programmes consisting of either six times mancozeb or three times strobilurin applications were evaluated in a commercial citrus orchard. Results obtained during this studied showed that the application of composting products resulted in increased ascospore release and disease severity. The strobilurin and mancozeb programmes differed in the rate of leaf litter decomposition and ascospore numbers but not disease incidence. Leaf litter in strobilurin programmes decomposed slower and released less ascospores than those from mancozeb programmes. The effect of leaf litter treatments can have a negative effect on ascospore numbers and disease incidence in South Africa while fungicide programmes did affect ascospore numbers. Pre-blossom applications might influence leaf infections but was not clearly demonstrated in this study. Enhanced leaf litter decomposition should not coincide with infection periods and the effect of pruning material in orchards on ascospore numbers needs to be determined.

## Opsomming

Hierdie studie het op aktiwiteite om inokulumvlakke te verlaag gefokus, as deel van 'n holistiese beheerprogram om die voorkoms van sitrus swartvlek te verminder. Dit verskil van standaard praktyke om slegs vrugte teen infeksie te beskerm deur gebruik te maak van spuitprogramme met swamdoders. Die effek van bespuitings met benomyl of urea voor blom, die behandeling van blaarafval met 'n komposterings-aktiveerder en swamdoderprogramme bestaande uit ses mankoseb bespuitings of drie strobilurien bespuitings om infeksie van vrugte te verhoed, is in 'n kommersiële sitrusboord vergelyk. Resultate tydens die studie het getoon dat die toediening van komposteringsprodukte verhoogde spoorvrystelling tydens somermaande as vrugte vatbaar is veroorsaak en verder ook dat die siekte intensiteit hoër is as waar geen blaarafval behandeling toegepas is nie. Die strobilurin en mancozeb programme het verskil t.o.v. die tempo van afbraak van blaarreste en askospor getalle maar nie siektevoorkoms nie. Blaar reste in strobilurin programme breek gewoonlik stadiger af en laat minder spore vry. Die effek van blaarreste behandeling in Suid Afrika kan 'n negatiewe effek op spoorgetalle en siektevoorkoms hê en terwyl swamdoderprogramme beslis 'n invloed op askospor getalle het. Voor-blom behandelings kan 'n invloed op blaarinfeksies hê, maar

is nie in die studie uitgewys nie. Versnelde kompostering van blare moet nie met infeksieperiodes ooreenstem nie en die effek van snoei reste op askosporvoorkoms moet bepaal word.

## Introduction

Citrus black spot is considered a major threat to citrus producing countries where the disease do not occur and strict phytosanitary control measures on imported fruit have been implemented by several countries in the European Union, as well as Japan and the USA. Infection is commercially prevented with standard fungicide spray programmes, which is usually very effective. However, due to a wide spectrum of factors that can influence disease epidemiology, poor control sometimes result in interception of fruit with citrus black spot symptoms. The latter pose a threat to the South African citrus export industry and market access for citrus from certain production areas in South Africa, with a direct, negative economic impact to producers.

Severity of plant disease is influenced by the susceptibility of the host, the level and fitness of inoculum and climatic conditions that favour production and dispersal of inoculum, infection and lesion development. Disease management in agriculture mostly focus on the host for identification and development of genetic resistance, and on the pathogen, where biocides are used to reduce inoculum, prevent infection, and/or inhibit lesion development. Very little can be done with regards to climatic conditions, especially if requirements for optimum production and disease development, are similar. Currently, citrus producers in South Africa are mainly dependent on the use of fungicides to protect fruit from infection for six months, between October and February. The current practice of extensive pruning, increased leaf litter presence on the orchard floor and the potential for increased production of inoculum on the leaf litter, may lead to high levels of black spot disease.

The concept of reducing inoculum levels to control apple scab disease have been studied intensively and was successful in a number of trials (Jespersion, 1995; Beresford, *et al.*, 2000; Carisse *et al.*, 2000; Sutton, *et al.*, 2000; Mondal & Timmer, 2003). By reducing inoculum levels in orchards, the spread of disease can be slowed down, disease pressure can be reduced, resulting in less successful infections. The latter will also have an effect on tempo of resistance development by high risk fungicide groups.

## Objectives

The objective was to study the effect of current disease control programmes and other production practices on available inoculum levels in order to find a holistic approach to citrus black spot disease control where efforts are focussed to reduce inoculum production and disease severity.

## Materials and methods

A trial was designed to determine the effect of several treatments on ascospore production and disease occurrence on fruit at harvest. These treatments included pre-blossom applications aimed to reduce infection of intact leaves, fungicide spray programmes for fruit protection and enhancing the decomposition process of leaf litter under the trees.

The trial was conducted in the Letsitele area in a 32-year-old Valencia orchard with a history of severe citrus black spot disease. All trees received standard commercial postharvest pruning. Just after pruning, mature leaves were picked from trees, placed between two plastic mesh grids and positioned under tree canopies for the aging and decomposition process.

Pre-blossom treatments: Benomyl (500 g/kg, WP) + BP medium oil (50 g + 300 ml/100 l water) or spray urea (1000 g/100 l water) were applied with the QMS experimental spray apparatus at 20 bar pressure, using hand held lances. Some treatments did not receive a pre-blossom treatment (Table 1).

Fruit protection treatments: All trees in the trial received either a mancozeb program, consisting of 6 applications of mancozeb (800 g/kg, WP) at 200 g / 100 l water, at 24-day intervals, or a strobilurin program, consisting of 3 applications of pyraclostrobin (500 g/l, EC) + mancozeb (800 g/kg, WP) + BP medium oil (10 ml + 150 g + 300 ml /100 l water), at 6-week intervals, applied with a commercial "Eagle" applicator.

Leaf litter treatment: Leaf litter under the tree canopies was sprayed with Compost Aid + Breakdown All (CaBa) at 2 g + 1.5 ml in 10 l of water with the QMS experimental spray applicator (equivalent to the commercial recommendation of 1 kg Compost Aid + 750 ml Breakdown All / ha 5000 l of water/ha).

**Layout:** Trees were divided into 21 sub-plots containing approximately 50 trees per treatment combination (7 rows by 8 trees). All treatments were randomly replicated twice except for treatments 10 (- Ma -) and 12 (-

St -), where only 1 replicate was possible due to lack of trees and replicates had to be taken from a single block of trees. Treatment numbers and description of applications are shown in Table 4.6.6.1.

**Ascospore evaluation:** Two sets per replicate of naturally fallen leaves as well as two sets of picked leaves placed in grids in November per replicate were analysed with a Kotzé Inoculum Monitor (KIM) to determine the effect of treatments on ascospore inoculum production on the remaining leaf litter mainly at the end of January and end of March. To determine the level of *Guignardia* ascospores produced on leaf litter samples, grids with leaves were dipped in hot water (50°C) for 5 min and placed in a Kotzé-inoculum-monitor (KIM). *Guignardia* ascospores, discharged over a period of 2 hours, were deposited on a Vaseline® coated microscope slide. After staining the slide with lacto-phenol-cotton-blue, ascospores in four lanes (45 mm long) on each microscope slide, representing approximately 180 mm<sup>2</sup> surface area, were counted, using a light microscope at 400x magnification and the total number of ascospores was recorded. Inoculum production were analysed as according to season, i.e. summer or autumn. Leaf litter in grids were also evaluated for the stage of decomposition (November and February). Leaf litter present on the ground was also evaluated for stage of decomposition in November and February. A scale from 1 - (no decomposition) to 5 - (totally decomposed) was attributed to the composition scale.

**Disease evaluation:** Two trees per replicate (n = 4) were evaluated separately for the presence of citrus black spot (CBS) fruit symptoms in 2009. The number of trees were increased to 4 per replicate block (n = 8) for the 2010 and 2011 disease evaluations. Two hundred fruits (20 fruits in 10 sectors) of each replicate tree were evaluated and classified into three disease categories, i.e. no lesions, 1 to 3 lesions and more than 3 lesions. The percentage fruit in each category was also determined. A disease index (DI) was calculated with the following equation:  $DI = ((\text{number of fruit with 1 to 3 lesions} \times 5) + (\text{number of fruit with more than 3 lesions} \times 10)) / 20$ .

#### 2008/2009 season

Pre-blossom benomyl and urea applications were done on the 10<sup>th</sup> of September 2008. Trees in some treatments did not receive the pre-blossom treatment (Table 1). Commercial mancozeb or strobilurin applications commenced on 15 October 2008. Pruning was completed late in October 2008 and therefore, leaf litter could only be treated with CaBa on 5 November 2008. Leaf analysis with the KIM was done in January and March 2009. Disease evaluation on harvested fruit was done in August 2009.

#### 2009/2010 season

Treatments in the 2009/2010 season were similar to the previous season and were repeated on the same plots. Pruning was completed in middle September 2009 and the pre blossom benomyl and urea applications were done at the end of September. Mancozeb and strobilurin programmes commenced on 9 October 2009. Leaf litter was treated with CaBa on 19 October 2009 and 15 January 2010. Two sets of naturally fallen leaves per replicate (n = 4) as well as two sets of picked leaves (n = 4), placed in grids in November 2009, were analysed with the KIM at the end of January and the end of March 2010. Leaf litter in grids were also rated for stage of decomposition in order to add more clarity on results obtained with the KIM. The stage of decomposition was evaluated on a scale from one (no decay) to five (totally decayed). Disease evaluation was done on harvested fruit in July 2010.

**Table 4.6.6.1.** Products and treatments applied to different plots.

Treatment no	Programme description*	Products applied		
		Pre-blossom application	Preventative fruit protection	Treatment of leaf litter
1	Be Ma CaBa	Benomyl	Mancozeb	CaBa
2	Be Ma -	Benomyl	Mancozeb	-
3	Be St CaBa	Benomyl	Strobilurin	CaBa
4	Be St -	Benomyl	Strobilurin	-
5	Ur Ma CaBa	Urea	Mancozeb	CaBa
6	Ur Ma -	Urea	Mancozeb	-
7	Ur St CaBa	Urea	Strobilurin	CaBa
8	Ur St -	Urea	Strobilurin	-
9	- Ma CaBa	-	Mancozeb	CaBa
10	- Ma -	-	Mancozeb	-
11	- St CaBa	-	Strobilurin	CaBa
12	- St -	-	Strobilurin	-

\*Be = benomyl, Ur = urea, Ma = mancozeb, St = strobilurin, CaBa = Compost Aid + Breakdown All

## 2010/2011 season

Treatments in the 2010/11 season differed from the previous seasons due to the fact that the pre-blossom applications were omitted. Fruit was harvested very late (mid-October) in 2010, after which pruning was done and completed on 18 October 2010. As it was already post-blossom and after the planned first mancozeb and strobilurin application dates, the pre-blossom benomyl and urea applications could not be done. The first mancozeb and strobilurin applications commenced on 21 October 2010. Follow-up mancozeb applications (200 g/100 l) were done on 11/11/2010, 2/12/2010, 22/12/2010, 26/01/2011 and 23/01/2011. Ortiva (250 g / kg, SC) + mancozeb + oil (20 ml + 150 g + 300 ml/100 l) were applied on 21/10/2010, 3/12/2010 and 18/01/2011 during this season. Leaf litter was treated with CaBa on 25 October 2010, 25 November 2010 and on 1 February 2011. The orchard was irrigated daily for 2 hours via micro-jet irrigation in order to enhanced leaf litter decomposition. Two sets of picked leaves per replicate (n = 4), were placed in grids in November 2010 and placed under respective tree canopies. Rating and analysis with the KIM was done at the end of December 2010 and the end of March 2011. Natural leaf litter under tree canopies, two sets per replicate (n = 4), were also rated for the stage of decomposition and analysed in the KIM in November 2010 and again in February 2011. Disease evaluation was done on harvested fruit early in October 2011. All trees in this orchard were removed in November 2011.

## Analysis

Data (ascospore counts and disease incidence) was analysed as multifactorial ANOVA with season as factor and the three pre-blossom treatments, the two fungicide treatments and two leaf litter treatments as factors. Results from treatments with interactions with a confidence level with a value of more than 95% (Fisher LSD test), is reported.

## **Results**

### Ascospores

There was a significant effect on the number of ascospores produced on picked leaves, placed in grids in the summer evaluation with significantly more ascospores trapped in 2009 where a mancozeb programme was applied and urea was applied at pre-blossom and no pre-blossom programme was applied in a strobilurin programme (Table 4.6.6.2). The rest of the treatment combinations produced significantly less ascospores with the 2010 season in general lower counts than 2009. Significantly more spores were trapped during 2009 in programmes that received Breakdown-All + Compost Aid treatments than the rest of the combinations (Table 4.6.6.3).

**Table 4.6.6.2.** Combined ascospore counts from grid-leaves during 2009 and 2010 summer seasons as affected by year, pre-blossom and fungicide programmes.

Year and programme combinations			Spore counts <sup>z</sup>
Year	Pre-blossom programmes	Fungicide programmes	
2009	None	Mancozeb	8026 bc
2009	None	Strobilurin	25626 ab
2009	Urea	Mancozeb	30890 a
2009	Urea	Strobilurin	92 c
2009	Benomyl	Mancozeb	392 c
2009	Benomyl	Strobilurin	106 c
2010	None	Mancozeb	39 c
2010	None	Strobilurin	0.3 c
2010	Urea	Mancozeb	13 c
2010	Urea	Strobilurin	0.8 c
2010	Benomyl	Mancozeb	97 c
2010	Benomyl	Strobilurin	16 c

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.3.** Combined ascospore counts from grid-leaves during 2009 and 2010 summer seasons as affected by year and leaf litter treatments.

Year and programme combination		Spore counts <sup>z</sup>
Year	Leaf litter treatment	
2009	Breakdown-All + Compost Aid	19930 a
2009	No treatment	1780 b
2010	Breakdown-All + Compost Aid	28 b
2010	No treatment	27 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

Spore counts were significantly higher during the autumn evaluation of leaves in grids during 2009 where a mancozeb programme was applied than the rest of the combinations. It is worthy to note that the spore counts from mancozeb treated plots were always higher than the strobilurin treated plots of corresponding years (Table 4.6.6.4). The pre-blossom programmes and leaf litter treatments had no significant effect on autumn spore counts from leaves in grids (Table 5 and 6).

**Table 4.6.6.4.** Combined ascospore counts from grid-leaves during 2009, 2010 and 2011 autumn seasons as affected by year and fungicide programmes.

Year and programme combination		Spore counts <sup>z</sup>
Year	Fungicide programme	
2009	Mancozeb	102 a
2009	Strobilurin	17 b
2010	Mancozeb	1.5 b
2010	Strobilurin	0.1 b
2011	Mancozeb	23 b
2011	Strobilurin	1.8 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.5.** Combined ascospore counts from grid-leaves during 2009, 2010 and 2011 autumn seasons as affected by pre-blossom programmes.

Pre-blossom programme	Spore counts <sup>z</sup>
Urea	28.0 a
Benomyl	27.8 a
No treatment	17.1 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.6.** Combined ascospore counts from grid-leaves during 2009, 2010 and 2011 autumn seasons as affected by leaf litter treatments.

Leaf litter treatment	Spore counts <sup>z</sup>
Breakdown-All + Compost Aid	29.1 a
No treatment	19.4 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

Significantly more spores were trapped during summer evaluations from collected leaf litter in 2011 in the pre-blossom untreated (benomyl) plots where a mancozeb fungicide programme were followed in which leaf litter was treated with Breakdown All + Compost Aid than the rest of the treatments (Table 4.6.6.7). Besides this significant higher spore release, no clear pattern was evident in relation to different treatment combinations.

Spore counts from leaf litter collected during the summer of 2011 was significantly higher in plots from mancozeb programmes that received leaf litter Breakdown All + Compost Aid treatments than most of the other combinations (Table 4.6.6.8). The counts were, however, not significantly more than the 2010 mancozeb treatment with no leaf litter treatments and the 2010 strobilurin treatment that received Breakdown-All + Compost Aid.

**Table 4.6.6.7.** Combined ascospore counts from naturally fallen leaf litter during 2010 and 2011 summer seasons as affected by year, pre-blossom (only in 2010) and fungicide programmes and leaf litter treatments.

Year and programme combination				Spore counts <sup>y</sup>
Year	Pre-blossom programme	Fungicide programme	Leaf litter treatment	
2010	None	Mancozeb	No treatment	77.0 b
2010	None	Mancozeb	Breakdown-All + Compost Aid	28.1 b
2010	None	Strobilurin	No treatment	9.5 b
2010	None	Strobilurin	Breakdown-All + Compost Aid	286.9 b
2010	Urea	Mancozeb	No treatment	188.6 b
2010	Urea	Mancozeb	Breakdown-All + Compost Aid	191.9 b
2010	Urea	Strobilurin	No treatment	5.3 b
2010	Urea	Strobilurin	Breakdown-All + Compost Aid	64.4 b
2010	Benomyl	Mancozeb	No treatment	293.8 b
2010	Benomyl	Mancozeb	Breakdown-All + Compost Aid	22.5 b
2010	Benomyl	Strobilurin	No treatment	45.6 b
2010	Benomyl	Strobilurin	Breakdown-All + Compost Aid	170.8 b
2011	None	Mancozeb	No treatment	0.0 b
2011	None	Mancozeb	Breakdown-All + Compost Aid	0.0 b
2011	None	Strobilurin	No treatment	0.5 b
2011	None	Strobilurin	Breakdown-All + Compost Aid	0.0 b
2011	None (Urea) <sup>z</sup>	Mancozeb	No treatment	45.5 b
2011	None (Urea) <sup>z</sup>	Mancozeb	Breakdown-All + Compost Aid	4.0 b
2011	None (Urea) <sup>z</sup>	Strobilurin	No treatment	3.8 b
2011	None (Urea) <sup>z</sup>	Strobilurin	Breakdown-All + Compost Aid	0.0 b
2011	None (Benomyl) <sup>z</sup>	Mancozeb	No treatment	0.0 b
2011	None (Benomyl) <sup>z</sup>	Mancozeb	Breakdown-All + Compost Aid	1387.5 a
2011	None (Benomyl) <sup>z</sup>	Strobilurin	No treatment	8.8 b
2011	None (Benomyl) <sup>z</sup>	Strobilurin	Breakdown-All + Compost Aid	0.0 b

<sup>y</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

<sup>z</sup>Due to late harvesting, no pre-blossom treatments were made in 2010 leading up to the 2011 analyses.

**Table 4.6.6.8.** Combined ascospore counts from naturally fallen leaf litter during 2010 and 2011 summer seasons as affected by year, fungicide programme and leaf litter treatments.

Year and programme combination			Spore counts <sup>z</sup>
Year	Fungicide programme	Leaf litter treatment	
2010	Mancozeb	No treatment	186.5 ab
2010	Mancozeb	Breakdown-All + Compost Aid	80.8 b
2010	Strobilurin	No treatment	24.5 b
2010	Strobilurin	Breakdown-All + Compost Aid	174.0 ab
2011	Mancozeb	No treatment	15.2 b
2011	Mancozeb	Breakdown-All + Compost Aid	464.3 a
2011	Strobilurin	No treatment	5.8 b
2011	Strobilurin	Breakdown-All + Compost Aid	0.2 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

The combined ascospore counts from naturally fallen leaf litter during 2010 autumn season in the mancozeb treatments were also much higher than the strobilurin treatments but as a result of variation between plots, this difference was not significant (Table 4.6.6.9).

**Table 4.6.6.9.** Combined ascospore counts from naturally fallen leaf litter during 2010 autumn seasons as affected by fungicide programmes.

Fungicide programme	Spore counts <sup>z</sup>
Mancozeb	592.1 a
Strobilurin	44.5 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ )

During the combined autumn ascospore counts for 2010 from collected leaf litter, the urea pre-blossom treatments had the highest spore counts followed by the benomyl and lastly the untreated plots. The counts did not differ significantly (Table 4.6.6.10). The Breakdown-All + Compost Aid leaf litter treatments also had much higher ascospore counts than the untreated plots but not significantly so (Table 4.6.6.11).

**Table 4.6.6.10.** Combined ascospore counts from naturally fallen leaf litter during 2010 autumn season as affected by pre-blossom treatments.

Pre-blossom programme	Spore counts <sup>z</sup>
Urea	569.2 a
Benomyl	315.0 a
No treatment	70.8 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.11.** Combined ascospore counts from naturally fallen leaf litter during 2010 autumn season as affected by leaf litter treatments.

Leaf litter treatment	Spore counts <sup>z</sup>
Breakdown-All + Compost Aid	462.1 a
No treatment	174.5 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

#### Leaf decomposition stage

The interactions of the fungicide programmes as well as year on stage of leaf decomposition of picked leaves that were placed in grids under the trees were significant during evaluation in February 2010 where the leaf litter from strobilurin plots were significantly less decomposed than those from the mancozeb treatment of the same year and the 2011 treatments (Table 4.6.6.12). Leaves in grids from the mancozeb programmes were more composted than those from strobilurin programmes. When the years are combined, this difference in decomposition stage was significant (Table 4.6.6.13). Leaves in grids were in a significantly more advanced stage of decomposition in February 2011 than in February 2010 (Table 4.6.6.14).

**Table 4.6.6.12.** The effect of the year (2010 and 2011) and fungicide programmes on stage of decomposition in February of picked leaves placed in grids under trees.

Year and programme combinations		Decomposition stage <sup>z</sup>
Year	Fungicide programme	
2010	Mancozeb	3.48 b
2010	Strobilurin	2.65 a
2011	Mancozeb	3.55 b
2011	Strobilurin	3.68 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.13.** The effect of the fungicide programmes on stage of decomposition in February of picked leaves placed in grids under trees.

Fungicide programme	Decomposition stage <sup>z</sup>
Strobilurin	3.19 a
Mancozeb	3.51 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.4.6.14.** The effect of the year (2010 and 2011) on stage of decomposition in February of picked leaves placed in grids under trees.

Year	Decomposition stage <sup>z</sup>
2010	3.08 a
2011	3.61 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

During the March evaluation, the fungicide programmes as well as the year had a significant influence on the stage of decomposition with leaves from the strobilurin programmes significantly less decomposed than those from mancozeb programmes (Tables 4.4.6.15 and 4.4.6.16).

**Table 4.4.6.15.** The effect of the fungicide programmes on stage of decomposition in March of picked leaves placed in grids under trees.

Fungicide programme	Decomposition stage <sup>z</sup>
Strobilurin	3.91 a
Mancozeb	4.32 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.4.6.16.** The effect of the year (2010 and 2011) on stage of decomposition in March of picked leaves placed in grids under trees.

Year	Decomposition stage <sup>z</sup>
2010	3.77 a
2011	4.45 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

Leaf decomposition of naturally fallen leaves was significantly more advanced during November 2010 than November 2011 (Table 4.4.6.17).

The combined ratings of the year, fungicide programme and leaf litter treatments showed the 2010 mancozeb, Compost aid + Breakdown all programme to be significantly more composed than the rest of the programmes besides the 2010 strobilurin, no leaf litter programme in February. The 2010 decomposition of naturally fallen leaves was significantly more advanced during the February evaluation than the corresponding period the following season (Tables 4.4.6.18 and 4.4.6.19). In 2010, leaves from fungicide programmes that received Breakdown-All + Compost Aid were at a more advanced stage of decomposition than corresponding programmes of which leaf litter were not treated.

**Table 4.4.6.17.** The effect of the year on stage of decomposition in November of naturally fallen leaves (leaf litter).

Year	Decomposition stage <sup>z</sup>
2011	1.91 a
2010	2.69 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.4.6.18.** The effect of the year, fungicide programmes and leaf litter treatments, on stage of decomposition in February of naturally fallen leaves (leaf litter).

Year and programme combinations			Decomposition stage <sup>z</sup>
Year	Fungicide programme	Leaf litter treatment	
2010	Mancozeb	Breakdown-All + Compost Aid	3.00 d
2010	Mancozeb	None	2.00 b
2010	Strobilurin	Breakdown-All + Compost Aid	2.17 bc
2010	Strobilurin	None	2.50 cd
2011	Mancozeb	Breakdown-All + Compost Aid	1.08 a
2011	Mancozeb	None	1.27 a

2011	Strobilurin	Breakdown-All + Compost Aid	1.50 a
2011	Strobilurin	None	1.16 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.19.** The effect of the year on stage of decomposition in February of naturally fallen leaves (leaf litter).

Year	Decomposition stage <sup>z</sup>
2011	1.26 b
2010	2.40 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

#### Disease incidence

The mean percentage clean fruit over the evaluation period differed significantly regarding the pre-blossom treatments with the 2011 'No treatment (benomyl)' treatment with significantly more diseased fruit than the rest of the treatments in 2011 and previous years (Table 4.6.6.20). The 2009 season had significantly less diseased fruit than the other seasons in these combinations but not within the season. There was no difference in the percentage diseased fruit in the different fungicide programmes (Table 4.6.6.21) and leaf litter treatments (Table 4.6.6.22).

**Table 4.6.6.20.** The effect of pre-blossom treatments and season, on the percentage of CBS diseased fruit.

Year and programme combination		Disease Index <sup>y</sup>
Year	Pre-blossom treatment	
2009	No treatment	12.6 d
2009	Urea	10.3 d
2009	Benomyl	13.6 d
2010	No treatment	41.5 bc
2010	Urea	37.5 bc
2010	Benomyl	32.7 c
2011	No treatment	44.8 b
2011	No treatment (Urea) <sup>z</sup>	39.8 bc
2011	No treatment (Benomyl) <sup>z</sup>	62.4 a

<sup>y</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

<sup>z</sup>Due to late harvesting, no pre-blossom treatments were made in 2010 leading up to the 2011 analyses.

**Table 4.6.6.21.** The effect of fungicide programmes throughout the different growing seasons, on the percentage of CBS diseased fruit.

Fungicide programme	Percentage diseased fruit <sup>z</sup>
Mancozeb	33.2 a
Strobilurin	32.4 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.22.** The effect of leaf litter treatments throughout the different growing seasons, on the percentage of CBS diseased fruit.

Leaf litter treatment	Percentage diseased fruit <sup>z</sup>
Breakdown-All + Compost Aid	33.4 a
No treatment	32.2 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

The disease index (DI) differed significantly between seasons (DI with 2009 the lowest, significantly lower than both 2010 and 2011 seasons). The 2011 strobilurin programmes had significantly more disease than those in other seasons. The DI was also higher than that of the mancozeb programme for the year but not significantly so. In 2010, the DI of the mancozeb programme was higher than the strobilurin programme and

in 2009 the strobilurin programme was higher than the mancozeb programme but not significantly so (Table 4.6.6.23).

**Table 4.6.6.23.** The effect of fungicide programmes and year, throughout the different growing seasons, on the disease index

Year and programme combination		Disease Index <sup>z</sup>
Year	Fungicide programme	
2009	Mancozeb	5.7 d
2009	Strobilurin	12.8 d
2010	Mancozeb	39.2 bc
2010	Strobilurin	33.7 c
2011	Mancozeb	43.4 ab
2011	Strobilurin	50.0 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

The combined DI of the interaction between year and pre-blossom treatments had significant differences with the 2011 No treatment (benomyl) treatment having the highest DI (Table 4.6.6.24). Due to late harvest, no pre-blossom treatments were applied which may have contributed to this increased disease presence. In 2010, the untreated plots had a higher DI than the urea and benomyl treatments but not significantly so. In 2009, however, the untreated plots had the lowest DI.

**Table 4.6.6.24.** The effect of pre-blossom treatments and year on the disease index

Year and programme combination		Disease Index <sup>y</sup>
Year	Pre-blossom treatment	
2009	No treatment	5.7 d
2009	Urea	11.6 d
2009	Benomyl	10.3 d
2010	No treatment	40.9 bc
2010	Urea	36.7 bc
2010	Benomyl	31.8 c
2011	No treatment	42.0 b
2011	No treatment (Urea) <sup>z</sup>	37.5 bc
2011	No treatment (Benomyl) <sup>z</sup>	60.6 a

<sup>y</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

<sup>z</sup>Due to late harvesting, no pre-blossom treatments were made in 2010 leading up to the 2011 harvest.

Leaf litter treatments had a significant effect on DI over the trial period with the Breakdown All + Compost Aid showing significantly more disease than the treatments where leaf litter was not treated (Table 4.6.6.25). The fungicide programmes had no significant effect on the mean DI over the trial period (Table 4.6.6.26).

**Table 4.6.6.25.** The effect of leaf litter treatments on the disease index.

Leaf litter treatment	Disease Index <sup>z</sup>
Breakdown All + Compost Aid	33.5 a
No treatment	28.1 b

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

**Table 4.6.6.26.** The effect of fungicide programmes on the disease index.

Fungicide programme	Disease Index <sup>z</sup>
Strobilurin	32.1 a
Mancozeb	29.4 a

<sup>z</sup>Mean values in a column followed by the same letter are not significant different from each according to Fisher's t-test ( $P = 0.05$ ).

## Discussion and conclusion

The number of ascospores trapped from picked leaves placed under trees in grids, differed significantly between seasons. The reason for this difference between seasons can only be speculated about but the leaf infection rate of picked leaves, as well as the infection level and development stage of pseudothecia at the moment of KIM trapping evaluation would have played an important role. The highest ascospore numbers during the summer evaluation were counted in grids from plots that received pre-blossom urea and a mancozeb programme in 2009. In general, more spores were trapped in mancozeb programmes than strobilurin programmes during the different evaluation periods from grids as well as from natural leaf litter. In most cases, leaves from mancozeb programmes were also in a more advanced stage of decomposition than those from strobilurin programmes; those placed in grids, significantly so. Besides the fungicidal effect of strobilurin fungicides, that may attribute to this finding, the physiological effect of the fungicide group on plants, may also play a role, especially the so called greening or delayed senescence effect caused by strobilurin fungicides (Venancio *et al.*, 2003). Fallen leaves under trees may have, therefore, been in a different stage of decomposition with pseudothecia at different stages of development as shown in the decomposition stages.

Compost Aid + Breakdown All treatment of leaf litter caused increased spore release from picked leaves in grids; significantly so in 2009 during summer evaluations. This effect was still present, but not so prominent later during autumn evaluations. In 2010, the Compost Aid + Breakdown All did significantly contribute to the stage of decomposition of natural leaf litter in mancozeb treated plots compared to corresponding plots that did not receive leaf litter treatments. The effect of enhanced composting of leaf litter by applying of products like Compos Aid + Breakdown All may not be as effective in South Africa as in Brazil (Belotte *et al.*, 2009) as a result of lower rainfall, different climate and slower decomposing processes. Leaf composting stage seems to play a critical role in optimal spore production and ascospore release. Enhanced composting may, for instance, only occur after peak spore release and not eliminate ascospore bearing leaf litter prior to infection periods as the case with a perennial crop like apples and apple scab (Beresford *et al.*, 2000; Carisse *et al.*, 2000; Jespersen, 1995). In citrus, leaves also drop over an extended period of time and trees do not lose all their leaves within a short time.

The pre-blossom benomyl or urea applications did not influence the stage of leaf decomposition (results not shown). Pre-blossom applications was not done during the last season as a result of late harvest, after flowering, which should be taken into account in interpreting the results. It had, however, an effect on ascospore ejection during summer evaluation of leaves in grids but not consistent within the different fungicide programmes. Within the mancozeb programmes, the benomyl pre-blossom treatments had significantly less ascospores detected than the urea pre-blossom treatments. The strobilurin programmes that received pre-blossom urea, in contrast, had the lowest spore counts with the untreated plots the highest.

There has been a general increase in the incidence of citrus black spot fruit symptoms in the orchard over the trial period despite fungicide programmes generally effective to prevent ascospore initiated fruit infections. In old orchards with trees in poor condition, as the case in the trial orchard, conidia produced on dead twigs, may be an important source of fruit infection (Spósito *et al.*, 2011; Swart and van Broekhuizen, 2004). The importance of infected fruit overlapping with the new crop was also found to be an important inoculum source by Spósito *et al.* (2011). Fruit from the trial orchard was only harvested after the fruit set in the 2010/2011 season, thus serving as additional inoculum for the 2011 harvest season.

Within the 2009 and 2010 seasons, when pre-blossom treatments were applied, none of the treatments had a significant effect on the percentage diseased fruit. The fungicide programmes and leaf litter treatments had no effect on percentage diseased fruit. The disease index, which indicates the severity of infection, showed an interaction between the season and fungicide programmes applied. Significant differences were found between seasons but not within seasons. The strobilurin programme, however, had a higher disease index than the mancozeb programme in two of the three seasons. The pre-blossom programme year combinations showed an effect on disease index between seasons, but only the 2011 no treatment (benomyl) trial blocks had a significantly higher index than the other treatments that year. No pre-blossom applications were done during the 2010/11 season and the effect was not the same in the previous seasons when benomyl pre-blossom programmes was followed. Over the trial period, the pre-blossom and fungicide programmes did not had an effect on disease incidence but the leaf litter treatments did, with plots treated with Compost Aid + Breakdown All having a significant higher disease index than plots that was not treated.

Fungicide programmes had an influence on spore release and leaf decomposition but did not influence the disease presence and severity. The pre-blossom programmes gave varying results. Leaf litter treatment with Compos Aid + Breakdown All enhanced spore release and did result in increased disease severity as indicated by disease index.

Micro and drip irrigation, only wets a part of the leaf litter, and therefore can also play a role in the variability of leaf litter in different stages of decomposition. The KIM system is useful in determining the presence of ripe ascospores in leaf litter at a specific moment but cannot determine continuous inoculum data for an extended period of time. In this study, continuous monitoring of ascospore counts during the infection period would probably have resulted in more useful data; however due to the trial layout and large number of treatment combinations this was not possible. Reis *et al.* (2006) found a strong relationship between disease severity and total rainfall and no relationship between disease severity and leaf wetness or ascospore numbers.

Disease severity on fruit might not necessarily correlate with ascospore inoculum levels, measured at any specific time during the growing season, since disease severity is normally a cumulative result of all the successful infections during the growing season. Pseudothecia are produced in fallen and decomposing leaves, and ascospores of *G. citricarpa* can be produced during the entire year. Results of several years of inoculum trapping in commercial citrus orchards show that ascospores are produced abundantly for at least 7 months after petal drop in South Africa (QMS Ascospore Data) and at least for 5 months after petal drop in Brazil (Reis, *et al.*, 2006). The observation of elevated ascospore numbers was mostly associated with rain events and not necessarily with amount of rain. Disease severity normally correlates with the number of duration of infection periods, predominately determined by extended wetting periods.

Ascospore release in orchards routinely monitored by QMS Agri Science shows a consistent increase in the ascospore release over the last number of years. Strobilurin programmes are applied in the orchards monitored which, according to the findings in this study, should lower ascospore numbers. One of the cultural practices that changed over the period is the chipping and use of pruned material as mulches in the orchards. Leaf litter is normally seen as the primary source of ascospores. However, high numbers of ascospores were detected with the KIM system, on dried-out twigs and branches 7 months after removal of the experimental orchard. Material other than leaves may be important sources of ascospores. Another practice that changed is the replacement of benomyl programmes by strobilurin programmes.

#### **Further objectives (milestones) and work plan**

The trial orchard has been removed in 2011. Results from this trial should be used to compile a more realistic trial on a semi-commercial scale in orchards with healthier trees and less diverse situations. Disease pressure should not be a parameter to evaluate. In orchard spore monitors should be used to monitor ascospore release in an orchard where different cultural practices are employed, i.e. removal of pruned material from the orchard vs. chipping and mulching. The difference between spore release in strobilurin and mancozeb programmes can also be monitored. The presence of ascospores on dead wood in trees as well as on chipped pruned material should also be determined with KIM monitoring.

#### **Technology transfer**

Results will be presented on the biennial CRI Symposium in August 2012.

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**4.6.7 PROGRESS REPORT: Epidemiology and pest risk assessment of *Guignardia citricarpa***  
Experiment 1026 (April 2011 - March 2014) by Paul Fourie, Vaughan Hattingh and Tian Schutte (CRI)

**Summary**

Citrus Black Spot is the most important citrus disease in South Africa, especially given its impact on market access. A considerable amount of effort and *ad hoc* research is conducted on an ongoing basis to service market access to these markets. This project formalises the *ad hoc* research and will focus on developing and improving a model for *Guignardia pseudothecium* maturation and ascospore dispersal based on meso-climatic weather data. On this topic, initial modelling research was completed and an article was provisionally accepted for publication in a leading scientific journal. Additionally, three CRI-researchers are collaborating on a project funded by the Florida citrus industry in USA to develop a quantitative pest risk assessment of *Guignardia citricarpa*, with special emphasis on the fresh fruit pathway. Two workshops were held in Florida and substantial progress was made by identifying the various steps in the model, assigning probabilities to these steps and identifying research gaps. These research gaps are currently being addressed in this project, and include data collection to improve the models and to distinguish between the CBS pathogen and endophytic *Guignardia* sp., new modelling approaches and surveys to determine efficacy of CBS orchard and packhouse control measures.

**Opsomming**

Sitrus swartvlek is die belangrikste sitrus-siekte in Suid-Afrika, veral gegewe sy impak op marktoegang. Baie aandag en *ad hoc* navorsing is onlangs hieraan gespandeer. Hierdie projek formaliseer die *ad hoc* navorsing en sal fokus op die ontwikkeling en verbetering van 'n model vir *Guignardia pseudotesium* rypwording en spoorvrystelling gebasseer op meso- en mikroklimate data. Op hierdie onderwerp is aanvanklike modelering afgehandel en 'n artikel voorwaardelik aanvaar in 'n toonaangewende wetenskaplike joernaal. Verder is sekere CRI navorsers betrokke in 'n samewerkingsprojek wat deur die Florida sitrusbedryf in VSA befonds word. Hierdie doelwit beoog om 'n kwantitatiewe pes risiko analiese vir *Guignardia citricarpa*, met spesifieke fokus op vars vrugte as verspreidingsweg, te ontwikkel. Twee werkwinkels is gehou, en noemenswaardige vordering is gemaak, spesifiek deur die verskillende stappe in die model te identifiseer, moontlikhede van die stappe te kwantifiseer, en gapings in die beskikbare kennis te identifiseer. Navorsing is tans onder weg om nuwe data in te samel ter verbetering van die modelle en om tussen die swartvlek patogeen en 'n endofitiese *Guignardia* sp. te onderskei, nuwe modellering-strategië te beproef, asook ondersoek om die sukses van swartvlek beheer in boorde en pakhuse te kwantifiseer.

**4.7 CRI DIAGNOSTIC CENTRE** (Lorika Beukes, Elbie Liebenberg, Elaine Basson, Vongani Rikhotso and Timothy Zulu - CRI)

<b>Analysis</b>	<b>Citrus nurseries</b>	<b>Commercial samples</b>	<b>Other crops</b>	<b>Research samples</b>	<b>River Bioscience</b>
Nematodes: Roots	9	916	53	1559	
Nematode: Soil	1	40	52	1594	
<i>Phytophthora</i>	2115	845	42	778	
Water	102	13	3		
Black spot identification (PCR)		20		49	
Black spot benzimidazole resistance		6			
Fungal isolations		7	5		
Citrus greening (PCR)		13			
Soil dilution plating		1			
Fruit and foliar identification		3	3		
Quality control					43

**Citrus Accredited Nurseries**

It is compulsory for all citrus nurseries participating in the Citrus Improvement Scheme to submit samples for *Phytophthora* analysis on a quarterly basis. The irrigation water must also be tested for *Phytophthora* by making use of the spore trap method. In total, 2115 nursery samples were received by the Diagnostic Centre for *Phytophthora* analyses. Of these samples, 15.6% tested positive. In addition to soil and water

samples, nurseries are required to send root samples once a year to test for the presence of *Tylenchulus semipenetrans*. For the nematode root samples, 11.1% tested positive and for the nematode soil samples 0.0% tested positive.

### Commercial samples

Samples were received from the following citrus growing areas: Mpumalanga, Eastern Cape, Western Cape, Swaziland, Limpopo and Gauteng. Most of the samples received from citrus growers were analysed for *Phytophthora nicotianae* and the citrus nematode, *T. semipenetrans*. Forty-one percent of the 916 samples analysed for citrus nematode had counts above the threshold value of 1000 females per 10 g of roots, and nematicide treatments were recommended. Sixty one percent of the 845 samples analysed for *Phytophthora* tested positive.

### Other crops

Nematode counts were done on soil or root samples of cotton, leather leaf fern, watermelon, wheat, potatoes, pumpkin and peppadews. Nematodes found present on these crops included: *Tylenchorhynchus*, *Hemicycliophora*, *Pratylenchus*, *Scutellonema*, *Xiphinema Criconemoides*, *Trichodorus*, *Meloidogyne*, *Helicotylenchus* and *Longidorus*. *Phytophthora* and *Pythium* analyses were done on macadamia, leather-leaf fern, avocado, coco peat, coffee beans, apricots and peppadews. The diagnostic centre analysed twenty soil samples from macadamia nurseries and seven from avocado nurseries for the presence of *Phytophthora cinnamomi*.

### Research samples

Nematode and *Phytophthora* analysis were done on 1594 samples from experimental trials. PCR protocols for the identification of citrus black spot form part of services provided by the Diagnostic Centre.

### River Bioscience

Quality control tests were conducted on River Bioscience samples. These tests included virus enumeration, dose-response bioassays and total bacterial counts of their product, Cryptogran. In total, 43 samples were analysed and results reported to River Bioscience.

**CRI DIAGNOSTIESE SENTRUM** (Lorika Beukes, Elbie Liebenberg, Elaine Basson, Vongani Rikhotso en Timothy Zulu - CRI)

Ontleding	Sitrus kwekerie	Kommersiële monsters	Ander gewasse	Navorsings -monsters	River Bioscience
Aalwurms: Wortels	9	916	53	1559	
Aalwurms: Grond	1	40	52	1594	
<i>Phytophthora</i>	2115	845	42	778	
Water	102	13	3		
Swartvlek (PKR)		20		49	
Swartvlek benzimidazole bestandheid		6			
Swamisolasies		7	5		
Sitrusvergroeningsiekte (PKR)		13			
Grondverdunningsplate		1			
Vrug en blaarsiektes		3	3		
Kwaliteitsbeheer					43

### Sitrus Geakkrediteerde Kwekerie

Dit is verpligtend vir al die sitruskwekerie wat aan die Sitrus Verbeteringskema deelneem om kwartaalliks monsters vir *Phytophthora* te laat ontleed. Die besproeiingswater moet ook deur middel van die spoorlokval metode vir *Phytophthora* getoets word. In totaal 2115 monsters is deur die Diagnostiese Sentrum vir *Phytophthora* ontleding ontvang, waarvan 15.6% positief getoets het. Benewens die water en grondmonsters, moet kwekerie een keer per jaar 'n wortelmonster instuur om vir die teenwoordigheid van *Tylenchulus semipenetrans* te toets. Van die 9 wortelmonsters wat ontvang is, het 11.1% positief vir die teenwoordigheid van *T. penetrans* getoets en van die 1 grondmonsters het 0.0% positief getoets.

## **Kommersiële monsters**

Monsters is uit die volgende sitrusverbouingsareas ontvang: Wes-Kaap, Mpumalanga, Limpopo, Swaziland, Gauteng en Oos-Kaap. Die meeste van die monsters wat van sitrusprodusente ontvang is, is vir *Phytophthora nicotianae* en die sitrusaalwurm, *Tylenchulus semipenetrans*, ontleed. Een-en-veertig persent van die 916 aalwurmmonsters wat ontleed is, het tellings hoër as die drempelwaarde van 1000 wyfies per 10 g wortels gehad. Aalwurmdoderbehandelings is aanbeveel. Een-en-sestig persent van die 845 monsters wat vir *Phytophthora* ontleed is, het positief getoets.

## **Ander Gewasse**

Aalwurmtellings is op grond- of wortelmonsters van katoen, leerblaar varing, waatlemoen, graan, aartappels, pampoens en peppadews. Aalwurms teenwoordig gevind op hierdie gewasse sluit in: *Tylenchorhynchus*, *Hemicycliophora*, *Pratylenchus*, *Scutellonema*, *Xiphinema Criconemoides*, *Trichodorus*, *Meloidogyne*, *Helicotylenchus* en *Longidorus*. Makadamia, leerblaar varing, avokado en kokosneuthaar, appelkose, koffiebone en peppadews monsters is vir *Phytophthora* en *Pythium* ontleed. Die diagnostiese sentrum het twintig monsters vanaf macadamia kwekerie en sewe monsters vanaf avokado kwekerie vir *Phytophthora cinnamomi* ontleed.

## **Navorsingsmonsters**

Aalwurm en *Phytophthora* ontleding is op 1594 monsters afkomstig uit navorsingsprojekte om aalwurmdoders te toets, gedoen. PKR protokolle vir die identifikasie van sitrus swartvlek is geoptimeer en word vervolgens as 'n diens binne die diagnostiese sentrum gelewer.

## **River Bioscience**

River Bioscience kwaliteitsbeheer toetse sluit virus tellings, dosis-reaksie toetse en totale bakteriese tellings van hulle produk, Cryptogran, in. In totaal is 43 monsters ontleed en resultate aan River Bioscience gestuur.

## 5 PROGRAMME: HORTICULTURE

### 5.1 PROGRAMME SUMMARY

By Tim G Grout (Manager: Research & Technical)

Recently there has been a lot of emphasis on the state of the economy in our markets and discussions on how to reduce costs in the cold chain, so it is easy to overlook possible savings that can be gained by doing the basics right and avoiding unnecessary losses. As profit margins shrink, sufficient production per hectare becomes critical and water management and avoidance of stress must be prioritised. Water is likely to become increasingly scarce in the future so research on its efficient use is being increased and other supplements that may reduce stress or improve root systems are being investigated. Alternate bearing can mean that citrus is unprofitable every second year but research has shown that foliar carbohydrate levels just before harvest can be used as a tool in managing this problem. Further progress has been made in reducing losses from peteca spot, now that ethylene is known to play a key role, and results from research on other rind condition problems such as chilling injury and rind pitting have reduced losses from these disorders when certain practices are implemented. Creasing remains a complex challenge but it was shown that foliar sprays of calcium do not contribute to calcium levels in the flavedo and that soil treatments would be required for this purpose. Continuing research on reversed air flow in shipping containers has confirmed earlier results of more rapid cooling at a lower energy cost. With the formation of CGA's Cultivar Company, the impartial cultivar and rootstock evaluations that CRI conducts were brought into this programme as part of CRI's research division. These evaluations are being conducted in citrus production regions throughout South Africa and Swaziland in order to determine where the best production and quality can be obtained with new cultivars that will carry this industry into the future.

### PROGRAMOPSOMMING

Daar is onlangs baie klem gelê op die stand van die ekonomie in ons markte en besprekings oor hoe om kostes in die koue ketting te verminder. Dit is dus maklik om moontlike besparings wat behaal kan word deur die basiese reg te doen en onnodige verliese te vermy oor die hoof te sien. Soos die winsgrense krimp, word hoë produksie per hektaar al belangriker en moet die bestuur van water en vermyding van stres geprioritiseer word. Water gaan waarskynlik in die toekoms al hoe skaarser raak, so navorsing oor die doeltreffende gebruik daarvan het toegeneem en ander aanvullings wat stres kan verminder of wortelstelsels verbeter, word ondersoek. Alternatiewe drag kan te weeg bring dat sitrus elke tweede jaar nie winsgewend is nie, maar navorsing het getoon dat koolhidraatvlakke van blare net voor oes, as 'n hulpmiddel gebruik kan word om hierdie probleem te bestuur. Verdere vordering is gemaak in die vermindering van verliese weens peteka kol nadat dit aan die lig gekom het dat etileen 'n belangrike rol speel. Resultate van navorsing op ander skildefekte soos koueskade en gepokte skil het getoon dat verliese verminder kan word deur die implementering van sekere praktyke. Kraakskil bly egter 'n komplekse uitdaging, maar daar is bewys dat blaarbespuitings van kalsium nie bydra tot kalsiumvlakke in die flavedo nie en dat grondbehandelings vir hierdie doel nodig sal wees. Voortgesette navorsing oor omgekeerde lugvloei in skeepshouers het vroeëre resultate van vinniger verkoeling teen 'n laer energiekoste bevestig. Met die stigting van die CGA se kultivarmaatskappy is die onpartydige kultivar en onderstok evalueringe wat CRI gedoen het by hierdie program, as deel van CRI se navorsing-afdeling, ingesluit. Hierdie evaluasies word in sitrus produserende streke regoor Suid-Afrika en Swaziland gedoen, ten einde te bepaal waar die beste produksie en kwaliteit met nuwe kultivars verkry kan word wat die bedryf die toekoms moet inlei.

### 5.2 PROJECT: RIND CONDITION

Project coordinator: Paul Cronjé (CRI-SU)

#### 5.2.1 Project summary

All aspects affecting the incidence of rind disorders remain a strong focus area in the Horticulture programme due to the negative economic impact on grower returns. On several of the physiological disorders, significant advances were made during the previous season. The importance of good colour development and the protection from carotenoids during cold sterilisation to the flavedo were illustrated by the lack of chilling injury in well-coloured 'Star Ruby' grapefruit (5.2.2). A similar reduction to rind breakdown in well-coloured 'nules Clementine' mandarin was recorded in previous seasons, illustrating the need to make a distinction based on fruit colour for specific markets. In addition, it was shown that TBZ (known to reduce non-chilling rind pitting) also reduced the incidence of chilling injury if applied in a warm water bath (5.2.2). The results on peteca spot of lemons confirmed previous seasons' reports of an effect of ethylene metabolism in the rind on determine fruit susceptibility to this postharvest disorder. Preharvest manipulations of the fruit ethylene synthesis with Ethephon and AVG did reduce the incidence in 2011 (5.2.3, 5.2.5). Rind pitting and staining of 'nadorcott' mandarin was shown to be aggravated by dehydration of the fruit after harvest and before

waxing. It is therefore important to reduce the water loss from the fruit after harvest to avoid these disorders. In addition, fruit grown on 'Rough lemon' rootstock were more susceptible compared to 'Carrizo citrange' (5.2.4). In an experiment to test new techniques to manipulate mineral nutrient allocation in the tree (5.2.5), it was shown that fruit thinning agents (Maxim and Corasil P) could possibly increase the Ca and Mg levels in the flavedo as well as decrease the K (5.2.6). In a detailed experiment on creasing, it was concluded that foliar Ca applications during fruit development are as yet not an effective strategy to increase fruit rind Ca content. In addition, the impact of season climatic variation on fruit susceptibility to this complex disorder was highlighted by the low creasing incidence during the high rainfall season in the Eastern Cape in 2011 (5.2.7).

## Projekopsomming

Navorsing op verskeie aspekte wat betrokke kan wees in die voorkoms van fisiologiese skildefekte, bly 'n strek fokus area van die Vrugkwaliteit portefeulje. In die voorafgaande seisoen is daar vordering gemaak in verskeie van die skildefekte. Die feit dat goed opgekleurde 'Star Ruby' pomelo's nie koueskade ontwikkel nie, wys weereens die belangrike beskermende aksie van karotene in die voorkoming van skildefekte uit. Die resultaat stem ooreen met die laer skilafbraak wat voorkom in goed opgekleurde 'hules Clemetine' mandaryn, en ondersteun die argument vir kleursortering vir verskillende markte. Daar was ook bevind dat TBZ, bekend vir die vermindering van "non-chilling rind pitting" koueskade van Nawel lemoene verminder as dit in 'n warm bad toegedien word. Die resultate van proewe om peteka kol was bevestigend tot die vorige seisoen se data. Voor-oes toediening van Ethephon en AVG, wat beide die vrug etileen metabolisme beïnvloed, het gelei tot verlaagde voorkoms van peteka. Gepokteskil van 'nadorcott' mandaryne word vererger as die vrugte vog verloor in die periode tussen pluk en verpakking, en daar moet gelet word op die inkorporering van enige maatreël om die vogverlies te beperk in die na-oes ketting. Daar was ook gevind dat onderstamkeuse die voorkoms van skildefekte beïnvloed en 'Groweskil suurlemoen' onderstam het tot 'n hoër insidensie gelei as 'Carizzo citrange'. Die invloed van uitdunmiddels (Maxim en Corasil P) is geëvalueer i.t.v. hulle invloed op akkumulering van voedings minerale, en beide het gelei tot 'n verhoging van Ca en Mg asook 'n verlaging van K in die flavedo. 'n Intensiewe eksperiment om kraakskil te verminder is daar gevind dat blaarbespuitings met verskillende Ca bevattende middels nie betekenisvol die Ca vlakke in die skil verhoog nie. Gedurende die eksperimente is die oorkoepelende effek van klimaat op die voorkoms van kraakskil weer uitgelig, na uiters lae voorkoms was in die 2011 wat gekenmerk was deur baie hoë reënval syfers.

### 5.2.2 PROGRESS REPORT: Development of postharvest treatments to prevent chilling injury in various citrus species

Experiment 832 (2005/6-2011/12) by PJR Cronje (CRI-SU)

#### Summary

Cold disinfestation protocols, e.g. a mandatory  $-0.5^{\circ}\text{C}$  for 24 days to certain markets, reduce the quality of fruit due to the development of chilling injury (CI) symptoms in the fruit rind. There is also an indication that the same cultivar produced in different microclimates, i.e. colder vs. warmer, do differ in susceptibility to chilling injury, with fruit in colder areas being more susceptible. The further experiment in this project aimed to determine the effect of different postharvest fungicides i.e. Benzimidazole, Benazid<sup>®</sup>, Thiabendazole (Tecto<sup>®</sup>) and Thiabendazole (ICA-TBZ<sup>®</sup>), on the chilling injury susceptibility of 'Washington Navel' oranges (*Citrus sinensis* L. Osb.) in warm and cold baths. The results indicated that the Tecto<sup>®</sup> treatments were most effective in reducing the incidence and severity of chilling injury and were more effective when used in warm water than in cold water. Further research will focus on how to optimize postharvest application in the citrus packhouse. In addition to postharvest treatments the importance of the rind condition at harvest is thought to be critical in determining the susceptibility to CI. This aspect was studied in 'Star Ruby' grapefruit and a clear correlation between amount of lycopene in the flavedo and CI susceptibility was seen. Lycopene synthesis in shaded grapefruit rind is significantly higher compared with sun-exposed fruit. The direct influence of sunlight on this synthesis pathway is unknown, however, the extremely high antioxidant capacity of lycopene is thought to be central in protecting the fruit against the development of chilling damage.

#### Opsomming

Die uitvoer van sitrusvrugte na spesifieke markte vereis 'n temperatuur protokol waartydens die vrugte vir 24 dae aan  $-0.5^{\circ}\text{C}$  blootgestel moet word, om te verseker alle insekklawes word gedood. Die behandeling lei tot hoë vlakke van koueskade simptome nl. gepokteskil of verbruining van die flavedo. Dit wil bleik of daar wel 'n impak is van mikroklimaat op kouegevoeligheid en vrugte uit koue dele van 'n vallei was meer vatbaar. 'n Verder eksperiment in die projek het ten doel gehad om die effek te bepaal van verskillende na-oes swamdodders i.e. Benzimidazole, Benazid<sup>®</sup>, Thiabendazole (Tecto<sup>®</sup>) en Thiabendazole (ICA-TBZ<sup>®</sup>), op die

vatbaarheid van koueskade van 'Washington' Navel lemoene (*Citrus sinensis* L. Osb.) wat opgelos in warm of koue baddens. Daarna is ook bepaal of die gebruik van verskillende konsentrasies van die mees suksesvolle swamdoder in warm of koue baddens die vatbaarheid van koueskade van 'navel' vrugte verminder. Die resultate het aangedui dat Tecto<sup>®</sup> behandeling die mees suksesvol was in die vermindering van koueskade voorkoms en die behandeling was meer effektief in warm water as in koue water. Verdere navorsing sal fokus om die na-oes toediening in sitrus pakhuis te optimaliseer. In verder proewe is die belangrike invloed van voor-oes toestande tydens vergroei op skilkwaliteit en kouegevoeligheid getoets. 'Star Ruby' pomelo wat in die direkte son ontwikkel, het 'n baie lae skil konsentrasie van likopeen, teenoor vrugte wat in die skadu ontwikkel het. Hierdie goed gekleurde vrugte is egter betekenisvol minder gevoelig vir koueskade vermoedelik a.g.v. die likopene se antioksidatiewe eienskappe wat die selmembrane beskerm tydens lae tempertuurstres. Die rede vir die hoër likopeen sintese in die skadu (binne in die blaardak) is onbekend en verskil van ander karotene in die sitruskil.

### 5.2.3 PROGRESS REPORT: Effect of different chemical applications on development of Peteca spot in lemons

Experiment 833 (2006/7-2012/3) by PJR Cronje (CRI-SU)

#### Summary

Peteca spot (PS) of lemon is a postharvest physiological disorder resulting in the collapse of the oil gland. Subsequently the oil leaks into the adjacent tissue and causes a darkened depression or sunken area. The occurrence can be severe, resulting in substantial economic losses without any specific pre- or postharvest practises that could be implemented to avoid or significantly reduce the incidence. PS occurs in all production areas of South Africa and is thought to be the result of the immature rind being subjected to postharvest stress associated with high CO<sub>2</sub>, the packing line and wax application. Although earlier reports linked PS to an imbalance of calcium in the rind this hypothesis is currently not universally accepted. Over several seasons the incidence of PS was investigated to identify factors that influence the incidence of the disorder. The first observation was the highly erratic incidence between seasons as well as between orchards. In addition, PS incidence varies significantly within an orchard in a season, with the early fruit being highly susceptible. In experiments to identify postharvest factors which influence the PS incidence, ethylene (3 ppm) and CO<sub>2</sub> (1%) were applied in a continuous flow-through system, at 20°C for 3 days. The CO<sub>2</sub> treatments resulted in significantly higher incidence compared to the control (air) or ethylene treatments. Following on from these results, postharvest Ethephon (2-Chloroethyl phosphoric acid) (200 mg/L and 400 mg/L) and AVG (aminoethoxy-vinylglycine) (400 mg/L and 800 mg/L) applications to fruit resulted in a significant reduction in PS. In the subsequent season, the same treatments were applied in an orchard one week before harvest and a similar reduction in PS was recorded for the Ethephon and AVG treatments. Ethylene synthesis by these fruit was also measured after harvest and a transient spike was recorded in fruit receiving the two treatments, but was absent in the control fruit. The results collected over several seasons could indicate a protective action of ethylene to reduce the rind sensitivity to PS. It is hypothesised that if the internal ethylene synthesis is increased in sensitive fruit i.e. immature fruit, a reduction in PS can occur due to a protective action.

#### Opsomming

Petaka kol (PK) van suurlemoen is 'n na-oes fisiologiese skildefek waar die olieklier in skeur en lek die olie uit in die omliggende weefsel en lei tot 'n donker versonke letsel in die skil. Die voorkoms kan uiters hoog wees en lei tot ernstige finansiële verliese, en daar bestaan tans nie 'n voorkomings of beheer maatreël nie. PK kom voor in alle suurlemoen areas in SA en daar word vermoed dat onvolwasse vrugteskille wat aan na-oes stres (soos hoë CO<sub>2</sub>, verpakking en waks aanwending) blootgestel word, lei tot 'n verhoogde voorkoms. Alhoewel vorige navorsing op die invloed van Ca-wanbalanse gedui het, word die teorie tans nie aanvaar nie. Oor verskeie seisoene was daar gepoog om die voorkoms van PK en die faktore wat dit beïnvloed te bepaal. Die eerste waarneming is die uiters wisselvallige voorkoms tussen seisoen en boorde. Verder meer verskil die voorkoms drasties in 'n boorde oor die seisoen, en is veral die vroeë pluk uiters sensitief vir PK. In 'n eksperiment om die direkte impak van CO<sub>2</sub> (1%) en etileen (3 ppm) te bepaal is die gasse asook lug in 'n geslote sisteem toegedien vir 3 dae teen 20°C. Die CO<sub>2</sub> het tot 'n betekenisvolle verhoging gelei in vergelyking met die kontrole en etileen gas. In aansluiting by die data is na-oes Ethephon (2-Chloroethyl phosphoric suur) (200 mg/L en 400 mg/L) en AVG (aminoethoxy-vinylglycine) (400 mg/L en 800 mg/L) toegedien op vrugte en 'n verlaging in albei behandelings is gesien. In die daaropvolgende jaar is die selfde middels een week voor oes gespuit en weereens is 'n verlaging in PK voorkoms gedokumenteer. Die etileen produksie van die laaste vrugte is gemeet en 'n tydelike styging in etileen produksie in die Ethephon en AVG (alhoewel laer as die Ethephon) is gemeet, waar so 'n piek afwesig was in die kontrolevrugte. In geheel gesien kan uit die data afgelei word dat 'n hoër vlak van interne geproduseerde etileen in die skil toe 'n verlaagde sensitiwiteit tot PK in die skil lei a.g.v. 'n onbekende beskermende funksie.

#### 5.2.4 **PROGRESS REPORT: Studies on aspects concerning rind pitting/staining citrus fruit** Experiment 958 (2009/10 – 2013/4) by PJR Cronje (CRI)

##### **Summary**

Postharvest physiological rind disorders, such as staining and pitting, affect all citrus cultivars and have a significantly negative impact on return on investment for producers. Fluctuations in rind water balance, as influenced by ambient conditions during handling may play a major part in inducing rind disorders. The aim of this research was to determine if the type of rootstock (known to influence water supply to fruit) and postharvest handling practices affect the incidence of pitting and staining in 'nadorcott' mandarin (*Citrus reticulata* Blanco) rind. Fruit were harvested at optimum maturity from two adjacent commercial 'nadorcott' mandarin orchards, grafted either on rough lemon or Carrizo citrange rootstocks and either immediately waxed and kept at ambient conditions or first dehydrated for 4 days at 20°C prior to waxing, or dehydrated before being subjected to a high RH (>90%) prior to waxing and storage at ambient conditions. After 14 days the fruit were evaluated for incidence of rind disorders and loss of rind firmness. The results indicated a significantly higher susceptibility of fruit from rough lemon rootstocks compared to fruit from Carrizo citrange. The postharvest dehydration prior to wax application induced significantly higher levels of rind disorders compared to fruit that was waxed within 24 hours after harvest. The data concurs with findings on different citrus rind disorders where a dramatic water loss, due to high vapour pressure deficit (VPD) resulted in an inadequate adjustment of the water status of the rind, leading to cellular collapse and tissue damage. It is hypothesized that rough lemon rootstocks, result in a rind with less of an ability to prevent water loss and therefore a higher rind disorder development. In addition, postharvest handling practices could aggravate the incidence of rind disorders. Therefore known and implementable postharvest practices such as removal of field heat and reduction of fruit VPD could decrease citrus postharvest rind disorders.

##### **Opsomming**

Na-oes fisiologiese skildefekte soos gepoketeskil en "staining", affekteer alle sitrus kultivars en het 'n negatiewe invloed op winsgewendheid. Daar word vermoed dat die verandering in die hoeveelheid vog in die vrugskil, soos beïnvloed deur na-oes hantering, 'n belangrike aandeel het in die indusering van skildefekte. Die doel van die eksperiment was om te bepaal of die onderstam asook uitdroging van die skil na pluk die voorkoms van skildefekte beïnvloed in 'nadorcott' mandaryn (*Citrus reticulata* Blanco) skil. Die vrugte is geoes van twee aangrensende 'nadorcott' boorde geplant op 'Carrizo citrange' en 'Growweskil suurlemoen' onderstamme. Na-oes is die vrugte verdeel in behandelings en is of dadelik gewaks en opberg teen hoë RH en kamer temperatuur of is eers vir 4 dae teen 20°C gedehidreer voor hoë RH (>90%) opberging. Na 14 dae is die vrugte geëvalueer vir die voorkoms van skildefekte. Die vrugte van die 'Growweskil'-boord het betekenisvol meer skildefekte gelewer in vergelyking met die 'Carrizo citrange'. Die vrugte wat gedehidreer was voor waks aanwending het meer gewigsverlies getoon wat korrespondeer met die betekenisvolle hoër voorkoms van skildefekte. Die data bevestig gerapporteerde resultate op ander sitrus kultivars, waar 'n wesenlike vogverlies a.g.v. hoë dampdruk verskil (VPD), tot hoër skildefekte sal lei na die skil nie die vogbalans kan regstel nie, en sel-verval tot die gevolg het. Uit die data kan die hipotese ontwikkel word dat 'Growweskil suurlemoene' onderstam die vogbalans of vermoë of die vogbalans te handhaaf, van die 'nadorcott' vrug negatief beïnvloed en lei tot meer skildefekte. Daar word aanbeveel dat enige na-oes praktyk, tussen pluk en waks aanwending wat vogverlies sal beperk geïmplementeer moet word. Verder meer word dit streng aanbeveel dat die tyd tussen pluk, waks aanwending en verkoeling so kort moontlik gehou word om die voorkoms van die defekte te beperk.

#### 5.2.5 **PROGRESS REPORT: Development of laboratory-based biochemical methods to determine the physiological condition of the citrus fruit flavedo** Experiment 962 (2009/10-2011/12) by PJR Cronje (CRI-SU)

##### **Summary**

Detailed biochemical studies of the citrus rind are necessary to understand the impact of environmental and management constraints on rind condition. To employ any new or established method to analyse a change in plant cell biochemistry requires a critical evaluation of the protocol to determine the optimal conditions and technique for extraction, purification and quantification of a biochemical compound. This is especially true if such a compound is extracted from citrus flavedo, which is very rich in secondary metabolites, e.g. essential oils and phenolics, which can distort the analysis values. Two methods were developed during the 2010/11 season, viz. a spectrophotometric method and an extraction and HPLC analysis method for lycopene and  $\beta$ -carotene content in the flavedo. The first method was improved from earlier work done on lycopene content in the fruit pulp at Outspan Citrus Centre. The second method was developed during a research visit to IATA, Valencia, from the method they currently use in carotenoid synthesis research. The

spectrophotometric method was used to analyse the lycopene content of fruit sampled in 2010. During 2011 the methods to measure ethylene and ACC were developed and tested for lemon fruit. All the full methods are available from the researcher.

## Opsomming

In die navorsingsprojekte van verskeie skildefekte is dit belangrik om die impak van omgewings en bestuursaspekte te kan bepaal op biochemiese prosesse in die skil. Om bekende asook nuwe ontledings metodes, soos gebruik in plantfisiologie in die skilkondisie-navorsing projekte in te sluit moet dit kritiese geëvalueer word t.o.v. ekstraksie en kwantitatiewe bepaling van spesifieke verbindings. Die rede hiervoor is dat die flavedo baie ryk aan verskeie sekondêre metaboliete is wat ontledings resultate kan beïnvloed. Gedurende 2010 is 2 metode ontwikkel en verfyn nl. 'n spektrofotometer, asook 'n HPLC protokol om likopeen en  $\beta$ -karoteen te ekstraheer en analiseer. Die spektrofotometries metode is 'n verbeterde metode van die ontwikkel in Outspan Sitrus sentrum om lycopene konsentrasie in 'Star Ruby' pomelo pulp te bepaal. Die 2<sup>de</sup> metode is met behulp van Drs. Zacarias en Rodrigo van IATA-Valencia ontwikkel gedurende 'n navorsings besoek aan die instansie. Die 1<sup>ste</sup> metode is al gebruik gedurende 2011 om die 2010 monsters te ontleed. Beide die volledige metodes is beskikbaar van die navorser. Gedurende 2011 is die metode om etileen en ACC vlakke te bepaal op 'n gas chromatograaf suksesvol ontwikkel en getoets op suurlemoen vrugte.

### 5.2.6 **PROGRESS REPORT: Increasing Ca and Mg content in the flavedo using novel techniques** Experiment 978 (2010/1-2011/2) by PJR Cronje (CRI-SU)

#### Summary

In previous studies it was determined that the content of the two important mineral nutrients Ca and Mg, could be sub-optimal in the flavedo of 'nules Clementine' mandarin fruit resulting in a reduction in rind condition and ultimately in rind breakdown. This mineral nutrient deficiency is thought to be related to the increased sensitivity of the fruit rind to physiological disorders. These suboptimal nutrient levels have been shown to develop during fruit growth and development and are influenced by position in the tree canopy. Canopy position microclimate affects transpiration which in turn appears to be the cause of mineral nutrient imbalances in different canopy positions. Therefore, by manipulating and improving the sink strength of the rind by reducing vegetative growth, it may be possible to increase the allocation of nutrients to the rind, specifically Ca and Mg, and to correct nutrient imbalances, thereby reducing rind breakdown. By application of Maxim and Corasil P, a change in some mineral nutrients' accumulation patterns in the fruit rind were seen viz. an increase in Ca and decrease in K. Although these agro-chemicals are known to reduce fruit load it is thought that they could have influenced the fruit sink positively.

## Opsomming

In 'n afgehandelde uitgebreide projek, waarop gefokus was op skilafbraak van 'nules Clementine' mandaryne, is vasgestel dat die Mg en Ca inhoud in die flavedo laer is in swakker vrugte. Die vrugte was ook meer vatbaar vir die ontwikkeling van skilafbraak en die posisie van die vrug in die boom (binne teenoor buite) bepaal tot 'n groot mate die minerale samestelling in die flavedo. Twee uitdunmiddels nl. Maxim en Corasil P is soos voorgeskryf gespuit op 'nules Clementine' mandaryn bome in Citrusdal (November) waarna die vrugte geoes was in Mei voor skil en blaarmonsters ontleed is. Die ontledings toon dat verskeie minerale elemente se konsentrasie verander in die skil in vergelyking met die kontrole behandelings nl. verlaging in K en verhoging in Mg. Die resultate gee 'n baie goeie aanduiding dat daar wel aan die skink-bron balans ten gunste van die skil verander is.

### 5.2.7 **FINAL REPORT: Influence of calcium foliar sprays on creasing (albedo breakdown)** Experiment 1002 (August 2010 – July 2011) by J T Vahrmeijer (CRI)

#### Summary

Many fruit and vegetable disorders are related to calcium (Ca) deficiencies. Soil and spray applications of Ca have been reported to ameliorate disorders such as fruit-cracking of sweet oranges, rind puffiness and rind-pitting, although with varied success. A field trial to evaluate the influence of Ca on rind condition was initiated in Addo in the Eastern Cape where creasing occurs regularly. Calcium chloride ( $\text{CaCl}_2$ ), calcium nitrate ( $\text{CaNO}_3$ ) and calcium nitrate with humic acid (HA) were sprayed on 'Cara-Cara' navel oranges. No evidence was found that foliar-applied Ca reduced creasing in this trial. The reason is that during this season there was a low incidence of creasing, not only in this specific orchard, but also in the area. It was

also found that no significant increase ( $P \leq 0.05$ ) in the Ca-content of the leaves and fruit were obtained with the foliar application of different Ca-formulations.

## Opsomming

Kalsium (Ca)-tekorte is die oorsaak van verlaging in kwaliteit van verskeie vrug- en groentetipes. Navorsing het getoon dat grond- en blaartoedings van Ca 'n verlaging in die voorkoms van skildefekte soos kraakskil het, alhoewel met wisselvallige welslae. 'n Veldproef om die invloed van Ca op skildefekte (bv. kraakskil) te evalueer, is uitgevoer in Addo in die Oos-Kaap waar kraakskil algemeen voorkom. Kalsiumchloried ( $\text{CaCl}_2$ ), kalsiumnitraat ( $\text{CaNO}_3$ ) en kalsiumnitraat met humiensuur (HS) is as 'n blaarbespuiting op 'Cara-Cara' nawelmoene in die Addo-omgewing toegedien. Geen bewys dat blaarbespuitings met die genoemde Ca-formulasies die voorkoms van kraakskil verminder, is gevind nie. Die rede was dat tydens die eksperiment, kraakskil in die boord en omgewing minimaal voorgekom het. Daar is ook gevind dat die Ca-inhoud van die blare en vrugte nie betekenisvol verhoog ( $P \leq 0.05$ ) is met blaarbespuiting van verskillende Ca-formulasies nie.

## Introduction

Many fruit and vegetable disorders are related to Ca deficiencies. Soil and spray applications of Ca have been reported to ameliorate disorders such as fruit-cracking of sweet oranges (Jiankai, Jiezhong, Heqing et al 1994), rind puffiness (Kawase 1984) and rind-pitting (Zaragoza, Almela, Tadeo et al 1996), although with varied success. Orange rind showing symptoms of albedo breakdown (crease) is frequently low in Ca- and high in K/Ca- and Mg/Ca-ratios. Seasonal trends for this phenomenon were also observed in 'Bellamy' navel orange (Storey and Treeby 2000). Calcium concentration (whole fruit, rind and albedo) increased during stage I and early stage II of fruit development (Jackson 1999) and then progressively decreased (Storey and Treeby 2000). In the pulp these trends were reversed. The K/Ca-ratio of whole fruit and rind initially decreased during stage I and then remained constant or increased slightly. In contrast, the K/Ca-ratio of the pulp increased linearly during most of fruit development (Storey and Treeby 2000).

Calcium is poorly transported in the phloem and is mainly restricted to xylem flow in the unidirectional transpiration stream (Mengel and Kirkby 2001). As a result, deficiency symptoms are always more pronounced in young tissue (Salisbury and Ross 1992). Results published on the nutrient distribution in sweet orange trees indicated that Ca-concentration in older leaves and woody tissues was much greater than those in other parts of the tree (Mattos, Quaggio, Cantarella et al 2003). The high concentration of Ca in the rind and the low concentration in the pulp during most of the fruit development, can be attributed to low mobility of Ca in the phloem (Marschner 1995). The pulp is hydraulically isolated from the vasculature of the outer parts of segments and rind (Koch and Avigne 1990), therefore it is not a site of high water flow derived from xylem sap, as the leaves, and to a lesser extent, the rinds of fruits are (Storey and Treeby 2000).

Control measures for creasing (also called, albedo breakdown) are currently based on the applications of gibberellic acid ( $\text{GA}_3$ ) (Agusti, Martinez-Fuentes, and Mesejo 2002). Navels in the Eastern Cape region of South Africa are subjected to a high tendency of creasing and  $\text{GA}_3$  is sprayed in late summer, however, this only delays creasing (Bower 2004).

In the first report on the foliar spray of a 1% Ca solution of  $\text{CaCl}_2$ ,  $\text{CaNO}_3$  or  $\text{CaCO}_3$  to reduce the incidence of creasing in citrus fruits, Treeby and Storey (2002) concluded that Ca sprays could be used as an alternative to  $\text{GA}_3$  to reduce creasing in orchards in Australia. Using another approach, Bower (2004) concluded that creasing occurs due to a lack of adequate pectin formation and cross-linking and therefore factors restricting pectin synthesis, should be identified and corrected.

## Objectives

### Seasonal changes in nutrient concentration

The seasonal changes in nutrient concentrations of the structural parts of fruit (whole fruit, pulp, rind, albedo) and their relationship with the incidence of creasing will be investigated.

### Calcium sprays applied to single fruit

The objective of this study is to determine whether Ca sprays could potentially reduce the incidence of creasing in navel oranges in South Africa.

### Evaluation of different calcium formulation sprays on creasing

The influence of different Ca-formulations on creasing and Ca-concentrations in the different structural parts of the fruit (whole fruit, pulp, rind, albedo) will be evaluated.

### Comparison between Ca and GA<sub>3</sub> spraying on creasing

The effect of Ca-sprays on creasing will be compared with GA<sub>3</sub> applications.

### **Materials and methods**

The trial commenced in July 2010 and ended in June 2011. The experimental site was on the farm of Dave Gerber, in the vicinity of Addo. Thirty-six trees in a commercial block of 12-year old 'Cara Cara' navels grafted on Carizzo citrange rootstocks were tagged in a randomised complete block design with six blocks of six trees each. In each block two trees were sprayed with a handgun every 7 - 18 days with 1% Ca prepared as CaCl<sub>2</sub>, CaNO<sub>3</sub> and CaNO<sub>3</sub> with humic acid (HA). During the season (December) the Ca-spray concentration was reduced to 0.4% Ca. Cultivation practices common to the area were followed with leaf samples taken at the beginning of the trial in February 2011 and in May 2011. The leaves selected for leaf analyses were the new leaves that developed during the season. Fruit samples were picked in June 2011 and assessed for albedo breakdown. The leaf and fruit samples were cleaned from surface contaminants, dried and stored in a cold room at 2°C. The fruit samples were separated into the pulp, flavedo and albedo. At the end of the trial all samples were batch analysed to determine the Ca-, Mg- and K-content. Data were analysed by analysis of variance as outlined by SAS (SAS<sup>®</sup> Institute, inc., 2002-2008) and the treatment means were compared using the least significant difference test (LSD, P≤0.05) according to the F-test.

### **Results and discussion**

#### Seasonal change in nutrient concentration

Climate plays an important role in the incidence and severity of creasing, which causes great variability from one season to the next (Fourie and Joubert 1957 and Holtzhausen 1981). The role of temperature in the development of creasing is not clear. Creasing was correlated to a temperature range during the early fruit development stage (Jones et al. 1967), but these results could not be repeated by Gambetta et al. (2000). However, a positive correlation between creasing and the average maximum and minimum temperatures prior to flowering was found (Ali et al., 2000). High mean relative humidity from full bloom until physiological fruit drop was also related to a higher incidence of creasing (Gambetta et al., 2000). More detail information on the factors that influence creasing can be found in the MSc dissertation of Phiri (2010).

In Table 5.2.7.1 a summary of rainfall data of the last 4 years is presented. From these data it is clear that during the trial period abnormally high rainfall (728.2 mm) was measured.

**Table 5.2.7.1.** Summary of the annual rainfall in mm.

Month	2008	2009	2010	2011
January	31	10.6	61.4	20.7
February	20.2	63.4	22	96.7
March	19.4	10.4	62	168.6
April	34.2	52	48.4	46.4
May	2.6	7.6	4.8	85.4
June	10.4	8.6	21.4	115.5
July	2.2	31	7	89.4
August	26.8	4.4	6.8	59
September	1.6	4	8.4	2.4
October	21.4	69.8	84.4	13
November	93.6	5.8	35.8	35.8
December	17.2	14.2	52.6	49
<b>Total</b>	<b>280.6</b>	<b>281.8</b>	<b>415</b>	<b>781.9</b>

To increase the potential for creasing in the experimental block the 'Cara-Cara' which is a mid-maturing navel (late May to mid-June) was harvested at the end of June. However, the 2010/2011 season was characterised by a very low incidence of creasing in the Addo region. Information received from the pack house of the Sundays River Citrus Co-op indicated that only 4% of the fruit was rejected due to rind damage that includes wind and thrip damage and rind disorders such as creasing during this time. Results from the trial plots showed that the incidence of creasing was less than 1% for all treatments and no significant difference (P≤0.05) in the creasing for the different treatments was found.

It was also found that the solutions containing a high concentration of Ca as CaCl<sub>2</sub>, CaNO<sub>3</sub> or CaNO<sub>3</sub>+HA and frequent spray interval (7-10 days) caused some spray damage. The same effects were reported by Bramlage (1994) and Treeby and Storey (2002) for sprays with a high concentration of CaCl<sub>2</sub> that caused leaf damage, seen as browning and death of the leaf margins and fruit drop. Therefore, the Ca-concentration in the spraying mixture was reduced and the spraying interval increased to 14-18 days. In Table 5.2.7.2 the Ca-content of the leaves sprayed with different Ca-formulations, at different time intervals, are shown. These results indicate that there was no significant increase ( $P \leq 0.05$ ) in the Ca-content of the leaves over time and no significant difference ( $P \leq 0.05$ ) in the Ca-content of the leaves for the different Ca-formulations used as foliar sprays.

**Table 5.2.7.2.** Calcium content of the 'Cara-Cara' navel orange leaves during the 2011 season.

	CaNO <sub>3</sub>	CaCl <sub>2</sub>	CaNO <sub>3</sub> + HA	Control
<b>Date</b>	<b>mgkg<sup>-1</sup></b>			
<b>Jun-10</b>				32005.4 <sup>a</sup>
<b>Feb-11</b>	32613.5 <sup>a**</sup>	34904.4 <sup>a</sup>	31998.6 <sup>a</sup>	32405.2 <sup>a</sup>
<b>May-11</b>	32706.2 <sup>a</sup>	33696.2 <sup>a</sup>	32092.7 <sup>a</sup>	30152.7 <sup>a</sup>
<b>Sig (P≤0.05)*</b>	NS	NS	NS	NS

\*Indicates difference in values for the different dates within a given Ca-formulation. NS indicates difference was not significant at  $P \leq 0.05$ .

\*\*Different letters indicate significant differences ( $P \leq 0.05$ ) for the different Ca-formulations.

This lack of Ca uptake into the leaves could be due to wax on the fruit and leaves that reduces the effectiveness of Ca uptake (Peryea, 1994; Saure, 2004). The high rainfall frequency may also have played a role in lowering Ca uptake by reducing the contact time between the foliar applied Ca and the leaves of the trees. In Table 5.2.7.3 the Ca, K and Mg content of leaves, which received no Ca sprays, are given at different time intervals.

**Table 5.2.7.3.** Calcium, potassium and magnesium content of untreated leaves.

	Ca	K	Mg
<b>Date</b>	<b>mgkg<sup>-1</sup></b>		
<b>Nov-10</b>	32005.4 <sup>a**</sup>	6751.6 <sup>b</sup>	3639.4 <sup>c</sup>
<b>Feb-11</b>	32405.2 <sup>a</sup>	6717.9 <sup>b</sup>	3620.1 <sup>c</sup>
<b>May-11</b>	30152.7 <sup>a</sup>	6322.3 <sup>b</sup>	3565.6 <sup>c</sup>
<b>Sig (P≤0.05)*</b>	NS	*	NS

\*Indicates difference in values for the different dates for a given element. NS indicates difference was not significant at  $P \leq 0.05$ . K-content for May-11 is significant lower than K-content for Nov-10 and Feb-11.

\*\*Different letters within rows indicate significant differences ( $P \leq 0.05$ ).

These results indicate that there was a slight but non-significant decrease in the Ca and Mg content of the leaves with time but a significant decrease in K content during the season. No correlation between nutrient content of the leaves and creasing incidence was found.

#### Calcium spray to a single fruit

Due to the high concentration calcium and frequent spray interval (7-10 days) some fruit drop occurred. Therefore, fruit was only harvested at the end of the season and no effort was made to differentiate between the fruit from different positions in the tree.

In Table 5.2.7.4 the Ca-content of the different fruit parts sprayed with the different Ca-formulations is presented. The Ca-content of the flavedo and albedo had the highest Ca-concentration with the pulp containing the lowest Ca-concentration which is in accordance with the findings of Storey and Treeby (2002). They postulated that the low Ca-concentration in the pulp can be attributed to the low mobility of Ca in the

phloem (Marshner, 1995) and limited transport of Ca through the xylem sap because the pulp is hydraulically isolated from the vasculature of the outer parts of segments and rind (Koch and Avigne, 1990).

**Table 5.2.7.4.** The influence of calcium formulations on the calcium content of different fruit parts of 'Cara-Cara' navel orange, at commercial fruit maturity.

	Pulp	Flavedo	Albedo
CaNO <sub>3</sub>	2541.2 <sup>a*</sup>	4203.6 <sup>a</sup>	3658.7 <sup>a</sup>
CaCl <sub>2</sub>	2324.3 <sup>a</sup>	4236.0 <sup>a</sup>	4909.4 <sup>b</sup>
CaNO <sub>3</sub> + HA	2575.8 <sup>a</sup>	4007.2 <sup>a</sup>	3673.3 <sup>a</sup>
Control	2640.5 <sup>a</sup>	4423.6 <sup>a</sup>	4230.7 <sup>a</sup>

\*Different letters within columns indicate significant differences (P≤0.05).

There were no significant differences (P≤0.05) between the Ca-concentration in the flavedo or albedo and Ca-formulation, except for CaCl<sub>2</sub> that was significant higher. These results are in accordance with the results of Treeby and Storey (2002) that also found no significant differences in the Ca-content of the rind applied either as CaCl<sub>2</sub> or CaNO<sub>3</sub>.

In Table 5.2.7.5 the Ca, K and Mg content of the different fruit parts is presented. The different elements (Ca, K and Mg) were respectively of the same order for the different fruit parts with the general order of abundance of K>Ca>Mg, which corresponds with results from Storey and Treeby (2000). No correlation was found between the nutrient concentration in the fruit and creasing.

**Table 5.2.7.5.** Calcium, potassium and magnesium content of different fruit parts of non-treated 'Cara-Cara' Navel orange fruit at commercial maturity

Fruit part	Ca	K	Mg
	mgkg <sup>-1</sup>		
Pulp	2640.5 <sup>b**</sup>	6612.2 <sup>a</sup>	553.4 <sup>c</sup>
Flavedo	4423.6 <sup>b</sup>	6892.0 <sup>a</sup>	566.6 <sup>c</sup>
Albedo	4230.7 <sup>b</sup>	6560.3 <sup>a</sup>	553.7 <sup>c</sup>
Sig (P≤0.05)*	*	NS	NS

\*Indicates difference in values for the different fruit parts for a given element. NS indicates difference was not significant at P≤0.05. Ca-content of pulp differs significantly from the Ca-content of the flavedo and albedo.

\*\*Different letters within rows indicate significant differences (P≤0.05).

#### Evaluation of different calcium formulations

For both the leaf and fruit analyses no significant difference in the Ca-content in the leaves and fruit for the different Ca-formulations could be found. It was also found that there was no significant increase in the Ca-content for the leaves and fruit when the trees were sprayed with different Ca-formulations.

#### Comparison between Ca and GA<sub>3</sub> spraying on albedo breakdown

No GA<sub>3</sub> was sprayed due to the high frequency of rainfall received during the season.

#### **Conclusion**

Although Ca may play a role in reducing creasing in citrus, no evidence was found with this trial due to the low incidence of creasing, not only in this specific orchard but also in the Addo area. It was also found that no significant increase in the Ca-content of the leaves and fruit occurred due to the foliar application of different Ca-formulations. Therefore, foliar Ca applications to increase the Ca-content of leaves and fruit are most probably not effective, and Ca uptake via healthy roots should be seen as the main source of this important mineral nutrient.

#### **Technology transfer**

Results of this trial will be discussed with the different study groups.

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### 5.3 PROJECT: FRUIT PRODUCTION AND QUALITY

Project coordinator (Acting): Tim G Grout (CRI)

#### 5.3.1 Project summary

The subjects covered by this project are fundamental to producing profitable citrus and include a wide range of investigations. The size of the navel opening on navel oranges is not of critical importance but large openings are sometimes culled and they do harbour some insect pests. A spray of 2,4-D early in the season has been found to reduce the size of this opening (5.3.2). Farmers have had questions about the role of silicon in citrus for years. Research has already shown that there is no difference between different formulations used for foliar applications and that none of these are as effective as soil applications. A practical method of measuring silicon levels was determined and is now being used to evaluate any role that silicon may play in frost tolerance, crop yield or fruit quality (5.3.3). Alternate bearing in late mandarins is being investigated and it has been found that carbohydrate levels in leaves shortly before harvest give a good indication of whether the next season will be an “on” or “off” year. Now treatments such as the time of harvest, fruit thinning and pruning are being applied to determine their effect on leaf starch/sugar levels and therefore on flower number, fruit set and fruit number per tree (5.3.4). Collaborative research with the Water Research Commission is providing valuable information on the latest techniques for measuring water usage and understanding the demands of mature citrus trees. This research may open the door to a more in-depth

study on citrus water requirements at different ages and phenological stages (5.3.5). Research on fruit splitting has shown that 2,4-D and NPK treatments are giving positive results (5.3.6). Another question that growers have often asked is whether there is any benefit to applying humic acids to the soil. This is now also under investigation (5.3.7). These and other fruit production and quality issues will continue to be investigated.

## Projekopsomming

Die onderwerpe wat in hierdie projek hanteer word is fundamenteel vir winsgewende verbouing van sitrus en sluit 'n verskeidenheid van ondersoeke in. Die grootte van die nawel opening van nawel lemoene is nie van kritieke belang nie, maar groot openinge kan soms tot hoër uitskot lei en hul dien as skuilplek van insekplae. 'n Bespuiting van 2,4-D vroeg in die seisoen is gevind om die grootte van die opening te verklein (5.3.2). Vir jare het produsente vrae oor die rol van silikon in sitrus. Navorsing het reeds bewys dat daar geen verskil tussen die verskillende formulasies is wat as blaartoedienings gebruik word nie en dat geen van hierdie formulasies so effektief soos grondtoedienings is nie. 'n Praktiese metode vir die vasstelling van silikonvlakke is ontwikkel en word nou gebruik om enige rol wat silikon mag speel in vriestoleransie, oes opbrengs of vrugkwaliteit te evalueer (5.3.3). Alternatiewe drag by laat mandaryne word ondersoek en daar is gevind dat die koolhidraatvlakke in blare, kort voor oes, 'n goeie aanduiding gee van of die volgende seisoen 'n "op" of "af" jaar gaan wees. Behandelings soos die tyd van oes, uitdunning van vrugte en snoei word nou toegepas om die uitwerking daarvan op stysel/suiker vlakke in blare te bepaal en gevolglik ook blomgetalle, vrugset en aantal vrugte per boom (5.3.4). Gesamentlike navorsing met die Waternavorsingskommissie verskaf tans waardevolle inligting oor die nutste tegnieke om watergebruik te meet en om die eise van volwasse sitrusbome te verstaan. Hierdie navorsing kan die deur oopmaak vir 'n meer in-diepte studie op die waterbehoefte van sitrus op verskillende ouderdomme en fenologiese stadiums (5.3.5). Navorsing op vrugsplit het getoon dat 2,4-D en NPK behandelings positiewe resultate lewer (5.3.6). Nog 'n vraag wat produsente dikwels vra is of daar enige voordeel is aan die grond-toediening van humiedsuur. Dit word ook nou ondersoek (5.3.7). Daar sal met ondersoeke na hierdie en ander vrugproduksie en kwaliteit aspekte voortgegaan word.

### 5.3.2 FINAL REPORT: Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on the size of the navel end opening of navel oranges

Experiment 935 (April 2007-March 2012) by Stephan Verreyne (CRI-SU)

#### Summary

Fruit with large open navel-ends are culled in the packhouse which reduces the export packout. The main objective of this study was to determine the effect of different timings and concentrations of 2,4-D on the navel-end opening, to determine if there are differences in efficacy between the amine or ester formulations and to determine whether lower concentrations of 2,4-D than 15 ppm are also as effective as 15 ppm applied at full bloom (FB). The study was conducted on Autumn Gold navel in Heidelberg, Newhall navel in Citrusdal, Robyn navel in Clanwilliam and Washington navel in Citrusdal. It seems that 10 ppm 2,4-D at FB is as effective as 15 ppm for both amine and ester formulations with no differences between the formulations and without any negative effects on external and internal fruit quality. Harvesting of trials for the 2010/11 season will commence in June 2011. In addition, residue trials were sprayed in October 2010 and residue samples collected for the purpose of future registration of 2,4-D in order to reduce the size of the navel-end of navel oranges. No detectable levels of 2,4-D were recorded in the fruit analyzed from the different experimental sites and treatments at commercial harvest time.

#### Opsomming

Vrugte met groot nawel-ent openinge word uitgegooi in die pakhuis en verlaag die uitvoer persentasie. Die doel van die studie was om die effek van verskillende tye van toediening en konsentrasies van 2,4-D op die grootte van die nawelent opening te bepaal en om te bepaal of daar verskille in die effektiwiteit van die amien en ester formulasies is asook om te bepaal of laer konsentrasies as 15 dpm 2,4-D net so effektief is as 15 dpm toegedien by volblom. Die studie is uitgevoer op Autumn Gold nawel lemoene in Heidelberg, Newhall nawel in Citrusdal, Robyn nawel in Clanwilliam en Washington nawel in Citrusdal. Dit lyk of 10 dpm 2,4-D by volblom net so effektief is as 15 dpm ook vir beide die amien en ester formulasies en dat daar geen verskille is tussen die ester en amien formulasies nie. Daar was ook geen negatiewe effekte op eksterne en interne vrugkwaliteit. Die oes van die 2010/11 proewe sal begin in Junie 2011. Residu proewe is ook gespuit in Oktober 2010 en residu monsters was versamel met die oog op toekomstige registrasie van 2,4-D vir die doel om nawel-ente kleiner te maak. Daar was in geen van die behandelings wat in die plukvenster geoes was enige 2,4-D residu's waargeneem nie.

## Introduction

The size of the navel-end opening is one of the parameters that is evaluated for external fruit quality in navel oranges. Fruit with large open navel-ends are culled in the packhouse which reduces the export packout (Verreynne, 2008). The maximum acceptable navel-end opening diameter for export fruit is 20 mm (Grout, 1992). The presence of large navel-end openings also causes certain problems such as the higher incidence of stylar-end decay in fruit with large navel-end openings (Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939, 1941). Physiological disorders such as fruit splitting are more common in fruit with large navel-end openings (Krezdorn, 1969; Lima and Davies, 1984a; Lima et al., 1980; Wager, 1939) and large navel-end openings also provide an entry point and harboring place for insects making it difficult to control them (Soule and Grierson, 1986). Some of the factors that influence the size of the navel-end opening are, the weather after fruit set (Grout, 1992; Wager, 1939), abnormal water relations (O'Connell, 2006; Wager 1939), the bearing position of fruit (Lima and Davies, 1984a; Wager 1939) and insect damage (Moore et al., 2007; Wager, 1939).

The synthetic auxin 2,4-D is used as a plant growth regulator to influence plant growth and development in citrus production by manipulating key physiological processes both in the orchard and the packhouse (Lovatt, 2005; Stover et al., 2000; Wright, 2004). The main commercial uses of 2,4-D in citrus production are: to increase fruit size (Anthony and Coggins, 1999; Guardiola, 1997), prolong harvest time (Coggins, 1981; Sarooshi, 1982) and postharvest calyx retention (Cronjé et al., 2005; Singh et al., 1977; Wright, 2004). It is used at low concentrations thereby posing low risk to both man and the environment whilst leaving no hazardous residues (El-Otmani et al., 2000; Monselise, 1979).

The reduction in the size of the navel-end opening by 2,4-D was first reported by Krezdorn (1969) who showed that dipping flowers in a combination of 20 mg·L<sup>-1</sup> 2,4-D and 250 mg·L<sup>-1</sup> GA reduced the size of the navel-end opening. Recently Gardiazabal (2006) and Saavedra (2006) reported that 2,4-D applied at full bloom (FB) reduced the size of the navel-end opening of navel oranges and increased the percentage of fruit with closed navel-ends. Gardiazabal (2006) reported that 20 mg·L<sup>-1</sup> 2,4-D applied on 'Lane Late' navels in Chile at FB resulted in 49% closed navels compared to 3% in the control and reduced the navel-end size to 4.8 mm compared to 12 mm in the control. Similarly, Saavedra (2006) reported that 20 mg·L<sup>-1</sup> 2,4-D applied at FB on 'Lane Late' navels increased the percentage of closed navel-ends to 38.1% compared to 25.9% in the control and reduced the size of the navel-end to 6.8 mm compared to 8.6 mm in the control. Application of 2,4-D also reduced the percentage of fruit with split navel-ends (Saavedra, 2006).

Preliminary studies in South Africa showed that the application of 25 mg·L<sup>-1</sup> 2,4-D at 100% petal drop increased the percentage of closed navel-ends and reduced the average navel-end size in 'Palmer', 'Robyn' and 'Lane Late' navel oranges (Verreynne, 2008). The application of 2,4-D increased the percentage of closed navel-ends by 30% in 'Palmer' navel, 24% in 'Robyn' navel and 39% in 'Lane Late' navel (Verreynne, 2008). The mode of action appears to be related to the delay in style abscission, thereby keeping the navel-end closed (Verreynne, 2008).

The reduction in the size of the navel-end opening would bring several advantages to the grower such as higher export packouts, more effective insect control and a reduction in both fruit splitting and stylar-end decay (Verreynne, 2008).

## Objectives

- A. To determine the effect of different timings and concentrations of 2,4-D on the navel-end opening on different cultivars in different production areas in South Africa.
- B. To determine the effect of 2,4-D application on fruit quality and yield.
- C. To determine whether there are differences in efficacy between the amine and ester formulations.
- D. To determine if lower concentrations of 2,4-D than 15 ppm are also as effective as 15 ppm applied at FB.

## Materials and methods

To determine whether there are differences in efficacy between the amine or ester formulations and to determine if lower concentrations of 2,4-D than 15 ppm are also as effective as 15 ppm applied at FB (best treatment so far), different concentration of both the amine and ester 2,4-D was used at different timings (FB and petal drop (PD)). The study was conducted on Autumn Gold navel in Heidelberg, Newhall and Navelina navel in Citrusdal, Robyn navel in Clanwilliam and Washington navel in Citrusdal. The specific treatments are presented in the different data tables.

At commercial harvest, a full lug box (average 80 fruit) was collected from all sectors of each replicate. Fruit diameter and navel-end size was measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan) on site. Yield (kg) was determined per tree replicate for Newhall navel orange by harvesting all the fruit and recording the weight on an electronic scale (W22 Series, UWE Co, Hsin Tien, Taiwan). A sub sample of 12 fruit as taken and the following evaluations were done. Fruit diameter, fruit height and pedicel diameter were measured using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). Fruit shape was determined by calculating a ratio of fruit diameter: fruit height. Fruit rind colour was determined based on the no. 34 CRI colour chart for oranges [Citrus Research International (CRI), 2004], with 8 being dark green and 1 a fully developed orange colour. Navel-end colour was evaluated on a scale of 0 to 4 with 4 being a dark green navel-end and 0 a fully coloured navel-end. Creasing incidence (%) was calculated by dividing the number of creased fruit by the total number of fruit evaluated. Fruit were cut into half along the equatorial plane for internal quality determinations. Rind thickness at the sides of the fruit were measured for each fruit using an electronic calliper (CD-6" C, Mitutoyo Corp, Tokyo, Japan). Fruit were also scored for any visible symptoms of granulation. The fruit were then juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth and the juice percentage was determined by dividing the weight of the juice by the total fruit weight. °Brix from the extracted juice was determined using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan). Titratable acidity (TA) expressed as citric acid content was determined by titrating 20 ml of the extracted juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator. The °Brix:TA ratio was calculated by dividing the °Brix values by the TA values.

The residue analysis was done by Hearshaw and Kinnes Analytical Laboratory (Pty) Ltd. on fruit that were sprayed after full bloom and harvested at commercial harvest.

## Results and discussion

Although not significantly, all the treatments increased the percentage closed navel-ends by 14 to 20% in Autumn Gold navel orange (Table 5.3.2.1). None of the treatments affected the Brix (sugar content), TA or the sugar: acid ratio. The application of 15 ppm 2,4-D ester at PD significantly reduced the juice content. None of the treatments affected the fruit shape, fruit colour (Table 5.3.2.2) or the other external fruit quality parameters significantly compared to the control (Table 5.3.2.3).

**Table 5.3.2.1.** Effect of different 2,4-D treatments on the percentage closed navel-ends and the internal fruit quality of Autumn Gold navel in Heidelberg.

Treatments	% closed navel-ends	Brix	Juice content	Acid	Ratio
Control	24.2	11.8	46.9 a <sup>z</sup>	1.20	9.9
10 ppm amine at FB	49.5	10.9	46.7 a	1.06	10.4
10 ppm ester at FB	38.7	11.1	46.3 a	1.10	10.2
15 ppm amine at FB	48.4	11.3	46.1 a	1.20	9.6
15 ppm ester at FB	46.1	11.0	44.9 a	1.08	10.5
15 ppm ester at PD	54.1	11.3	42.3 b	1.28	8.9
P-value	0.2746	0.2456	0.0003	0.0933	0.1244

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.2.** Effect of different 2,4-D treatments on the fruit shape and colour of Autumn Gold navel in Heidelberg.

Treatments	Diameter	Height	Shape	Colour	Navel end colour
Control	79.65	79.92	1.00 ab <sup>z</sup>	2.2	1.5
10 ppm amine at FB	78.77	78.50	1.00 a	2.5	1.5
10 ppm ester at FB	79.02	79.25	1.00 ab	2.3	1.4
15 ppm amine at FB	78.80	78.36	1.01 a	2.1	1.4
15 ppm ester at FB	79.40	78.98	1.01 a	2.0	1.5
15 ppm ester at PD	80.40	81.79	0.99 b	2.3	1.6
P-value	0.9475	0.5453	0.0473	0.0782	0.8637

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.3.** Effect of different 2,4-D treatments on the external fruit quality of Autumn Gold navel in Heidelberg.

Treatments	Pedicle diameter	Rind coarseness	Peel thickness	Creasing
Control	3.40	1.20	4.11	2.1
10 ppm amine at FB	3.40	1.33	4.22	6.3
10 ppm ester at FB	3.23	1.34	4.34	10.4
15 ppm amine at FB	3.16	1.28	4.04	6.3
15 ppm ester at FB	3.28	1.32	4.42	1.0
15 ppm ester at PD	3.34	1.24	4.21	9.4
P-value	0.1523	0.7544	0.1597	0.4737

<sup>2</sup>Means with a different letter differ significantly at the 5% level (LSD)

Only 15 ppm 2,4-D (amine) at FB and 15 ppm 2,4-D (ester) at PD significantly increased the percentage closed navel-ends by 19.8 and 24.2%, respectively in Newhall navel oranges (Table 5.3.2.4). None of the treatments significantly affected fruit diameter, the average navel-end size, yield (kg/tree) or fruit number per tree compared to the control. None of the treatments significantly affected the Brix (sugar content), titratable acidity, the sugar: acid ratio or the juice content (Table 5.3.2.5). None of the treatments affected the fruit shape, fruit colour (Table 5.3.2.6) or the other external fruit quality parameters significantly compared to the control (Table 5.3.2.7).

**Table 5.3.2.4.** Effect of different 2,4-D treatments on the percentage closed navel-ends, fruit size, yield and total fruit number of Newhall navel in Citrusdal.

Treatments	% closed navel-ends	Diameter	Navel-end size	Yield	Fruit number
Control	18.0 c <sup>2</sup>	77.03	5.86	135.7	510
10 ppm ester at FB	30.3 abc	77.38	5.01	133.1	516
15 ppm ester at FB	23.6 c	74.94	5.34	137.3	554
15 ppm amine at FB	37.8 ab	77.98	3.98	134.0	511
25 ppm ester at FB	25.7 bc	75.19	4.53	139.0	578
15 ppm ester at PD	42.2 a	77.34	4.45	126.4	490
P-value	0.0047	0.7318	0.2396	0.9652	0.8288

<sup>2</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.5.** Effect of different 2,4-D treatments on the internal fruit quality of Newhall navel in Citrusdal.

Treatments	Brix	Juice content	Acid	Ratio
Control	9.9	41.1	0.82	12.2
10 ppm ester at FB	10.3	39.8	0.85	12.3
15 ppm ester at FB	10.3	42.7	0.89	12.1
15 ppm amine at FB	10.5	39.2	0.85	12.2
25 ppm ester at FB	10.4	38.8	0.85	12.3
15 ppm ester at PD	11.0	43.6	0.85	12.9
P-value	0.2740	0.1471	0.9438	0.8696

<sup>2</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.6.** Effect of different 2,4-D treatments on fruit shape and colour of Newhall navel in Citrusdal.

Treatments	Diameter	Height	Shape	Colour	Navel end colour
Control	78.68	82.62	0.96	1.3	0.4
10 ppm ester at FB	78.38	83.30	0.94	1.1	0.4
15 ppm ester at FB	77.99	82.80	0.95	1.3	0.4
15 ppm amine at FB	79.47	83.58	0.95	1.4	0.6
25 ppm ester at FB	78.44	81.47	0.97	1.3	0.5
15 ppm ester at PD	78.58	82.86	0.95	1.4	0.5
P-value	0.9862	0.9390	0.6608	0.3393	0.3937

<sup>2</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.7.** Effect of different 2,4-D treatments on the external fruit quality of Newhall navel in Citrusdal.

Treatments	Pedicle diameter	Rind coarseness	Peel thickness	Creasing
Control	3.09	1.4	5.94	22.9
10 ppm ester at FB	3.20	1.5	5.72	26.0
15 ppm ester at FB	2.79	1.6	6.08	17.7
15 ppm amine at FB	3.03	1.5	5.84	9.4
25 ppm ester at FB	3.12	1.7	6.13	24.0
15 ppm ester at PD	3.14	1.5	5.45	18.8
P-value	0.1006	0.3685	0.1219	0.3089

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

All the treatments significantly increased the percentage closed navel-ends by 26.5 to 45.1%, in Robyn navel orange with no differences among treatments (Table 5.3.2.8). None of the treatments significantly affected the Brix (sugar content), TA and the sugar: acid ratio. All the treatments, except for 15 ppm 2,4-D (ester) applied at PD significantly increased juice content compared to the control. None of the treatments affected the fruit shape and fruit colour (Table 5.3.2.9), peel thickness or creasing incidence (Table 5.3.2.10), while 15 and 20 ppm 2,4-D (ester) at FB and 15 ppm 2,4-D (ester) at PD increased pedicle diameter (Table 5.3.2.10). The application of 15 ppm 2,4-D amine and ester at FB and 15 ppm 2,4-D (ester) at PD resulted in significantly coarser rinds.

**Table 5.3.2.8.** Effect of different 2,4-D treatments on the percentage closed navel-ends and the internal fruit quality of Robyn navel in Clanwilliam.

Treatments	% closed navel-ends	Brix	Juice content	Acid	Ratio
Control	38.2 b <sup>z</sup>	9.8	37.4 d	1.10	8.8
10 ppm ester at FB	66.0 a	9.7	40.6 bc	1.07	9.2
15 ppm amine at FB	82.4 a	9.9	42.3 ab	1.04	9.6
15 ppm ester at FB	69.7 a	10.1	42.5 a	1.05	9.6
20 ppm ester at FB	83.3 a	9.9	40.2 c	1.09	9.1
15 ppm ester at PD	64.7 a	9.8	38.9 cd	1.04	9.5
P-value	0.0028	0.5683	0.0001	0.2131	0.0900

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.9.** Effect of different 2,4-D treatments on fruit shape and colour of Robyn navel in Clanwilliam.

Treatments	Diameter	Height	Shape	Colour	Navel end colour
Control	74.86	73.28	1.02	2.4	1.3
10 ppm ester at FB	75.83	74.96	1.02	2.7	1.4
15 ppm amine at FB	75.26	74.14	1.02	2.6	1.5
15 ppm ester at FB	74.62	73.83	1.01	2.4	1.2
20 ppm ester at FB	73.86	72.30	1.03	2.8	1.5
15 ppm ester at PD	74.56	74.69	1.00	2.8	1.4
P-value	0.7056	0.3599	0.2046	0.3266	0.3659

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.10.** Effect of different 2,4-D treatments on the external fruit quality of Robyn navel in Clanwilliam.

Treatments	Pedicle diameter	Rind coarseness	Peel thickness	Creasing
Control	3.28 c <sup>z</sup>	1.2 c	5.36	4.2
10 ppm ester at FB	3.38 bc	1.3 bc	5.66	2.1
15 ppm amine at FB	3.26 c	1.6 a	5.46	4.2
15 ppm ester at FB	3.48 ab	1.5 ab	5.28	2.1
20 ppm ester at FB	3.54 ab	1.4 abc	5.62	2.1
15 ppm ester at PD	3.58 a	1.5 ab	5.67	4.2
P-value	0.0040	0.0324	0.1076	0.9069

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

All the treatments, except for 15 ppm 2,4-D (ester) at PD, significantly increased the percentage closed navel-ends by 9.9 to 17.3%, in Washington navel orange with no differences among treatments (Table 5.3.2.11). None of the treatments significantly affected the Brix (sugar content), TA and the sugar: acid ratio.

5 ppm, 15 ppm and 20 ppm 2,4-D (ester) at FB significantly increased juice content compared to the control. None of the treatments affected the fruit shape and fruit colour, but 15 and 20 ppm 2,4-D (ester) at FB and 15 ppm (ester) at PD resulted in greener navel-ends (Table 5.3.2.12). None of the treatments affected the external fruit quality parameters (Table 5.3.2.13).

**Table 5.3.2.11.** Effect of different 2,4-D treatments on the percentage closed navel-ends and the internal fruit quality of Washington navel in Citrusdal.

Treatments	% closed navel-ends	Brix	Juice content	Acid	Ratio
Control	3.8 b <sup>z</sup>	11.2	39.5 c	0.94	12.0
5 ppm ester at FB	15.9 a	10.6	50.5 a	0.92	11.7
10 ppm ester at FB	21.1 a	10.4	43.2 bc	0.91	11.5
15 ppm ester at FB	17.5 a	11.1	45.3 ab	0.90	12.5
20 ppm ester at FB	13.7 a	10.7	45.5 ab	0.88	12.3
15 ppm ester at PD	4.4 b	10.9	42.1 bc	0.88	12.6
P-value	0.0023	0.0745	0.0086	0.8529	0.4858

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.12.** Effect of different 2,4-D treatments on fruit shape and colour of Washington navel in Citrusdal.

Treatments	Diameter	Height	Shape	Colour	Navel end colour
Control	77.43	78.28	0.99	1.7	0.7 c <sup>z</sup>
5 ppm ester at FB	77.08	77.75	1.00	1.7	0.7 bc
10 ppm ester at FB	78.04	79.12	0.99	1.7	0.8 abc
15 ppm ester at FB	77.35	77.96	1.00	1.6	0.9 a
20 ppm ester at FB	77.11	78.58	0.98	1.5	1.0 a
15 ppm ester at PD	77.7	79.26	0.98	1.4	0.9 ab
P-value	0.9644	0.9042	0.7811	0.1556	0.0417

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.13.** Effect of different 2,4-D treatments on the external fruit quality of Washington navel in Citrusdal.

Treatments	Pedicle diameter	Rind coarseness	Peel thickness	Creasing
Control	3.35	1.3	5.62	27.8 a <sup>z</sup>
5 ppm ester at FB	3.15	1.5	5.70	16.7 ab
10 ppm ester at FB	3.24	1.6	5.48	8.3 b
15 ppm ester at FB	3.44	1.2	5.57	19.5 ab
20 ppm ester at FB	3.26	1.3	5.34	11.1 b
15 ppm ester at PD	3.45	1.5	5.31	9.7 b
P-value	0.1542	0.1602	0.3503	0.0511

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

Although not significantly, all the treatments increased the percentage closed navel-ends by 7.9 to 20.7% in Navelina navel orange (Table 5.3.2.14). No granulation was visible in any of the fruit sampled in all the trials.

**Table 5.3.2.14.** Effect of different 2,4-D treatments on the percentage closed navel-ends and the internal fruit quality of Navelina navels in Citrusdal.

Treatments	% closed navel-ends
Control	9.8
10 ppm amine at FB	17.7
10 ppm ester at FB	25.2
15 ppm amine at FB	30.5
15 ppm ester at FB	20.6
15 ppm ester at PD	20.0
P-value	0.0958

<sup>z</sup>Means with a different letter differ significantly at the 5% level (LSD)

**Table 5.3.2.15.** Summary of experimental sites during 2011/12 season where samples to determine the residues of the preharvest 2,4-D sprays.

Experimental site	Spray date	Harvest date
Newhall' -ALG-Citrusdal	6 Oct 2010	Harvest: 9-16 June 2011
Robyn , Twaktuin-Clanwilliam	7 Oct 2010	Harvest: 29 July 2011
Newhall navels Addo, Avoca	28 Sept 2010	Harvest: 15 June 2011 2011

**Table 5.3.2.16.** Results of residue analysis of the 2,4-D preharvest applications on Navel fruit.

Treatment	No of replications	2,4-D residue (mg/kg) at harvest
<b>Experimental site</b>		
Citrusdal- ALG -Newhall	5	0
Clanwillilliam – Robyn	5	0
Clanwillilliam – Twaktuin- Newhall	5	0
Addo- Newhall	5	0
<b>Type of 2,4-D</b>		
30 ppm Amino (ALG, Newhall)	1	0
30 ppm Ester (ALG Newhall)	1	0
<b>Time of sample</b>		
Small Roby fruit, harvested just after spraying	5	0.145

The residues of the 2,4-D was determined by Hearshaw and Kinnes Analytical Laboratory (Pty) Ltd. The data indicate that in none of the fruit sampled at harvest on the various experimental sites, could the 2,4-D be detected (Table 5.3.2.16). The only fruit that had detectable 2,4-D were fruit harvested shortly after spraying, which indicated that the treatment applications were successful.

The reasons for the lack of any residues could be the long period between application and harvest as well as the very low concentrations of 2,4-D used.

## Conclusion

The data indicate that 10 ppm 2,4-D at FB is as effective as 15 ppm for both amine and ester formulations, without any negative effects on external and internal fruit quality. In addition it seems that these treatments do not result in any residues at harvest.

## Future research

No further research required.

## Technology transfer

### Full length proceedings

J.S. Verreyne, Mupambi, G., 2010. Effects of 2,4-D on the Size of the Navel End Opening and Fruit Quality of Navel Oranges. XI International Symposium on Plant Bioregulators in Fruit Production, Bologna, Italy. Acta Horticulturae 884: 745-751.

### Grower presentations

Verreyne, S. and Giverson Mupambi. Effects of 2,4-D on the size of the navel-end opening and fruit quality of navel oranges. 6<sup>th</sup> Citrus Research Symposium, Citrus Research International, Drakensberg, South Africa, 15-18 August 2010.

Verreyne, S. Effects of 2,4-D on the size of the navel-end opening and fruit quality of navel oranges. Terason Citrus training, Wellington. 3 August 2010.

Verreyne, S. Effects of 2,4-D on the size of the navel-end opening and fruit quality of navel oranges. UAP Citrus training, Paarl. 27 August 2010.

Verreyne, S. Effects of 2,4-D on the size of the navel-end opening and fruit quality of navel oranges National Farming Convention and Expo. Groblersdal. 30 Sept. 2010.

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### 5.3.3 PROGRESS REPORT: The evaluation of silicon absorption in citrus

Experiment 974 (2009/12 – 2012/03) by JT Vahrmeijer (CRI) and NM Asanzi (UP)

#### Summary

Silicon (Si) is the second most abundant element in the earth's crust after oxygen and is also found in plants in concentrations equivalent to certain macronutrients such as Ca, Mg and P. A silicon extraction procedure for citrus plant material was validated by using microwave assisted digestion and the Si recovery from this method was in the range of 81-96%. Silicon concentration in plant material was determined with an Induced couple plasma optical emission spectrum (ICP-OES) and a spectrophotometer (colorimetric analysis). A correlation of 98% was found between the results of the ICP-OES and the spectrophotometer. Therefore, Si concentrations can be determined with an ICP-OES, which consists of a simpler analytical procedure. Results also indicated that mature leaves tend to be 65% higher in Si than young leaves. During winter less Si was taken up by citrus plants than during summer, implying that season plays a role in Si uptake. Silicon concentration in the leaves is also time and concentration dependent. The Si content was significantly higher in citrus plants when Si was applied to the roots than when it was applied to the leaves. Results from foliar applied Si also indicates that Si accumulates unevenly in the cytoplasm of leaves and that no significant difference between types of Si formulation ( $\text{Si(OH)}_4$  and  $\text{K}_2\text{SiO}_3$ ) and Si concentration in the leaves exists. The use of chlorophyll fluorescence measurements failed to indicate any plant stress conditions for citrus trees at low temperatures and therefore the use of this method in detecting low temperature stress needs further investigation.

#### Opsomming

Silikon (Si) is die element, na suurstof, wat die tweede meeste in die aarde se kors voorkom. Silikon kom ook in konsentrasies soortgelyk as ander makrovoedingselemente soos, Ca, Mg en P in plante voor. 'n Si ekstraksie prosedure vir plantmateriaal is gevalideer deur gebruik te maak van 'n mikrogolf geassisteerde verteringsprosedure. Herwinning van Si deur die gebruik van hierdie metode was tussen 81 en 91%. Die Si-konsentrasie in plantmateriaal is met behulp van geïnduseerde gekoppelde plasma optiese emissie spektrum (ICP-OES) asook 'n spektrofotometer (kolometriese ontleding) bepaal. Die korrelasie in Si-konsentrasie tussen die twee metodes was 98%. Dus Si-konsentrasie in plantmateriaal kan met die ICP-OES, wat minder analitiese stappe bevat, gemeet word. Resultate het ook getoon dat ouer blare 65% meer Si bevat as jonger blare en dat Si gedurende die winter minder as gedurende die somer opgeneem word. Silikon konsentrasie in die blare word ook deur tyd en konsentrasie beïnvloed. 'n Toename in Si-konsentrasie in sitrus blare is met tyd en toedieningskonsentrasie waargeneem. Die Si-inhoud van sitrusplante was betekenisvol hoër vir plante waar die Si in grond toegedien is as vir die plante waar die Si as 'n blaarbespuiting toegedien is. Resultate van blaartoegedien Si dui daarop dat die Si oneweredig in die sitoplasma van die blaar akkumuleer. Daar is ook geen betekenisvolle verskille in die konsentrasie Si in die blare vir die verskillende Si-formulasies ( $\text{Si(OH)}_4$  en  $\text{K}_2\text{SiO}_3$ ) wat as blaarbespuitings toegedien is nie. Chlorofil-fluorensensie het geen stres toestande in sitrusbome wat by lae temperature groei aangedui nie en daarom moet hierdie metode om stres toestande in sitrusbome wat by lae temperature groei, verder ondersoek word.

### 5.3.4 PROGRESS REPORT: Effect of leaf carbohydrate concentrations on flowering and fruit set of alternate bearing late mandarins

Experiment 981 (April 2010- March 2011) by Stephan Verreyne (CRI-SU), Schalk van der Merwe (SU), Paul Cronje (CRI at SU).

#### Summary

Alternate bearing is a physiological phenomenon that occurs in late mandarin citrus fruit trees. This study addresses the basic physiological mechanism involved in alternate bearing, which is thought to be closely related to the energy balance within the tree and therefore carbohydrate levels in the various parts of the tree during the different phenological growth stages. Ten pairs of healthy "off" and "on" Nadorcott late mandarin (*Citrus reticulata*) trees of uniform size were used for the study from which ten monthly leaf samples from

each "on" and "off" tree were taken from the spring flush vegetative shoots. Leaves were analysed for starch and sugar. In addition, ten shoots per tree were tagged and flower number and fruit set percentage was determined at the appropriate time, as well as crop load (kg fruit per tree) at harvest in August. The leaf carbohydrate levels were determined for one season and the information can now be linked to the different phenological stages of Nadorcott mandarins to determine critical periods during the year in terms of starch reserve levels. The biggest difference in leaf starch levels was observed during April, indicating that this is the most important period for the tree in terms of starch reserve levels. Return bloom for "on" trees was less than half compared to "off" trees. It is evident that differences in yield can have a large influence on the phenology of Nadorcott mandarin trees and that this could be related to leaf starch levels at a critical stage during the season.

## Opsomming

Alternerende drag is 'n fisiologiese verskynsel wat in mandaryn-tipe sitrusbome voorkom wat laat in die seisoen geoes word. Hierdie studie ondersoek die basiese fenologiese meganisme wat betrokke is by alternerende drag, wat vermoedelik nou verband hou met die energiebalans in die boom self en daarom dus ook met die koolhidraatvlakke in die onderskeie dele van die boom gedurende die verskillende fenologiese groeistadia. Tien paar gesonde "af" en "aan" Nadorcott mandarynbome (*Citrus reticulata*) van uniforme grootte, was in die studie gebruik. Maandeliks is van elke "aan"- en "af"-boom tien blaarmonsters van die lente groei se vegetatiewe lote geneem. Blare se stysel- en suikerinhoud is geanaliseer. Daarby is tien lote per boom gemerk en die aantal blomme te tel en persentasie vrugset is op die gepaste tyd bepaal. Die drag se gewig (kg vrugte per boom) is tydens oestyd in Augustus bepaal. Die koolhidraatvlakke in die blare is vir een seisoen bepaal en die inligting kan nou verbind word met die verskillende fenologiese stadia van Nadorcott mandaryne om kritiese periodes gedurende die jaar in terme van die vlakke van styselreserwes te bepaal. Die grootste verskil in blare se styselvlakke is in April waargeneem wat daarop dui dat hierdie periode vir die boom die belangrikste is in terme van die vlakke van styselreserwes. Blomintensiteit die volgende seisoen vir "aan"-bome was minder as die helfte in vergelyke met dié van "af"-bome. Dit is duidelik dat verskille in opbrengs 'n groot invloed kan hê op die fenologie van Nadorcott mandarynbome en dat dit verband kan hou met styselvlakke in die blare tydens 'n kritiese stadium gedurende die seisoen.

### 5.3.5 PROGRESS REPORT: A novel approach to water and nutrient management in citrus

Experiment 986 (August 2010 – April 2012): J.T. Vahrmeijer (CRI), N.J. Taylor (UP) and H. Pienaar (UP)

## Summary

For efficient irrigation management in citrus, it is important to be able to accurately determine the evapotranspiration (ET) of orchards. By knowing the amount of water that leaves the plant-soil interface, the irrigation manager can replenish the profile with the correct amount of water and therefore prevent drought stress or water logged conditions. Two experimental sites were selected to monitor water use and nutrient concentration in the groundwater of a citrus orchard. The one site is in the Western Cape on the farm Patrysberg in Citrusdal (Rustenburg Navels) and the other one in Mpumalanga on Riverside Farms in Malelane (Midnight Valentias). Tree attributes are determined throughout the season: These include stem circumferences, canopy dimensions, wood characteristics, leaf area index (LAI), stomatal conductance and leaf water potential. Fruit have been harvested at the experimental site in Citrusdal and information on the yield, fruit size, fruit weight, Brix content, acid percentage and the Brix and acid ratio of the fruit were obtained. Wetting front detectors (WFD) were used to monitor nutrient movement in the soil and the data was collected by the farm manager. Tree water use (transpiration) was measured using the heat pulse velocity (HPV) technique. The data ( $\text{cm}\cdot\text{hr}^{-1}$ ) collected is patched and scaled up to ( $\text{L}\cdot\text{tree}^{-1}\cdot\text{day}^{-1}$ ) and then converted to orchard level ( $\text{mm}\cdot\text{day}^{-1}$ ). Eddy covariance measurements determining evapotranspiration have been collected over different time periods to validate the HPV technique. Satellite remote sensing information was received from the project 'Water Use Surveillance and Ecological Economic Modelling of Agro-Ecosystems in the Sandveld region, Western Cape. This information is based on the SEBAL model and will be compared with measured sap flow for field validation.

## Opsomming

Vir doeltreffende besproeiingsbestuur in sitrus, is dit belangrik om evapotranspirasie (ET) akkuraat te bepaal. Inligting oor waterverliese kan gebruik word om die regte hoeveelheid besproeiingswater toe te dien om droogtespanning of oor-besproeiing van die sitrusbome te voorkom. Twee eksperimente om die water gebruik van sitrusbome te monitor, is geïnisieer. Een op die plaas Patrysberg in Citrusdal, Wes-Kaap (Rustenburg Nawels) en die ander een op Riverside Farms in Malelane, Mpumalanga (Midnight Valentias). Inligting oor stamomtrek, blaardak-dimensies, eienskappe van die hout, blaaroppervlakte, stomata-geleiding

en blaarwaterpotensiaal word gereeld deur die seisoen ingesamel. In Citrusdal is inligting oor die oes-opbrengs, vruggrootheid, vruggewig, Brix-inhoud, suurpersentasie en die Brix-suur verhouding, versamel. Die beweging van plantvoedingselemente in die grondprofiel is met behulp van 'n benattingsfront-detektor (BFD) gemonitor en die data is deur die plaasbestuurder versamel. Die watergebruik van sitrusbome (transpirasie) is met behulp van die hitte-pulse-snelheid (HPS) tegniek bepaal. Die gemete waardes ( $\text{cm}\cdot\text{hr}^{-1}$ ) is eers omgeskakel na boomwatergebruik ( $\text{L}\cdot\text{boom}^{-1}\cdot\text{dag}^{-1}$ ) en daarna uitgebrei na boordwatergebruik ( $\text{mm}\cdot\text{dag}^{-1}$ ). ET is gedurende vensterperiodes met behulp van die Eddy-kovariante-tegniek bepaal. Hierdie inligting sal gebruik word om die HPS-tegniek te kalibreer en te valideer. Inligting van satellietbeelde is van die projek 'Water Use Surveillance and Ecological Economic Modelling of Agro-Ecosystems in the Sandveld region, Western Cape' deur die LNR verskaf. Die SEBAL model is gebruik om ET met behulp van satellietbeelde te voorspel en sal met die HPS-tegniek vergelyk word.

### 5.3.6 PROGRESS REPORT: Effect of 2,4-D on fruit splitting and fruit size of citrus

Experiment 1027 (November 2010 - March 2011): Stephan Verreyne (CRI at SU), Jakkie Stander (SU) and Paul Cronje (CRI at SU)

#### Summary

Fruit splitting is a physiological disorder in citrus that results in cracking of the rind usually from the stylar-end resulting in fruit drop. The objective of the study is to evaluate the effect of 2,4-D and potassium nitrate on fruit splitting and fruit size of both oranges and mandarins. The following treatments were applied on Marisol Clementines in Paarl and Citrusdal, Mor in Paarl, Or in De Doorns, and Midnight Valencia in Citrusdal: an untreated control, 2,4-D amine applied after physiological fruit drop (APFD), 5% Bonus NPK (potassium nitrate) APFD, 2% Ca ( $\text{NO}_3$ )<sub>2</sub> APFD, 2,4-D amine + 5% Bonus NPK APFD, 2,4-D amine + 2% Ca ( $\text{NO}_3$ )<sub>2</sub> APFD, 2,4-D amine in January or 2,4-D amine in February. All 2,4-D treatments were applied at 10 ppm. Only the combination of 10 ppm 2,4-D amine and 5% Bonus NPK applied after the physiological fruit drop significantly reduced the number of split fruit per tree by 62%, whereas only 10 ppm 2,4-D amine applied after physiological fruit drop and the combination of 10 ppm 2,4-D amine and 5% Bonus NPK applied after the physiological fruit drop significantly improved fruit growth rate. All the treatments applied after physiological fruit drop resulted in fruit with significantly coarser rinds, but none of the treatments had a significant effect on the internal fruit quality. It is suspected that these treatments increase the rind strength in the blossom-end of the fruit.

#### Opsomming

Vrugsplit is 'n fisiologiese abnormaliteit in sitrus wat veroorsaak dat die skil bars gewoonlik by die stylend en vrugte dan afval. Die doel van die studie is om die effek van 2,4-D en kaliumnitraat op vrugsplit en vruggrootheid van lemoene en mandaryne te evalueer. Die volgende behandelings is toegedien op Marisol Clementines in die Paarl en Citrusdal, Mor in die Paarl, Or in De Doorns, en Midnight Valencia in Citrusdal: 'n onbehandelde kontrole, 2,4-D amien na fisiologiese vrugval (APFD), 5% Bonus NPK (kaliumnitraat) APFD, 2% Ca ( $\text{NO}_3$ )<sub>2</sub> APFD, 2,4-D amien + 5% Bonus NPK APFD, 2,4-D amien + 2% Ca ( $\text{NO}_3$ )<sub>2</sub> APFD, 2,4-D amien in Januarie of 2,4-D amien in Februarie. Alle 2,4-D behandelings is toegedien teen 10 dpm. Slegs die kombinasie van 10 dpm 2,4-D amien en 5% Bonus NPK toegedien na fisiologiese vrugval het die aantal splitvrugte per boom betekenisvol verlaag met 62%, en slegs 10 dpm 2,4-D amien toegedien na fisiologiese vrugval en die kombinasie van 10 dpm 2,4-D amien en 5% Bonus NPK toegedien na fisiologiese vrugval het vruggroeiempo betekenisvol verhoog. Al die behandelings wat na fisiologiese vrugval toegedien is, het growwer skille tot gevolg gehad, maar geen behandelings het 'n betekenisvolle effek op die interne kwaliteit gehad nie. Die behandelings verhoog vermoedelik die sterkte van die skil aan die blom-end kant van die vrug.

### 5.3.7 PROGRESS REPORT: Preliminary study on the effect of humic acids on fertiliser application in citrus

Experiment 1028 (March 2011 – April 2012): JT Vahrmeijer (CRI) and A Gatabazi (UP)

#### Summary

The influence of potassium humates and fulvates on the leaching of nitrogen (N), phosphate (P) and potassium (K) was evaluated under laboratory conditions and in pot trials. Two soils (0-20 cm) with different textures (low and high clay content) were collected at the experimental farm of the University of Pretoria and the particle distribution and chemical content were determined.

Leaching studies were done in the laboratory in specially designed static-tension soil columns consisting of Plexiglas (0.1 m diameter and 0.3 m high) that were filled to a height of 0.17 m at a bulk density of 1386

kg.m<sup>-3</sup>. The soils were respectively mixed with K-humates and fulvates (two types of K-humates and one fulvate) equivalent to a rate of 200 kg.ha<sup>-1</sup>. Two fertiliser application rates were used in this experiment, 100% and 75% of the recommended N, P and K application rates. A known amount of water was added and the leachate was collected, this was repeated three times. The N, P and K concentrations were determined in the leachate and in the soils at the end of the trial.

The pot trials were done at the University of Pretoria's experimental farm. Ten liter plastic bags were filled with soils with respectively low and high clay content. Small Valencia citrus trees were planted in the bags and left for two months to acclimatize. K-humates and fulvates (two types of K-humates and one fulvate) equivalent to a rate of 200 kg.ha<sup>-1</sup> were respectively applied to the soils. The pots were arranged in a complete randomized block design (CRBD) with ten treatments and four replicates. N, P and K were applied to the soil at 75% of the recommended fertiliser application rate. A known amount of water was added and the leachate was collected. The N, P and K concentration were determined in the leachate and in the soils at the end of the trial. The plants were harvested and the N, P and K will be determined in the leaves, roots, bark and stems.

## Opsomming

Die invloed van K-humate en fulvate op die loging van stikstof (N), fosfaat (P) en kalium (K) in verskillende gronde is onder laboratoriumtoestande en met behulp van potproewe geëvalueer. In hierdie eksperiment is twee verskillende gronde (lae en hoë klei-inhoud), wat vanaf die proefplaas van die Universiteit van Pretoria versamel is, gebruik. Die gronde is chemies ontleed en die deeltjiegrootte-verspreiding is bepaal.

Logingstudies is in die laboratorium in spesiaal ontwerpte statiese grondkolomme uitgevoer. Die kolomme het bestaan uit Plexiglas (0.1 m deursnee en 0.3 m hoog) wat met grond tot 'n hoogte van 0.17 m gevul is sodat 'n bruto-digtheid van 1368 kg.m<sup>-3</sup> verkry is. Hierdie gronde is respektiewelik met K-humate en fulvaat, ekwivalent aan 200 kg.ha<sup>-1</sup>, gemeng (twee tipes K-humate en een fulvaat). Twee kunsmistoedienings, 100% en 75% van die aanbevole N, P en K toediening, is gebruik. 'n Bekende hoeveelheid water is bygevoeg en die logingswater is versamel. Hierdie prosedure is drie keer herhaal. Aan die einde van die eksperiment is die N, P en K konsentrasie in die logingswater en die grond bepaal.

Potproewe is by die proefplaas van die Universiteit van Pretoria uitgevoer. Tien liter plastiese sakke is respektiewelik met lae en hoë klei-inhoudgronde gevul. Klein Valencia sitrus boompies is in die sakke geplant en vir twee maande geklimatiseer. Daarna is K-humate en fulvate (twee tipes K-humate en een fulvaat) ekwivalent aan 200 kg.ha<sup>-1</sup>, respektiewelik aan die gronde in die potte toegedien. Die eksperiment het bestaan uit 10 behandelings met vier herhalings, wat in 'n volledige willekeurige blok ontwerp gerangskik is. N, P en K is teen 75% van die aanbevole hoeveelheid toegedien. 'n Bekende hoeveelheid water is bygevoeg en die logingswater is versamel. Hierdie prosedure is drie keer herhaal. Aan die einde van die eksperiment is die N, P en K konsentrasie van die logingswater en die grond bepaal. Die plantmateriaal is versamel en die N, P en K inhoud van die blare, wortels, bas en stingels sal bepaal word.

## 5.4 PROJECT: COLD CHAIN MANAGEMENT AND PACKAGING

Project coordinator: Malcolm Dodd (SU)

### 5.4.1 Project summary

The only levy-funded research in this project is that involving structural modification of ship containers to change the direction of air flow and increase their cooling efficiency (5.4.2). Data collected in this research has been processed to give a comparison between the cooling characteristics of the standard A15C carton and the so called "Super vent" carton. Using identical containers, citrus fruit in the standard carton took 7 days and 10 hours to cool from ambient ( $\pm 20^{\circ}\text{C}$ ) to close to set point of  $3.5^{\circ}\text{C}$ . When this work was repeated using Super Vent cartons with the same fruit kind and starting temperature the cooling time was reduced to 6 days. So just by changing the carton type (at no extra cost) the pre-cooling time can be reduced by one day with the concomitant benefit to product quality and reduction in energy foot print. The reversing of air flow in the containers has already been shown to increase efficiency and reduce cooling time and it is hoped that this can be commercialised in the near future.

### Projekopsomming

Die enigste navorsing van hierdie projek wat deur die heffing befonds word, is dit wat te doen het met strukturele veranderinge aan skeepshouers om die rigting van lugvloei te verander om die effektiwiteit van verkoeling te verhoog (5.4.2). Data wat tydens hierdie navorsing versamel is, is verwerk om 'n vergelyking tussen verkoelingseienskappe van die standaard A15C karton en die sogenaamde "Super vent" kanton te

tref. In identiese houers het dit 7 dae en 10 ure geneem om sitrusvrugte in die standaard karton vanaf kamertemperatuur ( $\pm 20^{\circ}\text{C}$ ) tot naby aan die stelpunt van  $3.5^{\circ}\text{C}$  te verkoel. Toe hierdie werk met die Super Vent kartonne, met dieselfde soort vrugte en begintemperatuur herhaal is, het die verkoelingstydperk na 6 dae verminder. Om net die kartontipe te verander (geen ekstra kostes) kan die voorverkoelingstyd met een dag verkort word met die gepaardgaande voordeel van produkgehalte en vermindering in die energie voetspoor. Die omkeer van lugvloei in houers het reeds getoon om effektiwiteit te verhoog en verkoelingstyd te verminder en daar word gehoop dat dit in die nabye toekoms gekommersialiseer kan word.

#### 5.4.2 **PROGRESS REPORT: Energy and temperature optimisation in refrigerated shipping containers**

Experiment C1/09 (Jan 2010 – Dec 2012) by M.C. Dodd (SU)

##### **Summary**

The citrus industry now exports over 80% of its fruit in refrigerated shipping containers. It is thus imperative that the industry ensures that the temperature and humidity management of the citrus in these containers is optimised. To this end new technology which manages the air flow within refrigerated shipping containers called Reversed Air Flow (RAF) has been tested in back to back trials (identical fruit packaged in the same cartons) placed in a container converted to RAF and a standard container. The purpose of this technology is, through managing the distribution of chilled air inside the containers, to improve the pulp temperature of fruit and relative humidity in the storage air. As this research would benefit all fruit kinds, funding has been received from all but the subtropical fruit industries. So far back to back trials have been conducted on apples, pears, plums, grapes and citrus. The technology has shown that the fruit temperatures can be managed closer to the set point and with less pulp temperature variability within the container. The concept of ambient temperature loading has been tested on citrus and the technology reduces the time to pre-cool the citrus from seven days to four days. The relative humidity is commodity driven, but in all cases the level was improved by the technology. Initial measurements on power consumption show that new technology reduces the energy consumed. A simulated "Steri" protocol trial has been conducted and this showed that the RAF technology was able to keep the fruit pulp temperature very close to the set point temperature.

##### **Opsomming**

Die sitrusbedryf voer nou meer as 80% vrugte in verkoelde skeepshouers uit. Dit is dus noodsaaklik dat die bedryf die temperatuur- en humiditeitsbeheer van sitrus in sulke houers verseker. Om dit te bewerkstellig is nuwe lugvloei-tegnologie, bekend as Omgekeerde Lugvloei (OL), in opeenvolgende proefnemings (identiese vrugte verpak in dieselfde kartonne) getoets – in 'n houer wat na OL omskep is sowel as in 'n standaard houer. Die doel van hierdie tegnologie is om die vrugtemperatuur en relatiewe humiditeit in die houer te verbeter deur doeltreffender verspreiding van verkoelde lug. Hierdie navorsing kan alle vrugtesoorte bevoordeel en dit word tans deur al die sektore behalwe die subtropiese bedryf gefinansier. Tot dusver is opeenvolgende proefnemings op appels, pere, pruime, tafeldruiwe en sitrus uitgevoer. Dié tegnologie het getoon dat die temperatuur van die vrugte nader aan die stelpunt gehandhaaf kan word en met minder afwykings binne die houer. Die konsep om vrugte teen kamertemperatuur te laai is getoets op sitrus en dié tegnologie verkort die voorverkoelingstyd van sitrus van sewe na vier dae. Relatiewe humiditeit is produkgedrewe, maar hierdie tegnologie het die relatiewe humiditeitsvlak in alle gevalle verbeter. Aanvanklike metings toon dat die nuwe tegnologie kragverbruik verminder. 'n Nagebootsde 'Steri'-protokol toets is uitgevoer en dit het getoon dat die OL-tegnologie in staat was om vrugtemperatuur baie na aan die stelpunt temperatuur te handhaaf.

#### 5.5 **PROJECT: CULTIVAR AND ROOTSTOCK EVALUATIONS**

Project coordinator (Acting): Tim G Grout (CRI)

##### 5.5.1 **Project summary**

Growing citrus is a long-term investment and both consumer preferences and even the climate may change during the lifetime of a tree. It is therefore critical that we keep up with the latest market trends and know in what region we get the best quality and production with the latest cultivars. A case in point is the Limpopo Seedless Valencia which had unacceptably low acid levels in the Weipe area near the Limpopo River but good internal quality near Citrusdal. Valencia types still account for 42% of the area planted to citrus (CGA statistics) so finding superior selections is important. Evaluations were conducted in hot-humid and hot-dry inland areas (5.5.2, 5.5.3, 5.5.4, 5.5.5, 5.5.7, 5.5.8) and in some cases included a range of new rootstocks. Valencias were also evaluated in cold production regions in both the Eastern and Western Cape provinces (5.5.12, 5.5.13). Navel oranges comprise 24% of citrus hectareage and were evaluated in cold production regions of the Western and Eastern Cape (5.5.18, 5.5.19, 5.5.20, 5.5.21). Although some growers are

replacing their grapefruit with other citrus types or other crops, grapefruit still account for 16% of citrus hectareage and a rootstock trial is continuing (5.5.6). The remaining evaluations are being done on soft citrus. Satsuma mandarins are being evaluated in the East Cape Midlands (5.5.9) and in the Western Cape (5.5.10), where Clementines are also under investigation (5.5.11). All other soft citrus trials are on mandarin hybrids (5.5.14, 5.5.15, 5.5.16, 5.5.17), some highlighting the importance of different rootstocks with Swingle delaying colour in at least two selections.

## **Projekopsomming**

Sitrus produksie is 'n langtermyn belegging en beide verbruikers voorkeur en selfs klimaat mag verander gedurende die leeftyd van 'n boom. Om hierdie rede is dit krities om op hoogte te bly van die huidige mark trend, en bewus te wees van in watter area ons die beste kwaliteit en produksie kry met die nuutste kultivars. 'n Praktiese voorbeeld is die Limpopo Saadlose Valencia wat onaanvaarbare lae suur vlakke in die Weipe area naby die Limpopo Rivier gelewer het, in teenstelling met goeie interne kwaliteit naby Citrusdal. Valencia seleksies beslaan steeds 42% van die areas waar sitrus aageplant is (CGA statistiek), wat die belangrikheid van uitstekende seleksies beklemtoon. Evaluasies was uitgevoer in warm-vogtige en warm-droë binnelandse areas (5.5.2, 5.5.3, 5.5.4, 5.5.5, 5.5.7, 5.5.8), en in sekere gevalle was 'n reeks nuwe onderstamme ingesluit. Valencias was ook ge-evalueer in koue produksie areas in beide die Oos-en Wes Kaap provinsies (5.5.12, 5.5.13). Nawels beslaan 24% van die sitrus aanplantings en was in die koue produksie areas van die Wes-en Oos Kaap ge-evalueer (5.5.18, 5.5.19, 5.5.20, 5.5.21). Alhoewel sommige produsente besig is om hulle pomelo's met ander sitrus tipes of ander gewasse te vervang, beslaan pomelo's steeds 16% van die sitrus aanplantings en 'n onderstam proef duur voort (5.5.6). Die oorblywende evaluasies word op sagte sitrus gedoen. Satsuma manderyne word in die Oos Kaap se Middellande (5.5.9) en in die Wes Kaap (5.5.10) ge-evalueer, waar Clementines ook ondersoek word (5.5.11). Alle ander sagte sitrus proewe is op Manderyn hibriede (5.5.14, 5.5.15, 5.5.16, 5.5.17), verskeie toon die belangrikheid van verskillende onderstamme met byvoorbeeld Swingle wat kleurontwikkeling vertraag in ten minste twee seleksies.

### **5.5.2 PROGRESS REPORT: Evaluation of Valencia selections in the hot humid inland areas (Onderberg)**

Experiment 75 A by J. Joubert (CRI)

#### **Summary**

Recommendations for selections that performed well in this season, according to optimum maturity from early to late in this hot, humid production area are as follows. Start the season off with Turkey which matures first, but bear in mind that the selection has a sensitive rind. Do not hang the fruit too long because the optimal picking period is no longer than 4-6 weeks. Benny 2 would follow, with good production and fruit size. Alpha and Midnight represent the middle of the Valencia season for this area, followed by McClean SL producing large size and seedless fruit. The Valencia season ends with the late selections, including Lavalley 2, producing large fruit, excellent yield and promising internal quality.

The experimental/semi-commercial selections performed well, including Jassie, Henrietta, Louisa and Skilderkrans. These selections should be included in future plantings when more and better information becomes available.

#### **Opsomming**

Aanbevelings vir seleksies wat goed presteer het vir hierdie seisoen volgens optimum rypheid wat van vroeg tot laat strek vir hierdie warm vogtige produksie area, is soos volg. Begin die seisoen met Turkey wat eerste ryp word, wees net versigtig om nie die seleksie te lank te hang nie. Baie skil probleme kan ontwikkel, want die optimum oes tydperk strek oor gemiddeld vier weke, ses weke maksimum dan moet die vrugte af wees. Benny 2 kan dan volg wat goeie produksie en vruggrootte produseer. Alpha of Midnight verteenwoordig die middel van die Valensia seisoen vir hierdie area, gevolg deur McClean SL wat uitstekend vaar met goeie vruggroote en saadlose vrugte. Die Valencia seisoen kan dan afgesluit word met die laat seleksies soos Lavalley 2 wat groot vrugte produseer, goeie oes verseker met belowende interne kwaliteit.

Die eksperimentele/semi-kommersiele seleksies wat goed presteer sluit in Jassie, Henrietta, Louisa en Skilderkrans. Hierdie seleksies kan in die toekoms ingesluit word soos meer en beter inligting beskikbaar word.

### 5.5.3 **PROGRESS REPORT: Evaluation of Valencia selections in the hot dry inland areas (Letsitele)** Experiment 75 B by J. Joubert (CRI)

#### **Summary**

The season starts with early selections and proceeds to the late maturing selections suitable for this hot-dry production area. Recommendations have therefore been made accordingly. The season starts with Turkey, producing large fruit size with good internal quality and soft fibre. Do not hang fruit too long on the trees because severe rind problems will result and high numbers of fruit will be lost. The optimal picking window will be within the first four weeks of peak maturity. Benny 2 follows after Turkey with good production and medium to large fruit size. Midnight 1 covers the middle of the Valencia season with good internal quality fruit, large fruit size, smooth rind and low seed counts per fruit. Lavalley 1 is currently the latest maturing Valencia selection that is being planted semi-commercially; developing excellent fruit size and yield.

There is a series of experimental/semi-commercial selections that have also been included in the hot production areas. The selection range will follow from early, mid, to late-maturing options. The season starts with Bend 8A1, followed by Bend 8A2. The middle of the Valencia season will be complimented by Jassie and Henrietta, delivering good production and internal quality fruit. Louisa matures more towards the end of the Valencia season, followed by Ruby and Skilderkrans. Late in the season you could possibly add Moosrivier Late 1 and 2 to the options, when more information becomes available from future evaluations.

#### **Opsomming**

Die seisoen word van vroeg tot laat rypwordende seleksies opgedeel in die warm droë produksie area en aanbevelings is soos volg. Die seisoen kan begin word met Turkey, wat groot vrugte produseer met goeie interne kwaliteit en sagte vessel. Moenie vrugte te lank hang nie, baie skil bederf sal intree wat groot verliese tot gevolg het. Optimum plukvenster is binne die eerste vier weke van piek rypheid. Benny 2 volg na Turkey met goeie produksie en medium tot groot vuggrootte. Midnight 1 vul die middel van die Valencia seisoen met goeie interne kwaliteit vrugte, groot vuggrootte, gladde skille en lae saadtellings per vrug. Lavalley 1 is tot op datum die laatste Valencia seleksie wat semi-kommersieel aangeplant word, met uitstekende vuggrootte en goeie produksie op die bome.

Daar is 'n reeks eksperimentele/semi-kommersiele seleksies wat ook vir die warm produksie areas ingesluit is. Hier volg die seleksies van vroeg, middle tot laat rypwordend. Die seisoen kan begin word met Bend 8A1, gevolg deur Bend 8A2. Die middle van die Valencia seisoen kan aangevul word met Jassie en Henrietta, wat goeie produksie lewer, asook goeie kwaliteit vrugte. Louisa word meer aan die einde van die Valencia seisoen ryp, gevolg deur Ruby en Skilderkrans. Laat in die seisoen kan aangevul word met Moosrivier Late 1 en 2, soos meer inligting beskikbaar word uit verdere evaluasies.

### 5.5.4 **PROGRESS REPORT: Evaluation of Valencia selections in the hot humid inland areas (Swaziland)** Experiment 740A by J. Joubert (CRI)

#### **Summary**

This production area is classified as hot and humid and the establishment of Valencia and Grapefruit selections is very favourable. Turkey, followed by Portgate one week later, represent the two early selections in this trial. However, three-year-old Turkey trees in the commercial orchards in this area already have Blackspot problems, so look out for this on young trees and adapt spray programmes accordingly. Alpha, followed by Jassie qualify as the mid-maturing selections of the Valencia season. Alpha performed very well, with good internal quality fruit and fruit size. Jassie as an experimental selection looks promising. Delta was the control for this trial, and fits in just after the middle of the season. Now McClean SL follows as one of the later maturing selections, with good production and internal quality, as well as seedless fruit. Ruby was the last selection to evaluate, with good internal quality and deep red internal colour, a niche-market would need to be established. Smaller fruit size and seeds are negative characteristics of Ruby.

#### **Opsomming**

Die produksie area word beskou as warm in kombinasie met vogtige klimaat en maak die verbouing van Valencia en Pomelo varieteite baie gunstig. Turkey, gevolg deur Portgate een week later, maak die twee vroeë seleksies van hierdie proef uit. Turkey het in die kommersiële boorde in hierdie area op driejarige ouderdom reeds swartvlek probleme getoon, hou die jong bome dus goed dop en pas spuitprogramme aan. Alpha, gevolg deur Jassie kwalifiseer as die mid-rypwordende seleksies van die Valencia seisoen. Alpha

presteer baie goed, met goeie interne kwaliteit en vruggrootte. Jassie as eksperimentele seleksie lyk baie belowend. Delta was die kontrole in hierdie proef, en pas in net na die middel van die seisoen. Dan volg McClean saadloos wat een van die later rypwordende seleksies uitmaak, met goeie produksie en interne kwaliteit, asook saadlose vrugte. Ruby was die laatste seleksie om te evalueer, en met goeie interne kwaliteit en diep rooi interne kleur, moet daar 'n nis-mark voor gevind word. Soms kan kleiner vruggrootte en saad probleme by Ruby veroorsaak.

#### **5.5.5 PROGRESS REPORT: Evaluation of Valencias on new imported rootstocks in the Malelane area**

Experiment 416 A by J. Joubert (CRI)

##### **Summary**

Midnight has proved to be compatible with Sunki x Beneke 812, a hybrid rootstock cross between a Sunki mandarin and Beneke trifoliolate. The internal quality produced on the Midnight and Sunki combination was very good, fruit developed medium to large fruit size and the yield on the trees light due to smaller tree size.

Delta seems to be compatible with Sunki x Beneke 812, HRS 802 and FF-6 rootstocks at this trial site. The bud-union between the rootstock and scion was fairly smooth without any growth tips. The fruit size on Sunki 812 was bigger than on HRS 802 and FF-6. The Delta x Sunki combination developed a smaller tree than with HRS 802 and FF-6. The yield on HRS 802 and FF-6 was better; the lighter crop on Sunki was due to smaller tree size and larger fruit size production.

Evaluations to date show that these rootstocks could be of value to citrus producers, particularly Sunki 812, should high pH levels and calcareous soils be a problem. Sunki 812 was selected for its high tolerance to Phytophthora, citrus nematodes and tristeza, as well as better tolerance of high pH and calcareous soils.

##### **Opsomming**

Midnight het bewys dis verenigbaar met Sunki x Beneke 812, 'n hibried onderstam kruisig tussen Sunki mandarin en Beneke trifoliaat. Die interne kwaliteit wat deur die Midnight op Sunki kombinasie geproduseer is, was baie goed, vrugte het medium tot groot vruggrootte ontwikkel en die oes op die bome was ligter a.g.v. die kleiner boom grootte.

Delta toon vereenigbaarheid met Sunki x Beneke 812, HRS 802 en FF-6 onderstamme vir hierdie proef perseel. Die entlas tussen die onderstam en bostam was glad met geen tekens van groeipunte nie. Die vruggrootte op Sunki 812 was groter gewees in vergelyking met HRS 802 en FF-6. Die oes op HRS 802 en FF-6 was beter; die ligter oes op Sunki kan toegeskryf word aan die kleiner boom grootte en groter vruggrootte produksie.

Evaluasies tot op datum toon aan dat hierdie onderstamme waardevol kan wees vir die sitrus produsente, meer spesifiek Sunki 812, waar hoë pH vlakke en kalkagtige gronde voorkom. Sunki 812 was vir sy hoë verdraagsaamheid teen Phytophthora, sitrus aalwurms en tristeza, asook beter weerstand vir hoër pH en kalkagtige gronde geselekteer.

#### **5.5.6 PROGRESS REPORT: Evaluation of Grapefruit varieties on different rootstocks in the Swaziland area**

Experiment 416 B by J. Joubert (CRI)

##### **Summary**

Marsh, Star Ruby and Nelruby on all four rootstocks performed well this season in regard to yield production on the tree. Marsh on Swingle produced the best crop. Nelruby on Swingle recovered extremely well and produced a yield six times higher this season than last year. Taking tree volume into consideration, C35 must be credited for the ability, in combination with all three selections, to produce on average the same yield as MxT and X639, but with trees one third smaller in size.

Nelruby produced the highest Brix: acid ratio for this trial, but Star Ruby developed the highest juice percentage. The general fruit quality went down for this season when compared to the 2010 season. Marsh went from second best quality last year, to third best and a low Brix: acid ratio of 6.51.

The general tendency for fruit size was heading towards smaller fruit, where more fruit was produced in total, but smaller size. This scenario is typical where a heavier crop was produced on the trees; the fruit size will decrease. Marsh developed the biggest fruit size and Star Ruby the smallest on X639.

Note: The trees in this trial are generally smaller than expected due to problems encountered with the irrigation scheme.

### Opsomming

Marsh, Star Ruby en Nelruby op al vier onderstam kombinasies het goed presteer hierdie seisoen t.o.v. oes produksie op die bome. Die hoogste oes is deur Marsh behaal op Swingle. Nelruby het uitstekend herstel op Swingle met 'n produksie gemiddeld ses keer hoër as die vorige seisoen. As die boomvolume in ag geneem word, moet C35 baie hoog aangeskryf word by al die kombinasies, want die bome is 'n derde kleiner en lewer gemiddeld dieselfde opbrengs as MxT en X639.

Nelruby het die hoogste Brix: suur verhouding vir hierdie proef behaal, maar Star Ruby het die hoogste sap persentasie gelewer. Die algemene vrug kwaliteit vir hierdie seisoen het gedaal in vergelyking met die 2010 seisoen. Marsh het van tweede beste uitgesak na die laaste posisie, met die laagste Brix: suur verhouding van 6.51.

Die algemene tendens wat vruggrootte aanbetref het 'n daling aangedui waar meer vrugte in total geproduseer was, maar by kleiner tellings. Hierdie is 'n scenario waar meer vrugte op die bome, kleiner vruggrootte kan veroorsaak. Marsh het die grootste vrugte geproduseer en Star Ruby die kleinste op X639.

Nota: Die bome in hierdie proef is oor die algemeen kleiner as normaal a.g.v. probleme wat met die besproeiings skema ondervind was.

### 5.5.7 PROGRESS REPORT: Evaluation of various Valencia selections on different rootstocks in the Komatipoort area

Experiment 590 B by J. Joubert (CRI)

### Summary

Delta produced the best internal quality on the dwarfing rootstocks C35, Koethen and Terrabella, except for Carrizo developing a medium size tree. The Brix: acid ratio on these rootstocks was all above 11. The fruit size for Delta increased and three rootstock combinations peaked at count 72. Crop yield increased and averaged 39 kg/tree. There were no incompatibility problems on the rootstock combinations visible.

McClellan seedless produced good internal quality on all the rootstock combinations and C35 was the exception where the Brix: acid ratio measured above 10 (11.8%). The fruit size was optimal for Valencia production with peaks between counts 56 and 72. X639 bore the best yield on the trees, setting 80.6kg and the average production on all the combinations improved to 43.6 kg/tree.

Midnight produced on three rootstocks a Brix: acid ratio above 10, including Carrizo (11.05), Terrabella (10.46) and X639 (10.69). Fruit size distribution peaked at count 56, except for X639 at count 72. Crop yield also increased for this season with an average crop of 23 kg/tree, although the yields were low when taken into consideration that 39 kg/tree was the best (C35).

Portsgate developed good internal qualities and Carrizo (11.89), Koethen (11.62) and Swingle 10.69) produced Brix: acid levels above 10. The lowest Brix: acid ratio was on MxT with 9.15. The fruit size on the different combinations varied completely from count 56 to 105/125, with the smallest fruit size on X639. Portsgate on X639 bore 73.1 kg/tree, explaining the smaller fruit size on this rootstock. The average yield this season was 30 kg per tree.

### Opsomming

Delta se interne kwaliteit het die beste gevaar op die verdwergende onderstamme C35, Koethen en Terrabella, behalwe vir Carrizo wat 'n medium boomgrootte lewer. Die Brix: suur verhoudings op hierdie onderstamme was almal bo 11 gewees. Delta se vruggrootte het toegeneem en drie van die kombinasies het gepiek by telling 72. Oesproduksie se gemiddeld het na 39 kg/boom toegeneem. Geen onverenigbaarheids tekens was by enige van die kombinasies sigbaar nie.

McClellan saadloos het goeie interne kwaliteit met die verskillende onderstam kombinasies behaal, maar C35 was die enigste onderstam waar die Brix: suur verhouding bokant 10 was (11.8). Die vruggroote was optimaal vir Valencia produksie met pieke by telling 56 en 72. X639 het die beste oes op die bome gelewer met 80.6 kg en die gemiddelde produksie vir al die kombinasies het ook verbeter tot 43.6 kg/boom.

Midnight het op drie onderstamme 'n Brix: suur verhouding bo 10 geproduseer, wat Carrizo (11.05), Terabella (10.46) en X639 (10.69) insluit. Vruggroote verspreiding het gepiek by telling 56, behalwe vir X639 met telling 72. Oesproduksie het ook vir hierdie seisoen toegeneem met 'n gemiddeld van 23 kg/boom, maar die opbrengste was laag wanneer in aanmerking geneem word dat 39 kg/boom die hoogste was (C35). Warm temperature tydens blomset in hierdie produksie area het 'n groot invloed op die prestasie.

Portsgate se interne kwaliteit was goed gewees en Carrizo (11.89), Koethen (11.62) en Swingle (10.69) het Brix: suur verhoudings bo 10 gelewer. Die laagste Brix: suur verhouding was op MxT gewees met 9.15. Die vruggroote van hierdie kombinasies het baie gewissel van telling 56 to 105/125, met die kleinste vrugte op X639. Portsgate op X639 het 73.1 kg/boom geproduseer, wat moontlik kan verklaar hoekom die vruggroote op hierdie onderstam die kleinste was. Die gemiddelde opbrengs vir hierdie seisoen was 30 kg/boom gewees.

#### **5.5.8 FINAL REPORT: Evaluation of Limpopo Seedless Valencia on four different rootstocks in the Weipe area** Experiment 900 by J. Joubert (CRI)

##### **Summary**

Limpopo seedless performed the best in combination with Roughlemon. There were no incompatibility problems on any of the four rootstocks visible at this trial. The internal quality was marginal with only Rough lemon above the minimum requirements, Brix was acceptable and acids repeatedly low. The low acids seem to be a cultivar characteristic. Yields on Carrizo, Swingle and X639, except for Rough lemon was light to very light, resulting in large fruit size. Rough lemon with the lowest acid levels in this trial, indicates the dangers of producing fruit that will not comply with the export standards. Although having optimal yield and fruit size, these facts must be taken into consideration.

This trial was stopped after this season and will be replaced with a new one, including a wide range of rootstocks in combination with Weipe seedless, representing the latest selection available. Sunki x Beneke rootstock will be included and was specifically selected for replant soils with high salinity and pH.

##### **Opsomming**

Limpopo saadloos het die beste vertoon in kombinasie met Growweskil suurlemoen. Daar was geen onverenigbaarheids tekens by enige van die vier onderstamme sigbaar nie. Die interne kwaliteit was marginaal met slegs Growweskil bo die minimum sap vlakke, Brix was aanvaarbaar en sure weer laag. Die lae suurinhoud blyk soos 'n kultivar eienskap te wees. Die oes op Carrizo, Swingle en X639, behalwe vir Growweskil was lig tot baie lig gewees, met groter vrugte tot gevolg. Growweskil met die laagste suurvlakke vir hierdie proef toon die gevaartekens aan vir vrugte wat nie aan die uitvoer standaard gaan voldoen nie. Alhoewel die produksie en vruggroote optimal was, kan hierdie feite nie buite rekening gelaat word nie.

Hierdie proef word gestaak en sal vervang word deur 'n groter verskeidenheid onderstamme in kombinasie met Weipe saadloos, wat die nuutste beskikbare seleksie verteenwoordig. Sunki x Beneke onderstam word ook ingesluit, wat spesifiek vir herplant gronde met hoër vry kalk en pH geselekteer was.

#### **5.5.9 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (East Cape Midlands)** Experiment 57A by R. Fenwick (CRI)

##### **Summary**

The following recommendation can be made according to the results obtained from the 2011 season. The season should start with either Miyagawa Wase or Miho Wase which mature a few days apart. Although the fruit on Miyagawa Wase is on average one count larger than Miho Wase, both had excellent yields. These can be followed by Okitsu Wase and Kuno, which had slightly lower yields than Miho Wase and Miyagawa Wase with Okitsu Wase having smaller fruit. The Satsuma season ends with the late selections which are Dobashi Beni and lastly Ueno, both having similar yields, although the high acid levels in Ueno could assist in its hanging ability and extend the picking season slightly. However, the picking periods should be kept

short, approximately 2 to 3 weeks to maintain a good internal quality standard and to avoid fruit becoming puffy. All Satsuma selections would require degreening after harvest as all were internally mature but without the external colour.

### **Opsomming**

Na aanleiding van die 2011 seisoen se resultate, kan die volgende aanbevelings gemaak word. Die Satsuma seisoen moet begin word met Miyagawa Wase of Miho Wase. Hierdie seleksies se rypwordings tyd verskil met net 'n paar dae. Miyagawa Wase se vruggrootte was gemiddeld een telling groter as Miho Wase, maar albei het uitstekende opbrengs gehad. Daarna volg Okitsu Wase en Kuno, alhoewel die opbrengs van hierdie seleksies effens laer was as Miho Wase en Miyagawa Wase. Okitsu Wase het kleiner vruggroote gehad. Die Satsuma seisoen kan afgesluit word met die laat seleksies, Dobashi Beni en Ueno, albei met soortgelyke opbrengste. Ueno se hoë suurvlaakke dui aan dat hierdie seleksie vir 'n langer tydperk kan hang en die seisoen verleng. Daar word aanbeveel om nie die oes periode langer as 2 tot 3 weke te verleng nie, om die hoë interne gehalte te behou en powwerige vrugte te voorkom. Alle Satsuma seleksies het ontgroening nodig gehad as gevolg van vertraagde eksterne kleur.

#### **5.5.10 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Satsuma mandarins in a cold production region (Western Cape)**

Experiment 57D by R. Fenwick (CRI)

### **Summary**

This is a late Satsuma trial. Although Ueno Satsuma was shown to be the earliest maturing variety in this instance, it is not recommended as the results are not indicative of the selection in other production regions where it is regarded as one of the latest selections. The results obtained from the previous year in the same area also differ with the current results. The season should rather start with Aoshima, which has good yields but should not be kept too long on trees as acid drops rapidly towards end of the season. This can then be followed up with Kuno and then Ohtsu, both with good yields and good internal quality. Either Dobashi Beni or Owari would be next with Owari slightly later than Dobashi Beni, but both having excellent yields. The latest Satsuma selection is Imamura, although it is not recommended at this stage due to vigorous trees and poor yields this season. Colour development and internal quality was good but further evaluations will be required to establish whether this selection is suitable to the industry. Picking periods should be limited to 2 to 3 weeks to maintain a good internal quality standard and to avoid fruit becoming puffy. All Satsuma selections would require degreening after harvest, although there were some selections that had slightly better colour than others when internally mature.

### **Opsomming**

Hierdie is 'n laat Satsuma proef. Ueno was die seleksie wat eerste rypgeword het, maar dit is nie die geval in boorde in ander kommersiele areas nie, waar dit bekend is as een van die laaste seleksies. Die resultate van die vorige seisoen verskil van die huidige inligting. Aoshima se rypwordings tyd is volgende, met goeie opbrengs, alhoewel die seleksie nie te lank op die bome moet bly nie as gevolg van die vinnige daling in suurvlaakke. Kuno en Ohtsu volg, albei met goeie opbrengs en interne gehalte. Dobashi Beni of Owari sal volgende wees, met Owari wat effens later is as Dobashi Beni, alhoewel albei uitstekende opbrengs geproduseer het. Imamura is die laaste Satsuma seleksie, maar word nie aanbeveel nie as gevolg van die groeikragtige bome en swak opbrengs. Kleur het goed gevorder en interne gehalte was goed, maar hierdie seleksie sal verder ge-evalueer moet word om vas te stel of dit enige waarde inhou vir die sitrusbedryf. Dit is aanbeveel dat die pluk periode nie langer as 2 tot 3 weke moet wees nie, om hoë interne gehalte te behou en powwerige vrugte te vermy. Alle Satsuma seleksies was ontgroen, as gevolg van vertraagde eksterne kleur.

#### **5.5.11 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Clementine mandarins in a cold production region (Western Cape)**

Experiment 1000D by R. Fenwick (CRI)

### **Summary**

The Early Clementine would be a possible option to start off the Clementine season, with fruit much flatter than the other selections as well as having good size, internal quality and yields (although splitting of fruit was present on the trees). This could be followed by Clemenpons, which may have slightly smaller fruit and poor colour development, but has very good internal quality with similar yields to Early Clementine. Marisol Clementine follows closely with very good yields, good internal quality and better fruit size than Clemenpons,

although stylar end splitting was present which may account for some fruit losses. Nules Clementine will end the Clementine season as fruit hangs well on trees.

### **Opsomming**

Die vroeë Clementine is 'n moontlike opsie om die Clementine seisoen mee te begin. Die vrugvorm is platter as die ander seleksies en vruggrootte is goed. Interne gehalte en opbrengs was baie goed, maar daar was vrug-split op van die vrugte merkbaar. Clemenpons volg met effens kleiner vrugte en swakker eksterne kleur vordering; alhoewel interne gehalte goed was met soortgelyke opbrengs. Marisol se rywordings tyd is volgende, met baie goeie opbrengs, goeie interne gehalte en beter vruggroote as Clemenpons, alhoewel die split van die vrugte verliese veroorsaak. Nules sal die Clementine seisoen eindig en vrugte kan goed hang aan die bome.

#### **5.5.12 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia Oranges in a cold production region (Sundays River Valley)**

Experiment 75C by R. Fenwick (CRI)

### **Summary**

The results from the 2011 season show that Midnight Valencia had good yields and matures earlier than Turkey, although it is on Volckameriana. Turkey had the best internal quality and also good yields. After Turkey the selection that matures next is Delicia, which had good yields but poorer internal quality. The season ends with Delta Valencia, also with good yields and internal quality. Fruit size on Midnight, Turkey and Delicia was good but fruit size on Delta was small. For all selections, colour development occurred before fruit were internally mature. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders, especially for Turkey Valencia.

### **Opsomming**

Die resultate van die 2011 seisoen het bewys dat Midnight Valencia goeie opbrengs gehad het en dat dit voor Turkey rygeword het, selfs met Volckameriana as onderstam. Turkey het die beste interne gehalte gehad met goeie opbrengs. Na Turkey was dit Delicia gewees wat goeie opbrengs behaal het, maar swak interne kwaliteit. Delta Valencia eindig die seisoen met goeie opbrengste en interne kwaliteit. Vruggrootte was goed gewees vir Midnight, Turkey en Delicia, maar klein vir Delta. Kleur het goed gevorder in alle seleksies en vrugte was opgekleur voor vrugte intern ryp was. Aanbevelings is dat die pluk periode nie langer as 2 tot 3 weke moet neem nie om goeie interne kwaliteit te verseker en skil probleme te vermy.

#### **5.5.13 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Valencia Oranges in a cold production region (Western Cape)**

Experiment 75D by R. Fenwick (CRI)

### **Summary**

The results from the 2011 season show that Limpopo SL is the earliest Valencia to mature with good internal quality, small to medium fruit size, moderate yield and thicker than usual rinds. Midnight 1 is the next to mature with good internal quality, small to medium fruit size and good yield. Benny 1 and 2 mature together and bring the Valencia season to an end. Both cultivars had very good yields, excellent internal quality and good fruit size, however, seed is prevalent in both with Benny 1 having more seed than Benny 2. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

### **Opsomming**

Die 2011 seisoen wys Limpopo SL was die vroegste Valencia seleksie om ryp te word. Interne kwaliteit was goed, maar vruggrootte was klein en die skil dikker as gewoonlik. Midnight 1 volg met goeie interne kwaliteit, gemiddelle vruggrootte en goeie opbrengs. Benny 1 en Benny 2 word saam ryp en eindig die Valencia seisoen vir hierdie proef. Albei seleksies het baie goeie opbrengs, uitstekende interne kwaliteit en goeie vruggrootte. Saad is gevind in albei seleksies, maar die meeste in Benny 1. Die aanbeveling is om nie die oes periode langer as 2 tot 3 weke te maak nie, om goeie interne kwaliteit te behou en skil probleme te vermy.

**5.5.14 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (East Cape Midlands)**  
Experiment 997A by R. Fenwick (CRI)

**Summary**

The results of the 2011 season show that Nadorcott on X639 had the largest fruit size, excellent yield and matured the earliest. This was followed by Nadorcott on Swingle with slightly smaller fruit and delayed external colour but with excellent yields. Or matured next, with very good yields but small fruit relative to the other selections, although Brix levels were the highest. This was followed by Mor, with good fruit size, good yields but lowest Brix levels relative to the other selections as well as the highest incidence of seed in fruit. The last to mature was Nadorcott on Carrizo which also had excellent yields, high Brix levels and good fruit size. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

**Opsomming**

Die resultate van die 2011 seisoen het bewys dat Nadorcott op X639 die grootste vrugte geproduseer het met 'n uitstekende opbrengs, en die seleksie was eerste ryp vir die seisoen. Nadorcott op Swingle was volgende, met effens kleiner vrugte, maar die hoogste Brix vlakke. Mor is volgende met goeie vruggrootte, goeie opbrengs maar die laagste Brix vlakke en meeste saad teenoor die ander seleksies. Nadorcott op Carrizo was die laaste seleksie van die seisoen, ook met uitstekende opbrengs, hoë Brix vlakke en goeie vruggrootte. Daar word aanbeveel om nie die oesperiode langer as 2 tot 3 weke te verleng nie om goeie interne kwaliteit te verseker en minimum skil probleme.

**5.5.15 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Sundays River Valley)**  
Experiment 997B by R. Fenwick (CRI)

**Summary**

The results of the 2011 season show that HE Mandarin was the earliest of the selections to mature. Fruit size was largest and yields were average. This is attributed to heavy pruning. African Sunset was next to mature in the season, also with relatively average yields and very large fruit. African Sunset had the lowest Brix levels. Valley Gold and Clemcott end the Mandarin Hybrid season, both yielding excellent quality fruit with very good yields, however, Clemcott had larger fruit and the greatest number of seed. Acid held well on Clemcott. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

**Opsomming**

Die uitslae van die 2011 seisoen het bewys dat HE Mandarin die vroegste van die seleksies rypgeword het. Vrugte was die grootste en opbrengs was gemiddeld, 'n moontlike resultaat van die swaar snoeiwerk op die bome. African Sunset het gevolg met 'n gemiddelde opbrengs, baie groot vrugte en die laagste Brix vlakke van die seleksies. Valley Gold en Clemcott word laaste ryp, albei met uitstekende interne gehalte en baie goeie opbrengs, alhoewel Clemcott die grootste vrugte gehad het met die meeste saad. Suurvlakke het goed gehou op Clemcott. Dit is aanbeveel dat die pluk periode nie langer as 2 tot 3 weke neem nie om goeie interne gehalte standarde te behou en weg te bly van na-oes skil probleme.

**5.5.16 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (Gamtoos River Valley)**  
Experiment 997C by R. Fenwick (CRI)

**Summary**

The results of the 2011 season show that Cape Naartjie 1 was the first to mature with excellent yields and large fruit size. Tasty 1 was next to mature with good to very good yields, but fruit size was extra large and it had an unacceptable amount of seeds. Cape Naartjie 2 follows the Tasty 1 with slightly delayed external colour but excellent yields and good fruit size. Tasty 2 matures next with excellent yields, good fruit size and intense orange-red coloured rind, however, the fruit tends to be externally overmature (puffy) when internals are still immature and also has a high incidence of seediness. The last two selections are ultra late as a result of high acid levels. The MinneolaX, which matures second last also has deep red-orange colour, but with large fruit size and good yields with very high numbers of seed. OrangeX is the latest maturing selection

and has average yields, large fruit size and high seed levels. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders, especially for the Tasty 2 Mandarin.

### **Opsomming**

Die uitslae van die 2011 seisoen toon aan dat die Kaap Naartjie 1 die vroegste seleksie is met uitstekende opbrengs en groot vruggrootte. Tasty 1 is volgende op die lys met goeie tot baie goeie produksie, alhoewel vruggrootte baie groot is en die hoeveelheid saad onaanvaarbaar was. Kaap Naartjie 2 volg met effens vertraagde eksterne kleur ontwikkeling, maar uitstekende opbrengs en goeie vruggrootte. Tasty 2 word volgende ryp met uitstekende opbrengs, goeie vruggrootte en helder, diep oranje-rooi skil kleur. Ongelukkig word die vrugte ekstern oorryp en powwerig, met die interne kwaliteit ver van gereed asook baie saad. Die laaste twee seleksies is baie laat as gevolg van die hoë suurvlaakte. Minneola X is die tweede laaste seleksie wat ryp word met groot vrugte, goeie opbrengs en baie saad per vrug. Orange X is die laatste seleksie met 'n gemiddelde opbrengs, groot vruggrootte en heelwat saad. Die pluk periode moet nie langer as 2 tot 3 weke wees nie, om goeie interne gehalte standarde te behou en skil probleme te vermy.

#### **5.5.17 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Mandarin hybrids in a cold production region (South Western Cape)**

Experiment 997E by R. Fenwick (CRI)

### **Summary**

The results from the 2011 season show that Swingle should not be recommended as a rootstock for Sweet Spring as it delays colour development. Rootstocks inducing improved colour development are recommended. Larger fruit were of poorer quality than smaller fruit and were internally mature earlier than the smaller fruit. The quality of the Sweet Spring will improve as the trees get older, as is indicated by the results of the older trees. Yields for Sweet Spring are excellent. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

### **Opsomming**

Swingle word nie aanbeveel as 'n onderstam vir die Sweet Spring nie. Dit is as gevolg van die laat kleur ontwikkeling wat hierdie onderstam induseer. Onderstamme wat vroeër kleur induseer word aanbeveel. Die bevinding was dat groter vrugte 'n swakker interne kwaliteit gelever het as die kleiner vrugte, asook vroeër intern ryp as die kleiner vrugte. Bome is nog jonk so interne gehalte behoort beter te wees soos die bome ouer word. Opbrengs was uitstekend gewees. Die pluk periode moet nie langer as 2 tot 3 weke wees nie om goeie interne gehalte standarde te behou en skilprobleme te voorkom.

#### **5.5.18 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Navel oranges in a cold production region (Sundays River Valley)**

Experiment 998B by R. Fenwick (CRI)

### **Summary**

The early selections had poor acid levels and weak colour development. Yields were similar for all the early selections. Newhall (CC) was the earliest selection to mature and had good Brix levels. Fukumoto (TC) matured next with the highest Brix levels of all the earlier selections. Lina (CC) and Lina (TC) matured at the same time and also had Brix levels above 10.0%. Newhall (TC) matured next and had the poorest Brix levels of the early selections. Tulegold was next to mature and had poor Brix levels with the smallest fruit size of the early selections, followed by Fukumoto (CC) with acceptable Brix. Washington was the latest to mature and had acceptable Brix levels but the poorest colour development. For the late selections, Robyn was the earliest to mature with very good yields but poor colour development and lowest internal quality. Cambria was next to mature, with slightly higher yields but better colour and slightly higher internal quality. Lane Late matured next with very good yields, but improved colour development and the second best internal quality of the late selections. Witkrans was next to mature, with slightly less yields and acceptable internal quality but the best colour development of the late navel selections. This was followed by Autumn Gold; with very good yields, good internal quality but poor colour development. Powell Summer was the second latest to mature with good yields but had acceptable colour development and the best internal quality compared to the other selections. Glen Ora Late was the latest selection with acceptable internal quality but lowest yields in relation to the other late selections. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

## Opsomming

Swak kleur ontwikkeling en laë suurvlaekke het voorgekom by die vroeë seleksies. Die vroegste seleksie was Newhall (CC) wat baie goeie Brix vlakke gelewer het. Fukumoto (TC) se rypwordings tyd was volgende en het die hoogste Brix vlakke gehad van al die vroeë seleksies. Lina (CC) en Lina (TC) se Brix vlakke was bo 10.0 en was volgende ryp. Lina was gevolg deur Newhall (TC) wat die swakste Brix vlakke gehad het van al die vroeë seleksies. Tulegold was volgende en het die kleinste vrugte geproduseer van die vroeë seleksies met swak Brix vlakke, gevolg deur Fukumoto (CC) met aanvaarbare Brix vlakke. Die vroeë seleksies sluit af met Washington. Uit die laat seleksies was Robyn nawel die eerste ryp en het baie goeie opbrengste gelewer, maar met swak eksterne kleur ontwikkeling en die laagste interne kwaliteit. Cambria was volgende met effens beter opbrengste en interne gehalte, as ook beter eksterne kleur. Dit was gevolg deur Lane Late nawel wat die tweede beste interne gehalte gehad het, saam met baie goeie opbrengs en verbeterde eksterne kleur. Witkrans se rypwordings tyd is volgende met effens ligter drag, aanvaarbare interne gehalte en die beste kleur ontwikkeling van al die laat seleksies. Autumn Gold is volgende met baie goeie opbrengs, goeie interne gehalte maar swak kleur ontwikkeling. Powell Summer was die tweede laaste seleksie om ryp te word. Hierdie seleksie het goeie opbrengste geproduseer met aanvaarbare kleur ontwikkeling en interne gehalte, moontlik die beste van al die ander seleksies. Die laatste seleksie om ryp te word is Glen Ora Late. Die interne gehalte was aanvaarbaar, maar die drag op die bome was die swakste van al die seleksies. Die pluk periode moet nie langer as 2 tot 3 weke wees nie om goeie interne gehalte standarde te behou en naes skil probleme te vermy.

### 5.5.19 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Navel oranges in a cold production region (Western Cape)** Experiment 998D by R. Fenwick (CRI)

#### Summary

Yields on the young trial trees were lower than average since it was the first crop for these trees. Acid levels were low although Brix levels were higher for the young trees. Lina was the first to mature and had the highest Brix levels and best colour development, although fruit size was smallest. Glen Ora Late and Letaba Early matured at the same time, with Glen Ora Late having larger fruit and poorer colour but similar Brix to Letaba Early. Yields and internal quality were better on the older trees with excellent colour development. Witkrans was the first to mature with good Brix levels but smallest fruit compared to the other selections. Royal Late was next to mature with good fruit size and the highest Brix levels compared to the other selections. Lane Late matured next and also had good fruit size and Brix levels. Glen Ora Late was the latest maturing selection with the largest fruit size but lowest Brix levels. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

## Opsomming

Opbrengs op die jong bome was laër as gewoonlik, moontlik as gevolg boom ouderdom. Suurvlaekke was laag alhoewel Brix vlakke heelwat beter was vir die jong bome. Die seisoen begin met Lina nawel, die seleksie het die beste kleur en hoogste Brix vlakke geproduseer, alhoewel vruggrootte die kleinste was. Glen Ora Laat en Letaba Early was volgende gewees met dieselfde rypwordingstyd. Glen Ora Laat het groter vrugte en swakker eksterne kleur ontwikkel, maar met dieselfde Brix vlakke. Op ouer bome was die interne gehalte, kleur ontwikkeling en drag baie beter. Witkrans was eerste om ryp te word van die later seleksies, met die kleinste vruggrootte maar goeie interne gehalte. Royal Late het gevolg met beter vruggrootte en die hoogste Brix vlakke van al die seleksies. Lane Late volg na Royal Late met goeie vruggrootte en interne gehalte. Glen Ora Late is die seleksie wat die laatste ryp geword het van al die seleksies met die grootste vruggrootte en laagste Brix vlakke. Die pluk periode moet nie langer as 2 tot 3 weke wees nie, dit verseker goeie interne gehalte en voorkom skil probleme.

### 5.5.20 **PROGRESS REPORT: Cultivar characteristics and climatic suitability of Experimental Navel oranges in a cold production region (Sundays River Valley)** Experiment 1001A by R. Fenwick (CRI)

#### Summary

Habata Early was first to mature with good internal quality and deep orange rind but poor yield, mostly attributed to poor tree health. This is followed by the EH Navel, which has good yields, Deep red-orange rind and acceptable Brix but poor acid levels. Palmer navel (control) matures next followed by 99 Navel which has excellent colour development, soft internal flesh but poor yields, low Brix and high acid. HE Late was the

latest to mature with good yields and the best internal quality of all the selections. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

### **Opsomming**

Habata Early was die vroegste seleksie om ryp te word. Interne gehalte was goed, met 'n diep oranje-rooi skil kleur. Opbrengs was swak, maar kan toegeskryf word aan die slegte toestand van die bome. Die EH Nawel volg, met beter opbrengste, diep oranje-rooi skilkleur en aanvaarbare Brix vlakke, maar lae suurvlakke. Palmer is volgende, gevolg deur 99 Nawel. Hierdie seleksie het goeie eksterne kleur ontwikkeling, sagte vesel, swak opbrengs, lae Brix en hoë suur vlakke. HE Late was die laatste seleksie van die seisoen met goeie opbrengs en die beste interne gehalte van al die seleksies. Die pluk periode moet nie langer as 2 tot 3 weke wees nie om goeie interne kwaliteit te verseker met minimum skil probleme.

#### **5.5.21 PROGRESS REPORT: Cultivar characteristics and climatic suitability of Experimental navel oranges in a cold production region (Gamtoos River Valley)**

Experiment 1001B by R. Fenwick (CRI)

### **Summary**

Patensie Early is the earliest maturing navel selection in South Africa and has maintained its earliness for four years. Internal qualities are good but colour development was not good this season. Fisher navel is next to mature and has very good yields, fruit size, very good internal quality and good colour. EDP1 matures next with excellent colour development, good yields and fruit size. However, internal quality was poor due to low acid content. Navel ends are completely closed. KS Navel matures after Cambria and has very good yields, good fruit size, smooth rind and good internal quality. Suitangi is the next to mature and also has a smooth rind, but has the best internal quality of all selections, however, yields are slightly lower and fruit size slightly smaller. Dansweet matures next with slightly lower yields than Suitangi but also with good internal quality and fruit size. Lazy Boy is the latest selection and has excellent internal quality, good colour development and good fruit size and above average yields. Picking periods should not be longer than 3 - 4 weeks to maintain a good internal quality standard and to avoid rind disorders.

### **Opsomming**

Die vroegste nawel seleksie in Suid Afrika vir die afgelope vier jaar is Patensie Early. Interne gehaltes is goed; maar kleur ontwikkeling was nie goed vir die 2011 seisoen nie. Fischer nawel is volgende met baie goeie opbrengs, vruggrootte, baie goeie interne gehalte en goeie eksterne kleur. EDP1 was volgende, met uitstekende kleur ontwikkeling, goeie opbrengs en goeie vruggrootte. Interne gehalte was swak as gevolg van die lae suurvlak en die nawel-ente was groot oop. KS nawel se rypwordings tyd is na Cambria en het baie goeie opbrengs, vruggrootte, gladde skil en goeie interne gehalte. Suitangi is volgende, ook met 'n gladde skil, maar met die beste interne gehalte van al die seleksies, alhoewel opbrengste effens laër was en met kleiner vruggrootte. Dansweet is volgende om ryp te word met effens laër opbrengs as Suitangie, maar goeie interne gehalte en beter vruggrootte. Die Lazy Boy is die laatste seleksie en het uitstekende interne gehalte, goeie kleur ontwikkeling, goeie vruggrootte en bo-gemiddelde produksie. Die pluk periode moet nie langer as 2 tot 3 weke wees nie om goeie interne kwaliteit te verseker sonder skil probleme.

6 **CITRUS IMPROVEMENT SCHEME (CIS) JANUARY TO DECEMBER 2011**  
By M.M.N. du Toit, M. le Roux, L. Olivier, P.H. Fourie, J.H.J. Breytenbach, G. Cook and S.P. van Vuuren

**SUMMARY**

6.1 **Budwood**

A total of 2,950,328 buds were supplied locally by the Citrus Foundation Block (CFB) during January to December 2011 (Tables 6.1.1 & 6.1.2). This is 175,596 less buds than in 2010. Eureka is the most popular cultivar for the second consecutive year. Star Ruby, the most popular cultivar in 2009, fifth most popular in 2010, has decreased in popularity and does not rank amongst the ten most popular cultivars of 2011. Midnight was the second most popular cultivar during 2009 and 2010, but has lost its ranking during 2011 to Nadorcott 1 (Table 6.1.3). A total of 35,050 buds were exported to neighbouring countries (Table 6.1.1c).

6.2 **Seed**

During January to December 2011 an amount of 2743 litres of seed were supplied locally of which 350 litres were stock from the previous season. During January to December 2010, 3220 litres of seed were supplied locally, of which 487 litres were stock from the previous season (Table 6.2.1). Only 437 litres of seed were exported during 2011, which is 788 litres less than in 2010 (Table 6.2.1c).

6.3 **Production**

The remaining 2,475 seedlings in greenhouse four were budded in December 2011. The total number of increase trees that were budded during 2011 is 11,708. Greenhouse five was erected and 312 interim replacement mother trees were established here. The remainder of the greenhouse is being prepared and will be used for the establishment of multiplication trees.

6.4 **Tree Certification**

There were 1,255,056 trees certified during 2011. This is 1,045,977 less trees than in 2010 and 188,211 less than in 2009. It is expected that a large number of trees will be certified during the next quarter (Table 6.4.1).

6.5 **Nursery Certification**

Twenty-one nurseries were visited during 2011 audits and were certified (7.5.1).

6.6 **Statutory Improvement Scheme**

Communications with the Department have not progressed.

6.7 **Protective zone surrounding the Citrus Foundation Block**

The legislation declaring a radius of 5 km around the CFB as a citrus free area has been published in the Government Gazette on 21 January 2011. Orders to remove citrus trees were issued by DAFF and feedback were received that some residents have already started to remove the citrus trees.

6.8 **Establish and maintain a virus-free gene source at CRI (Experiment 790)**

Shoot tip grafting (STG) is used to eliminate graft transmissible pathogens from citrus material before introduction into the Citrus Improvement Scheme. During the current year 14 new selections were submitted for STG by various clients and a further 33 are in the process pipeline from previous years. Twenty five of the latter are in the process of indexing. Virus-free material is pre-immunised with a suitable *Citrus tristeza virus* source before it is supplied to the Citrus Foundation Block (CFB) at Uitenhage. A virus-free gene source is maintained in an insect-free tunnel at CRI. Ten new cultivars and selections from 2010 were supplied to the CFB and they were added to the gene source block, which now consists of a total of 261 cultivars and selections.

6.9 **Diagnostic services for graft transmissible diseases (Experiment 796)**

The success of the Citrus Improvement Scheme (CIS) relies on the diagnostic detection of pathogens, the elimination thereof, and the maintenance and distribution of healthy propagation material. Primarily biological

indexing is done to establish whether graft transmissible disease agents are present. Molecular diagnostic techniques are now also used to supplement the biological indexing results. We report on these ongoing diagnostic activities of the CIS. The mother trees at the CFB are indexed every two years on a rotating basis for the presence of severe CTV strains and the presence of citrus viroids (CVd). The presence of severe CTV in 150 mother trees was evaluated this year. Due to the detection of CVd at the CFB in a few instances, it was decided to index all mother trees at the CFB for CVd infection in 2011. Four cultivars tested positive for CVds and RT-PCR identified CVd-II in two cultivars and CVd-IV in the other two cultivars. Sequencing of the PCR products obtained with CVd-II positive sources was done and all sources were shown to be CVd-IIa, the non-Cachexia inducing variant. Subsequent to the findings, the tests were repeated by re-sampling of the nucleus block trees, all mother trees and the multiplication blocks of the implicated cultivars.

#### **6.10 Restructuring of the Citrus Improvement Scheme**

South Africa's Citrus Improvement Scheme (CIS) was praised as world-class by an international panel during the 2010 review. Several aspects were identified to ensure sustained and improved services. These included several infrastructural changes to address space constraints, increased funding to address more extensive diagnostic services, insufficient human resource allocation to CIS administration and Biosecurity, and urgent succession planning for experienced staff nearing retirement age, specifically the current CIS-Manager, Thys du Toit, and CRI's coordinator for CIS services, Fanie van Vuuren.

The following restructuring/positions were approved for CIS support: Paul Fourie (CIS-Manager), Thys du Toit (CFB Manager), Zama Maqutu (molecular diagnostician), Loderick Silinda (general assistant), and Louise Olivier (Administration Assistant at CFB). Additionally, the following members of the Disease Management team will continue servicing the CIS in terms of plant pathology, diagnostics and biosecurity: Fanie van Vuuren, Glynnis Cook, MC Pretorius, Kobus Breytenbach, Doctor Silinda and the new diagnostician, Elaine Basson.

### **OPSOMMING**

#### **6.1 Okuleerhout**

'n Totaal van 2,950,328 okuleerhout ogies is deur die Sitrus Grondvesblok (SGB) vanaf Januarie tot Desember 2011 verskaf (Tabelle 6.1.1 & 6.1.2). Dit is 149,346 minder ogies as in 2010. Eureka is die afgelope twee jaar die mees populêre kultivar. Star Ruby, die mees populêre kultivar in 2009 en die vyfde in 2010, het afgeneem in populariteit en verskyn tans nie op die ranglys nie. Midnight is die tweede mees populêre kultivar gedurende 2009 en 2010, maar het sy plek in 2011 aan Nadorcott 1 afgestaan (Tabel 6.1.3). Daar is 35,050 okuleerhout ogies na die buurlande uitgevoer (Tabel 6.1.1c).

#### **6.2 Saad**

Gedurende Januarie tot Desember 2011 is daar 2743 liter saad plaaslik verskaf, waarvan 350 liter voorraad van die vorige seisoen was. Gedurende Januarie tot Desember 2010 is daar 3220 liter saad plaaslik verskaf en 487 liter hiervan was voorraad van die vorige seisoen (Tabel 6.2.1). Daar was 427 liter saad gedurende 2011 uitgevoer en dit was 788 liter minder as in 2010 (Tabel 6.2.1c).

#### **6.3 Produksie**

Die oorblywende 2,475 saailinge is in Desember 2011 geokuleer en daar was gedurende 2011 altesaam 11,708 vermeerderingsbome in kweekhuis vier geokuleer. Kweekhuis vyf is opgerig waarin 312 vervangings moederbome gevestig is. Die oorblywende gedeelte van die kweekhuise word voorberei en sal vir die vestiging van vermeerderingsbome gebruik word.

#### **6.4 Boomsertifisering**

Daar is 1,225,056 bome gesertifiseer in 2011. Dit is 1,045,977 minder bome as in 2010 en 188,211 minder as in 2009. Daar word verwag dat 'n groot aantal bome in die volgende kwartaal gesertifiseer sal word.

#### **6.5 Kwekery Registrasie**

Een-en-twintig kwekerye is gedurende 2011 besoek en is almal gesertifiseer (Tabel 7.5.1).

## 6.6 Statutêre Verbeteringskema

Die samesprekinge met die Departement het nie verder gevorder nie.

## 6.7 Beskernde sone rondom die Sitrus Grondvesblok

Die wetgewing, wat 'n sitrus-vrye sone van 'n 5 km radius rondom die Sitrus Grondvesblok verklaar, is in die Staatskoerant op 21 Januarie 2011 gepubliseer. Vernietigingsopdragte is deur DAFF uitgereik en terugvoering is ontvang dat daar reeds inwoners is wat hul bome verwyder het.

## 6.8 Vestig en onderhoud van 'n virus-vrye genebron by CRI (Eksperiment 790)

Groeipuntenting (GPE) word gebruik om sitrus materiaal te vrywaar van ent-oordraagbare patogene voor toevoeging tot die Sitrusverbeteringskema se genebron. Gedurende die jaar is 14 nuwe seleksies ingedien vir GPE deur verskillende kliënte en 'n verdere 33 van vorige introduksies is in die pyplyn. Vyf-en-twintig van laasgenoemde word reeds geïndekseer. Virusvrye materiaal word met 'n toepaslike "*Citrus tristeza virus*" bron gepreïmuniseer voordat dit aan die Sitrus Grondvesblok (GVB) by Uitenhage vrygestel word. Virusvrye boompies van verskillende kultivars en seleksies word as 'n genebron in 'n insek-vrye tunnel by CRI bewaar. Tien nuwe seleksies van 2010 is aan die GVB voorsien en die word by die genebron gevoeg, wat tans uit 261 kultivars en seleksies bestaan.

## 6.9 Diagnostiese dienste vir ent-oordraagbare siektes (Eksperiment 796)

Die sukses van die Sitrusverbeteringskema (SVS) berus op 'n fitosanitêre program wat op 'n diagnostiese bepaling van die teenwoordigheid van skadelike patogene gebaseer is. Vir die skema behels dit beide die eliminerings van patogene en die onderhoud en verpreiding van gesonde voortplantingsmateriaal. Indeksering vir die teenwoordigheid van entoedraagbare siektes in plantmateriaal word hoofsaaklik deur biologiese indeksering op indikatorplante gedoen. Molekulêre diagnostiese tegnieke word nou aanvullend tot die biologiese indeksering toegepas. Daar word hier verslag gelewer op die voortdurende diagnostiese aktiwiteite van die SVS. Die moederbome by die Sitrus Grondvesblok word op 'n rotasie basis elke tweede jaar geher-indekseer om te bepaal of enige strawwe CTV rasse, of enige viroïede (CVd), in die moedermateriaal voorkom. Die virulensie van die CTV in 150 moederbome is gedurende die jaar getoets. Weens die opsporing van viroïede in die Sitrus Grodvesblok (SGB) in enkel gevalle, is daar besluit om gedurende 2011 die SGB moederbome volledig te her-indekseer vir die teenwoordigheid van CVd. Vier kultivars het positief getoets vir CVd en die teenwoordigheid van CVd-II in twee kultivars en CVd-IV in die ander twee kultivars is bevestig met PKR. DNS volgorde-bepalings van die PKR produkte van die CVd-II positiewe bronne is ook gedoen en bevestig dat die bronne, CVd-IIa is, en dus nie bronne wat Cachexia siekte veroorsaak nie. Na afloop van die bevindinge, is die toetse herhaal deur her-monsterneming van die kernblok bome, alle moederbome en die vermeerderingsblokke van die betrokke kultivars.

## 6.10 Herstrukturering van die Sitrus Verbeteringskema

Suid-Afrika se Sitrus Verbeteringskema (SVS) is as wêreldklas deur 'n internasionale paneel gedurende die 2010 inspeksie geprys. Verskeie aspekte is egter geïdentifiseer wat volhoubare en verbeterde dienste sal verseker. Hierdie het verskeie infrastrukturele veranderinge ingesluit, ten einde die volgende aan te spreek: ruimte-bepanking, verhoogde befondsing ten einde meer uitgebreide diagnostiese dienste aan te spreek (insluitend die mees onlangse verskaffing van molekulêre diagnostiese dienste), onvoldoende menslike hulpbron allokering aan SVS administrasie en Biosekuriteit, en dringende opvolgingsbeplanning vir ervare personeel wat aftree-ouderdom nader, spesifiek die huidige SVS bestuurder, Thys du Toit, en CRI se koördineerder vir SVS dienste, Dr. Fanie van Vuuren.

Die volgende herstrukturering/posisies is gevolglik vir SVS ondersteuning goedgekeur: Paul Fourie (SVS bestuurder), Thys du Toit (SGB bestuurder), Zama Maqutu (molekulêre diagnostikus), Loderick Silinda (algemene assistent), en Louise Olivier (administratiewe assistent by SGB). Verder, sal die volgende lede van die Siektebestuurspan voortgaan om die SVS in terme van plantpatologie, diagnostiese dienste en biosekuriteit te diens: Fanie van Vuuren, Glynnis Cook, MC Pretorius, Kobus Breytenbach, Doctor Silinda en die nuwe diagnostikus, Elaine Basson.

## 6.1 Budwood

A total of 2,950,328 buds were supplied locally by the Citrus Foundation Block (CFB) during January to December 2011 (Tables 6.1.1 & 6.1.2). This is 175,596 less buds than in 2010. Eureka is the most popular cultivar for the second consecutive year. Star Ruby, the most popular cultivar in 2009, fifth most popular in 2010, has decreased in popularity and does not rank amongst the ten most popular cultivars of 2011. Midnight was the second most popular cultivar during 2009 and 2010, but has lost its ranking during 2011 to Nadorcott 1 (Table 6.1.3). A total of 35,050 buds were exported to neighbouring countries (Table 6.1.1c).

**Table 6.1.1.** Budwood supplied during January to December 2009-2011.

### a) Summary of Budwood Supplied

Area	2009	2010	2011	Total
South Africa	2 843 365	3 125 924	2 950 328	8 919 617
Exported	50 500	8 800	35 050	94 350
	<b>2 893 865</b>	<b>3 134 724</b>	<b>2 985 378</b>	<b>9 013 967</b>

### b) Budwood Supplied in South Africa per Area

Area	2009	2010	2011	Total
Eastern Cape	488 763	641 168	615 506	1 745 437
KwaZulu Natal	45 000	52 200	38 500	135 700
Limpopo	1 171 717	1 360 420	1 091 293	3 623 430
Mpumalanga	302 936	287 421	264 510	854 867
North West Province	119 150	100 890	141 350	361 390
Northern Cape	225 190	173 785	134 550	533 525
Western Cape	490 609	510 040	664 619	1 665 268
	<b>2 843 365</b>	<b>3 125 924</b>	<b>2 950 328</b>	<b>8 919 617</b>

### c) Budwood Exported

Area	2009	2010	2011	Total
Namibia		4 500	10 150	14 650
Botswana	6 300			6 300
Swaziland		4 300		4 300
Zimbabwe	44 200		24 900	69 100
	<b>50 500</b>	<b>8 800</b>	<b>35 050</b>	<b>94 350</b>

### d) Budwood Supplied per Variety

Variety	2009	%	2010	%	2011	%
Clementine	25 550	0.88%	53 005	1.69%	86 402	2.89%
Ellendale	1 150	0.04%	3 900	0.12%	1 400	0.05%
Grapefruit	562 350	19.43%	233 790	7.46%	58 545	1.96%
Grapefruit Hybrid		0.00%	4 000	0.13%		0.00%
Kumquat	10 900	0.38%	6 400	0.20%	10 850	0.36%
Lemon	296 295	10.24%	554 950	17.70%	782 103	26.20%
Lime	10 820	0.37%	38 050	1.21%	25 300	0.85%
Mandarin Hybrid	579 419	20.02%	723 042	23.07%	657 413	22.02%
Midseason	55	0.00%	348	0.01%	942	0.03%
Navel	708 140	24.47%	690 083	22.01%	533 938	17.89%
Ornamental	500	0.02%	1 872	0.06%	2 160	0.07%
Pummelo	1 000	0.03%	1 130	0.04%	650	0.02%
Rootstock	76	0.00%	50	0.00%	80	0.00%
Satsuma	82 105	2.84%	112 503	3.59%	218 165	7.31%
Seville	300	0.01%	3 532	0.11%	1 000	0.03%
Valencia	615 205	21.26%	708 069	22.59%	606 430	20.31%
	<b>2 893 865</b>	<b>100.00%</b>	<b>3 134 724</b>	<b>100.00%</b>	<b>2 985 378</b>	<b>100.00%</b>

**Table 6.1.2.** Budwood Supplied per area and variety during January to December 2009-2011.

Area	Year	Clementine	Ellendale	Grapefruit	Kumquat	Lemon	Lime	Mandarin Hybrid	Midseason	Navel	Ornamental	Pummelo	Rootstock	Satsuma	Seville	Valencia	Total
Eastern Cape	2009	300		29 900		92 321	450	165 117		160 395				21 030	250	19 000	488 763
	2010	9 180		19 710	200	197 886	200	138 508	300	146 280				28 200		100 704	641 168
	2011	4 300		800	150	256 510	2 050	91 713	415	135 371			80	45 390		78 727	615 506
KwaZulu-Natal	2009			4 000		11 500	2 500	5 500		15 500						6 000	45 000
	2010	200		200	300	18 800	4 500	3 800		15 800	800			300	200	7 300	52 200
	2011	300		1 200	300	7 200	200	8 700		13 900	400			3 100		3 200	38 500
Limpopo	2009	1 900		227 220	8 800	116 300	1 300	173 212		274 500	400	1 000		22 900		344 185	1 171 717
	2010	2 000		97 140		252 850	15 000	284 150	48	278 102		4 900		30 500		395 730	1 360 420
	2011	32 000		9 815	1 000	255 700	5 200	185 160		186 030	350			65 900		350 138	1 091 293
Mpumalanga	2009	670		115 520		17 060	520	16 990	55	69 355			26	3 100		79 640	302 936
	2010	540		45 860	1 000	15 760	4 900	25 809		89 042	100	160	50	5 000		99 200	287 421
	2011	2 000		36 270	5 800	57 625	5 900	38 965	40	55 470		650		9 000		52 790	264 510
Namibia	2010							1 500		1 500						1 500	4 500
	2011			500		100	50	3 000		4 500						2 000	10 150
North-West Province	2009	1 500			750	21 900	750	26 200		46 700				3 350		18 000	119 150
	2010	4 000		1 500	3 000	7 300	4 000	20 900		31 040				4 000		25 150	100 890
	2011			1 200	2 100	41 200	4 000	27 100	300	45 050	200			2 000		18 200	141 350
Northern Cape	2009	30		126 350	400	7 300	500	51 500		18 610	100			1 000		19 400	225 190
	2010	3 400		60 400		19 620		20 605		25 460	500			5 000		38 800	173 785
	2011	4 300	800	5 000	500	30 500		34 400		24 180	1 000			5 000		28 870	134 550
Botswana	2009							2 100		2 100						2 100	6 300
Swaziland	2010			4 300													4 300
Western Cape	2009	21 150	1 150	59 360	950	29 014	3 500	128 800		116 480			50	30 725	50	99 380	490 609
	2010	33 685	3 900	4 680	1 900	42 734	9 450	227 770		102 859	472	70		39 503	3 332	39 685	510 040
	2011	43 502	600	860	1 000	133 268	7 900	268 375	187	69 437	210			87 775	1 000	50 505	664 619
Zimbabwe	2009					900	1 300	10 000		4 500						27 500	44 200
	2011			2 900												22 000	24 900
<b>Total</b>		<b>164 957</b>	<b>6 450</b>	<b>854 685</b>	<b>28 150</b>	<b>1 633 348</b>	<b>74 170</b>	<b>1 959 874</b>	<b>1 345</b>	<b>1 932 161</b>	<b>4 532</b>	<b>6 780</b>	<b>206</b>	<b>412 773</b>	<b>4 832</b>	<b>1 929 704</b>	<b>9 013 967</b>

**2011** | 2 985 378

**2010** | 3 134 724

**2009** | 2 893 865

**Total** | 9 013 967

**Table 6.1.3.** Top 10 Cultivars according to Budwood Supplied: January to December 2009-2011.

2011			
Variety	Cultivar	QTY	%
Lemon	Eureka	575 877	19.29%
Mandarin	Nadorcott 1	259 292	8.69%
Valencia	Midnight	219 425	7.35%
Valencia	Late	165 533	5.54%
Mandarin	Nova	163 267	5.47%
Satsuma	Miho Wase	108 048	3.62%
Lemon	Lisbon	107 626	3.61%
Navel	Bahianinha	106 690	3.57%
Navel	Chislett M7	106 470	3.57%
Satsuma	Sonet	100 372	3.36%
<b>Total Top 10</b>		<b>1 912 600</b>	<b>64.07%</b>
<b>Total</b>		<b>2 985 378</b>	<b>100.00%</b>

2010			
Variety	Cultivar	QTY	%
Lemon	Eureka	353 186	11.27%
Valencia	Midnight	305 570	9.75%
Mandarin	Nova	233 202	7.44%
Navel	Bahianinha	184 425	5.88%
Grapefruit	Star Ruby	181 900	5.80%
Navel	Palmer	118 465	3.78%
Navel	Washington	113 416	3.62%
Valencia	Late	104 330	3.33%
Mandarin	Valley Gold (B17)	101 401	3.23%
Lemon	Genoa	92 160	2.94%
<b>Total Top 10</b>		<b>1 788 055</b>	<b>57.04%</b>
<b>Total</b>		<b>3 134 724</b>	<b>100.00%</b>

2009			
Variety	Cultivar	QTY	%
Grapefruit	Star Ruby	534 370	18.47%
Valencia	Midnight	215 820	7.46%
Lemon	Eureka	207 455	7.17%
Navel	Bahianinha	196 600	6.79%
Mandarin	Nova	191 289	6.61%
Mandarin	Nadorcott 1	179 048	6.19%
Navel	Washington	135 170	4.67%
Navel	Palmer	114 270	3.95%
Navel	Cambria	114 035	3.94%
Valencia	Du Roi	90 330	3.12%
<b>Total Top 10</b>		<b>1 978 387</b>	<b>68.36%</b>
<b>Total</b>		<b>2 893 865</b>	<b>100.00%</b>

## 6.2 Seed

During January to December 2011 an amount of 2743 litres of seed were supplied locally of which 350 litres were stock from the previous season. During January to December 2010, 3220 litres of seed were supplied locally, of which 487 litres were stock from the previous season (Table 6.2.1). Only 437 litres of seed were exported during 2011, which is 788 litres less than in 2010 (Table 6.2.1c).

**Table 6.2.1.** Seed Supplied: January to December 2009-2011.

### a) Summary of Seed Supplied

Area	2009	2010	2011	Total
South Africa	2 429	3 220	2 743	8 392
Exported	3 945	1 225	437	5 607
	<b>6 374</b>	<b>4 445</b>	<b>3 180</b>	<b>13 998</b>

### b) Seed Supplied in South Africa

Area	2009	2010	2011	Total
Eastern Cape	328.5	258	343	930
Free State	2.5	-	-	3
KwaZulu Natal	34	19	14	67
Limpopo	1370	2080	1689	5 139
Mpumalanga	50	48	43	141
North West Province	37	235	204	476
Northern Cape	93.5	18	58	170
Western Cape	513	562	392	1 467
	<b>2 429</b>	<b>3 220</b>	<b>2 743</b>	<b>8 392</b>

### c) Seed Exports

Area	2009	2010	2011	Total
Australia/NZ	5	5	135	145
Caribbean	16	10	8	34
Europe	150	-	72	222
Far East	3639	1016	-	4 655
Other African States	135	105.5	21	262
South America	-	88	201	289
	<b>3 945</b>	<b>1 225</b>	<b>437</b>	<b>5 607</b>

### d) Seed supplied per Cultivar

Cultivar	2009	%	2010	%	2011	%
C35 citrange	727	11.40%	500	11.25%	413	12.99%
Carrizo citrange	2 398	37.63%	1784	40.14%	1250	39.31%
Cleopatra mandarin	-	0.00%	-	0.00%	2	0.06%
Flying Dragon	5	0.08%	2	0.04%	208	6.54%
Minneola x Trifoliolate	167	2.62%	138	3.10%	88	2.77%
Rough lemon	307	4.81%	419.5	9.44%	317	9.97%
Sunki x Beneke	-	0.00%	2	0.04%	-	0.00%
Swingle citrumelo	960	15.07%	824	18.54%	526	16.54%
Troyer citrange	1 330	20.87%	521	11.72%	60	1.89%
Volkameriana	172	2.70%	46	1.03%	33	1.04%
X639	299	4.70%	185	4.16%	268	8.43%
Yuma citrange	8	0.12%	23	0.52%	15	0.47%
	<b>6 374</b>	<b>100.00%</b>	<b>4 445</b>	<b>100.00%</b>	<b>3 180</b>	<b>100.00%</b>

## 6.3 Production

The remaining 2,475 seedlings in greenhouse four were budded in December 2011. The total number of increase trees that were budded during 2011 is 11,708. Greenhouse five was erected and 312 interim replacement mother trees were established here. The remainder of the greenhouse is being prepared and will be used for the establishment of multiplication trees.

## 6.4 Tree Certification

There were 1,255,056 trees certified during 2011. This is 1,045,977 less trees than in 2010 and 188,211 less than in 2009. It is expected that a large number of trees will be certified during the next quarter (Table 6.4.1).

**Table 6.4.1** Tree Certification: January to December 2009-2011.

Variety	Year	Botswana	Eastern Cape	Gauteng	KwaZulu-Natal	Limpopo	Mozambique	Mpumalanga	Namibia	North-West Province	Northern Cape	Other African States	Swaziland	Western Cape	Zimbabwe	Total
Clementine	2009		1 276						1 000	4 487				21 805		28 568
	2010	10	50			1 615				200		200		1 101		3 176
	2011		1 050						1 670					8 830		11 550
Ellendale	2009									1 750				403		2 153
	2010													200		200
Grapefruit	2009		21 878		11 110	51 199		47 570		2 905	70 027		20 980	9 610		235 279
	2010	70	21 653		42 000	190 884	2	98 751	225	150	25 550		43 828	59 904		483 017
	2011		15 642		1 300	50 761		22 935			119 384		3 000	1 272		214 294
Grapefruit Hybrid	2009									100						100
	2010	20				120		4 510				100				4 750
	2011					1 400			11							1 411
Kumquat	2009		200			1 000				8 000				580		9 780
	2010					4 932								100		5 032
Lemon	2009		50 561			21 060		4 000	500	41 508	2 000			16 830		136 459
	2010	10	108 289			70 414	20	46 379		28 240	2	300		6 370		260 024
	2011		71 204		2 200	39 114		37 230	5 281	13 500	5 515		2 900	19 063		196 007
Lime	2009					2 000		1 104		6 500				1 050		10 654
	2010	20		400		5 610		555	30			50		2 745		9 410
	2011					1 702			2 208				1 500	150		5 560
Mandarin Hybrid	2009		50 154		1 700	33 270		15 198	1 500	37 443				99 338		238 603
	2010	1 385	99 874	1 500		175 761		29 009	635	35 140	16 265	480	10 000	39 025		409 074
	2011		63 663			19 059		18 810	2 760	6 730	26 361			87 374		224 757
Midseason	2009			760						1 000						1 760
Navel	2009		116 033			26 814		59 058		55 603	13 805	5 000		64 377		340 690
	2010	1 540	159 111	40	1 600	169 010		131 998	205	44 297	56	26 800	4 175	26 279		565 111
	2011		137 184		940	60 049		30 005	5 322	2 130	18 186			27 754		281 570
Rootstock	2009						1 095									1 095
Satsuma	2009		10 308					500		5 845	2 450			15 502		34 605
	2010		3 525			11 160		14 798		581				380	6 610	37 054
	2011		15 928			23 122		9 160		50	1 030			12 597		61 887
Seville	2009													620		620
Valencia	2009		63 960			113 955		122 395	1 500	37 074	11 725	10 000		42 292		402 901
	2010	978	35 806	660	7 200	259 657	3 250	138 219	4 899	19 564	9 944	10 800		31 008	2 200	524 185
	2011		32 480			97 570		58 390	375	5 394	19 279			44 532		258 020
<b>Total</b>		<b>4 033</b>	<b>1 079 829</b>	<b>3 360</b>	<b>68 050</b>	<b>1 431 238</b>	<b>3 272</b>	<b>891 669</b>	<b>28 121</b>	<b>358 191</b>	<b>341 579</b>	<b>53 730</b>	<b>86 383</b>	<b>641 091</b>	<b>8 810</b>	<b>4 999 356</b>

<b>2011</b>	<b>1 255 056</b>		<b>2010</b>	<b>2 301 033</b>		<b>2009</b>	<b>1 443 267</b>
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## 6.5 Nursery Certification

Twenty-one nurseries were visited during 2011 audits and were certified (Table 6.5.1).

**Table 6.5.1.** Certified Nurseries 2011.

Kwekery	Address	Town	Code	Contact	Telephone	Facsimile	Cell phone	E-mail
Apapanzi Kwekery	Posbus 147	KIRKWOOD	6120	Mnr C J Meiring	042 230 1483	042 230 0923	082 550 6210	nellis@srvalley.co.za
Casmarnursery	Posbus 3	MOOINOOI	0325	Mnr N Wenhold	014 574 3152	014 574 3798	082 881 4189	casmarnursery@absamail.co.za
Cederberg Tree Nursery	P O Box 69	SIMONDIUM	7670	Mev P Willemse	021 874 2630	021 874 2110	076 622 7007	teresac@topfruit.co.za
Du Roi Kwekery	Posbus 66	LETSITELE	0885	Mnr S le Roux	015 345 1650	015 345 1650	082 874 8040	smit@duroi.zo.za
Esselen Kwekery	Posbus 100	MALELANE	1320	Mnr L Esselen	013 790 0160	013 790 0492	083 325 0565	esselenk@mweb.co.za
Gamtoos Kwekery	Posbus 140	PATENSIE	6335	Me Joanie de Vos	042 283 0506	042 283 0978	072 260 9813	joanie@rikusld.co.za
BF Joubert Kwekery	Posbus 193	KIRKWOOD	6120	Mnr F Joubert	042 230 0309	042 230 0280	084 951 1922	bfjksweek@srvalley.co.za
H J Joubert Kwekery	Posbus 207	MONTAGU	6720	Mnr H J Joubert	023 614 2237	023 614 2237	082 578 5747	hopewell@breede.co.za
Letsitele Kwekery	Posbus 114	LETSITELE	0885	Mnr B Vorster	015 345 1600	015 345 1601	083 259 5590	mahela@mweb.co.za
Loskop Kwekery BK	Posbus 1101	MARBLE HALL	0450	Mnr J Odendaal	013 261 2736	086 623 0912	013 261 2735	kynomel@lantic.net
Mistkraal Nursery	P O Box 106	KIRKWOOD	6120	Mrs T Ferreira	042 230 0614	042 230 1461	082 789 5150	beans@srvalley.co.za
Ngwenya Kwekery	Posbus 36	MALELANE	1320	Mev M van der Merwe	013 790 3004	013 790 3480	082 418 7693	milaniemerwe@hotmail.com
Oranjerivier Kwekery	Posbus 875	KAKAMAS	8870	Mev B Rossouw	-	086 544 9691	0833060622	osk@vodamail.co.za
Paksaam Kwekery	Posbus 16	PATENSIE	6230	Mnr P Lamont	042 283 0201	042 283 0884	072 575 4471	paksaam@lantic.net
Sondagsrivier Kwekery	Posbus 304	KIRKWOOD	6120	Mnr F Olivier	042 230 0349	042 230 0510	083 227 6655	brenda@srvalley.co.za
Stargrow Kwekery	Posbus 189	CITRUSDAL	7340	Mnr M du Toit	022 921 2232	022 921 2747	082 563 0795	stargrowcitrus@alazon.co.za
Tulbagh Kwekery	Posbus 99	TULBAGH	6820	Mnr P B Roux	023 230 0694	023 230 1353	082 214 2520	admin@tulbaghnursery.co.za
Tweeling Kwekery	Posbus 190	KIRKWOOD	6120	Mnr J Potgieter	042 230 1408	042 230 1408	082 560 2179	tweeling@srvalley.co.za
Vaalharts Kwekery	Posbus 317	HARTSWATER	8570	Mnr E Greyling	053 474 0565	053 474 1926	082 948 2552	orange@lantic.net
Waterfall Nursery	P O Box 339	ADELAIDE	5760	Mr R van der Meulen	046 684 0738	046 684 1451	082 695 3433	waterfall@intekom.co.za
Witkrans Kwekery	Posbus 17	BOSHOEK	0301	Mnr J Grobler	014 573 3036	014 573 3036	082 922 1579	witkrans1@mweb.co.za
Produsola LDA	Messica Farm	Lake Chicamba, Manica Province, MOZAMBIQUE		Dave and Kathie Sole	+258 23 91 0045		+258 82 546 7255	info@produsola.com

## 6.6 Statutory Improvement Scheme

Communications with the Department have not progressed.

## 6.7 Protective zone surrounding the Citrus Foundation Block

The legislation declaring a radius of 5 km around the CFB as a citrus free area has been published in the Government Gazette on 21 January 2011. Destruction orders were issued by DAFF and feedback were received that some residents have already started to remove the citrus trees.

## 6.8 Establish and maintain a virus-free gene source at CRI

Experiment 790 by JHJ Breytenbach, S.P. van Vuuren and G. Cook (CRI)

The overall objective of the southern African Citrus Improvement Scheme (CIS) is to enhance the productivity of the industry by ensuring supply of the highest quality propagation material. Graft transmissible diseases (GTD) have detrimental effects on the growth and production of citrus trees and are responsible for stunting, decline, small fruit and a range of other harmful effects. Shoot tip grafting (STG) is the standard method for the elimination of pathogens (Navarro *et al.*, 1975). Some pathogens are more difficult to eliminate and heat therapy should be incorporated with the STG process (Roistacher, 1977). The STG technique was developed by Murashige *et al.* (1972) and improved by Navarro *et al.* (1975) and de Lange (1978). Some cultivars and selections of the virus-free gene source maintained at the ARC-ITSC have been duplicated in part at CRI Nelspruit as a back-up source. STG facilities at CRI are used to introduce new virus-free cultivars and selections which are added to the gene source after STG and indexing. Cross-protection for severe CTV infection is a function of the CIS and specific pre-immunising CTV sources are applied to all citrus varieties before supply to the CFB.

### Objectives

- Receive and introduce new cultivar selections.
- Do STG of new editions and index for GTD, ensure that they are virus-free.
- Maintain the virus-free gene source in an insect-free tunnel.
- Pre-immunise selections with a suitable cross-protecting *Citrus tristeza virus* (CTV) source before budwood supply to the Citrus Foundation Block (CFB) at Uitenhage.

### Materials and methods

In vitro cultured rootstocks: The standard method used for *in vitro* cultured rootstocks is to expose the cotyledons by removing the seed coat of Troyer citrange seed and surface sterilise in 1% sodium hypochlorite (NaOCl) for 10 minutes followed by three rinses in sterile distilled water. Three to four seeds are planted in growth tubes containing sterile Murashige and Skoog (MS) agar medium (Murashige & Skoog, 1962). Germination takes place at a constant temperature of 28°C in continuous darkness. When the seedlings have reached a height of 30 to 40 mm, they are stored at 4°C in darkness.

Scion preparation: Method 1; buds of the plant that should go through STG (source plant) are budded on a standard rootstock in the glasshouse. After the buds have grown and matured (approximately 3–4 months), the source plant is defoliated by hand to induce flushing. Ten to 14 days later, the new shoots are harvested and surface sterilised on a flow bench for 5 minutes in 0.52% NaOCl and then rinsed three times in sterile distilled water. Method 2; bud sticks from the source plant are cut in 50 mm lengths and surface sterilised by immersion for 10 minutes in 1% NaOCl containing a wetting agent. After 3 rinses in sterile distilled water the bud sticks are cultured in 250 ml glass bottles containing sterile wet sand. The cultures are incubated at 32°C and exposed to 16 h light/day. Ten to 14 days later new shoots are harvested and treated as in method 1.

STG: The seedling rootstock is aseptically decapitated about 50 mm above the cotyledons. The cotyledons and their auxiliary buds are removed and an inverted T incision is made, 1 mm vertically and 1 – 2 mm horizontally approximately 10 mm from the top. The cuts are made through the cortex to reach the cambium. A shoot tip consisting of the apical meristem and 2 to 3 leaf primordia is excised from the growth point of the collected shoots under a stereo microscope. The growth point with primordia is placed on the horizontal cut of the incision on the rootstock. The grafted plant is transferred to sterile MS liquid medium and cultured at constant 28°C exposed to 16 h light/day.

STG plant increase. The shoot tip will start growing 3 to 4 weeks after STG. The growing shoot tip is micro-grafted with the seedling rootstock onto a vigorous-growing virus-free rootstock in the glasshouse. After micro-grafting, it is closed by a plastic bag for 8 days. Buds for indexing are taken from this material once the graft has sufficiently grown.

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). Biological indexing results are now also confirmed with molecular diagnostic techniques. Reverse-Transcription Polymerase Chain Reaction (RT-PCR) is used to detect viroids, CPsV and ASGV. PCR is used to detect the bacterial pathogen causing citrus greening. Virus-free plants are maintained in an insect-free tunnel containing the gene sources from where material is taken, multiplied and pre-immunised with suitable CTV cross-protection sources (van Vuuren and Collins, 1990), prior to release to the CFB at Uitenhage.

## Results and discussion

Objective / Milestone	Achievement
<ul style="list-style-type: none"> <li>Receive and maintain new selections/cultivars.</li> </ul>	Ongoing: 33 brought forward from previous year; 14 new selections received in current year.
<ul style="list-style-type: none"> <li>Do shoot tip grafting (STG) of new selections/cultivars and index for graft transmissible diseases, to ensure they are virus-free.</li> </ul>	Ongoing: 391 STGs, 40 successful micro-grafts.
<ul style="list-style-type: none"> <li>Maintain the virus-free nucleus block in an insect-free tunnel.</li> </ul>	Ongoing: currently 261 cultivars and selections.
<ul style="list-style-type: none"> <li>Establish a pre-immunised source of the new selection/cultivar with a suitable CTV cross-protection source and supply budwood to the CFB.</li> </ul>	Ongoing: 8 additions from 2010 supplied to CFB.
<ul style="list-style-type: none"> <li>Re-index the virus-free selection every three years.</li> </ul>	Ongoing: Partly indexed for CVd and CPsV.

### STG:

STG at CRI was initiated in 2004 with the current facilities available in 2005. The introductions for STG and subsequent releases to the CFB from 2006 to date, is summarised in Table 6.8.1. Fourteen new selections of three cultivar groups were submitted by clients for STG in the current year and 33 brought forward from the previous year. During this report period a total of 391 STGs were done on these introductions, including failed grafts. Of these, 40 (10%) grew and were successfully micro-grafted. This is considered an acceptable success rate for STG in citrus.

Twenty five of the successful STGs have been indexed biologically while the remainder are duplicates (ex-plants) or are still too small for indexing. Eleven of these, 18 indexed negative for CTV, ASGV and CVd by biological indexing, seven tested positive for CVd and results of seven are still outstanding (Table 6.8.2). Twenty STGs were biologically indexed for CPsV and CID of which the results are still outstanding (Table 6.8.3). In general it takes 24 to 30 months to complete removal of GTD with STG followed by the scheduled indexing to ensure the virus-free status of the cultivar. However, delays can occur with elimination of diseases. The reason for these “difficult to remove” cases is unknown. Currently there is an ornamental source where the removal of CTV has been unsuccessful after several attempts and similarly removal of CVd with STG from one navel selection has been ineffective.

Confirmation of biological indexing by PCR on a number of STG submissions is reflected in Table 6.8.4. Eight STG submissions free of CTV, CVd, and ASGV were pre-immunised successfully and budwood was supplied to the CFB.

**Table 6.8.1.** STG submissions in the pipeline for graft transmissible disease elimination and indexing.

Cultivar/ Variety Group <sup>2</sup>	STG introductions and releases 2006 to 2010 <sup>1</sup>															
	2007			2008			2009			2010			2011			Balance
	Bf from 2006	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	Bf	New Introductions	Releases to CFB	
C	2	0	0	2	0	0	2	0	2	0	0	0	0	0	0	0
G	2	0	0	2	0	1	1	0	0	1	0	1	0	0	0	0
L	1	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0
Mi	7	0	0	7	0	2	5	0	4	1	0	2	1	0	0	1
Ma	1	0	0	1	1	1	1	1	0	2	3	2	3	0	2	1
N	24	2	1	25*	4	1	20**	6	7	19	6	3	22	11	4	29
R	2	0	0	2	0	1	1***	0	0	0	0	0	0	0	0	0
V	7	0	1	6	1	0	7***	2	1	6	0	0	6	2	2	6
Or	6	0	0	6	0	4	2	0	0	2	0	1	1	0	0	1
Rs	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	1
<b>Total</b>	<b>52</b>	<b>2</b>	<b>2</b>	<b>52</b>	<b>8</b>	<b>11</b>	<b>41</b>	<b>9</b>	<b>15</b>	<b>32</b>	<b>9</b>	<b>10</b>	<b>33</b>	<b>14</b>	<b>8</b>	<b>39</b>

<sup>1</sup> Bf = Brought forward from previous year; Balance = Balance for the current reporting year.

<sup>2</sup> Cultivar/variety group: C = Clementine; G = Grapefruit; L = Lemon; Mi = Midseason; Ma = Mandarin; N = Navel; R = Reticulata; V = Valencia; Or = Ornamental; Rs = Rootstock.

\* Seven navel selections withdrawn by owner.

\*\* Release of 1 navel selection withdrawn by client.

\*\*\* One Reticulata, 1 navel and 2 Valencia selections withdrawn by owners.

**Table 6.8.2.** STG submissions indexed biologically for CTV, ASGV and CVd.

Variety Group	Number of plants	Negative	Positive	Awaiting results
Navel	12	4	5	3
Midseason	5	4	1	-
Mandarin	3	2	-	1
Valencia	4	1	1	2
Clementine	1	-	-	1
Grapefruit	-	-	-	-
Lemon	-	-	-	-
Ornamental citrus	-	-	-	-
Rootstock	-	-	-	-
<b>Total</b>	<b>25</b>	<b>11</b>	<b>7</b>	<b>7</b>

**Table 6.8.3.** STG submissions indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive	Awaiting results
Navel	7	-	-	7
Midseason	2	-	-	2
Valencia	4	-	-	4
Reticulata	1	-	-	1
Mandarin	3	-	-	3
Grapefruit	1	-	-	1
Clementine	1	-	-	1
Lemon	1	-	-	1
Ornamental	-	-	-	-
Rootstock	-	-	-	-
<b>Total</b>	<b>20</b>			<b>20</b>

**Table 6.8.4.** STG plants indexed by PCR for CVd, ASGV and CPsV and Greening.

<b>Cultivars</b>	<b>CVd</b>	<b>ASGV</b>	<b>CPsV</b>	<b>Greening</b>
Navel	16	9	4	3
Midseason	5	1	1	1
Valencia	2	5	4	4
Reticulata	1	-	-	-
Mandarin	2	4	2	3
Grapefruit	-	1	-	1
Clementine	1	-	-	-
Lemon	-	-	-	-
Ornamental	5	-	-	-
Rootstock	-	1	1	1
<b>Total</b>	<b>32</b>	<b>21</b>	<b>12</b>	<b>13</b>

Maintaining the virus-free gene source:

The number of selections maintained at CRI is listed per cultivar/variety group in Table 6.8.5. The re-indexing of the nucleus block for CVd and CPsV with RT-PCR is reported in experiment 796. Eight new additions were made to the gene source this reporting year (Table 6.8.1). Two trees of each selection are maintained in the gene source and trees have to be re-budded to new rootstocks every five years as part of the routine maintenance. During the previous reporting period one of each selection was budded to a new rootstock and the remainder were done during this reporting period.

**Table 6.8.5.** The number of accessions per cultivar/variety group maintained at the CRI nucleus block.

<b>Variety Group</b>	<b>No. of selections maintained at CRI</b>
Clementine	23
Diverse (Citron, Sour orange, etc.)	2
Ellendale	4
Grapefruit	18
Kumquat	1
Lemon	20
Lime	4
Mandarin	4
Midseason	27
Navel	50
Ornamnetal	4
Pummelo	7
Reticulata	33
Rootstock	21
Satsuma	8
Valencia	43
<b>Total</b>	<b>261</b>

**Conclusion**

- Successful elimination of GTDs from new selections was achieved. On average it takes 30 months for the entire process from STG to final release (the quickest being 22 months) although some selections proved to be problematic and still remain infected despite repeated STG attempts over a 5-year period.
- Eight new selections were added to the gene source and also released to the CFB.
- Fourteen new selections were received this year for elimination of GTD and are in the STG process.

**Technology transfer**

None.

**Further objectives and work plan**Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2013 and Jan-Mar 2014

- Receive material.
- Bud to virus-free rootstocks and maintain at high temperature.
- Prepare liquid and solid Murashige & Skoog culture mediums.
- Prepare and plant seed in culture tubes with solid medium.

- Germinate seed in darkness.
- Store rootstocks at 4°C.
- Prepare rootstocks under stereo microscope under aseptic conditions.
- Collect new shoots from source maintained at high temperature.
- Prepare etiolated rootstock from culture tube.
- Under the stereo microscope, cut and place shoot tip on rootstock.
- Put the rootstock with shoot tip into a culture tube with liquid medium.
- Keep tubes in growth room (do weekly trimmings of rootstock suckers).
- Graft shoot tip with rootstock on virus-free rootstocks in the glasshouse.
- Let shoot tip grow for indexing.
- Index for graft transmissible agents.
- Pre-immunise rootstock with suitable cross protector.
- Bud virus-free shoot tip grafted material to pre-immunised rootstock.
- Do ELISA to confirm pre-immunisation.
- Multiply pre-immunised budwood on virus-free rootstocks.
- Supply budwood to Citrus Foundation block.
- Maintain virus-free material in nucleus block.

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## 6.9 Diagnostic services for graft transmissible diseases

Experiment 796 (October to December 2011) by J.H.J. Breytenbach, S.P. van Vuuren and G. Cook (CRI)

As with any commercial tree crop, citrus species are susceptible to various graft transmissible diseases (GTD) caused by viruses, viroids, bacteria and, in some cases, unidentified pathogens. The GTD affect the vigour, longevity of the trees, as well as the yield and quality of fruit. The framework of disease-free planting material is a phytosanitary programme based on diagnosis, detection and elimination of causal agents and maintenance and distribution of healthy propagation material. Shoot tip grafting (STG) is used to eliminate these diseases (Navarro, 1976) and is used in South Africa since 1977 (de Lange *et al.*, 1981).

Indexing, or establishing whether GTD disease agents are present in plant material, is done mostly by means of biological indicator plants. A range of virus-free plants are propagated in the glasshouse, each of which is used for detection of a specific graft transmissible pathogen. Previously only biological indexing was used for the detection of GTD following STG, but this is now supplemented with molecular based techniques, which target regions of the pathogen's nucleic acid are used to specifically identify the pathogen. These techniques such as Reverse-Transcription Polymerase Chain Reaction (RT-PCR), PCR and dot-blot have an enhanced sensitivity compared to symptom expression on indicators.

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 cross-protection source (Müller & Costa, 1987). Currently three CTV sources are used for cross-protection in the southern African Citrus Improvement Scheme (CIS) depending on the scion material to be protected (von Broembsen & Lee, 1988; van Vuuren *et al.*, 1993a; van Vuuren *et al.*, 1993b; van Vuuren *et al.*, 2000). ELISA is used to confirm pre-immunisation with CTV (Roistacher, 1991). The STG and pre-immunisation procedures have been improved to suite South

African conditions (Fourie & van Vuuren, 1993). Re-indexing of the mother trees, maintained at the Citrus Foundation Block (CFB), is done to ensure these trees remain free of graft transmissible pathogens and that the pre-immunising CTV remains mild within these cultivars. CTV severity indexing is done on an annual basis, indexing for Citrus viroids (CVd) is done biennially and other GTD are indexed every 10 years.

Indexing for GTD is also done to support growers where field problems are experienced and is necessary to ensure appropriate recommendations. Bud wood sent in by growers or collected during field visits, are budded to indicator plants and kept in the glasshouse at optimum temperatures according to the requirements for disease detection.

### Objectives

1. Biological and molecular indexing of material that went through STG.
2. Biological and molecular re-indexing of mother trees at the CFB.
3. Requests from growers and institutions to index suspected material for graft GTD.
4. Ad hoc indexing as required

### Materials and methods

Specific virus-free indicator plants are propagated from seed or clonally from virus-free material for the detection of the various graft transmissible diseases (GTD). These are maintained in an insect-free glasshouse and kept in stock until needed. When budwood for indexing is received, two buds are budded on each of three indicator seedlings for each disease. For CPsV indexing 4 buds are used to inoculate each of 3 indicator plants. Hereafter the plants are cut back to force new growth and kept in the glasshouse at a temperature required for symptom expression of the specific disease. Known positive and negative control samples are included. A minimum indexing time of 6 months is required for CTV, CVd, *Apple stem grooving virus* (tatter leaf) (ASGV) and greening, while 12 months are required for *Citrus psorosis virus* (CPsV) and Citrus Impietratura Disease (CID) indexing.

Field material is usually not suitable for the serological or molecular techniques (PCR, s-PAGE, DOT blots etc.), since the organisms are usually present in low concentrations or are poorly distributed, and therefore false negative results may be obtained. Field material is inoculated on suitable indicator plants at optimal temperatures in the glasshouse which are then tested at least 3 months after inoculation for a specific pathogen. Results are seen as a confirmation of the biological result.

### Results and discussion

Objective / Milestone	Achievement
<ul style="list-style-type: none"> <li>• Biological and molecular indexing of STG plants for CTV, CVd, ASGV, CPsV and CID.</li> </ul>	Achieved and ongoing.
<ul style="list-style-type: none"> <li>• Annual biological and molecular indexing of the CFB mother trees (every year for CTV severity; every third year for the presence of CVd; every 10 years for the presence of CPsV and ASGV).</li> </ul>	Achieved and ongoing. Additionally all mother trees at the CFB were indexed for CVd in this report period.
<ul style="list-style-type: none"> <li>• Indexing samples send in by growers and institutions using ELISA, PCR and biological indicators.</li> </ul>	General samples were received for indexing for possible CiLRV, CPsV, CVd and CTV infection. Analyses still underway.  SSR marker analysis for B17 investigation

#### 1. STG material

After the STG process, citrus cultivars undergo initial biological indexing for CTV, ASGV and CVd (Table 6.9.1). Once indexed negative for CTV, ASGV and CVd, a process which takes 6 months, the source is pre-immunised with a suitable CTV cross-protection source. After confirmation of positive pre-immunisation, budwood is supplied to the CFB to establish mother trees. The cultivar is then also introduced into the nucleus block (Table 6.9.4). Following interim releasing to the CFB, plants are further biologically indexed for CPsV and CID (Table 6.9.3). The presence of greening is continuously monitored since all the cultivars and selections, except the trifoliolate types, are self-indexed, but PCR is also done prior to final release to the CFB. Thirty two cultivars, further in the release process, were tested by PCR for the presence of CVd and 21 for the presence of ASGV, 12 for the presence of CPsV and 13 for Greening. All indexed negative for these pathogens (Table 6.9.2).

**Table 6.9.1.** Status of STG plants indexed biologically for CTV, ASGV and CVd<sup>1</sup>.

Cultivar	Number of plants	CTV			ASGV			CVDs		
		+	-	±	+	-	±	+	-	±
Navel	14	1	10	3	-	11	3	6	5	3
Midseason	5	-	5		-	5		1	4	
Mandarin	3	-	2	1	-	2	1	-	2	1
Valencia	4	-	2	2	-	2	2	1	1	2
Clementine	1	-	-	1	-	-	1	-	-	1
Lemon	-	-	-	-	-	-	-	-	-	-
Grapefruit	-	-	-	-	-	-	-	-	-	-
Ornamental citrus	-	-	-	-	-	-	-	-	-	-
Rootstock	-	-	-	-	-	-	-	-	-	-

<sup>1</sup> + = positive; ± = awaiting final results; - = negative.

**Table 6.9.2.** STG plants tested by PCR for CVd, ASGV, CPsV and Greening.

Cultivars	CVd	ASGV	CPsV	Greening
Navel	16	9	4	3
Midseason	5	1	1	1
Valencia	2	5	4	4
Reticulata	1	-	-	-
Mandarin	2	4	2	3
Grapefruit	-	1	-	1
Clementine	1	-	-	-
Lemon	-	-	-	-
Ornamental	5	-	-	-
Rootstock	-	1	1	1
<b>Total</b>	<b>32</b>	<b>21</b>	<b>12</b>	<b>13</b>

**Table 6.9.3.** STG plants indexed biologically for CPsV and CID.

Variety Group	Number of plants	Negative	Positive	Awaiting results
Navel	7	-	-	7
Midseason	2	-	-	2
Valencia	4	-	-	4
Reticulata	1	-	-	1
Mandarin	3	-	-	3
Grapefruit	1	-	-	1
Clementine	1	-	-	1
Lemon	1	-	-	1
Ornamental	-	-	-	-
Rootstock	-	-	-	-
<b>Total</b>	<b>20</b>			<b>20</b>

**Table 6.9.4.** Pre-immunisation status and new cultivar additions to the gene source and Foundation Block.

Cultivars	Number of plants pre-immunised	Pre-immunisation confirmed by ELISA	Pre-immunisation to be confirmed	Supplied to the CFB and additions to Nucleus Block
Navel	6	4	2	4
Midseason	-	-	-	-
Valencia	4	2	2	2
Reticulata	-	-	-	-
Mandarin	4	2	2	2
Grapefruit	-	-	-	-
Clementine	1	0	1	0
Lemon	-	-	-	-
Ornamental	-	-	-	-
Rootstock	-	-	-	-
<b>Total</b>	<b>15</b>	<b>8</b>	<b>7</b>	<b>8</b>

## 2. Re-indexing of mother trees at the CFB

During 2011 the CVd status of 363 mother trees of 90 cultivars were determined using biological indexing and PCR to ensure CVd-free status at the CFB. Results are presented in Table 6.9.5. Nine mother trees of 4 cultivars; Or 4, Valencia G5, Palmer Navel and Cambria R-2 were found positive and shown to contain CVd-II (Or 4, Valencia G5) and CVd-IV (Palmer Navel and Cambria R-2). RT-PCR analysis on the gene source trees of these cultivars confirmed the viroid-free status of all, except for Cambria R-2. The nucleus block testing was previously conducted on the ITSC source of this accession. Supply to the CFB was from CRI and the positive finding explains the CFB contamination. Sequencing of the PCR products obtained with CVd-II positive sources was done and these sources were shown to contain CVd-IIa, the non-Cachexia inducing variant. Subsequent to the findings, the tests were repeated by re-sampling of the nucleus block trees, all mother trees and the multiplication blocks of the implicated cultivars at the CFB and findings are presented in Table 6.9.6. The Valencia G5 and Palmer Navel infections were limited to single mother trees, which were not used for further multiplication and, no samples from multiplication blocks or other mother trees have tested positive. One multiplication block of Or 4 has tested positive, but the other negative. Diagnosis of the associated multiplication trees is ongoing and further sampling was done to confirm the findings.

The CTV severity status of 150 mother trees was evaluated during 2011 and results are presented in Table 6.9.7. The Mexican lime indicators showed the presence of severe CTV in six mother trees of two cultivars. It is suggested that these trees should be terminated as budwood sources. There is also evidence that 62 mother trees, mainly soft citrus cultivars, are CTV-free which indicates that pre-immunisation sources are potentially not appropriate. For this reason the pre-immunising CTV source for soft citrus has been changed from LMS 6 to GFMS 12. A field trial has been initiated to identify more suitable CTV sources for pre-immunisation.

**Table 6.9.5.** CVd indexing results of all the mother trees maintained at the CFB.

Cultivar	Number of mother trees	Number negative	Number positive
Nadorcott1	6	6	-
Morr 26	6	6	-
2PS LS Murcott	6	6	-
Cami	2	2	-
Haddas	3	3	-
Irradiated 122	3	3	-
Murcott x Clem	3	3	-
Nova	3	3	-
Valley Gold	6	6	-
Shasta Gold	2	2	-
Yosemite Gold	2	2	-
Tahoe Gold	2	2	-
Or 4	5	1	4
African Sunset	3	3	-
Ortanique Tangor	3	3	-
Nadorcott SL	3	2	-
Empress	3	3	-
Mandalate	3	3	-
Nova mutant	3	3	-
Tango	4	4	-
Clara	3	3	-
Gold Nuget	3	3	-
Tacle	3	3	-
Shani SL	3	3	-
Sweet Spring	3	3	-
B17	3	3	-
<b>Total Mandarins</b>	<b>89</b>	<b>85</b>	<b>4</b>
Tarocco Scire (nuc)	3	3	-
Tarocco Tapi	4	4	-
Tarocco Scire	3	3	-
<b>Total Midseasons</b>	<b>10</b>	<b>10</b>	<b>0</b>
Newhall	3	3	-
Chislett M7	3	3	-
Cambria 3	4	4	-
Rautenbach Late	5	5	-
Bahianinha	6	6	-
Navelina	6	6	-

Palmer	8	7	1
Autumn Gold	3	3	-
Barnfield Summer	3	3	-
Chislett Summer	3	3	-
Powell Summer	3	3	-
Summer Gold	3	3	-
Dream	3	3	-
Cambria K Tak	3	3	-
Washington	3	3	-
Clarke	3	3	-
Lane Late California	6	6	-
Fukumoto	6	6	-
Glen Ora Late	3	3	-
Lina	6	6	-
Cambria R-2	3	0	3
Fischer	3	3	-
Cara Cara	6	6	-
Letaba Early	4	4	-
Witkrans 3	6	6	-
<b>Total Navels</b>	<b>105</b>	<b>101</b>	<b>4</b>
Turkey	6	6	-
Kirkwood Red	3	3	-
Lavelle 2	3	3	-
McClellan SL	5	5	-
Delta	6	6	-
Late	6	6	-
McClellan	6	6	-
Midnight	6	6	-
G5	5	4	1
Bend 8A2	3	3	-
Bennie 2	6	6	-
Alpha	6	5	-
Midnight 1	1	1	-
Ruby	3	3	-
<b>Total Valencias</b>	<b>65</b>	<b>64</b>	<b>1</b>
Sonet	6	6	-
Kuno	4	4	-
Miho Wase	6	5	-
<b>Total Satsumas</b>	<b>16</b>	<b>16</b>	<b>0</b>
Mandered C1739	3	3	-
Esbal	3	3	-
Basol	3	3	-
Nules	6	6	-
<b>Total Clementines</b>	<b>15</b>	<b>15</b>	<b>0</b>
Flamingo H17	5	5	-
Star Ruby	4	4	-
Nelruby	3	3	-
Marsh	5	5	-
Rosé	3	3	-
Nartia	6	6	-
<b>Total Grapefruit</b>	<b>26</b>	<b>26</b>	<b>0</b>
Eureka seedless	6	6	-
Limoneira	6	6	-
Eureka	4	4	-
Lisbon	5	5	-
2PH Eureka	3	3	-
Genoa	3	3	-
Lemox (Triploid)	2	2	-
<b>Total Lemons</b>	<b>29</b>	<b>29</b>	<b>0</b>
Bears	5	5	-
<b>Total Limes</b>	<b>5</b>	<b>5</b>	<b>0</b>
Nagami	3	3	-
<b>Total Kumquats</b>	<b>3</b>	<b>3</b>	<b>0</b>
<b>Grand Total</b>	<b>363</b>	<b>354</b>	<b>9</b>

**Table 6.9.6.** CVd re-indexing results of positive accessions found at the CFB

<b>Accession</b>	<b>CIS code</b>	<b>Source description</b>	<b>CVd</b>
1627 (Or4) CRI-NB		NB-CRI	-
1627 (Or4)	MH04-R/56/P01	CFB mother tree	-
1627 (Or 4)-MH04-R/56	GH1-R2	CFB multiplication block	CVd-II
1627 (Or 4)-MH04-R/56	CRI-0004 (11/2008)	CFB multiplication block	-
1299 (Royal Late) CRI-NB		NB-CRI	CVd-IV
1299 Cambria-NCA-R-N/35	CRI 0005 (01/2009)	CFB multiplication block	CVd-IV
1299 Cambria-NCA-R-N/35	T1-R2	CFB multiplication block	CVd-IV
1553 (G5 Valencia)	VG5-V/27/335/P01	CFB mother tree	CVd-II
1553 (G5 Valencia)	VG5-V/27/335/P02	CFB mother tree	-
1553 (G5 Valencia)	VG5-V/27/335/P03	CFB mother tree	-
1553 (G5 Valencia)	VG5-V/27/335/P04	CFB mother tree	-
1553 (G5 Valencia)	VG5-V/27/335/P05	CFB mother tree	-
1553 (G5)-VG5-V/27	ex P04 ARC source 10/1998	CFB multiplication block	-
1553 (G5)-VG5-V/28	ex P05 ARC source 10/1998	CFB multiplication block	-
1072 (Palmer Navel) CRI-NB		NB-CRI	-
1072 (Palmer Navel)	NP-N/01/056/P01	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P02	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P03	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P04	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P05	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P06	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P07	CFB mother tree	-
1072 (Palmer Navel)	NP-N/01/056/P08	CFB mother tree	CVd-IV
1072 (Palmer-NP-N/01)	ex P04 ARC source 10/1998	CFB multiplication block	-
1072 (Palmer-NP-N/01)	ex P06 ARC source 10/1998	CFB multiplication block	-

**Table 6.9.7.** Mother trees at the CFB indexed for CTV severity.

Cultivar	Number of mother trees	Number of trees with severe SP <sup>+</sup>	Number of trees with mild CTV	Number of trees negative for CTV
Nadorcott SL	3	0	0	3
Empress	3	0	0	3
Mandalate	3	0	1	2
Nova mutant	3	0	1	2
Tango	4	0	0	4
Clara	3	0	2	1
Gold Nugget	3	0	0	3
Tacle	3	0	0	3
<b>Total Mandarins</b>	<b>25</b>	<b>0</b>	<b>4</b>	<b>21</b>
None	-	-	-	-
<b>Total Midseasons</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
Lane Late California	6	0	4	2
Fukumoto	6	0	0	6
Glen Ora Late	3	0	3	0
Lina	6	1	5	0
Cambria R-2	3	0	3	0
Fischer	3	0	3	0
Cara Cara	6	0	5	1
<b>Total Navels</b>	<b>33</b>	<b>1</b>	<b>23</b>	<b>9</b>
Delta	6	0	6	0
Late	6	0	6	0
McClellan	6	0	5	1
Midnight	6	0	6	0
G5	5	0	5	0
Bennie 2	6	0	6	0
Alpha	6	0	0	6
Midnight 1	4	0	0	4
Ruby	3	0	1	2
<b>Total Valencias</b>	<b>48</b>	<b>0</b>	<b>35</b>	<b>13</b>
Miho Wase	6	0	6	0
<b>Total Satsumas</b>	<b>6</b>	<b>0</b>	<b>6</b>	<b>0</b>
Basol	3	0	0	3
Nules	6	0	0	6
<b>Total Clementines</b>	<b>9</b>	<b>0</b>	<b>0</b>	<b>9</b>
Nartia	6	0	6	0
<b>Total Grapefruit</b>	<b>6</b>	<b>0</b>	<b>6</b>	<b>0</b>
Eureka seedless	6	0	1	5
Limoneira	5	0	5	0
Eureka	4	0	2	2
Lemox (Triploid)	3	0	0	3
<b>Total Lemons</b>	<b>18</b>	<b>0</b>	<b>8</b>	<b>10</b>
Bears	5	5	0	0
<b>Total Limes</b>	<b>5</b>	<b>5</b>	<b>0</b>	<b>0</b>
<b>Grand Total</b>	<b>150</b>	<b>6</b>	<b>82</b>	<b>62</b>

3. Change of the cross-protecting source from LMS 6 to GFMS 12 for soft citrus

Problems were encountered with the pre-immunisation of soft citrus with the LMS 6 source. Re-indexing using both, biological host indicators and ELISA, indicated that LMS 6 is not a suitable CTV source for pre-immunising soft citrus. At the CIS Advisory Committee meeting in 2007 it was agreed that a change should be made to another CTV source that is compatible with mandarin types. GFMS 12 CTV source was approved to be re-instated as the pre-immunising CTV source for soft citrus until a more suitable source is identified. It was decided that the search for a more suitable source should start immediately as an *ad hoc* experiment. The final report was in the 2008/2009 annual report and a new experiment (Exp. 968) was initiated in 2009/10 to evaluate the field performance of selected mild strain sources.

Eleven soft citrus cultivars were previously sent to the CFB for multiplication with GFMS12 as the replacement pre-immunising source and a further 32 soft citrus cultivars have been successfully pre-immunised this year and sent to the CFB for multiplication.

#### 4. Ad hoc indexing

##### 4.1. General indexing for growers

Citrus material submitted by growers or collected during visits, are indexed for specific diseases (**Table 8**). Once the results are available, they are communicated to the submitting parties.

**Table 6.9.8.** Indexing of material sent in by growers or collected during visits.

Disease	No of samples	Results
CTV	18	Analysis still underway
CVd	19	Analysis still underway
CPsV	1	Analysis still underway
CiLRV	2	Analysis still underway

##### 4.2. Citrus viroids: the first detection of CVd-IV in South Africa

Identification and initial surveys were completed in the previous reporting period and the findings were published as a disease note in the current report period. Field analysis is ongoing to monitor the presence of the viroid and possible symptom expression.

##### 4.3. Valley Gold (B17) investigations:

An investigation into the multiple sprouting and weak intercalations of Valley Gold (B17) observed by various nurseries/growers was initiated in September 2010 and reported in the 2011 season report. No pathogen was implicated in the problem. Molecular analysis using simple sequence repeat markers (SSR) was used in this report period and established that the problem-B17 was not B17, but another undetermined cultivar. Details of this analysis were reported elsewhere.

#### **Conclusion**

The value of frequent re-indexing of the CFB mother trees has again been demonstrated and the importance of tool sterilization, to prevent the spread of CVd, has again been accentuated. Efficient pathogen detection enables supply of healthy bud-wood to the industry and this project is vital to achieve this aim. Supportive molecular techniques have enhanced the service and ensure a greater dependability.

#### **Technology transfer**

Cook, G., S.P. van Vuuren, J. H. J. Breytenbach and B.Q. Manicom, 2012. Citrus Viroid IV Detected in *Citrus sinensis* and *C. reticulata* in South Africa. *Plant Disease* 96: 772.

#### **Further objectives and work plan**

##### Quarterly milestones for Apr-Jun, Jul-Sep, Oct-Dec 2013 and Jan-Mar 2014

- Propagate virus-free indicator plants, for the different graft transmissible diseases.
- Bud any budwood send in, to these indicator plants.
- Keep plants in the glasshouse according to the disease diagnostic requirements.
- Use ELISA and PCR for detection of the presence of a pathogen.
- Discussion of results to the party involved.
- Improvement and validation of diagnostic techniques and development of new techniques for indexing for the various graft transmissible diseases (on-going)
- Source virus-free seed of various herbaceous plants for a host range study. Determine optimum growing conditions and maintain seedlings, mechanically inoculate various "psorosis"-sources. Submit certain samples for electron microscopic examination.
- Perform double-stranded RNA isolations and poly-acrylamide gel electrophoresis (PAGE).

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#### 6.10 Restructuring of the Citrus Improvement Scheme

South Africa's Citrus Improvement Scheme (CIS) was lauded as world-class by international panel during the 2010 review. Several aspects were identified to ensure sustained and improved services. These included several infrastructural changes to address space constraints, increased funding to address more extensive diagnostic services (which includes the recently employed molecular diagnostics), insufficient human resource allocation to CIS administration and Biosecurity, and urgent succession planning for experienced staff nearing retirement age, specifically the current CIS-Manager, Thys du Toit, and CRI's coordinator for CIS services, Dr Fanie van Vuuren.

Succession planning for Fanie is progressing very well, with the appointment of Glynnis Cook as virologist in August 2009, and the recent employment of Zama Maqutu as molecular diagnostics technician in April 2011. Due to the increased CIS tasks at CRI-Nelspruit, CRI has also approved the permanent appointment of one additional assistant, Loderick Silinda, who has been working on contract for number of years. These appointments, together with collaboration with ARC's Dr Barry Manicom and newly appointed Dr Desmond Ncango and efficient diagnostic systems that were established between CRI and ARC should ensure sustained and improved diagnostic services for the CIS.

Succession planning for Thys du Toit, whose employment contract with CRI was extended for 3 years, will be addressed through the following restructuring, which was approved by the Boards of CRI and CGA in November 2011:

- Dr Paul Fourie, presently CRI's Programme Manager for Disease Management, will also assume the responsibilities of CIS Manager from 3 January 2012
- Thys du Toit will fill the position of Citrus Foundation Block (CFB) manager, while a new CFB manager will be appointed in April 2013 to be trained during the final year of Thys's employment
- Michelle le Roux, the CIS administrator, received additional support from the appointment of an Administration Assistant, Louise Olivier, who started working at the Foundation Block on 3 January 2012

Citrus Biosecurity and South Africa's ability to prevent spread of feared diseases (such as the national spread of graft transmissible and soilborne pathogens by means of infected nursery trees, and importantly the spread of African Citrus Greening disease to the Eastern Cape province), and in preventing incursion of feared exotic diseases and pests (for example Asiatic Citrus Greening and its vector Asian Citrus Psyllid) has also been bolstered by the above-mentioned restructuring. Additionally, through the anticipated future appointment of a technician in the Soilborne Disease project, MC Pretorius's time allocation to Biosecurity will increase to 20%, while an additional 15% of MC's time will be spent specifically on soilborne pathogen related services for the CIS.

The following positions are accordingly added for CIS support: Paul Fourie (CIS Manager), Thys du Toit (CFB Manager), Zama Maqutu (molecular diagnostician), Loderick Silinda (general assistant), and Louise Olivier (Administration Assistant at CFB). Additionally, the following members of the Disease Management team will continue servicing the CIS in terms of plant pathology, diagnostics and biosecurity: Fanie van Vuuren, Glynnis Cook, MC Pretorius, Kobus Breytenbach, Doctor Silinda and the new diagnostician, Elaine Basson.

## 7 INTERNATIONAL VISITS

### 7.1 P.J.R. CRONJÉ

#### 7.1.1 Research visit to CSIC-IATA, Valencia 5 January - 5 April 2011

##### Executive Summary

During January until March 2011 I spent 3 months in the CSIC- IATA laboratory of Dr Zacarías. The aim of this visit was to foster a relationship with the group of researchers at this facility that are doing research on various important aspects of rind condition of citrus fruit. During this time I was exposed to the use of new technology, as well as the incorporation of physiological measurement with molecular technologies. Two successful experiments were conducted during this period and a new collaborative project on lycopene synthesis and the influence on chilling susceptibility was initiated. As preliminary output from the visit a talk was presented from this information at the “Congreso Hispoani Luso De fisiolgia Vegetal”.

#### 1. Background

This research collaboration developed between the two parties, CISC-IATA and CRI-US, due to a shared research focus area i.e., postharvest disorders of citrus fruit, and in particular physiological disorders developing during export in the citrus fruit rind. In addition, Dr. Zacarías, the international leader in this increasingly important field of citriculture, was the external examiner of my PhD dissertation in 2009, after which he invited me to visit his research group, with the aim of starting a collaborative project. During the end of 2010 the funding was made available via the TipTop fund from the CGA/CRI, as well as the DRD travel grant from Stellenbosch University (SU) for a 3 month study visit to IATA in Valencia.

The advantages of the collaboration between these two parties are twofold: firstly, it enables the collection of two data sets in one year, due to the difference in northern and southern hemisphere citrus seasons, and secondly an exchange of research methodology and knowledge on physiological rind disorders can take place between the researchers.

The specific aim of this initial research visit was to expose me to new research techniques and methodology (in particular the integration of physiological measurements and molecular techniques) used in the rind disorder research at IATA. Secondly two experiments would be conducted in the 3 month period. Firstly, on the change of carotenoids in the flavedo as influenced by time and temperature and secondly, the effect of postharvest handling on the water balance of the flavedo and the impact on rind disorder incidence.

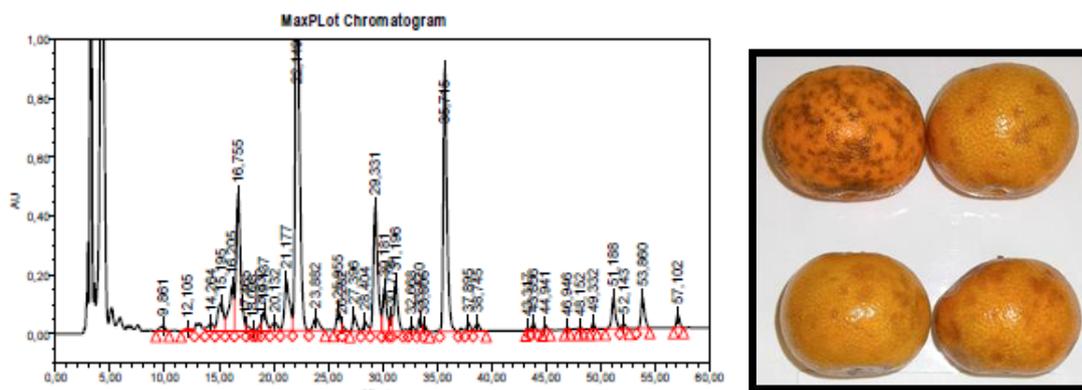
#### 2. Experimental work

##### Experiment 1: Carotenoid profiling of ‘Nules Clementine’ mandarin flavedo

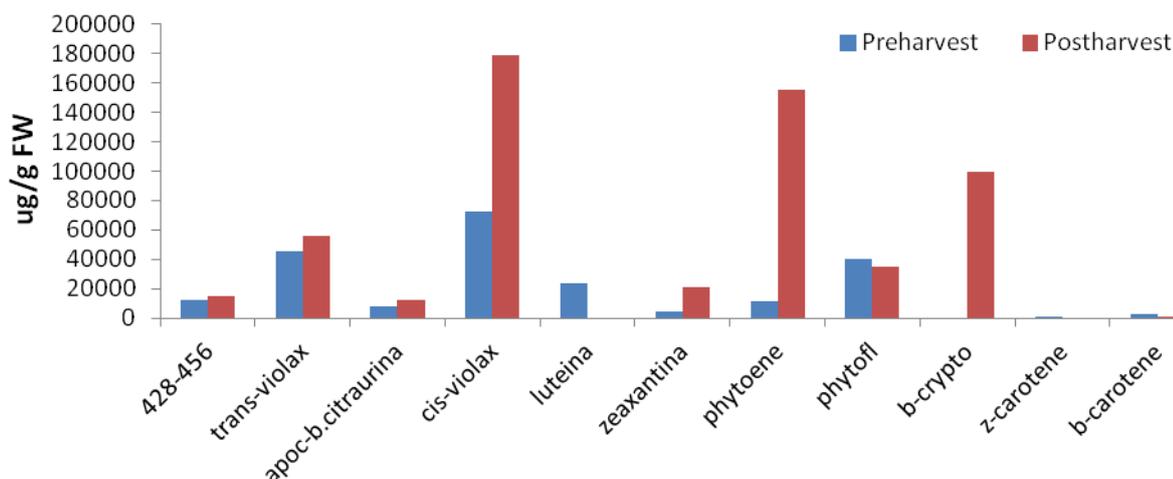
Collaborators: Drs. Maria-Jesus Rodrigo and Zacarías

This experiment involved analyzing material collected during my PhD study, which focused on elucidating the factors involved in the increased sensitivity to developing rind breakdown of ‘Nules Clementine’ mandarin. Rind breakdown is associated with the collapse of oil glands and leads to substantial postharvest losses. Low levels of carotenoid in the fruit rind resulted in significantly higher sensitivity to rind breakdown, which is thought to be related to a reduced anti-oxygen capacity (Cronje et al., 2011ab). Therefore, the aim was to ascertain if the carotenoid profile of sensitive (weak/yellow colour) fruit differ from non-sensitive (good/orange colour) fruit. The main collaborator was Dr M-J Rodrigo, a specialist in the carotenoid pathway, who assisted with the development of a detailed protocol to extract and purify all carotenoids from this tissue prior to quantitative analyses on the HPLC. This method is based on previous protocols developed by Dr. Rodrigo (Rodrigo et al., 2003; 2004; 2007). Preliminary interpretation of the data from these detailed and time-consuming analyses revealed that fruit position in the tree canopy *viz.* exposure to sun or developing in the shade, results in significantly different carotenoid profiles. In addition, these carotenoid profiles changed during cold shipment (-0.5°C vs. 7°C). These data could offer a better understanding of the different components that make-up the rind’s colour and its possible contribution to its physiological condition, as not all carotenoid species are thought to be equal in their antioxidant capacity.

*Potential output from this experiment:* The data are currently being analysed and will be prepared as a scientific article which will focus on the impact of time of harvest, canopy position and postharvest storage condition on carotenoid profile of ‘Nules Clementine’ mandarin.



**Figure 7.1.1.1.** Example of the complexity of carotenoid profiling. In addition of identifying the major peaks the ratio between the “yellow” and “orange” carotenoids is vital for adequate colour development.



**Figure 7.1.1.2.** Illustration of the influence of postharvest handling on carotenoid profiles of ‘Nules Clementine’ mandarin fruit, which are prone to develop rind breakdown in the cold chain

Experiment 2: Impact of water relations in the fruit rind on the incidence of physiological disorders  
 Collaborators: Drs Zacarías and Alférez

Postharvest physiological rind disorders such as pitting and staining develop in the flavedo of citrus fruit after harvest. It was shown by the IATA-group that sudden fluctuation in the relative humidity (RH) after harvest and prior to packing does influence the dehydration and re-hydration of the albedo and flavedo of ‘Navel’ orange and grapefruit (Alférez et al., 2003). Subsequent alteration of the water potential or its components (osmotic and turgor potentials) in the fruit rind was shown to play a major role in the development of staining and pitting in ‘Navel’ orange and grapefruit. It is thought that the resulting turgor difference between these components of the rind could induce and aggravate the incidence of rind pitting and staining (Alférez et al., 2008; 2010). In a commercial citrus packhouse, fruit are typically exposed to several hours or more of low %RH prior to high %RH conditions, i.e. degreening (95% RH), washing and waxing, which could contribute to the development of rind pitting or staining (Alférez et al., 2005). These changes in postharvest %RH which result in water content adjustments and water movement within the rind tissue, may underlie the morphological and structural alterations accompanying postharvest rind breakdown in ‘Navelate’ orange fruit (Alquezar et al., 2010).

The difference in rind morphology of mandarin-cultivars (reticulated) compared to ‘Navel’ orange is visibly evident in the thickness of the albedo. However, the hypotheses of water movement in the rind as discussed above have not been tested on the thin rinds of mandarin fruit. It is known that the new late mandarin cultivars do have a susceptibility to physiological disorders similar to rind staining or pitting, however, the development of these disorders is poorly understood in these cultivars.

During the visit to IATA a study was done on a ‘Navel’ orange and ‘Ortanique’ mandarin fruit at optimal harvest condition, to test how albedo thickness influences water movement in the rind under an RH-

fluctuating environment. In addition to linking physiological measurements (for example water, osmotic potential and ethylene production) with incidence of visible disorder symptoms, the expression of certain genes thought to be involved in cellular breakdown, such as phospholipase genes, was documented.

To measure water, osmotic and turgor potentials in flavedo and albedo during the experiments, 5 mm diameter disks from the equatorial area of fruit were excised by using a cork borer. Disks of 1 mm thick flavedo, external albedo (closest section to flavedo) and internal albedo (closest section to pulp) were sliced with a blade and placed in a sample chamber (C-52, Wescor Inc. Logan, UT) connected to a psychrometer switchbox (PS-10) and to a dew point microvoltmeter (HT-33T). Measurements were performed following the entire procedure as previously described (Alf rez et al., 2003).

To profile phospholipase gene expression in response to changes in water potential, RNA from flavedo was extracted following conventional methods currently used at the IATA postharvest research group in Valencia. Total RNA was treated with DNaseI (Ambion) to remove genomic DNA contamination. Specific primers were designed on partial sequences of the corresponding genes of interest available in public databases using the Genexpress software package. Then, a Roche LightCycler 2.0 Real-Time PCR system was utilized for one-step quantitative real-time RT-PCR analysis. Melting curve analysis was performed to confirm target-specific amplification. Primer concentration was individually optimized and standard curves were performed for assessing reaction efficiency and appropriate RNA concentration for each gene. The use of Citrus glyceraldehyde-3-phosphate-dehydrogenase (*CsGAPDH*) and Citrus *Actin* as reference genes was allowed to normalize experimental variability. By using LightCycler Software 4, data were compared to freshly harvested samples obtaining relative fold-expression values. The results confirmed the hypothesis that mandarin-type fruit reactions differ in response to the treatments (high and low % RH at constant temperature) compared to 'Navel' orange fruit.

#### Initial output from research visit

The initial physiological and rind disorder incidence data from the second experiment was prepared, submitted and accepted to be presented at the: "Congreso Hispano Luso De fisiolgia Vegetal": held in 22-42 June 2011 at Universitat Jaume I de Castell n (UJI). This is the primary meeting of the Spanish and Portuguese researchers of plant physiology (see abstract below).

#### Differential susceptibility to peel pitting in fruit from citrus cultivars with different albedo thickness

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Postharvest peel pitting at non-chilling temperatures is a physiological disorder that affects fruit from several citrus cultivars worldwide and diminishes commercial value of fruit. Evidences indicate that altered water relations in fruit peel induce this disorder during postharvest. Previous work showed that commercial packline or manual processing differentially altered water, osmotic and turgor potentials in the three layers of 'Marsh' grapefruit (*Citrus paradisi* Macf.) rind viz. flavedo, internal and external albedo. In this work we have studied the incidence of postharvest rind pitting in two cultivars with disparate albedo thickness. To this aim, we selected a variety with thin albedo, 'Ortanique' mandarin (*Citrus reticulata* Blanco) and a variety with thick albedo, 'Navelate' orange [*C. sinensis* L. (Osborne)]. Fruit from both cultivars were divided in lots and subjected to identical postharvest practices consisting of washing and/or waxing on a commercial packline, equipped with revolving brushes, where after the fruit were stored at either 45% relative humidity (RH) or 90% RH for 3 weeks. To serve as a comparative control additional fruit lots were washed manually and stored as above. Periodically, water loss, water, osmotic and turgor potentials were monitored and incidence of peel pitting was evaluated. The transpiration rate was higher in packline processed fruit than those manually processed fruit from both cultivars. However, only 'Navelate' fruit developed peel pitting after 21d. Peel pitting index was higher in packline than in manually processed fruit, and wax coating exacerbated this effect. Accordingly, water potential variations were more pronounced in wax-coated 'Navelate' orange fruit compared to 'Ortanique' mandarin fruit.

Our results suggest that water movement through the cell layers in the thick albedo of the 'Navelate' orange is related to postharvest peel pitting and support the notion that the inability of the peel to properly adjust its water status after a prolonged water stress could result in cellular collapse and tissue damage. We hypothesize that a thin albedo allows faster water adjustment in the peel and thereby preventing the cellular collapse due to water deficit.

**Acknowledgements:** Paul Cronjé was supported by Citrus Research International, Citrus Growers' Association of Southern Africa and the DRD Research Visit Grant of the Stellenbosch University, South Africa. F.A is the recipient of a Ramon y Cajal contract (MICINN and Fondo Social Europeo).



**Figure 7.1.1.3.** Illustration of the rind pitting seen in the 'Navel' orange and the lack thereof in the 'Ortanique' mandarin (right).

### Future collaboration between CSIC-IATA and CRI

A successful application for a new collaborative project between the two parties was put forward to the Postharvest Innovation fund from the DST. This two year project will focus on the lycopene content of 'Star Ruby' grapefruit as it has the highest anti-oxidant capacity of all carotenoids. This carotenoid prevents the development of chilling injury during export if prevalent at a high enough level in the flavedo. The first step will be to elucidate the influence of Mpumalanga and Northern Cape environmental (temperature and humidity) conditions on the lycopene synthesis-pathway. This will involve gene expression and profiling of the carotenoid species of this pathway to see which microclimatic conditions influence the rate of lycopene development.

The overall aim of this project, which will involve basic molecular gene expression studies in addition to fruit quality evaluation, is to successfully increase exportation of 'Star Ruby' grapefruit to new markets such as China and USA. The project is funded 50:50 by the Post Harvest Innovation fund and CRI for 2 years.

### Acknowledgements

I would like to express my gratitude to the CRI/CGA for the opportunity and funding that enabled me to spend this time at such a prestigious laboratory in this field of postharvest research. The experience gained in the laboratory as well as the friendships made during this period were more than I could hope for.

I would like to thank Drs. Zacarías, Rodrigo, Alférez and Marcos as well as all the postdocs and PhD students at the laboratory for the kindness shown to me during my stay at their laboratory on a scientific but also cultural level.

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IATA is part of the government supported CSIC research group of Spain and is situated on the campus of the Universidad de Valencia Burjasot Park Scientific.

In addition, to being a cutting edge-research facility the laboratory also has very friendly people.



## 7.2 G.C. SCHUTTE

### 7.2.1. Tour to Brazil and Argentina

#### Summary

I was invited by Nordox in Norway for an official visit to Brazil and Argentina to present talks on my work done with copper fungicides as used in SA for the control of Alternaria brown spot and black spot. The aim was to inspect their orchards for the status of citrus black spot, canker, Alternaria brown spot, Phytophthora branch canker and melanose. Furthermore, the aim was also to strengthen ties with the CBS researchers in both countries and to look for opportunities for cooperative research projects. Spray machines in operation were also investigated to determine how effective their spray applications are carried out.

#### Itinerary

Sunday 22 May	Travel: Johannesburg – Sao Paulo
Monday 23 May	Visit Fazenda Colorado citrus and dairy farm near
Tuesday 24 May	Present a talk at the INTA experimental station. Afterwards a meeting was held with Sergio Garran at INTA and Dr Guillermo Miguel Marco regarding CBS and market access to the EU. Also met Prof Victor Rodrigues of the University of UNNE and discussed joint research projects on Alternaria brown spot.
Wednesday 25 May	Travel to Tucuman, Argentina.
Thursday 26 May	Present a talk at Park Garden Hotel in Tucuman on cuprous oxide as used on citrus in SA. Tuesday 24 March Travelled to Montevideo and to BA.
Friday 27 May	Meetings with SA San Miguel researchers at their offices in the morning. Visited lemon orchards at Citromax. Inspect orchards with Phytophthora trunk and branch canker.
Saturday 28 May	Travel to BA.
Sunday 29 May	Return to SA.

#### Brazil

A field visit to Fazenda Colorado citrus farm was organized. A huge amount of trees were destroyed (Fig. 1) due to Asian greening and the impression is that they are losing the battle. Unfortunately neighbouring farmers are un-cooperative and do very little to prevent psylla infestations and do not remove or destroy any infected trees.



**Fig. 7.2.1.1.** Valencia orange trees with Asian greening. Orchards were sprayed with sulphur during the visit and it was interesting to see how poor the spray coverage was using the Jacto spray machine used.



**Fig. 7.2.2.2.** Jacto mist blower in operation in a Brazilian orchard spraying sulphur for the control of rust mite @ 2000 L/ha. A); inside fruit and leaves had no spray cover (B) while only the outside hanging fruit had a slight coverage of spray residue on the outward facing rind (C).

Only scab and some melanose were visible in this particular orchard. While driving through the orchards a huge amount of mulching was noticed under the trees. This practice is common throughout Brazil as they do not use micro-irrigation and through mulching, they prevent evaporation of water. What they do not realize is that mulching also prevents ascospore releases from the dead leaves on the orchard floor and if they can repeat this mulching exercise after winter, then the incidence of inoculums infections will be reduced. (Fig. 7.2.1.3).



**Fig. 7.2.1.3.** Weeds used as mulching between citrus trees within the rows used to minimize evaporation of water but can be useful for the control of CBS for the de-composition of dead leaves with inoculums on the orchard floor too.

#### Cuprous oxide seminar/symposium

A well attended meeting was held representing growers of about 450 000 ha of citrus in this region! Lectures covered topics on cuprous oxide used for the control of citrus canker, *Alternaria* brown spot and citrus black spot. The presenters were: Hector Mara and Alegre Sasson from Uruguay, Ian Lonie from Australia, Eduardo Feichtenberger and Antonio de Goes from Brazil and myself.

#### **Argentina**

##### Visit to Tucuman

The same lectures by the same people were presented to about 40 people at the Garden Park Hotel in Tucuman. Eduardo Feichtenberger and Antonio de Goes from Brazil were not present.

##### SA San Miguel

San Miguel's harvest was in full swing at the time of the visit. Some of the greener lemons had to be de-greened. Their pack-out is only in the mid-fifties and canker lesions on the fruit are still their biggest concern. Between 3-5% of the fruit had lesions on them on arrival at the packhouse. According to Sebastian Torres Posse, each percentage in pack-out is worth \$300 000 and only copper is used for the control of all their diseases. Because the culled fruit is used for the extraction of lemon oil for Coca Cola, they are not permitted to use mancozeb or any of the strobilurines that we use in SA because of the residue factor. Therefore an annual average of 4 and even 5 copper applications is normal in Tucuman.

Melanose was detected on all their trees, especially on the autumn flush. This resulted in leaf drop and a huge amount of dead twigs were observed in the top parts of the trees' canopies. This source of inoculum is

no problem to them because they hedge their trees and by doing so these twigs are removed from the trees and will therefore not cause further problems (Fig. 7.1.2.4).



**Fig. 7.1.2.4.** Die-back of lemon twigs due to melanose.

An interesting transport system of plastic crates are used in their orchards. These tracks are easily installed and cheap to make. Because there is no traffic between the trees, there is no soil compaction enabling a saving of fuel as tractors are not required to transport the fruit from the orchards during picking. They simply push the full crates to the end of each row where they are left to be collected at a later stage. Only two persons are needed to dislodge a crate from the tracks (Fig.7.1.2.5).



**Fig. 7.1.2.5.** A simply constructed rail system to remove crates of lemons from orchards. This prevents soil compaction between the rows.

#### Visit to Citromax

Lemon orchards were visited to observe the impact of copper and oil spray applications on fresh fruit. Oil rates of 0.125% are sprayed in October (with abamectin) and again in January, while 0.0125% is also added to the spray mix of mainly copper hydroxide in November and December to serve as an adjuvant. We believe that all the oil added to copper hydroxide aggravates the incidence of copper stippling which can result in up to 30% culling of fresh fruit (Fig. 7.1.2.6). It was noted that where cuprous oxide was sprayed in one of the

orchards, not only was less stippling observed, but more spray residues were also evident after having had 1700 mm rain during the past season.



**Fig. 7.1.2.6.** Stippling due to 4 copper hydroxide spray applications at Citromax.

During my previous visit to Citromax we visited a lemon orchard where numerous trees died due to *Phytophthora* trunk and branch canker. After treating their trees with Captan and Sporekill, the situation was reversed. On one tree it was found that the infection was curbed and the lesions therefore did not expand further on the trunk (Fig. 7). When the aerial photograph was taken, a lot of trees were dead and huge gaps existed, but replanting has since taken place and the trees are now about 4 years old. No new infections were detected which can be attributed to skirting of the younger trees to allow air movement and drying of the trunks to curb any further infections. If gumming is detected, a Captan + Sporekill treatment is immediately applied.

#### **General recommendations and conclusions**

- Copper fungicides are widely used in Brazil and Argentina for the control foliar diseases.
- Citrus canker is a widespread problem in both countries and copper is included in all their spray programmes. The consequence being that copper stippling is a huge problem which has an influence on pack-out.
- HLB is a serious problem in Brazil and they are trying to eradicate it by cutting down the trees and killing the rootstocks with herbicides.
- *Phytophthora citrophthora* (Pc) is a serious problem in certain lemon orchards along the mountain side in Tucuman where the climate is cooler and more suitable for the disease. Trunk paints consisting of Captan and Sporekill are effective in controlling the disease.
- Joint research projects were proposed between CRI and San Miguel in Tucuman.
- CBS isolates were also brought with to enlarge our culture collections for future research projects.



**Fig. 7.1.2.7.** Typical *Phytophthora citrophthora* branch canker symptoms on a lemon tree (right) in the orchard (left). The tree was saved after a Captan + Sporekill trunk paint was applied.

### 7.3 S.D. MOORE

#### 7.3.1 Visit to Kenya

##### Introduction

This visit took place from 6-12 November 2011 and was sponsored by River Bioscience. The 19<sup>th</sup> biennial meeting of the Association of African Insect Scientists (AAIS) took place from 8-11 November in Nairobi. Before this, visits were made to Dr Sunday Ekesi of ICIPE, Kenya Biologics and Kakuzi Estates in Thika, and Deka Citrus Plantations in Central Province. Information which is confidential to River Bioscience has been edited from this report.

##### Itinerary

Date/s	Destination	Institution/venue	Activity	Mode of travel
6 November	Nairobi	-	Travel via Johannesburg	Air
7 November	Nairobi	ICIPE	Visit Dr Sunday Ekesi	Road
8 November	Thika	Kenya Biologics	Discussion on exchange of virus products	Road
		Kakuzi Estates	Discuss pest issues & product needs	Road
		Deka Plantations	Inspect for and collect FCM; Discuss execution of Invader-b-Lok trial	Road
9-11 November	Nairobi	ICIPE	AAIS meeting	Road
12 November	Port Elizabeth	-	Travel via Johannesburg	Air

## Purpose of trip

1. To discuss possible collaboration with Dr Sunday Ekesi on FCM management in South Africa and Kenya.
2. To discuss exchange of virus products with Kenya Biologics and the process for importation of Cryptogran into Kenya.
3. To collect FCM infesting citrus at Deka Plantations and to discuss the layout and execution of an Invader-b-Lok trial for *Bactrocera invadens* control.
4. To participate in the 19<sup>th</sup> biennial meeting of the AAIS, and by so doing to:
  - a) Become familiar with the status of crop agriculture and pest and disease problems throughout Africa, particularly regarding pests such as false codling moth, bollworm and fruit fly.
  - b) Meet and connect with researchers working on relevant crops and pests.
  - c) Identify opportunities for RB products in Africa.
  - d) Identify needs for other product development in Africa.

## Dr Sunday Ekesi, ICIPE

Sunday reported that the pest status of FCM on avocados in Kenya had increased dramatically. He reported that he had substantial funding from the Finnish government to study the ecology of FCM on avocados, as part of a larger multi-national and multi-institutional programme on global warming. He also mentioned that he is very interested in working on management practices for FCM. We also discussed research collaboration and an application for funding from the Joint Research Grant under the South African/Kenya-Research Partnership Programme Bilateral Agreement. Unfortunately, private institutions are precluded from applying. However, Rhodes University have agreed that I may participate through my association with them. Sunday's main area of expertise is entomopathogenic fungi (EPF). Rhodes University currently has a CRI-funded study on the use of EPFs for control of subterranean life-stages of FCM. My expertise in baculoviruses, will assist ICIPE in testing Cryptogran for FCM control on avocados and citrus and possibly macadamias and pomegranates too. Sunday is very keen to test Cryptogran. As he is already working on FCM on avocados, some of the cost of conducting trials could be absorbed by his current project.

Sunday and his assistant, Dr Samira Feris are planning to visit South Africa in December 2011, mainly to visit CRI, Nelspruit. However, they will also visit CRI and RB in PE for a) further discussion on collaboration and importation and registration of Cryptogran, and b) to receive training on rearing of and field trials with FCM.

## Kenya Biologics

1. Kenya Biologics produce a bollworm NPV (same as Helicovir) and a diamond-back moth GV.
2. They have registered the NPV in Ghana and are awaiting registration in Kenya.
3. Exchange of viruses between RB and Kenya Biologics was discussed.
4. Chris Kolenberg is conducting a study on pheromone-based trapping for monitoring FCM in avocados. He obtains his pheromones, lures and yellow delta traps from a Korean supplier at a very good price. He did not want to divulge his supplier, as Kenya Biologics is considering venturing into the South African market. However it should not be too difficult to find this supplier.

## Kakuzi Estates

Kakuzi Estates is the largest avocado producer in Kenya. They also have several hundred hectares of macadamias, which will produce their first crop next year. They consider FCM as their number 1 problem on avocados and would desperately like to acquire Cryptogran. In the short term they plan to obtain Cryptogran from Tanzania, where it is legally imported. However, in the longer term, it is important that Cryptogran be registered in Kenya and supplied to them directly.

They are also interested in having access to the M3 and Invader-Lure and Invader-b-Lok. RB should therefore also ask Dr Sunday Ekesi of ICIPE, if he can conduct or organise registration trials with these products.

Although *Bi* occurs at Deka Plantations, just 17.5 km from Kakuzi on the tar road, Richard Collins (CEO of Kakuzi) claims that *Bi* is absent from Kakuzi. He says that they catch flies using methyl eugenol-loaded traps from Insect Science, and send these flies to the Nairobi Museum for identification. Thus far all identifications have been *Dacus* spp. They do not send flies to ICIPE for identification, even though Sunday Ekesi is the expert, as there appears to be a breakdown of trust between the two parties. Apparently ICIPE conducted trials which demonstrated that *Bi* can complete its life-cycle in very ripe avocados. As a result,

South Africa has halted all avocado exports from Kenya. According to Kakuzi, this finding and hence market closure is unjustified, as only hard unripe avocados are exported. *Bi* cannot complete its life-cycle in such avocados.

Despite this, Richard Collins indicated that it was important that Sunday Ekesi approach them to conduct trials, as they are the largest avocado producer in Kenya and are growing rapidly.

### **Deka Plantations**

According to the manager of Deka Plantations, they have around 20 000 citrus trees and about 10 000 mango trees. They also grow a few other smaller crops. According to the owner, Rahul Bidd, they farm about 160 ha. Their main citrus varieties are navel oranges, Satsumas and lemons. Due to the equatorial climate, there can be a number of overlapping fruit sets on the tree at any one time. However, the major crop sets in October and is harvested any time from the end of February to June.

A number of fallen fruit were collected and inspected for FCM. Only 15 individuals were collected and placed onto diet. If we were not restrained by time, we could have collected a lot more. Those individuals collected will be inspected for any possible virus infection. Further collections of FCM will be conducted by Deka staff and Chris Kolenberg of Kenya Biologics.

I also discussed with them a *Bi* trial which they had conducted for Tim Grout. I obtained a map for the farm, including trial blocks, and clarified some questions which Tim had for them. They did acknowledge that the trial "might not have been executed perfectly", but they were impressed with the reduction in fruit fly infestation in their citrus, which had contributed to a better harvest. They were willing to repeat the trial this season but said that they would need the trial material by January, as the season would be a lot earlier than the previous one.

### **AAIS – Key points from key papers**

The theme of the congress was "Biodiversity and sustainable development in Africa: contribution of insect science to the development of agriculture and improvement of human, animal and environmental health". The majority of talks were given in English. Only a few were given in French, either accompanied by English slides or English interpretation.

### **Plenary address: Integrated pest management in Africa in a climate change scenario – Prof Christian Borgemeister (Director General of ICIPE, Kenya)**

ICIPE is an inter-governmental organisation with involvement from 12 governments. It currently hosts 45 PhD graduates, post-doc researchers and visiting scientists; and has 50-70 MSc and PhD students in residence. ICIPE is active in 24 African countries. ICIPE currently has a large collaborative project, including Kenya, Tanzania and Ethiopia, to research the effect of climate change on IPM (especially biocontrol). Borgemeister believes that climate change could reduce the effectiveness of IPM strategies, leading to higher crop losses. This could be as a result of pest range expansion, faster life-cycles, disappearance of wild relatives of crops, decrease in soil fertility through erosion, and a reduction in capacity to control soil-borne pests. Borgemeister gave examples of coffee berry borer and *Bactrocera invadens* to support his arguments.

### **The efficiency of using different *Trichogramma* species and strains on the African bollworm, *Helicoverpa armigera* eggs – Sara Kehail (Agricultural Research Corporation, Sudan)**

In the Gezira scheme, only 10-45% parasitism was recorded, due to excessive pesticide usage. In the Rahad scheme, 71-77% parasitism was recorded. Five different species/strains of *Trichogramma* were compared. The average parasitized eggs per female were 13-54 for the different species/strains. The higher the number of parasitized eggs, the lower the emergence rate.

### **Impact of African weaver ant *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) against *Helopeltis* spp. and *Pseudotheraptus wayi*, key pests of cashew in Tanzania – M. Olotu (Mkwawa University College of Education, Tanzania)**

High presence of weaver ants significantly reduced pest pressure and damage.

### **Compatibility of *Metarhizium anisopliae* isolate ICIPE 69 with agrochemicals used in French bean production – S. Niassy (ICIPE, Kenya)**

Vegetative growth was higher in the control than for any of the treatments. Carbendazim completely suppressed vegetative growth. Abamectin, imidacloprid and thiomethoxam had no effect on conidia production. Carbendazim had the worst effect. Chlorpyrifos, azadirachtin, diazinon, copper hydroxide, L-

Cyhalothrin, propineb and spiromesifen caused some reduction in conidia. Effect on virulence was not measured. No synergism with imidacloprid against Western flower thrips was noted.

**Electrophysiological and behavioural responses of parasitoids to volatile semiochemicals in the headspace samples of maize exposed egg deposition – A. Tamiru (ICIPE, Kenya)**

Pest egg deposition was shown to change the volatile profile emitted by some strains of maize. This volatile change was shown to be detected by and attractive to parasitoids.

**Characterisation of Sudan strains of *Bacillus thuringiensis* pathogenic to the larvae of the house mosquito *Culex quinquefasciatus* – Gorashi Naieama (National Centre for Research, Sudan)**

39 *Bt* strains, which were shown to be morphologically and molecularly different, were isolated in Sudan. These were isolated from soil, stored product dusts and dead insects.

**Comportement d'oviposition de *Plutella xylostella* sur des choux protégé par des filets – Thibaud Martin (CIRAD, France)**

Nets were used to protect cabbages against moth pests. These were effective against diamond back moth and *Hellula* but not against *Spodoptera*. Pesticide usage was consequently reduced by 90% and yield was increased. The size of the mesh is important (1.5 x 1.5 mm). Even if the net is in contact with a cabbage leaf, DBM oviposition will be reduced, especially if given a choice. A commercial net (AgroNet™) treated with 1%  $\alpha$ -cypermethrin was also used. This acted as a deterrent, causing DBM adults to spend less time on the net.

**The impact of climate warming on insects: a case study of the southern green stink bug *Nezara viridula* in Japan – Kenji Fujisaki (Kyoto University, Japan)**

*Nv* is expanding northwards in Japan, threatening the extinction of *N. antennata* through interspecific non-reproductive copulation. The average global temperature is up by 0.3-0.6°C from the 19<sup>th</sup> century. This average is up by 0.13°C over the last 50 years. Over these 50 years, *Nv*'s range has shifted by 85 km (19 km/decade). This coincides with the shift of the isothermal line. *Nv* outcompetes *Na*, as it begins reproduction earlier, it has a higher fecundity and is multivoltine. It therefore has a higher reproductive rate under warming conditions. Climate warming will therefore lead to the extinction of *Na*. Organisms with the greatest risk of extinction are those with a low tolerance for warming, a limited acclimation ability and reduced dispersal.

**Susceptibility of some fruit species to natural infestation by fruit flies (Diptera: Tephritidae) in South Kordofan State, Sudan – Abdelaziz Gesmallah (University of Gezira, Sudan)**

Fruit flies were sampled on mango, guava, papaya and Annona in November. Fruit flies included *Bi*, *C. cosyra*, *C. capitata* and *C. quinaria*. In Sudan, 98-99.7% of all maggots infesting fruit were *Bi* (around 2% *C. cosyra*). In guava, this rose to 100% *Bi*. In northern Ethiopia, *Bi* made up 0.3% of the fruit fly assemblage and in southern Ethiopia, 60%. Papaya was free of *Bi*.

**Effects of aqueous extracts of Basil, *Ocimum basilicum* L., Sodom's apple, *Calotropis procera* Ait and Coriander, *Coriandrum sativum* L. On leaf miner, *Liriomyza* spp., on okra crop – Rehab Fadwal (University of Gezira, Sudan)**

Leaves of plants were dried, crushed, ground and stored at room temperature. Aqueous extraction (for 10 h) was then conducted. In field trials, water with gum Arabic and molasses was used as a control. Sprays (10% extract) were applied weekly for 4 weeks. All three extracts significantly reduced leaf miner infestation, with Sodom's apple being the most effective, followed by coriander and basil. The effect is primarily a repellent one.

**Dudutech – Jacinter Otieno (Dudutech, Nairobi)**

Dudutech is a manufacturer and supplier of biocontrol products. She reported that they have a granulovirus and a nucleopolyhedrovirus, but did not state for which pest species. She also mentioned that they have a mycorrhizal product and a vermicompost.

**Exploratory survey for natural enemies of *Rastrococcus iceryoides* green (Hemiptera: Pseudococcidae) in India and climatic matching to guide their introduction into Africa – MC Tanga (ICIPE, Kenya)**

*Rastrococcus invadens* and *R. iceryoides* are two invasive mealybug pests in Africa, coming from south Asia. Six parasitoids occur in Kenya and Tanzania, the most effective being *Anagyrus pseudococci*, with up to 21% parasitism. In Asia there are several species which can cause up to 40% parasitism. The best of these was *Praleurocerus viridis* and *Anagyrus chryos*. MAXENT and GARP (modelling software) were used to predict the suitability of Africa for these parasitoids, based on their distribution in Asia.

## **New tools for sustainable pest insect management from African biodiversity: delivery by plant extraction and GMOs – John Pickett (Rothamstead Research, UK)**

Oak Stump has been commercialised as a mosquito lure and bait and retails for \$14.99/box in the USA. Skatole is a plant derived oviposition pheromone. It is grown in Brazil and has been used successfully against *Culex* in Nigeria. Polygodial, isolated from water pepper (*Polygonum hydropiper*) is an antifeedant, protecting barley against aphids, replacing pyrethroids.

## **Formal contribution by Sean Moore to the AAIS programme:**

### **CONTROL OF FRUIT FLIES ON CITRUS USING ATTRACT AND KILL TECHNOLOGY**

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**Objectives:** Two species of fruit flies (Diptera: Tephritidae) are pests on citrus and other fruiting crops in South Africa, namely Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) and Natal fruit fly (*C. rosa* Karsch). A third species, the African invasive fruit fly (*Bactrocera invadens* Drew), has not yet established in South Africa but presents an impending risk. The objectives of the studies were to test the efficacy of attract and kill bait stations against these species. This represents the first studies demonstrating the ability of these bait stations (M3) to reduce fruit fly damage to citrus and the first study of the male annihilation technique (MAT) using methyl eugenol (Invader-b-Lok) for *B. invadens* in southern Africa.

**Methodology:** Three trials were conducted to compare control of *Ceratitis* spp using M3 fruit fly bait stations, baiting with protein hydrolysate and mercaptothion and GF-120. Comparisons were made through fruit fly trapping and evaluations of fruit damage and infestation, always in comparison with an untreated control. Trials for control of *B. invadens* were conducted in Namibia and Kenya using M3 bait stations, bait sprays and Invader-b-Lok. Efficacy was evaluated using BioLure traps which attract females.

**Results:** Although trap catches of *Ceratitis* spp remained high in the first trial, fruit damage and infestation were completely eliminated in M3 treated blocks. In the second trial, fruit fly damage was significantly reduced by M3 bait stations, which proved more effective than baiting. In the third trial, M3s caused a greater reduction in fruit fly numbers than did GF-120. For *B. invadens*, M3s alone and the M3s with Invader-b-Lok both resulted in commercial control and were as effective as weekly bait sprays alone or baiting plus Invader-b-Lok.

**Conclusions:** M3 fruit fly bait stations effectively controlled *Ceratitis* spp fruit flies and assisted in controlling *B. invadens* in combination with MAT blocks.

**Keywords:** *Ceratitis capitata*, *Ceratitis rosa*, *Bactrocera invadens*, attract and kill, M3 fruit fly bait station, methyl eugenol, male annihilation technique, Invader-b-Lok, citrus

A copy of the full programme and abstracts can be obtained from the writer.

## **Valuable discussions held with AAIS delegates:**

### **Dr. Nguya Maniania (ICRPE, Nairobi, Kenya)**

He is the discoverer of the *Metarhizium anisopliae* isolate (ICRPE 69) which is used in the Real IPM product which CRI is currently testing (against thrips and mealybug in field trials) and for which RB is trying to obtain distribution rights. I posed a number of questions to him and obtained the following answers:

1. The fungus should be applied at a concentration of  $10^{13}$  to  $10^{14}$  conidia per ha. It is possible that this should be higher on citrus.
2. The fungus should remain viable for 10-14 days after application in the field. It is possible that on a citrus tree, where there is good protection against UV-irradiation, this could be longer.
3. The fungus is only active between 15 and 30°C. Above 30°C it will die.
4. The oil-based formulation should be better for application on citrus.
5. No density-dependent (pest) trials have been conducted. He could therefore not comment on whether this is a limitation of the product. Incidentally, Sunday Ekesi, who has also worked extensively on the isolate, is of the opinion that it will be density dependent.

### **Miriam Karlsson (Addis Ababa University, Ethiopia)**

She is a Swedish post-doc, working on *Bi* in Ethiopia. Most of her work is in the field, with the main objective being to control the pest. She only recently started in Ethiopia and to date has only conducted interviews with farmers. I provided her with brochures for M3 and Invader-b-Lok and welcomed her to contact me or

RB should she want to acquire any products for trial purposes. I will also put her in contact with Marelize de Villiers as a possible collaborator on the CLIMEX study.

#### **Faisah Salah (University of Gezira, Sudan)**

She is one of Marelize de Villiers' collaborators in Sudan. She gave me some flies to take back to Marelize.

#### **Abdelaziz Gesmallah (University of Gezira, Sudan)**

He stated that *Bi* has been declared a national pest in Sudan. Therefore the government would pay for any control initiatives. He requested that RB supply him with Invader-b-Lok and M3s for three trial sites of about 5 ha each. He indicated that he could organise governmental involvement in such trials, which would draw high-level awareness to the efficacy and value of the products and expedite their registration.

#### **Robert Musundire (Chinhoyi University of Technology, Zimbabwe)**

He is a young and enthusiastic researcher, situated in Chinhoyi. He is willing and able to assist with monitoring for *Bi* in Zimbabwe if supplied with the material to do so. He indicated that he would be able to monitor or organise monitoring of traps in the following areas of Zimbabwe: Masvingo, Matabeleland South, Chiredzi, Chinhoyi and Chegutu.

#### **Jacinter Otieno (Dudutech, Kenya)**

Although she stated in her talk that they have two baculovirus products, subsequent discussion revealed that they do not have any virus products in production. According to Humboldt and Mary from Kenya Biologics, Dudutech previously tried to produce both bollworm NPV and diamond back moth GV, but did not succeed. I also enquired with Jacinter about EPNs. After phoning the Dudutech production department, she reported that they are currently producing *Steinernema feltiae* and a *Heterorhabditis* species. I believe that this is a solid-state production process. Ruth Vaughan, General Manager of Dudutech, has subsequently contacted me directly.

#### **Anges Yadouléon (CREC, Cotonou, Benin)**

He worked with Andy Cherry on the DBM GV several years ago. He reckoned that within about two days there was almost total UV breakdown of the virus. However, he did claim that they had obtained a commercial UV-protectant from the UK, named Coarse (spelling?). He advised me to follow up with Andy Cherry for exact details on the product.

#### **Visit to Nairobi Museum**

Three *Thaumatotibia/Cryptophlebia* species were found in the Nairobi Museum i.e. *T. batrachopa*, *C. peltastica* and another species which I do not know and whose specific name I cannot recall. The specimens were very old and were still labelled as *Argyroplote*, a name which was changed to *Cryptophlebia* many decades ago (and subsequently to *Thaumatotibia*, in the case of *batrachopa*). There were no FCM specimens in the museum. I promised to supply Esther Kioko (of National Museums of Kenya) with FCM specimens from South Africa and Kenya and literature on the reclassification of the species. In exchange, she agreed to supply specimens from their collection for Stellenbosch University's (Pia Addison) on morphological and molecular description of tortricid fruit pests.

#### **Value and summary of visit**

This was an extremely valuable visit. There was some good science presented at the AAIS meeting. However, the main benefit was the meetings before the AAIS Congress and one-on-one meetings with congress delegates. The importation of Cryptogran into Kenya and initiation of trials geared towards registration are imminent. An application for joint Kenyan-South African NRF funding to assist this is being prepared. This will include Kenyan expertise assisting with an FCM entomopathogenic fungus study in South Africa.

It appears that Sudan is ripe for the importation of Invader-b-Lok, Invader-Lure, and possibly M3s into this country. This should be pursued with urgency so as not to miss an opportunity.

Much benefit was also derived for CRI, in the identification of potential collaborators for monitoring of *Bi* in various parts of Africa, including Zimbabwe and Ethiopia, countries not currently being monitored within the CLIMEX study being conducted by Marelize de Villiers. Zimbabwe is also important for survey purposes for evaluation of South Africa's security (Aruna Manrakhan).

#### **Follow-up actions to be taken:**

1. Complete and submit application for NRF funding for ICIPE-CRI collaboration on microbial control of FCM in South Africa and Kenya.
2. Provide Sunday Ekesi with publications and information necessary for Cryptogran importation application.
3. Host Sunday Ekesi and Samira Feris for a) further discussion on collaboration and importation and registration of Cryptogran (into Kenya) and b) to train them on rearing of and field trials with FCM.
4. Rear field collected Kenyan FCM to adulthood; attempt to establish culture; send samples to Pia Adisson for CRI-funded descriptive study and to Nairobi Museum.
5. Send FCM trapping report to Kenya Biologics.
6. Obtain artificial diet recipe from Kenya Biologics or the name of the UK insect rearing specialist who gave them the diet and rearing technique.
7. Identify Korean supplier of pheromones and traps (for FCM).
8. Pursue sending of Invader-b-Lok and M3s to Sudan for field trials. Investigate feasibility of sending Aruna's new cone bait station instead of the M3.
9. Despatch Sudanese collected fruit flies to Marelize.
10. Provide Marelize and Aruna with contacts for monitoring of *Bi* elsewhere in Africa.

#### **Acknowledgements**

River Bioscience is thanked for funding and facilitating the trip. River Bioscience's Directors are thanked for authorising the trip. CRI is thanked for supporting the application.

#### **7.4 P.H. FOURIE**

##### **7.4.1 Attendance of Amercian Phytopathology Society congress, Hawaii, 6-10 August 2011**

Attendance of this congress was funded from Paul Fourie's THRIP supplementary funding at Stellenbosch University. The highly regarded American Phytopathology Society (APS) holds annual meetings, which are generally attended by >1000 delegates. This particular meeting was a joint meeting between APS and the International Association for the Plant Protection Sciences. Paul Fourie presented a talk titled "Modelling of *Guignardia pseudothecium* maturation and ascospore dispersal in citrus orchards". Good feedback was received by other citrus pathologists attending the meeting, including Profs Pete Timmer and Megan Dewdney from University of Florida. Collaboration was agreed with Prof Dewdney with regards to ongoing epidemiology research on Citrus Black Spot. Contact was also made with Alicia Kriss, a very promising PhD student from the research group of Prof Larry Madden, one of the world's leading epidemiologists. Future analysis of South African will investigate modelling approaches developed in Kriss's PhD study.

Apart from epidemiology, I had valuable discussions with Prof Wayne Wilcox (Cornell University; "Fungicide resistance testing and monitoring strategies: Good science and common mistakes") and Dr Helge Sierotzki (Syngenta; "Molecular methods for fungicide resistance detection") who presented in a special session on "Laboratory Methods for Detecting and Characterizing Fungicide Resistance". Another valuable talk in this session was by Prof Madden on "Sampling for detecting fungicide resistance". Valuable insights were gained in the conceptualisation of a current research objective, which aims at developing a real-time PCR assay to detect and quantify fungicide resistance in *Penicillium* population in SA packhouses. Most talks were recorded and submitted online for later viewing. This allowed colleagues and students to view slides and listen to talks of interest. The 294-page book of abstracts, which were later published in the journal *Phytopathology*, was also circulated.

## 8 VOORLIGTING 2011-12

Deur Hennie le Roux, Hannes Bester, Andrew Mbedzi en Melton Mulaudzi (CRI)

### 8.1 TEGNOLOGIEOORDRAGING deur Hennie le Roux en Hannes Bester (CRI)

#### 2011 Seisoen

Die finale volume wat gedurende 2011 uitgevoer was, was 96.5 miljoen kartonne, teenoor die aanvanklike skatting van 94.7 miljoen. Klimaats-omstandighede het veroorsaak dat die oesproses vertraag was. Die markte was vroeg onder druk en pryse laer as verwag, en die rand was sterk teenoor die meeste geldeenhede. Eers teen die einde van die seisoen het die prys in die mark weer kop opgetel en het die rand verswak teenoor die dollar en die euro, wat 'n effens beter situasie vir Valencias geskep het.

Die seisoen is ook gekenmerk deur swak gehalte, veral op Satsumas en nawels. Abnormale temperature en baie reën in sekere gebiede het tot sensitiewe skille en hoë bederf aanleiding gegee. Heelwat letsels, veral fisiologiese skildefekte, het op Satsumas voorgekom, asook op Clementines en Novas tot 'n mindere mate. Die skille was besonder dun en sensitief. Ondersoeke deur CRI het getoon dat die letsels nie deur enige chemiese middels veroorsaak is nie, maar eerder agv klimaatsfaktore in die boord geïnisieer was, wat dan tot uitdrukking gekom het nadat die vrugte ontgroen en gewaks is. Op suurlemoene was bruinvrot 'n groot probleem en klagtes van peteca het ook voorgekom. Groot volumes pomelo's, en self nawels, het in die koelkamers opgehoop agv stadige verkope. Dit het meebring dat vrugte teen baie lae pryse verhandel het en dat produsente in baie gevalle nie eers hul logistieke kostes kon delg nie.

Die seisoen is deur baie produsente en uitvoerders as die moeilikste nog beskou. Die markte was oor die algemeen oorvol en die vrugte het stadig verkoop, gevolglik het verliese agv bederf verder tot die probleem bygedra. Daar is baie inligting bekend om bederf te beheer, en selfs skildefekte te verminder, maar effektiewe toepassing daarvan bly 'n probleem in pakhuisse. Na die aanstelling van 'n na-oes voorligter, behoort hierdie probleme in die toekoms baie meer effektief aangespreek te word. Een van die grootste probleme van ons bedryf is dat volumes nie sinvol bestuur word nie, deurdat te groot volumes in die markte ingestuur word, in plaas daarvan dat die markte die vrugte trek.

#### 2012 Seisoen

Die skatting vir 2012 vir Suider-Afrika wat geoormerk word vir uitvoer, is 102.9 miljoen kartonne. Buiten vir pomelo-volumes wat laer is as verlede seisoen, is die skatting van al die ander variëteite effens hoër teenoor verlede jaar. Die skatting vir pomelo's is 15.2m, sagte sitrus 7.6m, suurlemoene 11.1m, nawels 22.9m en Valencias 46.1 miljoen kartonne.

Die aanduiding so vroeg in die jaar is dat dit weer 'n moeilike seisoen gaan wees in terme van bemerking. Die boodskap is duidelik tydens die afgelope voorseisoen pakhuiswerkswinkels oorgedra dat goeie kwaliteit en raklewe van uitvoervrugte nie onderhandelbaar is nie en dat marginale vrugte onder geen omstandighede vanjaar uitgevoer behoort te word nie. Alle pakmateriaal wat vir uitvoer gebruik word moet ook van hoogstaande gehalte wees en aan die voorgestelde spesifikasies voldoen. Dit is ook tydens die CMF vergadering beklemtoon. Die vruggrootte op pomelo's, nawels en Valencias is een telling kleiner as verlede seisoen, wat ook verdere druk op die markte kan plaas.

Die eerste Satsumas wat in die Wes-Kaap gepak is, toon weer tekens van skildefekte, veral ringbrand. Verder is verskeie klagtes aangehoor dat die vrugte sag voorkom. Verskeie produsente het aangedui dat hulle geen Satsumas verder gaan aanplant nie en oorweeg selfs om dit uit te haal.

#### Voorligting Herstrukturering

Daar was versoeke om weer deeglik te kyk na die huidige voorligtingsmodel binne CRI en te beplan vir alternatiewe behoeftes van produsente in die toekoms. Ten spyte van die feit dat die oorheersende gevoel in die bedryf was dat die huidige model effektief werk, sou alternatiewe modelle in ander sitrusproduserende lande nagevors word om te verseker dat CRI altyd die beste opsies aan die produsente kan bied.

Vraelyste is deur 'n gekontrakteerde konsultant aan 33 persone, waarvan 18 produsente was, gestuur om insette te kry sodat bepaal kon word of die huidige voorligtingsmodel binne CRI nog voldoen aan die behoeftes van die produsente. Die daaropvolgende verslag het getoon dat die oorheersende meerderheid positief op die huidige model gereageer het. Die bevindinge van die verslag was as volg:

- Only 17 of the 30 individuals to whom the questionnaire was sent responded with completed forms. This could be interpreted as satisfaction with the current system, lack of interest and apathy or disenchantment with CRI Extension. The data received was nevertheless sufficient to provide useful insights into the opinion of various sectors of the industry regarding the nature of the service Extension is providing in relation to that desired.
- The greatest challenges and threats facing growers and the industry at large include sanitary and phytosanitary issues, bio-security, the opening of new markets and matters relating to improved production efficiency.
- Those extension functions that are considered important and are being done well include the Cutting Edge, Bio-security support, Extension Briefs, Participation in programme committee meetings, Support for foreign visitors for market access and the Research Symposium.
- Work must be done to improve the level of service relating to the following important activities/services: Coordinating study groups, determining research priorities, cultivar interaction, engaging with the exporter technical panel, operating the CCCF, pack house extension, supporting the CIS and keeping the production guidelines updated.
- There is a high level of consensus that Extension is performing well and requires additional funding to improve its performance even further.
- However, it is important that grower expectations of the extension service be aligned with the ability of Extension to meet those expectations. This will require improving communication with growers and grower groupings.
- There is no need to change to a different extension model but the existing model should be adapted so that Extension can better meet the needs of the different constituencies identified in the assessment.

Voortspruitend uit die bevindinge van bogenoemde ondersoek het CRI Bestuur tydens 'n beplanningssessie in Januarie besluit dat daar gekyk moet word na die sukses wat bereik is met die Pakhuiswerkswinkels en dat daar drie Streekswerkswinkels gehou moet word in vyf areas nl. Limpopo, Mpumalanga, KwaZulu-Natal & Swaziland, die Oos Kaap en die Wes Kaap. Werkswinkel 1 sal handel oor die Lenteploegkompleks, Siektebestuur en Simposium terugvoer (Sept – Oktober). Werkswinkel 2 sal handel oor Na-oes Tegnieese Werkswinkel (Februarie).Werkswinkel 3 sal handel oor Besproeiing, Bemesting, Vrugssetstrategie & Kultivars.

Die take waarby CRI Voorligting dus betrokke sal wees in die toekoms is die volgende:

ACTION	REMARKS
<p><b>1. CRI Technology Transfer Groups</b> - also sometimes referred to as Study Groups (Extension responsible for convening to set Research Priorities)</p>	<p>27 Growing areas in South Africa                  2 growing areas in Zimbabwe                  1 growing area in Swaziland                  These groups will still have a chairman and a technical committee but their role will primarily be to determine the research needs (research priorities) for their area. If any of these groupings have a need to include a specific technical issue of only local relevance, they can call upon CRI Extension to coordinate this. CRI Extension will convene a round of TTG meetings in July/August to determine the research needs for the areas. These visits will also provide an opportunity for direct engagement between the EOs and individual growers in each area.                  In accordance with existing CRI procedures, the grower Research Priorities obtained from the TTGs are combined with Research Priorities emanating from the CRI Cold Chain Forum (specifically using the Exporters Technical Panel, Packaging Working Group and Packhouse Working Groups within the CRI-CCF</p>

	<p>to identify such needs) and supplied to the CRI Manager: Research and Technical. CRI uses these priorities as the basis for annually compiling and funding research projects to be conducted within the CRI Group (comprising CRI's in-house capacity, the Universities, the ARC, private research companies and any other suitable research partner locally and internationally). Extension is overall responsible for establishing the Research Priorities, which is of fundamental importance in ensuring that the industry's research and technical support capacity remains directed at that which is needed to ensure the long term competitiveness of the Southern African citrus grower and ensure that growers remain assured that their funds are well spent.</p>
<p><b>2. CRI Regional Extension Working Groups</b> (Extension responsible for convening these working groups three times per year)</p>	<p>Five CRI Regional Extension Working Groups will be constituted along the lines of (and incorporating) the existing 5 Regional Packhouse Working Groups:</p> <ul style="list-style-type: none"> <li>- CRI Limpopo Extension Working Group (including Zimbabwe)</li> <li>- Mpumalanga Extension Working Group (including Ngonini in Swaziland, North West and Gauteng)</li> <li>- KZN Extension Working Group (including the Swaziland Lowveld)</li> <li>- Eastern Cape Extension Working Group</li> <li>- Western &amp; Northern Cape Extension working Group (including the Free State)</li> </ul> <p>Two-day meetings will be held as is currently the case with the CRI Regional Packhouse Working Group meetings, with the meetings generally starting at lunch time on the first day and finishing at lunch time on the second day.</p> <p>1<sup>st</sup> round (September): Spring Pest complex meeting including soilborne diseases and fruit and foliar diseases. Every second year feedback from the CRI Research Symposium will be included.</p> <p>2<sup>nd</sup> round (February): CRI Regional Packhouse Working Group meetings (part of the CRI Cold Chain Forum).</p> <p>3<sup>rd</sup> round (June): Fertilisation, irrigation and fruit set strategies.</p> <p>Other topics will be included and addressed on request. Extension is responsible for ensuring that the results emanating from research are made available for implementation in the industry and this structure will in future be a valuable mechanism of achieving this.</p>
<p><b>3. CRI Cold Chain Forum - CRI-CCF</b> (Extension responsible for convening)</p>	<p>The CRI-CCF consists of four pillars: The Packaging Working Group, the Cold Chain Research Project, the Packhouse and Handling Panel, and the Exporters Technical Panel. There are two sub-committees, the Cooling Working Group and the Wax Committee, which slot into the Cold Chain Research Project. The five Regional Packhouse Working groups are convened as part of the Packhouse and Handling.</p> <p>The different groupings meet at least once a year. The five CRI Regional Packhouse Study Groups each have a pre-season meeting to keep the Packhouses informed of the latest developments before each packing season. This will now become one of the three rounds of annual CRI Regional Extension Working Group meetings. There are regular meetings with the Exporters Technical Panel during the season to stay informed on technical problems relating to exports. There is at least an annual update of the carton and pallet specifications/standards. The Cold Chain Research Project in turn integrates with CRI's other Research structures and the rest of the CRI Cold Chain Forum. CRI Extension provides the services of a packhouse extension specialist in support of the various components of the Forum. This Extension Officer visits citrus packhouses during the packing season if requested to assist them in implementing the procedures as recommended during the Packhouse study group meetings. Problems from the markets are also to be followed up. Packhouse extension is an integral part of extension to ensure that the fruit is treated properly from picking until it leaves the packhouse in order to prevent decay and to extend the shelf life. To achieve this, a close relationship is needed between the packhouses, the Exporters Technical Panel and Post Harvest Extension.</p>

	The Forum provides an over-arching structure for interfacing the four pillars and their sub-groupings with one another and other components of the industry (e.g. the Citrus Marketing Forum and the newly formed Logistics Forum).
<b>4. CRI Citrus Research Symposium</b> (Extension responsible for organising)	Every second year researchers from the CRI Group have the opportunity to give feedback on the research conducted during the previous two years. This includes researchers from CRI, the Universities, the ARC and private research organizations and any other relevant research service provider. The symposium provides a platform for researchers to present their results to the industry's primary layer of technology transfer. This constitutes a diverse grouping of organisations and individuals and includes producers, the SASCCON consultants, technical personal from the larger citrus estates and cooperatives, the agro-chemical and biocontrol industries, the citrus nursery industry, the ARC, the PPECB, government institutions such as DAFF and the Provincial Departments of Agriculture as well as private entrepreneurs involved in the citrus industry. The plan was originally to move the venues around to different citrus producing provinces. This was done for the first four symposia (from 2000 to 2006). However, the venue in KZN proved to be so popular that it was held there for the two previous symposia and will again be in 2012, but the future venues are open for change at the request of delegates. These symposia are attended by 400-500 delegates making this the largest Agricultural Research Symposium in Africa and has become a flagship for the technology transfer effort. Extension is responsible for staging this prestigious event.
<b>5. CRI Extension Briefs</b> (Extension is responsible for coordinating inputs and publication)	The SA Fruit Journal appears five times per year and goes to every citrus grower registered with the CGA. Each publication includes a CRI Extension Briefs section that informs producers of the latest updates with regard to citrus production for the next two months. Growers are kept updated on horticultural practices and are reminded of which pests and diseases can be expected during the coming months. They are updated about new registrations as well as registrations that have been withdrawn. Extension is responsible for coordinating researchers' inputs into and the placing of this section in each edition of the Journal.
<b>6. SA Fruit Journal Articles</b> (Extension contributes own publications & identifies topics for researcher articles)	Semi-scientific publications in the SAFJ help to keep producers informed of the latest research results and provide feedback from various industry technical meetings. Most producers do not have access to scientific publications and do not read the CRI annual reports. The SAFJ plays an important role in enabling growers to be aware of research developments and to know what is happening in the citrus industry in general.
<b>7. SASCCON interaction</b> (Extension facilitates)	Extension keeps close links with SASCCON to ensure that the same message goes to the growers. In 2007, when growers first agreed that Extension capacity should increase from one to three regional coordinators, only the second Extensionist was appointed. Instead of the third position individual SASCCON members were contracted on a part time basis to assist Extension in coordinating the programmes of the Technology Transfer Groups. The functioning of these SASCCON members as CRI appointees may not always have been adequately recognised by growers and the role played by CRI Extension thereby failing to be fully recognised. Provided the new levy adequately provides for the appointment of the outstanding third regional coordinator, SASCCON members will no longer be required to coordinate TTGs for CRI and the role of the TTGs will change. It will however remain important for Extension to retain ongoing close interaction with SASCCON to ensure that the same message is conveyed to growers.
<b>9. CRI Production Guidelines</b> (Extension responsible to ensure researchers keep guidelines updated)	The CRI Production Guidelines consist of four volumes that, with the exception of a few chapters, are frequently updated. The CRI Production Guidelines are the most comprehensive publication on citrus production that is available for Southern African citrus producers. The volumes are available in electronic format.

<b>10. CRI Cutting Edge</b> (Extension contributes)	The CRI Cutting Edge is an electronic means to get urgent information to producers. This enables quick grower reaction. The CRI Cutting Edge is currently the most successful way to reach the growers. Growers study the Cutting Edges as they are received and hold them in high regard.
<b>11. CRInet</b> (Extension contributes)	The CRnet is aimed at fulfilling a need which was created as a result of the absence of one on one extension. Growers can post their queries with pictures. Both researchers and other producers will then respond. Access is limited to Southern African residents.
<b>12. Orchard visits &amp; telephone calls</b> (Extension responds)	In the regulated era, a division of 50 full time extensionists was deployed to provide one on one extension to the industry that was then half its current size. Given the size of Extension today (two regional coordinators) it is simply not possible to contemplate any form of one on one extension. This remains one of the areas where grower expectations far exceed what is reasonable to expect Extension can deliver. Consequently Extension continues to receive daily requests for on-site visits and specific advice. Nonetheless having to deal with such enquiries is essential and producers are generally referred to parties that can assist in solving their problems. If this is not successful Extension will pay the grower a visit when next in the area, especially if the problem could hold a biosecurity risk.
<b>13. Research Programme Committee meetings</b> (Extension participates to ensure retention of research focus on industry needs)	Extension is part of each of these meetings to ensure that the grower needs are correctly understood and addressed. The Research needs of the industry cannot all be addressed because of a lack of personnel and funding. Extension is involved to ensure that the needs are appropriately prioritised and placed into perspective. Maintenance of a research focus on industry interests has historically been one of the greatest achievements of the Southern African citrus industry and Extension has a critically important role to play in ensuring that the relevance of research is maintained.
<b>14. Exporters Technical Panel interaction</b> (Extension participates & oversees priority identification)	Extension meets with the ETP during the season to monitor how the season is progressing and to deal with problems as soon as they occur. This role will in future be the responsibility of the Post Harvest Extension officer. The ETP is used by Extension to obtain exporter inputs into the research priorities. The ability to retain a research focus on market needs has historically been another key factor in the industry's sustained global competitiveness. Extension has a critically important role to ensure that this is maintained.
<b>15. Market Access &amp; Biosecurity</b> (Extension provides support)	Extension is involved with surveys such as those needed to get certain areas declared CBS free to gain market access into the USA. It is also involved in assisting DAFF with overseas delegates visiting South African citrus production areas to familiarize them with the local pest and disease complexes as a prelude to opening their markets to SA exports. Extension is involved in assisting the CIS and CRI management to ensure that biosecurity is taken care of. This involves keeping producers within Southern Africa informed of the exotic threats with regard to pests and diseases and includes interactions with relevant parties in neighbouring countries to ensure the biosecurity of the region (eg. stop the imports of trees from South America into the region).
<b>16. Citrus Marketing Forum interaction</b> (Extension participates)	Extension attends these meetings and gives inputs when needed. These meetings take place during the season. Extension is one of the means to carry the message from this forum to the growers and packhouses.
<b>17. Cultivar interaction</b> (Extension facilitates workshops)	The EOs have a worldwide network of contacts and as a result they have been instrumental in acquiring new cultivars and rootstocks for the Southern African citrus industry. Introducing new cultivars into the region is of the utmost importance. Extension has a role to play in facilitating the identification and introduction of new cultivars into the country, especially those with certain disease or pest resistance properties, something that ordinary cultivar management companies may overlook.
<b>18. CRI Transformation</b>	Because of the language differences the emerging citrus growers requested that separate study groups should be formed to accommodate them. Such groups

<b>TTGs</b> (Extension responsible for operating in accordance with CGA directives)	have been formed in the Limpopo province, the Eastern Cape and KwaZulu-Natal. There is still a need for the Transformation TTGs. They will therefore be kept and will be serviced by the Extension Coordinators until such time that the provincial departments of agriculture can take over. During the Transformation study group meetings, translations take place into at least one local language. The meetings are also of a practical nature and time is often spent in the orchards. Fewer topics are accommodated at a time in order for meetings not to become too drawn out.
<b>19. Transformation workshops</b> (Extension responsible) <b>CRI</b>	Two workshops are held per year in both the Limpopo and the Eastern Cape provinces. Topics that address the emerging growers specifically are addressed. Each of these workshops is attended by 80-120 emerging growers and could be compared with a mini symposium. The Extension coordinators will continue to assist with these workshops until the EOs of the Provincial Departments of Agriculture are in a position to take over.
<b>20. Transformation projects</b> (Extension provides technical support)	The Extension Coordinators provide support to these projects as the Extension Officers within the Provincial Departments of Agriculture often do not have the knowledge, the resources and in certain cases the willingness to assist the emerging farmers to become commercial growers.
<b>21. National &amp; International Societies</b> (Extension interacts)	Due to the expertise within Extension, the EOs are members of several international societies such as nematology, virology and plant pathology. An industry cannot just feed on the knowledge of other industries without participating in making a success of the societies involved. Extension maintains involvement with these societies and thereby remains up to date with relevant international technical developments.

#### CRI-Kouekettingforum (CRI-CCF)

Dawid Groenewald is begin November as koördineerder op 'n deelydse kontrakbasis binne Voorligting aangestel om die werksaamhede van beide die CRI-CCF en die Verpakkingswerkgroep te koördineer. Daar was die afgelope seisoen beduidend minder insidente tov verliese agv substandaard verpakkings-materiaal as in die voorafgaande seisoene.

Die akkreditasieproses vir kartonvervaardigers het gedurende 2011 begin, maar moes nog verfyn word tot die punt waar dit aan regstegniese vereistes kon voldoen, dws die stelsel moet nie leemtes laat wat dit in 'n hof aanvegbaar kan maak nie. Nie alle kartonvervaardigers het hul volle samewerking gegee nie, maar daar is hard gepoog om 'n regverdige, betroubare en maklike prakties uitvoerbare stelsel te implementeer. Hierdie proses het deur die loop van die seisoen momentum aangeneem en word as 'n groot stap in die regte rigting beskou.

Hoewel die voorseisoen pakhuiswerkswinkels weer gedurende Februarie en die eerste week van Maart in die vyf grootste produksiestreke gehou is, het dit duidelik geword dat die eenmalige beskikbaarstelling van hierdie inligting nie voldoende is om te verseker dat pakhuisse effektief funksioneer nie. Daar was 'n groot behoefte aan pakhuisbesoeke en een-tot-een voorligting aan individuele pakhuisse. Die aanstelling van Keith Lesar om as Na-oes Voorligter vanaf die begin van Junie 2011 binne CRI op te tree, was 'n groot stap vorentoe. Keith het in die onderskeie streke meer as 50 pakhuisse besoek om pakhuisse te evalueer en te adviseer rakende probleme in die pakhuisomgewing.

Tydens hierdie besoeke was die terugvoering van die produsente/pakhuisbestuur baie positief agv die feit dat CRI weer belangrike inligting/aanbevelings aan die pakhuisse op 'n een-op-een basis kon bespreek. Die bedryf was die afgelope seisoen weereens met baie skilprobleme en bederf toegeval agv wisselvalige omgewingstoestande, veral uitermatige reënval en temperatuur, asook vertraging in pluk van vrugte en die pluk van vrugte buite die plukvenster. Baie tyd is aan voorligting (telefonies en e-pos) bestee om hierdie aangeleenthede uit te sorteer. 'n "Checklist" (oorsiglys) is vir pakhuisse ontwerp, om elke pakhuis se rekordhouding en bestuur op datum te kry, asook om audits op die pakhuisse te doen. Die eerste proef-uitgawe van die "Postharvest Diseases – Illustrated" is voltooi en vir kommentaar uitgestuur.

Die begin van die 2012 seisoen en die pluk en verpakking van Satsumas het weer, soos tydens die 2011 seisoen, op die verkeerde voet afgeskop. Fitotoksiteit (brand) op die Satsumaskille, eers vanaf die Burgersfort gebied en daarna vanaf verskeie gebiede in die Wes-Kaap, is ontvang. In die meerderheid van die gevalle is brand op die Satsumas na “drench” alleen en/of saam met ontgroening van die vrugte waargeneem. Hoewel die verhoogde konsentrasie van guazatine moontlik aanleiding tot brand kon gee, is dit bewys dat die toediening van beide 500 en 1000dpm guazatine in die “drench” mengsel die vrugte, onder sekere omstandighede, kon brand. In die meeste gevalle waar brand op die Satsumas waargeneem is, is ongeregisteerde benatters en skuim teenmiddels gebruik, asook gevalle waar Philabuster saam in hierdie mengsels gebruik is. Een of meer middels, of kombinasie van middels, kon dus die reeds sensitiewe Satsumaskille gebrand het. Sekere produsente het hulle eie inisiatief gebruik en met behulp van boordproewe bewys dat die pluk van Satsumas gedurende die warmste ure van die dag (~ 10h00 – 14h00) tydens “drench” en/of ontgroening gebrand is, terwyl Satsumas wat gedurende die koeler ure van die dag gepluk is, nie brandsimptome getoon het nie. Dit wil dus voorkom of die probleem weer dieselfde tendens as in 2011 getoon het. As gevolg van uitermatige warm dae tydens pluk is die skille gepredisponer om meer gevoelig te wees vir fitotoksiteit na behandeling in die “drench” mengsels, alleen en/of saam met ontgroening.

’n Na-oes post-mortem is na afloop van die pakseisoen gehou waartydens ’n komitee gestig is om met die beplanning en tegniese insette vir die voorseisoen Pakhuiswerkswinkels vir 2012 te help. Bywoning van die voorseisoen Pakhuis-werkswinkels wat gedurende Februarie 2012 gehou is, was die hoogste sedert die stigting van die CRI-CCF aan die begin van 2007. Dit is deur ongeveer 650 persone bygewoon, wat dit ’n gesogte gebeurtenis vir borge maak agv die wye blootstelling wat hulle binne ’n kort tydjie kry. Tydens hierdie werkswinkels is groot klem op bederfbeheer en raklewe gelê en verskeie lesings is hieroor gedoen. Die volgende onderwerpe is gedurende elk van die twee-dag werkswinkels gedek:

- Terugvoer oor 2011 Seisoen:
  - Afkeurings en gehalte
  - ETP Markterugvoer
- Uitvoerregulasies:
  - Fitosanitiere regulasies (CBS en FCM)
  - Uitvoerstandaarde – 2012
- Terugvoer van pakhuisbesoeke van 2011
- Oorsig oor na-oes siektes en voor-oes beheer
- Na-oes hantering en pakhuissanitasie
- Fisiologiese skildefekte
- Swamdoderaanwending en residu-lading
- Swamdoder-opsies en weerstandsbestuur
- Residu-ontledings
- Praktiese wenke vir effektiewe waks-aanwending
- Effektiewe ontgroeningsriglyne
- Bestuur van kritiese beheerpunte
- Voedselveiligheid: Chemies en mikrobiologies
- CRI-Kouekettingforum:
  - Spesifikasies, protokolle en akkreditasie van kartonvervaardigers
  - Korrekte laai en vasmaak van palette op vragmotors en in houers
- Bemarkingsuitdagings vir die toekoms
- Logistiek: Uitdagings vir 2012
- Kartongewig: Wat is die oplossing?

Die beskikbaarheid van papier van die regte gehalte vir die vervaardiging van kartonne vir die 2012 oes is ’n bron tot baie groot kommer. Dawid Groenewald is deurlopend met die papiervervaardigers in gesprek om die probleem aan te spreek. Hoë-vlak samesprekings het alreeds op 7 Maart en 3 April 2012 plaasgevind en verdere opvolggesprekke is beplan. Verdere navorsing, in samewerking met een van die kartonvervaardigers, is ook aan die gang gesit om oplossings te probeer vind. Baie tyd word ook spandeer aan navrae van produsente oor pakmateriaal en verskaffers. ’n Ander bron van kommer is die beskikbaarheid van goeie kwaliteit palette en daar word ook baie tyd en aandag hieraan spandeer. Die gebruik van hoë kwaliteit pakmateriaal wat in alle

opsigte aan die voorgestelde spesifikasies voldoen is van kardinale belang. Die gebruik van minderwaardige kwaliteit pakmateriaal lei heel dikwels tot groot finansiële verliese en as gevolg daarvan verloor oorsese kopers hulle vertroue in sitrus afkomstig uit Suidelike Afrika.

### Studiegroepe

Die verskillende vakrigtings aan die produksiekant is die vorige paar jaar so goed gedek dat daar baie min versoeke vir studiegroepvergaderings vir 2011 gekom het. Die grootste behoefte was meer vir snoei, bemesting, besproeiing en dan die lentepaagkompleks in September. Die lentepaagkompleks is by al die studiegroepe in detail aangebied en produsente het die nuwe strategie vir die beheer van blaaspootjie en FCM baie goed ervaar, en indien dit nougeset toegepas word, hou dit groot belofte in. Daar is ook enkele gevalle van *Armilaria* wortel- en kraagvrot in die Oos- en Wes Kaap ondersoek. Hoewel hierdie swam nie in die groter geheel 'n noemenswaardige probleem is nie, is daar enkele boorde in die land waar dit vernietigend is.

'n Skrywe is aan die onderskeie Sitrusstudiegroepe se lede gestuur waarin al die CRI navorsingsprojekte vir 2011 aangetoon is. Produsente is versoek om hierdie inligting te bestudeer en dan te besin oor watter addisionele navorsingsbehoefte daar in die onderskeie sitrusproduserende gebiede voorkom. Hierdie behoeftes is aan die Tegniese komitee/Voorsitters van die studiegroepe oorgedra. Die Area voorligtingsbestuurders het in Junie met die verskillende Tegniese komitees geskakel en hierdie navorsingsbehoefte is saamgevat.

Dr Graham Barry het by verskeie van die studiegroepe werkwinkels gehou om te bepaal watter kultivars in die onderskeie gebiede kommersieel is, watter eksperimenteel is en wat die ryfwordingstye in die verskillende streke is.

River Bioscience het ook van die geleentheid gebruik gemaak om tydens die studiegroepvergaderings oor die lentepaagkompleks weereens al sy produkte onder die aandag van die produsente te bring. Terugvoer oor die effektiwiteit van Helicovir was deurgaans baie positief. Produsente is sterk aangemoedig om valletjies vir *Bactrocera invadens* op elke PUC uit te hang en deeglik te monitor.

Voorligting is betrokke by twee studiegroepe in Zimbabwe. Die Beitbrug studiegroep is in die suide en voer sowat 3,5 miljoen kartonne uit. Die Harare studiegroep verteenwoordig areas soos Chegutu, Mazoe en Mvurwi. Indien die blanke plase nie onteien was nie sou hierdie gebied waarskynlik sowat 4 miljoen kartonne uitgevoer het. Daar word tans waarskynlik nie veel meer as 100 000 kartonne uit die noordelike areas uitgevoer nie.

### **8.2 Studiegroepvoorsitters vir 2011-12**

<b>TTG/Studiegroep</b>	<b>Name/Naam</b>	<b>Tel. no/nr.</b>	<b>Email/Epos</b>
Baviaans	Phillip Dempsey	082 498 2778	phillipdempsey@southernfruit.co.za
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Benede-Oranjerivier (Kakamas)	Jacques de Wet Francois Reyneke	082 495 0632 082 771 6758	augpad@lantic.net francois@karsten.co.za
Breederivier	Sakkie Bruwer	083 226 2540	subtrop@netactive.co.za
Burgersfort	Shaun Ottey	082 448 4066	zalositrus@intekom.co.za
Citrusdal	Rynhardt Nel	083 647 3372	rynhardt@ghcitrus.com
Groblersdal/M. Hall	Pieter Engelbrecht	082 524 8925	pieter@dpet.co.za
Hoedspruit	Hannes Meintjies	082 460 5220	hannes@eden-fruit.com
Katrivier	Kierryn Keeton	083 656 8550	technical@katco.co.za

Knysna	John Stanwix	082 789 5051	knycit@mweb.co.za
Komatipoort	Dirk Horn	013-7937536 083 259 3359	sommerreg@soft.co.za
Letsitele	Eddie Vorster	083 629 4949	evmv@mweb.co.za
Malelane	Leon Esselen	013-790 0160	esselenk@mweb.co.za
Midnight Study Group	Evert Genis	083 230 4722	ejgenis@lantic.net
Nelspruit	Willem Kieviet	082 490 2991	wkieviet@vodamail.co.za
Nkwaleni	Mike Wafer	083 278 6150	michaelwafer@yahoo.com
Ohrigstad (Kaspersnek)	Ignus Bruyns	082 326 0537	ignus@kaspersnek.co.za
Paarl/Stellenbosch/ Swartland	Stephan Venter	083 670 8030	Stephan@insectscience.co.za
Patensie	Gerhard van Vuuren	071 684 8102	gerhardj@patensiecitrus.co.za
Pongola	André Barnard	083 229 8539	mhlali@idhweb.com
Rustenburg	Johan-Chris Grobler	082 922 1579	witkrans1@mweb.co.za
Southern Natal	Peter Button	082 488 8537	pbuttonuturenet.co.za
Sundays River	Dave Gerber	079 495 3162	technical@srcc.co.za
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Tshipise	Barend Vorster	082 651 2642	xmasbdy@lantic.net
Vaalharts (Hartswater)	Michael van Niekerk Danie Mathewson	082 948 2551 082 550 0293	orange@lantic.net saamfarm@lantic.net
Waterberg	Peter Pullinger	082 322 0964 014-7432850	prp@netactive.co.za
Weipe	Danie Erasmus	083 236 7798	depoweipe@lantic.net
Zimbabwe	John Perrott	09263 91223841 0726111478	johnperrott@zol.co.zw

### 8.3 Transformasie

#### LemonEx

Die CGA het vroeg in 2011 gepoog om die CRT direkteure in orde te kry sodat die JV tussen LemonEx en die NEF gevorm kon word. Hierdie proses was stadig en uiters frustrerend en het die aanvang van die BFS (Bankable Feasibility Study) vertraag. Tot tyd en wyl die JV nie gevorm was nie, kon die proses nie vorder nie. Tydens die samekoms van die Suurlemoen Fokus groep is die LemonEx projek weereens aan suurlemoenprodusente verduidelik. Die projek sou nie met varsrugprodusente kompeteer nie, nie wat betref uitvoere of binnelandse suurlemoen verkope nie. Al die vrugte sou gaan vir verwerking. PD Naidoo & Ass. was saam met Waterwese besig om elk van die gronde se waterregte te verifieer. Tydens 'n vergadering met die MEC vir Landbou is die boodskap ook duidelik aan die verskillende gemeenskappe (Trusts) oorgedra dat hulle hul huis in orde moes kry sodat LemonEx seker kon wees dat hulle met die regte entiteite onderhandel wat betref die eiendomsreg van die boorde waar die bome gevestig sou word.

Die LemonEx projek het egter 'n ernstige terugslag beleef deurdat sekere kommersiële suurlemoenprodusente beswaar aangeteken het teen die CGA se betrokkenheid by die projek. Daar is dus op 'n CGA direksievergadering besluit dat die CGA hulle van die projek sal onttrek. Dit is uiters jammer aangesien hierdie projek baie vir die CGA se beeld kon beteken wat Transformasie betref. 'n Tweede feit wat ter sprake is, is die feit dat die CGA die projek so sou kon beïnvloed dat die projek uitsluitlik vir verwerkings-vrugte sou produseer en dat dit nie sou meeding met die bestaande uitvoerprodusente of op die binnelandse mark nie. Met die onttrekking van die CGA aan die projek is daar geen sekerheid dat die projek nie varsvrugte as deel van hulle bemerkingsplan sal insluit nie. Hierdie sal dus die laaste terugvoer oor die projek wees.

#### Limpopo Bloedlemoenprojek

Tydens 'n besoek van die Limpopo MEC vir Landbou aan Italië was daar 'n versoek van die Italianers se kant dat die Limpopo provinsie bloedlemoene moes begin produseer vir uitvoer na Italië. Die Italianers was bereid om dit te befonds. Tydens 'n besoek deur Andrew Mbedzi, Johan Joubert en Hennie le Roux is die Limpopo Departement van Landbou ingelig dat die sitrusproduserende gebiede in die Limpopo provinsie nie geskik is vir die ontwikkeling van die bloedlemoene se kleur nie omdat die koue eenhede te laag is. Daar is egter gebiede soos Vaalharts of die Katriviervallei wat wel geskik mag wees. Voorligting was egter nie bereid om verder hierby betrokke te raak nie aangesien dit in 'n soortgelyke debakel as die LemonEx projek kon ontaard.

#### Thabazimbi sitrusprojek

Texas A&M sou graag betrokke wou raak by 'n sitrusprojek in Suid Afrika om agtergeblewe boere te help ontwikkel. Voorligting sien ook nie kans om hierby betrokke te raak alvorens die CGA direksie nie baie duidelik uitgespreek het waarby Voorligting betrokke mag wees wat betref Transformasie en waarby nie.

Andrew Mbedzi en Melton Malaudzi doen goeie werk onder die opkomende sitrus produsente onder moeilike omstandighede. Die wêreldwye tendens is dat kleiner produsente nie finansieel kan oorleef nie en uitgekoop word deur die groter rolspelers. In Suid Afrika poog ons om van hierdie klein opkomende sitrusprodusente te ondersteun ten spyte van die feit dat ons weet dit is nie lewensvatbaar nie. Die CGA en CRI sal moet besin oor 'n strategie van wat ons wil bereik.

In Badplaas het die onderskeie rolspelers besluit om voort te gaan om die lewensvatbaarheid van 'n suurlemoen verwerkingsprojek te ondersoek ongeag die feit dat die CGA en die NEF hulle daaraan onttrek het. Die IDC is besig met 'n voorlopige ondersoek.

Die transformasieproses in die Oos-Kaap sukkel om glad te verloop. Selfs in gevalle waar uitvoer-agente tevore betrokke was, deur geld voor te skiet en dan 'n sessie op die uitvoeroes te vat, sukkel die BEE-produsente, aangesien die agente nie meer kans sien om verder geld voor te skiet nie. Die huidige inisiatief van die regering om sitrusplase te herkapitaliseer het weereens misluk aangesien geen geld die betrokke ondernemings betyds bereik het om te verseker dat 'n uitvoer-oes vir die 2012 seisoen geproduseer kon word nie. In die meeste provinsies is die Departement van Landbou se Voorligtingsaksies 'n volslae mislukking agv 'n gebrek aan befondsing.

Grond word aan die BEE-produsente oorgedra en daarna word hulle aan hul eie lot oorgelaat totdat die bestaande produksie-eenheid ten gronde gegaan het, voordat daar weer aandag aan hulle gegee word. In meeste gevalle is die toestand van die plaas so swak dat dit geensins meer as 'n ekonomiese eenheid gereken kan word nie en die kostes om dit weer op te bou is wesentlik hoër as die koste om bloot die plaas van die begin af net deurlopend te onderhou het.

Verder word daar vergadering op vergadering met die betrokke rolspelers gehou, (van die BEE-produsente regdeur tot die regerings-amptenare wat betrokke is), om oplossings te vind, maar elke keer stap almal daar uit en verder gebeur daar niks nie. Niemand aanvaar enige verantwoordelikheid nie en niemand word tot verantwoording geroep nie.

In die praktyk beteken dit dat geen fitosanitêre plaas beheer word nie, wat 'n groot bedreiging vir die aangrensende kommersiële produsente word. Die BEE-produsente, wat veronderstel is om in 'n beter finansiële posisie te kom, verarm net verder, arbeiders verloor hul werk en die gemeenskap verarm mettertyd agv groter

werkloosheid. Die regering sal 'n model moet vind waar die regte kandidate, met die nodige kundigheid, geïdentifiseer moet word om die grond oor te neem, en waar die nodige fondse eers beskikbaar gehou word volgens 'n sinvolle begroting, voordat oornam van die grond kan plaasvind.

CRI is gevra om aanplantingsaanbevelings vir verskeie nuwe sitrusinisiatiewe te doen. Dit sluit in potensiele sitrusaanplantings in die Baberton en Swart Umfolozi gebiede. In Badplaas het die IDC besluit om voort te gaan met 'n lewensvatbaarheidstudie vir 'n suurlemoenprojek vir verwerking.

In die toekoms sal CRI Voorligting hulle beperk tot die verskaffing van tegniese inligting aan die Transformasieproses en nie direk poog om van Transformasie 'n sukses te maak deur by projekte betrokke te raak nie. Hierdie sal die verantwoordelikheid van die CGA bly.

#### Transformation Study Groups

The conducting of the study group sessions went well in Limpopo, Eastern Cape and Kwazulu Natal provinces. The following table indicates the dates, venues, number of attendances, presenters and the topics that were presented in the different study group sessions.

Date	Study Group	Venue	No of People	Presenter(s)	Topics
06/04/2011	Mopani Study Group	Mabunda Farm Giyani	41	A. Mbedzi	Importance of farm accreditation programmes (i.e. Global GAP, Natures Choice, Fairtrade, etc.)
				A. Mbedzi	Control of Fruit Fly
				A. Mbedzi	Control of False Codling Moth
06/04/2011	SRV Study Group	Luthando Trust	17	W. Kirkman	FF, FCM and White Woolly Fly control
07/04/2011	Patensie Study Group	Patensie ANC office	10	W. Kirkman	FF, FCM and White Woolly Fly control
				M. Mulaudzi	Soil and leaf analysis
08/04/2011	Vhembe Study Group	Tshirema Farm Mutele A	53	A. Mbedzi	The importance of soil and leaf analysis in planning the citrus fertilizer programme.
				A. Mbedzi	Control of Fruit Fly
				A. Mbedzi	Control of False Codling Moth
14/04/2011	KRV Study Group	Riverside T. Centre	25	W. Kirkman	FF, and White Woolly Fly control
				DR. Sean. Moore	FCM control
24/06/2011	Vhembe Study Group	Maswiri Farm Tshipise	36	Peter Koen	Packhouse Exposure Tour and Field Tour
				Peter Koen	Fruit Quality
28/06/2011	Patensie Study Group	Dankbaar CPA	20	Casper	Irrigation, fertilization and pruning of citrus
27/09/2011	SRV Study Group	Willow Tree Farm	16	A. Combrick J. Potgieter	Calibration of Spraying machine
30/06/2011	Waterberg Study Group	Ongerep Farm Lephale	13	A. Mbedzi	Harvesting of the citrus fruit
				A. Mbedzi	The importance of applying fertilizer in time
02/09/2011	Vhembe Study Group	Makonde Farm Thohoyandou	42	S. Aphane	Scouting of Citrus pests
				A. Mbedzi	Citrus Spring Pests Complex
08/09/2011	Mopani Study Group	Mariveni Farm Tzaneen	-	-	Postponed (Farmers were finishing up harvesting)
06/10/2011	Waterberg Study Group	Masas Farm Naboomspruit	23	A. Mbedzi	Citrus Rootstocks And Cultivars
				A. Mbedzi	Citrus Production Pests
13/10/2011	Patensie	Tobacco	10	Martina	FCM control

	Study Group	Office		Onderndaal	
				M. Mulaudzi	CBS prevention
20/10/2011	Mopani Study Group	Giyani Training Hall	32	A. Mbedzi	Control of Production pests
25/10/2011	KRV Study Group	Mpofu T. Centre	18	LLew Roberts	FCM control
				M. Mulaudzi	CBS prevention
04/11/2011	Vhembe Study Group	Bennde Mutale B	46	A. Mbedzi	Control of Production pests
				A. Mbedzi	Important of irrigation in citrus production
06/12/2011	Waterberg Study Group	Malesa Farm Warmbath	11	A. Mbedzi	Citrus Rootstocks And Cultivars
				A. Mbedzi	Citrus Production Pests
06/12/2011	Nkwaleni V. Study Group	Nkwaleni Hall	16	M. Mulaudzi	Visiting processing plant with commercial farmers
27/01/2012	Vhembe Study Group	Easy Farm Thohoyandou	26	V. Mtileni	Safe Use of Chemicals
				A.P. Mashau	Citrus Export protocols
				A. Mbedzi	Control of FF, FCM and BI
06/03/2012	Nkwaleni V. Study Group	Nkwaleni Hall	24	Dr. Aruna Manrakhani	Fruit Fly and BI control
				M. Mulaudzi	Importance of Soil and leaf analysis
08/03/2012	SRV Study Group	Willow Tree Farm	11	W. Kirkman	FF, FCM and White Woolly Fly control
				M. Mulaudzi	Importance of Soil and leaf analysis
09/03/2012	Patensie Study Group	Tobacco Office	12	W. Kirkman	FF, FCM and White Woolly Fly control
				M. Mulaudzi	Importance of Soil and leaf analysis

### Mentorship Programme

There is still no funding for the CGA mentorship programme from the provincial departments of agriculture except for the Eastern Cape Province. The Eastern Cape Province is the only one that continued to fund the mentorship programme. The CGA signed the service level agreement with the Eastern Cape Province department of agriculture in June 2011 regarding the funding of the mentorship programme. The contract was for a year and an amount of R667 945-00 was allocated for the mentorship programme. The department also promised to allocate 60% of R667 945-00, which is R400 767-00, for 2012 funding for the mentorship programme. The CGA Transformation desk will continue to engage with the other provincial departments of agriculture for the funding of the mentorship programme.

### Fruit of Success Publication

During 2005 the citrus industry published "Our Citrus Transforms", a publication illustrating the various transformation and empowerment projects within the South African citrus industry at the time. During the past six years there has been rapid development and growth in this field. Thus the time was ripe to take a fresh look at the current status of transformation within the industry and in 2011 the Fruits of Success Publication was compiled. This publication was launched on the morning of 31 January 2012 at the Willow Park Conference Centre in Johannesburg.

### Citrus Growers Development Chamber (CGDC)

The Citrus Growers Development Chamber nominated some of its members to represent the Chamber in other CGA structures and stakeholders in a meeting that was held there on 21 June 2011 at the Willow Park Conference Centre in Johannesburg.

Variety Focus Groups: Five members were nominated to represent the Chamber on the variety focus groups as follows:

<b>Name of CGDC Member</b>	<b>Variety Focus Group</b>	<b>Province (Where member comes from)</b>
Eric Nohamba	Navels	Eastern Cape
Lawrence Mgadle	Soft Citrus	Eastern Cape
Petros Shiba	Lemons	Mpumalanga
Samson Qomondi	Valencia	Limpopo
Mzo Makhanya	Grape Fruit	Kwazulu Natal

Citrus Marketing Forum: Three members were nominated to represent the Chamber at the Citrus Marketing Forum (CMF).

<b>Name of CGDC Member</b>	<b>Province (Where member comes from)</b>
M.J. Matlou	Gauteng
Khaya Katoo	Eastern Cape
Thompson Mankhili	Limpopo

Agrisa Transformation Forum: Three members were nominated to represent the Chamber on the Agrisa Transformation Forum.

<b>Name of CGDC Member</b>	<b>Province</b>
Hannes Hobbs	Eastern Cape
Mzo Makhanya	KZN
Thompson Mankhili	Limpopo

Logistic Committee: Israel Nemaorani was nominated to represent the Chamber on the Logistic Committee

The Chamber has also resolved to hold four meetings per year and these meeting will be rotated amongst the provinces.

#### Vhembe Citrus Information Day

Vhembe district held the Citrus Information day on 7 December 2011 at the Makhado local municipality show ground. The people who had an opportunity to sign the attendance register were 136, but the number of the people who were present at the citrus information day was more than that. This event was funded by the Limpopo Department of Agriculture (LDA) and the Citrus Growers Association of Southern Africa (CGA). The theme of the information day was the "Citrus Orchard Establishment" and featured presentations by CRI, LDA, AVCASA, CA, DAFF, ARC, Masingo Trading Pty Ltd and Brenco Feeds.

The following stakeholders were present to exhibit their products (i.e., pesticides, machinery, farm working tools, booklets, pamphlets, banners, etc.); NTK, ARC, Masingo, Citrus Academy, AVCASA, Agricura, CGA/CRI, Brenco, DAFF, LDA, Vhembe FET and all Limpopo District Municipalities.

#### 8.4 Biosekuriteit

*Bactrocera invadens*: Voorligting was behulpsaam met die monitoring van *Bactrocera invadens* in Botswana en Zimbabwe. Gedurende April is die gebied vanaf Zeerust via Gaborones en Francis Town tot by Plum Tree in Botswana gemonitor vir *Bactrocera*. Een vlieg is gevang in 'n valletjie in Francis Town. Valletjies is ook

uitgehang vanaf Plum Tree via Bulawayo, Masvingo, Burcenough Bridge en Mutare in Zimbabwe. Een Bi vlieg is gevang in Nyika en verskeie in Mutare. Valletjies wat in Mosambiek tussen Mutare en Chicamba gehang is, was negatief.

In totaal het Voorligting vier opnames gedoen om te bepaal wat die stand van *Bactrocera* in Zimbabwe is. Die vrugtevlug is in minstens sewe distrikte gevind. Met behulp van USAid sal die Zimbabiese regering in die toekoms in staat wees om self die land te monitor en sal dit dus nie verder nodig wees vir Voorligting om daarby betrokke te raak nie. As gevolg van die situasie is die produsente in die suide van Zimbabwe aangeraai om nie toe te laat dat vrugte vir versapping vanuit die noorde na die suide toe gestuur word nie.

Aanplantingsmateriaal: Produsola kwekery in Mosambiek is namens die Sitrusverbeteringskema geakkrediteer. Die kwekery produseer gesertifiseerde sitrusbome van hoë gehalte en kan aanbeveel word vir sitrusprodusente in Zimbabwe en Mosambiek.

In Angola is die invoer van bome uit Brasilië na Angola suksesvol gestop vir ongeveer twee jaar. Dit het egter onder Voorligting se aandag gekom dat daar weer bome die land ingekom het vanaf Brasilië en onder andere in die Sumbe area aangeplant is. Hierdie saak moet dringend aandag ontvang. Die Suid Afrikaanse sitrusbedryf moet alles in sy vermoë doen om te verhoed dat bome in Suider Afrika ingebring word vanaf 'n land waar siektes soos sitrus kanker, Asiatiese vergroening en Leprosis virus voorkom.

### Opsomming van Aktiwiteite

Datum	Studiegroep/ Aktiwiteit	Onderwerpe/Aksies	Betrokkenes/ Sprekers
1 Apr 2011	Chemcity	Organic SASOL fertilisers	Hennie le Roux
2 Apr 2011	Bactrocera invadens	Check trapping from Brits - Zeerust	Hennie le Roux
5 Apr 2011	Mimosa Sitrus	<i>Armillaria</i>	Hannes Bester
8 Apr 2011	Winterveldt BEE	Visit and give advice to project	Hennie le Roux
10-16 Apr 2011	Bi survey in Botswana , Zimbabwe & Mozambique	Bi trapping & sampling	Hennie le Roux
12-14 Apr 2011	Kursus	Finance for Non-financial Managers	Hannes Bester
13 Apr 2011	Produsola Citrus Nursery (Moz)	CIS accreditation	Hennie le Roux
19 Apr 2011	Mpumalanga MEC	Updating MEC on LemonEX progress	Hennie le Roux Andrew Mbedzi
20 Apr 2011	Vergroenings-vergadering	Agenda	Hennie le Roux Thys Du Toit Hannes Bester MC Pretorius
21 Apr 2011	Mimosa Sitrus	<i>Armillaria</i>	Hennie le Roux Hannes Bester
5 Mei 2011	Sondagsrivier	Pakhuisbesoeke: Deon Joubert, Pieter Nortje, Willem Bouwer	Hannes Bester Paul Fourie
10 Mei 2011	Sondagsrivier	Boordbesoeke met Steve Burdette en Craig Burne	Hannes Bester
11 Mei 2011	CFB	Besoek CFB met Aussies	Hannes Bester
	Patensie	Boordbesoeke met Craig Burne	Hannes Bester
15-19 Mei 2011	Stellenbosch	Nematology Symposium	Hennie le Roux MC Pretorius
17 May 2011	Robertson	Investigate <i>Armillaria</i> problems on Clementines	Hennie le Roux MC Pretorius
20 May 2011	Ciskastreet, Pretoria	LemonEx Meeting	Hennie le Roux

			Kobus Boshoff
23 May 2011	Magalies	Directors Training	Hennie le Roux
24 Mei 2011	Nelspruit	Voorligtingsvergadering	Hennie le Roux Keith Lesar Hannes Bester
25 Mei 2011	Transformasie Boeredag	Agenda	Hennie le Roux Hannes Bester Andrew Mbedzi
26 Mei 2011	CIP-vergadering	Diagnostiese Sentrum	Hennie le Roux Paul Fourie Hannes Bester MC Pretorius
26 Mei 2011	SASCCON	Jaarvergadering	Hennie le Roux Hannes Bester
1 Jun 2011	CCCF/CGA Vergadering	Vervoer en Hantering	Dawid Groenewald Justin Chadwick Frikkie van Wyk Mitchell Brooke Hannes Bester Paul Hardman
	Midlands Farm: Mooirivier	BEE Ontwikkeling	Hannes Bester
2 Jun 2011	Durban Inname-depots	Monitering van palette en hantering	Dawid Groenewald Frikkie van Wyk Hannes Bester
2-4 Jun 2011	Brazilians	Assess Pera & Hamlin plantings in Brits area	Hennie le Roux Carlos van Parys De Witt
6 Jun 2011	Chemcity	Organic Fertilizer trail at Casmar	Hennie le Roux
7-11 Jun 2011	EU Delegation	Accompany DAFF and EU delegation to Letsitele, Hoedspruit, Nelspruit citrus packhouses and orchards and KNP CRI Nelspruit	Hennie le Roux  Tim Grout Hennie le Roux Tian Schutte Glynnis Cook Lorika Beukes
7 Jun 2011	Patensie	Ondersoek letsels op Novas	Hannes Bester
9 Jun 2011	Patensie	Proef op Novas om oorsaak van letsels te bepaal	Hannes Bester
14 Jun 2011	Midnight Studiegroep NAFCO Meeting	Snoei Discussions on NAFCO Symposium	Hannes Bester Hennie le Roux
	Visit declining Nova & Du Roi orchards	Henry Pieters Piet Engelbrecht Trust	
15 Jun 2011	Waterberg Studiegroep	Snoei	Hannes Bester Hennie le Roux
21 Jun 2011	Pretoria Universiteit	Besproeiingsvergadering	Teunis Vahrmeijer Hennie le Roux Hannes Bester
22 Jun 2011	CMF Vergadering	Agenda	Vaughan Hattingh Hannes Bester Hennie le Roux
23 Jun 2011	Patensie Studiegroep	Snoei	Hannes Bester

	Rustenburg Studiegroep	Navorsingsprioriteite	Hennie le Roux
24 Jun 2011	Winterveldt BEE	Discussions with Magalies directors on Winterveldt and its production problems	Hennie le Roux
27 Jun 2011	Nelspruit Studiegroep	Navorsingsprioriteite	Hennie le Roux
28 Jun 2011	Korean visitors	Phytosanitary visit	Tim Grout Hennie le Roux DAFF
28 Jun 2011	Hoedspruit Studiegroep	Navorsingsprioriteite	Hennie le Roux
29 Jun 2011	Constantia Studiegroep	Navorsingsprioriteite	Hennie le Roux
30 Jun 2011	Wenen	Navorsingsprioriteite	Hennie le Roux
1 Jul 2011	Addo Wildsfees	Op uitnodiging van SAPPI en ABSA: onderhandel vir borge vir Simposium	Hannes Bester
4 Jul 2011	Swellendam SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Breederivier SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Pongola SG tegniese kommittee	Navorsingsprioriteite	Hennie le Roux
5 Jul 2011	Paarl/Stellenbosch & Swartland SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Citrusdal SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Burgersfort Pakhuise Naranja pakhuis Morone “ Waterval sitrus	Besoeke/konsultasie	Keith Lesar Arno Erasmus Suzel Serfontein (QMS)
6 Jul 2011	Benede-Oranjerivier SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
7 Jul 2011	Vergadering met Tal	ivm. Technidex sitrus wakse	Keith Lesar Arno Erasmus
	Malelane & Komati-poort SG Teg. Komm.	Navorsingsprioriteite	Hennie le Roux
8 Jul 2011	Vaalharts SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Burgersfort & Ohrigstad Teg. Komm	Navorsingsprioriteite	Hennie le Roux
11 Jul 2011	Waterberg SG Tegniese Komm.	Navorsingsprioriteite	Hennie le Roux
	Tshipise & Weipe SG Tegniese Komm	Navorsingsprioriteite	Hennie le Roux
	ClanWilliam Pakhuise Namaqualand sitrus Kleinvlei sitrus Groot Patrysvlei sitrus Clanfresh pakhuis	Besoeke/konsultasie	Keith Lesar Corrie Muller
12 Jul 2011	Nkwalini SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Citrusdal Pakhuise Goedehoop Koop Noordhoek pakhuis	Besoeke/konsultasie	Keith Lesar Corrie Muller

	New Season sitrus Groenkloof sitrus ALG sitrus Citrus Select pakhuis		
	Pontdrif	Ondersoek Phytophthora probleme	Hennie le Roux
13 Jul 2011	Piketburg Pakhuise Piketco	Besoeke/konsultasie	Keith Lesar Fred du Pont (Univeg)
14 Jul 2011	Sondagsrivier	Vergadering: M Mulaudzi	Hannes Bester Melton Mulaudzi
	Kirkwood	Citrogold	Hannes Bester Etienne Rabe Abs van Rooyen Peter Turner
	Casmar, Brits	Chemcity Organiese proewe	Hennie le Roux
	Paarl, Worcester, Franschoek Pakhuise Du Cap pakhuis Fruit 2 You pakhuis Franschoek Vrugte Verpakkers	Besoeke/konsultasie	Keith Lesar Fred du Pont
15 Jul 2011	Swellendam Pakhuise Swellenfruit Verpakkers Thornlands pakhuis Suiderland Sitrus	Besoeke/konsultasie	Keith Lesar Peter Moir
19 Jul 2011	Sondagsrivier SG tegniese kommittee	Navorsingsprioriteite	Hennie le Roux Hannes Bester
20 Jul 2011	Wes-Kaap CTA	Bemestingsvergadering	Hannes Bester Teunis Vahrmeijer Hennie le Roux Paul Cronje
21 Jul 2011	Badplaas	LemonEx projek	Hennie le Roux LED Waterwese DBSA Grondeienaars
25 Jul 2011	Fort Beaufort/Riverside Pakhuise Katco pakhuis Riverside pakhuis Eden Agri pakhuis	Besoeke/konsultasie	Keith Lesar Corrie Muller
26 Jul 2011	Katrivier SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	SRCC/Kirkwood Pakhuise SRCC pakhuis Unifrutti pakhuis Sun Citrus pakhuis	Besoeke/konsultasie	Keith Lesar Corrie Muller
27 Jul 2011	Sitrus Rand pakhuis Sun River Citrus Tregaron Citrus		
	Baviaans en Patensie SG tegniese kommittee	Navorsingsprioriteite	Hannes Bester
	Kirkwood/Patensie Pakhuise Endulini pakhuis	Besoeke/konsultasie	Keith Lesar Corrie Muller Hannes Bester
28 Jul 2011	Groblersdal SG Tegniese	Navorsingsprioriteite	Hennie le Roux

	Komm		Arno Erasmus
	Patensie Pakhuise Ventershoek Sitrus Southern Sitrus Kwaggaskloof pakhuis	Besoeke/konsultasie	Keith Lesar Corrie Muller
29 & 30 Jul 2011	Schoemanskloof Sitrusfees	CRI uitstalling	Johan Joubert Hennie le Roux
2 Aug 2011	CRI Raadsvergadering	Direksievergadering	Direksie Vaughan Hattingh Tim Grout Hennie le Roux
	Vergadering met Afrifert (Groblersdal)	Citrosol waks + guazatine	Keith Lesar Arno Erasmus
3 Aug 2011	ETP Vergadering	Agenda	Hannes Bester
	SVS Vergadering	Agenda	Thys du Toit Vaughan Hattingh Paul Fourie Fanie van Vuuren Hennie le Roux
5 Aug 2011	Casmar	LemonEx	Hennie le Roux
8 Aug 2011	Casmar	Chemcity bemestingsproef	Hennie le Roux
15 Aug 2011	LemonEx Vergadering Johannesburg	IDC, NEF, Landbank, Ciskastraat	Justin Chadwick Vaughan Hattingh Hennie le Roux
16 Aug 2011	Nelspruit SG Tegniese Kommittee	Navorsingsprioriteite	Hennie le Roux
17 Aug 2011	Real IPM Boeredag	Biologiese beheer produkte	Hennie le Roux
	Kirkwood: Pieter Nortje en Hannes De Waal	LemonEx	Hennie le Roux Hannes Bester
18 Aug 2011	Citrus Transformation Farmers Day - Addo	Addo	Melton Mulaudzi Andrew Mbedzi Hannes Bester Sean Moore Teunis Vahrmeijer Hennie le Rouxi
	Nelspruit, Malelane, Komatipoort Pakhuise MSK pakhuis-Malelane MSK pakhuis- Komatipoort Vergenoeg pakhuis Karino Koop	Besoeke/konsultasie	Keith Lesar Arno Erasmus Wilma du Plooy (JBT)
19 Aug 2011	Verpakkingswerkgroep vergadering	Agenda	Hannes Bester Hennie le Roux Keith Lesar Dawid Groenewald
23 Aug 2011	Sondagsrivier SG	Marktoegang Blaaspootjiebeheer FCM-beheer	Vaughan Hattingh Tim Grout Sean Moore Hannes Bester Wayne Kirkman Keith Danckwerts
24 Aug 2011	LemonEx vergadering	Grondeienaars	Hennie le Roux

25 Aug 2011	Hoedspruit SG	Lenteplaagkompleks Helicovir	Hennie le Roux Tim Grout Keith Danckwerts
29 Aug 2011	LemonEx vergadering: Kirkwood	LemonEx	Hennie le Roux Vaughan Hattingh Justin Chadwick Hannes Bester Fanie Viljoen Jock Danckwerts
31 Aug 2011	Burgersfort SG	Lenteplaagkompleks Helicovir	Tim Grout Keith Danckwerts Hennie le Roux
	Letsitele Pakhuise Bosveld Sitrus Merite pakhuis Mahela Boerdery	Besoeke/konsultasie	Keith Lesar Arno Erasmus Suzel Serfontein (QMS)
1 Sept 2011	Polokwane	Bloedlemoenprojek DAFF	Johan Joubert Andrew Mbedzi Hennie le Roux
	Hoedspruit Pakhuise Olifants Rivier Est. Morhia Sitrus Canyon Sitrus	Besoeke/konsultasie	Keith Lesar Arno Erasmus Suzel Serfontein (QMS)
2 Sept 2011	Groblersdal	NAFCO	Hennie le Roux Tim Grout Aruna Manrakhan Tian Schutte Johan Joubert
3 Sept 2011	Casmar	LemonEx	Hennie le Roux
7 Sept 2011	CGA Quality Workshop	Nawels Satsumas	Hannes Bester Vaughan Hattingh Hennie le Roux Keith Lesar Paul Cronje
	Vergadering met ICA	Stand van Sporekill en pakhuis bestandheid strategië met pyrimethanil	Keith Lesar Wouter Schreuder
8 Sept 2011	Swaziland SG	Spring Pest Complex	Tim Grout Hennie le Roux Chris Kellerman
9 Sept 2011	Onderberg- Dr H. Nel	Voorligtingsherstrukturering	Hennie le Roux Tim Grout
10 -13Sept 2011	Zimbabwe	Bacterocera invadens opname	Hennie le Roux
12 Sept 2011	Swellendam en Breederivier SG'e	Lenteplaagkompleks FCM Bemesting River Bioscience	Sean Moore Hannes Bester Teunis Vahrmeijer Keith Danckwerts
13 Sept 2011	Paarl/Stellenbosch & Swartland SG	Lenteplaagkompleks FCM Bemesting River Bioscience	Sean Moore Hannes Bester Teunis Vahrmeijer Keith Danckwerts
	Citrusdal SG	Lenteplaagkompleks FCM Bemesting	Sean Moore Hannes Bester Teunis Vahrmeijer

		River Bioscience	Keith Danckwerts
14 Sept 2011	Benede-Oranjerivier SG	Lenteplaagkompleks FCM Bemesting River Bioscience	Sean Moore Hannes Bester Teunis Vahrmeijer Keith Danckwerts
	Harare SG	Research Priorities Spring Pest Complex	Hennie le Roux Tim Grout
15 Sept 2011	Beitbrug SG	Research Priorities Spring Pest Complex	Hennie le Roux
	Vaalharts SG	Lenteplaagkompleks FCM Bemesting	Sean Moore Hannes Bester Teunis Vahrmeijer
19 Sept 2011	Katrivier SG	Spring Pest Complex FCM River Bioscience	Sean Moore Hannes Bester Keith Danckwerts
	Citrus Academy	Scouting Videos	Jacomien de Klerk Tim Grout Hennie le Roux
20 Sept 2011	Nkwalini SG	Spring Pest Complex FCM River Bioscience	Sean Moore Hannes Bester Keith Danckwerts
21 Sept 2011	Southern KZN SG	Spring Pest Complex FCM River Bioscience	Sean Moore Hannes Bester Keith Danckwerts
28 Sept 2011	Cultivar Development Research Committee Meeting	Agenda	Andy Lee Hennie le Roux Hannes Bester Johan Joubert Richard Fenwick Zondi
29 Sept 2011	Patensie SG	Lenteplaagkompleks FCM River Bioscience	Wayne Kirkman Hannes Bester Keith Danckwerts
	Baviaans SG	Lenteplaagkompleks FCM River Bioscience	Wayne Kirkman Hannes Bester Keith Danckwerts
	Disease Management Research Committee Meeting	Agenda	Paul Fourie Tim Grout Hennie le Roux
3 Okt 2011	Vergroening	Besoek van Prof Bové	Hennie le Roux Prof G. Pietersen
4 Okt 2011	CLFQM Committee meeting	Navorsingsprioriteite	Hennie le Roux Hannes Bester
5 Okt 2011	Besoek Vergroenings- boorde	Besoek met Bové	Hennie le Roux
6 Okt 2011	Nelspruit Studiegroep	Impak van Huanglongbing wêreldwyd	Prof Bové Hennie le Roux CRI personeel
7 Okt 2011	KNP vergadering	IOCV Kongres 2013	Prof Bové Hennie le Roux Prof G. Pietersen
8 Okt 2011	KNP	IOCV beplannings-vergadering	Prof Bové Hennie le Roux Prof G. Pietersen
9 Okt 2011	Swaziland	Visit Tamboti re IOCV in 2013	Prof Bove Hennie le Roux

10 Okt 2011	KZN	Visit Greening infected orchards in Melmoth	Prof Bové Hennie le Roux
11 Okt 2011	Stellenbosch	Vorming van CGA Kultivarmaatskappy	Vergadering Hennie le Roux
12 Okt 2011	Addo en Sondagsrivier-vallei	Besoek met Prof Bové	Hannes Bester
13 Okt 2011	Patensie	Besoek met Prof Bové	Hannes Bester
13 Okt 2011	Snykant 126	Latente Patogene	Keith Lesar
14 Okt 2011	Badplaas	Vergadering met Robert Nkozi en Fred Daniels	Hennie le Roux
14 Okt 2011	Franschhoek en Stellenbosch	Besoek met Prof Bové	Hannes Bester
20 Okt 2011	Marble Hall & Baltimore	Boordbesoeke om terugsterwing aan te spreek	Hennie le Roux Hannes Bester
21 Okt 2011	Polokwane	Boeredag	Hennie le Roux Hannes Bester Dawid Groenewald
24 Okt 2011	Nelspruit: Simposium beplannings-vergadering	Agenda	Hennie le Roux Hannes Bester Henry Skinner Jean De Gasperi Andrea Vinson
24 Okt 2011	Nelspruit Voorligtings - vergadering	Vordering van kontrak werk	Hennie le Roux Hannes Bester Keith Lesar
25 Okt 2011	SAPPI	Borgskap vir simposium Kontrakteer Dawid Groenewald	Hennie le Roux Hannes Bester Dawid Groenewald
26-27 Okt 2011	All Fresh Conference	Program	Hennie le Roux Hannes Bester
31 Okt 2011	Nelspruit	Marble Hall Sitrus vergadering	Keith Lesar Arno Erasmus
3 Nov 2011	CMF vergadering	Agenda	Dawid Groenewald Hannes Bester
6-10 Nov 11	Wes Kaap (Oudtshoorn & omliggende distrikte)	Swartvlekopname	Elma Carstens Hennie le Roux
15 Nov 2011	"Draft" Dokument van "Postharvest Diseases – Illustrated"	Hennie en Hannes kommentaar	Keith Lesar
17 Nov 2011	Heidelberg: Kobus De Kok	Terugsterwing op manderyne	Hannes Bester
17 Nov 2011	Hoedspruit Studiegroep	Na-oes Terugvoering vergadering	Keith Lesar Arno Erasmus Hennie le Roux
21 Nov 2011	Marble Hall Sitrus	Aanbiedinge oor bederfbeheer, pakhuis onderwerpe en besoek aan Pakhuis 1 en 2	Keith Lesar Arno Erasmus
22 Nov 2011	CRI staff meeting	Agenda	Hennie le Roux Hannes Bester Keith Lesar Dawid Groenewald
23 Nov 2011	CRI Raadsvergadering & CRI 10year function	Program	Hennie le Roux Hannes Bester Keith Lesar Dawid Groenewald

			Andrew Mbedzi Melton Mulaudzi
24 Nov 2011	Na-oes post-mortem	Agenda Beplanning van pakhuis werkswinkels	Hennie le Roux Hannes Bester Dawid Groenewald Keith Lesar Paul Fourie Arno Erasmus Paul Cronje
29 Nov 2011	Packaging Working Group	Agenda	Dawid Groenewald Hennie le Roux Hannes Bester Keith Lesar
30 Nov 2011	Ndandwa	BEE Meeting	Hennie le Roux
1 Des 2011	Polokwane	Limpopo DA meeting re MoU	Hennie le Roux Andrew Mbedzi
2 Des 2011	Brits	Magalies/ Winterveldt	Hennie le Roux
5-7 Des 2011	Nkwaleni	BEE Meeting & Visit to Nkwaleni Processors	Hennie le Roux Melton Malaudzi
7 Des 2011	Exporters Technical Panel meeting	Agenda	Hannes Bester Dawid Groenewald Keith Lesar
12 Dec 2011	Johannesburg	Interviews for DC appointment	Tim Grout Paul Fourie MC Pretorius Hennie le Roux
4 Jan 2012	Beplanningsvergadering met Patologie	Gideon van Zyl se landswye spuit aanbiedinge	Hennie le Roux Paul Fourie Gideon van Zyl
11 Jan 2012	Sondagsrivier-vallei Voorligtingstrategie- vergadering	Strategie tov voorligting	Hennie le Roux Hannes Bester
12 Jan 2012	Beplanningsvergadering	Pakhuis Werkswinkels	Hannes Bester Hennie le Roux
17 Jan 2012	Sondagsrivier Tegnieese Komitee	Studiegroep beplanning	Hannes Bester
17 Jan 2012	Nelspruit VLU	Suurlemoen & suurlemoenproduksie	Hennie le Roux
17 Jan 2012	Vergadering Johan Friis Waterval Sitrus Burgersfort	Chloordioksied installasie in pakhuis	Keith Lesar Arno Erasmus
18 Jan 2012	Boordbesoeke	Lydenburg	Hennie le Roux
20 Jan 2012	Skukuza	Inspekteer venue vir 2013 IOCV Kongress	Hennie le Roux Glynnis Cook Henry Skinner
23 Jan 2012	Transformasie	IDC	Hennie le Roux
24 Jan 2012	Boek van "Oorsig oor na- oes siektes"	Na Hannes en Hennie	Keith Lesar
26 Jan 2012	San Miguel	General citrus trends	Hennie le Roux Dirk Wissing
1 Feb 2012	Citrusdal Voorligting- strategievergadering	Strategie tov voorligting	Hannes Bester
1 Feb 2012	G2G	Citrus Processing	Hennie le Roux Venera Raffa

2 Feb 2012	Casmar		Discuss CGA tree deposit	Hennie le Roux Neville Wenhold
3 Feb 2012	Univ of Pretoria Stefan Kamburov		IOCV planning Kamburov's book	Hennie le Roux Gerhard Pietersen Lise Korsten Stefan Kamburov
6 Feb 2012	IOCV Meeting		Planning meeting with Going Africa	Gerhard Pietersen Glynnis Cook Fanie van Vuuren Hennie le Roux MC Pretorius
7 Feb 2012	Boordbesoeke		Baberton	Hennie le Roux Bruce Andrews
8 Feb 2012	7de Sitrusnavorsing- simposium		Beplanningsvergadering	Hennie le Roux Henry Skinner Jean de Gasperi
9 Feb 2012	Addo pakhuisbesoeke		Pakhuisbeplanning en ontwerp	Hannes Bester Melany Fischer
10 Feb 2012	Patensie pakhuisbesoeke		Pakhuisbeplanning en ontwerp	Hannes Bester Melany Fischer
14-15 Feb 2012	Limpopo Pakhuis- werkswinkels		Agenda	Hannes Bester Hennie le Roux Dawid Groenewald Keith Lesar Arno Erasmus
16-17 Feb 2012	Mpumalanga Pakhuis- werkswinkels		Agenda	Hannes Bester Hennie le Roux Dawid Groenewald Keith Lesar Arno Erasmus
20 Feb 2012	CGA-vergadering Patensie		Agenda	Vaughan Hattingh Hannes Bester
20 Feb 2012	CGA Vergadering Groblersdal		Agenda	CGA verteenwoordigers Hennie le Roux
21-22 Feb 2012	KZN & Swaziland Pakhuiswerkswinkels		Agenda	Hannes Bester Hennie le Roux Dawid Groenewald Keith Lesar Arno Erasmus Paul Cronje
23-24 Feb 2012	Oos-Kaap Pakhuis- werkswinkels		Agenda	Hannes Bester Hennie le Roux Dawid Groenewald Keith Lesar Arno Erasmus Paul Cronje
28 Feb 2012	CGA-vergadering Suid- Kaap		Agenda	Vaughan Hattingh Hannes Bester
28-29 Feb 2012	Wes-Kaap Pakhuis- werkswinkels		Agenda	Hannes Bester Hennie le Roux Dawid Groenewald Keith Lesar Arno Erasmus Paul Cronje
1 Mrt 2012	Vergadering met		Beskikbaarheid van die regte en	Dawid Groenewald.

	paletvervaardigers	goeie kwaliteit hout en die beheer van swamme.	
2 Mrt 2012	Vergadering met Karino Koop	Pakhuis behandelings	Keith Lesar
5 Mrt 2012	Vergadering met Joubert en Seuns en pakhuis en boord besoek	Pakhuis konsultasie/veranderinge in lyn en aanbevelings	Keith Lesar Arno Erasmus James Warrington
6-7 Mrt 2012	Bestuursvergadering	Agenda	Vaughan Hattingh Hennie le Roux Tim Grout Thys Du Toit Hannes Bester Sean Moore Paul Fourie
6 Mrt 2012	Vergadering in Letsitele.	Indringende gesprekke oor beskikbaarheid van papier vir uitvoer sitrus kartonne	Dawid Groenewald.
7 Mrt 2012	CRI	Grondgedraagde siektes	CRI Patoloë Hennie le Roux
8 Mrt 2012	Vergadering met Piet Engelbrecht Trust	Konsultasie ivm nuwe voorontgroening "drench" stelsel	Keith Lesar
8 Mrt 2012	CRI	Virologie	CRI Viroloë Hennie le Roux
12 Mrt 2012	Thai delegasie	DAFF Pretoria Tshipise	Hennie le Roux Mike Holtzhausen DAFF Peter Nicolson
13 Mrt 2012	Citrus Logistics Forum	Werkswinkel	Dawid Groenewald Hannes Bester
13 Mrt 2012	Thai delegasie	Boord besoeke	Hennie le Roux Mike Holtzhausen DAFF Fanie Viljoen
14 Mrt 2012	VFG-meetings	Agendas	Hannes Bester
14 Mrt 2012	Thai delegasie	CRI besoek Boord besoeke	Hennie le Roux Tim Grout Mike Holtzhausen DAFF
15 Mrt 2012	CMF vergadering	Agenda	Dawid Groenewald Hannes Bester
15 Mrt 2012	DOW Agrosience	Toekomstige registrasies	Hennie le Roux
15 Mrt 2012	Joubert en Seuns	Installasie van Chloor "Chip doser"	Keith Lesar Peter Bakker
20 Mrt 2012	Vergadering met Peter Bakker van Chemicorp	Bestuur van chloor "Chip doser" by Joubert en Seuns	Keith Lesar
20 Mrt 2012	Ohrigstadt	Nova SL terugsterwing	Hennie le Roux
21 Mrt 2012	Letsitele	Groep 91 Terugsterwing	Hennie le Roux
22 Mrt 2012	Burgersfordt	Glen Ora vrugset Studiegroepvergadering	Hennie le Roux Glynnis Cook Graham Barry
22 Mrt 2012	Nawel vrugmonsters by Diagnostiesesentrum	Identifikasie van bederf	Keith Lesar
23 Mrt 2012	Magalies Sitrus	Winterveldt BEE	Hennie le Roux
26 Mrt 2012	Prof Schumann CREC	Precision Farming & Florida HLB	Hennie le Roux
27 Mrt 2012	Prof Schumann (CREC)	Precision Farming & HLB	Hennie le Roux

	Univ Pretoria Boordbesoeke – Brits Boordbesoek Groblersdal		Gerhard Pietersen Jaco Burger
28 Mrt 2012	Prof Schumann (CREC) Boordbesoeke- Burgersfort & Schoemanskloof	Precision Farming & HLB	Hennie le Roux Andrew Cooper
28 en 29 Mrt 2012	Letsitele	Proewe met nuut- ontwikkelde papier om meer koste-effektiewe kartonne te verskaf	Dawid Groenewald.
28 Mrt 2012	Transformation meeting: Hankey	Phytosanitary issues Government support	Hannes Bester Melton Mulaudzi
29 Mrt 2012	Prof Schumann	Precision Farming & HLB	Hennie le Roux Tim Grout CRI personeel
30 Mrt 2012	Prof Schumann	Boordbesoeke Karino	Hennie le Roux James Warrington
30 Mrt 2012	CFB	Ogiehout-skattings	Thys Du Toit Hannes Bester
31 Mrt 2012	Prof Schumann (CREC)	Krugerwildtuin	Hennie le Roux

#### Summary of Extension Coordinators' Activities (April 2011 – March 2012)

Date	Venue/Place	Activity/Topics	Speakers/Extension Officer
04/04/2011	Makwarela	Preparations for the Vhembe Citrus Study Group session 1 to be held at Mutele on 8 <sup>th</sup> of April 2011	Andrew Mbedzi
05/04/2011	Kirkwood Aquapark Hall (SRV)	Casp Roadshow in the EC on CASP funding for 2011 and 2012 budget.	Melton Mulaudzi
06/04/2011	Mabunda Farm (Giyani)	Mopani Citrus Study Group session 1 on the Importance of Global GAP Accreditation	Andrew Mbedzi
06/04/2011	Luthando farming Trust (SRV)	SRV Citrus study group on Fruit fly, FCM, and Greening controls, citrus woolly fly and the importance of soil and leaf analysis.	Melton Mulaudzi
07/04/2011	Polokwane (Dept. of Economics Affairs)	Discussions on Plokwane Fresh Produce Hub by CGA, CRI, Farmers, Dept. of Transport, Dept. Agric. And Dept. Economic Affairs	Andrew Mbedzi
07/04/2011	Patensie ANC Office	Patensie citrus study group on Fruit fly, FCM, and greening controls, citrus woolly fly and the importance of soil and leaf analysis.	Melton Mulaudzi
08/04/2011	Tshirema Farm (Mutele)	Vhembe Citrus Study Group session 1 on the Importance of Soil and Leaf analysis in planning the Fertilization Program	Andrew Mbedzi
11/04/2011	Polokwane (Agri-Village 1)	Discussions on the finalization of the Citrus Field Day to be Held at the Zebediela Citrus Estate.	Andrew Mbedzi

12/04/2011	KAT River Farmers	Doing mentorship evaluation with Dr Richards Bates and Lukhanyo transformation Manager from CGA.	Melton Mulaudzi
13/04/2011	Patensie farmers	Doing mentorship evaluation with Dr Richards Bates and Lukhanyo transformation Manager from CGA.	Melton Mulaudzi
14/04/2011	KAT River (Riverside training centre)	KAT River citrus study group on Fruit fly, FCM, and Greening controls, citrus woolly fly and the importance of soil and leaf analysis.	Melton Mulaudzi
19/04/2011	Mpumalanga Govt. (MEC's Office)	Meeting MEC with Ndwandwa Community regarding the LemoneX Projects	Andrew Mbedzi
04/05/2011	Makonde Farm	Discussion with the beneficiaries and LDA officials regarding strategic partnership for Makonde farm	Andrew Mbedzi
04/05/2011	Topkat, Eden Jerico, Orange grange, Konzi	Taking Leaf and soil samples with Metula, Eric, Mandla and Ndzoyi	Melton Mulaudzi
05/05/2011	Bonnes Esperance Farm (Dendron)	Meeting with the beneficiaries discussing the possibility of planting citrus in the farm	Andrew Mbedzi
05/05/2011	Greenwood farm Lovers Retreat Farm	Sanitations of Orchards with Mbilase Weed control on young citrus trees	Melton Mulaudzi
09/05/2011	East London Airport	To fetch Mr Lukhanyo to attend the mentorship meeting at Bishop at Mr Mbaleni s office.	Melton Mulaudzi
10/05/2011	Bisho Agricultural Office	Attending meeting of signing the service level agreement with Lukhanyo, Mbaleni and the manager of Mbaleni.	Melton Mulaudzi
11/05/2011	Mpumalanga Govt. Offices	Accompanying Lukhanyo to meet with the mentorship programme officer	Andrew Mbedzi
11/05/2011	Jordan, Jerico, Eden, Konzi, Topkat and Greenwood	Follow up on citrus study group topics on FCM, Fruit fly, and BI controls, greening and soil and leaf analysis.	Melton Mulaudzi
12/05/2011	Dept. of Land Affairs	Sibonelo dispute meeting with Bruce, Lukhanyo, Beneficiaries and other stakeholders	Andrew Mbedzi
12/05/2011	Peddie farms	Monitor soil and leaf analysis, control of fruit fly, FCM, and orchard sanitation.	Melton Mulaudzi
17/05/2011	Khubvi Agric. Office	Follow-up meeting regarding the Strategic Partnership for Makonde Farm	Andrew Mbedzi
17/05/2011	Sundays river Valley farms	Monitor soil and leaf analysis, control of fruit fly, FCM, and orchard sanitation.	Melton Mulaudzi
19/05/2011	Zebediela Citrus Estate	Planning and Final preparations for the Citrus Field Day	Andrew Mbedzi
20/05/2011	Gonzana Torties	Fruit sampling	Melton Mulaudzi
25/05/2011	SRV (Mbuyiselo Farming Trust)	Attending commodity launching organised by Government	Melton Mulaudzi

31/05/2011	Lidell Farm	To discuss pruning and fertilisation of citrus after harvesting. Monitoring picking of Navels	Melton Mulaudzi
31/05/2011	Lettas Farm	To do aftercare services on newly planted Eureka lemon and to encourage the farmer to fertilize the newly planted lemon after winter.	Melton Mulaudzi
03/06/2011	Tzaneen	Preparations with farms in Tzaneen for the CGA Publication	Andrew Mbedzi
06/06/2011	Chauke's Farm	Problem of Phytophthora on new planted citrus trees	Andrew Mbedzi
07/06/2011	Nylstroom Govt. Offices	Waterberg Citrus Study group planning meeting with the extension officers and farmers	Andrew Mbedzi
07/06/2011	Peddie Farms	Growers' Interviews for the CGA Transformation Publication.	Melton Mulaudzi
08/06/2011	Mabunda Farm	Exposure tour for the Makonde Farmers and their Extension Officers	Andrew Mbedzi
08/06/2011	Sundays River Farms	Growers' Interviews for the CGA Transformation Publication.	Melton Mulaudzi
09/06/2011	Patensie Farms	Growers' Interviews for the CGA Transformation Publication.	Melton Mulaudzi
10/06/2011	KAT River and (Susan Herman)	Growers' Interviews for the CGA Transformation Publication.	Melton Mulaudzi
13/06/2011	Battlesden farm	To monitor the flood damage and also to help organise the picking of navels.	Melton Mulaudzi
21/06/2011	Johannesburg (Willow Park)	Citrus Growers Development Chamber (CGDC) meeting	Andrew Mbedzi Melton Mulaudzi
22/06/2011	Winterveldt Farm	Visited Winterveldt Farm to see the extent of the frost damage on young trees	Andrew Mbedzi
23/06/2011	Dept. of Land Affairs	Sibonelo Dispute meeting with Bruce, Beneficiaries, and other stakeholders	Andrew Mbedzi
24/06/2011	Maswiri Farm (Tshipise)	Vhembe Citrus Study Group session 2 of Quality of Citrus Fruit and Fertilization of the citrus trees.	Andrew Mbedzi
28/06/2011	Netshifhefhe Farm (Dzindi-T/Ndou)	Thulamela Local Municipality Commodity Information Day	Andrew Mbedzi
28/06/2011	Patensie (Dankbaar CPA)	Attending citrus study group on irrigation and fertilization of citrus trees.	Melton Mulaudzi
30/06/2011	Ongerep Farm (Lephalale)	Waterberg Citrus Study Group session 1 on Harvesting and Fertilization of the Citrus Trees	Andrew Mbedzi
01/07/2011	Jerico, Eden, and Lovers Retreat	Organizing PPECB training about Responsible use of Pesticides and monitoring picking.	Melton Mulaudzi
04/07/2011	Makhado Agric. Office	Planning the Exposure Tour to Mabunda Farm for the Dendron (Bonnes Esperance) Beneficiaries.	Andrew Mbedzi
04/07/2011	Mpofu training centre, Topkat and Lidell Farms	Attending responsible use of pesticides from AVCASA organized by PPECB SAPI 2 Programme	Melton Mulaudzi
05/07/2011	Tzaneen, X-Group Oficces	Partnership meeting between Makonde Farmers and the X-Group.	Andrew Mbedzi
06/07/2011	Ohrigstad, Matumi Orchard	To arrange for the CGA Publication with Farmers/Beneficiaries	Andrew Mbedzi

06/07/2011	Fort Beaufort-East London Air port – Fort Beaufort - Adelaide	To attend the Riverside Open Day and delivering document to be sign by JE Dekwerts as the Director of CGA board.	Melton Mulaudzi
07/07/2011	Makonde Farm	Meeting to discuss the Evaluation of the Farm by the X-Group, the Farmers and Andrew	Andrew Mbedzi
07/07/2011	Riverside P/house, Country Club and Kirkwood (SRV)	Attending open day and delivering document to be sign by Hobbs as the Director of CGA board	Melton Mulaudzi
08/07/2011	Patensie Three Pence	Having a meeting with Mr Ferreira so that he can become one of the mentors to mentor Three pence.	Melton Mulaudzi
12/07/2011	Lidell,Green wood and Topkat	Monitoring picking at green wood and discussion of pruning and Fertilisation at Lidell farm and Topkat farm.	Melton Mulaudzi
13/07/2011	Konzi farm, Jerico , Gonzana farm and Eden services Pack house	To discuss water spot on clementines and recap for Lawrence and Maqoma Sesiko with Shaun Brown who is their mentor.	Melton Mulaudzi
14/07/2011	Safe pack house at SRV, Khangela and Lenmore Farms	Planning the Grower Day at the Polo Club. Delivering learning materials and meeting Hannes Bester.	Melton Mulaudzi
19/07/2011	King Williams Town Crown Hotel	Preparation of grower day agenda, and transportation of farmers.	Melton Mulaudzi
21/07/2011	Mabunda Farm	Exposure tour to Mabunda Farm by Chalteka Farmers and Govt. E.O.'s	Andrew Mbedzi
22/07/2011	Makwarela Agric Offices	Meet the Vhembe District Coordinator to discuss the nomination of farms for CGA Publication	Andrew Mbedzi
25/07/2011	Mariveni, Alto Packhouse and Mabunda	Citrus Growers Association (CGA) Publication Interviews with Louise Brodie	Andrew Mbedzi
26/07/2011	Easy Farm, Masakona CPA, Ravele CPA	Citrus Growers Association (CGA) Publication Interviews with Louise Brodie	Andrew Mbedzi
27/07/2011	Levubu, Tshipise, and Messina	Citrus Growers Association (CGA) Publication Interviews with Louise Brodie	Andrew Mbedzi
28/07/2011	Zebediela Estat. Sunningdale and Pretoria	Citrus Growers Association (CGA) Publication Interview with Louise Brodie s	Andrew Mbedzi
29/07/2011	Winterveldt Farm	Citrus Growers Association (CGA) Publication Interviews with Louise Brodie	Andrew Mbedzi
01/08/2011	KZN Kwaleni Community Hall	Attending Financial Training at Kwaleni community hall and also do presentation about pack house management and Fertilisation.	Melton Mulaudzi
10/08/2011	Mpofu training centre, Jerico and Orange Grange	Organizing the venue for financial management training course. Monitoring harvesting at Jerico and arrangement of fertilization on none bearing trees.	Melton Mulaudzi

11/08/2011	Ratombo Farm	Assist Beneficiaries with the explanation of Citrus Academy Bursary/Pest Control	Andrew Mbedzi
11/08/2011	Oakdene, Konzi and Jerico	Visiting farms with Susan and monitoring picking at Jerico	Melton Mulaudzi
12/08/2011	Makwarela Agric. Offices	Vhembe Citrus Technical Committee meeting	Andrew Mbedzi
15/08/2011	DAFF Offices Pretoria	Mentorship meeting with the Office of the Chief Director	Andrew Mbedzi
16/08/2011	Riverside P/house, Eden Service pack house, SRV	Doing preparation of growers day and organizing carton boxes for display during this event at Polo Club (SRV)	Melton Mulaudzi
17/08/2011	Port Elizabeth Airport, Cri offices PE, Kronenhoff – Luthando farm	Fetching Andrew Mbedzi from the Airport, arranging CRI banners and River Bioscience banners for displayed during the Growers Day.	Melton Mulaudzi
18/08/2011	Sundays River Valley	Presentation of Worker Health and Safety at the Grower Day held at the Polo Club in SRV	Melton Mulaudzi Andrew Mbedzi
19/08/2011	SRV Kronenhoff-PE Greenacres Rural development and Land reform	Attending meeting about recapitalisation and the six farm to be purchased by Rural Development and Land Reform at Patensie.	Melton Mulaudzi
22/08/2011	Mpofu Training Centre, And Riverside	Attending PPECB Short course at Mpofu training centre And a visit to Dr Verena Blitzer of Riverside.	Melton Mulaudzi
23/08/2011	SRCC Pack house	Attending CRI Commercial citrus study group	Melton Mulaudzi
24/08/2011	Delmas	SAPIP/PPECB Farmers Information Day	Andrew Mbedzi
29/08/2011	White citrus farm, Jerico ,Eden	Monitoring Pruning and Fertilizer application	Melton Mulaudzi
30/08/2011	Jerusalem, Letas, Orange grange , Lovers retreat	Monitoring none bearing citrus at Lovers Retreat and newly planted Eureka lemon at Letas	Melton Mulaudzi
01/09/2011	Polokwane Agri-Village	Blood Oranges meeting at the Agri-Village Boardroom in Polokwane	Andrew Mbedzi
01/09/2011	Intaba Lodge	Attending calibration study group organize by Riverside advisory services at Ntaba Lodge	Melton Mulaudzi
05/09/2011	Savoy Hotel	Attending a Sappi 2 meeting regarding the election of committee members to deal with Provincial training activities.	Melton Mulaudzi
06/09/2011	Coutry Club	The event was organized by Riverside advisory Services. Concerning biological teas and compost tea.	Melton Mulaudzi
07/09/2011	Stellenbosch Hotel	Attending Citrus Growers Development Chamber meeting	Andrew Mbedzi Melton Mulaudzi
08/09/2011	ARC Stellenbosch	Attending Dept. of Agriculture Fisheries and Forestry Citrus Coordinating meeting	Andrew Mbedzi Melton Mulaudzi
09/09/2011	Riverside Pack house- Cape college	Organizing commercial study group with local Government Extension officers.	Melton Mulaudzi
12/09/2011	White citrus farm	Monitoring fertilisation and newly planted Newhall trees	Melton Mulaudzi

13/09/2011	Mariveni, Du Roi Nursery and Westfalia	Exposure tour to Mariveni, Du Roi Nursery and Westfalia by Bonnes Esperance (Chalteka Farmers)	Andrew Mbedzi
13/09/2011	King Williams Town, Greencares (PE)	Attending Recapitalization meeting with National chief of staff from DRDLR	Melton Mulaudzi
14/09/2011	Dan Staat Farm	To evaluate the extent of Deterioration of the citrus orchards	Andrew Mbedzi
16/09/2011	Pretoria	Meetings with the Agriseta, the Diplomatic Society and NAMC in Pretoria	Andrew Mbedzi
19/09/2011	KATCO East London	Attending Citrus study group organized by My line manager Hannes Bester about the Spring Pest Complex.	Melton Mulaudzi
20/09/2011	Blink water Eden Service and Cape College	Attending meeting on mentorship and cluster and Cooperative formation (Shaun Brown, Lukhanyo and myself)	Melton Mulaudzi
21/09/2011	Fort Beaufort Simillington Hotel / East London Airport	Accompany Mr Lukhanyo to airport	Melton Muaudzi
22/09/2011	Alice Kat – Blinkwater	Attending the cluster meeting with farmers who pack their fruits through Eden services.	Melton Mulaudzi
23/09/2011	Riverside Training Centre	Attending meeting between Metula Gladys and Riverside management regarding the recapitalisation.	Melton Mulaudzi
23/09/2011	Crossings	Meet Arend Venter and Bruce Andrews discussing the Transformation project of the FPEF.	Andrew Mbedzi
24/09/2011	Mpumalanga Govt. Offices	Meet the Chief Director discussing the Transformation project of the FPEF.	Andrew Mbedzi
27/09/2011	Sundays River Valley Sun orange Farm, Willow tree farm	Organizing beneficiaries to be part of Sundays River Citrus Study group.	Melton Mulaudzi
28/09/2011	Mpofu Training Centre	Attending food safety short course organised by SAPI 2 under PPECB programme	Melton Mulaudzi
29/09/2011	Battlesden farm Alice	Beneficiaries were busy applying the kohinor 350 sc at the rate of 10ml per liter. Advised to use protective clothing	Melton Mulaudzi
03/10/2011	Giyani	Re-planning for the Mopani Citrus Study Group sessions.	Andrew Mbedzi
04/10/2011	Makhado	Discussions on the Chalteka Community Farm (Dendron) cultivars.	Andrew Mbedzi
04/10/2011	Fort Hare University	Attending PPECB transformation farmer's field day	Melton Mulaudzi
05/10/2011	Peddie Citrus Farms	Monitoring bollworm	Melton Mulaudzi
06/10/2011	Masase, Naboomspruit.	Waterberg Citrus Study group session 2 on pests and disease, scouting and management (Control).	Andrew Mbedzi
11/10/2011	Zebediela Citrus Estate, Mokopane.	Government Extension Officers Training Workshop on Pests Control.	Andrew Mbedzi
13/10/2011	Easy Farm, Ravele and Masakona CPA's.	Fresh Produce Exporters Forum (FPEF) export program for emerging growers.	Andrew Mbedzi

13/10/2011	Patensie Tobacco offices	Attending citrus study group regarding FCM control by Martina Ordendaal and Citrus Black spot by Melton.	Melton Mulaudzi
14/10/2011	Riverside, Katco, Alice.	Organizing speakers for study group on the 25 <sup>th</sup> October 2011 at Mpofu training centre	Melton Mulaudzi
16/10/2011	Nkwaleni Valley	Visiting Nkwaleni Valley citrus farms and Dept. Of Agric offices	Andrew Mbedzi Melton Mulaudzi
17/10/2011	Nkwaleni Community Hall	Formation of Nkwaleni Valley Citrus Study group and technical committee	Andrew Mbedzi Melton Mulaudzi
18/10/2011	NKwaleni Valley	Visiting the farm and identifying citrus Black Spot and Sanitation challenges.	Andrew Mbedzi Melton Mulaudzi
20/10/2011	Letsitele Valley, Tzaneen.	Mopani Citrus Study group session 2 on citrus pests and diseases management.	Andrew Mbedzi
20/10/2011	Alice Kat- Jerico-Eden-Konzi-Gonzana	Monitoring bollworm	Melton Mulaudzi
21/10/2011	Bonnes Esprance, Dendron	Preparations for the plantings of the citrus plants with Chalteka community members and Makhado officers.	Andrew Mbedzi
24/10/2011	Alice Kat –Topkat-Greenwood- Letas-White citrus farm and Torties	Monitoring bollworm but garden snails have been identified at Topkat farm.	Melton Mulaudzi
25/10/2011	Mpofu training centre	Attending citrus study group concerning FCM control by Llew Roberts and Citrus Black Spot by Melton.	Melton Mulaudzi
26/10/2011	Torties,TopkatOakdene	Doing mentorship evaluation with Kat River farmers. Dr Bates recommended that the farmers should attend a Financial course.	Melton Mulaudzi
27/10/2011	Orange Grange-Lovers retreat-Battleden	Farmers and Riverside were discussing the handling of financial statements.	Melton Mulaudzi
03/11/2011	Agri-Village, Polokwane	Planning the Limpopo Provincial Citrus Technical Committee meeting that will take place on 1 <sup>st</sup> of December 2011.	Andrew Mbedzi
03/11/2011	Port Elizabeth Greenacres office	Attending meeting at DRDLR about farms on red as indicated on list submitted to Elton of DRDLR.	Melton Mulaudzi
04/11/2011	Makwarela, Vhembe	Vhembe Citrus Technical Committee meeting on the planning of the Vhembe Citrus Information Day.	Andrew Mbedzi
07/11/2011	Torties ,White citrus farm and Oakdene	Monitoring mainline irrigation at Torties farm and Oakdene.	Melton Mulaudzi
08/11/2011	Alice Kat Country Club- Gonzana farm-club Greenwood	Organizing farmers to attend financial short course organized by citrus academy on behalf of Agriseta	Melton Mulaudzi
09/11/2011	Country Club	Attending financial course organized by citrus academy	Melton Mulaudzi
10/11/2011	Nkwaleni Community Hall	Attending formation of study group and citrus technical committee for Nkwaleni Valley (Phase 2)	Andrew Mbedzi Melton Mulaudzi

14/11/2011	Torties, white citrus farm, Konzi, Eden,Green wood	Monitoring hail damage which occurred on the evening of the 13 <sup>th</sup> November 2011	Melton Mulaudzi
15/11/2011	Jordan Farm and Oakdene farm	Monitoring hail damage on bearing citrus and non-bearing trees.	Melton Mulaudzi
17/11/2011	Fort Hare	Attending meeting regarding planning of 2012 training offered by PPECB.	Melton Mulaudzi
18/11/2011	Makwarela, Vhembe	Vhembe Citrus Technical Committee meeting on the finalization of planning Vhembe Citrus Information Day to take place at Makhado Show-ground on 7 <sup>th</sup> of December 2011.	Andrew Mbedzi
18/11/2011	White, Jordan, Konzi, Jerico, Eden , Torties Jerusalem, Greenwood, and Oakdene	Monitoring and evaluation hail damage with Government Extension officer and a report was submitted to government	Melton Mulaudzi
22/11/2011	Fort Beaufort East London Air Port - Nespruit	Attending the 10 yrs anniversary of Citrus Research International	Melton Mulaudzi
24/11/2011	Nespruit	Attending the 10 yrs anniversary of Citrus Research International	Melton Mulaudzi
28/11/2011	PE DRDLR Offices	Meeting with Vivian Loest from DRDLR, Mr Nyokana from Dept of Agric. and Lukhanyo from CGA discussing recap and rescue plan from the Dept	Melton Mulaudzi
29/11/2011	Patensie Tobacco office	Explaining differences between Recap and Rescue plan to Board Member Phillip and the farmers.	Melton Mulaudzi
01/12/2011	Temo-Tower, Polokwane	Limpopo Provincial Citrus Technical Committee meeting on citrus farmers challenges.	Andrew Mbedzi
04/12/2011	Fort Beaufort-Blink water –East London	To attend farm visit and study group about formation of technical activities.	Melton Mulaudzi
05/12/2011	Thulwane farm, Rodney Mbuyazi, Vambela farm, Maweni and Intathakusa coop	Doing farm visit with government Extension officers and farmers. Encouraging farmers to sustain their citrus farm and also discussing government packages with them.	Melton Mulaudzi
06/12/2011	Bela-Bela, Warmbath.	Attend Waterberg Citrus Study group session 3 on fruit quality and Irrigation of citrus.	Andrew Mbedzi
06/12/2011	Nkwaleni Community hall	Planning the study group sessions and topics for the 2012 season.	Melton Mulaudzi
07/12/2011	Makhado Show-ground, Makhado.	Attend Vhembe Citrus Information Day on citrus establishment.	Andrew Mbedzi
07/12/2011	Richmond KZN	Attending farm assessments regarding the oranges still hanging in the orchard	Melton Mulaudzi
08/12/2011	Bennde Mutale.	Replanting of Bennde Mutale project and the planning of new cultivars.	Andrew Mbedzi
09/12/2011	Xikundu, Malamulele.	Follow-up on the Phytophthora challenge that was detected on small trees on the previous visit.	Andrew Mbedzi

12/12/2011	Alice KAT Office Fort Beaufort	Funding meeting with Mrs Nokulunga (PPECB), Shaun Brown, Mr Gongxeka (DRDLR) and growers.	Melton Mulaudzi
13/12/2011	SRCC office, SAFE P/house and Patensie	Recap funding meeting with Nokulunga (PPECB), beneficiaries at SRCC, Acting Manager Elton, Lukhanyo and Melton	Melton Mulaudzi
14/12/2011	PE- King Williams Town- FB	Accompany Lukhanyo to King Williams Town after meeting at Patensie Tobacco offices	Melton Mulaudzi
16/01/2012	Jerico, Konzi and Eden farm	Monitoring and assessing fruits damage by hail during November 2011	Melton Mulaudzi
17/01/2012	Country club	Preparing the Launching of Standard bank cluster group with Shaun Brown and Lena from Agric Academy.	Melton Mulaudzi
18/01/2012	Country club	Launch of cluster group with Mr Shaun Brown, Farmers from former Safe group and Capespan group.	Melton Mulaudzi
24/01/2012	Bennde Mutale	Assessment of Bennde Mutale Citrus Trees and Irrigation System.	Andrew Mbedzi
25/01/2012	DRDLR-Polokwane	Mentorship meeting between Dept. of Land Reform and CGA/CRI	Andrew Mbedzi
26/01/2012	Makwarela	Vhembe citrus technical committee meeting discussing assessment of Bennde Mutale and Study group.	Andrew Mbedzi
27/01/2012	Easy Farm	Vhembe citrus study group session 4 on FF and FCM	Andrew Mbedzi
30/01/2012	Willow Park JHB	Launch of Fruit of Success Book and Chamber meeting.	Andrew Mbedzi Melton Mulaudzi
01/02/2012	Sandton City	FPEF Initiative meeting with Lukhanyo and Arend Venter.	Andrew Mbedzi
06/02/2012	LED office, Polokwane	Sunningdale and Gillimburg meeting with Agriseta, LDA, LIMA, CGA/CRI and KPMG	Andrew Mbedzi
06/02/2012	Oakdene and Topkat Farm	Monitoring damage caused by garden snail at Oakdene farm. Attending vandalism of Hydrants at Topkat by community members.	Melton Mulaudzi
7/02/2012	Luthando Farm in SRV	Technical committee meeting with growers and Govt. E.O's planning study group session for 2012.	Melton Mulaudzi
08/02/2012	KZN/ Maweni	Discussing the financial status of Maweni farm and how to involve the project/farms into Recapitalisation programme with Lukhanyo, DRDLR and Beneficiaries	Melton Mulaudzi
13/02/2012	Hankey Pack house	Meeting with Klein Hoewe Boer Farm, Jaco and Khaya Katoo to discuss CBS at Klein Hoewe boer farm.	Melton Mulaudzi
14/02/2012	Patensie Tobacco office	Meeting with farmers to discuss the 2012 activities plan for Citrus study group	Melton Mulaudzi
14/02/2012	Tshipise	Citrus Pack-house Workshop	Andrew Mbedzi
15/02/2012	Tshipise	Citrus Pack-house Workshop	Andrew Mbedzi
16/02/2012	Loskop Dam	Citrus Pack-house Workshop	Andrew Mbedzi

16/02/2012	Eden Services	Meeting with Chinese, Shaun Brown and the farmers pertaining organic farming Markets.	Melton Mulaudzi
17/02/2012	Loskop Dam	Citrus Pack-house Workshop	Andrew Mbedzi
18/02/2012	Chauke's Farm	FPEF Initiatives and Checking water availability.	Andrew Mbedzi
20/02/2012	Edene Agri-service	Transformation meeting on pre-harvesting preparation of citrus fruit	Melton Mulaudzi
	Patensie	CGA Road show	Melton Mulaudzi
21/02/2012	Khangela Farm and Green Gables	Extension Services	Melton Mulaudzi
	Sundays River High School	Attending CGA Roadshows	Melton Mulaudzi
22/02/2012	Katco KAT River Valley	Attending CGA Roadshows	Melton Mulaudzi
23 to 24/02/2012	Summer Strand Hotel PE	Packhouse workshop	Melton Mulaudzi
28/02/2012	Lovers Retreat Farm, Gonzana farm and Battleden	Monitoring newly planted trees and advised them to put boards with cultivar planted, Rootstock used and the year the trees have been planted	Melton Mulaudzi
05/03/2012	Maweni farm and Vernon Tocknell	Discussing the challenges faced by Maweni project regarding irrigation pumps.	Melton Mulaudzi
06/03/2012	Ohrigstad and Letsitele	FPEF Initiatives-(briefing selected candidates)	Andrew Mbedzi
06/03/2012	Nkwaleni Community Hall (KZN)	Attending study group on control of BI, Fruit fly, FCM and soil and Leaf analysis with Dr. Aruna and Mr John Henry from CRI Nelspruit	Melton Mulaudzi
07/03/2012	Polokwane	FPEF Initiatives-(briefing selected candidates)	Andrew Mbedzi
07/03/2012	East London (ICC)	Attending Extension summit with Government Extension officers regarding the planning activities for 2012 projects in the Eastern Cape in connection with funding	Melton Mulaudzi
08/03/2012	Levubu	Coordination of BI Information Day	Andrew Mbedzi
08/03/2012	Sundays River Valley Willow tree farm	Attending Citrus Study Group with Wayne Kirkman on BI control, Fruit fly control, FCM, White woolly flies and soil and leaf analysis.	Melton Mulaudzi
09/03/2012	Patensie Tobacco office	Attending Citrus Study Group with Wayne Kirkman on BI control, Fruit fly control, FCM, White woolly flies and soil and leaf analysis	Melton Mulaudzi
12/03/2012	Torties farm , Konzi farm, Eden farm and Jerico farm	Monitoring bud mites on fruits	Melton Mulaudzi
13/03/2012	Riverside Training Centre & Willow Tree Farm	Meeting farmers at Kat River and Sundays River Valley to discuss establishing a pilot project regarding funding and training with Phistos from NAMC and Dawie Schotz	Melton Mulaudzi

19/03/2012	East London Air Port and Cape College	Attending Recapitalisation meeting with Mrs Peliwe and Mr Ncedisa, Citrus farmers, Govt. E.O's, Colin Painter and Shaun Brown.	Melton Mulaudzi
20/03/2012	Sihlangule Nkomashe Co-op	Attending presentation by Lukhanyo and handing over the manuals and CDs to the cooperative.	Melton Mulaudzi
26/03/2012	Polokwane	Discussion of the LDA 2012 Extension plan, Study Group, Information days and Citrus Field days	Andrew Mbedzi
27/03/2012	Nylstroom	Planning of the Waterberg citrus study groups and attendance of CRI citrus symposium.	Andrew Mbedzi
27/03/2012	PE Coega IDZ	Attending PPECB pre-season meeting with pack house managers, PPECB Officials and Farmers	Melton Mulaudzi
28/03/2012	Letsitele and Tarentaalrand	Improving relationship between farmers and extension officers	Andrew Mbedzi
28/03/2012	Hankey Pack house	Attending CBS meeting with Government officials from Daff , Provincial officials, CRI officials, CGA, Kouga Municipality officer from LED Office, Jaco and Farmers	Melton Mulaudzi
29/03/2012	King Williams Town	Presentation by Lukhanyo to Government official regarding the mentorship.	Melton Mulaudzi
30/03/2012	Grahastown	Accompany Lukhanyo to get transport from Grahamstown to PE	Melton Mulaudzi

## 8.5 Research Priorities for 2012

The research priorities for 2012 were determined during June – July 2011. It differed from the previous surveys in two ways, viz:

(1) A letter was sent to all the citrus producers who are on the CRI's Technology Transfer Group's (TTG) lists. In this writing all the Projects that were approved for research during 2011 were listed. It therefore for the first time gave the growers insight into which of their previous requests was actually addressed. Growers were requested to study these projects and to determine what additional research needed in their area still needed to be addressed. They should give it a weight from 1-3 where 3 would be the more urgent end of the scale. These research needs should then be forwarded to the Chairman and the Technical committee of each TTG.

(2) Once the Technical Committee had received all the research requests from the area they summarized it and a meeting was held between the Area Extension Manager and the Technical committees. The Area Extension Managers from the North and the South compiled the research needs and forwarded it to the Manager Research and Extension.

The research priorities were also determined at the five Packhouse study group meetings as well as the Exporters Technical Panel and the growers involved in the Transformation process. After the research projects were approved the Navel Forum called for a meeting during which additional research needs were determined. These needs will have to stand over until the next round of priorities.

The Research Priorities can be summarized as follows:

### 1. Disease Management

#### 1.1 Citrus Black spot

- In the past the highest priority was to ensure that the status of CBS was changed from a phytosanitary problem to a cosmetic problem. This required that certain research that was completed should be

published in refereed journals. The aim is still the same but the way in which it could be achieved was changed. The request by growers is that the USA should be opened up for all citrus producing areas in South Africa, with the hope that the EU would then accept the fact that fruit do not pose a threat as a pathway to spread the disease.

- Develop alternative spray programmes which is even more effective than the current programmes, if possible cheaper and that can be used to prevent resistance developing. It would be even better if these programmes could control *Alternaria* as well and if the oil used in these sprays could be replaced by other alternatives.
- Determine the critical period for CBS infection in the Eastern Cape.
- Develop alternative strategies to interrupt the diseases life cycle. This includes the destruction of inoculum (dead leaves) or genetic manipulation to build in resistance genes.

#### 1.2 Alternaria

- Alternative spray programmes to control *Alternaria* more effective with less numbers of sprays and more effective spraying techniques to reduce the volume of water needed to apply the chemicals effectively
- Screening of all new cultivars for *Alternaria* tolerance.

#### 1.3 Botrytis

- Spray programmes to control *Botrytis* on lemons during flowering.

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#### 1.4 Phytophthora citrophthora

- More effective control programmes.
- Screening of all new cultivars against *P.citrophthora*.

#### 1.5 Post – Harvest diseases

- Optimisation of fungicide treatments in the packhouse to be the most effective to protect the fruit and to prevent resistance developing.
- Optimizing the GRAS chemicals such as sodium bicarbonate, especially in an imazilil protection programme. (pH correlations, concentration, temperature, exposure times etc.)
- Develop techniques to use the quaternary ammonium products safely for exports to Japan.
- Development of wax standards.
- Registration of potassium phosphonate against *Phytophthora* brown rot.
- Alternatives for Guazatine.
- Determine the rate of residue breakdown of all products used in post harvest treatments.
- Determine the viability to pack citrus under certain conditions as chem. Free without causing decay problems.
- Control options for *Rhizopus*.

#### 1.6 Phytophthora root and collar rot

- Alternative control options.
- Screening of new rootstocks against *Phytophthora*
- More effective and safer phosphonate treatments
- Determine the effect of compost teas and commercially applied microbial applications against *Phytophthora* root rot.

#### 1.7 Citrus nematode

- Evaluation of pre-plant fumigation products on replant soils.
- Alternative control options (e.g. Imidacloprid?).

#### 1.8 Armillaria root and collar rot

- Develop control options for *Armillaria*.
- Test all commercial and experimental citrus rootstocks against *Armillaria*.

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#### 1.9 Root health

- Develop a more holistic approach to root health in general.

#### 1.10 Citrus Tristeza Virus

- Optimising cross protection.
- Evaluation of different cultivars in either the selection or suppression of different CTV strains.

#### 1.11 Citrus greening (Huanglongbing)

- Monitoring the spread of African greening towards the Eastern Cape citrus producing areas.
- Monitoring Kwa Zulu Natal for a possible introduction of Asian greening.
- Develop methods to cure greening infested trees.
- Study the role of alternative hosts in the epidemiology and spread of greening.
- Search for greening resistance through embryo rescuing.
- Search for greening protection using mild strain CTV.
- Investigate the transmission and infection of *Candidatus Liberibacter africanus* at different times of the season.

#### 1.12 Viroids

- Ensure that the CFB is free of all graft transmissible pathogens including the viroids.
- Study the effect of viroids on the horticultural characteristics of different cultivars.

## 2. **Integrated Pest Management**

### 2.1 False Codling Moth

- Develop more effective control methods. This includes the optimization of the SIT programme, more effective use of the granuloviruses, commercializing the entomopathogenic nematodes and the entomopathogenic fungi.
- Develop alternative mating disruption systems.
- Develop amelioration techniques to control FMC in fewer days during cold sterilization.
- Develop techniques to detect FMC on the packline.
- Develop techniques to enhance the release of parasites late in the season.

### 2.2 Fruit fly

#### 2.2.1 *Bactrocera invadens*

- Monitoring of *Bactrocera invadens* in South Africa, Zimbabwe, Botswana and Mozambique.
- Develop a risk mitigation strategy to be able to export citrus from areas where *Bactrocera* was detected. (on an orchard to orchard basis).
- Get all SADC countries together to deal with *Bactrocera* together. Get Zimbabwe to acknowledge the existence of the pest in that country.

#### 2.2.2 Mediterranean and Natal fruit fly

- Develop a more attractive attract and kill option than the M3 that will last as long but will reduce the number of traps /ha.
- Develop a M3 with both a male and a female attractant.
- Study the role of entomopathogenic nematodes and – fungi on fruit fly larvae in the soil.
- Register Spinosine to be used with Hymelure.

### 2.3 Mealybug

- Develop alternative control methods against mealy bug to replace products such as Dursban, Applaud, Ultracide and Tokuthion.
- Find biocontrol agents that can be used against mealy bug.
- Determine the possible role of entomopathogenic nematodes against mealy bugs.

### 2.4 Carob moth

- Study the morphology and control of carob moth in citrus.
- Study the effect of EPNs on carob moth.
- Monitoring of carob moth.

### 2.5 Fruit sucking moths

- Control of fruit sucking moths.

## 2.6 Leafhoppers

- Develop methods to control leafhopper.

## 2.7 Lepidopteran pests

- Determine the effect of imidacloprid on lepidopteran pests in citrus.

## 2.8 Ants

- Develop and commercialize ant baits for both pugnacious and brown house ants.

## 2.9 Thrips

- Develop alternative control options for thrips (Abamectin is overused but a registration @ 30 ml/100 litre is needed).

## 2.10 Mites

- Registration of a generic for Mitigate to reduce its price.
- Alternative control options to replace Acarol which is affordable and effective.

## 2.11 Red scale

- Alternative control options for imidacloprid especially on heavier soils.

## 2.12 Snails

- Control options more affordable than Moloxide.

## 2.13 Slugmoth

- Epidemiology and control.

## 2.14 Woolly white fly

- Alternative control options.

# 3. **Crop and Fruit Quality Management**

## 3.1 Rind condition

### 3.1.1 Peteca

- Effective control measures.

### 3.1.2 Creasing

- Effective control measures.
- Better understanding of the physiology of creasing.

### 3.1.3 Chilling injury

- Develop post harvest treatments to control chilling injury.

### 3.1.4 Rind breakdown

- Develop better methods to predict and control rind breakdown.
- Need alternatives for TBZ.

### 3.1.5 Blossom end clearing

- Develop a better understanding of the problem and ways to prevent it.

### 3.1.6 Cold damage

- Develop techniques to protect fruit going into steri-markets better.

### 3.1.7 Shelf life

- Develop techniques to extend the shelf life of citrus fruit.
- Method to quantify over ripeness and puffiness .

### 3.1.8 Tear staining

- Determine the cause of tear staining on Nadorcotts and how to prevent it.

### 3.1.9 Silica

- Determine the role silica can play to reduce all of the above damages.

## 3.2 Fruit Production & Quality

### 3.2.1 Flowering

- Alternating flower on Morr, orrs and Nadorcotts a problem.

### 3.2.2 Fruit set

- Fruit set a huge problem on Eureka Seedless! But also on Navels in the Eastern Cape and Deltas and Midnights in many citrus areas. Also with TSR in Nkwaleni. Effect of products such as Reflecto, Silica, Kaoline and Shade cloth on fruit set.
- 3.2.3 Fruit colour
  - A major problem in all the early varieties especially in the north.
- 3.2.4 Regrowth
  - A major problem especially when fruit set was poor. Need to register products such as Sunny, Cultar or Regalis to be tested.
  - Determine the MRLs for plant growth stimulants.
- 3.2.5 Pruning
  - Pruning techniques on late mandarins and lemons need to be developed.
- 3.2.6 Fertilisation
  - Recommendations needed to increase carbohydrate levels quicker after harvesting a large crop.
  - Manipulation of fertilization to decrease rind problems.
  - Role of humic and fulvic acids.
  - Role of silica.
  - Timing of first N applications on different cultivars.
  - Effect of Kelp products on rind integrity.
  - The influence of different formulations on the foliar uptake of elements
- 3.2.7 Internal quality
  - Ways to drop the acid levels.
  - Ways to increase the acid levels
  - Optimization of internal quality under OHS systems.
- 3.2.8 Sheepnose
  - Climate effect on sheep nose of grape fruit.
  - Effect of shade netting on sheep nose.
- 3.2.9 Cold damage
  - How to prevent frost damage (Frost Bite, Copper Silica etc).
  - Influence of rootstocks on frost damage.
- 3.2.10 Sunburn
  - Methods to reduce sun burn.
  - Alternatives for oil to reduce sun burn on grapefruit.
- 3.2.11 Fruit color
  - Methods to improve fruit color.
  - Methods to initiate earlier color.
  - Methods to intensify fruit color to prevent cold damage during cold steri.
- 3.2.12 Fruit size
  - Methods to increase fruit size on Deltas, Clementines and Rustenburg navels.
- 3.2.13 Water usage
  - The Water Research Council is looking at the water usage of the different fruit crops. They gave CRI the opportunity to be involved. This is important as this could affect water quotas in the future.
- 3.3 Cold Chain and Packaging
  - 3.3.1 Cold Chain Management
    - Investigate optimum shipping temperature and RH to control waste.
    - Updated manual annually for decay control (Production Guidelines & Booklet).
    - Publish article on Supervent carton in SAFJ.
    - Set time and temperature protocols for new varieties.
    - Investigate the correlation between variation in temperature on vessels and decay.
    - Determine the effect of cold sterilization on Star Ruby grapefruit to the USA.
    - Determine effect of forced air cooling on rind disorders.
    - Determine influence of loading at room temperature on decay and shelf life.
    - Determine the effect of wrapping of fruit on rind disorders and shelf life.
    - Determine optimum pre-cooling temperature to prevent excessive condensation during handling in port and loading of vessels.

- Determine optimum rate of cooling to restrict rind disorders.
- Investigate the variation and influence of temperature and humidity during transport with Tautliners vs flat bed trucks.
- Determine maximum CO<sub>2</sub>-levels during shipping.

### 3.3.2 Packaging and Palletizing

- Evaluate new pallets en set minimum specifications for pallets, including fungal en pest treatments. Ongoing project.
- Find alternative material to wood for manufacturing of pallets. Ongoing.
- Investigate stronger board combinations to replace end pieces. Ongoing.
- Set handling guidelines for all aspects of the cold chain. Ongoing.
- Set guidelines and specifications with photos for palletizing of all cartons (strapping, securing sheets, corner pieces, etc).
- Set guidelines to stabilize pallets on trucks.
- Evaluate different sizes and types of corner pieces. Ongoing for new corner pieces.
- Determine die influence of hi-cube pallets on physical losses throughout the cold chain.
- Accreditation process for packaging manufactures and service providers in the cold chain should be implemented.
- Set handling guidelines to prevent walking on pallets during loading of vessels and trucks.
- Use of short corner pieces instead of end pieces in open tops should be investigated.
- Set handling guidelines for all aspects of the cold chain. Cooling Working Group to finalize.
- Evaluation of fruit in Supervent cartons under cold sterilization. Ongoing.
- Develop control options for wood rotting fungi on pallets.
- Conduct a survey to determine the need for compliance audits.
- Investigate claims from certain overseas markets for under weight cartons.
- Investigate problems with wide range and duplication of carton codes.

## 4. **Cultivar Development**

### 4.1 Rootstocks

- More suitable rootstocks for high pH soils in general
- More suitable replant rootstocks for high pH soils
- More suitable dwarfing rootstocks on high pH soils
- Lemon Cultivar/ Rootstock trial for the Sundays River Valley
- Reintroduction of Flying Dragon from San Miguel to be tested on heavier soils.
- Evaluation of Argentinean rootstocks.

### 4.2 Cultivars

- Earlier and later Satsumas.
- Earlier and later Clementines.
- Late mandarins of which the plantings is not restricted.
- Late mandarins for the hotter areas.
- Navels that yield better with acceptable fruit size.
- Early navel with round fruit with good yields.
- Earlier and later Star Ruby selections.
- Early grapefruit which is not prone to sheeppnose.
- A better tasting red grapefruit.
- Earlier and later Valencia selections.
- Olinda Valencia to be reintroduced to the Foundation Block.

## **Summary of method in which research proposals are covering research needs**

### Disease Management Programme

- This programme is covering most of the requests put forward by the industry. The one request that was a number 1 priority and that was not met was the request by the Marble Hall/ Groblersdal area that there should be a comparison between material infected with diseases such as viroids and material that is disease-free to establish if for example earlier fruit colour was not induced by the stress caused by a

pathogen in the original material. This matter has been discussed in depth by the CIS committee as well as with world leaders such as Prof Bar Joseph from Israel, Dr Pat Barkley from Australia and Prof John da Graca from Texas and it was decided that no harmful pathogens will be allowed to form part of the CIS and therefore this research would be a waste of money.

- With regard to Post-Harvest decay the most important point set by the Packhouse Study Groups that is not addressed is to determine the rate of residue breakdown of the products used for post-harvest control.
- *Armillaria* is also not addressed sufficiently but that is because it is only a priority in a few areas.

#### Integrated Pest Management Programme

- As is the case with Disease Management the research proposals to cover the producers' research needs are brilliant. There can be no other citrus producing country where so much research is conducted by so few to cover so many pest problems which are threatening the future existence of the citrus industry.
- The way in which problems such as *Bactrocera* and FCM are addressed are much appreciated by the industry and though these problems have not yet been solved the progress made is appreciated.

#### Crop and Fruit Quality Management

- This is the Programme where there has been both major progress, e.g. with peteca and other rind disorders, but it is also the programme where not enough has been achieved over the last couple of years because of the focus being on Market Access issues and not enough investment in this programme. Fruit set is still not adequately addressed; creasing is still a major issue whereas the Onderberg can lose 40% of its grapefruit crop in certain years because of sheeplouse which is not addressed. Pruning of the late mandarins and lemons are not addressed and sunburn is still an issue. Cold damage in the orchard is addressed in one trial which is not enough. Frost damage has caused crop losses during three out of the last five years in some of the Limpopo districts. It is the Waterberg's no1 priority but no official trials are planned in that area. Colour initiation, fruit size and poor internal quality are also aspects that need more inputs.
- The CRI-CCF is perhaps one of the areas where most of the issues which are not addressed can be found. The appointment of Dawid Groenewald will hopefully help to solve this problem.

#### Cultivar Evaluations

- As the CGA has created a new company to do Cultivar Development, the CRI will in future only focus on Cultivar Evaluation. As new cultivars become available they will be included in the Cultivar Evaluation programme and the results are available to growers in the form of the Cultivar Fact Sheets. This programme is also producing the goods in spite of its small staff component.

8.6 THE RELATIVE FUNDING SUPPORT FOR RESEARCH PROGRAMMES AND PROJECTS FOR 2011-2012  
 By Tim G Grout (CRI)

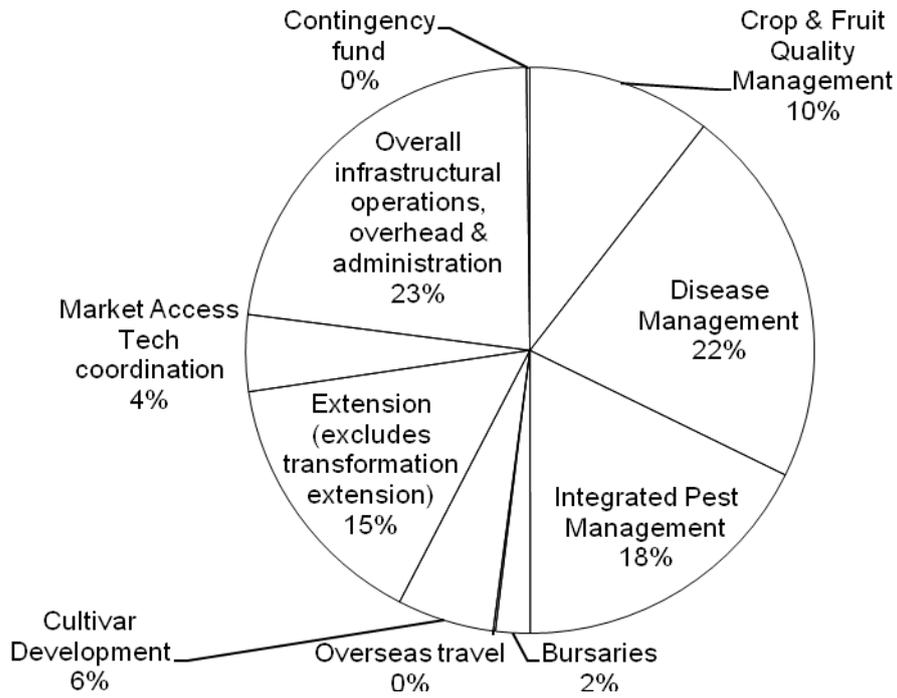


Fig. 8.6.1. Percentage funding in each CRI programme and rest of budget for 2011-12.

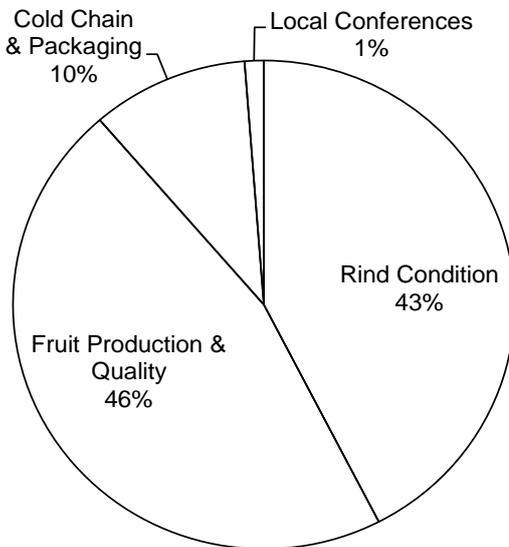
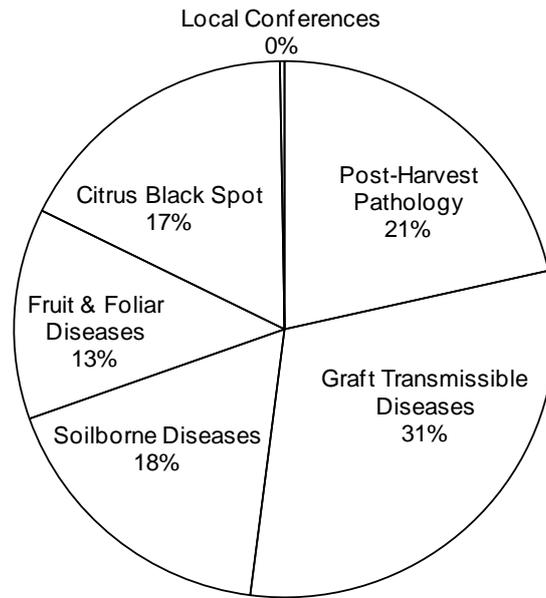
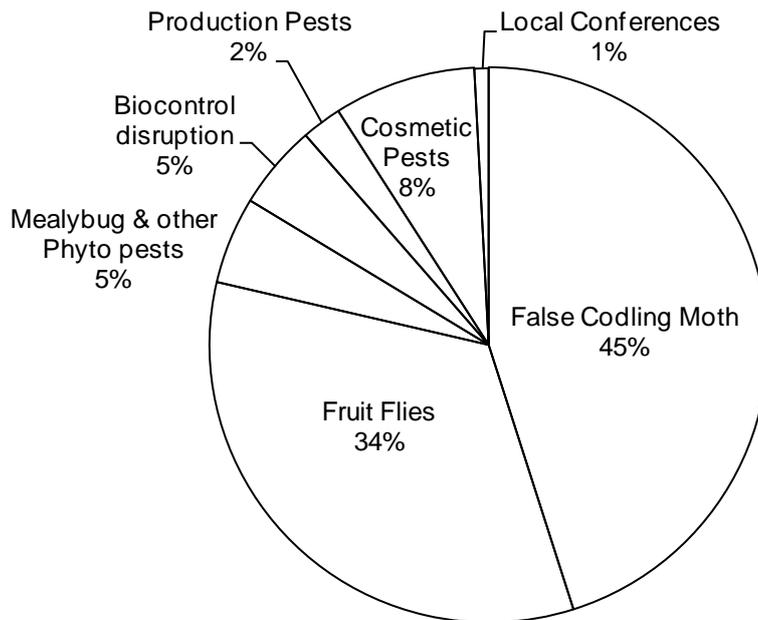


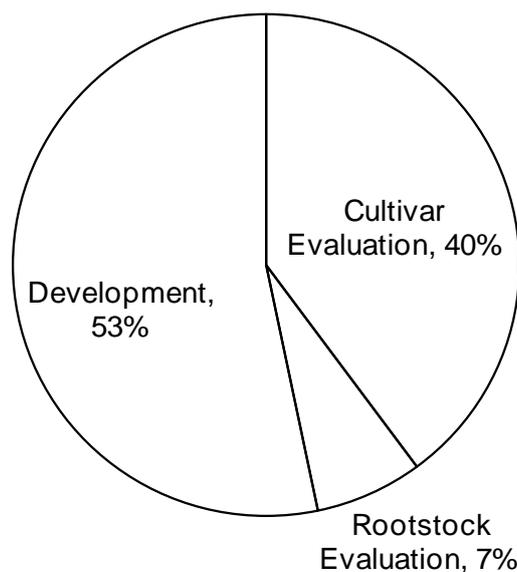
Fig. 8.6.2. Percentage funding to projects in the CRI research Programme: Crop and Fruit Quality Management for 2011-12.



**Fig. 8.6.3.** Percentage funding to projects in the CRI Research Programme: Disease Management for 2011-12.



**Fig. 8.6.4.** Percentage funding to projects in the CRI research programme: Integrated Pest Management for 2011-12.



**Fig. 8.6.5.** Percentage funding to projects in the CRI Research Programme: Cultivar Development and Evaluation for 2011-12.

#### 8.7 EXTENSION PRESENTATIONS BY CRI RESEARCHERS IN 2011-12

Name	Date	Place	Topic
Cronjé, PJR	Feb 2012	Packhouse Workshops: Gordonsbaai Letsitele Loskopdam Port Elizabeth	Influence of packhouse practices on rind condition
	16-20 Jan 2012	SASHS, Skukuza	<ul style="list-style-type: none"> <li>- Postharvest rind disorders of 'nardocott' Mandarin are affected by rootstock in addition to postharvest treatments</li> <li>- Novel usage of 2,4-D to increase citrus fruit quality</li> <li>- Phenology of alternate bearing 'nardocott' Mandarin trees</li> <li>- Postharvest application of thiabendazole reduces chilling injury of Navel citrus fruit</li> </ul>
Erasmus, A.	Feb 2012	Packhouse Workshops: Gordons Bay Letsitele Loskop dam Port Elizabeth Eshowe	Fungicide application & residue loading Fungicide options & resistance management Management of critical control points
Fourie, P.H.	Feb 2012	Packhouse Workshops: Gordons Bay Letsitele Loskop dam Port Elizabeth Eshowe	<ul style="list-style-type: none"> <li>- Three presentations: Swamdoderaanwending en residu-lading</li> <li>-Swamdoder-opsies en weerstandsbestuur</li> <li>-Bestuur van kritiese beheerpunte</li> </ul>
Grout, T.G.	15 Aug 2011	Malelane	Spring pest complex
	16 Aug 2011	Nelspruit	
	18 Aug 2011	Tshipise	
	19 Aug 2011	Mookgopong	

	23 Aug 2011	Sundays River Valley	Thrips management
	25 Aug 2011	Hoedspruit	Spring pest complex
	31 Aug 2011	Burgersfort	
	2 Sep 2011	NAFCO, Groblersdal	
	8 Sep 2011	Tambuti, Swaziland	
	13 Sep 2011	Harare, Zimbabwe	Citrus IPM
	13-15 Feb 2012	KZN, Mpumalanga	CGA road show
	20-24 Feb 2012	Limpopo, Zimbabwe	
Joubert, J.	26 Jun 2011	UK visit to CRI, Nelspruit	Presentation on pigmented fruit and grapefruit
	30-31 Jul 2011	Joubert & Seuns	Cultivar selections talk
	18 Aug 2011	DAFF	Presentation on Valencias and lemon selections for new development
	01 Sept 2011	Polokwane government	Presentation on pigmented midseason oranges (Tarocco, etc.)
	07 Sept 2011	Stellenbosch	Presentation at Navel Focus Group – Internal Quality with low acids discussion
Kirkman, W.	08 Feb 2011	Addo	SIT – Grower meeting
		Kirkwood	SIT – Grower meeting
	07 April 2011	Patensie	FCM, Fruit Fly & psylla – BEE grower meeting
	29 Sept 2011	Patensie PSB	Spring pest complex – Grower meeting
	29 Sept 2011	Patensie Private	Spring pest complex – Grower meeting
Lesar, K.H.	Feb 2012	Packhouse Workshops: Gordons Bay Letsitele Loskop dam Port Elizabeth	Amended version: Overview of Post-harvest diseases Pre- and Post-harvest handling of sanitation for decay control Degreening Guidelines
Manrakhan, A.	06 Mar 2011	Nkwaleni	Fruit Fly & <i>B. invadens</i> control
	02 Sept 2011	Groblersdal	Nafco
Moore, S.D.	14 Apr 2011	Fort Beaufort, BEE grower meeting	FCM, Fruit fly and psylla
	03 Jun 2011	Patensie (Chinchfords and Sainsburys)	IPM in citrus
	08 Jun 2011	Kirkwood, Grower study grp	FCM
	20-21 Jun 2011	Addo, Citrus Academy video production	IPM in citrus
	18 Aug 2011	Addo, BEE grower meeting	Spring pest complex
	23 Aug 2011	Addo, Grower meeting	FCM and thrips
	12 Sept 2011	Swellendam, Grower meeting	FCM and spring complex
	13 Sept 2011	Riebeek's Kasteel, Grower meeting	FCM and spring complex
		Citrusdal, Grower meeting	FCM and spring complex
	14 Sept 2011	Kakamas, Grower meeting	FCM and spring complex
	15 Sept 2011	Vaalharts, Grower meeting	FCM and spring complex
	19 Sept 2011	Fort Beaufort, Grower meeting	FCM and spring complex
	20 Sept 2011	Nkwaleni, Grower meeting	FCM and spring complex
	21 Sept 2011	Richmond, Grower meeting	FCM and spring complex
	22 Sept 2011	Letsitele, Grower meeting	SIT for FCM
	12 Oct 2011	Zebediela, BEE grower meeting with Citrus Academy	Pest monitoring course
Pretorius, M.C.	20 Apr 2011	Port Elizabeth	Greening and Armillaria meeting

	17 May 2011	Robertson	Investigate Armillaria problems on Clementines
	26 May 2011	Nelspruit	CIP Meeting
Schutte, G.C.	31 Aug 2011	Burgersfort	CBS
	2 Sept 2011	Nafco, Groblersdal	CBS
	6 Sept 2011	Letsitele	CBS
	8 Sept 2011	Malelane	CBS
	15 Sept 2011	BASF Boeredag	CBS
	19&20 Sept 2011	Nkwaleni	CBS
	28 Sept 2011	Hoedspruit	CBS
	29 Nov 2011	Nelspruit	CBS
Vahrmeijer, T.	18 Aug 2011	Addo	Citrus Transformation Farmers day
	12 Sept 2011	Swellendam	Fertilization
		Breederivier	Fertilization
	13 Sept 2011	Paarl/Stellen/Swartland and Citrusdal	Fertilization
	14 Sept 2011	Benede Oranjerivier	Fertilization
	15 Sept 2011	Vaalharts	Fertilization

## 8.8 OTHER MEANS OF TECHNOLOGY TRANSFER

### 8.8.1 SA Fruit Journal by Tim G Grout (CRI)

Every exporting citrus grower receives the SA Fruit Journal so it is one of the best means of transferring technology on technical issues. Bimonthly Extension Briefs are edited by Hennie le Roux and Hannes Bester and provide reminders of practices that need to be implemented at that time. All researchers contribute to these on a regular basis. In-depth, semi-scientific research articles are also provided that are usually of a practical nature and other topical or news articles are sometimes included. The citrus articles published in the SA Fruit Journal during 2011/2 are listed in Table 8.8.1.1. Due to the lag time of two months between submission of the articles and circulation of the journal, urgent information is circulated to growers as Cutting Edge or Snykant articles via CRInet and emails to the technology transfer groups.

**Table 8.8.1.1.** SA Fruit Journal articles by CRI Group members during 2011-12.

Issue	Article	Author
April/May 2011	Sunburn reduction on 'Miho Wase' Satsuma mandarin	S. Verreyne & S. van der Merwe
	Na-oes bederf van Sitrusvrugte: duur lesse uit die 2012 pakseisoen	J.J. Bester, H.F. le Roux, K. Lesar, A. Erasmus & P. Fourie
June/July 2011	Postharvest rind breakdown (RBD) of 'nules Clementine' mandarin: Symptom development and factors affecting incidence	P. Cronjé, M. Huysamer & G. Barry
	Voorseisoen pakhuiswerkwinkels wek groot belangstelling in 2011	H.F. le Roux & J.J. Bester
Aug/Sept 2011	Zebediela Citrus Field day	Mbedzi, A.
	Blossom-end clearing in grapefruit	S. Verreyne & K. Lesar
	Koolhidraat-bestuur Seminaar in Letsitele	J.J. Bester, J. Botha, H.F. le Roux & S. Verreyne
Oct/Nov 2011	No articles besides Extension Briefs	
Dec/Jan 2012	Sundays River Valley Citrus Field Day	
	The <i>Bactrocera invadens</i> surveillance programme in South Africa	A. Manrakhan, L. Brown, J-H. Venter, W. Stons & J-H. Daneel
	Entomopathogenic nematodes show excellent potential to control soil life stages of false codling moth and can use the adult moth for aerial transport over long distances	A.P. Malan & S.D. Moore
Feb/Mar 2012	Performance of Star Ruby Grapefruit on various rootstocks at Letaba Estates, Letsitele (2003 to 2007)	J. Joubert, A. Lee & R. Fenwick

Research on strawberries wins CRI's award at Science Expo	T.G. Grout
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### 8.8.2 CRI website by Tim G Grout (CRI)

During 2011/2 the numbers of unique visitors and total visits increased compared with the previous year and the numbers of pages and hits also increased. Most visits were from South African IP addresses followed by dot-net, German IP addresses and dot-com. Following these, the next highest numbers of hits were obtained from Turkey, Argentina and Australia. After Citrus Research International and CRI, *Bactrocera invadens* was the third most popular search phrase leading to our website. Statistics on usage are shown in Table 8.8.2.1.

**Table 8.8.2.1.** Visits and page requests on [www.cri.co.za](http://www.cri.co.za) since April 2011.

Month	Unique visitors	Number of visits	Pages	Hits	Bandwidth
Apr 2011	1089	1533	9014	24233	339.86 MB
May 2011	1058	1599	8924	23544	252.33 MB
Jun 2011	1005	1626	9139	23108	215.13 MB
Jul 2011	913	1540	8603	20188	209.82 MB
Aug 2011	948	1632	50240	63660	677.23 MB
Sep 2011	1063	1852	8865	20038	199.39 MB
Oct 2011	1034	1795	10089	21086	298.03 MB
Nov 2011	1101	1848	9633	19504	313.05 MB
Dec 2011	901	1609	7762	13723	190.60 MB
Jan 2012	996	1746	8904	19179	268.60 MB
Feb 2012	947	1490	8901	20553	306.23 MB
Mar 2012	971	1513	12452	23248	307.16 MB
<b>Total</b>	12026	19783	152526	292064	3577.43 MB

### 8.8.3 CRInet by Tim G Grout (CRI)

Usage of CRInet increased slightly over the report period due mostly to a few animated discussions in June 2011 and March 2012 (Table 8.8.3.1). It provides a good opportunity for growers to share opinions on any technical citrus topic. Membership has now passed 450.

**Table 8.8.3.1.** Numbers of messages circulated per month on CRInet.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2012	5	1	19										
2011	14	3	5	2	8	24	2	3	3	2	2	2	70
2010	0	1	5	3	2	0	6	12	9	4	9	3	54
2009	1	7	3	6	11	0	6	8	4	2	1	2	51
2008	3	6	1	8	5	2	7	3	3	5	3	4	50
2007	5	2	7	1	1	2	4	2	5	4	3	3	39
2006	18	3	1	2	13	9	9	2	1	2	13	2	75

### 8.8.4 Cutting Edge by Tim G Grout (CRI)

During 2011 the Cutting Edge had its 10<sup>th</sup> anniversary. Some growers consider it to be the most valuable means of communication from CRI, perhaps because it always contains urgent information and is to the point. Past issues of the Cutting Edge can be downloaded from the member area of the CRI website. Topics covered in 2011/2 are given in Table 8.8.4.1.

**Table 8.8.4.1.** Cutting Edge issues during 2011-12.

No.	Title	Issue	Author
116	Blossom-end clearing in grapefruit	May 2011	S. Verreynne & K.H. Lesar
117	Quality assurance and citrus export standards update	May 2011	P. Hardman (CGA)
118	Phyophthora brown rot warning Phytophthora bruinvrot waarskuwing	June 2011	M.C. Pretorius
119	Status of the invasive fruit fly- <i>Bactrocera invadens</i> - in South Africa	July 2011	A. Manrakhan & V. Hattingh

120	Food Safety Update	July 2011	P. Hardman (CGA)
121	Revised Terminology for the Citrus Improvement Scheme's Cultivar List	Aug 2011	CIS Advisory Committee
122	Interceptions of the exotic fruit fly, <i>Bactrocera invadens</i> , in Weipe and Groblersbrug areas, Limpopo province, South Africa	Aug 2011	A. Manrakhan & V. Hattingh
123	Food Safety Update	Sept 2011	P. Hardman (CGA)
124	Thrips management affects FCM populations	Sept 2011	S. Moore & T. Grout
125	CGA Consumer Assurance Update	Sept 2011	P. Hardman
126	Decay caused by the Latent Citrus Pathogens viz. Anthracnose, Diplodia and Phomopsis	Oct 2011	K.H. Lesar & P.Cronjé
127	The importance of molasses for FCM virus efficacy	Nov 2011	S. Moore & W. Kirkman
128	The essential foundation for successful FCM management	Dec 2011	S. Moore
129	Citrus Improvement Scheme	Dec 2011	S.R. Meyer (CGA)
130	Food Safety Update	Jan 2012	P. Hardman (CGA)
131	Update on Status of <i>Bactrocera invadens</i> in Limpopo Province, South Africa	Jan 2012	A. Manrakhan & V. Hattingh
132	Phytophthora warning	Jan 2012	M.C. Pretorius & H.F. le Roux
133	Sampling procedure and costs for <i>Phytophthora</i> and citrus nematode analysis and latest price list for services rendered by the Diagnostic Centre	Mar 2012	M.C. Pretorius & E. Liebenberg
134	Surveillance monitoring of <i>Bactrocera invadens</i> per registered Production Unit Code (PUC)	Mar 2012	A. Manrakhan & V. Hattingh

## 9 PUBLICATIONS IN 2011-12

### 9.1 REFEREED PUBLICATIONS (OR ISI RANKED JOURNALS)

- Basson, C.H., Nyamukondiwa, C. & Terblanche, J.S. 2012. Fitness costs of rapid cold-hardening in *Ceratitidis capitata*. *Evolution* 66(1): 296-304.
- Cronje, Paul J.R., Barry, Graham H. & Huysamer, Marius. 2011. Fruiting position during development of 'Nules Clementine' mandarin affects the concentration of K, Mg and Ca in the flavedo. *Scientia Horticulturae* 130:829–837.
- Cronje, Paul J.R., Barry, Graham H. & Huysamer. 2011. Postharvest rind breakdown of 'Nules Clementine' mandarin is influenced by ethylene application, storage temperature and storage duration. *Postharvest Biology and Technology* 60:192–201.
- Dzikiti, Sebinasi, Verreyne, Stephan J., Stuckens, Jan, Strever, Albert, Willem W. Verstraeten, Swenne, Rony, Theron, Karen I., Coppin, Pol. 2011. Seasonal variation in canopy reflectance and its application to determine the water status and water use by citrus trees in the Western Cape, South Africa. *Agricultural and Forest Meteorology* 151:1035–1044.
- Erasmus, Arno, Lennox, Cheryl L., Jordaan, Hennie, Smilanick, Joseph, L., Lesar, Keith, Fourie, Paul H. 2011. Imazalil residue loading and green mould control in citrus packhouses. *Postharvest Biology and Technology* 62:193–203.
- Goble, T.A., Dames, J.F., Hill, M.P. & Moore, S.D. 2011. Investigation of native isolates of entomopathogenic fungi for the biological control of three citrus pests. *Biocontrol Science and Technology*, 21(10):1193-1211.
- Grout, T.G., Daneel, J-H., Mohamed, S.A., Ekese, S., Nderitu, P.W., Stephen, P.R. & Hattingh, V. 2011. Cold susceptibility and disinfestation of *Bactrocera invadens* (Diptera: Tephritidae) in oranges. *J. Econ. Entomol.* 104(4):1180-1188.
- Grout, Tim G., Stephen, Peter R., Daneel, John Henry, Hattingh, Vaughan. 2011. Cold Treatment of *Ceratitidis capitata* (Diptera: Tephritidae) in Oranges Using a Larval Endpoint. *J. Econ. Entomol.* 104(4): 1174-1179
- Magwaza, Lembe S., Opara, Umezuruike Linus, Nieuwoudt, Hélène, Cronje, Paul J.R., Saeys Wouter, Nicolaï, Bart. 2012. NIR Spectroscopy Applications for Internal and External Quality Analysis of Citrus Fruit—A Review. *Food Bioprocess Technol.* 5(2):425-444.
- Malan, A., Knoetze, R., Moore, S.D. 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards in South Africa and their biocontrol potential against false codling moth. *Journal of Invertebrate Pathology* 108:115–125.
- Manrakhan, A. & Kotze, C. 2011. Attraction of *Ceratitidis capitata*, *C. rosa* and *C. cosyra* (Diptera: Tephritidae) to proteinaceous baits. *Journal of Applied Entomology*, 135:98-105.

- Manrakhan, A. Hattingh, V., Venter, J-H & Holtzhausen, M. 2011. Eradication of *Bactrocera invadens* (Diptera: Tephritidae) in Limpopo Province, South Africa. *African Entomology* 19(3):650-659.
- Pereira-da-Conceicao, L.L., Hill, M.P. & Moore, S.D. 2012. Development of a droplet-dose bioassay laboratory technique for *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *African Entomology* 20(1): 187-190.

## 9.2 SEMI-SCIENTIFIC PUBLICATIONS (other than SA Fruit Journal)

- Stuckens, J., Swennen, R.L., Coppin, P., Dzikiti, S., Verreyne, S. & W.W. Verstraeten. 2011. Extracting Physiological Info from a Hyperspectral Time Series of a Citrus Orchard. *Acta Hort* 919:11-18.
- Verreyne, J.S., Mupambi, G. 2010. Effects of 2,4-D on the size of the Navel End Opening and Fruit Quality of Navel Oranges. XI International Symposium on Plant Bioregulators in Fruit Production, Bologna, Italy. *Acta Horticulturae* 884: 745-751.

## 10 PRESENTATIONS AT SOCIETAL AND INTERNATIONAL CONGRESSES

- Chambers, C. & Moore, S.D. 2011. Commercial production of virus on mass reared insect hosts. In: Proceedings of the XVII Congress of the Entomological Society of Southern Africa, 3-6 July 2011, Bloemfontein, South Africa, p 24.
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